

Physiological and anatomical behaviour of two contrasting maize hybrids grown at high density sowing

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Abstract

This study evaluated the physiological and anatomical traits of two contrasting maize hybrids subjected to high density sowing in order to explain yield stability through yield component analysis and the relation between the grain yield capacities per plant according to individual growth. Experiment factors were two commercially important maize hybrids: DK190MGRR2 and DK670MG, which behave differently under stress, and two levels of plant density: 7 and 11 plant/m² high and recommended density to favour the conditions of water stress respectively. The experiment was carried out in the west of Argentina under rainfed conditions and five replications. Physiological parameters (pigments and endogenous content of phytohormones, injury of cell membranes, yield components) and anatomical parameters (stomatal behaviour, foliar and peduncles anatomy, xylem and phloem area in vascular bundles) were analyzed. In this study, both maize hybrids showed a differential response to both sowing densities. The DK670 showed an increased yield (24 and 10 % at 7 and 11 plant/m², respectively) due to enhanced individual yield components. This hybrid was differentiated by a higher stomatal density (7±1 in the abaxial side) and pigment amount (20%, mainly chl *a*) which led to a better photosynthetic ability. In addition, this hybrid showed the capacity to compensate damages derived from stress, mesophyll leaf thickness and closer bundles associated with tolerance to drought, and abundance in endogenous phytohormones to cope with stress. The description of these characteristics in response to different plant densities is novel results, especially those related to anatomical analysis under the different treatments. This research shows that the ability of hybrids to take advantage of abundance of resources at lower populations is an imperative need for over-seasonal potential yield accomplishment. This could help select more appropriate management practices and contribute to production areas.

Keywords: *Zea mays*; sowing density; maize hybrids; yield; morphophysiological responses.

Abbreviations: DK_Dekalb®; RR_Roundup® Resistant; ABA_abscisic acid; JA_jasmonic acid; IAA_indole acetic acid; SA_salicylic acid; ANOVA_Analysis of variance.

Introduction

Cereals constitute the basis for the world diet and among them; maize (*Zea mays* L.) is one of the most important. The increase in maize yield has been mainly associated with the increase in tolerance to different stresses (Tollenaar and Wu, 1999). Previous works show that modern hybrids that have been just released to the market are more tolerant to different stresses than old hybrids such as high density plants (Echarte et al., 2000; Ying et al., 2000; Tollenaar and Lee, 2002; Echarte et al., 2008). As a result, modern maize hybrids are more productive than the old ones when facing diverse quality environments (Campos et al., 2004), as in other cereals such as wheat (Slafer and Miralles, 1993; Alvaro et al., 2008). Maize grain yield is more affected by variations in plant population density than of other members of the grass family because of low tillering ability, monoecious floral organization, and the presence of a relatively short flowering period (Sangoi et al., 2002). The ideal plant population depends on several factors, e.g., water availability, soil fertility, hybrid maturity, and row spacing (Argenta et al., 2001). The use of lower plant densities delays canopy closure and decreases light interception, leading to high grain yield

per plant but low grain yield per unit area (Andrade et al., 1999). On the other hand, higher plant densities enhance interplant competition for assimilates, water and nutrients (Edmeades et al., 2000). High plant densities also stimulate barrenness and increase the anthesis-silking interval (Sangoi et al., 2001), thereby reducing kernel number per unit area, the main yield component of maize (Hall et al., 1980). Maize hybrids differ in their response to plant density (Andrade et al., 1996; Sangoi and Salvador, 1998; Echarte et al., 2000; Maddoni et al., 2001). Hybrids developed recently could withstand higher plant density levels than the older hybrids (Tollenaar, 1989). However, little is known about the morphoanatomic and ecophysiological processes and the yield components underlying these differences. Therefore, it is fundamental to be able to identify the different interactions, processes and mechanisms intended to optimize growth and economic yield, to increase production in a sustainable way (Andrade et al., 2005). The aim of this study was to evaluate and quantify the morphophysiological behavior of two maize hybrids submitted to high density sowing in order to explain yield stability, through the yield component analysis and the

relation between the grain yield capacities per plant according to individual growth.

Results

Chlorophyll and Carotenoid Content

The contents of the photosynthetic pigments were superior in the DK670 hybrid in both sowing densities, but there were no significant differences with respect to the DK190 hybrid at a density of 7 plants/m². Such contents at a density of 11 plants/m² were superior, but there were only significant differences between hybrids, resulting the DK670, the hybrid with higher *a* chlorophyll and carotenoids content (table 1).

Injury of cell membranes

The DK670 hybrid showed less injured cell membranes in both sowing densities with respect to the DK190 hybrid. The treatments in a density of 11 plants/m² increased the membrane injury significantly with respect to a density of 7 plants/m², in both maize hybrids (figure 1).

