

## **Agronomic performance of maize (*Zea mays* L.) populations segregating the polyembryony mutant**

### **Comportamiento agronómico de poblaciones de maíz (*Zea mays* L.) que segregan al mutante poliembrionía**

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#### **ABSTRACT**

The maize polyembryony (PEm) is phenotypically expressed when the seed germinates in two or more seedlings simultaneously, which in turn develops the capacity to overcome the close competition among sisters and neighboring plants. Because of that, it is thought that the inclusion of PEm in some new maize varieties can be useful looking for high yields and corn grain quality as a response to the global food demand. This research is about the PEm inheritance, the inclusion, recovery of polyembryony in segregating populations, and exploring their performance capacity. The foundation populations were the progenies from crosses among polyembryonic sources and inbred lines, producing several F<sub>1</sub> groups, and from each the proper F<sub>2</sub>, and G3 and G4 generations. The latter two were developed through successive positive assortative matings (AM+). G3 populations were used to generate diallel crossings, Griffing's method 4, and part of them were evaluated in a performance assay, using a complete block design with a split-split plot arrangement. Results supported a validation of the inheritance model proposed for this sort of polyembryony, which states that the trait is controlled by two independent loci, under epistatic interaction of the type "duplicate gene action". Moreover, the arbitrarily handling of sexual reproduction in F<sub>2</sub> plants and in G3 and G4 generations through positive assortative matings (AM+) increased the PEm frequency on an average up to 40 % in G4, departing from the 4.9 % in F<sub>2</sub>. Also, the performance assay shown a yield potential of the trait. The PEm mutant might be useful in maize production.

#### **Keywords**

*Zea mays* • polyembryony • inheritance model, performance assay • yield potential • plant density • fertilization doses

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## RESUMEN

La poliembrionía de maíz (PEm) se expresa fenotípicamente cuando la semilla germina en dos o más plántulas simultáneamente, lo que a su vez desarrolla la capacidad de superar la competencia cercana entre hermanas y plantas vecinas. Por eso, se cree que la inclusión de PEm en algunas variedades de maíz nuevas puede ser útil en busca de altos rendimientos y calidad de grano de maíz como respuesta a la demanda global de alimentos. Esta investigación trata sobre la herencia de PEm, la inclusión, la recuperación de la poliembrionía en poblaciones segregantes y la exploración de su capacidad de rendimiento. Las poblaciones de base fueron las progenies de cruzamientos entre fuentes poliembriónicas y líneas puras, produciendo varios grupos F1, F2, G3 y G4 apropiados. Los dos últimos se desarrollaron a través de apareamiento preferencial positivo (AM +). Las poblaciones G3 se utilizaron para generar cruza dialélicas, método 4 de Griffing, y parte de ellas fueron evaluadas en un ensayo de rendimiento, utilizando un diseño de bloques completos al azar con arreglo en parcelas subdivididas. Los resultados respaldaron una validación del modelo de herencia propuesto para este tipo de poliembrionía, en el cual se establece que el rasgo está controlado por dos loci independientes, bajo una interacción epistática del tipo "acción genética duplicada". Además, el manejo arbitrario de la reproducción sexual en plantas F2 y en generaciones G3 y G4 mediante apareamiento preferencial positivo (AM +) aumentó la frecuencia de PEm en un promedio de hasta 40% en G4, partiendo del 4,9% en F2. Además, el ensayo de rendimiento mostró potencial en los rasgos relacionados con el rendimiento. El mutante PEm podría ser útil en la producción de maíz.

### Palabras clave

*Zea mays* • poliembrionía • modelo de herencia, ensayo de rendimiento • potencial de rendimiento • densidad de plantas • dosis de fertilización

## INTRODUCTION

Maize polyembryony (PEm, hereinafter) is a heritable trait that can be observed since the embryonic stage, and is phenotypically manifested when the seed (caryopsis) germinated in two and up to five simultaneous plumules, which can be developed in two or more fruitful stems. The phenomenon has been reported by several authors from the twentieth century to date. Along the first half of last century, several reports about twin plants emerged from a single seed were published (25, 28, 29).

In later years, there were published reports on polyembryony corn from different research approaches; one of

those was related to the effect of the X-rays radiation applied on pollen grains of corn resulting in polyembryony appearance. Authors stressed that the highest doses of radiation increased up to 18% the polyembryony frequency (18). Another paper was about the discovery of twin plants in a local population of corn, and pointed out that there is a positive association between this phenomenon and higher contents of lysine and crude fat in grain content, compared to normal corn; the authors also indicated that the highest level of polyembryony frequency in a set of inbred lines derived from this population was 25% (22).

Moreover, the presence of twin plants was reported in a dwarf corn population, highlighting the positive response to selection to increase the twin frequency (up to 34% in 4 selection cycles) and reported that the parent-offspring heritability estimate was 67% (3). On the contrary, there are reports pointing that the two stems per seed trait could be determined by one or a few genes (24).

Studies in México on polyembryony maize have reported the existence of two populations of maize (called BAP for brachytic plants, high polyembryony frequency, and NAP for normal height plants, high polyembryony frequency) which concentrate the phenomenon on an average frequencies of 61 and 63% respectively, which are the highest values among those published so far (6).

There are several and different reports on the polyembryony inheritance, but most of them agree that the trait is genetically controlled by one or two genes. One of those reports is the case of the *ig* recessive gene (indeterminate gametophyte) found in a line of corn, which affects, among other reproductive phenomena, the generation of polyembryony, which appears in frequencies equal to or less than 6% (12). In another report it was stated that polyembryony corn is governed by the action of a single recessive gene (23).

