Selection of soybean F_3 populations for agronomic and physiological traits and vegetation indices using multivariate approaches

Selección de poblaciones de soja F_3 para los índices agronómicos, fisiológicos y de vegetación utilizando enfoques multivariados

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Abstract

Soybean is the most economically important oilseed in the world, being one of the main sources of oil and protein in human and animal food. The objective of this work was to select F_3 segregating populations for agronomic and physiological traits and vegetation indices (VIs). The experiment was carried out in the 2018/2019 crop seasons in a randomized block design with three replicates and 10 F_3 populations (P1, P2, P3, P4, P5, P6, P7, P8, P9, and P10). The evaluated traits were as fallows: number of days to maturity (NDM); number of pods per plant (NPP); number of nodes per plant (NNP); number of lateral stems per plant (NSP); grain yield (GY); photosynthesis (A); stomatal conductance (gs); internal CO_2 concentration (Ci); transpiration (E); NIR, Red-edge, Red and Green wavelengths; and NDVI and NDRE vegetation indices. Regarding the physiological traits evaluated in soybean F_3 populations, the P8 and P6 populations presented the highest averages for photosynthesis, while the P3, P10, and P9 populations presented the highest Ci, E, and gs. For VIs evaluated in soybean F_3 populations, the populations P7, P2, and P1 presented higher averages for NIR and Red-edge wavelengths. P5 and P4 showed a higher NDRE, and P8 showed a higher red wavelength. For agronomic traits, population P2 showed better results for the traits NNP and NSP. Populations P1 and P3 had a higher NDM. The P7 population presented the highest NPP, and the P6 population presented the highest GY.

Keywords
Glycine max • Segregating populations • NDVI • NDRE • photosynthetic efficiency

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Resumen

La soja es la oleaginosa más importante económicamente en el mundo, siendo una de las principales fuentes de aceite y proteína en la alimentación humana y animal. El objetivo de este trabajo fue seleccionar las poblaciones segregantes de soja F3 en función de las características agronómicas y fisiológicas y los índices de vegetación. El experimento se llevó a cabo en la zafra de 2018/2019 con un diseño de bloques al azar con tres repeticiones y diez poblaciones F3 (P1, P2, P3, P4, P5, P6, P7, P8, P9 y P10). Los caracteres evaluados fueron: número de días hasta la madurez (NDM); número de vainas por planta (NPP); número de nudos por planta (NNP); número de tallos laterales por planta (NSP); rendimiento de granos (GY); fotosíntesis (A); conductividad estomática (gs); concentración interna de CO_2 (Ci); transpiración (E); bandas NIR, Red-edge, Rojo y Verde; y los índices de vegetación NDVI y NDRE. Para los caracteres fisiológicos evaluados, las poblaciones P8 y P6 tuvieron las medias más altas de A, y las poblaciones P3, P10 y P9 tuvieron los valores más altos de Ci, E y gs. Para los índices de vegetación evaluados, las poblaciones P7, P2 y P1 presentaron medias más altas para el NIR y Red-edge, conjuntamente con las poblaciones P5 y P4 que presentaron NDRE más altos y la población P8 presentó longitudes de onda Rojas más altas. Para los caracteres agronómicos, la población P2 mostró mejores resultados para NNP y NSP. Las poblaciones P1 y P3 obtuvieron un mayor NDM. La población P7 mostró mayor NPP, y la población P6 mayor GY.

Palabras clave

Glycine max • Poblaciones segregantes • NDVI • NDRE • eficiencia fotosintética

Introduction

Soybean [*Glycine max* (L.) Merr.] is the most economically important oilseed in the world, as it is a valuable source of oil and protein in human and animal food. According to USDA data (32), world production in the 2018/2019 harvest was 362.075 million t in a cultivated area of 125.691 million hectares.

Brazil is currently the second-largest soybean producer in the world, and it is expected that it will increase its production by 2.6% per year compared to Argentina (2.1%) and the USA (1.0%) (23). The Cerrado is the biome where the majority of Brazil's soybean production is concentrated (27), with a cultivated area of 183,890.92 km^2, covering eight states: Minas Gerais, Goiás, Tocantins, Bahia, Maranhão, Mato Grosso, Mato Grosso do Sul, Piauí, and the Federal District (28).

