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Crop Management Options for Maximizing Maize Kernel Hardness

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ABSTRACT

Special hard endosperm maize (*Zea mays* L.) adapted for optimum dry milling yields is produced worldwide. Argentine flint maize is internationally known, and specific values for grain vitreousness, floaters, and test weight are demanded by the industry. Agricultural practices aimed to reach these standards, however, are not clear for farmers. Our general objective was to identify possible management options for maximizing the grain quality attributes described by these standards. We tested two flint and two dent kernel type genotypes under contrasting management options and environmental conditions (stand density, N fertilizer, defoliations, years), and studied their yield and grain quality response. Flint genotypes yielded less than dents across all tested field treatments (flint vs. dent, $P \leq 0.001$), with larger differences at the lowest yielding conditions. Large differences between kernel types, and for genotypes within each kernel type, were evident for all grain quality traits (test weight, floaters, vitreousness, 8 mm screen retention) and composition (protein, oil, starch). Low N fertilization levels and stressful situations during grain filling where the treatments reducing grain hardness and screen retention the most, especially for some genotypes. Other than genotype selection, adequate N availability and low stand density helped improve test weight, vitreousness, floaters, and screen retention, all traits relevant for maize dry milling industry.

Core Ideas

- Crop management options for maximizing maize kernel hardness are mostly unknown.
- Flint genotypes always yielded less than dented ones across a wide range of field treatment combinations.
- Stand density, N fertilization, and genotype selection are key management options for optimum grain quality.

Hard endosperm maize is highly demanded for dry-milling purposes. Hardness is a key grain quality attribute for the processing of several end use products (Mercier, 1994; Shandera et al., 1997). Reaching optimum grain quality standards is commonly feasible, but coupling this with high physical yields at farmer fields is challenging. Current regular soft endosperm dent hybrids out-yield hard endosperm kernel types at many production regions (Tamagno et al., 2015; U.S. Grains Council, 2006), so farmers are commonly paid premiums for producing special hard endosperm maize adapted for high dry-milling yields. Hard endosperm maize breeders selecting for higher yields at farmer fields have the limitation of maintaining minimum dry-milling quality standards. In addition to the strong genetic basis of kernel hardness (Watson 2003), the environment where the crop grows also affects kernel hardness and composition (Mestres et al., 1991; Blandino et al., 2010).

Argentine hard-endosperm flint maize, also called Plata maize, is internationally known for its kernel hardness (Eyherabide et al., 2004). Argentina has set minimum quality standards for this product, and trading agreements are subject to strict regulations to ensure a high quality grain is commercialized (MAGyP, 2015). The European Union issues special import permits for flint maize (European Commission, 1997) if the grain quality attains specific standards. At present the quality traits that a grain lot needs to meet for reaching the standard are: a minimum number of kernels with 50% or more of vitreous endosperm (92%), a maximum number of floaters at a standardized solution (25%), and a minimum test weight (76 kg hL^{-1}). Vitreousness is the proportion of kernels having more horny than floury endosperm, and is a key hardness attribute due to the differential density of horny vs. floury endosperm. Fields planted with hard endosperm flint genotypes not reaching minimum quality standards for optimum dry-milling yields are a possibility in Argentina or any other cropping system targeting hard endosperm grain, so farmers and coops need to combine adequate genotypes with specific crop management practices worldwide.

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There are very few studies reporting the effect of crop management options on yield and kernel hardness attributes described in international trading agreements. Cirilo et al. (2011) studied differences in yield and grain quality traits among flint genotypes grown at different environmental conditions. Their study evaluated only some of the traits described by the European Union trading standards (European Commission, 1997). Vitreousness, for example, was not included. Other previous studies on kernel hardness have highlighted available genotypic diversity (Mestres et al., 1991; Blandino et al., 2010) without considering crop management options.

Genotype selection, stand density, and N fertilizer management are among cropping options easily applicable by farmers. It is known that genotype differences in grain protein concentration have a strong correlation with dry-milling yield (Blandino et al., 2013). Although protein concentration comprises a low proportion of the total kernel composition (6–10%), it does play a significant role in influencing kernel hardness (Lending and Larkins, 1989; Chandrashekar and Mazhar, 1999; Fox and Manley, 2009; Holding and Larkins, 2006). Grain protein is strongly influenced by the genotype, but can also vary due to environmental conditions and soil N availability (Below, 2002; Uribelarrea et al., 2009). Although N fertilization is a common agronomic practice among farmers for reaching high yields, fertilizing maize crops is almost never targeted to modify final grain composition. Reductions in canopy stand density, on the other hand, are also known to increase maize grain protein concentration (Borrás et al., 2003), but the impact on kernel hardness is unknown.

We have recently described the link between endosperm zein profiles and kernel hardness (Gerde et al., 2016). In the present manuscript the objective was to identify field management options to achieve grain quality attributes needed to reach maize kernel hardness standards for helping farmers and coops. We focused on the specific traits used for exporting hard endosperm maize from Argentina to the European Union, but the implications are worldwide for any specialty hard endosperm maize produced for dry milling. We evaluated two flint and two dent kernel type genotypes widely used in Argentina under contrasting management options (stand density, N fertilizer use), and studied their yield and grain quality attributes relevant for dry milling. Two defoliation treatments were added, at flowering and at mid-grain filling, for testing how a severe stress during those stages would affect yield and grain quality in the different kernel types and genotypes.

Table 1. Description of the field treatments that were used for testing yield and grain quality response of two dent (AX887, DK747) and two flint (ACA2002, NT426) kernel type genotypes during two growing seasons (2012–2013 and 2013–2014).

