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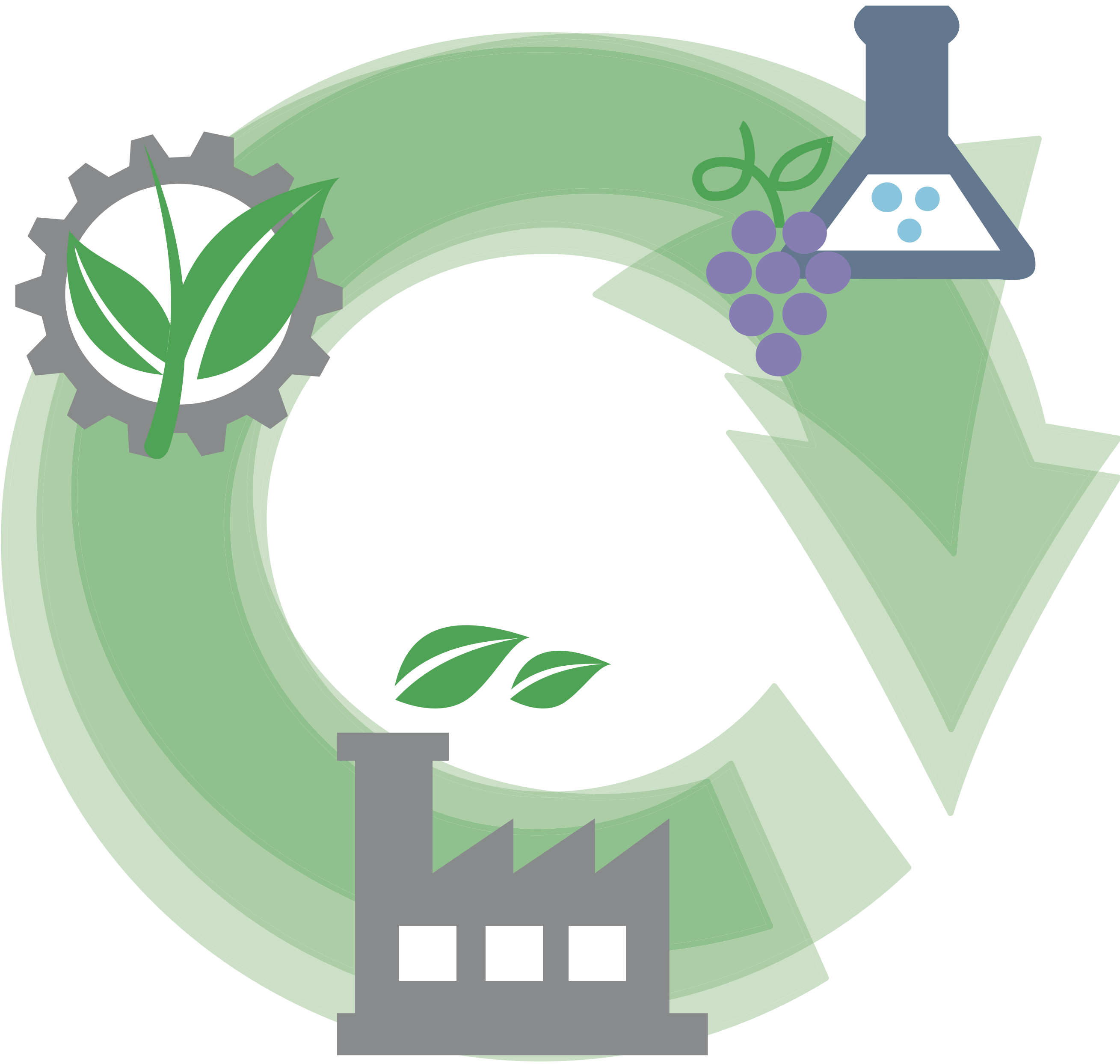

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Effect of plot size and plant spatial arrangement on the efficiency of family selection in sugarcane *(Saccharum officinarum)*

Efecto del tamaño de la parcela y la disposición espacial de la planta sobre la eficiencia de la selección familiar en la caña de azúcar *(Saccharum officinarum)*

Aparecido de Moraes [1], Matheus Henrique Silveira Mendes [2], Mauro Sérgio de Oliveira Leite [3], Regis de Castro Carvalho [2], Flávia Maria Avelar Gonçalves [2]

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Abstract

The purpose of this study was to identify the ideal sample size to represent the potential of a sugarcane family for identification of superior families, and, at the same time, determine which spatial arrangement may provide better phenotypic expression of the progeny of the sugarcane families tested. Thus, five full-sib families, each with 360 individuals, were evaluated in a randomized block design with three replications. The plants were placed in three different spatial arrangements in the row: 50 cm, 75 cm, and 100 cm between plants; with 150 cm between the rows. The bootstrap method was adopted to determine the ideal sample size, as well as the best spacing for evaluation. The 100-cm spacing provided the best mean values for number of stalks, mean stalk diameter, and estimated weight of stalks in the stool. The 75-cm spacing between plants within the row allowed better discrimination among the families for all the traits evaluated. At the 75-cm plant spacing, it was also possible to identify superior families with a sample of 30 plants from each plot and 3 reps in the trial.

Keywords
Plant breeding • experimental design • breeding strategy • bootstrap

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Resumen

El propósito de este estudio fue identificar el tamaño de muestra ideal que representa a una familia en su potencial, identificar familias superiores y, en paralelo, determinar qué disposición espacial puede tener una mayor precisión en cuanto a la selección de nuevas variedades de caña de azúcar. Para tal fin, se evaluaron cinco familias de hermanos completos, cada una con 360 individuos, en diseño de bloques al azar, con tres repeticiones y tres distancias diferentes entre las plantas en una misma hilera (50 cm, 75 cm y 100 cm). Se mantuvieron 150 cm entre hileras. Para determinar el tamaño ideal de la muestra, así como la mejor distancia para la evaluación, se adoptó el método bootstrap. Se observó que distancias de 100 cm resultaron en el mejor valor promedio de números de tallo, diámetro del tallo y peso estimado de los tallos en las heces. La separación de 75 cm entre las plantas permitió un mejor poder de discriminación entre las familias para todos los caracteres evaluados. Con 75 cm también fue posible identificar familias superiores con una muestra de 30 plantas en cada parcela y 3 repeticiones en el ensayo.

Palabras clave

Fitomejoramiento • diseño experimental • estrategia de mejoramiento • bootstrap

Introduction

Over the past 50 years, sugarcane yield has increased by approximately 40%, especially due to breeding. However, from eight to twelve years are required to obtain a new sugarcane cultivar through the breeding process (16). Continual efforts should be made to increase the efficiency of breeding programs, and early selection is an example of this (13).

Sugarcane selection starts with the identification and cloning of superior genotypes. Several steps are performed in this selection, ranging from the choice of desirable parents to quantification of genetic effects in expression of the trait evaluated (8, 16).

Desirable traits identified in superior genotypes are often controlled by many genes. Even relying on newly available tools (such as databases, parent history, and software), it is hard for breeders to precisely define the best parents for hybridizations (18, 21). Therefore, a large number of crosses are necessary to increase the chances of obtaining the best combinations for generating promising progenies.

In the early stages of clonal selection, superior families were identified, followed by mass selection of the best individuals within the families. This provided greater genetic gains and greater frequency of superior genotypes (5, 27). Sugarcane breeding programs in several countries have used this methodology (2, 5, 13).

Families are selected through collection of their phenotypic data. The best families for continuing selection can be inferred from analysis of this data. An evaluation is usually made from collection of samples to determine sugar content and stalk weight, and yield is estimated in tons of cane per hectare (TCH).

In the first stage of all sugarcane-breeding programs, seedlings are planted that come from seeds originating from the crosses made. These seedlings are planted at defined spacings in the row. The spacings are defined by each program and have consequences on phenotypic expression of traits, such as number, diameter, and height of stalks, which directly affect the family selection process. Several studies have aimed to identify the most adequate sample size for evaluating families. However, most of these studies were carried out using only one plant spacing in the row; other plant spacings were not tested. Family evaluation is one of the most expensive stages in a commercial breeding program. Thus, studies have sought to optimize resources through reducing plot size. According to Durner (1989), experimental plot size is a key decision for optimizing the use of the experimental area. This optimization allows genotype evaluation with a larger number of reps and sites. Some strategies have already been evaluated to check the viability of performing selection in the initial stages of a sugarcane-breeding program (19). It has already been observed that for traits related to sugar content, a one-row plot was as efficient as a two-row plot (12). In the literature, recommendations for sample size in sugarcane experiments range from 20 to 150 plants per family (1, 15). Since researchers' suggestions vary considerably, studies must be conducted to determine the ideal sample size to represent a family.

The aims of the present study were to identify the ideal sample size that represents the potential of a family and to determine the best spacing between plants to improve selection.

Materials and methods

The seeds of the sugarcane families used in this study were obtained from five biparental crosses made at the Canavialis hybridization site on the Vale das Flores farm, Maceió, AL, Brazil, during the 2014 season. These seeds were sent to the regional Canavialis site in Conchal, SP, where seedlings were produced. Each cross was used to produce 360 seedlings, which were sent to the regional station, the Destivale farm in Araçatuba, SP, with geographic coordinates of latitude 21° 6' 20.52" S and longitude 50° 28' 57" W, at an altitude of 430 m. Average temperature is 22.2°C, rainfall is 1,250 mm per year, and soil is classified as a *Latosol Rojo-Amarillo distrófico*.

Experiments were set up in September 2014 with plants spaced at 50 cm, 75 cm, and 100 cm in the row. Each experiment had a completely randomized block design, with six treatments (five families plus one standard commercial cultivar, RB867515), three replications, and a 1.5 m between-row spacing. The seedlings of the standard commercial check cultivar were produced through germination of single billets, like seeds in a greenhouse. This method makes the seedlings produce stools, which are similar to those produced by seeds (seedlings).

Conventional tillage for sugarcane was used for field planting. Furrows were opened and fertilizer was applied in the furrows at a dosage of 500 kg/ha of a 05-25-25 (N-P-K) formulation. Seedlings were planted manually at different plant spacings in the row. A distance of 2.5 m was maintained between plots, and borders of the experiment were planted with sugarcane to protect the perimeter. Sprinkler irrigation was performed three times at 10 mm each to ensure 100% seedling survival.

Data was collected in July 2015 from sugarcane at 10 months of age for the following traits: (i) number of stalks: obtained through counting the total number of stalks (fully developed) in each stool (seedling); (ii) stalk diameter: obtained by measuring three representative stalks in the stool at 1/3 stalk height with a digital caliper; (iii) stalk height: obtained by measuring three representative stalks in the stool from soil level to the base of the dewlap of the leaf +3.

Based on these traits, the mean stalk weights were estimated, obtained through the following biometric formula:

$$PE = d \times \pi \times AC \times (DC/2)^2$$ proposed by Chang and Milligan (1992),

where:

PE = estimated stalk weight;

d = the specific density of the stalk, whose suggested value is 1 g/cm^3;

π = the dimensional value of approximately 3.1415;

AC = the height of the stalk from its base to the last visible dewlap;

DC = stalk diameter expressed in centimeters, measured at 1/3 representative stalk height.

Along with stalk weight, stool weight was estimated, and subsequently, sugarcane stalk weight for the plots.

For statistical analysis, individual analyses of variance were performed for each trait in each spacing, and combined analyses of variance for all spacings. First, individual analyses of variance were performed for each trait within each spacing arrangement. Upon meeting the required premises, combined analyses were performed according to the following model:

$$y_{ijkl} = \mu + f_i + a_k + fa_{ik} + r_j(a_k) + f_i r_j(a_k) + d_{ijk}$$

where:

y_{ijkl} = is the observation of individual l, family i, block j, and spacing k;

μ = the general mean;

f_i = the effect of family i;

a_k = the effect of spacing k;

fa_{ik} = the interaction of family i and spacing k;

$r_j(a_k)$ = the effect of replication j within spacing k;
$f_i r_j(a_k)$ = the interaction effect of family i in replication j within each spacing k;
d_{ijk} = the effect of individual l within family i and spacing k.

Different scenarios were evaluated in each of the family evaluation experiments in the three different spacings, modifying the number of individuals in the plot from 1 to 40. For each scenario, 1000 bootstraps were performed, and each bootstrap simulated a new experiment. It should be noted that in each simulated experiment, bootstraps were performed with no replacements. With the simulated experiment, analysis of variance was performed using the fixed model, and some parameters were estimated as follows:

$$\%CVe = \frac{100\sqrt{S^2}}{\hat{m}}$$

where:
CVe = the value of the experimental coefficient of variation;
S^2 = the variance;
m = the mean of the trait;
probability value;

$$F_c = \frac{QM_G}{QM_E}$$

where*:*
F_c = the calculated F value;
QM_G = the mean square of the genotype;
QM_E = the mean square of the error;
repeatability;

$$\hat{r}_{\hat{g}g} = (1 - \frac{1}{F_c})^{0,5}$$

where:
$\hat{r}_{\hat{g}g}$ = the selective accuracy;
F_C = the calculated F value;
the ranking between the phenotypic means of the families in the simulated experiments was compared to the ranking of the three best families obtained in the experiment with the total number of plants in the plot.

The procedure was repeated for the three spacing arrangements. All data analysis was performed using R software (11).

Results and discussion

Significant differences among families were observed in analysis of the different spacings ($P < 0.05$) for number of stalks, stalk height, and stalk diameter; and for stalk weight, only the 75-cm spacing showed significant differences. These results show significant genetic variability among families, which allows successful selections.

The experimental coefficient of variation (CVe) observed for all traits at the three plant spacings are low in magnitude, showing good experimental quality. Most of the CVe's are below 10%, values indicated as low for agricultural experiments by Pimentel-Gomes (2009). Barbosa *et al.* (2005) and Leite *et al.* (2006) obtained similar values for the same traits in working with sugarcane families.

It is noteworthy that the combined analyses for all the traits analyzed showed a significant effect for the spacing and family sources of variation, but for the spacing × family interaction, only stalk height showed significant differences (table 1, page 5). According to Ramalho *et al.* (2012), non-significance of interaction means that the response of families coincided in the different spacings evaluated.

The purpose of this type of experiment is first to make selection among families and then proceed with individual selection within the best families. When significant differences were detected among the families evaluated, a means clustering test was performed (25). For number of stalks, only the 100-cm spacing exhibited three distinct clusters, and it had the highest mean family values. Stalk height also exhibited three distinct clusters at the 100-cm spacing, but the highest mean values were found at the 50-cm spacing. In the case of stalk diameter, all three spacings showed three distinct clusters, with the highest mean values of families for the 100-cm spacing. For stalk weight, there was no cluster formation by the Scott Knott test at 5% at the 50-cm spacing; however, at the 75-cm and 100-cm spacings, two distinct clusters were observed, and the highest mean values were observed at the 100-cm spacing. For stalk weight, which is the most important trait for sugarcane selection, the 75-cm and 100-cm spacings were the most efficient in discriminating families. The 75-cm spacing was able to discriminate the families in three distinct clusters (table 2, page 6). It is expected that the spacing that allows formation of a larger number of distinct clusters also allows greater possibility of discriminating families.

Table 1. Combined analysis of variance considering three different spacings among the plants in the row for the following traits for sugarcane families: number of stalks, stalk height (cm), stalk diameter (cm), and stalk weight (kg).

Tabla 1. Análisis conjunto de varianza considerando tres espacios diferentes entre las plantas en la línea para el número de caracteres de los tallos, la altura de los tallos (cm), el diámetro de los tallos (cm) y el peso de los tallos (kg) para las familias de caña de azúcar.

SV	DF	MS			
		No. of stalks	Height	Diameter	Weight
Spacing (S)	2	67.081 **	453.750 *	0.179 **	68.751 **
Family (F)	5	19.212 **	2418.790 **	0.463 **	5.949 **
S x F	10	0.908 ns	215.230 *	0.009 ns	0.926 ns
Error	30	0.674	93.050	0.006	0.782
Mean		10.357	208.049	2.221	8.906
CV (%)		9.610	6.129	4.226	11.060

ns : not significant; *and **: significant by the F-test at 5% and 1% probability, respectively.

ns : no significativo; *, ** Significativo por la prueba F, con 5% y 1% de probabilidad, respectivamente.

The data indicate that the ideal spacing among plants in the row is not the same for all individual traits, since this spacing varies depending on the trait concerned. For number of stalks, the 75-cm spacing discriminated more families, whereas for stalk height, the best spacing was 50 cm. In contrast, all three spacings showed efficient discrimination for stalk diameter. Stalk weight in the stools, known as TCH (tons of cane per hectare), is estimated from the three variables mentioned above and is the basis for yield estimates in sugarcane experiments. For TCH, the 75-cm spacing was the only one that exhibited three distinct clusters by the Scott-Knott test, proving to be more suitable for evaluation of families.

In a study carried out in the state of Louisiana, USA, comparing the efficiency of sugarcane selection methods at two different spacings (41 and 82 cm between plants in the row) and 180 cm between rows, Sousa-Vieira and Milligan (2009) concluded that the 82-cm spacing was more efficient in selection for weight of stools. That result corroborates the result in this study of the 75-cm spacing between plants in the row being the most promising. It is important to emphasize that the capacity for genotype discrimination is closely related to experimental accuracy (22). According to Pedrozo *et al.* (2008), experiments should be as accurate as possible to permit efficient selection. However, G × E interactions will always be present, and the family selected in one year will probably not be the best in another year.

Study of parameters through bootstrapping

A simulation method by bootstrapping was used to determine the ideal sample size for representing a family in a trial plot. Since the two main traits in this study were the number of stalks per stool and stalk weight, both of them were the target traits of the simulation study.

In an attempt to aid decision making on the minimum size of the plot, the following criteria were established to guide interpretation of the graphs obtained: a) The CV should be below 10%. Pimentel-Gomes (2009) consider that CVs below 10% are low in agricultural experiments. b) A probability value (*p* value) should be established at the significance level of 5% – the same applies to the F-test value. c) The repeatability value should be above 80%. According to Resende (2002), these values may be considered appropriate for this parameter. d)

Accuracy value should be above 80%. According to Resende (2007), for selective processes in plant breeding programs, values above 70% are considered high selective accuracy for experiments. e) The family rank values should coincide. Coincidence is obtained from comparing the rank of families in each bootstrapping scenario (1 to 39 plants) and the rank of families in total evaluation of the plot (40 plants). An acceptable coincidence value is 90% or higher.

Table 2. Clustering of means for the following traits for sugarcane families: number of stalks, stalk height, stalk diameter, and stalk weight, considering combined analysis for three different spacings (cm) between the plants in the row, carried out by the Scott-Knott test at 5% probability.

Tabla 2. Agrupación de promedios para caracteres número de tallos, altura de los tallos, diámetro del tallo y peso de los tallos para familias de caña de azúcar, considerando el análisis conjunto de tres espacios diferentes entre las plantas (cm) en la línea, realizado por la prueba de Scott-Knott al 5% de probabilidad.

Number of stalks								
50-cm spacing			**75-cm spacing**			**100-cm spacing**		
Family	Mean		Family	Mean		Family	Mean	
44	9.64	aC	44	12.66	aB	44	14.16	aA
57	9.17	aB	57	12.52	aA	57	13.44	aA
51	8.27	bC	51	11.06	bB	51	12.83	bA
49	7.73	bB	49	10.33	bA	66	11.81	bA
66	7.72	bC	66	10.01	bB	49	10.29	cA
Test.	6.68	bB	Test.	9.01	bA	Test.	9.09	cA
Stalk height								
50-cm spacing			**75-cm spacing**			**100-cm spacing**		
Family	Mean		Family	Mean		Family	Mean	
49	234.15	aA	Test.	233.55	aA	49	235.39	aA
51	226.32	aA	49	233.52	aA	Test.	224.28	aA
Test.	223.89	aA	51	225.06	aA	51	210.56	bA
57	220.21	aA	44	200.86	bA	66	204.79	bA
66	205.38	bA	57	198.72	bB	57	191.42	cB
44	198.43	bA	66	190.46	bA	44	181.85	cB
Stalk Diameter								
50-cm spacing			**75-cm spacing**			**100-cm spacing**		
Family	Mean		Family	Mean		Family	Mean	
Test.	2.55	aB	Test.	2.58	aB	Test.	2.8	aA
66	2.22	bB	66	2.21	bB	49	2.38	bA
49	2.09	cB	51	2.21	bA	66	2.36	bA
51	2.07	cB	49	2.12	bB	51	2.22	cA
57	2.01	cB	44	2	cA	57	2.15	cA
44	1.98	cA	57	1.93	cB	44	2.11	cA
Stalk weight								
50-cm spacing			**75-cm spacing**			**100-cm spacing**		
Family	Mean		Family	Mean		Family	Mean	
Test.	7.78	aB	Test.	11.14	aA	Test.	12.43	aA
57	6.91	aB	51	9.73	aA	51	10.96	bA
44	6.78	aB	44	8.99	bA	66	10.7	bA
51	6.77	aB	49	8.61	bB	49	10.55	bA
66	6.61	aB	57	8.58	bA	44	10.11	bA
49	6.35	aC	66	7.5	cB	57	9.81	bA

Means followed by the same lowercase letter in the column and by the same uppercase letter in the row constitute statistically homogeneous clusters by the Scott-Knott test ($p < 0.05$).

Los promedios seguidos de la misma letra minúscula en la columna y de la misma letra mayúscula en la línea constituyen un grupo estadísticamente homogéneo según la prueba de Scott-Knott (p <0,05).

For the CV for the number of stalks trait, the desirable value (below 10%) was achieved in 16-plant samples at a 75-cm spacing, and in 31-plant samples at a 50-cm spacing. At the 100-cm spacing, the desirable CV value was only reached in the entire 40-plant sample (figure 1). For stalk weight, the desirable value was achieved with 25 plants at the 75-cm spacing and 35 plants at the 50-cm spacing. For the 100-cm spacing, this value was only achieved with the entire 40-plant sample (figure 2).

Samples with 11 plants reached the *p* value of 5% significance at the 75-cm and 100-cm spacings for the number of stalks trait. At the 50-cm spacing, these *p* values were reached with 18 plants (figure 1). Regarding stalk weight, 25-plant samples already achieved this *p* value at the 75-cm spacing; the 50-cm and 100-cm spacings did not achieve this value. This indicates the lower efficiency of these spacing to discriminate families (figure 2).

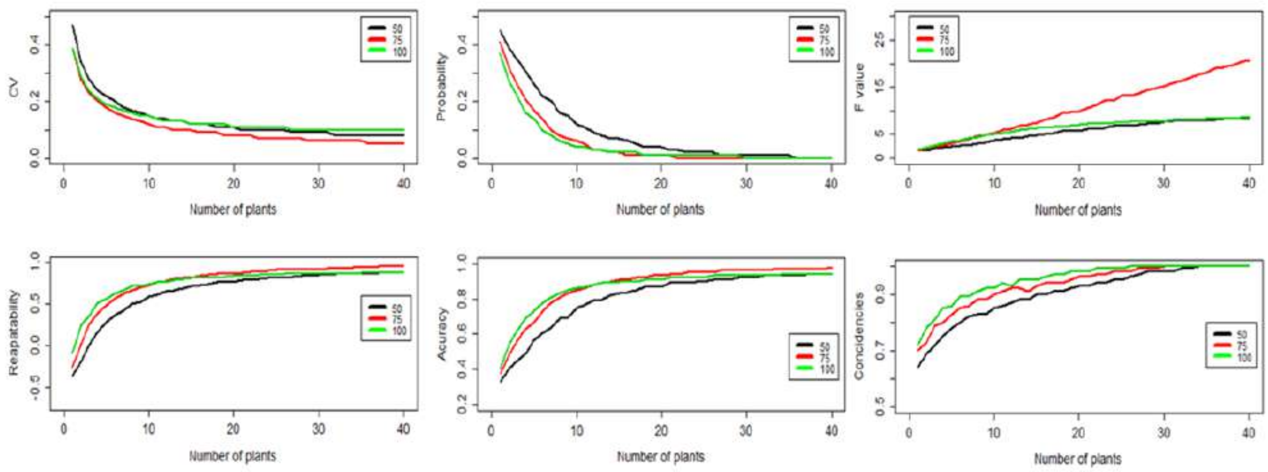

Figure 1. Mean of data from one thousand bootstrapping for the parameters of coefficient of variation (CV), probability, repeatability, F-value, and coincidence of family rank for each sample size (number of plants) for the number of stalks trait.

Figura 1. Promedio de datos del millar de bootstrapping para los parámetros Coeficiente de variación (CV), Probabilidad, Repetibilidad, Valor F y Coincidencia de las familias para cada tamaño de muestra (número de plantas) para el número de caracteres de los tallos.

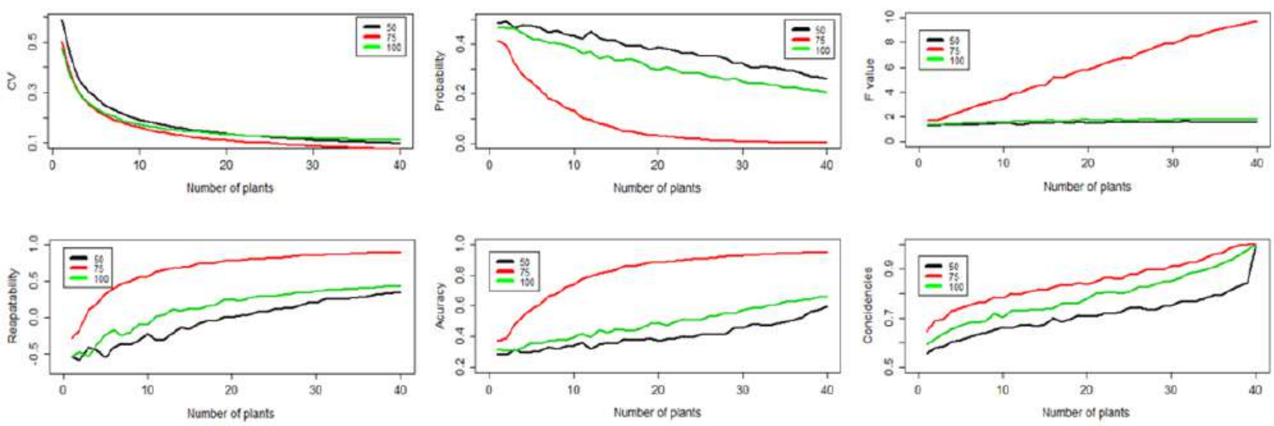

Figure 2. Mean of data from one thousand bootstrapping for parameters of the coefficient of variation (CV), probability, repeatability, F-value, and coincidence of family rank for each sample size (number of plants) for the stalk weight trait.

Figura 2. Promedio de datos del millar de bootstrapping para los parámetros Coeficiente de variación (CV), Probabilidad, Repetibilidad, Valor F y Coincidencia de las familias para cada tamaño de muestra (número de plantas) para el peso del carácter de los tallos.

Regarding the F-value calculated for number of stalks and stalk weight, greater increases were observed for the 75-cm spacing, due to the increase in the number of plants in the plot. For the other spacings, increases in the number of plants did not lead to substantial increases in the estimates of the F-value (figures 1 and 2, page 7).

Repeatability values above 80% were reached with 14-plant samples for the 75-cm and 100-cm spacings and with 23-plant samples for the 50-cm spacing. Accuracy values, also considering values above 80%, were reached with 8-plant samples for the 75-cm and 100-cm spacings, and with 13-plant samples for the 50-cm spacing (figure 1, page 7). For stalk weight, the repeatability values were reached with 30-plant samples for the 75-cm spacing. If selection were performed based on number of stalks, it would be possible to obtain high accuracy values (> 80%) with only 10 plants in the plot for the 75-cm and 100-cm spacings. It is important to emphasize that in these experiments, selection is usually performed considering the weight of stalks in the plot. For that trait, values above 80% were found at the 75-cm spacing with a minimum of 25 plants in the plot. At the 50-cm and 100-cm spacings, no high values of accuracy and repeatability were observed (figure 2, page 7). For the number of stalks and stalk weight traits, the 50-cm spacing between plants in the row was less efficient in discriminating families.

For family rank coincidence, values above 90% were observed with samples of 8 plants at the 100-cm spacing, 10 plants at the 75-cm spacing, and 16 plants at the 50-cm spacing. Regarding stalk weight, coincidence levels above 90% were observed when more than 35 plants were used in the plot for the 75-cm spacing (figure 2, page 7). For other spacings, the rank coincidences were always low. Mendes *et al.* (2014), working with eucalyptus, estimated rank coincidence above 90% and found 55 plants for plot size.

From the perspective of efficiency and accuracy of selection, the 50-cm spacing showed the least satisfactory results. When the 75-cm spacing is used, the number of plants for evaluation in the plot can be reduced from 40 to 15, without reducing the efficiency of selection. These results corroborate those obtained by Leite *et al.* (2009), who evaluated full-sib families of sugarcane and observed that 16 plants per plot would be enough to obtain reliable parameters for the traits under study.

Based on the results for weight of stalks in the stools (which is the most important trait since it includes all the previous traits, and recommendation of the highest yielding families is obtained from it), the minimum number of plants to be sampled that are sufficient for recommending the best families would be 30 plants for the 75-cm spacing and 35 plants for the 100-cm spacing. These would be the numbers of plants recommended to provide family rank coincidence values above 90% and accuracy values above 90% for the 75-cm spacing and above 70% for the 100-cm spacing. Leite, Peternelli, and Barbosa (2006), working with sugarcane families at a 50-cm spacing between plants and evaluating sugar content; the diameter, height, and number of stalks; and the mean weight of sugarcane stools, concluded that a sample of 16 plants per plot in a trial with six replications would be sufficient to represent the families. Espósito *et al.* (2012), also working with sugarcane families at a 50-cm spacing, estimated individual weights and concluded that a 30-plant sample per plot with three replications would be sufficient to indicate the best families.

In other species, such as Eucalyptus, it has already been confirmed that decreases in plot size from 100 to 25 plants does not affect accuracy of selection and experimental precision, and such decreases may reduce costs in the breeding program (17). Working with brachiaria grass, Dias *et al.* (2014) measured fresh biomass volume and carried out analysis through the bootstrapping method and the maximum curve of the coefficient of variation. They concluded that 3-m^2 plots were enough to ensure experimental precision when half-sib progenies were used. This reduction in plot size allows genotype evaluations in a larger number of places so the genotype × environment (GE) interaction can be more effectively determined. Gilbert *et al.* (2006) and Bernardo (2010) defended reduction in plot size and a larger number of sites and also observed the need for continuous evaluation of genotypes in several places in both the early steps and final steps of breeding programs.

From all these results and considering selective accuracy as the guiding parameter for decision making, the highest values obtained considering the number of stalks and weight of stalks in the stools were found at the 75-cm spacing. Comparing the bootstrapping results with those obtained in combined analysis of the three spacings, the 75-cm spacing had the highest power of discrimination among families, indicating that this spacing is the most efficient in evaluating sugarcane families for selection purposes. Souza-Vieira and Milligan (2009) argued that higher selection efficiency would be obtained when making use of larger spaces between plants, rather than more consolidated experiments. Those results are in accordance with the results of this study, where a less consolidated plant arrangement exhibited better capacity for discriminating families.

Conclusions

Considering the means test, the 75-cm and 100-cm spacings best discriminated the families for stool weight, the most important variable for indicating yield of families.

Considering the bootstrapping results and the 75-cm spacing, a 30-plant sample with three replications is enough to determine the family value.

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Mixed modeling for fiber yield genetic selection in sugarcane (*Saccharum officinarum*)

Selección genética de genotipos de caña de azúcar (*Saccharum officinarum*) hacia rendimiento de fibra mediante modelos mixtos

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Abstract

The current demand for clean and renewable energy has provoked considerable changes in the production system of agro-industrial companies. Bioelectricity generation through sugarcane bagasse burning has considerably risen in recent years. This work aimed to focus on sugarcane genetic selection for fiber productivity, using mixed linear modeling. The experiment was outlined in randomized blocks with four repetitions and sixteen genotypes. The evaluated traits were: cane tons per hectare, sucrose tons per hectare, fiber tons per hectare, fiber content and apparent sucrose content. Heritability coefficients suggested a significant genetic gain, while harmonic means of relative performance of predicted genotypic values allowed the identification of stable genotypes related to the traits evaluated, in four harvest cycles. The current agro-industrial demand for sugarcane varieties considers energy generation and sugar production with fiber content between 12% and 17%, and sucrose content near 13%. With this regard, genotypes EECAC 06, EECAC 03, EECAC 04 and EECAC 07 result interesting commercial cultivation options.

Keywords
biomass • bioenergy • bioelectricity • *Saccharum* spp. • REML/BLUP

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Resumen

La demanda actual de energías limpias y renovables ha provocado cambios considerables en el sistema productivo de las empresas agroindustriales. La generación de bioelectricidad mediante la quema de bagazo de caña de azúcar ha aumentado considerablemente en los últimos años. Este trabajo tuvo como objetivo centrarse en la selección de genotipos de caña de azúcar para la productividad de la fibra. El experimento se esbozó en bloques aleatorios con cuatro repeticiones y se evaluaron dieciséis genotipos. Las caracteristicas evaluadas fueron: toneladas de caña por hectárea, toneladas de sacarosa por hectárea, toneladas de fibra por hectárea, contenido de fibra y contenido aparente de sacarosa. Para la selección se utilizó la metodología de modelos lineales mixtos. Los coeficientes de heredabilidad sugieren una ganancia genética significativa y las medias armónicas de desempeños relativos de los valores genotípicos predichos permitieron la identificación de genotipos estables relacionados con las características evaluadas en cuatro ciclos de cosecha. Considerando la demanda actual de la agroindustria de la caña de azúcar por variedades con contenido de fibra entre 12% y 17% y contenido de sacarosa cercano al 13%, para generación de energía y producción de azúcar, los genotipos EECAC 06, EECAC 03, EECAC 04 y EECAC 07 se presentan como comerciales opciones de cultivo.

Palabras clave

biomassa • bioenergía • bioelectricidad • Saccharum spp. • REML / BLUP

Introduction

Sugarcane is an economically significant crop for various agro-industrial products like sugarcane distilled beverage cachaça, yeast, protein, pharmaceuticals, fodder for animal feed (1, 8, 20), and Brazilian main commercial products, sugar and ethanol, historically providing the greatest economic profitability (3).

The current worldwide higher demand for clean and renewable energy, coupled with growing concern about environmental issues, such as the greenhouse effect and water conservation, require considerable changes in the productive systems of small and large companies (2). Sugarcane biomass is one main alternative for diversifying the energetic matrix and reducing fossil fuels usage, especially in tropical and subtropical countries (10, 24). Nowadays, sugarcane bagasse is used for power generation. This makes sugar mills self-sustainable in electric power, and even able to supply the surplus electricity to surrounding power distribution companies (16). Stimulated by the Brazilian federal governmental RenovaBio program, and a favorable business environment, bioelectricity can potentially grow more than 50% by 2027 (16). This growing demand for biomass and bioenergy, directly dependent on bioelectricity and second-generation ethanol production, ultimately results from fiber content and composition of sugarcane varieties. This has inspired new breeding programs focusing on new varieties with higher fiber content (4, 23).

In this context, some studies have already selected families in phase T1, with no evaluation during ratoons (6, 19, 20, 23). However, in technical and practical terms, evaluating fiber productivity is key not only in plant cane, but mainly along ratoons, identifying the most stable clones and proceeding with commercial recommendations. In the state of Pernambuco, one traditional sugarcane producer in Brazil, no studies have approached this topic.

According to Tew and Cobil (2008), the currently cultivated varieties are constituted by about 12% fibers, 13% sugars and 75% water. These authors also state that genetic breeding could possibly develop new materials with up to 30% fiber, 5% sugar and 65% water. However, according to Fernandes Júnior *et al.* (2017), sugar mills are not yet ready to process biomass with more than 20% fiber, but newly developed varieties with around 17% fiber, and sucrose near 13%, would attend what the current sugar-energy infrastructure offers. These authors also state that this type of fiber-cane variety would also increase electricity production capacity, due to the higher fiber percentage. Consequently, bagasse heat/calorific power quality would also increase, without losses in sugar production. In view of the aforementioned needs, this work aimed to select sugarcane genotypes for potential fiber productivity, using mixed linear models.

Material and methods

Experimental design

Sixteen genotypes were evaluated: ten promising clones from the sugarcane genetic breeding Program of the Federal Rural University of Pernambuco, affiliated to the Inter-university Network for the Development of the Sugar and Energy Sector (PMGCA/UFRPE/RIDESA), and six standard commercial varieties (table 1).

Table 1. Sugarcane genotypes evaluated for fiber productivity.

Tabla 1. Genotipos de caña de azúcar evaluados para la productividad de la fibra

Genotypes	Obtaining Institution
1. RB813804*	UFRPE
2. RB863129*	UFRPE
3. RB92579*	UFAL (Federal University of Alagoas)
4. EECAC 01	UFRPE
5. EECAC 02	UFRPE
6. EECAC 03	UFRPE
7. EECAC 04	UFRPE
8. EECAC 05	UFRPE
9. EECAC 06	UFRPE
10. EECAC 07	UFRPE
11. EECAC 08	UFRPE
12. EECAC 09	UFRPE
13. EECAC 10	UFRPE
14. SP791011*	COPERSUCAR (Brazilian Sugar and Ethanol Cooperative)
15. SP813250*	COPERSUCAR
16. VAT90212*	SUGARMILL TRIUNFO, Alagoas

* Standard varieties.

* Variedades estándar.

The experiments were conducted in the agricultural area of Pumaty sugar mill, in the municipality of Joaquim Nabuco, in the state of Pernambuco, Brazil, at geographic coordinates 08°37'28"S and 35°32'00"W. A four-repeat randomized block design was used. The experimental plots were composed of five lines, with 8 m between lines, each plot having 40 m^2.

During four harvest seasons (plant crop and 1st, 2nd and 3rd ratoon crops), the following traits were evaluated: sugarcane tons per hectare (STH), sucrose tons per hectare (SucTH), fiber tons per hectare (FTH), fiber percentage (FP) and apparent sucrose percentage (SP).

Sugarcane tons per hectare (STH), sucrose tons per hectare (SucTH) and fiber tons per hectare (FTH) were obtained as:

$$STH = (\text{Total weight experimental plots} \times 10) / \text{plots area in } m^2;$$
$$SucTH = (STH \times SP) / 100;$$
$$FTH = (STH \times FP) / 100.$$

Fiber and apparent sucrose percentage were estimated in the laboratory according to Fernandes (4).

Statistical Analyses

Mixed models (REML/BLUP) estimated variance components and predicted genetic values, according to Resende (2007). Interactions between genotypes x harvest cycles (measurements) were evaluated via REML/BLUP and the statistical model 55 of the Selegen-REML/BLUP software (15), corresponding to:

$$y = Xm + Zg + Wp + Ti + e$$

where:

y = the data vector,

m = effect vector of harvest cycles-repetitions (fixed) combinations added to general mean,

g = genotypic effects vector (aleatory),

p = permanent environmental effect vector (plots, aleatory),

i = vector for genotypes x harvest cycles interaction,

e = error vector or residues (aleatory).

Capital letters represent incidence matrices for the cited effects. The m vector comprises all harvest cycles in all repetitions and adjusts simultaneously to repetitions effects, harvest cycles and harvest cycles interaction x repetitions.

Mean (E) and variance (Var) distributions and structures were:

$$E = \begin{bmatrix} y \\ g \\ p \\ i \\ e \end{bmatrix} = \begin{bmatrix} Xm \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix}; Var \begin{bmatrix} g \\ p \\ i \\ e \end{bmatrix} = \begin{bmatrix} 1\sigma_g^2 & 0 & 0 & 0 \\ 0 & 1\sigma_p^2 & 0 & 0 \\ 0 & 0 & 1\sigma_i^2 & 0 \\ 0 & 0 & 0 & 1\sigma_e^2 \end{bmatrix}$$

Through this model, variance components and genetic parameters were obtained. while genetic predictors (REML/BLUP) were free of genotypes x harvest cycles interaction, according to Resende (15).

Prediction of genotypic values gathering average interaction (gem) in the different Harvest cycles, is given by model: $(\hat{\mu} + \hat{g} + \hat{g}em)$

Calculated by the equation:

$$\hat{\mu}\left\{\left[\frac{\hat{\boldsymbol{\sigma}}_g^2 + \hat{\boldsymbol{\sigma}}_c^2}{\boldsymbol{n}}\right]\hat{\boldsymbol{\sigma}}_g^2\right\}\hat{\boldsymbol{g}}_i$$

where:

u = the overall average of all Harvest cycles;

$\hat{\sigma}_g^2$ = the genotypic variance;

$\hat{\sigma}_c^2$ = the variance of genotype x harvest cycles interaction;

n = the number of harvest cycles; and

$\hat{g}_i$ = the specific genotypic effect (genotype i).

Aiming to verify fiber and apparent sucrose content productivity and genotypic stability through harvest cycles, the harmonic means of relative performances of predicted genotypic values (HMRPPGV), were provided by:

$$HMRPPGV_i = n / (\Sigma_{j=1}^{n} 1/Vg_{ij}),$$

where:

n = harvest cycle number, where genotype i was evaluated

Vg_{ij} = the genotypic value of genotype i, in the harvest cycle j, expressed as mean proportion of each cycle.

The HMRPPGV values were multiplied by a general mean calculated for all environments (GM), resulting in the same magnitude order in which all traits were evaluated, with genetic values already discounted from instability, and capitalized by adaptability.

Genetic-statistical analyses were performed with Selegen software (15).

Results and discussion

Results regarding sugarcane tons per hectare, sucrose tons per hectare, fiber tons per hectare, fiber percentage and apparent sucrose percentage are shown in table 2 (page 15).

Deviance analysis revealed significant differences between genotypes for productivity traits, evaluated during four harvest cycles. These results indicate genetic variability between genotypes concerning the evaluated traits, implying the possibility of selecting superior genotypes, as previously found (14, 26).

High values of genotypic variance for permanent environmental variance were found for STH, SucTH and FTH. In relation to SP, the genotypic variance was higher than the permanent environmental variance and the genotype x harvest cycles interaction variance, suggesting that these traits have genetic over environmental predominance.

Medium and high genotypic correlations throughout harvest cycles suggest genotypic identification, whose phenotypic expression (SucTH and SP), was stable during harvest cycles with a greater predominant genetic component. Regarding SP, it might be inferred that the genotypes have a high potential for ratoon sucrose production. For Silva *et al.*

(2018), it is about regularity in the repetition of this important production component. For Oliveira *et al.* (2017), a complex part of the genotype x environment (harvest cycles) interaction causes traits such as STH and TFH to show a low genotypic correlation between harvest cycles.

Table 2. General mean, genetic traits, estimated variance components of sugarcane tons per hectare (STH), sucrose tons per hectare (SucTH), fiber tons per hectare (FTH), fiber percentage (FP) and apparent sucrose percentage (SP), evaluated in the agricultural area of Pumaty sugar mill, considering four harvest cycles.

Tabla 2. Media general, caracteres genéticos, componentes de varianza estimados de toneladas de caña de azúcar por hectárea (STH), toneladas de sacarosa por hectárea (SucTH), toneladas de fibra por hectárea (FTH), porcentaje de fibra (FP) y porcentaje de sacarosa aparente (SP), evaluados en el área agrícola del ingenio Pumaty, considerando cuatro ciclos de cosecha.

	Traits				
Genetic Parameters	STH ($t.ha^{-1}$)	SP (%)	FP (%)	SucTH ($t.ha^{-1}$)	FTH ($t.ha^{-1}$)
G_V	24.13	0.07	0.00	0.84	0.69
EP_V	1.98	0.02	0.00	0.10	0.02
GE_V	78.72	0.01	0.26	1.24	1.83
E_V	115.11	1.75	1.18	3.27	3.57
F_V	219.94	1.87	1.45	5.45	6.11
h_{2g}	0.11	0.04	0.00	0.15	0.11
r	0.12	0.05	0.00	0.17	0.11
R^2_E	0.08	0.01	0.00	0.01	0.00
R^2_{GM}	0.36	0.00	0.18	0.23	0.30
rgmed	0.23	0.82	0.00	0.40	0.27
h_{2mg}	0.52	0.66	0.02	0.69	0.57
General mean	79.26	14.83	15.77	11.88	12.34
Deviances	1265.32**	398.56**	343.45**	568.62**	588.06**

*, **Significant at 5 and 1%, respectively, according to deviance analysis. G_V: Genotypic variance. EP_V: Environmental variance. GE_V: Harvest cycles x genotype interaction. E_V: Temporary residual variance. F_V: Phenotypic individual variance. h_{2g}: Plots individual Heritability. r: repeatability. R^2_E: Environmental determination coefficient. R^2_{GE}: Determination coefficient for genotype x harvest cycle interaction effects. rgmed: Genotypic correlation through harvest cycle. h_{2mg}: mean genotype heritability.

*, ** Significativo al 5 y 1%, respectivamente, de acuerdo con la análisis de deviación. G_V: Varianza genotípica. EP_V: Varianza ambiental permanente. GE_V: Interacción genotipos x ciclos de cosecha. E_V: Varianza temporal residual. F_V: Varianza fenotípica individual. h_{2g}: Heredabilidad de parcelas individual. r: repetibilidad. R^2_E: Coeficiente de determinación ambiental permanente. R^2_{GE}: Coeficiente de determinación de los efectos de interación genotipos x ciclos de cosecha. rgmed: Correlación genotípica a través del ciclo de cosecha.. h_{2mg}: heredabilidad media de los genotipos.

Individual plots of heritability and repeatability coefficients showed average magnitude. Furthermore, determination coefficients of genotypes x harvest cycles interaction showed greater participation of this interaction in phenotypic variance genotypes.

A viable alternative for minimizing environmental effects would be to adopt a selection practice based on genotypes mean, given that environmental effects (harvest cycles) tend to cancel (11). Complementarily, Zeni Neto *et al.* (2008), said that considering an analysis of adaptability and stability would be important to identify stable genotypes.

As the mixed linear models methodology allows selection based on predicted genotypic values (9, 13), selection of the evaluated material could be based on the genotypic means of all harvest cycles. Such a procedure would provide greater selective accuracy, confirmed by the estimated genotype mean heritability for STH, SucTH and SP, which presented average magnitudes. For Garcia and Nogueira (2005), genotype mean heritability above 0.46 show higher selection potential. According to Rodrigues *et al.* (2011), it indicates phenotypic value reliability, as indicator for genetic value.

Observing the predicted genetic values (u+g+gem), the genotypes VAT90-212, SP81-3250, EECAC 04, EECAC 03 and SP79-1011 showed the highest values in harvest cycles and the highest genetic gains for STH (table 3).

Table 3. Estimated genetic gain on sugarcane tons per hectare (STH), sucrose tons per hectare (SucTH), fiber tons per hectare (FTH), fiber percentage (FP) and apparent sucrose percentage (SP) of top five sugarcane genotypes, as evaluated during the experiment conducted in the agricultural area of Pumaty sugar mill, considering four harvest cycles.

Tabla 3. Estimación de ganancia genética de los caracteres toneladas de caña de azúcar por hectárea (STH), toneladas de sacarosa por hectárea (SucTH), toneladas de fibra por hectárea (FTH), porcentaje de fibra (FP) y porcentaje de sacarosa aparente (SP), de los cinco mejores genotipos evaluados en el experimento realizado en el área agrícola del ingenio Pumaty, considerando cuatro ciclos de cosecha.

Sugarcane tons per hectare (STH)					
Genotypes	**G**	$\hat{\mu} + \hat{g}$	**Gain**	**New Mean**	$(\hat{\mu} + \hat{g} + \hat{g}em)$
VAT90-212	3.91	83.17	3.91	83.17	86.36
SP81-3250	2.73	82.00	3.32	82.58	84.23
EECAC 04	2.08	81.35	2.91	82.17	83.05
EECAC 03	1.91	81.17	2.66	81.92	82.73
SP79-1011	1.77	81.03	2.48	81.74	82.48
Sucrose tons per hectare (SucTH)					
VAT90-212	0.85	12.73	0.84	12.72	13.04
EECAC 04	0.71	12.60	0.80	12.66	12.86
SP81-3250	0.53	12.41	0.70	12.58	12.61
EECAC 08	0.46	12.34	0.64	12.52	12.51
EECAC 03	0.42	12.29	0.59	12.47	12.45
Fiber tons per hectare (FTH)					
SP81-3250	0.67	13.02	0.67	13.02	13.46
SP79-1011	0.50	12.85	0.59	12.93	13.18
EECAC 03	0.39	12.73	0.52	12.86	12.98
VAT90-212	0.32	12.66	0.47	12.81	12.88
EECAC 08	0.28	12.62	0.43	12.78	12.81
Fiber percentage (FP)					
SP79-1011	0.0063	15.77	0.0063	15.77	16.01
EECAC 06	0.0045	15.76	0.0054	15.77	15.94
SP81-3250	0.0041	15.76	0.0050	15.76	15.93
EECAC 09	0.0015	15.76	0.0041	15.76	15.82
RB813804	0.0012	15.76	0.0035	15.76	15.81
Apparent sucrose percentage (SP)					
EECAC 07	0.27	15.10	0.27	15.10	15.11
EECAC 04	0.24	15.06	0.25	15.08	15.07
EECAC 06	0.18	15.01	0.23	15.06	15.02
RB813804	0.05	14.88	0.19	15.01	14.88
EECAC 03	0.04	14.87	0.16	14.98	14.88

Mean components (individual BLUP). G: Genotypic effect. $\hat{\mu} + \hat{g}$: Predicted genotypic values free from environmental interaction (Harvest cycles). $(\hat{\mu} + \hat{g} + \hat{g}em)$: Average genotypic value in the harvest cycles. This parameter capitalizes on an average interaction with all the harvest cycles evaluated.

Componentes medios (BLUP individual). G: efecto genotípico. $\hat{\mu} + \hat{g}$: Valores genotípicos previstos libres de interacción ambiental (ciclos de cosecha). $(\hat{\mu} + \hat{g} + \hat{g}em)$:Valor genotípico medio en los ciclos de cosecha. Este parámetro capitaliza una interacción promedio con todos los ciclos de cosecha evaluados.

Regarding FTH, the following genotypes stood out: SP81-3250, SP79-1011, EECAC 03, VAT90-212 and EECAC 08. For FIB, SP79-1011 EECAC 06, SP81-3250, EECAC 09 and RB813804 showed a tendency, however not significant, something normally expected given low heritability. Finally, regarding PC f, the genotypes EECAC 07, EECAC 04, EECAC 06, RB813804 and EECAC 03 were the most important.

The relative performance of predicted genotypic values (HMRPPGV) on sixteen sugarcane genotypes for sugarcane tons per hectare (STH), sucrose tons per hectare (SucTH), fiber tons per hectare (FTH), fiber percentage (FP) and apparent sucrose percentage (SP) are presented in table 4 (page 17).

Table 4. Harmonic means of relative performance of predicted genotypic values (HMRPPGV and HMRPPGV*GM) for sixteen sugarcane genotypes. Traits: sugarcane tons per hectare (STH), sucrose tons per hectare (SucTH), fiber tons per hectare (FTH), fiber percentage (FP) and apparent sucrose percentage (SP) in experiments conducted in the agricultural area of Pumaty sugar mill, considering four harvest cycles.

Tabla 4. Medias armónicas de actuaciones relativas de valores genotípicos predichos (HMRPPGV and HMRPPGV*GM) de dieciséis genotipos de caña de azúcar de los caracteres toneladas de caña de azúcar por hectárea (STH), toneladas de sacarosa por hectárea (SucTH), toneladas de fibra por hectárea (FTH), porcentaje de fibra (FP) y porcentaje de sacarosa aparente (SP), evaluados en el experimento realizado en el área agrícola del ingenio Pumaty, considerando cuatro ciclos de cosecha.

	Traits					
	STH (t.ha^{-1})		SucTH (t.ha^{-1})		FTH (t.ha^{-1})	
Genotypes	**A**	**B**	**A**	**B**	**A**	**B**
VAT90-212	1.06	84.22	1.07	12.80	1.02	12.65
SP81-3250	1.06	83.69	1.06	12.65	1.07	13.32
EECAC 03	1.05	83.38	1.05	12.54	1.05	13.05
EECAC 04	1.05	83.18	1.08	12.88	1.03	12.76
SP79-1011	1.04	83.05	1.04	12.35	1.07	13.32
EECAC 08	1.03	81.99	1.04	12.43	1.02	12.65
RB863129	1.01	80.50	1.02	12.20	1.00	12.51
EECAC 02	1.00	79.98	0.98	11.72	1.01	12.51
EECAC 09	0.99	79.22	0.96	11.41	1.00	12.38
RB813804	0.99	78.89	1.00	11.99	1.00	12.38
RB92579	0.99	78.56	1.00	11.89	0.99	12.27
EECAC 06	0.97	77.59	0.98	11.75	0.99	12.26
EECAC 07	0.97	77.48	0.99	11.87	0.96	11.93
EECAC 01	0.96	76.55	0.94	11.21	0.97	11.98
EECAC 05	0.88	70.08	0.86	10.33	0.89	10.99
EECAC 10	0.88	64.36	0.80	9.55	0.79	9.86

	FP (%)		SP (%)	
	A	**B**	**A**	**B**
VAT90-212	0.98	15.49	1.00	14.83
SP81-3250	1.01	15.92	1.00	14.86
EECAC 03	1.00	15.81	1.00	14.88
EECAC 04	1.00	15.64	1.01	15.07
SP79-1011	1.01	16.01	1.00	14.84
EECAC 08	1.00	15.62	0.97	14.53
RB863129	0.99	15.68	1.00	14.86
EECAC 02	1.00	15.77	0.99	14.69
EECAC 09	1.00	15.80	0.98	14.64
RB813804	1.00	15.80	1.00	14.88
RB92579	1.00	15.77	1.00	14.86
EECAC 06	1.01	16.01	1.01	15.02
EECAC 07	0.99	15.65	1.01	15.12
EECAC 01	1.00	15.78	0.99	14.70
EECAC 05	1.00	15.80	0.98	14.66
EECAC 10	0.99	15.61	0.99	14.77

A. HMRPPGV / PADRVG
B. HMPRVG*GM / PADRVG*MA

The highest genotypic (HMRPPGV) selection (table 4), implied, simultaneously, adaptability, stability and productivity (18).

The highest harmonic means for STH were obtained for the genotypes VAT90-212, SP81-3250, EECAC 03, EECAC 04 and SP79-1011. For SucTH, the highest harmonic means were obtained for EECAC 04, VAT90-212, SP81-3250, EECAC 03 and SP79-1011. For FTH, the genotypes SP81-3250, SP79-1011, EECAC 03, EECAC 04 and EECAC 08 showed the highest harmonic means. For FP, the EECAC 06, SP79-1011, SP81-3250, EECAC 03 and EECAC 09, and finally, for the trait SP, the genotypes EECAC 07, EECAC 06, EECAC 04, EECAC 03 and RB3804 resulted the best.

The genotypes EECAC 05, EECAC 01, EECAC 09 and RB92579 showed higher stability in fiber yield. However, they did not stand out in terms of TCH, TSH and TFH. These genotypes could be used as parents in hybridization approaches, transmitting high fiber productivity to possible progenies.

The general mean for fiber percentage (15.77%) was the same obtained by Fernandes Júnior *et al.* (2017). However, the general mean for apparent sucrose content was considerably higher (14.83%). This difference in SP values may be due to the fact that these authors evaluated some families from crosses with *Saccharum spontaneum* species along with other materials combining high rusticity and low sucrose content.

It is worth mentioning that the best family selected by Fernades Júnior *et al.* (2017) presented a fiber content of 17%, while sucrose apparent percentage was 11.33%. These results evidence the difficulty to simultaneously concentrate high fiber and sucrose content in a genotype, since these parameters are not correlated (0.06 correlation, (21)). Therefore, in order to "optimize" these traits in genotypes obtained by artificial hybridization, several selection cycles are necessary.,

In the present work, considering sugar mill demands (varieties with fiber content between 12 and 17% and sucrose content about 13%), genotypes EECAC 06, EECAC 03, EECAC 04 and EECAC 07 could be commercially exploited (table 4, page 17). EECAC 03 genotype also showed higher fiber productivity per hectare. These genotypes presented higher genotypic stability and adaptability, for all traits and throughout the harvest cycles.

Conclusion

Mixed models constitute an efficient tool for sugarcane selection focused on fiber and sucrose production. This methodology provides significant genetic gains based on predicted genetic values free from interaction with harvest cycles.

The evaluated genotypes present high fiber and sucrose productivity, genotypic adaptability and stability throughout harvest cycles, indicating longevity in the sugarcane crop.

Genotypes EECAC 06, EECAC 04, EECAC 03 and EECAC 08 presented favorable fiber and sucrose percentages, towards greater capacity for bioenergy and sugar production.

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Tropicalization of canola (*Brassica napus* L.): commercial hybrids show potential for cultivation in the Brazilian Cerrado

Tropicalización de canola (*Brassica napus* L.): híbridos comerciales muestran potencial de cultivo en el Cerrado brasileño

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Abstract

Given its high energetic efficiency, canola (*Brassica napus L.*) constitutes a potential biodiesel feedstock. However, since this crop is native to temperate regions, Brazil has not yet consolidated its production. Thus, the objective of this study was to evaluate agronomic performance of canola hybrids grown in the tropical Brazilian Cerrado (15° 35'18" S, 47° 43'57" W at 999 m), aiming to select promising candidates for crop expansion. Our hypothesis states that obtaining high canola yields in Cerrado conditions is possible given a favorable agronomic adaptation of the species to this environment. Eight commercial canola hybrids were evaluated for seven agronomic traits in three experiments. Data were subjected to joint ANOVA and Tukey test. Grain yield of the evaluated hybrids resulted higher than the national average, including southern plantations. Hyola 61 and Hyola 76 hybrids outperformed regarding grain yield, followed by Diamond which, in addition to high productivity, exhibited a short crop cycle, even in rainfed conditions. Given these characteristics, Diamond hybrid can be considered the best option for crop rotation system. These promising results reveal the adaptability and cultivation potential of these hybrids in the Brazilian Cerrado.

Keywords
Brassica • crop cycle • oilseed • productivity

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Resumen

La canola es una materia prima potencial para la producción de biodiesel debido a sus altos niveles de eficiencia energética. Sin embargo, Brasil aún no ha consolidado su producción pues se desarrolla en las regiones templadas. Este estudio tuvo la hipótesis de que es posible obtener altos rendimientos de canola en condiciones de Cerrado. Así, el objetivo de este estudio fue evaluar el comportamiento agronómico de los híbridos de canola en las condiciones tropicales de la Sierra brasileña - Cerrado - (15 ° 35'18 "S, 47 ° 43'57" W a 999 m) para seleccionar candidatos que contribuyan con la expansión del cultivo de canola en el país. Se evaluaron ocho híbridos comerciales de canola para siete características agronómicas, en tres experimentos. Los datos obtenidos para cada variable se sometieron a análisis conjunto de varianza y se compararon las medias mediante la prueba de Tukey. El rendimiento de grano de los híbridos evaluados fue superior al promedio nacional, que incluye plantaciones solo en la región sur. Los híbridos que destacaron en productividad de grano fueron Hyola 61 y Hyola 76 seguidos por Diamond, que además de su alta productividad, tuvo un ciclo de cultivo más corto incluso en condiciones de secano. Por sus características, el híbrido Diamond puede considerarse la mejor opción para ser incluido en el sistema de rotación de cultivos. Estos resultados prometedores revelan la adaptabilidad y el potencial de cultivo de estos híbridos en el Cerrado Brasileño.

Palabras clave

Brassica • ciclo de cultivo • oleaginosas • productividad

Introduction

Canola (*Brassica napus L.*) is a Brassicaceae oilseed developed by genetic enhancement of two rapeseed species: *Brassicas oleracea* and *Brassica rapa* (7). Besides being responsible for 15% of worldwide edible vegetable oil production, it integrates a select feedstock group producing 95% of biodiesel, globally (8). Thus, canola outstands as an alternative to oil-sourced dependence, increasing renewable energy and reducing greenhouse gas emissions.

Despite its potential, canola is not fully consolidated in Brazil since its cultivation is indicated within 35° and 55° latitudes, in temperate regions. Currently, Brazilian production is concentrated in the south portion of the country, with over 90% of production located in Rio Grande do Sul (3). However, studies have shown that this oilseed could be produced in a tropical area such as Cerrado (15° 35'18" S, 47° 43'57" W at 999 m) (1, 13). Additionally, canola has the advantage of being an interesting Safrinha crop, where short-cycle annual crops such as soybeans and maize can grow in crop rotation systems. Incorporating canola in the soybean/maize system could also reduce disease incidence and enable winter production of vegetable oils when large areas of cultivation remain unoccupied.

Thus, canola tropicalization in Cerrado would expand oil production for energy purposes and human consumption, generating job positions and reducing deforestation. However, canola cropping in the Cerrado, requires evaluating hybrid agronomic performance, assisting decision making and choosing appropriate management, and technologies and equipment. Therefore, our objective was to evaluate agronomic performance of canola hybrids in the tropical Brazilian Cerrado, selecting promising candidates to contribute to crop expansion.

Materials and methods

Experiment installation and conduction

Eight spring commercial canola hybrids were evaluated: Hyola 50, Hyola 61, Hyola 76, Hyola 433, Hyola 571, Hyola 575, ALHT B4 and Diamond. Experiments I and II were irrigated by a fixed sprinkler, applying a total of 163 and 520 mm water, respectively, throughout the whole experiment. Experiment III received 182 mm of irrigation just before flowering onset.

Experiment I was conducted from June 17 to September 14, 2017, experiment II from April 23 to September 6, and experiment III from April 11 to August 24, 2019, in the experimental area of Embrapa Cerrados, located at 15° 35'18" S, 47° 43'57" W, at 999 masl.

The soil is classified as a Red Latosol with high clay content. According to Köppen & Geiger (1936), the regional climate is tropical with dry winter and rainy summer (Aw), with an average annual temperature of 21 °C, relative humidity of 68% and average annual rainfall of 1,668 mm. Average temperature during experiments was 21.2 °C, with total rainfall of 9.2 mm during experiment I and 403.5 mm during experiments II and III (figures 1 and 2).

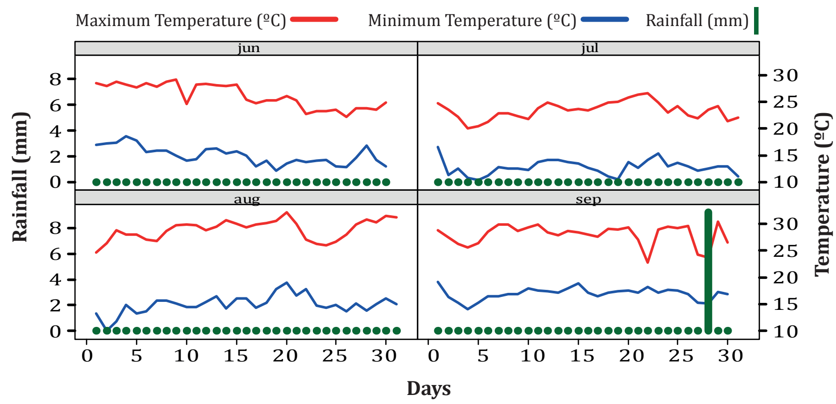

Figure 1. Precipitation and maximum/minimum temperatures during Experiment I. Brasília, DF, 2017.

Figura 1. Precipitación, temperatura máxima y mínima durante el experimento I de canola. Brasilia, DF, 2017.

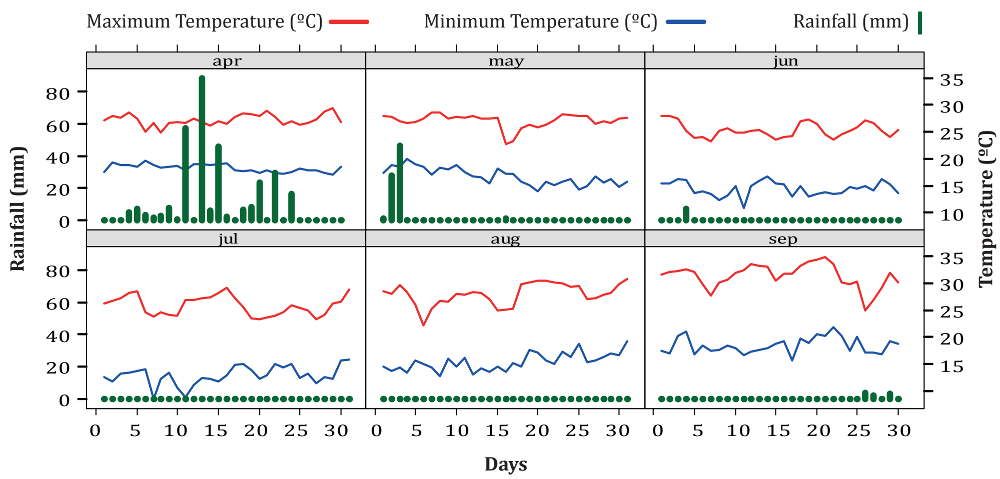

Figure 2. Precipitation and maximum/minimum temperatures during Experiments II and III. Brasília, DF, 2019.

Figura 2 Precipitación, temperatura máxima y mínima durante el experimento II y III de canola. Brasilia, DF, 2019.

The eight genotypes were distributed in a randomized block design (RBD) with four replications. In experiment I, each plot consisting of 16 lines, 5 m long and spaced 0.17 m, had a population density of 40 plants.m^{-2} and a total area of 656 m^2. Experiments II and III, consisted of plots with 8 lines, 5 m long, spaced 0.17 m, with a population density of 40 plants.m^{-2} and a total area of 450 m^2.

Seven agronomic traits were evaluated: i) number of days to flowering onset (NDF, days): number of days from sowing to flowering onset, in which 50% of the plants presented at least one flower; ii) cycle (CY, days): days from sowing to harvest; iii) pod length (PL, cm), average length of five random pods derived from five randomly chosen plants; iv) pod mass

(PM, g): average weigh of the randomly chosen pods with grains; v) number of grains per pod (NGP): average grains from five random pods; vi) mass of 1000 (thousand) grains (M1000G, g): weight of 1000 grains from five plants; vii) grain yield (PROD, $kg.ha^{-1}$): total grain weight in relation to plot area.

Statistical analysis

After checking normality and homoscedasticity by Shapiro-Wilk and Bartlett tests respectively, data were subjected to ANOVA ($p<0.05$). Mean comparison was assessed by Tukey test ($p<0.05$).

The following statistical model was used:

$$Y_{ijk} = m_k + H_i + B_k + A_j + HA_{ij} + e_{ijk}$$

where:

Yijk = effect of observing the hybrid i on repetition j in k environment (experiments);
m_k = general environment average in k environment;
H_i = hybrid effect;
B_k = block effect;
A_j = environmental effect;
HA_{ij} = interaction effect of hybrid i and environment j;
e_{ijk} = random error.

The following genetic parameters were estimated: quadratic genotypic component (Φ_g^2); quadratic component of the hybrid x environment interaction (Φ_{ga}^2); residual variance (σ_r^2); genotypic determination coefficient (R^2); intraclass correlation (r); genetic variation coefficient (CVg (%)) and CVg/CVe ratio. All analyses were performed with Genes software (4).

Results and discussion

Significant differences were detected for NDF, PL and NGP (table 1, page 24), indicating variability between hybrids. A similar result was previously detected by Zare and Sharafzadeh (2012) for the same traits.

Significant environmental variation for all traits, except PL, evidenced strong environmental influence. CY, PL and NGP showed significant differences for hybrid-environment interaction (HxE), indicating different environmental performance for all hybrids.

Heritability (H^2) constitutes the proportion of genetic variance in total phenotypic variance. It allows estimating genetic gains in selection cycles, and for fixed treatments, as in the present study, it represents a determination coefficient (R^2) (14). For NDF, H^2 resulted in 0.92, followed by 0.72 for PL, meaning that these traits are less influenced by the environment than the others. Other studies have also reported high R^2 magnitudes (above 0.89) for NDF (12, 15, 16). Meanwhile, NGP and M1000G presented intermediate R^2 (0.68 and 0.57, respectively), as previously found (9, 11). The lowest R^2 values detected in this study (less than 0.50), indicated a stronger environmental influence on those traits.

Regarding PL (table 2, page 24), ALHT B4 and Diamond hybrids did not significantly vary among environments. As for NGP, three of the eight genotypes (Hyola 50, Hyola 433 and Diamond) had significant environmental variation.

When analyzing within each environment, Diamond hybrid presented the shortest cycle in the three experiments (ranging from 102 to 114 days), being, however, not statistically different from the rest in Experiment I. In general, considering that canola varies from 107 to 166 days (6) these evaluated genotypes can be classified as short-cycle hybrids. Precocity may be associated with climatic conditions, such as high temperature (approximately 32.5 °C in experiment I and 33.5 °C in experiments II and III) and long photoperiod. Canola hybrids with a shorter cycle are vital for incorporating canola in crop rotation systems in Brazilian Cerrado, allowing the species to benefit from the end of rainy periods and reducing mechanized irrigation.

Table 1. ANOVA and genetic parameters for number of days to flowering onset (NDF); cycle (CY); pod length (PL); pod weight (PM); number of grains per pod (NGP); weight of 1000 (thousand) grains (M1000G) and grain yield (GY) in eight canola genotypes grown in Cerrado, Brazil.

Tabla 1. Análisis de varianza conjunta y parámetros genéticos de los experimentos para las características: número de días para iniciar la floración (NDF); ciclo (CY); longitud de la vaina (PL); masa de la vaina (PM); número de granos por vaina (NGP); peso de 1000 (mil) granos (M1000G) y rendimiento de grano (GY) en ocho genotipos de canola.

SV	DF	Medium squares						
		NDF	CY	PL	PM	NGP	M1000G	GY
Block	3	28.07	9.31	0.22	920.72	9.96	0.10	2,009,995.54
Hybrids	7	151.04**	210.66	1.16*	1,689.23	103.19*	0.19	772,174.77
Environments	2	7,044.66**	728.51**	0.10	203,947.91*	271.32**	2.41**	28,461,036.04**
HxE	14	12.92	76.26**	0.31*	929.78	32.74**	0.08	675,616.09
Residue	69	12.76	10.78	0.14	1640.02	12.42	0.11	553,632.35
Mean		50.14	120.58	5.48	83.24	20.94	3.26	23.93
CVe(%)		7.12	2.72	6.89	48.64	16.82	10.08	31.09
Φ_g^2		11.51	11.20	0.07	63.29	5.87	0.01	8,046.56
Φ_{ga}^2		0.03	14.32	0.04	0.00	4.45	0.00	26,683.94
σ_r^2		12.76	10.78	0.14	1,640.02	12.42	0.11	553,632.36
R^2		91.45	63.80	72.91	44.96	68.27	57.66	12.50
r		47.36	30.85	28.19	4.09	25.83	8.09	1.37
CVg(%)		6.77	2.78	4.86	9.56	11.57	2.90	3.75
CVg/CVe		0.95	1.02	0.71	0.20	0.68	0.29	0.12

Sources of Variation (SV); Degrees of Freedom (DF); Hybrid x Environment Interaction (HxE), Environmental Variation Coefficient (CVe(%)); Genotypic Quadratic Component (Φ_g^2); Quadratic Component of Genotype x Environment Interaction (Φ_{ga}^2); Residual Variance (σ_r^2); Genotypic coefficient of determination (R^2); Intraclass Correlation (r); Genetic Variation Coefficient (CVg(%)) and CVg/CVe ratio (CVg/CVe); **,* Significant at 1% ($p<0.01$) and 5% ($p<0.05$) probability, respectively.

Fuentes de variación (SV); Grados de libertad (DF); Interacción Genotipo x Ambiente (GxE), Coeficiente de Variación Ambiental (CVe (%)); Componente cuadrático genotípico (Φ_g^2); Componente cuadrático de la interacción genotipo x ambiente (Φ_{ga}^2); Varianza residual (σ_r^2); Coeficiente de determinación genotípico (R^2); Correlación intraclase (r); Coeficiente de variación genética (CVg (%)) y relación CVg / CVe (CVg / CVe); **, * Significativo al 1% ($p <0,01$) y al 5% ($p <0,05$) de probabilidad, respectivamente.

Table 2. Tukey media comparison test for the characteristics cycle (CY, days), pod length (PL, cm) and number of grains per pod (NGP) in eight canola genotypes in the three experiments.

Tabla 2. Prueba comparación de medias de Tukey para el ciclo de características (CY, días), longitud de vaina (PL, cm) y número de granos por vaina (NGP) en ocho genotipos de canola en los tres experimentos evaluados.

Genotypes	CY						PL						NGP					
	Exp. I		Exp. II		Exp. III		Exp. I		Exp. II		Exp. III		Exp. I		Exp. II		Exp. III	
Hyola 50	117.5	Ba	27.0	Abc	125.5	Aa	5.6	Aa	5.8	Aa	5.6	Aabc	21.5	Aa	27.7	Aa	21.8	Aab
Hyola 61	117.3	Ba	133.5	Aab	118.5	Bab	5.4	Aa	5.7	Aa	5.5	Aabc	17.9	Ba	24.3	Aa	21.6	Abab
Hyola 76	117.3	Ca	135.0	Aa	126.0	Ba	5.5	Aa	5.4	Aab	5.3	Abc	19.8	Aa	22.2	Aa	19.4	Aab
Hyola 433	117.0	Aa	120.5	Ac	117.3	Ab	5.5	Aa	5.6	Aab	5.0	Ac	19.5	Aa	25.1	Aa	8.7	Bc
Hyola 571	117.3	Aa	122.5	Ac	122.3	Aab	5.2	Aa	4.7	Ab	4.9	Ac	16.3	Aa	21.1	Aa	17.0	Abc
Hyola 575	117.0	Aa	122.8	Ac	118.5	Aab	5.2	Aa	5.3	Aab	5.3	Abc	15.4	Aa	20.7	Aa	17.1	Ab
ALHT B4	117.0	Ba	125.8	Abc	122.3	ABab	5.9	Aa	5.8	Aa	6.2	Aab	21.0	Aa	26.3	Aa	23.0	Aab
Diamond	114.0	Ba	120.5	Ac	102.0	Cc	5.8	Aba	5.1	ABab	6.3	Aa	21.6	Aa	27.1	Aa	26.9	Aa

Means followed by the same lowercase letters vertically and the same uppercase letters horizontally constitute a statistically homogeneous group.

Las medias seguidas de las mismas letras minúsculas verticalmente y las mismas letras mayúsculas horizontalmente constituyen un grupo estadísticamente homogéneo.

The NGP significantly varied across all three experiments, with the hybrids Diamond, Hyola 50, ALHT B4, Hyola 433, Hyola 76 and Hyola 61 exhibiting the highest number of grains. For this trait, Diamond presented the higher values in experiments II and III, agreeing with the 17-24 grains per pod, previously observed in different canola genotypes (5, 15).

Grain yield resulted promising (figure 3) since all averages resulted higher (above 1,500 $kg.ha^{-1}$) than the national grain yield average (1,429 $kg.ha^{-1}$) (2), expecting Hyola 571, Hyola 433, ALHT B4 and Hyola 50 hybrids, that resulted below the national average in Experiment III.

The Hyola 61 hybrid showed the highest grain yield (2,926 $kg.ha^{-1}$), followed by Diamond hybrid (2,816 $kg.ha^{-1}$) in experiment I. Hyola 61 had previously exhibited high grain yield stability under both water deficit and intense cold (6). Diamond also outstood in experiment II for grain yield (3,356 $kg.ha^{-1}$), surpassed only by Hyola 76 (4,179 $kg.ha^{-1}$) and Hyola 571 (3,930 $kg.ha^{-1}$). In experiment III, Diamond (1,977 $kg.ha^{-1}$) showed the highest grain yield, followed by Hyola 61 (1,821 $kg.ha^{-1}$) and Hyola 76 (1,637 $kg.ha^{-1}$).

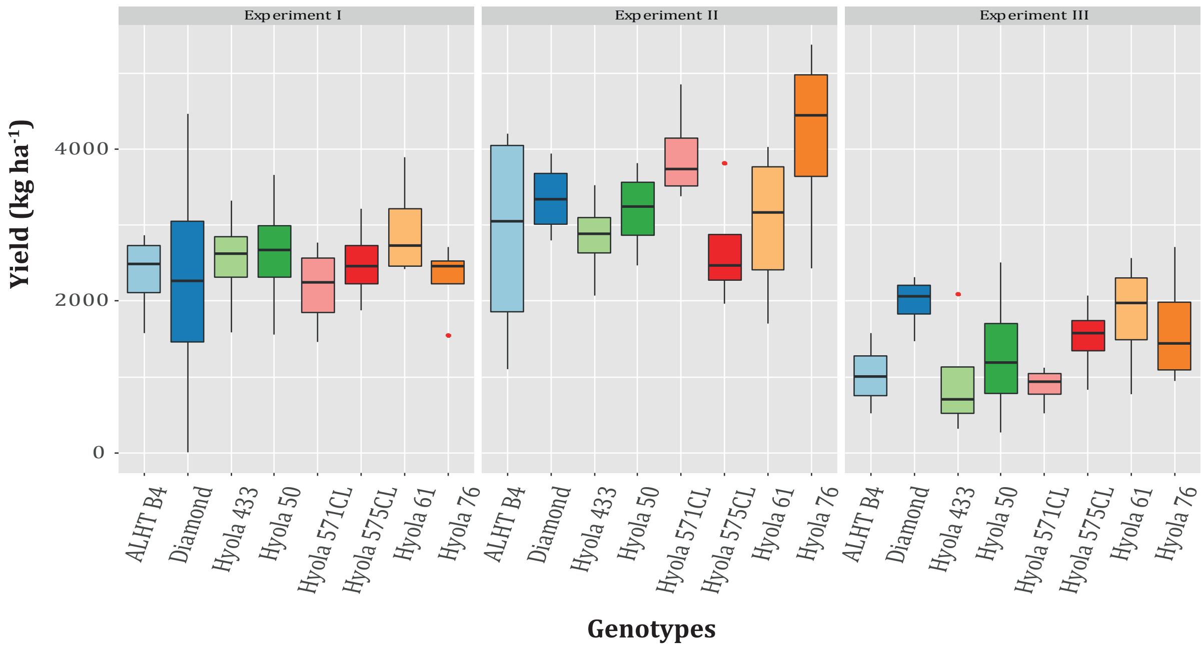

Figure 3. Boxplot from experiments I, II and III for grain yield in eight canola genotypes grown in Cerrado, Brazil.

Figura 3. Diagrama de caja de los experimentos I, II y III para el rendimiento de grano en ocho genotipos de canola cultivados en Cerrado, Brasil.

Conclusion

In summary, observing average estimates, we conclude that Hyola 61 and Hyola 76 outperformed with higher grain yield, followed by Diamond, with high production and shorter cycle. Due to these two key characteristics, Diamond can be considered the best option for crop rotation systems. Therefore, both hybrids constitute valuable alternatives for the Brazilian Cerrado.

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Prioritization of vigor QTL-associated genes for future genome-directed *Vitis* breeding

Priorización de genes asociados a QTLs de vigor para futuros planes de mejoramiento dirigido en *Vitis*

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Abstract

Vigor control in grapevine may become especially important under climate change. A better understanding of gene-phenotype relationships is required in order to exploit plant genomics for breeding purposes. This research aims to use quantitative trait loci (QTLs) for vigor identified in the progeny from a cross of Ramsey (*Vitis champinii*) × Riparia Gloire (*V. riparia*). Genes located 700 kb up and downstream from each QTL position were interrogated for functional enrichment through ShinyGO online tool, based on the gene ontology annotation of *Vitis vinifera* PN40024. Key biological processes like phloem and xylem development, cell cycle, response to hormones, amino acid transport, tissue development, sugar metabolism, nitrogen transport, and stress/immune responses, showed functional enrichment. Integral response to light and auxin might be required for fine molecular tuning of vegetative growth in *Vitis*. Fifty out of 1318 candidate genes were prioritized, reducing their amount to a manageable number of candidates genes for further directed breeding strategies.

Keywords
biological processes • gene ontology • QTL map • vigor • gene prioritization

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Resumen

El control del vigor en vid es un factor de gran importancia en el contexto de cambio climático actual. Es necesario desarrollar una mejor comprensión de la relación gen-fenotipo para la asistencia del mejoramiento vegetal mediante genómica. En este trabajo se utilizó la información obtenida de un mapeo de QTLs para vigor realizado con la progenie de Ramsey (*Vitis champinii*) × Riparia Gloire (*V. riparia*). De acuerdo a la anotación ontológica de Vitis vinifera PN40024, los genes ubicados 700 kb por encima y por debajo de cada marcador fueron interrogados para determinar enriquecimiento funcional a través de la herramienta online ShinyGO. Distintos procesos clave en la definición de vigor mostraron enriquecimientos altamente significativos, tales como desarrollo tisular (floema y xilema), ciclo celular, transporte de aminoácidos (nitrógeno) metabolismo de azúcares y respuesta a hormonas, a estrés e inmune. La respuesta integral a la luz y la producción de auxinas parece modular molecularmente el crecimiento vegetativo en *Vitis*.

Palabras clave
proceso biológico • ontología génica • mapeo de QTL • vigor • priorización de genes

Introduction

The understanding of genotype-phenotype associations constitute a major challenge for plant scientists. The causal association between variation of a certain trait and genotypic differences is the foundation for developing targeted strategies to be used in molecular breeding (1).

Vigor is considered as the propensity to assimilate, store, and/or use non-structural carbohydrates to produce large canopies. It is associated with high metabolism and rapid shoot growth (17). The main processes that account for vigor are: 1- carbon assimilation or photosynthesis, 2- cell growth, by division and expansion, and 3- leaf area development, for the reception of CO_2 and light. All these complex processes contribute to what is known as a highly quantitative trait.

There is increasing interest in understanding the genetic basis of grapevine vigor and biomass production, given its substantial impact on yield, water management, plant health, and fruit quality. Advances in understanding the genetic bases of complex traits through genetic mapping and quantitative trait locus (QTL) analysis have linked certain complex phenotypes to specific regions of chromosomes, and helped to identify the action, number, and precise location of these regions. The Ramsey (*Vitis champinii*) × Riparia Gloire (*V. riparia*) linkage map was developed by Lowe and Walker (2006) with the goal of mapping traits like biotic resistance, drought tolerance and vigor. We recently reported QTLs for vigor in this progeny that are associated with leaf area, specific leaf area, field canopy biomass and several partitioning indices (12). However, the genes behind these quantitative traits have not been identified. Here, we analyze the function of genes surrounding the QTLs based on gene location and the Gene Ontology (GO) annotation of the *V. vinifera* genome. GO is a computational representation of the functions of protein and non-coding RNA molecules produced by genes from many different organisms. It can be used for interpreting large-scale molecular biology experiments to gain insight into the structure, function, and dynamics of an organism. This approach was proposed by Correa *et al.* (2014) when identifying QTLs and candidate genes associated with cluster architecture in grapevines, and later by Ritcher *et al.* (2019), when approaching cluster architecture and revealing genes enriched by physical projection of the QTLs onto the PN40024 reference genome sequence.

In this paper, we communicate the results of a GO functional enrichment to prioritize candidate genes responsible for vigor that interrogated 1318 genes obtained from the QTL mapping of the Ramsey x Riparia Gloire progeny. We also attempt to elucidate the putative role of genes in terms of physiological and biochemical processes and identify priority genes for further targeted breeding approaches.

Materials and Methods

Six highly significant QTLs for vigor components from the Ramsey x Riparia Gloire map were selected based on their LOD values above the genome wide significance level (12). The BLAST server against the *V. vinifera* PN40024 genome (https://urgi.versailles.inra.fr/blast/) was then used to locate the hybridization regions of PCR primers targeting the simple sequence repeat (SSR) markers linked to the selected QTLs (table 1, page 30-31). The coordinates 700 kb upstream and downstream of primer hybridization on the reference sequence were used to identify the genes within the region flanked by those limits. The 700 kb limit was established considering that candidate genes should be no farther than 3-4 cM from the marker, since the *V. vinifera* genome has been estimated at about 475 Mb and 1 cM equals 200-300 kb (3). For that purpose, a script was developed to search for annotated genes flanking the QTL regions in the annotation file of the *V. vinifera* PN40024 genome.

The annotated genes found with the script were then used to interrogate for functional enrichment through ShinyGO online tool version 0.61 (8). For the set of genes contained in the QTL regions associated with vigor, the occurrence of associated gene ontology terms (GO terms) was statistically evaluated for overrepresentation through an hypergeometric test retrieving those GOterms with statistical significance (False Discovery Rate < 0.05) when comparing their percentage of occurrence with the percentage of each GOterm in the whole annotated genome. Integral plots that associate gene locations in chromosomes with enriched GO categories were performed with shinyCircos (22).

Results

In this work, GO functional enrichment allowed the identification of genes involved in key processes related to vigor and prioritization reduced the number of candidate genes from 1318 to 50.

Our results show that associations between overrepresented GO terms and vigor helped to rank candidate genes, based on their putative function. Phloem and xylem development, cell cycle, response to hormones, tissue development, aminoacid and nitrogen transport, sugar metabolism and immune responses, all showed functional enrichment (figure 1 page 32; table 1, page 30-31). On chromosome 1, most identified genes encode for aminoacid transmembrane transporters (figure 1 page 32; table 1, page 30-31). In addition, the single gene found on chromosome 3 influences the enrichment for functions related to transport of organic acids and nitrogen compounds.

Two important transcription factors (TFs) related to photomorphogenesis (TF 104879018) and auxins (TF 104879021) were identified on chromosome 4 (figure 1 page 32; table 1, page 30-31). TFs are especially interesting because they control the expression of several genes. Genes in chromosome 10 are involved in biotic and abiotic stress responses (figure 1 page 32; table 1, page 30-31). Another transcription factor related to this processes, identified in table 1 (page 30-31) as 100243518, appears significant in this chromosome. On chromosomes 14 and 19, we found the most varied group of genes in terms of function (figure 1 page 32; table 1, page 30-31), including, once again, genes encoding for nitrogen transport, that are key for growth, cell cycle regulation and development (13, 19). Many genes encode for proteins involved in phloem and xylem development. This was the function with major enrichment (figure 1 page 32; table 1, page 30-31). It represents a key aspect for growth, as transport of water, nutrients, proteins and sugars is vital for the plant to develop.

Discussion

Although the analysis used the *V. vinifera* PN40024 genome, due to its superior characterization and annotation, sequence alignment with *V. riparia* Gloire (9) of chromosomes with significant QTLs averaged 96,77 % (data not shown). Also, Liang *et al.* (2019) determined a 97.34-97.65% identity through whole genome comparison of *V. vinifera* PN40024 with two *V. riparia* accessions.

Table 1. Prioritized genes for vigor based on Gene Ontology enrichment analysis and hypothetical function of predicted proteins. SSR markers associated with selected QTLs are shown with chromosome number.

Tabla 1. Genes priorizados para vigor basados en enriquecimiento por ontología génica y sus respectivas funciones proteicas hipotéticas. Los marcadores SSR asociados con los QTLs elegidos se identifican por número de cromosoma.

Chrom # Marker ID	Locus	Protein/Accession	Function
1 ssrVvUCH29	LOC100240857	Lysine histidine transporter 1 (LHT1)	Amino acid transmembrane transport
	LOC100247632	LHT1	Id.
	LOC100247728	LHT2	Id.
	LOC100257870	LHT1	Id.
	LOC100264875	LHT1	Id.
	LOC100244217	Signal recognition particle subunit SRP72	Targeting secretory proteins to rough endoplasmic reticulum membrane. SRP-dependent cotranslational protein targeting to membrane
	LOC100254597	Protein kish-like	Intracellular transport. Protein secretion
	LOC100256355	ATP-dependent DNA helicase Q-like 5	DNA recombination, repair, replication
3 CTG1030395*[1]	LOC100265163	Vacuolar-sorting receptor 3	Protein targeting to vacuole
4 CTG1011026*[2]	LOC100242237	GTP-binding nuclear prot. Ran-3-like	Nucleocytoplasmic transport. Import prot. into the nucleus and RNA export
	LOC100247365	GTP-binding nuclear prot. Ran-3	Id.
	LOC100243952	Stromal cell-derived factor 2-like prot.	Innate immune response. Defense response to bacteria and fungi
	LOC100249173	Ammonium transporter 3 member 1	Ammonium transmembrane transport
	LOC100254226	Overexpressor of cationic peroxidase 3	1. Innate immune response. Response to bacteria, fungi, ABA, jasmonic acid and water deprivation
	LOC100262771	RNA-dependent RNA polymerase 6	RNA silencing pathway and generation of small interfering RNAs
	LOC100268069	Uncharacterized prot.	Protein import into chloroplast stroma
	LOC104879018	Transcription factor HY5	Red/far red signalling pathway. Regulation of photomorphogenesis
	LOC104879021	Auxin-responsive prot. IAA28	Repression of early auxin response genes at low auxin concentrations
10 VrZAG64	LOC100243518	Transcription factor VOZ1	Response to heat, cold, salt, drought, and light. Defense to bacteria, incompatible interaction
	LOC100243637	MACPF domain-containing prot. NSL1	Hypersensitive response. Inmune response
	LOC100245146	Uncharacterized prot.	Regulation of systemic acquired resistance (SAR) and transcription. Histone modification
	LOC100247033	Homeobox-DDT domain protein RLT1	Regulation of transcription. Regulation of transition from vegetative to reproductive phase
	LOC100255452	Mitochondrial arginine transporter BAC1	Nitrogen compound transport. Mitochondrial transmembrane transport

*[1] CTG1030395 (5'–3: FW-TCCCTACAATCTCATCGCAA, RV-CATGGCTCAAGAGAGTGCAA)

*[2] CTG1011026 (5'–3: FW-GAAGAACACCACAGCAAGCA, RV-AAAATGCACAATCTCCCACC)

Additional information is available at http://www.ncbi.nlm.nih.gov/gene and www.uniprot.org. Locus with lowest FDR values and the highest QTL LOD scores have been shaded.

Información adicional se encuentra disponible en http://www.ncbi.nlm.nih.gov/gene and www.uniprot.org. Los locus con menor FDR y valores mayores de LOD score en el mapa de QTLs, se ven sombreados.

Table 1 (cont.). Prioritized genes for vigor based on Gene Ontology enrichment analysis and hypothetical function of predicted proteins. SSR markers associated with selected QTLs are shown with chromosome number.

Tabla 1(cont.). Genes priorizados para vigor basados en enroqiecimiento por ontología génica y sus respectivas funciones proteicas hipotéticas. Los marcadores SSR asociados con los QTLs elegidos se identifican por número de cromosoma.

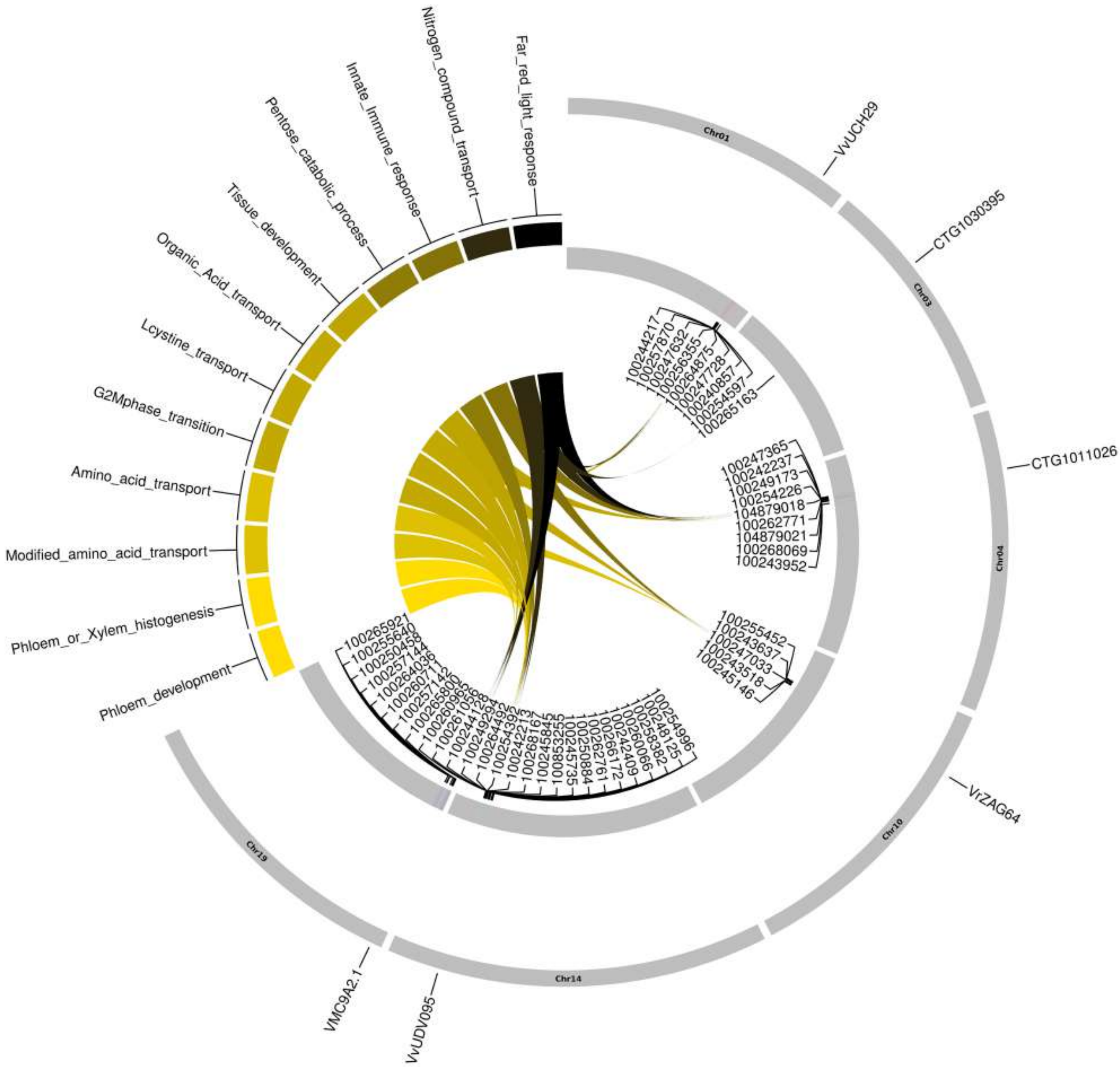
Chrom # Marker ID	Locus	Protein/Accession	Function
14 UDV-095	LOC100242409	Ribulose-phosphate 3-epimerase, cytoplasmic isoform	Carbohydrate metabolic process. Catalitic activity
	LOC100242213	Sieve element occlusion B-like/	Phloem development
	LOC100244128	Sieve element occlusion B	Id.
	LOC100245845	Sieve element occlusion B	Id.
	LOC100249294	Sieve element occlusion B	Id.
	LOC100254392	Sieve element occlusion B-like	Id.
	LOC100261056	Sieve element occlusion B	Id.
	LOC100264492	Sieve element occlusion B	Id.
	LOC100268167	Sieve element occlusion B-like	Id.
	LOC100245735	Cystinosin homolog	L-cystine transmembrane transport
	LOC100250884	Cystinosin homolog	Id.
	LOC100248125	Mitochondrial import receptor subunit TOM20	Protein insertion into mitochondrial outer membrane
	LOC100254996	Transport prot Sec61 subunit gamma	Protein transmembrane transport
	LOC100258382	Transcription and mRNA export factor SUS1	Nitrogen compound transport. Regulation of transcription
	LOC100260066	Chloride channel ClC4.	Nitrate transmembrane transport. Chloride transport
	LOC100260965	Ras-related protein RABB1b	Nitrogen compound transport. Intracellular protein transport
	LOC100262761	LHT1	Amino acid transmembrane transport
	LOC100266172	COP9 signalosome complex subunit 3	Positive regulation of G2/M transition of mitotic cell cycle.Protein catabolic process
	LOC100853255	RPM1-interacting prot. 4	Defense response signaling pathway. AvrRpt-cleavage domain-containing prot
19 VMC9A2.1	LOC100250458	Transport prot Sec61 subunit alpha	Protein transmembrane transport
	LOC100255640	IRK-interacting protein	Negative gravitropism. Response to light
	LOC100257142	Probable sodium/metabolite cotransporter BASS1 chloroplastic	Nitrogen compound transport. Panthotenate import across plasmamembrane
	LOC100257144	Septum-promoting GTP-binding protein 1	GTPase activity. Intracellular protein transport
	LOC100260711	B-box zinc finger protein 22	Anthocyanin-and chlorophyll biosynthetic process. Chloroplast organization. Photomorphogenesis. Regulation of transcription
	LOC100264036	TOM1-like protein 2	Intracelullar protein transport
	LOC100265800	Ribokinase	Phosphorylation of ribose, can then be used for sythesis of nucleotides and aminoacids, or in pentose phosphate pathway
	LOC100265921	Ras-related protein Rab11D-like	GTPase activity. Hyperosmotic salinity response. Vesicle mediated transport

*[1] CTG1030395 (5'–3: FW-TCCCTACAATCTCATCGCAA, RV-CATGGCTCAAGAGAGTGCAA)

*[2] CTG1011026 (5'–3: FW-GAAGAACACCACAGCAAGCA, RV-AAAATGCACAATCTCCCACC)

Additional information is available at http://www.ncbi.nlm.nih.gov/gene and www.uniprot.org. Locus with lowest FDR values and the highest QTL LOD scores have been shaded.