Anatomic analysis

A greater number of stomata were observed in the abaxial leaf face, in both hybrids and in both sowing densities. In the adaxial face, there were not significant differences between the hybrids in both sowing densities. The stomatal density in the DK670 hybrid abaxial face was superior to that of the DK190 hybrid, at a density of 7 plants/m², without any variations at a sowing density of 11 plants/m², but the DK190 hybrid increased the stomatal density reaching similar values of the DK670 hybrid (figure 2). Both maize hybrids showed a ‘Kranz’ foliar anatomy, typical of the Panicoideae grass subfamily, C4 species. Vascular bundles are surrounded by a sheath composed of uniform, thin-walled parenchymal cells, containing numerous amyloplasts. Surrounding the parenchymal sheath is a chlorophyllous mesophyll formed by cells radially arranged. The average thickness of the mesophyll in the DK190 hybrid leaf was 27% (25.07 µm) bigger with respect to the DK670 hybrid and it was kept at a high sowing density; while the DK670 hybrid mesophyll thickness increased 14% (12.8µm), when sowing density increases. The distance between the vascular bundles was only reduced (18%) in the DK190 hybrid at a density of 7 plants/m² while the distance in the DK670 hybrid was similar in both sowing densities. Significant differences were not observed in the xylem and phloem areas in the peduncles vascular bundles of the ear in both maize hybrids and in both sowing densities (figure 3a and b). Despite this, it is important to point out that a clear increasing tendency in the amount of xylem was observed at a higher density.

Content of ABA, JA, IAA and SA

The endogenous content of all the analyzed phytohormones, abscisic, jasmonic, indole acetic and salicylic acid in the DK670 hybrid was significantly higher with respect to the DK190 hybrid (table2). There were not significant differences between sowing density conditions.

Yield and yield components

The DK670 hybrid had the greatest weight of the grains per ear with respect to the DK190 hybrid, with a highly significant difference in the treatment at a density of 7

plants/m². There were not significant differences between hybrids under the same sowing density condition regarding the number of grains per ear, but in both hybrids this number was significantly reduced at a density of 11 plants/m². The ear length was also significantly reduced by the sowing density increase, while the DK670 showed a greater ear length with respect to the DK190 hybrid. The ear width did not show significant differences between the hybrids and the different sowing densities (table 3). It could be observed that the interaction of both maize hybrids and the sowing density was statistically significant only for grain yield. The number of grains/m² was affected significantly only for the production of grains while the grains individual weight was altered by the hybrid and density (table 4). After the analysis of the effect of different density treatments about the number of grains, it could be determined that the lowest densities created the greatest number of grains without statistical differentiation, while the highest densities produced the lowest number of grains (table 5). In relation to the grains weight, it could be observed that the highest density created a lower weight while the DK 670 produced heavier grains than the DK 190 (table 5). In figure 4a, it can be observed that before a decrease of sowing density, there is a greater production of the DK 670 hybrid. Within a higher plant density both hybrids showed a marked reduction of grain production as a consequence of a great fall in the yield per plant (figure 4b). When evaluating the relation between grains yield and sowing density through a polynomial function of second order; parameters for each hybrid were obtained through the regression analysis, with statistical significant differences between them (table 6). Therefore, the function for the DK670 hybrid ($P=671.98+40.76D-5.28D^2$) and for the DK 190 ($P=-6.87+163.80D-10.82D^2$) can be reconstructed.

Discussion

The DK670 hybrid showed the highest pigment content, and it was even significantly superior under higher sowing density conditions. This result gives such maize hybrid a greater capacity to compensate stress conditions, since the highest density indicates an environmental stress in the plants (Fontanetto et al., 2001). Besides, the great carotenoid content could be a factor that contributes to keeping the photosynthetic integrity structure, specially, the chloroplast, since they are considered non-enzymatic antioxidants (Foyer and Harbinson, 1994). Therefore, the DK670 hybrid could have a better photosynthetic activity, entailing a possible higher accumulation of dry matter in crop products (Thomas and Howarth, 2000). Moreover, the DK670 hybrid showed less injury in the cell membranes and in both sowing densities. These results reinforce the idea that this hybrid could increase the capacity to compensate damages derived from stress. The stability of the cell membrane has a critical role in keeping the metabolic activities of the cell under stress conditions, being this, one of the main components of tolerance (Bewley, 1979). The number of stomata in the adaxial surface in comparison with the abaxial one is a distinctive characteristic of different species (Tichá, 1982), but it was proved that it is sensitive to change in certain plant growth stages or in response to environmental stimulus (Piña, 1994). A greater number of stomata on the leaf reverse were observed in this study, in both hybrids and both sowing densities. Particularly, the DK670 hybrid stomata density was superior in comparison with the DK190 hybrid at a density of 7 plants/m². A higher density of stomata of smaller size is related to an increase in the diffusive resistance of the lamina

Table 1. Average values of chlorophylls and carotenoid ($\mu\text{g/g}$ fresh weight leaf) of the DK670 and DK190 maize hybrids in a plant density of 7 and 11 plants/ m^2 . Values within a row followed by asterisk (*) mean significant differences, with $P \leq 0.05$ for the Fisher a test.

	DK670		DK190	
	7	11	7	11
Plant Density	7	11	7	11
Chlorophyll a	231.02	282.00*	188.39	217.42
Chlorophyll b	130.00	151.06	109.32	144.74
Total Chlorophyll	361.03	433.06	297.71	383.08
Carotene	0.462	0.634*	0.470	0.551

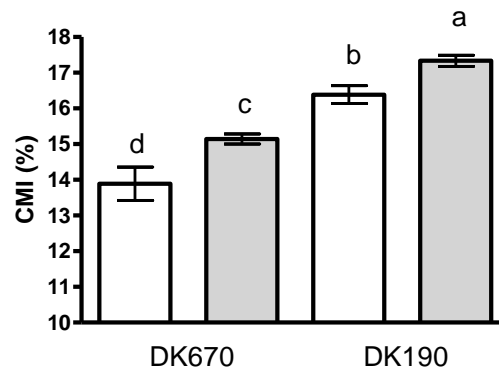


Fig1. Cellular Membrane Injury (CMI) (%) in leaves of the DK670 and DK190 maize hybrids in a plant density of 7 and 11 plants/ m^2 (light and dark bars, respectively). Different letters mean significant differences at $P \leq 0.05$ for the Fisher test.