Treatments	Stand density	Nitrogen availability	Defoliation timing
	plant m^{-2}	kg N ha^{-1}	
Low N	8	85	–
Control	8	155	–
High nitrogen	8	250	–
Low stand density	5	155	–
Defoliation flowering	8	155	10–12 d before anthesis
Defoliation grain filling	8	155	20 d after anthesis

MATERIALS AND METHODS

Experiments were conducted at Campo Experimental Villarino located at Zavalla (33°1′ S, 60°53′ W), Santa Fe, Argentina, during years 2012–2013 and 2013–2014 (Years 1 and 2, respectively). The soil was a silty clay loam Vertic Argiudoll, Roldán series. Planting dates were 21 Sept. 2012 and 2 Oct. 2013. Both field experiments were arranged following a completely randomized block design with four replicates. Experiments were all rainfed, sown using no-tillage conditions with soybean [*Glycine max* (L.) Merr.] as previous crop, and following common agricultural practices at the region. Weeds and pests were controlled with common agronomic practices. Insect pressure was minimized by planting early, and specifically monitored and controlled throughout the season for minimizing any possible effect. Individual plot replicates were four rows with 0.52 m row spacing and 6 m long.

Rainfall from planting date to physiological maturity was different across years. Both years showed similar rainfall distribution but the total rainfall amount was quite different (681 and 391 mm for Years 1 and 2, respectively). Average temperatures were also different (21.1 and 23.0°C for Years 1 and 2, respectively), making Year 2 a warmer and dryer growing season.

Field treatments consisted in variations of different agricultural practices, like genotypes, stand density, and N fertilization (Table 1). In addition, two severe plant growth reductions were tested by imposing two defoliations at different moments of the crop cycle (pre-flowering and grain filling). We tested two commercial hard endosperm non-genetically modified organism (GMO) flint (ACA2002 and NT426) and two regular GMO dent (AX887 and DK747) kernel type genotypes for understanding possible differential responses and interactions with specific genotypes. Genotypes represent widely planted hybrids for both kernel types at the central Argentinean region.

At planting monoammonium phosphate (MAP) (10–50–0, N–P–K) was applied at a rate of 20 kg ha^{-1} for all plots, and after this three N treatment levels were arranged: (i) a low N treatment, (ii) an intermediate N treatment, and (iii) a high N one. Soil samples (0–60 cm) were taken before planting, and analyzed for $N\text{-}NO_3$ (0–60 cm). Nitrogen was applied as urea (49–0–0, N–P–K) for reaching three fertilization levels. In the low N level, no N source other than MAP was used. At this treatment the soil reached, on average across years, 85 kg N ha^{-1} (N from the soil at planting plus N from MAP). At the intermediate level urea was applied for adjusting the soil N level to 155 kg N ha^{-1} (N from the soil at planting plus MAP plus urea). At the high N level the soil was adjusted for reaching 250 kg N ha^{-1} (N from the soil at planting plus MAP plus urea). Urea was broadcasted over the plots soon after thinning (V3–V4).

Plots were all over planted and hand-thinned at V3 (Abendroth et al., 2011) to the stand density that corresponded to each plot (Table 1). Defoliation treatments consisted in manually removing 70 to 80% of plant green leaf area. Two defoliation treatments were imposed, one of them 10 to 15 d before anthesis (flowering treatment) and the other 20 d after anthesis (grain-filling treatment). In both situations the upper four leaves of all plants within the plot were left. These treatments were similar to Borrás et al. (2009).

Grain Yield and Its Numerical Components

At harvest maturity the two central rows of each plot were manually harvested and used for grain yield, kernel number per unit area and average individual kernel weight determination. Grain yield was corrected to 14.5 g 100 g^{-1} moisture basis. Individual kernel weight was determined by weighing two sets of 100 kernels per plot, and kernel number per unit land area was calculated using yield and average individual kernel weight.

Physical Properties for Grain Quality and Grain Composition

Grain starch, protein, and oil percentages were determined by near infrared (NIR) spectroscopy using an Infratec 1241 instrument (Foss, Hillerod, Denmark) as in Borrás et al. (2002) using 400 g of grain per plot. Values are reported in dry basis.

Test weight (kg hL^{-1}) was determined using a Schopper chondrometer. Samples were previously homogenized with a sample homogenizer (MAGyP, 2015).

Percent floaters (%) was determined introducing a 100 kernel aliquot in an aqueous solution of $NaNO_3$ (density: 1.25 g cm^{-3}) at 35°C, and thoroughly shaken every 30 s for 5 min to eliminate bubbles. At the end of this time period floating kernels were counted and reported as percentage. This test was done five times per sample. A low percentage of floating kernels is known to represent grain samples with high kernel density and hardness (Robutti et al., 2000).

To determine vitreousness (%) 400 kernels per plot were longitudinally dissected and visually inspected. The percentage of kernels that were not indented in the crown, that had central starchy endosperm completely surrounded by horny endosperm, and horny endosperm representing 50% or more of the endosperm were considered vitreous kernels, and reported as percentage over the total number of inspected kernels. For a particular maize lot to be considered as flint, percent kernels vitreousness needs to be above 95%, however there is a 3% tolerance that sets the limit value at 92% (MAGyP, 2015).

The proportion of kernels sized over 8 mm was determined using a Ro-Tap like sieve shaker (Zonytest, Rey & Ronzoni, Rosario, Argentina). A 100 g grain aliquot was loaded on top of an 8 mm round hole stackable standard sieve. The weight of the aliquots retained before and after the 8-mm sieve was determined after 2 min shaking. The percentage (%) of grains retained by the 8-mm sieve over the total sample was reported (Cirilo et al., 2011).