Two of the most recent proposals on maize polyembryony inheritance are: 1) the one that claims that polyembryony is controlled by two loci in epistatic interaction, duplicated gene action type, leading to an  $F_2$  with two phenotypic classes, in proportions 15: 1, normal plants –to- polyembryonic plants. In this type of epistasis, the presence of just one dominant allele of any of the loci is enough to express the normal plant phenotype, therefore, the polyembryony cases are caused by the

action of two loci in homozygous recessive condition. Besides that, PEm is accompanied by the phenomenon of incomplete penetrance, which states the proportion of the polyembryonic genotypes that should be expressed by varying the amount of 10 to 50%, all depending on the source of the exotic germplasm with whom the polyembryonic populations have been crossed (5, 26). The second proposal states that the phenotypic expression of twins stems corn seedlings could be an evidence of an epigenetic mechanism given that the twin trait has shown one of the epimutants characteristics, which is referred to the ability to be reversible, it is to say that they can return to the original mutated phenotype that, in this case, is the normal plant single stem condition (17).

Some researchers have found the association between polyembryony and the corn grain nutritional quality. In one of the early works about this matter, it was said that the trait was associated with the grain content of higher crude protein and higher levels of lysine and crude fat compared to the one found in common corn nutrimental contents (22). Another work on nutrient contents in PEm corn grains, using progenies from populations BAP and NAP, has shown that they contain higher levels of crude fat, lysine, oleic and linoleic fatty acids than those found in samples of common maize grains (8, 30).

### **Hypothesis and Objectives**

In the context of maize polyembryony, and taking into account that populations with the inclusion of the PEm trait are capable to generate more dry matter per seed sowed, and to gather more nutrients per grain, mainly crude fat and the amino acids lysine and tryptophan, it is pertinent to make research on the possible use of this PEm mutant to generate variation

that might be useful to generate new genotypes of maize for possible agronomic use, and under the hypothesis that the PEm trait is inherited and easily recuperated, and that it has the potential to increase corn productivity, This paper is intended to reports on:

- 1) to validate the PEm inheritance model proposed by Rebolloza *et al.* (2011);
- 2) to show a methodology to recover the PEm from segregating maize populations;
- 3) to explore the potential production capacity of maize populations segregating the polyembryony trait.

## MATERIALS AND METHODS

This research was carried out in a series of three experiments; two of them were conducted at the facilities of the Universidad Autónoma Agraria Antonio Narro (UAAAN) located at Buenavista, Saltillo, state of Coahuila, México, whose geographical coordinates are: 25 ° 21' N, and 101 ° 02' W, with an altitude of 1756 m a.s.l.

### First experiment

Crossbreeding between polyembryonic corn (BAP, for brachytic plants and NAP, for normal height plants) with

several inbred lines, representatives of common corn germplasm, No-PEm, was performed, which in turn resulted in nine segregating polyembryonic populations (table 1) this was generated at UAAAN, having 24 furrows for both NAP and BAP, and 2 furrows for each inbred line. The furrows were 0.8 m apart and 12 m long, the distance among plants was 0.17 m.

In the following cycle the various F<sub>1</sub> were advanced to F<sub>2</sub> by means of crossing plant to plant within each group. In order to determine the PEm frequencies, 600 seeds of each of the F<sub>1</sub> and F<sub>2</sub> groups were sowed under greenhouse conditions using a complete random design, three replicates, 200 per replicate.

The seeds were placed in polystyrene germination trays, 200 cavities, with the following dimensions: 67 x 34 x 7cm. The sowing substrate was a mixture of forest soil and peat moss, in a 2: 1 v / v ratio. The trays were placed on brick rails to facilitate the drainage and aeration of the roots of the seedlings. The watering of the trays was done manually, on daily bases along the experimental trials (3 weeks).

Data from the segregating PEm genotypes in F<sub>2</sub> were analyzed using a Chi-square test of goodness of fit for the 15: 1 hypothesis.

**Table 1.** Original crosses between NAP or BAP with inbred lines.

**Tabla 1.** Cruzas originales entre NAP o BAP con líneas endogámicas.

ID	Cross	ID	Cross
A	NAP x CML-78	F	NAP x AN-Tep-3
B	NAP x AN-7	G	NAP x AN-CS-8
C	BAP x CML-78	H	BAP x AN-Tep-3
D	BAP x AN-7	I	BAP x AN-CS-8
E	NAP x AN-255-18-19		

### Second experiment

To generate the diallel crossings, it was decided to handle the pollination through phenotypic assortative matings (AM+) thought as the most adequate pairing system. This pollination method was applied from the  $F_2$  to the advanced cycles (G3 and G4, respectively). Seeds from these  $F_2$  were sowed in germination trays in greenhouse, and only those seedlings with the PEm phenotype were transplanted into pots with a capacity of 20 L. Once flowering was reached, (AM+) pollination was practiced among plants of the same group. The seeds from each of these groups represented the G3 populations.

The G3 populations were used as the parental of the G4 progenies. These groups were generated at the experimental station of the National Research Institute of Forestry, Agricultural and Livestock (INIFAP) located in the city of Rio Bravo, state of Tamaulipas, Mexico (25 ° 59'N and 98° 06'W, 139 m a.s.l.). The experiment was aimed to generate diallel crosses using the nine G3 populations by applying the mating design, method 4 (Griffing, 1958), planted (a seed by stroke) in February and harvest in June 2015. The resulting progeny from the crosses represented the fourth generation (G4). A second way to generate G4 progenies was using representative samples of the nine G3 populations which were handled through (AM+) pollinations within each group. So that, we got two kinds of G4 level populations, one for the diallel hybrids evaluation, and the second one to have G4 seeds to continue the process of grading up the PEm frequency to reach the G5 populations.

The experimental settings to generate the G4 populations were as follows, the genetic materials were placed in plots of 8 furrows, 14 m long, 0.8 m between furrows, and 20 cm between plants. The fertilizer dose was 140-40-00, applied

at planting. For pest control, permethrin was used; Weeds were controlled with Pendimethalin and Atrazine. Prior to sowing, the land was irrigated by floods in order to obtain a moist soil in field capacity. After sowing, three irrigations were applied on the demand for plants.

The generations were observed through the mean values in order to see the progressive PEm gain per generation, each genotype.