Información adicional se encuentra disponible en http://www.ncbi.nlm.nih.gov/gene and www.uniprot.org. Los locus con menor FDR y valores mayores de LOD score en el mapa de QTLs, se ven sombreados.



Lines connect genes to enriched GO terms (ranging from FDR 0.034 in black, to FDR $1x10^{-9}$ in gold).
Las líneas conetan los genes con los términos de enriquecimiento desde FDR 0,034 en negro hasta FDR $1x10^{-9}$ en dorado).

Figure 1. Chromosome location of vigor-associated genes identified through GO functional enrichment. Genes and SSR markers showing GO categories enrichments (FDR<0.05) are indicated on chromosomes of *V vinifera.*

Figura 1. Ubicación cromosómica de genes asociados a vigor identificados por enriquecimiento funcional GO (FDR<0,05) y marcadores SSR en los cromosomas de *V vinifera.*

As mentioned, on chromosome 1, most identified genes encode for aminoacid transmembrane transporters (figure 1; table 1, page 30-31). Transmembrane aminoacid transport is associated with enhanced growth and high rates of protein synthesis (20). Nitrogen can be taken up from soil in various forms, being one of them amino acids, and is of considerable importance in vigor control, yield and berry quality (7). The fact that these genes are closely located, suggests some kind of structural regulation. It has been observed that root elongation and enlargement in the rootstock 110R, partly depend on transcriptomic regulations of sugar and protein transporter genes *SWEET* and *NRT1/PTR* in roots. This was found to facilitate carbohydrate and nitrogen accumulation, providing essential energy to 110R roots under drought (21).

In relation to the two important TFs related to photomorphogenesis and auxins identified on chromosome 4, they are particularly intriguing given that they control the expression of several genes. The particularity of having both TFs in the same chromosomal region increases the probability of a synergistic response through coregulation. Indeed, the coordination between TFs in response to light and auxins was well established in *Arabidopsis thaliana* by Halliday, *et al.* (2009), who showed that light regulates growth of distant tissues from the site of light exposure through auxin production. Something similar was observed in *A. thaliana* by Hornitschek, *et al.* (2012), where phytochrome interacting factors 4 and 5 (*PIF4* and *PIF5*) regulated elongation growth by controlling the expression of genes that encode for auxin biosynthesis and signaling. Interestingly, TF 104879018 in our study is homologous to *HY5* of *A. thaliana*, which is found downstream in the signaling cascade of *PIF1*/*PIF3* (10). *HY5* promotes growth, especially through photosynthesis induction and higher nutrient uptake by roots. The other prioritized TF 104879021, is a homolog to the auxin-responsive protein IAA28 of *A. thaliana*, which plays a role in regulation of lateral root growth. In grapevines, kaolin, a mineral that reflects radiation from the leaf surface, produced an increase in IAA content. This treatment also caused higher values of stomatal conductance, net CO_2 assimilation rate, intrinsic water use efficiency, and a slight decrease in ABA (5). These results might be supported by the same mechanism connecting growth hormones and light interception. Therefore, vigor in grapevines may partly depend on promotion of photosynthesis, lateral root development and nitrate uptake, and these processes may be associated through the expression of genes downstream-regulated by TF 104879018 and TF 104879021.

Regarding the genes and TF found in chromosome 10, involved in biotic and abiotic stress responses, some authors have shown significant correlation between vigor and tolerance/susceptibility to diseases that could induce different defense responses in the host plant (2). Vigorous plants may have developed stronger immune responses to defend themselves.

Considering our findings in chromosomes 14 and 19, many genes encode for proteins involved in phloem and xylem development. This function showed the major enrichment (figure 1 page 32; table 1, page 30-31), representing a key aspect for growth, as transport of water, nutrients, proteins and sugars is vital for the plant to develop. These functions are tightly correlated to auxins and soluble carbohydrates seasonal dynamics, since cambium activity and xylem/phloem development respond to this signaling in woody species. It has been observed that IAA and soluble carbohydrate dynamics directly affect xylem and phloem formation in trees (6). In addition, gibberellins increase carbon allocation to different organs by inducing accumulation of non-structural carbohydrates in leaves, enhancement of phloem area and expression of sugar transporters (16).

Our results may lead to deeper gene selection strategies, aiming at choosing a smaller number of genes. Candidates with the smallest enrichment FDR values that are associated with QTLs that explain the highest percentages of variation, constitute interesting targets. For quantitative characters, where positive feedbacks cause large effects, significant explanatory effects from 10% to 20%, may result in impressive effects. This strategy considers that both approaches, QTL mapping and GO enrichment, work at different levels. While QTLs indentify regions on chromosomes containing genes encoding for a certain trait, the enrichment process takes into account all the genes involved in a pathway related to that trait. In processes where numerous genes are involved, a meaningful change should include many of them. Consequently, transcription factors could produce phenotype differences even at higher FDR values. In our work, this last effort selected 16 genes and 4 TRFs as plausible candidates for further breeding studies (table 1, page 30-31). Further functional genomic studies should weigh the importance of these selected genes on the final phenotype.

Conclusions

In conclusion, this analysis allowed the detection of plausible candidate genes encoding for the components of key processes governing vegetative growth in *Vitis*. The analysis allowed the reduction of candidate gene number based on marker proximity and functional enrichment, clearly demonstrating a suitable shortcut for target-directed genome-guided breeding strategies. This approach is particularly useful when a map is not densely saturated.

Two TFs, which potentially enhance growth by relating light response to hormone activation, and then to photosynthesis and morphogenesis, are strong candidates for targeted breeding. The nitrogen transport encoding genes would allow this light/hormone promoted growth by facilitating amino acid/protein synthesis and transport. Phloem and xylem related genes would also be part of this process by enabling water and nutrient transport. All these functions need to be tightly correlated, since auxins and soluble carbohydrates seasonal dynamics play key roles in tissue growth, cambium activity and xylem/phloem development. Gene characterization in individuals of the Ramsey × Riparia Gloire progeny will be the topic for future research.

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"We respectfully remember Dr. Hernán Vila, who lost his battle against COVID-19 in October 2020 and dedicate this article to his memory"

Germination and development of M_1 seedlings of two *Selliera radicans* Cav. accessions subjected to gamma radiation

Germinación y desarrollo de plántulas en M_1 de dos accesiones de *Selliera radicans* Cav. sometidas a radiación gamma

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Abstract

Selliera radicans is a creeping plant native to Chile, New Zealand and Australia. It is increasingly used in the ornamental industry, and there is interest in breeding it to create commercial varieties. The aim of this study was to determine the effects of different doses of gamma radiation applied to the seeds on the germination and development of seedlings (M_1) and the LD_{50} of two accessions of *Selliera radicans* for use in the induction of mutations. Seeds of the Vichuquén and La Serena accessions were exposed to 0, 100, 200, 300, 400, 500, 600 and 700 Gy from a ^{60}Co source. Weekly germination percentages along with seedling numbers and lengths were recorded. Vichuquén seeds were more sensitive to this physical agent. The LD_{50} was 243.9 Gy for Vichuquén and 445.6 Gy for La Serena. Seedling lengths reached almost 4 mm for Vichuquén and 11.3 mm for La Serena at 12 weeks after sowing. Doses lower than 200 Gy are recommended since higher doses do not allow the development of seedlings to the extended cotyledon stage.

Keywords

marsh weed • LD_{50} • seedling survival • M_1

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Resumen

Selliera radicans es una planta rastrera nativa de Chile, Nueva Zelandia y Australia. Su uso en la industria ornamental ha ido creciendo y hay interés en su mejoramiento genético para crear variedades comerciales. El objetivo del estudio fue determinar la dosis LD_{50} y el efecto de distintas dosis de radiación gamma aplicadas a las semillas sobre la germinación y el desarrollo de plántulas (M_1) en dos accesiones de *Selliera radicans*, para su uso en la inducción de mutaciones. Semillas de las accesiones "Vichuquén" y "La Serena" fueron expuestas a 0, 100, 200, 300, 400, 500, 600 y 700 Gy desde una fuente de ^{60}Co. Se registró el porcentaje de germinación semanal además del número y longitud de las plántulas formadas. La accesión de Vichuquén fue más sensible a la radicación. La LD_{50} fue de 243,9 Gy para Vichuquén y 445,6 Gy para La Serena. Las plántulas alcanzaron longitudes cercanas a los 4 mm para Vichuquén y 11,3 mm para La Serena a las 12 semanas desde la siembra. Se recomienda el uso de dosis menores a 200 Gy, pues dosis superiores no permiten la formación de plántulas en fase de cotiledón extendido.

Palabras clave
maleza de marismas • LD_{50} • sobrevivencia de plántulas • M_1

Introduction

Genetic improvement via the induction of mutation has been applied to various plant species that have nutritional importance or ornamental interest (27, 42). To induce mutations, physical and chemical agents, or a combination of both, have been used. Determining the mean lethal dose (LD_{50}) is essential (54). The LD_{50} value represents the amount of radiation or chemical agent absorbed with which 50% of the exposed population survives or that causes 50% growth reduction (7). Mutagens must be used in accordance with the aforementioned doses, since a greater (5) spectrum of mutations is generated; moreover, lower doses (less than 300 Gy) allow recovery of an adequate number of useful mutants compared to higher doses (29).

Compared to other types of radiation, the induction of mutations by gamma radiation has prevailed due to its wide availability, versatility and greater effectiveness at generating a wide range of mutations (9). The penetration of gamma rays into plant tissues is strong and uniform, which results in its high potential for plant breeding (28, 33). Exposure to ionizing radiation activates a series of steps between the initial absorption of energy and the final biological injury. The main and most important direct targets of radiation are water molecules, which lead to the generation of chain reactions that produce reactive oxygen species (ROS). These radicals are toxic at high levels and can react rapidly with various types of macromolecules, including lipids, proteins and, in particular, DNA, leading to cell damage and cell death (14). Data from the International Atomic Energy Agency (26) through 2017 revealed that 3,251 registered plant varieties were generated from induced mutations, of which 49.4% were obtained by gamma radiation.

The *Selliera* genus consists of three perennial rhizomatous herbaceous species that are currently recognized as a single polymorphic group (44). The species *Selliera radicans* Cav. has a rosette-type growth habit with whole, green fleshy leaves (22). The species is used as an ornamental plant for low-maintenance coverings, and due to its ability to be propagated by stolons, it is used in the creation of green roofs (51). This species can tolerate strongly saline soils that have an electrical conductivity > 20 dS m^{-1} and a pH ranging from 5 to 10 (3). Due to the scarcity of good-quality water resources required by ornamental species, especially those used for forage, the use of this type of species represents a great opportunity (12). The Universidad de Talca (UTalca) and the Pontificia Universidad Católica de Valparaíso (PUCV) currently have a collection consisting of 11 accessions from different areas of Chile; most of these accessions were described by Meza *et al*. (2015).

The aim of this study was to determine the effects of different doses of gamma radiation on the seed germination and development of seedlings (M_1) and the LD_{50} of two accessions of *Selliera radicans* for use in the induction of mutations.

Materials and methods

Plant material

Fresh seeds were extracted from the dried mature fruits of individual plants that were maintained as an in vivo clonal collection of plants, were collected from different zones in Chile and had been propagated for three generations. The selected accessions for this study were Vichuquén and La Serena.

Irradiation tests

Radiation was carried out on the premises of the Comisión Chilena de Energía Nuclear (CCHEN), La Reina, Santiago, Metropolitan Region, Chile. A ^{60}Co Gammacell 220R irradiator was used. The irradiation geometry was previously determined, and via Fricke dosimetry, the necessary time of exposure to apply the required doses was also determined. To determine the LD_{50}, seven doses were applied: 100, 200, 300, 400, 500, 600 and 700 Gy. A nonirradiated seed control group was also included. For each dose, 100 seeds were used. The seeds were sown one day after irradiation.

Sowing and germination conditions

The seeds were placed in Petri (10 cm) dishes on filter paper that was moistened with disinfectant solution (1 g L^{-1} captan); four replications of 25 seeds were used for each dose. The seeds were stratified for two weeks at 8°C in darkness according to Schiappacasse *et al*. (2017). Afterwards, the Petri dishes were transferred to germination conditions, which were 20°C (± 2°C) and artificial lighting for 24 h per day at 80 μmol m^{-2} s^{-1}. Germination was recorded weekly for 12 weeks. Seeds that produced a radicle equal to or greater than 2 mm were considered germinated (50).

Seedling growth and development conditions

Seedling size was determined by measuring the total length and shoot and root length from the apex of the cotyledons to the distal root end via images of the seedlings. Subsequently, all the seedlings were planted in trays filled with peat and perlite at a ratio of 1:1 (v/v); the substrate was amended with a Multicote© granular fertilizer (5 g L^{-1}). The seedlings were then kept under greenhouse conditions (21.2°C and 58.1% relative humidity [RH]). Two hundred days after germination, the final length of the seedlings was measured by digital image analysis using ImageJ© software, and the percent survival, in accordance with dose, was determined.

Statistical analysis

The tests were conducted in accordance with a completely randomized model; for both accessions, each dose was applied to a total of 100 seeds (4 replications with 25 seeds for each replication in a Petri dish). Germination curves were adjusted for each combination of dose and accession separately. The germination rate at time *t* was modeled using a log-logistic model with three parameters via the drc package in R software version 3.4.3 (48).

The parameter *d* indicates the maximum proportion of germination, *e* is the median time of germination (when F (t) = 0.50), and *b* is proportional to the slope of $F(t)$ when $t = e$. The estimation and verification procedures of the models are based on the use of time data for the event of interest, in this case, germination, as described by Ritz *et al*. (2015).

To determine the LD_{50}, a dispersion graph was constructed that included the cumulative germination at the end of the test (y-axis) in response to the applied radiation dose (x-axis). A linear relationship between both variables was calculated, and the LD_{50} value was then determined. The results of the survival lengths and percentages were processed with Minitab 17 statistical software. The seedling length values were transformed to 1/x values (41). The data were examined via analysis of variance (ANOVA), and means were compared by Tukey's test. For all the analyses, a significance level of 0.05 was used.

Results

The results of the model of each accession are shown in table 1 and table 2. For the Vichuquén accession, it was only possible to model the 300 Gy dose due to the large number of seeds that did not germinate at higher doses; the lower radiation doses had a stimulatory effect. For the La Serena accession model (table 2), parameter *d* (maximum germination ratio) tended to decrease as the dose increased, and parameter *e* (mean germination time relative to parameter *d*) increased as the dose increased.

Table 1. Estimates of the parameters (*e*, *d* y -*b*) and standard errors (SEs) of the log-logistic model as a function of the dose of gamma radiation for accession Vichuquén of *Selliera radicans* (1).

Tabla 1. Estimaciones de parámetros (*e*, *d* y -*b*) y error estándar (ES) del modelo log-logistic en función de las dosis de radiación gama para la accesión Vichuquén de *Selliera radicans* modelo (1).

Dose (Gy)	t_{50} (weeks)		Maximum germination (upper limit)		Slope at inflection	
	e	SE	*d*	SE	-*b*	SE
0	3.29	1.12	0.85	0.14	1.30	0.28
100	2.19	0.34	0.72	0.06	1.74	0.30
200	14.58	34.37	0.95	1.91	1.36	0.63
300	29.84	77.18	1.06	5.75	2.46	1.32

Table 2. Estimates of the parameters (*e*, *d* y -*b*) and standard errors (SEs) of the log-logistic model as a function of the dose of gamma radiation for accession La Serena of *Selliera radicans* (2).

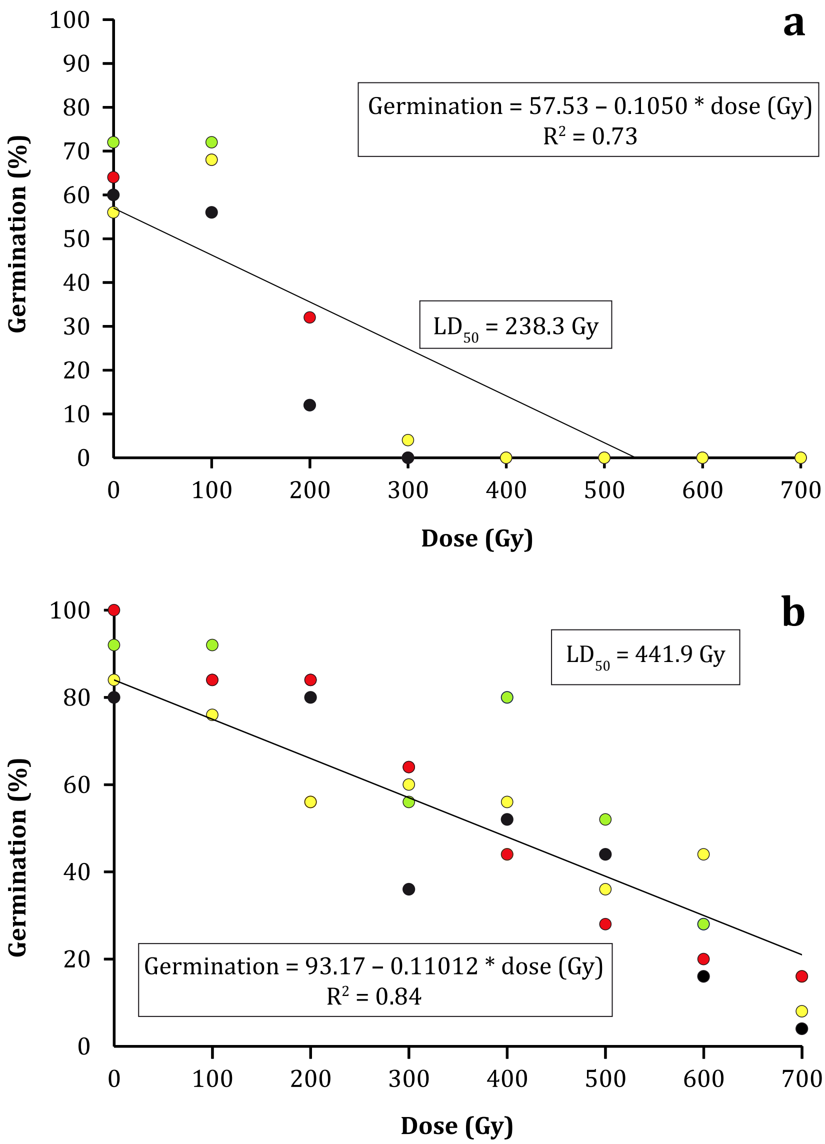
Tabla 2. Estimaciones de parámetros (*e*, *d* y -*b*) y error estándar (ES) del modelo log-logistic función de las dosis de radiación gama para la accesión La Serena de *Selliera radicans* modelo (2).

Dose (Gy)	t_{50} (weeks)		Maximum germination (upper limit)		Slope at inflection	
	e	SE	*d*	SE	-*b*	SE
0	1.05	0.08	0.89	0.03	2.48	0.36
100	1.04	0.06	0.82	0.03	3.73	0.55
200	0.99	0.06	0.76	0.04	3.73	0.61
300	1.65	0.17	0.55	0.05	2.32	0.39
400	2.46	0.17	0.59	0.05	3.42	0.46
500	2.52	0.14	0.40	0.04	5.00	0.77
600	2.32	0.40	0.28	0.05	2.15	0.52
700	2.63	0.28	0.11	0.03	5.26	1.55

Under the control conditions, the germination percentage was 65% for the Vichuquén accession and 89% for the La Serena accession. The Vichuquén accession reached its maximal germination during the eighth week (at the 100 Gy dose); on the other hand, the La Serena accession reached its maximal germination during the fourth week (at 0 Gy). The increase in dose had a negative effect on germination. When comparing both accessions, Vichuquén was more radiosensitive, since doses greater than 200 Gy were drastically harmful, and doses greater than 300 Gy resulted in no survival. For this accession, there was a stimulatory effect on germination at a dose of 100 Gy; these results were similar to those of the control (table 1).

Compared with the Vichuquén accession, the La Serena accession was more radiotolerant, having higher values in the germination curves. In agreement with the results of the other accession, the most harmful dose for La Serena was 700 Gy, but in this case, total lethality was not reached at any dose (table 2, page 39).

The differences in the germination curves of both accessions were converted into calculated LD_{50} values, as shown in figure 1a. The greater radiosensitivity of the Vichuquén accession was reflected in its LD_{50} value of 238.3 Gy, which was lower than that calculated for the La Serena accession (441.9 Gy).



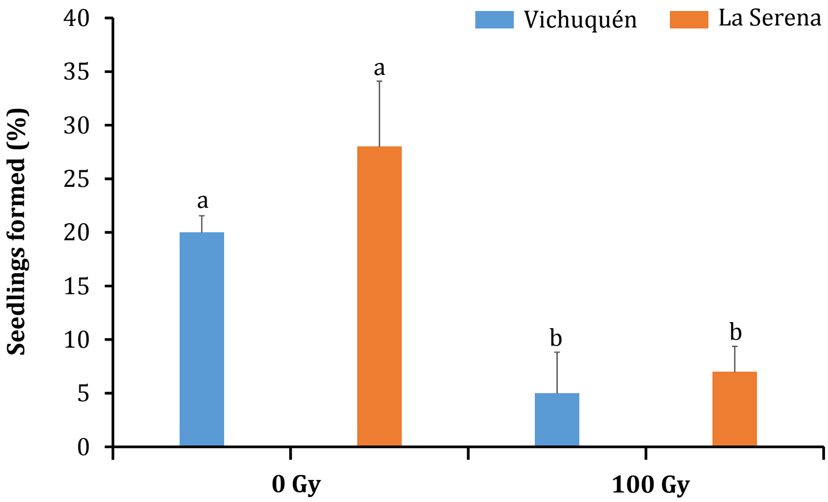
The percentage of germination used corresponding to week 8 (4 replicas of 25 seeds each).The boxes within the graphs show the equation of the trend lines and the LD_{50} values. Figure *a* corresponds to accession Vichuquén; figure b corresponds to accession La Serena.

El porcentaje de germinación utilizado corresponde a la semana 8 (4 réplicas de 25 semillas cada una). Los recuadros dentro del gráfico muestran la ecuación calculada y el valor estimado de LD_{50}. La figura *a* corresponde a la accesión Vichuquén y la figura *b* a la accesión La Serena.

Figure 1. Radiosensitivity curve for *Selliera radicans* accessions Vichuquén and La Serena.

Figura 1. Curva de radiosensibilidad para las accesiones Vichuquén y La Serena de *Selliera radicans.*

Although germination was observed for both accessions in response to different doses, survival and growth occurred only with the 0 and 100 Gy doses, and dose significantly affected the percentage of seedlings formed (figure 2, page 41).



The bars indicate the standard deviation, and different letters denote significant differences (Tukey test, α = 0.05%).

Las barras indican la desviación estándar y las letras diferentes indican diferencias significativas (Test de Tukey, α = 0,05%).

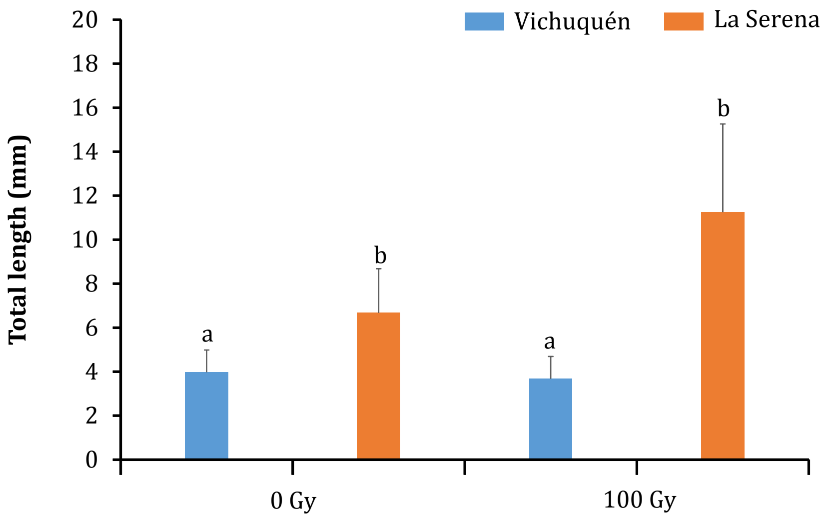
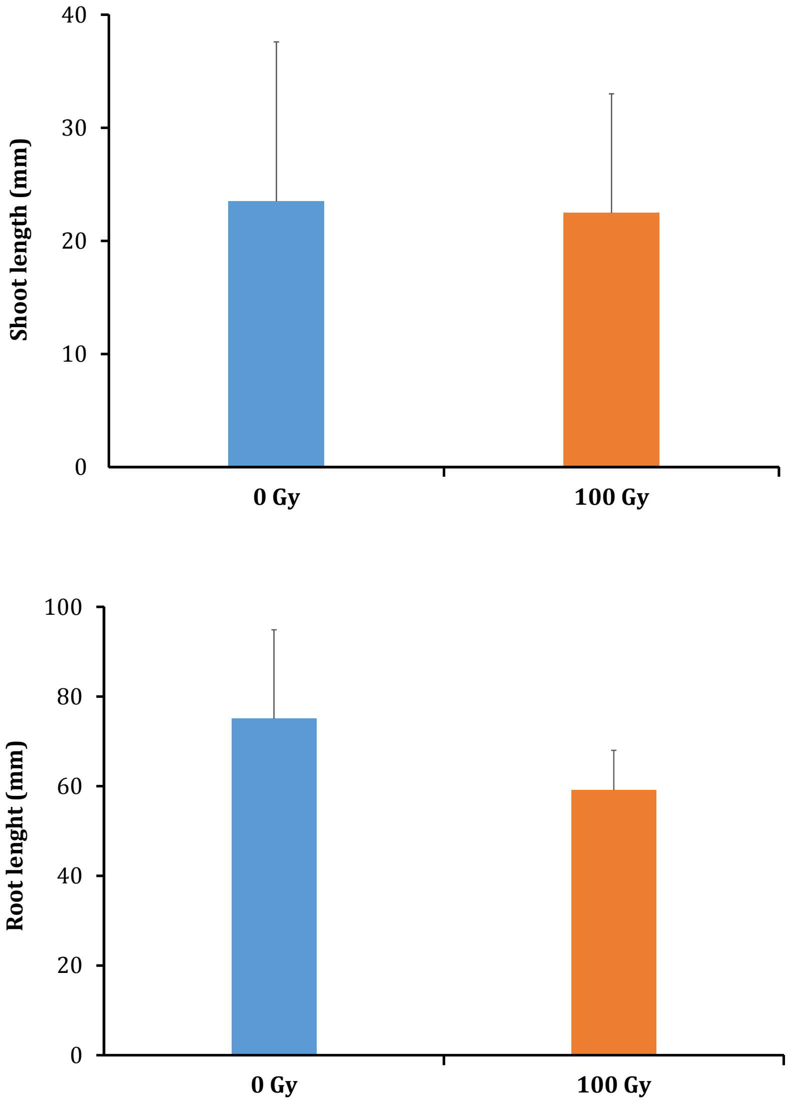


Figure 2. Percentage and length of seedlings formed by the *Selliera radicans* accessions Vichuquén and La Serena. The percentage of seedlings was estimated in terms of the total seeds of each replication (25 seeds) at 12 weeks after sowing.

Figura 2. Porcentaje y longitud de plántulas formadas en las accesiones Vichuquén y la Serena de *Selliera radicans.* El porcentaje de plántulas fue estimado con base en el total de semillas de cada réplica (25 semillas) a las 12 semanas posteriores a la siembra.

At the end of 12 weeks, the length of the control seedlings of the Vichuquén accession did not surpass 4 mm, and those of La Serena were significantly longer. Compared with the control dose, the 100 Gy dose had a stimulatory effect that resulted in longer La Serena seedlings. No mutations with obvious morphological effects, such as changes in leaf color or deformities in the formed seedlings, were observed in the M_1 seeds.

At 200 days after transplanting, data are only available for the Vichuquén accession since the seedlings from La Serena did not survive the radiation stress. The survival percentage according to dose reached 55% for the control and 60% for 100 Gy with respect to the total number of transplanted seedlings, with no significant differences. Regarding the values achieved in terms of leaf and root length, there were no significant differences between the control dose and the 100 Gy dose. The total length (foliar and root lengths) of the seedlings after 200 days showed an approximately 30-fold increase with respect to the initial seedling size (figure 3, page 42).



The measurements correspond to all the seedlings formed at 200 days after transplanting. The bars indicate the standard deviation.

Las medidas corresponden a todas las plántulas formadas 200 días después del trasplante. Las barras indican la desviación estándar.

Figure 3. Foliar and root length for the *Selliera radicans* accession Vichuquén.

Figura 3. Longitud foliar y radicular de *Selliera radicans* accesión Vichuquén.

Discussion

At higher doses of gamma radiation, a loss of viability was observed in the seeds of *Selliera radicans*, especially for the Vichuquén accession, which resulted in a decreased germination percentage (tables 1 and 2, page 39). This phenomenon may be related to the frequency of chromosomal damage, as an increased dose may be responsible for a lower germination potential and subsequent reduced growth (31, 37, 39). Gamma radiation-induced DNA damage results in the activation of different mechanisms to identify both the type and level of damage caused and drive both the repair and separation of damaged bases (17, 18). Depending on the radiosensitivity of each genotype, the levels of damage to the cells and cell protection involve a cascade of events that imply the activity of a series of enzymes and genes (25).

Ionizing radiation triggers a series of enzymatic defense systems (peroxidases and superoxide dismutases) and nonenzymatic systems in plants; these systems are involved in the compensatory mechanisms of free radical inhibition formed by oxidative stress after exposure to gamma radiation (2, 10, 21).

Oxidative stress at certain intensities can be repaired at the cellular level; however, gamma radiation affects the rate of germination (tables 1 and 2, page 39) and causes less growth as the dose increases. This pattern has also been reported in *Chenopodium quinoa* (19), *Zea mays* (36) and *Solanum melongena* (8). The 100 Gy dose stimulated the germination of the Vichuquén accession (table 1, page 39). Similar results in response to 100 Gy doses have been reported for *Terminalia arjuna* (1) and *Moluccella laevis* (40).

The stimulating effects of radiation on germination are still being studied. Some theories point to stimulating effects on enzymatic activity and on the synthesis of nucleic acids or the activation of protein synthesis; these effects occur during the initial stage of germination after irradiation of the seed, accelerating cell division and directly or indirectly stimulating auxin-response genes (7, 35).

Because the ability to repair damaged DNA has a strong genetic component, the studied accessions have specific radio sensitivities in terms of germination (figure 1, page 40). Differences in LD_{50} values have been recorded for other species at the variety or accession level (4, 30, 40, 52). In *Vigna unguiculata*, the LD_{50} value varied between 165 and 689 Gy depending on the genotype used (23).

Compared to that at the 100 Gy dose, the formation of seedlings in response to the 0 Gy dose was evidently greater (figure 2, page 41). It has been observed that at an early stage, growth is interrupted since the seedlings that form cannot survive after a certain period, which is probably due to DNA breakage and the inability to repair the damage (11, 20, 53). The prolongation of the growth interruption period can vary from a few minutes to several days, mainly according to the irradiation dose and the intrinsic radiosensitivity of the organism (34).

The imbalance in the development from the emergence of the radicle to the formation of a seedling may be related to an interruption of the cell cycle in G2/M phase during somatic cell division and/or to damage within the genome (45). In addition, imbibition is not affected by mutagenesis; this process has a relatively more pronounced effect on the subsequent development of the primary structures of seedlings, which can guarantee their survival (16). Interruption of the growth of the radicle and the zone of active cell division frequently (15) results in abnormal cells that have chromosomal aberrations (6).

The La Serena seedlings that survived the 100 Gy dose were longer than the control seedlings. A similar phenomenon was reported in response to gamma irradiation of *Vigna unguiculata* seeds at this same dose; this irradiation increased the vigor of M_1 seedlings in terms of foliar area and plant height at six weeks (43).

The ability of radiation to increase secondary metabolites may be because gamma radiation is a potent inducer of metabolites that have known activities against a broad spectrum of plant stresses, particularly oxidative stresses (47).

Considering the doses that allowed the formation of true viable seedlings, future applications of gamma radiation in this species should focus on doses lower than 200 Gy. In general, most mutated genes are recessive; it has been reported that the frequency of recessive mutations ranges from 90 to 100% and that for dominant mutations ranges from 0 to 6% (16). From the M_2 generation onwards, the resultant individuals are genetically diverse due to phenotypic segregation, which allows extensive selection from generations M_2 to M_5 to be carried out (24).

One of the most crucial requirements when inducing mutations is the selection of effective doses of mutagenic agents applied to the parental materials. This fact indicates why the significant differences between the LD_{50} values established in this study represent an important factor for subsequent irradiations, as the range of doses that can be used is limited (32). *Selliera radicans* was shown to be very sensitive to gamma radiation; in this respect, there may be a relationship between genome size and radiation sensitivity (13).

The ornamental potential of this species could be improved by gamma radiation, as many attractive characteristics that can be modified, such as growth habit and flower color and shape, are easily observable if they are present in plants generated after mutagenic treatment (46).

Conclusions

For the Vichuquén accession, the LD_{50} value for M_1 seed germination was 238.3 Gy; for La Serena, it was 441.9 Gy. However, *Selliera radicans* was highly sensitive to gamma radiation, so seedling formation occurred only at a dose of 100 Gy because the seedlings of La Serena did not survive after 200 days. At low doses, there was a stimulatory effect that could be studied in future research to accelerate the germination process. This beneficial effect did not translate into an increase in the foliar or root length of the seedlings formed at the dose of 100 Gy for the Vichuquén accession.

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Implementation of ice plant (*Mesembryanthemum crystallinum* L.) production under semi-controlled conditions

Implementación de la producción de lechuga glacial (*Mesembryanthemum crystallinum* L.) bajo condiciones semicontroladas

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Abstract

Ice plant (*Mesembryanthemum crystallinum* L.) is regarded as a drought and saline stress-tolerant plant with many biological properties, and especially valued in gourmet cuisine. The objective of this work was to find an optimum plant cultivation mode to produce edible parts under greenhouse conditions. Three soilless media were evaluated: peat, vermiculite and hydroponic culture. Peat pot culture yielded reduced biomass. Vermiculite and hydroponics, however, led optimum *M. crystallinum* growth. Plants grown in vermiculite and irrigated with nutrient solution presented a significantly higher yield. In fact, vermiculite-grown plants presented enhanced leaf area and leaf fresh weight as well as high foliar N, Mg, Mn, Fe, Na, chlorophyll and carotenoid concentrations. To conclude, greater succulence and Na concentration in edible parts of the vermiculite-grown glacier lettuce can offer consumers a more interesting taste, consistency and improved nutrient contents.

Keywords
Mesembryanthemum crystallinum • hydroponic culture • substrates • pot culture

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Resumen

La lechuga glacial (M*esembryanthemum crystallinum* L.) muestra tolerancia a los estreses salino e hídrico, y posee diversas actividades biológicas que han permitido su revalorización como planta comestible en la alta cocina. El objetivo del presente trabajo fue evaluar el óptimo modo de cultivo para la producción de hojas de *M. crystallinum* bajo condiciones de invernadero*.* Para ello, se compararon tres formas de cultivo sin suelo: turba, vermiculita y cultivo hidropónico. El crecimiento en maceta con turba no resultó ser adecuado para la lechuga glacial, provocando una baja producción de biomasa. Sin embargo, tanto el cultivo en vermiculita como hidropónico permitieron un óptimo crecimiento de *M. crystallinum,* mostrando una producción significativamente superior las plantas crecidas en vermiculita mediante riego con solución nutritiva. La lechuga glacial desarrollada en vermiculita incrementó su área y materia fresca foliar, junto con elevadas concentraciones de N, Mg, Mn, Fe, Na, clorofilas y carotenoides en hojas. Además, el aumento de la suculencia y la concentración de Na en las partes comestibles de las plantas cultivadas en vermiculita pueden ofrecer un sabor, consistencia y contenido en nutrientes de mayor interés para el consumidor.

Palabras clave

Mesembryanthemum crystallinum • cultivo hidropónico • sustratos • cultivo en maceta

Introduction

Mesembryanthemum crystallinum, commonly known as ice plant or glacier lettuce, is regarded as a stress-tolerant plant. During the dry season and periods of water stress, the photosynthesis mode switches from C_3 to Crassulacean Acid Metabolism (CAM) (9). This halophytic plant possesses specialised trichomes called epidermal bladder cells, which have various functions including: water storage, salt accumulation, protection from UV rays and a role in plant defence (7). When mature plants die, the stored salt is leached into the soil thereby dramatically increasing salinity, making it difficult for other native species to grow (10, 20, 32). However, due to its ability to accumulate salt, the plant has been employed for soil desalination and proposed for bioremediation (2). The ice plant can also rapidly absorb soil moisture and help to build up high nitrate levels (13).

The ice plant's numerous biological properties include antioxidant activities, performed by betacyanin and other flavonoids, as well as antimicrobial activities (18, 25). Leaves and stems are used raw or cooked. With its succulent, mellow, slightly salty-tasting leaves, the species is considered a fashionable plant sold in delicatessen shops (16, 30). The product, however, is highly perishable. The wild nature of this species suggests it could be cultivated throughout the year, with little watering and care, based on a different maintenance approach (1). Therefore, the objective was to conduct a greenhouse production experiment and assess optimum ice plant growth conditions in soilless media.

Materials and methods

Plant material and growth conditions

Mesembryanthemum crystallinum seeds were collected from wild plants in Alicante (in South East Spain). They were surface disinfected with 0.5% NaClO for 2 h and pre-hydrated with aerated, distilled water for 22 h. Germination subsequently took place in vermiculite, hydrated with distilled water and maintained in a growth chamber at 24°C day/night (D/N) air temperature (T) and 70% D/N relative humidity (RH) (29). Chamber light conditions were 16 h light-8 h dark cycle with photosynthetically active radiation of 400 μmol $m^{-2}s^{-1}$, provided by a combination of fluorescent tubes (Philips TLD 36W/83, Germany and Silvana F36W/GRO, USA). After 20 days, seedlings were transferred to a greenhouse under semi-controlled conditions of T D/N: 25/18°C; RH D/N: 60/80% and received natural daylight (mean photosynthetic photon flux rate of 400 μmol $m^{-2} s^{-1}$) (3) (Photo 1, page 49). During the transplant, plants were divided into three homogeneous groups of 10 plants. Each group underwent different growth conditions: 1) seedlings transplanted to plastic peat pots (V=1 L) watered weekly with 300 mL demineralised water; 2) seedlings inserted into 1 L plastic vermiculite pots watered weekly with 300 mL Hoagland nutrient solution (17); 3) seedlings grown in hydroponic containers (V=1 L) with Hoagland aerated nutrient solution, which was replaced weekly. Hoagland solution has been widely used for glacier lettuce growth (3, 5, 24, 29). Peat was mainly composed of pine bark, with 86% of organic matter based on dry weight, 49 mS m^{-1} conductivity, pH 7 and grain size <10 mm. After 45 days, the plants were harvested and the different determinations were performed. At the end of the experiment, the electrical conductivity (EC) of the hydroponic, vermiculite and peat culture was 1.38, 0.57 and 0.63 mS cm^{-1}, respectively.

Photo 1. Experimental site and ice plants grown under different mode of cultivation.

Foto 1. Unidad de experimentación vegetal y plantas de lechuga glacial crecidas bajo diferentes modos de cultivo.

Growth parameters

Plant dry weight (DW) was determined after oven drying at 80°C until constant weight. Shoot and root lengths were also measured. Leaf area was measured using the app "Easy Leaf Area Free" (12), while Specific leaf area (SLA) was calculated as the ratio of the leaf area to leaf dry weight.

Water status

Leaf relative water content (RWC) was calculated according to Weatherley's method (1950), using the following equation:

$$RWC\ (\%) = (FW-DW) / (TW-DW) \times 100$$

where:
FW = fresh weight,
TW = turgid weight,
DW = dry weight of the tissue, respectively.

This determination was performed on expanded young leaves collected at noon. Foliar succulence was measured according to Atzori *et al.* (2017) as the ratio of leaf FW to leaf area.

Photosynthetic pigments

Foliar photosynthetic pigment concentration was determined in young, recently expanded leaves collected at noon, as described in Sesták *et al.* (1971). Samples (20 mg FW) were placed in 5 ml of 96% ethanol at 80° C for 10 minutes to extract the pigments. The absorbance of the extracts was spectrophotometrically measured and the equations reported by Lichtenthaler (1987) were used to calculate chlorophyll and carotenoid concentrations.

Mineral analysis

Leaf samples (0.5 g DW) were dry-ashed and dissolved in HCl in accordance with Duque (1971). Phosphorus, potassium, calcium, magnesium, manganese, zinc, iron and sodium concentrations were determined using a Perkin Elmer Optima 4300 inductively coupled plasma optical emission spectroscopy (ICP-OES) (Perkin Elmer, USA). The ICP-OES operating parameters were: radio frequency power 1300 W, nebulizer flow 0.85 L min^{-1}, nebulizer pressure 206.84 kPa, auxiliary gas flow 0.2 L min^{-1}, sample introduction 1 mL min^{-1} and three replicates per sample. Total nitrogen and carbon were quantified after leaf DW combustion (950°C) with pure oxygen using an elemental analyser with a thermal conductivity detector (TruSpec CN, Leco, USA).

Statistics

To compare all three treatments, a one-way analysis of variance (ANOVA) (SPSS v.26, IBM Corp., USA) was conducted. The means ± standard deviation (SD) were calculated. When the *F*-ratio was significant ($p<0.05$), Duncan's multiple range test was applied. When only two treatments were compared, Student's *t*-test was performed. Significance levels were always set at 5%.

Results

Growth parameters of *Mesembryanthemum crystallinum* showed significant differences according to the culture medium (table 1). Plants in vermiculite presented greater shoot growth and leaf DW than those under hydroponic conditions, but the difference was especially notable compared to peat-grown plants. Vermiculite treatment also enhanced ice plant root length and biomass, while peat growth presented significantly low values. In fact, peat pot culture did not seem to be an adequate substrate to cultivate *M. crystallinum.* Despite the same environmental conditions and age, plants did not grow properly. Therefore, it was not possible to obtain sufficient plant matter from the peat treatment to perform further determinations such as: RWC, succulence, photosynthetic pigments or mineral analysis. On the other hand, vermiculite-grown plants exhibited a significant increase in leaf area compared to the hydroponic culture, despite their similar SLA values.

Table 1. Growth parameters in ice plants grown under different mode of cultivation.

Tabla 1. Parámetros de crecimiento de lechuga glacial crecida bajo diferentes modos de cultivo.

Treatment	[1]Shoot DW (g $plant^{-1}$)	[1]Leaf DW (g $plant^{-1}$)	[1]Root DW (g $plant^{-1}$)	[2]Leaf area (cm^2)	[1]SLA (cm^2 g^{-1})	[2]Shoot height (cm)	[2]Root length (cm)
Hydroponic	0.94±0.14 b	0.84±0.14 b	0.15±0.03 b	50,22±9,04 b	76,26±12,75 a	6.41±1.50 b	24.47±3.60 b
Peat	ND	ND	ND	0.87±0.14 c	ND	0.80±0.21 c	4.13±0.96 c
Vermiculite	2.12±0.36 a	1.83±0.29 a	0.32±0.07 a	133.65±19.14 a	72,47±16.94 a	10.25±2.04 a	29.36±5.16 a

[1]Means (n=10) ± SD were compared with Student-*t* test to analyze the differences between two of plant culture conditions. [2]Means (n=10) ± SD were compared with Duncan's test. Within each column, data followed by the same letter indicated that values did not differ significantly ($p≥0.05$). DW: dry weight; ND: not detected.

[1]Para la comparación de dos condiciones de cultivo vegetal, las medias (n=10) ± DS fueron analizadas mediante el test *t*-Student. [2] Las medias (n=10) ± DS fueron analizadas mediante el test de Duncan. Dentro de cada columna, el valor acompañado por una misma letra indica que las medias no difieren significativamente ($p≥0,05$). DW: materia seca; ND: no detectado.

Regarding leaf water status, ice plant foliar RWC did not differ according to the cultivation mode (table 2, page 51). Vermiculite-grown plants, however, presented the highest edible leaf FW production values together with greater succulence. As for photosynthetic pigments (table 3, page 51), both chlorophyll and carotenoid concentrations increased in vermiculite-grown plants.

Foliar mineral concentration is summarised in tables 4 and 5 (page 51). While C concentration did not differ among the plants grown in different media, foliar N concentration was higher in the ice plant cultivated in vermiculite (table 4, page 51). Moreover, vermiculite-grown plants had significantly higher Mg (table 4, page 51), Mn, Fe and Na (table 5, page 51) concentrations compared to the hydroponic treatment. Nevertheless, K and Zn concentrations were greater in plants grown in hydroponics, while P and Ca contents were similar across all treatments.

Table 2. Leaf fresh weight (FW), relative water content (RWC) and succulence in ice plants grown under different mode of cultivation.

Tabla 2. Materia fresca foliar, contenido hídrico relativo y suculencia de hojas de lechuga glacial crecida bajo diferentes modos de cultivo.

Treatment	Leaf FW (g)	RWC (%)	Succulence (g FW cm^{-2})
Hydroponic	23.43±4.78 b	78.15±15.40 a	0.45±0.05 b
Vermiculite	66.53±16.19 a	88.18±7.48 a	0.51±0.04 a

Means (n=10) ± SD were compared with Student-*t* test. Within each column, data followed by the same letter indicated that values did not differ significantly ($p \geq 0.05$).

Las medias (n=10) ± DS fueron analizadas mediante el test *t*-Student. Dentro de cada columna, el valor acompañado por una misma letra indica que las medias no difieren significativamente ($p \geq 0,05$).

Table 3. Leaf photosynthetic pigment concentration in ice plants grown under different mode of cultivation.

Tabla 3. Concentración foliar de pigmentos fotosintéticos de lechuga glacial crecida bajo diferentes modos de cultivo.

Treatment	Photosynthetic pigments (mg g^{-1} leaf DW)	
	Chlorophylls	Carotenoids
Hydroponic	15.74±1,99 b	2.85±0.40 b
Vermiculite	20.07±3.08 a	3.84±0.92 a

Means (n=10) ± SD were compared with Student-*t* test. Within each column, data followed by the same letter indicated that values did not differ significantly ($p \geq 0.05$). DW: dry weight.

Las medias (n=10) ± DS fueron analizadas mediante el test *t*-Student. Dentro de cada columna, el valor acompañado por una misma letra indica que las medias no difieren significativamente ($p \geq 0,05$). DW: materia seca.

Table 4. Foliar concentrations of macronutrients in ice plants grown under different mode of cultivation.

Tabla 4. Concentración foliar de macronutrientes de lechuga glacial crecida bajo diferentes modos de cultivo.

Treatment	C (mg g^{-1} DW)	N (mg g^{-1} DW)	P (mg g^{-1} DW)	K (mg g^{-1} DW)	Ca (mg g^{-1} DW)	Mg (mg g^{-1} DW)
Hydroponic	395.36±26.61 a	52.11±7.64 b	4.57±1.33 a	124.46±27.11 a	4.83±0.74 a	4.86±1.77 b
Vermiculite	373.91±35.75 a	64.18±3.79 a	4.20±0.46 a	75.05±13.50 b	4.84±0.85 a	13.26±1.32 a

Means (n=10) ± SD were compared with Student-*t* test. Within each column, data followed by the same letter indicated that values did not differ significantly ($p \geq 0.05$). DW: dry weight.

Las medias (n=10) ± DS fueron analizadas mediante el test *t*-Student. Dentro de cada columna, el valor acompañado por una misma letra indica que las medias no difieren significativamente ($p \geq 0,05$). DW: materia seca.

Table 5. Foliar concentrations of micronutrients and sodium in ice plants grown under different mode of cultivation.

Tabla 5. Concentración foliar de micronutrientes de lechuga glacial crecida bajo diferentes modos de cultivo.

Treatment	Mn (μg g^{-1} DW)	Zn (μg g^{-1} DW)	Fe (μg g^{-1} DW)	Na (mg g^{-1} DW)
Hydroponic	84.43±15,93 b	129.34±25.84 a	128.66±44.36 b	5.26±1.47 b
Vermiculite	107.91±29.18 a	64.72±16.44 b	1509.37±339.51 a	31.97±8.97 a

Means (n=10) ± SD were compared with Student-*t* test. Within each column, data followed by the same letter indicated that values did not differ significantly ($p \geq 0.05$). DW: dry weight.

Las medias (n=10) ± DS fueron analizadas mediante el test *t*-Student. Dentro de cada columna, el valor acompañado por una misma letra indica que las medias no difieren significativamente ($p \geq 0,05$). DW: materia seca.

Discussion

Peat is a widely used substrate for plant cultivation. In fact, *Mesembryanthemum crystallinum* has been successfully grown in peat-based substrate or mixed with vermiculite (3, 4, 8). However, our results showed that peat was not a suitable medium for ice plant pot-growing. Plant growth was very poor, making it impossible to determine many parameters and leading to significantly reduced shoot length, root length and leaf area. These results could be given by the fact that peat-grown plants were irrigated with demineralised water, while those grown in vermiculite and hydroponics received Hoagland nutrient solution. This may have generated an essential nutrient deficit in peat-grown plants, since the limited 1 L potting space increases nutrient needs (28). Moreover, according to Morales and Casanova (2015), peat is poorly drained. This probably leads to insufficient root oxygenation, causing growth limitations.

On the other hand, hydroponic and vermiculite cultures led to optimum *M. crystallinum* growth*.* In addition, vermiculite-grown plants irrigated with Hoagland nutrient solution presented a significantly greater yield. Vermiculite has a high cation exchange capacity (19), while in hydroponics, the nutrients are very accessible (22). It is worth noting that the hydroponic culture's EC levels at the end of the experiment were more than two times greater than that of the other two substrates; despite this, EC levels were within an optimum range regarding root water and nutrient absorption (15). In addition, the nutrient solution was replaced every week in order to ensure good nutrient balance in the hydroponic culture.

Vermiculite-grown plants presented higher values across all growth parameters, except for SLA. The reason may be that the nutrient intake of vermiculite-grown ice plants is greater due to longer roots and higher root DM, which would improve water and nutrient acquisition and/or use efficiency (26). In fact, plants grown in vermiculite presented higher leaf N, Mg, Mn and Fe concentrations.

Regarding the water status, cultivation mode was not found to influence foliar RWC in any way. On the contrary, leaf succulence improved in vermiculite-grown plants, probably due to greater nutrient intake than in hydroponics. Increases in succulence, that is, water content per unit area, allows osmotic adjustment (14). According to our results, vermiculite-grown *Mesembryanthemum* leaves also exhibited higher Na concentrations. The latter increases the salty taste of leaves and is highly appreciated by consumers. In fact, leaf succulence and glistening bladder cells, in particular, provide the edible leaves with a taste, consistency and appearance that make ice plants particularly valued by consumers (6). In addition, Agarie *et al.* (2007) worked on an ice plant mutant lacking epidermal bladder cells (EBCs). The authors concluded that EBCs contribute to succulence by serving as a water storage reservoir. They also contribute to salt tolerance by maintaining ion sequestration and homeostasis within photosynthetically active tissues of *M. crystallinum*.

Significant differences were found regarding pigment concentrations, with enhanced levels in total chlorophylls and carotenoids of vermiculite-grown leaves. This fact could imply that ice plants grown in vermiculite have a greater photosynthetic capacity, together with a greater allocation of energy in thermal dissipation over photochemistry (6).

Conclusions

Clear evidence was found that vermiculite pot culture irrigated with nutrient solution allows obtaining *M. crystallinum* yield for edible purposes. Under greenhouse conditions, this cultivation mode leads to greater biomass - including the production of edible fresh leaves - than hydroponics or peat culture. In addition, increased leaf succulence and Na concentration of vermiculite-grown plants offer consumers a more interesting taste, consistency and appearance.

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Optimal plot size for experimentation of common beans (*Phaseolus vulgaris* L.) in the northern region of Minas Gerais, Brazil

Tamaño de parcela óptimo para la experimentación de frijoles (*Phaseolus vulgaris* L.) comunes en la región norte de Minas Gerais, Brasil

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Abstract

The objective was to estimate the optimal plot size for field experiments with the common bean (*Phaseolus vulgaris* L.) using the modified maximum curvature method. A uniformity trial was carried out with the cultivar BRSFC-402 sown at a spacing of 0.5 m between plant rows and 10 plants per meter within the row. Final bean stand, mean number of pods per plant, mean number of grain per pod, mean 100-grain weight, and grain yield were evaluated. Measurement were taken from 20 central rows measuring 20 m in length, totaling 4,000 plants on an area of 200 m^2. A basic unit (0.5 m^2) was a row containing 10 plants. Four hundred basic units were combined to form 14 plot shapes. The methods relative information and modified maximum curvature were used to define the optimal shape and size of plots, respectively, for experimental evaluation with the common bean. As the plot should be suitable for efficient evaluation of all evaluated characteristics, the optimal plot size consisted of five basic units (25 plants) and was shaped as five rows x one basic unit per row.

Keywords
simulation • experimental precision • experimental design • *Phaseolus vulgaris* L.

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Resumen

El objetivo fue evaluar el tamaño mínimo de parcelas experimentales para el frijol común (*Phaseolus vulgaris* L.) utilizando el método de curvatura máxima modificado. El experimento consistió en una prueba de uniformidad en un suelo Latosol amarillo-rojo con la temporada de lluvias marcada por el verano con el cultivar BRSFC-402 sembrado a un espaciamiento de 0,5 m entre hileras de plantas y 10 plantas por metro dentro de la hilera. Para las mediciones se consideraron 20 hileras centrales de 20 m de longitud, totalizando 4.000 plantas en un área de 200 m^2. Se evaluó el rodal final de frijol, el número medio de vainas por planta, el número medio de granos por vaina, el peso medio de 100 granos y el rendimiento de grano. En las evaluaciones, cada fila con 10 plantas se consideró una unidad básica (0,5 m^2), que asciende a 400 unidades básicas cuyas dimensiones se combinaron en 14 formas de parcela. Se utilizaron los métodos de información relativa y curvatura máxima modificada para obtener la mejor forma y el tamaño de parcela más apropiado, respectivamente, para la evaluación experimental con frijol común. Considerando que la parcela óptima debería permitir una evaluación eficiente de todas las características evaluadas, el tamaño de parcela apropiado fue de cinco UB (25 plantas) en el formato de cinco filas x una UB por fila.

Palabras clave

simulación • precisión experimental • diseño experimental • *Phaseolus vulgaris* L.

Introduction

Common bean (*Phaseolus vulgaris* L.) is widely grown in both small and large areas in Brazil, where it plays an important role socially, culturally and economically (22). Although initially recognized as a subsistence crop, common bean production has been growing; therefore, several techniques have been adopted in its cultivation, including irrigation (17), biostimulant (1), soil cover, genetic parameters (23) and improved crop practices (15).

Brazil, the third world's largest producer of common beans, produced 3.3 million tons from 3.1 million hectares in 2017/2018, with an average yield of 1,043 kg ha^{-1} (9). As the crop is an important source of dietary protein, it plays a key role in food security. Accordingly, field experiments have been carried out across the country to test different cropping systems. Due to uneven soil and climate conditions, large experimental error is produced in field experiments, thereby decreasing the statistical power in verifying minimal significant differences between treatments.

The size of experimental plots may vary depending on the soil heterogeneity throughout the experimental area and intrinsic characteristics of each crop (12). Several aspects should be taken into account when determining the optimal plot size, such as crop type, number of treatments, technology, available land, and financial resources (30). Experimenters usually define the plot size based on site-specific conditions, available area or their personal research experience.

The plot size directly influences the experimental precision. Currently, plot sizes having four to six plant per row and measuring four to five meters long are used in most field studies with common beans. However, depending on the nature of the study, the number of experimental plots may be too large, so that field experimentation becomes virtually unfeasible. Smaller plot sizes may come as a solution but to the detriment of experimental precision.

Therefore, plot size and number of replicates should be optimally determined to obtain greater experimental precision (31). To do so, several methods have been used to determine the size and shape of the experimental plots. Among them, the Modified Maximum Curvature Method (MMCM) (20) stands out. This method algebraically determines through a regression equation the optimal relationship between plot sizes and their respective coefficients of variation (27). With this model, one can minimize experimental error, optimize resources, and ensure maximum precision (8).

The objective of this work was to estimate, using the Modified Maximum Curvature Method, the optimal plot size for determining yield components and the final plant stand of the common bean, with the aim of optimizing the use of resources without compromising the reliability of data obtained from experiments with this legume in the northern region of Minas Gerais, Brazil.

Material and methods

The study was conducted at the Experimental Farm of the Universidade Estadual de Montes Claros (UNIMONTES), located in the municipality of Janaúba, northern Minas Gerais state, Brazil (15° 48' 32"S, 43° 19' 3"W, and altitude of 533 m). The region's climate is Aw type, which corresponds to rainy summers and dry winters, according to the Köppen's classification. In the experimental area, the soil was classified as yellow-red Latosol (Ferralsols, WRB / FAO; Oxisols, Soil Taxonomy). The rainy season is normally marked by long dry spells in the middle of the season, followed by intense heat (25-35°C), strong sunlight and low relative humidity.

The experiment consisted of a uniformity trial with the cultivar BRSFC-402 sown at a spacing of 0.5 m between plant rows and 10 plants per meter within the row. Twenty 20-m-long central rows were considered for measurements, totaling 4,000 plants on an area of 200 m^2.

We evaluated grain yield (kg ha^{-1}), measured with a field scale and corresponding to the total mass of grains per basic unit (BU); final plant stand (FS), given by different BU combinations; mean number of pods per plant (NPP), obtained by direct counting; mean number of grains per pod (NGP), and mean 100-grain weight (M100). Number of grains per pod and M100 were estimated using a sample of five plants within each BU.

At harvest, the number of beans (grains) per plant in each BU was counted to allow the estimation of the final stand (FS). Then, results were converted to number of grains per hectare. The plants harvested in each BU were identified and taken to the laboratory, where the total number of pods of these plants was counted to estimate the average number of pods per plant (NVP).

The pods were threshed and the beans were counted to estimate the average number of grains per pod (NGP) by dividing the total number of grains by the total number of pods of each BU. The 100-grain weight (M100) was estimated by weighing three samples containing 100 beans collected from each BU, which were corrected to 13% humidity. Grain yield was estimated by weighing all the beans produced in each BU, and results were expressed as kg ha^{-1} and corrected to 13% humidity.

For each evaluated characteristic, we considered only BU arrangements that allowed plants to cover the entire experimental area. Thus, the basic units were combined in 36 plot sizes and 14 plot shapes with the largest relative information (RI) for the same BU size. The RI model considers the effect of the plot shape – width and length – on experimental precision. Thus, 14 plot sizes were obtained by combining BUs to form rectangular plots along the crop rows (table 1, page 58).

Using the Modified Maximum Curvature Method (MMCM), adapted by Meier & Lessman (1971), the point of maximum curvature or optimal plot size was determined by a potential regression equation.

$$y = \frac{a^b}{x} \quad (1)$$

where:
y = coefficient of variation,
x = plot size in basic units and a and b are constants of the model.

The point of maximum curvature was estimated by the following equation:

$$X_0 = \left[\frac{\hat{A}^2\hat{B}^2(2\hat{B}+1)}{\hat{B}+2}\right]^{\frac{1}{(2+2B)}} \quad (2)$$

where:
X_0 = value of the abscissa corresponding to the point of maximum curvature,
i.e. = the estimator of the optimal plot size
$\hat{A}$ and $\hat{B}$ = the respective estimates of A and B, which are constants of the equation.

Table 1. Number of plots (NP), plot shape, number of rows (NR), number of basic units per row (BUR), number of basic units (NBU) and total plot area (TPA).

Tabla 1. Número de parcelas (NP), formato, número de filas (NR) y de unidades básicas por fila (BUR), número de unidades básicas (NBU) y área de parcela total (TPA).

Identification	NP	Shape	NR	BUR	Dimensions		NBU	TPA (m^2)
					Width (m)	Length (m)		
A	2	Rectangular	10	20	5	20	200	100.00
B	4	Rectangular	10	10	5	10	100	50.00
C	5	Rectangular	20	4	10	4	80	40.00
D	8	Rectangular	5	10	2.5	10	50	25.00
E	10	Rectangular	10	4	5	4	40	20.00
F	16	Rectangular	5	5	2.5	5	25	12.50
G	20	Row	20	1	10	1	20	10.00
H	25	Rectangular	4	4	2	4	16	8.00
I	40	Row	10	1	5	1	10	5.00
J	50	Rectangular	4	2	2	2	8	4.00
K	80	Row	5	1	2.5	1	5	2.50
L	100	Rectangular	2	2	1	2	4	2.00
M	200	Row	1	2	0.5	2	2	1.00
N	400	Row	1	1	0.5	1	1	0.50

Relative information is determined in percentage by the ratio of variances: the variance of the plot composed of one BU (V1) and the comparable variance (Vc) obtained by dividing the variance by its corresponding plot size in BU (19).

$$IR(\%) = \frac{V_1}{V_c} x\ 100 \quad (3)$$

Using the RI method, we initially considered the variance of grain yield, FS, NPP, NGP and M100, between the X BU areas of each plot shape defined on the experimental field:

$$S^2{}_x = \frac{\sum_i (X_i - M(X))^2}{NP - 1} \quad (4)$$

$$M(X) = \frac{\sum_i X_i}{NP} \quad (5)$$

where:

X_i = the studied variable of the i-eth plot,

M = the mean of the variable measured in the plots with a size of X BUs; and NP is the number of plots with a size of X BUs.

Statistical analyzes to determine plot sizes by MMCM were built using Excel® based on a routine proposed by Donato *et al.* (2008, 2018). The models were selected according to the significance of the coefficients and the highest coefficients of variation and adjusted coefficients of variation, obtained through the analysis of variance and the t test in the R software (10). To determine plot shapes by the relative information method, simulation routines were built and run on Excel®.

Results and discussion

Of 36 tested plot shapes, 14 having greater relative information (RI) were selected. Therefore, the shapes and their respective RIs were determined for final plant stand, grains per pod, 100-grain weight, number of pods per plant, and yield.

Changes in relative information are not directly related to plot size in basic units, but rather to the shape of the plot (31). Due to the way the method works, the plot shaped as 1 R x 1 BU/R has the largest relative information for all analyzed variables and is used as reference (table 2). Therefore, based on results presented in table 2, the 5 R x 1 BU/R plot shape, with five BUs, ensures RI values closer to 100% for all studied variables, so this plot shape is the most suitable for field experiments with the common bean.

Table 2. Variables anallyzed for the shape of the plot for experimental evaluations with common bean.

Tabla 2. Variables analizadas para la forma de la parcela para evaluaciones experimentales con frijol común.

Plot shape (row or rectangular), dimensions (row and basic unit per row), number of basic units (BUs), number of plots (NP), plot area and relative information (%) with various shapes for stand, grains per pod (GP), 100-grain weight (MG), yield (Y) and pods per plant (PP).

Formato de trazado (fila o rectangular), dimensiones (fila y unidad básica por fila), número de unidades básicas (BU), número de trazado (NP), área de trazado e información relativa (%) con varios formatos para soporte, granos por vaina (GP), masa de 100 granos (MG), rendimiento (Y) y vainas por planta (PP).

Type (Shape)	Dimension	X BU	N° of plots repetitions	Area m^2	RI (%)				
	R x BU/R				Stand	GP	MG	Y	PP
Row	1 x 1	1	400	0.50	100	100	100	100	100
Row	1 x 2	2	200	1.00	96.09	90.75	91.58	92.49	79.46
Rectangular	2 x 2	4	100	2.00	82.22	92.70	96.12	87.91	71.04
Row	5 x 1	5	80	2.50	99.34	92.53	82.28	90.59	76.95
Rectangular	4 x 2	8	50	4.00	79.09	94.03	95.82	83.30	64.13
Row	10 x 1	10	40	5.00	86.06	99.06	66.38	74.68	56.14
Rectangular	4 x 4	16	25	8.00	64.07	86.13	96.58	79.97	47.10
Row	20 x 1	20	20	10.00	98.17	98.41	84.76	90.09	84.49
Rectangular	5 x 5	25	16	12.50	84.95	95.82	87.71	71.76	37.44
Rectangular	10 x 4	40	10	20.00	65.19	68.35	78.38	73.79	22.33
Rectangular	5 x 10	50	8	25.00	86.59	99.45	91.42	45.41	24.49
Rectangular	20 x 4	80	5	40.00	76.55	83.52	78.66	50.87	40.30
Rectangular	10 x 10	100	4	50.00	61.56	69.48	66.70	88.23	11.99

By grouping the 14 plot sizes, the lowest and highest coefficients of variation (CV) were associated with the combinations with the largest and lowest number of BUs, respectively (table 3, page 60). The relationship between these quantities tends to be inversely proportional because larger plots usually correlate negatively with lower coefficients of variation. An inverse relationship may occur on highly heterogeneous soils or with the use of unstable plant material; in both cases, large plots may produce high coefficients of variation (12).

In field trials, CV indicates experimental precision. Plot sizes with lower CVs are recommended for experimental purposes (8). The inverse behavior between plot size (BU) and coefficient of variation (table 3, page 60) has also been found in several similar studies (4, 8, 27, 30).

Figure 1 (page 61) depicts the relationship between the coefficient of variation and plot size in BU. The highest estimates of the optimal plot sizes or maximum curvature point (XMC) were associated with yield (figure 1A, page 61) and final plant stand (figure 1B, page 61), both having five BUs, followed by the number of pods per plant (figure 1C, page 61), number of grains per pod (figure 1D, page 61) and 100-grain weight (figure 1E, page 61), with approximate plot sizes of four, four and two BUs, respectively.

Table 3. Common bean yield evaluation in a 400 BU uniformity test (1 BU = 0,5 m^2).

Tabla 3. Evaluación del rendimiento del frijol común en una prueba de uniformidad de 400 BU (1 BU = 0,5 m^2).

Number of plots (NP), with sizes (rows (Xr) × basic units per rows (Xpr^{-1}), basic units (BU), area (m^2) and respective estimates of means (m), variance (s^2), standard deviation (SD) and coefficient of variation (CV).

Número de parcelas (NP), con tamaños (filas (Xr) × unidades básicas por filas (Xpr^{-1}), unidades básicas (BU), área (m^2) y estimaciones respectivas de medias (m), varianza (s^2), desviación estándar (DE) y coeficiente de variación (CV).

NP	Xr	Xpr^{-1}	BU	Area	Means	S^2	SD	CV
2	10	20	200	100.00	822606.29	499166682.29	22342.04	2.72
4	10	10	100	50.00	411303.14	94972178.64	9745.37	2.37
5	20	4	80	40.00	329042.52	131766325.79	11478.95	3.49
8	5	10	50	25.00	205651.57	92265277.75	9605.48	4.67
10	10	4	40	20.00	164521.26	45420918.68	6739.50	4.10
16	5	5	25	12.50	102825.79	29190348.24	5402.81	5.25
20	20	1	20	10.00	82260.63	18601763.44	4312.98	5.24
25	4	4	16	8.00	65808.50	16765227.58	4094.54	6.22
40	10	1	10	5.00	41130.31	11220117.29	3349.64	8.14
50	4	2	8	4.00	32904.25	8047571.77	2836.82	8.62
80	5	1	5	2.50	20565.16	4624810.23	2150.54	10.46
100	2	2	4	2.00	16452.13	3812557.38	1952.58	11.87
200	1	2	2	1.00	8226.06	1811875.07	1346.06	16.36
400	1	1	1	0.50	4113.03	837909.60	915.37	22.26

The coefficient of determination of optimal plot sizes ranged from 0.7366 to 0.9621 for the common bean's evaluated phenotypic descriptors (figure 1, page 61), with the best fit associated with yield (figure 1A, page 61), followed by final stand (figure 1B, page 61), 100-grain weight (figure 1E, page 61), beans per pod (figure 1D, page 61) and pods per plant (figure 1C, page 61). The coefficients of determination were high enough to ensure adequate precision in defining the plot size.

Using the MMCM, Santos *et al.* (2015) determined different plot sizes for sunflower and reported R^2 values ranging from 0.8806 to 0.9648 for optimal plot sizes. With this same estimator, R^2, Cargnelutti Filho *et al.* (2018) identified the optimal plot size (Xo, in m^2) with a coefficient of 0.9989 to assess fresh mass of turnips. For experimental evaluation of cactus pear, cv. Gigante, Guimarães *et al.* (2019) determined different plot sizes with R^2 between 0.8082 and 0.9352. These results reaffirm the high adjustment quality of MMCM.

Appropriate plot sizes varied with respect to the evaluated characteristics, with estimates of Xo and X falling between 1.64 and 5.00 and two and five BUs, respectively (figure 1, page 61). The smallest estimated plot size was associated with the 100-grain weight (2 BUs), followed by pods per plant and grains per pods (4 BUs), and by yield and final plant stand (5 BUs) (figure 1, page 61). The MMCM, besides estimating the optimal plot size determined by the critical level, also presents intermediate sizes, as found in several studies (6, 16, 18, 28).

Nevertheless, keeping reducing the coefficient of variation beyond the critical point is not beneficial to experimental precision, because, from the point of maximum curvature on, the coefficient of variation assumes a linear behavior (28).

The use of suboptimal or divergent plots may result in low experimental precision, higher experimental errors and high operational cost (3, 5, 8 13, 14, 25). In this study, depending on the investigated agronomic trait, plot size ranged from two to five BUs for field trials with the common bean. Similar results for the same crop was reported in the state of Mato Grosso, Brazil, with 3 m^2 or 60 plants (29).

*** Significant at a 0% level of significance (p < 0.001).

*** Significativo a un nivel de significancia de 0% (p <0,001).

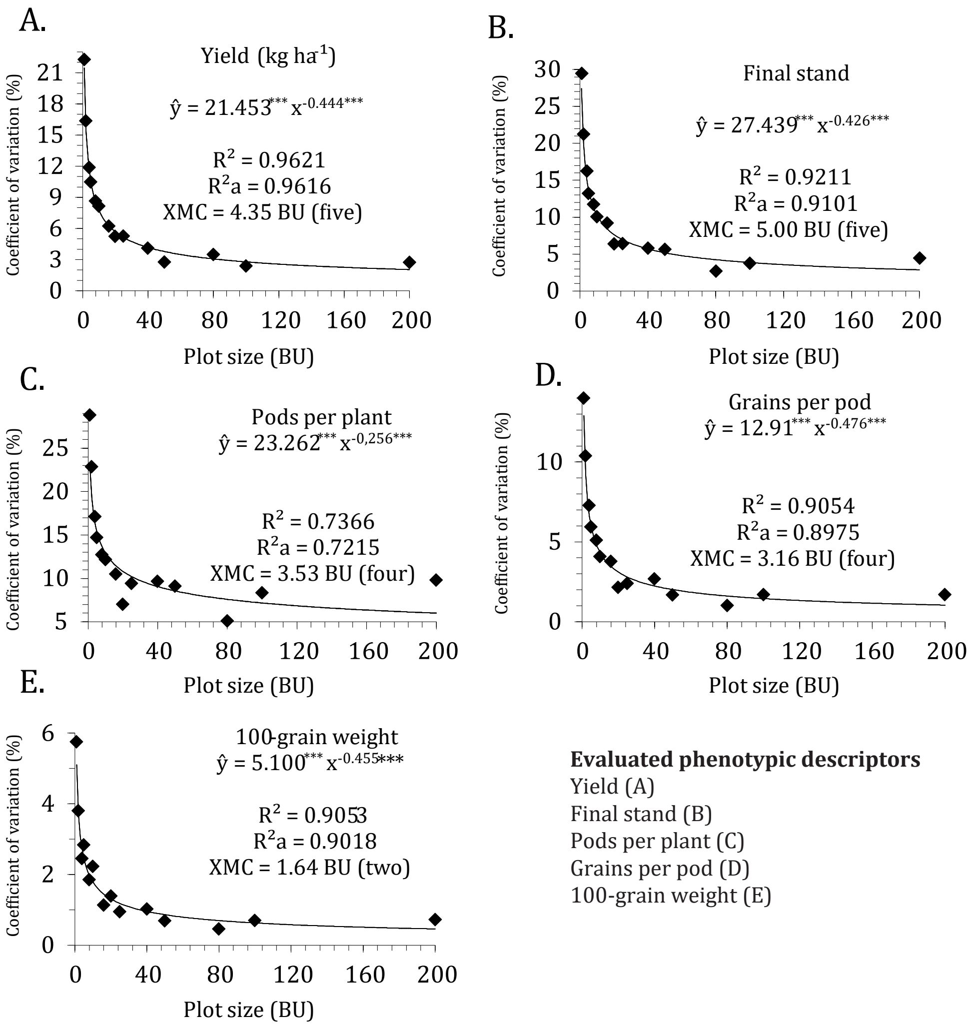

Figure 1. Graphical representation between coefficient of variation (CV) and plot size in basic unit (BU) to estimate optimal plot size (XMC) of yield (A), final plant stand (B), pods per plant (C), grains per pod (D) and 100-grain weight (E) for the common bean.

Figura 1. Representación gráfica entre el coeficiente de variación (CV) y el tamaño de la parcela en la unidad básica (BU) para estimar el tamaño óptimo de la parcela (XMC) de productividad (A), soporte (B), vainas por planta (C), granos por vaina (D) y masa de 100 granos (E) para frijol común.

Studies with common bean have been developed with plots of various sizes, with areas of 24 m^2 (4), 100 m^2 (21), 72 m^2 (26) and 24 m^2 (2). These plot sizes are defined based on factors including available resources, the researcher's experience, and limitations on the experimental area. However, 5 m^2 plots ensure significant precision for experimental evaluation, thereby optimizing agricultural inputs and human resources. Thus, in line with the results obtained in this work, the experimental area can be optimized with significant reduction in plot size.

Field experiments with the common bean should be designed with five BUs, because all traits evaluated in this work are measured together. Moreover, the modified maximum curvature method, despite being a procedure with algebraic determination (7), tends to estimate smaller plots when compared to other methods (12, 32). By following this statistical recommendation, the experimenter will gain efficiency in the use of resources without compromising the reliability of collected data.

Conclusions

Yield and final plant stand require larger plot sizes to be evaluated with higher experimental precision. For experimental evaluation of common beans involving grain yield and its primary components (final stand, number of pods per plant, number of grains per pod and 100- grain weight), the plot size should be composed of five basic units in the northern region of Minas Gerais, Brazil.

The plot shape of five-rows x one BU/row, with five BU, ensures relative information values closer to 100% for all evaluated variables. This shape is suitable for experimental purposes with the common bean in the northern region of Minas Gerais, Brazil.

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Does foliar nicotinamide application affect second-crop corn (*Zea mays*)?

¿La aplicación foliar de nicotinamida afecta al cultivo de maíz (*Zea mays*) de segunda?

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Abstract

The largest corn yield in Brazil is currently provided from the second-crop, which is the most susceptible period to climatic adversities occurring during crop development. Thus, introducing beneficial elements for maintaining the adequate development of the plant can help producers in obtaining higher grain yields. Among studied elements, nicotinamide has potential use since it is associated with accumulating secondary metabolites and manifesting defense metabolism in plants. This study aimed to evaluate the influence of nicotinamide applied in different doses (one or two applications) on the biometric and productive characteristics of corn. The treatments were composed of the number of nicotinamide applications (one or two) and five doses (0, 50, 100, 150, and 200 mg L^{-1}). Plant diameter, plant height, ear insertion height, leaf area and dry matter, grain yield, and 100-kernel mass were evaluated. It was found that doses close to 100 mg L^{-1} resulted in increases in vegetative and reproductive development, regardless of the number of applications. In this way, the foliar nicotinamide application positively influences the biometric and productive characteristics of second-crop corn.

Keywords
Zea mays • niacin • biostimulant • vitamin B3

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Resumen

El mayor rendimiento de maíz en Brasil se encuentra actualmente en la segunda cosecha, que es el período más susceptible a las adversidades climáticas que ocurren durante el ciclo. Por lo tanto, la introducción de elementos beneficiosos para mantener el desarrollo adecuado de la planta puede ayudar a los productores a obtener mayores rendimientos de grano. Entre los elementos estudiados, la nicotinamida tiene un uso potencial ya que está asociada con la acumulación de metabolitos secundarios y la manifestación del metabolismo de defensa en las plantas. El objetivo de este trabajo fue evaluar la influencia de la nicotinamida aplicada en diferentes dosis (una o dos aplicaciones) sobre las características biométricas y productivas del maíz. Los tratamientos fueron compuestos por el número de aplicaciones de nicotinamida (una o dos) y cinco dosis (0, 50, 100, 150 y 200 mg L^{-1}). Se evaluaron el diámetro de la planta, la altura de la planta, la altura de inserción de la mazorca, el área de la hoja y la materia seca, el rendimiento y la masa de 100 granos. Se encontró que dosis cercanas a 100 mg L^{-1} resultaron en aumentos en el desarrollo vegetativo y reproductivo, independientemente del número de aplicaciones. De esta manera, la aplicación foliar de nicotinamida influye positivamente en las características biométricas y productivas de maíz de segunda cosecha.

Palabras clave

Zea mays • niacina • bioestimulante • vitamina B3

Introduction

Corn is the largest cereal produced in the world, with approximately 1.06 billion tons produced in 2016. The United States, China, Brazil, and Argentina are the largest producers, accounting for about 68% of world production (10). In Brazil, corn is the second most important crop in agricultural production and the main alternative to soybean cultivation, with a production estimate of 88.6 million tons in the 2017/2018 harvest (7).

With the largest portion of corn production coming from the second-crop (about 71.1%) (7), one of the current challenges is to maintain crop productivity, even under the occurrence of adverse conditions. In this sense, we are looking for products or elements with a protective character or biostimulants that can maintain adequate development of the plant. Among the studied elements are B vitamins, which have the desired actions against barriers imposed on corn production (13, 18).

Vitamins are necessary to maintain normal growth and the proper development of organisms. These elements act as coenzyme systems and therefore play an important role in regulating metabolism. Vitamins may be limiting factors in plant development (5), acting on the plant's defense mechanisms (12).

Among vitamins that occur in plant tissues, nicotinamide is a constituent of pyridine dinucleotide coenzymes NADH and NADPH, which are directly associated with some enzymatic redox reactions in living cells. The nicotinamide concentration may increase in plants after situations that cause oxidative stress and induce defensive metabolism (6).

Nicotinamide is an element associated with stress which regulates secondary metabolite accumulation and induces defense metabolism manifestation in plants (6). The effects of nicotinamide are observed in vegetative growth via cell expansion, reserve accumulation, and productive characteristics improvements under adverse environmental conditions, such as water deficit and soil salinity (2, 9). Also, for cereal crops, it is observed that nicotinamide affects vegetative and reproductive development, as in the case of upland rice (19).

The study is based on the information and under the hypothesis that the exogenous application of nicotinamide has a positive effect on the vegetative and reproductive characteristics of second-crop corn. This study aimed to evaluate the influence of this vitamin in different doses and the number of applications on the biometric and productive characteristics of second-crop corn.

Material and methods

The study was carried out in the second crop of the 2016/2017 harvest, in the experimental area of the Federal University of Mato Grosso do Sul, in Chapadão do Sul campus, MS, at 18°5’46” S, 52°62’99” W, and altitude of 820 m. The soil of the experimental area is classified as a Latossolo Vermelho distrofico or Oxisol.

The climate of the region is Aw-type, according to the Köppen classification, defined as tropical humid with a rainy season in the summer and dry in the winter, with an average annual rainfall of 1850 mm and average daily temperature from 13°C to 28°C (8). The rainfall values and average temperature (figure 1) in the experimental area during the experiment evidenced that there were water limitations and thermal variations during the crop cycle.

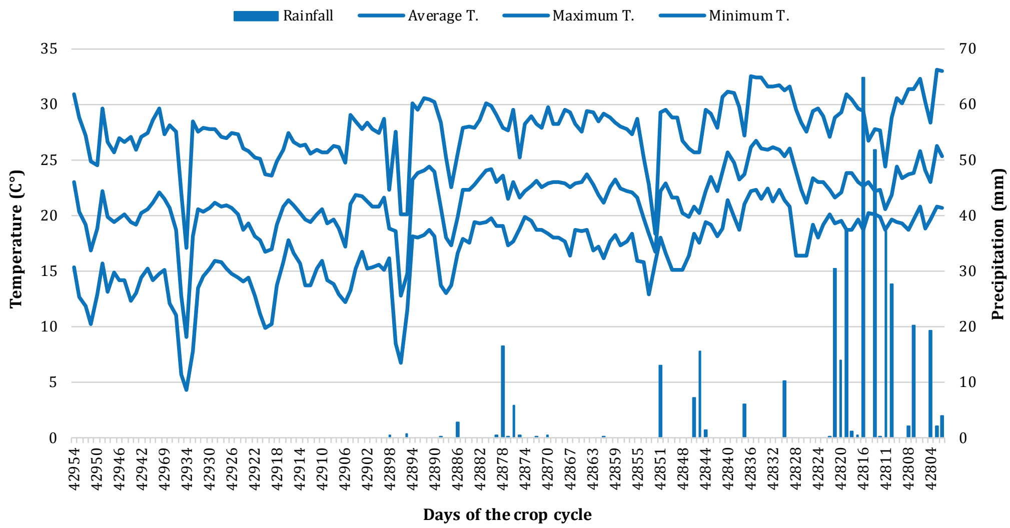


Figure 1. Rainfall (mm), maximum, average, and minimum temperature during the experimental period. 2016/2017 harvest.

Figura 1. Precipitación (mm), temperatura máxima, media y mínima en °C, para el período de cultivo de maíz durante la cosecha de 2017.

A randomized complete block design with four replicates was used. The treatments were arranged in a 2 x 5 factorial scheme. The number of applications (one or two) and five nicotinamide doses (0, 50, 100, 150, and 200 mg L^{-1}) were evaluated. The first application was performed at the V3 growth stage, and the second at the V5 growth stage. The experimental plots consisted of five rows, spaced 0.45 m apart, and 5.0 meters long. The evaluations were carried out in the two central, excluding 1.0 meter at each end.

The hybrid Pioneer 30S31 was sown on March 08, 2017. Fertilization in the sowing furrow was performed with a dose of 150 kg ha^{-1} of K_2O, with KCl as a source. The topdressing fertilization consisted of an application of 60 kg ha^{-1} of N, using urea as a source (45% N) at 20 and 30 days after sowing. Weed, pest, and disease controls were carried out as recommended by EMBRAPA for the corn crop.

The biometric characteristics of stem diameter, plant height, ear insertion height, leaf area, and dry matter were evaluated. A digital caliper was used to measure stem diameter at the base of the plant, close to the ground. Five plants per plot were evaluated for plant height and ear insertion height via a topographic survey for data collection .

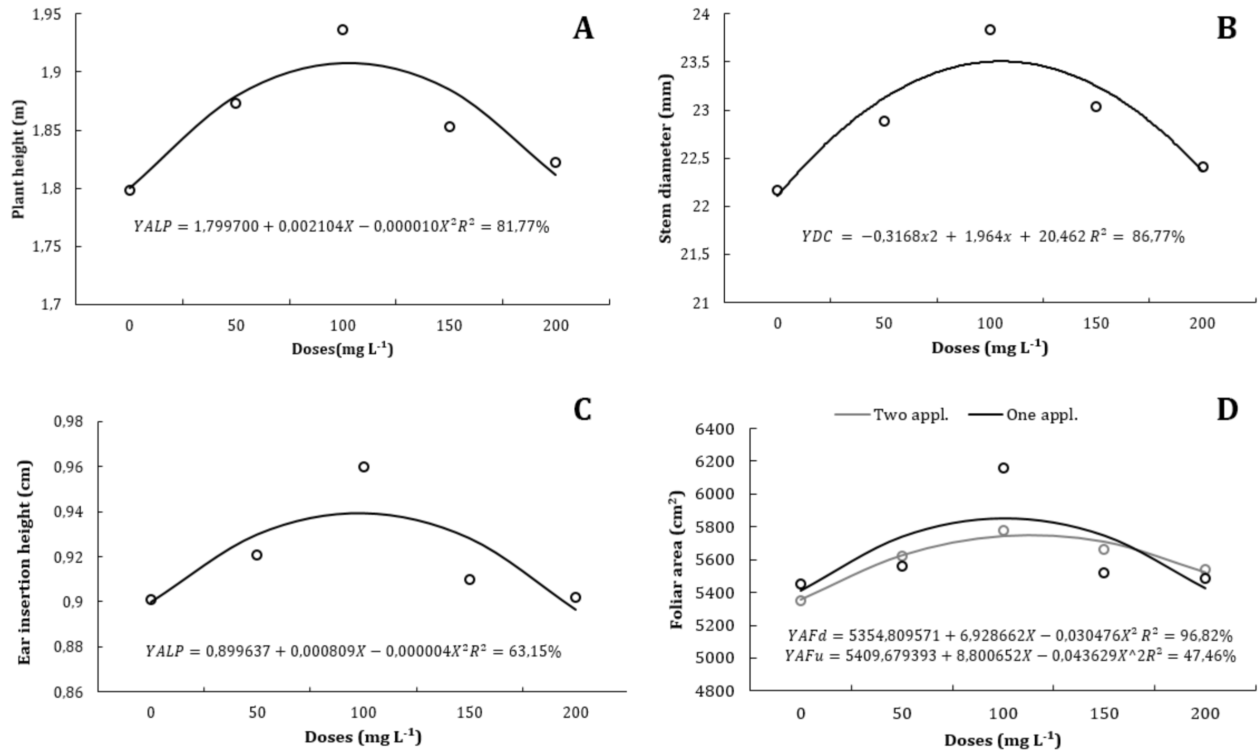
The number of leaves and leaf area of the plants were also measured through a portable meter (CI-203, CID Bioscience, Camas, Washington, USA). Finally, the dry matter of the leaves was obtained after drying in a forced air circulation oven at 65°C until obtaining a constant matter.

For productive characteristics evaluations, the ears were harvested manually in sequence, aiming at separating husk, grain, and cobs. After the threshing, the 100-kernel mass was evaluated by separating and weighing them on a semi-analytical balance. Grain yield was obtained by weighing the grains harvested in the useful area of each plot (2.7 m^2) and adjusting the moisture to 13%.

The data were submitted to analysis of variance. The means from the number of applications were compared by the Tukey test at 5% probability. The means from the nicotinamide doses were evaluated by regression analysis at a 5% probability.

Results and discussion

For corn plant height (PH), it was observed that there was positive quadratic behavior up to the maximum calculated dose of 105.2 mg L^{-1}, with a decrease in this characteristic after this point. Moreover, there was an increase of 5.6% and 1.4% regarding the treatment without nicotinamide application and the 50 mg L^{-1} application, respectively. However, for doses above the maximum point, there was a decrease in plant height up to the maximum dose used (figure 2a).



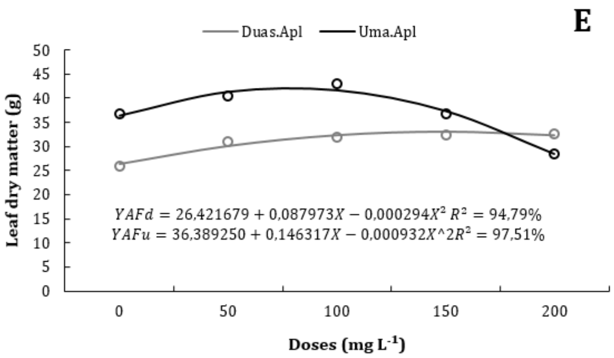


Figure 2. Plant height (a), stem diameter (b), ear insertion height (c), leaf area (d), and leaf dry matter (e) of corn plants submitted to applications of nicotinamide doses.

Figura 2. Altura de la planta (a), diámetro del tallo (b), altura de inserción de la mazorca (c), área de la hoja (d) y materia seca de la hoja (e) de las plantas de maíz sometidas a dosis de nicotinamida.

In a study combining humic acids with nicotinamide, it was verified a responsive effect in using nicotinamide for height growth in wheat plants (*Triticum durum*) (9). These authors attributed this positive effect to the fact that nicotinamide improves cell elongation, also protecting photosynthetic pigments, which explain the increase observed up to 100 mg L^{-1} of nicotinamide in corn plants in this study. Similarly, another study tested the foliar nicotinamide application in quinoa plants (*Chenopodium quinoa*) and found an increase in the length of the progressive branches in doses varying from 0 to 100 mg L^{-1} (1).

The highest stem diameter (SD) was estimated at the dose of 104.7 mg L-1. The SD value was 23.5 mm at this dose, 5.9% larger than the control, and 1.6% higher than the application of 50 mg L^{-1}. However, doses above the maximum point promoted decreases in the stem diameter of the plants (figure 2b, page 67).

Studying the growth of corn hybrids in the state of Goiás, it was found a maximum SD of 23.44 mm in the Feroz hybrid, similar to that obtained in the present study with the nicotinamide application of 100 mg L^{-1} (4). These values are attributed to thick and resistant stalks, being sufficiently strong against the breaking and lodging of corn plants (20).

By applying 0, 200, and 400 mg L^{-1} nicotinamide, it was verified an increase in fava bean (*Vicia faba* L.) growth (2), thus corroborating this work. As already stated in the literature, the use of nicotinamide stimulates cell expansion and division, and the accumulation of reserves (9), possibly justifying favoring thicker stalks in corn plants.

Ear insertion height (EIA) was found to be below 1 m, regardless of the applied nicotinamide doses (figure 2c, page 67). Compared with the control, the dose of 101.1 mg L^{-1} provided 4.2% higher ear insertion height. This result can be related to the plant height (PH) since the same quadratic behavior was observed, thus the height of the plants directly influences the height that the ears will be inserted.

In several corn cultivars, it has been shown that the ear insertion height influences the plants' lodging and breaking, where the highest frequency of these events was observed in plants in which the insertions occurred above 0.90 m. Thus, considering that corn plants were favored by increasing nicotinamide doses due to cellular elongation (9). It is possible to infer that these plants are more susceptible to breaking by wind, thus conferring a negative characteristic since the higher the plant height, the higher the ear insertion (14).

Regarding the leaf area, it was verified that only one nicotinamide application gave better results than two applications of 100 mg L^{-1} of nicotinamide (figure 2d, page 67) was used.

With two applications, there were increases in leaf area up to the dose of 100 mg L^{-1} of nicotinamide, with maximum point estimated at 113.7 mg L^{-1} (5748.68 cm^2). In relation to the control (0.0 mg L^{-1}), doses of 50, 100, 150, and 200 mg L^{-1} provided increases of 5.0%, 8.0%, 5.9% and 3.49% in the leaf area, respectively. Whereas increases of 2.0%, 13.1%, 1.3%, and 0.7% were observed for the same doses with one nicotinamide application, and the maximum point was obtained at 100.9 mg L^{-1}. Also, there was a significant difference between the number of applications when the nicotinamide dose of 100 mg L^{-1} was applied, and the single application exceeded the split dosage by 1.9%.

In general, the leaf area is linked to the photosynthesis potential of a plant; since the plant yield will be proportionally higher, the larger its area of photoassimilates production (3). Taking into account that the yield of corn kernels is dependent on the efficiency of using solar radiation for accumulating biomass (11), the increase in the nicotinamide doses up to 100 mg L^{-1} favored the leaf increase, independently from the number of applications.

With increasing niacin doses (vitamin B3) and thiamine (vitamin B1) applied to mustard plants (*Brassica juncea* L.), it was obtained similar results to those found in this study with quadratic behavior of the leaf area, but only decreasing at doses above 400 mg L^{-1} (17). The treatment of plants with B vitamins favors accumulating foliar carbohydrates stimulating their expansion (16), as well as promoting an increase in chlorophylls and carotenoids (9), increasing plant productivity.

For the leaf dry matter (DLM), it was verified a maximum value of 42.13 g when an estimated single dose of 78.66 mg L^{-1} was applied (figure 2e, page 67). The increase was 13.64% in comparison with the control treatment. After this point, there were progressive decreases up to 200 mg L^{-1}, obtaining DLM of 32.6% lower when compared to the maximum point. With two applications, there were constant increases of DLM up to the estimated peak of 33.00 g, obtained with the maximum calculated nicotinamide dose of 149.6 mg L^{-1}. Regarding the control, the maximum DLM point was 19.94% higher.

These results were similar to those obtained for mustard plants; the highest value was obtained in the 400 mg L^{-1} application of niacin and thiamine (17). For bean plants, it was observed an increased dry matter yield with nicotinamide (200 and 400 mg L^{-1}) for both plants cultivated without stress and for plants irrigated with saline water at 50 mM NaCl and 100 mM NaCl (2). Results favoring the accumulation of dry matter by nicotinamide application have also been reported for quinoa plants (1).

About corn yield under the effect of foliar nicotinamide application, it is possible to observe similar behavior for both one and two applications, but smaller when only applied once in doses of 150 and 200 mg L^{-1} (figure 3a). The estimated maximum yield point of 6181.08 kg ha^{-1} was obtained with a nicotinamide application at the dose of 96.65 mg L^{-1}. In contrast, the maximum estimated yield for two applications was 6203.64 kg ha^{-1} in the application of 80.48 mg L^{-1}.

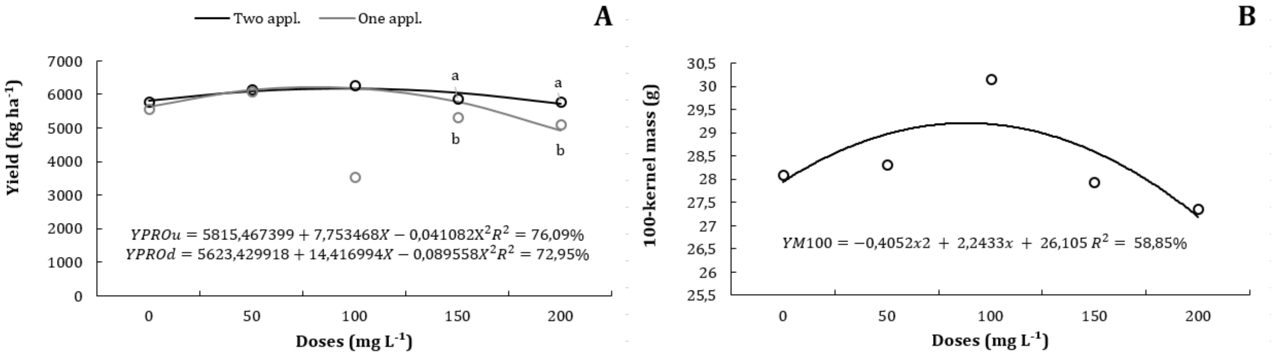


Figure 3. Productivity (a) and 100-kernel mass (b) of corn plants submitted to nicotinamide application.

Figura 3. Productividad (a) y masa de 100 granos (b) de plantas de maíz sometidas a la aplicación de nicotinamida.

About the estimated maximum values, two nicotinamide applications promoted an increase of 0.36% on grain yield when compared to one application; however, with the use of 16.73% less of the product through the foliar application, it is therefore inferred that the split nicotinamide application may give satisfactory results in yield.

The corn yield in Brazil is around 5000 kg ha^{-1} and 5500 kg ha^{-1} in the first and second crop (7), respectively. The nicotinamide application promoted gains at doses of 50 mg L^{-1} and 100 mg L^{-1}, and yields close to the national averages were observed for the other treatments.

An increase in wheat grain yield of approximately 11.0% was observed when using recommended doses of fertilizers in association with nicotinamide, to the detriment of only applying fertilizer doses (9). In the present study, there was an increase of 9.7 and 6.9% of grain yield with one and two applications of nicotinamide when compared to the control treatments.

For the 100-kernel mass (M100), a similar pattern to that obtained in the leaf dry matter was observed, in which one nicotinamide application was positively superior in accumulating M100 up to 150 mg L^{-1}. The only dose in which the sequence of two applications was more suitable was 200 mg L^{-1} of nicotinamide. The estimated maximum point for two applications was 78.66 mg L^{-1} of nicotinamide. With one application, this point was 149.6 mg L^{-1}, with quadratic behavior, as observed for leaf dry matter (figure 3b).

