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Research paper

Sowing date and maize grain quality for dry milling

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ABSTRACT

Argentina is the single exporter of non-gmo hard endosperm maize to the European Union, and is internationally known for its grain hardness. This special hard endosperm maize supply chain follows strict regulations to ensure a high quality grain. Specific values for test weight, flotation index, grain vitreousness, and screen retention are demanded by the dry milling industry. Central temperate Argentinean production system is currently changing to later sowings, and there is limited information on the effect of contrasting sowing dates over specific grain quality attributes of interest for the industry. In this study we explored the effects of delaying maize sowing dates from September-October to December on maize dry milling grain quality in the central temperate area. Eighteen commercial genotypes differing in grain hardness were sown during two growing seasons and two sowing dates. Measured traits were grain yield, individual grain weight, dry milling quality (test weight, floaters, vitreousness, 8 mm screen retention), and composition (oil, protein, starch). Grain yield varied significantly among genotypes ($p < 0.001$), and semi-dents showed higher yields when compared to hard endosperm flints (13 110 and 11 463 kg ha^{-1}, respectively). Early and late sown maize yielded 12 737 kg ha^{-1} and 11 003 kg ha^{-1}, respectively. Significant genotype differences were observed for all grain quality and composition attributes. Delaying the sowing date from September-October to December had minimum effects on physical grain quality traits, only evident at some genotypes (significant sowing date x genotype interaction for most traits). Genotype to genotype differences in grain quality and composition were larger than variations between sowing dates. Grain hardness was strongly determined by the genotype, making genotype selection a critical management option for attaining high quality at any sowing date. It is evident that high dry milling quality can be obtained with adequate genotypes also at later sowings.

1. Introduction

Argentinean maize production is around 33 million tons per year (FAO, 2014). Most of the planted area, near 5 million hectares, is occupied with soft endosperm semi-dent gmo (genetically modified organism) genotypes. At the same time Argentina produces 130 000–150 000 ha (average last 10 years) of hard endosperm non-gmo maize for dry milling, also known as flint or plata maize. This production results in a yearly average of 360 thousand metric tons of flint maize exported to the European Union during the last decade (Greco and Martí Ribes, 2016). Argentina is currently the single maize exporter of non-gmo flint maize to the European Union, and special import permits for flint maize are used if the grain quality attains specific standards (European Commission, 1997).

Flint maize is known to present a high proportion of vitreous or hard endosperm, smooth crown, and orange pigmentation. Its physicochemical characteristics make it a preferred raw material for the dry milling industry (Litchfield and Shove, 1990; Rooney and Serna Saldívar, 2003). It is highly demanded because of its high milling yields of large endosperm grits, and the particular quality that it provides to a wide variety of end use products such as corn flakes, snacks, and other textured ingredients (Macke et al., 2016). Their characteristic color and specific cooking functional properties are quality attributes highly desirable by the food industry (Kuiper, 2014).

Hard endosperm maize genotypes are currently yielding in the field 10–20% less than most dent (or semi-dent) genotypes (Tamagno et al., 2015, 2016), and premiums are paid to farmers for covering this yield gap. Flint non-gmo production fields are produced using contracts between farmers and industry, and are subject to strict regulations to ensure a high quality grain (MAGyP, 2015). The physical standards that a grain lot needs to reach for optimum quality are: a minimum test weight (76 kg hL^{-1}), a maximum number of floaters at a standardized solution (25%), and a minimum number of grains with 50% or more of vitreous endosperm (92%). Vitreousness is the proportion of grains having more horny than floury endosperm, and is a key attribute for the milling industry. Screen retention is also contemplated in many

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contracts, and industry demands most grains to be retained in an 8 mm round sieve (ideally > 50%) to achieve optimum milling quality. High test weight, low floaters percentage, high vitreous to floury endosperm ratio, and high screen retention are all attributes related to high dry milling yields (Kirleis and Stroshine, 1990; Cirilo et al., 2011; Blandino et al., 2013). These attributes are especially relevant for recovering a large proportion of large flaking grits after milling.