that leads to a reduction in the foliar transpiration (Roth, 1990). This is why it is commonly associated with species adapted to growing in dry environments (Fahn and Cutler 1992, García and Lapp 2001, 2004). It is important to mention that the differences found in the stomatal density were not observed when evaluating the stomatal rates. These data coincide with the ones reported by Metcalfe and Chalk (1979), who mention that the size of stomata is usually associated with the stomatal density, where the small stomata have a high stomatal density and the big ones have a low one. However, the big stomata have a high stomatal rate related to their stomata size. Notwithstanding, according to Weng and Chen (1998) the stomatal density is positively related to a greater photosynthetic capacity. Therefore, a higher stomatal density in DK670, together with the higher amount of pigment and lower membranes injury, could be suggesting this greater photosynthetic capacity. At a higher sowing density, the DK670 hybrid increased the average thickness of the leaf mesophyll, which could indicate that under more unfavorable conditions of light effect this hybrid could increase its photosynthetic efficiency (Roth 1992). Distance between the bundles was shorter in the DK190 hybrid at a density of 7 plants/ m^2 . These reductions are a hint of organ better vascularization, since a denser veins connection can be created (Fahn and Cutler, 1992). This characteristic of great importance under conditions of limited water availability is lost in this hybrid under higher sowing density conditions, while the DK190 hybrid showed similar distance in both densities. The fact that the bundles are closer could be because of a greater parenchymal tissue volume, due to a higher number of cells or a higher volume each (García and Jáuregui, 2008). The xylem and phloem areas of the peduncle vascular bundles of the ear were similar in both maize hybrids and in both sowing densities. It is important to stand out that in the obtained samples in this study, the ABA

endogenous levels were superior in the DK670 hybrid, but this did not change the phloem area, as observed in previous studies with maize plants treated with ABA exogenously, which had a 40% phloem area increase in the peduncle vascular bundles of the ear (Travaglia et al., 2012). The fact that the DK670 hybrid had a greater content of all the phytohormones analyzed can offer a better resistance condition against an unfavorable environmental situation. It is already known that the sequential action of the JA, SA and ABA phytohormones plays a main role in the signal response of the plants facing environmental factors (O'Donnell et al., 2003). The higher levels of these maize plants hormones would allow different environmental stimulus in the DK670 hybrid, without demanding more energy for its biosynthesis, with respect to DK190. Selection of sowing density in maize is a decision of great influence on its yield, since this crop shows little foliar and reproductive plasticity (Cirilo, 2004). In this study, both maize hybrids showed a differential response to both sowing densities. The combination of density effect on the number of grains and density on the grains weight explains that the hybrid per density interaction is significant on the production of grains per area. It is clear that under the climatic conditions this experiment was carried out, sowing density increase caused higher stress, meaning a lower grain yield as a consequence of a big reduction of yield per plant, independently of the hybrid used. Within a higher plant density, the grain yield was reduced, which usually occurs with all hybrids in general (Kamara et al., 2006). This yield reduction together with the higher sowing density would indicate environmental stress in the plants, due to a reduction of the radiation, nutrients and soil water (Zhang et al., 2006). The maize hybrid and the plant density affected the length and diameter of the ear, a fact that was already reported with similar results (Zhang et al., 2006; Seyed Sharifi et al., 2009; Lashkari et al., 2011).

Table 2. Phytohormones content (ng/g DW): auxins (IAA), abscisic acid (ABA), salicylic acid (SA) and jasmonic acid (JA), in hybrid maize DK670 and DK190. Data represent the means and the asterisk (*) mean significant differences at $P \leq 0.05$, for the Fisher test.

Phytohormone	Hybrid	
	DK670	DK190
IAA	216235*	62721
ABA	27290*	187
SA	2413*	165
JA	928*	466

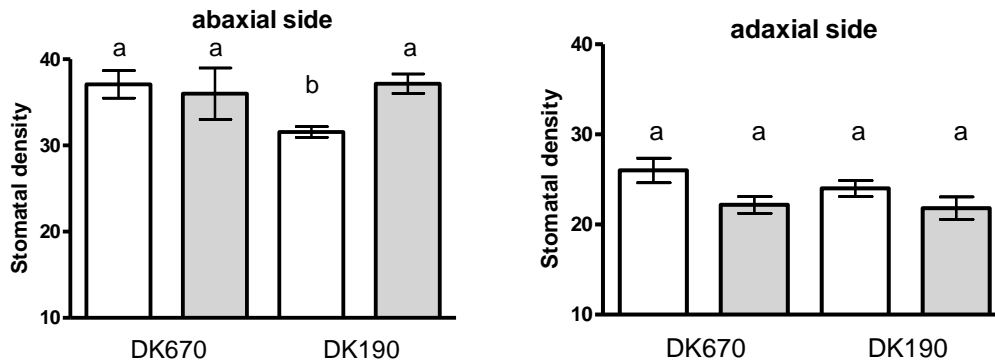


Fig 2. Stomatal density (number of stomata/mm²) on the abaxial and adaxial leaf sides of the DK670 and DK190 maize hybrids in a plant density of 7 and 11 plants/m² (light and dark bars respectively). Different letters mean significant differences at $P \leq 0.05$ for the Fisher test.