### Third experiment

The assay for agronomic performance of some of the diallel hybrids was carried out at Buenavista, UAAAN. The experiment was designed as a 2 x 2 factorial traits arrangement. The A Factor was related to population density (moderated size: 73 000 and high size: 93 000 plants  $ha^{-1}$ ), and the B Factor was about fertilization doses (low 120:80:00 and high 240: 90: 00 N:P:K), all their combinations were applied on populations. For this experiment, seven genetic materials were used, as follows: four PEm G4 segregating populations originated from the diallel, the NAP population, used as a reference for polyembryony, one open-pollinated variety (TUX), and one commercial hybrid (CAI) (table 2, page 6). The last two materials were used as controls. The trial was established under the design of a randomized complete block with split-split plot arrangement.

The experiment display was as follows: planting was established in plots of two rows, 4 meters long, and three repetitions (blocks). Sowing date: July 11, 2015, and harvest in December 12, the same year. The treatments were the possible combination of two factors, two levels each, applied on the genetic materials. Pests were chemically controlled through the applications of Carbofuran, Permethrin and Metamidophos.

**Table 2.** Maize genotypes used in the assay, factors: population density and fertilization, two levels each.

**Tabla 2.** Genotipos de maíz utilizados en el ensayo de rendimiento, factores: densidad de población y dosis de fertilización, dos niveles cada uno.

ID	G4 Populations, identified by their initial cross
AxE	(NAP x CML 78) x (NAP x AN-255-18-19)
CxE	(BAP x CML 78) x (NAP x AN-255-18-19)
ExF	(NAP x AN-255-18-19) x (NAP x AN-Tep-3)
GxH	(NAP x AN-CS-8) x (BAP x AN-Tep-3)
NAP	Reference population for polyembryony
TUX	Tuxpeño variety HOC (sample from CIMMYT)
CAI	Commercial hybrid "Cayman" (from Asgrow)

Weeds were controlled by both chemical and mechanical procedures, the first with atrazine and 2, 4-D, and the second by using hoe and tractor. It was sowed on dry soil, and watering immediately afterwards by means of irrigation tape, and the same procedure for subsequent irrigations, according to crop water requirements.

Data on the response variable were taken properly at the required time, according to the variable definition. The harvest of each material was done manually.

The resulting data was analyzed by the appropriate analysis of variance accordingly with the experimental design used, in

doing so, it was used the statistical package SAS version 9.1. In cases where there were statistical differences in any of the variation sources, interaction graphs were performed using the statistical package STATISTICA version 10. There were six response variables, one was in regard to sexual maturity (Days to male flowering), two were taken as descriptors of plant type (plant and ear's height), other two were used to qualify plant and ear health (rotten ears and fusarium infected ears), and lastly the yield variable.

Model of the experimental design: a randomized complete block with split-split plot arrangement <sup>1</sup>.

$$^1 Y_{ijkl} = \mu + t_i + b_j + (tb)_{ij} + t_k + (tt)_{ik} + (ttb)_{ijk} + t_l + (tt)_{il} + (tt)_{kl} + (ttt)_{ikl} + \epsilon_{ijkl}$$

**Where:**

$Y_{ijkl}$  = the corresponding observation i-th main treatment  
 k-th secondary treatment  
 l-th tertiary treatment in the j-th block  
 $\mu$  = general mean  
 $t_i$  = effect of i-th main treatment  
 $b_j$  = effect j-th block  
 $(tb)_{ij}$  = error (a), at the level of the main plot  
 $t_k$  = effect secondary treatment k-th  
 $(tt)_{ik}$  = effect of interaction ik-th t x t  
 $(ttb)_{ijk}$  = is the error (b) subplot level

t = l-th effect of tertiary treatment  
 $(tt)_{il}$  = effect of interaction il-th t x t  
 $(tt)_{kl}$  = effect of the kl-th interaction t x t  
 $(ttt)_{ikl}$  = effect of the ikl-th txtxt interaction  
 $\epsilon_{ijkl}$  = is the error (c), the subplot level  
 $t_p, t_q, (tt)_{ikp}, t_r, (tt)_{ip}, (tt)_{kp}, (ttt)_{ikl}$  they are considered fixed effects: i= 1, 2,..., I treatments t (densities)  
 j= 1, 2,..., J blocks b (Repeats)  
 k= 1, 2,..., K treatments t (Fertilizations)  
 l= 1, 2,..., L treatments t (G = crossings)

## RESULTS AND DISCUSSION

### Validation of the PEm inheritance

The Chi Square tests for the pre-established hypothesis 15 to 1, normal, to polyembryonic plants, in the several F<sub>2</sub> groups are presented in Table 3. As it can be seen, the PEm segregations were accordingly to the hypothesis, as the ones presented by Rebolloza *et al.* (2011). In spite of that, the Mendelian genetic analysis underlined a diverse response capacity taking into account the hybrid combination and the source of the parental pollinator. It is instructive to say that in a test of this type ( $X^2$ , 1 degree of freedom,  $\alpha = 0.05$ ), the hypothetical extreme values of the  $X^2$  can be from 0 to less than 3.84.

The PEm segregation proportions across populations, measured under greenhouse, ranged from 3.7 to 5.8%.

On the other hand, the results obtained for the trials under field conditions, the PEm proportions ranged from 3.3 to 4.4%, all of them within the 15: 1 hypothesis. However, it is clear that there were deviations from the optimal expected value of 6.25%. This might be due to certain degree of incomplete penetrance of the genes. Moreover, there was observed a parallelism among the relative magnitudes of proportions between the two kinds of experiments, the crossings with higher values under greenhouse were also higher on field (table 3).

One way to test about the data similarities observed for the proportions of the different crossings is the application of a Homogeneity Chi square. This was run with the proper data (table 4, page 8).

**Table 3.** Goodness-of-fit Chi Square test of F<sub>2</sub> populations that segregates the double recessive PEm trait.

**Tabla 3.** Prueba de Chi cuadrado de bondad de ajuste de poblaciones F<sub>2</sub> que segregan el rasgo de PEm doble recesivo.

