

## **Seed production and crosses among maize (*Zea mays* L.) accessions of the Magdalena department of Colombia**

### **Producción de semilla y cruzamientos entre accesiones de maíz (*Zea mays* L.) del departamento del Magdalena, Colombia**

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#### **Abstract**

Genetic resources of maize are important for breeding programs. From maize accessions of department of Magdalena-Colombia, under the soil and climatic conditions of Experimental Center for Agricultural and Forestry from the Universidad del Magdalena located in Santa Marta (74° 07' - 74° 12' W and 11° 11' - 11° 15' N) at 2 MASL, a program of crosses was initiated. The objectives were to obtain inbred lines from ten accessions of maize and generate 45 F1 hybrids directly between them. The production of the inbred lines was only possible with eight accessions; accession 35 from San Pedro de la Sierra (1397 MASL) did not flower under conditions of Santa Marta and the accession 91 from Fundación (62 MASL) flowered but, it did not form grains. Accessions 34 and 90 produced little seed unlike the accession 92 which produced abundant seeds. Overall, the development of the ears produced by auto-pollination was poor and with low grain formation. The amount of seed was different between F1 crosses; only four accessions: 3202, 88, 89 and 92 had the ability to cross and produce hybrid seed, unlike accessions 34, 35 and 91 that showed poor adaptation and inability to cross.

**Key words:** Climate, cross, pollination, crossbreeding.

#### **Resumen**

A partir de accesiones de maíz con pasaporte del departamento del Magdalena, Colombia, se inició un programa de cruzamientos bajo las condiciones edafoclimáticas del Centro Experimental Agrícola y Forestal de la Universidad del Magdalena (74° 07' - 74° 12' O y 11° 11' - 11° 15' N) ubicado en la ciudad de Santa Marta, Colombia, a 2 m.s.n.m. El objetivo en el trabajo fue obtener líneas endocriadas a partir de 10 accesiones de maíz y formar 45 híbridos F1 directos entre ellas. La producción de las líneas endocriadas sólo fue posible con ocho de las accesiones; la accesión 35 procedente de San Pedro de la Sierra (1397 m.s.n.m.) no floreció bajo las condiciones del ensayo y la accesión 91 procedente de Fundación (62 m.s.n.m.) floreció, pero no formó semilla. Las accesiones 34 y 90 produjeron poca semilla, a diferencia de la accesión 92, que produjo abundante semilla. En general el desarrollo de las mazorcas producidas por autofecundación fue pobre y con baja formación de grano. La cantidad de semilla en F1 fue diferente entre las cruces. Las accesiones 3202, 88, 89 y 92 fueron buenos parentales con habilidad para cruzarse y producir

semilla híbrida, a diferencia de las accesiones 34, 35 y 91 que mostraron pobre adaptación y escasa habilidad en los cruzamientos. Las condiciones edafoclimáticas del Centro Experimental Agrícola y Forestal de la Universidad del Magdalena no son adecuadas para producir semillas en programas de cruzamientos dirigidos de maíz.

**Palabras clave:** *Zea mays*, clima, cruzamiento, polinización, mejoramiento convergente.

## Introduction

Corn (*Zea mays*) is an important crop in Colombia, where is adapted to several regions with different agroclimatic and socioeconomic conditions. The grain is produced from La Guajira till the Amazon and, from the Pacific till the Western Plain; it is grown from sea level till 3000 MASL, in areas with precipitation less than 300 mm in La Guajira till 10,000 mm in Choco (Polanía and Méndez, 2012). The white and yellow type are the most cultivated ones, the first one for human consumption and the second one mainly for industrial uses in the preparation of concentrates for breeding and fattening of animals (Polanía and Méndez, 2012).

The knowledge on the use of the new variety or hybrid that will be developed in traditional breeding programs is essential to direct the goal of corn breeding, so, in traditional agriculture variety are used whereas in commercial agriculture hybrids are sown. Therefore, if the goal is to get varieties or improve the existing ones is used to use methods for recurrent population breeding and, if the need is for hybrids, hybridation methods are used (Vargas, 2010). In both methods, it is required to develop inbred lines, which are symbolize by the "S" letter and are obtained by controlled auto-pollination, a process which genetic goal is to increase homozygosity in the plant traits, being the visual effect of the endogamy the loss of vigor in the plant.