Maize grain hardness has an important genetic control (Williams et al., 2009; Gerde et al., 2016). However, the crop growing environment can also affect maize final grain quality and composition (Borrás et al., 2002; Fox and Manley, 2009; Cirilo et al., 2011; Tamagno et al., 2016). It is relevant that farmers and cooperatives combine adequate genotypes with specific crop management practices for minimizing the risk of not reaching market quality standards for hard endosperm maize. Genotype selection, stand density, sowing date, and N fertilizer are among cropping options easily applicable by farmers.

The Argentinean maize production system has changed drastically in the last years, especially in relation to variations in sowing date. The sowing date for the central temperate region has moved from late September and early October to December. Late sown maize locates the critical flowering period for yield definition (Andrade et al., 1999) under conditions of less evaporative demand and higher probability of rainfall compared to earlier traditional sowings. However, they are able to complete the crop cycle before the first killing frost. Early sowing dates have been traditionally associated with higher maize yields (Cirilo and Andrade, 1994; Mercau and Otegui, 2014) and lower insect pest incidence, specifically *Diatraea saccharalis* and *Spodoptera frugiperda* (Gil et al., 2010; Mercau and Otegui, 2014). But, under these later sowings farmers are obtaining acceptable yields with higher yield stability, and currently most maize in Argentina is planted under late sowings (PAS, 2015). At present, specific crop management options for late sowing (*e.g.*, stand density, planting date, soil P and N management, genotype selection) are becoming available (Mercau and Otegui, 2014; Gambin et al., 2016). However, information regarding grain quality changes like grain hardness and grain dry milling quality is scarce, especially in relation to variations in traits the industry is interested. Preliminary data showed sowing date not affecting grain hardness (Gerde et al., 2017) in our region. A recent study by Cerrudo et al. (2017) reported decreases in grain quality, referred as grain coarse to fine ratio, for dry milling under late sowings, but tested later sowings in latitudes where crops will normally experience a killing frost before physiological maturity. None of these previous studies described the specific grain quality standards used by the supply chain.

In the present study we explored the consequences of delayed maize sowing dates from September-October to December over maize dry milling grain quality in the central temperate region. Analyzed traits focused on those 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 at any temperate environment.

2. Materials and methods

2.1. Crop management

A field experiment was conducted at the Campo Experimental Villarino, Facultad de Ciencias Agrarias, Universidad Nacional de Rosario, in Zavalla, Santa Fe, Argentina (33°1′S, 60°53′W). The experiment was sown during two growing seasons (2014/2015 and 2015/2016, years 1 and 2, respectively) and two sowing dates within each season. Sowing dates were 29 September and 18 December during year 1, and 14 October and 19 of December during year 2.

Field experiments were arranged following a completely randomized design with four replicates. Plots were 4 rows with 6 m long and 0.52 m of inter row spacing. A uniform stand density of 8 plants m^{-2} was used, and plots were always overplanted and thinned at V2-V3 (Abendroth et al., 2011). All measurements were done at the two central rows. Soil samples (0–60 cm) were taken before sowing and analyzed for $N\text{-}NO_3$. At sowing, monoammonium phosphate (MAP, 10–50–0, N–P–K) was applied at a rate of 120 kg ha^{-1} to all plots. The experimental area was fertilized with N using urea (46-0-0, N-P-K) at different rates for reaching 160 kg N ha^{-1} of N from soil sample plus added N. Urea was broadcasted manually over the plots from V4 to V6. Trials were kept free of weeds and pests throughout the growing season. Weeds were controlled by spraying commercially recommended maize herbicides, and also periodically removed by hand whenever necessary. Insect pressure of *D. saccharalis* and *S. frugiperda* were specifically monitored and controlled with recommended products for minimizing any possible effects.

Rainfall from sowing to physiological maturity was 464 and 342 mm (year 1) and 504 and 654 mm (year 2) for early and late sowings, respectively. Average temperatures were 20.9 and 21.9 °C (year 1) and 22.3 and 22.1 °C (year 2) in early and late sowings, respectively. These values are within expected ones based on average historic data for the last 30 years (Table 2). All crops reached physiological maturity before the first killing frost was evident. During both years harvest took place in mid-March and late-May for early and late sowings, respectively.

