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Genotypic Differences among Argentinean Maize Hybrids in Yield Response to Stand Density

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ABSTRACT

Maize (*Zea mays* L.) stand density selection is an important management practice because yield is maximized at a particular optimum value. Optimum stand density (OSD) varies across environments, and many have argued that current commercial genotypes differ in their optimum stand density for a similar environment. We tested this concept by planting 11 Argentinean commercial genotypes from four seed companies at a range of stand densities (1, 8, and 16 plants m^{-2}) in two environments. Genotypes differed in their yield response to changes in stand density, and their OSD varied from 7.3 to 11.9 plants m^{-2}. Yield of tested genotypes was similar at the lowest stand density but different at the highest density, indicating no differences in potential yield per plant but significant differences in crowding tolerance. When using a crop growth and biomass partitioning framework for understanding kernel set differences among genotypes in their response to stand density, hybrids differed in most measured traits, showing differential strategies for coping with stress tolerance. Under high stand density conditions, genotypic strategies for avoiding barrenness were key for hybrid tolerance to crowding stress. We conclude that stand density management needs to take into account not only the environment but also the specific genotype, especially under high density management systems.

Maize grain yield has a parabolic response to stand density changes, and there is an optimum stand density that maximizes yield (Echarte et al., 2000; Sangoi et al., 2002; Sarlangue et al., 2007). This is widely known by crop managers, physiologists, and breeders; together with the concept that this optimum stand density varies with the environment (e.g., N and water availability). Better environments have maximum yields at higher stand densities (Al-Kaisi and Yin, 2003). This creates a need to decide which stand density is needed at each environment (Reeves and Cox, 2013; Van Roekel and Coulter, 2011, 2012; Robles et al., 2012). Recently, however, many have argued that stand density management also needs to consider the specific genotype because commercial hybrids differ in their optimum stand density for similar environments (Sarlangue et al., 2007).

Maize grain yield response to stand density changes is usually dissected in two components, potential yield per plant and tolerance to crowding stress. The last component has been successfully enhanced by breeding and is responsible for most yield improvements (Russell, 1991; Tollenaar and Wu, 1999; Duvick and Cassman, 1999; Sangoi et al., 2002; Duvick et al., 2004; Tokatlidis and Koutroubas, 2004; O'Neill et al., 2004). There is evidence that the first component, potential yield per plant, has increased (Ci et al., 2011; Luque et al., 2006) or not altered at all (Duvick and Cassman, 1999; Duvick et al., 2004; Tollenaar and Wu, 1999; Sangoi et al., 2002). It is accepted that greater crowding tolerance of modern genotypes allows using higher stand densities when compared to older ones (Tollenaar et al., 1994; Tollenaar and Wu, 1999; Duvick et al., 2004; Lee and Tollenaar, 2007). This has steadily increased the stand density farmers are using. Sarlangue et al. (2007) found differences in optimum stand density when current commercial genotypes with different canopy structure and maturity were compared, short-season hybrids showed reduced plant size and higher optimum stand densities.

At the individual plant level yield is mostly correlated to changes in kernel number per plant (KNP; Andrade et al., 1999; Vega et al., 2001). The number of established kernels depends on the accumulation of ear biomass (EB) around the flowering period and in the efficiency this biomass is used for setting kernels (Fig. 1A). The accumulation of EB depends on total plant growth (plant growth rate around flowering, PGR; Otegui and Bonhomme, 1998; Andrade et al., 1999) and in the partitioning of this plant biomass to the developing ear (Vega et al., 2001; Echarte et al., 2004; Fig. 1B).

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Abbreviations: C_{EB}, curvature of ear biomass accumulated at 15 days after anthesis vs. plant growth rate around flowering; C_{KN}, curvature of kernel number per plant vs. ear biomass accumulated at 15 days after anthesis; CVPGR, coefficient of variation of plant growth rate around flowering; EB, ear biomass accumulated at 15 days after anthesis; IS_{EB}, initial slope of plant growth rate around flowering vs. ear biomass accumulated at 15 days after anthesis; IS_{KN}, initial slope of kernel number per plant vs. ear biomass accumulated at 15 days after anthesis; KNP, kernel number per plant; OSD, optimum stand density; PGR, plant growth rate around flowering; PGR_b, base plant growth rate for ear biomass accumulation.