Once formed, the inbred lines have different uses in the breeding programs, among them, to identify the best endogamic lines by evaluation with a wide genetic based

genotype (Top-Cross) in different environments, to identify those promising ones in specific environments and exploit its hybrid vigor or heterosis using diallelic crosses (Vallejo and Estrada, 2002). By using the Top-Cross is identified the general combining ability of the S line and, with the diallelic cross is studied the general and specific combining ability. The inbred lines can be improved by crossing those that show complementary phenotypic characteristics like disease resistance. The backcross is another way of improving the inbred lines that are used to develop synthetic varieties using polycrossings (Ramírez, 2006).

The hybrid development in corn with endogamic lines is a pioneer method in respect to the other crops, its importance in agriculture and economy has been big, there has been development of simple, double and three-way cross hybrids (Vargas, 2010). In the local breeding programs several objectives are pursue at a time, such as adaptability of the germplasm to specific conditions of the farmers and the fitness to marginal environments, the seed acceptance by the farmers, the economic stability and other characteristics (Paliwal, 2001).

Corn has been breed *in situ*, based on the use perception and the needs of the farmer and consumer. In this case the developed cultivars are known as ancestral, local or native varieties. They are a source of agronomical importance that affects the local fitness, economic stability and the farmer sustainability. Different to the breed varieties, for which the main goal is yield in

favorable environments, the local materials are source of resistance to fungi, insects or abiotic stress (Vargas, 2010).

This study was done with the objective to develop  $S_1$  lines between 10 native corn accessions and to obtain  $F_1$  hybrid seeds between them, to later, evaluate and estimate the genetic advance for grain yield and identify the families with good agronomical behavior, that can be used as varieties and/or sources to get inbred lines; also, to generate information to continue the breeding process of this cultivated populations in the Department of Magdalena. In this article are highlighted the results for seed production by the assisted pollination processes between native accessions.

### Materials and methods

The research was done at the Experimental Center for Agriculture and Forestry of the Universidad del Magdalena (74° 07' – 74° 12' W and 11° 11' – 11° 15' N), Santa Marta, Colombia. The average temperature at the experiment time was 29 °C, rainfall 453 mm, solar brightness 45 h, wind speed 3 m/seg, soil texture is sandy clay loam (Vásquez, 2009; IDEAM, 2012).

Seeds were obtained from the Collection of Colombian Corn from the Universidad

Nacional de Colombia – Palmira (Table 1). These seeds were introduced in the Plant Breeding Program of the Universidad del Magdalena in 2011. For that, its multiplication started through parental crosses, similarly self-pollinations ( $S_1$ ) and directed crosses between them ( $F_1$  hybrids) were made without previous self-pollinations.

### Seed multiplication and development of $S_1$ lines in ten accessions of native corn

The ten accessions were sown in plots of two lines of 4 m and distance between plants 0.25 m. Sowings were done on a staggered manner, three times during the experimental time, with sowings every week to guarantee the pollen availability between accessions at the moment of crossings. The plots were fertilized with bocashi and nitrogen (170 kg/ha), phosphorus (35 kg/ha) and potassium (175 kg/ha) that were incorporated into the soil. Irrigation was done at the vegetative time till 15 days after flowering. The control of the armyworm (*Spodoptera frugiperda*) was done manually at the beginning and later, with *Bacillus thuringiensis* (1000 g/ha), it was applied Lorsban (1 l/ha) when the number of individuals increased. For weed control, especially *Cyperus rotundus*, Roundup (5

**Table 1.** Native accessions of corn collected in the Department of Magdalena, Colombia.

Entry No.	Site of collection	Longitude	Latitude	Altitude (MASL)	Race	Color	No. BG
3202	—	—	—	—	Clavo	Amarillo	ZmMagColCIM3202
11	Fundación	74 11	10 3100	53	Cariaco	Amarillo	ZmColCIM3132
34	San Pedro de la sierra	74 0247	10 5424	1397	Guiura	Morado y amarillo	ZmColCIM 3125
35	San Pedro de la sierra	74 0247	10 5424	1397	Guiura	Morado y amarillo	ZmColCIM 3121
87	Pivijay	74 23008	10 27455	24	Clavo	Amarillo	ZmMagCol1001
88	Fundación	74 11195	10 31231	49	Clavo	Amarillo	ZmMagCol1002
89	Fundación	74 11070	10 31236	62	Carioco	Amarillo	ZmMagCol1003
90	Fundación	74 11070	10 31236	62	Clavo	Amarillo	ZmMagCol1004
91	Fundación	74 11070	10 31236	62	Clavo	Blanco	ZmMagCol1005
92	Ciénaga	74 05403	10 53591	65	Clavo	Amarillo	ZmMagCol1006

**No BG:** Identification according to the Universidad Nacional de Colombia- Palmira

l/ha) was applied as preemergence control and then it was used scythe. Complementary management practices were hilling, twice, and foliar fertilization at vegetative stage using Nutrifoliar completo (2 l/ha).