The lowest number of grains per ear at a higher sowing density can be the result of a lower flower primordia formation, a poor pollination as a result of a flowering asynchrony, or due to the grains abortion after fertilization (Daynard and Muldoon, 1983; Tetio-Kangho and Gardner, 1988). These results coincide with the ones obtained by Zhang et al. (2006), Seyed Sharifi et al. (2009) and Lashkari et al. (2011) who reported that the highest and lowest values of grains per ear were obtained at a sowing density of 7 and 13 plants/m² respectively. In this study, the DK670 hybrid showed an increase in most individual yield components with respect to the DK190 hybrid, resulting in increased yield. The relation between the yield of grains and the sowing density in maize is related to the polynomial function of second order, being this relation a characteristic of the genotype and its interaction with the environmental surrounding (Andrade et al. 1996). The results described help us understand that under the environmental conditions of this experiment, the DK670 hybrid has mechanisms that allow a higher production than the DK190 hybrid, mainly at a lower density. This higher production can be explained in terms of the grains greater weight. Under adverse conditions, the DK670 hybrid possibly keeps better physiological conditions during the grain filling which allows its individual weight increase when low sowing densities are used. As far as the yield by density interaction is concerned, major emphasis is commonly given on high populations and the same is obvious in this study. Modern hybrids are largely reliant on high populations. Instead, good performance at low populations is essential to cope with the highly diverse environments and is drawn from a number of recent studies (Norwood 2001; Kiniry et al. 2002; Blumenthal et al., 2003; Shanahan et al. 2004; Duvick, 2005; Tokatlidis et al., 2011; Berzsenyi and Tokatlidis, 2012; Tokatlidis, 2013).

Materials and Methods

Plant materials

The maize hybrids DK190MGRR2 and DK670MG were selected because of their commercial importance in the area under study and based on studies in which important genetic differences were detected before the behaviour against stress, having to elucidate other aspects associated with different environmental conditions (De Santa Eduvigis, 2010). The characterizations of both maize hybrids are listed in Table 7 (Tecnocampo, 2010).

Experimental design and management

Field research was conducted in 2010 and 2011 growing season in the central semiarid Pampa region, in the south of the province of San Luis, Buena Esperanza (34° 45'S, 65° 15'O), Argentina. The total rainfall received at this site was 396.9 mm/crop cycle and the average temperature was 17.4° C. An estimation of the relationship between rainfall and evapotranspiration reports a strong moisture deficit recorded throughout the year. Weed, pests and diseases control were done in a timely manner. It was fertilized according to the recommendations from the soil analysis and the area common practices, to avoid possible nutritional inferences. Plot area was 7x2.8m with 4 rows per plot. Blocks were separated by 1m unplanted distances. The experiment was laid out in randomized complete block design with factorial arrangement and five replications. Experiment factors were two maize hybrids: DK190MGRR2 and DK670MG, and two levels of plant density: 7 and 11 plant/m². The 7 plant/m² sowing density is the recommended one for this region.

Table 3. Weight (g) and number of grains/ear and length and diameter ear (cm) at harvest, of the DK670 and DK190 maize hybrids in a plant density of 7 and 11 plants/m². Data represent the means and different letters mean significant differences at $P \leq 0.05$ for the Fisher test.

	DK670		DK190	
	7	11	7	11
Plant Density	7	11	7	11
Weight grains	137.24 a	100.73 b	101.16 b	91.81 b
Nº grains	484 a	396 b	470 a	402 b
Length ear	14.88 a	13.15 c	13.83 b	12.67 c
Diameter ear	13.87 a	13.58 a	13.48 a	13.10 a

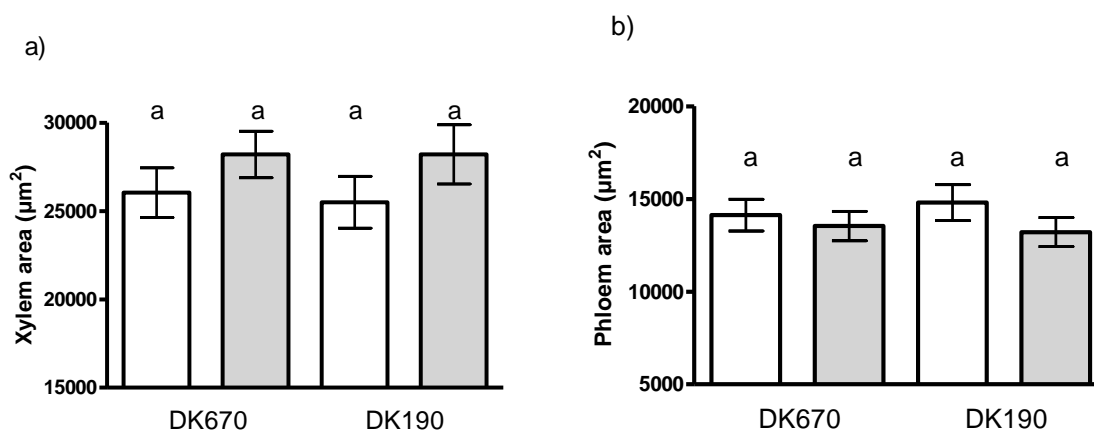


Fig 3. Area (µm²) of the vascular tissues, a) Xylem and b) Phloem of the peduncle vascular bundles of the DK670 and DK190 maize hybrids in a plant density of 7 and 11 plants/m² (light and dark bars respectively). Different letters mean significant differences at $P \leq 0.05$ for the Fisher test.