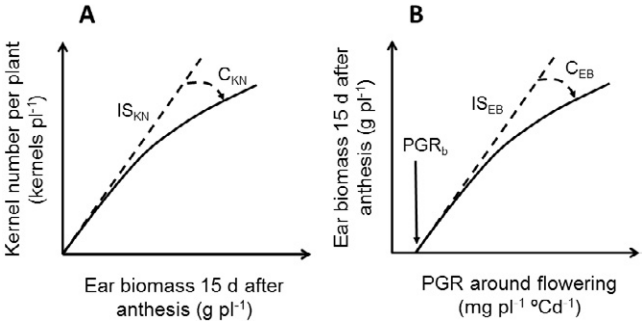

Fig. 1. Relationship between (A) kernel number per plant (KNP) and accumulated ear biomass 15 days after anthesis (EB) and (B) between EB and plant growth rate around flowering (PGR). IS_{KN}: initial slope of the ear biomass 15 days after anthesis vs. kernel number per plant relationship and C_{KN}: curvature degree of the ear biomass 15 days after anthesis vs. kernel number per plant relationship, PGR_b: base plant growth rate around flowering for ear biomass 15 days after anthesis, IS_{EB}: initial slope of the relationship between plant growth rate around flowering and ear biomass 15 days after anthesis, C_{EB}: curvature of the plant growth rate around flowering vs. ear biomass 15 days after anthesis relationship. Figures are based on Borrás et al. (2007, 2009).

There are several reports describing genotypic variability in traits like PGR, biomass partition to ears during this period, and kernel set per unit of accumulated EB between older and modern genotypes (Tollenaar et al., 1994; Echarte et al., 2000, 2004; Luque et al., 2006; D'Andrea et al., 2009) or between different types of maize (popcorns vs. dents, Severini et al., 2011; inbred lines vs. hybrids, Borrás et al., 2009). Genotypic differences in these processes create the need to describe KNP as a function of PGR as a two-step process (Fig. 1). But, reports describing variability among current commercial genotypes for these traits is scarce, and usually involve few genotypes (Pagano et al., 2007; Rossini et al., 2011). We hypothesize these differences are behind reported genotypic differences in optimum stand density among current commercial genotypes.

The objectives of the present study were: (i) to test if a set of current Argentinean commercial hybrids from different seed companies differ in their response to changes in stand density and quantify such differences, (ii) to determine if genotypic differences are related to yield potential per plant and/or to crowding tolerance, and (iii) to determine which physiological mechanisms are underlying genotype differences in stand density response. We used a crop physiology model with an individual plant approach in an attempt to describe the mechanisms behind genotypic differences in stand density response and genotype × stand density interactions for grain yield.

MATERIALS AND METHODS

Site and Crop Management

Experiments were conducted at Campo Experimental Villarino located in Zavalla (33°1′ S, 60°53′ W), Provincia de Santa Fe, Argentina, during 2011/2012 (Year 1) and 2012/2013 (Year 2). The soil was a silty clay loam vertic argiudoll, Roldán series. Planting dates were 17 Oct. 2011 and 23 Sept. 2012. Both experiments were planted under no-tillage conditions and the previous crop was always soybean [*Glycine max* (L.) Merr.]. Fertilizer (20–0–0–16, N–P–K–S) was broadcast 4 to 5 d before planting with a rate of 100 kg N ha^{-1}, and MAP (10–50–0–0) was applied at planting with a rate of 20 kg N ha^{-1}. The experiments were conducted under rain-fed conditions, except in Year 1 when 35 mm of water were applied at flowering with a sprinkler irrigation system. Year 1 was a dry year with 452 mm rainfall during the entire growing season, while Year 2 had 682 mm rainfall during the same period. During the flowering period, about 30 d around flowering, rainfall was 107 and 216 mm for Years 1 and 2, respectively. Weeds were controlled using standard agronomic practices and manually removed whenever necessary.

Plant Material and Experimental Design

Eleven commercial Argentinean maize hybrids (all single cross hybrids) with similar maturity group (116–126) were tested at three stand densities: low, intermediate, and high (1, 8, and 16 plants m^{-2}, respectively). The concept was not to have a large number of stand densities tested but to use stand densities that are contrasting so that the isolated plant and the plant under severe stress are considered in this analysis. Hybrids corresponding to four seed companies were NK860, NK900 (Syngenta), P1979, P2069Y, and P2053Y (Pioneer-DuPont), DK670, DK692 (Dekalb-Monsanto), AX852, AX886, AX887, and NPreCom (Nidera). NPreCom was a pre-commercial genotype from Nidera at the time the experiments were conducted. Maturity group for each genotype was 116 for AX852; 117 for DK670; 118 for DK692; 119 for P1979, AX886, AX887, and NPreCom; 120 for P2069 and P2053; 122 for NK860; and 126 for NK900. Despite maturity groups reported by seed companies were slightly different, genotypes flowered and attained physiological maturity within a limited time period. During Year 1 all genotypes flowered within a 7 d time window, and attained physiological maturity within 13 d. During Year 2 the flowering window was 5 d, and physiological maturity range was 10 d across genotypes. Experiments were arranged in randomized complete block design with three replicates. Plots were six rows for the lowest stand density and four rows for intermediate and high stand densities. Rows were 0.52 m apart and 6 m long. Plots were overplanted and manually thinned at V2 to V3 ligulated leaves stage (Ritchie et al., 1993).