Statistical Analysis

For analyzing the effects of kernel type, genotype within each kernel type, treatments, year, and all possible interactions we did an ANOVA fitting a general linear model with genotypes nested within kernel type using the GLM Procedure (SAS Institute, 1999). Assumptions for ANOVA (normality and homogeneity) were satisfied by all traits. Statistical differences for the significant sources of variation were tested using LSD at the 5% level.

To study differences between kernel types (flint or dent) in their yield response to the environments generated by the different field treatments an environmental index analysis was conducted following Finlay and Wilkinson (1963). Here the environmental index is the average yield of all genotypes tested at each particular environment. In our specific experiment we tested all genotypes under 12 environments (six treatments under 2 yr). We also fitted a segmented bilineal regression (Eq. [1] and [2]) between the relative flint kernel type yield in relation to the dent kernel type yield and the environmental index as determined by Finlay and Wilkinson (1963):

$$Y = aX + b \text{ when } X < c \quad [1]$$

$$Y = d \text{ when } X \geq c \quad [2]$$

where Y is the relative flint kernel type yield in relation to the dent kernel type yield, X is the environmental index yield for each particular environment, and a, b, c, and d are parameters of the regression model.

RESULTS

Nitrogen Effects

Higher N availability increased grain yield, varying from 6884 to 8511 kg ha^{-1} in low and high N treatments, respectively (Table 2). However, N fertilization treatments affected yield differently each year (significant year × treatment interaction, $P \leq 0.01$). During the higher yielding Year 1 all genotypes presented a positive yield response to N increases, while the same trend was not observed in Year 2 (Table 2). Although there were significant yield differences between flints and dents ($P \leq 0.001$), and between genotypes within each kernel type ($P \leq 0.001$), there was no N treatment × kernel type interaction ($P \geq 0.05$), showing the yield of both flints and dents responded similarly to N treatments.

Harvested kernel number per unit land area showed mostly similar significant effects than the yield response (Table 2). Significant effects were year, kernel type (higher harvested kernel number for dents and lower for flints), and genotype within kernel type (Table 2), and significant interactions were year × N treatment and year × genotype within kernel type ($P \leq 0.01$).

Kernel weight was significantly affected by kernel type, N fertilization, and genotype within kernel type, showing that flints had lower kernel weights than dents, that kernel weight was lower at reduced N availability treatments, and that genotypes within each kernel type had different kernel weights (Table 2). Significant interactions showed dents reducing their kernel weight more than flints at the lower yielding Year 2 (significant year × kernel type interaction, $P \leq 0.05$), and that N treatments affected kernel weight of genotypes within each kernel type differently (significant N fertilization × genotype within kernel type, $P \leq 0.001$). As such, the lower yield at limited N treatments was related to reductions in harvested kernel number and average kernel weight.

Kernel protein concentration was positively affected by N availability, and although genotype differences within each kernel type were evident ($P \leq 0.001$) flint genotypes had more protein concentration than dents ($P \leq 0.001$). Differences between years created large protein concentration differences, and there were significant year interactions with kernel type and genotype within kernel type, mostly because kernel type and genotype within kernel type differences were larger during the higher yielding Year 1 (Table 2). However, N treatments affected kernel protein concentration similarly to both kernel types and N fertilization increased kernel protein

Table 2. Nitrogen fertilization effects over yield, kernel number, kernel weight, grain protein, starch and oil concentration, test weight, floaters, vitreousness and 8 mm screen retention for two dent (AX887 and DK747) and two flint (ACA2002 and NT426) genotypes grown during two seasons (Years 1 and 2). See N fertilization levels in Table 1.