The multiplication of seeds was done by paternal crosses and  $S_1$  lines were obtained by self-pollination of the plant; the obtained crosses were identified to know which ones were paternal crosses and which were self-pollinations. At harvesting time, at the maturity time, ears were harvested, air dried under shade, then seeds were isolated and counted before placing them on paper and plastic bags, spray them with Vitavax and stored them in the fridge with identification tags.

### Formation of $F_1$ hybrid seeds and diallelic crosses 10 x 10

In this stage a similar sowing as the one previously described was done using the same agronomical rules for harvesting and storing the seeds. The 10 accessions were sown in rows at 4 m distance and plant distance was 0.25 m. Sowings were done three times on the duration of the experiment to guarantee the pollen availability. The crosses were directed, following a diallelic 10 x 10 scheme (Figure 1), on a program of 45 direct crosses.

The crosses were done at early hours in the morning (between 6:00 and 7:00 a.m.) before wind started. The stigmas were covered with paper bags before they emerged to avoid non-controlled pollination. Pollen was directly taken from the anthers and placed on the corresponding flower with

receptive stigmas (Picture 1). After the pollen was spread on the stigma, the crossing was covered with paper bags and was identified with a tag. This process was replicated the day after and, to avoid the non-controlled pollination the bag covered the stigmas till viability was lost. The ears labelled with tags were harvested when the grain was at the milky stage.

## Results and discussion

### Increments in seed and formation of $S_1$ line in 10 native accessions

The percentages of seed germination of the accessions were different ( $P < 0.05$ ). Two groups were observed, one with high germination percentage and other with reduced viability on the seed (Table 2). Among the last ones were found the genotypes of the accessions from San Pedro (34 y 35) and Fundación (92 y 90). In the accessions 34 and 35 (San Pedro), 90 (Fundación) and 3202 (unknown origin place) it was not possible to increase the seed by parental crossings. The accession 11 (Fundación) had a low seed production by the parental method giving only 20 seeds.

The development of inbred lines, self-pollination ( $S_1$ ), was possible in eight accessions and in only two of them (91 and 35) there was no  $S_1$  seed. From the accession 35 it was not possible to get seed by the parental method or by self-pollination; to the opposite, the accession 87 (from Pivijay), 88 (Fundación) and 92 (Cienaga)