In an evaluation of the agronomic performance of 11 corn hybrids in the central-west region, it was verified a mass of 100 kernels varying between 24.95 g and 36.34 g, similar to the results obtained with two nicotinamide applications, ranging from 25.99 g (0 mg L^{-1}) and 36.34 g mg L^{-1}). However, one application at the dose of 100 mg L^{-1} (42.92 g) had better results than the most productive hybrid of the cited study, hybrid 20A55. Nicotinamide participates in the $NADP^+$ (Nicotinamide adenine dinucleotide phosphate) photosynthesis electron acceptor. Thus, the external application of this substance can positively influence an increase in the photosynthetic process and photoassimilate production, used in the final stage of kernel filling (14), therefore explaining the reason its use is indicated up to a certain dose.

Conclusion

Foliar nicotinamide application, at a dose of 100 mg L^{-1} and conducted as a single spray, positively influences the biometric and productive characteristics of second-crop corn.

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Crop coefficient estimated by degree-days for 'Marandu' palisadegrass and mixed forage

Coeficiente de cultivo estimado por grados-día para el pasto en empalizada 'Marandu' y el forraje mixto

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Abstract

Considering profitability in pasture-based systems, investigating parameters affecting crop coefficients for irrigation management becomes important. In this experiment, we determined the crop coefficient of 'Marandu' palisadegrass based on accumulated degree-days and estimated plant water consumption under single ('Marandu' palisadegrass) and mixed ('Marandu' palisadegrass + black oats + Italian ryegrass) cropping regimes. The research was conducted at the Luiz de Queiroz College of Agriculture in Piracicaba, São Paulo, Brazil, between 2016 and 2017. Evapotranspiration was assessed using weighing lysimeters while crop evapotranspiration was calculated using mean weight variation. Reference evapotranspiration and degree-days were estimated. Data were obtained from an automated weather station. Equations and regression models relating crop coefficient with accumulated degree-days were generated for two seasons (spring/summer and autumn/winter) and evaluated for two year-cycles, from 2015 to 2018. The results showed better prediction accuracy for the single cropping system in spring/summer 2017–18.

Keywords
degree-days evapotranspiration • water consumption • forage

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Resumen

Considerando la importancia económica de los pastos, existe una necesidad de investigar los parámetros que afectan los coeficientes de cultivo utilizados para el manejo del riego. En este experimento, nuestro objetivo fue determinar el coeficiente de cultivo del pasto 'Marandu' en función de la acumulación de grados-días y estimar el consumo de agua del cultivo puro (pasto 'Marandu') y del cultivo mixto (pasto 'Marandu' + avena negra + raigrás italiano) regímenes de cultivo. La investigación se llevó a cabo en la Escuela de Agricultura Luiz de Queiroz en Piracicaba/SP, Brasil, entre 2016 y 2017, en este periodo se registró la evapotranspiración utilizando lisímetros de pesaje. La evapotranspiración del cultivo se calculó utilizando la variación de peso promedio registrada por lisímetros. Los datos de una estación meteorológica automatizada se utilizaron para estimar la evapotranspiración de referencia y calcular los grados-días. Se generaron ecuaciones y modelos de regresión con relación al coeficiente de cultivo y los grados-días acumulados durante dos períodos estacionales (primavera/verano y otoño/invierno). Los modelos matemáticos se probaron durante dos ciclos anuales, de 2015 a 2018 y mostraron mejores resultados, en términos de precisión y exactitud, en el sistema de cultivo único en primavera/verano en los años 2017/18.

Palabras clave

grados-días • evapotranspiración • consumo de agua • forraje

Introduction

Intensive agriculture has improved productivity by investing in several crop-management practices, including irrigation and fertilization (12, 14, 36). Since pastures are major fodder sources for cattle in Brazil, intensification of pasture production turns key for the cultivation and management of this crop.

Occupying a vast territory, pastures are subjected to a wide range of environmental conditions, particularly temperature, light, and rainfall. Outranged environmental magnitudes for pasture production compromise vegetative development, expressed as low biomass accumulation and marked production seasonality (20, 23, 33). Meteorological conditions prevailing during winter impair tropical and subtropical forage crops, whereas spring/summer favor high forage productivity (25, 28).

In order to reduce fertilization and counteract low pasture yields during winter, cultivation of temperate forage species has been identified as an alternative for these cultivation systems. Good results have been reported for forage nutritional value and animal production (13). However, mixed cropping systems, where two or more species are co-cultivated using the overseeding method, cause changes in crop water requirements. Therefore, estimating these changes in irrigated pasture systems turns essential.

Adequate information on plant and microclimate interaction constitutes one major factor for accurate irrigation management (6, 29). Crop water requirements can be calculated after plant water losses through transpiration and soil evaporation, termed crop evapotranspiration (ET_c). Estimated reference evapotranspiration (ET_o) calculated using meteorological data, is then converted to ET_c using a crop coefficient (K_c) as a correction factor, while the precise ET_c can be determined using a weighing lysimeter (4).

In established pastures, the K_c varies depending on crop growth and landscape, such as after sowing, seed germination, or forage grazing (7, 17, 18, 27, 31). However, as the grazing cycle is defined by species, climate, and animal characteristics, this approach turns imprecise and avoided for tropical forage crops.

Air temperature is used to calculate degree-days, expressing available thermal units for crop growth. Accumulated degree-days (*ADD*) are also used to estimate the correct K_c for irrigation management. Thus, K_c-as estimated by *ADD*-varies with ambient temperature, influencing crop growth and cycle (16). Therefore, alternative methods for K_c estimation may be particularly useful for the dynamics of irrigated pastures. Therefore, in this study and using the weighing-lysimeter method, we aimed to measure K_c for growth cycles of 'Marandu' forage, a palisadegrass cultivar (*Urochloa brizantha*). The study took place during 2016 and 2017 and developed an adjustable regression model relating K_c and *ADD* during the four seasons, evaluated when applied to previous and subsequent years.

Materials and methods

Experimental site and soil classification

The field experiment was conducted at the Luiz de Queiroz College of Agriculture (ESALQ/USP), Piracicaba, São Paulo (latitude 22°42' S, longitude 47°38' W, altitude 546 m) in Brazil. The soil is classified as Ferralic Nitisol (38), with 48.6% clay, 32.5% sand, and 18.9% silt (0-0.4 m).

Soil chemical characteristics were determined prior experiment: pH = 5.1, $P_{(resin)}$ = 52 mg dm^{-3}, K = 0.69 $cmol_c$ dm^{-3}, Ca = 2.6 $cmol_c$ dm^{-3}, Mg = 1.4 $cmol_c$ dm^{-3}, H + Al = 3.65 $cmol_c$ dm^{-3}, Al = 0.2 $cmol_c$ dm^{-3}, and CEC = 8.34 $cmol_c$ dm^{-3}. Soil preparation included plowing and harrowing, weed control, Ph correction, and fertilization adjustment.

Forage cultivation

The experimental area included two, 144 m^2 plots. The area surrounding the experimental plots was covered with irrigated pastures and sugarcane. Only one close field had bare soil. Reading errors lead by bare soil, affecting evapotranspiration, were identified and excluded from daily calculations after verifying hot air mass entrances.

Initially, both plots were direct-seeded by broadcasting 15 kg ha^{-1} of 'Marandu' palisadegrass. Similar crop management practices were followed in both plots. After crop establishment (~70 days), the grass was cut to a standard height of 0.15 m above soil surface using a brush cutter. In autumn, one plot was kept for a single cropping system under 'Marandu' palisadegrass. Meanwhile, and to create a mixed cropping system, the other plot was direct-seeded (broadcasting) with 100 kg seeds ha^{-1} of black oats (*Avena strigosa* 'Embrapa 29 (Garoa)') and 60 kg seeds ha^{-1} Italian ryegrass (*Lolium multiflorum* 'Fepagro São Gabriel'). This created a pasture comprising three different forage crops during autumn and winter.

Cutting cycles were grouped according to seasons: summer (February to March 2016, and December 2016 to January 2017), autumn (April to June 2016), winter (July 2016 to October 2016), and spring (October to December 2016). After each cut, the plots were fertilized with 80 kg ha^{-1} urea during spring and summer and 50 kg ha^{-1} during autumn and winter. The Average total forage yield (*TFY*) was calculated as the sum of leaf and stem dry mass (DM), after kiln drying.

Forage cutting in the single-cropped plot ('Marandu' palisadegrass), was performed every 28 days during spring and summer, extended to 40 days during autumn and winter. In the mixed-cropped plot ('Marandu' palisadegrass + black oats + ryegrass), harvest was performed at intervals ranging from 24 to 40 days when the canopy had exceeding 95% light interception, measured with a LAI-2000 Plant Canopy Analyzer (LI-COR Environmental, Lincoln, Nebraska USA).

All experimental plots were irrigated with a conventional sprinkler system, operating via a sectoral mechanism at a flow rate of 590 L h^{-1} and running at a pressure of 245 kPa. The quantity of water applied was determined by weighing-lysimeter readings (in L/m^2 or mm), considering the lysimeter depth (z = 0.58 m) as the maximum crop root depth. In tropical forages, most roots are detected down to 0.60 m, and 70% of all roots are concentrated within the first 0.30 m depth (2, 21, 34).

An irrigation interval kept soil moisture content (SMC) over 70% (recommended for K_c estimate), between field capacity (θ_{fc}) and lowest soil water content (θ_{wp}). It was measured within 15 bars to standard atmospheric pressure (SMC ≥ 0.7 [θ_{fc} – θ_{wp}]), guaranteeing null soil water deficit, and accurate K_c data.

Evapotranspiration and accumulated degree-days

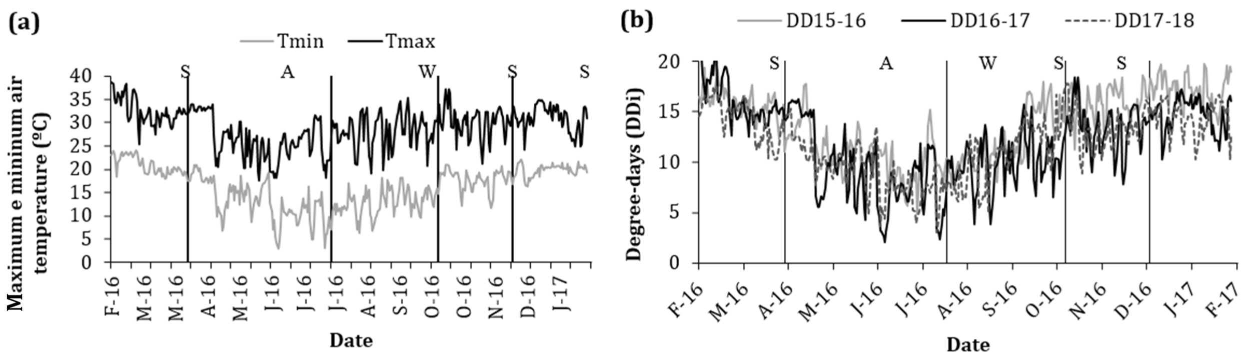
Crop evapotranspiration (ET_c) in the experimental plots was estimated using two lysimeters, made of a PVC box (500 L), of 1.22 m^2 and 0.58 m depth (26), with an automatic drainage system. Both sets were calibrated and controlled by a datalogger program (26). Lysimeter measurements were recorded every 15 min and converted into 1-day interval data. Values exceeding 0.20 mm in a 15 min interval, caused by excessive rainfall or evapotranspiration, were excluded. Brief periods of high evapotranspiration and consequent ET_c fluctuations arose during hot transient air masses originated from exposed soil and arriving mainly from the north, northwest, west, and occasionally, from the southwest.

Daily degree-days (DD_i) were calculated based on temperature (22). Equation 1 was used when crop basal temperature (T_b) was lower than minimum daily temperature (T_{min}), and eq. 2, when T_b exceeded T_{min}. Meteorological data were obtained from the ESALQ Meteorological Station, located 100 m from the experimental area. Data for $T_{min,}$ and T_{max} are shown in figure 1. Reference evapotranspiration (ET_o) (Penman-Monteith model, 2, 3) for different cycles is shown in table 1 (page 75).

$$DD_i = \frac{(T_{max} + T_{min})}{2} - T_b \quad (1)$$

$$DD_i = \frac{[(T_{max} - T_b)^2]}{2(T_{max} - T_{min})} \quad (2)$$

In equations 1 and 2:
DD_i = daily degree-days,
T_{max} = maximum daily temperature in °C,
T_{min} = minimum daily temperature in °C (figure 1a),
T_b = basal crop temperature for the single-cropped 'Marandu' palisadegrass plot, which was considered to be 10.6 °C (26). This same basal temperature was applied for plots with palisadegrass mixed with black oats and Italian ryegrass.



Graphs are divided sequentially according to season (Summer, Autumn, Winter, Spring).
En ambas figuras, los gráficos se dividen secuencialmente según estación (verano, otoño, invierno, primavera).

Figure 1. a. Minimum (Tmin) and maximum (Tmax) air temperatures during 2016 - 2017. **b.** Daily degree-days (DD) during 2015 - 2016 (DD15-16), 2016 - 2017 (DD16-17), and 2017 - 2018 (DD17-18) in Piracicaba, São Paulo, Brazil.

Figura 1. a. Temperaturas mínimas (Tmin) y máximas (Tmax) del aire durante 2016 - 2017; **b.** Los grados-días (DD) diarios durante los períodos 2015 -2016 (DD15-16), 2016 - 2017 (DD16-17) y 2017 - 2018 (DD17-18) en Piracicaba, São Paulo, Brasil.

It should be noted that *ADD* was also estimated for the original cropping cycles (28 days for spring/summer and 40 days for autumn/winter), with 11 productive cycles for single-cropped 'Marandu' palisadegrass and five cycles of the same forage in the overseeded mixed-cropping regime ('Marandu' palisadegrass + black oats + ryegrass).

The summed DD values for each cropping cycle comprised all accumulated degree-days (*ADD*). The K_c values were obtained as ET_c/ET_o. Daily values of K_c were averaged over 4-day intervals obtaining mean daily values and reducing possible distortions due to water flow within the lysimeters. The K_c values of the studied forage crops were grouped as follows: single-cropped pasture during spring-summer (Sp/Su) and autumn-winter (A/W), and the overseeded mixed-cropped pasture during A/W. Subsequently, daily K_c means were correlated with *ADD* for each cutting cycle verifying if *ADD* could be used in K_c determinations.

Table 1. Reference evapotranspiration (ETo) for each period from 2016 to 2017 in Piracicaba/SP, Brazil.

Tabla 1. Evapotranspiración de referencia (ETo) por período de 2016 a 2017 en Piracicaba/SP, Brasil.

Date cycles (month/day)		ET_o (mmcycle^{-1})	ET_o (min-max daily)
Su	02/11 – 03/09/2016	90.6	0.9 - 5.3
Su	03/10 - 04/06/2016	101.6	1.4 - 5.3
A	04/07 - 05/04/2016	100.3	1.1 - 4.9
A	05/05 – 06/13/2016	79.6	0.6 - 3.3
A	06/14 – 07/23/2016	97.8	1.2 - 3.7
W	07/24 - 09/01/2016	143.4	1.2 - 4.8
W	09/02 - 10/11/2016	164.5	1.0 - 5.9
Sp	10/12 - 11/08/2016	118.4	0.5 - 6.7
Sp	11/09 - 12/06/2016	122.1	0.9 - 6.3
Su	12/07/2016 - 01/03/2017	128.2	2.9 - 6.4
Su	01/04 - 01/31/2017	120.2	0.7 - 6.1
Date cycles (month/day)		**ET_o (mmcycle^{-1})**	**ET_o (min-max daily)**
05/05 -06/13/2016		79.6	0.6 - 3.3
06/14 - 07/11/2016		67.2	1.2 - 3.7
07/12 - 08/04/2016		66.8	1.2 - 4.0
08/05 -09/05/2016		123.2	1.7 - 4.8
09/06 - 10/07/2016		129.1	0.8 - 5.6

Single cropping cycles are divided sequentially according to season (Su = summer, A = autumn, W = winter, Sp = spring).

Los ciclos para un solo cultivo se dividen secuencialmente según la temporada (Su = verano, A = otoño, W = invierno, Sp = primavera).

Statistical analysis

K_c and *ADD* data were correlated using SigmaPlot software for regression analysis, with equations adjusted for the entire experimental period according to seasons. For 2015-2016 and 2017-2018 periods (prior and after the experiment, respectively), climate data was collected from a weather station located near the experimental area. For both periods, second-order polynomial models (K_c *vs. ADD*) were generated ($R^2 \geq 0.97$). K_c was estimated according to eq3, based on the calculated *ADD* in the simulated sequences for 2015-2016 and 2017-2018.

$$K_c = a + b \cdot ADD + c \cdot ADD^2 \tag{3}$$

Degree-days were counted from February 11, 2016 to January 31, 2017 (355 days), according to the original experimental period. The same periods were adopted for 2015-2016 and 2017-2018.

Using the generated regression models, K_c values were estimated for these years. Estimation accuracy was evaluated by the mean error (*ME*; eq 4), the root mean square error (*RMSE*; eq 5), Willmott's concordance index *d* (37), and confidence index *c* (10, eq 6 and 7).

$$ME = \left(\frac{1}{n}\right)\sum_{i=1}^{n}(E_i - O_i) \tag{4}$$

$$RMSE = \sqrt{\left[\left(\frac{1}{n}\right)\sum_{i=1}^{n}(E_i - O_i)^2\right]} \tag{5}$$

$$d = 1 - \frac{\sum_{i=1}^{n}(E_i - O_i)^2}{\sum_{i=1}^{n}\left(\left|E_i - \overline{O}\right| + \left|O_i - \overline{O}\right|\right)^2} \tag{6}$$

$$\boldsymbol{c = \sqrt{R^2} \times d} \tag{7}$$

In the equations:
n = the number of data points,
E_i = the estimated data,
O_i = the observed data.

Results

Regression curves for K_c means *vs. ADD* in single-cropped 'Marandu' palisadegrass were divided into two periods: Sp/Su and A/W, as shown in figure 2. For both periods, second-order polynomial models (K_c vs. *ADD*) were generated ($R^2 \geq 0.97$). During A/W, maximum K_c ($K_{c\,max}$ = 0.82) was obtained at an *ADD* of 160 °C·days (°C × days, degree-days), whereas in Sp/Su, $K_{c\,max}$ reached 0.90 at an *ADD* of 280 °C·days (figure 2).

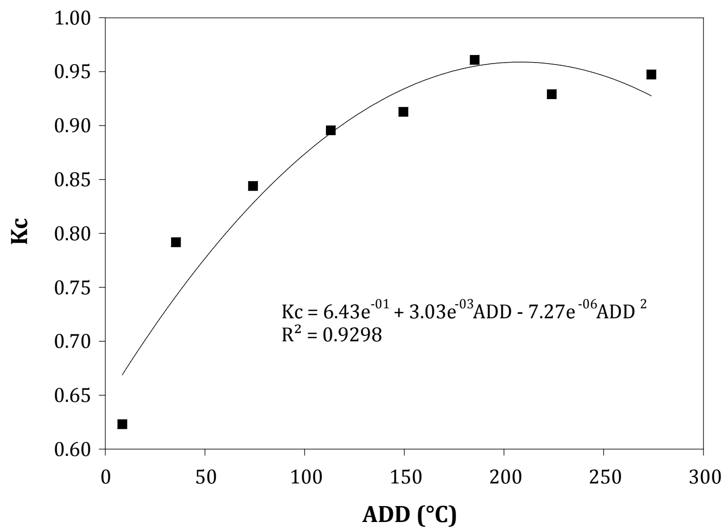


Figure 2. Empirical models for accumulated degree-days (ADD) and crop coefficient (*Kc*) during spring (Sp) and summer (Su) (ADD_{SpSu} *vs.* Kc_{SpSu}) (●); and autumn (A) winter (W) (ADD_{AW} *vs.* Kc_{AW}) (○). Equation parameters (a, b, c) and coefficient of determination (R^2) for single-cropped Marandu palisadegrass during the experimental period February 2016 to January 2017; Piracicaba, São Paulo, Brazil.

Figura 2. Modelos empíricos de coeficiente de cultivo (Kc) y grados-días acumulados (ADD) durante la primavera (Sp) y el verano (Su) (ADD_{SpSu} vs. Kc_{SpSu}) (●); y otoño (A) invierno (W) (ADD_{AW} vs. Kc_{AW}) (○). Parámetros de la ecuación (a, b, c) y coeficiente de determinación (R^2) para el pasto Marandu de un solo cultivo durante el experimento (febrero de 2016 a enero de 2017); Piracicaba, São Paulo, Brasil.

Mean minimum K_c values ($K_{c\,min}$) determined at the beginning of the study periods were 0.50 for Sp/Ss and 0.67 for A/W. *ADD* values for spring and autumn were 375.82 °C·days and 355.25 °C·days, respectively (figure 2). ET_c was largely ranged during Sp/Su (0.54-6.57) than during the A/W (0.64-5.62), as shown in table 2, page 77.

K_c mean values obtained in the present study were lower than those previously reported, but observed K_{cmax} under 'Marandu' palisadegrass in summer, reached 1.20. In addition, except for cycle 1 (establishment), each cycle reached a similar productive potential to that observed by other authors (table 2, page 77). The *TFY* values for the single-cropping system in each season were 13927.9 kg DM ha^{-1} (summer), 5803.6 kg DM ha^{-1} (spring), 5624.6 kg DM ha^{-1} (autumn), and 4187.8 kg DM ha^{-1} (winter). These values sum a total of 29543.9 kg DM ha^{-1} yr^{-1} in the single-cropping system. The *TFY* in the mixed-cropping system was 11343.74 kg DM ha^{-1} as recorded in a 155-day period (table 2, page 77).

Table 2. Reference evapotranspiration (ETo) and crop evapotranspiration for single-cropped 'Marandu' palisadegrass (ETc_sc) and for palisadegrass overseeded with black oats and Italian ryegrass (ETc_o), and total forage yield (*TFY*) in kg of dry mass (DM) per each period from 2016 to 2017 in Piracicaba/SP, Brazil.

Tabla 2. Evapotranspiración de cultivos para pasto Marandu de un solo cultivo (ETc_sc) y para pasto Marandu sobre sembrado con avena negra y de raigrás italiano(ETc_o) y rendimiento total de forraje (TFY) en kg materia seca (DM) para cada período de 2016 a 2017 en Piracicaba/SP, Brasil.

Cycles for single cropping are sequentially divided according to season (Su = summer, A = autumn, W = winter, Sp = spring).

Los ciclos para un solo cultivo se dividen secuencialmente según la temporada (Su = verano, A = otoño, W = invierno, Sp = primavera).

single-cropped palisadegrass				
Date cycles (month/day)		ET_c*_sc* (mm cycle^{-1})	ET_c*_sc* (min-max daily)	*TFY* (kg DM ha^{-1} cycle^{-1})
Su	02/11 – 03/09/2016	74.1	1.7 - 3.8	1708.3
Su	03/10 - 04/06/2016	65.6	1.3 - 3.4	2542.9
A	04/07 - 05/04/2016	83.9	2.0 - 4.0	2307.1
A	05/05 – 06/13/2016	--	--	1998.8
A	06/14 – 07/23/2016	--	--	1318.7
W	07/24 - 09/01/2016	127.6	1.8 - 4.1	1617.3
W	09/02 - 10/11/2016	129.3	1.2 - 5.2	2570.5
Sp	10/12 - 11/08/2016	101.9	1.6 - 6.5	2580.8
Sp	11/09 - 12/06/2016	106.8	2.1 - 5.7	3222.8
Su	12/07/2016 - 01/03/2017	116.2	1.5 - 8.0	4951.5
Su	01/04 - 01/31/2017	90.0	1.0 - 7.2	4725.2
palisadegrass overseeded with black oats and Italian ryegrass				
Date cycles (month/day)		ET_c*_o* (mm cycle^{1})	ET_c*_o* (min-max daily)	*TFY* (kg DM ha^{-1} cycle^{-1})
05/05 -06/13/2016		81.0	0.9 - 4.4	1827.37
06/14 - 07/11/2016		65.4	0.2 - 4.6	1735.87
07/12 - 08/04/2016		69.9	1.8 - 4.4	2748.9
08/05 -09/05/2016		105.5	1.6 - 5.0	2554.8
09/06 - 10/07/2016		111.3	1.8 - 5.1	2476.8

In figure 3 (page 78), mean K_c for palisadegrass mixed of black oats and Italian ryegrass was similar to that obtained for the single-cropped system. However, at the end of the cycle, K_c values did not decrease, as previously seen for the single-cropped pasture during AW (figure 2, page 76).

The K_c *vs. ADDa* equation (figure 2, page 76 and figure 3, page 78) was used for K_c estimates based on calculated *ADD* in the simulated sequences for the years 2015-2016 and 2017–2018, as shown in table 3 (page 78). The estimated K_c resulted in R^2 exceeding 0.92 for both Sp/Su and A/W periods, resulting in greater accuracy (higher coefficient *d*) and lower error in the Sp/Su estimation. Regarding the second year, 2017-2018, R^2 exceeded 0.95, improving the Sp/Su estimation (table 3, page 78).

Error analysis determined the Willmott's index (table 3, page 78). Mean errors (*ME*) were negative, indicating differences between model estimations and observed data in the field experiment. Observed values were higher than estimated values (with the largest error of 0.0641) in the single-cropped system during A/W. In the same period, the largest root mean square error (*RMSE*) was 0.11.

During the experimental year, *ADD* values reached approximately 404 °C·days (DD during spring and summer, and 360 °C·days during autumn and winter. In the previous annual period (2015-2016), mean values for Sp/Su and A/W were 443 and 438 °C·days, respectively, whereas in the subsequent annual period (2017-2018), ADD reached 383 and 379 °C·days, respectively.

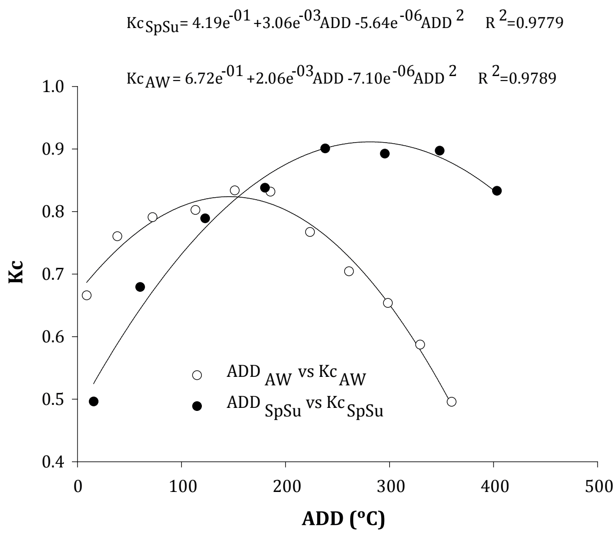


Figure 3. Empirical models of accumulated degree-days (ADD) and crop coefficient (K_c) during autumn/winter for palisadegrass mixed of black oats and Italian ryegrass (■), Piracicaba, São Paulo, Brazil.

Figura 3. Modelos empíricos de coeficiente de cultivo (K_c) y grados-días acumulados (ADD) durante el otoño/invierno para pastos mixtos de avena negra y raigrás italiano (■), Piracicaba, São Paulo, Brazil.

Table 3. Determination coefficient (R^2*), Willmott's index (d), Camargo and Sentelhas, (1997) index (c), mean error (ME), and root mean squared error (RMSE) of empirical models of K_c estimated from accumulated degree-days over 2 years. Observed *vs.* simulated K_c values for Piracicaba/SP, Brazil.

Tabla 3. Coeficiente de determinación (R^2*), índice de Willmott (d), Camargo y Sentelhas, (1997) índice (c), error medio (ME) y error cuadrático medio (RMSE) de modelos empíricos de Kc estimados a partir de grados-días acumulados durante 2 años. Valores de Kc observados *versus* simulados para Piracicaba/SP, Brasil.

2015/16	R^2*	*d*	*c*	*ME*	*RMSE*
spring/summer	0.9296	0.9797	0.9446	-0.0098	0.0361
autumn/winter	0.9248	0.8630	0.8300	-0.0641	0.1059
intercropped	0.9569	0.9597	0.9307	-0.0016	0.0384
2017/18	**R^2**	***d***	***c***	***ME***	***RMSE***
springer/summer	0.9754	0.9937	0.9814	-0.0009	0.0205
autumn/winter	0.9589	0.9625	0.9425	-0.0276	0.0452
intercropped	0.9569	0.9767	0.9388	-0.0077	0.0299

Discussion

The decreasing K_c values for single-cropped 'Marandu' palisadegrass during autumn and winter (figure 2, page 76) may be related to low biomass accumulation and extensive leaf senescence during this phase, leading to reduced tropical forage production and lower water requirements. In this regard, some authors have shown that even in irrigated cropping systems, biomass yield in colder seasons (A/W) is approximately 50% lower than that produced during warmer periods (1).

The coefficients of determination (R^2) for the observed *vs.* estimated data in 2017-2018 were higher than those in 2015-2016, keeping high accuracy for both periods. According to the categories for the *c* index (10), the present model can be considered *excellent*, except for simulations obtained for A/W, 2015-2016.

The Sp/Su and A/W periods showed a different ADD value for $K_{c\ max}$ obtention, with a greater maximum for Sp/Su. Other related studies on tropical forages have presented $K_{c\ max}$ values of 1.04 to 1.25 for *Megathyrsus maximus* 'Tanzania'; 1.04 to 1.54 for *Megathyrsus maximus* 'Mombaça'; and 1.33 for *Urochloa brizantha* 'Piatã' (5, 9, 15, 19). In our study, curves of K_c *vs. ADD* were constructed by grouping mean K_c values for Sp/Su, resulting in an average $K_{c\ max}$ value of 0.90, while the original data indicated a $K_{c\ max}$ value of 1.27. This difference can be explained by the plant -atmosphere interaction-a concept known as coupling-observed by other authors (30, 32) throughout the year. During the beginning of the Sp/Su cycles, high temperatures may have resulted in the crop-atmosphere decoupling, making evapotranspiration largely determined by solar radiation, resulting in lower K_c values. This differs from the beginning of the A/W period, when temperature, solar radiation, air humidity and cloud cover tend to be lower. These physiological parameters need to be more accurately determined in future studies. Literature on tropical pasture reports the optimum and maximum temperatures for vegetative development to be 40 °C, and 45 °C, respectively (24). Thus, given that maximum daily temperature recorded over the studied years was between 37.3 °C and 38.7 °C an influence on growth is not considered a significant factor in this study.

During autumn and winter, K_c models for the intercropped forage showed different behaviors compared to the models for single-cropped 'Marandu' palisadegrass (figure 3, page 78). In a well-managed intercropped system, water use efficiency (in terms of forage yield and water consumption) may be higher than in a single-cropped system (11, 39). Furthermore, the use of climatically adapted species may guarantee stable productivity through the intercropping cycles (35).

Estimation models applied to the 2016–2017 experimental cycle of K_c versus *ADD* for single-cropped 'Marandu' palisadegrass in the Sp/Su and A/W periods showed high precision ($R^2 \geq 0.97$). In addition, during winter, the mixed-cropping system reached an R^2 ±0.93.

When these equations were tested against two other experimental-year data sets considering ADD value as an input parameter, they showed high precision (R^2 between 0.93 and 0.98), accuracy (*d* values from 0.86 to 0.99), and confidence (*c* values between 0.83 and 0.98). A higher error was detected during A/W given that, during this period, the tropical pastures showed major productivity variances. K_c and *ADD* in pasture-irrigation management can be widely adopted given their versatility to cope with grazing rotation (8).

Conclusions

Seasonal *Kc* was measured by the weighing-lysimeter method for growth cycles of the palisadegrass 'Marandu' cultivar and the overseeded pasture of palisadegrass, black oat, and Italian ryegrass. When tested via regression modeling and estimated by accumulated degree-days (*ADD)* this model showed accurate results. The equations developed for the spring/summer season and the intercropped system showed higher correlations than autumn/winter. The models can be used to estimate K_c with *ADD,* only requiring minimum and maximum air temperature measurements for *Kc* estimate, easy in irrigation management.

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Changes in physiological and biochemical parameters during the growth and development of guava fruit (*Psidium guajava*) grown in Vietnam

Cambios en los parámetros fisiológicos y bioquímicos durante el crecimiento y el desarrollo de la fruta de guayaba (*Psidium guajava*) cultivada en Vietnam

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Abstract

This research examined the ripening time of guava fruit to provide a scientific basis for better harvesting and preservation of these fruits. Biochemical research methods were used to analyse changes in physiological and biochemical parameters according to the growth and development of guava fruit. The fruit took 14 weeks after anthesis to reach its maximum size in terms of length and diameter. The chlorophyll content in guava peel peaked after 10 weeks, decreasing until week 15. The content of carotenoids, which was low at fruit formation, rose rapidly until fruit ripening, while the vitamin C and reducing sugar contents increased continuously and peaked at week 14. A gradual increase was seen in the starch and total organic acid contents from the beginning of fruit formation, with peaks at 10 weeks, followed by a moderate downward trend. The pectin content showed the same trend, as it declined gradually after peaking at 12 weeks. The first 4 weeks showed an increase in the tannin content, which decreased afterward. The study results show that guava fruit should be harvested after physiological maturity and before ripening completely (14 weeks) to ensure that the nutritional value of the fruit is maintained during storage.

Keywords
guava fruit • biochemical parameters • physiological parameters • ripening

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Resumen

Esta investigación examinó el tiempo de maduración de la guayaba para proporcionar una base científica para una mejor cosecha y conservación de estas frutas. Se utilizaron métodos de investigación bioquímica para analizar los cambios en los parámetros fisiológicos y bioquímicos de acuerdo con el crecimiento y desarrollo de la guayaba. El fruto tardó 14 semanas después de la antesis en alcanzar su tamaño máximo en cuanto a longitud y diámetro. El contenido de clorofila en la cáscara de guayaba alcanzó su punto máximo después de 10 semanas, disminuyendo hasta la semana 15. El contenido de carotenoides, que era bajo en la formación de la fruta, aumentó rápidamente hasta la maduración de la fruta, mientras que el contenido de vitamina C y azúcar reductor aumentó continuamente y alcanzó su punto máximo en la semana 14. Se observó un aumento gradual en el contenido de almidón y ácido orgánico total desde el inicio de la formación del fruto, con picos a las 10 semanas, seguido de una moderada tendencia a la baja. El contenido de pectina mostró la misma tendencia, ya que disminuyó gradualmente después de alcanzar su punto máximo a las 12 semanas. Las primeras 4 semanas mostraron un aumento en el contenido de taninos, que luego disminuyó. Los resultados del estudio muestran que la guayaba debe cosecharse después de la madurez fisiológica y antes de madurar por completo (14 semanas) para garantizar que el valor nutricional de la fruta se mantenga durante el almacenamiento.

Palabras clave
fruto de guayaba • parámetros bioquímicos • parámetros fisiológicos • maduración

Introduction

Guava is a relatively common tropical fruit grown in many tropical and subtropical regions of the world (16), such as in the United States, Asia, Africa and Oceanía. *Psidium guajava* is a species of the myrtle family (Myrtaceae) that is widely planted. In addition, there are some other *Psidium* species, such as *Psidium guineensis* and *Psidium acutangulum*. Guavas have been grown in many countries around the world with high yields. According to statistics released in 2019 (4), guava production in the world achieved 46.5 million tons, and the largest producer was India, followed by China and Thailand. The guava has many different shapes, such as circle or oval, depending on the species, and also has high nutritional value. Guava fruit contains a large amount of vitamin A, vitamin B, vitamin C, minerals, polyphenolic and flavonoid compounds that play an important role in preventing cancer, ageing, etc. (2, 4). Additionally, antioxidant, anticholesterol and anticancer effects have been reported. As a result of its economic and medicinal benefits, guava is becoming an important crop in many countries around the world. Changes in physiological and biochemical parameters during the ripening of guava fruit take place over a relatively short period of time. Consequently, guava has a very short shelf life, which in turn leads to difficulties in transportation and storage (10). Thus, a comparative study on various physiological and biochemical parameters associated with fruit quality between different guava cultivars will provide great value, especially for selecting a guava cultivar for the region, which could provide superior-quality fruit. Changes in sugar, pectin, and antioxidants of guava fruit at various stages of maturity and ripening were reported by several studies from different locations (19, 23). These are important criteria for determining the optimum stage of fruit harvesting to obtain quality fruits with extended shelf life.

One of the world's largest guava fruit exporters is Vietnam, in which Xa Li guava varieties are widely grown and have significant economic value. Because of the economic benefits, gardeners have invested in planting guava trees in addition to fruit production in many provinces and cities. As a result of this investment, the number of products made from guava trees and fruit has increased nationwide. During the production process, fruit collection and preservation steps are crucial to ensure the quality of the fruit and may have direct effects on consumers (18). Therefore, it is necessary to understand the physiological and biochemical indicators of guava fruit from formation to fruit ripening to improve the quality, storage, and processing characteristics of the fruit. This research aims to determine the physiological ripening time of guavas to help consumers better use and preserve guavas.

Materials and methods

Research materials

Xa Li guava was harvested in Haiduong, Vietnam (20°56′00″N and 106°19′00″E). Physiological and biochemical parameters were analysed at the Plant Laboratory, Hongduc University and the Department of Plant Physiology and Application, Hanoi National University of Education, Vietnam.

Sample collection method

Samples were collected from March to June following the mixed sampling method across the experimental area. We collected samples at many points on various trees. These plants grew normally and were free from pests. At each stage of the study, we collected samples from all plants, with 05 fruits per tree. A part of the sample was used to immediately analyse the contents of pigments and vitamin C, while the rest was stored at -80°C for later analysis of other parameters.

Determination of fruit length and diameter

At an interval of approximately 2 weeks after anthesis, the length and diameter of fruit were measured until the fruit ripened, and afterward it was harvested (figure 1). Fruit length and diameter were measured from randomly selected fruit during the study. The length from the base of fruit to the base of the calyx and diameter at the largest bulge of fruit from both sides were measured with the help of Panme calipers. The mean values of length and diameter are presented in centimetres (19).

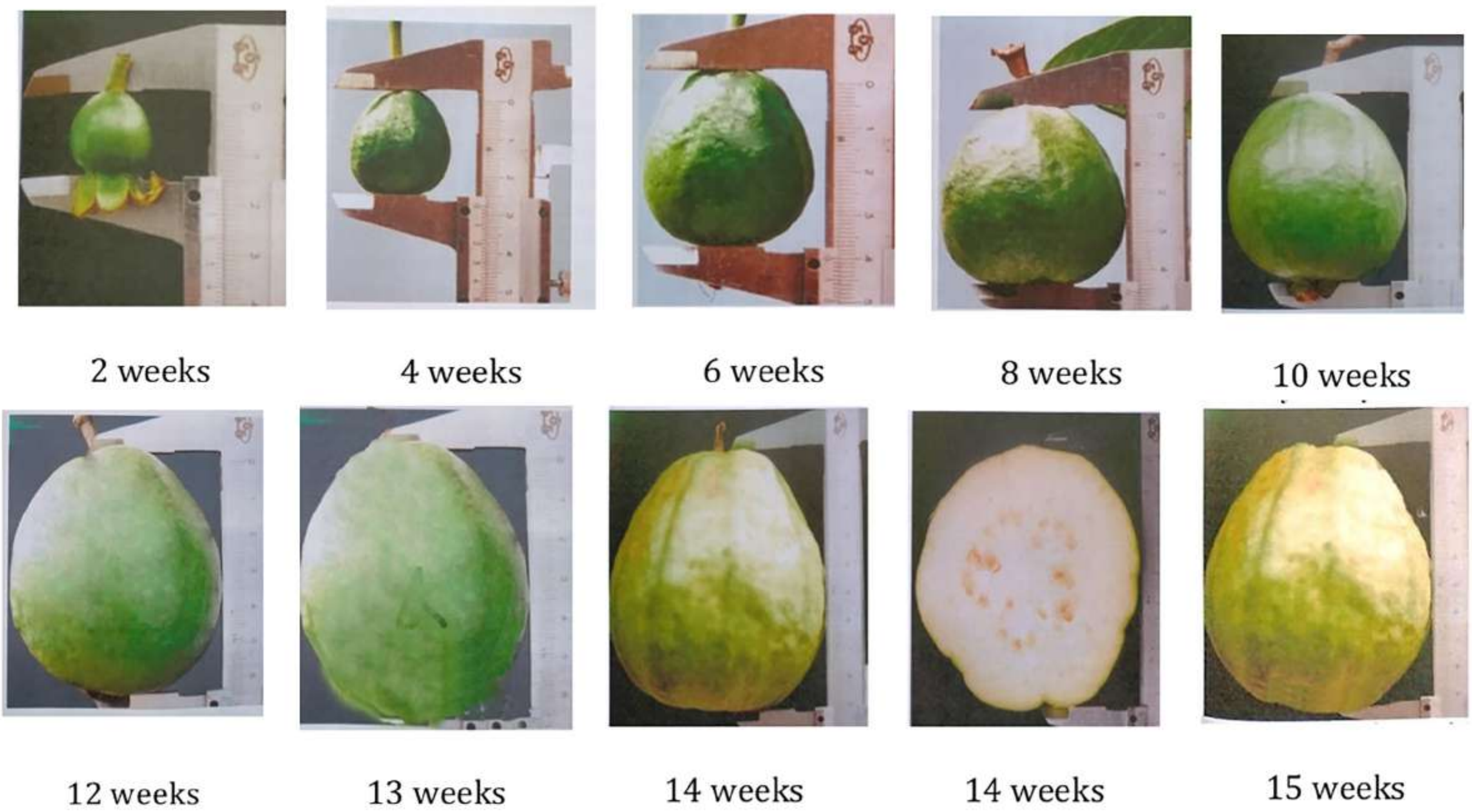

Figure 1. The growth and development of guava fruit

Figura 1. El crecimiento y desarrollo de la guayaba.

Determination of the pigment content in the peel by the spectral method (14)

The chlorophyll content was calculated by the following formula: C_a (mg/l) = 9.784 x E_{662} - 0.990 x E_{644}. C_b (mg/l) = 21.426 x E_{644} - 4.650 x E_{662}. The carotenoid content was calculated by the formula $C_{carotenoids}$ (mg/l) = 4.695 x $E_{440.5}$ - 0.268 x $C_{(a+b)}$. Then, the pigment content per 1 g of fresh fruit peel was calculated by the formula:

$$X = \frac{C \times V}{g \times 1000}$$

where:

E_{662}, E_{644} and $E_{440.5}$ = the results of measuring the chlorophyll at wavelengths of 662 nm, 644 nm and 440.5 nm

C_a, C_b, C_{a+b} = the contents of chlorophyll *a, b* and total chlorophyll, respectively

X = the content of chlorophyll in 1 g of fresh fruit peel

C = the chlorophyll content of the pigment extract (mg/l)

V = the volume of pigment extract (10 ml)

g = the sample mass (g).

Determination of reducing sugar and starch contents by the Bertrand method (8)

The reducing sugar content (*X %*) was calculated with the following formula:

$$X = \frac{a \times V_1 \times 100}{V \times g \times 1000}$$

The starch content (*Y%*) was calculated by the following formula:

$$Y = \frac{a \times V_1 \times 100 \times 0.9}{V_2 \times g}$$

where:

V = the volume of the diluted sample solution (ml)

V_1 = the volume of the analysed sample solution (ml)

V_2 = the volume of diluted sample solution (ml)

a = the weight (mg) of glucose obtained

g = the weight of the analysed sample (g)

100 = the conversion factor to %

1000 = the coefficient of conversion from *g* to *mg*

0.9 = the coefficient of conversion from glucose into starch.

Determination of the pectin content by the calcium pectate precipitation method (17)

The amount of pectin required for saponification (*X*) was calculated by the following formula:

$$X = \frac{a \times V_2}{V_1}$$

where:

a = the weight of pectin introduced into the solution (g)

V_1 = the volume of the initial pectin solution (ml)

V_2 = the volume of pectin solution required for saponification (ml).

The content of pectin (*Y %*) was calculated by the following formula:

$$Y = \frac{b \times 0.92 \times 100}{X}$$

where:

b = the amount of calcium pectate precipitate (g)

X = the amount of pectin required for saponification (g)

0.92 = the transfer coefficient except for the calcium content of the precipitate

100 = the conversion factor to express the results in %.

Determination of the tannin content by the Leventhal method (8)
The tannin content was calculated by the formula:

$$X(\%) = \frac{(a-b)\times V \times k \times 100}{V_1 \times g}$$

where:
X = the tannin content (%)
a = the volume of KMnO4 used for titration in the flask (ml)
b = the volume of KMnO4 used for titration in the control vessel (ml)
V = the total volume of extract (ml)
V_1 = the volume of the analysed extract (ml)
g = the weight of the analysed sample (g)
k = the tannin coefficient = 0.00582.

Determination of the total organic acid content (12)
The total organic acid content was calculated by the following formula:

$$X = \frac{a \times V_1 \times 100}{V_2 \times g}$$

where:
X = the amount of total organic acid present in the extract
a = the amount of 0.1 N NaOH titrate (ml)
g = the amount of analytical sample (g)
V_1 = the total volume of the extract (ml)
V_2 = the volume to be titrated (ml).

Determination of the vitamin C content by the titration method (3)
The vitamin C content (*X mg/100g*) was calculated by the following formula:

$$X = \frac{V \times V_1 \times 0.00088 \times 100}{V_2 \times g}$$

where:
V = the volume of diluted sample solution (ml)
V_1 = the volume of 0.01 N I2 solution (ml)
V_2 = the volume of analysed solution (ml)
g = the weight of the sample (g)
0.00088 = the weight (g) of vitamin C, which was equivalent to 1 ml of 0.01 N I2.

Methods of data analysis

The experiments were conducted three times, and the results are presented as the mean and standard deviation (SD) (1). The results were subjected to analysis of variance (ANOVA) of the data to determine the F ratio. If the value of the F ratio was greater than the appropriate critical F distribution ($p < 0.05$), the hypothesis was rejected, and it is assumed that at least one of the group means was significantly different from the other group means. The data were compared with Tukey's test using IRRISTAT software for Windows computers.

Results and discussion

The data in table 1 (page 87) indicate that during the growth and development of guava, the length and diameter changed significantly. From 2 weeks to 15 weeks after anthesis, fruit length increased 5.94-fold (1.515 cm to 8.991 cm), while fruit diameter increased 5.43-fold (1.462 cm to 7.934 cm). In particular, a sharp increase in fruit size was observed during the period from 2 weeks to 12 weeks. This was due to an increase in both the number and size of cells in the guava fruit. From 12 to 15 weeks, the fruit reached the maximum value for the cultivar under the study conditions (at 14 weeks, fruit length was 8.882 cm, fruit diameter was 7.934 cm). After 14 weeks, the fruit size increased very slowly and remained almost unchanged. The ripening process of guava fruit is marked by the colour change of the fruit pods. During this study, we observed that at 14 weeks after anthesis, the guava fruit peel began to show the first yellow spot. Therefore, it can be said that 14 weeks is the physiological maturity stage of guava. The growth of Xa Li guava showed a similar pattern to that of other guava varieties reported previously (22, 24).

Table 1. Changes in length and diameter of guava fruits and pigment contents in guava peels.

Tabla 1. Cambios en la longitud, el diámetro de la guayaba y el contenido de pigmento en la cáscara de las guayabas.

In the same data column, values with similar letters represent non-significant differences, values with different letters represent significant differences ($p < 0.05$).

En la misma columna de datos, los valores con letras similares representan diferencias no significativas, los valores con letras diferentes representan diferencias significativas ($p < 0,05$).

Age of fruit	Length (cm)	Diameter (cm)	Chlorophyll a content (mg/g fresh peel)	Chlorophyll b content (mg/g fresh peel)	Carotenoid content (mg/g fresh peel)
2 weeks	1.515f ± 0.035	1.462f ± 0.033	0.039d ± 0.001	0.053f ± 0.003	0.012f ± 0.001
4 weeks	2.659e ± 0.041	2.536e ± 0.045	0.054c ± 0.003	0.085d ± 0.004	0.019f ± 0.001
6 weeks	3.952d ± 0.046	3.816d ± 0.091	0.082b ± 0.002	0.123c ± 0.002	0.029e ± 0.001
8 weeks	4.657c ± 0.071	4.421c ± 0.067	0.117a ± 0.002	0.249a ± 0.001	0.035e ± 0.003
10 weeks	5.182c ± 0.093	4.984c ± 0.073	0.131a ± 0.001	0.275a ± 0.005	0.051d ± 0.001
12 weeks	7.289b ± 0.135	6.062b ± 0.104	0.093b ± 0.001	0.183b ± 0.001	0.084c ± 0.002
13 weeks	8.417a ± 0.123	7.219a ± 0.095	0.052c ± 0.002	0.105c ± 0.002	0.126b ± 0.001
14 weeks	8.882a ± 0.087	7.928a ± 0.112	0.042d ± 0.003	0.069e ± 0.001	0.163a ± 0.002
15 weeks	8.991a ± 0.128	7.934a ± 0.069	0.028e ± 0.001	0.051f ± 0.003	0.172a ± 0.004

During the first weeks, the chlorophyll content in guava peels was low. The chlorophyll a content was 0.039 mg/g fresh peel, and the chlorophyll *b* content was 0.053 mg/g fresh peel at 2 weeks after anthesis. From 2 to 10 weeks, the chlorophyll content increased rapidly and reached the highest value at 10 weeks (chlorophyll a was 0.131 mg/g fresh peel, chlorophyll *b* was 0.275 mg/g fresh peel). After 10 weeks, the contents of chlorophyll *a* and chlorophyll *b* gradually decreased and then decreased rapidly to 15 weeks because fruits began to move to the stage of ripening, at which time chlorophyll was decomposed and carotenoid pigments were synthesized. Some studies suggest that the maturation of some fruits is related to chlorophyll resolution; for example, during litchi colouration, a visible degreening process associated with chlorophyll degradation has been reported (13, 26). This result is consistent with several studies stating that chlorophyll breakdown is associated with the maturity of some kinds of fruits (11, 27).

The carotenoid content in guava peel increased with the growth and development of the fruit. In the first week, a low carotenoid content (0.012 mg/g fresh peel) was observed (table 1). From 2 to 10 weeks, the content of carotenoids increased slowly and then increased rapidly with the maturity stage of the fruit. At week 15, the content of carotenoids reached 0.172 mg/g fresh peel. At the early stage, the fruit is primarily green due to the large amount of chlorophyll that obscures carotenoids; when entering the ripening process, the yellow colour of carotenoids becomes more apparent due to chlorophyll breakdown (7). This result is consistent with the finding that ripening in tomatoes is accompanied by significant increases in carotenoids (21).

Guava fruit contain carbohydrates such as sugars and starches (5) with varying levels from fruit formation to maturity. The results in table 2 (page 88) show that in the early period of guava fruit development (first 2 weeks), the reducing sugar content was relatively low, reaching 2.026% of the weight of fresh fruit. From 2 to 12 weeks, the content of reducing sugars increased slowly and reached 3.547% when the fruit was 12 weeks old. During the fruit development period from 12 to 15 weeks, the content of reducing sugar increased rapidly and reached 6.204% when the fruit was 14 weeks old. These research results are consistent with research on total sugar, which increase rapidly in the later stages of fruit development (19). At week 15, the reducing sugar content decreased to 6.184% of the fresh fruit weight, indicating a decrease in fruit quality .

When the fruit had just formed, the starch content reached 3.921% of the weight of fresh fruit (2 weeks). The highest starch content was 6.494% at 10 weeks after anthesis (table 2, page 88). This is the time when fruits tend to accumulate nutrients in preparation for the ripening process. After 10 weeks, the content of starch in the fruit decreased due to the strong metabolism in the fruit. At week 15, the content of starch decreased to 1.975% of the weight of fresh fruit. During this period, the activity of the α-amylase enzyme also increased. Due to increased α-amylase enzyme activity, starch converts into the sugar required for respiration. From the unripe stage to the ripe stage, the starch content declined. When the fruit enters the ripening period, starch decomposes into sugar to increase the amount of reducing sugar to create sweetness in the fruit (28).

Table 2. Contents of reducing sugar, starch, pectin, and tannin in guava fruits.

Tabla 2. Contenido de azúcar reductor, almidón, pectina y tanino en guayabas.

In the same data column, values with similar letters represent non-significant differences, values with different letters represent significant differences ($p < 0.05$).

En la misma columna de datos, los valores con letras similares representan diferencias no significativas, los valores con letras diferentes representan diferencias significativas ($p < 0,05$).

Age of fruit	Reducing sugar content (% weight of fresh fruit)	Starch content (% weight of fresh fruit)	Pectin content (% weight of fresh fruit)	Tannin content (% weight of fresh fruit)
2 weeks	2.026c ± 0.021	3.921c ± 0.074	0.238d ± 0.012	2.382a ± 0.023
4 weeks	2.400c ± 0.022	4.274c ± 0.042	0.294d ± 0.009	2.798a ± 0.050
6 weeks	2.481c ± 0.024	5.196b ± 0.038	0.368c ± 0.014	2.176ab ± 0.044
8 weeks	2.807c ± 0.015	5.761ab ± 0.094	0.427c ± 0.010	1.615bc ± 0.028
10 weeks	2.897c ± 0.034	6.494a ± 0.048	0.519b ± 0.007	1.128cd ± 0.013
12 weeks	3.547b ± 0.026	5.784ab ± 0.023	0.605a ± 0.009	0.756de ± 0.018
13 weeks	5.583a ± 0.038	3.968c ± 0.025	0.572ab ± 0.021	0.445e ± 0.024
14 weeks	6.204a ± 0.012	2.597d ± 0.036	0.435c ± 0.018	0.418ef ± 0.009
15 weeks	6.184a ± 0.031	1.975e ± 0.043	0.418c ± 0.007	0.358f ± 0.013

The pectin content increased rapidly from week 2 to week 12 (from 0.238% to 0.605% of fresh fruit weight) and decreased in the period from week 12 to week 15 (from 0.605% to 0.418%), making the fruits softer. The pectin content decreased at a later stage due to the increased activity of the pectinase enzyme, which reduced the pectin content in the fruit. The activity of the pectinase enzyme was associated with the softening of the fruit along with an increase in soluble pectin (15).

The tannin content in guava fruit was relatively high at 2 weeks (reaching 2.382%). The high tannin content in the early growth period of fruit made it acrid. The tannin content in guava gradually decreased at first and rapidly decreased at 9 weeks (from weeks 4 to 13). In the period of fruit maturity, the tannin content decreased to only 0.358% at week 15, indicating that the guava fruits were soft and not acrid. This decline was due to tannins being decomposed into pyrogallol and CO_2 when the fruit entered the physiological ripening stage (9).

In plant cells, organic acids can be found in the free form, as ammonium salts or esters. Organic acids are characterized by their weakly acidic properties. These compounds are necessary for aerobic metabolism and as flavour constituents that contribute to fruit quality, organoleptic properties and fruit acidity (25).

The data in table 3 (page 89) show that at the stage when fruit started to develop, the organic acid content was 68.428 mg/100 g fresh fruit. During the growth period from 2 to 10 weeks, the total organic acid content increased gradually and reached the highest value of 136.667 mg/100 g fresh fruit after 10 weeks. Degradation of protein and lipids leads to the production of intermediate products such as amino acids, increasing the organic acid content. During the fruit growth period from 10 to 15 weeks, the organic acid content decreased because organic acids are used in respiration (6). On the other hand, energy continues to be needed for the biosynthesis of fruit-specific ripening substances such as enzymes for hydrolysis, esters to create fruit aromas during the ripening period and synthesis of sugars to create fruit sweetness, resulting in the decrease in the total acid content (20).

Vitamin C is synthesized by plants and is a main source of nutrition for humans. The vitamin C content is an important indicator to assess the nutritional value of many fruits. The content of vitamin C from 2 to 12 weeks increased rapidly. This is the major period of fruit development, and the accumulation of vitamin C along with other nutrients takes place at this time. After 12 weeks, the vitamin C content continued to increase, but at a slower rate, and the highest value (205.134 mg/100 g fresh fruit) was reached at week 14; thereafter, the vitamin C content decreased. Changes in the vitamin C content of guava fruits at different stages of maturity are related to different metabolic pathways and the enzymatic activity of decomposition (23).

Table 3. The content of total organic acid and vitamin C in guava fruits.

Tabla 3. El contenido de ácido orgánico total y vitamina C en la guayaba.

Age of fruit	Total organic acid content *(mg/100g fresh fruit)*	Vitamin C content *(mg/100g fresh fruit)*
2 weeks	68.428d ± 0.972	89.625d ± 0.652
4 weeks	82.376c ± 1.261	94.429d ± 0.931
6 weeks	113.209b ± 2.467	105.762d ± 1.347
8 weeks	129.742a ± 2.138	123.384c ± 2.125
10 weeks	136.667a ± 1.575	138.415c ± 1.628
12 weeks	133.243a ± 0.937	162.749b ± 1.015
13 weeks	105.309b ± 1.208	184.635b ± 2.437
14 weeks	81.115c ± 1.025	205.134a ± 2.015
15 weeks	74.823d ± 0.874	203.472a ± 1.108

In the same data column, values with similar letters represent non-significant differences, values with different letters represent significant differences ($p < 0.05$).

En la misma columna de datos, los valores con letras similares representan diferencias no significativas, los valores con letras diferentes representan diferencias significativas ($p < 0,05$).

Conclusion

The guava fruits gradually changed from green to light yellow at 14 weeks after anthesis due to decreased chlorophyll and increased carotenoid contents, at which time, the fruit size was maximum and almost unchanged, and most of the main components of the fruit such as vitamin C and reducing sugars were at their highest values, while some such as pectin and tannins showed low contents, making the fruit taste better. In this study, guava fruit reached physiological maturity at 14 weeks. Therefore, this is the most appropriate time to harvest guava fruits for excellent quality. If harvested earlier or later, the quality of guava fruit will decrease significantly.

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Collard greens and chicory intercropping efficiency as a function of chicory (*Cichorium intybus*) transplant time

Eficiencia de los cultivos intercalados de col y achicoria en función del tiempo de transplante de la achicoria (*Cichorium intybus*)

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Abstract

Vegetable intercropping has advantages over single cultivation in terms of less environmental impact. However, to convince farmers to adopt this production system, it is necessary to prove greater efficiency in the production of more food per unit area and therefore an increase in productivity. An experiment was carried out aiming to evaluate the effect of the chicory transplant time in intercrops with collard greens on crop yields and land use efficiency index (LUE). The experimental design was a randomized block, with nine treatments in a 2 × 4 + 1 factorial scheme, and four replications. Crop systems (intercrop and monoculture) and chicory transplant time (0, 14, 28 and 42 days after transplant (DAT) of collard greens) were evaluated. The collard greens yield increased as the chicory transplant time was delayed. The total and per harvest yields of chicory were not influenced by its transplant time. Regardless of chicory transplant time, collard greens and chicory intercropping provided greater LUE than their monocultures and reached the maximum value (52% higher) when the chicory was transplanted 42 days after collard greens.

Keywords

Brassica oleracea L. var. *acephala* • *Cichorium intybus* • crop systems • intercropping feasibility • land use efficiency

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Resumen

El cultivo intercalado de hortalizas tiene ventajas sobre el monocultivo en cuanto a su menor impacto ambiental. Sin embargo, para convencer al productor de que adopte este sistema de producción, es necesario demostrar mayor eficiencia en la producción de más alimentos por unidad de superficie, y por tanto, un aumento de la productividad. Se llevó a cabo un experimento con el fin de evaluar el efecto del tiempo de trasplante de la achicoria en los cultivos intercalados con la col en el rendimiento de los cultivos y el índice de eficiencia del uso de área (LUE). El diseño experimental fue en bloques aleatorios, con nueve tratamientos en un esquema factorial 2 x 4 + 1, y cuatro repeticiones. Se evaluaron los sistemas de cultivo (intercalado y monocultivo) y el tiempo de trasplante de la achicoria (0, 14, 28 y 42 días después del trasplante (DAT) de la col). El rendimiento de la col aumentó al retrasarse el tiempo de trasplante de la achicoria. El rendimiento total y por cosecha de la achicoria no se vio influido por su tiempo de trasplante. Independientemente del tiempo de trasplante de la achicoria, la col y el cultivo intercalado de achicoria proporcionaron una mayor LUE en comparación con los sistemas de monocultivos, y alcanzaron el valor máximo (52% más alto) cuando la achicoria se trasplantó 42 días después de la col.

Palabras clave

Brassica oleracea L. var. *acephala* • *Cichorium intybus* • sistemas de cultivo • viabilidad de los cultivos intercalados • eficiencia del uso de área

Introduction

Vegetable production is an activity characterized by intense soil management and exposure, and intensive use of water and fertilizers, which provide considerable environmental impact (16). Developing technologies that allow the rational use of land for food production is therefore necessary, and intercropping is an available technology that can assist in production but which has less environmental impact, mainly due to complementary use of resources in time and space among different species (10, 13). In addition, intercropped cultivation allows a reduction of production costs, making the activity better remunerated and the producer more competitive in the market (6, 7, 9, 11).

Intercropped cultivation efficiency depends on the species involved in the system and the planting time of the second species, because these factors affect the period for which the species coexist and, consequently, the spatial and/or temporal complementarity, with impacts on crop yields and on the whole system (5, 6, 8).

Cecílio Filho *et al.* (2011, 2015) observed that land use efficiency decreased on increasing the transplanting time between tomato and lettuce (2011) and between cucumber and lettuce (2015), due to increased shading of tomato and cucumber on lettuce. The longer the period between transplantations, the larger were the tomato and cucumber plants when the lettuce was transplanted. Consequently, less solar radiation was available to the lettuce that grew under the tomato and cucumber canopy. Similarly, Ohse *et al.* (2012) evaluated the agronomic viability of broccoli and lettuce intercropping and they verified that the yield of lettuce was affected by its transplanting time. The authors obtained the best result when lettuce was transplanted on the same day as broccoli. Consequently, the effect of lettuce transplant time impacted intercropping efficiency in the same way.

This study aims to evaluate the yields for intercropping between collard greens (*Brassica oleracea* var. *acephala*) and chicory (*Cichorium intybus*), two vegetables commonly grown by small farmers (family farming), which can easily incorporate the technology of an intercropping system to replace the monoculture system of those crops. Also, the two species have different characteristics such as size, height and foliar architecture, which are important to the success of intercropping (5, 12, 14).

No studies about collard greens and chicory intercropping have been found in the literature. However, Cecílio Filho *et al.* (2017) studied the effect of New Zealand spinach (*Tetragonia expansa*) transplanting time in intercropping with collard greens. The authors observed that the New Zealand spinach, regardless of the time at which it is transplanted in relation to collard greens, does not affect collard greens yield. However, collard greens cause a loss of New Zealand spinach yield, regardless of the spinach transplanting time, by about 13.5% in relation to the yield obtained in monoculture. It is expected that a simple change of the intercropped species can alter the crop system viability. In this case, chicory and New Zealand spinach have differences such as size and height, kind and velocity of growth, and architecture among other characteristics, which can cause different effects on spatial and/or temporal complementarity in intercropping with collard greens. Our hypothesis is that the production of collard greens and chicory in an intercropping system has greater land use efficiency than in monoculture systems and that the chicory transplanting time in the intercropping affects the yields of both species in the system.

Thus, this study was performed with the aim to evaluate the agronomic efficiency of collard greens and chicory intercropping as a function of the chicory transplant time in relation to that of collard greens.

Materials and methods

Characterization of experimental site

The experiment was carried out in the field at São Paulo State University (21°14'39'' S, 48°17'10'' W; 575 m a. s. l.) from May to November 2018.

The soil was classified as a Eutrudox with sand = 253 g kg^{-1}, silt = 132 g kg^{-1} and clay = 615 g kg^{-1}. The chemical attributes before installation of the experiment were: pH ($CaCl_2$) 5.6; 17 g dm^{-3} organic matter; 31 mg dm^{-3} P (resin); 3.3 $mmol_c$ dm^{-3} K; 22 $mmol_c$ dm^{-3} Ca; 10 $mmol_c$ dm^{-3} Mg; and soil base saturation = 71%.

During the experimental period, the climate parameters were: relative humidity 63.9%, rainfall 330 mm and average temperature 22°C, with maximum and minimum averages of 29.8 and 16.2°C, respectively.

Treatments and experimental design

Nine treatments were evaluated as result of combining two crop systems (intercropping and monoculture) and four chicory transplanting times (0, 14, 24 and 42 days after transplanting collard greens - DATCG). The treatments were arranged in a 2 × 4 + 1 factorial scheme, in a randomized block, with four replications. The additional treatment corresponded to collard greens monoculture. Collard greens was considered as the main crop and chicory as the secondary crop.

Each experimental unit (3.36 m^2) contained 14 collard greens plants and 70 chicory plants. Two rows of collard greens were transplanted in the centre of the bed, with 0.75 m between rows and 0.40 m between plants in a row. Five rows of chicory were transplanted to the bed, being three from their between the collard greens rows with 0.25 m spacing between rows and 0.20 m between plants in a row. The useful area for the evaluation of the experiment corresponded to the plants in the centre of the bed excluding two collard greens plants from each end of the row. For evaluation of chicory, three central rows were considered, excluding 0.40 m at the beginning and end of each row. In total, 30 chicory plants and 10 collard greens plants were evaluated (figure 1, page 94).

Installation of experiment

According to the results of the soil analysis, liming was carried out, applying lime to increase the soil base saturation to 80%. Planting and covering fertilization for both crops were performed according to Trani and Raij (1997) and Trani *et al.* (2018). At the planting time, 40, 320 and 80 kg ha^{-1} of nitrogen (urea), phosphorus (simple superphosphate) and potassium (potassium chloride) were supplied, respectively.

The 'HS-20' collard greens seedlings were transplanted on a single date and the 'Pão de Açúcar' chicory seedlings were transplanted on the dates established in the treatments. At the time of chicory transplanting at 0, 14, 28 and 42 DATCG, the height of collard greens plants was measured and corresponded to 4.6, 11.9, 19.3 and 39.9 cm, respectively. The chicory seedlings were formed on several dates in order to obtain plants with four leaves for all treatments (transplanting times).

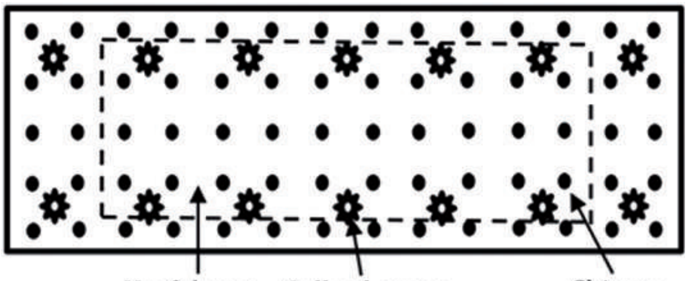

Figure 1. Graphical representation of an experimental unit of intercropping system showing the arrangement of crops.

Figura 1. Representación gráfica de una unidad experimental de sistema de cultivo intercalado en la disposición de los cultivos.

The cover fertilization for collard greens was carried out by applying 40 kg ha^{-1} of N (urea) and 20 kg ha^{-1} of K_2O (potassium chloride) every 15 days after the transplant until completing 45 days. For the chicory, 30 kg ha^{-1} of N and 20 kg ha^{-1} K_2O were applied at 14 and 24 days after transplanting the chicory and immediately after each harvest for both cultures. The experiment was irrigated periodically by spraying throughout the crop cycle. Weed control was performed by manual weeding, weekly.

Every 10 days, leaves of collard greens were harvested, totalling 14 harvests. The chicory was harvested when the head was formed, which happened twice.

Characteristics evaluated

Yield (kg ha^{-1}): obtained by summing the harvests carried out throughout the crop cycle.

Relative yield (RY %): obtained by the ratio between crop yields in intercropping and in monoculture. RY was proposed by Wit and Bergh (1965) and calculated from the following equations:

$$RY_{cg} = \frac{Y_{12}}{Y_{11}} \times 100 \qquad RY_c = \frac{Y_{21}}{Y_{22}} \times 100$$

where:

RYcg and RYc correspond to relative yields of collard greens and chicory, respectively

Y_{12} = collard greens yield when intercropped with chicory

Y_{11} = collard greens yield obtained in monoculture

Y_{21} = chicory yield when intercropped with collard greens

Y_{22} = chicory yield obtained in monoculture.

Land use efficiency (LUE): obtained by the equation proposed by Willey (1979):

$$LUE = RY_{cg} + RY_c$$

Statistical analysis

The average value for monocultures (blocks) was considered as the denominator of the indices, as recommended by Bezerra Neto *et al.* (2012).

The data of total and per harvest yields of collard greens and chicory were subjected to variance analysis (F test) and, when significant, the means of the intercropped and monoculture systems were compared by the Tukey test at 5%. For collard greens, a complete randomized block design was used, with five treatments (four intercrops and one monoculture) and four replications. For chicory, a complete randomized block design was used in a 2 × 4 factorial scheme, with two cultivation systems and four transplant times. For LUE and RY indices, a randomized complete block design was used, with four treatments (intercrops). The regression equations were obtained according to the chicory transplanting time. In all analyses, the AgroEstat statistical program was used (2).

Results

Collard greens yield

The total and per harvest yields were influenced by the crop system (table 1). The collard greens total yield in the intercropping system established by chicory transplant at 42 DATCG did not differ from the yield obtained in monoculture. On the other hand, the collard greens total yields obtained in intercropping systems established with chicory transplanted at 0, 14 and 28 DATCG were lower than that obtained in monoculture, reaching 36% less when both crops were transplanted on the same day (table 1).

In the partial harvests, the yields of intercropped crops sometimes did not differ from those in monoculture; sometimes they differed, sometimes being higher or lower (table 1). Table II shows adjusted polynomial equations for total and per harvest yields of collard greens when intercropped with chicory. The collard greens total yield increased linearly as the chicory transplant was performed later in relation to the collard greens transplant. The collard greens yield increased by 438.11 kg ha^{-1} for each day of delaying the chicory transplant (table 2, page 96).

Table 1. Summary of variance analysis for total yield (TY) and yield per harvest (HY) (kg ha^{-1}) of collard greens as a function of crop system.

Tabla 1. Resumen del análisis de varianza del rendimiento total (TY) y el rendimiento por cosecha (HY) de la col en función del sistema de cultivo.

Source of variation	TY	HY1	HY2	HY3	HY4
	kg ha^{-1}				
Treatment	11.73**	1.48ns	25.68**	8.07**	16.82**
CV %	10.49	25.30	23.97	23.40	13.07
I-0 DATCG	47870.69c	5007.05a	1242.84b	2157.11c	3317.81c
I-14 DATCG	54029.20bc	5518.49a	1099.98b	2646.39bc	5235.63b
I-28 DATCG	58037.71bc	6542.76a	1603.55b	3977.80ab	4932.07b
I-42 DATCG	66979.71ab	7080.84a	1747.12b	4517.79ab	6667.76a
Monoculture	75384.59 a	7185.61a	3985.66a	4999.93a	4092.80bc
Source of variation	**HY5**	**HY6**	**HY7**	**HY8**	**HY9**
	kg ha^{-1}				
Treatment	11.90**	9.33**	2.44ns	4.60*	1.88ns
CV %	14.89	13.10	21.02	21.45	21.61
I-0 DATCG	5074.92b	5903.49b	4664.21a	2778.53b	3389.24a
I-14 DATCG	4999.93b	5678.49b	5875.63a	3178.53b	4024.94a
I-28 DATCG	5928.48b	5585.63b	5360.64a	3421.38ab	4371.36a
I-42 DATCG	8353.45a	7089.48ab	5414.20a	3628.76ab	4295.18a
Monoculture	8289.16a	8685.58a	7428.46a	4960.64a	5128.50a
Source of variation	**HY10**	**HY11**	**HY12**	**HY13**	**HY14**
	kg ha^{-1}				
Treatment	5.89*	3.24ns	7.62**	2.17ns	0.90ns
CV %	15.77	20.05	18.58	19.72	24.20
I-0 DATCG	3839.23b	3985.67a	2042.83b	2414.25a	2053.54a
I-14 DATCG	4257.08b	4249.94a	2466.63b	2790.43a	2007.11a
I-28 DATCG	4378.50ab	3642.81a	2271.40b	3389.24a	2632.10a
I-42 DATCG	5128.49ab	5574.92a	2207.11b	2857.10a	2417.82a
Monoculture	6146.34a	5171.35a	3657.09a	3439.23a	2214.25a

The number beside HY corresponds to the collard greens harvest number (*e.g.* HY1 = Yield of the first harvest); I = Intercropping; DATCG = days after transplant of collard greens; means followed the same letter in a column do not differ by Tukey test (p > 0.05); F Test: ns: not significant; *: p ≤ 0.05; ** p ≤ 0.01.

El número delante de HY corresponde al número de la cosecha de col (ej.: HY1 = rendimiento de la primera cosecha) I = Intercambio de cultivos; DATCG = días después del transplante de col; las medias seguidas de la misma letra en la columna no difieren por la prueba de Tukey (p > 0,05); Prueba F: ns: no significativo; *: p ≤ 0,05; ** p ≤ 0,01.

Table 2. Adjusted equations, significance (F values) and determination coefficients (R^2) for yields per harvest (HY) and total yield (TY) of collard greens as a function of the chicory transplanting time.

Tabla 2. Ecuaciones ajustadas, significancia (valores F) y coeficientes de determinación (R^2) para los rendimientos por cosecha (HY) y el rendimiento total (TY) de la col en función del tiempo de trasplante de la achicoria.

Yield	Equations	R^2	F
HY1	no adjust	-	-
HY2	no adjust	-	-
HY3	y = 2062.7535 + 60.0961x	0.96	18.23**
HY4	y = 3575.3720 + 69.6164x	0.84	42.97**
HY5	y = 50599.56500 – 57.0398x + 3.1887x2	0.99	7.76**
HY6	no adjust	-	-
HY7	no adjust	-	-
HY8	no adjust	-	-
HY9	no adjust	-	-
HY10	y = 3802.4447 + 28.4943x	0.92	5.64*
HY11	no adjust	-	-
HY12	no adjust	-	-
HY13	no adjust	-	-
HY14	no adjust	-	-
TY	y = 47528.9923 + 438.11105x	0.98	15.04**

The number beside of HY corresponds to the collard greens harvest number (*e.g.* HY1 = yield of the first harvest);*: p ≤ 0.05; **p ≤ 0.01.

El número delante de HY corresponde al número de la cosecha de col (Ej.: HY1 = rendimiento de la primera cosecha);*: p ≤ 0,05; **p ≤ 0,01.

Chicory yield

The total and per harvest yields of chicory were not influenced by the chicory transplant time or by interaction of the evaluated factors, but they were influenced by the crop systems. Monoculture produced more than the intercropping system (table 3). There was no adjustment of regression equations for chicory yield (total and per harvest) according to the chicory transplanting time.

Table 3. Summary of variance analysis for total yield (TY) and per harvest yield (HY) of chicory as a function of crop system (CS) and chicory transplanting time (CTT) in relation to collard greens transplant.

Tabla 3. Resumen del análisis de varianza del rendimiento total (TY) y por cosecha (HY) de la achicoria en función del sistema de cultivo (CS) y el tiempo de trasplante de la achicoria (CTT) en relación con la col.

Source of variation	TY		HY1		HY2	
	F value					
CS	111.26**		206.94**		36.38**	
CTT	0.85ns		0.67ns		1.75ns	
CS x CTT	0.10ns		0.52ns		0.39ns	
CV %	11.2		8.18		19.69	
	Yield (kg ha^{-1})					
	TY		**HY1**		**HY2**	
	I	**M**	**I**	**M**	**I**	**M**
	44068.40b	67330.73a	24208.86b	36917.00a	19859.55b	30413.72a

The number beside HY corresponds to the chicory harvest number (*e.g.* HY1 = yield of the first harvest); I = Intercropping; M = Monoculture; F test: ns: not significant; ** p ≤ 0.01; means followed by the same letters in a row do not differ by Tukey test (p > 0.05).

El número delante de HY corresponde al número de cosecha de achicoria (Ej.: HY1 = rendimiento de la primera cosecha); I = Intercambio de cultivos; M = Monocultivo; prueba de F: ns: no significativo; ** p ≤ 0,01; las medias seguidas de las mismas letras en la fila no difieren por la prueba de Tukey (p > 0,05).

Relative yields and land use efficiency

RYcg was influenced by treatments, which was not observed for RYc (table 4). RYcg increased linearly as function of chicory transplanting time and reached 28% more when the chicory transplant happened 42 DATCG in relation to the RYcg obtained when both crops were transplanted on the same day.

Table 4. Summary of variance analysis for relative yield of collard greens (RYcg), relative yield of chicory (RYc) and land use efficiency (LUE) as a function of chicory transplanting time (CTT) in relation to collard greens transplant.

Tabla 4. Resumen del análisis de variaciones del rendimiento relativo de la col (RYcg), el rendimiento relativo de la achicoria (RYc) y la eficiencia del uso de la tierra (LUE) en función del tiempo de trasplante de la achicoria (CTT) en relación con el trasplante de la col.

Source of variation	RYcg (%)	RYC (%)	LUE
F value			
CTT	5.5*	0.17ns	2.96ns
CV %	12.47	13.29	8.22
kg ha^{-1}			
0 DATCG	63.50b	67.33a	1.31a
14 DATCG	71.67ab	63.41a	1.35a
28 DATCG	76.99ab	66.52a	1.44a
42 DATCG	88.85a	64.52a	1.53a

DATCG = days after transplant of collard greens; F Test: ns: not significant; *: $p \leq 0.05$; means followed by the same letter in a column do not differ by Tukey test ($p > 0.05$).

DATCG = días después del transplante de col; Prueba F: ns: no significativo; *: $p \leq 0,05$; las medias seguidas de la misma letra en la columna no difieren por la prueba de Tukey ($p > 0,05$).

Therefore, the later the chicory transplant, the higher the intercropped collard greens yield and the nearer it is to the monoculture yield (figure 2).

Regarding LUE, there was no effect of the treatments (table 4), but there was a significant adjustment of the linear equation (figure 2).

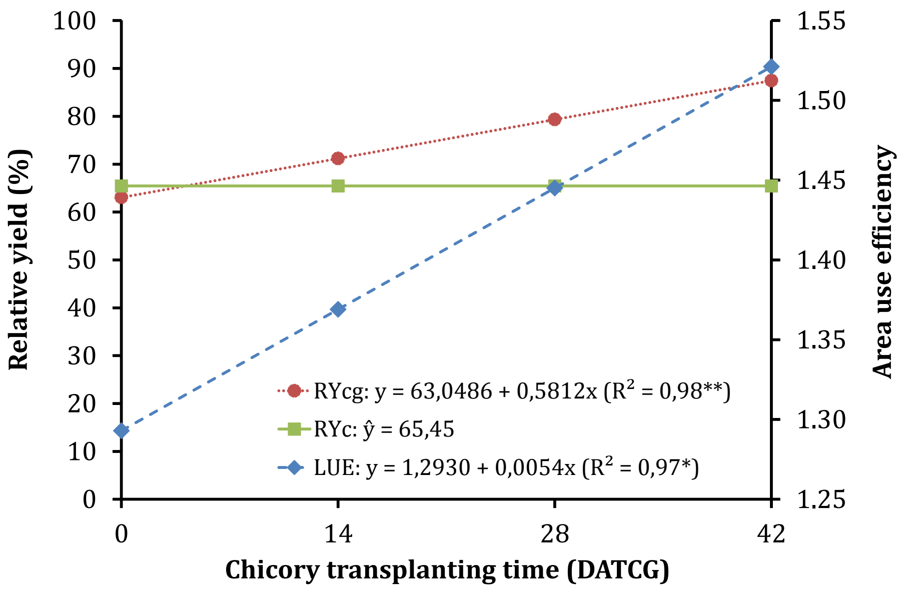

Figure 2. Relative yield of collard greens (RYcg) and chicory (RYc) and land use efficiency (LUE) as a function of chicory transplanting time in relation to collard greens transplant (DATCG).

Figura 2. Rendimiento relativo y eficiencia en el uso de la tierra (LUE) de la col (RYcg) y la achicoria (RYc) en función del tiempo de trasplante de la achicoria en relación con el trasplante de la col (DATCG).

Discussion

According to the results, the chicory affected the collard greens yield, especially when both species were transplanted on the same day. The chicory grew faster than the collard greens and quickly occupied the area. Due to that and the proximity to collard greens plants, the chicory leaves intercepted the solar radiation and reduced the availability of this resource for collard greens plants. However, the later the chicory was transplanted, the weaker its interference with the collard greens since that was itself more developed. When chicory was transplanted at 14, 28 and 42 DATCG, the height of the collard greens plants was 11.9, 19.3 and 39.9 cm, respectively, while when transplanted at 0 DATCG it was 4.6 cm.

The delay in the chicory transplant determined lower interference capacity of the chicory in the interception of solar radiation to the collard green. In an intercropping system, competition between plants is greater for light than for water and nutrients (3), which are adequately supplied to crops according to the management of the production system.

The results observed for collard greens and chicory intercropping differ from those found by Cecílio Filho *et al.* (2017), who evaluated collard greens and New Zealand spinach intercropping. The authors observed that total and per harvest yield of collard greens was influenced neither by crop system (intercropping and monoculture) nor by New Zealand spinach transplanting time (0 to 98 DATCG). Therefore, the secondary species associated with collard greens determines the collard greens performance, since in the collard greens and chicory intercropping system, the chicory grew faster. On the other hand, according to Cecílio Filho *et al.* (2017), collard greens grows faster than New Zealand spinach and quickly positions its photosynthetic canopy above the stratum occupied by the spinach, which has prostrate growth. Then, the collard greens intercepted the incident solar radiation, causing shading and negatively impacting the chicory yield. Despite the chicory yield reduction was no observed harmful to the commercial aspect.

The results for interspecific competition between collard greens and chicory for light corroborate the results observed for tomato–lettuce intercropping by Cecílio Filho *et al.* (2011), broccoli-lettuce intercropping by Ohse *et al.* (2012), cucumber-lettuce intercropping by Cecílio Filho *et al.* (2015) and collard green–New Zealand spinach intercropping by Cecílio Filho *et al.* (2017). These authors observed that, similar to what happened with chicory in relation to collard greens, the photosynthetic process and, consequently, growth of lettuce and spinach was harmed, due to their low height and shading by broccoli, tomato and cucumber, respectively. In the present study, the highest chicory yield was obtained in the monoculture system and, unlike the intercropping systems mentioned above, there was no effect of the time at which chicory was transplanted on its yield.

The RYcg and RYc indices were less than 1, showing that there was competition between species. The lowest RYcg happened when both crops were transplanted on the same day, determined by greater interference of the chicory on collard greens.

All collard greens–chicory intercropping systems showed a LUE greater than 1, which reflects the complementarity of the species, *i.e.*, there was an advantage in food production per unit area in the intercropping system due to the better use of environmental resources (1). The LUE was at a maximum (1.52) when chicory transplant was performed at 42 DATCG, meaning that 1 ha in the intercropping system yielded the same quantity of food (collard greens and chicory) as 1.52 ha in the monoculture system.

Conclusion

The later chicory transplanting is performed, the greater the collard greens yield.

The chicory total yield is not influenced by chicory transplanting time in relation to collard greens transplanting time.

Collard greens–chicory intercropping promotes greater LUE than their monocultures and the efficiency is at a maximum (+52%) when the chicory transplant is performed at 42 DATCG.

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Lotus tenuis and *Schedonorus arundinaceus* co-culture exposed to defoliation and water stress

Co-cultivo de *Lotus tenuis* y *Schedonorus arundinaceus* ante defoliación y estrés hídrico

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Abstract

The present study aimed to investigate the effect of defoliation frequency (low and high) and water stress (excess or deficit) on biomass production, P and N nutrition, and symbiosis with native soil microorganisms on a *Lotus tenuis* and *Schedonorus arundinaceus* co-culture in a pot experiment. Combined effects of defoliation frequency and water stress affected plant accumulated shoot biomass. *L. tenuis* root biomass decreased in response to defoliation and water stress, while *S. arundinaceus* root biomass was similar between non-defoliated and defoliated plants, at all water levels. Low and high frequencies of defoliation in a waterlogged soil can be considered the most stressful scenario for *L. tenuis* and *S. arundinaceus* co-culture. Colonization of arbuscular mycorrhizal fungi in *L. tenuis* roots and dark septate endophytes colonization in *S. arundinaceus* roots were affected by both factors, whereas arbuscular mycorrhizal colonization in *S. arundinaceus* was affected only by water stress. Both plants tolerated defoliation and water stress due to the interaction between the translocation of nutrients and carbon compounds from roots to shoots, and P and N absorption (plus N_2 fixation in *L. tenuis*).

Keywords
Lotus tenuis - Schedonorus arundinaceus co-culture • P and N nutrition • water stress • defoliation • native soil microorganisms

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Resumen

El presente estudio tuvo como objetivo investigar el efecto de la frecuencia de defoliación (baja y alta) y el estrés hídrico (exceso y déficit) sobre la producción de biomasa, la nutrición P y N y la simbiosis con microorganismos edáficos nativos en un co-cultivo de *Lotus tenuis* y *Schedonorus arundinaceus* en un ensayo en macetas. Los efectos combinados de la frecuencia de defoliación y el estrés hídrico afectaron la biomasa acumulada del vástago. La biomasa radical de *L. tenuis* disminuyó en respuesta a la defoliación y estrés hídrico, mientras que en *S. arundinaceus* fue similar entre no defoliadas y defoliadas en todos los niveles hídricos. La defoliación (baja o alta frecuencia) en un suelo inundado puede ser considerada el escenario con mayor estrés para el co-cultivo de *L. tenuis* y *S. arundinaceus*. La colonización micorrícica arbuscular en las raíces de *L. tenuis* y por endofitos septados oscuros en las raíces de *S. arundinaceus* fueron afectadas por ambos factores, mientras que la colonización micorrícica arbuscular en *S. arundinaceus* fue solo afectada por el estrés hídrico. Ambas especies toleraron defoliación y estrés hídrico debido a la interacción entre la traslocación de nutrientes y compuestos carbonados desde la raíz hacia el vástago y la absorción de P y N (más el N_2 fijado en *L. tenuis*).

Palabras clave

Co-cultivo *Lotus tenuis - Schedonorus arundinaceus* • nutrición P y N • estrés hídrico • defoliación • microorganismos edáficos nativos

Introduction

The performance of legume-grass mixtures has been evaluated in several grassland ecosystems. This plant combination is especially advantageous under conditions of stress and low availability of environmental resources (4, 7). Legumes can contribute to increasing grass forage yield, substituting added inorganic N-fertilizers through symbiotic N_2 fixation, enhancing environmental stress tolerance, and increasing its nutritional value (4, 21).

In the pampas grasslands of the Province of Buenos Aires (Argentina), lowlands occupy a significant proportion of the total production area for dairy cattle. These grasslands are dominated by a range of perennial native grasses (6, 24), with lack of legumes and subjected to periodic droughts or floods. In addition, their soils show high levels of salinity and sodicity (9). In these areas, species persistence within the plant community depends on their tolerance to a combination of stressful conditions, on their ability to absorb and mobilize nutrients, and on soil water content, the latter of which modifies nutrient availability for plant growth (11).

Lotus tenuis Waldst. & Kit. is a much-appreciated legume given its high forage nutritional value for beef and dairy cattle in nutrient-deficient soils (5). In addition, *L. tenuis* is tolerant to water stress (10, 29) and to defoliation in saline-sodic soils (11). *Schedonorus arundinaceus* (Schreb.) Dumort is a grass that tolerates saline conditions and grazing (1). *Lotus tenuis* and *S. arundinaceus* co-culture has been reported to be a sustainable alternative for forage quality improvement (21). However, little is known about its performance under the combined effect of defoliation and water stress. These stressful conditions may allow evaluating tolerance mechanisms and persistence of the *L. tenuis - S. arundinaceus* co-culture under adverse conditions. The impact of high-defoliation frequencies on both plants under soil water deficit or soil water excess is expected to be different from that under well-watered conditions.

As known, removal of photosynthetic biomass, by either grazing or defoliation, alters plant carbon balance, forcing physiological changes within the plant. The production of new plant tissue depends on the availability of nutrient reserves and on plant ability to efficiently absorb soil nutrients (19, 28). During postdefoliation regrowth, rapid carbohydrate degradation and resynthesis occurs in roots (18). In addition, 40 to 60% of root total N is mobilized to meet shoot N demand during early regrowth (18). In relation to P nutrition, a previous study has demonstrated that grass defoliation tolerance depends on P reserves and that P reutilization and remobilization provide tolerance to defoliation by promoting compensatory growth under P-deficient conditions (23). This pattern suggests that the effect of nutritional reserves on grass regrowth depends on the level of those reserves, as shown for N reserves by Hamilton *et al.* (14). With respect to legumes in particular, a previous study has shown that *L. tenuis* plants defoliated up to 75% of their aerial biomass are able to compensate total absorbed P and N by non-defoliated plants after a 30-day recovery period (11). This is associated with higher rates of shoot growth in defoliated plants compared to non-defoliated ones, along with a sufficient level of absorbed nutrients necessary to sustain shoot regrowth (11). It is necessary to emphasize that, to estimate changes in forage quality in a legume-grass co-culture under stress, studying P and N metabolism in conjunction, is important.

It is known that conditions that alter root growth, reduce plant photosynthetic capacity, and modify soil environment, may also affect the symbiotic relationship between plants and soil microorganisms (3). In addition, defoliation and soil-water content may influence arbuscular mycorrhizal (AM) fungal colonization, and AM fungi may, in turn and under stress conditions, alter plant competitive ability to improve plant nutrition and water status. Dark septate endophytic (DSE) fungi not only may function as pathogens or saprophytes, but also may form mutualistic associations similar to those of mycorrhizae (17). Several studies have shown that DSE fungi improve plant growth and N and P nutrition (17, 22). DSE fungal colonization has been shown to respond to herbivory, nutrient availability, and habitat in a contrasting manner compared to the pattern observed with AM fungi (12, 26). Although several studies have described the effect of defoliation by cutting-either under natural or controlled conditions-on the growth of different plant species in monoculture, few investigations have evaluated the ability of legume-grass mixtures to associate with AM or DSE fungi. Such an approach to evaluate the dynamics of symbiosis with native soil microorganisms, especially under abiotic-stress conditions, is highly desirable.

Based on all the above, this study aimed to investigate the combined effect of different defoliation frequencies and water stresses on the performance of *L. tenuis* and *S. arundinaceus* grown in co-culture in a saline-sodic soil. The hypotheses were that, at high defoliation frequency plus water stress conditions, (i) both plant species are not able to compensate clipped shoot biomass, and (ii) AM fungal colonization in both plant species decreases and DSE fungal colonization in *S. arundinaceus* roots increases. The prediction was that, under these stress conditions, regrowth would be low, as a result of a drastic reduction of root biomass, which decreases nutrient absorption and carbon reserves, and affects the symbiotic relationship between plants and native soil microorganisms.

Materials and methods

Soil characteristics and experimental setup

The soil was a Typic Natraqualf from San Vicente (Buenos Aires, Argentina). The plant community in the sampling site was dominated by *L. tenuis*, *Distichlis spicata* (L.) Greene, *Paspalum vaginatum* Swartz, *Eleocharis viridans* Kük. ex Oken, and *Cynodon dactylon* (L.) Pers. The soil is characterized by: pH 9.53, electrical conductivity 10.10 dS/m, Na^+ 19.45 $cmol_c$/kg, available P 8.72 mg/kg (Bray I), total carbon 9.10 g/kg, and total N 1.02 g/kg (9). The soil was air-dried and sieved through a 2-mm mesh screen.

Seeds of *L. tenuis* cv. Esmeralda and *S. arundinaceus* cv. Arizona were surface-sterilized and pregerminated under sterile conditions. Fifty closed-bottom 1.6-L pots were filled with 950 g of air-dried soil. Three seedlings of each plant species were planted together in each pot and the soil surface was covered with 1 cm of sterilized sand to conserve moisture. During the experimental period, pots were placed in a greenhouse at a mean relative humidity of 65 ± 15%, mean temperature of 30 ± 5°C and 20 ± 3°C during days and nights, a photoperiod ranging from 10 to 12 h, and a photon-flux density at midday of 900-1,300 μmol $m^{-2}s^{-1}$. Pots were randomized and daily rotated to minimize potential gradient effects.

After 62 days of growth in soil at 80% of field capacity (46.10% w/w, 33 kPa), five pots were harvested (initial plants) and the remaining pots were subjected to a combination of two defoliation frequencies and two water stress conditions (water excess and deficit). Defoliation treatments comprised removing all plant material 4 cm above ground level at different time intervals over a 28-day period, starting with defoliation of all plants, except controls (non-defoliated plants under all irrigation conditions), at the beginning of this period. *L. tenuis* and *S. arundinaceus* were defoliated by clipping at a frequency of 2 and 4 times representing intervals of 2 and 1 weeks between defoliations, respectively. The 2-time frequency is here referred to as low defoliation frequency (LF) and the 4-time frequency as high defoliation frequency (HF). Water-stress treatments consisted in subjecting plants to water excess (WE), water deficit (WD), or 80% of field capacity (WW- well watered plants) for a 28-day period. The WE treatment consisted in maintaining a water level of 1 cm above the soil surface, whereas the WD treatment consisted in keeping soil moisture at 24.50 w/w (1,488 kPa), near the permanent-wilting-point level of soil hydration (1,500 kPa). Control pots contained non-defoliated plants maintained at the three different water levels (WW, WE, and WD). Five replicates were assigned to each combination of defoliation frequency and water treatment. The water status of pots was examined daily by weighing them and the amount of water lost was replaced. At the end of the 28 days of treatment, all plants were harvested (final plants).

Plant yield and analytical determinations in plant tissue and soil

After each harvest (initial and final plants), *L. tenuis* and *S. arundinaceus* biomasses were separated into shoots and roots. All shoot biomasses of each plant species removed during the defoliation and water treatments were dried and weighed to be later included in the shoot fraction of the corresponding plant at the end of the experiment, determining accumulated shoot dry weight. Shoots and roots of each plant species were oven-dried at 70°C for 48 h and weighed. A portion of fresh root was used to measure AM fungal colonization in both plant species and number of rhizobia nodules in *L. tenuis* roots. For each species, defoliation or stress-tolerance efficiency (STE) for each water level was calculated using the following equation:

STE (%) = (dry biomass of defoliated plant/ dry biomass of non-defoliated plant) x 100

Shoots and roots of each species were digested separately in sulfuric acid to determine P content by a modification of the Murphy Riley method and N content by the Kjeldahl method (16). Total absorbed N per nodule of rhizobia was calculated (10). Soil P availability was measured by Bray and Kurtz. The root fraction colonized by AM fungi (mycorrhizal colonization index) and the root fraction colonized by DSE fungi (DSE colonization index) were assessed following McGonigle *et al.* (1990). Septate, melanized or hyaline hyphae and microsclerotia were classified as DSE fungi (2). Rhizobia nodules were counted in whole fresh root system under a binocular stereomicroscope (7.5x). Results were analyzed by ANOVA with Tukey's mean separation test ($P \leq 0.05$) using InfoStat statistical software (8).

Results and Discussion

Plant growth

Under each combination of treatments (water level+defoliation) studied in a saline-sodic soil, *L. tenuis* contributed near 80% of the total biomass produced by the co-culture (figure 1a, page 104). *Lotus tenuis* defoliated plants (low and high frequency) were not able to compensate for the shoot biomass produced by non-defoliated plants under well watered conditions through tissue regrowth (defoliation frequency mean value of STE: 59.7%) (figure 1a, page 104 and table 1, page 104). Compensation of removed shoot tissue, especially after frequent defoliation, requires large amounts of energy investment, which is derived from reallocating energy stored in the remnant shoot and root tissue (18), resulting in reduced shoot biomass and root growth (figure 1b, page 104 and table 1, page 104). Unlike *L. tenuis*, *S. arundinaceus* produced similar amounts of accumulated shoot biomass in defoliated and non-defoliated plants under well watered conditions (figure 1a, page 104 and table 1, page 104).

Different letters indicate significant differences (*P*< *0.05*) for each plant species among treatments according to Tukey's test.

Letras diferentes indican diferencias significativas (*P*< *0,05*) para cada especie entre tratamientos según test de Tukey.

DW, dry weight; ND, no defoliation; LF, low defoliation frequency; HF, high defoliation frequency; WW, well watered; WE, water excess; WD, water deficit. Mean (N=5)± SE.

DW, peso seco; ND, no defoliada; LF, baja frecuencia de defoliación; HF, alta frecuencia de defoliación; WW, bien regada; WE, exceso hídrico; WD, déficit hídrico. Media (N=5)± EE.