This is why an increased density of 50% higher than the one recommended was evaluated in order to favour water stress conditions. This is why the following variables were evaluated in different phenological stages, from anthesis to physiological maturity for each hybrid, using Zadocks's scale (Zadocks et al., 1974).

Measurements of traits

For pigment measurement, 50 mg fresh weight (FW) of the flag leaf was homogenized in a mortar with 10 ml of 80% acetone. The homogenate was loaded into Eppendorf tubes, and after 1 h at 4°C to allow pigment extraction, it was centrifuged (twice) for 5 min at 5000 rpm. Aliquots were taken and chlorophyll a and b levels were measured by spectrophotometry at 650 and 665 nm, respectively. Five millilitres of 1 M NaOH and 15 ml of diethyl ether were added to the total volume. Carotene content was assessed from the ether fraction by spectrophotometry at 450 nm (modified from Mac Kinney, 1938). In order to determine the cellular membrane injury, leaf pieces were quickly washed three times and then immersed in 10 ml of deionized water for 24 h at 10° C. The electrical conductivity was measured and then the leaf tissues were killed by autoclaving for 15 min, cooled to 25° C, and the electrical conductivity was measured for the second time. Membrane injury was evaluated as the percentage injury index following the Sullivan's formula (1971), $J = [1 - (1 - T_1/T_2) / (1 - C_1/C_2)] \times 100\%$, where C_1 and C_2 represent conductivity measurements of control samples before and after autoclaving respectively; and T_1 and T_2 represent conductivity measurements of water-stressed samples before and after autoclaving, respectively. For stomatal behaviour a layer of acrylic (synthetic nail amend) was brushed on both adaxial and abaxial sides of the leaf, allowed to dry for a few seconds, then carefully extracted and mounted for microscope observation (D'Ambriogo de Argüeso, 1986). A standard Zeiss model 16

microscope was used to assess the histological preparations, and photomicrographs were taken with a Zeiss Axiophot microscope equipped with image capture and digitization (AxioVision 4.3, with camera AxioCam HRc). Numbers of stomata (s) and epidermal cells (e) for each film strip were counted. The leaf stomatal index was estimated using the formula $[s/(e + s)] \times 100$. The leaf stomatal density was expressed as the number of stomata per unit leaf area (Salisbury, 1928). Cross-sections of flag leaf and the peduncles of the upper female inflorescences were collected for the anatomic analysis. Samples were obtained at R1 and R3 respectively. The sections were processed using microtechnical methods, following Johansen's recommendations (1940), which involved the use of tissues in dehydration, infiltration and paraffin embedding, rotary microtome cutting, staining, and observation. A standard Zeiss Model 16 microscope was used to assess the histological preparations, and photomicrographs were taken with a Zeiss Axiophot microscope with an AxioVision 4.3 image capture and a digitalization piece of equipment and an AxioCam HRc camera. In the cross-section of the leaf mesophyll, the thickness and the distance between the vascular bundles was determined. In the cross-section of the peduncle, the vascular bundles were counted and, in these bundles, the area covered by the phloem and xylem - phloem relationship were estimated with an Iproplus area integrator. In order to determine the phytohormone content, 200 mg of lyophilized leaves were crushed with N liquid and then homogenized with 5 ml of distilled water, demonized and acidified with Acetic Acid at 1%. The corresponding internal standard was added (IAA, ABA, SA, JA) (Olchemim, Check Republic). After filtering and evaporating at total reduction, it was dissolved in 50 µl of methanol 100 %. The determinations were done in an LC-ESI-MS-MS Alliance piece of equipment (Waters 2695) as described by Zhou et al. (2003). The data were expressed as ng/g of sample. At harvest, the spike length and width as

Table 4. Probability value ANOVA for grain yield, number of grains per unit area and individual grain weight. P-values ≤ 0.005 were considered significant. CV: Coefficient of Variation.

	Grain yield	N° grains/m ²	Grain weight
Hybrid	0.0001	0.4659	0.0002
Density	0.0001	0.0001	0.0370
Hybrid/Density	0.0001	0.2961	0.1712
CV	6.21	9.94	8.28