Eighteen maize hybrids from different seed companies were evaluated (Table 1). At present, farmers are using the same relative maturities for early and late sowings (Mercau and Otegui, 2014; Gambin et al., 2016), and tested hybrids are common commercial genotypes cropped by farmers in the region, representing a range of endosperm hardness. Five hybrids were regular semi-dent grain type, and thirteen were hard endosperm flint grain type. These thirteen flint hybrids are currently used by both local dry milling industry and exporters.

2.2. Grain yield

At commercial maturity, the two central rows of each plot were harvested and used for determining grain yield, average individual grain weight, and all other phenotypic traits. Yield is presented on a 14.5% moisture basis. Individual grain weight was determined by weighing two sets of 100 grains per plot, and average weight per grain calculated.

2.3. Grain quality and composition

Test weight, floaters percentage, and vitreousness were determined according to the methods approved by SENASA (MAGyP, 2015) and the

Table 1
Description of the 18 genotypes tested during two growing seasons and two sowing dates within each season (September-October and December).

Genotype	Grain type	Relative maturity
ACA2002	Flint	128
ACA2002BT	Flint	128
ACA514	Flint	116
ACA530	Flint	131
AX7822TD/TG	Semi-dent	117
AX8010	Flint	118
CyR7325	Flint	124
DK692VT3Pro	Semi-dent	119
DK7210VT3Pro	Semi-dent	122
Mill522	Flint	126
NK940TGPLUS	Flint	126
NK960TD/TG	Semi-dent	128
NT426	Flint	126
NT426BT	Flint	126
NT525	Flint	125
NT525BT	Flint	125
P1780HR	Semi-dent	117
SPS2866	Flint	127

Table 2
Meteorological description (average rainfall and temperature) for each sowing date within each year, together with the historic data (last 30 years) for Zavalla, Santa Fe, Argentina.

Season	Sowing date	Rainfall mm	Temperature °C
Year 1	Early	464	20.9
	Late	342	21.9
Year 2	Early	504	22.3
	Late	654	22.1
Historic	Early	522	21.7
	Late	514	22.4

European Commission for flint maize imports (European Commission, 1997) after a minimum sample cleaning.

Test weight was determined after grain sample homogenization (MAGyP, 2015) using a Schopper chondrometer (Cuenca, Rosario, Argentina). Results are expressed in kg hL^{-1}.

Floaters percentage (%) was measured introducing 100 grains in a $NaNO_3$ solution (density: 1.25 g cm^{-3}) at 35 °C, thoroughly shaken every 30 s for 5 min to eliminate bubbles. At the end of this time period floating grains were counted and reported as percentage. Grain moisture concentration ranged from 12 to 14.5%, always below 14.5%. The test was done two times per field replicate, following Tamagno et al. (2016).

To determine vitreousness (%) 400 grains per plot were longitudinally dissected and visually inspected. The percentage of grains 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 grains. The number of grains complying with these three conditions was divided by the total number of grains, and expressed as percentage vitreousness. For a particular maize lot to be considered as flint, percent grain vitreousness needs to be above 95%, although there is a 3% tolerance that sets the limit value at 92% (MAGyP, 2015).

The proportion of grains sized over 8 mm was measured by using a Ro-Tap like sieve shaker (Zonytest, Rey & Ronzoni, 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 two minutes shaking. This test was done twice per sample. Screen retention is basically a sizing grain test to determine the proportion of grains over a specific round hole. The percentage (%) of grains retained by the 8 mm sieve over the total sample was reported (Tamagno et al., 2016).

Grain starch, protein, and oil percentages were determined using near infrared spectroscopy with an Infratec 1241 (Foss, Hillerød, Denmark) as in Borrás et al. (2002), using 400 g of grain per plot. Prediction errors for our calibration curves were 0.2, 0.3 and 1.5% for oil, protein and starch, respectively. Values are reported on a dry weight basis.