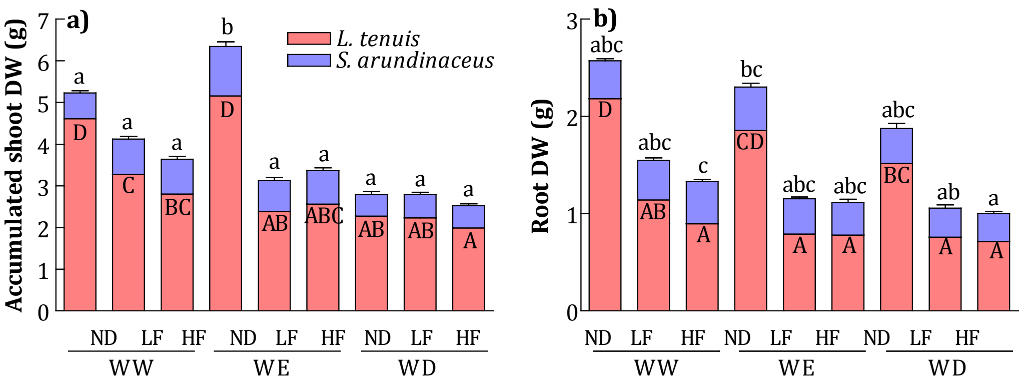


Figure 1. Biomass (a, b) of *Lotus tenuis and Schedonorus arundinaceus* grown in co-culture under a combination of different defoliation frequencies and water stress.

Figura 1. Biomasa (a, b) de *Lotus tenuis* y *Schedonorus arundinaceus* crecidas en co-cultivo ante la combinación de diferentes frecuencias de defoliación y estrés hídrico.

Table 1. Results of the two-way ANOVA used to evaluate the effects of defoliation frequencies (D) and water treatments (W) on different variables.

Tabla 1. Resultados del ANOVA de dos vías usado para evaluar el efecto de la frecuencia de defoliación (D) y estrés hídrico (W) sobre diferentes variables.

Variable	L. tenuis						S. arundinaceus					
	D		W		D xW		D		W		D xW	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Accumulated shoot DW	35.3	***	40.5	***	8.0	***	1.3	ns	18.5	***	7.54	***
Root DW	104.9	***	13.3	***	1.8	ns	1.5	ns	7.9	**	2.1	ns
STE plant	2.3	ns	22.8	***	1.3	ns	0.01	ns	27.1	***	0.2	ns
Plant P content	120.0	***	209.4	***	96.0	***	15.3	***	133.2	***	45.5	***
Plant N content	18.1	***	27.5	***	0.4	ns	20.9	***	6.9	***	6.4	***
N total/nodules	3.7	*	29.5	***	5.2	**						
MC index	25.5	***	69.9	***	9.8	***	2.0	ns	99.6	***	0.2	ns
DSE colonization index							27.7	***	61.9	***	25.6	***
Number of nodules/FW	0.9	ns	13.6	***	0.5	ns						

F values and significant differences: *, *P*<0.05; **, *P*<0.01; ***, *P*<0.001; ns, *P* >0.05.

DW, dry weight; FW, fresh weight; MC index, mycorrhizal colonization index.

Valores de *F* y diferencias significativas: *, *P*<0,05; **, *P*<0,01; ***, *P*<0,001; ns, *P* >0,05. DW, peso seco; FW, peso fresco; MC index, índice de colonización micorrícica.

Since *S. arundinaceus* was able to compensate for biomass clipping by increasing the allocated biomass below the cutting height (data not shown) and maintaining root growth relative to that of non-defoliated plants, defoliation at the two frequencies actually resulted in enhanced plant growth (figure 1a, b and table 1). These results are consistent with the highest values of STE recorded for this species (defoliation frequency mean value of 126.5%), which showed high tolerance to frequent defoliation under well watered conditions.

Under the water excess treatment, *L. tenuis* and *S. arundinaceus* non-defoliated plants recorded the highest accumulated shoot biomass (5.16 g for *L. tenuis* and 1.18 g for *S. arundinaceus*) (figure 1a and table 1). In agreement with that previously described by several authors (11, 27), the adaptation of both species to water excess conditions may be due to an increased soil nutrient availability (11) along with the consequent reduction in soil salinity and sodicity as a result of soil water excess (11). In the present study, the soil of non-defoliated plants under water excess showed a significant increase in available P with regard to the well watered condition (9.72 mg/kg and 12.23 mg/kg for well watered and water excess, respectively). The combination of water excess plus defoliation treatments resulted in a decrease in accumulated shoot and root biomasses of both plant species. These results were associated with lowest efficiencies in tolerating stress (figure 1 a, b, page 104). Mean defoliation frequency of STE was 46.5% for *L. tenuis* and 68.9% for *S. arundinaceus* (table 1, page 104). Both low and high defoliation frequencies in a waterlogged soil can be considered as the most stressful scenario for a *L. tenuis* and *S. arundinaceus* co-culture. The low STE of both species may be associated with a marked loss of nutrients, which affected plant ability to recover the defoliated biomass when defoliation frequency increased, or with decreases in root growth and soil P availability (defoliation frequency mean value of 10.43 mg/kg).

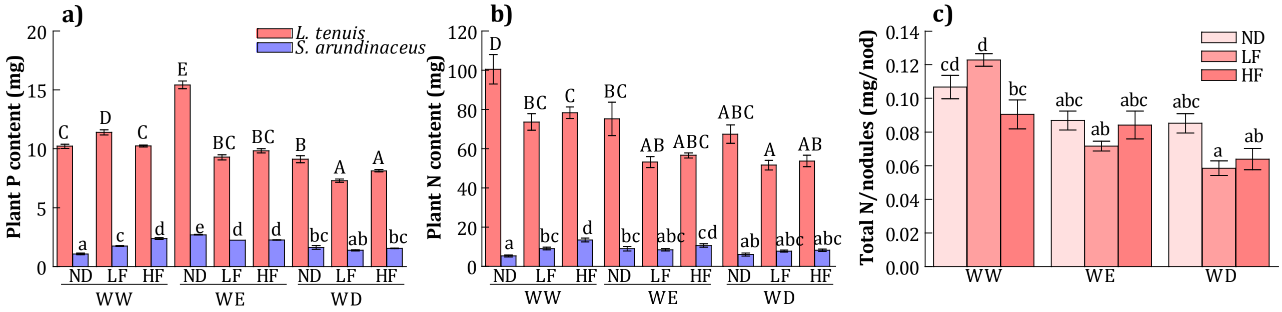
Under water deficit, non-defoliated and defoliated plants of both species produced similar quantities of shoot biomass (figure 1 a, b and table 1, page 104). A reduction in soil moisture resulted in a decrease in soil P availability (mean value for the combination of defoliation and water treatment of 8.39 mg/kg), affecting plant growth and P absorption. Defoliated plants growing under water deficit, however, have an adaptive advantage because a lower proportion of aerial biomass leads to an improvement in plant water balance and an avoidance of excessive losses of water through transpiration compared to non-defoliated plants. In these species, defoliation may alleviate the deleterious effect of water deficit on shoot biomass accumulation, enabling defoliated plants to compensate for the quantity of shoot biomass produced by control plants under drought, whose response becomes manifest in a high STE percentage (figure 1a, page 104). Mean defoliation frequency of STE was 75.2% for *L. tenuis* and 95.5% for *S. arundinaceus* (table 1, page 104). Loss of apical dominance after defoliation and basal meristem activation may stimulate such clipped stems to develop more biomass (15). Moreover, an increase in the photochemical efficiency and photosynthetic capability of remnant leaves below a cutting induces a compensatory growth response through an increase in photosynthates (27).

Defoliation generally results in reduced root growth (15). Root biomass loss in *L. tenuis* in response to defoliation frequency, especially in combination with water stress, would be attributable to such a decrease in root growth, a diversion of assimilated substances, and a mobilization of P and N from roots to regrowing shoot tissues (figure 1b and table 1, page 104). In contrast, the strategy of defoliated *S. arundinaceus* plants was to maintain root growth after low and high defoliation frequencies independently of water status, similar to the respective non-defoliated ones (figure 1b and table 1, page 104).

P and N nutrition

Lotus tenuis defoliated plants under well watered conditions were able to compensate total absorbed P by non-defoliated plants (figure 2a, page 106 and table 1, page 104). This occurred in association with an internal recycling of nutrients from necrotic roots and/or an additional amount of absorbed soil P satisfying shoot regrowth demands. Under water stress plus defoliation, defoliated plants were not able to compensate total absorbed P by non-defoliated plants (figure 2a, page 106 and table 1, page 104). In this case, the plant strategy focuses on preserving the root system through increasing P allocation to roots while limiting P transport to shoots. This strategy was reflected by a decrease in shoot regrowth and plant P content under water stress and defoliation.

Defoliation induces a reduction in the N uptake and N_2 fixation capacity of forage legumes (14) until carbon supply to roots is restored. In the present study, plant N content in *L. tenuis* under defoliation decreased regardless of water status (figure 2b, page 106 and table 1, page 104). Total absorbed N per nodule and number of nodules in *L. tenuis* roots was similar under defoliation in all irrigation conditions (figure 2c, 3c, page 106 and table 1, page 104). These results indicate that the N_2-fixing efficiency of roots of defoliated plants was similar to that of non-defoliated plants. In this situation, defoliated plants mobilized N from roots to shoots to sustain the growth of the latter.



Treatment symbols and statistical notes are the same as those for figure 1 (page 104).

Los símbolos de tratamientos y notas estadísticas son iguales a los de la figura 1 (pág. 104).

Figure 2. Plant P (a) and N (b) content in *Lotus tenuis and Schedonorus arundinaceus* grown in co-culture under a combination of different defoliation frequencies and water stress. Figure c shows total N per nodule of *L. tenuis*.

Figura 2. Contenido de P y N en las plantas de *Lotus tenuis* y *Schedonorus arundinaceus* crecidas en co-cultivo ante la combinación de diferentes frecuencias de defoliación y estrés hídrico en un suelo salino-sódico. La figura c muestra el N total por nódulo de *L. tenuis*.

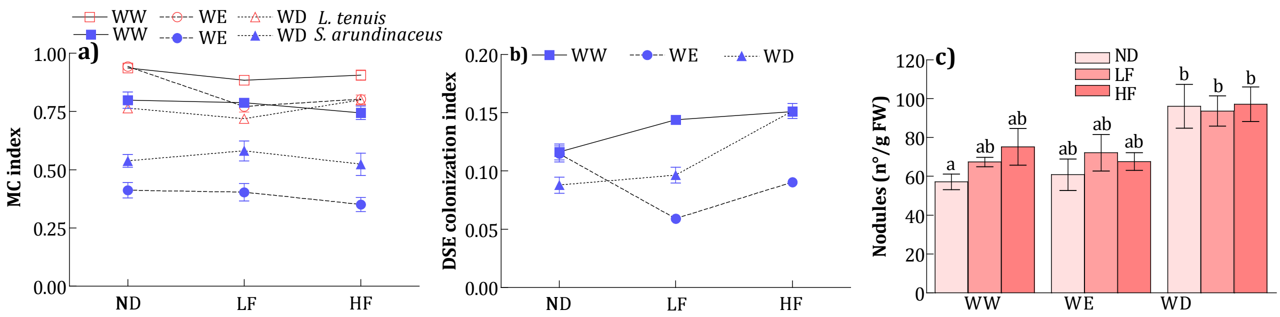
Schedonorus arundinaceus exhibited a similar pattern of plant P and N content in all water treatments (figure 2 a, b and table 1, page 104). In this species, root growth maintenance after defoliation allowed plants to maintain or increase soil P and N uptake in response to increased shoot demands. Iqbal *et al.* (2012) reported that P and N reallocation from roots to shoots would appear to be an essential component of *S. arundinaceus* tolerance to stressful conditions.

The results of plant growth of both species at high defoliation frequency and water excess are consistent with the proposed hypothesis, but responses to water deficit are inconsistent. In fact, both plant species showed higher STE after the combination of defoliation and water deficit than after that of defoliation and water excess.

Fungal colonization and root nodulation

Defoliation may influence plant function through changes in AM fungal colonization (3). Variations in the mycorrhizal response to the loss of above-ground biomass have been suggested to be due to the amount of tissue removed and root growth rate (13). Despite the decrease here observed in root biomass of *L. tenuis* defoliated plants (figure 1b, page 104), defoliation frequency increased the AM colonization index in plant roots under well watered and water deficit conditions (figure 3a, page 107 and table 1, page 104). This result contrasts the proposed hypothesis that defoliation decreases AM colonization. A positive effect on AM colonization may be a consequence of increased nutrient demand by defoliated plants (25). In contrast, the AM colonization in *L. tenuis* roots was affected by the defoliation plus water excess treatment (figure 3a, page 107), where the combination of stresses seemed to be the most stressful scenario for *L. tenuis* growth (figure 1b, page 104). The decrease in AM colonization in defoliated plants was in line with a reduction in root biomass. AM colonization in *S. arundinaceus* roots under water stress and defoliation was minimal (figure 3a, page 107 and table 1, page 104), and similar quantities of root biomass were observed in the three water treatments evaluated (figure 1b, page 104). Barto and Rilling (2010) found similar results and proposed that root biomass resilience to defoliation may explain the lack of effect on AM colonization of grasses.

In the present study, *S. arundinaceus* roots were co-colonized by AM fungi and DSE (figure 3b, page 107 and table 1, page 104). In agreement with the proposed hypothesis, DSE colonization was affected by defoliation frequency, water stress, and the combination of both stresses. Root DSE colonization in defoliated plants grown on well watered and water-deficit soils increased compared to non-defoliated plants, whereas that under water excess, decreased. Since DSE colonization reached lower values than AM colonization, it may be that AM colonization limits DSE colonization due to competition for host carbohydrates. In a previous field study, *S. arundinaceus* roots were much more extensively colonized by AM fungi when grown with *L. tenuis* in co-culture than when grown in monoculture (21).



Treatment symbols and statistical notes are the same as those for figure 1 (page 104).
Red symbols correspond to *L. tenuis* and blue ones to *S. arundinaceus*.
Los símbolos de tratamientos y notas estadísticas son iguales a los de la figura 1 (pág. 104).
Los símbolos rojos corresponden a *L. tenuis* y los azules a *S. arundinaceus.*

Figure 3. Mycorrhizal colonization (MC) index (a) in both plant species, DSE colonization index in *Schedonorus arundinaceus* (b) and number of nodules in *Lotus tenuis* roots (c). FW, fresh weight.

Figura 3. Índice de colonización micorrícica (MC) (a) en las raíces de ambas especies, índice de colonización de DSE en las raíces de *Schedonorus arundinaceus* (b) y número de nódulos en las raíces de *Lotus tenuis* (c). FW, peso fresco.

The presence of *L. tenuis* in mixed communities is crucial to improve not only grassland quality for beef production (5), but also to maintain the AM fungal community, especially under water-stress conditions (9, 11). The high percent of AM colonization in *L. tenuis* roots may be another advantage over the less extensively colonized *S. arundinaceus* roots, accessing soil moisture and nutrients for plant regrowth, mainly under water deficit, given that *S. arundinaceus* is somewhat resistant to drought (1).

Conclusions

These results showed that *L. tenuis* and *S. arundinaceus* tolerated defoliation+water stress in a saline-sodic soil through reutilization and remobilization of P and N reserves from roots to shoots, the concomitant soil P and N uptake capacity, and the additional N fixed by rhizobia in *L. tenuis* roots. The presence of *L. tenuis* in mixtures is crucial to maintain AM fungal communities, especially under water-stress conditions. Promoting the presence of *L. tenuis* through low defoliation frequency would improve forage yield and quality with the maintenance of AM symbiosis in legume–grass communities. The present work is a first step for future studies under field conditions promoting better management recommendations of forage production in saline-sodic soils of lowlands in the pampas grasslands.

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Mycorrhizal fungi and phosphate fertilization in the production of *Euterpe edulis* seedlings

Hongos micorrícicos y fertilización con fosfato en la producción de plántulas de *Euterpe edulis*

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Abstract

The present study evaluated the effect of arbuscular mycorrhizal fungi (AMF) inoculation on growth and nutrition of *Euterpe edulis* seedlings, supplemented or not with phosphate fertilization. The experiment was conducted in a greenhouse. The randomized block design, consisted of a 3x2 factorial arrangement and 4 replicates, with two phosphorus doses (0 and 50 mg dm^{-3} of soil), two microbiological treatments (*Rhizophagus clarus; Claroideoglomus etunicatum; R. clarus + C. etunicatum*) and control (without fungus). Sowing and inoculation occurred concurrently in 2 kg plastic bags. Height, collar diameter, leaf area, dry shoot mass, macronutrient content and mycorrhizal colonization percentage were determined after 226 days. Regarding mycorrhizal colonization percentage, *R. clarus* resulted significantly beneficial for the production of *E. edulis* seedlings. In the absence of phosphate fertilization, *R. clarus* and mixed inoculum increased all biometric variables and macronutrient contents in seedlings. Therefore, it is concluded that AMF inoculations provide beneficial effects for growth and nutrition of *E. edulis* seedlings, resulting in more vigorous plants at a low-cost strategy.

Keywords
Euterpe edulis • mycorrhizal fungi • plant growth • mineral nutrition • seedling quality

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Resumen

El presente estudio evaluó el efecto de la inoculación de hongos micorrízicos arbusculares (AMF) en el crecimiento y la nutrición de plántulas de *Euterpe edulis* en ausencia y presencia de fertilización con fosfato. El experimento se realizó en invernadero. El diseño de bloques al azar, consistió en un arreglo factorial 3x2, con 4 repeticiones, dos dosis de fósforo (0 y 50 mg dm^{-3} de suelo), dos tratamientos microbiológicos (*Rhizophagus clarus*; *Claroideoglomus etunicatum*; *R. clarus* + *C. etunicatum*) y un testigo (sin hongos). La siembra y la inoculación ocurrieron simultáneamente en bolsas de plástico de 2 kg. Después de 226 días, se determinó altura, diámetro de cuello, área foliar, masa seca del brote, el contenido de macronutrientes y el porcentaje de colonización micorrízica. *R. clarus* resultó superior, considerándose entonces una especie fúngica beneficiosa para las plántulas de *E. edulis*. En ausencia de fertilización con fosfato, *R. clarus* y el inóculo mixto aumentaron todas las variables biométricas en comparación con el testigo. Además, se observó un aumento en el contenido de macronutrientes en las plántulas.

Palabras clave

Euterpe edulis • hongos micorrízicos • crecimiento de la planta • nutrición mineral • calidad de plántulas

Introduction

Euterpe edulis is a palm species, native to Brazil, that grows in the Northeastern, Midwestern, Southeastern and Southern regions, predominantly in the Cerrado and Atlantic Forest biomes (11). These biomes are part of 35 worldwide biodiversity hotspots, being a priority for the biodiversity conservation process (13).

Palm trees have suffered a predatory and, in most cases, illegal form of exploitation. Even though a sustainable plan is included in the Official List of Brazilian Species Threatened with Extinction since 2008, drastic reductions in its populations have been reported (14).

E. edulis, popularly known as Juçara, is a symbolic species of the Atlantic Forest with ecological, cultural, and economic value (6). Brazil outstands as the largest producer and exporter of canned hearts of palm worldwide. In addition to heart of palm, Juçara also produces rounded fruits, with bright black colored pulp covering the seeds that, after pulping, is freshly consumed or used for different types of drinks, ice cream, sweets and sauces (6). Palm fruit pulp has high nutritional value, with considerable content of proteins, sugars, fibers, and a lipid fraction with high content of polyunsaturated fatty acids, oleic acid, low content of saturated lipids and high content of phenolic compounds (4).

The sustainable exploitation of Juçara has been stimulated by pulp production, product with similar properties to *Euterpe oleraceae* Açaí pulp (23) has aroused the interest of the food industry, increasing the demand for seedlings, representing up to 73% of the costs of Juçara palm production (6). Studies on planting, management and seedling production techniques, as well as ecology and plant growth dynamics, are required for sustainable exploration and reintroduction of the species in its habitat (15).

Among seedling production techniques, inoculation with arbuscular mycorrhizal fungi (AMF) has proved strategic to produce Juçara palm seedlings in nurseries (Sgrott *et al.*, 2012; Moreira *et al.*, 2016). Inoculation of seedlings with mycorrhizal fungus usually results in rapid plant growth, with greater absorption of nutrients and enhanced tolerance to environmental stresses after field transplantation (16, 17).

Although records have shown positive effects of mycorrhizal inoculation on Juçara palm seedlings, no study has yet shown the effect of phosphorus (P) doses on plant initial growth and nutritional status. However, AMF colonization and efficiency are influenced by the availability of this nutrient in the soil (23).

In this sense, Souza *et al.* (2011) suggested that studies aiming to obtain higher plant absorption efficiency and mycorrhizal symbiosis under low P availability, are essential to ensure sustainability of the Brazilian agribusiness. Thus, this work aimed to evaluate the effect of AMF inoculation on growth and nutrition of Juçara palm seedlings, with and without phosphate fertilization, verifying technical applicability in the production of Juçara palm seedlings.

Material and methods

Experimental location and substrate preparation

The experiment was conducted in a greenhouse at the Federal University of Espírito Santo, municipality of Alegre, State of Espírito Santo, Brazil, (20°47'1" S, 41°36'56" W, 640 m a. s. l.), between April and December 2018. Arbuscular mycorrhizal fungi (AMF) species *Rhizophagus clarus* Nicolson & Schenck and *Claroideoglomus etunicatum* Becker & Gerd, were obtained from the inoculum bank of the Soil Microbiology Sector - Laboratory of Soils, "Darcy Ribeiro" State University of Northern Rio de Janeiro (UENF), municipality of Campos dos Goytacazes-RJ, Brazil. Inocula were multiplied in association with *Brachiaria brizantha* for 90 days and grown in 5-kg capacity pots filled with previously sterilized soil.

For sowing, *B. brizantha* seeds were disinfected in 0.5% hypochlorite solution for 15 minutes and washed with deionized water four consecutive times. After 90 days, shoots were cut and pots were covered with kraft paper bags without irrigation for 30 days, facilitating fungi sporulation. Subsequently, soil mixture holding colonized roots and AMF spores was kept in cold chamber at 4°C until the experiment was conducted.

The substrate constituted a mixture of soil (collected at 0-20 cm depth, sieved in 2 mm mesh), and sand at 1: 2 ratio (v/v). Subsequently, this substrate was sterilized twice in autoclave (121°C for one hour), eliminating native fungi. After sterilization, chemical analysis of the substrate was carried out at the Laboratory of Soil Analysis, Federal Rural University of Rio de Janeiro (UFRRJ), campus of Campos dos Goytacazes-RJ. Table 1 shows substrate chemical characteristics.

Table 1. Substrate chemical analysis after sterilization.

Tabla 1. Análisis químico del sustrato esterilizado.

pH	OM	P	K	Ca	Mg	Na	Al	H+Al	SB	CTC	Fe	Cu	Zn	Mn
H_2O	g dm^{-3}	mg dm^{-3}		$cmol_c$ dm^{-3}							mg dm^{-3}			
6.3	16.4	6	50,0	1.9	1.0	0,09	0.0	1.2	3.1	3.1	42.5	0.6	3.7	89.1

OM = Organic matter. / OM = Materia orgánica.

P doses were applied with KH_2PO_4. Alternatively, Potassium (K) was increased to 113 mg dm^{-3} by supplying K_2SO_4 in treatments without P. After fertilization, the substrate was transferred to plastic bags and incubated for 45 days. After this period, P analysis was performed in all treatments with Mehlich^{-1} extractor, obtaining the following results: 6 and 36 mg dm^{-3} for P doses of 0 and 50 mg dm^{-3} respectively.

Inoculum preparation, inoculation and seed germination

E. edulis seeds were obtained from the processing of Juçara palm fruits, from the Pedro Menegardo Bortolotti industry located at São Vicente, rural area of Rio Novo do Sul-ES, Brazil.

On 05/22/2018, three seeds were sown at approximately one centimeter depth. Concomitantly, in treatments having AMF, 30 cm^3 bag^{-1} were inoculated, at approximately 5 cm depth. Control treatment received no inoculation.

The experiment followed a randomized block design, 2x4 factorial arrangement and four replicates, three microbiological treatments with *Rhizophagus clarus* and *Claroideoglomus etunicatum*, mixed inoculum (*Rhizophagus clarus + Claroideoglomus etunicatum*) and control without AMF, in the absence and presence of P (50 mg kg^{-1}).

According to greenhouse temperature and humidity conditions, daily irrigations maintained almost constant substrate moisture. At 102 days after sowing, thinning was performed, leaving two seedlings per seedling bag. Concomitantly, at 81, 116 and 151 days after sowing, 20 mg dm^{-3} nitrogen (N), as ammonium nitrate (NH_4NO_3) was applied to all treatments. Later, at 171, 185 and 203 days after sowing, Hoagland and Arnon (1950) nutrient solution without P, was also added.

Chemical, biometric and quality analysis of seedlings

At 226 days after sowing, concluding the experiment, leaf area (LA) was measured with a leaf area meter (model LI-3100 LICOR, Lincoln, NE, USA). Subsequently, shoots were packed in paper bags and placed in a forced-air ventilation oven at 70°C for 7 days until constant mass. Then, dry shoot mass (DSM) was determined using a digital scale (± 0.001)). Later, the material was crushed in a Wiley mill (20 mesh sieve), and hermetically packed in sealed plastic tubes. Finally, macronutrient levels were determined by chemical analysis.

N was determined after sulfuric digestion, by the method of Nessler (5). P, K, calcium (Ca), magnesium (Mg) and sulfur (S) levels were determined using Shimadzu® plasma (ICPE-9000) after digestion with HNO_3 and H_2O_2, in an open digestion system (17). Nutrient contents were calculated by multiplying nutrient concentrations by the respective dry shoot mass.

Fragments of the finest roots were collected and stored in 50% ethyl alcohol for later determination of mycorrhizal colonization percentage according to Koske and Gemma (1989), and adapted by Sgrott *et al.* (2012), since according to these authors, the original method resulted in dark roots impairing proper visualization of fungal structures. The method was applied as follows: 1) roots were immersed in 10% KOH solution for 72 hours (solution change every 24 hours); 2) roots immersed in KOH were placed in water bath at 90°C for 60 minutes; 3) roots were washed under tap water and immersed in alkaline H_2O_2 solution for 30 minutes; 4) after washing, roots were placed in 1% HCl solution for 20 minutes; 5) After removing HCl, roots were covered with 0.05% trypan blue solution and left for another 60 minutes in water bath (90°C); 6) roots were kept in distilled water at 4°C until analysis. To verify root colonization, ten (10) segments of thin roots of approximately 1 cm were placed by tweezers after staining on the slide. A few drops of PVLG (polyvinyl-lacto-glycerol) were added and covered with cover slip. Then, samples were observed with a 100x optical microscope, evaluating fungal structures (hyphae, arbuscules and vesicles) based on Giovanetti and Moose (1980).

Data obtained were compared by ANOVA, and Tukey test at 5%, using the SANEST software (27).

Results and discussion

Mycorrhizal colonization percentage

Mycorrhizal colonization (%) of Juçara palm roots was significantly affected by fungus and P doses. Colonization ranged from 18.33% for treatment with *C. etunicatum* at 50 mg dm^{-3} P, to 58.33% for treatment with *R. clarus* at 0 mg dm^{-3} P. Mycorrhizal colonization was not detected in control treatments, assuring any undesired contamination (table 2).

Table 2. Mycorrhizal colonization percentage of Juçara palm seedlings as a function of arbuscular mycorrhizal fungi (AMF) and phosphorus (P) content at 226 days after sowing.

Tabla 2. Porcentaje de colonización micorrízica de plántulas de palma Juçara en función de hongos micorrízicos arbusculares (AMF) y fertilización con fósforo 226 días después de la siembra.

P doses (mg dm^{-3})	Treatments				
	IN0	IN1	IN2	IN3	Mean
	%				
0	0.00bA	58.33aA	0.00bB	55.00aA	28.33
50	0.00cA	40.00aB	18.33bA	35.00aB	23.33
Average	0.00	49.17	9.17	45.00	25.83
CV (%)	12.91				

IN1 = *Rhizophagus clarus*; IN2 = *Claroideoglomus etunicatum*; IN3 = mixed inoculum (*Rhizophagus clarus* + *Claroideoglomus etunicatum*); IN0 = control, without fungus. Different lowercase letter in rows (AMF) and uppercase in columns (P doses) show statistical differences by Tukey test ($P < 0.05$).

IN1 = *Rhizophagus clarus*; IN2 = *Claroideoglomus etunicatum*; IN3 = inóculo mixto (*Rhizophagus clarus* + *Claroideoglomus etunicatum*); IN0 = control, sin hongos. Distimtas letras minúsculas en filas (AMF) y mayúsculas en columnas (P doses) muestran diferencias significativas según la prueba de Tukey ($P < 0,05$).

At 0 mg dm^{-3} P, no mycorrhizal colonization occurred with *C. etunicatum*, suggesting that this isolate causes no infection in Juçara palm roots under low P availability. In fact, *C. etunicatum* structures were only detected under 50 mg dm^{-3} P (table 2, page 112). On the contrary, treatments with *R. clarus* and mixed inoculum showed higher colonization rates without phosphate fertilization and lower colonization when P was added to the soil. Decreasing mycorrhizal colonization percentage with increased P is normal, and, in many cases related to plant nutritional status (3).

Previously, Sgrott *et al.* (2012) and Moreira *et al.* (2016) had reported difficulty in quantifying mycorrhizal colonization in Juçara palm seedlings due to the anatomical characteristics of *E. edulis* roots. Moreira *et al.* (2016) stated that studies aiming at identifying AMF in *E. edulis* roots should consider longer seedling cultivation times. Following this recommendation, this study postponed evaluations over six months after inoculation, achieving effective mycorrhizal colonization percentages between 18 and 58%, depending on fungal isolates and P doses applied. Root colonization percentages of palm species reported in the literature range from 13 to 53% in *Bactris gasipaes* (20, 23), 4 to 33% in *Desmoncus orthacanthos* (18), 27 to 86% in *Phoenix dactylifera* (8), and 4 to 4.2% in *Astrocaryum mexicanum* (16).

E. edulis plants inoculated with AMF accumulated higher N, P, K, Ca and Mg in shoots and roots (15).

Shoot biometry

Significant effects of fungal treatments and P doses applied were verified for the following biometric variables: shoot length (H), shoot diameter (SD), leaf area (LA) and dry shoot mass (table 3).

Table 3. Shoot length (H), shoot diameter, (SD), leaf area (LA) and dry shoot mass (DSM) of Juçara palm seedling as a function of arbuscular mycorrhizal fungi (AMF) and phosphorus content (P), 226 days after sowing.

Tabla 3. Altura del brote (H), diámetro del brote (SD), área foliar (LA) y biomasa seca del brote (DSM) de la plántula de palma Juçara en función de hongos micorrízicos arbusculares (HMA) y contenido de fósforo (P), 226 días después de la siembra.

Treatments	H (cm)		SD (mm)		LA (cm^2)		DSM (g)	
	0 P	50 P	0 P	50 P	0 P	50 P	0 P	50 P
IN0	21.33bB	26.71aA	7.31bB	9.16abA	75.71bB	131.60aA	0.98bB	1.70abA
IN1	27.28aA	27.70aA	8.87aB	9.85aA	136.04bA	147.30aA	1.60aB	1.79aA
IN2	22.48bB	25.86aA	6.91bB	8.68bA	80.98aB	123.90aA	1.02bB	1.52bA
IN3	26.83aA	27.08aA	8.83aB	9.28abA	119.57aA	137.60 aA	1.53aB	1.73abA
Average	25.66		8.61		119.08		1.48	
CV (%)	5.46		5.53		11.79		8.38	

IN1 = *Rhizophagus clarus*; IN2 = *Claroideoglomus etunicatum*; IN3 = mixed inoculum (*Rhizophagus clarus* + *Claroideoglomus etunicatum*); IN0 = control, without fungus. Different lowercase letter in rows (AMF) and uppercase in columns (P doses) show statistical difference by Tukey test (P < 0.05).

IN1 = *Rhizophagus clarus*; IN2 = *Claroideoglomus etunicatum*; IN3 = inóculo mixto (*Rhizophagus clarus* + *Claroideoglomus etunicatum*); IN0 = control, sin hongos. Distintas letras minúsculas en filas (AMF) y mayúsculas en columnas (P doses) muestran diferencias significativas según la prueba de Tukey (P < 0,05).

Plants inoculated with *R. clarus* (IN1) and *R. clarus + C. etunicatum* mixed inoculum (IN3) increased shoot length (H) even in the absence of P (table 3). At P dose of 50 mg dm^{-3}, different inoculated and non-inoculated plants showed no significant difference in this variable. However, these treatments exhibited average lengths close to 30 cm, the commercial recommendation for the species according to Aguiar *et al.* (2002) (table 3).

Average H was 25.66 cm, with variations between 21.33 cm (minimum for control treatment at 0 mg dm^{-3} P), and 27.70 cm (maximum for *R. clarus* treatment at 50 mg dm^{-3} P). In the absence of phosphate fertilization, fungal treatments containing *R. clarus* (IN1) and mixed inoculum did not differ from each other and showed the highest increases in H, 28% and 26%, respectively, when compared to control.

In the absence of P, fungal treatment with *C. etunicatum* (IN2) did not statistically differ from control, presenting the lowest average H, 21.33 cm and 22.48 cm, respectively. Under phosphate fertilization, fungal treatments did not differ from each other (table 3, page 113).

The H values obtained in this work are higher than those obtained by Sgrott *et al.* (2012) and Moreira *et al.* (2016) when evaluating the effect of AMF on initial growth of *E. edulis*. At 160 days after inoculation, Sgrott *et al.* (2012) recorded average H of 15.52 cm for treatments inoculated with an AMF mixture (*Acaulospora koskei, Scutellospora heterogama, Gigaspora albida, R. clarum*), and 15.70 cm for control, with no significant differences between treatments. Moreira *et al.* (2016) recorded average H ranging between 16.15 cm and 18.25 cm at 180 days after inoculation, with the shortest stem recorded for control treatment and the longest for fungal treatment with AMF soil spores collected using the on-farm method, and finding no differences between treatments as well.

The absence of mycorrhizal effect on early stages of seedling growth can be explained by the large Juçara palm seeds. Venturi and Paulilo (1998) evaluated depletion of seed reserves and the effect of mineral nutrition on *E. edulis* seedlings finding that during initial growth stages, within the first five months after sowing, seedlings still depended on seed reserves.

Considering that the time required to reach these indexes is six to eight months (22), these results demonstrate the positive effects of inoculation with mycorrhizal fungi on growth of Juçara palm seedlings. Treatments with *R. clarus* (IN1) and mixed inoculum reached average values close to those recommended for seedling commercialization, in less time than expected.

Regarding stem diameter (SD), no significant difference was found among fungal treatments (AMFs) regardless P content (table 3, page 113). However, when not inoculated, plants showed higher average SD under phosphate fertilization than in the absence of P. Seedling diameter is one of the most important quality standards (25). According to Duarte *et al.* (2015), seedlings with adequate SD show improved shoot growth balance. Larger diameters indicate better uptake and translocation of nutrients to the plant. According to Silva *et al.* (2015), although no precise standard for Juçara seedlings commercialization is defined, seedlings with a minimum SD of 5 mm are recommended, requiring six to eight months of nursery. Contrasting results show, on one side, no significant difference in SD of *E. edulis* seedlings 160 days after inoculation with AMF (Sgrott *et al*., 2012). On the other side, Moreira *et al.* (2016) did observe significant differences for this variable 180 days after inoculation with AMF, showing that, regardless of AMF used, the presence of mycorrhiza increased seedling diameter when compared to control treatment, without inoculation.

The LA of Juçara palm seedlings in the absence of P showed significant differences among treatments inoculated with *R. clarus* and mixed inoculum, obtaining the highest averages of 136.04 and 119.57 cm^2, with 80% and 58% increase, respectively (table 3, page 113). Control and treatment with *C. etunicatum* (IN2) did not differ at 0 mg dm^{-3} P, showing the smallest values, 75.71 and 80.98 cm^2, respectively (table 3, page 113).

In the presence of phosphate fertilization, treatments did not differ from each other. However, a significant increase in LA was observed for IN1 and IN3 treatments with *C. etunicatum*, (74% and 64%, respectively), at 0 mg dm^{-3} P (table 3, page 113). These results hold mechanistic importance, since LA expresses the size of the photosynthetic apparatus, influencing carbon assimilation, plant growth and development (16). In *Bactris gasipaes* seedlings, LA of inoculated plants was significantly higher than that of non-inoculated plants, resulting in more developed plants and, therefore, earlier planting capacity and better performance expectations under field conditions (24). In contrast, assessing AMF contribution to initial growth and P absorption of *Desmoncus orthacanthos* Martius palm, Ramos-Zapata *et al.* (2006) observed that LA significantly responded to P treatments, but not to mycorrhizal treatment.

Dry shoot biomass (DSM) of Juçara seedlings inoculated with *R. clarus* and mixed inoculum, without P, was significantly higher than in control treatment, with 64% and 57% increments, respectively (table 3, page 113). DSM of control plants and those inoculated with *C. etunicatum* (IN2) resulted in the lowest averages, 0.98 and 1.02 g, respectively, not differing significantly from each other at 0 mg dm^{-3} P (table 3, page 113).

Under phosphate fertilization, average DSM of plants with *R. clarus* (IN1) (1.79 g) significantly differed from those with *C. etunicatum* (IN2) holding the lowest average (1.52 g), and not differing from the other treatments under the same P dose. The addition of 50 mg dm^{-3} of P increased seedling DSM (table 3, page 113).

When producing *E. edulis* seedlings inoculated with mycorrhizal fungi, Sgrott *et al.* (2012) found no significant difference in DSM five months after inoculation under nursery conditions. However, after 24 months under field conditions, previously inoculated seedlings showed almost 50% increases in dry shoot biomass in relation to non-inoculated plants. Later, Moreira *et al.* (2016) evaluated initial growth of *E. edulis* seedlings inoculated with AMF and found that, regardless of the AMF species, inoculated seedlings showed higher dry shoot biomass than control treatment.

According to Rodrigues *et al.* (2018), greater plant growth after inoculation with mycorrhizal fungi reduces seedling production time and enhances initial growth after field planting. For heart of palm seedlings inoculated with AMF, Silva *et al.* (1998) recorded 140% increase in dry shoot biomass in relation to non-inoculated treatment.

Macronutrient content

Shoot nutrient content expresses the ability of AMF to increase soil nutrient absorption. In this work, N, P, K, Ca, Mg and S contents in dry shoot biomass of Juçara palm seedlings were influenced by P doses and fungal treatments (table 4).

Table 4. Macronutrient contents, (mg $plant^{-1}$), of Juçara palm seedling as a function of arbuscular mycorrhizal fungi (AMF) and phosphorus content (P), 226 days after sowing.

Tabla 4. Contenido de macronutrientes, (mg $planta^{-1}$), de plántula de palma Juçara en función de hongos micorrízicos arbusculares (HMA) y contenido de fósforo (P), 226 días después de la siembra.

Treatments	N		P		K	
	(mg $plant^{-1}$)					
	0 P	50 P	0 P	50 P	0 P	50 P
IN0	13.03bB	24.17aA	0.63bB	2.36bA	12.21bB	20.64aA
IN1	20.93aB	26.50aA	2.20aB	3.00aA	19.71aA	21.47aA
IN2	13.90bB	22.40aA	0.66bB	2.01aA	13.40bB	17.80aA
IN3	20.30aB	25.86aA	2.05aB	2.77aA	20.61aA	21.04aA
General average	20.89		1.96		18.36	
CV (%)	10.40		10.20		12.40	
Treatments	Ca		Mg		S	
	(mg $plant^{-1}$)					
	0 P	50 P	0 P	50 P	0 P	50 P
IN0	6.38bB	12.90aA	2.93bB	6.21bA	2.66bB	3.86aA
IN1	10.91aB	13.12aA	5.65aB	6.63aA	4.33aA	4.35aA
IN2	6.87bB	12.00aA	2.95bB	5.48bA	2.68bB	3.49aA
IN3	10.92aA	12.53aA	5.49aB	6.28aA	4.07aA	4.10aA
General average	10.70		5.20		3.69	
CV (%)	10.40		11.20		8.92	

IN1 = *Rhizophagus clarus*; IN2 = *Claroideoglomus etunicatum*; IN3 = mixed inoculum (*Rhizophagus clarus* + *Claroideoglomus etunicatum*); IN0 = control, without fungus. Different lowercase letter in rows (AMF) and uppercase in columns (P doses) show statistical differences by Tukey test ($P < 0.05$).

IN1 = *Rhizophagus clarus*; IN2 = *Claroideoglomus etunicatum*; IN3 = inóculo mixto (*Rhizophagus clarus* + *Claroideoglomus etunicatum*); IN0 = control, sin hongos. Distimtas letras minúsculas en filas (AMF) y mayúsculas en columnas (P doses) muestran diferencias significativas según la prueba de Tukey ($P < 0,05$).

In the absence of phosphate fertilization, macronutrient contents were statistically higher in Juçara seedlings inoculated with *R. clarus* (IN1) and mixed inoculum (IN3), than in control. Macronutrient content increments for treatments with *R. clarus* and mixed inoculum were: 61% and 56% for N; 249% and 225% for P; 61% and 69% for K; 71% and 71% for Ca; 93% and 87% for Mg; and 63% and 53% for S, respectively, when compared to control treatment (without inoculum at P dose of 0 mg dm^{-3}) (table 4).

N, K, Ca and S contents were not influenced by fungal treatments at 50 mg dm^{-3} *P. Meanwhile*, treatments with *R. clarus* (IN1) and mixed inoculum did not differ for P content but were statistically superior to control treatments without inoculation and with *C. etunicatum* (IN2) at 50 mg dm^{-3} P (table 4, page 115).

At 50 mg dm^{-3} P, macronutrient contents of all treatments increased. However, in treatments with *R. claruse* and mixed inoculum, P application did not affect K and S contents (table 4, page 115). This was also observed for Ca contents in treatments with mixed inoculum (table 4, page 115). These results demonstrated that using mycorrhizal fungi was effective even in the absence of phosphate fertilization, evidencing economy of inputs for seedling production.

Moreira *et al.* (2016) recorded greater N, P, K, Ca and Mg accumulation in DSM of Juçara seedlings six months after inoculation with AMF, demonstrating the significant effect of this association for plant nutrition. Sgrott *et al.* (2012) also evidenced several benefits of mycorrhizal inoculation of *E. edulis* seedlings but did not evaluate shoot nutrient contents. Before, Lima *et al.* (2014) had evaluated growth of *E. edulis* seedlings in response to different P doses 12 months after phosphate application and concluded that P did not change plant nutrient content, finding the following nutrient absorption sequence in shoots: N> Ca> K> Mg> P> S, but not quantifying these nutrients in plants.

Studies involving associations between mycorrhizal fungi and plants have proven to be important. AMF improve plant nutritional status, promoted by a more efficient soil exploration, easier access to soil micropores by fungal hyphae and consequent higher nutrient absorption capacity. In addition, hyphae produce and exude organic compounds that facilitate phosphate solubilization, increasing nutrients availability (17). In this sense, Ait-El-Mokhtar *et al.* (2019) reported the importance of AMF in stabilizing K, P and Ca levels, and also Ca / Na, K / Na ratios in *Phoenix dactylifera* L. plants submitted to salt stress. This greater nutrient accumulation in plant tissues provides higher seedling quality and, consequently, greater chance of survival. In this respect, the use of inoculants with AMF results in more vigorous plants obtained by a low-cost production strategy. Moreira *et al.* (2016) reported that these benefits are even more significant for plants like *E. edulis*, since the species, now threatened with extinction, has difficulties germinating and stabilize in the field.

Conclusions

AMF, a biological agent of mutualistic associations with plants, constitutes an innovative approach to sustainable agriculture, contributing to increase plant survival rate, a key-factor for the successful reintroduction and conservation of *E. edulis*, as well as for its commercial exploitation.

Fungal species *R. clarus* and mixed inoculum (*R. clarus* + *C. etunicatum*) have high infectious potential, promote greater growth, and shoot dry matter accumulation of *E. edulis* plants.

Fungal species *R. clarus* and mixed inoculum (*R. clarus* + *C. etunicatum*) show associative preference with roots of *E. edulis* seedlings.

Inoculation with *R. clarus* and mixed inoculum (*R. clarus* + *C. etunicatum*), in the absence of P, contributes to greater N, P, K, Ca, Mg and S accumulation in *E. edulis* plants.

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Competitive ability of canola (*Brassica napus* var. oleifera) hybrids with black oat (*Avena strigosa*) in a subtropical environment

Habilidad competitiva de híbridos de canola (*Brassica napus* var. oleifera) contra avena negra (*Avena strigosa*) en un ambiente subtropical

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Abstract

The objective of this study was to assess the competitive ability of canola (*Brassica napus* var. oleifera) hybrids in competition with black oat (*Avena strigosa*) in a subtropical environment. The experiments were conducted in a greenhouse where canola hybrids 'Hyola 61,' 'Hyola 76,' 'Hyola 433,' and 'Hyola 571' were tested individually for their competitive performance with black oat. The plant proportion between black oat and the canola hybrid was changed (100%:0%; 75%:25%; 50%:50%; 25%:75%; and 0%:100%) while keeping the total population of plants constant (20 plants plot^{-1}). Photosynthesis rate (μmol m^{-2} s^{-1}), internal CO_2 concentration (μmol mol^{-1}), and transpiration rate (mol H_2O m^{-2} s^{-1}) were assessed using an infrared gas analyzer 55 days after emergence. Leaf area (m^2 m^{-2}) and dry matter (g m^{-2}) were also assessed on the same day. The data set was analyzed by the replacement series method for competition studies. There was evidence of intense competition between canola and black oat, independent of plant proportion. The competitive ability of canola was distinct among hybrids; Hyola 571 performed better than the others in the competition against black oat. Choosing the most competitive hybrid, such as Hyola 571, helps maintain high canola grain yield levels in areas infested with black oat.

Keywords
Brassica napus var. oleifera • *Avena strigosa* • competitive interaction • replacement series method • aggressiveness

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Resumen

El objetivo de este estudio fue evaluar la capacidad competitiva de los híbridos de canola (*Brassica napus* var. oleifera) en competencia con la avena negra (*Avena strigosa*), en un ambiente subtropical. Los experimentos se llevaron a cabo en un invernadero donde los híbridos de canola Hyola 61, Hyola 76, Hyola 433 y Hyola 571 fueron probadas individualmente por su habilidad competitiva contra la avena negra. Se cambió la proporción de plantas (100%: 0%; 75%: 25%; 50%: 50%; 25%: 75% y 0%: 100%) manteniendo constante la población total de plantas (20 plantas parcela $^{-1}$). Se evaluaron el área foliar (AF: m^2 m^{-2}) y la materia seca (DM: g m^{-2}) además de la tasa de fotosíntesis (A: µmol m^{-2} s^{-1}), la concentración interna de CO_2 (Ci: µmol mol^{-1}) y la tasa de transpiración (E: mol H_2O m^{-2} s^{-1}) se evaluaron utilizando un analizador de gases en el infrarrojo (IRGA) 55 días después de la emergencia. Se registró una intensa competencia entre la canola y la avena negra. La capacidad competitiva de los híbridos de canola fue distinta; Hyola 571 se desempeñó mejor que los otros, siendo una opción para mantener altos niveles de rendimiento en condiciones competitivas con avena negra. Elegir híbridos más competitivos como el Hyola 571 ayuda a mantener altos niveles de rendimiento de canola en áreas infestadas con avena negra.

Palabras clave

Brassica napus var. oleifera, • *Avena strigosa* • interacción competitiva • método de serie sustitutiva • agresividad

Introduction

Canola is a source for the production of edible oil and biofuel. In Southern Brazil, grain production fields have shown increases in the cultivation of canola (*Brassica napus* var. oleifera (Mönch) Delile) in winter. The state of Rio Grande do Sul (RS) is the largest canola producer in the country, being responsible for about 85% of the total Brazilian production, with average grain yields of 1,422 kg ha^{-1} (8). This productivity is much lower than that obtained in countries that have grain yields of about 4,000 kg ha^{-1}, such as Belgium, Germany, Denmark, and Chile (13). This difference may be associated with cultivation technologies such as better pest control (diseases, insects, and weeds), harvesting technology, superior genotypes, and climatic conditions that favor plant performance.

One of the factors that canola producers are facing is the interference caused by weeds, since there are few herbicides registered for this crop in Brazil. Among the main weed species infesting canola are turnip (*Lolium multiflorum* Lam.) and black oat (*Avena strigosa* Schreb.), which are very competitive for water, light, CO_2, and nutrients. Furthermore, in many cases, these weeds are resistant to ALS, ACCase, or EPSPs, inhibiting herbicides (1, 10, 30) and making them harder to control (5).

Canola causes allelopathic suppression on germination and seedling development of both weeds and mulching species when cultivated under no-tillage; this agronomic trait allows for better competitive ability. Thus, it is an excellent alternative to manage weeds when cultivated in succession to summer crops, such as maize, soybean, and common bean (18, 32). Furthermore, there is strong evidence of differences in competitive ability among both varieties of the same crop and among distinct genotypes of wild species (3, 19).

Black oat is sown as mulching for the production of grains/seeds and for animal feed; however, in many cases, natural reseeding occurs, and that is when it becomes a weed in winter crops. The extract of *A. strigosa* may have an allelopathic effect on some crops and weeds, but the first studies were inconclusive (30). Black oats are known to perform better than barley (*Hordeum vulgare* L.) in competition (16), as evidenced by its ability to suppress other crops. The competition dynamics between black oat and canola is not yet well defined in subtropical production systems, such as those found in Southern Brazil and neighboring regions of Uruguay, Argentina, Paraguay, and Chile.

Weeds, if not properly controlled, reduce both grain yield and quality, in addition to hosting insects and diseases and hampering mechanized harvest (17). Experiments with replacement series are used to assess weed interference on crops by helping to understand how distinct plant species perform when grown in a community (26). The development of strategies to minimize the effects of weeds on crops is essential in the agriculture since there is an environmental need to reduce pesticide applications while maintaining high grain yields to guarantee the economic sustainability of farming.

Therefore, the objective of this study was to assess the competitive ability of canola hybrids in competition with black oat in a subtropical environment.

Material and methods

The experiments were conducted in a greenhouse in the agricultural year 2015/16. Experimental units consisted of 8 L plastic pots filled with Humic Oxisol (31), previously corrected and fertilized according to the technical recommendation for canola (36) and watered to maintain field capacity. A completely randomized design was used, as the greenhouse was considered sufficiently homogeneous, with four replications. The canola hybrids 'Hyola 61,' 'Hyola 76,' 'Hyola 433,' and 'Hyola 571' were tested individually for their competitive performance with black oat. There were no attacks, pests, or diseases on the plants throughout the study.

Preliminary experiments were carried out, both for canola hybrids and for black oat grown in monoculture to determine the minimum plant density in which the final dry mass becomes constant. For this, densities of 1, 2, 4, 8, 16, 24, 32, 40, 48, 56, and 64 plants pot^{-1} were used (equivalent to 24, 48, 96, 192, 384, 576, 768, 960, 1,152, 1,344, and 1,536 plants m^{-2}, respectively). The constant final production was obtained with 20 plants pot^{-1}, for all hybrids tested in competition with the weed, equivalent to 481 plants m^{-2} (data not shown). The constant final production obtained in the preliminary experiment was higher than what is usually found in field conditions; this may occur because the objective in the field is the production of grains and not competition or the accumulation of dry mass, as the case in the greenhouse.

In the second phase of experimentation, four experiments with replacement series were installed to assess the competitiveness of canola hybrids with black oat, in different combinations, with distinct plant proportions (20:0, 15:5, 10:10, 5:15, and 0:20 plants pot^{-1}, or 100%:0%;75%:25%; 50%:50%; 25%:75%, and 0%:100%), maintaining the total population of plants at 20 plants pot^{-1}.

Physiological variables were assessed using an infrared gas analyzer (IRGA), model LCA4 Pro SD (ADC Bioscience, UK), 55 days after emergence (DAE) to evaluate a completely developed leaf in the middle third of canola plants; for black oats, readings were performed on a fully developed leaf in the top third of the plant. The photosynthesis rate (A: µmol m^{-2} s^{-1}), internal CO_2 concentration (Ci: µmol mol^{-1}), and transpiration rate (E: mol H_2O m m^{-2} s^{-1}) were evaluated.

After physiological assessment, leaf area (AF: m^2 m^{-2}) was assessed with a portable leaf area meter (model CI-203 BioScience). Plants were also collected, packed in paper bags, and dried in an air circulating oven at 65^{o}C until a constant mass, for later weighing and determination of dry mass (DM: g m^{-2}).

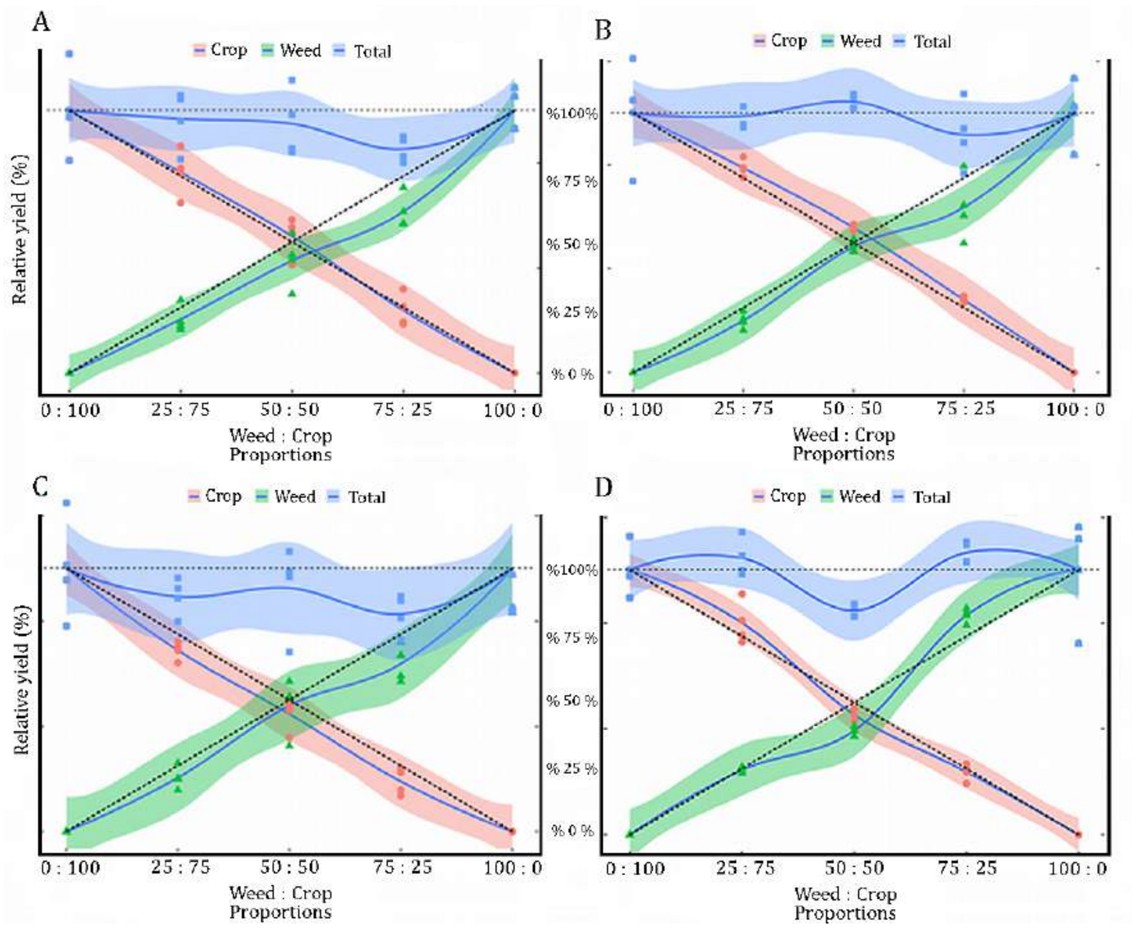
The expected values are indicated in the graphs as dotted straight lines, while the observed values are shown with solid regression lines. This analysis was based on the construction of a diagram based on the variations in relative (PR) and total (PRT) productivity, following this equation with the two tested species: $PR_{(A)} = P_A\, A_{MIX} / A_{MON}$, where P_A is the proportion of species A in the mixture; Amix is the productivity of species A in the mixture; and Amon is the productivity of species A in monoculture. When PR_A was a straight line (observed = expected), the competitive ability of both species was equal. A PR below that expected indicated harm to the growth of the species, and a PR above that expected indicated a benefit to the growth of the species (4).

For the PRT values, the equation was $PRT = PR_A + PR_B$. When PRT = 100%, there was competition for the same resources; values of PRT > 100% indicated that competition was avoided, and values of PRT < 100% indicated mutual damage for growth (26). Differences between treatments for estimated and observed values of PR and PRT were compared by their 95% confidence intervals of the means (colored bands around the regressions) and were considered distinct when the estimated values were not in the range of the 95% confidence intervals for the observed means (7).

The relative competitiveness index (CR), relative clustering coefficient (K), and aggressiveness (A) were obtained, as well as their standard errors. CR was the comparative growth of a species in relation to the other under competition. K indicated the relative dominance of one species over another, and A determined which species was more aggressive in the competition. Species 1 was more competitive than species 2 when $CR_1 > 1$, $K_1 > K_2$, and $A > 0$, and vice-versa (11). To calculate these indices, the 50%:50% species proportion was used, with the following equations: $CR_1 = PR_1 / PR_2$; $CR_2 = PR_2 / PR_1$; $K_1 = PR_1 /(100 - PR_1)$; $K_2 = PR_2 /(100 - PR_2)$; $A_1 = PR_1 - PR_2$; and $A_2 = PR_2 - PR_1$, as suggested by other studies. The significances of CR and A were measured by the one-way T-test and were considered significant when they differed ($p \leq 0.05$) from 1 and 0, respectively. Differences between K_1 and K_2 were compared by the two-way T-test with Welch criteria and considered significant when $p \leq 0.05$. All graphics and coefficients were obtained using the statistical software environment 'R' (27).

Results and discussion

The relative yield of the photosynthesis rate (A) did not differ consistently from the expected values in most situations (figure 1).



Dashed lines represent the expected values; solid lines represent the observed values, with their respective 95% confidence intervals.

Las líneas discontinuas representan los valores esperados; las líneas continuas representan los valores observados, con los respectivos intervalos de confianza a un 95%.

Figure 1. Relative yield (%) for photosynthesis rate (A) of canola (●) hybrids (A: Hyola 61; B: Hyola 76; C: Hyola 433; D: Hyola 531) and black oat (▲) and for the whole plant community (■) as a function of plant proportion.

Figura 1. Rendimiento relativo (%) para la tasa de fotosíntesis (A) de híbridos de canola (●) (A: Hyola 61; B: Hyola 76; C: Hyola 433; D: Hyola 531) y avena negra (▲) y para toda la comunidad de plantas (■) en proporción de plantas.

Only hybrids Hyola 61 and 76 presented lower photosynthetic rates when in smaller proportions in the mixture (less than 25% of the plants in the stand; figures 1A; 1B, page 122). The photosynthetic behavior of Hyola 433 (figure 1C, page 122) did not differ from the expected values (dotted lines), while Hyola 571 (figure 1D, page 122) showed increased competition levels with black oats when their plant stands were equal. In this situation, the competition for the same environmental resources intensified.

There was no difference in the photosynthesis rate when canola, under any competition level, was compared to the respective control treatment free of competition, and it ranged between 15.68 and 21.04 μmol m^{-2} s^{-1} (table 1). Similar results were also observed by Naderikharaji *et al.* (2008), when observing the effect of radiance and drought stress on the photosynthetic rate of four canola cultivars, respectively. The results showed that the values of net photosynthesis were similar even with the use of different varieties.

Table 1. Physiological responses of canola (*Brassica napus*) hybrids under competition with black oat (*Avena strigosa*), in terms of photosynthesis rate (A), internal CO_2 concentration (Ci), and transpiration rate (E) in experiments conducted by the substitutive series method.

Tabla 1. Respuestas fisiológicas de híbridos de canola (*Brassica napus*) en competencia con avena negra (*Avena strigosa*), en términos de tasa de fotosíntesis (A), concentración interna de CO_2 (Ci) y tasa de transpiración (E) en experimentos realizados por el método de serie sustitutiva.

Plant proportions (Crop: Weed)	Physiological parameters		
	A	Ci	E
		Hyola 61	
100:0 (T)	17.89	246.67	5.65
75:25	18.23	237.00	5.08
50:50	18.66	244.50	5.92
25:75	17.07	258.25	5.62
C.V (%)	19.80	8.80	18.50
		Hyola 76	
100:0 (T)	16.79	233.5	5.67
75:25	17.66	253.50	6.37
50:50	18.75	246.50	6.67
25:75	18.87	253.33	6.64
C.V (%)	10.70	5.80	12.90
		Hyola 433	
100:0 (T)	20.63	277.00	5.44
75:25	18.94	269.75	4.54
50:50	18.44	250.00*	5.42
25:75	15.68	255.50*	5.37
C.V (%)	19.40	4.6	21.40
		Hyola 571	
100:0 (T)	19.71	260.25	6.69
75:25	21.04	256.75	6.68
50:50	17.89	253.67	6.35
25:75	18.73	212.19*	5.58*
C.V (%)	11.10	5.1	11.00

* Means differ from the respective control treatment (T) according to Dunnett's test ($p \leq 0.05$). * Las medias difieren del tratamiento control respectivo (T) según la prueba de Dunnett ($p \leq 0,05$).

The relative yield based on the CO_2 concentration in the leaf mesophyll (Ci) presented narrower confidence intervals (figure 2, page 124) compared to the photosynthesis rate (figure 1, page 122). There were no consistent changes in relative Ci yield among cultivars. Hyola 61 had a lower performance than the competitor when it represented 75% of the plant stand (figure 2A, page 124), and Hyola 76 performed better than the competitor in the same situation (figure 2B, page 124). Hyola 433 was harmed when the plant stand equaled the competitor (figure 2C, page 124), while Hyola 571 was harmed when it represented only 25% of the stand (figure 2D, page 124).

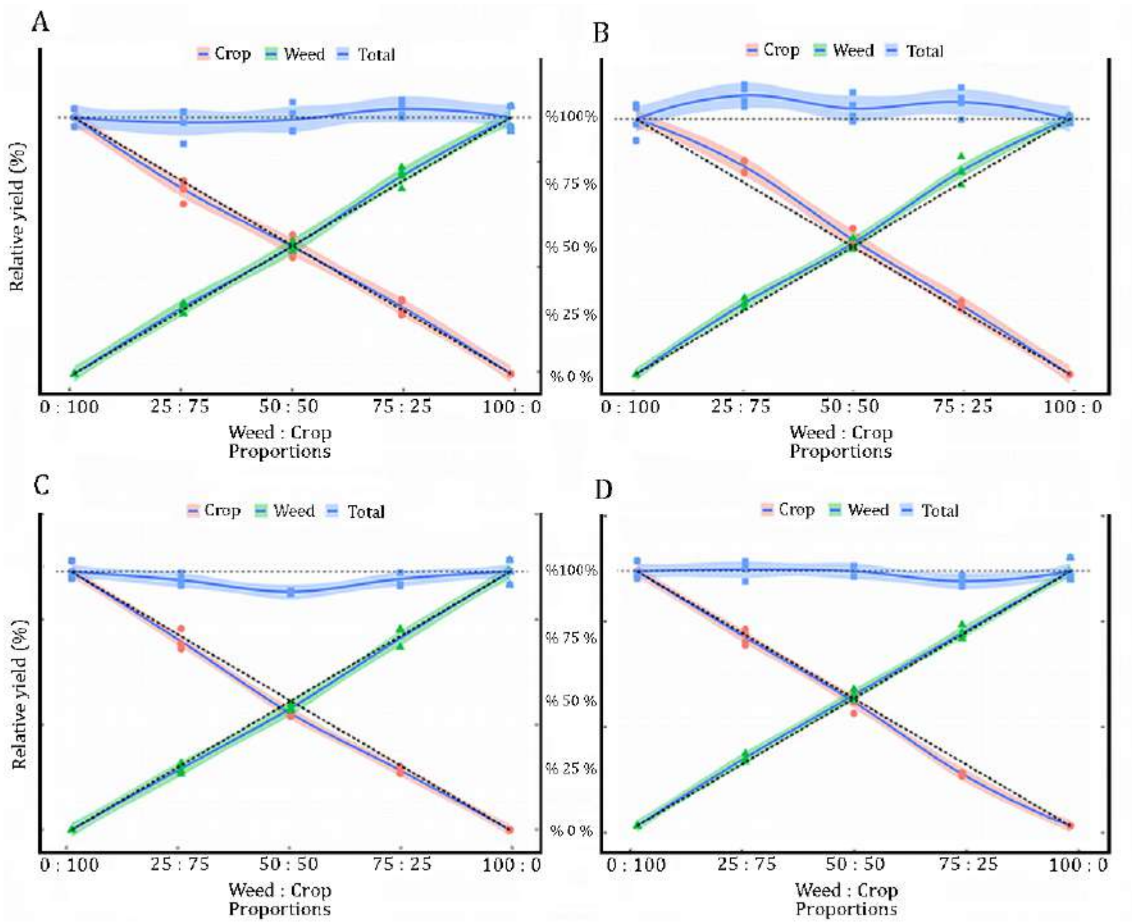


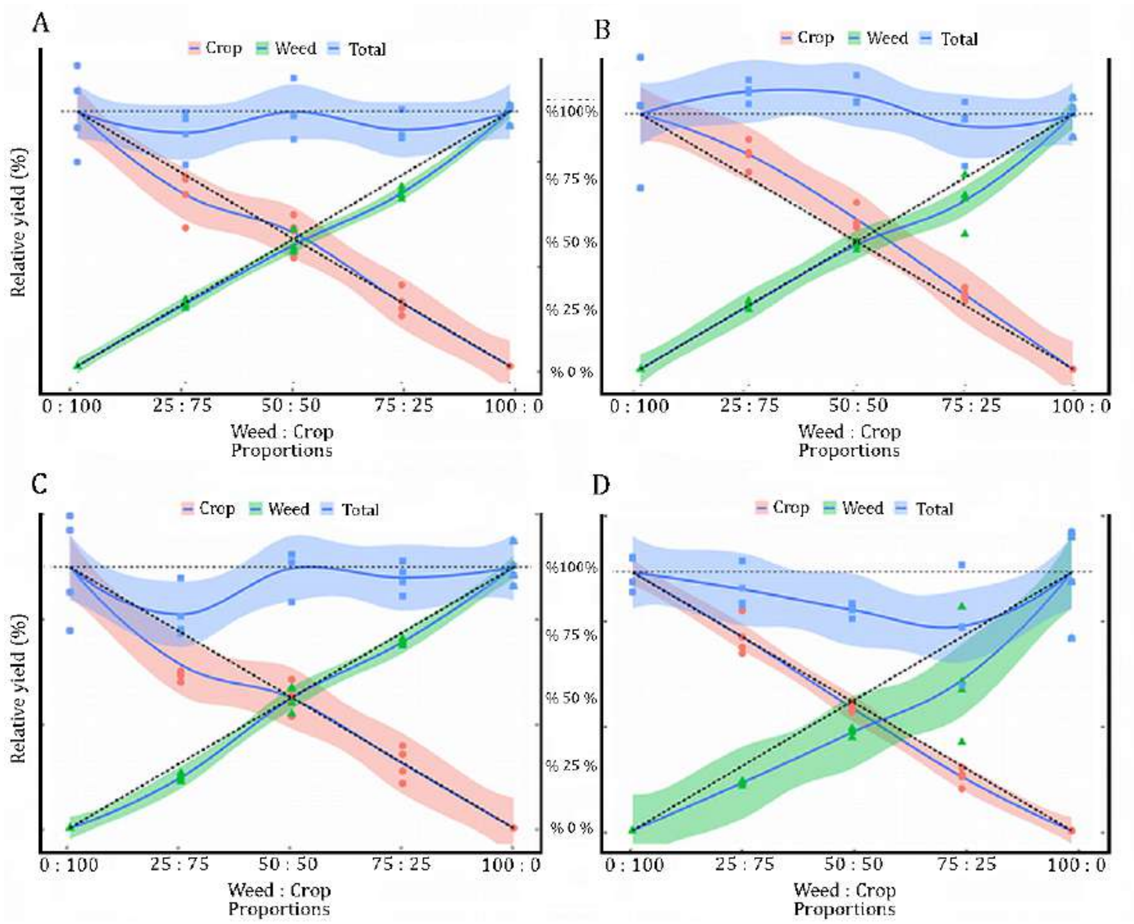
Figure 2. Relative yield (%) for the internal CO_2 concentration (Ci) of canola (●) hybrids (A: Hyola 61; B: Hyola 76; C: Hyola 433; D: Hyola 531), black oat (▲) and for the whole plant community (■) as a function of plant proportion.

Figura 2. Rendimiento relativo (%) para la concentración interna de CO_2 (Ci) de híbridos de canola (●) (A: Hyola 61; B: Hyola 76; C: Hyola 433; D: Hyola 531); avena negra (▲) y para toda la comunidad de plantas (■) en función de la proporción de plantas.

The internal CO_2 concentration of the leaf (Ci) depends greatly on A (35); the higher the A, the lower the Ci is expected to be, as the CO_2 is consumed at higher rates by photosynthesis. The photosynthesis rate was barely changed by competition levels (figure 1, page 122); thus, it was expected that Ci would not be changed (figure 2). The narrow confidence interval may indicate that Ci is a reliable marker for inferences about relative physiological yield (28). The CO_2 concentration in canola leaves ranged between 212.19 and 277.00 μmol mol^{-1} (table 1, page 123).

Values of 390 ppm (390 μmol mol^{-1}) can be considered as usual CO_2 concentrations in free air in the Southern hemisphere (30); thus, between 113 and 178 ppm CO_2 were consistently consumed by the photosynthetic metabolism of canola (table 1, page 123). In this study, competition did not affect the physiological metabolism of the plant. One possible explanation for this lack of change in the studied proportions can be attributed to the rapid carboxylation and subsequent transformation of CO_2 into glucose, which can be evidenced by the increase in photosynthetic rate. Similar behavior was observed in competitive studies of soybeans with barnyard grass (6).

Competition also did not affect the relative plant yields based on transpiration rates (E); as for most situations, no effects of competition were reported (figure 3, page 125). There was, however, a consistent decrease in the physiology of the competitor species (black oat) when it represented 75% of the plant stand (figures 3A; 3B; 3D, page 125).



Dashed lines represent the expected values; solid lines represent the observed values, with the respective 95% confidence intervals.

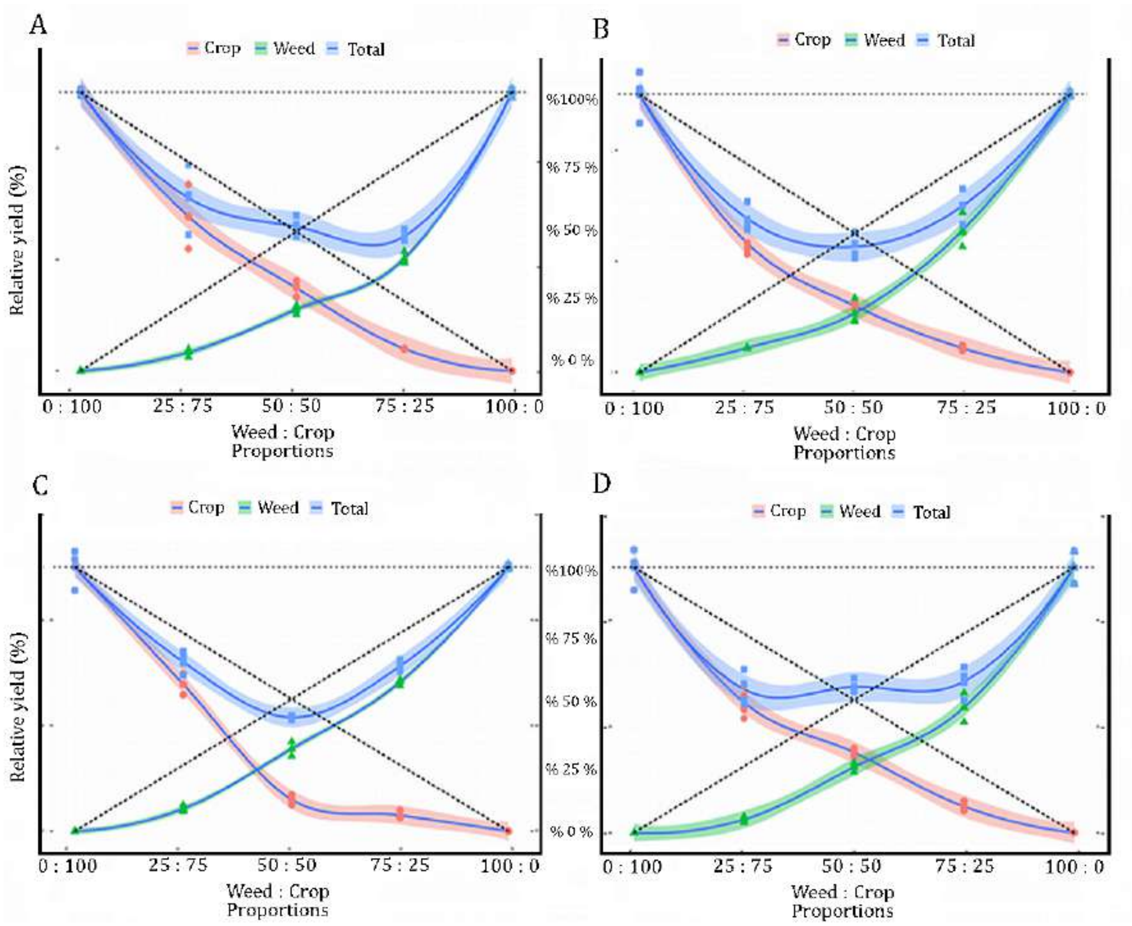
Las líneas discontinuas representan los valores esperados; las líneas continuas representan los valores observados, con los respectivos intervalos de confianza a un 95%.

Figure 3. Relative yield (%) for transpiration rate (E) of canola (●) hybrids (A: Hyola 61; B: Hyola 76; C: Hyola 433; D : Hyola 571); black oat (▲) and for the whole plant community (■) as a function of plant proportion.

Figura 3. Rendimiento relativo (%) de la tasa de transpiración (E) de híbridos de canola (●) (A: Hyola 61; B: Hyola 76; C: Hyola 433; D: Hyola 571); avena negra (▲) y para toda la comunidad de plantas (■) en función de la proporción de plantas.

From the point of view of the competitor, this is an indication that a few plants of canola may trigger competition sensing in black oats; in other words, black oat is capable of noticing the competition (16), but the data in this work suggest that it is not capable of overcoming canola easily in competition. Canola, however, may be harmed in the competition process if some environmental resource or a mismanaged cultural practice promotes advantages to the competitor (25). Absolute transpiration rates averaged 4.54-6.69 mol H_2O m^{-2} s^{-1} (table 1, page 123).

In contrast to the physiological parameters (figure 1, page 122, figure 2, page 124 and figure 3), the relative yield of canola based on leaf area (figure 4, page 126) was affected by competition for all genotypes. The competitor (black oat) was most seriously affected by the crop (canola) when the latter represented between 50 and 75% of the plant stand. Canola was more sensitive to competition when it represented 25-50% of plant stand (figure 4, page 126). Both species were more harmed by compatible combinations. Decreases in leaf area yield ranged between 22 and 40% for the competitor in the more harmful plant combinations. Similarly, the most damaging competition levels for canola were at complementary (opposite) densities (figure 4, page 126).



Dashed lines represent the expected values; solid lines represent the observed values, with the respective 95% confidence intervals.
Las líneas discontinuas representan los valores esperados; las líneas continuas representan los valores observados, con los respectivos intervalos de confianza a un 95%.

Figure 4. Relative yield (%) for leaf area (AF) of canola (●) hybrids (A: Hyola 61; B: Hyola 76; C: Hyola 433; D: Hyola 531); black oat (▲) and for the whole plant community (■) as a function of plant proportion.

Figura 4. Rendimiento relativo (%) para el área foliar (AF) de híbridos de canola (●) (A: Hyola 61; B: Hyola 76; C: Hyola 433; D: Hyola 531); avena negra (▲) y para toda la comunidad de plantas (■) en función de la proporción de plantas.

The community behavior for leaf area showed strong competition for the same resources since both competitors were seriously harmed in their interaction (figure 4).

The community lost between 44 and 57% of the total leaf area canopy when plants were grown together, compared to the monoculture stands. Competition for essential resources is also an important aspect of integrated weed management. The canola crop represents a tool capable of reducing the presence of some spontaneous species and, through crop rotation, delays the emergence of resistant weeds due to allelopathic effects (22). Winter cereals, when competing for resources, invest their photoassimilates in the formation of stalks (height) as a strategy to capture more luminosity; consequently, there is a decrease in the development of leaf area and dry mass (29).

Leaf area (table 2, page 127) was presented in terms of m^2 of leaf area per m^2 of soil to correspond to leaf area index (LAI), a growth analysis parameter (24). The leaf area index differed for all treatments and genotypes compared to their respective control treatments. Hyola 61, 76, and 433 averaged LAI's of 6.16, while Hyola 571 averaged an LAI of 2.31. Overall, leaf area decreases reached about 65% when all canola varieties were faced with the most intense competition levels with black oats (table 2, page 127).

Table 2. Biometrical responses of canola (*Brassica napus*) hybrids under competition with black oat (*Avena strigosa*), in terms of leaf area (AF) and dry matter (DM) in experiments conducted by the substitutive series method.

Tabla 2. Respuestas biométricas de los híbridos de canola (*Brassica napus*) en competencia con avena negra (*Avena strigosa*), en términos de área foliar (AF) y materia seca (DM) en experimentos realizados por el método de serie sustitutiva.

Plant proportions (Crop:Weed)	Biometrical responses	
	Leaf area (cm^2 pot^{-1})	Dry mass (g pot^{-1})
	Hyola 61	
100:0 (T)	2870.29	19.64
75:25	2120.41*	13.22*
50:50	1708.37*	8.12*
25:75	913.63*	4.84*
C.V (%)	11.3	13.10
	Hyola 76	
100:0 (T)	2876.63	19.3
75:25	1785.33 *	12.41*
50:50	1377.43 *	9.43*
25:75	1008.99 *	6.74*
C.V (%)	9.3	21.58
	Hyola 433	
100:0 (T)	2601.72	18.46
75:25	1929.73*	12.44*
50:50	620.75*	7.41*
25:75	628.23*	3.75*
C.V (%)	9.9	11.40
	Hyola 571	
100:0 (T)	1046.36	18.06
75:25	685.42*	13.93*
50:50	633.54*	7.56*
25:75	412.28*	4.20*
C.V (%)	9.9	8.30

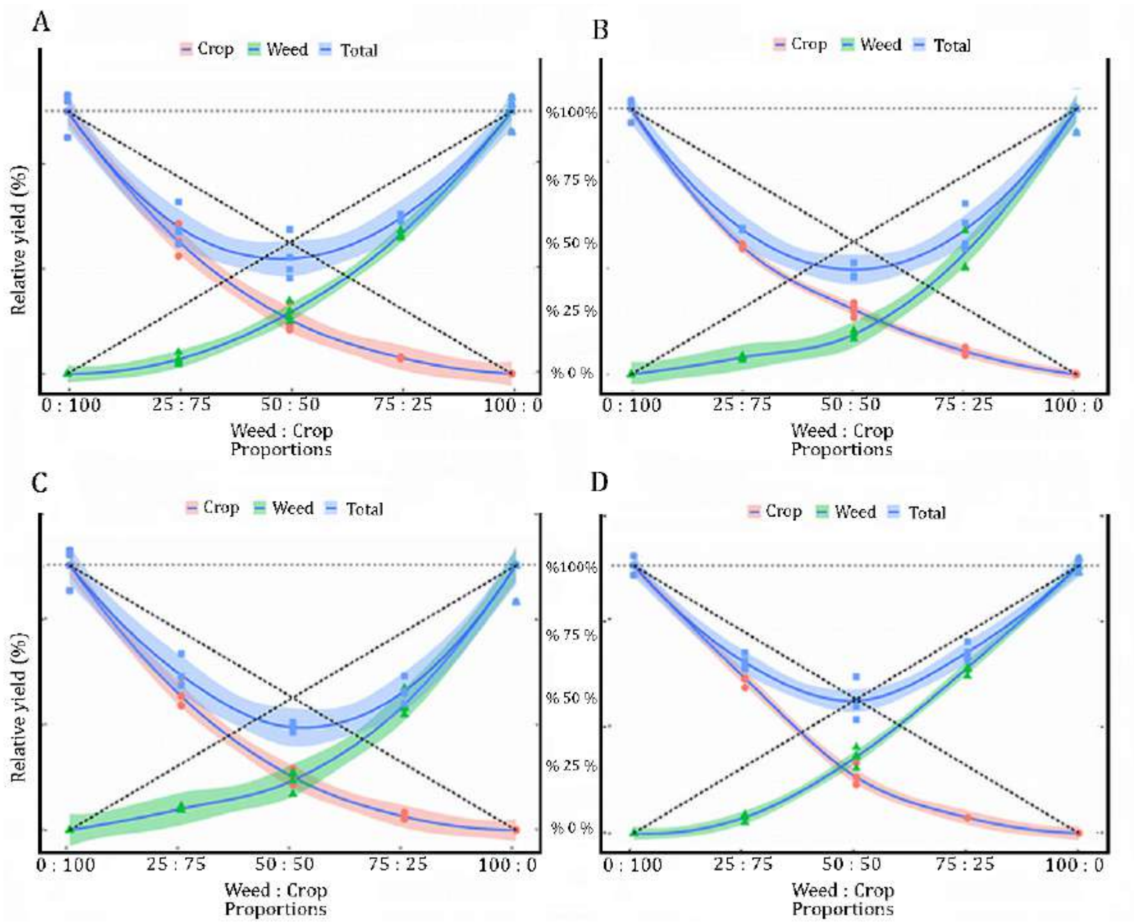
* Means differ from the respective control treatment (T) according to Dunnett's test ($p \leq 0.05$). * Las medias difieren del tratamiento control respectivo (T) según la prueba de Dunnett ($p \leq 0,05$).

The mass accumulation of canola was consistent with the final grain production (20); thus, any weed species interference will result in loss of yield. With the development of more competitive hybrids, it has been possible to use more aggressive species, obtaining a higher final grain yield in the presence of weeds compared to less competitive hybrids (33). In field conditions, the use of more competitive hybrids can decrease up to 50% of the accumulation of biomass in weeds (21). In competitive studies with ryegrass (*Lolium multiflorum*), which is also a Poaceae, the behavior between canola varieties determined the survival strategy generated by the competition (34); for example, the hybrid Hyola 571 invests in taller plants, allowing greater capture of solar radiation (22). In this study, the difference in architecture between species showed greater differentials, where the canola was more efficient in capturing light when compared to some Poaceae species (33, 36).

For dry mass (figure 5, page 128), the relative yield presented similar behavior as that reported for leaf area, as these two variables were co-dependent. In general terms, canola was harmed with any degree of competition with black oats, with more intense impacts being reported when it represented 25-50% of the plant stand (figure 5 A-D, page 128).

For all genotypes, 50% of the environment (overall) relative yield was decreased by competition, proving that the interaction of canola and black oats is harmful for both species. Plant dry mass accumulation (table 2) also followed that reported for leaf area, where all competition levels for all canola genotypes differed from their respective control treatment according to Dunnett's test.

Studies on the capacity of dry matter accumulation are important tools to determine the impact of competition between crops and weeds. Photosynthesis is directly related to this production of dry mass, since the productive capacity depends on the photosynthetic efficiency of the crop, combined with the speed with which resources are captured (14).



Dashed lines represent the expected values; solid lines represent the observed values, with the respective 95% confidence intervals.

Las líneas discontinuas representan los valores esperados; las líneas continuas representan los valores observados, con los respectivos intervalos de confianza a un 95%.

Figure 5. Relative yield (%) for dry mass (DM) of canola (●) hybrids (A: Hyola 61; B: Hyola 76; C: Hyola 433; D: Hyola 531); black oat (▲) and for the whole plant community (■) as a function of plant proportion.

Figura 5. Rendimiento relativo (%) para la masa seca (MS) de híbridos de canola (●) (A: Hyola 61; B: Hyola 76; C: Hyola 433; D: Hyola 531); avena negra (▲) y para el toda la comunidad de plantas (■) en función de la proporción de plantas.

While the stress levels imposed by competition were not enough to decrease photosynthesis rates (figure 1, page 122), they did decrease leaf expansion (figure 4, page 126). Physiologically, lower stress levels tended to decrease photosynthesis while maintaining the leaf expansion rate. In contrast, intense stress also decreased leaf area, as plants strive to surpass competition, in some cases by elongation of the culm or stem to the detriment of leaf expansion (9, 14, 21). Thus, more competitive canola genotypes were supposed to maintain photosynthesis rates even under competition, compared to less competitive ones (23). Furthermore, the relationship between net assimilation rate and leaf area expansion is not always proportional.

The canola genotypes also suffered a relative decrease compared to the black oat average in the studied proportions, suggesting that the crop had greater competition ability than the weed. Maximum dry mass reductions due to competition reached 60-75% for all varieties.

The relative competition index (CR) of canola (table 3, page 129) indicated that the crop was more competitive than black oats when it differed from ‘1’ by the one-way t-test (15). This was reported for Hyola 76 and 571 for all variables except Ci, and for the leaf area of Hyola 433 (table 3, page 129). This endorses Hyola 76 and 571 as more competitive with black oat than Hyola 61 and 433. The joint study of the values of CR, K (dominance), and C (competitiveness) represents the reliability between the studied competitiveness (6).

Table 3. Competitive parameters of canola (*Brassica napus*) hybrids under competition with black oat (*Avena strigosa*), in terms of relative competitiveness (CR), clustering coefficient (K), and aggressiveness (A) in experiments conducted by the substitutive series method.

Tabla 3. Parámetros competitivos de híbridos de canola (*Brassica napus*) en competencia con avena negra (*Avena strigosa*), en términos de competitividad relativa (CR), coeficiente de agrupamiento (K) y agresividad (A) en experimentos realizados por el método de serie sustitutiva.

Variables	CR	Kx (canola)	Ky (black oat)	A
	Photosynthesis rate (A)			
Hyola 61 x black oat	1.286 ± 0.137	1.250 ± 0.317	0.784 ± 0.145	0.093 ± 0.058
Hyola 76 x black oat	1.153 ± 0.024*	1.266 ± 0.024*	0.945 ± 0.044	0.073 ± 0.010*
Hyola 433 x black oat	0.949 ± 0.050	0.823 ± 0.093	0.973 ± 0.177	-0.032 ± 0.023
Hyola 571 x black oat	1.156 ± 0.032*	0.832 ± 0.032*	0.648 ± 0.021	0.061 ± 0.012*
Internal CO_2 concentration				
Hyola 61 x black oat	0.996 ± 0.032	0.993 ± 0.085	0.990 ± 0.030	-0.002 ± 0.016
Hyola 76 x black oat	1.029 ± 0.024	1.126 ± 0.075	1.057 ± 0.048	0.015 ± 0.012
Hyola 433 x black oat	0.958 ± 0.021	0.823 ± 0.015*	0.892 ± 0.021	-0.020 ± 0.010
Hyola 571 x black oat	0.952 ± 0.043	0.956 ± 0.059	1.055 ± 0.038	-0.026 ± 0.023
Transpiration rate (E)				
Hyola 61 x black oat	1.102 ± 0.068	1.132 ± 0.150	0.916 ± 0.081	0.048 ± 0.031
Hyola 76 x black oat	1.206 ± 0.042*	1.448 ± 0.148*	0.951 ± 0.026	0.100 ± 0.021*
Hyola 433 x black oat	1.004 ± 0.063	1.013 ± 0.120	1.004 ± 0.093	0.000 ± 0.031
Hyola 571 x black oat	1.252 ± 0.026*	0.907 ± 0.031*	0.613 ± 0.019	0.095 ± 0.009*
Leaf area (AF)				
Hyola 61 x black oat	1.351 ± 0.058*	0.425 ± 0.024*	0.283 ± 0.011	0.077 ± 0.011*
Hyola 76 x black oat	1.147 ± 0.086	0.315 ± 0.008	0.273 ± 0.032	0.026 ± 0.018
Hyola 433 x black oat	0.387 ± 0.039*	0.136 ± 0.01*	0.454 ± 0.024	-0.192 ± 0.019*
Hyola 571 x black oat	1.228 ± 0.035*	0.435 ± 0.013*	0.328 ± 0.013	0.056 ± 0.007*
Shoot dry mass (DM)				
Hyola 61 x black oat	0.883 ± 0.034*	0.264 ±0.039*	0.304 ± 0.029	-0.026 ± 0.007*
Hyola 76 x black oat	1.632 ± 0.088*	0.324 ± 0.020*	0.177 ± 0.009	0.094 ± 0.012
Hyola 433 x black oat	1.132 ± 0.206	0.252 ± 0.020	0.232 ± 0.027	0.013 ± 0.031
Hyola 571 x black oat	0.732 ± 0.038*	0.267 ± 0.032*	0.401 ± 0.031	-0.076 ± 0.011*

CR: * = differs from "1" by the one-way t-test, at 5% probability; K_x K_y : * = K_x differs from K_y by the two-way t-test, at 5% probability; A : * = differs from "0" by the one-way t-test, at 5% probability.
CR: * = difiere de "1" en la prueba t unidireccional, con una probabilidad del 5%; Kx Ky: * = Kx difiere de Ky en la prueba t de dos vías, con un 5% probabilidad; A: * = difiere de "0" en la prueba t unidireccional, con un 5% de probabilidad.

The CR index relates the comparison between the growth of species, relating $CR_{(canola)}$ to $CR_{(black\ oat)}$ Therefore, canola was considered more competitive than black oats when CR > 1 (11) and vice versa. In a study with canola and turnip, Galon *et al.* (2015) showed that CR = 1.59 (leaf area), demonstrating a greater ability of the hybrid Hyola 433 in relation to the others tested.

The clustering coefficient (K) reports, in a loose definition, the ability of different plants of a given species to aggregate efforts aiming to surpass the competition imposed by plants of a different genetic background, including genotype, ecotype, biotype, or species, as discussed by Concenço (2016). K is superior for the crop (K_{crop}) when it differs from $K_{competitor}$ (18), as found for Hyola 61 in AF and MS, Hyola 76 and 571 in A, E, AF, and MS, and Hyola 433 in Ci and AF (table 3). This corroborates the CR values, where overall Hyola 76 and 571 performed better. Similar results were also observed by Durigon *et al.* (2019) when working with the canola hybrid Hyola 571 and turnip (*Raphanus sativus*). The greater index of dominance of a species indicates greater efficiency in the apprehension of resources of the environment, thus, greater growth and development in conditions of competition (2).

The aggressiveness (A) determines how intense the attack of the crop is against the competitor, or how fast it responds to competition Durigon *et al.* (2019). Not considering Ci, where there was no difference for any genotype, Hyola 571 was more aggressive than black oat for all variables. Hyola 61 surpassed the competitor for AF and DM, and Hyola 76 performed better for A, E and DM. Hyola 433 performed better than the competitor only for AF (table 3). The hybrid Hyola 571 was highlighted as the most aggressive canola genotype among those evaluated.

Conclusions

The results found evidence of intense competition between canola and black oat, independent of plant proportion, and the competitive ability of canola was distinct among hybrids. The hybrid Hyola 571 performed better than the others, followed by Hyola 76. Hyola 433 was the most susceptible competition with black oat. As the competition between canola and black oat is intense, weed control should be effective and respect the critical period of weed interference in canola. The choice for most competitive hybrids, such as Hyola 571, can help in the task of keeping high canola grain yield levels in areas infested with black oat.

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Assessment of fodder corn grown under surface and subsurface drip irrigation in Mendoza, Argentina

Evaluación del maíz forrajero regado por goteo superficial y subterráneo en Mendoza, Argentina

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Abstract

Surface (DI) and subsurface (SDI) drip irrigation constitute one advantageous system that increases both irrigation and water use efficiency. The objective of this research was to assess and compare the response of fodder corn to DI and two different depths of SDI, in Mendoza. We used a factorial experimental design with randomized blocks and repeated measures. Tests were run in two consecutive cycles. Germination percentage (GP), yield, water use efficiency (WUE) and water productivity (WP), were assessed. High yields ranging from 70,214 to 105,771 kg ha^{-1} of green matter and 10,020 to 22,476 kg ha^{-1} of dry matter (DM) were obtained in both cycles, respectively. DM after the first sowing was significantly higher in both cycles under SDI than under DI. No significant differences in WP, WUE, GP or soil moisture (SM) could be found among treatments, but significant differences were found in SM (p=<0.0001) between the first soil layer and the other two layers.

Keywords

Zea mays L. • subsurface drip irrigation • localized irrigation • yield • water use efficiency • water productivity

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Resumen

El riego por goteo -superficial (RG) y subterráneo (RGS)- es uno de los medios para aumentar la eficiencia de riego y del uso de agua. El objetivo de la investigación fue evaluar en Mendoza, la respuesta de maíz forrajero a tratamientos de riego por goteo a dos profundidades respecto del goteo superficial. El diseño experimental fue factorial con parcelas completamente al azar y medidas repetidas en el tiempo. Los ensayos se realizaron en dos ciclos consecutivos. Se evaluó el porcentaje de germinación (PG), el rendimiento, la eficiencia de uso del agua (WUE) y la productividad (WP). Se lograron altos rendimientos: 70,214 a 105,771 kg ha^{-1} de materia verde y 10,020 a 22,476 kg ha^{-1} de materia seca (MS), respectivamente en ambos ciclos. La producción de MS de la primera siembra resultó significativamente mayor para los dos tratamientos de riego por goteo subterráneo respecto del riego por goteo superficial. No se encontraron diferencias significativas en la WP ni en la WUE. El PG y la humedad del suelo (HS), no presentaron diferencias entre tratamientos y sí hubo diferencias significativas (p=<0,0001) de la HS entre el primer estrato de suelo y los otros dos restantes.

Palabras clave

Zea mays L. • riego por goteo enterrado • riego localizado • rendimiento • eficiencia del uso de agua • productividad del agua

Introduction

Corn cultivation is of great economic importance worldwide. It is valued for human food, fodder, and raw material for different industries. By 2026, production of fodder corn is expected to achieve 695 million tons, representing an increase of 86 million tons (38). Corn production estimates for Argentina, for the 2020-2021 period, predict 50 million tons from a cultivated area of 7 million hectares (7). Production of fodder corn in Mendoza has increased after the expansion of local cattle farming as consequence of the increase of agriculture in Argentina, which has displaced cattle raising to extra-Pampean regions (21). According to the Los Andes newspaper (2017), this production grew by 40% in a cultivated area of about 3,500 ha in Valle de Uco and San Rafael, located in the central and southern oases of Mendoza.

Currently, increasing surface and groundwater use efficiency (WUE), meeting water demand (for agricultural, industrial, and urban uses) while avoiding conflicts among users (15), has become crucial. Additionally, climate change scenarios predict increasing extreme events in a large part of the planet that may lead to reduced water availability (35). Agriculture is a major water consumer; current irrigation efficiency should be improved for water conservation and availability for other purposes (51). In the province of Mendoza, water availability is a limiting factor for agricultural expansion due to large water demands from human consumption (drinkable water), industry, and hydropower generation (31).

According to the FAO (2015), 40,000 hectares of Mendoza's 268,300 irrigated area are under DI (44), and 3,750 hectares are irrigated with center pivot systems (32). In Mendoza, SI mean application efficiency (AE) is 64% without drainage, and 39% with drainage. The AE for DI and center pivot irrigation is 90%, and 80%, respectively (44). In this context, localized irrigation is an essential technology package for competitive production, both in terms of quantity and quality (18). Localized irrigation systems contribute to a substantial increase in efficiency since they allow watering according to crop needs, reducing deep percolation and runoff at the furrow end (45). However, evaporation losses can still be detected.

Subsurface drip irrigation (SDI) reduces evaporation and is highly effective (23), but its maximum efficiency will depend on water meeting crop requirements (13). In Mendoza, SDI could be implemented for corn production, a rotation alternative to extensive and intensive crops. Nevertheless, in arid areas like Mendoza (poorly structured saline and alkaline soils with reduced organic matter), where integrated irrigation is required, SDI could affect seedling emergence and increase soil salinization. In this sense, such areas should be assessed for sustainability.