ID	Greenhouse					Field				
	Ple	PIPEm	Pln	Chi2	Prob.	Ple	PIPEm	Pln	Chi2	Prob.
A	184	8	176	1.1	0.30-0.20	181	6	175	2.66	0.20-0.10
B	191	11	180	0.1	0.90-0.70	189	8	181	1.31	0.30-0.20
C	193	10	183	0.4	0.70-0.50	192	7	185	2.22	0.20-0.10
D	188	7	181	2.1	0.20-0.10	185	7	178	1.92	0.20-0.10
E	189	11	178	0.1	0.90-0.70	183	11	172	1.1	0.30-0.20
F	185	8	177	1.2	0.30-0.20	179	6	173	2.57	0.20-0.10
G	189	9	180	0.7	0.50-0.30	184	8	176	1.14	0.30-0.20
H	196	10	186	0.4	0.70-0.50	194	8	186	1.5	0.30-0.20
I	182	9	173	0.6	0.50-0.30	186	8	178	1.20	0.30-0.20

Sample size = 200 seeds; Ple = seedlings emerged; PIPEm = Polyembryonic seedlings; Pln = One seedling per seed germinated, or Normal seedlings; Chi2= Chi-square test; Prob. = Probability

Tamaño de muestra = 200 semillas; Ple = Plántulas emergidas; PIPEm = Plántulas poliembriónicas; Pln = Una plántula por semilla germinada, o Plántulas normales; Chi2 = Prueba de Chi cuadrado; Prob. = Probabilidad

**Table 4.** Homogeneity test for the nine populations.  
**Tabla 4.** Prueba de homogeneidad para las nueve poblaciones.

	Greenhouse			Field		
	Chi-squares	DF	Probability	Chi-squares	D.F.	Probability
Totals	6.58	9		15.62	9	
Summed data	5.36	1	0.05-0.01	12.91	1	< .001
Homogeneity	1.22	8	1-0.95	2.71	8	1-0.95

DF = Degrees of freedom. / DF = Grados de libertad.

The Chi square calculations led to an accepted Homogeneity test, signifying that the data are homogeneous; however, it is necessary to note that the important source for the test, the “summed data”, is not in the range of the accepted limit, which invalid the idea of homogeneous data and cannot be added for a homogeneity test. The diverse amount of proportions observed in the nine  $F_2$  populations might be an indication of the occurrence of the claimed incomplete penetrance upon the PEm phenomena, mainly due to the differences sources of pollinators.

#### Increasing PEm frequency

The increasing amount of PEm frequencies throughout the  $F_1$  to G4 (succeeding four generations) is shown in table 5 (page 9). As expected, and given the recessive condition of the trait, the  $F_1$  progeny were seeds that germinated in solely individual plants, which is the normal phenotypic condition of common (normal) maize.

The pollination handling of the PEm segregating populations through assortative mating (AM+) was intended for a rapid increase of the trait frequency, in spite of the incidence of incomplete penetrance.

The average PEm frequency grew 3 times by the G3, and 8 times by G4 departing from the  $F_2$ . However, it is necessary to pay attention to the mating procedure. From the  $F_2$  groups, one can take only plants that show phenotypically the double or more sister plants grew from one seed. It is reasonable to think that these plants have the double homozygous recessive PEm genes that is to say those genes are fixed.

As it can be seen from the data shown here, that is not the case. Besides that, it is necessary to state that the (AM+) method won't affect the PEm genes conditions because of they are fixed, but it will have an effect on the rest of the genome at least in two ways, one because a degree of inbreeding is generated which mean a growing homozygosis within each population, and second, it has an effect on the genetic recombination that take place over generations, which could disarticulate the action(s) of the genetic factors that causes the so-named “incomplete penetrance” that reduce in some degree the phenotypic appearance of polyembryony limiting the expression of the double recessive homozygote. This disarticulation might facilitate to certain degree the major cases of polyembryony in BAP and NAP populations.



It is clear from the obtained data (table 5) that the two procedures, selecting only PEm plants and applying the (AM+) along progressing generations, the PEm percentages reached up to 20 and 40% by the third and fourth generations, respectively. It is convenient to say that polyembryony frequencies in the reference populations were observed in the range from 54 to 74%. The actual knowledge about this matter can raise two possible explanations, one is about the occurrence of incomplete penetrance of the PEm genes (Rebolloza *et al.*, 2011) and the other is on a possible pangenetic effect on the trait (Meráz-Fonseca *et al.*, 2015). We are leaned to though in terms of incomplete penetrance of the PEm genes.

The inclusion of No-PEm witnesses and the reference populations (BAP and NAP) in all experiments was intended to check for the occurrence and non-occurrence polyembryony phenotypes. The common maize genotypes will show always one seedling per seed germinated. However, the seeds from F<sub>2</sub> or more advances segregating groups will show cases of seedlings with the PEm phenotype. The average PEm frequency in populations BAP and NAP calculated across generations, green-

house and field data, was 62.7%, which is in agreement with previous reports (5, 6, 8, 26).

### Agronomic performance

With regard to the assay of yield data, the general results from the analysis of variance appear in table 6 (page 10). From these information, it can be seen that only the Genotypes (G) source showed statistical differences in all the response variables, this may reflect the diverse genetic condition among the segregating PEm groups. Related to Density (D) statistical differences were detected in health variables; this was probably due to the specific conditions of climate-environment generated by the high number of plants. The Fertilization (F) had a significant influence on all variables, except YIELD, which could indicate that nutrition greatly impacted the plants grow and development.

In cases of double interactions arising between Genotype with Density or Fertilization factors (table 6, page 10), it can be seen that the genotypes responded in a different way given the particular conditions of one or other factor, this outstandingly appreciated in variables such as precocity, height and health.

**Table 5.** The PEm average proportion per generation, pooled data across populations.  
**Tabla 5.** Proporción promedio de PEm por generación, datos agrupados entre poblaciones.