2.4. Statistical analysis

Data were analyzed using linear mixed-effects models (nlme package; Pinheiro et al., 2016) in R (R Core Team, 2016, version 3.3.0). For each trait, the model considered sowing date, genotype, and sowing date x genotype interaction as fixed effects and year as random effect. We also accounted for the hierarchical data structure by considering genotype nested in sowing date, and sowing date nested in year in the random term. Models were fitted using the restricted maximum likelihood method (Zuur et al., 2009).

We checked the Gaussian and homoscedasticity assumptions for the standardized residuals of the models with graphical analysis (Zuur et al., 2009). Depending on the trait, variance heterogeneity was found across sowing dates, genotypes or years. Heterogeneity was incorporated into the models using the varIdent variance structure (Zuur

Table 3
Genotype and sowing date effects over grain yield, grain weight, test weight, flotation index, vitreousness, 8 mm screen retention, oil, protein, and starch grain concentration for the 18 genotypes tested. Only main effects are described here. A full description of all genotypes at each sowing date is available as Supplemental Information (Table SI). Oil, protein and starch concentrations are reported in a dry weight basis.

Sowing date	Genotype	Yield kg ha^{-1}	Grain weight mg $grain^{-1}$	Test weight kg hL^{-1}	Flotation index %	Vitreousness %	8 mm screen retention %	Oil %	Protein %	Starch %
Early		12 737	302	79.1	9	66	41	4.9	9.2	71.0
Late		11 003	286	79.1	9	62	36	4.9	8.6	72.0
	DK7210VT3Pro	14 064	308	77.2	36	2	44	4.5	7.9	72.6
	AX7822TD/TG	13 531	317	77.0	23	3	42	4.2	8.2	72.9
	DK692VT3Pro	12 983	285	78.2	16	12	49	4.7	8.5	72.5
	P1780HR	12 646	303	77.3	33	3	42	4.3	8.5	72.1
	AX8010	12 601	299	79.4	3	83	44	4.8	8.9	71.5
	NT525BT	12 516	300	79.7	5	47	32	4.6	7.8	72.6
	NK960TD/TG	12 326	302	79.3	3	82	30	5.6	9.1	70.1
	NT426BT	12 264	279	80.3	1	97	19	5.6	8.8	70.7
	NT525	11 954	306	80.5	4	59	30	4.6	8.2	72.9
	SPS2866	11 911	298	78.2	6	60	30	5.3	9.1	70.7
	NK940TGPLUS	11 568	284	78.6	4	75	10	5.3	9.3	70.6
	NT426	11 510	267	79.8	2	95	14	5.5	9.0	70.8
	ACA514	11 234	291	79.7	5	88	52	4.6	8.9	71.9
	Mill522	10 971	299	80.5	2	94	66	4.9	9.7	70.6
	ACA2002BT	10 749	288	79.3	5	80	41	5.0	9.7	71.2
	CyR7325	10 648	291	79.7	3	89	45	4.9	9.1	71.7
	ACA530	10 578	307	80.5	2	93	69	5.1	10.0	70.0
	ACA2002	10 514	278	79.3	5	86	40	5.0	9.5	71.4
	Semi-dent	13 110	303	77.8	22	20	42	4.7	8.4	72.0
	Flint	11 463	291	79.6	4	80	38	5.0	9.1	71.3
Genotype (G)		***	***	***	***	***	***	***	***	***
Sowing date (SD)		ns	ns	ns	ns	ns	ns	ns	ns	ns
G x SD			***(1304)[†]	***(19)	**(0.71)	*(6)	**(10)	***(10)	***(0.2)	***(0.5)

*, **, *** significant at $p < 0.05$, 0.01, and 0.001 respectively, ns: not significant.
[†] Numbers in parentheses represent the least significant differences (LSD) of the means at $p \leq 0.05$.