Background information

Previous research on corn using laterals spaced at 1.5, 2.3 and 3.0 m and buried at depths of 0.40-0.45 m, showed that the highest yields were obtained at 1.5 m (23). These authors point out that this system is expensive but explain that using a wider lateral spacing may lower costs. Severina *et al.* (2016) state that laterals represent 70% of a project's initial cost. For corn grown in clay soils, a 1.4 m spacing between laterals produced higher yields than 0.70 or 2.10 m spacing (10). Regarding depth, Lamm and Trooien (2005) tested 0.20, 0.30, 0.41, 0.51 and 0.61 m without finding significant differences in yield. However, they noticed that, when compared to a four-year production, yields were significantly lower with laterals buried at 0.61 m. In brief, depth defines soil moisture distribution and affects optimum seed germination. In arid areas with integrated irrigation, too deep laterals hinder seed germination. Even though corn can reach effective rooting depths of 2.8 m or more during maturity, in regions with stratified soils and low organic matter content, like Mendoza, rooting depths are in-between 1.5 and 2 m (46).

On the other hand, few references on SDI refer to arid areas with integrated irrigation, where seed germination may be jeopardized by the lack of proper moisture. Lamm and Trooien (2005) and Camp (1998) state that if irrigation is required for seed germination or plant growth, SDI may not be the best method. Additionally, Neufeld *et al.* (2004) consider that seed germination with SDI systems is highly site-specific, depending on soil texture, spacing, lateral depth and emitter flow.

Objectives

• Assess green and dry matter yields, water use efficiencies (WUE) and water productivity (WP) of fodder corn irrigated with DI and SDI.

• Compare field emergence percentages of fodder corn with DI and SDI.

Materials and Methods

A factorial experimental design was applied to a completely randomized plot with replicated measurements. Three treatments were applied: surface drip irrigation (T1), subsurface drip irrigation at 0.20 m (T2), and subsurface drip irrigation at 0.40m (T3), with four replications and two crop cycles. Yields were measured after 30, 45, 60, 75 and 95 days in the first cycle and after 30, 45, 60 and 105 days in the second cycle.

The plots were 3 m wide and 50 m long. Three irrigation laterals were spaced at 1 m, with 6 rows of plants at 0.25 m on both sides of each lateral. The two central rows of plants constituted the minimum plot size and the four remaining rows -2 on each side- the edging. Fodder corn (Aca vg 48rr2 variety) was planted at 0.30 m between plants and 0.50 m between rows. Two seeds were placed at each sowing point achieving a sowing density of 133,333 plants per hectare. DI and SDI with 2.1 L h^{-1} emitters placed 0.50 m apart, administered 4.2 mm.h^{-1}. Weed control was carried out by one glyphosate application. Fertigation was performed through 3 applications of 50 kg of nitrogen and 45 kg of phosphorous per hectare 15, 30 and 45 days after sowing.

Irrigation distribution uniformity (DU) (11) was measured in the four DI replications with the middle lateral of each plot in four positions: head, 1/3, 2/3 and end. Also, salinity expressed as apparent electrical conductivity (ECa), possible electrical conductivity, probable salts, pH, chlorides, carbonates, bicarbonates, sulfates, calcium and magnesium (Ca^{+2}+Mg^{+2}), sodium (Na^{+}) and Sodium Adsorption Ratio (SAR) were determined for the irrigation water. All analyses were run using official and standard methods (3). Reference crop evapotranspiration (ETo) was calculated according to Penman-Monteith (1) and the CROPWAT-FAO 8.0 model (48). ETo was affected by a reduction coefficient due to a decrease in solar radiation caused by the anti-hail netting that covered the field. Daily soil moisture balance included: reference crop evapotranspiration (ETo), crop coefficient (kc), effective rainfall (Ppe), crop evapotranspiration (ETc), root depth (D) and allowable depletion level (AD) (17).

A daily irrigation schedule was controlled by gravimetric soil moisture determination and properly installed sensors. Irrigation timing was determined after soil water status and drip lines water application intensity, affected by the uniformity coefficient. Water application intensity was calculated as a quotient between emitter average flow and the irrigated area by each emitter.

Before sowing, a 1 m deep test pit was dug in the middle of the plot and apparent soil density was determined using the core method (6) at three depths: 0-0.20, 0.20-0.40 and 0.40-0.60 m. For organic matter, fertility and soil salinity assessment, random sub-samples were collected from different points throughout the plot.

Soil samples were obtained from six points and three different depths: 0-0.30; 0.30-0.60 and 0.60-0.90 m. Texture was determined with the sedimentation volume method (37). A densitometry study determined sand, lime and clay percentages (9). Before sowing and after the last harvest, infiltration tests were run at three points in the plot (head, middle and end) using the double-ring infiltrometer method (19).

Soil moisture was determined by two methods: a) *Decagon* 10HS sensors in six points (2 per treatment and at 3 depths: 0.30, 0.60 and 0.90 m) recorded soil moisture hourly during the two crop cycles; b) soil moisture was randomly and weekly monitored at the three above-mentioned depths (0.25 m from the irrigation lateral at the center of each replication) by the gravimetric method with a soil auger and a weighing bottle. Soil moisture sensors provided accurate and continuous records at the already stated depths. On the other hand, weekly gravimetric measurements were conducted by randomly sampling the whole profile between 0-0.30, 0.30-0.60 and 0.60-0.90 m. WUE and WP for green and dry matter were determined at the end of each cycle using the following equations (32, 49):

$$\mathbf{WUE} = \frac{\mathbf{Y}}{\mathbf{(I)+(Ppe)}} \pm \text{moisture difference} \quad (1)$$

Where:
WUE = water use efficiency (kg m^{-3}),
Y = Yield (kg),
I = Irrigation (m^3),
Ppe =Effective precipitation (m^3).

$$\mathbf{WP}=\frac{\mathrm{Y}}{\mathrm{ETc}} \quad (2)$$

Where:
WP= Water productivity (kg m^{-3}),
Y= Yield (kg),
ETc= Crop evapotranspiration (m^3).

Results were analyzed with InfoStat 2017 statistical software (14). For yield and moisture data, "General and Mixed Linear Models", with first-order autoregressive structure, were used among the errors of the same plot since the periods were not equidistant. Corrected means were compared by LSD Fisher test ($p \leq 0.05$). For efficiency, productivity, and germination, ANOVA was conducted, and means were analyzed by Duncan's multiple comparison test ($p \leq 0.05$).

Results and Discussion

Table 1 (page 136) shows the main chemical parameters of the irrigation water. According to Riverside classification, modified by Thorne and Peterson (4, 50), water is classified as C-3 (medium salinity), *i.e.*, moderately saline, and suitable for irrigating all crops. In terms of Na^+ concentrations, classified as S1. Its high pH may lead to precipitates that might cause emitter clogging.

Sediment volume estimation revealed silty loam and silty clay. Soil densitometry confirmed silty clay texture. The soil was classified as non-saline, non-sodic and moderately alkaline. Table 2 (page 136) shows the main soil parameters. Stony subsoil was found below a depth of 0.60 m with 23% of stones. This was considered when calculating available water depth. Contents of N^+ and P^+ were within the acceptable range. K^+ resulted high according to the Departamento de Edafología de la Facultad de Ciencias Agrarias de la UNCuyo.

Table 1. Chemical parameters of irrigation water.

Tabla 1. Parámetros químicos del agua de riego.

Parameter	Value
Salinity expressed as apparent electrical conductivity CEA [dS m^{-1}]	0.78
Salinity expressed as probable electrical conductivity CEP [dS m^{-1}]	0.97
Probable salts [me L^{-1}]	8.0
pH	8.1
Chlorides (Cl^{-1}) [me L^{-1}]	1.6
Carbonates (CO_3^{-2}) [me L^{-1}]	0
Bicarbonates (HCO_3^{-1}) [me L^{-1}]	2.1
Sulfates (SO_4^{-2}) [me L^{-1}]	5.3
Calcium (Ca^{+2}) [me L^{-1}]	5
Magnesium (Mg^{+2}) [me L^{-1}]	2.4
Sodium (Na^{+}) [me L^{-1}]	1.7
SAR	0.9
Adjusted SAR	1

Table 2. Main soil parameters.

Tabla 2. Principales parámetros del suelo.

Field capacity from soil moisture characteristic curve (g% g dry soil)	29.3 – 33.7
Permanent wilting point from soil moisture characteristic curve (g% g dry soil)	11.2 -15.2
Total nitrogen (N) (ppm)	952
Phosphorous (P)(ppm)	6.1
Exchangeable potassium (K^{+}) (ppm)	340
Organic matter (%)	15
Electrical conductivity (dS.m^{-1})	1.3
Calcium + Magnesium (Ca^{2} + Mg^{+2}) (me L^{-1})	11.6
Sodium (Na^{+}) (me L^{-1})	1.8
Chloride (Cl^{-}) (me L^{-1})	3.5
SAR	0.8
Basic infiltration (mm h^{-1})	13 (±11)
Infiltration at 100 minutes ($I_{100,}$ min)	66 (±59)

Emitter average flow rate ranged between 2.12-2.2 L h^{-1} and distribution uniformity varied from 92% to 95%. Table 3 shows crop main water balance parameters.

Table 3. Main data on both sowings.

Tabla 3. Principales datos de las dos siembras realizadas.

Sowing and harvest dates	First (sowing) (10/21/17 to 2/2/18)	Second (sowing) (2/13/18 to 5/23/18)
ETo (mm)	486	304
Adjusted ETo (mm)	464	290
ETc (mm)	417	251
Total irrigation water depth (mm)	289.5	197.4
Total rainfall (mm)	165.6	16.7
Effective rainfall (mm)	101	7
Water used (mm)	417	251
Soil moisture variation (mm)	26.5	46.6

Figure 1 shows, for T3, soil moisture content using sensors and weekly gravimetric sampling during the first sowing. Bars represent effective rainfall or irrigation in mm. In this treatment, moisture was recorded by the sensor at 0.60 m given that the irrigation lateral is at 0.40 m. At 0.3 m, the sensor showed greater variability since it was affected by soil evaporation and crop water use, while at 0.90 m, a layer of less moisture-retentive soil results in less water content. In general, there is good correspondence between moisture records (sensors and gravimetric samplings) and soil wetting, either by rainfall or by irrigation. From early December, the three sensors detected soil moisture reductions due to higher crop water use, coinciding with significant crop production.

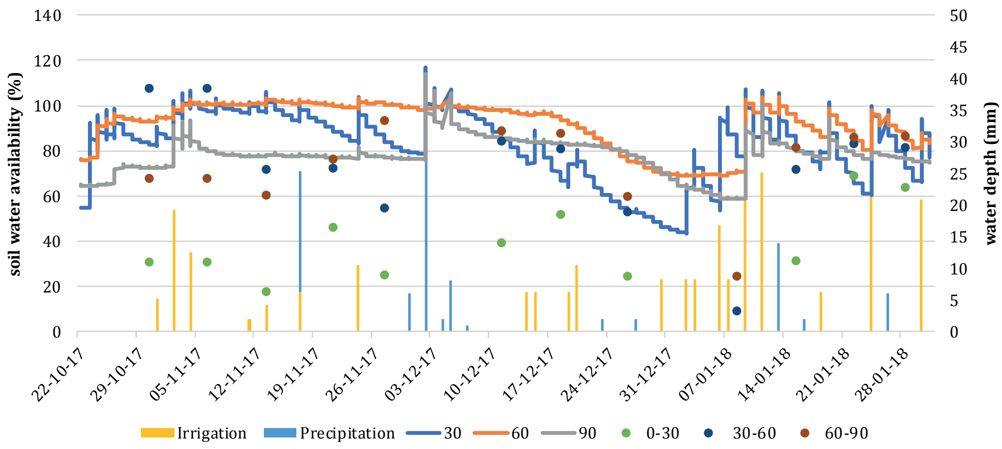

Figure 1. Variations in soil water availability measured at three depths (continuous line of sensors and gravimetric points) and rainfall and irrigation records during the first sowing.

Figura 1. Variación del agua disponible en el suelo medida a tres profundidades (línea continua (sensores) y puntos (gravimetría)) y registro de lluvias y riegos de la primera siembra.

Wetting patterns around a surface drip line relative to a subsurface line, coincided with lateral proximity (20). On the other hand, root distribution around drip lines coincides with wetting patterns. Andreau *et al.* (2012) found that larger wet bulbs and higher moisture contents were obtained with subsurface drip irrigation than with surface drip irrigation.

Germination percentage

The highest germination percentage was found with T 1 (97%) and the lowest (93%) with T2 and T3. Although differences were not statistically significant, germination percentages obtained with this irrigation method is one debated issue and considered a limiting factor (table 4).

Table 4. Mean emergence percentage (%) during the two sowings.

Tabla 4. Promedio del porcentaje de emergencia (%) durante las dos siembras.

Sowing/Treatment	1	2	3	Average
1	96	96	94	95
2	98	90	92	93
Mean	97	93	93	
Overall Mean	94			

The relationship between drip line depth and emergence percentage has been a matter of great concern among researchers around the world. SDI could hinder direct seeding given the minimum ascending movement of water affecting emergence, especially in coarse-textured soils (5). Emergence percentage is mostly affected by the distance between seeds and drip lines, closely related to lateral depth (40, 41). In California, less than 10 percent of farmers adopted SDI with laterals at depths of up to 10 cm for seed germination (12). For emergence uniformity with SDI, an adequate amount of water should be applied around the seed at sowing (8, 26). Sprinkler irrigation systems may ensure germination by placing subsurface laterals on V-shaped impermeable materials, increasing the wetted width, reducing deep percolation and, finally, allowing germination. After seed germination, a deeper drip line does offer many advantages.

Green and dry matter yields

As shown in Table 5, significant differences were only found for dry matter (table 6). Yields from the first sowing were significantly higher in relation to buried drip irrigation. In this sense, another experiment with fodder corn compared drip irrigation with lines buried at 0.40 m and different lateral spacing (0.8-0.9 y 1,0), with surface irrigation. The study found green matter yields of 70,190- 55,370 and 42,400 kg ha^{-1} with subsurface irrigation, and 27,800 kg ha^{-1} with surface irrigation for a sowing density of 104,000 plants per hectare (29). These results reveal that fodder production with surface irrigation was 150, 96 and 56 % less than with drip irrigation using laterals spaced at 0.8, 0.9 and 1.0 m respectively. On the other hand, for this study, mean yields from the first and second sowings (105,771 - 70,214 kg ha^{-1}) (table 5) were higher than those reported by Montemayor *et al.* (2007). With respect to dry matter forage, these authors obtained maximum yields of 20,190 kg ha^{-1} with laterals spaced at 0.8 m and yields of 15,880 - 12,160 kg ha^{-1} with laterals spaced at 0.9 and 1.0 m, respectively, while 8,080 kg ha^{-1} were obtained with surface irrigation. In the first sowing, with 1 m spacing, we obtained from 19,165 to 24,681 kg ha^{1}. However, in the second sowing, crop cycle could not be completed due to the onset of winter, obtaining 8,823 to 11,439 kg ha^{-1} (table 6). At the same time, yields of fodder corn were higher than those reported by Lamm *et al.* (1992) and Oktem *et al.* (2003) with subsurface drip irrigation. These authors pointed out that their results were influenced by lateral spacing, plant density and irrigation water depth. Additionally, according to Montemayor *et al.* (2006) lateral depth did not affect corn fodder weight. In another study in Mendoza, two corn fodder tests using surface irrigation recorded mean green matter yields of 58,390-73,760 kg ha^{-1} during the 2015-2016 season and mean dry matter yields of 21,789-23,493 kg ha^{-1} in 2016-2017 (43). These green yields were smaller than those recorded in this test. However, dry matter resulted similarly to those obtained from the first sowing, probably given to irrigation method, sowing density and corn variety.

Table 5. Mean green matter yield, (kg ha^{1}).

Tabla 5. Promedio del rendimiento en materia verde en kg ha^{-1}.

Sowing/Treatment	1	2	3	Mean
1	95,575 (a)	108,488 (a)	113,250 (a)	105,771
2	74,635 (a)	74,415 (a)	61,593 (a)	70,214
Mean	85,105	91,451	87,421	
Overall Mean	87,993			

Table 6. Mean Dry Matter Yield, (kg ha^{1}).

Tabla 6. Promedio del Rendimiento en materia seca en kg ha^{-1}.

Sowing/Treatment	1	2	3	Mean
1	19,165 (b)	23,582 (a)	24,681 (a)	22,476
2	11,439 (a)	9,798 (a)	8,823 (a)	10,020
Mean	15,302	16,690	16,752	
Overall Mean	16,248			

Water Use Efficiency (WUE) and Water Productivity (WP)

The highest WUE value was obtained under T2 in the second sowing (37 kg m^{-3}) while the lowest value was recorded under T1 in the first sowing (27.2 kg m^{-3}). On average, treatments yielded 29.7 kg m^{-3} in the first sowing and 34.8 kg m^{-3} in the second sowing. The overall mean was 32.2 kg m^{-3}. As for dry matter, the highest WUE value reached 6.89 kg m^{-3} in the first sowing under T3, while the lowest resulted in 4.37 kg m^{-3} for the second sowing, under T3. The overall mean under all treatments and sowings reached 5.64 kg m^{-3}.

Table 7 shows the effect of WP on green matter. The highest value (27.14 kg m^{-3}) was obtained under T3 while the lowest (22.91 kg m^{-3}) was found under T1. In the second sowing date, the highest WP (29.70 kg m^{-3}) was achieved under T1 and the lowest (24.51 kg m^{-3}) under T3, with an overall mean of 26.65 kg m^{-3}.

Table 7. Water productivity based on green matter (kg m^{-3}) in both sowing dates.

Tabla 7. Productividad del agua en materia verde (kg m^{-3}) para las dos fechas de siembra.

Sowing/Treatment	1	2	3	Mean
1	22.91(a)	26.00(a)	27.14(a)	25.35(a)
2	29.70(a)	29.61(a)	24.51(a)	27.94(a)
Mean	**26.31(a)**	**27.81(a)**	**25.83(a)**	
Overall Mean	**26.65**			

Regarding dry matter, table 8 summarizes WP data for both sowing dates. The highest value for the first sowing corresponded to T3 (5.92 kg m^{-3}) and the lowest to T1 (4.59 kg m^{-3}). Analyzing all treatments in both sowing dates, revealed the best result under T2 (4.78 kg m^{-3}), followed by T3 (4.72 kg m^{-3}). The lowest value was obtained under T1 (4.57 kg m^{-3}).

Table 8. Water productivity based on green matter (kg m^{-3}) in both sowing dates.

Tabla 8. Productividad del agua en materia Seca (kg m^{-3}) en las dos siembras.

Sowing/Treatment	1	2	3	Mean
1	4.59(a)	5.65(a)	5.92(a)	**5,39(a)**
2	4.55(a)	3.90(a)	3.51(a)	**3,99(a)**
Mean	**4.57(a)**	**4.78(a)**	**4.72(a)**	
Overall Mean	**4.69**			

With respect to WUE and WP, no significant differences were found among treatments. Given that WUE and WP are usually expressed as grain yield, few references mention these specific parameters for green fodder. Yescas *et al.* (2015) found dry matter WUE values of 2.84 - 3.24 kg m^{-3} in fodder corn under subsurface irrigation at a depth of 0.40 cm. Other tests ranged from 2.0 to 4.5 kg m^{-3} for WUE expressed as dry matter (29, 47). Montemayor *et al.* (2012) reported that dry matter WUE for fodder corn under arid conditions and subsurface drip irrigation was 4.07 kg m^{-3}, lower than the 6.89 kg m^{-3} found in this study. Previously, these authors had also compared subsurface drip irrigation and furrow irrigation, reporting WUE values of 2.9 kg m^{-3} with drip tape buried at 0.25, 0.35 and 0.45 m, and of 2.0 kg m^{-3} with furrow irrigation (28). Statistically, no significant differences were found among the three drip tape depths, though substantial differences were observed between subsurface drip irrigation and furrow irrigation. Lamm and Trooien (2003) found that WUE, in individual years, was not significantly affected by lateral depth, but when analyzed during the four-year test, lines buried at 0.51 and 0.61 m used less water. According to Payero *et al.* (2008), researchers may generally mention "increasing WUE" as a desirable objective. In some cases, they refer to WP, in others to WUE or other water use indicators, such as yield/ total water (rain + irrigation + variations in soil water storage).

Increasing field WP or WUE must follow both biophysical crop response, and economic factors. Even though this study did not evidence emergence effects on yield and WUE caused by inadequate irrigation, this might easily occur. According to Pablo *et al.* (2007), corn WUE was higher with drip lines buried at 15 cm than at 20 – 30 cm given better soil wetting during pre-emergence growth stages. Dripline depth will depend on the crop and the sowing/transplant method. Thus, laterals buried at 20 cm may suffice to irrigate transplanted tomatoes but not for crop sowing (34).

Conclusion

Germination percentage was not affected by lateral depth (between the surface and a depth of 40 cm). No significant differences were found in green matter between treatments in either crop cycle. Significant differences were found for dry matter. Both SDI treatments had the highest yields when compared to DI treatments. Dry matter represented 21% and 14% of green matter in the first and second sowing dates, respectively. Even though no significant differences were found in WUE (with and without rainfall) or WP indicators, high crop water use was achieved regarding green and dry matter. WUE values were between 12 and 41 % higher than those cited. Although results about subsurface drip irrigation in agricultural plots under integrated irrigation are encouraging, it should be borne in mind that they are contingent upon soil type and crop. It should also be noted that these irrigation practices involve deep plowing, only possible between buried laterals. Otherwise, they should be avoided altogether, no plowing at all, or else applying the concept of direct sowing in intensive agriculture.

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Modeling natural mortality for different plant densities in dendroenergetic trials

Modelación de la mortalidad natural en diferentes densidades de plantación en ensayos dendroenergéticos

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Abstract

Mortality was modeled for the *Acacia melanoxylon*, *Eucalyptus camaldulensis*, and *Eucalyptus nitens* species at plant densities of 5,000, 7,500, and 10,000 trees ha^{-1} in a biomass production trial for dendroenergetic purposes. One setup was based on individual tree level and two modeling alternatives were evaluated involving four survival probability equations and eight difference equations. Individual tree survival modeling considered a logistics model, which is a linear combination of variables per tree at current time t and previous time as estimator. These were the main variables of the competition index variation and the basal area growth variation between the current and the previous growth period. The survival probability alternative involved state variables of the stand like age, dominant height, and average square diameter as predictors, while the difference equations were fitted according to age-based changes only. The stand level models showed better results than the individual tree models, and in general, the mortality models based on difference equations presented better precision indicators and parsimony. The relative mortality rate was constant, *i.e.*, $(\partial N / \partial E)/ N = \alpha$, and varied between species, revealing greater mortality, consecutively, in *E. nitens*, *A. melanoxylon*, and *E. camaldulensis*. Although mortality tended to be higher at greater plantation densities, stand density did not significantly affect the fitted models' parameters.

Keywords

Survival probability models • difference equations • relative mortality rate • dendroenergy

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Resumen

Se modeló la mortalidad para tres especies (*Acacia melanoxylon*, *Eucalyptus camaldulensis*, *Eucalyptus nitens*) en tres densidades de plantación (5000, 7500 y 10000 árboles ha^{-1}) en un ensayo de producción de biomasa con fines dendroenergéticos. Se evaluó una modelación basada en el nivel de árbol individual y dos alternativas de modelización de la mortalidad: cuatro ecuaciones de probabilidad de supervivencia y ocho ecuaciones diferenciales Se consideró un modelo logístico para el modelado de supervivencia de árboles individuales, este es una combinación lineal de variables a nivel de árbol individual en el momento actual t y en el momento anterior como estimador, siendo las principales variables la variación del índice de competencia y la variación del crecimiento del área basal entre el periodo de crecimiento actual y el anterior. En la alternativa de probabilidad de supervivencia se utilizaron como predictores las variables de estado de rodal (edad, altura dominante, diámetro cuadrado medio), mientras que las ecuaciones diferenciales se ajustaron únicamente en función de los cambios basados en la edad. Los modelos de niveles de rodales mostraron mejores resultados que los modelos de árboles individuales y, en general, los modelos de mortalidad basados en ecuaciones diferenciales presentaron mejores indicadores de precisión y parsimonia. La tasa de mortalidad relativa fue constante, *i.e.* $(\partial N / \partial E)/ N = \alpha$, y varió entre las especies, revelando una mayor mortalidad, consecutivamente, en *E. nitens*, *A. melanoxylon* y *E. camaldulensis*. Aunque la mortalidad tendía a ser mayor a mayores densidades de plantación, la densidad de rodales no afectaba significativamente a los parámetros de los modelos ajustados.

Palabras clave

Modelos de probabilidad de supervivencia • ecuaciones diferenciales • tasa de mortalidad relativa • dendroenergía

Introduction

Forestry decision-making is often based mainly on stand growth and yield (14), and the prediction of these variables has been under constant study. At present, stand growth and yield are predicted with mathematical models in function of state variables such as number of trees, basal area, dominant height, and others (10). In growth models, the number of trees per unit of area is expressed in terms of mortality or survival (8). Stand mortality is a key component of growth and yield models, and its prediction is essential for obtaining precise estimates of state variables such as volume and biomass.

Modeling mortality can be done at the individual tree or surface unit levels. The first option is difficult due to its high variability (8, 20, 21, 26), and this problem is augmented by a limited database and when a wide range of growing conditions is not available (9). Modeling mortality at the stand level, however, is more robust since it only considers mortality generated by competition, excluding mortality generated by causes such as attacks by biological or other external agents (7, 8, 12).

Modeling mortality at the individual tree level is generally done with logistics models (6, 17, 24, 25, 26). At the surface unit level, probability equations of the shape $P = \left(1 + e^{\beta X}\right)^{-1}$ are used, where βX is a linear combination of parameters b_i and state variables of stand x_i , including crop age, average square diameter (D_q), crown diameter, average height, and dominant height. Another way to model mortality at the surface unit level is with difference equations. This alternative involves the relative mortality rate ($\partial N / \partial E$), which is assumed to be closely related to the current stand age (E), density in a previous period (N), and site quality (3, 5, 27). Although relative mortality rates are generally related to various stand state variables such as stand density, age, dominant height, basal area, *DQ*, volume, and biomass, the literature recommends avoiding the use of variables derived from trunk diameter (basal area, D_q, volume, biomass) as interventions like thinning substantially weaken the relationship of these variables with stand density (8, 12).

In several studies, mortality has been modeled at the surface unit level using either difference equations or survival probability equations. Zhao *et al.* (2007) noted that both require taking measurements throughout crop life and that long periods of time may pass in which no mortality is recorded in the stand, leading to a lack of convergence for these models (24). The properties of difference equations include consistency, mortality rates lacking variance, and near-zero asymptotic limits of stand density (3, 24, 27). The research presented in this paper was carried out with traditional crops, *i.e.*, in stands created for sawable or pulpable products and, to date, no such research has been published regarding crops for biomass production for dendroenergetic generation. Intraspecific competition mainly for water and nutrients should occur much earlier for short-rotation crops, usually established in high-density plantations, than for traditional crops. Therefore, the former should also manifest individual mortality at earlier ages.

In Chile, crops intended for biomass production have only been established recently in experimental areas. Crop potential for operational scale and the best strategy for growth and yield projection remain unknown. The objective of this paper is to model stand mortality in the first 40 months of growth for three dendroenergetic crops: *Acacia melanoxylon* R. Br., *Eucalyptus camaldulensis* Dehnh., and *Eucalyptus nitens* (Deane & Maiden), established at plantation densities of 5,000, 7,500, and 10,000 trees ha^{-1}.

Material and methods

Trial characteristics and location

The trial was established in August 2007 in the rainfed interior of Ñuble Region, Ninhue locality, Chile. The site presented nutritional and water limitations and was predominately characterized by low yield forest plantations dedicated to pulp or sawtimber production. The site was previously occupied by a 24-year-old *Pinus radiata* D. Don plantation, registered mean annual rainfall of 1,324 mm and minimum average, medium average and maximum average annual temperatures of 0.0 °C, 11.3 °C, and 23.5 °C, respectively. Soils were of the Cauquenes soil family series derived from granitic rocks and classified as mesic Ultic Palexeralfs (Alfisol). They were deep (> 100 cm), well drained and with a well evolved clay texture profile (2). The terrain had a rolling to abrupt topography but the slope in the study area did not exceed 5%.

Prior to establishing the trial, the site was prepared by removing the previous crop's trunks. The soil was then deep-plowed to 80 cm in the form of a grid using a Caterpillar D8K and leaving 60 cm space between rows. A chemical weed control substance was applied, consisting of pre- and post-plantation applications of a mixture containing 4 kg ha^{-1} Glyphosate-Roundup Max, 1.5 kg ha^{-1} simazine, and 2.5 kg ha^{-1} atrazine. Post-plantation fertilization was done in circles 25 cm from the tree trunks using 30 g boronatrocalcite, 150 g diamonic phosphate, and 50 g Sul-Po-Mag. The trial was protected with a perimeter fence to keep out animals; the fence was made of galvanized 5014 mesh, buried 0.3 m and measured approximately 1.2 m above-ground height.

The trial was established using a completely randomized block design with three replicates. Each block was a square with 75 m per side (5,625 m^2), made up of nine experimental units with 25 m per side (625 m^2). Each unit, in turn, consisted of a border-effect buffer zone and a square core of 49 useful trees. *A. melanoxylon*, *E. camaldulensis*, and *E. nitens* were planted in each block at densities of 5,000, 7,500, and 10,000 trees ha^{-1}.

Measurement of variables

We carried out eight measurements: in October and December 2007, and in July and December 2008, 2009, and 2010. On each occasion, we recorded the trunk diameter at ground level (D; 0.1 m above ground) and the diameter at breast height (DBH) when the tree was taller than 1.3 m, crown diameter, and total height of all live trees in each experimental unit's core.

Since the analysis revealed some experimental units with high mortality rates evidently associated with external factors not related to intraspecific competition, we used only the most representative plots defined as those with the lowest mortality rates, *i.e.*, where mortality was generated from competition for site resources caused by tree growth.

Modeling mortality

We modeled mortality at two levels, first using individual tree and stand data. For individual trees, we used the logistics model approach in the form of $P = e^{\beta X} / (1 + e^{\beta X})$. In this equation, βX is a linear combination of individual tree variables at current time t and previous time $t-1$, $\beta X = b_1 \Delta IC + b_2 \Delta g + b_3 E + b_N I_N$. Here, ΔIC is the competition index variation between the current growth period and the previous one. Likewise, Δg is the variation of basal area growth in both periods, E is age expressed in months and I_N the stocks incorporated as dummy variable. The competition index was calculated using the Hegyi model (11) and implemented via the Siplab package in R software (9). Δg was calculated according to differences between average increment and the last period's increment in total height $\left(\left(h_t - h_{t-1}\right)/\left(E_t - E_{t-1}\right)\right)/\left(h_{t-1} / E_{t-1}\right)$. We postulated the growth rate decrease and hence the competition index in measurement to instance $t-1$ would be an estimator of mortality in later instance t. The logistics model was set to classify the death of a tree with a probability value less than 0.5.

We followed two routes for mortality modeling at the stand level. One comprised survival probability models, *i.e.* $P = (1 + e^{\beta X})^{-1}$, where P represents the proportion of live trees in each plot in relation to the 49 initially established ones, and βX is the linear combination of parameters β and independent variables X to estimate X. For linear combinations of βX in the survival probability models, we used equations:

$$\beta X = b_0 + b_1 E \quad (1)$$
$$\beta X = b_0 + b_1 E + b_2 D_q \quad (2)$$
$$\beta X = b_0 + b_1 E + b_2 H_{dom} \quad (3)$$
$$\beta X = b_0 + b_1 E + b_2 D_q + b_3 H_{dom} \quad (4)$$

where:
E = the age of the crop expressed in months,
D_q = the mean square diameter,
H_{dom} = the dominant height,
b_0, b_1, b_2, and b_3 = the parameters to estimate. Dominant height was calculated as the average height of the five trees with the greatest *D* per plot.

Difference equations were also used to model mortality. Zhao *et al.* (2007) related relative mortality to age and/or site quality, following the $\left(\partial N / \partial E\right)/ N = \alpha N^{\beta} f(site) f(age)$ shape, an equation in which $\partial N / \partial E$ represents the mortality rate at age *E*, $f(site)$ and $f(age)$ are functions of site quality and age, respectively, and α and β are parameters. We analyzed eight of the 28 models proposed by Zhao *et al.* (2007) for modeling mortality in the trial. Only one site was used in this study; thus, the expression was reduced to $\left(\partial N / \partial E\right)/ N = \alpha N^{\beta} f(age)$. Moreover, assuming the relative mortality rate was not related to age ($f(age) = 1$) and that its decline was independent of plantation density ($\beta = 0$), the expression of mortality rate $\left(\partial N / \partial E\right)/ N = \alpha N^{\beta} f(site) f(age)$ was reduced to $\left(\partial N / \partial E\right)/ N = \alpha$, implying the relative mortality rate was constant (α) at any age. In its initial condition, this expression was $N = N_1$ when $E = E_1$ and should be the difference equation for predicting $N = N_2$ at $E = E_2$. Therefore, integrating $\int dN / N = \int \alpha \, dE$, we got $N = e^{(\alpha E)}$ and evaluating the difference equation we got $N_2 = N_1 \, e^{b_1 (E_2 - E_1)}$ (model [1]). In models [2] to [8], the initial assumption changed regarding the relative mortality rate, but its development was identical. All these functions were used previously to model mortality in adult stands with densities ranging from 740 to 1,970 trees ha^{-1} (4, 15, 16) (table 1, page 147).

To determine the level of intraspecific competition to which the trial was subjected, we analyzed the relationship of stand density-D_q following the methodology proposed by Wittwer *et al.* (1997). These authors suggested incrementing the intercept 1.96 times the standard deviation of the model to determine the mortality curve of the maximum site load. The fitting were done for each species and plantation density.

Table 1. Mathematical expressions of difference equations and their respective mortality models.

Tabla 1. Expresiones matemáticas de las ecuaciones diferenciales y sus respectivos modelos de mortalidad.

Relative mortality rate	Difference equation	#
$\frac{\partial N / \partial E}{N} = \alpha$	$N_2 = N_1 \, e^{b_1 (E_2 - E_1)}$	(5)
$\frac{\partial N / \partial E}{N} = \alpha E^{\beta}$	$N_2 = N_1 \, e^{b_1 (E_2^{b2} - E_1^{b1})}$	(6)
$\frac{\partial N / \partial E}{N} = \alpha + \frac{\beta}{E}$	$N_2 = N_1 \, e^{b_1 (E_2 - E_1)} \left(\frac{E_2}{E_1} \right)^{b_2}$	(7)
$\frac{\partial N / \partial E}{N} = \alpha \beta^{E}$	$N_2 = N_1 \, e^{b_1 (b_2^{E2} - b_1^{E1})}$	(8)
$\frac{\partial dN / \partial E}{E} = \alpha N^{\beta}$	$N_2 = \left[N_1^{b_0} + b_1 (E_2 - E_1) \right]^{1/b_0}$	(9)
$\frac{\partial N / \partial E}{E} = \alpha N^{\beta} E^{\gamma}$	$N_2 = \left[N_1^{b_0} + b_1 \left(E_2^{b2} - E_1^{b2} \right) \right]^{1/b_0}$	(10)
$\frac{\partial N / \partial E}{E} = \alpha N^{\beta} \left(1 + \frac{\gamma}{E} \right)$	$N_2 = \left[N_1^{b_0} + b_1 (E_2 - E_1) + b_2 \ln \left(\frac{E_2}{E_1} \right) \right]^{1/b_0}$	(11)
$\frac{\partial N / \partial E}{E} = \alpha N^{\beta} \gamma^{E}$	$N_2 = \left[N_1^{b_0} + b_1 \left(b_2^{E_2} - b_2^{E_1} \right) \right]^{1/b_0}$	(12)

Data analysis

For the individual tree mortality models, we analyzed the fit accuracy $\text{Acc} = (Positive + Negative) / Total$, positive prediction value $\text{PPV} = Positive / Total$ and negative prediction value $\text{NPV} = Positive / Total$. The metrics were calculated from confusion matrix denoting the proportion of well classified trees, the proportion of living well classified trees, and the proportion of dead well classified trees, respectively.

On the other hand, the stand level mortality models being analyzed were fitted independently for each species and plantation density level. The precision of the fitting was determined based on the root mean square error, calculated as $RMSE = \sqrt{sse / (n - p)}$. Hence, model selection was done using Akaike's index, calculated as $AIC = n \ln (sse / n) + 2p$. Under these criteria, n is the number of observations corresponding to all measurement opportunities, p the number of model parameters, and sse the sum of the squared errors, calculated as $sse = N^2 \sum_{i=1}^{n} (P_i - \hat{P}_i)^2$ and $sse = \sum_{i=1}^{n} (N_i - \hat{N}_i)^2$ for the mortality probability models and the difference equations, respectively. In turn, the effect of plantation density on the shape of the mortality equations was evaluated through the extra sum of squares, which was determined using Fisher's distribution F-test, calculated as $F = ((sse_s - sse_c) / (p_c - p_s)) / (sse_c / (n - p_c))$ (22); the sub-indexes of this test indicated the use of simple (s) and complex (c) models. Both modeling strategies were compared according to accuracy indexes obtained in the individual tree and stand level models.

Results

Individual tree modeling showed potential to predict the mortality rate, with the ΔIC and Δg variables being significant, denoting that those variables were valid estimators in the model, also permitting describing the dynamic of the competition index in successive periods (figure 1). This approach allowed to fit a model by species, and the different plantations were incorporated as dummy variables. The best modeling accuracy was achieved for *A. melanoxylon* with 88.6%, followed by *E. camaldulensis* and *E. nitens* (table 2, page 149). For all species, the model showed better performance to estimate living trees than dead trees, expressed as positive prediction value (PPV), as opposed to negative prediction value (NPV). In the case for *E. camaldulensis*, the model's performance to estimate individual tree mortality was deficient, achieving only 23.7% of accuracy. Although the individual tree method had high accuracy and good ability to estimate live tress, NPV values suggested discarding this analysis approach.

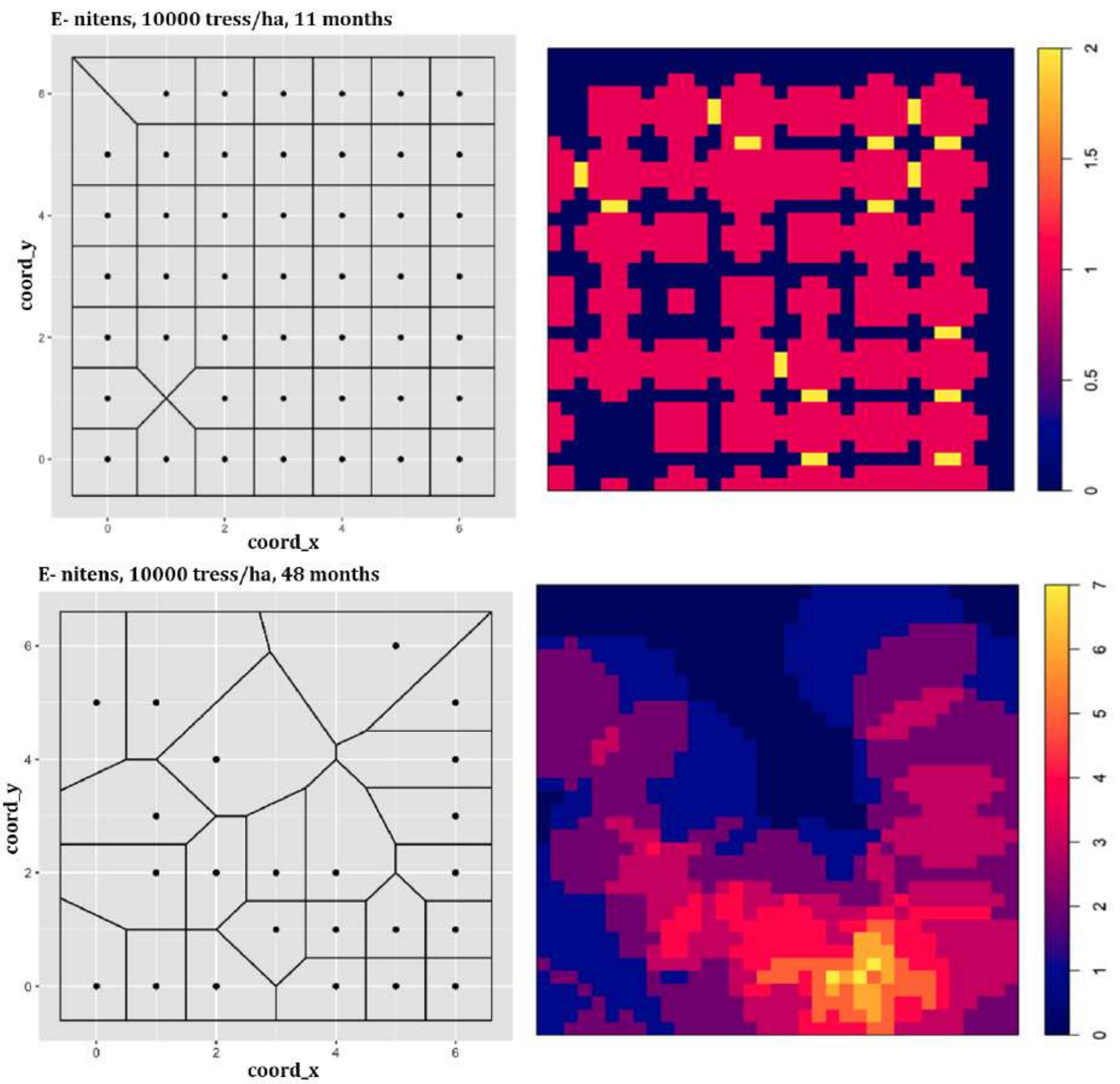

Figure 1. Left: Voronoi triangulation for *E. nitens*; the available space to grow at initial density of 10,000 trees ha^{-1} for ages of 11 and 48 months. Right: Heatmap for the Hegyi competition index in both growth periods.

Figura 1. Izquierda: Triangulación de Voronoi para *E. nitens*; espacio disponible para crecer en la densidad de plantación de 10.000 árboles ha^{-1} para la edad de 11 y 48 meses. Derecha: Mapa de calor para el índice de competencia de Hegyi en ambos periodos de crecimiento.

Table 2. Estimated parameters for the individual tree mortality model.

Tabla 2. Parámetros estimados para el modelo de mortalidad de árboles individuales.

Species	ΔIC	Δg	Month	D5,000	D7,500	D10,000	Accuracy (%)	PPV (%)	NPV (%)
A. melanoxylon	43.2532	1.5282	-0.0163	-149.6014	-52.7733	-21.9799	88.6	92.6	55.8
E. camaldulensis	46.0678	0.6168	-0.0001	-159.2877	-56.7218	-22.2876	61.3	96.2	23.7
E. nitens	41.5435	3.2034	-0.0327	-143.3300	-50.7747	-21.8756	58.1	71.4	52.2

Although model [4] was the most precise of the probability equations (table 3, page 150), followed consecutively by models [2], [1], and [3], model [2] was the most appropriate for modeling survival probability in terms of parsimony, followed by models [1], [4], and [3]. Although model [4] was more precise, it required the estimation of four parameters, which is one more than model [2], indicating that $Hdom$ was not a variable that, in the presence of the age and D_q variables, significantly reduced the estimation error. Mortality modeling using this methodology presented many non-significant estimates of the parameters. Apparently, the applicability of this method was conditioned by the number of observations. In this study, variables were only measured eight times, the last time 40 months after establishing the trial. These scant measurements caused the limited significance of the parameters estimated in the mortality probability equations.

Age was the variable with the greatest explanatory capacity in the survival probability equations (table 3, page 150). Although model [1] did not have the best precision indicators (*RMSE*), its results were similar to those obtained by model [2] in terms of parsimony (*AIC*), with age and D_q incorporated into its polynomial. In model [1], parameter b_1, associated with crop age, was significant in most cases. When incorporating other predictive variables into the survival probability models ([2], [3], [4]), no parameter was significant.

Model [1], with which we obtained the best results in terms of parsimony, showed that the probability of survival varied between plantation densities and had an inverse relationship with plantation density (table 3, page 150). The results only indicated a significant effect of density on the model parameters for the *Eucalyptus* species, and an independent fitting was required for each level of plantation density. Nonetheless, for *E. camaldulensis*, the lack of convergence of the model affected the interpretation of the test and the suggestion of fitting for each plantation density of this species should not be considered, since the observed mortality rate was low and null for 5,000 trees ha^{-1} (table 3, page 150 y figure 2, page 151).

Difference equation [5] showed the best results for both precision and parsimony (table 4, page 152), followed by models [7] and [6], which gave good *RMSE* and *AIC* indicators. Table 4 (page 152) shows only the three best fits obtained; the results of models [8] and [9] were excluded due to low precision, as were the results of models [10], [11], and [12], which had convergence problems, likely due to the high number of parameters in the models and the scant number of measurements available. Despite these limitations, difference equation [5] modeled satisfactorily the decline in tree numbers, and all its parameters were significant. In general, the models of the difference equations that converged showed mostly significant parameters.

Observed relative mortality rate remained constant during the first 40 months of growth, *i.e.*, $\left(\partial N / \partial E\right) / N = \alpha$. This assumption was implicit in model [5] (table 4, page 152) and, therefore, this equation sufficed for obtaining precise mortality rate estimates. Model [7], which showed precision and parsimony indicators similar to model [5], assumed a relative mortality rate of $\left(\partial N / \partial E\right) / N = \alpha + \beta / E$. This assumption was adequate for predicting the mortality rate, although the estimation of this model showed a greater error. Model [6] was also fitted satisfactorily but showed greater prediction errors, assuming the relative mortality rate varied non-linearly in the function of crop age ($\left(\partial N / \partial E\right) / N = \alpha E^{\beta}$). Therefore, of the eight difference equations tested, model [5] yielded the most precise for the first 40 months of growth, indicating that mortality was constant regardless of age. The precision of functions [6] and [7] was probably obscured by the scant number of observations available. Logically, mortality should be closely related to crop age, whether expressed in shape change or slope change that would eventually be manifested at some age of the crop. This effect was implicit in these equations.

Table 3. Estimated parameters using the method of estimating survival probability equations.

Tabla 3. Parámetros estimados utilizando el método de estimación de ecuaciones de probabilidad de supervivencia.

Model	Species	Density (trees ha^{-1})	b_0	b_1	b_2	b_3	*RMSE*	*AIC*	*P (F)*
1	*A. melanoxylon*	5,000	0.01251ns	-0.00725**			146.5	81.5	
		7,500	0.02189ns	-0.00808**			333.4	94.7	
		10,000	0.03193ns	-0.00869**			388.2	97.1	
		Average	0.02160**	-0.00799**			227.1	66.7	0.8335
	E. camaldulensis	5,000	---	---			---	---	
		7,500	0.00206ns	-0.00025ns			48.4	63.8	
		10,000	-0.00248ns	-0.00066**			85.6	72.9	
		Average	-0.00074ns	-0.00032**			63.4	24.8	<0.0001
	E. nitens	5,000	0.04966ns	-0.01244**			444.5	99.2	
		7,500	0.04012ns	-0.01161**			526.5	102.0	
		10,000	0.08705ns	-0.02348**			1503.7	118.7	
		Average	0.05760ns	-0.01580**			783.7	108.5	0.0114
2	*A. melanoxylon*	5,000	0.00792ns	-0.00069ns	-0.00369**		109.0	78.8	
		7,500	0.02500ns	-0.01006**	0.00209ns		328.6	96.4	
		10,000	0.01032ns	0.01234ns	-0.01795ns		312.0	95.6	
		Average	0.02160**	-0.00808**	0.00007ns		230.9	69.2	0.2635
	E. camaldulensis	5,000	---	---	---		---	---	
		7,500	0.00089ns	-0.00094ns	0.00052ns		44.4	64.4	
		10,000	0.00105ns	0.00058**	-0.00098**		40.8	63.0	
		Average	-0.00006ns	-0.00001ns	-0.00024ns		63.9	27.1	<0.0001
	E. nitens	5,000	0.04478ns	-0.02003ns	0.00402ns		431.4	100.8	
		7,500	0.03418ns	-0.01706ns	0.00355ns		506.3	103.3	
		10,000	0.06905ns	-0.04409**	0.01302ns		1399.7	119.6	
		Average	0.04560ns	-0.02952**	0.00828**		745.4	108.9	0.0726
4	*A. melanoxylon*	5,000	0.00550ns	-0.00021ns	-0.00447ns	0.00007ns	108.4	80.7	
		7,500	0.02071ns	-0.01218**	-0.00436ns	0.00080ns	313.9	97.7	
		10,000	0.00899ns	0.01248ns	-0.01851ns	0.00005ns	311.7	97.6	
		Average	0.02371**	-0.00746**	0.00074ns	-0.00014**	233.3	71.6	0.4427
	E. camaldulensis	5,000	---	---	---	---	---	---	
		7,500	0.00253ns	-0.00058ns	0.00100ns	-0.00009ns	41.1	65.1	
		10,000	0.00136ns	0.00057**	-0.00082**	-0.00002ns	40.8	65.0	
		Average	0.00327**	0.00034ns	0.00060**	-0.00014**	37.3	10.2	0.0011
	E. nitens	5,000	0.01444ns	0.03132ns	-0.00160ns	-0.00265**	296.9	96.8	
		7,500	0.03997ns	-0.02643ns	0.00213ns	0.00096ns	476.9	104.4	
		10,000	0.06591ns	-0.03985ns	0.02103ns	-0.00168ns	1355.3	121.1	
		Average	0.05153ns	-0.03284**	0.00572ns	0.00061ns	745.9	110.9	0.0693

In general, precision (*RMSE* and *AIC*) diminished along with plantation density, a result shown in the three best difference equations (table 4, page 152). Of these, model [5] indicated an inverse relationship of parameter b_1 with plantation density. This was shown by the greater relative mortality rate at the highest plantation densities, which was reasonable if we assumed greater intraspecific competition, which conditions crop survival, on plots established with more trees per surface unit. The effect of plantation density on the parameters of model [5] was manifested in this tendency, but no significant reduction of the residual variation in the fitted models independently by plantation density levels was found in relation to average fitting. Therefore, up to 40 months of age, modeling mortality through difference equations could be done by averaging, *i.e.*, without considering the effect of the plantation density.

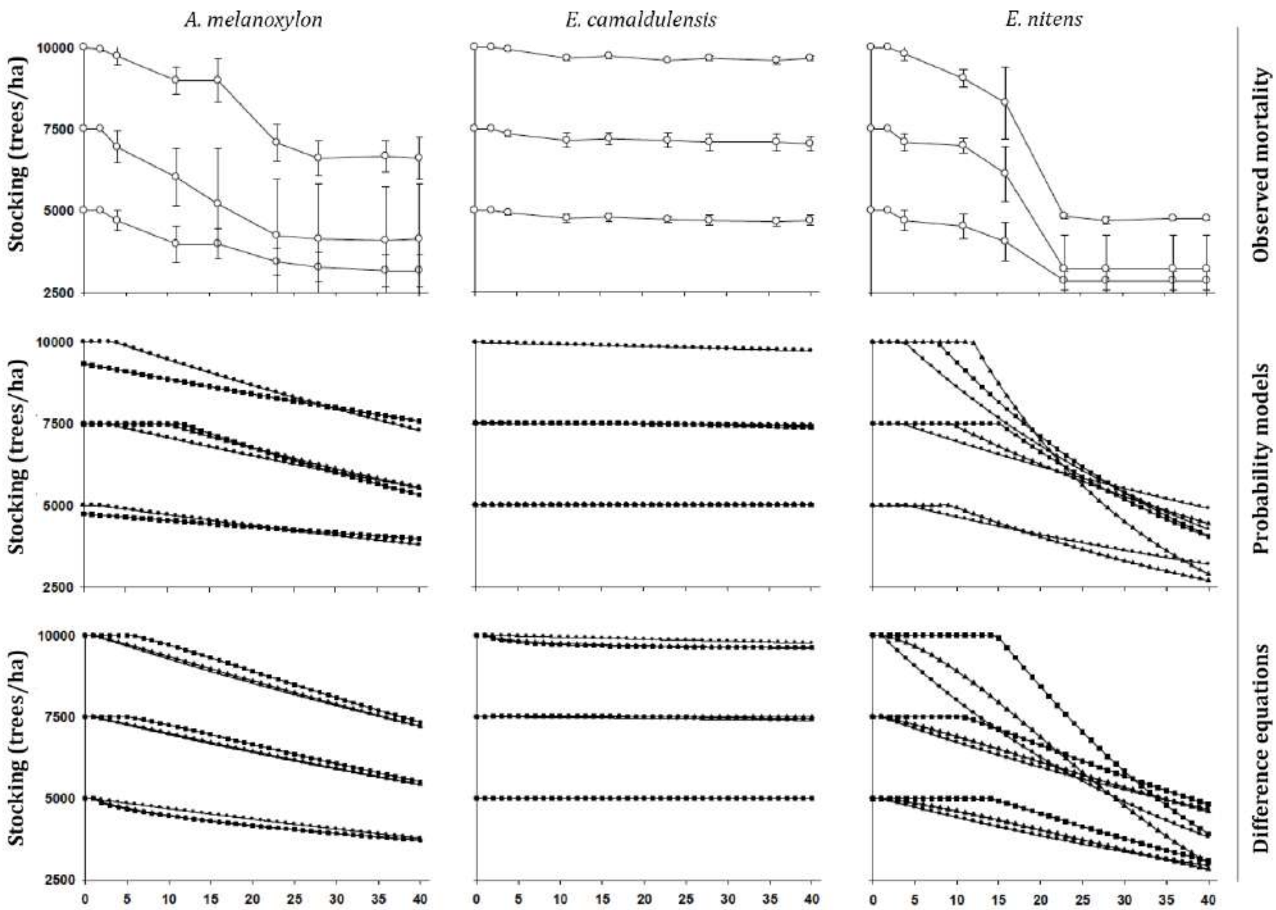

Figure 2. Mortality modeling based on the survival probability functions for models 1 (○), 2 (▲), and 4 (■) and the difference equations for models 5 (○), 6 (▲), and 7 (■).

Figura 2. Modelación de la mortalidad basada en las funciones de probabilidad de supervivencia para los modelos 1 (○), 2 (▲) y 4 (■) y ecuaciones diferenciales para los modelos 5 (○), 6 (▲) y 7 (■).

Some equations were deficient at estimating mortality in the first growth stages (figure 2). Model [4] underestimated the number of *A. melanoxylon* trees at the time of establishing the crop. For this same species, model [1], the most precise of the probability equations, overestimated density in the first growth stages at densities of 7,500 and 10,000 trees ha^{-1}. In those cases, restricting the estimate to the value of the nominal stand density caused the curve to flatten. On the contrary, the difference equations did not underestimate the tree numbers at the time of establishing the plantation for any of the three species, although this type of model did register cases of overestimation in the first growth stages, for example for *A. melanoxylon* and *E. nitens*. Normally, mortality was not generated in the crops' early stages due to a lack of competition. However, semi-annual records of these rapidly growing crops revealed a strong rather than a gradual decline in the number of trees between the fourth and fifth measurements, *i.e.*, 16 to 23 months after establishment (figure 2). This hindered the predictive capacity of both the probability and the difference equation models, leading to underestimating tree numbers when there was no mortality.

Table 4. Estimated parameters in the best three fitted models using the difference equations method.

Tabla 4. Parámetros estimados en los tres mejores modelos ajustados mediante el método de ecuaciones diferenciales.

Model	Species	Density (trees ha^{-1})	b_1	b_2	*RMSE*	*AIC*	*P (F)*
5	*A. melanoxylon*	5,000	-0.00713**		138.6	79.8	
		7,500	-0.00826**		365.0	95.3	
		10,000	-0.00836**		441.6	98.4	
		Average	-0.00816**		349.1	246.9	0.9989
	E. camaldulensis	5,000	---		---	---	
		7,500	-0.00035**		55.8	65.3	
		10,000	-0.00064**		68.2	68.5	
		Average	-0.00046**		53.1	167.8	0.9812
	E. nitens	5,000	-0.01358**		524.1	101.1	
		7,500	-0.01213**		618.5	103.8	
		10,000	-0.02462**		1519.0	118.1	
		Average	-0.01870**		1039.0	292.7	0.9799
6	*A. melanoxylon*	5,000	-0.03919**	0.57708**	133.4	80.0	
		7,500	-0.00654**	1.06228**	393.9	97.3	
		10,000	-0.00592**	1.09264**	476.0	100.3	
		Average	-0.00771**	1.01520**	358.1	248.9	0.9999
	E. camaldulensis	5,000	---	---	---	---	
		7,500	-0.00007ns	-8.79169ns	62.5	67.9	
		10,000	-0.00506ns	0.01031ns	56.2	66.2	
		Average	-0.03379ns	0.14439ns	51.7	167.6	0.9948
	E. nitens	5,000	-0.00336**	1.39080**	556.0	102.8	
		7,500	-0.00511**	1.23824**	662.3	105.6	
		10,000	-0.00257**	1.66598**	1552.9	119.3	
		Average	-0.00362**	1.46969**	1038.6	293.6	0.9991
7	*A. melanoxylon*	5,000	-0.00474ns	-0.02997ns	141.8	81.0	
		7,500	-0.01028**	0.02556**	389.3	97.1	
		10,000	-0.01065**	0.02856**	468.2	100.1	
		Average	-0.00972**	0.01957**	355.0	248.5	0.9999
	E. camaldulensis	5,000	---	---	---	---	
		7,500	-0.00069ns	0.00482ns	58.9	66.9	
		10,000	0.00038ns	-0.01430**	54.3	65.6	
		Average	-0.00001ns	-0.00635ns	51.8	167.7	0.9869
	E. nitens	5,000	-0.02319**	0.11493**	537.4	102.3	
		7,500	-0.01852**	0.07650ns	643.9	105.2	
		10,000	-0.04643**	0.23722**	1492.3	118.6	
		Average	-0.03289**	-0.16137**	1002.3	292.1	0.9990

Discussion

The best method for modeling mortality in these dendroenergetic trials was with models derived from difference equations. These models, in general, presented greater precision of fit and showed a greater number of significant parameters. The mortality models derived from the probability functions presented many non-significant parameters. Therefore, the estimates were not reliable and had limited applicability in growth and yield models. The difference equations also presented non-significant parameters, but a lesser number. This inconvenience was apparently connected to the number of observations available for the study, since the hypothesis tests for the parameters in the non-linear models were known to be strongly conditioned by sample size (19).

Other authors have also evaluated the two alternatives for modeling the mortality analyzed here. For example, Zhao *et al.* (2007) modeled the probability of survival based on a probability model using age, basal area, site index, and stand density from the immediately preceding measurement period as predictive variables; all the parameters were found to be significant. Based on those results, the authors recommended only incorporating age and basal area as predictive variables in survival probability models, since this latter variable indicated the competition level. The results of that study were in agreement with ours, since our best model contained the explanatory variables age and D_q as a competition indicator. The recommendation of other authors to avoid the use of expressions derived from the diameter (*e.g.* D_q or basal area), since these variables are highly susceptible to forestry interventions (8), did not apply to this type of crop as it was not subjected to forestry interventions such as thinning. Nor did the use of dominant height seem advisable when dealing with crops destined for biomass production. The utility of this variable is dubious, considering that the proportion of trees is not a clear indicator of site quality due to crop age. At early ages, the intrinsic quality of the site did not show in tree growth. Rather, activities such as site preparation and/or other forestry activities were the main factors that condition early-age crop establishment, growth, and survival.

Although this study obtained similar precision with both methods, the best difference equation [5] was more precise than the best survival probability equation [2]. Therefore, we recommend using models based on difference equations to model the mortality of crops intended for dendroenergetic generation. Moreover, study results showed these models had an advantage over the survival probability models in terms of parsimony and the number of significant parameters. Zhao *et al.* (2007) evaluated these two alternatives in adult stands of *Pinus taeda* Engelm., establishing good precision results with difference equations. In this study, the best results were obtained using the $(\partial N/\partial E)/N = (c_0 + c_1)N^{\beta}\delta^{E}$ and $(\partial N/\partial E)/N = N^{\beta}(\alpha/Site + \delta/E)$ models. Given that the current study was conducted at only one site, it was not possible to incorporate site quality as a predictive variable in the difference equation. Therefore, the best results were obtained assuming the relative mortality rate was proportional to age in the shapes of $(\partial N/\partial E)/N = \alpha$ and $(\partial N/\partial E)/N = \alpha E^{\beta}$. These assumptions also were in line with other studies, in which, given the objective of simplifying the development of mortality models, it was assumed that relative mortality rates were independent of site quality (1, 13).

No mortality was recorded in the first measurement carried out in this study, like what happened with adult stands: often, during long intervals of time in the first growth stages, no crop mortality was registered. Between 36.7% and 44.6% of initial time intervals did not show mortality, according to Zhao *et al.* (2007). The authors noted that this problem was the main cause of a lack of convergence in the non-linear mortality models. Woollons (1998) recommended extracting data that do not present mortality, since these complicate the estimation and can cause the model to underestimate the stand's mortality rate. Other authors noted that by maintaining only the information presented by mortality, the model can overestimate the mortality rate (6, 24). In this study, the longitudinal extraction of information was not required since mortality was manifested as of the fourth month of the trial.

Mortality rates observed for this crop varied widely between species (figure 3, page 154). The experimental units established with *E. camaldulensis* showed the lowest mortality rates, followed by *A. melanoxylon* and *E. nitens*. Apparently, *E. nitens* showed the highest level of stress due to strong intraspecific competition, which was expressed in greater mortality rates. The model proposed by Wittwer *et al.* (1997) indicated that the slope of the relationship of density-D_q in *A. melanoxylon* was -0.015, -0.062, and -0.204 for 5,000, 7,500, and 10,000 trees ha^{-1}, respectively, and -0.286, -0.311, and -0.393 for 5,000, 7,500, and 10,000 trees ha^{-1} for *E. nitens*, respectively. For *E. camaldulensis*, slope value was close to zero, since mortality for this species was near zero (figure 3, page 154). But these results were heavily conditioned by the high persistence of these species. Empirical data from this trial showed a large proportion of individuals in a clearly suppressed social class. Sandoval *et al.* (2012) analyzed the evolution of the diameter distributions in this trial, reporting that the distribution tended to be more platykurtic in the measure that the crop grew, but smaller-diameter classes were constantly causing a skew towards the left of the distribution. This revealed the high persistence of these species, which conditioned the relationship of stand density with size and allowed the observed mortality to be attributed to the levels of intraspecific competition generated in the trial.

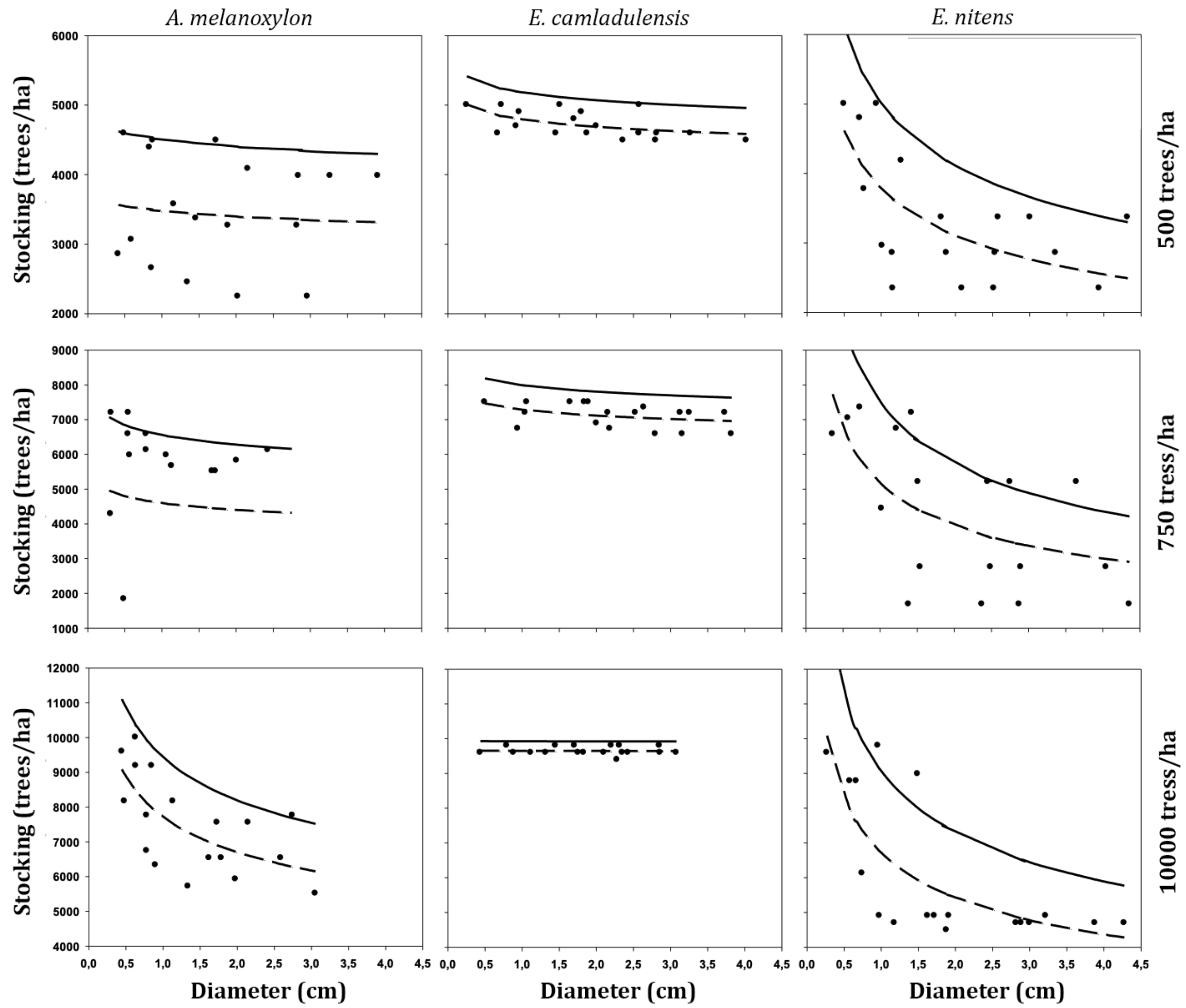

Figure 3. Relationship between average and maximum size-density (solid line = stand density index maximum; dashed line = stand density index average).

Figura 3. Relación entre tamaño-densidad media y máxima (línea sólida = índice de densidad del rodal máximo; línea segmentada = índice promedio de densidad del rodal.

Conclusions

Forty months after the establishment of the crop, *E. camaldulensis* was the species that had the greatest survival rate, followed by *A. melanoxylon* and *E. nitens*. Evidently, *E. camaldulensis* is a highly persistent species, unlike the other two. Nonetheless, another study conducted with these crops showed low biomass yields for *E. camaldulensis* and *A. melanoxylon*, indicating that, despite the high mortality rates of *E. nitens*, this is an interesting species to consider for establishment as a dendroenergetic crop.

The mortality models derived from difference equations showed, in general, greater precision than the survival probability equations. The former was also advantageous in terms of parameter significance. Therefore, study results indicated that difference equations should be used for modeling the mortality of crops intended for dendroenergetic purposes. The survival probability equations showed estimation difficulties, apparently due to the limited number of observations.

Of the eight difference equations tested, model [5] was the most appropriate for modeling the mortality of dendroenergetic crops. Although models [5], [6], and [7] presented a similar estimation error, model [5] surpassed them in terms of parsimony. These results showed that the relative mortality rate remained constant, which was well-described by relationship $(\partial N / \partial E) / N = \alpha$.

There was no statistical evidence of a significant effect of plantation density on the mortality models based on the difference equations. Plantation density level fitting was not justified in the three best models. The results showed a constant relative mortality rate, which indicated that the rate of decline in number of trees was similar between plantation densities. Although the results clearly showed that the rate of decline in the number of trees increased at greater plantation density, this difference was slight and not significant. Moreover, this result was probably also influenced by the high variability of the information registered at this stage of the crop.

The relative mortality rate was well modeled by difference equation [5], which assumed a constant, predictable decline in the number of trees based on crop age. Density-size relationship analysis showed the site was still not at its maximum load capacity. Nonetheless, this result was heavily conditioned by the high level of persistence of the species. Therefore, mortality modeling is recommended for crops established at high plantation densities and, with these species, this may be done following a classification of the social stratum, excluding suppressed trees from the analysis. Thus, including dead and suppressed trees as “social mortality” could improve the density-size relationship and the estimate of the number of live trees would then concentrate on individuals of greater interest in terms of biomass yield.

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Root length density (RLD) of oil palm (*Elaeis guineensis* Jacq) in a haplic Luvisol in Chiapas, Mexico

Densidad de longitud de raíces (DLR) en palma de aceite (*Elaeis guineensis* Jacq) en un Luvisol de Chiapas, México

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Abstract

The tight relationship between root architecture and uptake capacity of soil water and minerals, is well established. Support roots, generally long-lived, perform support functions such as transportation and food storage. Absorbing roots, thin and short-lived, absorb nutrients and regulate plant metabolism. Roots distribution in the soil profile is crucial for plant development. It optimizes resource usage and ensures a prompt response to seasonal changes. This work aimed to study the vertical distribution of the root system of nine-year-old oil palms in a haplic Luvisol, low fertility, moderately acidic, with Nitrogen (N) and Potassium (K) deficiency, average content of Phosphorous (P), and medium to low Cation Exchange Capacity (CEC). Using the cylinder method, soil samples were collected every 10 cm and down to 150 cm of soil depth, from each cardinal side of three soil profiles. The results showed that oil palms had good root development. Most roots (73%) were found in the first 30 cm of soil, with a predominance of fine roots (78%). At 50 cm in depth, fine roots represented 88%, thin roots, 67% and medium roots, 94%. Further study should assess root length density at 15, 20, 25, and 30 years.

Keywords
fine roots • number of roots • soil profile • absorption of nutrients

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Resumen

Existe una relación entre la arquitectura de las raíces (patrones de ramificación y de anclaje) y la captación de recursos hídricos y minerales del suelo: las raíces de soporte, longevas, realizan funciones de apoyo, transporte y almacenamiento de alimentos; las de alimentación, delgadas y de vida corta, aseguran la absorción y el metabolismo de la planta. La distribución de las raíces en el perfil es crucial para el buen desarrollo y la optimización de los recursos disponibles y asegura una pronta respuesta a cambios estacionales. El objetivo fue estudiar la distribución vertical de raíces de palma de aceite de nueve años de edad en un Luvisol háplico de fertilidad baja, pH moderadamente ácido, deficiente de N, con contenido medio de P, muy deficiente de K y CIC baja a media en la profundidad. Mediante el método del cilindro se tomaron muestras de suelo cada 10 cm hasta 150 en cuatro caras de tres perfiles. Los resultados mostraron un buen desarrollo radical de la palma; la mayor cantidad de raíces (73%) se encontró los primeros 30 cm, predominando las raíces finas (78%); en los 50 cm de profundidad se ubicaron: 88% de raíces finas, 67% de delgadas y 94% de medias. Se necesitan más estudios para observar cómo se comporta la densidad de longitud de raíces a los 15, 20, 25 y 30 años de edad de las plantas.

Palabras Clave
raíces finas • número de raíces • perfil edáfico • absorción de nutrientes

Introduction

Besides being the main nutrient-fixing organ in plants, roots are responsible for soil water and nutrient uptake. Their absorption capacity is directly related to their developmental stage. Branching and penetration are root prime morphological characteristics supporting plant development in moisture deficient soils. A greater or lesser root development determines water absorption rates, and influences important physiological processes such as photosynthesis, respiration, and cellular elongation, among other metabolic activities (1, 12, 18).

Roots play a vital role in crop yield. The mentioned water and nutrient uptake directly depends on transport efficiency of root cell plasma membranes, root architecture, nutrient transport regulation, and root growth (14, 19). In this sense, Jackson *et al*. (2008) have recognized the close relationship between root architecture (branching and penetration patterns) and soil water and mineral uptake. They also agree with Jenik *et al.* (1964), who classified the root system into a) '*macrorhizae*', including long-lived support roots, which carry out most support, transport, and storage functions; and b) *'brachyrhizae'* or feeder roots, generally thin, short-lived and carrying out absorption and metabolic functions (rhizosynthesis). Holdaway *et al*. (2011) argued that roots are denser when subjected to moisture and nutrient restrictions. In agreement, a slightly higher proportion of fine roots was found in sites with low nutrient content and penetration restrictions. It has also been reported that tree root production generally follows aerial growth patterns, tending to stabilize with canopy closing (7, 38).

In oil palms (*Elaeis guineensis* Jacq.), the highest proportion of absorbing roots is located in the superficial horizons, at depths greater than 45 cm (4). Fine roots are generally found between 0 and 20 cm of soil depth, while thin and medium roots are found between 20 and 45 cm. From that depth downwards, the proportion of roots per unit volume of soil decreases sharply (3, 6, 33). Oil palm root system is characterized by stable and continuous growth. From germination and during the first year, fine roots grow 1 cm day^{-1}. When the plant reaches adulthood (>10 years), fine rooting drops to 0.1 cm day^{-1}(13). Vertical growth patterns of oil palms show a constant direction, conditioned by gravity, whereas horizontal growth shows either upward or downward direction. This is partly determined by emergence times and environmental conditions such as light, temperature, pH, and oxygen availability, among others (14). Subsoil root growth and development may also be affected by mechanical or chemical properties such as acidity and high water table (17, 31).

Root activity is based on biomass and root length (26, 37), which indirectly reflect plant absorbing capacity (18, 33, 35). Root length density (RLD), *i.e.* root length (cm) and diameter (fine, thin, medium) in a specific area, is associated with soil aeration and water/

nutrient absorption and transport (28). Root vertical distribution allows quick responses to seasonal changes affecting water and nutrient availability.

Unlike the reasonably well studied production rate of aerial biomass, information on root biomass remains rather limited, mainly due to methodological issues (21). Therefore, the present study aimed to describe vertical distribution of different types of oil palm roots and its association with soil bulk density in a haplic Luvisol soil, a deep and imperfectly drained soil with a short waterlogging period.

Materials and methods

Fieldwork was carried out in a 9 year old commercial oil palm (*Elaeis guineensis* Jacq.) plantation, with an area of 1-30-00 ha, in the ejido Filadelfia (17°37'10'' N and 91°55'50'' W), municipality of Palenque, Chiapas, Mexico. The cultivated genotype was hybrid Deli x Avros. The plantation was established at 9 x 9 m (planted in a triangular pitch arrangement). Mean annual precipitation and temperature are 2500 mm and 27 °C, respectively, and average altitude of 29 meters above sea level (9).

The soil was described and classified after Cuanalo (1990). A 1 kg sample was taken from each horizon for analysis at the Laboratory of Water, Soil and Plants of the Graduate College, Tabasco Campus. The following parameters were determined in all soil samples: organic matter (OM) using Walkley and Black method; pH by water potentiometry (1:2), and texture by Bouyoucos. Ca^{2+}, Mg^{2+}, K^{+}, Na^{+} were extracted using 1 N ammonium acetate at pH 7.0 and quantified by spectrophotometry. Extractable P was obtained by the Olsen method, and total N by the semi-micro Kjeldahl method. All analyzes were performed as proposed by the Official Mexican Standard (2002). The soil was classified according to the World Reference Base for Soil Resources based on physicochemical analysis results and soil profile descriptions (36).

For root and soil bulk density, three wells were dug to a depth of 1.5 m at representative points of the studied area, in between plants, equidistant from the nearest palms. Samples were taken from each of the four cardinal sides (north-south-east-west) of each soil profile, every 10 cm, using the cylinder method (33). Fifteen 270.5 cm^3 samples, were taken from each cardinal side, 60 per profile. Root vertical distribution was observed on these samples.

For each sample, roots were carefully washed, measured (length and diameter) and oven-dried at 70° C to constant weight. Later, they were weighed with precision balance (± 0.0001 g). According to diameter, roots were classified as: fine (<1 mm), thin (1-3 mm) and medium (3-10 mm) (5). Root length density (RLD) was calculated after Moreno *et al.* (2005):

$$RLD = \text{Root Length}/\text{Soil Volume}$$

where:

root length = expressed in km and soil volume in m^3.

Soil samples used to estimate bulk density were dried in a forced circulation oven (Venticell) at 60°C to constant weight, for approximately 72 hours. Estimation was made according to:

$$DA = \text{Soil weight}/\text{soil volume}$$

where:

soil weight = expressed in g and soil volume in cm^3.

The resulting data were subjected to ANOVA, multiple mean comparisons (Tukey α= 0.5), and correlation analysis using the SAS statistical package (2004), version 9.1.

Results and discussion

Soil classification and description

The studied soil was a haplic Luvisol (LVha) with a B argic horizon. Sandy, clayey and loamy textured. According to the NOM-021-RECNAT-2000, mildly acidic in the first 30 cm depth and moderately alkaline at 30-60 cm depth (table 1, page 160). At both depth levels, total N content is low, while P content is medium in the first 30 cm and low in the 30-60 cm level. K shows very low values at both depths. Exchangeable Ca and Mg show medium and high concentrations, respectively, while cation exchange capacity (CIC) is low in the first 30 cm of soil depth, reaching medium level at 30 to 60 cm depth.

Table 1. Physicochemical properties of a Haplic luvisol cultivated with oil palm in Ejido Filadelfia, Palenque, Chiapas, Mexico.

Tabla 1. Propiedades fisicoquímicas del Luvisol háplico cultivado con palma de aceite en el Ejido Filadelfia, Palenque, Chiapas, México.

Parameter	Unit	Depth (cm)	
		0-30	30-60
Acidity	pH	5.63	7.43
CIC	$Cmol^{+}kg^{-1}$	9.1	16.55
MO	%	1.65	0.95
Nt	%	0.08	0.07
P-Olsen	mg kg^{-1}	5.55	1.25
K	$Cmol^{+}$ kg^{-1}	0.07	0.07
Ca	$Cmol^{+}$ kg^{-1}	6.25	11.65
Mg	$Cmol^{+}$ kg^{-1}	4.2	5
Na	$Cmol^{+}$ kg^{-1}	0.1	0.24
B	mg kg^{-1}	15.67	7.79
Zn	mg kg^{-1}	66.06	0.5

Physiographically, Haplic Luvisols are located in flat areas with slightly convex slopes (less than 0.5%). Their parent material corresponds to alluvial sediments influenced by marsh sediments, both from the Pleistocene. They are deep soils with a thin A horizon, loamy-sand textured and dark-brown colored. Imperfectly drained, but with a waterlogging period shorter than that of gleyic Luvisols. In these soils, distance to the water table and humidity regime are more important than low permeability (26). Haplic luvisols found in Savannah of Palenque are usually covered by forests and agricultural lands (26). This region hosts major rubber (*Hevea brasiliensis* L.) and oil palm (*Elaeis guineensis* Jacq.) plantations, grazing lands, and, during a certain period each year, watermelon (*Citrullus lanatus* Thunb.), Haplic luvisols are optimal soils for oil palm cultivation due to their depth (> 150cm), over 50% base saturation, and pH of 5.5-6.6 (16). In the present study, deficiencies in primary macronutrients were detected. Other important soil parameters, such as pH and CEC, indicated that oil palms can grow and develop without toxicity problems and that chemical fertilizers correcting the mentioned deficiencies can be retained in the exchange complex (16, 20, 30). Soil organic matter was low at the two sampled depths (0-30 and 30-60 cm). Although low organic matter is characteristic of oil palm plantations in the area, this could be a limiting factor, considering the role of OM in soil physical, chemical, and biological fertility. Regarding physical factors, and given soil structure, oil palm roots were not able to fully breathe and grow.

Vertical root distribution

Figure 1 (page 161) shows that root length density (RLD) decreased as soil depth increased. The highest number of oil palm roots (73%) was found in the first 30 cm, with 78% of fine roots, in agreement with Barrios *et al.* (2003). Although most oil palm roots are found in surface horizons, roots can still be found at depths of up to 1.5-5 m (37).

Fine roots resulted the most abundant through the entire soil profile, evidencing good absorption and metabolic activity (10, 37). The largest number (341) of fine roots was found in the first 10 cm, followed by thin roots (11) and medium roots (figure 2, page 161).

Roots: RzF = fine; RzD = thin; RzM = medium.

Raíces: RzF = finas, RzD = delgadas, RzM = medias.

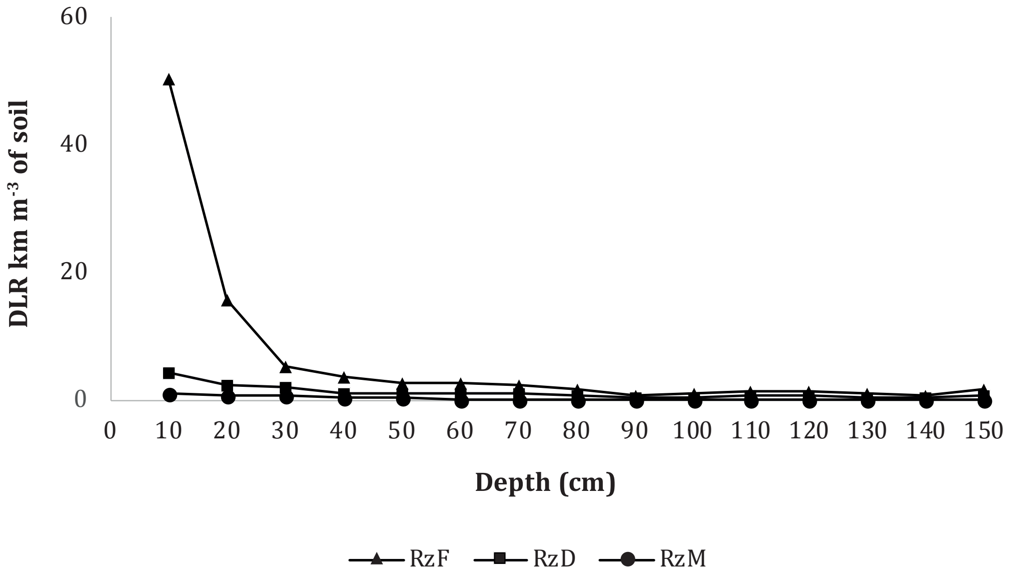

Figure 1. Root length density (km m^{-3}) of oil palms cultivated in a haplic Luvisol in Palenque, Mexico.

Figura 1. Densidad de longitud de raíces (km m^{-3}) de palma de aceite cultivada en un Luvisol háplico de Palenque, México.

Roots: RzF = fine; RzD = thin; RzM = medium.

Raíces: RzF = finas, RzD = delgadas, RzM = medias.

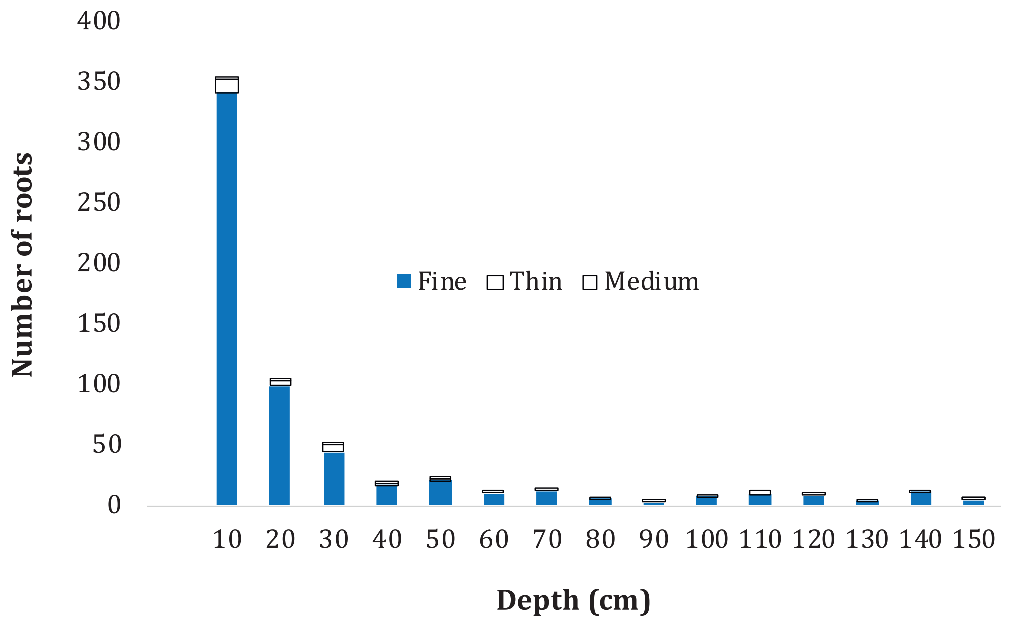

Figure 2. Distribution of the number of oil palm roots growing in a haplic Luvisol in Palenque, Mexico.

Figura 2. Distribución de número de raíces de palma de aceite cultivada en un Luvisol háplico de Palenque, México.

Table 2 shows comparison of means for RLD, for p ≤ 0.05. In the first 10 cm depth numerous, mainly fine roots were observed. Significant statistical differences in RLD values were found between the first 10 cm and the other depths for each type of roots (fine, thin and medium). As depth increased, RLD values became more homogeneous. These results differ from previous studiesfinding most roots at greater depths, in the first 45 cm (6, 33), or deeper (2, 23), even for other tree species (18, 22, 25). Bulk density did not show significant differences among depths (table 2), nor a relationship with DLR. The African palm, due to its high capacity for radical exploration at high depths, may contribute to high soil bulk density levels through root decomposition (15, 29).

Table 2. Root Length Density (RLD km m^{-3}) according to diameter, depth and bulk density in an oil palm plantation on haplic Luvisol, Palenque, Mexico.

Tabla 2. Densidad de Longitud de Raíces (DLR km m^{-3}) según diámetro, profundidad y densidad aparente en una plantación de palma de aceite cultivada en un Luvisol háplico de Palenque, México.

Depth (cm)	Bulk density (t m^{-3})	Fine	Thin	Medium
		(km m^{-3})		
10	1.14 a	50.20 a	4.37 a	1.17 a
20	1.10 a	15.55 b	2.34 b	0.68 ab
30	1.09 a	5.25 c	2.13 cb	0.54 abc
40	1.14 a	3.59 c	1.00 cbd	0.38 bc
50	1.17 a	2.74 c	0.98 cbd	0.23 bc
60	1.10 a	2.64 c	0.94 cbd	0.17 bc
70	1.14 a	2.29 c	0.94 cbd	0.11 bc
80	1.21 a	1.61 c	0.71 cd	0.06 bc
90	1.12 a	0.84 c	0.49 d	0.00 c
100	1.14 a	1.13 c	0.52 d	0.00 c
110	1.18 a	1.32 c	0.53 d	0.04 bc
120	1.10 a	1.50 c	0.62 cd	0.05 bc
130	1.17 a	1.00 c	0.51 d	0.00 c
140	1.03 a	0.82 c	0.38 d	0.00 c
150	1.23 a	1.62 c	0.83 cbd	0.06 bc

N = 12. Different letters within columns indicate significant differences (Tukey α = 0.05).

N = 12. Letras diferentes dentro de columnas indican diferencias significativas (Tukey α = 0,05).

Significant differences were found between number of fine (NFR) and thin roots (NTR) in the first 10 cm, and deeper. However, no significant differences in number of medium roots (NMR) (table 3, page 163) could be observed. The first 50 cm of soil depth contained 88% of total fine roots, 67% of total thin roots, and 94% of total medium roots. The number of medium roots decreased between 60 and 140 cm, showing up again at 150 cm.

The results showed that fine roots were distributed throughout the entire soil profile, evidencing high nutrient-absorption and metabolic activities, resulting in good palm oil yields, despite soil limitations. In Indonesia, where this palm is cultivated on millions of hectares, biomass yield is of great importance for carbon capture and degraded soils restoration (34).

Table 3. Number of roots (NR) by depth, diameter and bulk density in oil palms grown in a haplic Luvisol soil in Palenque, Mexico.

Tabla 3. Número de raíces (NR) por profundidad, diámetro y densidad aparente en palma de aceite cultivada sobre un suelo Luvisol háplico de Palenque, México.

Depth (cm)	Bulk density ($t\ m^{-3}$)	Fine	Thin	Medium
10	1.14 a	341.33 a	10.00 a	0.667 b
20	1.10 a	98.33 b	4.00 b	0.333 b
30	1.09 a	43.67 bc	6.00 ab	3.000 a
40	1.14 a	16.33 c	1.667 b	0.667 b
50	1.17 a	20.67 c	1.333 b	0.333 b
60	1.10 a	9.67 c	0.667 b	0.000 b
70	1.14 a	12.00 c	1.00 b	0.000 b
80	1.21 a	5.00 c	0.333 b	0.000 b
90	1.12 a	2.67 c	0.667 b	0.000 b
100	1.14 a	7.00 c	0.333 b	0.000 b
110	1.18 a	9.33 c	3.00 b	0.000 b
120	1.10 a	8.00 c	2.333 b	0.000 b
130	1.17 a	3.67 c	0.667 b	0.000 b
140	1.03 a	10.67 c	1.667 b	0.000 b
150	1.23 a	4.67 c	0.667 b	0.333 b

N = 12. Different letters within columns indicate significant differences (Tukey α = 0.05).

N = 12. Letras diferentes dentro de columnas indican diferencias significativas (Tukey α = 0,05).

Conclusions

The studied soil was classified as a haplic Luvisol. The highest number of oil palm roots was found in the first 30 cm of soil (73%), most of them being fine roots (78%). The first 50 cm contained 88, 67 and 94% of total fine, thin, and medium roots, respectively. Further study is required to assess the behavior of root length density over the years, for 15, 20, 25 and 30 years old plants.

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Implantation of corporate social responsibility measures in the horticulture in Mexico and Spain

Implantación de medidas de responsabilidad social corporativa en la horticultura de México y España

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Abstract

This work presents the results of a Delphi survey aimed at experts in the horticultural activity in Mexico and Spain, to find out the main stakeholders and factors that influence the adoption of Corporate Social Responsibility (CSR) measures and standards, as well as the limitations in their implementation. The Delphi analysis concluded that companies in Mexico and Spain adopt CSR measures and standards mainly at the request of destination supermarkets in the US and Europe, which hold the greatest power in the global value chain. In addition, it is identified that with the adoption of CSR, companies seek to improve their image in the field and gain competitive advantages in the market. Among the limitations are the lack of business culture, ignorance of the protocols, and high administrative burden, especially among smaller production companies. Experts consider that CSR measures and standards have a positive impact on the field. The research is completed with a SWOT analysis (Weaknesses, Threats, Strengths, and Opportunities), providing a ranking list of great interest for the design of competitive improvements.

Keywords
Corporate Social Responsibility • standards • horticulture • Delphi • Mexico • Spain

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Resumen

Este trabajo presenta los resultados de una encuesta Delphi dirigida a expertos de la actividad hortícola de México y España, con el objetivo de conocer los principales grupos de interés (*stakeholders*) y factores que influyen en la adopción de medidas y estándares de Responsabilidad Social Corporativa (RSC) así como las limitaciones para implementarlas. El análisis Delphi permitió concluir que las empresas de México y España adoptan medidas y estándares de RSC principalmente por requerimiento de los supermercados de destino en EE. UU. y Europa, quienes ostentan el mayor poder en la cadena global de valor. Además, se identifica que con la adopción de RSC buscan mejorar la imagen de la empresa en la actividad y ganar ventajas competitivas en el mercado. Entre las limitaciones están la falta de cultura empresarial, el desconocimiento de los protocolos y la alta carga administrativa, en especial entre las empresas productoras de menor tamaño. Los expertos consideran que las medidas y estándares de RSC tienen un impacto positivo en el campo. Se finaliza el estudio con un análisis DAFO (Debilidades, Amenazas, Fortalezas y Oportunidades), aportando un valioso ranking de interés para el diseño de mejoras competitivas en la actividad hortícola de ambos países.

Palabras clave

Responsabilidad Social Corporativa • estándares • horticultura • Delphi • México • España

Introduction

In Southeastern Spain (Region of Murcia and Almería) and Northwestern Mexico (Sinaloa and Baja California), there are two important horticultural production areas which commonly supply to the European and American markets, outside the summer season. Intensive horticulture in these areas has been developed alongside the implementation of numerous measures and standards of Corporate Social Responsibility (CSR), which are common among the production companies that lead the international agricultural sector.

The CSR was first used in Howard R. Bowen's book "Social Responsibilities of Businessmen" in 1953. Later, in 1979, Archie Carroll defined CSR as what society expects of companies in economic, ethical, legal, and philanthropic matters. Currently, there are more than thirty-seven definitions of CSR in the academy (10). The concept used in this research is that of the ISO 26000, one of the most accepted worldwide. They define the CSR as the responsibility that companies have over the actions, they carry out concerning society and the environment, for which communication with stakeholders is transcendental (26).

In the implementation of CSR, the stakeholder theory explains how companies are obliged to respond to society. Stakeholders are groups or individuals who can benefit or be harmed by the action of the company. Stakeholders can be grouped into two categories: the first one, which includes employees, owners, customers, the local community, and suppliers, is vital for the endurance of the company. The second one includes groups or individuals such as the government, NGOs, and competitors that can be affected by the corporation (10, 15).

According to Esteban (2007), the theory of stakeholders is relevant because it allows the transition from theory to practice in the field of social responsibility. This theory sees the intertwining of relationships that a company is subject to, which is diverse. On the other hand, the existence and recognition of stakeholders have allowed companies to articulate various CSR actions in their management, sometimes voluntarily, sometimes as a strategy to obtain a better reputation, and with it minimize risks. Companies also articulate CSR action pressured by non-governmental organizations, competitors, or even by international regulations (3). At the same time, the benefits of being a responsible company are notable, for instance, making decisions in the present ensures long-term permanence; as many government regulations are avoided by anticipating new demands (11).

In this sense, Porter and Kramer (2006) indicate that companies must adopt CSR measures to obtain competitive advantages over other companies, such as improving the company's image and reputation, or ensuring its permanence in the market. For this, the company must strategically select what actions and CSR measures to develop. These authors consider the stakeholder theory to be a wrong approach on exercising CSR.

For Luhmann and Theuvsen (2016) the development of CSR measures in the agribusiness sector is determined by the size of the company, its degree of internationalization, brand orientation, and its position in the value chain. In the agricultural export activity, specifically, Food Retail Markets (FRM) have developed several standards to avoid certain risks that could damage their reputation or image (5, 16, 19, 21, 24). The growing importance of new standards and norms in agricultural systems around the world should not be forgotten, as pointed out by Handschuch *et al.* (2013); in the case of export-oriented countries, abiding those standards and norms ensures continuous access to the main markets.

The private social responsibility standards and measures imposed by the FRM are set considering their global purchasing power, especially in developed countries; for instance, the United Kingdom, where five retailers owned more than 75% of the market share by the end of the 1990s (Freidberg, 2007). This purchasing power allows companies to impose numerous seemingly “voluntary” buying rules on producers, which, if breached, will cause them to not be accepted as suppliers; a similar case can be observed in agro-export companies in the two production zones studied simultaneously in Mexico and Spain.

The decade of the 1990s reported a couple of international incidents related with the lack of food safety in fresh vegetables, which had a lot of media coverage, helped developed countries to achieve an important milestone in food safety regulations (2). This led national governments to make retailers responsible for food safety. In turn, retailers handled their agricultural supplies responsibly through quality standards and traceability, among others (30). Today, private standards have become a market entry requirement for producers, in both Europe and the US, because of environmental and social justice problems that governments have been unable or unwilling to solve (6, 17, 20, 39). The FRM seeks to ensure compliance with national and/or international food safety, quality, labor, and environmental regulations through certifications, setting higher standards than those established by national organizations. Nowadays, the agribusiness sector has a plethora of CSR standards (35).

The objective of this work is to analyze which factors and/or stakeholders drive the development and implementation of CSR measures, as well as the limiting factors for their development, through the Delphi methodology applied to a panel of experts from the horticulture activity in Spain and Mexico. As a secondary objective, it is discussed whether these CSR measures lead to labor conditions and environmental improvements.

The hypothesis underlying this work is that mainly the value chain, controlled by retailers, drives the implementation of CSR measures and standards in the export-oriented horticultural activity in both Mexico and Spain. By adopting measures and standards of social responsibility, agricultural companies seek to gain competitive advantages, remain in the market, and improve the reputation of the sector. On the other hand, the limitations of CSR are the lack of business culture and the administrative burden.

Another hypothesis in this work is that implementing both measures and standards of Corporate Social Responsibility is considered positive for the improvement of labor, human rights, and environmental practices, more as a strategic objective than as a means for immediate benefits for the community. Although there is still a long way to go, the contribution of this work lies in analyzing the implementation of CSR measures and standards in two distant but similar agricultural countries: Mexico and Spain. These countries, despite having different markets, such as North America and Europe as the final destination for their products, share many similarities in the development of CSR initiatives, implemented in their production systems in the last two decades.