The constant increase in soybean production is due to the growing investment in research and technology added to the sharing of technical information by public and private companies, improving the efficiency of crop management (13). In addition to yield, soybean genetic breeding programs have focused on increased photosynthetic efficiency (2, 12, 16, 31). Several studies have shown a positive linear association between photosynthetic parameters and crop yield (2). However, there is still scarce research in the literature on breeding for physiological traits, especially on the association of physiological and agronomic traits (31). Studies addressing the selection of superior genotypes for physiological traits related to higher photosynthetic and water-use efficiencies, for example, are crucial for the choice of parents and selection strategies in breeding programs for abiotic stress. Additionally, knowledge about the association of physiological and agronomic traits contributes to greater efficiency of the programs by identifying auxiliary traits that can be used in indirect selection.

A set of traits that can be used as an auxiliary in the selection of superior genotypes for agronomic and physiological traits are the vegetation indices (VIs), which are mathematical ratios between different reflected wavelengths (3). The VIs have been applied in monitoring crop status and predicting yields (29). A vegetation index used for crop monitoring is the Normalized Difference Vegetation Index (NDVI), which allows the determination of the density of photosynthetically active foliar biomass per area unit (7). It stands out for relating the biophysical parameters of vegetation cover (18). The increase in NDVI is proportional to the degree of greenness of the crop, which allows determining if the crop is denser or in full growth vigor (4). Another vegetation index used is the Normalized Difference Red Edge (NDRE). This index can provide a better analysis of crops at more advanced stages, with the possibility of more accurately measuring the canopy, unlike the NDVI (30).

This study hypothesized that soybean-segregating populations have differences in agronomic and physiological traits and vegetation indices. Thus, the objectives of this study were: i) to evaluate the association between different trait groups, ii) to select promising F_3 soybean populations for each trait group, and iii) to recommend promising crosses for obtaining superior offspring for the traits evaluated.

Material and Methods

The experiment was carried out at the experimental field of the Federal University of Mato Grosso do Sul, Campus of Chapadão do Sul (18°46'26" S, 52°37'28" W and the average altitude of 810 m), from October 2018 to February 2019. The region's climate, according to the Köppen classification, is Aw, with rainy summers and dry winters. The soil of the experimental area is dystrophic Red Latossolo, whose attributes obtained by chemical analysis are: pH ($CaCl_2$) = 4.8; organic matter = 17.6 g dm^{-3}; P = 5.0 mg dm^{-3}; H + Al = 5.3; K = 69.0 mg dm^{-3}; Ca= 1.6 cmol dm^{-3}; Mg = 0.5 cmol dm^{-3}; cationic exchange capacity = 7.6 cmol dm^{-3}; base saturation = 30%. The proportions of clay, sand and silt were 46, 46, and 8%, respectively.

Conventional tillage was used for soil preparation to implement the experiment. The opening and fertilization of the rows were performed mechanically with a John Deere® seeder of five rows at 0.45 m spacing between rows. The furrow fertilization used was 300 kg ha^{-1} of the formulation 04-14-08. Seeding was performed manually with the distribution of 15 seeds m^{-1}. The seeds were treated with Piraclotrobina + Metil Tiofanato fungicides and Fipronil insecticide in a the proportion of 200 mL of the commercial product for every 100 kg of seeds to ensure protection against the attack of pests and soil fungi. For biological nitrogen fixation (BNF), the seeds were inoculated with bacteria of the genus *Bradyrhizobium* spp. using a proportion of 200 mL of concentrated liquid inoculant for every 100 kg of seeds.

The experimental design used was randomized blocks with three replicates and 10 F_3 populations (P1, P2, P3, P4, P5, P6, P7, P8, P9, and P10). The experimental unit consisted of three three-meter lines for each treatment, spaced 0.45 m apart. Segregating populations were obtained by the bulk method in previous generations. Table 1 contains the genealogy of each population. The control of weeds, pests, and diseases was carried out according to technical recommendations for the crop (24).