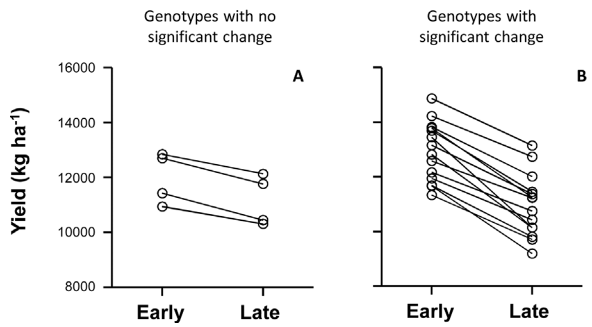

Fig. 1. Relationship between grain yield and sowing date for genotypes not showing a significant change with sowing date (Fig. 1A) and for those that did have a significant sowing date effect (Fig. 1B) ($p < 0.05$). Symbols indicate the average of each genotype at each sowing date during two growing seasons.

et al., 2009). Thus, each sowing date, genotype or year is allowed to have a different variance. Models with and without variance structure were compared using the log-likelihood ratio test. Multiple comparisons between means were conducted using the predict means function in R (Luo et al., 2014). R^2 of models for most traits were higher than 0.80, indicating they properly described observed data.

3. Results

3.1. Yield

Grain yield showed significant genotype differences ($p < 0.001$), varying from 10 514–14 064 kg ha^{-1} (Table 3). Sowing date had no effect over grain yield ($p > 0.05$; Table 3), but there was a significant genotype x sowing date interaction ($p < 0.001$; Table 3) showing that yield of different genotypes responded differently to sowing date changes (Fig. 1). On average, early and late sown maize yielded 12 737 kg ha^{-1} and 11 003 kg ha^{-1}, respectively (Table 3), and only four genotypes (NT525BT, NT426BT, Mil522, and CyR7325) showed no yield changes across sowing dates (Fig. 1A). On average, flint genotypes yielded ca. 87% of semi-dented ones across sowing dates, where semi-dents and flint genotypes yielded 13 110 and 11 463 kg ha^{-1}, respectively (Table 3). The yield difference between flints and semi-dent genotypes was similar across sowing dates (88 and 87%, for early and late sowing dates, respectively).

Individual grain weight was different across genotypes ($p < 0.001$), and varied from 267 to 317 mg grain^{-1} (Table 3). Sowing date had no effect over individual grain weight ($p > 0.05$; Table 3), but the genotype x sowing date interaction was significant ($p < 0.001$; Table 3). This interaction showed genotypes responded differently with delaying sowing date (Fig. 3A and B). On average across years and sowing dates, flint and semi-dent genotypes had 291 and 303 mg grain^{-1}, respectively (Table 3).

3.2. Grain quality and composition

Physical grain quality for dry milling was tested using four different traits: test weight, flotation index, grain vitreousness, and screen retention. Grain composition was evaluated by measuring grain oil, protein, and starch concentration.

Test weight showed significant genotype to genotype differences ($p < 0.001$; Table 3). Flint genotypes showed higher values than semi-dented ones (79.6 and 77.8 kg hL^{-1}, respectively). No sowing date effect was evident on test weight ($p > 0.05$; Table 3), and some genotypes responded differently (significant genotype x sowing date interaction, $p < 0.01$; Table 3). The interaction was related to ACA2002, the single genotype that significantly reduced its test weight with delayed sowings (Fig. 2A and B). All evaluated genotypes reached the minimum test weight value (76 kg hL^{-1}) for attaining high quality maize (MAGyP, 2015) at early and late sowings.

Flotation index showed significant genotype differences ($p < 0.001$; Table 3). No differences were evident over flotation index among sowing dates ($p > 0.05$), and a significant genotype x sowing date interaction was evident ($p < 0.05$; Table 3). No flint genotype showed significant changes in flotation index due to changes in sowing date (Fig. 2C and D). The significant genotype x sowing date interaction was related to three semi-dent genotypes (DK7210, DK692, and P1780; Fig. 2D) changing their value at later sowings. Larger genotype differences in flotation index were evident within semi-dented genotypes (Table 3). Flint genotypes had all very similar flotation index values, and were always below the maximum floaters percentage imposed by the norm (MAGyP, 2015; Table 3) at both sowing dates.