Year	Kernel type	Genotype	Treatment	Yield	Kernel no.	Kernel wt.	Protein	Starch	Oil	Test wt.	Floaters	Vitreousness	8 mm sieves
				kg ha^{-1}	kernel m^{-2}	mg kernel^{-1}	g 100 g^{-1}			kg hL^{-1}	%		
1	Dent	AX887	Low N	10,040	4243	239	7.3	68.8	4.5	77	57	5	25
			Control	11,838	4370	272	8.6	67.9	4.5	78	24	5	24
			High N	13,824	4561	303	9.0	67.2	4.7	79	18	7	34
		DK747	Low N	9,614	3738	262	7.9	67.6	4.6	77	49	6	26
			Control	12,556	4413	285	8.8	67.5	4.7	77	38	12	29
			High N	12,969	4377	263	9.7	68.0	4.7	77	40	6	31
	Flint	ACA2002	Low N	6,326	2888	251	8.6	67.5	5.2	77	32	74	40
			Control	9,021	3775	241	9.6	66.2	5.2	79	16	92	31
			High N	9,501	3727	256	9.8	66.6	5.0	78	17	89	43
		NT426	Low N	7,919	3624	218	8.1	66.2	5.6	79	17	87	11
			Control	9,705	4168	233	8.8	66.7	5.4	79	14	94	18
			High N	10,780	4135	229	9.8	65.9	5.6	79	12	96	12
2	Dent	AX887	Low N	6,392	2882	225	9.3	73.6	4,3	78	38	20	14
			Control	5,730	2252	255	10.8	67.6	4.5	79	19	50	28
			High N	5,979	2231	267	11.5	71.9	4.8	79	10	60	30
		DK747	Low N	5,889	2300	258	9.6	71.3	4.3	79	44	11	35
			Control	6,850	2556	267	10.8	71.7	4.5	79	19	20	30
			High N	6,741	2723	247	11.0	63.8	4.5	78	31	20	22
	Flint	ACA2002	Low N	3,363	1389	247	11.3	67.8	4.7	78	14	92	28
			Control	2,137	840	254	12.8	64.0	5.1	79	9	96	35
			High N	2,785	1077	261	12.9	68.8	5.0	78	11	96	37
		NT426	Low N	5,528	2505	218	9.9	65.2	5.6	80	6	93	12
			Control	6,032	2588	233	11.4	67.1	5.7	79	5	97	15
			High N	5,506	2335	236	11.6	65.6	5.7	79	8	96	32
1				10,341	4007	254	8.8	67.2	5.0	78	28	48	27
2				5,244	2140	247	11.1	68.2	4.9	79	18	63	27
	Dent			9,035	2754	262	9.5	68.9	4.6	78	32	19	27
	Flint			6,550	3380	240	10.4	66.5	5.3	79	13	92	26
		AX887		8,967	3423	260	9.4	69.5	4.6	78	28	25	26
		DK747		9,103	3334	264	9.6	68.3	4.6	78	37	13	29
		ACA2002		5,522	2283	252	10.8	66.8	5.0	78	17	90	36
		NT426		7,578	3226	228	9.9	66.1	5.6	79	10	94	17
			Low N	6,884	2921	240	9.0	68.5	4.9	78	32	49	24
			Control	7,984	3120	255	10.2	67.3	5.0	78	18	58	26
			High N	8,511	3146	258	10.6	67.2	5.0	78	18	59	30
Year (Y)				***	***	ns†	***	ns	*	***	***(5)	***	ns
Kernel type (KT)				***(450)‡	***(192)	***	***	**(1.4)	***	***	***	***	ns
Treatment (T)				**	ns	**	***(0.2)	ns	**	*	***	***	*
Genotype (kernel type) [G(KT)]				***	***	***	***	ns	***	***	**(7)	***	***
Y × KT				ns	ns	*(11)	**(0.3)	ns	ns	**(1)	ns	***	ns
Y × T				***(780)	**(333)	ns	ns	ns	**(0.1)	ns	ns	ns	ns
Y × G(KT)				**(901)	***(384)	ns	***(0.4)	ns	**(0.1)	ns	ns	***(7)	ns
KT × T				ns	ns	ns	ns	ns	*(0.1)	ns	**(8)	ns	ns
T × G(KT)				ns	ns	***(119)	ns	ns	*(0.2)	**(1)	ns	ns	ns
Y × KT × T				ns	ns	ns	ns	ns	ns	ns	ns	***(8)	ns
Y × T × G(KT)				ns	ns	ns	ns	ns	ns	ns	ns	ns	*(8)

* Significant at $P \leq 0.05$.

** Significant at $P \leq 0.01$.

*** Significant at $P \leq 0.001$.

† ns: nonsignificant.

‡ Numbers in parentheses represent the least significant differences (LSD) of the means at $P \leq 0.05$.

concentration both years. Protein concentration was maximized at the highest N availability treatment, and was significantly different between the low, intermediate, and high N levels (Table 2).

Grain starch concentration was only affected by kernel type, where dents had significantly more starch than flints (Table 2). Oil concentration was higher in flints than dents, increased at higher N availability, was slightly higher during Year 1, and showed significant genotype within kernel type differences (mostly due to the significantly higher values of genotype NT426). Significant kernel type × N fertilization interaction showed dents increasing their oil concentration more than flints at higher N rates (dents increased from 4.4 to 4.7 g 100 g^{-1} while flints from 4.7 to 4.8 g 100 g^{-1} when comparing low and high N conditions, respectively). A significant year × N fertilization interaction showed that grain oil concentration increased more during the lower yielding Year 2 with higher N availability than during Year 1 (Table 2).

Grain quality for dry milling was tested using four different traits: test weight, floaters, vitreousness, and screen retention. The four traits showed a significant positive N treatment effect, improving when N availability was increased.

Test weight was mostly affected by kernel type, where flints had higher values than dents, and genotypes within kernel type were also different (Table 2). Flint genotype NT426 had higher test weight than ACA2002. Nitrogen fertilization increased test weight values, but no differences in test weight response was evident for kernel types (no significant N treatment × kernel type interaction, $P \geq 0.05$). A significant year × kernel type interaction showed differences between flints and dents were higher during Year 1 than during Year 2. The genotype AX887 increased more its test weight than DK747 in response to higher N availability, supported by the significant treatment × genotype within kernel type interaction ($P \leq 0.01$).

Floaters percentage was significantly different between kernel types and genotypes within each kernel type (Table 2). Higher N availability reduced floaters percentage, and this reduction was larger in dents than in flints (significant N fertilization × kernel type interaction, $P \leq 0.01$). The lower yielding Year 2 had lower floaters percentage, but this did not interact with any other treatment effect.

Screen retention was only affected by N treatment and genotype within kernel type (Table 2). Flints and dents had similar screen retention values. There was a significant triple year × N treatment × genotype within kernel type interaction ($P \leq 0.05$), but this interaction explained <7% of the total variability.

In brief, increasing N availability increased yield, protein concentration, and all the grain quality attributes related to dry milling in both kernel types (flints and dents). The overall grain quality was not only different between the extreme N fertilization treatments, but also with the intermediate level.

Stand Density Effects

Similarly to N fertilization treatments, modifying canopy stand density also showed a strong year interaction effect over yield ($P \leq 0.001$). During the higher yielding Year 1 reducing stand density negatively affected grain yield, while the opposite effect was observed during the lower yielding Year 2. Averaged across kernel types yields during Year 1 were 10,780 and 9604 kg ha^{-1} for the average and low stand densities, respectively, while during Year 2 yields were 5187 and 11,131 kg ha^{-1} for the average and low stand densities, respectively.