Table 1. Genealogy of the 10 F_3 soybean populations and relative maturity group (RMG) of IPRO cultivars used as genitors.

Tabla 1. Genealogía de las 10 poblaciones de soja F_3 y el grupo de madurez relativa (RMG) de los cultivares IPRO utilizados como genitores.

Population	Male Genitor		Female Genitor	
	Cultivar	RMG	Cultivar	RMG
P1	BMX Prisma	7.5	SYN 13671	7.1
P2	BMX Bônus	7.9	SYN 13671	7.1
P3	BMX Flecha	6.6	SYN 13671	7.1
P4	M6410	6.4	SYN 13671	7.1
P5	NS 6909	6.9	BMX Ponta	6.1
P6	BMX Prisma	7.5	DM 6563 RSF	6.3
P7	BMX Bônus	7.9	DM 6563 RSF	6.3
P8	BMX Flecha	6.6	DM 6563 RSF	6.3
P9	M6410	6.4	DM 6563 RSF	6.3
P10	M7739	7.7	DM 6563 RSF	6.3

At 60 days after the emergency (DAE), the physiological traits were analyzed with the portable photosynthesis analyzer (IRGA), ADC 6400 LICOR model. Photosynthesis photon flux of 1000 µmol m^{-2} s^{-1} and ambient CO_2 concentrations were used (372 ± 10 mol s^{-1} m^{-2}). The evaluations were performed at 8:30 a.m., without cloudiness, in five plants of each experimental unit, and the following physiological traits were obtained: net photosynthesis (A, mmol CO_2 m^{-2} s^{-1}), transpiration (E, mmol H_2O s^{-1} m^{-2}), stomatal conductance (gs, mmol m^{-2} s^{-1}), and internal CO_2 concentration (Ci, mmol m^{-2} s^{-1}).

In addition to these variables, the following vegetation indices (VIs) were assessed at 60 DAE: NDVI (Normalized Difference Vegetation Index) and NDRE (Normalized Difference Red Edge Index). The Sensefly eBee RTK fixed-wing remotely piloted aircraft (RPA) was used, with autonomous take-off, flight, and landing control. The eBee is equipped with the Parrot Sequoia multispectral sensor. The Sequoia multispectral sensor acquires the reflectance in the wavelengths of the green (550 nm - Green), red (660 nm - Red), red-edge (735 nm), and near-infrared (790 nm - NIR), having a brightness sensor that allows the calibration of the acquired values. The images were mosaiced and orthorectified by the Pix4Dmapper software. The positional accuracy of the orthoimages was checked with ground control points (GCP), raised with RTK (Real Time Kinematic). The VIs studied as a function of the higher correlation with plant mass, according to Raper and Varco (2015), are contained in table 2.

Table 2. Abbreviations, equations and references of the vegetation indices calculated by the Sequoia multispectral sensor.

Tabla 2. Abreviatura, ecuaciones y referencias de los índices de vegetación calculados por el uso del sensor multiespectral Sequoia.

Abbreviation	Vegetation Index	Equation	References
NDVI	Vegetation Index by Normalized Difference	$\frac{(R_{NIR} - R_{RED})}{(R_{NIR} + R_{RED})}$	Rouse *et al.* (1974)
NDRE	Normalized Difference Red Edge (*Red-edge*)	$\frac{(R_{NIR} - R_{EDGE})}{(R_{NIR} + R_{EDGE})}$	Gitelson and Merzlyak (1994)

R_{NIR}: near-infrared reflectance; R_{RED}: red reflectance; R_{EDGE}: red-edge reflectance.

R_{NIR}: reflectancia en el infrarrojo próximo; R_{RED}: reflectancia en el rojo; R_{EDGE}: reflectancia en el red-edge.

The agronomic traits evaluated were: number of days to maturity (NDM), number of pods per plant (NPP), number of nodes per plant (NNP), number of lateral stems per plant (NSP), and grain yield (GY). NDM corresponded to the number of days between germination and maturity in more than 50% of plants in the plot. For evaluation of the other traits, five plants were randomly selected in each experimental unit due to the low availability of seeds. After counting the NPP, NNP, and NSP traits, GY was obtained by individual harvesting and tracking of these plants, with the mass expressed in g $plant^{-1}$ after correcting the grain moisture for 13%. Figure 1 (page 26) shows the climatic conditions in the experiment.