Phenotypic Measurements

Measurements were done on 10, 15, and 20 plants (low, intermediate, and high stand density plots, respectively) from the central rows of each plot. These plants were tagged at random 15 d before 50% anthesis and were always selected in one, two, or three sets of consecutive plants. Nondestructive allometric models (Vega et al., 2000) were used to estimate plant biomass at the pre- and post-flowering stages for all tagged plants. The allometric approach was used to document biomass accumulation, biomass partitioning around the flowering period, and kernel set at the individual plant level. This technique provides an accurate representation of plant biomass corresponding to tagged plants remaining in the field until harvest (Vega et al., 2000; Echarte et al., 2004; Gambín et al., 2008; Borrás et al., 2009). Allometric models were developed with 18 additional tagged plants per genotype per year from border rows. Pre-flowering biomass samples were taken –15 d before 50% anthesis. The pre-flowering model was based on the linear regression between shoot biomass and stem volume (Vega et al., 2001; Gambín et al., 2008). Stem volume was calculated from plant height (ground level up to the uppermost leaf collar)

and stem diameter at the base of the stalk. The post-flowering biomass sample was made 15 d after 50% anthesis, and the model utilized stem volume and maximum diameter of every ear with husks showing silks. This was fitted using a multiple linear regression analysis (Vega et al., 2001; Borrás and Otegui, 2001; Gambín et al., 2008). Biomass was determined after cutting plants and drying them in a forced-air oven at 65°C for at least 7 d. During the post-flowering sampling ears with visible silks were separated, and used to estimate accumulated EB 15 d after 50% anthesis.

Plant growth rate around flowering (mg plant^{-1} °C d^{-1}) was calculated as the difference between post-flowering and pre-flowering plant biomass (mg plant^{-1}) divided by the accumulated thermal time between sample dates. Daily thermal time values were calculated using a base temperature of 8°C. Plant growth rate plant-to-plant variability was determined on a plot basis using the individual PGR data from tagged plants and represented by the coefficient of variation of plant growth rate (CV_{PGR}).

Ears from tagged plants were harvested at physiological maturity. Kernels per plant were counted manually. Ears were dried in a forced-air oven and shelled individually. Kernel weight was calculated as the ratio between individual plant yield and kernel number. Yield values were corrected to 145 g kg^{-1} moisture; plant yield was the average between 10, 15 or 20 tagged plants per plot. Plants with less than 10 kernels at maturity were considered barren (Tollenaar et al., 1992). Barrenness was calculated as the percentage of barren plants per plot and crop yield was calculated as the product between average plant yield and plot stand density (1, 8 or 16 plants m^{-2}).

Following Tollenaar (1989) OSD was estimated adapting the equation proposed by Duncan (1958; Eq. [1]):

$$OSD = -1/(0.932 \times b) \quad [1]$$

The parameter b of the equation is the slope of linear regression (Eq. [2]). The constant was changed from 2.303 to 0.932 to transform data from plants per acre to plants per meter square. A linear regression model was fit for each genotype × year × replicate combination to relate natural logarithm of individual plant yield ($\ln y$) with stand density (Duncan, 1958; Eq. [2]) using (Graph Pad Prism V5.0, GraphPad Prism, 2007). The r^2 values ranged from 0.77 to 0.99, replicates were used for an ANOVA test.

$$\ln y = a + b\,x \quad [2]$$

Individual plant yields were calculated using the $\ln y$ values (Eq. [3]), and crop yield at optimum stand density (Yosd) was calculated as the product between individual plant yield at optimum stand density (yOSD and calculated OSD (Eq. [4]):

$$y\text{OSD} = \exp^{\ln y} \quad [3]$$

$$Y\text{osd} = \text{OSD} \times y\text{OSD} \quad [4]$$

We described the relationship of KNP with PGR around flowering for the 11 commercial hybrids using an individual plant approach. According to this, KNP is related to the accumulated EB 15 d after anthesis and this accumulated EB depends on the PGR around the flowering period. Both relationships were described by an hyperbolic function with their specific parameters (Fig. 1; Eq. [5], [6], [7]). Descriptive parameters of the models are PGR_b, IS_{EB}, C_{EB}, IS_{KN}, and C_{KN} (Fig. 1). Models were fit to each genotype × replicate × year combination including in the same curve the three stand densities utilized within each replicate, so parameters were estimated for each genotype × replicate × year combination. Replicates were used for an ANOVA test, R^2 values ranged from 0.66 to 0.97.

$$EB = 0 \text{ if } PGR \le PGR_b \quad [5]$$

$$EB = [IS_{EB} \times (PGR - PGR_b)] / [1 + CEB \times (PGR - PGR_b)] \text{ if } PGR > PGR_b \quad [6]$$

$$KNP = (IS_{KN} \times PGR)/[1 + (C_{KN} \times PGR)] \quad [7]$$

Statistical Analysis

Analysis of variance was conducted for all traits. The model included year and genotypes (and stand density when corresponding) as fixed factors. Blocks (nested within year) and all the interactions including blocks were considered random factors. Data were analyzed using the MIXED procedure of SAS (SAS Institute, 1999, Cary, NC). Means were compared with a LSD test at the 0.05 probability level.