Stand density response showed a moderate interaction with genotype within kernel type (Table 3), but this effect explained only 2% of the total variability.

Stand density yield effects were matched by changes in harvested kernel number per land area, also showing a year × stand density treatment significant interaction ($P \leq 0.001$, Table 3). Contrarily, kernel weight was increased by stand density reductions during both years, with no kernel type interaction but only a moderate genotype within kernel type interaction ($P \leq 0.05$, explaining only 2% of the total variability).

Reducing stand density increased kernel protein concentration, but only during the higher yielding Year 1 (year × stand density significant interaction, $P \leq 0.001$). Also, although both kernel types (flints and dents) increased their protein concentration whenever stand density was reduced, the effect was larger for dents (kernel type × stand density treatment significant interaction, $P \leq 0.01$).

Stand density had no effect over kernel starch concentrations (Table 3), and kernel oil concentration was also not affected by stand density changes, only by kernel type and genotype within kernel type (Table 3).

Reducing stand density had very positive effects over kernel hardness attributes. It increased grain test weight, reduced floaters, and increased screen retention values (Table 3). Vitreousness was mostly not affected by stand density changes, and although the stand density treatment interacted significantly with year, kernel type and year × kernel type, none of these three interactions explained more than 2% of total variability. Vitreousness variability was mostly explained by kernel type (91% of total variability).

Reducing stand density increased screen retention more in dents than in flints (kernel type × stand density significant interaction, $P \leq 0.05$), and a significant stand density × year interaction showed that although the effect of reducing stand density was always positive the effect was larger during the higher yielding Year 1.

In brief, reducing stand density had a yield response dependent on the environmental condition, but showed positive grain quality responses. Dents seemed to be more responsive than flints in terms of improving their grain quality to stand density reductions, as evidenced by their larger kernel weight, protein concentration, vitreousness, and screen retention differential responses (Table 3).

Defoliation Effects

Yield was severely affected by both defoliation treatments (Table 4). Averaging across years defoliating the crop during flowering affected yield more than defoliating the crop during grain filling. However, when years are individualized both defoliation treatments affected yield mostly during Year 1. There was no defoliation × kernel type significant interaction over yield, but only a defoliation × genotype within kernel type significant interaction (Table 4). This showed that not all genotypes responded similarly, but their differential yield response was not related to kernel type.

Table 3. Stand density (control and low stand density were 8 and 5 plants m^{-2}, respectively) effects over yield, kernel number, kernel weight, grain protein, starch and oil concentration, test weight, floaters, vitreousness and 8 mm screen retention for two dent (AX887 and DK747) and two flint (ACA2002 and NT426) genotypes grown during two seasons (Years 1 and 2).

Year	Kernel type	Genotype	Treatment	Yield	Kernel no.	Kernel wt.	Protein	Starch	Oil	Test wt.	Floaters	Vitreousness	8 mm sieves
				kg ha^{-1}	kernel m^{-2}	mg $kernel^{-1}$	g 100 g^{-1}			kg hL^{-1}	%		
1	Dent	AX887	Control	11,838	4370	272	8.6	67.9	4.5	78	24	5	24
			Low SD	11,171	3376	331	9.5	67.3	4.6	78	8	9	49
		DK747	Control	12,556	4413	285	8.8	67.5	4.7	77	38	12	29
			Low SD	9,804	3106	316	10.2	66.6	4.6	78	39	10	52
	Flint	ACA2002	Control	9,021	3775	241	9.6	66.2	5.2	79	16	92	31
			Low SD	8,317	2858	291	10.4	66.0	5.2	79	13	94	56
		NT426	Control	9,705	4168	233	8.8	66.7	5.4	79	14	94	18
			Low SD	9,124	3512	259	9.6	65.4	5.6	80	8	95	21
2	Dent	AX887	Control	5,730	2252	255	10.8	67.6	4.5	79	19	50	28
			Low SD	13,070	4270	306	10.9	69.7	4.6	79	10	25	39
		DK747	Control	6,850	2556	267	10.8	71.7	4.5	79	19	20	30
			Low SD	11,963	4074	294	11.1	69.1	4.6	79	14	13	38
	Flint	ACA2002	Control	2,137	840	254	12.8	64.0	5.1	79	9	96	35
			Low SD	8,853	3434	257	12.3	73.8	4.8	79	8	94	30
		NT426	Control	6,032	2588	233	11.4	67.1	5.7	79	5	97	15
			Low SD	10,639	4105	260	11.2	67.4	5.8	80	4	97	15
1				10,192	3697	279	9.4	66.7	5.0	79	20	51	35
2				8,159	3015	266	11.4	68.8	5.0	79	11	62	29
	Dent			10,373	3552	291	10.1	68.4	4.6	78	21	18	36
	Flint			7,979	3160	254	10.8	67.1	5.4	79	10	95	28
		AX887		10,452	3567	291	10.0	68.1	4.6	79	15	22	35
		DK747		10,293	3537	291	10.2	68.7	4.6	78	28	14	37
		ACA2002		7,082	2727	261	11.3	67.5	5.1	79	12	94	38
		NT426		8,875	3593	246	10.3	66.7	5.6	80	8	96	17
			Control	7,984	3120	255	10.2	67.3	5.0	78	18	58	26
			Low SD	10,368	3592	289	10.7	68.2	5.0	79	13	55	38
Year (Y)				***	***	***	***	*(1.5)	ns†	***(1)	***	***	**
Kernel type (KT)				***(455)‡	***(164)	***	***	ns	***(0.1)	***(1)	***(4)	***	***
Treatment (T)				***	***	***	***	ns	ns	*(1)	ns	ns	***
Genotype (kernel type) [G(KT)]				***	***(232)	*	***	ns	***	ns	**	*	***(5)
Y × KT				ns	ns	*(8)	***(0.2)	ns	ns	ns	ns	***(4)	ns
Y × T				***(789)	***(284)	*(10)	***(0.2)	ns	ns	ns	ns	*(5)	***(6)
Y × G(KT)				*(911)	ns	ns	ns	ns	**(0.1)	ns	*(8)	***(6)	ns
KT × T				ns	ns	*(10)	**(0.2)	ns	ns	ns	ns	*(5)	*(6)
T × G(KT)				*(1115)	ns	*(14)	ns	ns	ns	ns	ns	ns	ns
Y × KT × T				ns	ns	ns	ns	ns	ns	ns	ns	*(7)	ns
Y × T × G(KT)				ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