Initially, to verify the presence of genetic variability among soybean populations, a multivariate analysis of variance (MANOVA) was performed. Afterward, principal component analysis was performed to verify the interrelationship between the populations and each group of traits evaluated (physiological, agronomic, and vegetation indices). Finally, to verify the relationship between each group of traits evaluated, factor analysis was performed. All analyses were performed with the free platform R.

Results and Discussion

Table 3 (page 26) shows the results of the multivariate analysis of variance (MANOVA). The approximation of the Fischer F test indicates that there is a difference between the F_3 soybean populations considering all the traits evaluated simultaneously. These results reveal genetic variability between populations, an essential factor for selecting the best genotypes for each group of traits.

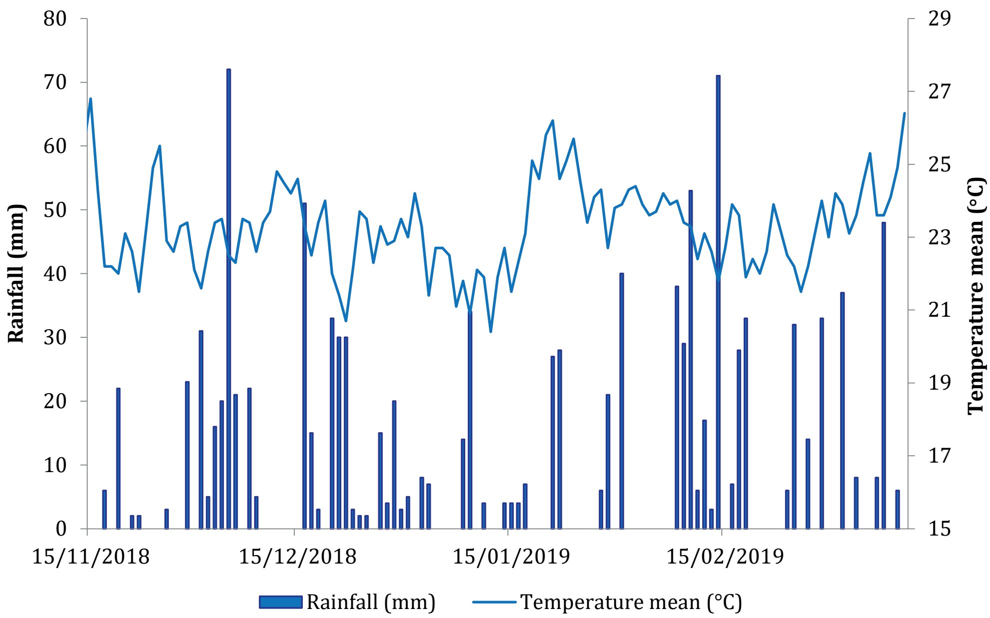

Figure 1. Climatic conditions in the experiment

Figura 1. Condiciones climáticas durante el experimento.

Table 3. Multivariate analysis of variance (MANOVA) for soybean F_3 populations simultaneously considering all traits evaluated.

Tabla 3. Análisis de varianza multivariante (MANOVA) para poblaciones F_3 de soja considerando simultáneamente todos los rasgos evaluados.

Source of variation	Degrees of freedom	Pillai test	Approximation F-test
Intercept	1	0.99	1665.95*
Blocks	3	1.11	1.64*
Populations F_3	9	2.85	1.66*
Residuals	27		

*: significant by F-test at 5% of probability.

*: significativo por prueba F al 5% de probabilidad.

The PCA applied to the physiological traits gathered 99.50% of the variance in the first two principal components (figure 2, page 27). Mingoti (2005) recommends that for the use of this analysis, the first two principal components gather at least 80% of the variation. Group 1 was formed by the populations P4, P2, P6, P8, P5, P1, and P7, and from these, P8 and P6 stood out for having higher net photosynthesis. The photosynthetically active radiation incident is related to the efficiency at which a plant intercepts radiation and converts it into biomass and the efficiency at which biomass is converted into seeds, affecting the crop yield (34). Thus, the selection of soybean populations with higher photosynthesis is one way to increase grain yield.