Genetic materials	Greenhouse data (%)				Field data (%)			
	F1	F2	G3	G4	F1	F2	G3	G4
Populations (PEm segregates)	0	4.9	19.7	42.2	0	3.9	2.1	38.6
Diallel crosses				41.0				42.6
Reference populations (average from BAP and NAP)	66.5	70.5	55.9	57.9	53.6	nd	73.6	60.6
Controls (TUX and CAI)	nd	nd	0	0	nd	0	0	0

nd = no data available. / nd = no hay datos disponibles.

**Table 6.** Mean squares analysis of variance for agronomic variables corresponding to PEm genotypes and their witnesses.**Tabla 6.** Cuadrados medios del análisis de varianza para variables agronómicas correspondientes a genotipos de PEm y sus testigos.

SV	DF	MF	PH	EH	ROTE <sup>‡</sup>	FUSE <sup>‡</sup>	YIELD
REP	2	4.37	5.58	12.73	3.79	2.81	4.52
D	1	0.30	0.43	34.07	357.53 *	293.07 **	73.36
Error a	2	1.23	8.18	5.65	4.84	1.96	5.95
F	1	152.01 **	9472.19 **	2568.57 **	604.29 **	1052.23 **	45.03
D x F	1	0.11	192.01	59.17	2.64	63.27 **	5.30
Error b	4	1.70	29.07	15.70	4.05	0.88	6.19
G	6	75.71 **	1540.37 **	1187.98 **	205.51 **	168.72 **	96.12 **
D x G	6	2.99 **	36.36 *	51.68 **	29.88 **	53.26 **	8.64
F x G	6	2.65 **	182.20 **	41.08 *	18.86 **	23.25 **	2.97
D x F x G	6	1.41	98.66 **	76.70 **	19.09 **	19.68 **	5.72
Error c	48	0.71	14.29	14.57	3.78	2.89	5.68
CV (a)		1.40	1.30	2.00	12.10	9.20	18.70
CV (b)		1.70	2.50	3.30	11.10	6.20	19.10
CV (c)		1.10	1.70	3.20	10.70	11.20	18.30
R <sup>2</sup>		95	97	94	94	96	76

SV = Source of variation; REP = Replicates; D = Density; F = Fertilization; G = Genotype; CV = Coefficient of variation; R<sup>2</sup> = coefficient of determination; DF = Degrees of freedom; MF = Male flowering; PH = Plant height; EH = Ear height; ROTE = Rotten ears; FUSE = Fusarium in ear; YIELD = Yield; † Variables with transformed data using angular or inverse sine method.

SV = Fuente de variación; REP = Repetición; D = Densidad; F = Fertilización; G = Genotipo; CV = Coeficiente de variación; R<sup>2</sup> = coeficiente de determinación; DF = Grados de libertad; MF = Floración masculina; PH = Altura de la planta; EH = Altura de mazorca; ROTE = mazorcas podridas; FUSE = Fusarium en mazorca; YIELD = Rendimiento; † Variables con datos transformados usando el método de seno angular o inverso.

The three factors interaction, Density x Fertilization x Genotype showed statistically differences only for plant type and health variables. The variation coefficients, considering the three types of error in the analysis, were quite acceptable (less than 20%). Because of the statistical differences found, all the response variables are discussed through interaction graphs in figures.

Among the four PEm segregating genotypes coming from the diallel crosses, the GH hybrid was the most early considering male flowering (MF). This condition had

connection with the significance of Density x Genotype interaction under the high population density level, and in a similar manner in the Fertilizer x Genotype on the side of moderated fertilization. Overall, the average values of segregating genotypes were later (4 days) than the TUX variety and the commercial hybrid CAI, but earlier than the reference population (2 days) which means that the initial hybridization between polyembryonic populations and non-related genotypes has an effect in the progeny earliness.

In general, the TUX genotype was the earliest ( $p \leq 0.01$ ). On the other hand, and according to the factor Fertilizer it can be observed that the supply of high fertilization tends to increase days to male flowering, but the opposite was observed when the moderate fertilization was applied (figure 1, page 12). These results differed from those published by Ortiz *et al.* (2013) who detected no significance under different fertilization levels (150, 250 and 350 kg ha<sup>-1</sup>) on the variable "days to silking". However, when considering the effect of population densities regarding MF, the results of this study are in agreement with those published by Ortiz *et al.* (2014) who detected no significant differences on this variable when three population densities (60, 75 and 90 thousand plants ha<sup>-1</sup>) were evaluated.

In regard to the PH variable (figure 2, page 12) the cross GH had the highest plant height average in both variation sources Genotype, Density x Genotype with the moderate density level, and Fertilization x Genotype under the high fertilization level. On the other hand, the triple interaction Density x Fertilization x Genotype had shown that the cross EF had the highest value when moderate density and high fertilization.

Plant height for the segregating polyembryony genotypes was 10% lower than the reference population NAP, and the commercial hybrid CAI, but 6% higher than the variety TUX, which can be considered desirable because a moderate height can reduce stalk lodging while presenting greater volume of dry matter. The TUX genotype showed the shortest plant height (204 cm average).

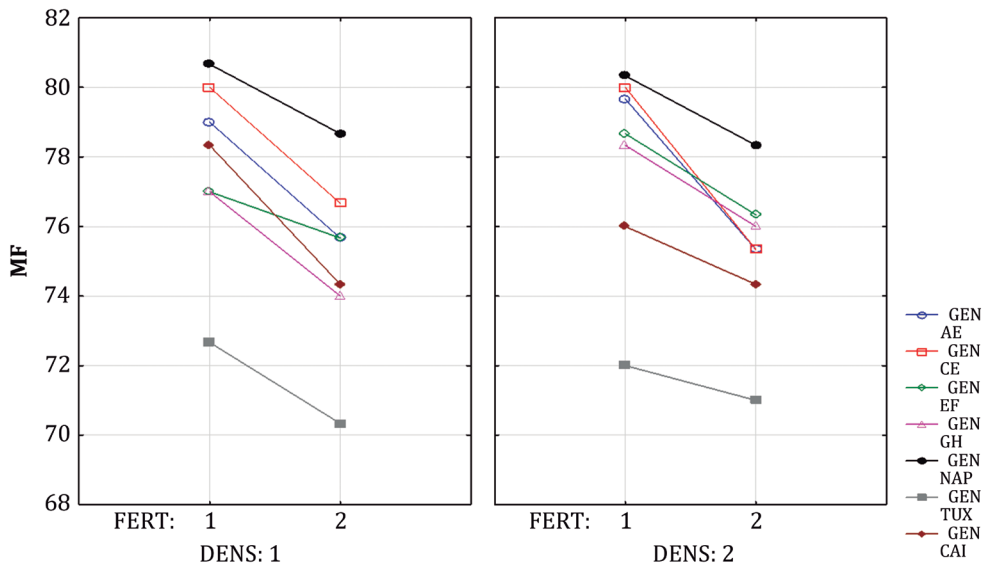
High fertilization tended to increase plant height (10% on an average); this is because the effect of nitrogen can promote cell proliferation and stimu-