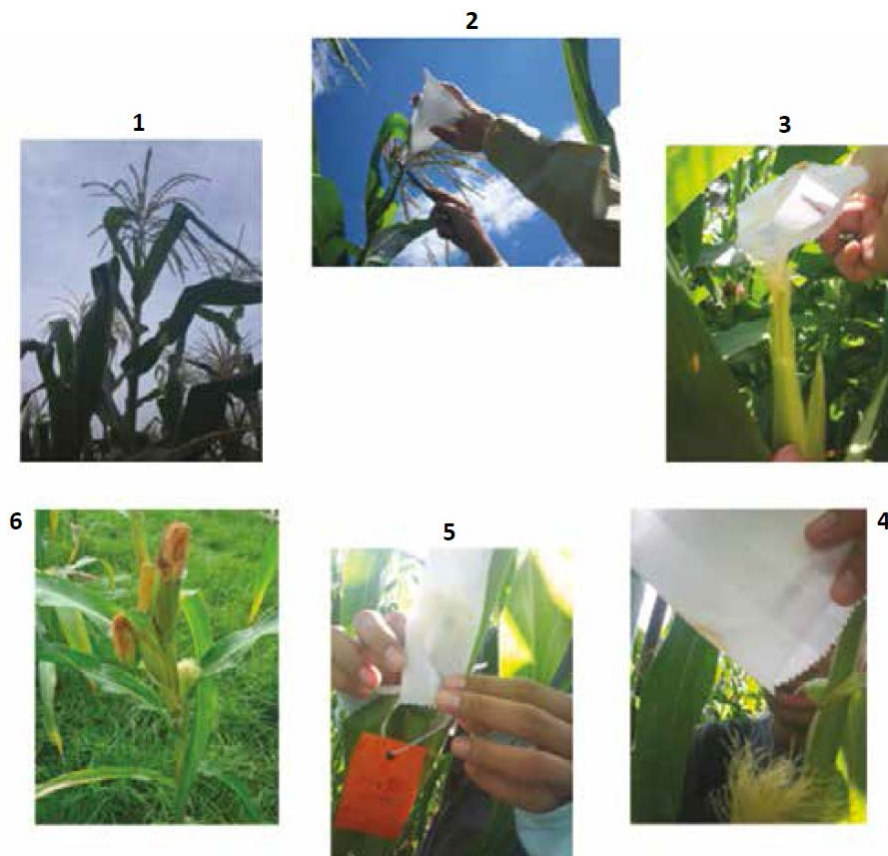
	1	2	3	4	5	6	7	8	9	10
1 (02)	1X1	2X1	3X1	4X1	5X1	6X1	7X1	8X1	9X1	10X1
2 (11)		2X2	3X2	4X2	5X2	6X2	7X2	8X2	9X2	10X2
3 (34)			3X3	4X3	5X3	6X3	7X3	8X3	9X3	10X3
4 (35)				4X4	5X4	6X4	7X4	8X4	9X4	10X4
5 (87)					5X5	6X5	7X5	8X5	9X5	10X5
6 (88)						6X6	7X6	8X6	9X6	10X6
7 (89)							7X7	8X7	9X7	10X7
8 (90)								8X8	9X8	10X8
9 (91)									9X9	10X9
10 (92)										10X10

Figure 1. Diallelic crossig 10 x 10 between native genotypes of corn.

showed good seed increases by both methods. These results show the absence of an uniform behavior of the accessions due to their different environmental origins into

the Department of Magdalena (Picture 2).

The accession 91 (Fundación) did not produced seed by self-pollination ( $S_1$ ), whereas the accessions 34 (San Pedro) and



**Picture 1.** Formation of directed crosses in corn. (1) Mature spikes. (2) Pollen collection. (3) Uncoverage of receptive stigmas. (4) Pollination. (5) Crossing identification. (6) Development of ears after fertilization.

**Table 2.** Germination percentages and increases in seeds of native corn from the Germplasm Bank of the Universidad Nacional de Colombia - Palmira.

Accession (No.)	Replicates <sup>a</sup>	Germinación (%)	Amount of seeds	
			Parentals	S1
3202	2	97.50 a*	0	599
11	2	97.50 a	20	270
89	2	92.50 a	160	209
91	2	90.00 a	235	0
88	2	87.50 a	346	304
87	2	85.00 a	300	351
35	2	75.00 b	0	0
92	2	75.00 b	502	882
90	2	72.50 b	0	130
34	2	55.00 b	0	108

a. = Number of replicates, 20 seeds were sown per accession in each replicate.

\* Means with similar letters are not statistically different ( $P > 0.05$ ).

Parentals = Crossing method to multiply seeds, expressed as the number of obtained seeds.

S1 = Number of seeds produced by self-fecundation.



**Picture 2.** Ear formation as product of the crosses between sister plants of native accessions of corn from the Department of Magdalena, Colombia.

90 (Fundación) produced low amount of seeds by endogamy (Picture 3). In general terms, both the ear formation and the grain production were scarce. All the grains of the ears were stored and only the seeds attacked by plagues and diseases or, the badly formed ears, were discarded.

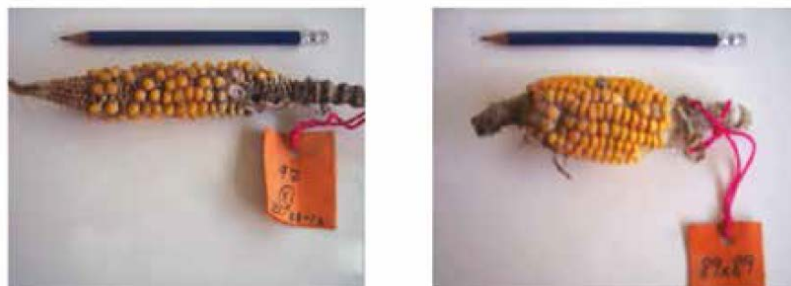
### **Formation of F<sub>1</sub> hybrid seed and 10 x 10 diallelic cross**