Group 2 gathered the populations P3, P10 and P9, which stood out for having the highest Ci, E and gs. Stomatal conductance (gs) is a measure of the ratio between the passage of carbon dioxide (CO_2) entering and water vapor leaving the leaf stomata (21). The importance of gs on initial responses to water stress has been reported in the literature (5, 8, 14). The change in gs is the first response to a water deficit, hence limiting photosynthesis (8). This phenomenon was also demonstrated by Oliveira *et al.* (2005), who found that despite the interaction of several factors acting on the stomata and consequently on gs, the effect of drought stress on this parameter is evident, which means that the stomatal conductance can be used as an indicator of water deficiency.

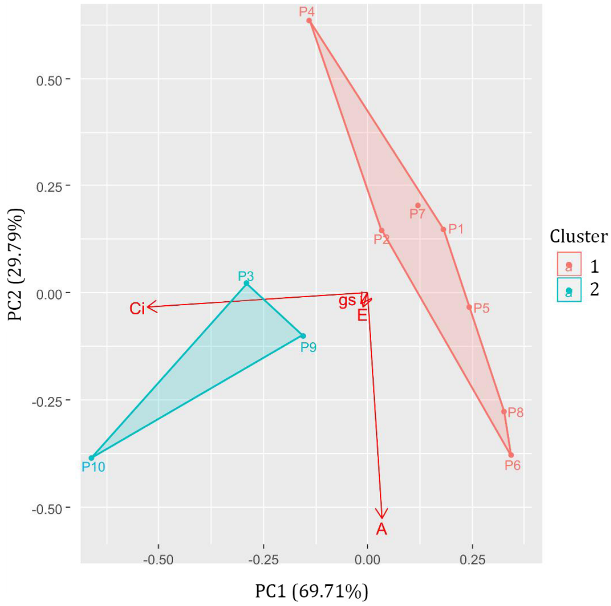

Figure 2. Principal component analysis (PCA) applied to physiological traits (net photosynthesis - A, transpiration - E, stomatal conductance - gs and internal CO_2 concentration - Ci) evaluated in F_3 soybean populations.

Figura 2. Análisis de componentes principales (ACP) aplicado a los caracteres fisiológicos (fotosíntesis - A, transpiración – E, conductividad estomatal – gs y concentración interna de CO_2 - Ci) evaluados en las poblaciones de soja F_3.

The principal component analysis applied to the wavelength and vegetation indices gathered 95.47% of the variance in the first two principal components (figure 3). The populations P7, P2, and P1, are in group 1 and presented a higher mean for NIR and Red-edge. Group 2 was composed of the populations P10, P5, P8, P6, P9, P4, and P3, of which P5 and P4 had the highest NDRE and P8 presented the highest red wavelength.

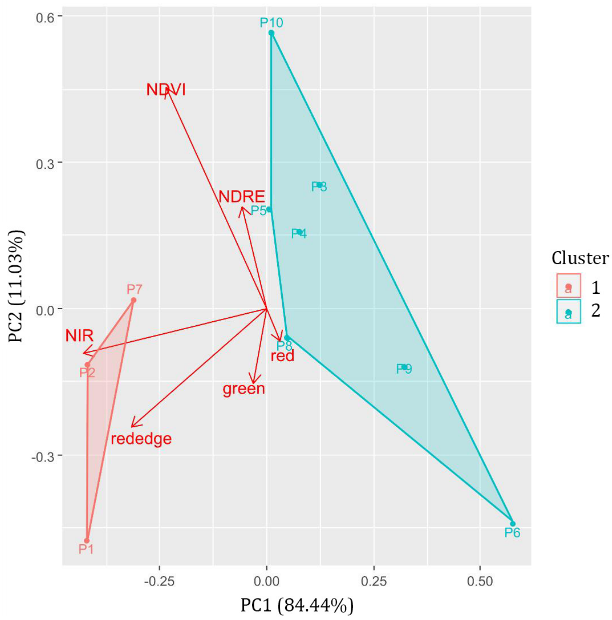

Figure 3. Principal component analysis applied to vegetation indices (green, red, rededge, NIR, NDVI and NDRE) evaluated in F_3 soybean populations.