RESULTS

Yield Response to Stand Density Changes

There were significant yield differences between years ($p < 0.001$), stand densities ($p < 0.001$), and genotypes ($p < 0.001$) (Table 1), and significant interactions were year × stand density ($p < 0.001$) and genotype × stand density ($p < 0.01$). Interactions genotype × year and genotype × year × stand density were not significant. The interactions showed that genotypes yielded differently at the stand densities tested but their response was not different across years, nor did year affect the genotype × stand density interaction. These results also indicate that stand density did not have the same effect on yield between years (Table 1).

When comparing years, Year 2 showed significantly higher yields than Year 1 (mean = 6.3 vs. 7.9 Mg ha^{-1} for Years 1 and 2, respectively). Among stand densities, the intermediate stand density had the highest average yield during both years (Table 1). Yields were very different at the highest stand density when Years 1 and 2 are compared (Table 1).

At the low stand density there were no significant ($p > 0.05$) yield differences between genotypes. All genotypes had similar yields at this low stand density, varying from 2.5 to 3.3 Mg ha^{-1} (Table 1). The opposite was observed at the highest stand density, where significant differences in yield showed large genotypic differences, ranging from 5.7 to 9.9 Mg ha^{-1} (Table 1).

Genotypic Differences in Optimum Stand Density

Because genotypes showed a differential response to stand density changes, we calculated the OSD that maximized yield for each evaluated genotype at each year (Table 2). Significant differences

Table 1. Grain yield of commercial maize hybrids grown at three stand densities (1, 8, and 16 plants m^{-2}) in 2 yr.

Treatment	1 plant m^{-2}	8 plants m^{-2}	16 plants m^{-2}
		Mg ha^{-1}	
Year 1	3.2	10.6	5.1
Year 2	2.5	10.9	10.1
NK860	2.9	10.3	6.6
NK900	3.3	9.7	5.7
P1979Y	2.7	10.2	6.8
P2069Y	2.7	10.2	6.1
P2053Y	3.0	11.3	7.8
DK670	2.9	11.5	9.7
DK692	2.8	10.7	9.5
AX852	2.5	10.9	8.3
AX886	2.8	11.0	6.1
AX887	2.9	11.6	7.1
NPreCom	3.0	10.9	9.9
Year (Y)		***	
Genotype (G)		***	
G × Y		ns†	
Stand density (SD)		***	
Y × SD		*** (0.8)‡	
G × SD		** (1.4)	
G × Y × SD		ns	

** $p < 0.01$.

*** $p < 0.001$.

† ns = not significant at the 0.05 probability level.

‡ Data in parenthesis represent the LSD value for $p \leq 0.05$.

Table 2. Optimum stand density and estimated grain yield at optimum stand density for 11 genotypes grown in 2 yr. Optimum stand density was calculated following Duncan (1958). Details regarding the calculation of the estimated yield at the optimum stand density are described in the text.

Treatment	Optimum stand density	Yield at optimum stand density
	plants m^{-2}	Mg ha^{-1}
Year 1	7.0	10.9
Year 2	12.2	12.8
NK860	8.7	11.2
NK900	7.3	11.2
P1979Y	9.6	11.2
P2069Y	8.8	10.4
P2053Y	9.9	12.5
DK670	11.9	13.5
DK692	11.4	12.7
AX852	10.1	11.6
AX886	8.3	11.1
AX887	8.9	11.8
NPreCom	10.5	13.3
Year (Y)	** (1.6)†	** (0.6)
Genotype (G)	* (2.1)	** (1.1)
Y × G	ns‡	ns

* $p < 0.05$.

** $p < 0.01$.

† Data in parenthesis represent the LSD value for $p \leq 0.05$.

‡ ns = not significant at the 0.05 probability level.

in OSD among genotypes ($p < 0.05$) and years ($p < 0.01$) were observed. The genotype × year interaction was nonsignificant ($p > 0.05$), showing that the genotypic ranking for OSD was the same for both environmental conditions. Genotypes showed an OSD that ranged from 7.3 to 11.9 plants m^{-2} (Table 2).