Vitreousness showed significant genotype differences, ranging from 2 to 97% ($p < 0.001$; Table 3). Sowing date had no effect over grain vitreousness ($p > 0.05$; Table 3), but some genotypes did change their values across sowing dates (significant genotype x sowing date interaction, $p < 0.01$; Table 3). The interaction was related to genotypes ACA2002, NT525, and NT525BT (Fig. 2E and F). Changing the sowing date had mostly no effect over grain vitreousness for most genotypes (Fig. 2E), showing that genotype selection is critical for this trait. Although flint genotypes showed higher vitreousness than semi-dented genotypes, only four flint genotypes reached the minimum value of 92% (MAGyP, 2015) at both sowing dates (NT426BT, NT426, Mil522 y ACA530).

Screen retention also showed significant genotype differences ($p < 0.001$; Table 3), and there was a significant genotype x sowing date interaction showing that not all genotypes responded similarly to changes in their sowing date ($p < 0.001$; Table 3). This was related to two genotypes only (ACA2002 and ACA514). From Fig. 2G and H it becomes evident that genotype selection is highly relevant for this trait. On the other hand, only three hard endosperm flint genotypes (ACA514, Mil522, and ACA530) reached values considered optimum for dry milling (ideally > 50%; Table 3). On average across years and sowing dates, semi-dents and flint genotypes had similar screen retention values (42 and 38%, respectively). But, flint genotypes showed more screen retention variability, ranging from 10 to 69%, while semi-dents ranged from 30 to 49%.

Grain oil concentration showed significant genotype differences, ranging from 4.2 to 5.6% ($P < 0.001$; Table 3), and some genotypes did change their grain oil concentration at different sowing dates (significant sowing date x genotype interaction; $p < 0.001$; Table 3; Fig. 3C and D). When discriminating between flints and semi-dent genotypes, flints had higher grain oil concentrations (5.0%) when compared to semi-dents (4.7%).

Grain protein concentration showed significant genotype to genotype differences ($p < 0.001$; Table 3). Sowing date had no effect over grain protein concentration ($p > 0.05$; Table 3) but most genotypes did show some changes (significant sowing date x genotype interaction; $p < 0.001$; Table 3). In general genotypes reduced their grain protein concentration with delayed sowing date (Fig. 3E and F), and only five genotypes (SPS2866, Mil522, ACA2002, ACA2002BT, and ACA530) had no variation in grain protein concentration between sowing dates (Fig. 3E and F). When averaged across genotypes, grain protein concentration was 9.2 and 8.6% for early and late sowings, respectively (Table 3). When averaged across sowing dates, flint genotypes had higher values than semi-dents (9.1 and 8.4%, respectively).

Grain starch concentration showed clear genotype differences ($p < 0.001$; Table 3), and a significant genotype x sowing date interaction was evident ($p < 0.001$; Table 3). On average across sowing dates genotypes ranged from 70.0 to 72.9%, and the tendency was to have higher grain starch concentration at the later sowing date, but only five genotypes (AX7822TD/TG, DK692VT3Pro, NT525BT, NK940TGPLUS, and CyR7325) showed a significant increase (Fig. 3G and H). Semi-dented genotypes had, on average, slightly more grain starch concentration than flint ones (72.0 and 71.3%, respectively).