Figura 3. Análisis de componentes principales aplicado a los índices de vegetación (green, red, rededge, NIR, NDVI and NDRE) evaluados en las poblaciones de soja F_3.

Most vegetation indices relate reflectance in the visible and near-infrared wavelength ranges to minimize variability caused by external elements (18). The higher the density of vegetation cover in an area (19) and the higher the presence of water in the plant's cellular structures, the higher will be the reflectance in the near-infrared wavelength (25). Thus, higher NIR values indicate more vigorous plants in terms of water status.

Healthy and dense vegetation are related to the high values of NDVI and NDRE, which are reflected in low values of red reflectance (Red and Red-edge) and high values of NIR reflectance (33). The Red-edge band provides information about the chlorophyll content in the plant (6) so that when there is more chlorophyll, the Red-edge wavelength becomes longer (9). Furthermore, the Red and Red-edge wavelengths are used for the photosynthetic process, and there is a greater absorption of these wavelengths by the plants (25)

The principal component analysis applied to the agronomic traits gathered 98.91% of the variance in the first two principal components (figure 4). Populations P1, P7, P5, P6, P2, and P3 are included in group 1, where P2 showed better results for NNP and NSP. The higher the number of nodes in a plant, the greater the number of stems, and hence the higher the number of leaves that will intercept solar radiation, which in turn will be converted into energy used for the formation of seeds, reflecting on grain yield of a crop. P1 and P3 populations obtained a higher NDM, which is not desired in the region due to the interest in carrying out a second season, aiming at higher profitability. The P7 presented higher NPP, and P6 had higher grain yield (GY), which is desired by farmers. Populations P4, P10, P9 and P8 made up group 2 and did not present high means for the agronomic traits evaluated.

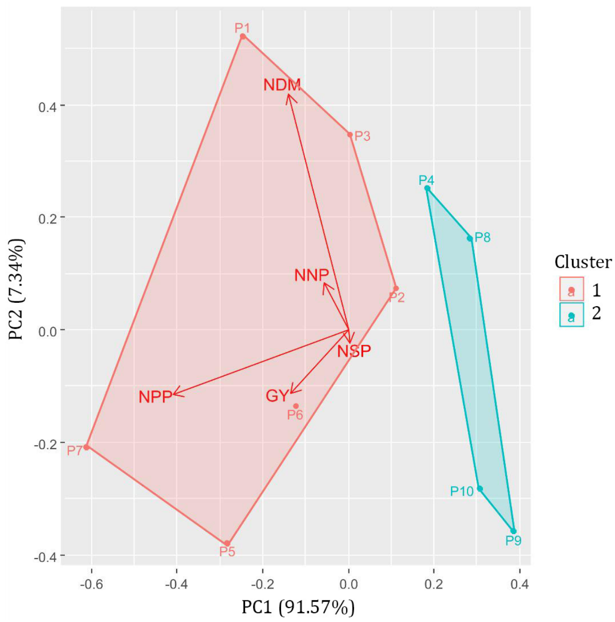

Figure 4. Principal component analysis applied to agronomic traits (number of days to maturity - NDM, number of pods per plant - NPP, number of nodes per plant - NNP, number of lateral stems per plant - NSP and grain yield - GY) evaluated in F_3 soybean populations.

Figura 4. Análisis de los componentes principales aplicado a los caracteres agronómicos (número de días hasta la madurez – NDM, número de vainas por planta – NPP, número de nudos por planta – NNP, número de tallos laterales por planta – NSP and rendimiento de granos - GY) evaluados en las poblaciones de soja F_3.

The results previously reported suggest that new crossings can be performed between plants from the P6 population (higher GY) with plants from P8, P9, and P10 (higher photosynthetic efficiency). High yield is the main target for soybean breeding programs, and studies have shown higher crop yield as photosynthetic efficiency parameters increase (11). Thus, obtaining genotypes that gather favorable traits for production and photosynthetic efficiency is of great importance for soybean improvement.