Materials and methods

A questionnaire was designed and applied to a panel of experts from the horticultural activity in Mexico and Spain following the Delphi methodology, which was developed iteratively in two rounds. The objective was to know the experts´ qualified opinion on the motivations that lead to the implementation of CSR measures and standards, their limitations, and the situation of the fruit and vegetable export activity in Mexico and Spain.

The qualitative and exploratory Delphi methodology is defined as a systematic and iterative process aimed at obtaining expert group opinions with the objective of reaching consensus (28, 29). It is used to obtain the qualified assessment from a low number of knowledgeable respondents in the object of study, which allows identifying similar trends or aspects where there is a consensus. The possibility that in a 2nd round respondents can modify their responses given in the first submission, allows them to reinforce their agreement or disagreement and include relevant aspects that were not initially considered, providing information about their needs or priorities (27). One of the disadvantages of this approach is the risk of respondents losing interest in participating in the second or later rounds, which is considered normal (27).

In recent decades, the Delphi method has become a tool used to analyze complex realities in the agri-food field in Spain and Mexico. These realities are food consumption (12), agricultural policy analysis (32), design components as a non-technological innovation (22) and the evaluation of the sustainability of production systems and rural areas (1, 33), among others.

The questionnaire designed to carry out the first round of the Delphi method in this work included statements that had to be assessed according to the Likert scale of 1 to 5 (1 Strongly Disagree and 5 Strongly Agree), and open-ended questions to generate new options to be assessed in the second round. The questions included in that first round were the result of the opinions expressed in previously conducted personal interviews with some experts, but they were mostly taken from the literature review on CSR in the agricultural sector, as previously mentioned in the introductory heading. Prior to the first contact with the experts, a pilot questionnaire was conducted with only two experts in October 2017, one in Spain and one in Mexico.

The applied questionnaire consisted of four blocks. The first block gathered information about the participating experts such as years of activity in the sector and their level of knowledge in marketing and CSR. The second block gathered information regarding their opinion and motivations for taking CSR initiatives. In the third block, trends in standards, initiatives, and the challenges in the fruit and vegetables export sector in both economies were analyzed using the SWOT analysis (Strengths, Weaknesses, Opportunities, and Threats)

For this Delphi, a varied group of participants was gathered in order to ensure the diversity of opinions. The first Delphi round took place in November 2017, in which 40 specialists and technicians participated: 16 experts from Mexico and 8 from Spain, with 24 respondents. The second round of the Delphi was sent in February 2018, obtaining responses from 20 experts: 13 from Mexico and 7 from Spain. It should be noted that the adequate number of experts for a Delphi must be small, ranging from 7 to 30 participants (Landeta, 1999), so the total number of experts participating in this work was considered appropriate. The joint consideration of all responses was valuable, as was the analysis of the individual view that experts from both countries had about certain respects.

As noted, the second round provided the panel of experts who responded to the first round with all the statistical results achieved by the group (mean, mode, and frequency of the answers to all questions from the first submission) in each of the questions. This allows the group of experts to change or maintain their assessments and then assess the new items proposed by the group, as well as the possible questions or answers incorporated into the questionnaire after the first round. A third round was discarded because the responses in the second round achieved an adequate level of stability, as measured by the Coefficient of Variation and the study of the reduction of the Interquartile Range, and because of the significant loss of participants that would occur with a new round (Landeta, 1999).

Characterization of the experts

Out of the 20 experts who were formerly interviewed and responded to the questionnaire in the second round, 13 were from Mexico and 7 from Spain; all of them with various professional backgrounds, which guaranteed the diversity of views. 3 experts were farmers, 2 were field technicians, 1 was the quality manager in an agricultural company, 1 was the manager in the commercial area, 1 was an agricultural company manager, 1 was the representative from a marketing company, 3 were representatives of the distributors' association, 3 were researchers, 2 were certification consultants, and 2 were government officials in the horticultural field. The respondents were mostly men (65%), all of them with university studies (65%) or postgraduate studies (35%), with an average age of 43 years, and with more than 16 years of experience in the sector. Concerning the level of knowledge in marketing and the CSR measures and standards in the agricultural sector, all technicians demonstrated a "high" level of knowledge on these three aspects, which the questionnaire focused on, and they are analyzed in the following sections.

Results and discussion

According to the experts' responses in the second round, horticultural activity is a leader in the application of measures and standards of Corporate Social Responsibility (CSR) within the national agriculture, both in Mexico and Spain. This is due to the consensus in the understanding that those measures and standards are an "imposition by customers and supermarket chains" of their products' destination countries, the fact that 85% of the panel of expert estimated it, confirms the arguments described in the literature (9, 18). Other motivations, such as "the need for the activity to be more environmentally, socially, and economically sustainable" (70%) and the "concern for workers" (60%), are also considered important; these last claims have reached a consensus, while dissent is high in considering that the "positive results of investing in CSR shares" (50%).

Sixty-five percent of the consulted experts consider the adoption of CSR actions "positive" for the horticultural activity, with 40% of them pointing out positive and negative elements. Regarding some considerations described in the literature, there is a high level of consensus to understand such actions, as shown in table 1 (page 170). First there is the adoption of CSR actions allows companies to "gain competitive advantages," with 90% of experts "agreeing or totally agreeing" with that claim, which according to the literature is supposed to be the one that achieves a higher average rating and a median response of 5, equivalent to fully agreeing with it. In second place there is "demands of the distribution chains, which demand and value the efforts of CSR" with 85% of responses; this affirmation could be ranked as the second most important motivation due to the slightly lower than average score it obtained in Spain, with average scores higher than 4, equivalent to agreeing with the statement. It is followed by "improving the image or general reputation of the company within the sector "in third place, then "responding to the demands of end consumers, who demand and value the CSR effort" in forth, and finally "to stay in the market, following the trends that are set in it" in fifth place.

The latter, in which the level of agreement is slightly lower, refers to how the adoption of CSR can allow companies to obtain greater profitability. Other reasons, such as "the company's concern for wanting to take better care of workers and contribute to social welfare," "being concerned about the environment," and "responding to the legal framework and achieving tax benefits", are not relevant issues showing a low level of consensus.

The Delphi survey confirmed the initial hypothesis for this study. There are several stakeholders and important factors in the adoption of CSR standards and measures to consider; among the most important factors are the influence of the distribution chains of food retail in the export's destination countries, improving the image of the companies, and obtaining competitive advantages. It should be noted that the two horticultural production areas studied are part of global food chains in which the power of retail distribution is remarkably high, so they face a lot of competition, for this reason they have become leaders in the implementation of CSR standards.

When comparing the reasons for implementing CSR actions in Mexico and Spain, it is possible to identify that in Mexico the main reason is "to respond to the requirements of the distribution chains, which demand and value CSR efforts". In contrast, Spanish experts considered the aforementioned reason important; however, they believe that the strongest reason to implement CSR actions is "to improve the image or general reputation of the company within the sector". This finding is similar to that of Martos-Pedrero *et al.* (2019) who indicate that CSR measures in the agricultural activity in Almeria have a positive impact on the companies' reputation, economic performance, and their relationship with stakeholders. Meanwhile, Briones Peñalver *et al.* (2017) indicate that CSR measures in the agribusiness in the Region of Murcia have a positive impact on its development, which in turn, positively influences its economic performance.

Table 1. Main reasons that lead companies in the exporting horticulture in Mexico and Spain to implement CSR initiatives.

Tabla 1. Principales motivos que llevan a las empresas de la horticultura de exportación de México y España a implantar iniciativas de RSC.

Source: Own elaboration from the Delphi Survey (2nd round).

Fuente: Elaboración propia.

Valuation from 1 to 5: 1. Not at all Important; 2. Unimportant; 3. Neutral; 4. Important; 5. Very important.	Mexico		Spain		Total	
	Me	**Md**	**Me**	**Md**	**Me**	**Md**
Gaining competitive advantages.	4.4	5.0	4.6	5.0	4.5	5.0
Responding to the demands of distribution chains, which demand and value the efforts of CSR.	4.5	5.0	4.3	5.0	4.5	5.0
Improving the overall image or reputation of the company within the sector.	4.1	4.0	4.7	5.0	4.3	5.0
Responding to the demands of end consumers, who demand and value the efforts of CSR.	4.2	5.0	4.0	4.0	4.1	4.5
Staying in the market, following its trends.	4.0	4.0	4.1	4.0	4.1	4.0
Getting higher profitability.	3.8	4.0	3.9	4.0	3.9	4.0
Responding to the company's concern for workers to contribute to social welfare.	3.3	4.0	4.0	4.0	3.6	4.0
Complying with the demands of a legal framework developed in the adoption of CSR initiatives.	3.5	4.0	3.7	4.0	3.6	4.0
Responding to the company's concern about its impact on the environment.	3.0	3.0	4.3	4.0	3.5	4.0
Being able to access tax benefits and/or access public funding linked to the CSR measures adopted.	2.6	2.0	3.3	3.0	2.9	3.0
Me= Mean. Md=Median.						

There is a notable difference in the responses regarding the reasons to implement CSR measures; in Spain the "concern of the company for the impact on the environment" is better valued with an average of 4.3 while in Mexico it is valued with 3.0 points. This may mean that in the former country there is a bigger concern about this issue than in the latter. In Mexico it is believed that the adoption of CSR measures means not "being able" to access tax benefits and/ or public funding linked to adopting CSR measures, while in Spain they remain neutral about it.

Among the negative arguments about the implementation of CSR initiatives described in the two rounds of interviews, experts pointed out that companies are sometimes "more concerned with their profit than the social or environmental impact they cause". Some experts believe that these are "simulated practices done to comply with customers' demands", considering that there are many entrepreneurs who "are not really concerned or interested in the subject", and that "if managed poorly, these initiatives can generate confrontations and problems".

Regarding the limitations or obstacles companies in the fruit and vegetable agro-export sector of Mexico and Spain face when adopting CSR measures, the level of consensus is high in considering that the "lack of business culture" (with a 90 % of experts ``agree or totally agree" with this statement), "unawareness of the subject" (80%) or the "high administrative burden" (80%) limit the implementation of more measures (table 2, page 171), confirming that the expert panel's considerations were already described in the literature. Considering the relevance given to them by practically all the experts, (they reached an average score close to 4), these three issues are the main obstacles, while the lack of public programs for their promotion was not relevant, or their cost outweighs the benefits.

According to the experts' opinion (table 3, page 171), the CSR initiatives that help make the greatest progress within the export horticultural activity studied, are those referred to as "Labor Practices" (18 out of the 20 experts chose this answer out of the three more advanced answers available). This result differs with De Castro *et al.* (2017) who mention that the adoption of standards in the grape sector in the Region of Murcia (Spain) does not improve labor. On the other hand, the Mexican researchers Avendaño *et al.* (2015) and Villafan and Ayala (2014) indicate that in the agricultural sector in the states of Baja California and Michoacan (Mexico) there are still labor and environmental issues to be worked on. According to table 3 (page 171), the experts interviewed in this Delphi are of the opinion that the fruit and vegetable companies have made progress in the area of CSR, especially in the labor, environmental, and human rights areas both in Mexico and in Spain. This confirms the second hypothesis of this work, the standards and measures of CSR have a positive impact in the sustainability of horticultural activity.

Table 2. Main limitations faced by exporting horticulture companies in Mexico and Spain to develop CSR measures.

Tabla 2. Principales limitaciones a las que se enfrentan las empresas de la horticultura de exportación en México y España para desarrollar medidas de RSC.

Source: Own elaboration from the Delphi Survey (2nd round).

Fuente: Elaboración propia.

*1. Not at all Important; 2. Unimportant; 3. Neutral; 4. Important; 5. Very important.	Mexico		Spain		Total	
	Me	**Md**	**Me**	**Md**	**Me**	**Md**
The lack of "business culture" about the importance of the CSR.	4.2	4.0	4.3	4.0	4.3	4.0
The high administrative burden and the lack of personnel implementing more CSR measures.	4.0	4.0	4.0	4.0	4.0	4.0
Ignorance on the subject.	3.8	4.0	3.9	4.0	3.9	4.0
The lack of public programs to broadcast and promote the CSR, with no government co-responsibility.	3.4	4.0	3.9	4.0	3.6	4.0
The economic cost of CSR measures, which reduce the farmers and marketers' profits.	3.5	4.0	3.1	3.0	3.4	4.0
The activity's characteristics: long work time and a low price of the product.	3.2	3.0	3.7	4.0	3.4	3.5
Me= Mean. Md=Median.						

Table 3. Areas with greater advances in CSR in companies of the exporting horticulture in Mexico and Spain.

Tabla 3. Ámbitos con mayores avances en materia de RSC en las empresas de la horticultura de exportación México y España.

Source: Own elaboration from the Delphi Survey (2nd round).

Fuente: Elaboración propia.

AXES OF ISO 26 000	AF			RF%		
	Mexico	**Spain**	**Total**	**Mexico**	**Spain**	**Total**
Labor Practices.	12	7	18	92.3	100.0	95.0
The Environment.	6	7	14	46.2	100.0	65.0
Human Rights.	9	2	11	69.2	28.6	55.0
Organizational Governance.	6	1	7	46.2	14.3	35.0
Community Involvement and Development.	3	2	5	23.1	28.6	25.0
Consumer Issues.	3	2	5	23.1	28.6	25.0
Fair Operating Practices.	0	0	0	0.0	0.0	0.0
AF= Absolute Frequency. RF (%) = Relative Frequency. Total=Mexico and Spain.						

The level of consensus is high (85%) in considering that large supermarket chains, especially transnationals value their suppliers and agricultural enterprises, with a median of 5 (table 4, page 172), equivalent to fully agreeing with this assertion. Eighty percent of the panel agrees or totally agrees that the main reason supermarkets value CSR practices is because they "do not want to be involved in boycotts or controversies" for buying from fruit companies that badly perform food safety practices, with a median of 5.0 and an average of 4.4. However, experts from Mexico and Spain indicate that national supermarkets, either Mexican or Spanish, depending on the nationality of the expert, do not value CSR practices because consumers do not either, since they are only interested in paying a lower price. The experts dissented with that statement, as well as with the consideration that the price criterion dictates in which supermarket chain to purchase.

As for whether or not end consumers are aware of and value the efforts of companies in the horticultural activity adopting CSR practices, a level of dissent is detected among experts depending on their nationality. While the majority of the experts, globally, point out that end consumers lack awareness and do not value CSR measures, among the expert group in Spain this assessment shows a higher level of consensus (71.4%) (table 5, page 172). This difference in the results may be pointing that American consumers have greater concern and knowledge about such certifications, or perhaps that they pay more attention to food products when they come from a third world country. For US consumers, these products are not normatively integrated as in the European Union and might be produced in countries of lower levels of development such as Mexico. Thus, the private standards such as CSR could ensure that third countries meet the minimum requirement established by the market. This is not the case for the Spanish vegetables that are consumed in other European countries, since they share production regulations.

According to the majority of the panel of experts (90%), there are niche markets that do value CSR initiatives in the agri-food activity (table 6). However, there is a low level of consensus regarding if the end consumers' application and value of CSR is marginal (60%) or if there is a lack of diffusion about the niche markets that do value CSR.

Table 4. Assessment of the supermarket chains to companies of the exporting horticulture in Mexico and Spain who are socially responsible.

Tabla 4. Valoración de las grandes cadenas de supermercados a las empresas de la horticultura de exportación en México y España socialmente responsables.

* 1. Not at all Important; 2. Unimportant; 3. Neutral; 4. Important; 5. Very important.	RF (%)					Me	Md
	1	2	3	4	5		
Transnational supermarkets value CSR and require their suppliers to comply with these kinds of practices.	5.0	5.0	5.0	30.0	55.0	4.3	5.0
Supermarkets value CSR practices because they do not want to be involved in boycotting or controversy.	0.0	5.0	15.0	20.0	60.0	4.4	5.0
Supermarkets in my country do not value CSR, because consumers are only interested in paying low prices.	5.0	20.0	20.0	40.0	15.0	3.4	4.0
Supermarkets do not value CSR because they usually just try to buy from cheaper sellers.	5.0	50.0	15.0	25.0	5.0	2.8	2.0

Source: Own elaboration from the Delphi Survey (2nd round).

Fuente: Elaboración propia.

Table 5. Knowledge and assessment of end consumers on the CSR efforts of exporting horticulture companies in Mexico and Spain.

Tabla 5. Conocimiento y valoración de los consumidores finales sobre los esfuerzos en materia de RSC de las empresas de la horticultura de exportación en México y España.

	AF			RF (%)		
	Mexico	Spain	Total	Mexico	Spain	Total
Yes	6	2	8	46.2	28.6	40.0
No	7	5	12	53.8	71.4	60.0
Total	13	7	20	100.0	100.0	100.0

Source: Own elaboration from the Delphi Survey (2nd round).

Fuente: Elaboración propia.

Table 6. Global assessment of what type of consumers know and value the CSR efforts of companies of the exporting horticulture in Mexico and Spain.

Tabla 6. Valoración global sobre qué tipo de consumidores conocen y valoran en general los esfuerzos en materia de RSC de las empresas de la horticultura de exportación en México y España.

*1. Not at all Important; 2. Unimportant; 3. Neutral; 4. Important; 5. Very important.	RF (%)					Me	Md
	1	2	3	4	5		
CSR is valued in some niche markets, but others are only interested in price.	0.0	10.0	0.0	40.0	50.0	4.3	4.5
Consumers do not value CSR in general and those who do are still a marginal sector.	5.0	10.0	25.0	40.0	20.0	3.6	4.0
Consumers do not rate CSR in general because of its lack of promotion and dissemination.	5.0	10.0	30.0	40.0	15.0	3.5	4.0

Source: Own elaboration from the Delphi Survey (2nd round).

Fuente: Elaboración propia.

To reiterate, the differences between the horticultural activities in Spain and Mexico are the reason why the responses obtained in the assessment that the expert panel made (Mexico / Spain / Both) regarding the level of development of the CSR measures in both agro-export sectors differ. The panel's assessment about the level of development of CSR measures within the fruit and vegetable production (table 7, page 173) - on a scale of 1 to 10 - reached a notable average assessment similar in both production systems (6.6 /6.9 /6.7), higher in both countries than the assessment obtained from other sectors – whatever they may be – in each country (5.7 /6.1 /5.8). This denotes the high levels of CSR implementation within the highly globalized agro-export sector. However, the experts from Mexico and Spain acknowledge that the CSR implementation in the horticultural activity studied, despite being prominent among companies in the country, is far from the levels of development that CSR practices reach in companies in other developed sectors (7.8 /7.0 /7.5), such as the US or EU countries other than Spain.

Table 7. Assessment of companies of the exporting horticulture in Mexico and Spain against other sectors and countries.

Tabla 7. Valoración en materia de RSC de la horticultura de exportación en México y España frente a otros sectores y países.

Source: Own elaboration from the Delphi Survey (2nd round).

Fuente: Elaboración propia.

Valuation from 1 to 10, corresponding 10 to the highest excellence	Mexico					Spain					Total
	Me	**Md**	**Mo**	**Max**	**Min**	**Me**	**Md**	**Mo**	**Max**	**Min**	**Md**
The company in your sector (of the country).	6.6	7	8	8	4	6.9	7	8	8	5	6.7
Businesses in general (of the country).	5.7	6	7	7	3	6.1	6	6	8	4	5.8
Companies in general (from other developed countries), such as from the EU or US.	7.8	8	8	9	6	7.0	7	7	8	6	7.5
Me=Mean. Md=Median. Mo=Mode. Max=Maximum. Min=Minimum. Total= Mexico and Spain.											

In the opinion of the experts interviewed, the adoption of CSR measures is considered by companies in the horticulture sector in their respective countries as a “strategic expenditure” (69.2%/71.4%/70.0%) in (Mexico/Spain/Both). Other less selected responses were considering CSR measures to be an “investment, albeit unprofitable” (20.8%), an “expense necessary to contribute to the well-being of the company” (17.3%) or a “medium- and long-term profitable investment” (10.3%). Other opinions proposed by experts in the first round such as “it being an unnecessary expense”, “a profitable short-term investment” or “a way to hide or disguise industry problems” (table 8), were not considered which indicates a unanimous disagreement.

Table 8. Assessment of the exporting horticulture in Mexico and Spain about the adoption of CSR initiatives.

Tabla 8. Valoración de la horticultura de exportación en México y España sobre la adopción de acciones de RSC.

Source: Own elaboration from the Delphi Survey (2nd round).

Fuente: Elaboración propia.

	AF			RF(%)		
	Mexico	**Spain**	**Total**	**Mexico**	**Spain**	**Total**
A strategic expense for the company.	9	5	14	69.2	71.4	70.0
An expense necessary to contribute to the well-being of society.	4	1	5	30.8	14.3	25.0
An investment, albeit unprofitable.	4	2	6	30.8	28.6	30.0
A profitable investment in the medium and long term	2	1	3	15.4	14.3	15.0
Others: "a disguise for problem sectors".	1	0	1	7.7	0.0	5.0
A profitable investment in the short term.	0	0	0	0.0	0.0	0.0
An unnecessary expense.	0	0	0	0.0	0.0	0.0

These results are not in accordance with the considerations shown in the literature, as is the case of the study “Panorama of Social Responsibility in Mexico” done by the Agency Responsible (2019), according to which 36.8% of Mexican companies see the CSR as “a very profitable investment in the short, medium, and long term”. Twenty-seven-point five percent of the experts regard it as “a necessary expense to contribute to the well-being of society" and 21.1% of them as a long-term profitable investment, and only 6.6% see it as a strategic expense for the company, this being the most relevant response by the panel of experts consulted in this work.

Another question in this study explored if "there are incentives for small businesses to take social responsibility actions". The panel of experts from both countries answered "yes", in the case of Spain the panel agreed unanimously and in the case of Mexico 84.6% of the panel agreed. In this regard, authors such as Hartmann (2011) point out that the main reason for medium and small enterprises to adopt CSR measures is the direct pressure of supply chains, unlike large enterprises, in which CSR measures originate or are initiated by non-governmental bodies (NGOs) and activist consumers, who are concerned about the sustainability of the sector and their consumption.

Then, taking advantage of the potential of the Delphi technique to propose predictive analyses, the panel of experts was asked in the first round to indicate what the most frequently implemented CSR standards and initiatives were in the sector. Proceeding to the second round with the objective of finding out which of CSR standards and initiatives will develop more in the coming years by means of a trend analysis. As can be seen in table 9, experts observed a plethora of standards, initiatives, platforms that fruit and vegetable producers can adopt.

Table 9. CSR initiatives and standards implemented in the exporting horticulture in Mexico and Spain with the most growth potential in the coming years.

Tabla 9. Iniciativas y estándares de RSC implantadas en la horticultura de exportación en México y España con más potencial de crecimiento en los próximos años.

	AF			RF%		
	Mexico	**Spain**	**Total**	**Mexico**	**Spain**	**Total**
GRASP	2	5	7	15.4%	71.4%	35.0%
Fair Trade	6	0	6	46.2%	0.0%	30.0%
Rainforest Alliance	5	0	5	38.5%	0.0%	25.0%
GRI (Global Report Initiative)	2	3	5	15.4%	42.9%	25.0%
Eleven River	4	0	4	30.8%	0.0%	20.0%
ESR (CEMEFI)	3	0	3	23.1%	0.0%	15.0%
SEDEX	2	1	3	15.4%	14.3%	15.0%
United Nations Global Compact	1	2	3	7.7%	28.6%	15.0%
Most demanding environmental production standards.	0	3	3	0.0%	42.9%	15.0%
DEAR	2	0	2	15.4%	0.0%	10.0%
BSCI	2	0	2	15.4%	0.0%	10.0%
SA 8000	2	0	2	15.4%	0.0%	10.0%
DEALTI (Mexico)	2	0	2	15.4%	0.0%	10.0%

Source: Own elaboration from the Delphi Survey (2nd round).

Fuente: Elaboración propia.

According to the panel of respondents, the belief of the growth potential GRASP (GLOBAL-GAP Risk Assessment on Social Practice) and the development of reports based on GRI (Global Reporting Initiative) in the case of Spain, which are expected to be joined by more environmental standards, are the most demanding. In the case of Mexico the adoption of the Fair Trade [1], Rainforest Alliance, and other standards, such as ESR of Mexican Center for Philanthropy (Centro Mexicano para la Filantropía, CEMEFI), Eleven River -specific to the state of Sinaloa- and DEAR (Distintivo de Empresa Agrícola Responsable, Responsible Agricultural Company Badge) promoted by the Mexican government is predicted. In addition, companies in Mexico and Spain in the horticultural sector have joined the United Nations Global Compact Initiative and the SEDEX (Supplier Ethical Data Exchange) electronic platform.

In conclusion, a plethora of standards and initiatives, some common to both markets, co-exist; which are particularly varied in the case of Mexico. Some of these standards and initiatives are similar in both agro-export centers despite the presence of increasingly global and interconnected markets. According to Busch and Bain (2004), private standards prevail

[1] For many researchers Fair Trade is not considered a CSR standard. However, experts as Poetz *et al.*, 2013 consider that Fair Trade is an opportunity to differentiate the product in high quality markets and niches, but at the same time, it has been institutionalized as a mechanism to address both environmental and social problems in the production and consumption of promoting fair international trade rules.

over public standards in the international food trade and in the free market economy in general. The two horticultural systems studied (Mexico and Spain) are a clear example of the globalization process of the agri-food system and the international expansion of quality standards promoted by large distribution chains, a process that has been studied internationally in other areas of food production (25). It should not be forgotten that the growing importance of new standards and norms in agricultural systems around the world, as pointed out by Handschuch *et al.* (2013), is due to the countries' attempt to ensure their continuous access to the main profitable markets, as is the case of export-oriented countries

Finally, the expert panel gave their assessment on the activity's Strengths, Weaknesses, Opportunities, and Threats (SWOT) as ranking of the three most important points according to the expert panel, as shown in table 10. Both countries' Strengths are being prominent product areas and having access to international distribution channels, with both agro-export enclaves having extensive experience in the production and international marketing of vegetables. As Opportunities, both Mexico and Spain highlight the potential for access to niche export markets, and those that particularly value the Social Responsibility of their companies and production systems. As Threats, there is a consensus among the Spanish experts that limiting factors in southeastern Europe is mainly the lack of water and general natural resources in both areas of intensive production in winter times, as well as the increasing international competition to expand fruit and vegetable production.

Table 10. Main Strengths, Opportunities, Weaknesses and Threats of the exporting horticulture in Mexico and Spain (Top 3).

Tabla 10. Principales Debilidades, Amenazas, Fortalezas y Oportunidades de la horticultura de exportación en México y España (Top 3).

Strengths	Mexico		Spain		Weaknesses	Mexico		Spain	
	Me	Md	Me	Md		Me	Md	Me	Md
Outstanding production area in several crops.	4.5	5.0	4.4	5.0	High and increasing production costs.	3.8	4.0	4.6	5.0
Access to international distribution channels.	4.5	5.0	4.4	5.0	Perishable production	4.2	4.0	4.0	4.0
Companies' adaptability.	4.4	5.0	4.4	5.0	Low competitiveness of small producers.	3.6	4.0	3.7	3.0
Opportunities	**Mexico**		**Spain**		**Threats**	**Mexico**		**Spain**	
	Me	**Md**	**Me**	**Md**		**Me**	**Md**	**Me**	**Md**
New international markets.	4.5	5.0	4.1	4.0	Water scarcity and loss of natural resources.	4.4	5.0	5.0	5.0
High-quality export market niches.	4.8	5.0	4.3	4.0	Climate Change.	4.2	5.0	4.3	4.0
Export niches that value the CRS.	4.3	5.0	4.1	4.0	Growing foreign competition.	4.0	4.0	4.0	4.0
1 Not at all Important; 2. Unimportant; 3. Neutral; 4. Important; 5.Very important.									

Source: Own elaboration from the Delphi Survey (2nd round).
Fuente: Elaboración propia.

Conclusions

The main reason to implement food safety and CRS standards, certifications, and initiatives in the agro-export fruit and vegetable activity of Mexico and Spain is linked to the demands of the large chains of North American and European supermarkets. These supermarkets are the end consumers of the agro-export activity in Mexico and Spain, so great effort is made to safeguard them from risks that could damage their reputation and image.

The development of CSR is positive for companies in the horticultural sector in terms of reputation, due to their demand to access the increasingly standardized and connected international markets, which makes them stand out in the development of these initiatives within the country. Labor and environmental practices are the areas in which CSR develops most despite the limitations that hinder further development of measures aimed at making their production systems more sustainable, especially in small and medium-sized enterprises that have fewer resources and incentives in the short and medium-term.

However, access to massive commercial export channels requires more effort to develop voluntary certifications that ensure that their production systems are increasingly sustainable (socially, economically, and environmentally) to identify and avoid risks. The panel of experts considers the aforementioned a strategic issue for companies in the sector. The opinions gathered from horticultural activity experts from Mexico and Spain describe the process of proliferation of food safety, quality, and social responsibility standards applied in the sector to identify trends and opportunities, in addition to contrasting the motivations, limitations, and challenges faced by the agro-export sector of these two countries.

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Modeling the adoption of a garlic (*Allium sativum* L.) variety in Mexico through survival analysis

Modelando la adopción de una variedad de ajo (*Allium sativum* L.) en México mediante análisis de supervivencia

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Abstract

The objective of this research was to analyze adoption over time of the improved garlic variety CEZAC 06, in North-Central Mexico, determining factors associated with this adoption process through survival analysis (SA). The data was collected at farm level, through a questionnaire administered to 80 garlic farmers in Zacatecas, Mexico, in 2019. Among respondents, 62.5 % of the farmers adopted CEZAC 06 during the first two years after it was introduced. The factors affecting the adoption process were: farmer age, how long the farmer had been in business, number of hectares for garlic production, yield, number of college-educated family members, income from crop farming, income from garlic farming, from the federal government and belonging to any type of membership to an organization. Improving garlic bulb yield and quality requires an adequate extension program communication system that provides for farmers to receive updated and reliable information on the importance of technological innovation. Survival analysis can evaluate changes in explanatory factors between farmers and other changes over time, thus addressing decision-making and adoption processes for improved seeds.

Keywords
Allium sativum L. • improved variety • adoption speed • adoption factors • extension programs communication system

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Resumen

El objetivo de esta investigación fue analizar la adopción en el tiempo de la variedad mejorada de ajo CEZAC 06 y los factores asociados con este proceso de adopción mediante el análisis de supervivencia (AS), en el Norte-Centro de México. Los datos a nivel de finca fueron recolectados en 2019 a través de un cuestionario administrado a 80 agricultores de ajo en Zacatecas, México. Los resultados mostraron que el 62,5 % de los agricultores que adoptaron la variedad CEZAC 06 lo hicieron en los dos primeros años después de que la conocieron por primera vez. Las variables que influyeron en la adopción fueron: edad, tiempo dedicado a la siembra de ajo, número de hectáreas producidas con ajo, rendimiento, familiares con estudios universitarios, ingresos obtenidos de la agricultura, ingresos obtenidos de la siembra de ajo, número de cursos tomados sobre temas agrarios, apoyos federales y pertenecer a alguna organización. Mejorar el rendimiento y sobre todo la calidad del bulbo requiere de un sistema de extensión adecuado que permitan a los agricultores recibir información actualizada y confiable sobre la importancia de la innovación tecnológica. El análisis de supervivencia puede analizar los cambios en los factores explicativos en relación con los agricultores y otros cambios a lo largo del tiempo, abordando así el proceso de toma de decisiones y adopción de semillas mejoradas.

Palabras clave

Allium sativum L. • variedad mejorada • velocidad de adopción • factores para la adopción • programas de extensión

Introduction

In 2018, total garlic (*Allium sativum* L.) production in Mexico was 94,692.19 tons, with an approximate market value of $ 71,830,621.88, of which 60% took place in Zacatecas (54) giving it a promising potential for producing improved garlic varieties. However, most farmers in this area still use native garlic genotypes. The socio-economic importance of garlic farming lies in the labor needs for successful cultivation (requires 180 to 210 field workers per crop cycle) and production happens during the autumn-winter cultivation season, when few alternatives for employment are available in rural areas.

Garlic is primarily used as seasoning or food condiment and it is consumed in the form of fresh cloves, bulbs, after dehydration and processed in various ways (61). In Mexico, consumption per capita in 2018 reached 400 g per person (22).

Several factors limit crop productivity in Mexico. One is the absence of a seed production program, which leaves farmers no choice but to use seeds that come from different sources. Most of the time, farmers have no idea where these seeds were produced. This leaves farmers with little to no certainty that the seeds being used were produced in plots under controlled phytosanitary conditions. Uncertain sanitation causes pest and disease spread, with resulting financial losses for the producers and increased production costs (59). Another factor associated with limited crop productivity is consistent drop in seed quality over time. Bigger garlic bulbs usually enter the market, since customers value them at a higher price. Such market behavior forces farmers to use below-average sized garlic bulbs as seeds, leading to steady decline in seed quality over time. This also lowers the potential for greater garlic yields in future harvest seasons (32). Although there are elements that reveal the importance of this crop, the reality is that in Mexico, and especially in Zacatecas, the lack of varieties in the region is a major limitation to productivity. Solving this problem would require farmers to adopt new garlic varieties as an alternative to traditional methods (42). Planting improved garlic varieties suited to the region would increase productivity and garlic yield (50).

Adoption can be defined as integrating innovation in everyday agricultural activities carried out by farmers for a prolonged period (15). There are three broad categories that limit technological adoption in developing countries: 1) farmer characteristics and behavior 2) characteristics and advantages of new technology and 3) institutional aspects (55).

The Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP-México), as the institution responsible for supporting national agricultural development, faces several challenges. These challenges include seeking, validating, transmitting, and providing the means for technological adoption in benefit of agricultural and forestry producers. In 2011, the Zacatecas Experimental Field generated a new garlic variety called CEZAC 06. Advantages of this variety include higher yields, consistently round bulbs, fewer cloves per bulb and homogeneous growth. By using CEZAC 06 in commercial plots, performance has been improved by 9% to 17% and has reached yields of up to 30 t ha^{-1} (42). This variety is a productive and profitable alternative for garlic farming in the North-Central region of Mexico.

However, the adoption rate of improved varieties has been low, especially among small producers (44). A wide range of factors can affect a farmer's ability to adopt technologies, such as intrinsic conditions, socio-economic, cultural, institutional, and political variables (4). Another adverse factor is seed price. Despite this, evidence suggests that small producers are willing to use improved varieties if yields are higher and innovations are affordable, as shown in studies in Zimbabwe and Kenya (31).

The economic analysis of technological adoption has sought to explain how it is affected by factors such as characteristics and personal aspects of the producer, information quality, risk, uncertainty, institutional limitations, infrastructure, and availability of inputs (15, 17, 28). This is because a new technology is often associated with risk and uncertainty regarding its use or application, the appropriateness of an implementation scale, suitability with the environment, and most importantly, the perception and producer expectations as the end user of new technology (1, 18, 60). Adoption of agricultural technologies has been associated with multiple benefits including higher income, reduced poverty (26), improved nutrition and lower food prices.

No studies on garlic cultivation address adoption of new varieties nor the time it takes farmers to adopt a technological innovation. In this context, we evaluated adoption behavior for the improved garlic (*Allium sativum* L.) variety known as CEZAC 06 and other factors associated with the adoption process in Northern-Central Mexico through survival analysis (SA). We evaluated the hypothesis that whether farmers adopt depends on the type of technology under consideration. Thus, a greater perceived utility results in a shorter adoption time.

This work also contributes to the scarce literature on the application of survival analysis to agricultural technologies. It is expected to provide a basis for improved intervention on agricultural policies that guide production and aid in transferring new technologies. The results may also be helpful to farmers considering adopting new garlic varieties, to retailers seeking to satisfy customer needs, and to those who manage garlic supply chains.

Methods and materials

Description of the technological innovation: improved variety CEZAC 06

The garlic plant (*Allium sativum* L.*)* CEZAC 06 grows in a vertical fashion; it has an average height of 43 cm, grows an average of eighteen leaves measuring 23.99 mm wide and 45.88 cm long approximately. It also has a robust stem that resembles a strong plant; the presence of a floral scape is a characteristic of this variety. The bulbs are covered by white cataphylls with vertical streaks of violet-pink color. The average number of cloves per bulb is 16 and the cloves are screamy white, individually covered by a pink wrapping leaf. Cloves are radially distributed and arranged but inserted into the stem. The cultivation cycle is 220 days, and which is part of the advantages (42). No studies specifically address the economic impact of the CEAC 06 variety. However, Reveles *et al.* (2011) mentioned the CEZAC 06 variety as an alternative for garlic producers in the region due to the homogenous shape and size of the bulb, which command a greater sale price.

Area under study

The state of Zacatecas is in the North-Central part of Mexico, with coordinates between 25° 09'and 21° 01' North latitude and between 100° 48' and 104° 20' West longitude at an altitude of 2230 m above sea level. The average annual temperature is > 18°C, with June being the hottest month and January being the coldest (20). The state has a population of 1.6 million of which half live in rural areas. Although the state´s economy is based on agricultural production, this sector is considered of low economic development since 72% of the population has a monthly income of less than two minimum wages (21). However, in terms of gross domestic product (GPD), agriculture contributes 22% to 25% thus becoming the main economic activity. The value of agricultural activities in the last production cycle was ~ 15.3 million pesos, which represented ~ 68% of the total value of the sector in the state (54). Thus, Zacatecas exports > 772 thousand tons of agricultural products to the rest of Mexico, including beans, dry chili, guava, peach, prickly pear, vine, and garlic (46).

In Zacatecas 3,548.50 hectares of garlic seeds were planted in 2018 (54), which ranked the State among the main producers nationwide. The average yield in Zacatecas was 16.46 t ha^{-1}, with a statewide production of 56,423.61 t. Among the top producers, the municipalities of Calera, Villa de Cos, Guadalupe, and Panuco, utilized about 84% of the total land s area designated for this crop (43) (figure 1).

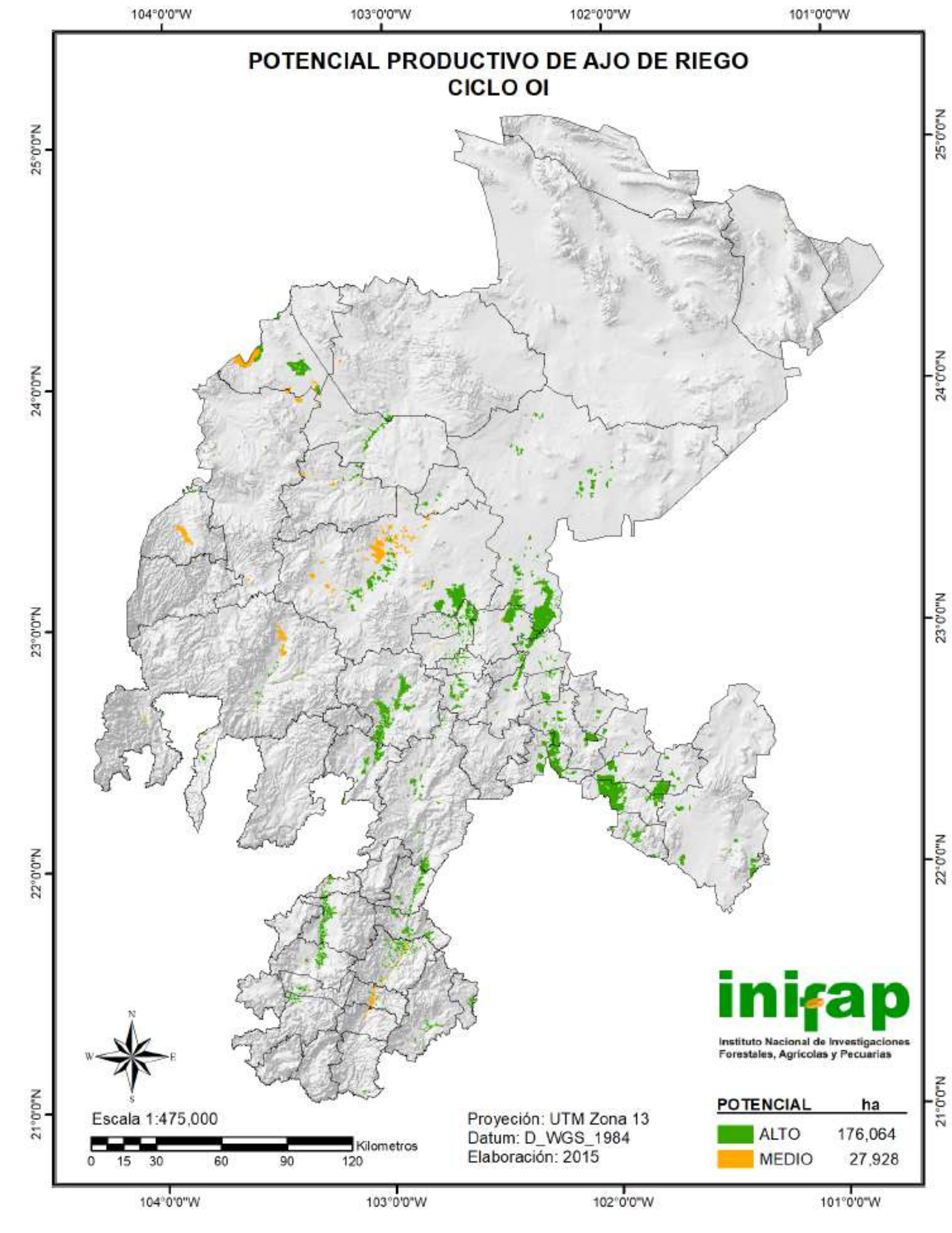

Figure 1. Areas with promising potential for garlic production in Zacatecas.

Figura 1. Zona con potencial para la producción de ajo en Zacatecas.

Defining the sample size

Data was obtained through a survey administered to 80 farmers during August and October of 2019. The sample was stratified by seed variety (creole or improved) and region (post district). Interviewing took place in regions with promising potential for garlic production including Calera, Villa de Cos and Guadalupe and Panuco.

Participants and sample size were determined according to the registry of agricultural producers in the state´s census, which at the time of the study, had 100 farmers registered.

Sample size was calculated as a finite population with a significance level (NS) of 95% and an error of 0.05% (33, 47). This sample size is similar to other studies that have analyzed adoption of technological innovations through survival analysis (5, 14, 23).

Methodological framework

Several research on medical, agricultural, economic, and psychological topics focuses on estimating the time elapsed until an event of interest takes place. This is called survival analysis (SA) or duration analysis (DA). Frequently, the data in consideration for SA tends to violate a normal assumption, lacks completeness, exist censored observations, and occurrence of the event of interest depends on external factors. Therefore, most of the usual statistical tests are not applicable. To study the relationship between survival and external variables, a set of statistical concepts, tools and techniques that allow us to model the length of time until the event occurs was used.

Survival analysis has been used in different areas. In medicine, it has been used to study time until recovery from a disease and in other fields in estimating durability of household appliances or machine failure. In agriculture, survival analysis has been used to model the adoption of sustainable technology, conservation of tillage, improved varieties, fertilizers, and herbicides (33, 37, 38, 62).

In this manuscript, we used duration analysis as appropriate to this research's objectives and to the characteristics of the data (heterogeneous population, censored observations that do not follow a normal distribution and the presence of an external variable that can affect time until adoption).

In general, DA has three relevant functions: survival function, probability density and hazard function.

Let T be a non-negative random variable (r.v.) that measures time until an event of interest occurs. Suppose that t is a realization of T. Let us consider a random sample of n duration times $t_1<t_2<...<t_n$. If $f(t)$ denotes the probability density function (PDF) of the random variable T, we define the distribution of duration as the cumulative distribution function (CDF); that is:

$$F(t) = P[T \leq t] = \int_{-\infty}^{t} f(r)dr \tag{1}$$

Equation (1) determines the probability that T is less than or equal to t. However, in DA, determining the probability that T will survive until at least t is the objective. Thus, the probability is determined by the survival function $S(t)$, defined as:

$$S(t)=1\text{-}F(t) \tag{2}$$

The hazard function $h(t)$ is defined as the probability that a farmer adopts the improved technology at time t, if up until time t, adoption has not occurred. That is:

$$h(t) = \lim_{\Delta t \to 0} \frac{P[t \leq T \leq t + \Delta t | T > t]}{\Delta t} \tag{3}$$

There is a well-defined relationship between $f(t)$, $F(t)$, $S(t)$ and $h(t)$. In fact, if any of these is known, the others can be determined:

$$h(t) = \frac{f(t)}{S(t)} = \frac{d\left(\frac{F(t)}{dt}\right)}{S(t)} = -\frac{d}{dt} ln(S(t)) \tag{4}$$

Let T denote failure time and $\boldsymbol{x} = (x_1, ..., x_k)'$ represent a vector of available covariates (economic and non-economic variables may be expected to influence and alter the distribution of duration). Modelling and determining the relationship between T and **x** is of interest.

When including additional explanatory variables in DA, the hazard function needs to be redefined and reformulated as being a conditional function on these variables:

$$h(t, \boldsymbol{\theta}, \boldsymbol{x}, \boldsymbol{\beta}) = \lambda_0(t, \boldsymbol{\theta})\boldsymbol{\lambda}(\boldsymbol{x}, \boldsymbol{\beta}) \quad (5)$$

where:

β = a vector of unknown parameters,

$\boldsymbol{x}$ = a vector of explanatory variables that may include time-invariant and time-varying variables

$\boldsymbol{\theta}$ = a vector of parameters of the hazard rate.

From (5), it is notable that the hazard function $h(t, \boldsymbol{x}, \boldsymbol{\theta}, \boldsymbol{\beta})$ can be split into two components. The first component is the baseline hazard function $\lambda_0(t, \theta)$ which is equal to the hazard when all covariates are zero and therefore does not depend on individual characteristics. This component captures the way the hazard rate varies along duration. The second component is the part of hazard that depends on the subject's characteristics $\lambda_0(\boldsymbol{x}, \boldsymbol{\beta})$.

A widely used specification in survival regression allows the hazard function $\lambda_0(t, \theta) = \lambda_0(t)$ to be multiplied by $\lambda_0(\boldsymbol{x}, \boldsymbol{\beta}) = exp\ (\boldsymbol{x'}\,\boldsymbol{\beta})$.

The survival model is:

$$h(t, |\boldsymbol{x}) = \lambda(t)exp(\boldsymbol{x\beta}) = \lambda(t)exp(x_1\beta_1 + \cdots + x_k\beta_k) \quad (6)$$

The model (6) regression formulation is called the proportional hazards (PH) model (6). Since λ (t) can be left completely unspecified, (6) is a semiparametric model.

The Cox's semiparametric model has been widely used in analysis of survival data to explain the effect of explanatory variables on hazard rates. The advantage of a semiparametric model is that no assumptions must be made about the shape of the hazard function.

In general regression notation, the log hazard can be used as the property of response evaluated at time T, which allows distribution and regression components to be isolated and evaluated. The PH model can be linearized with respect to $\boldsymbol{x'}\,\boldsymbol{\beta}$ using the following identity:

$$log\ \lambda(t|\boldsymbol{x}) = log\ \lambda(t) + \boldsymbol{x\beta} \quad (7)$$

Or

$$log\ \lambda(t|\boldsymbol{x}) = log\ \lambda(t) + x_1\beta_1 + \cdots + x_k\beta_k \quad (8)$$

The interpretation of the Cox model is not directly made with the estimated coefficient $\hat{\beta}_i$, but instead through $exp(\hat{\beta}_i)$ and is similar to that performed in logistic regression. If $\hat{\beta}_i$ is the estimated coefficient corresponding to the variable x_i (continuous variable), $exp(\hat{\beta}_i)$ represents the relative risk when x_i increases one unit, keeping all other variables constant. For dichotomous variables, $exp(\hat{\beta}_i)$ is an estimator of the hazard ratio (hazard ratio = RR) and is interpreted as the increase in risk derived from the presence $x_i = 1$ of each covariate in relation to absence $x_i = 0$. The estimation procedure is based on the partial likelihood function; more details are available in Cox (1972).

Information analysis

The objective in duration analysis is determining the time elapsed until an event of interest occurs (40). In the context of technological adoption, this transition is regarded from the moment the technology is known until adoption happens. Through duration analysis, behavioral models are created in which personal options and technological dynamics are analyzed to be incorporated as part of the elements for adoption (4). As roles for explaining technological adoption.

For this article´s purposes, the year in which the farmer is introduced to CEZAC 06 was established as the start date and the year in which adoption occurred as the finish date or period. In certain cases, farmers had not adopted the new variety at the time the study was completed although adoption could occur afterwards. Thus, they were censored to the right, meaning, the final analysis date equals the time in which the survey was administered.

Regarding independent variables, adoption can depend on a broad set of determinants which include characteristics related to innovation, politics, economics, expectations, hierarchical structure, socio-economic atmosphere, opinions, objectives, and perceived impact (17, 23, 48). A dummy variable was included given a significant increase in adoption that occurred in 2015, thus, this variable has a value of one if adoption happened after 2015 and a value of zero otherwise.

Two types of statistical analysis were conducted: parametric and non-parametric. Non-parametric analysis of adoption intervals, which considers the nature of censored data, was carried out using the Kaplan-Meier estimated survival function. This information allowed suggestion of appropriate functional forms for a parametric analysis (27). Furthermore, this method helps represent adoption speed of different technologies and facilitates comparisons among sampled individuals in different populations. The Kaplan-Meier survival curves for each variable were obtained and the log-rank test was used at a confidence level of α = 0.05 to determine whether curves plotted were the same. Parametric analysis considered all variables collected in the survey and were analyzed through the proportional hazard Cox's model (1972), highlighting variables that significantly influenced adoption. In addition, the likelihood ratio test, Wald test and Score test (achievenk) were applied. Data analysis was completed using R (R Core Team).

Results

Descriptive analysis of hypothetical variables

Descriptive statistics of primary variables that influence time required for farmers to adopt CEZAC 06 were determined (table 1).

Table 1. Description of the variables used for statistical analysis (n = 80).

Tabla 1. Descripción de las variables utilizadas en el análisis estadístico (n = 80).

Covariates	Non-adopters or Censored (*n*= 20)		Adopters (*n*= 60)		Total (*n*=80)	
	Mean	Std.	Mean	Std.	Mean	Std.
Dependent Variable						
Duration	Years between introduction to CEZAC 06 until adoption					
Explanatory Variables						
Farmer age in years	53.3	11.9	40	14.3	43.3	14.8
Land tenure (1: Ejido land, 2: Small property, 3: Rented	2.9	1.4	1.1	0.5	1.6	1.1
Farmer education (1: Elementary education; 2: Secondary education; 3: High school; 4: College; 5: Postgraduate studies; 6: Other)	1.6	0.9	2.7	1.14	2.4	1.1
Family members with college education (1: Yes, 2: No)	0.1	0.3	1.4	0.4	1	0.7
Way in which technology became known (1: INIFAP, 2: Demonstrative plot 3: Pamphlet, 4: Technician, 5: Another farmer, 6: A family member)	5.5	0.5	1.7	1.1	2.7	1.9
Household size (Continuous)	4	0.6	5.2	1.6	4.9	1.5
Number of family workers (Continuous)	0	0	1.5	0.8	1.1	0.9
Income from agriculture (%)	22	0.1	91	0.1	74	0.3
Income from garlic (%)	22	0.1	31	0.1	28	0.1
Government aid (1: Yes, 2: No)	1	0	1.3	0.4	1.2	0.4
Courses taken on agricultural technology (Continuous)	0.3	0.4	1.3	1.7	1.1	1.5
Hectares planted with garlic (Continuous)	6.6	3.8	21.6	19.1	17.8	17.8
Tons per hectare (Continuous)	8.2	1.6	15.3	3.2	13.5	4.2
Garlic price per tons in euro (Continuous)	521.7	43.8	15016.6	578.6	564.3	57.6
Member of an organization (1: Yes, 2: No)	1.9	0.3	1	0.2	1.2	0.4
Products with added value (1: Yes, 2: No)	2	0	1.9	0.2	1.9	0.2
Technological innovations are necessary in its cultivation (1: Completely disagree; 2: Disagree; 3: Neutral; 4: Agree; 5: Completely agree)	5	0	4.9	0.1	4.9	0.1
Risk attitude (1: risk averse, 2: risk-cautious, 3: risk loving)	2	1	2	1	2	1

The 80 farmers were divided into two groups at the time of surveying, 25% were censored (non-adopters) and the rest (75 %) were adopters. Adopters had some college education, and the average age and household size was 40 years old and five members, respectively. These farmers were introduced to the improved seed through INIFAP, have attended CEZAC 06 courses, and own ~ 21 hectares of land that produce 5 t ha^{-1}. Non-adopters only enjoyed of elementary education, and their average age and household size was 53.3 years old and 4 members, respectively. Unlike adopters, they became aware of CEZAC 06 through another farmer, had not attended courses, and only had an available land area of seven hectares that produce 8 t ha^{-1}.

Econometric Analysis

The Kaplan-Meier method allowed the length of time that farmers wait before adopting the CEZAC 06 garlic variety to be more closely examined (figure 2). The horizontal axis shows the number of years elapsed since the technology was first known until the year that the variety was adopted, and the vertical axis shows the respective probabilities. The curve shows that 62.5% of the farmers adopted the improved garlic variety in the second year after they were first introduced to it (figure 2).

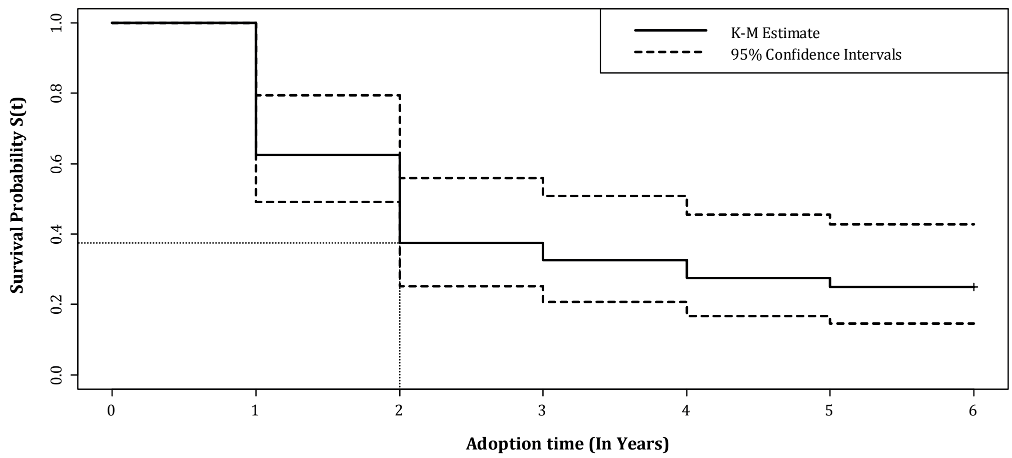

Figure 2. Kaplan-Meier survival curve.

Figura 2. Curva de supervivencia de Kaplan-Meier.

The previous statement is confirmed by the cumulative hazard function (figure 3), where in the third year, the cumulative hazard of adoption is 0.80. Farmers who received information from a trained person (agricultural technician) or researcher adopted the variety.

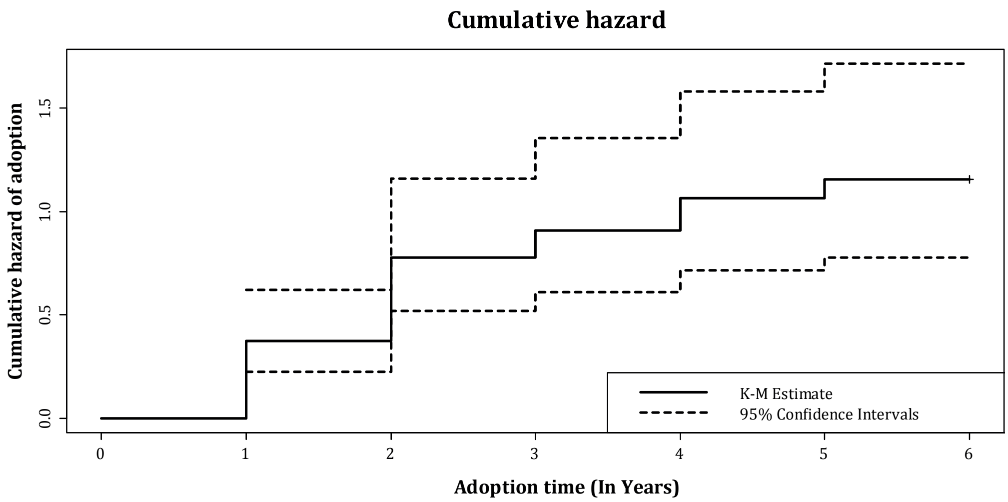

Figure 3. Accumulated risk curve.

Figura 3. Curva de riesgo acumulado.

Demonstration plots also played an important role in adoption (figure 4).

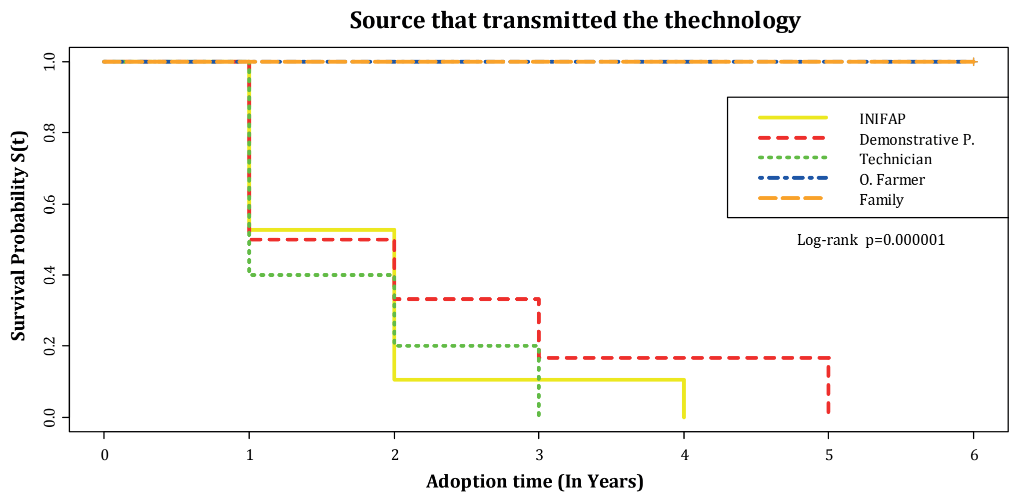

Figure 4. Kaplan-Meier survival curve by source of information on the technology.
Figura 4. Curva de supervivencia de Kaplan-Meier por fuente de información sobre la tecnología.

The willingness of farmers to take the risk associated with adopting a new variety of garlic is an important consideration, given the uncertainty regarding several factors such as seed cost, cost of additional materials, and the application of new agricultural practices (figure 5).

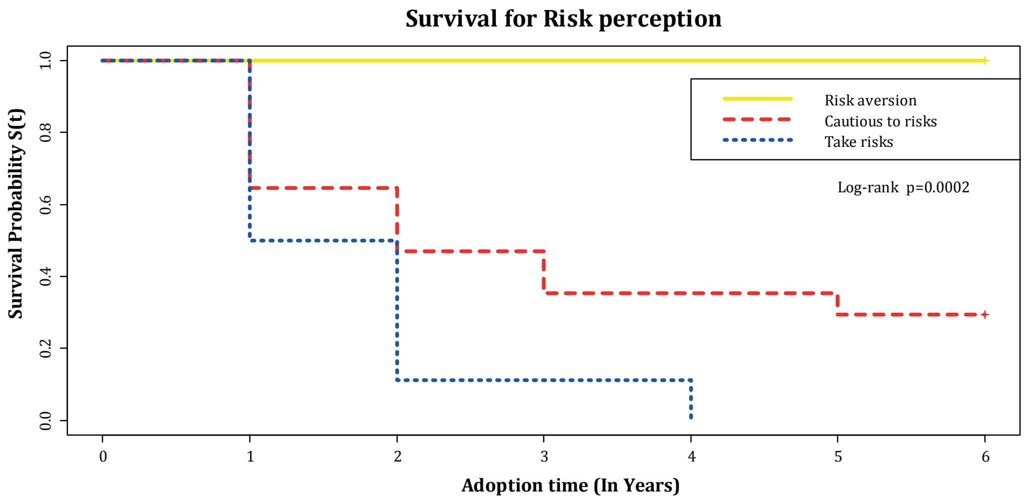

Figure 5. Kaplan-Meier survival curve based on risk perceptions.
Figura 5. Curva de supervivencia de Kaplan-Meier basada en percepciones de riesgo.

The time to adoption is associated with different combinations of covariates collected from the survey. The backward steps method was followed to determine the final list of variables to be included in the model (49). At an α = 0.05 confidence level, the null hypothesis stating that all coefficients are jointly equal to zero was rejected.

This method allowed construction of the best PH Cox´s model, with eleven covariates associated with adoption of the CEZAC 06 variety among garlic farmers and explained 98% of the variation in adoption time. The predictor based on age increases the probability of adoption by a third (table 2, page 187). In addition, there is a greater possibility of adoption by farmers whose head of household is between 30 and 43 years old. The number of years spent garlic farming also increases the probability of adoption. Farmers who considered the increase in yield were more than six times more likely to adopt when predictive yield was not considered.

Farmers with college-educated relatives were three times more willing to risk adoption than those with no college-educated family members or relatives. The variables describing income from agriculture and garlic cultivation, respectively, showed a probability five times greater and almost double for adoption, respectively. Attendance at classes and conferences on agricultural issues are also factors that influenced adoption. The variable aid received increased the probability of adopting by three times, which indicates that a greater availability of government aid increased the speed of adoption.

Table 2. Cox proportional model analysis of adoption of the variety CEZAC 06 (*n* = 80).

Tabla 2. Resultados del modelo proporcional de Cox sobre la adopción de la variedad CEZAC 06 (n = 80).

Significance level: *** p < 0.001; ** p < 0.01, * p < 0.05.

Nivel de significancia: *** p < 0,001; ** p < 0,01, * p < 0,05.

Variables	β	exp (β)	p-value
Head of household's age	-1.24	0.28	0.000***
Year when started garlic farming	-1.29	0.27	0.000***
Hectares	-0.52	0.59	0.085*
Yield	1.81	6.15	0.002**
Family members with college education	1.14	3.14	0.027*
Income percentage from agriculture	1.62	5.09	0.024*
Income percentage from garlic farming	0.56	1.76	0.021*
Attendance at workshops and conferences on agricultural matters	-1.50	0.22	0.001**
Government aid	1.37	3.94	0.011*
Requesting agricultural information from government offices	-1.00	0.36	0.054**
Member of an organization	-3.35	0.03	0.001**
R-square: 0.98			
Likelihood ratio test: 65.22 on 11 df, p = 1e-09 Wald: 29.18 on 11 df, p =0.002 Score (logrank) test: 46.83 on 11 df, p = 1e-06			

The predictors estimated by the PH Cox´s model (table 2) predict that, in four years, 90% of the farmers will improved seeds (figure 6).

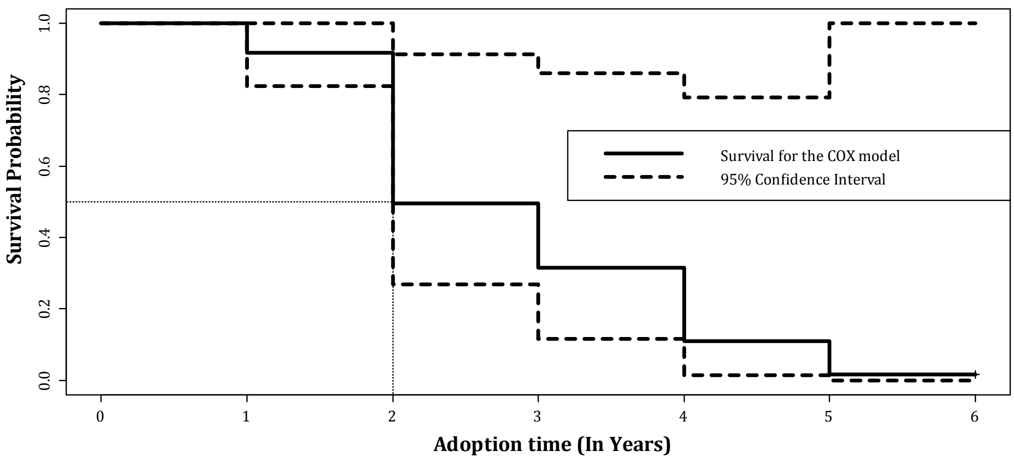

Figure 6. Survival curve using Cox-model variables.

Figura 6. Curva de supervivencia utilizando variables del modelo de Cox.

Discussion

The positive effect that education has on adoption of new technology coincide with studies that indicate "… farmers with a better level of education are more likely to adopt new agricultural technologies" (52, 53). Adoption is also positively associated with relatives with more education (6), something that motivates adoption and can potentially result in larger plots and higher income. Attendance at classes and conferences on agricultural issues influenced adoption. In this sense, various authors (24, 25, 41) argue that the link between different parties through participation in courses and technical assistance could be more important than education level in predicting technology adoption.

As expected from previous findings, younger farmers were quicker in adopting new technology, in comparison to older farmers, who lean towards traditional farming practices (16). Age can be a decisive factor in adopting new technologies (45). Furthermore, the years spent on garlic farming increases the probability of adoption. Experienced farmers are more likely to adopt technological innovations (11). Technology adoption is a process that requires the participation of several components, among which are the producer and decision-maker. Making a decision regarding adoption is influenced by several factors that require time to resolve before adoption can take place (9). Mexican farmers demonstrate only a moderate trend toward change over time given a general attitude of mistrust toward non-traditional agricultural practices (49). However, adoption of a technological innovation is fast when farmers perceive immediate benefits, as with hybrid corn in Ethiopia, where 50% of farmers adopted it within two years (3). Timely information of good quality regarding new technology influences the farmer's decision to adopt it (29).

Results are consistent with other literature that highlights the importance of non-economic factors that play a role in technology adoption such as access to technological information, trust, and perceived utility of various information sources (56). Extension programs given by trained personnel are potentially effective in spreading new technologies intended to increase productivity and improve rural poverty conditions (36, 41). Demonstration plots or field trips are the fastest way to communicate technological information to small farmers, followed by agricultural instructors (35). In the same way, preferences toward risk are a factor in adopting improved varieties (7, 16, 30). Thus, adoption of one component of a technological package increases the probability that farmers will adopt other essential components (57). Although adopting improved varieties contributes to increased productivity, the use must be complemented with other innovations and materials that allow them to express their full genetic potential.

Yield increase is expected to increase income, which is important not only for purchasing production materials, but also for acquiring more land, hiring more labor, and buying other non-productive assets that could help expand the crop (2).

The economic constraint model sets forth that endowment of resources is the main obstacle in short-term adoption (39, 51). Thus, farmer confidence in external public support can positively influence adoption of improved seeds. Relevant theoretical and empirical factors reported in previous studies were identified. At theoretical level, adoption of a technology follows an S-shaped sigmoidal process (19) and occurs after a certain period; in this study, after the second and third year. In addition, factors such as knowledge on the new technology were decisive for adoption to occur, which is consistent with other studies in Mexico (10, 12, 13, 58).

Age is another determinant of technology adoption that is repeated in many empirical studies, setting forth that younger producers are more likely to adopt new technologies. Finally, results agree with Mottaleb (2018) in terms of greater technology adoption as the result of initial support in the form of subsidies and technical support as a facilitator.

Conclusions

This work evaluates adoption time of the garlic variety, CEZAC 06, and the factors that influenced this decision among farmers. CEZAC 06 was adopted by 62.5% of farmers by the second year after they were first introduced to it. The decision to adopt the improved variety was significantly affected by age, years in the garlic farming industry, available hectares for production, yield, college-educated family members, income from agriculture, income from garlic farming, number of courses taken on agrarian topics, federal aid, and membership to an organization. Increased adoption of CEZAC 06 will increase yield and rural farmers could improve the quality of the bulb as a marketable surplus. This would increase income and improve household well-being. Technological innovations that significantly increase income are adopted more quickly.

The analysis also suggested that new technologies should be transmitted at higher rates to increase adoption. This can be done by implementing courses aimed at farmers with low educational backgrounds, small plots of land and low productivity levels. Courses must be short, dynamic and avoid technical language while highlighting income and production benefits derived from technological adoption. Promoting adoption of CEZAC 06 among producers will boost productivity of vegetable patches and better satisfy market demand. Government institutions play an important role by granting aid, a key investment when promoting use of efficient agricultural technologies. This work contributes to the scarce literature on the application of survival analysis to agricultural technologies. Future research should more deeply evaluate risk attitudes and extension programs.

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The role of fuel prices in spatial price transmission between horticultural markets: empirical analysis from a developing country

El rol del precio de combustible en la transmisión espacial de precios en mercados hortícolas de países en desarrollo

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Abstract

This article aims to analyze how fuel prices impact spatial price transmission between two Chilean horticultural wholesale markets. We implement a regime-dependent VECM where price transmission parameters depend on dynamics imposed by a stationary exogenous variable (fuel price). We identified two price transmission regimes characterized by different equilibrium relationships and short-run adjustment processes. This implies that fuel prices affect price transmission elasticities and intermarket adjustment speeds. Our results show increasing marketing costs as farm to market distance grows. This impact depends on each product's attributes.

Keywords
Price transmission • fuel prices • horticultural markets • developing countries • Chile

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Resumen

Este artículo tiene como objetivo analizar cómo los precios del combustible influyen en la transmisión espacial de precios entre dos mercados mayoristas hortícolas en Chile. Implementamos un modelo de corrección de errores de vector dependiente del régimen, donde los parámetros de transmisión de precios dependen de la dinámica impuesta por una variable exógena estacionaria (precio del combustible). Identificamos dos regímenes de transmisión de precios caracterizados por diferentes relaciones de equilibrio y procesos de ajuste a corto plazo. Esto implica que los precios del combustible afectan las elasticidades de transmisión de precios y la velocidad de los ajustes entre los mercados. Nuestros resultados muestran un patrón de aumento de los costos de comercialización a medida que crece la distancia desde el origen hasta el mercado. Este impacto depende de los atributos de cada producto.

Palabras clave

Transmisión de precios • precios de combustible • mercados hortícolas • países en desarrollo • Chile

Introduction

Price is the main mechanism for spatially integrating various marketing chain levels (5, 13, 18). Price transmission (PrT) refers to the process based through which markets for a homogeneous commodity at spatially separated locations share long-run information (6, 12). Price transmission has been widely analyzed in the context of the "Law of One Price," which hypothesizes that if two markets are linked by trade and are efficient, the price differential between them is equal to the cost of carrying out trade between them. Prices are consequently thought of as being connected by a stable long-run equilibrium, with attraction forces of this equilibrium resulting in the correction of temporal deviations that occur due to supply or demand shocks. Ejrnæs and Persson (2000) identified three reasons for intermarket price transmission failure, including prohibitive transaction costs[1] (TAC).

[1] TAC raises the price effectively paid by buyers and lower the price effectively received by sellers of a good, creating a "price band" within which some households find it unprofitable to either sell or buy. TAC includes per-unit costs of accessing markets associated with transportation and imperfect information.

Transport costs (TPC) are the expenses a farmer incurs when it transfers its products or other assets to another location. They are the most relevant TACs in price formation for all traded agricultural products (9). As recent global food price spikes' causes are debated, some stress fuel prices' role on PrT along the agricultural marketing chain (MC) (3). Consumer produce demand is known to be price-sensitive, so fuel price surges could significantly affect fresh produce consumption, providing exceptional insight into their impact (22, 27).

Our method builds on the Gonzalo- Pitarakis approach (2006), incorporating a stationary threshold exogenous variable. We use fuel prices as a threshold variable (ThVar), allowing PrT elasticity and adjustment speed to differ across different trade regimes. This approach seems especially apt in irregular PrT settings where using seasonal dummy variables to account for seasonal equilibrium relationship variation might lack flexibility (20). It is also unique in explicitly incorporating an exogenous ThVar in the price adjustment, and allows us to derive conclusions on whether fuel price level implications on the horizontal PrT mechanism between the main Chilean HC wholesale markets (WM) by trade, namely: Santiago and Talca. We consider the most planted vegetables in Chile (corn, tomato, onion and carrot). We analyze with a unique continuous weekly price series for each product and market, and hypothesize that the long-run price relationship and adjustment process between both markets switches between different regimes (or display threshold cointegration behavior) depending on fuel price levels.

Spatial market integration

Fackler and Goodwin (2001) define market integration as a measure of how well demand and supply shocks of a commodity in a given market are transmitted to other markets. Many studies on market integration, both spatially and vertically, studied factors that impede price signal pass-through bet-ween markets in developing countries, where poor infrastructure, transport and communication services create large marketing margins due to high costs of delivering locally produced commodities to domestic markets for internal consumption or to the border for export (4, 16, 29). When 16 developing countries are analyzed for a mix of monthly and annual consumer, wholesale, and producer agricultural prices, Conforti (2004) finds that world-domestic PrT for Latin American is higher than for their African counterparts. The work of González-Rivera and Helfand (2001) reinforces these conclusions. They analyzed Brazilian rice market integration and found that distance and quality contribute to forming transfer costs between major local markets.

These studies examine various market interaction channels: linkages among regions, infrastructure, qualities and recently, the effect of agricultural price volatility on the agricultural value chain. They effectively show interdependence, but lack insights into the causes of exogenous-driving factors in stock market integration of specific sectors in Latin America. This paper fills in the void of the current literature by accounting for exogenous variables' effect on the PrT process. This assumption may not always be justified because HC market chains are characterized by a fixed-proportion relationship among farm inputs, wholesale products, and final retail products, as well as an inelastic short-run supply due to perishability at the timing of growing seasons (26). As a result, the margin between wholesale and farm prices can be interpreted as the major cost of produce at the wholesale level. Consequently, production costs, and therefore gross margins, avoid interseasonal disruptions Most vegetables' short post-harvest life also limits the ability to arbitrate prices and obtain seasonal advantages over other products. This paper also analyzes horizontal PrT processes between relevant HC WM among the most planted vegetables in Chile. To achieve this, we implement a regime depen-dent PrT model allowing a variation of the price elasticity and speed of adjustment according to the level of a stationary exogenous variable (Chilean fuel price). There is still no clear evidence on fuel prices' effect on the spatial integration process between HC WM within developing countries. Since fresh produce undergoes minimal processing and packaging, the main component of that cost is likely to be transport fuel (9). Therefore, the role played by fuel prices offers another method to analyze TPCs' effect on intermarket spatial integration levels.

Transport costs and horticultural prices

All commodities have fuel costs. Other food production and marketing sectors may use more energy, but transport remains extremely dependent on oil-based fuels (15). Chile is a net crude oil importer exposed to foreign markets' volatility. Until 2013, the upward price evolution after the US sub-prime crisis and subsequent global financial crisis was due to various external events. On the other hand, from the break in early 2013, specific events such as OPEC's agreement to lower production quotas and the rise of shale oil put greater pressure on the price until now. However, at the aggregate level Chilean prices' behavior when compared with international prices has remained more stable. This is probably due to a fuel price stabilization system intended to reduce the volatility of the fuel prices to which the domestic market is exposed[2].

Most of TAC among Chilean domestic WM is likely to be transport fuel. Accordingly, interest in fuel prices' potential HC market impact spans various economic research areas. First, questions abound as to why retail food price volatility has increased since the recent global oil price crises, further exacerbating food price fluctuations (14). Second, with health advocates recommending increased produce consumption, researchers and policymakers are investigating the driving factors of their consumption, particularly regarding their price formation (30). Third, all commodities have fuel as a cost. Fourth, the economics of PrT on food systems among developing countries are highly sensitive to fuel variations. Therefore, it is necessary to analyze how fuel price spikes affect rural producers' welfare level (as produce costs) (9).

[2] More information regarding this policy can be obtained from http://www.minenergia.cl/archivos_bajar/Documentos/Agenda_Energetica/Agenda_de_Energia-Documento_Completo_Ingles.pdf

Methods

We implement a three-stage empirical strategy nested with both the stationary (fuel prices) and non-stationary (market prices) character of the time series. After determining the integration order of the series, we assess the linear cointegration relationships through the Johansen *et al.* (2000) trace test, followed by applying the Gonzalo-Pitarakis (2006) approach, who proposed that long-run equilibrium relationships between two markets may change according to the level of a stationary variable imposed exogeneously. Here, we test the linear cointegration null against the threshold cointegration between MR and 7R WM. In this approach, the null hypothesis of linear cointegration is:

$$y_t=\alpha_0+\alpha_1 * x_t+u_t \quad [1]$$

against the alternative hypothesis of cointegration with threshold effects:

$$y_t=(\alpha_0+\alpha_1 * x_t)+(\lambda_0+\lambda_1 * x_t)I(q_{t-d}>\gamma)+u_t \quad [2]$$

where:
y_t = Talca prices,
x_t = Santiago prices
u_t and v_t = scalar and p-vector valued stationary disturbance terms respectively,
q_{t-d} with $d \geq 1$ = a stationary ThVar lagged by d periods,
$I(q_{t-d}>\gamma)$ = an indicator function that equals one if $q_{t-d}>\gamma$, and zero otherwise.

Gonzalo & Pitarakis (2006) propose a supLM test based on the following statistic:

$$LM_T(\gamma)= 1/\sigma_0^2\, u'MX\gamma\, (X'_\gamma MX_\gamma)-1X'_\gamma Mu \quad [3]$$

where:
$M = I - X(X'X)-1X'$,
X stacks all values of x_t in the linear model [1],
$X\gamma$ stacks the values of x_t corresponding to the criterion $q_t>\gamma$ in the non-linear model [2].
T = the length of the full sample,
u = the residual,
σ_0^2 = the residual variance of the linear model [1].

The LM test statistic $LM\ T(\gamma)$ is calculated for all possible values of the ThVar q_t. The supLM test statistic is given by:

$$\sup LM=\sup_{\gamma\in\Gamma} LMT(\lambda) \quad [4]$$

Critical values for this test statistic are taken from Andrews (1993). Similarly to Götz and von Cramon-Taubadel (2008), we estimate an unrestricted, regime-specific ECM by including dummy variables defined by the indicator function $I(q_{t-d}>\gamma)$ corresponding to the threshold determined by the supLM test. This ECM takes the form:

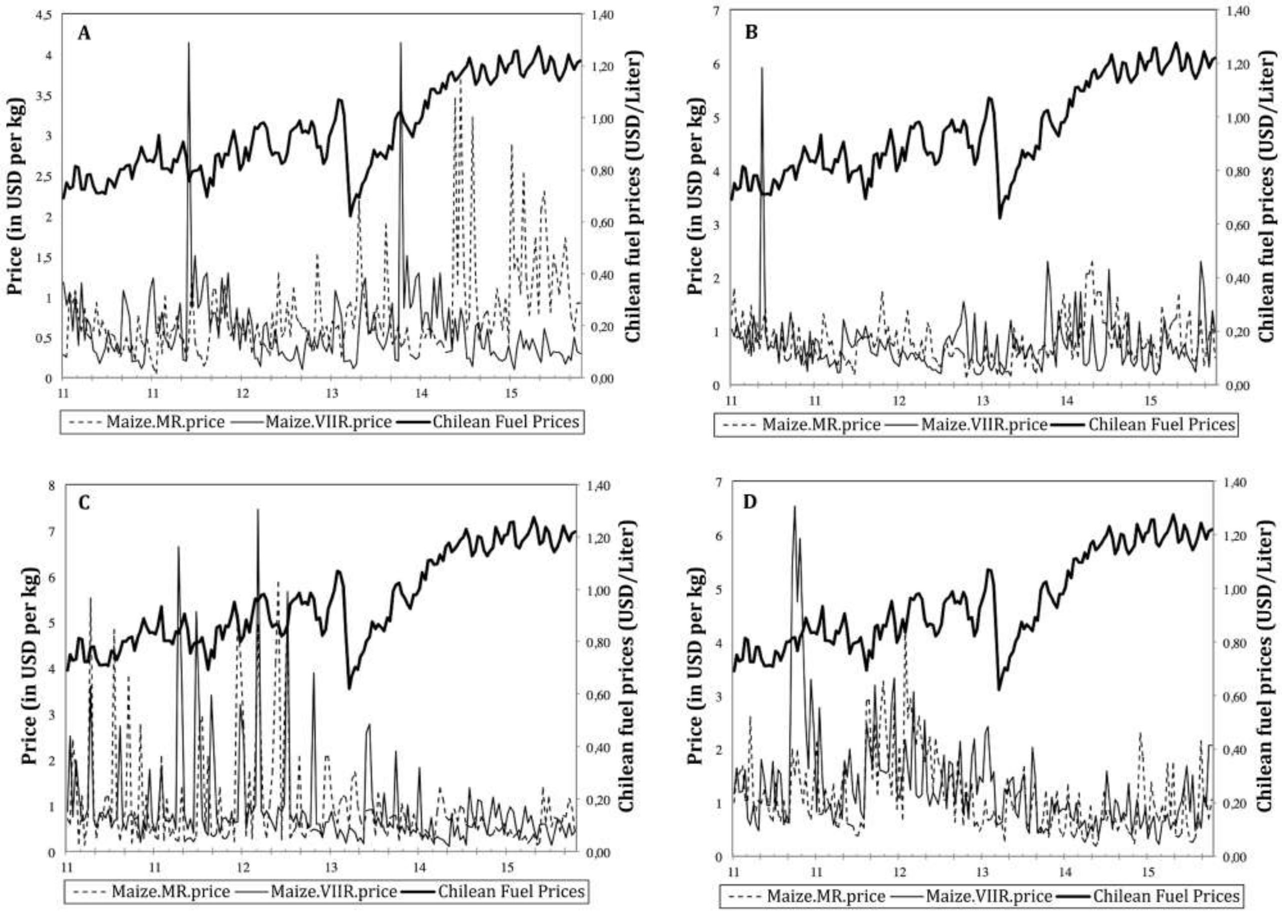
$$\Delta y_t=\beta_0+\delta_0 * I(q_{t-d}>\gamma)+\sum_{m=1}^{K}(\beta_{1m}\Delta x_{t-m+1}+\delta_{1m}\Delta x_{t-m+1} * I(q_{t-d}>\gamma)) \quad [5]$$

$$+\sum_{n=1}^{L}(\beta_{2n}\Delta y_{t-n}+\delta_{2m} * \Delta y_{t-n} * I(q_{t-d}>\gamma))+\beta_3 * y_{t-1}+\delta_3 * y_{t-1} * I(q_{t-d}>\gamma)+\beta_4 * x_{t-1}$$

$$+\delta_4 * x_{t-1} * I(q_{t-d}>\gamma)+\varepsilon_t$$

Data description

According to the Chilean government's Agricultural Research and Policies Office (hereafter ODEPA) wholesalemarkets concentrate 79% of Chilean domestic HC product trade. Among them, the *Central de Abastecimiento Lo Valledor* located in Santiago (Metropolitan region), hereafter MR, and the *Parque Mayorista* located in Talca (7th region), hereafter 7R, account for 61% of domestic annual traded volume. In particular, the marketing system through the *Central de Abastecimiento Lo Valledor* concentrates a majority percentage of the country's total horticultural marketing. This market has an area of 29 hectares, with 1,500 commercial premises and yards to receive 2,600 truckloads of products. It is visited daily by 30,000 people and 5,000 buyer vehicles. Transactions are characterized by being direct between the seller and the buyer, most of whom are small and medium-sized farmers and traders, with intermediaries and distributors from regional centers joining this group, which makes it possible to obtain benefits in determining final prices for consumers (figure 1, page 197).



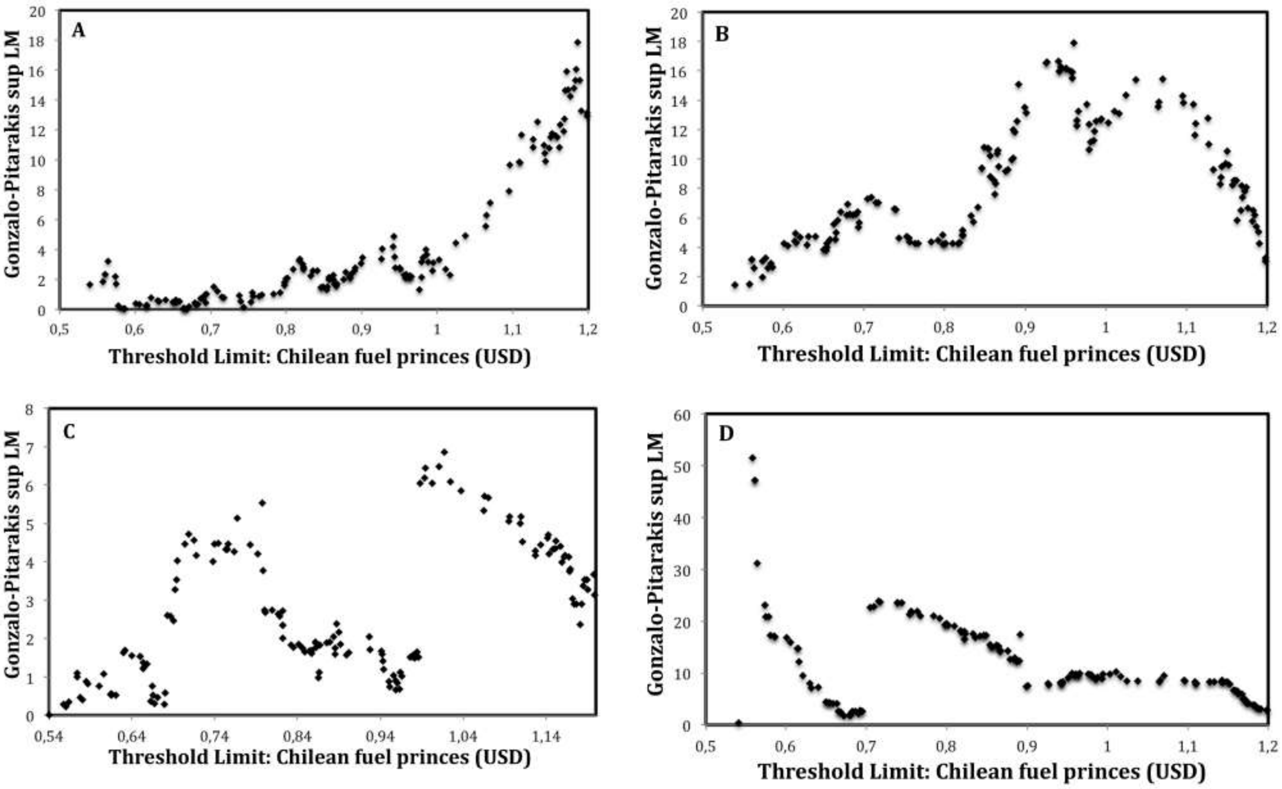
Source: ODEPA database. / Fuente: base de datos ODEPA.

Figure 1 A-D. Prices of the WM of MR and 7R (in USD per kg) over 2009-2015.

Figura 1 A-D. Precios de mercado mayorista para RM 7R (en USD por kg) durante 2009-2015.

Given their relevance, we consider the weekly wholesale prices (in USD/Kg) from 1-2011 to 12-2015 in MR and 7R. We consider the four most planted vegetables in Chile, accounting for 38% of total cultivated vegetable surface in Chile, 28% of total domestic trade (in value) and 18% of total producer units (23) (figure 2, page 198). For fuel prices (hereafter CHF), we use Chilean real prices. The data was obtained from ODEPA, the Chilean Statistical Office, the Chilean Institute for Agricultural Development and the National Petroleum Company of Chile. These series are expressed in USD (for vegetables) and USD/Liter (for Chilean fuel retail prices).

Initially, price developments show typical seasonal variation and price differences. This behavior of irregular seasonal PrT is typical for vegetable markets (20). Most price developments between MR and 7R follow similar patterns over time. Those patterns suggest the 7R market follows MR market price developments. This implies that MR prices could partially determine 7R prices. The Gonzalo-Pitarakis (2006) approach can capture price series non-linearities due to seasonal reversals in trade flow direction and non-constant TACs. Practically, TACs and trade flow reversals can modify arbitrage conditions and the nature of PrT and intermarket integration. Therefore, the observed reversal in trade flow due to seasonal supply source changes implies a variation in inter-market transportation costs (TACs' main component), suggesting the likelihood of the presence of threshold effects in price dynamics in the Chilean vegetable marketing system.



Figures 2 A-D. A) Corn (MR/7R); B) Tomato (MR/7R); C) Onion (MR/7R); D) Carrot (MR/7R).
Figuras 2 A-D. A) Maíz (RM/7R); B) Tomate (RM/7R); C) Cebolla (RM/7R); D) Zanahoria (RM/7R).

Results

Time series properties of data

MR and 7R wholesale prices for corn, tomato, onion and carrot, as well as CHF were tested for unit roots. The fuel price variable proved to be stationary at least with 10% level of significance (ADF statistics -0.48 and KPSS statistics 0.34) and 5% level of significance (PP statistics -1.99). Table 1 (page 199) reports the descriptive statistics and unit root tests results for the price series.

Since MR and 7R prices are non-stationary, cointegration techniques can be used to model these long-run inter-market relationships. The Johansen *et al.* (2000) trace test was applied, suggesting the presence of cointegration across the sample. The null hypothesis of a single cointegration vector cannot be rejected when bi-variate price pairs are considered. Accordingly, we conclude that MR and 7R markets are linked by one cointegrating vector at 5% level of significance, thus determining an equilibrium relationship between them.

Next, we test fuel prices' role in horizontal spatial PrT between MR and 7R markets. We use the Gonzalo-Pitarakis (2006) method for each vegetable price between MR and 7R markets, where 7R price = f (MR price). It seems plausible that long-run price relationships between MR and 7R display different patterns depending on CHF. Estimated sup LM-test statistic (equation 6) and the threshold values (ThVal) are presented in figures 2 A-D.

Generally, we confirm fuel prices' role in horizontal PrT between key Chilean HC WM. All regime dependent VEC models appear in table 2 (page 199).

Table 1. Description of covariates considered in this study.
Tabla 1. Descripción de las covariables consideradas en este estudio.

	Carrot MR price	Carrot 7R Price	Corn MR Price	Corn 7R Price	Onion MR Price	Onion 7R Price	Tomato MR Price	Tomato 7R Price	CHF
Share on total surface^	5%		17%		7%		9%		-
Sample size (N)	174	174	174	174	174	174	174	174	174
Mean	1.11	1.30	0.77	0.59	0.95	0.98	0.75	0.79	0.91
Standard Deviation	0.71	0.95	0.58	0.50	1.08	1.09	0.56	0.41	0.21
Minimum	0.19	0.23	0.06	0.10	0.11	0.14	0.19	0.14	0.54
Maximum	4.35	6.53	3.68	4.14	7.45	5.87	5.92	2.30	1.28
Skewness	1.37	2.70	2.53	4.18	3.52	2.64	4.89	1.03	0.01
Kurtosis	5.26	12.84	11.07	29.05	17.23	10.08	42.47	4.08	1.78
ADF test *Ho=Non Stationarity Process*	-0.26	+0.44	-0.13	-0.17	-0.18	+0.14	-0.09	-0.06	-0.48*
KPSS test *Ho=Stationarity Process*	0.77***	0.75***	0.62**	1.01***	1.71***	0.93***	0.79***	0.82***	0.34
Phillips Perron test *Ho=Non Stationarity Process*	-5.61	-6.78	-9.34	-5.13	-9.88	-10.74	-7.63	-8.01	-1.99**

Note: *, ** and *** denote significance at the 0.10, 0.05 and 0.01 levels, respectively. ^ with respect to the total Chilean horticultural planted area.
Nota: *, ** y *** denotan significancia en los niveles 0,10, 0,05 y 0,01, respectivamente. ^ con respecto al área hortícola total plantada chilena.

Table 2. Estimates for regime dependent VEC models following Gonzalo & Pitarakis method (2006).
Tabla 2. Estimaciones para modelos VEC dependientes del régimen siguiendo el método de Gonzalo y Pitarakis (2006).

	Corn (7R price = f (MR price))		**Tomato** (7R price = f (MR price))		**Onion** (7R price = f (MR price))		**Onion** (MR price = f (7R price))		**Carrot** (7R price = f (MR price))	
	Regime 1	Regime 2	Regime 1	Regime 2	Regime 1	Regime 2	Regime 1	Regime 2	Regime 1	Regime 2
Johansen trace test	0.052 (0 CIV)		0.061 (0 CIV)		0.011 (0 CIV)		0.022 (0 CIV)		0.049 (0 CIV)	
p-value (CIV#)	0.204 (1 CIV)		0.605 (1 CIV)		0.444 (1 CIV)		0.581 (1 CIV)		0.227 (1 CIV)	
Number of observations	150	24	101	73	117	57	39	135	1	173
Maximum LM statistic	17834		17877		6858		7041		50511	
Threshold limits (γ)	1.18		0.96		1.01		1.17		0.56	
Price Transmission Elasticity (β)	0.473	0.150	0.218	0.089	0.010	0.122	-0.062	0.212	0.473	0.149
Speed of Adjustment (α)	-0.091	-0.737	-0.200	-0.377	-0.065	-0.029	-0.099	-0.147	-0.016	-0.376
Mean of price difference	0.046	0.631	0.012	0.005	-0.202	0.075	-0.940	0.251	0.046	0.631
(standard error)	(0.132)	(0.225)	(0.065)	(0.189)	(0.170)	(0.115)	(0.462)	(0.160)	(0.132)	(0.225)
Likelihood ratio test	43665	55332	38768	39556	41224	40667	43754	40001	41442	56881
(p-value)	(<0.01)	(<0.01)	(<0.01)	(<0.01)	(<0.01)	(<0.01)	(<0.01)	(<0.01)	(<0.01)	(<0.01)

Discussion

A three stage method was applied to assess fuel prices' role in the wholesale price relationship bet-ween the MR and 7R HC markets. The cointegration test showed a strong long-run relationship between them. PrT analysis coefficients (α and β) suggest that, apart from onion, the price relation-ship is one-way (7R price only error corrects while MR dominates price). Also, the speed of adjustment coefficients are significant for all variables, thus reinforcing the hyphotesis of a causality pattern from the Santiago market (Central) to Talca market (Regional). These findings support the idea that regardless of the quantity produced in Talca, the major effect of price adjustment is a result of the high demand and market concentration of the Central wholesale market rather than the favourable productive conditions in Talca. These results mirror many empirical studies, which highlight market power's role on PrT among agricultural markets (1, 25). One major implication is that asymmetric central market price hikes that would cut marketing margins are passed to regional markets faster than producer price decreases that widen marketing margins. The results emphasize the presence of feedback between different markets, and support the imperfect PrT between central and regional markets.

For most price pairs, the Gonzalo-Pitarakis (2006) exogenous threshold driven cointegration confirmed non-linear results, displayed in two trade-share dependent long-run price elasticity relationships, and we asume that the differences between each regime mainly depend on the product traded and the fuel prices. Accordingly, we can confirm the important role played by Chilean fuel prices in horizontal PrT between the MR and 7R markets. We depart from the idea that differences between each regime mainly depend on each product's attributes and trade volume. One practical implication is that fuel prices affect wholesale prices through TPC, and there is a pattern of increasing marketing costs as farm to market distance grows. This acceptance follows previous studies on market integration within fresh produce (20).

When each product is analyzed, we found that corn presents its maximum $LM\gamma$ statistics with a (ThVal) of 17.834. The first regime has 150 observations and is active with CHF <1.18 USD/liter. It shows a price transmission elasticity (β) of 0.473 and speed of adjustment (α) of -0.091. This regime could characterize the fall harvest season when ±55% of corn offered in the MR comes from 7R. The second, larger regime pools 24 observations and is active with CHF>1.18 USD/l. This regime probably considers the origin from other regions further south than 7R. When compared with other vegetables, the effect of the ThVar is the highest among all variables, that is, lower fuel prices mostly affect the margin in which the trade between markets is profitable. It confirms our suspicion that the reaction on arbitrage for yearly crops [3] (products with low producer prices) is more elastic than cash crops [4] (characterized by higher price segmentation). These findings fit with other works (11, 26).

[3] Yearly crops: Plant that perform their entire life cycle from seed to flower to seed within a single growing season.

[4] Cash crops: plant cultivated to be sold to gain profit from the sale.

Tomatoes have a very stable supply in Chilean markets, representing 70% of all greenhouse production in Chile and complement outdoor production whenever it cannot supply the market (23). Despite its high sensitivity to winter frost, fresh tomato is present in the Chilean market throughout the year (28). The wide range of growing conditions offered nationwide allows its cultivation across most of the country. Currently, during the April-September period, the regions supply most tomatoes offered in MR markets. These attributes are expressed in the regime structure observed in this work. The larger regime has 101 observations, with β=0.218 and α=-0.200. In this regime, the maximum $LM\gamma$ statistics correspond to a ThVal of 17.877 and is active with CHF<0.96 USD/l.