* Significant at $P \leq 0.05$.

** Significant at $P \leq 0.01$.

*** Significant at $P \leq 0.001$.

† ns: nonsignificant.

‡ Numbers in parentheses represent the least significant differences (LSD) of the means at $P \leq 0.05$.

Yield reductions due to defoliations at the flowering period were related to changes in the number of kernels harvested, while defoliations during grain filling were mostly related to changes in kernel weight. Only for kernel weight there was a defoliation treatment × kernel type significant interaction, and was related to a higher kernel weight reduction in dents than in flints at the grain-filling defoliation treatment (270 and 240 mg $kernel^{-1}$ for dents and flints at control treatment, and 220 and 205 mg $kernel^{-1}$ for dents and flints at the grain-filling defoliation treatment).

Kernel protein concentration was reduced by the grain-filling defoliation and not by the flowering defoliation (Table 4). However the defoliation treatments showed significant interactions with kernel type, year, and genotype within kernel type, each of these effects explained <4% of the total variability. This showed that year, kernel type, and genotype within kernel type were the most relevant effects (explaining 60, 14, and 7% of the total variability, respectively) for kernel protein concentration.

Starch concentration was not affected by defoliation treatments, but only by kernel type (Table 4). Contrarily, kernel

Table 4. Defoliation effects at flowering (Def. FL) and at grain filling (Def. GF) over yield, kernel number, kernel weight, grain protein, starch and oil concentration, test weight, floaters, vitreousness and 8 mm screen retention for two dent (AX887 and DK747) and two flint (ACA2002 and NT426) genotypes grown during two seasons (Years 1 and 2).

Year	Kernel type	Genotype	Treatment	Yield	Kernel no.	Kernel wt.	Protein	Starch	Oil	Test wt.	Floaters	Vitreousness	8 mm sieves
				kg ha^{-1}	kernel m^{-2}	mg kernel^{-1}	g 100 g^{-1}			kg hL^{-1}	%		
1	Dent	AX887	Control	11,838	4370	272	8.6	67.9	4.5	78	24	5	24
			Def. FL	7,414	2723	262	8.3	67.9	4.6	78	28	13	27
			Def. GF	7,827	3979	198	8.5	68.3	4.3	75	75	4	12
		DK747	Control	12,556	4413	285	8.8	67.5	4.7	77	38	12	29
			Def. FL	5,962	2183	275	9.5	67.4	4.6	78	36	17	38
			Def. GF	8,065	3826	210	7.9	68.9	4.3	74	90	1	17
	Flint	ACA2002	Control	9,021	3775	241	9.6	66.2	5.2	79	16	92	31
			Def. FL	4,332	1848	247	10.2	66.3	5.0	78	23	86	45
			Def. GF	5,834	3234	189	9.2	72.7	4.6	75	41	68	17
		NT426	Control	9,705	4168	233	8.8	66.7	5.4	79	14	94	18
			Def. FL	5,264	2219	237	9.7	64.7	5.8	80	6	97	22
			Def. GF	7,239	4006	180	8.6	67.0	5.3	77	32	67	8
2	Dent	AX887	Control	5,730	2252	255	10.8	67.6	4.5	79	19	50	28
			Def. FL	5,818	2294	253	10.1	70.3	4.5	79	12	49	23
			Def. GF	5,777	2391	243	10.5	69.6	4.4	78	20	43	21
		DK747	Control	6,850	2556	267	10.8	71.7	4.5	79	19	20	30
			Def. FL	5,583	2317	241	10.1	73.0	4.4	79	54	14	20
			Def. GF	6,483	2879	225	10.2	72.4	4.3	77	59	9	18
	Flint	ACA2002	Control	2,137	840	254	12.8	64.0	5.1	79	9	96	35
			Def. FL	3,180	1249	255	11.9	69.2	4.7	79	9	94	42
			Def. GF	2,359	1068	221	12.2	67.6	4.9	77	14	94	28
		NT426	Control	6,032	2588	233	11.4	67.1	5.7	79	5	97	15
			Def. FL	3,653	1518	247	11.1	66.9	5.7	80	5	98	26
			Def. GF	5,919	2587	228	10.5	66.4	5.5	80	8	97	15
1				7,921	3453	236	9.0	67.7	4.9	77	35	46	24
2				4,960	2056	244	11.0	68.8	4.9	79	19	63	25
	Dent			7,492	3022	249	9.5	69.4	4.5	78	40	20	24
	Flint			5,390	2422	230	10.5	67.1	5.2	79	15	90	25
		AX887		7,401	3014	247	9.5	68.6	4.5	78	30	27	23
		DK747		7,583	3029	251	9.6	77.1	4.5	77	49	12	25
		ACA2002		4,477	1893	235	11.0	77.7	4.9	78	19	88	33
		NT426		6,302	2906	226	10.0	79.2	5.6	79	12	92	17
			Control	7,984	3120	255	10.2	78.4	5.0	79	18	58	26
			Def. FL	5,151	2045	252	10.1	79.0	4.9	79	22	59	30
			Def. GF	6,188	2981	212	9.7	76.5	4.7	77	42	48	17
Year (Y)				***	***	*	***	ns†	ns	***	***	***	ns
Kernel type (KT)				***	***	***	***	*(1.6)	***(0.1)	***	***	***	ns
Treatment (T)				***	***	***	***	ns	***	***	***	***	***
Genotype (kernel type) [G(KT)]				***	***	ns	***	ns	***	***	***(8)	***	***(4)
Y × KT				ns	**(215)	**(8)	**(0.2)	ns	ns	*(1)	ns	***(4)	ns
Y × T				***(653)‡	***(264)	***(10)	***(0.3)	ns	**(0.1)	***(1)	***(9)	***(5)	**(5)
Y × G(KT)				**(754)	**(304)	ns	*(0.3)	ns	*(0.1)	ns	ns	***(6)	ns
KT × T				ns	ns	*(10)	ns	ns	ns	ns	*(9)	ns	*(5)
T × G(KT)				**(923)	*(373)	ns	**(0.4)	ns	**(0.2)	*(1)	ns	ns	ns
Y × KT × T				ns	ns	ns	ns	ns	ns	ns	ns	**(7)	ns
Y × T × G(KT)				ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