Figure 5 demonstrates the factor analysis applied to the agronomic and physiological traits and vegetation indices evaluated in F_3 soybean populations. This analysis highlighted the positive correlation between NDVI and NDRE with NIR and red-edge bands. These results were expected since the NIR band is included in the calculation of these indices.

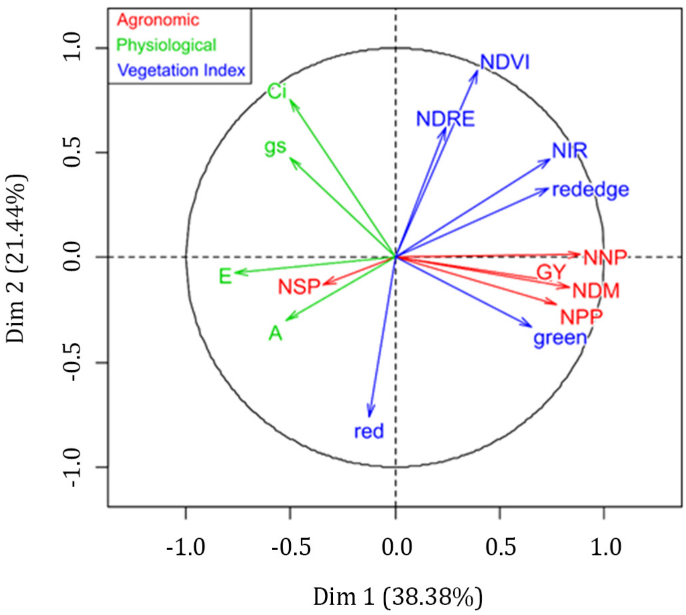

Figure 5. Factor analysis applied to the agronomic and physiological traits and vegetation indices evaluated in F_3 soybean populations.

Figura 5. Análisis factorial aplicado a los caracteres agronómicos y fisiológicos y a los índices de vegetación evaluados en las poblaciones de soja F_3.

Ci correlated positively with gs. The CO_2 concentration is a regulatory factor for the stomatal conductance (1). The higher the Ci, the greater the opening of the stomas for the exit of water vapor, consequently increasing the gs. Transpiration (E) and photosynthesis (A) correlated positively with the NSP and the red band. These results indicate that when soybean populations have more lateral stems and hence a more massive canopy, their photosynthetic capacity is increased, which this is related to the red band. Gates *et al.* (1965) reported that when there is a higher chlorophyll content in the plant; the red wavelength becomes longer. Therefore, for future studies, we recommend using vegetation indices containing this band to select soybean populations with higher photosynthetic efficiency, since these indices present a variability measurement better than the NDVI (30).

The other agronomic traits evaluated (NNP, GY, NDM, and NPP) correlated with the green band. These results indicate that the longer the wavelength reflected in this band, the higher the cycle and grain yield of soybean populations. The green band is more sensitive to detecting different nutritional levels than the red band, providing better identification of chlorophyll content in the plant (26). However, the longer the crop cycle, the higher the grain yield.

Conclusions

For the physiological traits evaluated in F_3 soybean populations, the P8 and P6 populations presented higher means for photosynthesis, while the P3, P10 and P9 populations presented the highest values of internal CO_2 concentration, transpiration, and stomatal conductance.

For the vegetation indices evaluated in F_3 soybean populations, P7, P2, and P1 presented higher means for near-infrared and Red-edge wavelengths, while P5 and P4 populations presented higher NDRE vegetation indices and P8 population presented higher mean red wavelength.

For the agronomic traits, the population P2 showed better results for the traits number of nodes per plant and the number of lateral stems per plant. Populations P1 and P3 obtained a higher number of days for maturity. Population P7 showed a higher number of pods per plant, and the population P6 higher grain yield. The results reported here suggest that new crossings can be performed between plants from P6 (higher grain yield) with P8. P9 and P10 (higher photosynthetic efficiency).

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