lates the growth of grasses (16). These results are similar to the ones published by Gökmen *et al.* (2001) who mentioned that the fertilization showed significant differences in plant height, obtaining the maximum height with the highest dose of N (250 kg ha<sup>-1</sup>), while the lowest values were recorded when the nitrogen fertilization was 50 kg ha<sup>-1</sup>.

The superior ear height (variable EH) in corn is of economic importance because of the plant standability which describes the ability of a plant to remain erect until the crop is harvested. In general, a high EH placement corresponds to a high plant height (PH), but it is the EH/PH rate that matters. It is known that a ratio of about 50% favors the plant standability (32), and avoid lodging.

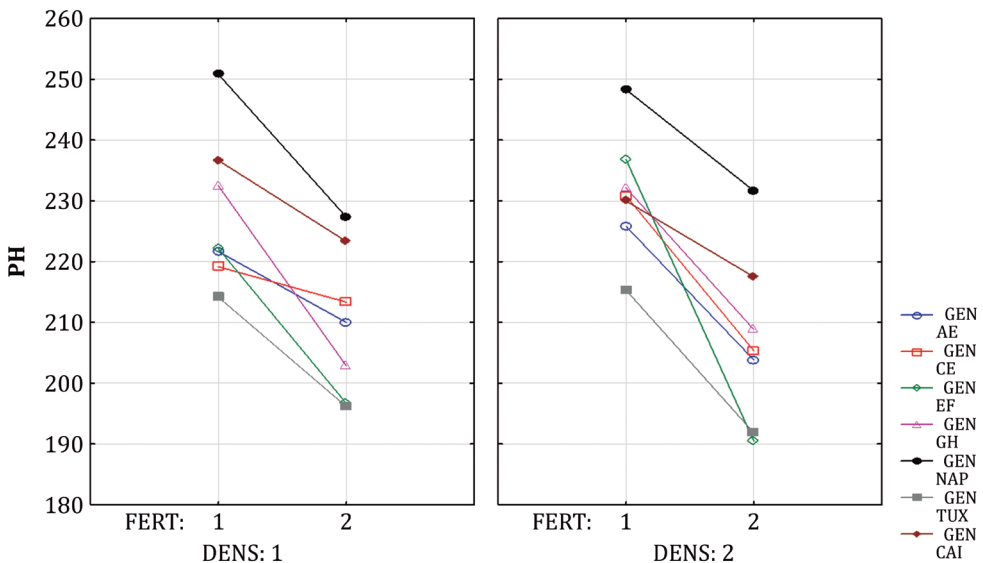
On the crosses, the lowest values of the presented ear height, AE in source Genotype, GH on interaction Density x Genotype with moderate density, whereas in interaction, Fertilization x Genotype, the EF crosses showed lower on fertilization moderate; this influenced for the two genotypes (GH and EF) presented the lowest value in triple interaction Genotype x Fertilization x Density with density and moderate fertilization. The genotype that stood out was TUX since it had the lowest value.

The crosses segregating PEm presented EH intermediate values, which were 15% less height than NAP, the reference genotype, but 7% more than the TUX and CAI. This condition might lead to reduce lodging, and favoring some yield trait components. In this research there was found that fertilization levels had an impact on EH (figure 3, page 13), however, this is not the case with the report published by Ortiz *et al.* (2013) who did not find statistical differences between ear height and fertilization dosages.



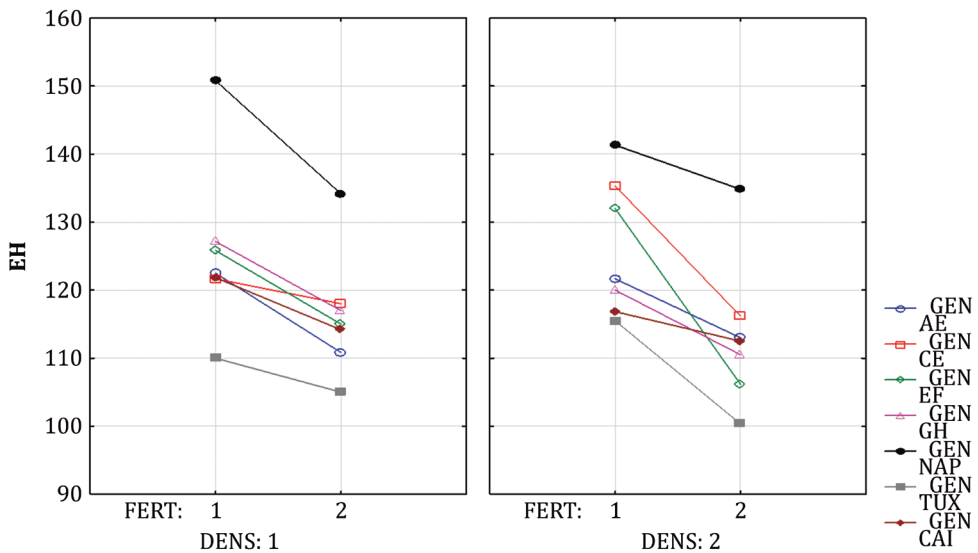
**Figure 1.** Days to male flowering (MF) means, two population densities, two fertilization doses and seven genotypes.