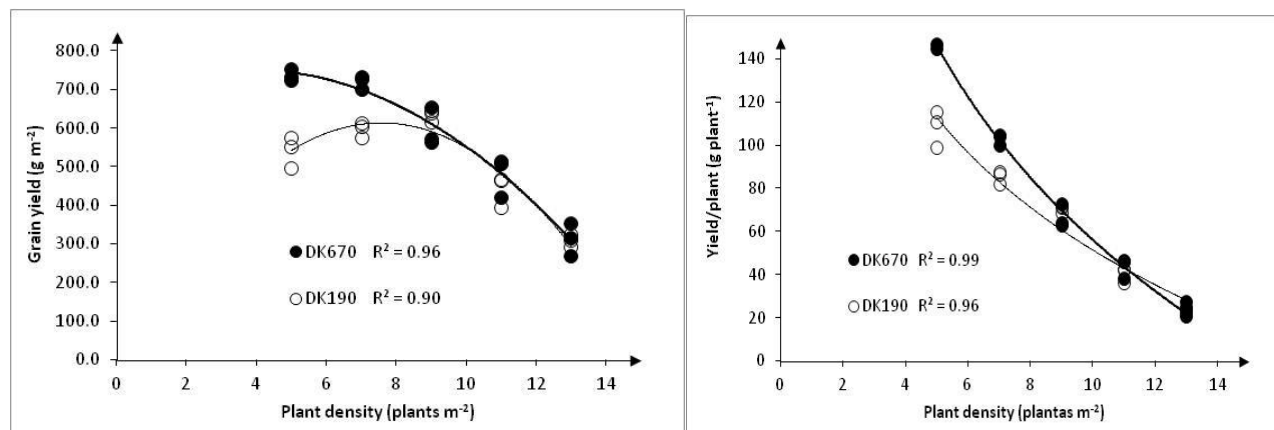


Fig 4. Relationship between: grain yield and plant density (a), and between yield/plant and plant density (b), in hybrid maize DK670 (full circles) and DK190 (empty circles). Circle values are measured data and lines are polynomial regression adjusted to 5% probability.

Table 5. Number of grains per unit area and individual grain weight. Data represent the means for hybrid (DK 670 and DK 190) and density (5, 7, 9, 11, 13 plants/m²). Fisher's Least Significant Difference (LSD) test, P-values ≤ 0.005 .

	...N° grains/m ²	Grains weight
DK 670	2425 a	0.2326 a
DK 190	2492 a	0.2021 b
DMS	187	0.0138
5 plants/m ²	2791 a	0.2294 a
7 plants/m ²	2882 a	0.2290 a
9 plantas/m ²	2929 a	0.2105 a
11 plants/m ²	2119 b	0.2196 a
13 plants/m ²	1575 c	0.1983 b
LSD	296	0.0218

Table 6. The estimated value of each parameter of the model and p value of the non-linear regression analysis, considering the inferior values to 0.05 significant.

Parameter	Valor	p-valor
β_0	332.55	0.0005
$\beta_1 D$	102.28	<0.0001
$\beta_2 D^2$	-8.05	<0.0001
$\beta_3 H$	339.43	0.0004
$\beta_4 HD$	-61.52	0.0044
$\beta_5 HD^2$	2.77	0.0168

P is the production/m² maize grains; D is the maize plant/m² density sowed; H is the dummy variable to add the hybrid effect to the function; β_0 , β_1 , β_2 , β_3 , β_4 and β_5 , are the model parameters.

Table 7. Characterization of maize hybrids.

Hybrid	DK190 MGRR2	DK670 MG
Hybrid type	Simple (F1)	Simple (F1)
Cycle	intermediate	intermediate
Thermal requirements Ve-VT	675 °C/days	650 °C/days
Thermal requirements Ve-R1	696 °C/days	660 °C/days
Relative maturity	121 days	117 days
Grain	Hard, orange	Hard, orange
Drought tolerance	medium	high

well as the number and weight of grains per spikes of all the treatments were evaluated. In order to establish the interaction between the DK 670 and 190 maize hybrids, and the sowing density, grain yield measurements per area and plant, for each hybrid in 5 different densities, as well as the number of grains per area and the individual grains weight.

Statistical analyses

The results were analyzed for variance using the InfoStat statistical analysis software (professional version 1.1), and the LSD Fisher 5% test was used to compare differences among treatments. In the tables, different letters mean significant differences with $P \leq 0.05$. In the figures, bars represent standard errors of the mean value. Besides, the non linear regression analysis for the parameters estimation of the production function according to sowing density and its interaction with the hybrid, adjusted to the polynomial formula of second order: $P = \beta_0 + \beta_1 D + \beta_2 D^2 + \beta_3 H + \beta_4 HD + \beta_5 HD^2$ where, P is the production/m² maize grains; D is the maize plant/m² density sowed; H is the dummy variable to add the hybrid effect to the function; $\beta_0, \beta_1, \beta_2, \beta_3, \beta_4$ and β_5 , are the model parameters.

Conclusions

Considering that for every production system there is a population that maximizes grain yield, in this study, it can be concluded that there were differences between modern maize

hybrids, and that the highest yield of grains was obtained by the DK670 hybrid at a density of 7 plants/m², along with tolerance to high populations improved individual performance. This suggests that such hybrid under this sowing condition can be recommended to be used in the region. The description of morphophysiological behavior of maize hybrids grown at high density sowing are novel results, especially those related with anatomic analysis. This research shows that the ability of hybrids to take advantage of abundance in resources at lower populations is an imperative need for over-season potential yield accomplishment and that breeding density-independent hybrids help in maize yield under field-grown. This could help select more appropriate management practices, implying an interesting contribution to the productive area.

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References

Alvaro F, Isidro J, Villegas D, García del Moral LF, Royo C (2008) Old and modern durum wheat varieties from Italy and Spain differ in spike components. *Field Crop Res.* 106: 86–93.
 Andrade F, Cirilo A, Uhart S, Otegui M (1996) *Ecofisiología del cultivo de maíz*. Dekalb Press. Requerimientos hídricos. Capítulo. 5: 121-146.