As genotypes showed significant differences in OSD we calculated their yields at its genotype specific optimum to understand if each genotype planted at this stand density still gave significant differences in yield (Table 2). Calculated yields at OSDs were also different across genotypes ($p < 0.01$) and years ($p < 0.001$). Year 2 showed higher optimum stand density values when compared to Year 1, and the average yields at this optimum stand density were also higher than Year 1 (average of 12.8 vs. 10.2 Mg ha^{-1} for Years 2 and 1, respectively). We found significant and positive correlations between OSD and yield at OSD ($p < 0.001$, $r^2 = 0.72$, $n = 22$) and yield at high stand density and OSD ($p < 0.001$, $r^2 = 0.93$, $n = 22$).

As such, our results showed that (i) genotypes must be planted at different stand densities because their optimum values are quite different and (ii) even if genotypes are planted at the specific stand density that maximizes their yield they still yield differently.

Grain Yield Components

Kernel number per unit area and kernel weight showed significant differences between years ($p < 0.05$ and $p < 0.001$ for kernel weight and kernel number, respectively), genotypes ($p < 0.01$ and $p < 0.001$ for kernel weight and kernel number, respectively) and stand densities ($p < 0.001$) (Table 3). For kernel weight none of the interactions were significant ($p > 0.05$). For kernel number the interactions year × stand density, genotype × stand density and genotype × year were significant ($p < 0.01$).

Kernel number per square meter and grain yield showed similar variations across treatments, and were highly correlated when pooling all data ($p < 0.001$, $r^2 = 0.92$, $n = 66$). Although individual kernel weight was also affected by treatments and correlated to yield ($p < 0.01$, $r^2 = 0.11$, $n = 66$), grain yield was more related to changes in kernel number than to individual kernel weight ($r^2 = 0.92$ vs. $r^2 = 0.11$ for kernel number and kernel weight, respectively).

Crop Physiological Mechanisms behind Kernel Number Differences

As kernel number was the most important yield component affecting yield variations across stand density, year, and genotype treatments, we further analyzed several physiological mechanisms related to kernel number determination. We were mostly interested in differences in plant growth around the flowering period, in the partitioning of this biomass to the developing ear around flowering, and on the efficiency of the different genotypes to set kernels per unit of EB accumulation.

As stand density increased from 1 to 16 plants m^{-2} there was a significant ($p < 0.001$) decline in PGR, a decline in the accumulated EB at 15 d after anthesis, a reduction in KNP, and an increase in CV_{PGR} (Table 4). In most cases these changes interacted with the genotype and the year.

Individual plant growth varied across genotypes, years and stand densities, and a genotype × year × stand density interaction was evident ($p < 0.01$), showing that the ranking and relative difference among treatments was not always similar

Table 3. Average kernel weight and kernel number per square meter for 11 commercial genotypes tested at three contrasting stand densities (1, 8, and 16 plants m^{-2}) in two experimental years (Years 1 and 2).

Treatment		Kernel weight	Kernel number
		mg kernel^{-1}	kernels m^{-2}
Year 1		284	
Year 2		267	
1 pl m^{-2}		316	
8 pl m^{-2}		277	
16 pl m^{-2}		233	
NK860		280	
NK900		253	
P1979Y		264	
P2069Y		256	
P2053Y		310	
DK670		298	
DK692		262	
AX852		298	
AX886		262	
AX887		274	
NPreCom		274	
NK860	Year 1		1930
	Year 2		2980
NK900	Year 1		1933
	Year 2		3173
P1979Y	Year 1		2066
	Year 2		3126
P2069Y	Year 1		2190
	Year 2		2855
P2053Y	Year 1		2229
	Year 2		2825
DK670	Year 1		2483
	Year 2		3261
DK692	Year 1		2517
	Year 2		3467
AX852	Year 1		2393
	Year 2		2808
AX886	Year 1		2055
	Year 2		3353
AX887	Year 1		2224
	Year 2		3492
NPreCom	Year 1		2650
	Year 2		3404
NK860	1 pl m^{-2}		862
	8 pl m^{-2}		3563
	16 pl m^{-2}		2849
NK900	1 pl m^{-2}		1079
	8 pl m^{-2}		3672
	16 pl m^{-2}		2908
P1979Y	1 pl m^{-2}		969
	8 pl m^{-2}		3790
	16 pl m^{-2}		3030
P2069Y	1 pl m^{-2}		914
	8 pl m^{-2}		3635
	16 pl m^{-2}		3019
P2053Y	1 pl m^{-2}		872
	8 pl m^{-2}		3458
	16 pl m^{-2}		3252
DK670	1 pl m^{-2}		919
	8 pl m^{-2}		3871
	16 pl m^{-2}		3827
DK692	1 pl m^{-2}		1033
	8 pl m^{-2}		4037
	16 pl m^{-2}		3905
AX852	1 pl m^{-2}		734
	8 pl m^{-2}		3637
	16 pl m^{-2}		3431
AX886	1 pl m^{-2}		899
	8 pl m^{-2}		4318
	16 pl m^{-2}		2896
AX887	1 pl m^{-2}		860
	8 pl m^{-2}		4451
	16 pl m^{-2}		3262
NPreCom	1 pl m^{-2}		1014
	8 pl m^{-2}		3851
	16 pl m^{-2}		4217
Year 1	1 pl m^{-2}		986
	8 pl m^{-2}		3658
	16 pl m^{-2}		2085
Year 2	1 pl m^{-2}		860
	8 pl m^{-2}		4046
	16 pl m^{-2}		4569
Year (Y)		* (11)†	***
Genotype (G)		** (23)	***
G x Y		ns‡	** (293)
Stand density (SD)		*** (11)	***
Y x SD		ns	*** (385)
G x SD		ns	*** (463)
G x Y x SD		ns	ns

* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.
† Data in parenthesis represent the LSD value for $p \leq 0.05$.
‡ ns = not significant at the 0.05 probability level.

Continued next column

(Table 4). It is evident that some commercial genotypes do have higher PGRs than others. For example, genotype DK670 was always among those hybrids with the highest PGR across stand densities and years, while AX852 was the one that showed the lowest PGRs, especially at the highest stand density.

We analyzed the plant to plant variability in PGR by calculating the coeffienct of variation of the plants within the canopy (CV_{PGR}). Overall, the plant to plant variability was higher as stand density increased, and differences among genotypes, years, and stand densities were evident. The genotype × stand density and year × stand density interactions were also significant for CV_{PGR} ($p < 0.05$; Table 4).

Table 4. Individual plant growth rate around flowering (PGR) and their coefficient of variation (CVPGR), accumulated ears biomass 15 days after anthesis (EB), kernel number per plant (KNP) and barrenness for the 11 genotypes tested at three stand densities (1, 8, and 16 plants m^{-2}) and grown during 2 yr.

Genotype	Stand density	PGR		CV_{PGR}		EB		KNP		Barrenness	
		Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
	plant m^{-2}	– mg plant^{-1} °Cd^{-1} –		———% ———		– g plant^{-1} –		– kernels plant $^{-1}$ –		——— % ———	
NK860	1	418	539	16	15	56	48	958	767	0	0
NK900		459	484	11	13	56	32	1104	1053	0	0
P1979Y		462	410	17	16	58	38	1133	804	0	0
P2069Y		458	396	22	19	70	42	999	829	0	0
P2053Y		473	464	17	19	44	51	996	747	0	0
DK670		549	514	13	28	48	38	997	841	0	0
DK692		384	480	22	31	64	39	1078	988	0	0
AX852		420	605	17	25	36	45	756	712	0	0
AX886		480	591	18	12	50	47	947	850	0	0
AX887		448	588	13	19	63	57	835	885	0	0
NPreCom		434	447	14	17	38	47	1041	986	0	0
NK860	8	194	212	26	31	20	19	406	508	0	0
NK900		197	217	28	19	23	17	412	506	0	2
P1979Y		176	198	34	16	20	18	409	538	7	2
P2069Y		204	196	33	22	20	17	458	450	2	2
P2053Y		183	227	29	24	15	19	421	444	0	0
DK670		278	285	25	25	19	17	477	491	0	0
DK692		198	238	24	24	25	19	520	490	0	0
AX852		165	256	36	27	15	21	432	477	2	0
AX886		158	256	35	27	14	19	524	556	4	0
AX887		191	305	24	27	17	17	502	611	0	0
NPreCom		220	226	27	23	14	19	469	494	0	0
NK860	16	79	124	54	32	6	12	99	257	45	5
NK900		61	133	63	26	6	12	87	276	41	4
P1979Y		88	140	45	24	7	11	112	267	49	11
P2069Y		85	121	68	33	3	9	119	258	52	17
P2053Y		90	147	36	34	3	9	145	261	29	2
DK670		76	172	63	27	5	10	165	313	24	3
DK692		81	168	43	26	6	12	145	343	38	0
AX852		53	92	87	72	7	10	185	244	22	4
AX886		71	137	58	45	4	10	64	298	75	7
AX887		91	135	51	42	4	8	114	294	49	9
NPreCom		86	145	38	30	6	10	197	330	21	0
Year (Y)		**		**		**		ns†		***	
Genotype (G)		***		***		***		***		***	
G × Y		***		ns		***		ns		ns	
Stand density (SD)		***		***		***		***		***	
Y × SD		ns		*** (4)‡		***		*** (51)		***	
G × SD		***		*** (8)		***		*** (54)		***	
G × Y × SD		** (50)		ns		*** (5)		ns		* (9)	

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$.

† ns = not significant at the 0.05 probability level.

‡ Data in parenthesis represent the LSD value for $p \leq 0.05$.