On the contrary, the second regime pools 73 observations, with CHF>0.93 USD/l, β=0.089 and α=-0.377. The aforementioned probably reflects October prices, representative of early spring production, that show the greatest variation and a downward trend. This is due to the increase in the area under greenhouses and the higher production in the north of the country, which supplies, in addition to the markets of the Metropolitan Region, the south of the country. Thus, tomato trucks arrive directly to these wholesale markets from the north of Chile.

Onions are the second vegetable in acreage and production value in Chile. Since 2010, ±10,000 Ha/year has been planted, with ±40% for early and mid-season onions and 60% dedicated to "storing" onions (24). Previous evidence suggests that storing capacity could restrict PrT magnitude due to a smoothing effect of arbitrage on the price formation process (1).

Onions present the second largest differences in the number of observations between the larger regime (117 observations) and smaller regime (57 observations). This product presents the highest value of threshold exogenous variable with a fuel price of 1.08 USD. The maximum $LM\gamma$ statistics correspond to a ThVal of 6.858. The first regime contains 117 observations, and is active at CHF<1.01 USD, with β=0.010 and α=-0.065. The second regime pools 57 observations, at CHF>1.01 USD/l, with β=0.122 and α=-0.029. The characterization of the larger regime corresponds to market conditions when stored onions are supplied to WM. During this regime the price relationship is unidirectional with the MR price, not error correcting, and thus dominating the 7R price. Consequently, the initial price level for the new harvest is set in the MR and transmitted to regional markets allowing relatively high price differences that give margin to profitable arbitrage opportunities. Accordingly, during this period, the effect of CHF on wholesale margins and consumer prices is expected to be lower than other vegetables considered here.

70% of carrots are cultivated in the central region, which has two planting seasons (March-May and October-November). Their post-harvest life is very short (3 weeks on average). The maximum $LM\gamma$statistics corresponds to a ThVal of 50.511. The larger regime cointains 173 observations, and is activated at CHF>0.56 USD/liter. It shows β=0.149 and α=0.376. The shorter regime is active at CHF<0.56 USD/liter, however it pools only 1 observation with β=0.473 and α=-0.016. Two practical implications can be derived from the important difference in observation numbers. First, CHF could not act as an efficient trimming parameter in a threshold cointegration model (independent of the effect this variable has on PrT parameters) and, second, the arbitrage advantages from seasonality effects are more difficult to obtain. With this in mind, another vein for discussion can be developed. It focuses on the price differences mean, which is the mean of price changes over the pairs and regimes. These statistics equal 0.046 in the first regime, but 0.631 in the larger regime, further suggesting higher volatility of traded quantities during harvest season. This situation has two consequences: a) a more sensitive response of wholesale prices to CHF increases (expressed in the largest mean of price difference between both regimes), and b) relatively low profitability level relative to other species.

Overall, we confirm that the effect of fuel price increases on wholesale prices varies by product attributes. For vegetables with multiple growing sources and weak seasonality, fuel price effects are significant but vary across geographic markets and seasons. For vegetables with clear growing seasons, fuel price effects are more constant, with a discernible relationship between price increases, seasona-lity and perishability.

Our discussion to this point has focused on comparing PrT parameters and CHF changes across origins and markets. We assume that these parameters provide a measure to compare how product attributes affect intermarket trade flows. Examining the seasonal nature across products and regions, we can summarize different agribusiness implications into the importance of CHF in fresh produce prices:

-Price differences across products help clarify how much the impact of CHF varies. They suggest that TPC may help mitigate wholesale price volatility across products and possibly within WM across time. As the fuel share of wholesale prices increases, volatility potential deriving from alternative supply-side sources decreases.

-Both sensitivity to fuel prices and the margins themselves increase with distance. Thus, an important portion of the wholesale margin could be related to fuel costs for transport.

-Fuel price volatility can lead to substantial geographic variation in wholesale market produce prices.

-Fuel prices affect wholesale prices through TPC and there is a pattern of increasing marketing costs as farm to market distance grows. These findings corroborate previous work conducted on fuel and energy impacts on food prices (7).

Conclusions and limitations

In this paper, different methodological approaches were applied for an in-depth assessment of the role played by fuel prices on the wholesale price relationship between two major Chilean horticultural markets. We consider the most planted vegetables in Chile. To analyze, we use a unique continuous weekly price series for each product and market.

The goal was to identify the role played by CHF in HC price market integration by including an exogenous ThVar.

For most products, we found evidence for CHF's role in horizontal PrT between MR and 7R markets. This situation supports the idea that regardless of quantities traded in regional markets, the major effect of price adjustment is a result of the high demand and market concentration of a central market. Also, we confirm that fuel prices affect wholesale prices through TPC and that there is a pattern of increasing marketing costs as the distance from origin to market grows. Another type of threshold VECM could be considered to investigate short-run dynamics and to allow for asymmetric adjustments in response to positive and negative price shocks, especially endogeneity's effect of handling costs on prce transmission parameters, since commodity flows between markets are often unidirectional. Thus, transportation infrastructure and handling facilities may be better suited for one-way commodity flows. Thresholds may be asymmetric as transports cost more in one direction than the other.

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Chinese consumers' purchase intention of fresh cherries: Modeling of relations between satisfaction and perceived quality

Intención de compra en cerezas frescas de los consumidores chinos: modelando relaciones entre satisfacción y calidad percibida

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Abstract

The purpose of this study was to model Chinese consumers' intention to purchase fresh cherries through direct and indirect relations and mediating role between perceived intrinsic quality, perceived extrinsic quality and satisfaction. The analytical method used was a structural equation modeling (SEM). Surveys were applied to 388 buyers in three traditional markets in Beijing, China. A confirmatory factor analysis was performed, which presented an adequate goodness of fit to accept the model, according to the literature, which confirmed the relations. The results showed, that perceived extrinsic quality and satisfaction are directly and significantly related to Chinese consumers' intention to purchase fresh cherries. Additionally, an indirect and significant relationship was found between perceived intrinsic quality and purchase intention, through the mediating role of satisfaction.

Keywords
consumers • fresh cherries • perceived quality • satisfaction • purchase intention • China

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Resumen

El propósito de este estudio fue modelar la intención de compra de los consumidores chinos de cerezas frescas a través de relaciones directas e indirectas y un papel mediador entre calidad intrínseca percibida, calidad extrínseca percibida y satisfacción. El método analítico utilizado fue un modelado de ecuaciones estructurales (SEM). Se aplicaron encuestas a 388 compradores en tres mercados tradicionales en Beijing, China. Se realizó un análisis factorial confirmatorio, el cual presentó una bondad de ajuste adecuada para aceptar el modelo, según la literatura, confirmando las relaciones. Los resultados mostraron, que la calidad extrínseca percibida y la satisfacción están directa y significativamente relacionados con la intención de consumidores chinos de comprar cerezas frescas. Adicionalmente, se encontró una relación indirecta y significativa entre la calidad percibida intrínseca y la intención de compra, a través del papel mediador de la satisfacción.

Palabras clave

consumidores • cerezas frescas • calidad percibida • satisfacción • intención de compra • China

Introduction

Chile is the main supplier of fresh cherries in China, which receives more than 85% of the total exports of this fruit (6). An important leap forward considering that more than 10 years ago it was the 22nd supplier for the fruit industry in China (35). In addition, in 2018 Chile exported more than one hundred thousand tons of fresh cherries to China, almost quadruple the tons for all of 2012 (32). Such rapid growth can raise questions about certain factors and behavioral patterns that can provide information about the reasons for fruit consumption and, in particular, fresh cherries in China. Yet few studies have been published about the Chinese consumer generally and the way in which they buy food (19). It has been stated that the Chinese consumer's behavior is essentially different from other countries due to its unique cultural, social and economic roots (3).

From this perspective, concepts such as purchase intention have been used in the specialized marketing literature as a measurement for predicting behavior and subsequent repurchase. The concept reflects consumers' likely behavior in future purchase decisions in the short term (7). Purchase intention is the possibility that determines customers' willingness to buy the product; the greater the chances, the stronger the purchase intention (25). It is crucial to recognize customers' purchase intentions, since their behavior can generally be predicted according to their intention Hsu *et al*. (2017) suggest that purchase intention is a combination of the concentration of consumers in the purchase of a product and the likelikhood of buying. For a complete representation of consumers' purchase behavior, their attitudes, preferences, motivations and perceptions of income must be considered. It is also noted that purchase intention is a future projection of the consumer's behavior, which will significantly help form his/her attitudes (15).

In this area, the fresh fruit market in China has grown significantly, driven by growing demand from consumers increasingly concerned about healthy diets, food quality requirements and availability of fruit all year round (40), which has opened the Chinese domestic market to influence by international trade (3).

Although, perceived quality cannot be determined objectively, there are currently four main approaches to explain it: The economics of information approach, the means-ends chain approach, integrative approaches and multi-attribute approaches (12, 28), with the last being the one used in this study. The multi-attribute approach understands quality as a dichotomous phenomenon between intrinsic and extrinsic cues (12, 19, 33, 48).

Intrinsic signals measure the quality objectively. These qualities pervade the product with their functionality and are related to its physical appearance (1, 30). According to Olson and Jacoby (1972), the intrinsic attributes are specific to each product, disappear when they are consumed and cannot be altered without changing the nature of the product itself (1, 5). Conversely, extrinsic signals are not physically part of the product, but they represent information related to the product, which can be modified externally, for example use by dates, information related to the origin and information related to production and processing practices (20, 30). Generally, high perceived quality reflects superiority, greater reliability or greater functionality of a product. Therefore, when consumers favorably perceive the quality of a product, they are more willing to buy it, *i.e.*, a greater potential intention to determine the mentioned behavior (11).

Satisfaction is the psychological state where the emotions that contain unconfirmed expectations are combined with the consumer's previous feelings about consumption experiences (23). It is considered a cognitive and affective response by consumers to a food product in a purchase context (14). The important factors for consumer satisfaction when consuming foods appear before, during and after eating. For example, before eating, the important factors are: expectations and desires based on memories of previous food experiences and the context in which the meal is perceived (4). One of the most studied approaches in the literature is the paradigm or theory of the disconfirmation of expectations. This theory indicates that disconfirmation is a post-purchase process, in which the consumer establishes a comparison between the results obtained and the previously created expectations, and it is the difference between these two magnitudes that leads the consumer to form judgments about a product or service (42). Consequently, customer satisfaction can lead to a later behavioral intention as a purchase and a positive communication. Several authors have shown a direct relation between consumer satisfaction and the consumer's behavioral intention (22).

Additionally, the perceived quality of and satisfaction with food are highly correlated, with the former concept being a precursor of the latter (12, 14, 15, 31, 36).

Therefore, the main aim of this study was to model the purchase intention of Chinese consumers of fresh cherries through direct and indirect relations and mediating role between intrinsic perceived quality, extrinsic perceived quality and satisfaction. On this basis, the following hypotheses were tested:

H_1: Perceived intrinsic quality has a direct and significant relation with the satisfaction of the Chinese consumer of fresh cherries.

H_2: Perceived extrinsic quality has a direct and significant relation with the satisfaction of the Chinese consumer of fresh cherries.

H_3: Perceived intrinsic quality has a direct and significant relation with the purchase intention of the Chinese consumer of fresh cherries.

H_4: Perceived extrinsic quality has a direct and significant relation with the purchase intention of the Chinese consumer of fresh cherries.

H_5: Perceived intrinsic quality has an indirect and significant relation with the purchase intention of the Chinese consumer of fresh cherries through the mediating role of satisfaction.

H_6: Perceived extrinsic quality has an indirect and significant relation with the purchase intention of the Chinese consumer of fresh cherries through the mediating role of satisfaction.

H_7: Satisfaction has a direct and significant relation with the purchase intention of the Chinese consumer of fresh cherries.

The structural model is shown in figure 1.

Indirect relations: H_5 and H_6 are provided in the body of the text. Direct relations; H_3 and H_4 are provided in the body of the text.

Relaciones indirectas: H_5 y H_6 se proporcionan en el cuerpo del texto. Relaciones directas; H_3 y H_4 se proporcionan en el cuerpo del texto.

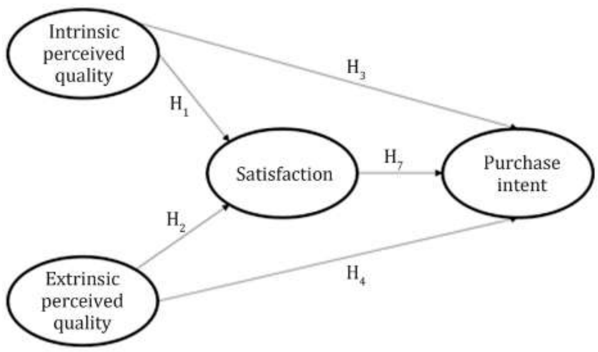

Figure 1. Causal model on purchase intention in Chinese consumers of cherries.

Figura 1. Modelo causal de intención de compra en consumidores chinos de cerezas.

Materials and Methods

Sample and questionnaire

A survey was applied to a non-probability convenience sample in Beijing, China (12, 28, 37) comprised of 388 people of Chinese nationality. The survey was developed in Chile in Spanish and then sent to China for translation. After the completion of this process by the specialized agency in Chinese, possible issues were identified and the writing was modified to adjust to the local cultural contexts in terms of vocabulary, idiomatic and syntactic equivalence where it was corrected and translated again back into Spanish. The two versions were compared, ensuring their consistency.

To improve the survey-taking process, a user manual was designed in Chinese, which was meant to instruct and guide the Chinese surveyors, reducing the likelihood of errors in the data collection due to reports of similar studies conducted in China, warning of people there being less familiar with surveys (19). The field work was conducted in two phases: the first consisted of applying a pretest, where 30 individuals were surveyed in the Yuyan Daxue market in Haidian district in Beijing, through which errors were detected that could affect the result, and these surveys were thus excluded from the analysis. The second stage consisted of applying the survey on a massive scale. The buyers of fresh cherries in three characteristic sectors of Beijing were defined as the sampling unit: i) the Sanyuanli food market, with 125 valid surveys, located in the international sector, is ample, with a good infrastructure and location, and has fruits of different origins and qualities; ii) the Wuluju market, with 128 valid surveys, located in the city's southwest, is in a sector far from the international area and focuses more on suburban life, and the prices of fruits are lower than in the Sanyuanli market; and iii) the Wudaokou sector, with 135 valid surveys, in the Haidian district, has a young population, as it is located near Tsinghua University and the Beijing Language and Culture University. Data were collected on different days.

The evaluation instrument included questions corresponding to perceived extrinsic quality (PEQ), perceived intrinsic quality (PIQ), satisfaction (ST) and purchase intention (PI). The method used to collect the information was quantitative based on a structured survey of 16 statements, (1: not important; 5: very important) for perceived quality, 5 statements for satisfaction (1: strongly disagree, 5: strongly agree), and 6 statements for purchase intention (1: strongly disagree, 5: strongly agree).

For the construct PIQ, attributes were used based on the studies by Zeithaml (1988), Grunert *et al*. (2015) and Chiang *et al*. (2018). The items used were: color, size, aroma, juiciness, flavor, texture and freshness. For the construct PEQ, attributes were used based on the studies by Ness *et al*. (2010), Grunert *et al*. (2015) and Chiang *et al*. (2018). The items considered in this study were: price, region of origin, seller's recommendation, seals of quality, commercial brand, packaging and bulk sale.

The construct satisfaction included statements used in previous studies by Sanzo *et al*. (2003), Espejel *et al*. (2009) and Chiang *et al*. (2018). The statements included in the study were: I feel satisfied with the product, I feel happy with the product, the product fulfills my purchase expectations, I am satisfied with the seller of the product and the product satisfies my needs.

Finally, for purchase intention, statements were included such as: I intend to continue buying the product (15, 21); if I couldn't find the product in my usual store, I would look for it another one (17); I want to buy the product (41); I intend to increase the size of the purchases (11); I intend to increase the frequency of purchases (11), and I am going to make an effort to buy the product in the near future (46).

To perform the exploratory factor and confirmatory factor analyses and the structural equation model, the software SPSS Statistics v.21 was used with its complement Amos, employing maximum likelihood extraction (27).

Exploratory and confirmatory factor analyses

The exploration of dimensions, Kaiser-Meyer-Olkin (KMO) index and Bartlett's test of sphericity (BTS) were applied, which must be significant, *i.e.*, less than 0.05, being obtained from the reduction of dimensions (44). In the confirmatory analysis, two tests of construct reliability were determined: Cronbach's alpha, which measures the internal consistency of the constructs individually, and the composite reliability index (CRI), which considers the interrelations of the constructs, both with an accepted minimum value of 0.7 (8). In order to obtain the validity of the latent variables, a convergent validity analysis was performed. The convergent validity is verified through the average variance extracted (AVE) of the constructs, with the recommended minimum of 0.5, with which it may be stated that the variables explain more than 50% of the variance of their constructs, there being convergent validity (38).

Structural equation model

Once these tests were completed, the goodness of fit of the model was estimated. For this study, the following was determined: a) Relative Chi (X2/df), which must be less than 2, b) Mean root error of approximation (RMSEA), with the maximum accepted value less than 0.05, c) Index Goodness of Fit (GFI), which must be greater than 0.95, d) Comparative Adjustment Index (CFI), which must be greater than 0.95, and e) Average Goodness of Fit Index (AFGI), which must have higher values at 0.80 (29).

Finally, based on the approach suggested by Baron and Kenny (1986), the *bootstrap* technique was used to evaluate direct and indirect relations as well as the mediating role of the variables on purchase intention with a 95% confidence level and a P value less than 0.05.

Results and discussion

With respect to the sociodemographic profile of the sample, table 1 illustrates greater participation in the purchase and consumption of fresh cherries by women. Generally, they are more aware of the implications of foods for health and the benefits of eating healthful foods (34).

Table 1. Sociodemographic profile of the sample.

Tabla 1. Perfil sociodemográfico de la muestra.

Item	Type	Frequency	Percentage
Gender	Male	172	44.3%
	Female	216	55.7%
Age	Between18 and 24 years	116	29.9%
	Between 25 and 34 years	120	30.9%
	Between 35 and 49 years	76	19.6%
	Between 50 and 64 years	51	13.1%
	More than 64 years	25	6.4%
Monthly income	Less than 4000 (RMB)	151	38.9%
	Between 4000 and 4999 (RMB)	25	6.4%
	Between 5000 and 6000 (RMB)	39	10.3%
	More than 6000 (RMB)	173	44.3%
Education level	Primary education	7	1.8%
	Secondary education	43	11.1%
	Technical professional	49	12.6%
	University education	289	74.5%

Williams, Ball and Crawford (2010), indicates that women have a greater gustatory preference for fruits and vegetables, and even have greater self-efficacy when it comes to following a healthy diet. Conversely, men worldwide consume fewer fruits and vegetables than what is recommended (2, 13). With respect to monthly family income, Florkowski *et al.* (2014) states that consumers with a higher income have greater chances of acquiring good quality fresh fruits. One the other hand, for purchaser of imported food in supermarkets of Beijing, Grunert *et al.* (2015) quantify a mean monthly family income of 7897 renminbi (RMB) and over 84 percent of those surveyed have a higher education. This is consistent with the results found in this research, especially if the data were collected in high-income sectors and with many universities.

Given that the sample obtained a value of 0.848 on the Kaiser-Mayer-Olkin index, a result above the minimum of 0.5 established by Williams, Brown and Onsman (2010), it was valid to perform a factorial analysis.

Bartlett's test of sphericity was 0.00, indicating that the correlation matrix is not an identity matrix. The individual reliability of the indicators (table 2) determined that the observable variables of Chinese origin (ATRI01), Chilean origin (ATRI02) and US origin (ATRI03) were significant for the construct PEQ, and the variables texture (ATRI10), size/caliber (ATRI11) and color (ATRI16) for the construct PIQ.

Table 2. Exploration of dimensions.

Tabla 2. Exploración de dimensiones.

Construct	Attribute	Code	Critical ratio
Perceived extrinsic quality (PEQ)	Origin China	ATR101	nd
	Origin Chile	ATRI02	15.307***
	Origin U.S.A	ATRI03	15.664***
	Seller's recommendation	ATRI04	ns
	Packaging	ATRI05	ns
	Bulk	ATRI06	ns
	Commercial brand	ATRI07	ns
	Seals of quality	ATRI08	ns
	Price	ATRI09	ns
Perceived Intrinsic Quality (PIQ)	Texture	ATRI10	nd
	Size /caliber	ATRI11	9.851***
	Aroma	ATRI12	ns
	Juiciness	ATRI13	ns
	Taste	ATRI14	ns
	Freshness	ATRI15	ns
	Color	ATRI16	9.971***
Satisfaction (ST)	I feel satisfied with the fresh cherries purchased	ATRI17	nd
	I feel happy with the cherries acquired	ATRI18	16.353***
	The fresh cherries meet my expectations	ATRI19	18.202***
	I feel satisfied with the seller of fresh cherries	ATRI20	14.447***
	The fresh cherries satisfy my needs	ATRI21	18.207***
Purchase Intention (PI)	I intend to increase the frequency of purchases of fresh cherries	ATRI22	nd
	I want to buy fresh cherries	ATRI23	18.716***
	If I couldn't find the product in my usual store, I would look for it another one	ATRI24	ns
	I intend to buy a greater amount of fresh cherries	ATRI25	20.668***
	I intend to continue buying fresh cherries	ATRI26	ns
	I am going to make an effort to buy fresh cherries in the near future	ATRI27	21.297***

***p< 0.05. nd: not determined, because this regression coefficient was adjusted to 1 to identify the model. ns: not incorporated as a variable to the model because it is insignificant.

*** p< 0,05. nd: no determinado, porque este coeficiente de regresión se ajustó a 1 para identificar el modelo.

ns: no incorporado como variable al modelo porque es insignificante.

Intrinsic quality results are consistent with another study on fruits like goji conducted in China by Yao *et al*. (2018), who reported that the Chinese consumer prefers fruits associated with good quality based on color and texture. Liu and Niyongira (2015) also detected that color generates the perception of safety that Chinese consumers have about foods. In another study, Sun (2010) established that the buyers of fresh cherries at online food markets prefer cherries according to their size and country of origin.

The indicators I feel satisfied with the fresh cherries purchased (ATRI17), I feel happy with the cherries acquired (ATRI18), the fresh cherries meet my expectations (ATRI19), I feel satisfied with the seller of fresh cherries (ATRI20) and the fresh cherries satisfy my needs (ATRI21) were part of the construct satisfaction, confirming the studies conducted on foods by Espejel *et al.* (2009) and Bech-Larsen and Tsalis (2018), which yielded results similar to those found in this study. Finally, the construct purchase intention was comprised of the indicators: I intend to increase the frequency of purchases of fresh cherries (ATRI22), I want to buy fresh cherries (ATRI23), I intend to buy a greater amount of fresh cherries (ATRI25) and I am going to make an effort to buy fresh cherries in the near future (ATRI27), obtaining results similar to the studies by Buaprommee and Polyorat (2016) on meats and Fandos and Flavián (2006) on food products with denomination of origin.

The reliability of the constructs was determined in the confirmatory analysis (table 3). Cronbach's alpha was above 0.7 for the five constructs; thus, they can be reliably measured individually. At the same time, the composite reliability determined that the constructs were reliable to measure as a whole. The convergent validity of the construct indicated that the attributes of the latent variables share more than 50% of the explained variance. The goodness of fit of the model was good and fulfilled what was stipulated in the literature: X^2/d.f = 1.887, RMSEA= 0.048, GFI= 0.940 and AFGI= 0.915 and finally, CFI= 0.971.

Table 3. Reliability and convergent validity of the constructs.

Tabla 3. Fiabilidad y validez convergente de los constructos.

Construct	Cronbach's α	Composite reliability	Average variance extracted
Perceived intrinsic quality	0.745	0.751	0.503
Perceived extrinsic quality	0.860	0.863	0.679
Satisfaction	0.891	0.895	0.633
Purchase intention	0.889	0.890	0.672

With respect to the direct and indirect relationships between the variables (table 4), it was found with 95% confidence (p <0.05) that only the intrinsic perceived quality in fresh cherries is directly and significantly related to the satisfaction of Chinese consumers, generating indirect effects on the purchase intention through the mediating role of satisfaction, which is opposed to the results obtained in extrinsic perceived quality. Other authors such as Ness *et al*. (2010), achieve partial support in the indirect relationship between perceived quality and behavioral intentions, possibly due to the behavioral duality of intrinsic and extrinsic perceived quality.

Table 4. Type of mediation. Direct, indirect path coefficients and totals by construct.

Tabla 4. Tipo de mediación. Coeficientes de trayectoria directa, indirecta y totales por constructo.

Construct	Satisfaction			Purchase intention		
	DE	IE	TE	DE	IE	TE
Perceived intrinsic quality	0.333*	nd	0.330*	Ns	0.175*	0.175*
Perceived extrinsic quality	ns	nd	ns	0.267*	ns	0.267*
Satisfaction	nd	nd	nd	0.228*	nd	0.228*

* p<0.05. nd not determined.
ns: not significant. DE: Direct effect. IE: Indirect effect. TE: Total effect.

* p< 0,05. nd no determinado.
ns: no significativo. DE: Efecto directo. IE: Efecto indirecto. TE: Efecto total.

It was found statistically that there is a direct and significant relation between the PIQ and satisfaction. Fan *et al*. (2017) and Chiang *et al*. (2018) showed that the signals of intrinsic quality present a direct and significant relation with Chinese consumers' satisfaction when preferring fresh fruits. This statistical relation was also demonstrated in other food types, *e.g.*, Espejel *et al*. (2009) in ham and Grunert *et al*. (2015) in pork ribs in China. This result means that hypothesis H_1 is accepted.

There was no statistical significance between PEQ and satisfaction, which means hypothesis H_2 is rejected. In this respect, studies like those conducted by Espejel and Fandos (2009) on wine and Chiang *et al.* (2018) on fresh cherries concluded that there is no positive and significant relation between PEQ and satisfaction; however, Grunert *et al.* (2015) determined that only in a narrow age group do the extrinsic attributes reach significance in Chinese consumer satisfaction.

Little information is available in the scientific literature linking PIQ and PEQ purchase intention; rather, various authors analyze it as a perceived general quality. For example, Buaprommee and Polyorat (2016) demonstrated that there is no direct and significant relation between perceived quality and purchase intention in Thai consumers of red meats; however, Ness *et al*. (2010) concluded that in 5 of 6 European countries a direct and significant relation does exist between the two variables. The differences in the results obtained by both authors might not only be due to cultural diversity, but also because there was no dissociation between extrinsic and intrinsic attributes. The results obtained in this study determined that PIQ presents no direct and significant relation with purchase intention, a result in conflict with that obtained in PEQ. Therefore, hypothesis H_3 is rejected and H_4 is accepted.

A similar context occurs when the indirect relations of PIQ and PEQ on satisfaction are measured. Ness *et al*. (2010) determined that perceived quality has an indirect relation on the behavioral intentions of European consumers of fruits like tomatoes. Also, in the services sector, researchers like Wu and Li (2017) demonstrated that quality is a precursor of satisfaction and a good predictor of behavioral intentions. In this study it was demonstrated statistically that PIQ has an indirect and significant bearing on purchase intention through the mediating role of satisfaction; therefore, hypothesis H_5 is accepted, on the other hand, no indirect and significant influence was found between PEQ and purchase intention as a result of the mediating role of satisfaction. Therefore, H_6 is rejected.

Satisfaction has a direct and significant relation on the purchase intention of Chinese consumers. Thus, hypothesis H_7 is accepted. This is consistent with studies conducted by Espejel and Fandos (2009) on wines, Ness *et al*. (2010) on tomatoes and Konuk (2018) on organic foods, which shows a direct and significant relation between satisfaction and purchase intention.

In addition, the results obtained from hypotheses $H_{1,}$ $H_{5,}$ and H_7 confirm that there is complete mediation between PIQ and purchase intention from the mediating role of satisfaction. The results of this research seem to be in line with the marketing initiatives developed by ASOEX, enhancing the attributes of intrinsic quality, color and size, and linking it to celebrations and traditions, through activities such as "Super Cherries days", "Chilean Cherries dance" and "Cherries from Chile", activities that have also been enhanced by public policies and agreements such as the free trade agreement between the two countries in force since 2005.

The limitations of this study, considering the non-probabilistic nature of the sample, are that the results cannot be generalized and that the sample is not representative according to the population distribution.

Conclusions

The main aim of this study was to model the purchase intention of Chinese consumers of fresh cherries through direct and indirect relations and mediating role between perceived intrinsic quality, perceived extrinsic quality and satisfaction. It was found that Chinese consumers' satisfaction with fresh cherries is related directly and significantly to purchase intention, and that in turn satisfaction acted as a mediator of the intrinsic perceived quality variable, indirectly and significantly affecting purchase intention.

The study also found a direct and significant relationship between the latent variable of perceived extrinsic quality with purchase intention, and although it is not explained by the mediating role of satisfaction, it manages to be the variable with the best correlation in total effects on the intention to purchase.

The attributes that comprise the latent variables are worthy of study for exporters of fresh cherries to China, as the country of origin stands out for perceived extrinsic quality, and the color, size and texture for perceived intrinsic quality. It is important that these attributes are not neglected, because combined they can help generate the construction of quality in consumers, who will be willing to acquire fresh cherries when they perceive a greater value and useful life of the product than for the money they pay.

In summary, the exporter of fresh cherries should focus his strategy on the intrinsic attributes of the product that directly satisfy the Chinese consumers (color, texture and size). These attributes have a direct and significant relationship on the purchase intention these consumers, through the latent variable of satisfaction. On the other hand, the exporters should highlight the origin attribute (such as Chilean origin), because it shows direct and significant relationship with the intention of purchase, through of latent variable of perceived extrinsic quality.

Future studies could include in the model variables related to the consumer's purchase intention such as: loyalty, sacrifice and perceived risk. Additionally, studying the moderating role of these variables in consumers can be a feasible suggestion to delve more deeply into the study discussed here.

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Evaluation of tolerance to *Fusarium oxysporum* and *Fusarium solani* in Virginia-type tobacco (*Nicotiana tabacum* L.) varieties under controlled conditions in Northwestern Argentina

Evaluación de la tolerancia a *Fusarium oxysporum* and *Fusarium solani* en variedades de tabaco (*Nicotiana tabacum* L.) tipo Virginia bajo condiciones controladas en el noroeste de Argentina

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Abstract

The production of tobacco (*Nicotiana tabacum* L.) in Argentina is centered in the northwestern region (NWA), where the incidence of root rots and stem diseases caused by *Fusarium* spp. has increased considerably in recent years. This study aimed to evaluate the pathogenicity levels of isolates of the *F. oxysporum* and *F. solani* complexes in different varieties of Virginia Type tobacco. The commercial varieties MB47, PVH229, NC71, K346, K326, and K394 were inoculated with six isolates of both complexes. The variables evaluated were the incidence and severity of the symptoms. The area under the disease progress curves (AUDPC) was calculated and subjected to analysis of variance (ANOVA). Also, disease epidemiological models were fitted to the experimental data. The MB47 variety was significantly less infected and the varieties K346, K326, and K394 had the highest AUDPC means, showing susceptibility to the isolates. The disease intensity curves were adequately described by the monomolecular and logistic models. The results provide, for the first time, information about the levels of tolerance to vascular wilt and root rot under controlled conditions for the main varieties of Virginia-type tobacco grown in NWA.

Keywords

tolerance • *Nicotiana tabacum* L. • FOSC • FSSC • soil-borne diseases • pathogenicity

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Resumen

La producción de Tabaco (*Nicotiana tabacum* L.) en Argentina se concentra en la Región Noroeste (NOA), donde la incidencia de las enfermedades radiculares y de tallo causadas por *Fusarium* spp. han aumentado en los últimos años. Este estudio, tuvo como objetivo evaluar los niveles de patogenicidad de aislamientos de los complejos *F. oxysporum* y *F. solani* en diferentes variedades de tabaco tipo Virginia. Las variedades evaluadas fueron: MB47, PVH229, NC71, K346, K326 y K394 que se inocularon con seis aislamientos de ambos complejos. Las variables evaluadas fueron incidencia (I) y severidad (S). Se calculó el área bajo las curvas de progreso de la enfermedad (ABCPE) y se sometió a análisis de varianza (ANOVA). Además, se ajustaron los datos experimentales a modelos epidemiológicos del proceso de enfermedad. La variedad MB47 resultó significativamente menos infectada y las variedades K346, K326, K394 presentaron los valores de ABCPE más altos, mostrando un comportamiento susceptible. Las curvas de intensidad de la enfermedad se describieron adecuadamente mediante los modelos monomolecular y logístico. Los resultados generados en el presente estudio proporcionan por primera vez información sobre los niveles de tolerancia al marchitamiento vascular y podredumbre radicular en condiciones controladas para las principales variedades de tabaco cultivadas en NOA.

Palabras clave

tolerancia • *Nicotiana tabacum* L. • FOSC • FSSC • enfermedades de hongos de suelo • patogenicidad

Introduction

Tobacco (*Nicotiana tabacum* L.) is an economically important crop cultivated in more than 125 countries worldwide. Argentina, which is among the top ten producers, cultivates 43,815 hectares of tobacco every year, mainly in the northwestern region of the country. Virginia-type tobacco varieties represent 74% of the national production, while the other 26% corresponds to the Burley and Tobacco Creole types (28).

Fusarium wilt, caused by members of the *Fusarium oxysporum* species complex (FOSC), is a widespread disease that causes severe damage in tobacco-producing areas of Argentina and many countries around the world (24, 33). The tobacco wilt-causing *Fusarium* species have been designated as *F. oxysporum* f. sp. *nicotianae*, *F. oxysporum* f. sp. *batatas,* and *F. oxysporum* f. sp. *vasinfectum* based on their ability to cause disease on multiple hosts such as sweet potato, cotton, and tobacco (1, 2, 19, 32). Disease symptoms caused by members of FOSC may appear in the field, manifesting slow yellowing and drying of the leaves, sometimes along one side of the tobacco plant (24, 33). This pathogen causes crop losses of up to 15-20% of tobacco production (18, 30). On the other hand, Fusarium root rot is caused by members of the *Fusarium solani species* complex (FSSC). Disease symptoms include chlorosis and wilt, progressing from the lowest to the highest leaves. Members of FOSC and FSSC have been associated with tobacco wilt and root rot in Northwestern Argentina (NWA) (4) and other tobacco-growing regions worldwide (8, 36).

Soil-borne diseases are difficult to control, making it essential to adopt integrated management strategies. The most effective control of Fusarium wilt and root rot has been the development and use of resistant or tolerant tobacco varieties (19). In Argentina, commercial varieties with genetic resistance are not available; instead, tolerant varieties that contribute to reducing infection and minimizing yield losses are used. It has recently been reported that pathogenic isolates of FOSC and FSSC from NWA differed in their virulence levels when tested under controlled conditions in tobacco plants (4). However, the existence of specific interactions between isolates and tobacco cultivars remains unknown.

No information is available on the level of *Fusarium* inoculum concentrations in local soils cropped with tobacco or on the response of the varieties to these diseases. Chlamydospores are the main form of inoculum in the field, although the fungus can also produce microconidia and macroconidia (22). Once present in a field, the fungus persists for years in the absence of a susceptible host. Understanding the relationship between disease incidence and pathogen variability may allow the development of effective management strategies and a better prediction of the disease progression over time, in a context of sustainable cropping. The objectives of this study were (1) to evaluate the pathogenicity levels of isolates of FOSC and FSSC recovered from the main tobacco-growing area of Argentina; and (2) to evaluate six Virginia-Type varieties of tobacco for their resistance to FOSC and FSSC under controlled conditions.

Materials and methods

Fungal isolates and inoculum production

Six isolates of FOSC and FSSC, previously characterized (4), were used to evaluate wilt and root rot resistance on tobacco plants (table 1). Monoconidial cultures of the six tobacco pathogenic *Fusarium* spp. isolates were selected from the fungal collection of "Laboratorio de Sanidad Vegetal" INTA-EEA-Salta Microbial Collection, Argentina. The isolates were initially recovered from tobacco plants showing wilt and root rot symptoms in the main Virginia-type tobacco-growing area of Argentina.

Table 1. Origin and features of the six isolates of FOSC and FSSC, recovered from the main Virginia-type tobacco-growing area in Argentina, used in this study.

Tabla 1. Origen y características de los seis aislamientos de FOSC y FSSC, recuperados de las principales zonas de cultivo de tabaco tipo Virginia en Argentina, utilizados en este estudio.

Isolate	Species (GenBank#) [a]	Geographic origin (latitude, longitude)	Pathogenicity [b]
		FOSC	
Fo15	*F. oxysporum* f. sp. *vasinfectum* (MF327631)	La Caravana, Jujuy, Argentina (24°28´12" S; 65°5´32" W)	HP
Fo27	*F. oxysporum* f. sp. *batatas* (MF327609)	Alto Verde, Jujuy, Argentina (24°26´39" S; 65°7´18" W)	HP
Fo33	*F. oxysporum* f. sp. *batatas* (MF327613)	Alto Verde, Jujuy, Argentina (24°26´40" S; 65°7´02" W)	HP
		FSSC	
Fs44	*F. solani* (MF327639)	Alto Verde, Jujuy, Argentina (24°27´39" S; 65°7´08" W)	HP
Fs55	*F. solani* (MF327646)	La Merced, Salta, Argentina (24°58´ 43" S; 65°28´18" W)	HP
Fs98	*F. solani* FSSC5 (MF327665)	Vaqueros, Salta, Argentina (24°41´17" S; 65°23´55" W)	MP

[a] Species determined based on EF1-α sequence analysis, GenBank#: GenBank accession number
[b] HP: Highly pathogenic, MP: moderately pathogenic. Isolates were characterized in a previous study (Berruezo *et al.* 2018).

[a] Especies determinadas con base en el análisis de las secuencias de EF1-α. GenBank#: GenBank número de acceso.
[b] HP: altamente patogénicos, MP: moderadamente patogénicos. Los aislamientos fueron caracterizados en estudios previos (Berruezo *et al.* 2018).

For inoculum preparation, fungal colonies were grown on potato dextrose agar (PDA) at 25°C in the dark for five days (17). Microconidia suspensions were obtained by adding 10 mL of sterile distilled water to the cultures and rubbing the culture surfaces with a sterile glass rod. The suspensions were filtered through sterilized cotton, and microconidia were quantified microscopically using a hemocytometer (11).

Tobacco plant inoculation

Six commercial tobacco varieties (MB47, PVH2291, NC71, K346, K326, and K394) were evaluated. These genotypes represent the varieties most commonly used in NWA. Tobacco seeds were seeded under a hotbed with a sterile substrate (autoclaved for 30 minutes at 120°C, on two consecutive days); the seedlings were grown at 25 ± 2°C with a 12 h photoperiod (32). When the plants had grown four true leaves, they were transplanted.

The experiment was conducted following a factorial treatment. The experimental design consisted of two factors: tobacco variety (six levels) and Fusarium isolate (six levels), in a completely randomized design. Data from the two independent pathogenicity tests were combined and analyzed as one.

Each plastic pot (final volume of 400 g) containing sterile sand and mulch (autoclaved for 30 minutes at 120°C, on two consecutive days) was used as the substrate mixed in a proportion of 1/1 (vol/vol). The inoculum concentration (1 x 10^6 microconidia/g of the substrate) of each *Fusarium oxysporum (Fo)* and *Fusarium solani (Fs)* isolate was used. Six pots of each variety were inoculated with the deposition of 1 mL of each conidial suspension (31). Plants were maintained for 30 days in a growth chamber at 25 ± 2°C with a 12 h photoperiod. The pots were drenched periodically with sterile distilled water in order to keep the humidity of the substrate. Six pots with a non-inoculated sterile substrate of each variety were used as control.

Disease assessment

Disease progressions were analyzed by the incidence (I) and severity (S) of typical wilt and root rot symptoms. Each plant was assessed for symptom severity on a scale of 0-4 at 5-day intervals. To evaluate wilt (FOSC), the scale proposed by LaMondia and Taylor (1987) was used (0 = 0%, 1 = 1-33%, 2 = 34-66%, 3 = 67-100%, 4 = dead plants). Roots were rated for Fusarium root rot (FSSC) symptoms following a five-class rating scale (0 = no lesions, 1 = small root lesions, 2 = central root lesions, 3 = large root lesions, 4 = dead plant). The disease severity index (DSI) was calculated for each isolate using the following formula: $(n_1)+(n_2x2)+(n_3x3)+(n_4x4)/n_o+n_1+n_2+n_3+n_4$, where n_0 is the number of plants in category 0 of the scale, n_1 is the number of plants in category 1, n_2 is the number of plants in category 2, n_3 is the number of plants in category 3, and n_4 is the number of plants in category 4 (15, 35). The number of healthy plants was recorded at a 5-day interval for 30 days post-inoculation (5, 10, 15, 20, 25, and 30 dpi).

Finally, the plants were cut across the stem to verify the vascular discoloration, superficially disinfected, placed onto PDA plates, and incubated for ten days. The pathogen was reisolated from all symptomatic tissue fulfilling Koch's postulates.

Data analyses

The incidence was related to the time to describe a disease progress curve. The area under the disease progress curve (AUDPC) was calculated through the polygon method (7), subjected to analysis of variance (ANOVA), and compared using the Fisher LSD test ($\alpha = 0.05$).

Additionally, the incidence data was linearized, and linear regression analysis was performed to obtain the parameters of three epidemiological models: monomolecular, logistic, and Gompertz (25). The residuals and the graphic adjustment of the experimental data and of the coefficient of determination (R^2), as well as the mean squared residue, were considered for the model selection. The apparent infection rate parameter (slope) of the equation for each repetition was determined from linearized data. The slopes were then analyzed by ANOVA and used to construct a simulated disease progress curve for each isolate evaluated (initial incidence considered $y_0 = 0.0001$; $y_0* = \ln [(y_0 / (1-y_0)])$. All data analyses and model adjustments were performed using the InfoStat software (13).

Results

Tobacco variety-*Fusarium* isolate interaction

All six *Fusarium* isolates tested were pathogenic and produced different symptoms on tobacco plants depending on the species used. FOSC isolates produced mainly wilting, chlorosis, and growth reduction in tobacco plants (Supplementary Figure 1); in contrast, FSSC isolates caused root rot, with characteristic necrotic lesions and root rot symptoms (Supplementary Figure 2).

There were highly significant statistical differences in disease severity for tobacco varieties (genotypes) ($p < 0.0001$) and for variety x isolate interaction ($p < 0.01$) (table 2, page 218). These results clearly suggest that the disease progression may vary according to the *Fusarium* isolate and tobacco varieties.

Table 2. Mean squares from the analysis of variance for AUDPC values for the six varieties of tobacco evaluated at 30 days post-inoculation with six isolates of *Fo* and *Fs* and the interactions between them.

Tabla 2. Análisis de varianza para los valores de ABCPE para las seis variedades de tabaco evaluadas a los 30 días después de la inoculación con los seis aislamientos de *Fo* y *Fs* y las interacciones entre ellos.

Source	df	Mean squares	p-*value*
Model	71	65.08	<0.0001
Variety	5	250.55	<0.0001
Isolate	5	39.13	0.0516
Variety*Isolate	25	33.17	0.0095
Error	144	18.84	
Total	215		

Aggressiveness of *Fusarium* isolates

All the isolates caused high disease incidence in tobacco plants after 30 dpi. Significant differences ($p < 0.05$) were observed in the mean DSI scores between isolates (table 3). The highest DSI scores were registered for Fo27 and Fo15, while the lowest DSI score was found for Fs98. As expected, the results obtained from the DSI scores were related to the mean AUDPC values of the isolates. These results suggest a differential behavior of *Fusarium* isolates for disease development on tobacco plants. Tobacco varieties differed significantly ($p < 0.05$) based on the mean DSI scores. K394 and NC71 showed the highest and lowest DSI scores, respectively.

Table 3. Mean Disease Severity Index (DSI) and the AUDPC registered at 30 days post-inoculation for the tobacco varieties and *Fo* and *Fs* isolates evaluated.

Tabla 3. Media del Índice de Severidad de Enfermedad (DSI) y ABCPE registrados a los 30 días después de la inoculación para las variedades de tabaco y los aislamientos *Fo* y *Fs* evaluados.

Varieties	DSI	AUDPC (VC) [a]		Isolates	DSI	AUDPC (VC)	
MB47	2.68 b	7.95 (0.44)	e	Fo15	2.99 c	11.88 (0.35)	ab
PVH2291	2.87 c	11.18 (0.46)	cd	Fo27	3.06 d	12.60 (0.44)	a
NC71	2.28 a*	9.36 (0.49)	de	Fo33	2.84 b	10.42 (0.37)	b
K346	2.91 d	14.89 (0.56)	a	Fs44	2.84 b	12.69 (0.47)	a
K326	2.94 d	12.83 (0.31)	bc	Fs55	2.82 ab	11.92 (0.52)	ab
K394	3.63 e	13.60 (0.46)	ab	Fs98	2.74 a	10.30 (0.49)	b

[a] Different letters indicate statistically significant differences ($p \leq 0.05$). Comparison by the Fisher LSD test ($\alpha = 0.05$). VC: Variation coefficient.

[a] Diferentes letras indican diferencias estadísticamente significativas ($p \leq 0,05$). Comparación por prueba de Fisher LSD ($\alpha = 0,05$). CV: coeficiente de variación.

Varietal performance of *Fusarium* infection

All tobacco varieties showed significantly different levels of tolerance against the infection caused by the six *Fusarium* isolates evaluated. MB47 and NC71 were significantly less infected than the other varieties, which registered low AUDPC values (figure 1, page 219).

Besides, MB47 resulted in a greater degree of tolerance to FOSC isolates, followed by PVH2291 and NC71. For FSSC isolates, the varieties NC71 and MB47 exhibited better behavior under controlled conditions. In contrast, K346, K326, and K394 had high AUDPC scores, resulting in susceptible behavior for all the isolates of *Fusarium* analyzed (table 4, page 219).

Model adjustment

The disease intensity curves were adequately described by the monomolecular and logistic models (table 5, page 220). These models were the ones that were best adjusted based on the residue graphs and adjusted determination coefficients (R^2). The equation that represents the incidence (y) as a function of time (t) was calculated as follows: $y = 1- (1-y_0) \exp(-rt)$; then the equation that defines the logistic model was $y = 1 / [1 + \{-\ln y_0 / (1- y_0) + rt\}]$. To control the fulfillment of the assumptions of the analysis, we requested the student residual *vs.* predicted graphs, and Q-Q plot to confirm the normality of the model data.

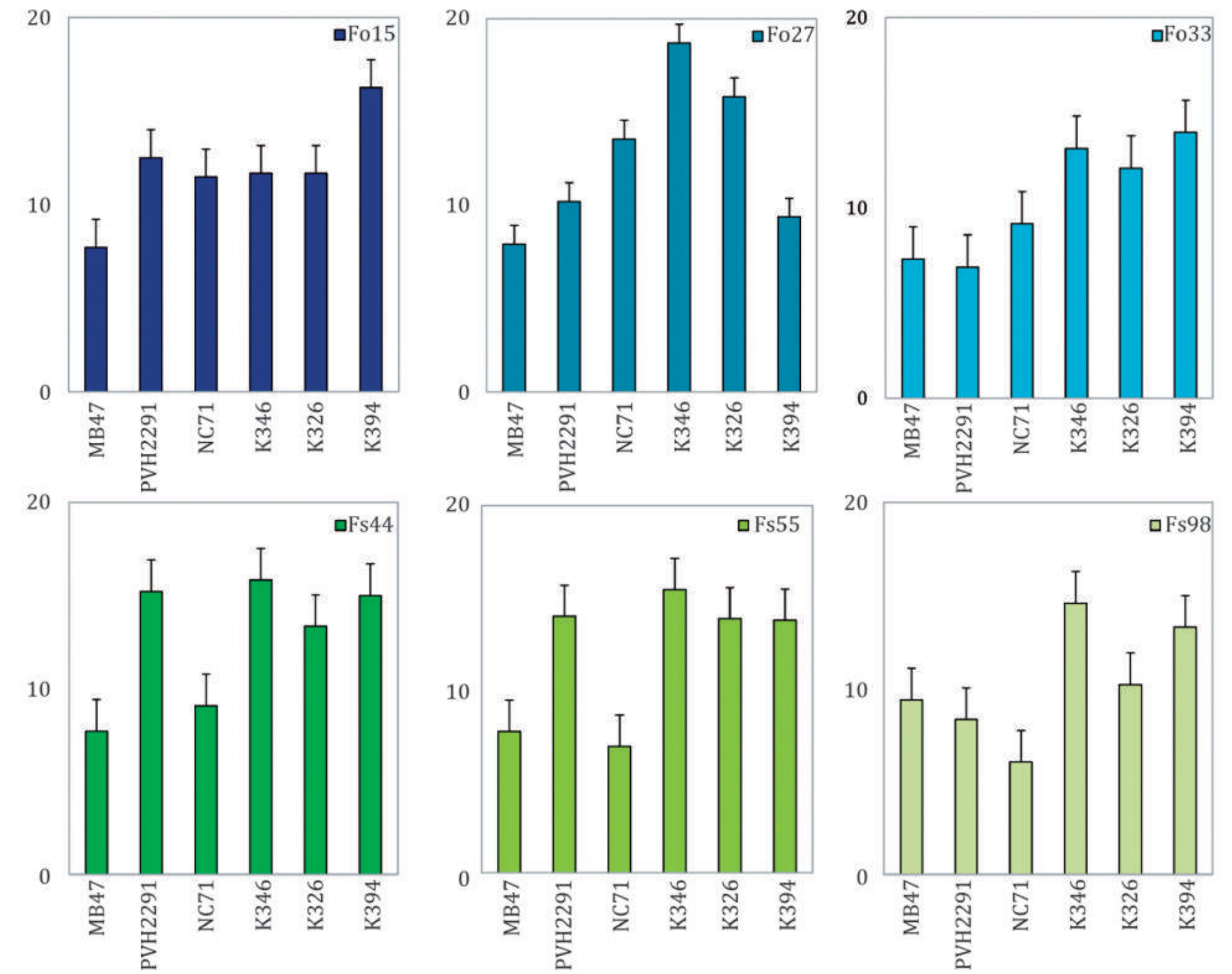

Figure 1. Interaction between the *Fusarium oxysporum* and *Fusarium solani* isolates and the six tobacco varieties evaluated (Fisher's LSD test, α = 0.05). Vertical bars represent the standard deviation.

Figura 1. Interacción entre los aislamientos *Fusarium oxysporum* and *Fusarium solani* y las seis variedades de Tabaco evaluadas (prueba de Fisher's LSD, α = 0,05). Barras verticales representan la desviación estándar.

Table 4. *Fo* and *Fs* isolates evaluated and mean of the AUDPC registered at 30 days post-inoculation.

Tabla 4. Aislamientos de *Fo* y *Fs* evaluados y media del ABCPE registrado a los 30 días después de la inoculación.

Fusarium Isolates	**Fo15**	**Fo27**	**Fo33**	**Fs44**	**Fs55**	**Fs98**
Varieties						
MB47	7.71	7.92	7.31	7.71	7.72	9.38
PVH2291	12.5	10.20	6.88	15.21	13.96	8.33
NC71	11.47	13.56	9.17	9.06	6.88	6.04
K346	11.67	18.71	13.12	15.83	15.42	14.58
K326	11.68	15.83	12.08	13.33	13.83	10.21
K394	16.25	9.38	13.96	14.99	13.75	13.29
[1]Mean	11.88	12.6	10.42	12.69	11.92	10.30
[2]LSD ($P \leq 0.05$)	6.18	6.55	4.53	8.00	7.75	7.58

[1] Mean values of the AUDCP. Comparison by the Fisher LSD test (α = 0.05). [2]LSD: Least significant difference to compare *Fusarium* wilt and root rot.

[1] Media del ABCPE. Comparación por prueba de Fisher LSD (α = 0,05). [2]LSD: Diferencia menos significativa para comparar el marchitamiento por *Fusarium* y podredumbre radicular.

[a] Adjusted models; [b] apparent infection rate; [c] adjusted coefficient of determination; [d] mean square error; [e] p probability associated with the model.

[a] Ajuste del modelo; [b] tasa de infección aparente; [c] coeficiente de determinación ajustado; [d] cuadrado medio del error; [e] probabilidad asociada al modelo.

Table 5. Statistical parameters applied for model selection and description of the *F. oxysporum* and *F. solani* complexes.

Tabla 5. Parámetros estadísticos aplicados para la selección del modelo y descripción de los complejos *F. oxysporum* y *F. solani*.

Isolates	Models[a]	r[b]	R[c]	MSE[d]	p(m)[e]
Fo15	Monomolecular	0.01	0.90	0.02	0.0022
Fo27	Logistic	0.03	0.92	0.02	0.0007
Fo33	Logistic	0.05	0.47	0.02	0.0097
Fs 44	Monomolecular	0.05	0.93	0.05	0.0003
Fs55	Monomolecular	0.05	0.85	0.06	0.0210
Fs98	Monomolecular	0.06	0.9	0.05	0.0007

Figure 2 presents the adjusted curves for FOSC and FSSC. Different curves in the same plot represent each of the three *Fusarium* isolates belonging to each complex. Using these models, it was confirmed that the final amount of disease increases in a monomolecular model in most of the isolates. The slopes of the adjusted equations for each isolate concentration were evaluated by ANOVA (VC= 4.44), and the results are shown in table 6.

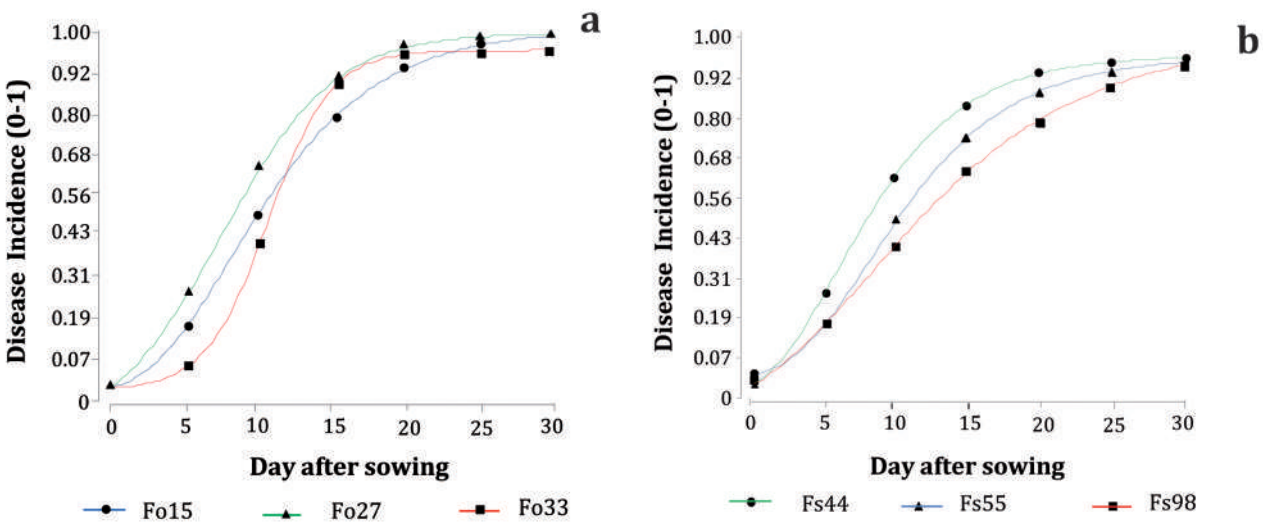

Figure 2. Progression of Fusarium wilt and root rot in six varieties of Virginia-type tobacco. a) *Fusarium oxysporum* and b) *Fusarium solani*.

Figura 2. Progreso del marchitamiento vascular por *Fusarium* y podredumbre radicular en las seis variedades de Tabaco tipo Virginia, a) *Fusarium oxysporum* y b) *Fusarium solani*.

*Different letters indicate statistically significant differences ($p \leq 0.05$). Comparison by the Fisher LSD test (α = 0.05).

*Letras diferentes indican diferencias estadísticamente significativas (p ≤ 0,05). Comparación por prueba de Fisher LSD (α = 0,05).

Table 6. Analysis of the variance of the mean slope coefficients for the linearized model.

Tabla 6. Análisis de la varianza de los coeficientes de pendiente media para el modelo linealizado.

Isolates	Models [a]	r [b]	R [c]	MSE [d]	p(m) [e]
Fo15	Monomolecular	0.01	0.90	0.02	0.0022
Fo27	Logistic	0.03	0.92	0.02	0.0007
Fo33	Logistic	0.05	0.47	0.02	0.0097
Fs 44	Monomolecular	0.05	0.93	0.05	0.0003
Fs55	Monomolecular	0.05	0.85	0.06	0.0210
Fs98	Monomolecular	0.06	0.9	0.05	0.0007

Discussion

This work represents the first analysis of the behavior of commercial varieties of tobacco against pathogenic isolates of the *Fusarium oxysporum* species complex (FOSC) and *Fusarium solani species* complex (FSSC) in Argentina. The varieties exhibited differential behavior against the isolates. FOSC isolates were associated with typical wilting symptoms, like slow yellowing and occasional drying of the leaves along one side of the plant. The presence of *Fusarium solani* has been recently detected for the first time in the region as a pathogen causing stunting, wilting, necrosis, and death in the tobacco plant (4).

The most effective and sustainable control against wilt and root rot diseases caused by soil-borne pathogens resides in the use of resistant genotypes. However, the existence of a narrow genetic basis among commonly used tobacco varieties has been established (37, 38). The situation of flue-cured tobacco may be an extreme example of the impact of stringent quality requirements and conservative breeding strategies on the narrow genetic base of germplasm pools. Many of the cultivars recently developed involved crosses with K326, a widely cultivated variety with high quality and performance but susceptible to pathogenic soil fungi (29). In this study, K394 under controlled conditions resulted in the variety with the highest DSI and AUDPC, in spite of it being registered as moderately tolerant to pathogenic soil fungi (10).

Modern breeding strategies are also impacting genetic diversity through the wide-scale release of F1 hybrid tobacco cultivars with cytoplasmic male sterility (23). In the present study, the varieties with the best performance were the hybrids MB47, PVH2291, and NC71. The relatively new hybrid variety MB47, registered as tolerant to *Fusarium oxysporum*, showed low DSI and AUDPC for FOSC and FSSC. In addition, the hybrid variety PVH2291 performed well for the FOSC, while NC71 did so for the FSSC. In a previous study, the molecular analysis of 17 Virginia-type tobacco varieties from northwestern Argentina, based on microsatellite markers, revealed that these three varieties showed greater genetic divergence than the rest (12). Therefore, we highlight the importance of knowing the degree of tolerance of the varieties to be cultivated. This knowledge, along with the determination of the inoculum amount present in the soil, will allow an effective selection of the variety to be incorporated into the production process.

Varied levels of susceptibility of tobacco varieties to *Fusarium oxysporum*, with different degrees of severity between the Virginia and Burley types, have been reported (34). However, there are no reports for members of FSSC associated with tobacco in the NWA region. There are reports for FSSC for other crops such as beans, passion fruit, and soybean (6, 9, 26). In soybean cultivation, this behavior was also observed with *Fusarium graminearum* in tests under controlled conditions (5). A recent study reported transgenic lines of tobacco with significantly increased resistance to *Fusarium solani*, with no wilting or root rot symptoms after 30 dpi (3). However, genetic resistance to black shank (*Phytophthora parasitica*) and bacterial wilt (*Ralstonia solanacearum*) was only incorporated into some varieties of tobacco (23). Furthermore, the tobacco varieties used in the NWA region show differential susceptibility to root rot (*Rhizoctonia solani)* (27). The main Virginia-type tobacco varieties cultivated in the NWA exhibited varying degrees of tolerance to FOSC and FSSC, manifesting the characteristic symptoms of each disease. In the varieties studied, no resistance mechanism is at work, but varying degrees of tolerance have been shown, which do not interfere with the growth of the host, tolerate the infection and have adequate performance (13). In the field, variable tolerance levels are commonly observed with very high inoculum potentials and favorable environmental conditions (20).

In the present study, AUDPC was found to be a useful parameter in comparing the incidence of the disease over time to describe the behavior of commercial varieties versus isolates from both complexes. Similar results were observed in chickpea, where the AUDPC made it possible to identify the behavior of varieties and races of *F. oxysporum* f sp. *ciceris* under controlled conditions (30). The disease incidence data of the isolates evaluated were adjusted to monomolecular and logistic growth models. Madden *et al.* (2007) relate the monomolecular model to monocyclic epidemics, where the inoculum comes from previous epidemics, while they associated the logistic model with polycyclic epidemics, where there is inoculum movement from diseased to healthy plants. In the present study, the adjustment of only three isolates to the logistic models can be attributed to inoculum density in soil, and root growth with or without lesion expansion on roots might affect the dynamics of root disease epidemics (16, 18).

This is the first study providing useful information on the relationship established between pathogen aggression and degree of tolerance in tobacco varieties in Argentina. The results revealed that the infection caused by *Fusarium* isolates depends on the identification of the inoculum present in the soil and the degree of tolerance of the varieties used. Given the great variability observed in the *Fusarium* complexes studied, it would be interesting to increase the number of isolates and the tobacco genotypes evaluated and to add other possible interactions in the pathosystem, such as the interaction with nematodes, to identify resistant genotypes.

Conclusions

The varieties that exhibited the best performance under controlled conditions were determined, and the genetic materials used were characterized. The existence of a narrow genetic base in the commonly used tobacco varieties requires the search for new sources of resistance to the main diseases that affect the crop in the region. The results found suggest that the varieties NC71, MB47, and PVH2291 should be incorporated into breeding programs to obtain genotypes with higher levels of tolerance to vascular wilting and root rot. However, field studies are necessary to determine the health behavior and yield of the varieties evaluated under growing conditions. In addition, it would be of great interest to carry out additional studies to determine the density of inoculum present in tobacco soils for both complexes to establish disease control strategies.

Supplementary Figures

https://drive.google.com/file/d/13k1VdyONBwVn44hyE3pxW38sb_MIJQjC/view?usp=sharing

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Predation capacity and larval development of *Ceraeochrysa claveri* (Neuroptera: Chrysopidae) fed with *Raoiella indica* (Acari: Tenuipalpidae)

Capacidad de depredación y desarrollo larval de *Ceraeochrysa claveri* (Neuroptera: Chrysopidae) alimentado con *Raoiella indica* (Acari: Tenuipalpidae)

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Nota científica

Abstract

Ceraeochrysa claveri Navás (Neuroptera: Chrysopidae) is a predator found in several agricultural ecosystems and feeds on insects and phytophagous mites. Its high reproductive potential and forage capacity makes it a candidate for biological control of agricultural pests. *Raoiella indica* Hirst (Acari: Tenuipalpidae) is an important pest that can damage several species of palms, in particular, *Cocos nucifera* L. Given the scarcity of available knowledge about the biological aspects of Chrysopidae fed with phytophagous mites, the present work aimed to study the larval development of *C. claveri* fed mainly with *R. indica*, in order to obtain information that would be of help in the integrated management of this pest. The evaluation was performed in the F0 generation. Larva 3 is the instar that consumes the most mites (F value = 32.99; P > 0.0001) (L3: 46.80 ± 10.12 a; L2: 9.80 ± 1.23 b; L1: 9.40 ± 1.58 b). *C. claveri* did not complete larval development when fed only with *R. indica*. Larval instars L1, L2 and L3 lived 7.4 ± 2.2, 7.6 ± 1.9 and 9.0 ± 3.9 days, respectively. The larvae that reached the pupal stage failed to grow further. When adding *Sitotroga cerealella* Olivier (Lepidoptera: Gelechidae) eggs to the diet, the development lasted 7.9 ± 0.2, 7.4 ± 0.8, 6.5 ± 0.9 and 13.6 ± 0.9 days for L1, L2, L3 and pupae, respectively. The adults lived on average 6.7 ± 4.9 days. The sexual ratio was rt = 0.42. According to the conditions under which the experiment was carried out, it can be inferred is that *C. claveri* cannot complete its development by feeding only on *R. indica*, and that it thus consumes this mite as an occasional prey.

Keywords
predator • biological control • red mite • phytophagous • biological cycle

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Resumen

Ceraeochrysa claveri Navás (Neuroptera: Chrysopidae) es un depredador que se encuentra en varios ecosistemas agrícolas y se alimenta de insectos y ácaros fitófagos. Su alto potencial reproductivo y capacidad depredadora lo convierten en un candidato para el control biológico de plagas agrícolas. *Raoiella indica* Hirst (Acari: Tenuipalpidae) es una plaga importante que puede dañar varias especies de palmeras, en particular, *Cocos nucifera* L. Debido a la escasa información sobre aspectos biológicos de crisópidos alimentados con ácaros fitófagos, se planteó el presente estudio con el objetivo de conocer el desarrollo larval de *C. claveri* teniendo como presa principal a *R. indica*. Esta información es necesaria para considerarlo en el manejo integrado de esta plaga. La evaluación se realizó sobre la generación F0. La larva 3 es el instar que mayor cantidad de ácaros consume (F value = 32,99; P > 0,0001) (L3: 46,80 ± 10,12 a; L2: 9,80 ± 1,23 b; L1: 9,40 ± 1,58 b). *C. claveri* no completó su desarrollo larval al alimentarse únicamente con *R. indica* viviendo los ínstares larvales 7,4 ± 2,2; 7,6 ± 1,9 y 9,0 ± 3,9 para L1, L2 y L3 respectivamente. Las larvas que llegaron al estado de pupa no lograron superar esta etapa. Al adicionar huevo de *Sitotroga cerealella* Olivier (Lepidoptera: Gelechidae) a la dieta de L3, el desarrollo fue 7,9 ± 0,2; 7,4 ± 0,8; 6,5 ± 0,9 y 13,6 ± 0,9 días para L1, L2, L3 y pupa respectivamente. El adulto vivió en promedio 6,7 ± 4,9 d. La relación sexual fue rs = 0,42. De acuerdo con las condiciones en las que se realizó el experimento, se puede inferir que *C. claveri* no completa su desarrollo al alimentarse únicamente con *R. indica* y en consecuencia toma a este ácaro como presa ocasional.

Palabras clave
depredador • control biológico • ácaro rojo • fitófago • ciclo biológico

Introduction

The introduction of exotic species represents a threat to the biodiversity of ecosystems and a serious problem for agriculture (13, 25).

The red palm mite, *Raoiella indica* Hirst 1924 (Acari: Tenuipalpidae) is an exotic pest native to India (30). In the American continent, it was first detected in 2004 in the Caribbean, in the Island of Martinique (9). Since then, it spread throughout most of the islands of the region (9, 24). In 2008, *R. indica* appeared in Venezuela (36) and Florida, USA, (23); in 2009 it was recorded in Mexico (17).

The coconut palm (*Cocos nucifera* L.) is the main host of *R. indica*, although its host list has almost 70 species (6), *R. indica* causes damage to coconut palms by inserting its chelicerae through stomatal openings in order to feed on the cellular content of the mesophyll (19). The greatest damage has been observed during the nursery phase, even leading to the death of plants. In adult plants, the damages are more evident in mature leaves, which turn yellowish and can dry up completely, reducing the plant's photosynthetic rate and causing flower abortion, which affects the coconut yield (24, 29).

The control of *R. indica* has been carried out mainly through the use of chemical products, but given the height of some of its hosts and its presence in areas where this type of control is problematic, such as tourist and residential areas, the use of natural enemies can be considered a promising alternative (5, 11, 12).

Chrysopidae species are excellent predators that feed on a wide variety of phytophagous insects and mites that are found on plant leaves (1, 10, 34). Peña *et al.* (2009) reported species of Chrysopidae feeding on *R. indica* in Trinidad and Tobago, Puerto Rico and Florida, United States. Carrillo *et al.* (2011) identified two species of Chrysopidae, including *Ceraeochrysa claveri* (Navás, 1911) (Neuroptera: Chrysopidae), that fed on *R. indica*. Contreras-Bermúndez *et al.* (2017) also reported five species of Chrysopidae, including *C. claveri*, that naturally fed on *R. indica* in the area of Tecomán, Colima, Mexico.

Ceraeochrysa claveri is an important predator of phytophagous pests in many Neotropical agroecosystems and because of a broad prey range, high voracity, high search capacity and short developmental time, *C. claveri* can be considered an efficient biological control agent (1).

Based on the information presented above and given the little information available on the biology and predatory activity of Chrysopidae on phytophagous mites, the present study aimed to study the predatory capacity, larval development and longevity of adults of *C. claveri* when having *R. indica* as its main prey. The information obtained would be useful for the integrated management of this pest.

Materials and methods

A *C. claveri* colony was established using the method developed by the Entomophagous Insects Department of the Centro Nacional de Referencia de Control Biológico (IE-CNRCB) (22). The insects were collected in a commercial coconut palm crop located on the malecon of Real-Pascuales, Municipality of Tecomán, Colima, Mexico (18°50’56.43’’ N; 103°57’15.08’’ W). The mites were collected in a commercial coconut palm crop located in the Amela Lagoon, of the same municipality (18°49’39.60’’ N; 103°47’08.83’’ W).

Prior to the study, *C. claveri* was identified using the keys of Tauber *et al.* (2000). The identity of *R. indica* was confirmed by molecular analysis (genomic DNA extraction using the HotSHOT method) at the Molecular Biology Laboratory (MBL) of the CNRCB (32, 35). The specimens were deposited in the Entomophagous Insect Collection (CIE) of the CNRCB.

The evaluations were performed on the F0 generation of *C. claveri* in a laboratory of the IE-CNRCB located in the city of Tecomán, Colima, Mexico (18°55’37.62’’ N and 103°53’ 01.45’’ W; 45 m a. s. l.) under the following conditions: 25 ± 2°C, 60-70% RH and 14:10 h LD.

Predation capacity

The predation capacity of *C. claveri* was evaluated using the sand paper method, which consisted of a Petri dish (5 cm in diameter) with a disk of sterile wet filter paper at the bottom, covered by a disk of banana leaf (5 cm in diameter), on top of which were placed 50 individuals of *R. indica*. An hour later, a predator was introduced to each experimental arena. Before starting the experiment, the larvae were fasted for 12 h. The first larval instar was used 24 h after emerging from the egg; the second and third instars were used 24 h after the immediately previous instar change. The number of individuals consumed in a period of 5 h was determined. The individuals consumed by the predator were not replaced during the experiment. Ten repetitions were performed in a breeding chamber under the environmental conditions mentioned above.

The number of individuals consumed by each instar was analysed by ANOVA. When significant differences were observed, a Tukey’s multiple comparison test ($\alpha = 0.05$) was applied (28).

Larval development

Two larval development assays were conducted. The first consisted of a cohort of 129 larvae with 24 h of having emerged, which were placed individually in Petri dishes whose bottom was covered with a 5-cm-diameter banana leaf. The first and second instars were fed with 50 individuals of *R. indica*; the third with 100. The second assay consisted of a cohort of 90 larvae with 24 h of having emerged, which were fed using the method described above. To evaluate the effect of diet on larval development, 100 eggs of *Sitotroga cerealella* (Oliver, 1789) (Lepidoptera: Gelechiidae) were added to larva 3 in the second trial. Every 24 h observations were made and the food was changed until the pupae were obtained. The presence of larval exuviae was taken into account to estimate the duration of each biological stage in days; survival was also recorded. The pupa was defined by the presence of the cocoon and culminated in the emergence of the adult.

The emerging adults were individually transferred to Styrofoam cups of 8 cm in diameter and 6 cm in height to determine their longevity. To facilitate aeration and to prevent insects from escaping, a 4-cm-diameter opening was made in the lid and covered with organza cloth. A 1.5-cm-diameter opening was made on the lower side of the cup; it was plugged with cotton and moistened daily to supply water to the adult. To feed the adults, a piece of 1.5 X 2 cm bond paper impregnated in the artificial diet (honey, brewer’s yeast, pollen, ascorbic acid and spirulina) was placed inside the cup every third day (22).

The data were recorded per day and the survival and development time of the biological stages were evaluated. It allowed to determine the mortality rate (qx = Death rate [dx/lx]) and life expectancy (ex = Life expectancy [Tx/lx]), where lx = proportion of survivors at the beginning of each stage (Nx/N0), dx = number of deaths between stages lx and lx + 1, and Tx = Time left to live until extinction (inverse summation lx). The sex ratio was determined by the formula rs = number of females/number of females + number of males (31).

Results and discussion

Predation capacity: the statistical analysis showed a significant (α = 0.05) difference between instars (F value = 32.99; P < 0.0001) indicating a greater consumption by third instar larvae (table 1).

Table 1. Average consumption of *Raoiella indica* by the F0 generation of *Ceraeochrysa claveri* after 5 h of evaluation (25 ± 2°C, 60-70% RH, 14:10 LD).

Tabla 1. Consumo promedio de la generación F0 de *Ceraeochrysa claveri* alimentada con *Raoiella indica* a las 5 h de evaluación (25 ± 2°C, 60 - 70 % HR, 14:10 LO).

Instar	Mites consumed (Mean ± SD)
Larva 1	9.40 ± 1.58 b
Larva 2	9.80 ± 1.23 b
Larva 3	46.80 ± 10.12 a

* Means with the same letter are statistically the same.

* Medias con la misma letra estadísticamente son iguales.

Although species of the family Chrysopidae have been reported feeding on mites, we found no previous study on predation capacity against mites. For this reason, the data obtained in the present study was compared with other species and other preys. The greatest voracity was recorded in the third instar larvae (larva 3). Guarín (2003) and Velásquez (2004) mentioned that the third larval instar of Chrysopidae species shows a voracious appetite, as well as a high degree of cannibalism. In agreement with it, Ferreira-Almeida *et al*. (2009) reported that third instar larvae of *C. claveri* consumed 80% of preys when fed with *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). Similarly, when evaluating *Ceraeochrysa cincta* (Schneider) and *C. valida* Banks (Neuroptera: Chrysopidae) fed with *Diaphorina citri* Kuwayama (Hemiptera: Liividae), Pacheco-Rueda *et al.* (2015) and Palomares-Pérez *et al.* (2016) concluded that third instar larvae are the most voracious. Probably, the greater voracity of third instar larvae is a behaviour associated with the larger size of these larvae (26) and the need to accumulate and store molecules, such as lipids, proteins and carbohydrates, which will be used for keeping the insect alive during the diapause and for nutrition afterwards.

Larval development. Table 2 (page 229), shows the data on larval development. It shows that all individuals in the third instar died when feeding only on *R. indica* (table 2a, page 229). When eggs of *S. cerealella* were added to the diet of third instar larvae of *C. claveri* they were able to surpass the larval and pupal stages and reach adulthood in 35.4 ± 2.8 d, with a survival rate of 13.3% (table 2b, page 229).

Table 2 (page 229) shows that the duration of the first instar (Larva 1) is similar to that of the second instar (Larva 2), unlike the third instar (Larva 3), which lasts longer but is shortened when *S. cerealella* eggs are added to the diet (table 2b, page 229). This may be due to the nutritional quality provided by *S. cerealella* eggs compared to *R. indica*. Soffiantini-Lira and De Luna-Batista (2006) mentioned that a shorter life cycle is a consequence of good nutrition.

Ferreira-Almeida *et al*. (2009) reported shorter periods for the three larval instars of *C. claveri* when fed with different preys; they concluded that this was due to good nutrition. Santa-Cecília *et al.* (1997) evaluated the performance of *Ceraeochrysa cubana* (Hagen) (Neuroptera: Chrysopidae) with different preys and found that the quality of food ingested by larvae affected their biological development.

Table 2. Larval development in days of the F0 generation of *Ceraeochrysa claveri* fed with *Raoiella indica* and with eggs of *Sitotroga cerealella* at 25 ± 2°C, 60-70% RH and 14:10 h LD.

Tabla 2. Desarrollo larval en días de la generación F0 de *Ceraeochrysa claveri* alimentado con *Raoiella indica* y con huevo de *Sitotroga cerealella* a 25 ± 2°C, 60-70% HR y 14:10 h. LO.

Instar	*C. claveri* fed with *R. indica* (a)					*C. claveri* fed with *R. indica* and eggs of *S. cerealella* (b)				
	n	Mean ± SD	Range	q_x	e_x	n	Mean ± SD	Range	q_x	e_x
Larva 1	129	7.4 ± 2.2	5-13	0.5	1.1	90	7.9 ± 0.2	7-8	0.5	1.5
Larva 2	61	7.6 ± 1.9	7-14	0.8	0.7	40	7.4 ± 0.8	7-8	0.4	1.7
Larva 3	12	9.0 ± 3.9	3-14	1.0	0.5	23	6.5 ± 0.9	3-9	0.4	1.6
Pupa	0	0	0	0	0	14	13.6 ± 0.9	11-14	0.1	1.3
Adult	0	0	0	0	0	12	6.7 ± 4.9	1-16	1	0.5

n = Individuals evaluated; SD = Standard deviation; qx= Mortality rate; ex = Life expectancy.

n=Individuos evaluados; SD=Desviación estándar; q_x =Tasa de mortalidad; e_x = Esperanza de vida.

The mortality rate was high in larva 2, and in larva 3, total death occurred when the three larval instars were fed only with *R. indica*. Possibly the size and number of prey offered did not provide the necessary amounts of nutrients to allow the full development of the larvae, the good formation of the pupae and as a result, give rise to adults. When adding *S. cerealella* eggs to the diet of the larva 3, 12 individuals of 90 reached the adult state, living on average 6.7 ± 4.9 d (table 2b), this time was not sufficient to exceed the period of pre-oviposition (10.6 ± 0.51 days) (8) as a consequence, there was no offspring. The data suggest that *C. claveri* can take *R. indica* as an secundary prey since feeding on this mite alone it could not complete its cycle, unlike the results obtained by Carrillo *et al.* (2011), where they found that *C. claveri* complete its cycle, although adults don't get to reproduce.

The results obtained in the present study indicate that increasing the quality of food in the diet of the third instar larvae provides sufficient nutrients to complete the larval and pupal stage, but requires better feeding, possibly during the first two instars, for the adult to develop fully.

Although the sex ratio (rs = 0.42) indicates a greater number of males, it is close to the 50/50 ratio reported by Núñez (1988). However, a biased sex ratio to males may favor fertility by increasing the possibility of female encounters with virgin males, which would result in increased egg production (16).

The type of feeding during the larval period is crucial for preimaginal development and can affect the time of development, survival, fertility and longevity in the adult stage (3, 15). Santa-Cecília *et al.* (1997), Auad *et al.* (2001) and Ferreira-Almeida *et al*. (2009) mentioned that the quality of the food ingested by larvae of *C. cincta*, *C. cubana* and *C. claveri* can interfere with the development and survival of adults.

Conclusions

According to the information obtained, *C. claveri* is unable to complete its larval development using *R. indica* as the only prey and therefore, this mite can be considered as a secondary prey.

Its high voracity, especially in the third stage, demonstrates its potential to be included in a biological control program for *R. indica*.

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Effect of rearing system and sex on the composition and fatty acid profile of *Andinoacara rivulatus* meat from Ecuador

Efecto del sistema de producción y sexo en la composición y perfil de ácidos grasos de la carne de *Andinoacara rivulatus* criada en Ecuador

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Abstract

This study evaluated the influence of rearing system (cultured vs. wild rearing systems), and fish sex on carcass and flesh traits of *A. rivulatus,* raised in Ecuador. Three hundred mature specimens from *A. rivulatus* were captured, 150 from each origin. Slaughter yield and dress-out resulted similar for both rearing system, and average fillet yield for cultured fish was significantly higher than for wild fish, while cooking loss was significantly lower. Dress-out was significantly higher in females. Significant differences were found in wet percentage, ash, fat and protein content in both rearing systems, but only wet percentage was significantly affected by sex. Palmitic, oleic and arachidonic acids had the maximum percentage of saturated (SFA) and mono/poly unsaturated (MUFA/PUFA) fatty acids, respectively. In cultured and wild fish, differentiations were also found in PUFA/SFA, docosahexaenoic acid (DHA)/eicosapentaenoic acid (EPA), atherogenicity (IA), and thrombogenicity (IT) indices, along with P, K, Mg, Cu and Fe. Sex and rearing system significantly influenced most of the analyzed characteristics of carcass and flesh of *A. rivulatus.* Fillet high yield and its proximate composition categorize *A. rivulatus* as a suitable food. These results provide valuable nutritional information about native species for consumers in Ecuador.

Keywords

freshwater fish • flesh attributes • proximate analysis • minerals

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Resumen

Se evaluó la influencia del sistema de producción y el sexo en las características de la canal y la carne de *A. rivulatus* criado en Ecuador. Trescientos especímenes maduros de *A. rivulatus* fueron capturados, 150 de cada sistema de producción. El rendimiento de sacrificio y la canal fueron similares para ambos sistemas de producción y el rendimiento promedio de filete para los peces cultivados fue significativamente mayor en comparación con el pescado salvaje, mientras que la pérdida por cocción fue significativamente menor. El rendimiento a la canal fue significativamente mayor en hembras que en machos. Se encontraron diferencias significativas en el contenido de humedad, cenizas, grasas y proteínas en ambos sistemas de producción, pero solo la humedad se vio afectada significativamente por el sexo. Los ácidos palmítico, oleico y araquidónico presentaron el porcentaje máximo de ácidos grasos saturados y mono y poliinsaturados, respectivamente. En peces cultivados y silvestres también se encontró que difieren en los índices de PUFA / SFA, ácido docosahexaenoico (DHA) / ácido eicosapentaenoico (EPA) y aterogenicidad (IA) y trombogenicidad (IT). Hubo diferencias significativas en P, K, Mg, Cu y Fe. El sistema de cría y el sexo influyen significativamente en la mayoría de las características analizadas de la canal y la carne de A. *rivulatus*. El alto rendimiento de filete y su composición próxima categorizan a *A. rivulatus* como un alimento adecuado. Estos resultados aportan información nutricional valiosa de especies nativas para los consumidores de Ecuador.

Palabras clave

pez de agua dulce • características de la carne • análisis proximal • minerales

Introduction

Fish is an important functional food for approximately three billion people (46). It provides high quality protein, essential minerals (31) and polyunsaturated long chain omega-3 fatty acids, especially eicosapentaenoic and docosahexaenoic acids, with high nutritive value (12). Therefore, fish quality (in terms of freshness and the mentioned nutritive value) has become a strategic priority for the industry, and its inclusion in the human diet is essential (30).

In Ecuador, fishery contributes 7% of total animal protein supply. Sixty five percent comes from capture fisheries, and the remaining 35% from aquaculture. This last activity is source of employment and foreign exchange, while contributing to rural settlement and food security (15). Also, rural aquaculture has become a key component of rural livelihoods in situations where increasing population, environmental degradation, or loss of access, limit catches from wild fisheries (14). The fish species mainly caught on the coastal and Ecuadorian Amazon areas are *Cichlasoma festae, Andinoacara rivulatus*, *Prochilodus magdalenae*, *Brycon alburnus*, *Leporinus ecuadoriensis*, *Hoplias microlepis* and *Lebiasina bimaculata,* among others (15). *A. rivulatus* (syn. *Aequidens rivulatus*) or Vieja Azul, whose meat is highly appreciated by local consumers, is a colorful fish from the cichlid family distributed in the coastal waters from the Tumbes River (Perú) to the Esmeraldas River (Ecuador). They are found in low flow environments, although some inhabit more fast-flowing rivers. Its diet is mainly composed of insects and crustaceans (17). Adult males and females may reach lengths of 30 cm.

In order to produce and preserve this native species, a conservation programme was developed by the Subsecretary of Aquaculture of the Ministry of Agriculture, Livestock, Aquaculture and Fisheries (MAGAP, according to its initials in Spanish). In addition to improving environmental conditions and reducing pollution, this program must support aquaculture development. According to Solórzano Armijos (2016), knowledge of wild populations is necessary for improving chemical, physical and nutritional traits, as well as sensory profiles in the farmed specimens (21, 23, 39). Although morphological comparisons between cultured and wild fishes from several species have already been approached by several authors (7, 19, 21, 44), differences based on nutritional composition among cultured and wild *A. rivulatus* stocks, have not been studied yet. According to Mazon *et al.* (2018, 2020, 2021) and Gonzalez *et al.* (2017), productive yields of native species were influenced by genetic and environmental factors, like sex and rearing system (cultured *vs.* wild production). Consequently, this study aimed to evaluate the effect of rearing system and sex on the carcass and fillet traits, fatty acid composition and nutritional value of muscle tissue from *A. rivulatus*.

Materials and methods

Study area and experimental fish

The study was carried out in three areas of the Babahoyo River and a fish farm Center located in the Province Los Rios (Ecuador). The area has a tropical climate with average temperature of 25°C, annual rainfall of 2,400 mm and relative humidity of 82%. Water salinity did not exceed 0.1%, pH was 7.0-7.3 and temperature ranged between 19.7 and 24.7°C. Dissolved oxygen in the river and fish farm was 6.8 and 8.9 mg/l, respectively. Conductivity values were about 145 mS/cm.

Three hundred healthy adult specimens, male and female, of *A. rivulatus* were obtained at random from catches throughout year 2017. The sample included 300 adult specimens from Babahoyo River; 150 wild captured by local fishermen using nets, and 150 cultured. Farm rearing was carried out as follows: Three hundred and sixty initial specimens were cultured in net cages fixed in a pond bypass of the Babahoyo River. Eighteen net cages were fixed in a surface of 1 m^2, submerged 1 m, and filled with 20 fish per m^2, according to Rodriguez *et al.* (2017). In the farm, food was distributed three times/day adjusting consumption to 1.5% biomass. The diet was 32% crude protein, 7% fat, 5% crude fiber, 9% ash and 12% moisture. It was elaborated with cereal by-products, soybean meal, fish meal, corn protein concentrate, lecithin, vegetable oil, calcium carbonate, calcium phosphate, antioxidants and a premix of vitamins and minerals. From these 360 cultured fish, 150 were then sampled. Meanwhile, wild fish ate natural food based on insects and crustaceans. Males and females were morphologically differentiated.

All individuals were healthy adult fish, 2 to 3 years old, according to the number of scales, (13). In this research, no significant differences were found in number of scales according to rearing system and sex (7) ranging between 18.90 to 19.38 scales. Therefore, fish age was not considered a fixed factor.

After being caught, the specimens were kept in glass containers with 200 l dechlorinated tap water and continuous aeration. They were transported alive and introduced into two masonry tanks (capacity of 500 L, dissolved oxygen = 6.20 ± 0.0 mg/L, temperature = 20.5 ± 0.2°C and pH = 5.6 ± 0.1). All fishes rested for 48 h before the experiment, with a fasting period of 24 h before stunning. For the experiment, water level in the tank was reduced by half; all fishes were quickly caught with a net and transferred to a plastic box (100 L), containing a mixture of 40 L of ice and 40 L of water (0.8°C), for stunning (about 20 min). Once death was certified, the fishes were identified, pH and biometric traits were performed and the specimens, in an undistorted condition, were stored in individual plastic bags at 0 ± 2°C in ice, until further analysis and processing.

pH determination

Muscle pH was determined in duplicate after death (pH_0), at 2 hours (pH_2) and 12 hours (pH_{12}) *post mortem* by inserting a pH electrode (portable meat pHmetre, HI99163, Hanna Instruments Ltd, UK) approximately 1 cm into the fillet's right dorsal-cranial portion. The instrument was frequently calibrated using pH 4.01 and pH 7.00 buffers, and the electrode regularly cleaned to obtain consistent results.

Biometric and yield parameters

After *rigor mortis*, approximately 24 h *post mortem*, the fishes were weighed (bodyweight), measured (total and standard length) and dissected with a scalpel and scissors. Fins, scales, head, entrails, bones and fillet were removed and weighed. Head, guts and skin + fins yield was calculated according to Rutten *et al.* (2004). In addition, slaughter yield, dress-out, fillet yield and condition factor (K) were estimated by the following equations:

$$Slaughter\ yield\ (\%) = \frac{\text{Gutted body weight}}{\text{Bodyweight}} x100\ ;\ Dress\ out\ (\%) = \frac{\text{Bodyweight} - \text{Gutted body weight}}{\text{Gutted body weight}} x100$$

$$Fillet\ yield\ (\%) = \frac{\text{Fillet weight}}{\text{Bodyweight}} x100\ ;\ Condition\ factor = \frac{\text{Bodyweight}}{\text{Standard length}^3} x100$$

Flesh quality

After 45 minutes of filleting, surface colour of the internal side of right fillets was recorded at three positions using a portable colourimeter (Lutron RGB-1002 Chroma Meter) equipped with light source C and a 2° observed angle, calibrated to a white standard. Colour variables were: L* (lightness, +L* = white, -L* = black), a* (red-green chromaticity, +a* = red, -a* = green) and b* (yellow-blue chromaticity, +b* = yellow, -b* = blue) as recommended by CIE (1976). For each fillet, three measurements (along the fillet) were done. Water holding capacity was determined according to Grau & Hamm (1953), in two ways: drip loss and cooking loss. To determine drip loss, two pieces of 5 mm × 10 mm × 20 mm fresh muscle, were cut. These cubes were carefully suspended with a pin on the inside of a bottle cap, not touching either side of the bottle, and stored for 24 h at 2 ±°C. The amount of drip measured between 24 h and 48 h *post mortem* was expressed as percentage of the initial weight: $Drip\ loss\ (\%) = \frac{\text{Final weight}}{\text{Initial weight}} x100$. To evaluate cooking loss, the samples (approximately 30 g) were trimmed of external fat, weighed, placed in a polyethylene bag and immersed in a water bath (JP Selecta, Barcelona, Spain) at 80°C until internal temperature achieved 70°C. Temperature was repeatedly monitored by a Type flexible high-temperature thermocouple (Hanna, Instruments, EE.UU) inserted into the geometric centre of each piece. Once the samples were cooled at room temperature (approximately 15°C) for 40 min and gently dried on filter paper, they were re-weighed. Cooking loss percentage was calculated as follows:

Proximate composition and fatty acid profile

For proximate composition analysis, *A. rivulatus* fillets were homogenized using a 20.000 rpm grinder. Crude protein and fat content were measured by the block digestion method (UNE 55-020), ash was obtained at 550°C for 24 h (ISO R-936), and wet percentage was determined by drying at 103°C for 24 h (ISO R-1442) according to AOAC (2000). Fat percentage was measured according to the Soxhlet method (ISO R-1443) using a Foss Tecator AB Soxtec 2050. Analyses were determined in duplicate and expressed as mg/100g of raw meat.

Skinned and deboned muscles from individual fish, were blended into homogeneous flesh for total lipid extraction with chloroform/methanol (2:1 v/v) containing 0.01% of Butylated hydroxytoluene (BHT) as antioxidant (16). The organic solvent was evaporated under nitrogen stream and lipid content was determined gravimetrically. Aliquots of extracted lipids were converted to fatty acid methyl esters (FAME) according to Chistie (1993). FAME were separated and identified on GC Perkin Elmer Clarus 500 gas chromatograph with a flame ionization detector (FID) equipped with a TR-FAME capillary column (30 m x 0.25 mm i.d., 0.25 µm film thickness, Shinwa Inc.), using helium as carrier gas at a flow rate of 0.5 ml/min. Both injector and detector were maintained at 250 and 260°C, respectively. Oven temperature was programmed at 100°C, followed by increasing steps of 2°C/min until 220°C, with a final hold time of 20 min. Individual fatty acids were identified by comparing their retention times with those of a standard fatty acid mix Sulpeco 37 (Sigma Chemical Co. Ltd., Poole, UK). Nonadecanoic acid methyl ester (19:0 ME) was used as internal standard. Individual fatty acids were expressed as percentage of total identified fatty acids, and as mg/g muscle raw tissue. They were grouped as follows: saturated fatty acid (SFA), monounsaturated (MUFA), polyunsaturated fatty acid (PUFA), *n*-6 and *n*-3. The PUFA/SFA, DHA/EPA, $\sum n$-6/$\sum n$-3 ratios, atherogenicity (IA) and thrombogenicity (IT) indices were also calculated. IA indicates the relationship between the sum of the main saturated fatty acids and that of the main classes of unsaturated, the former being considered pro-atherogenic (favouring the adhesion of lipids to cells of the immunological and circulatory system), and the latter anti-atherogenic (inhibiting plaque aggregation and diminishing levels of esterified fatty acid, cholesterol, and phospholipids, preventing micro and macro coronary diseases). Finally, IT shows clotting tendency in blood vessels (42). IA and IT indices were calculated by using the Ulbricht and Southgate (1991) equations as follows:

$$IA = \frac{(C18{:}0) + (4 \times C14{:}0) + (C16{:}0)}{(PUFA\ n{-}6\ and\ n{-}3) + MUFA)}$$

$$IT = \frac{(C14{:}0) + (C16{:}0) + (C18{:}0)}{(0.5 \times MUFA) + (0.5 \times PUFA\ n-6) + (3 \times PUFA\ n-3) + (PUFA\ n-3/PUFA\ n-6)}$$

Statistical analysis

A total of 300 fish flesh samples were analysed according to different parameters. Normality and homoscedasticity were checked with Kolmogorov-Smirnoff and Levene tests, respectively. Then, general linear models (GLM) with post hoc Tukey test determined effects of rearing system (wild and cultured) and sex (male and female) on carcass and fillet traits, proximate composition, fatty acid composition and trace mineral content. Differences were considered statistically significant for $p < 0.05$. All statistical analyses were done using Statistica 12.0 for Windows.

Results and discussion

Biometric and yield parameters

Live weight was significantly higher in the wild population (table 1), which seems to indicate that artificial feeding should be improved. However, the cultivated population had a greater total length, probably a morphological adaptation to this aquatic habitat. These results partially agree with Vreven *et al.* (1998) who also indicated that confinement of domesticated fish affects their growth rate, but contrasting with our results as they observed no body elongation, along with a higher K value. Sex had a non-significant influence on both variables, attributable to the fact that catch size is conditioned by consumer demand. Rearing system and sex had a significant effect on the K factor, in agreement with González *et al.* (2016) in *Cichlasoma festae*. All wild fish and cultivated males presented higher values than all those cultivated and females. The K values obtained in this study, indicators of the status of *A. rivulatus* aquatic ecosystems, were higher than that recorded by Anene (2005) in four cichlid fish (4.9), and those registered for González *et al.* (2016) in *Cichlasoma festae* (3.01 and 3.62, for cultured and wild specimens, respectively) in similar habitats to those of the present study. This evidences a greater feeding capacity than the species previously mentioned.

Table 1. Biometric measurements and yield parameters (mean value ± SE) by rearing system and sex of *A. rivulatus*.

Tabla 1. Medidas biométricas y rendimientos (media ± ES) en cada sistema y sexo de *A. rivulatus*.

	System		Sex		P-value[1]		
	Cultured (n=150) (A)	Wild (n=150) (B)	Males (n=187) (C)	Females (n=113) (D)	System (A x B)	Sex (C x D)	System * Sex
Body weight (g)	153.59±1.80	160.59±2.62	159.34±2.51	154.86±1.99	*	n.s.	n.s.
Total length (cm)	18.91±0.10	18.32±0.12	18.54±0.14	18.69±0.13	*	n.s.	n.s.
Standard length (cm)	14.57±0.16	14.37±0.15	14.30±0.11	14.44±0.12	n.s.	n.s.	n.s.
K	5.14±0.12	5.54±0.12	5.55±0.13	5.15±0.10	*	*	**
Head %	31.98±0.46	39.39±0.46	36.53±0.46	33.83±0.46	***	***	n.s.
Gut %	5.02±0.15	4.82±0.09	4.65±0.12	5.20±0.12	n.s.	**	***
Skin + fin %	32.03±0.52	28.35±0.51	29.51±0.52	30.87±0.55	***	n.s.	***
Slaughter yield (%)	95.02±0.14	95.25±0.09	95.28±0.12	94.99±0.12	n.s.	n.s.	n.s.
Dress-out (%)	5.32±0.17	5.07±0.11	4.89±0.13	5.51±0.14	n.s.	**	***
Fillet yield %	31.94±0.46	29.09±0.36	30.15±0.47	30.88±0.38	***	n.s.	**

[1] * p < 0.05; ** p < 0.01; *** p < 0.001; n.s.: not significant.

[1] * p < 0,05; ** p < 0,01; *** p < 0,001; n.s.: no significativo.

Rearing system significantly affected head (higher in wild specimens), skin + fin and fillet yield percentages (higher in cultivated specimens), in accordance with Gonzalez *et al.* (2017). Meanwhile, sex significantly affected head and slaughter + end (higher in males), and gut and dress-out percentage (higher in females). Head percentage was significantly higher in wild fish, while skin + fins and fillet percentages were higher in cultured specimens. These results were similar to those found by Gonzalez *et al.* (2016) for *Cichlasoma festae* in Ecuador. Differences between systems could be attributed to fish high phenotypic plasticity, greater than any other vertebrates (6).