**Figura 1.** Medias de la variable días a floración masculina (MF), dos densidades de población, dos dosis de fertilización y siete genotipos.



**Figure 2.** Means of plant height variable (PH) on two densities, with two different fertilization doses in seven genotypes.

**Figura 2.** Medias de la variable de altura de la planta (PH), dos densidades, con dos dosis diferentes de fertilización en siete genotipos.



**Figure 3.** Means of the ear height variable (EH) two densities, with two different fertilization doses in seven genotypes.

**Figura 3.** Medias de la variable altura de mazorca (EH), dos densidades, con dos dosis diferentes de fertilización en siete genotipos.

As it can be checked, the Density factor was not of impact on MF, PH and EH. According to the analysis of variance (table 4, page 8) no statistical significance was presented on these variables. Similar results are presented by Khan *et al.* (2003) who reported that the increased population density in maize has no effect on those variables. On the other hand, the high level of fertilization had an important impact on the three variables, increasing their values.

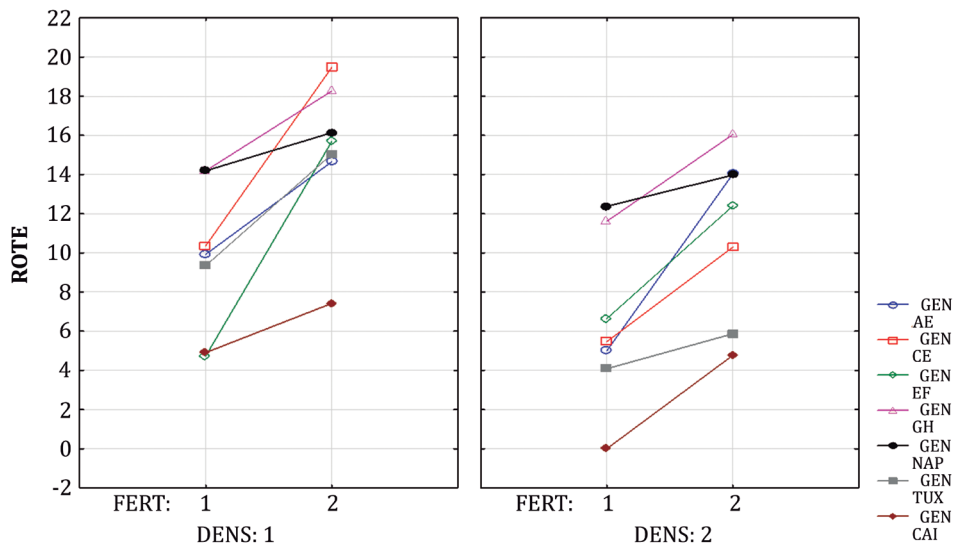
Analyzing the ROTE data, it can be observed that almost all the model effects resulted statistically significant (table 4, page 8; figure 4, page 15). In addition, three segregating genotypes polyembryony (AE, CE and EF) obtained 25% less rotten ears than the reference population NAP. The high population density provoked more rotten ear cases; meanwhile the high fertilization tends to reduce them. The moderate population density presented 30% less damage.

The notable presence of rot cases could be due because the high density contributed to maintain high humidity in the foliage, which may have triggered the development of diseases. These results coincide with those presented by Blandino *et al.* (2008) who indicated that plant density affected the percentage of rotten grains, and stated that plots with the higher number of plants (82 thousands  $ha^{-1}$ ) had a higher severity (+43%) than plots with lower plant density (65 thousands  $ha^{-1}$ ). In this sense, Dodd (1983) mentions that, when using a corn plant density greater than the optimal population increases competition for light, water and nutrients causing reductions in the root volume, the number of ears and the number of grains per ear, also increases the intensity of root and stem rots which favors the lodging both root and stem as well as ear rots percentage.

In regard to fertilization, the high level had a positive effect as it reduced the ROTE by 39% due to the greater ability to provide nutrition for growth and development; this condition is in agreement with the results published by Huber (1989) and Blandino (2008) who mentioned that plants receiving proper mineral nutrition in general are significantly more tolerant to diseases.

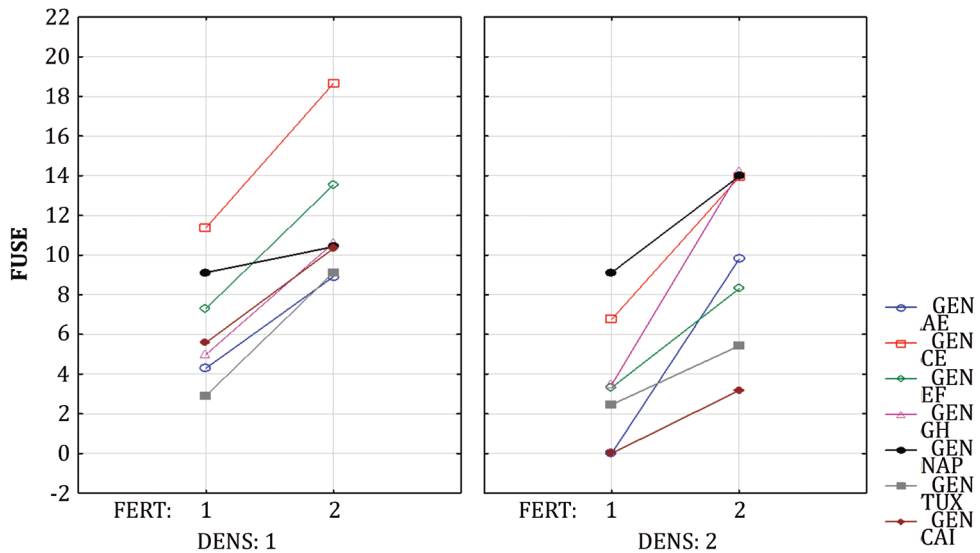
FUSE is generally one of the variables that have negative impact on the crop yield, and in this research it presented statistical differences in all sources of variation (table 4, page 8). As it can be seen, the high density plant population had a negative effect on all genotypes because of this variable (figure 5, page 15). The higher fusarium damage in all genotypes was presented with the combination high population density and moderate fertilization. Moderate Density was the best with 26% less FUSE, but even better was the effect of high fertilization which reduced the percentage of this variable by 53%. With these results it is not surprising that the interaction Density x Fertilization presenting lower incidence of the disease (61% less FUSE) with the combination moderate density and high fertilization. The stated results coincide with those published by Lozano and Diaz (2002) who found that the highest density (100 thousand plants ha<sup>-1</sup>) had 28.6% more ear fusarium than the intermediate and low population densities (75 and 50 thousand plants ha<sup>-1</sup>). Moreover, Huber (1997) mentions that the severity of most plant diseases can be diminished by improvements in handling fertilization. On the contrary, Martinez *et al.* (2005) working on these issues in maize, concluded that nitrogen fertilization and density have no effect on the quality of the seed.