Accumulated EB around the flowering period showed a significant ($p < 0.05$) genotype × stand density × year interaction. However, it was evident that the higher the stand density the lower the accumulated EB per plant. The lowest EB accumulations were achieved at the highest stand density of 16 plants m^{-2} during Year 1, where the lowest KNP and the highest proportion of barren plants were observed (Table 4).

As such, results showed that (i) at similar environmental conditions (years and/or stand densities) genotypes showed significant differences in plant growth and its variability (Table 4), (ii) that similar changes in stand density affected the genotypes differently, and (iii) that changes in plant growth and its variability resulted in different accumulated EB and barrenness, affecting KNP (Table 4). By grouping all data at the individual plant level, KNP was significantly

Table 5. Descriptive parameters of the crop physiology model that relates kernel number per plant (KNP) with plant growth rate (PGR) around flowering, for 11 genotypes in 2 yr.

Genotype	PGR_b†		IS_{EB}		C_{EB}		IS_{KN}		C_{KN}	
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
	– mg plant^{-1} °Cd^{-1} –		– mg EB mg plant^{-1} °Cd^{-1} –		– mg^{-1} plant^{-1} °Cd^{-1} –		– KNP gr EB^{-1} –		– mg^{-1} plant^{-1} °Cd^{-1} –	
NK860	47‡	0	158	98	0	0	21	32	4	20
NK900	15	15	132	102	0	1	19	31	0	0
P1979Y	45	58	161	164	0	22	22	33	2	13
P2069Y	92	55	200	136	0	0	29	35	14	18
P2053Y	64	95	141	160	1	0	35	33	12	21
DK670	34	42	87	81	0	23	44	39	36	23
DK692	50	88	183	166	0	1	25	41	8	23
AX852	0	0	106	106	1	1	35	33	20	27
AX886	54	12	138	80	0	14	48	44	29	29
AX887	84	69	176	102	0	0	62	54	62	40
NPreCom	33	60	92	128	0	0	38	35	10	15
Year (Y)	ns§		**		** (2)¶		ns		ns	
Genotype (G)	***		***		ns		*** (9)		** (16)	
G x Y	*** (24)		** (29)		ns		ns		ns	

** $p < 0.01$.

*** $p < 0.001$.

† PGR_b: base plant growth rate around flowering for ear biomass accumulation, IS_{EB}: initial slope of the relationship between plant growth rate and ear biomass accumulated at 15 days after anthesis, C_{EB}: curvature degree of the plant growth rate vs. ear biomass relationship, IS_{KN}: initial slope of the ear biomass vs. kernal number per plant relationship and C_{KN}: curvature degree of the ear biomass vs. kernal number per plant relationship.

‡ Each value is an average of three replicates; years were separately analyzed because there was a significant genotype × year interaction in at least one parameter.

§ ns = not significant at the 0.05 probability level.

¶ Data in parenthesis represent the LSD value for $p \leq 0.05$.

correlated with accumulated EB ($p < 0.001$, $r^2 = 0.86$, $n = 66$). And this accumulated EB was consistently correlated with PGR ($p < 0.001$, $r^2 = 0.82$, $n = 66$).

We further analyzed the different genotype-specific parameters describing the relationships between (i) accumulated EB 15 d after anthesis and the number of kernels per plant (Fig. 1A), and (ii) PGR and the accumulation of EB (Fig. 1B).

Several of these traits describing the relation between PGR and EB showed an important environmental component; a significant ($p < 0.01$) genotype × year interaction for traits PGR_b and IS_{EB} was evident (Table 5). Likewise, genotypes showed significant ($p < 0.001$) differences in both PGR_b, with values ranging from 0 to 95 mg plant^{-1} °C d^{-1}, and IS_{EB}, with values ranging from 80 to 200 mg EB mg plant^{-1} °C d^{-1}. Contrary to this, we did not detect any significant genotype or genotype × year interaction effects for the curvilinearity parameter (C_{EB}), but only a year effect ($p < 0.01$, Table 5). The lowest PGR_b values were detected for AX852 and NK900 at both experimental years (Table 5). Furthermore, there was a significant correlation between PGR_b and IS_{EB} ($p < 0.001$, $r^2 = 0.48$, $n = 22$).

Regarding the relation between accumulated EB at the end of the flowering period and KNP, there were significant genotypic differences for IS_{KN} ($p < 0.001$) and C_K ($p < 0.01$) (Table 5). No significant year or genotype × year interaction effects (Table 5) were evident for any of the two traits. Averaging across years, IS_{KN} values ranged from 25 to 58 kernels g^{-1} of accumulated EB and C_{KN} ranged from 0 to 51 mg^{-1} plants^{-1} C°d^{-1} (Table 5).

No unique trait (PGR, CV_{PGR}, PGR_b, IS_{EB}, C_{EB}, IS_{KN}, and C_{KN}) was responsible for differences in crowding tolerance among tested genotypes (Table 1).