Flesh quality and proximate composition

Flesh quality traits of *A. rivulatus* are shown in table 2. The pH_0, suitable index of meat quality (25), constitutes the most important factor influencing meat texture. Minor changes in pH dramatically modify both its physical and sensory properties.

Table 2. Flesh quality traits, water holding capacity (WHC), and proximate composition (g/100 g wet weight) (mean value ± SE) by rearing system and sex of *A. rivulatus*.

Table 2. Características de calidad de la carne y capacidad de retención de agua (WHC) y composición proximal (g/100 g peso húmedo) (media ± ES) en cada sistema y sexo de *A. rivulatus.*

	System		Sex		P-value[1]		
	Cultured (n=150) (A)	Wild (n=150) (B)	Males (n=187) (C)	Females (n=113) (D)	System A x B	Sex C x D	System * Sex
pH_0	6.74±0.03	7.05±0.02	6.88±0.03	6.89±0.03	***	n.s.	n.s.
pH_2	6.53±0.02	6.61±0.02	6.58±0.02	6.58±0.02	***	n.s.	n.s.
pH_{12}	6.15±0.02	6.23±0.02	6.18±0.02	6.23±0.03	*	n.s.	n.s.
L*	56.55±0.87	55.88±0.87	53.89±0.88	58.40±0.43	n.s.	***	n.s.
a*	2.82±0.19	2.83±0.18	2.57±0.27	3.41±0.29	n.s.	**	n.s.
b*	2.56±0.22	2.74±0.16	2.82±0.21	2.49±0.16	n.s.	n.s.	n.s.
Drip loss (%)	2.86±0.06	2.75±0.05	2.78±0.05	2.83±0.05	n.s.	n.s.	n.s.
Cooking loss (%)	28.35±0.39	35.21±0.45	32.24±0.52	31.31±0.46	***	n.s.	n.s.
Wet %	72.09±0.34	75.91±0.12	73.08±0.36	74.91±0.19	***	***	n.s.
Ash %	1.60±0.01	1.50±0.01	1.56±0.01	1.54±0.01	***	n.s.	n.s.
Fat %	4.29±0.03	4.16±0.03	4.22±0.03	4.24±0.03	**	n.s.	n.s.
Protein %	23.81±0.18	21.03±0.08	22.47±0.20	22.37±0.16	***	n.s.	n.s.

pH0 = pH at slaughter; pH_2 = pH at 2 hours *post mortem*; pH_{12} = pH at 12 hours *post mortem*; L*, a* and b* = instrumental parameters color (CIE L*. a*. b*)

[1] * p < 0.05; ** p < 0.01; *** p < 0.001; n.s.: not significant.

pH0 = pH al sacrificio; pH_2 = pH a las 2 horas *post mortem*; pH_{12} = pH a las 12 horas *post mortem*; L*, a* and b* = parámetros del color (CIE L*. a*. b*)

[1] * p < 0,05; ** p < 0,01; *** p < 0,001; n.s.: no significativo.

In the first 12 hours *post mortem*, pH values were lower in wild specimens than in cultivated ones (0.83 *vs.* 0.59), in accordance with Robb *et al.* (2000) and Roth *et al.* (2009) who reported that muscle pH displayed a rapid decline during the aforementioned period. However, this drop was linear and progressive, in disagreement with González *et al.* (2017), who had indicated a harsh drop during the first 2 hours *post mortem*. Even though pH values were adjusted by the temperature sensor of the pHmetre used, temperature may have generated differences in pH. High values were associated with small and stressed animals (8, 28) and influenced by rearing system, with higher values in wild specimens, to which capture procedure could contribute.

Drip loss resulted similar to the obtained by González *et al.* (2017), although lower than those reported by Intarak *et al.* (2015) in Panga fish (*Pangasius bocourti* Sauvage), who obtained values from 4.88% to 2.88%, with significant decreases as live weight increased. None of the studied factors, rearing system or sex, had significant influence on drip loss. Cooking loss was similar to the recorded by Gonzalez *et al.* (2017) in *Cichlasoma festae*. These authors did not find system-attributable differences, while in our study wild specimens flesh showed significantly higher values, possibly due to higher wet content.

Fish composition is affected by several factors such as size, temperature, salinity, rearing system and feeding, among others (18). According to Chandrashetkar and Desthale (1993), normal variations between fish constituents are: 66-81% wet, 16-21% protein, 0.2-25% fat and 1.2-1.5% ash. Proximate analysis (table 2, page 237) resulted similar to that reported by Mashaii *et al.* (2012) for ash (1.38-1.59%), wet percentage (75.2-76.9%) and total fat (2.48-4.88%), while resulted higher than protein content (17.4-17.9%) in cultured *Oncorhynchus mykiss*. Concerning crude protein content, our results were also higher than those obtained for *Cyprinus carpio* (16% wet weight) (14) and for *Cichlasoma festae* (17.33% wet weight) (20) but similar to the 18.4-20.8% reported for the *Cichlidae* family (36). Gonzalez-Artola (2004) classified fish according to fat content, into lean fish (fat less than 2%), low-fat fish (fat 2-4%), medium fat fish (fat 4-8%) and high-fat fish (fat more 8%). Thus, based on this classification, *A. rivulatus* could be considered a medium fat fish. In agreement with previous studies (1, 5, 20, 31), rearing system significantly influenced every analyzed variable. Cultured specimens possessed higher ash, fat and protein, and lower wet percentage than wild ones. Female fish flesh had higher wet percentage than males. Differences between culture and wild specimens could probably be due to a high dietary protein level in cultured fish feed.

Fatty acid profile

Fish body composition suffers changes in response to diet and environmental conditions. Differences in fatty acid composition between rearing systems could be attributable to differences between cultured and wild specimens. Significant differences in fatty acid content were found between cultured and wild fish, except for ratio *n*-3/*n*-6, which was similar between both systems (table 3, page 239). However, sex had little influence on fatty acids profile. SFA and PUFA percentages were higher in cultured fish, whereas MUFA content, was lower, probably given to the low content of oleic and palmitoleic acids in cultured fish feed. Assimilation patterns of dietary fatty acids in fish muscle reflect the content of dietary lipid sources (1). The major fatty acids identified in both fish were palmitic, oleic, stearic, arachidonic, docosahexaenoic and docosapentaenoic acids.

Palmitic acid was the primary saturated fatty acid, contributing, approximately, with 58.6% and 60.8% of total SFA content for cultured and wild fish, respectively, lower than those obtained by Alasalvar *et al.* (2002). Meat from cultured specimens had a higher SFA content than that of wild ones (40.18% *vs.* 38.33%), in accordance with Łuczynsk *et al.* (2014). Thus, the remaining fatty acids found in both fish (about 56%) were mono and polyunsaturated fatty acids.

Oleic acid was identified as the primary MUFA in both rearing systems, significantly higher in wild fish, in accordance with Alasalvar *et al.* (2010). This fatty acid has an exogenous origin, probably owed to the diet (27).

Regarding PUFA content, significantly higher levels were found for cultured fish. According to Martínez *et al.* (2010) and Busetto *et al.* (2008), *n*-6 fatty acid content was significantly higher in cultured fish. Arachidonic acid was the dominant fatty acid in both fish, significantly higher in wild fish and greater than that obtained by Łuczynsk *et al.* (2014), Jabeen *et al.* (2011) and Martínez *et al.* (2010). This acid is a prostaglandin and thromboxane precursor (37), probably facilitating blood clotting by attaching to endothelial cells during wound healing.

In the *n*-3 fatty acids family, docosahexaenoic and docosapentaenoic acids were the most abundant, achieving greater values than for *C. festae* in similar climatic conditions (20). However, eicosapentaenoic acid values were lower than those obtained by Gonzalez *et al.* (2017) for *C. festae* in Ecuador. Given that DHA and EPA are key components for human healthy diets (29), suitable choices of dietary lipids in cultured fish would allow improving fatty acids profile, especially in *n*-3 PUFAs.

In our study, no differences between rearing systems were found in *n*-3/*n*-6 ratio (1.32 for all data). These values were higher than those obtained by González *et al.* (2017) in *C. festae* and Hoseini *et al.* (2013), in farmed *Hypophthalmichthys nobilis* and *Ctenopharyngodon idella*. Simopoulos (2008) suggested that *n*-3/*n*-6 ratio should be kept between 1:1 and 1:4.

Nutritional quality was evaluated through atherogenicity (IA) and thrombogenicity (IT) indices determining the potential impact on human health. Mean values of IA and IT indices were 0.48 and 0.41 for cultured fish and 0.43 and 0.39 for wild specimens, lower than the reported by Šimat *et al.* (2015). However, in contrast with Šimat *et al.* (2015), in cultured fish, IA and IT were significantly higher.

Table 3. Fatty acid composition (mean value ± SE) according to rearing system and sex of *A. rivulatus*.

Table 3. Composición de ácidos grasos (media ± ES) en cada sistema y sexo de *A. rivulatus*.

	System		Sex		P-value[1]		
	Cultured (n=150) (A)	Wild (n=150) (B)	Males (n=187) (C)	Females (n=113) (D)	System A x B	Sex C x D	System * Sex
Myristic 14:0	1.71±0.01	1.45±0.01	1.59±0.01	1.58±0.01	***	n.s.	n.s.
Palmitic 16:0	22.38±0.02	22.32±0.02	22.35±0.04	22.43±0.05	*	n.s.	n.s.
Margaric 17:0	1.26±0.00	1.14±0.01	1.21±0.01	1.19±0.02	***	n.s.	n.s.
Stearic 18:0	12.83±0.01	11.92±0.016	12.37±0.04	12.38±0.03	***	n.s.	n.s.
∑ SFA	38.19±0.03	36.89±0.05	37.53±0.07	37.56±0.06	***	n.s.	n.s.
Palmitoleic 16:1 *n*-9	3.35±0.02	4.57±0.02	3.88±0.01	4.03±0.05	***	***	n.s.
Oleic 18:1 *n*-9	17.00±0.01	17.81±0.03	17.38±0.04	17.43±0.05	***	n.s.	n.s.
∑ MUFA	20.36±0.014	22.37±0.03	21.32±0.08	21.36±0.09	***	n.s.	n.s.
Linoleic 18:2 *n*-6	3.21±0.01	2.86±0.02	3.07±0.03	2.99±0.02	***	***	n.s.
Stearidonic 18:4 *n*-3	1.23±0.01	1.02±0.01	1.13±0.02	1.12±0.02	***	n.s.	n.s.
Arachidonic 20:4 *n*-6 (ARA)	10.19±0.01	11.548±0.02	10.87±0.06	10.86±0.05	***	n.s.	n.s.
Eicosapentaenoic 20:5 *n*-3 (EPA)	1.77±0.01	2.32±0.02	2.06±0.02	2.04±0.02	***	n.s.	n.s.
Heneicosapentaenoic 21:5 *n*-3	1.93±0.01	2.12±0.01	2.05±0.02	2.05±0.02	***	n.s.	n.s.
Adrenic 22:4 *n*-6	4.490±0.01	3.19±0.04	3.81±0.06	3.82±0.06	***	n.s.	n.s.
Docosatetraenoic 22:4 *n*-3	1.68±0.01	2.16±0.02	1.92±0.02	1.94±0.02	***	n.s.	n.s.
Docosapentaenoic 22:5 *n*-3 (DPA)	8.33±0.02	5.79±0.04	7.15±0.10	6.98±0.11	***	n.s.	n.s.
Docosahexaenoic 22:6 *n*-3 (DHA)	8.61±0.01	9.72±0.02	9.16±0.05	9.17±0.050	***	n.s.	n.s.
∑ *n*-6	17.88±0.01	17.55±0.04	17.75±0.03	17.68±0.03	***	*	n.s.
∑ *n*-3	23.57±0.02	23.19±0.06	23.49±0.03	23.32±0.04	***	*	n.s.
∑ PUFA	41.45±0.02	40.74±0.11	41.21±0.11	40.95±0.14	***	n.s.	n.s.
∑ PUFA/∑ SFA	0.99±0.02	0.99±0.02	0.99±0.02	0.99±0.02	***	n.s.	n.s.
DHA/EPA	4.81±0.03	4.22±0.05	4.51±0.06	4.56±0.03	***	n.s.	n.s.
∑ *n*-3/∑ *n*-6	1.32±0.01	1.33±0.01	1.33±0.01	1.32±0.01	n.s.	n.s.	n.s.
Atherogenicity index (AI)	0.48±0.01	0.43±0.01	0.46±0.01	0.46±0.01	***	n.s.	n.s.
Thrombogenicity index (TI)	0.41±0.01	0.39±0.01	0.40±0.01	0.40±0.01	***	n.s.	***
Hipo/hypercholesterolemic	2.04±0.01	2.10±0.02	2.08±0.06	2.06±0.06	***	n.s.	n.s.

SFAs. saturated fatty acids; MUFAs. monounsaturated fatty acids; PUFAs. polyunsaturated fatty acids.

[1] * $p < 0.05$; *** $p < 0.001$; n.s.: not significant.

SFAs. Ácidos grasos saturados; MUFAs. Ácidos grasos monoinsaturados; PUFAs. Ácidos grasos poliinsaturados.

[1] * p < 0,05; *** p < 0,001; n.s.: no significativo.

Conclusions

Rearing system and sex significantly influence most of the analyzed characteristics of carcass and flesh of *A. rivulatus.* High fillet yield and its proximate composition categorize *A. rivulatus* as a suitable food. The results obtained from this study indicate that proximate composition of fillet of cultured *A. rivulatus* is more adequate than those of wild *A. rivulatus,* since cultured fish contains higher *n*-3 and *n*-6 PUFA percentages and adequate *n*-3/*n*-6 ratio.

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Effects of different energy source diets, as corn substitutes, on carcass characteristics and meat quality of feedlot lambs

Efectos de diferentes dietas con fuentes de energía, como sustituto del maíz, sobre las características de la canal y la calidad de la carne de los corderos de engorde

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Abstract

This study evaluated the effects of different energy sources, as corn substitutes, on the carcass characteristics and meat quality of lambs. Twenty-four intact ram lambs were identified, weighed, and assigned to one of four dietary treatments: corn, citrus pulp, rice bran, and soybean hulls. The ram lambs were confined in feedlots, and when they reached 30 kg of live weight, they were slaughtered. A completely randomized design with different replicates was used for the analysis, along with the Tukey-5% test. For hot and cold carcass weight, rice bran produced lower values than the other dietary treatments. For the red color content of the meat, citrus pulp presented superior values when compared to corn, whereas meat and fat color, loin eye area, and objective tenderness did not differ between treatments. Different energetic sources used to replace corn in ram lambs finishing diets did not affect the proximate composition and quality of the meat.

Keywords
sheep • citrus pulp • rice bran • soybean • termination

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Resumen

Este estudio evaluó los efectos de diferentes fuentes de energía, como sustitutos del maíz, sobre la calidad de la carcasa y la carne de los corderos. Se identificaron, pesaron y distribuyeron 24 corderos machos no castrados en cuatro tratamientos: maíz, pulpa de cítricos, salvado de arroz y cáscaras de soya. Los corderos fueron confinados en corrales de engorde y cuando alcanzaron los 30 kg de peso vivo fueron sacrificados. Para el análisis de resultados se utilizó un diseño completamente aleatorio con diferentes réplicas y se utilizó la prueba de Tukey-5%. Para las variables de peso de la carcasa caliente y fría, el salvado de arroz fue inferior a los otros tratamientos. Para el color rojo de la carne, la pulpa de los cítricos presentó un resultado superior al maíz, mientras que el color de la carne y la grasa, la ternura objetiva y el área del ojo del lomo no difirieron entre tratamientos. Las diferentes fuentes de energía utilizadas en las dietas de terminación de corderos no alteraron la composición química de la carne ni las características de calidad.

Palabras clave
ovino • pulpa de cítricos • salvado de arroz • soya • terminación

Introduction

Throughout the Brazilian territory, sheep production chains have demonstrated growth in production and consumer acceptability of sheep meat. The supply chain has shown flock growth rates due to the expansion of domestic and foreign markets. To maintain market supply, the feedlot management system has often been used by producers mainly due to the low availability and quality of forages during the drought season (24).

Research has shown satisfactory results from this system, mainly as a strategy to minimize the impacts of forage shortage, but also to allow animal fattening during the off-season. In addition, this system enables a reduction in slaughter age and improves meat supply during the off-season (11, 13, 17, 31). However, the costs of finishing lambs in this system are linked to productivity and are directly associated with high costs of energy and protein concentrates. Therefore, the search for feeds that can improve productivity and economic indices in livestock farming systems without affecting animal performance has been a continuous challenge among researchers.

In general, cereal grains, especially corn, are the main source of energy in animal diets. However, the greater availability of by-products has increased the interest for these alternative sources to substitute corn, with the aim of lowering the cost per kilogram produced (26). Nonetheless, it is necessary to study the effects of these by-products on the final product to be delivered to consumers. Therefore, the objective of this study was to evaluate the effects of by-products as corn substitutes in feedlot lamb diets on carcass characteristics and meat quality.

Material and Methods

The work was conducted in accordance with ethical standards and approved by the ethics and biosafety committee, protocol number 987, of the University of Western São Paulo, in the municipality of Presidente Prudente, São Paulo, Brazil, latitude 22°07 North and longitude 51°23', with an average annual temperature of 28°C and with an average annual precipitation of 1400 to 1500 mm, characterized by a hot climate with dry winter (Unoeste -Metrological Station-SP).

Twenty-four intact ram lambs, Highlander crossbreed, with an average weight of 18 kg + 1,61, were randomly distributed in four dietary treatments (T): corn (TC - control), citrus pulp (TCP), rice bran (TRB), and soybean hulls (TSH). All diets were isoenergetic (71% NDT) and isoproteic (21% CP) (table 1, page 245), Animals were housed in individual pens (1.20 x 1.0 m), with concrete floor covered with wood shavings, equipped with individual feeder and drinker and mineral salt *ad libitum*.

Table 1. Percentage of ingredients and proximate composition of diets used in the feedlot of crossbreed Highlander ram lambs.

Tabla 1. Porcentaje de ingredientes y composición aproximada de las dietas utilizadas en los cebaderos de corderos cruzados Highlander.

Ingredients	Dietary treatments (%)			
	TC	TCP	TRB	TSH
Corn	23.5	0.0	0.0	0.0
Citrus pulp	0.0	26.0	0.0	0.0
Rice Meal	0.0	0.0	25.0	0.0
Soybean Hull	0.0	0.0	0.0	27.0
Soybean Meal	27.0	28.0	22.0	26.0
Wheat Meal	21.0	15.0	25.5	16.5
Limestone	1.5	1.5	1.5	1.5
Oil	0.0	3.5	0.0	3.0
Hay	27	26	26	26
Composition				
Crude Protein	20.20	19.74	20.32	20.75
Crude Fiber	14.43	16.58	19.21	20.24
Mineral Matter	6.66	7.21	9.29	8.26
Ether Extract	2.34	5.95	3.55	5.16
TDN[1]	71.76	72.46	66.23	67.12

[1] Total digestible nutrients estimated according to Cappelle *et al.* 2001.

[1] Nutrientes digeribles totales estimado según Cappelle *et al.* 2001.

Endoparasite control was done with ivermectin, 1 mL per 50 kg body weight, during the entire stay of animals in the feedlot strategically using test of counting the eggs per gram of feces (OPG). Adaptation to diet and facilities lasted 14 days and the experimental period an average of 53 days.

Animals were weighed every 14 days to obtain daily weight gain and, consequently, to adjust the feed offered. The lambs were slaughtered when they reached 30 kg of body weight.

Prior to slaughter, body condition (BC) was determined by the method developed in England by Russel *et al.* (1969) that is based on palpation of the lumbar region to determine the amount of fat and muscle observed in the angle formed by the dorsal and transverse processes. Values from 1 to 5 were assigned, in which 1 represented a cachectic animal and 5 denoted an obese animal.

After solid fasting for 16 h, the animals were weighed to obtain pre-slaughter body weight (PSBW) and then stunned by an electric discharge of 0.50 A for eight seconds, according to the methodology proposed by Gomide and Ramos (2006) and following the standards for humane slaughter.

After evisceration, carcasses were weighed to obtain hot carcass weight (HCW) and transferred two hours later to a cold chamber at 4°C, where they remained for 24 h. Carcasses were hung from the tendons on appropriate hooks, maintaining the tarsometatarsal joints at a distance of 17 cm. At the end of this period, cold carcass weight (CCW), carcass commercial yield (CCY = CCW/PSBW × 100), and cooling loss (CL = HCW - (CCW/HCW) × 100) were obtained. To obtain the carcass compactness index (CCI = CCW/internal carcass length), the internal length of the carcass (ILC, maximum distance between the anterior border of the ischiopubic symphysis and the anterior edge of the first rib at its midpoint) measured with a measuring tape was obtained according to Sañudo and Sierra (1986).

In addition, subjective evaluations of the carcass were performed for fat cover (FATCOV) (1 for lean and 5 for very fat), carcass conformation (CC) (1 for poor and 5 for very good), meat color (MEATCOL) (1 for pink and 3 for red), and fat consistency (FATCON) (1 for firm and 3 for soft).

Carcasses were divided in the middle with a longitudinal cut in the cranial-caudal direction, and the loin eye area (LEA) was obtained by exposing the *longissimus dorsi* muscle after a transverse section on the left carcass side, between the 12th and 13th ribs, outlining it on tracing paper. The area was then determined as square centimeters of the image using AUTOCAD® software. Using a caliper, the following measurements were performed on the loin: maximum depth (MD), maximum width of the loin (MW), greatest fat thickness (GFT), and lowest fat thickness (LFT). Subcutaneous fat thickness was measured at the final third of the muscle, from the backbone, perpendicular to the muscle, using a digital caliper.

The loin was then removed from the left half of the carcasses, and after 30 min blooming, color was evaluated for myoglobin oxygenation, using a portable Color reader CR-10 Konica Minolta® (Konica Minolta, Chiyoda, Tokyo, Japan). The components L* (lightness/darkness), a* (redness/greenness component), and b* (yellowness/blueness component) were expressed in the CIELAB* color system.

Loin chops (2.54 cm) were cut, and the pH was assessed using a Hanna® pH meter. Moisture, ash, protein (N × 6.25), and fat content were determined according to AOAC methods (2000): moisture by AOAC Method 950.46; protein by estimating the nitrogen content using the Kjeldahl method (AOAC Method 920.152); ash by incineration at 525°C (AOAC Method 940.26); and fat by the Soxhlet method (AOAC Method 963.15).

Cooking for objective tenderness and sensory analysis was performed by thawing the loin chops for 24 h at 5 °C and grilling to 71 °C (1). The objective tenderness was measured using a CT3 Texture Analyzer Brookfield® (Brookfield Engineering, Middleboro, MA, USA) with a 3 mm thick Warner-Bratzler blade. The cores were sheared with a C3 Texture Analyzer Brookfield® (Brookfield Engineering) with a Warner-Bratzler blade.

Thawing loss and cooking loss were calculated from chops used for objective tenderness according to the following formulas respectively: Thawing loss % = (Thawed weight of the chop × 100)/Frozen weight of the chop); Cooking loss % = (Grilled weight of the chop × 100)/Raw weight of the chop).

A completely randomized design with different replicates was used with the Tukey-5% test for the composition of the means, using the ASSISTAT Program.

Results and Discussion

Variables for daily gain, pre-slaughter body weight, hot carcass weight, cold carcass weight, cooling loss, commercial carcass yield, carcass index, and confinement period are reported in table 2.

Table 2. Estimated means and coefficients of variation for performance and carcass characteristics of feedlot crossbreed Highlander lambs fed with different energy sources.

Tabla 2. Medias estimadas y coeficientes de variación para el rendimiento y la canal de los corderos Highlander tratados en corral de engorde con diferentes fuentes de energía.

Variables	Dietary treatments				CV%	Effect
	Corn	Citrus pulp	Rice bran	Soybean hulls		
DG (kg/day)	0.233[ab]	0.254[a]	0.175[b]	0.211[ab]	20.60	*
PSBW (kg)	31.63[a]	32.24[a]	29.98[a]	30.78[a]	6.37	ns
HCW (kg)	14.66[a]	15.40[a]	12.66[b]	14.75[a]	6.64	**
CCW (kg)	13.33[a]	14.24[a]	11.40[b]	13.55[a]	7.79	**
CL (%)	1.95[a]	2.19[a]	1.44[a]	1.99[a]	24.84	ns
CCY (%)	42.29[ab]	44.13[a]	38.03[b]	44.01[a]	63.57	**
CCI (kg/cm^3)	0.237[ab]	0.251[ab]	0.215[b]	0.253[a]	8.96	*
FP (day)	51.16[ab]	48.40[b]	59.33[a]	52[ab]	11.78	*

** p < 0.01. *p <0.05. ns: not significant (p>= .05). Means followed by different letters in a line differ statistically from each other. DG = daily gain. PSBW = pre-slaughter body weight. HCW=hotcarcassweight. CCW = cold carcass weight. CL = cooling loss. CCY = commercial carcass yield. CCI = carcass compactness index. FP = feedlot period.

** p <0,01. * p <0,05. no significativo (p> = ,05). Las medias seguidas de una letra diferente en la línea difieren estadísticamente entre sí. DG = ganancia diaria. PSBW = peso vivo antes del sacrificio. HCW = peso de la canal caliente. CCW = peso de la canal fría. WLC = pérdida de peso por enfriamiento. CCY = rendimiento comercial en canal. CCI = índice de compacidad de la carcasa. FP = período de encierro.

For daily gain means, the citrus pulp diet (0.254 kg/day) and the rice diet (0.175 kg/day) differed from each other ($p<0.05$) but were statistically similar to corn and soybean hulls. The lowest daily gains for the rice bran treatment can be explained by the reduction in the digestibility of nutrients, especially proteins (12, 30, 33). Tabeidian and Sadeghi (2008), studying different levels of rice bran in lamb diets observed a linear reduction in daily gain with increasing levels of rice bran in the diets.

For hot carcass weight and cold carcass weight, the rice bran treatment produced significantly ($p<0.05$) lower weights than the other dietary treatments. These results have a direct correlation with the least daily gains observed for animals fed this treatment, which is also reflected in hot carcass weight and cold carcass weight. Weight percentage loss from cooling had no significant effect on feedlot finished crossbreed Highlander lambs receiving diets with different energy sources.

The carcass compactness index significantly differed between the rice bran and soybean hull dietary treatments. Animals fed soybean hulls presented the highest carcass compactness index values. This index is a strong indicator of the conformation of the carcass, since it evaluates the amount of muscular tissue deposited in the carcass in a unit of length (23). The commercial carcass yield values for animals fed rice bran were similar to those observed for animals fed corn but were statistically different when compared to other dietary treatments.

The feedlot period for the diet containing rice bran was 59.33 days longer ($p<0.05$) and was the longest of all dietary treatments. According to lamb research with rice bran in the diet, this ingredient has a negative effect on feed conversion (30), and animals must ingest more food in order to reach slaughter weight, requiring more days in the feedlot.

The estimated means and coefficients of variation for carcass composition, fat cover, fat consistency, and meat color of crossbreed Highlander lambs fed with different energy sources are presented in table 3, however the supplementation with different energy foods did not influence these variables. However, no statistical differences were observed between the dietary treatments ($p>0.05$) for any of the variables.

Table 3. Estimated means and coefficients of variation (CV) for subjective evaluations of carcass conformation, fat cover, fat consistency, and meat color of Highlander lambs fed diets with different energy sources.

Tabla 3. Medias estimadas y coeficiente de variación (CV) para las evaluaciones subjetivas de la conformación de la canal, la cobertura de grasa, la consistencia de la grasa y el color de la carne de corderos Highlander alimentados con dietas con diferentes fuentes de energía.

Variables	Dietary treatments				CV%	Effect
	Corn	Citrus pulp	Rice bran	Soybean hulls		
CC[1]	2.500	2.400	2.583	2.250	20.336	ns
FATCOV[2]	2.833	2.600	2.250	2.250	19.158	ns
FATCON[3]	1.66	1.700	1.750	1.500	19.678	ns
MEATCOL[4]	1.583	1.600	1.750	1.500	12.621	ns

ns = not significant (p >= 0.05). CC = carcass conformation. FATCOV = fat cover. FATCON = fat consistency. MEATCOL = meat color. [1]1(poor) to 5 (very good). [2]1 (lean) to 5 (very fat). [3]1 (firm) to 3 (soft). [4]1 (pink) to 3 (red).

NS = No significativo (p >= 0,05). CC = conformación de la canal. FATCOV = cobertura de grasa.FATCON=consistencia grasa. MEATCOL = color de la carne. [1]1 (pobre) a 5 (muybueno).[2]1(magro)a 5 (muy graso). [3]1 (firme) a 3 (suave). [4]1 (rosa) a 3 (rojo).

Zundt *et al.* (2010), in an experiment conducted with crossbreed Highlanders lambs slaughtered at 30 kg, found values of 2.50, 1.41, and 1.50 for FATCOV, FATCON, and MEATCOL respectively, which are similar to the results in this research. Values for fat cover observed in this study are lower than the 3 mm suggested in the literature as desirable fat cover in the sheep carcass to prevent losses during the cooling period (7, 20).

Measurements of maximum depth (MD), maximum width of the loin (MW), greatest fat thickness (GFT), and lowest fat thickness (LFT) are shown in table 4 (page 248). There were no significant differences ($p> 0.05$) between dietary treatments on the measured variables.

ns = not significant (p >= 0.05). MD = maximum depth. MW = maximum width of the loin. GFT = greatest fat thickness. LFT = lowest fat thickness.

ns = No significativo (p >= 0,05). MD = longitud del lomo más larga. MW = longitud del lomo más corta. GFT = mayor espesor de grasa. LFT = menor espesor de grasa.

Table 4. Estimated means and coefficients of variation (CV) for loin measurements of Highlander lambs fed diets with different energy sources.

Tabla 4. Medias estimadas y coeficientes de variación (CV) para la longitud del lomo y el grosor de la grasa en dietas con diferentes alimentos energéticos en corderos.

Variables	Dietary treatments				CV%	Effect
	Corn	Citrus pulp	Rice bran	Soybean hulls		
MD (mm)	50.563	50.976	49.401	48.921	9.538	ns
MW (mm)	27.633	28.846	22.736	24.745	16.745	ns
GFT (mm)	1.993	2.038	1.278	1.741	46.415	ns
LFT (mm)	1.008	0.806	0.491	0.596	58.978	ns

Loin maximum depth measurements are used to estimate the amount of muscle in the carcass and have a high correlation with the loin eye area and conformation. The loin measurements were not influenced by treatments (P>0.05), and the values obtained for the maximum depth in this study were lower than those reported by Neres *et al.* (2000) and Lombardi *et al.* (2010) of 55.58 mm and 54.08 mm, respectively.

Mean values for meat and fat color, pH, fat thickness (FT), loin eye area (LEA), cooking loss (CL), and objective tenderness (WBSF) of crossbreed Highlander lambs finished with different energy sources are listed in table 5.

*p<0.05. Means followed by different letters a line differ statistically from each other; ns = not significant (p >= 0.05). FT = fat thickness. LEA = loin eye area. CL = cooking loss. WBSF = objective tenderness.

*p<0,05. Las medias seguidas de una letra diferente en la línea difieren estadísticamente entre sí. ns = No significativo (p> = 0,05). FT = espesor de grasa. LEA = área de los ojos del lomo. CL = pérdida de peso por cocinar. WBSF = ternura objetiva.

Table 5 Estimated means and coefficients of variation (CV) of color, pH, fat thickness, loin eye area, cooking loss, and WBSF of the *Longissimus thoracis et lumborum* muscle from Highlander lambs fed diets with different energy sources.

Tabla 5. Medias estimadas y coeficientes de variación (CV) del color, pH, el grosor de la grasa, el área de los ojos del lomo, la pérdida de cocción y la ternura de la carne del músculo *Longissimus thoracis et lumborum* de corderos terminados con diferentes fuentes de energía.

Variables	Dietary treatments				CV %	Effects
	Corn	Citrus pulp	Rice bran	Soybean hulls		
Meat Redness a*	13.59^{b}	16.23^{a}	14.77^{ab}	14.66^{ab}	9.41	*
Meat Yellowness b*	6.29^{a}	7.12^{a}	6.25^{a}	6.51^{a}	17.70	ns
Meat Lightness L*	40.87^{a}	42.19^{a}	41.14^{a}	41.06^{a}	6.20	ns
Fat Redness a*	6.67^{a}	9.50^{a}	8.26^{a}	8.85^{a}	34.18	ns
Fat Yellowness b*	10.09^{a}	11.72^{a}	9.93^{a}	11.49^{a}	21.30	ns
Fat Lightness L*	66.19^{a}	69.12^{a}	67.22^{a}	66.95^{a}	6.10	ns
pH	5.64^{a}	5.70^{a}	5.76^{a}	5.79^{a}	3.26	ns
FT (mm)	2.53^{a}	2.64^{a}	2.02^{a}	3.41^{a}	34.88	ns
LEA (cm^2)	11.92^{a}	12.69^{a}	10.41^{a}	12.79^{a}	18.28	ns
CL (%)	22.58^{ab}	24.71^{ab}	26.90^{a}	21.91^{b}	11.10	*
WBSF (kgf/cm^2)	5.62^{a}	5.70^{a}	6.30^{a}	5.60^{a}	17.28	ns

According to the literature, the color values for sheep meat range from L* 31.36 to 38.0, a* 12.27 to 18.01, and b* 3.34 to 5.65 (6). Although no statistical differences were observed for parameters L* and b*, the values measured in this study were higher than those suggested in the literature. These findings could be related to the slaughter age of the animals, as young animals have more water on the muscle, resulting in the highest values for L* (25). For a* values, a statistical difference was observed between the corn and citrus pulp diets (p>0.05). This difference can be explained by the capacity of citrus pulp to improve the oxidative stability of meat (15). Fat color parameters for L*, a*, and b* did not differ (p>0.05) between dietary treatments.

The ideal pH for meat ranges between 5.5 and 5.8 (28). In this study, no statistical differences (p>0.05) were observed between diets, and the values measured (5.72) indicated a proper resolution of *rigor mortis*.

No differences were observed in fat thickness between dietary treatments (p>0.05), and the mean values measured in this study (1.75 mm) were considered unsatisfactory. A minimum fat coverage of 3 mm is necessary to protect the carcass from water loss during refrigeration. The minimum fat thickness also protects the carcass from cold-induced burning during the freezing process (19). In a study on citrus pulp diets, Henrique *et al.* (2004) observed a linear reduction in this variable with the addition of up to 55% of citrus pulp to the cattle diet and attributed this effect to the lower energy value of citrus pulp when compared to corn (21). This reduction was not observed in the present study and can be related to the fact that the citrus pulp diet received inclusion of oil. The lower deposition of fat in the carcass from animals fed exclusively with citrus pulp can also be a result of differences in volatile fatty acids produced during ruminal fermentation (8).

For the loin eye area (11.92 cm^2), no statistical differences were observed between dietary treatments. Zundt *et al.* (2010) also working with crossbreed Highlander lambs with a slaughter weight of 30 kg, observed lower area values (10.89 cm^2) than the ones observed in this study.

A statistically significant difference was observed in cooking loss (p<0.05). The soybean hulls diet presented lower loss (21.91%) when compared to the rice bran diet (26.9%), but with no statistical difference from the other treatments. Values for cooking loss in the literature vary between 33% and 44% (4) for small ruminants, which are higher than the values observed in this study. When evaluating different diet compositions for lambs, Atsbha *et al.* (2021) reported cooking loss values similar to those observed in this study, with an average of 24.18% to 26.12%.

For objective tenderness, the observed mean values were 5.81 kgf, and the results corroborate those of Costa *et al.* (2011). The meat from this study was classified as soft (5, 32).

Table 6 presents the mean results and coefficients of variation (CV%) for proximate composition of *Longissimus thoracis et lumborum* muscle of crossbreed Highlander lambs fed different energy sources as corn substitutes.

The results revealed no effect of different dietary treatments (p>0.05) on moisture, protein, and ash content of the meat, whereas ethereal extract content differed between dietary treatments (p<0.05). The citrus pulp diet had the lowest percentage of ethereal extract, and Caparra *et al.* (2007) observed an ethereal extract values decreased when the inclusion of citrus pulp increased.

Table 6. Means and coefficients of variation (CV) for proximate composition of the *Longissimus thoracis et lumborum* muscle of Highlander lambs fed diets with different energy sources.

Tabla 6. Media y coeficiente de variación (CV) para la composición aproximada de la carne del músculo *Longissimus thoracis et lumborum* de corderos Highlander terminados con diferentes fuentes de energía.

Variables (%)	Dietary treatments				CV(%)	Effect
	Corn	Citrus pulp	Rice bran	Soybean hulls		
Moisture	75.97^a	76.30a	77.01 ^{a}	76.13^a	1.15	ns
Protein	20.49^a	19,49^a	19.39 ^{a}	20,79^a	4.04	ns
Ethereal Extract	1.16^a	1.07^b	1.15^{ab}	1.18^a	5.24	*
Ashes	1.94^a	2.66^a	1.81 ^{a}	2.05^a	33.32	ns

ns = not significant (p >= 0.05). * p < .05. Means followed by different letters in a line differ statistically from each other.

ns = No significativo (p> = 0,05). * p <,05. Las medias seguidas de una letra diferente en la línea difieren estadísticamente entre sí.

Conclusion

The use of different energy sources in ram lamb finishing diets did not change the proximate composition and quality of the meat. The inclusion values used in this study are recommended, although the use of rice bran as a substitute for corn should be carefully evaluated, as its inclusion provided less daily weight gain, consequently more days of feedlot for the animals.

Disclosure of interest

The authors declare that they have no conflict of interest regarding the work presented.

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Effect of thermosonication on enzymatic oxidation and physicochemical properties of soursop (*Annona muricata*) pulp

Efecto de la termosonicación en la oxidación enzimática, y las propiedades físico-químicas de la pulpa de guanábana (*Annona muricata*)

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Abstract

Soursop is an exotic tropical fruit with marked commercial importance. However, its post-harvest presents losses of around 30%. The objective of this research was to study the effect of thermosonication (TS) on physicochemical properties and enzymatic oxidation of soursop pulp. Four treatments were evaluated: control (0 min), ultrasound at 20 °C (US20), 40 °C (US40), and 60 °C (US60) for 25 minutes. Concentration of vitamin C, and peroxidase (POD) and polyphenoloxidase (PPO) inactivation in the soursop pulp, were assessed. The results indicate a loss of vitamin C of 2.63%; 13.16% and 28.95% for temperatures at 20, 40 and 60 °C, respectively. On the other hand, partial inactivation of 48.41%; 69.83% and 74.24% of POD, and 56.38%; 74.47% and 81.91% of PPO enzymes were achieved at temperatures of 20, 40 and 60 °C, respectively. Finally, it was concluded that ultrasound combined with heat (TS) can inactivate POD and PPO enzymes in the soursop pulp, achieving considerable retention of vitamin C.

Keywords
pH • Soluble solids total • Polyphenoloxidase • Peroxidase • Vitamin C

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Resumen

La guanábana es una fruta tropical exótica tiene una marcada importancia comercial, sin embargo, presenta pérdidas de alrededor del 30% en poscosecha. El objetivo del trabajo fue estudiar el efecto de la termosonicación (TS) sobre propiedades fisicoquímicas y la oxidación enzimática de la pulpa de guanábana. Los tratamientos evaluados fueron control (0 min), ultrasonido a 20 °C (US20), a 40 °C (US40), y a 60 °C (US60) durante 25 minutos. Se evaluaron principalmente la concentración de vitamina C, la inactivación de la peroxidasa (POD) y la polifenoloxidasa (PPO) en la pulpa de guanábana. Los resultados indican que se observó una pérdida de vitamina C en un 2,63; 13,16 y 28,95% para temperaturas a 20, 40 y 60 °C, respectivamente. Por otro lado, una inactivación parcial de las enzimas, POD, en 48,41; 69,83 y 74,24% y para PPO en 56,38; el 74,47 y el 81,91% se logró a temperaturas de 20, 40 y 60 ° C, respectivamente. Finalmente, se demostró que el ultrasonido combinado con calor (TS) inactivan en un gran porcentaje las enzimas POD y PPO en la pulpa de guanábana logrando importantes niveles de retención de la vitamina C.

Palabras clave

pH • sólidos solubles totales • polifeniloxidasa • peroxidase • vitamina C

Introduction

Quality of minimally processed fruits is widely valued by consumers. During elaboration, the enzymes Peroxidase (POD) and Polyphenoloxidase (PPO) trigger enzymatic processes affecting product natural color. According to Raimbault *et al.* (2010) these two enzymes produce quinones after oxidation of phenolic decolorizing compounds. In order to increase shelf life and improve product appearance, inactivation of these two enzymes must be total or partial (>70%). According to Soysal (2008) and Goyeneche *et al*. (2013) fruit and vegetable products subjected to thermal treatments at 60 and 95 °C stop enzymatic browning after protein inactivation, whereas Castro *et al.* (2008) reported that along with inactivation, it may also degrade nutrients such as vitamin C. In this sense, the demand for fresh products has prompted research to find non-thermal treatments such as ultrasound (US), an inaudible acoustic wave frequency higher than 20kHz (33). Islam *et al.* (2014) and Abid *et al.* (2014) evaluated the effects of US enzymatic inactivation and indicated that acoustic waves alter enzymatic tertiary structure leading to biological activity loss. In another work, Sulaiman *et al.* (2015) studied the effect of thermal US on PPO inactivation observing an increase in the inactivation rate after 10 minutes of US. Jang and Moon (2011) studied the effects of US and ascorbic acid on PPO and POD activity in fresh apples during storage. Liu *et al.* (2017) studied the effect of US on PPO in eastern sweet melon, reporting that high intensity US generated enzymatic conformational changes, causing inactivation.

Soursop is an exotic fruit with high levels of bioactive compounds, and great agro-industrial potential given its high recovery yields and excellent sensory characteristics (3). However, as stated, this fruit is susceptible to enzymatic browning (6). In Colombia, browning is one main rejection factor, generating losses of around 30% in post-harvest handling of this fruit. Therefore, research in this matter is required. The objective of this work was to study the effect of thermosonication (TS) on the physicochemical properties and enzymatic oxidation of soursop pulp.

Materials and methods

Chemicals

Phosphate buffer (pH 7.5), polyvinylpolypyrrolidone (PVPP), triton X-100, potassium iodide [90%], and hydrochloric acid [37%] (V/V) were obtained from Merck. Guaiacol [98%], hydrogen peroxide [30%], catechol [99%] and Arsenic (III) oxide [99.995%] were purchased from Sigma–Aldrich.

Pulp preparation

Five kg of fresh ripen soursop (*Annona muricata*) were purchased from a local supermarket in the city of Cúcuta, Colombia. These fruits were immediately taken to the Research Laboratory of the Food Innovations Group of the University of Pamplona, Villa del Rosario, Colombia. After peeling, the seeds were manually removed. Then, the pulp was vacuum packed (95%) and stored under refrigeration (4 °C) until further processing or analysis.

Treatment with thermosonication (TS)

Fruit pulp was divided in four samples, control (0 min), ultrasonic at 20 °C (US20), ultrasonic at 40 °C (US40), and ultrasonic at 60 °C (US60). Pulp samples (150 g) were vacuum-packed at 95%, and treated in an ultrasonic bath system (Elmasonic E equipment Model: E60H), with 40 kHz frequency and maximum ultrasonic power of 100 W. Internal dimension: 300 mm x 151 mm x 150 mm. All treatments were carried out in the dark, immediately cooled by immersion in an ice water bath and stored at 4 °C until further analysis.

Physicochemical properties

Total soluble solids (SST) were measured by refractometry (ATC-FG113), and pH was measured using an Orion 210ª electronic pH meter previously calibrated following AOAC (1990). Vitamin C was quantified by titration of ascorbic acid according to Santos and Daghastanli (2001).

Peroxidase (POD) extraction and activity

Enzymatic extraction was carried out after Silva *et al.* (2015) modified. Twenty g of soursop pulp were mixed with 100 mL distilled water, and centrifuged at 10000 g, 7 °C, for 10 minutes. Then, 1.5 ml of the extract was mixed with 320 ɥL of guaiacol 5% (V / V) and 160 ɥL of H_2O_2 [0.147 M] (V / V). Absorbance was recorded using a spectrophotometer (ThermoSpectronic Genesys 20) at 470 nm, every 20 seconds for 4 minutes, at 25 °C. Absorbance was related as a function of time. Enzymatic activity was obtained by equation 1:

$$\text{Eq. 1 } AE/e = (M) / (Ke),$$

where:
AE / e = enzymatic activity of the treated or not treated sample,
M = rate of reaction progress (ΔAbs / Δtim), and Ke: extinction coefficient.
Residual POD activity (%), resulted from equation 2:

$$\text{Eq. 2 } AER\ (\%) = ((\Delta E/Ao)*100),$$

where:
ΔE = enzymatic activity of the thermosonicated sample,
Ao = enzymatic activity of the control sample (untreated),
AER = residual enzymatic activity.

Oxidation caused by hydrogen peroxide reduces Guayacol to Tetraguayacol, generating brown coloration. According to Liburdi *et al.* (2019), the molar extinction coefficient of tetraguayacol is 26.6 $mM^{-1}cm^{-1}$ for an absorbance of 470 nm. This value is used to calculate enzymatic activity of POD after treatments with TS using eq. 1 and 2.

Polyphenoloxidase (PPO) extraction and activity

Enzymatic extraction was carried out according to Campo and Gélvez (2011) with modifications. Ten g of soursop pulp were mixed with 25 mL of 0.2M phosphate buffer solution (pH 6.5) containing 4% polyvinylpolypyrrolidone (PVPP) and 1% Triton X-100, homogenized for 3 minutes and then centrifuged (10000 rpm / 30 min at 4 °C). Seventy-five µL of the extract were extracted and mixed with 3 mL of 0.007M catechol in 0.05M phosphate buffer (pH 6.5) as enzyme substrate. Absorbance was recorded using a spectrophotometer (ThermoSpectronic Genesys 20) at 420 nm, every 20 seconds, for 4 minutes, at 25 °C. Absorbance was related as a function of time. This value is used to calculate enzymatic activity of PPO after treatments with TS using eq.1 and 2, calculating residual activity of PPO (%). According to Palmer (1963), Catecol is reduced to Benzoquinone by O_2 with an extinction coefficient of 2.6 $M^{-1}cm^{-1}$ at 420 nm.

Statistical analysis

Results are expressed as means ± standard deviations. Treatment effects (control, US20, US40, and US60) on individual dependent variables (PPO and POD activity, Vitamin C, total solids and pH) were evaluated by one-way ANOVA. Significant differences between means were determined by the Tukey pairwise comparison test at a significance level of $P < 0.05$. Statistical analyses were conducted using the SPSS 18 program for Windows. All treatments were carried out in triplicate.

Results and discussion

Analysis of physicochemical properties

Figure 1 shows pH values of the treated and untreated samples. This variable increased significantly ($p < 0.05$) after TS and temperature due to cavitation, after breaking pulp hydroxyl bonds (OH^-). These results were also reported by Avalos *et al.* (2015) and Campo *et al.* (2011), indicating that with US in exposure time range of 15 and 30 minutes, a slight increase in cherimoya pulp pH, occurred. In contrast, Porras *et al.* (2011), reported a decrease in pH in mango pulp for times greater than 30 minutes. In the present study, pH reduces PPO and POD enzymatic actions, allowing pulp stability. In addition to the inactivation of these enzymes, the OH- group is associated with antimicrobial and antioxidant activities that allow preserving and improving the shelf life of food (2). According to Avalos *et al.* (2015), this slight increase is related to the release of volatile aromatic substances given cavitation and temperature effects in the TS treatment, favoring organoleptic properties of soursop pulp.

MC= control samples, US20= ultrasonic at 20 °C, US40= ultrasonic at 40 °C, and US60= ultrasonic at 60 °C.

MC= muestra control, US20 = ultrasónico a 20 °C, US40 = ultrasónico a 40 °C y US60 = ultrasónico a 60 °C.

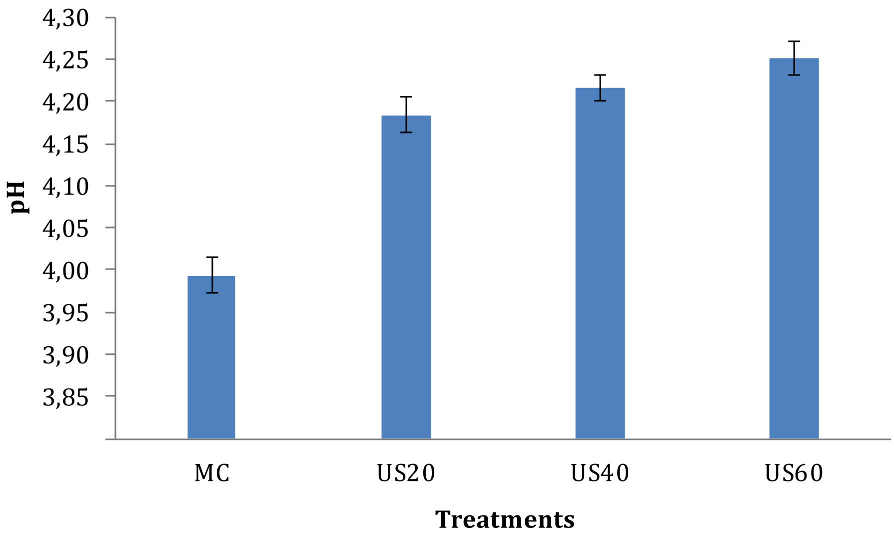

Figure 1. Soursop pulp pH treated and untreated with thermosonication at different temperatures.

Figura 1. Valores de pH de pulpa de guanábana tratados y no tratados con termosonicación a diferentes temperaturas.

Figure 2 (page 256) shows SST (°Brix) of the treated and untreated samples. SST increased significantly ($p < 0.05$) after TS and temperature, as observed for mango pulp, apple juice and cherimoya pulp, (1, 5, 25). During ultrasound treatments, polysaccharide diffusion coefficient and solubility increased, mainly due to cavitation in the plant matrix, fracturing cell walls (32). An alternative explanation states that increasing SST (°Brix) is given by micro evaporation of water during ultrasonic cavitation (17, 25). According to Makino *et al.* (1983), cavitation causes a dissociation of water molecules into hydrogen ions (H^+) and hydroxyl groups (OH^-) increasing pulp ° Brix. Figure 3 (page 256) shows vitamin C values of the treated and untreated samples.

MC= control samples, US20= ultrasonic at 20 °C, US40= ultrasonic at 40 °C, and US60= ultrasonic at 60 °C.

MC= muestra control, US20 = ultrasónico a 20 °C, US40 = ultrasónico a 40 °C y US60 = ultrasónico a 60 °C.

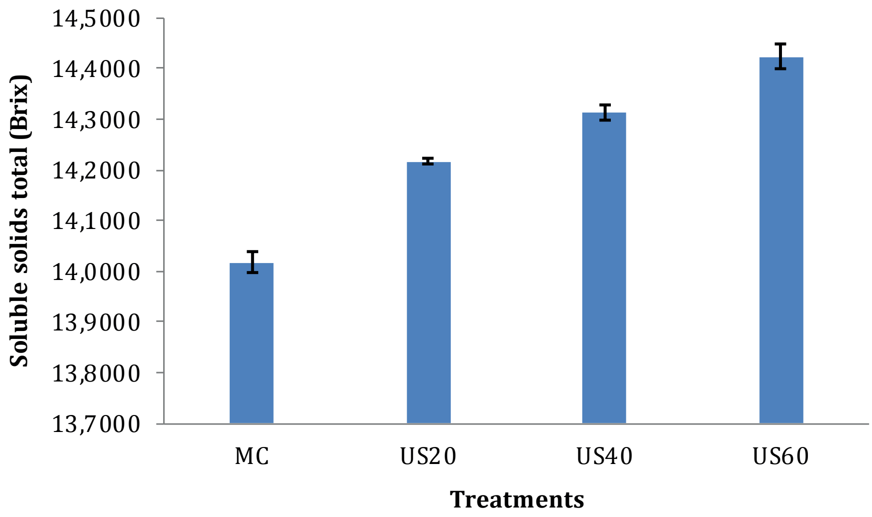

Figure 2. Total soluble solids of soursop pulp treated and not treated with thermosonication at different temperatures.

Figura 2. Valores de sólidos solubles totales de pulpa de guanábana tratada y no tratada con termosonicación a diferentes temperaturas.

MC= control samples, US20= ultrasonic at 20 °C, US40= ultrasonic at 40 °C, and US60= ultrasonic at 60 °C.

MC= muestra control, US20 = ultrasónico a 20 °C, US40 = ultrasónico a 40 °C y US60 = ultrasónico a 60 °C.

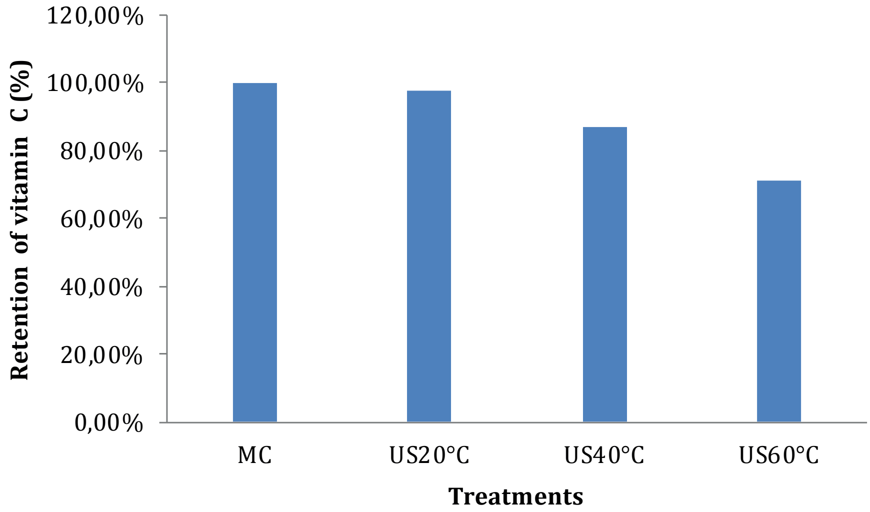

Figure 3. Vitamin C before and after thermosonication.

Figura 3. Vitamina C presente en la muestra antes y después del tratamiento con termosonicación.

Significant differences *($p < 0.05$)* in vitamin C retention among control sample and treatments were found with temperatures around 40 and 60 °C. The lowest vitamin C retention value (71.05%) occurred for TS around 60 °C, followed by TS at 40 °C with 86.84%. On the contrary, no significant differences could be found for TS at 20 °C. Our results agree with those reported by Dabir and Ananthanarayan (2018) in custard apple. These authors found no significant effect between treated and untreated samples with US at room temperatures. However, Abid *et al.* (2014) indicated that under TS, when temperature raised above 40 °C, a significant loss of vitamin C occurred. The synergistic effect of radical formation (OH^- and H^+) and temperature during thermosonication, triggers hydrolysis, decarboxylation, regrouping and cyclization reactions, initiating the formation of compounds like 2,3-diketogulonic, 3-hydroxy acids -2-pirone, and 2-furoic, losing nutritional value (23).

Effects of thermosonication on POD and PPO enzymatic activity in soursop

During fruit processing and storage, PPO and POD enzymes can affect product final quality. PPO is involved in the oxidation of polyphenols to o-quinones, which subsequently polymerize into undesirable brown, red, or black pigments in the presence of oxygen (18). These authors also comment that POD, an antioxidant enzyme, catalyzes the conversion of hydrogen peroxide to water using polyphenols as hydrogen donors. Figure 4 shows POD reaction before and after the treatment. Its reaction rate decreases with TS and temperature. POD residual enzymatic activity under TS around 20 °C, is 52.59% compared to the control sample, whereas when temperature is increased, only 30.17 and 25.86% are observed at 40 and 60 °C, respectively (figure 4A). Other studies also report a reduction in POD activity after ultrasound. In bayberry juice, residual activity levels resulted in between 9.49-73.14%, while in pear juice, inactivation levels reached between 4.30-43.2% (9, 27).

MC= control samples, US20 = ultrasonic at 20 °C, US40 = ultrasonic at 40 °C and US60 = ultrasonic at 60 °C.

MC=Muestra control, US20 = ultrasónico a 20 °C, US40 = ultrasónico a 40 °C y US60 = ultrasónico a 60 °C.

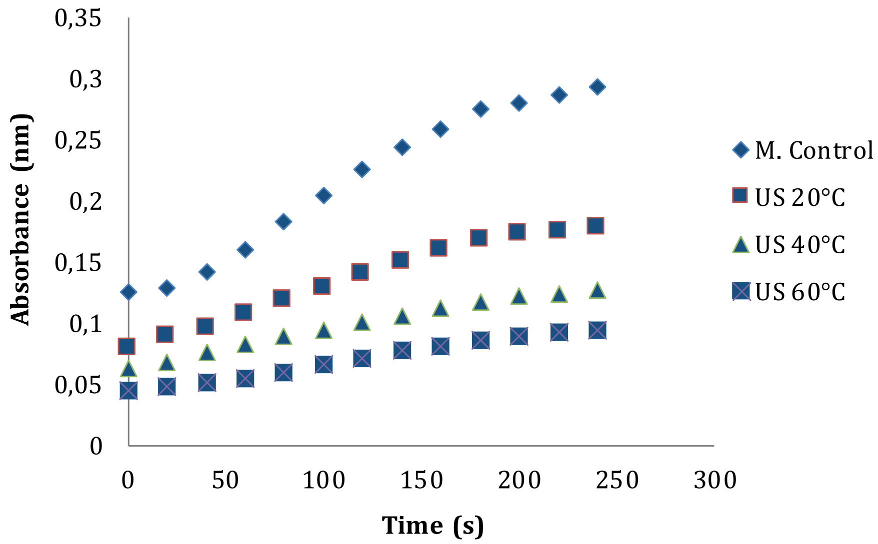

Figure 4. Progression curve of (Abs *vs* Time) soursop peroxidase reaction before and after TS treatments.

Figura 4. Curva de progreso de la reacción (Abs *vs* Time) de la peroxidasa de guanábana antes y después de los tratamientos con TS.

MC= control samples, US20= ultrasonic at 20 °C, US40= ultrasonic at 40 °C, and US60= ultrasonic at 60 °C.

MC= muestra control, US20 = ultrasónico a 20 °C, US40 = ultrasónico a 40 °C y US60 = ultrasónico a 60 °C.

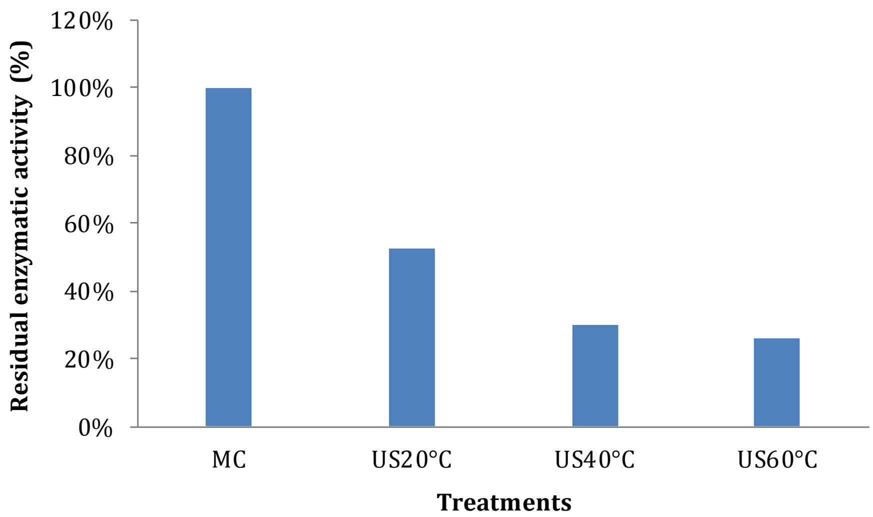

Figure 4A. Residual enzymatic activity of peroxidase in soursop after thermosonication at different temperatures.

Figura 4A. Actividad enzimática residual de peroxidasa en guanábana después del tratamiento con termosonicación a diferentes temperaturas.

On the other hand, figure 5 shows PPO behaviour before and after the treatment. PPO reaction rate also decreased with TS and temperature. Its residual enzymatic activity with TS around 20 °C, was 43.62% compared to the control sample, whereas when temperature is increased, only 25.53 and 18.09% were observed for 40 and 60 ° C, respectively (figure 5A). Accordingly, in bayberry juice, registered residual activity levels reached between 0.08 and 53.23%, while in pear juice reported inactivation levels resulted between 1.91-89.33% (9, 27).

MC= control samples, US20= ultrasonic at 20 °C, US40= ultrasonic at 40 °C, and US60= ultrasonic at 60 °C.

MC= muestra control, US20 = ultrasónico a 20 °C, US40 = ultrasónico a 40 °C y US60 = ultrasónico a 60 °C.

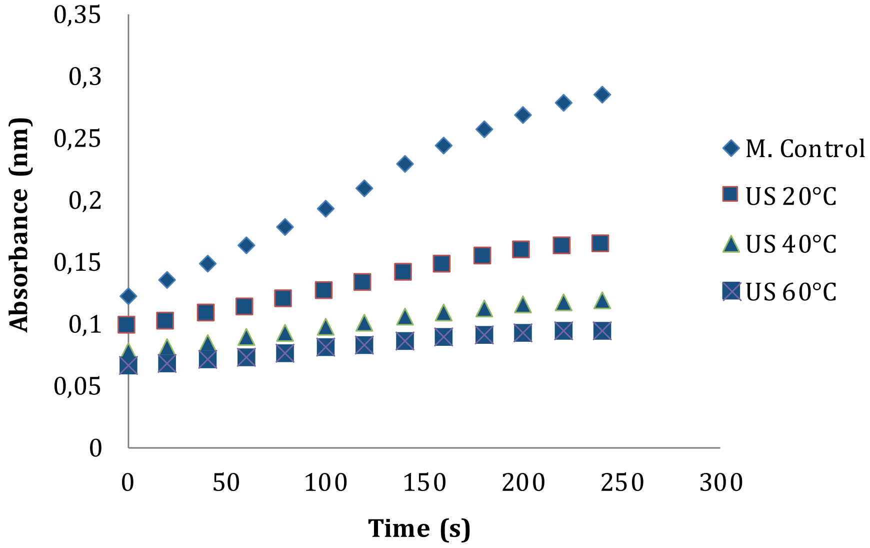

Figure 5. Progression curve of the reaction (Abs *vs* Time) of soursop polyphenoloxidase before and after TS.

Figura 5. Curva de progreso de la reacción (Abs *vs* Tim) de la polifenoloxidasa de guanábana antes y después de los tratamientos con TS.

MC= control samples, US20= ultrasonic at 20 °C, US40= ultrasonic at 40 °C, and US60= ultrasonic at 60 °C.

MC= muestra control, US20 = ultrasónico a 20 °C, US40 = ultrasónico a 40 °C y US60 = ultrasónico a 60 °C.

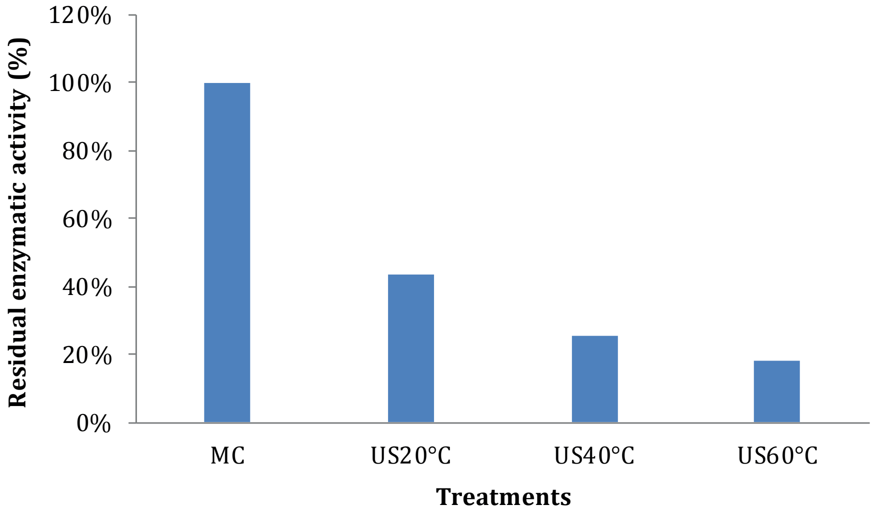

Figure 5A. Residual enzymatic activity of polyphenoloxidase in soursop after thermosonication at different temperatures.

Figura 5A. Actividad enzimática residual de polifenoloxidasa en guanábana después del tratamiento con termosonicación a diferentes temperaturas.

According to Ercan and Soysal (2011) ultrasonic generated heat, and exposure time leading to increased sample temperatures, allow the reduction of POD and PPO activities. When US is combined with temperature, a synergic effect generates enzymatic dissociation of the prosthetic group, significantly decreasing *(p<0.05)* the content of the structure of the helix/alpha and causing conformational changes in the secondary and tertiary structures of POD and PPO after cavitation effect. This finally leads to partial or total protein denaturation (14, 20, 22).

Anaya-Esparza *et al.* (2017) reported that US generates micro-streaming phenomena that can interrupt polypeptide Van der Waals interactions and hydrogen bonds. In addition, free radicals generated during US can react with disulfide bonds, destabilizing the enzymatic conformation, or oxidizing amino acids related to enzymatic catalytic activity and stability, such as tryptophan, tyrosine, histidine, and cysteine. According to Dabir and Ananthanarayan (2018), complete inactivation of POD and PPO is achieved at 5 minutes under an ultrasonic power of 85 W and 90 W respectively, followed by 21.6% loss of vitamin C. In this study, TS at 40 °C resulted the most efficient treatment inactivating POD in 69.83% and PPO, in 74.47%, while retaining 86.84% vitamin C. On the other hand, TS and its lower temperatures (<60 °C), favoured vitamin C retention, in comparison with conventional thermal treatments using temperatures higher than 70 °C. According to Burdurlu *et al.* (2006), greater temperature decreases vitamin C.

Conclusions

TS treatment in soursop pulp (*Annona muricata*) affects its physicochemical properties, increases pH and soluble solids, while decreases vitamin C retention. The evaluated TS treatments inactivated POD and PPO enzymes in a range of 48.41-74.24% and 56.38-81.91%, respectively. Maximum POD (69.83%) and PPO (74.47%) inactivation were reached with 40 kHz and 40 °C TS, keeping vitamin C at 86.84%. Further research on POD and PPO inactivation in soursop is needed, Studies demonstrating that TS could be more efficient than blanching and bleaching, should be developed. Given that lower temperatures do not need? additional chemicals, this constitutes a product and environmentally friendly proposal.

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Water loss and chemical composition of cactus pear genotypes submitted to post-harvest storage periods

La pérdida de agua y composición química de las variedades de pera de cactus forrajeras sometidas a períodos de almacenamiento posteriores a la cosecha

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Abstract

The objective of this study was to evaluate water loss and chemical composition of cactus pear genotypes submitted to post-harvest storage periods. The experimental design adopted was a the completely randomized, in a 3 × 5 factorial arrangement, with three cactus pear genotypes and five storage periods, and ten replications. The cactus pear genotypes [Doce, Baiana and Orelha de Elefante Mexicana (OEM)] were harvested after 2 years of cultivation under rainfed conditions, and stored in a ventilated shed (0, 15, 30, 45 and 60 days). The genotype Baiana showed greater water reduction in the stored cladodes when compared to the other genotypes. All cactus pear genotypes showed reduction in crude protein and carbohydrates in the storage period of 60 days. There were no losses of nutrients, dry matter and ether extract during the storage periods for the genotype Doce. There was increase in the fiber content of the cladodes of all stored genotypes. During the storage period of the cladodes of all genotypes, there was reduction in the contents of Ca, Mg and Cu. The cactus pear genotypes Doce and OEM can be stored for up to 60 days after harvest.

Keywords
conservation • forage • *Nopalea cochinillifera* • *Opuntia tuna*

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Resumen

El objetivo de este estudio fue evaluar la pérdida de agua y la composición química de las variedades de pera de cactus forraje sometidas a períodos de almacenamiento posteriores a la cosecha. El diseño experimental adoptado fue un diseño completamente al azar, en un arreglo factorial 3 × 5, que consistía en tres variedades de pera de cactus forrajero y cinco períodos de almacenamiento, con diez repeticiones. Las variedades de pera de cactus forrajero [Doce, Baiana y Orelha de Elefante Mexicana(OEM)] se cosecharon después de 2 años de siembra en condiciones de secano y se almacenaron en un cobertizo ventilado (0, 15, 30, 45 y 60 días). El genotipo Baiana mostró una mayor reducción de agua en los cladodios almacenados en relación con otros genotipos. Todos los genotipos de pera de cactus mostraron una reducción en proteínas crudas y carbohidratos en el período de almacenamiento de 60 días. No hubo pérdidas de nutrientes, materia seca y extracto de éter durante el período de almacenamiento para el genotipo Doce. Hubo un aumento en el contenido de fibra en los cladodios para todos los genotipos almacenados. Durante el período de almacenamiento para cladodios de todos los genotipos, hubo una reducción en el contenido de Ca, Mg y Cu. Las variedades de pera de cactus Doce y OEM se pueden almacenar hasta 60 días después de la cosecha.

Palabras clave

conservación • forraje • *Nopalea acochinillifera* • *Opuntia tuna*

Introduction

The cactus pear is an important resource for animal feeding due to its rusticity and production potential even with low water availability, especially in the semi-arid region of Brazil, as this plant presents high forage supply capacity when compared to the native vegetation of the caatinga (14), mainly during the dry season.

When dealing with historical aspects of cactus pear, the literature points to Mexico as its origin point (7). The cactus pear species most used as forage belong mainly to the genera *Opuntia* and *Nopalea* (10), which present variable chemical composition according to the period of the year, plant age, cladode order, genotype, fertilization management, cultivation spacing, and other factors (6). It is of a forage that has low contents of dry matter (63.7 g kg^{-1}), crude protein (54.2 g kg^{-1}), neutral detergent fiber (283.0 g kg^{-1}) and acid detergent fiber (209.3 g kg^{-1}) (4). However, it is rich in non-fibrous carbohydrates (473.6 g kg^{-1}) and total digestible nutrients, presenting high water content, and high animal acceptability. The cactus pear is also rich in vitamins A, complex B and C, and minerals such as calcium, magnesium, sodium, potassium, and 17 types of amino acids (13, 17).

Cactus pear is also considered a strategic forage reserve and, depending on the needs of the producer and climatic conditions, the harvest frequency may vary. Depending on the crop structure, the cactus pear can be harvested manually and transported by animal traction to its place of use. In general, this operation is carried out daily, increasing production costs (23). Another factor that jeopardizes the harvest is the lack of pattern of the orchard, hindering management practices, such as fertilization. Also, direct grazing might compromise its cultivation, due to damages caused to the plants by the animals (3).

Post-harvest storage may be an alternative to lower the costs of harvesting and transportation of the material, however the storage must be done in a shady or covered place as long as it is ventilated. However, few information is available in the literature on post-harvest storage of cactus pear genotypes and its effects on the nutritional value and animal performance. It was hypothesized that the cactus pear genotypes stored for up to 60 days post-harvest would not present changes in their chemical and mineral composition enough to reduce their quality. Thus, the objective was to evaluate water loss and chemical composition of the cactus pear genotypes Doce, Baiana and Orelha de Elefante Mexicana submitted to up to 60 days of storage post-harvest.

Material and methods

Experimental site

The experiment was carried out at the Experimental Farm Alvorada of Gurguéia, which belongs to the Federal University of Piauí (UFPI), in Alvorada of Gurguéia, Piauí, Brazil. The city of Alvorada of Gurguéia is located at latitude 08°25'28" South and longitude 43°46'38" West, at an altitude of 281 m. The region has a semi-arid climate, with a dry period of approximately eight months (1).

Experimental design

The experimental design adopted was the completely randomized, in a 3 × 5 factorial arrangement, with three genotypes of cactus pear (Doce, Baiana and Orelha de Elefante Mexicana) and five storage periods (0, 15, 30, 45 and 60 days), in ten replications.

Planting and fertilization

Before planting the cactus pear genotypes, a soil sample was collected for analysis and chemical characterization of the 0-20-cm layer, which was carried out at the Soil Analysis Center of the UFPI, Bom Jesus, Piauí, Brazil. It was not necessary to perform soil correction based on soil base saturation and crop requirement. The basic fertilization consisted of the application of 50 kg ha^{-1} of nitrogen as urea (45% N), 50 kg ha^{-1} of potassium as potassium chloride (48% K_2O) and 30 kg ha^{-1} of phosphorus as single superphosphate (18% P_2O_5).

The spacing used for the cultivation of the cactus pear genotypes was 1.5m × 0.1m, in a density of 66,133 plants ha^{-1}. Three genotypes of cactus pear were planted in December 2013: *Nopalea cochinillifera*, genotypes Doce and Baiana, and *Opuntia tuna*, genotype Orelha de Elefante Mexicana (OEM). After 2 years of cultivation under rainfed conditions, the harvest was performed on December 6th, 2015. The cactus pear was harvested manually, using a 14-inches Tramontina® knife preserving a residual cladode area (23), and stored in a ventilated shed on wooden pallets, with a height of approximately 10 cm from the floor, storing 200 cladodes from each genotype, thus providing a random choice, for a period of up to 60 days of post-harvest.

Quantification of water loss

After harvesting, ten cladodes of each genotype were selected and separated for weighing, which were alwas the same cladodes to be weighed according to the storage periods. In order to measure the amount of water lost by the catus pear genotypes, it was used a digital electronic scale, with capacity of 1 g to 5 kg, model Sf-400 UNICASA®. The calculation of water loss was made through the difference between the weight of the current period and the previous period.

The cladodes of the cactus pear genotypes were subjected to natural drying due to the conditions of rainfall (mm), air relative humidity (%) and room temperature (°C). The accumulated precipitation during the entire experimental period was 387 mm. The maximum and minimum values of air relative humidity were 93.5% and 33%, respectively. The maximum temperature values ranged from 25.6°C to 38.4°C, and the minimum temperature values ranged from 19.2°C and 24.2°C.

Chemical composition

Ten cladodes of each genotype and each treatment (0, 15, 30, 45 and 60 days of storage) were randomly collected in plastic bags, duly identified and taken to the Animal Nutrition Laboratory of the UFPI, where they were chopped, weighed and taken to a forced ventilation oven at a temperature of 65°C until reaching constant weight. Later, the pre-dried samples were ground in a "Thomas Wiley" stationary mill, model SP-32 SPLAPOR® with a 1.0-mm mesh sieve, and packed in containers with covers for chemical analysis. The contents of dry matter (DM) (Method n° 934.01), mineral matter (MM) (Method n° 930.05), crude protein (CP) (Method n° 981.10) and ether extract (EE) (Method n° 920.29) were determined (2). In the analysis for the determination of neutral detergent fiber (NDF) and acid detergent fiber (ADF), it was adopted a methodology with modifications proposed by the Ankon device manual of the Ankon Technology Corporation (25). Lignin was determined on the ADF residue with 72% sulfuric acid (25). For the estimation of total carbohydrates (TCHO) the following equation was used: TCHO = 100 - (%CP + %EE + %MM) (24). The non-fiber carbohydrates (NFC) were estimated through the equation: NFC = 100 - %MM - %CP - %EE - %NDF (11).

Mineral composition

In the analysis of mineral composition, after nitric-perchloric digestion, the phosphorus (P) contents were determined by UV/VIS spectrophotometry at 660nm, by reading the blue color intensity of the phosphomolybdic complex produced by the reduction of molybdate with ascorbic acid in a spectrophotometer model IL-592 EVEN®. Whereas the contents of potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn), sodium (Na) and copper (Cu) were determined by atomic absorption spectrophotometry (AAS), in a spectrophotometer model AA240FS VARIAN® (20), in the Soil Analysis Center of the UFPI.

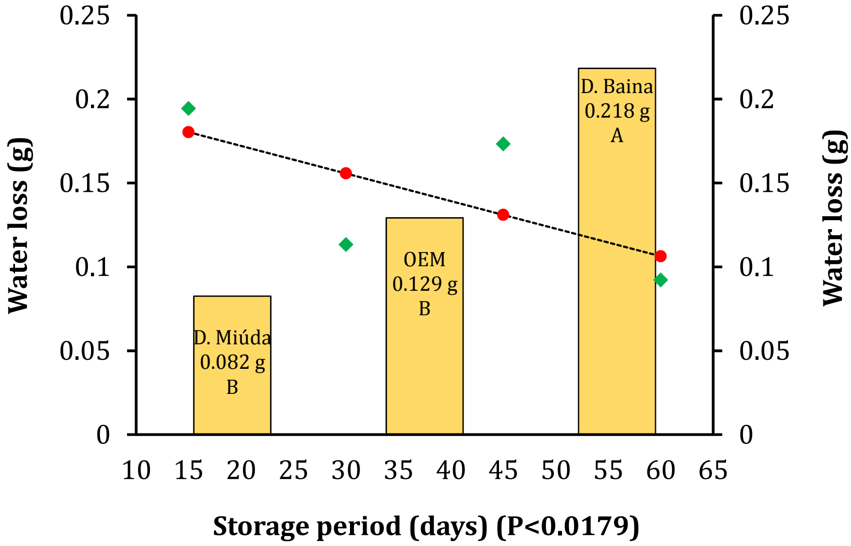
Statistical analysis

The results were submitted to analysis of variance and regression analysis (storage period), by the Scott-Knott test (cactus pear genotypes), at a level of 5% of significance, using the software SISVAR version 5.0 developed by the Federal University of Lavras (8).

Results

Quantification of water loss

Regarding the analysis of water loss of the stored cladodes, there was no effect (P=0.2589) of the interaction between the factors genotypes and storage periods (figure 1). It was observed a significant difference between the cactus pear genotypes for water loss (P=0.0004), in which the genotype Baiana presented the highest water loss (0.218 g). There was a linear reduction (P=0.018) of water loss in all genotypes as the storage period advanced, with wate loss being observed only after 15 days os post-harvest.



-------- Quadractic linear effect. ● Estimate of periods values. ◆ Actual value of water loss from each evaluation; means followed by different uppercase letters, differ by the Scott-Knott test (P<0.05).

-------- Efecto lineal cuadrático. ● Estimación de los valores de los períodos. ◆ Valor real de la pérdida de agua para cada evaluación; Las medias seguidas de letras mayúsculas diferentes, difieren según la prueba de Scott-Knott (P <0,05).

Figure 1. Water loss of the cactus pear genotypes during post-harvest storage periods.
Figura 1. La pérdida de agua de las variedades de pera de cactus durante los períodos de almacenamiento posteriores a la cosecha.

Chemical composition

It was observed significant effect (P<0.05) of the interaction between cactos pear genotypes and storage periods on the variables dry matter, crude protein, ether extract, mineral matter, organic matter and non-fiber carbohydrates (table 1).

Table 1. Chemical composition of cactus pear genotypes submitted to different storage periods.

Tabla 1. Composición química de las variedades de pera de cactus forraje sometidas a diferentes períodos de almacenamiento.

Genotype	Storage periods (days)					Mean	P-value
	0	15	30	45	60		
Dry Matter (DM) (g kg^{-1})							
Doce	124.56A	122.50A	135.33A	105.37A	124.93A	122.54	0.1683^{ns}
Baiana	113.97B	114.13A	110.47B	102.70A	103.80B	109.01	0.0106*
OEM[2]	104.87B	117.27A	118.77B	108.93A	102.77B	110.52	0.2890^{ns}
Mean	114.47	117.97	121.52	105.67	110.50		
Crude Protein (CP)[1]							
Doce	34.77C	34.40C	34.63B	32.43B	32.60B	33.77	0.0100*
Baiana	40.97B	36.60B	35.80B	34.20B	33.50B	36.21	<0.0001*
OEM[2]	52.57A	52.37A	55.83A	54.33A	56.00A	54.22	0.0006*
Mean	42.77	41.12	42.09	40.32	40.70		
Ether Extract (EE)[1]							
Doce	14.30B	14.20B	13.17B	11.67B	16.03A	14.05	0.4384^{ns}
Baiana	17.37A	17.40A	21.43A	15.10A	16.13A	17.49	0.1790^{ns}
OEM[2]	11.90B	13.80B	10.83B	15.60A	16.90A	13.63	0.0061*
Mean	14.52	15.13	15.14	14.12	16.36		
Mineral Matter (MM)[1]							
Doce	128.80A	159.20A	150.60B	161.17A	156.77B	151.31	<0.0001*
Baiana	133.20A	135.37B	154.07B	165.40A	161.60A	149.93	<0.0001*
OEM[2]	114.67B	129.97C	167.60A	161.07A	152.67B	145.19	<0.0001*
Mean	125.56	141.51	157.42	162.54	157.01		
Organic Matter (OM)[1]							
Doce	871.20B	840.80C	849.40A	838.83A	843.23A	848.69	<0.0001*
Baiana	866.80B	864.63B	845.93A	834.60A	838.40B	850.07	<0.0001*
OEM[2]	885.37A	870.07A	832.40B	838.97A	847.37A	854.83	<0.0001*
Mean	874.45	858.50	842.58	837.47	843.00		
Total Carbohydrates (TCHO)[1]							
Doce	822.13A	792.20B	801.67A	794.70A	793.77A	800.89	<0.0001*
Baiana	808.47B	810.63A	788.73B	785.23B	788.80A	796.37	<0.0001*
OEM[2]	820.87A	803.90A	765.73C	769.00C	775.30B	786.96	<0.0001*
Mean	817.16	802.24	785.38	782.98	785.96		
Non-Fiber Carbohydrates (NFC)[1]							
Doce	597.47	561.93	547.63	516.00	530.60	550.73A	-
Baiana	554.37	562.70	517.33	508.33	524.17	533.38B	-
OEM[2]	562.93	549.57	504.93	495.57	505.57	523.71C	-
Mean	568.47	551.37	535.94	520.50	505.06		<0.0001*

[1]g kg^{-1} on DM basis; [2]OEM: Orelha de Elefante Mexicana; P-value: linear regression for the storage periods, *significant at 5%; ^{ns}non-significant at 5%; Means followed by different uppercase letters in the columns are statistically different by the Scott-Knott test (P<0.05).

[1]g kg^{-1} en base DM; 2 OEM: Oreja de elefante mexicano; Valor P: regresión lineal para los períodos de almacenamiento, * significativo al 5%; ^{ns}non-significativo hasta el 5%; Las medias seguidas de letras mayúsculas diferentes en las columnas, difieren estadísticamente según la prueba de Scott-Knott (P <0,05).

Regarding the DM content, only the genotype Baiana had significant effect (P=0.0106) of the storage periods, presenting the highest content at 15 days (114.13 g kg^{-1}), and decreasing over time (table 1). Regarding the CP content, a linear increase (P=0.0006) was observed for the genotype OEM, and linear decrease for the genotypes Doce and Baiana (table 1).The genotype OEM also presented the highest CP contents (54.2 ± 0.07 g kg^{-1}) in all storage periods and a linear increase in the EE content (P=0.0061).

There was a linear increase in MM (P<0.0001) and decrese in OM, TCHO and NFC variables in the different storage periods (table 1). The genotype OEM presented the lowest levels of MM except for 30 days of storage (167.60 ± 0.17 g kg^{-1}). The increase in the MM content in this period can be explained by the wearing of OM caused by higher temperatures up to 29 days during the entire studied period.

Regarding the chemical composition of the cell wall, there was a significant difference between the cactus pear genotypes for NDF, ADF and lignin (table 2). The different storage periods caused these variabes to present a linear increase. The genotype Doce presented the lowest levels of NDF and ADF (250.15 ± 0.64 g kg^{-1} and 203.40 ± 0.61 g kg^{-1}, respectively). The genotype OEM presented the highest lignin contents when compared to the others (48.37 ± 0.35 g kg^{-1}). There was no significant difference between the cactus pear genotypes for hemicellulose and cellulose (table 2). Nevertheless, hemicellulose presented a linear increase (P=0.0016) in the storage periods.

Table 2. Chemical composition of the cell wall of cactus pear submitted to different storage periods.

Tabla 2. Composición química de la pared celular de la pera del cactus forrajero sometida a diferentes períodos de almacenamiento.

Genotype	Storage periods (days)					Mean	P-value
	0	15	30	45	60		
Neutral Detergent Fiber (NDF)[1]							
Doce	224.63	230.30	254.03	278.70	263.10	250.15B	-
Baiana	254.07	247.97	271.40	276.93	264.63	263.00A	-
OEM[2]	257.97	254.30	260.80	273.43	269.73	263.25A	-
Mean	245.56	244.19	262.08	276.36	265.82		<0.0001*
Acid Detergent Fiber (ADF)[1]							
Doce	189.67	197.53	211.53	213.23	205.03	203.40B	-
Baiana	197.47	209.20	216.03	211.93	208.67	208.66B	-
OEM[2]	213.07	221.53	210.57	222.87	221.97	218.00A	-
Mean	200.07	209.42	212.71	216.01	211.89		0.0108*
Lignin[1]							
Doce	42.07	36.33	35.77	48.03	41.73	40.79B	-
Baiana	35.83	37.93	37.10	41.80	33.50	37.23B	-
OEM[2]	40.90	47.07	47.97	53.53	52.37	48.37A	-
Mean	39.60	40.44	40.28	47.79	42.53		0.0496*
Hemicellulose[1]							
Doce	34.9	32.7	42.5	65.4	58.0	46.7A	-
Baiana	56.5	38.7	55.3	65.0	55.9	54.3A	-
OEM[2]	44.8	32.7	50.2	50.6	47.7	45.2A	-
Mean	40.2	44.5	48.7	53.0	57.2		0.0016*
Cellulose[1]							
Doce	147.6	161.2	175.7	165.1	163.3	162.6A	-
Baiana	161.6	171.3	178.9	170.1	175.1	171.4A	-
OEM[2]	172.1	174.4	162.5	169.3	169.6	169.6A	-
Mean	160.4	169.0	172.4	168.2	169.3		-

[1]g kg^{-1} on DM basis; [2]OEM: Orelha de Elefante Mexicana; P-value: linear regression for the storage periods, *significant at 5%; [ns]non-significant at 5%; Means followed by different uppercase letters in the columns are statistically different by the Scott-Knott test (P<0.05).

[1]g kg^{-1} en base DM; [2]OEM: Oreja de elefante mexicano; Valor P: regresión lineal para los períodos de almacenamiento, * significativo al 5%; [ns]non-significativo hasta el 5%; Las medias seguidas de letras mayúsculas diferentes en las columnas, difieren estadísticamente según la prueba de Scott-Knott (P <0,05).

Mineral composition

As for the composition of macro and micronutrients of the cactus pear genotypes in the different storage periods, there was effect (P<0.05) of the the storage periods on the studied variables, exception for potassium (K). Regarding the isolated effects of the cactus pear genotypes, potassium (K), calcium (Ca), manganese (Mn) and sodium (Na) did not have significant effect (table 3, page 267).

The Mg content statistically differed among genotypes, where OEM presented the highest content in comparison to the genotypes Doce and Baiana (4.83 ± 0.32 g kg^{-1}) (table 3, page 267). In addition, it presented linear reduction (P=0.0005) in the storage periods. There was a significant difference between the genotypes for Cu and Zn. The Cu contents presented linear reduction and Zn presented linear increase, in the sorage periods. However, the genotype Doce had no effect (P=0.9531) on Zn in the post-harvest storage periods (table 3, page 267).

Table 3. Composition of macronutrients and micronutrients of cactus pear genotypes submitted to different storage periods.

Tabla 3. Composición de macronutrientes y micronutrientes de las variedades de pera de cactus forraje sometidas a diferentes períodos de almacenamiento.

Genotype	Storage periods (days)					Mean	P-value
	0	15	30	45	60		
			Calcium (Ca) (g kg^{-1})[1]				
Doce	26.55	34.51	31.46	26.45	27.63	29.32A	-
Baiana	29.14	32.70	29.73	28.14	26.64	29.27A	-
OEM[2]	29.18	31.81	26.22	26.52	18.45	26.43A	-
Mean	28.29	33.00	29.14	27.04	24.24		0.0016*
			Phosphorus (P) (g kg^{-1})[1]				
Doce	0.55	0.82	0.77	0.90	0.86	0.78B	-
Baiana	0.92	0.89	1.14	0.93	0.85	0.94A	-
OEM[2]	0.87	0.92	1.07	0.96	1.19	1.00A	-
Mean	0.78	0.87	0.99	0.93	0.97		0.0130*
			Magnesium (Mg) (g kg^{-1})[1]				
Doce	2.97	3.92	3.26	3.47	2.84	3.29B	-
Baiana	4.28	3.84	3.35	3.03	3.31	3.56B	-
OEM[2]	5.42	5.12	4.98	4.80	3.83	4.83A	-
Mean	4.35	4.12	3.89	3.66	3.43		0.0005*
			Sodium (Na) (g kg^{-1})[1]				
Doce	0.04	0.04	0.06	0.07	0.03	0.05A	-
Baiana	0.03	0.04	0.04	0.05	0.02	0.04A	-
OEM[2]	0.03	0.05	0.05	0.05	0.03	0.04A	-
Mean	0.03	0.04	0.05	0.06	0.03		0.9945^{ns}
			Potassium (K) (g kg^{-1})[1]				
Doce	20.17	28.36	24.55	28.94	25.46	25.50	-
Baiana	28.02	23.30	28.28	26.02	29.33	26.99	-
OEM[2]	24.67	27.52	28.87	22.45	29.17	26.53	-
Mean	24.29	26.39	27.23	25.81	27.99		-
			Copper (Cu) (mg kg^{-1})[1]				
Doce	9.98A	5.39B	5.07A	7.07A	2.21A	5.94	0.0090*
Baiana	6.11A	14.70A	9.56A	5.18A	1.92A	7.49	0.0011*
OEM[2]	10.11A	14.05A	7.18A	7.18A	6.54A	8.98	0.0077*
Mean	8.73	11.38	7.27	6.42	3.56		-
			Iron (Fe) (mg kg^{-1})[1]				
Doce	16.78	14.30	24.99	32.63	22.97	22.33B	-
Baiana	14.28	15.62	21.26	24.74	15.49	18.29B	-
OEM[2]	35.75	48.03	40.19	49.94	34.38	41.66A	-
Mean	22.27	25.98	28.82	35.77	24.28		0.1208^{ns}
			Zinc (Zn) (mg kg^{-1})[1]				
Doce	14.75A	8.84A	4.71B	15.68A	11.48B	11.09	0.9531^{ns}
Baiana	8.10B	6.03A	5.11B	11.45A	12.64B	8.68	0.0077*
OEM[2]	11.56A	6.67A	14.08A	13.84A	19.01A	13.03	0.0002*
Mean	11.47	7.18	7.97	13.66	14.38		
			Manganese (Mn) (mg kg^{-1})[1]				
Doce	73.75	60.17	78.58	132.58	157.00	100.42A	-
Baiana	71.50	66.00	80.50	135.75	117.50	94.25A	-
OEM[2]	37.67	72.75	118.17	129.2	145.41	100.68A	-
Mean	60.97	66.31	92.42	132.58	139.97		<0.0001*

[1]g kg^{-1} on DM basis; [2]OEM: Orelha de Elefante Mexicana; P-value: linear regression for the storage periods, *significant at 5%; ^{ns}non-significant at 5%; Means followed by different uppercase letters in the columns are statistically different by the Scott-Knott test (P<0.05).

[1]g kg^{-1} en base DM; [2]OEM: Oreja de elefante mexicano; Valor P: regresión lineal para los períodos de almacenamiento, * significativo al 5%; ^{ns}non-significativo hasta el 5%; Las medias seguidas de letras mayúsculas diferentes en las columnas, difieren estadísticamente según la prueba de Scott-Knott (P <0,05).

For Fe, there was no effect (P=0.1208) of the storage periods. The cactus pear genotypes presented significant differences, with OEM showing the highest content (41.66 ± 4.73 mg kg^{-1}) in all storage periods. Initially, the Mn content did not present significant difference between the genotypes, but the mean values along the storage periods showed linear increase (P<0.05), with concentration effect of this nutrient caused by the water loss.

Discussion

The loss of water (figure 1, page 264) in the genotype Baiana is related to the respiratory activities through plant evapotranspiration. Plants with crassulacean acid metabolism (CAM) avoid water loss through evaporation by keeping the stomata closed during the day (12), thus reducing gas exchange. However, after harvesting, if opened, the stomata can no longer close. The stomatal opening throughout the entire storage period was confirmed by the emergence of new sprouts of the cactus pear genotypes, mainly in the *Nopalea* genus ones. The opening of the stomata may increase immediately after harvest (9). With stomata open, the vapor pressure deficit between the forage and the air is high, but thanks to their structural and functional characteristics, cacti are able to consume and lose the minimum water amount they store (18), even with the stomata opened. The increase in ambient temperature promotes an exponential increase in respiration, since the higher the temperature, the greater the metabolic activity of the tissues (15).

Research testing the effect of the post-harvest storage period on DM content and chemical composition of the cactus pear, did not find significant difference between the genotypes Gigante and Redonda up to 16 days of storage (19), but for the genotype Doce, the period of 12 days presented higher contents than day 0.

The water loss resulted in a concentration of CP in OEM (table 1, page 265) due to the respiratory activity of the plant, which results in a decrease in the soluble carbohydrate contents, while CP concentrations are not affected by respiration, increasing in proportional terms. That cactus pear from the *Opuntia* genus presented higher CP content than *Nopalea*, ranging from 54.2 to 52.1 g kg^{-1} for Gigante and Redonda, respectively, and 43.1 g kg^{-1} for Doce (4). Differently from the present study, researchers did not find statistical difference for EE up to 21 days of storage post-harvest in OEM (23). Among the genotypes, Baiana presented the highest levels of EE up to 45 days. As lipids are the most susceptible constituents to chemical degradation and directly influence the drying and preservability of the stored product, this similarly justifies the highest water and DM losses in this genotype (16).

The decrease in carbohydrates is attributed to the shoots that appeared during the post-harvest storage period, due to the dilution effect, caused by the increase in new cladodes, which are poor in structural carbohydrates (22). As in hay making, unfavorable environmental conditions lead to the loss of soluble carbohydrates through continuous cellular respiration, and when the cladodes are stored with moisture content higher than the recommended levels (between 150 and 180 g kg^{-1} of DM), they easily heat up and result in increased levels of NDF, ADF and lignin (5). The increase in MM content in this period can be explained by the wearing of OM caused by higher temperatures up to 30 days during the entire study period.

When considering the plant development stage, there is an increase in the contents of structural compounds, such as hemicellulose, cellulose and lignin and consequent decrease in cellular content (table 2, page 266). Researchers reported higher lignin contents in the genotypes Gigante and Redonda in comparison to Doce, and did not find significant differences for hemicellulose and cellulose contents, in Gigante, Redonda and Miúda in different crop densities (4). Put Silva *et al.* (2017) found a significant difference of crude fiber in genotype Doce between the periods 0 and 12 days of storage, with contents of 123.4 and 96.5 g kg^{-1}, but not differing from the other periods (4, 8 and 16 days).

The water loss over time may have led to the concentration of P contents, (table 3, page 267) since the genotypes Baiana and OEM lost more water and presented the highest concentrations of P (0.94 and 1.00 g kg^{-1}, respectively). The reduction in the Ca content over time may be related to the emergence of shoots in the post-harvest storage. In addition, Ca influences the structure and strength of the cell wall. Reserchers found levels of Ca of 17.0 and 28.2 g kg^{-1} and P of 0.7 and 0.8 g kg^{-1} in cactus pear Gigante at 390 and 620 days after planting, respectively, in the treatment without fertilization, which are values very similar to those found in the present study (21).

Sprouts over the storage period may have influenced the decrease in Mg (table 3, page 267) since it is rapidly transferred to young plants. In contrast, there was no difference for K and Na contents in the studied genotypes, nor for the post-harvest storage periods. Studies evaluating the mineral composition in the harvest of cactus pear cladodes under different spacing and chemical fertilization, also did not find significant difference for K and Na (21). Santos *et al.* (1992) noted that differences in the Fe content can be explained by the interactions of Fe with other nutrients, notably phosphorus, zinc, molybdenum and copper, which are common. There is little information available in the literature on macro and micronutrient contents in cactus pear and its effects on production, especially when it comes to storage for long post-harvest periods. In general, the reduction in some minerals during post-harvest storage may be due to the action of microorganisms, which degrade part of the constituents over time.

Conclusions

The cactus pear genotypes Doce and Orelha de Elefante Mexicana (OEM) can be stored for a period up to 60 days after harvest, with minimum losses of water content and maintainance of a stable chemical composition, mainly regaring to the variables dry matter, crude protein, ether extract, sodium and potassium.

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