* Significant at $P \leq 0.05$.

** Significant at $P \leq 0.01$.

*** Significant at $P \leq 0.001$.

† ns: nonsignificant.

‡ Numbers in parentheses represent the least significant differences (LSD) of the means at $P \leq 0.05$.

oil concentration was significantly reduced by the grain-filling defoliation ($P \leq 0.001$), and the treatment affected genotypes differently (significant treatment × genotype within kernel type effect, $P \leq 0.01$) evidenced by the high oil concentration reduction in ACA2002 during both years.

Defoliating the crop during flowering showed no effect over grain quality traits, while defoliating the crop during grain filling severely reduced test weight and vitreousness, and increased floaters percentage (Table 4). Interestingly, floaters percentage increased more in dents than in flints under the grain-filling defoliation treatment (significant kernel type × defoliation interaction, $P \leq 0.05$). Screen retention showed a kernel type × defoliation treatment significant interaction ($P \leq 0.05$), related to an increased screen retention at the flowering defoliation that was mostly evident for the flint genotypes (Table 4). All grain quality changes generated by the defoliation treatments showed to reduce grain quality more in the higher yielding Year 1 (year × defoliation treatment significant interaction for test weight, vitreousness, floaters, and screen retention; Table 4).

In brief, the higher yield reduction was observed with the flowering defoliation treatment, but had negligible grain composition and quality effects. Defoliating the crops during grain filling reduced yield moderately but severely affected all four grain quality traits.

Yield Differences between Flints and Dents across Tested Environments

Last, we tested differences between kernel types in their yield response across the different treatment combinations following an environmental index approach. Here the environmental index is calculated as the average yield of all genotypes tested at that each particular treatment × year combination (Fig. 1).

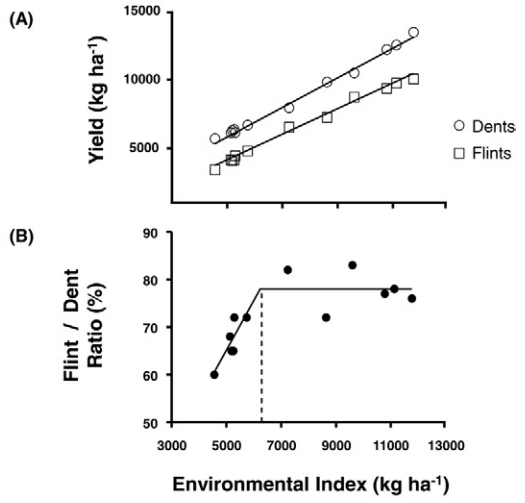

Fig. 1. (A) Relationship between yield and environmental index for dent (empty circles; r^2: 0.89; $P \leq 0.001$; n: 12) and flint (empty squares; r^2: 0.76; $P \leq 0.001$; n = 12) kernel type genotypes. The environmental index is the average yield of all genotypes tested at that each particular treatment × year combination, following Finlay and Wilkinson (1963). (B) Relationship between the relative yield of flint genotypes as a proportion of dent kernel type genotypes yield and environmental index (r^2: 0.79; $P \leq 0.05$; n = 12). At Fig. 1A the linear functions for dent kernel type is y: 1.06 × x + 633, and for flint kernel type is y: 0.93 × x – 629. At Fig. 1B the parameters of the bi-linear model are a, 0.0104; b, 12.85; c, 6250; and d, 77.97.

Flint kernel type always presented lower grain yields than dents across all the environmental conditions explored with our field treatments (Fig. 1A). A relatively constant yield difference of 1000 to 2000 kg ha^{-1} was evident between kernel types across a wide environmental index ranging from 5000 to 12,000 kg ha^{-1}. However, when estimating the performance of flint kernel type genotypes calculated as a percentage of the yield attained by dent kernel type genotypes it was evident that under poor environmental conditions flint and dent kernel types showed proportionally larger yield differences than under higher environmental index scenarios (Fig. 1B). Above an environmental index threshold of 6250 kg ha^{-1} we observed a mostly constant relative yield difference between kernel types, and a larger difference below this threshold. As such, relative yield differences between kernel types depended on the explored environmental index (Fig. 1B).