The amount of hybrid seed produced in the 45 direct crossings following the 10 x 10 diallelic scheme is shown in the Table 3. The parentals (accessions) 88, 3202, 92 and 89 produced seed, both, by the parental and endogamic methods and, as parentals, they showed the capacity for crossing between them, which indicates that there is a genetical identity. Not all the parentals were

crossed and formed grains, for example, the accessions 34, 35 of cold zones and 91 of hot areas participated in three or four crosses; similarly was the behavior of the accessions 87 and 90 that were crossed with five of the other parentals and the amount of F<sub>1</sub> seed obtained was lower than the average (Picture 4).

### **Discussion**

Regeneration is a technique used to obtain fresh seed with high viability (Rao *et al.*, 2007). The results of the seed germination percentage coming from the Germoplasm Bank of Colombian Maize were variable (Table 2). Cerovich and Miranda (2004) found that the corn seed loss longevity by the effect of storing and, as practices to pre



**Picture 3.** Ear formation as product of the manual directed self-crossing crosses between native accessions of corn from the Department of Magdalena, Colombia.

**Table 3.** Number of corn seeds obtained by 10 x 10 diallelic crosses and ability of the parentals to cross and produce seed.

										Number of seeds produced per parental				Ability of the parental for crossing and seed production			
										1	2 = CTE	1-2	1	2 = CTE	1-2		
87	34	88	11	3202	35	90	91	92	89	€Pi.	€Pi./n-2	€Pi/n(n-2)	Índice	€Pi	€Pi/n-2	€Pi/n(n-2)	Índice
87	0	180	26	82	0	126	0	0	9	423	52.875	109.35	-56.475	5	0.625	0.7375	-0.1125
34		245	0	0	0	0	92	237	110	684	85.5	109.35	-23.85	4	0.5	0.7375	-0.2375
88			449	175	75	84	253	122	63	1646	205.75	109.35	96.4	9	1.125	0.7375	0.3875
11				71	0	0	6	105	82	739	92.375	109.35	-16.975	6	0.75	0.7375	0.0125
3202					70	108	263	344	237	1350	168.75	109.35	59.4	8	1	0.7375	0.2625
35						0	0	84	0	229	28.625	109.35	-80.725	3	0.375	0.7375	-0.3625
90							0	115	286	719	89.875	109.35	-19.475	5	0.625	0.7375	-0.1125
91								51	0	665	83.125	109.35	-26.225	4	0.5	0.7375	-0.2375
92									224	1282	160.25	109.35	50.9	8	1	0.7375	0.2625
89										1011	126.375	109.35	17.025	7	0.875	0.7375	0.1375
€P. =										8748	—	—	€P. =	59	—	—	—

€Pi.= Total number of seeds produced by parental (accession) *i* in the crosses where it participates.

1 = €Pi./n-2 = Mean of the number of seeds produced per parental (accession) *i* in the crosses where it participates.

2 = T= €Pi/n(n-2) General mean of the number of seeds produced in all the crosses, it is an index =subtraction of the cells 1 and 2. Positive values are over the mean, negative values are below the mean.

€Pi.= Number of crosses with seed production where the parental (accession) *i* participates.

1 = €Pi./n-2 = Mean of the number of seeds produced per parental (accession) *i* in the crosses where it participates.

2 = T= €Pi/n(n-2) General mean of the number of seeds produced in all the crosses, it is an index =subtraction of the cells 1 and 2. Positive values are over the mean, negative values are below the mean.

serve them it is suggested to store high quality seeds and, to control humidity and storage temperature. These authors considered that the genetic characteristics of the species under storage change between species, cultivars of the same species, batches and even between individuals of the same batch. It is known that corn has an intermediate longevity and that sweet corn has mayor problems at storage than the white and yellow corn. The accessions in this work included all the types, besides the purple accessions that showed low viability levels.