Andrade F, Vega C, Uhart S, Cirilo A, Cantero M, Valentinuz O (1999) Kernel number determination in maize. *Crop Sci.* 39: 453-459.
 Argenta G, Silva PRF, Sangoi L (2001) Arranjo de plantas em milho: análise do estado da arte. *Ci Rur.* 31: 1075-1084.
 Berzsenyi Z, Tokatlidis IS (2012) Density Dependence Rather Than Maturity Determines Hybrid Selection in Dryland Maize Production. *Agron J.* 104(2):331–336.
 Bewley JD (1979) Physiological aspects of desiccation tolerance. *Ann Rev Plant Physiol.* 30: 195-238.
 Blumenthal JM, Lyon DJ, Stroup W (2003) Optimal Plant Population and Nitrogen Fertility for Dryland Corn in Western Nebraska. *Agron J.* 95:878–883.
 Campos H, Cooper M, Habben J, Edmeades G, Schussler J (2004) Improving drought tolerance in maize: view from industry. *Field Crop Res.* 90: 19-34.
 Cirilo AG (2004) Fecha de Siembra y Rendimiento en Maíz. *IDIA XXI. Ed. INTA. N° 6:* 122-127.
 D'Ambrigiolo de Argüeso A (1986) *Manual de técnicas en histología Vegetal. Hemisferio Sur* (ed.). Buenos Aires, Argentina.
 Daynard T, Muldoon J (1983) Plant-to-plant variability of maize plants grown at different densities. *Can J Plant Sci.* 63: 45-59.
 De Santa Eduvigis JM (2010) *Potencial de rendimiento y tolerancia a sequía en híbridos de maíz*. Tesis para Título de Magister Scientiae en Producción Vegetal Facultad de Ciencias Agrarias, Universidad Nacional de Mar Del Plata.
 Duvick DN (2005) The contribution of breeding to yield advances in maize (*Zea mays* L.). *Adv Agron.* 86: 83-145.
 Echarte L, Luque S, Andrade F, Sadras V, Cirilo A, Otegui M, Vega C (2000) Response of maize kernel number to plant density in Argentinean hybrids released between 1965 and 1993. *Field Crops Res.* 68: 1-8.
 Echarte L, Rothstein S, Tollenaar M (2008) The response of leaf photosynthesis and dry matter accumulation to nitrogen supply in an older and a newer maize hybrid. *Crop Sci.* 48: 656-665.
 Edmeades GO, Bolanos J, Elings A, Ribaut JM, Banziger M (2000) The role and regulation of the anthesis/silking interval in maize. In: Westgate, M.E. Boote, K.J. (eds.), *Physiology and Modeling Kernel Set in Maize*. CSSA. Madison, WI, pp: 43-73.
 Fahn D, Cutler D (1992) Xerophytes. *Encyclopedia of plant anatomy XIII*. Gebruder Borntraeger. Berlin. 177.
 Fontanetto H, Vivas H, Keller O (2001) INTA Anuario Agronomía. Eficiencia del uso del nitrógeno en maíz con siembra directa. Efecto de la densidad de siembra.
 Foyer C, Harbinson J (1994) Oxygen metabolism and the regulation of photosynthetic electron transport. In: Foyer C, Mullineaux P, (eds.). *Causes of photooxidative stress and amelioration of defense systems in plants*. Boca Raton, FL: CRC Press, 1-42.
 García M, Jáuregui D (2008) Efecto de la salinización con NaCl o Na₂SO₄ sobre la anatomía foliar en dos genotipos de caña de azúcar (*Saccharum* spp.) con tolerancia salina diferencial. *Ernstia.* 18(1): 89-105.
 Garcia M, Lapp M (2001) Morfoanatomía foliar en tres biotipos de *Pithecellobium unguis-cati* (L.) Bentham creciendo en distintas comunidades vegetales. *Phyton.* 20: 147-158.
 Garcia M, Lapp M (2004) Anatomía foliar en especímenes de *Oyedaea verbessinoides* DC. (Asteraceae) creciendo en tres ambientes distintos. *Ernstia.* 15: 129-143.