DISCUSSION

Yield differences between flint and dent kernel types are in general agreement with our previous observations (Tamagno et al., 2015), showing that flint kernel type are on average yielding approximately 75 to 90% of dents yield. However, in the present study the relative yield difference between flints and dents increased at the poorest yielding conditions (low N fertilization levels, defoliation at flowering). The result describing that flint kernel type genotypes have a lower capacity to cope with environmental stress when compared to dents indicates that farmers should avoid low yielding environments for producing this specialty maize because these environments maximize the relative yield differences between both kernel types. Most important, these were also the environments with the poorest grain quality. The use of target environments for discarding which specific flint genotypes have poorer yield and quality tolerance to stressful conditions could be implemented (Cooper et al., 2014). Common alternatives for distinguishing subquality flint genotypes are high stand density, water deficit, or reduced levels of soil N (Beck et al., 1996; Chapman and Edmeades, 1999; Edmeades et al., 1999; Bänziger et al., 2002; Troyer and Rosenbrook, 1983).

Field treatments allowed testing yield and kernel quality responses to common agricultural practices, like stand density and N fertilization. Yield response to N applications was less for flints when compared to dents resulting from a differential efficiency related to their lower yield potential. Averaging across genotypes and years, the fertilizer efficiencies for flints were 13 and 8 kg grain per kg N added as fertilizer for the intermediate and high N fertilized treatments, respectively, and for dent kernel type they were 18 and 12 kg grain per kg N added as fertilizer for the intermediate and high N fertilized treatments, respectively. This differential efficiency could be related to kernel type differences in kernel protein concentration, but protein differences were quite minor (average of 9.6 vs. 10.5 g 100 g^{-1} for dents and flints, respectively). They are most surely related to their intrinsic physiological yield determination differences, where flint kernel types are known to have less plant growth (Tamagno et al., 2015). Genotypic differences among Argentinean commercial dent and semi-dent kernel type genotypes in yield response to N availability are well

known (D'Andrea et al., 2008), so differences between flint and dent kernel types reported in our study are not surprising.

From a crop management perspective N fertilization levels increased not only yield but also all grain quality attributes. Increasing N levels increased test weight, vitreousness, and screen retention, and decreased floaters percentage. These results agree with previous research describing similar results (Mestres and Matencio, 1996; Cirilo et al., 2011), not only for physical properties but for dry-milling performance for endosperm grits. An important outcome of our study is that grain hardness attributes were maximized at N fertilization levels that were higher than the ones needed for maximum yield. During Year 2 N fertilization had no effect on crop yields but did increase kernel hardness and kernel protein concentration, showing the importance that this management option has for increasing grain quality. Our results are helping understand the critical role crop management decisions have over grain composition and quality beside genotype selection (Yuan and Flores, 1996; Duarte et al., 2005; Blandino et al., 2010, 2013; Cirilo et al., 2011).

The least kernel hardness values were reported from the defoliation treatment at the grain-filling period. It is widely accepted that any reduction in plant growth during this stage in maize disrupts the plant source-sink balance, severely affecting final kernel weight, yield, and composition (Borrás et al., 2002, 2004). A reduced plant growth during the flowering period, on the contrary, severely affected yield but not grain quality to the same degree (Table 4). During grain-filling maize N accumulation is quite large (Pearson and Jacobs, 1987), and roots are actively competing with growing kernels for available assimilates (Pan et al., 1986, 1995). In our present experiments defoliation treatments during the effective grain-filling period affected individual kernel weight, protein concentration, and all grain quality attributes related to kernel hardness. It is evident that any severe plant growth reduction during grain-filling needs to be avoided as much as possible when managing crops, not only because of their yield but also for possible grain physical quality penalties.

The two growing seasons created large differences in yield and grain quality, and management strategies interacted with the growing season generating different optimum strategies depending on the year (e.g., stand density and yield). The drier and warmer second experimental year generated lower yields with higher vitreousness and test weight, and lower floaters percentage, but reduced screen retention values. Because many management decisions are made before or around planting, farmers need tools for estimating what type of growing season the crop will be experiencing. In this regard the use of accurate weather forecasts is becoming indispensable (Podestá et al., 2002).

Kernel hardness is known to negatively affect animal digestibility and the efficiency of gain in feedlot cattle (Corona et al., 2006; Jaeger et al., 2006). Argentinean non-GMO flint maize is mostly intended for human consumption. It is a potential vehicle for delivering nutrients to human diet. Nutritional aspects in crops are a current concern for plant breeders who are facing the challenge to create more nutritious crops without affecting yield performance (Morris and Sands, 2006). Achieving this objective is not easy, it requires a joint effort from different research areas in which plant breeding needs to interact with general agronomy and food scientists (Diepenbrock and Gore, 2015). At the present study we characterized environment and management effects in yield and kernel hardness contributing to a more efficient production of a staple crop.

CONCLUSIONS

Dent genotypes yielded significantly more than flints at every field management condition tested. However, the relative difference between flints and dents was significantly larger at the poorest yielding environments (Fig. 1). Therefore, farmers should avoid using flint kernel type genotypes at these conditions. Relevant genotype yield differences within each kernel type makes genotype selection a critical management option.

Genotypes within flint kernel type showed significant differences in several grain quality traits, and field conditions changed their kernel composition and grain quality for dry milling. Low N fertilization and stressful situations during grain filling affected grain hardness the most.

Adequate N availability and low stand density helped improve vitreousness, floaters, and screen retention. These two management practices, together with genotype selection, are critical for minimizing the risk of not reaching market quality standards for hard endosperm maize.

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