The diversity of the accessions origin and the contrasting weather at those places make it difficult for the seed regeneration and cross formation at the Agricultural and Forestal Center. The place of origin of the accessions was diverse and it is from Fundación, Pivijay, Ciénaga till San Pedro de la Sierra (Table 1). The Department of Magdalena has a contrasting topography,

so, Santa Marta is placed at 2 MASL and the weather at the Experimental Center is classified as dessert. Bermejo and Jiménez (1976) make reference to the agro climatological conditions of this place, where rain is irregular and it is necessary to complement with programmed irrigation. Accessions 34 and 35 from San Pedro de la Sierra were not adapted to the edaphoclimatic conditions of the Center, where the sowing for pollinations was established, due, possibly, to the fact that at their original place the cold weather conditions prevail, then, the flowering time of both genotypes requires suitable levels of light and heat to induce the masculine and feminine flowers emergence. Rincón *et al.* (2006) point that the combination of high temperature and drought cause reduction in photosynthesis and in consequence in grain production. The high temperatures at the initial state of the grain filling period have negative effects in the individual weight of



**Picture 4.** Ear formation as product of the crossings between native accessions of corn from the Department of Magdalena, Colombia. The tag numbers correspond to the evaluated accessions.

grain, which is attributed to the reduction on the starch concentration.

The accessions 11, 90 and 91 coming from Fundación produced a reduced amount of seeds. This place is characterized for a temperature similar to the one in Santa Marta, where the Experimental Center is located, but the rainfall regimes are higher in Fundación. The IDEAM records place the Caribbean region on an annual rainfall range between 500 and 2000 mm

and Santa Marta between 500 and 1000 mm. The city recorded less than 50 rainy days in 2012 and in the period from December to April less than four days with rain; the periods with more rainy days were May, June and October with 5 to 10 rainy days. According to the data of the Simón Bolívar station, in 2012 in the rain in Santa Marta was 453 mm and the minimum water requirements for corn are 500 mm distributed along the crop cycle.



When drought happens by the time of crop establishment, the corn seedlings die; if the drought happens at the vegetative stage the leaf area and plant development are reduced, additionally of accelerating the leaf senescence and, if the drought occurs at flowering time yield is affected because the reserve formation is reduced, the number of grains per plant is reduced due to low fertilization or developmental arrest of the fertilized ovules, stamen growth is retarded (Lafitte, 2001). Rincón *et al.* (2006) consider that in corn temperatures higher than 35 °C together with a low relative humidity caused stigma desiccation; whereas temperature that is higher than 38 °C reduced the pollen viability. Based on the results of Rincón *et al.* (2007) it has been suggested that for each centigrade in temperature above the optimal (25 °C) the grain yield is reduced 3 to 4, a physiological behavior of the plant that explains why the low seed production in the Experimental Center, a place with weather stressful conditions for corn crops. In the F1 seed was also observed a similar trend to produce less seed when the multiplication was done (Table 3). According to Carballo and Hernández (n.d.) an ear from a local variety produces 400 grains approximately.

In the accession 91 the feminine flowers were scarce and the plant high was more than 2.5 m, making it difficult to manipulate the pollen and stigmas. The IDEAM (2012) recorded a wind speed for Santa Marta of 3 m/sec and the highest values were 4 m/sec in February and March, conditions that favored the constant leaf movement and pollen loss in corn.

In Santa Marta the air temperature is constant without contrasting variations. The weather is characterized as hot and in the period of June – September is slightly hotter (38 °C) than the other months of the year. The weather station Simón Bolívar recorded for 2012 a range of temperature, annual average, between 27 and 29 °C and, maximum temperature between 34 °C and 38 °C. According to Lafitte (2001) the corn in tropical zones is often exposed to higher temperatures than the optimal. When the

leaves freely transpire its temperature is 1 or 2 °C below the air temperature, avoiding tissue damage. The photosynthesis is reduced at leaf temperatures higher than 40 °C and the damage is accelerated at high temperatures causing the death of tissues. In corn, the higher photosynthetic capacity is presented at flowering, therefore, the assimilable matter available during this time is a critical factor that determines grain yield and, a reduced carbon and nitrogen flux towards the under developed grain is important since they determine its size. The pollen viability is reduced importantly above 35 °C, affecting directly the pollination. The high temperatures also increase the grain filling rate and shorten the duration of that period. The effects of the high temperatures are confused due to the water deficit.

## Conclusions

- The weather conditions at the Agricultural and Forestry Experimental Center of the Universidad de Magdalena were not suitable to produce abundant seeds from the directed crosses in native corn from the Department of Magdalena.
- The accessions 3202, 88, 89 and 92 were shown as good parentals in the breeding programs, showing high capacity for combination and seed production.

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