DISCUSSION

We did not find genotypic differences in potential yield per plant. Crowding stress tolerance was responsible for genotypic yield and OSD differences. The lack of differences in potential yield per plant is in agreement with previously published data for other environments (Tollenaar and Wu, 1999; Duvick and Cassman, 1999; Duvick et al., 2004) showing maximum yield per plant remained constant throughout years. Results from Luque et al. (2006) described improvements in yield potential per plant with breeding selection for Argentinian maize hybrids. As such, it seems that in Argentina there have been improvements in potential yield per plant (Luque et al., 2006) but if a set of current commercial genotypes are tested differences are negligible (Table 1).

With crowding stress genotypic yield differences were explained by differences in barrenness: the correlation between yield and barrenness in such conditions was significant and negative; $p < 0.001$, $r^2 = 0.86$, $n = 22$ (Table 4). Barrenness has always been considered an important secondary trait for drought and N stress tolerance selection, since it has: (i) high correlation with yield, (ii) high heritability, and (iii) the correlation with yield and heritability increases in stressful conditions (Bänziger et al., 1997, 2000; Chapman and Edmeades, 1999; Zaidi et al., 2004; Mansfield and Mumm, 2014). Barreness is directly related to the proportion of plants from the canopy growing less than the PGR_b. Hence, genotypic differences in barrenness (and consequently in crowding tolerance) are the result from genotypic differences in: (i) PGR under stress conditions, (ii) PGR_b, and/or (iii) plant to plant variability in growth of the plants within the canopy (CV_{PGR}). Differences among genotypes in these three traits were evident. We have detected that some genotypes

have higher PGR at high stand density, that there are genotypic differences in PGR_b, and that some genotypes have more uniform PGR than others (Tables 4 and 5). It is relevant to recall the importance of uniform canopies at stressful conditions (Tollenaar and Wu, 1999), as experienced at high stand densities (Table 4).

The correlation between yield at high stand density and OSD indicated that the OSD resulted almost exclusively from crowding tolerance. That is, genotypes with higher crowding tolerance supported greater stand densities with lower changes in their individual plant yield. Consequently their OSD and their yield at OSD were higher (Table 2). An important conclusion from our study is that among current commercial Argentinean maize hybrid differences in OSD are determined by genotypic differences in crowding tolerance. This assertion is consistent with those documented in retrospective studies, where crowding tolerance and OSD were both increased over time (Tollenaar et al., 1992; Duvick and Cassman, 1999; Tollenaar and Lee, 2002; Duvick et al., 2004). Another important conclusion is that no single trait measured in this study explained differences among genotypes in crowding tolerance (Fig. 1, Tables 4 and 5).

Although a single point estimate of the OSD can be calculated, this is of limited interest to growers it they are quite similar. When calculating a point estimate differences among hybrids can be related to slight differences in the regression parameters. It is more informative to growers to have a range of OSDs that produce near maximum net returns. When looking at the optimum calculated stand densities in this way, many hybrids may not differ in their response to stand density if values are within a limited range. However, the calculated values showed by the commercial hybrids tested (7.3–11.9 plant m^{-2}, Table 2) indicated that there is a clear need for farmers to adjust the stand density to their specific hybrid.

Finally, we demonstrated the existence of genotypic variability in most eco-physiological mechanisms involved in kernel set among current commercial maize hybrids (Table 5), which helped explain genotypic differences in yield response to stand density. Genotypic variability for such mechanisms exist among inbred lines (Echarte and Tollenaar, 2006; D'Andrea et al., 2009). However, information regarding the inbred line to hybrid correlation in yield response to stand density changes, and inheritance of the traits we analyzed, is limited. Such information exists for N (D'Andrea et al., 2013), but not for stand density. This is relevant for product development. Any information on parental inbred lines that is indicative of derived hybrid performance is highly desirable, as hybrid-specific stand density recommendations should to be provided by seed companies. Such information may help eliminate the need for doing testcrosses and conducting extensive trials that seem necessary based on the high genotype × stand density interaction (Table 2).

CONCLUSIONS

Current Argentinean commercial hybrids differ in their yield response to changes in stand density. Yield of tested genotypes was similar at low stand density but different at high densities. This indicated no variability in potential yield per plant but high variability in crowding tolerance. Genotypes must be planted at a particular stand density to maximize their yield.

When using a crop growth and biomass partitioning framework for understanding kernel set differences among genotypes in their response to stand density, hybrids differed in most measured traits. Genotypes showed differential strategies for coping with stress tolerance. Under high stand density conditions barrenness was key for hybrid tolerance to crowding stress. Tolerant genotypes had less barrenness values through lower PGR_b values, higher PGR at stressful conditions, and/or lower plant-to-plant variability in plant growth.

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