Finally, and regarding to the YIELD variable, the analysis of variance detected statistically differences only for the factor Genotypes. In this context, it can be observed that the PEm segregating groups were better than the reference population (NAP) up to 18% more t ha<sup>-1</sup> and two of them (crosses AE and CE) were slightly higher than the range TUX having 2 and 4% more yield, and as it was expected, the CAI genotype presented the highest yield. Although there was no statistical significance, it can be seen (table 4, page 8, and figure 6, page 16) that under high fertilization doses, the genotypes tend to be more productive (12% t ha<sup>-1</sup> higher); this coincides with Osborne *et al.* (2002) and Yasari *et al.* (2012) which mention that with high levels of nitrogen grain yield increases, having a positive effect on the number of seeds per ear and the weight of the seed of maize hybrids. The lack of effect of population density on YIELD could be attributed to that by increasing the population density it decreases yield per plant, increased number of barren plants as a result of competition effect, so that the moderate density would be the most appropriate. These results are in agreement with that of Karlem and Camp (1985) and Sangoi (2000), who mentioned that the yield per unit area, responds to the increase in plant density up to a peak and decreases when the density exceeded that point, giving an optimum type curve. On the other hand, Bruns and Abbas (2005) mentioned in a publication that using a low density (71760 plants ha<sup>-1</sup>) with furrows at 76 cm wide, the maximum yields were obtained (10.3 t ha<sup>-1</sup>) when comparing with those yields with higher population densities (82160, 92560 and 102960 plants ha<sup>-1</sup>).



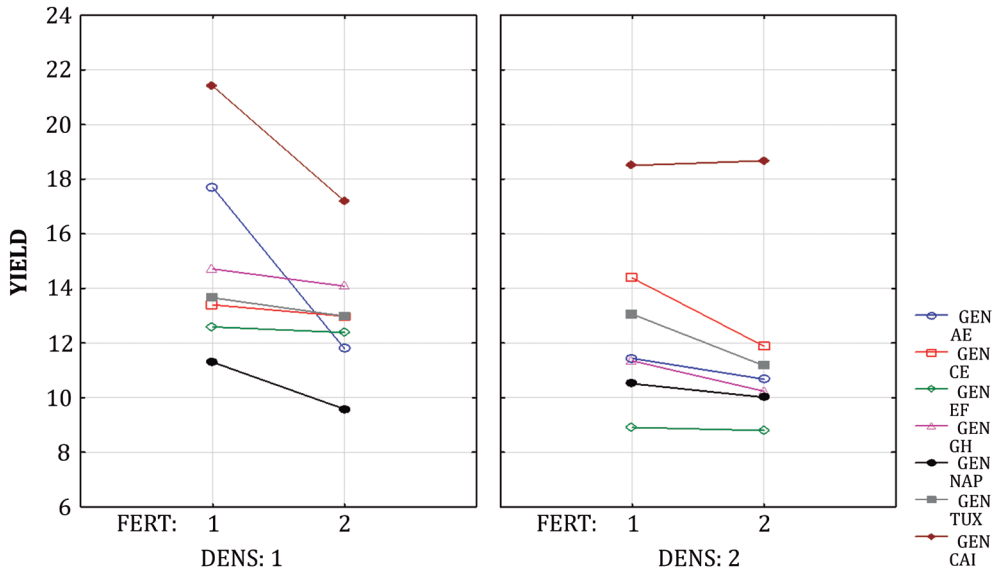
**Figure 4.** Means of the ear rot variable (ROTE) two densities, with two different fertilization doses in seven genotypes.

**Figura 4.** Medias de la variable mazorcas podridas (ROTE), dos densidades, con dos dosis diferentes de fertilización en siete genotipos.



**Figure 5.** Mean of the fusarium ear variable (FUSE) two densities, with two different fertilization doses in seven genotypes.

**Figura 5.** Media de la variable fusarium en mazorca (FUSE), dos densidades, con dos dosis diferentes de fertilización en siete genotipos.



**Figure 6.** Means of the yield variable (YIELD) two densities, with two different fertilization doses in seven genotypes.

**Figura 6.** Media de la variable de rendimiento (YIELD), dos densidades, con dos dosis de fertilización diferentes en siete genotipos.

## CONCLUSIONS

Maize polyembryony is controlled by two epistatic loci, double recessive homozygous, and it can be handled in such a way that the trait is easily recovered through assortative mating within genotypic groups who segregates the trait. Also, it is proposed that PEm is affected by the incomplete penetrance phenomena. The agronomic performance of segregating genotypes showed to be competitive when was compared with the control genotypes, which presented the extreme values for

most of the variables. The less competitive values corresponded to the reference population of high polyembryony (NAP) meanwhile the better values were shown by the commercial hybrid (CAI). However, it is advisable to carry out a more extensive experimentation, including a greater number of segregating genotypes, larger plots, and several different environments to size sufficiently the agronomic potential in using the maize PEm mutant.



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