- Hall AJ, Ginzo HD, Lemcoff JH, Soriano A (1980) Influence of drought during pollen-shedding on flowering, growth and yield of maize. *J Agron Crop Sci.* 149: 287-298.
- Johansen D (1940) Plant microtechnique. McGraw-Hill Book Co. Inc. New York. XI + pp 523.
- Kamara A, Menkir A, Kureh I, Omoigui L, Ekeleme F (2006) Performance of old and new maize hybrids grown at high plant densities in the tropical Guinea savanna. *Commun Biometry Crop Sci.* 1 (1): 41-48.
- Kiniry JR, Xie Y, Gerik TJ (2002) Similarity of maize seed number responses for a diverse set of sites. *Agronomie.* 22:265-272.
- Norwood CA (2001) Dryland corn in western Kansas: Effect of hybrid maturity, planting date and plant population. *Agron J.* 93: 540-547.
- Konuskan O (2000) Effects of plant density on yield and yield related characters of some maize hybrids grown in hatay conditions as 2nd crop. M.Sc. Thesis, Science Institute, Madurai Kamaraj University, p. 71.
- Lashkari M, Madani H, Ardakani M, Golzardi F, Zargari K (2011) Effect of plant density on yield and yield components of different corn (*Zea mays* L.) hybrids. *American-Eurasian J Agric Environ Sci.* 10(3): 450-457.
- Mac Kinney G (1938) Some absorption spectra of leaf extract. *Plant Physiol.* 13: 128-140.
- Maddonni GA, Otegui ME, Cirilo AG (2001) Plant population density, row spacing and hybrid effects on maize canopy architecture and light attenuation. *Field Crop Res.* 71: 183-193.
- Metcalf CR, Chalk L (1979) *Anatomy of the Dicotyledons.* I. Clarendon Press, Oxford.
- O'Donnell P, Schmelz, E, Moussatche P, Lund S, Jones J, Klee H (2003) Susceptible to intolerance: a range of hormonal actions in a susceptible *Arabidopsis* pathogen response. *Plant J.* 33: 245-257.
- Piña JM (1994) El cultivo del chícharo (*Pisum sativum* L.): Su respuesta bajo condiciones de acolchado de suelos y azufre elemental. Tesis de Licenciatura en Biología. División de Ciencias Biológicas, ICCAC. Saltillo, Coah.
- Roth I (1990) Leaf structure of a Venezuelan cloud forest in relation to the microclimate. *Gebruder Borntraeger, Berlin, Germany.* p 244.
- Roth I (1992) Anatomía de las plantas superiores. Tercera edición. Ediciones de la Biblioteca. Universidad Central de Venezuela. Caracas pp 273-287.
- Salisbury E (1928) On the causes and ecological significance of stomatal frequency with special reference to woodland flora. *Philosophical Transactions of the Royal Society* 216: 1-65.
- Sangoi L (2001) Understanding plant density effects on maize growth and development: an important issue to maximize grain yield. *Ciencia Rural.* 31(1): 159-168.
- Sangoi L, Gracietti MA, Rampazzo C, Bianchetti, P (2002) Response of Brazilian maize hybrids from different ears to changes in plant density. *Field Crop Res.* 79: 39-51.
- Sangoi L, Salvador RJ (1998) Influence of plant height and leaf number on maize production at high plant densities. *Pseq Agrop Bras.* 33: 297-306.
- Seyed Sharifi R, Sedghi M, Gholipouri A (2009) Effect of population density on yield and yield attributes of maize hybrids. *Res J Biol Sci.* 4 (4): 375-390.
- Shanahan JF, Doerge TA, Johnson JJ, Vigil MF (2004) Feasibility of site-specific management of corn hybrids and plant densities in the great plains. *Precis Agric.* 5:207-225.
- Tecnocampo (2010) Insumos estratégicos, semillas de maíz. <http://www.tecnocampo.com/website/map.html>
- Tokatlidis IS, Has V, Melidis V, Has I, Mylonas I, Evgenidis G (2011) Maize hybrids less dependent on high plant densities improve resource-use efficiency in rainfed and irrigated conditions. *Field Crops Res.* 120: 345-351.
- Slafer GA, Miralles DJ (1993) Fruiting efficiency in 3 bread wheat (*Triticum aestivum*) cultivars released at different eras -number of grains per spike and grain weight. *J Agron Crop Sci.* 170: 251-260.
- Sullivan C (1971) Technique for measuring plant drought injury and resistance in crops. (ed) K.I. Larson & J.D. Eastin, Madison, Wisconsin; Crop Science Society of America, 1-18.
- Tetio-Kangho F, Gardner F (1988) Responses of maize to plant population density. II Reproductive development, yield and yield adjustments. *Agron J.* 80: 935-940.
- Thomas H, Howarth C (2000) Five ways to stay green. *J Exp Bot.* 51: 329-337.
- Tichá I (1982) Photosynthetic characteristics during ontogenesis of leaves. 7. Stomata density and sizes. *Photosynthetica.* 16: 375-471.
- Tokatlidis IS (2013) Adapting maize crop to climate change. *Agron Sustain Develop.* 33:63-79.
- Tollenaar M (1989) Genetic improvement in grain yield of commercial maize hybrids grown in Ontario from 1959-1988. *Crop Sci.* 29: 1365-1371.
- Tollenaar M, Lee E (2002) Yield potential, yield stability and stress tolerance in maize. *Field Crops Res.* 75: 161-169.
- Tollenaar M, Wu J (1999) Yield improvement in temperate maize is attributable to greater stress tolerant. *Crop Sci.* 39: 1597-1604.
- Travaglia C, Balboa G, Espósito G, Reinoso H (2012) ABA action on the production and redistribution of field-grown maize carbohydrates in semiarid regions. *Plant Growth Regulation* 67: 27-34.
- Weng JH, Chen CY (1998) Stomatal frequency associated with an sterase band in rice genotypes. <http://probe.nalusda.gov:8000/otherdocs/rgn/rgn5/V5IV26.html>
- Ying J, Lee E, Tollenaar M (2000) Response of maize leaf photosynthesis to low temperature during the grain-filling period. *Field Crops Res.* 68: 87-96.
- Zadocks J, Chang, T, Konzak C (1974) A decimal code for the growth stage of cereals. *Weed Res.* 14: 415-421.
- Zhang J, Dong, S, Wang K, Hu C, Liu P (2006) Effects of shading on the growth, development and grain yield of summer maize. *Ying Yong Sheng Tai Xue Bao Chinese.* 17(4): 657-662.
- Zhou R, Squires TM, Ambrose SJ, Abrams SR, Ross AR, Cutler AJ (2003) Rapid extraction of ABA and its metabolites for liquid chromatography-tandem mass spectrometry analysis. *J Chromatogr.* 1010: 75-85.