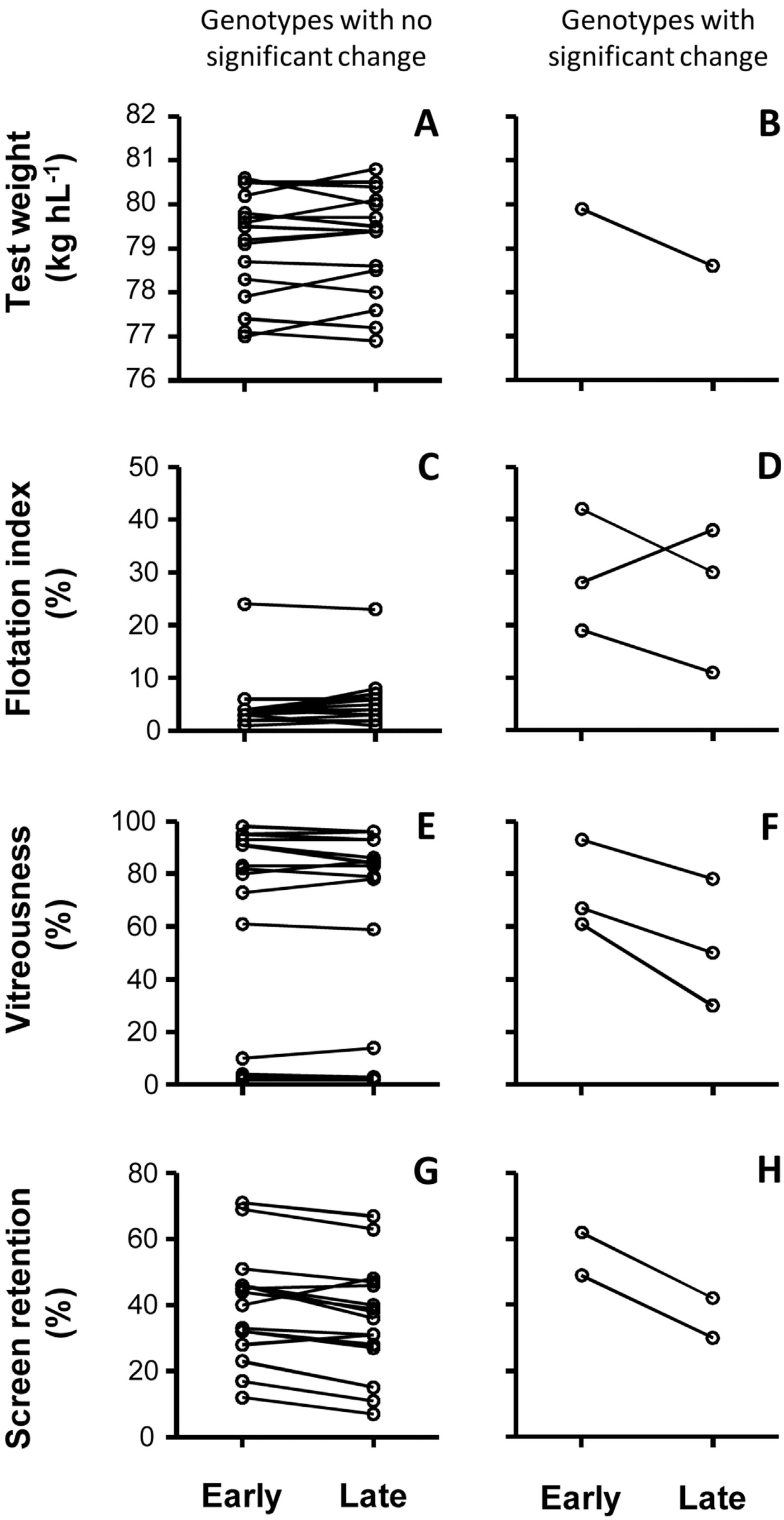

Fig. 2. Relationship between grain hardness attributes (test weight, flotation index, vitreousness, and screen retention) and sowing date for genotypes not showing a significant changes with sowing date (Fig. 2A, C, E, and F) and for those that did have a significant change due to sowing date (Fig. 2B, D, F, and H) ($p < 0.05$). Symbols indicate the average of each genotype at each sowing date during two growing seasons.

In brief, all grain quality and composition traits showed significant genotype differences. A three month delay in sowing date affected grain quality and composition in some, but not all, genotypes (Table 3). Grain hardness traits like test weight, flotation index, vitreousness, and screen retention showed sowing date had no effect in most genotypes. Several genotypes reached optimum dry milling grain quality at early and late sowings.

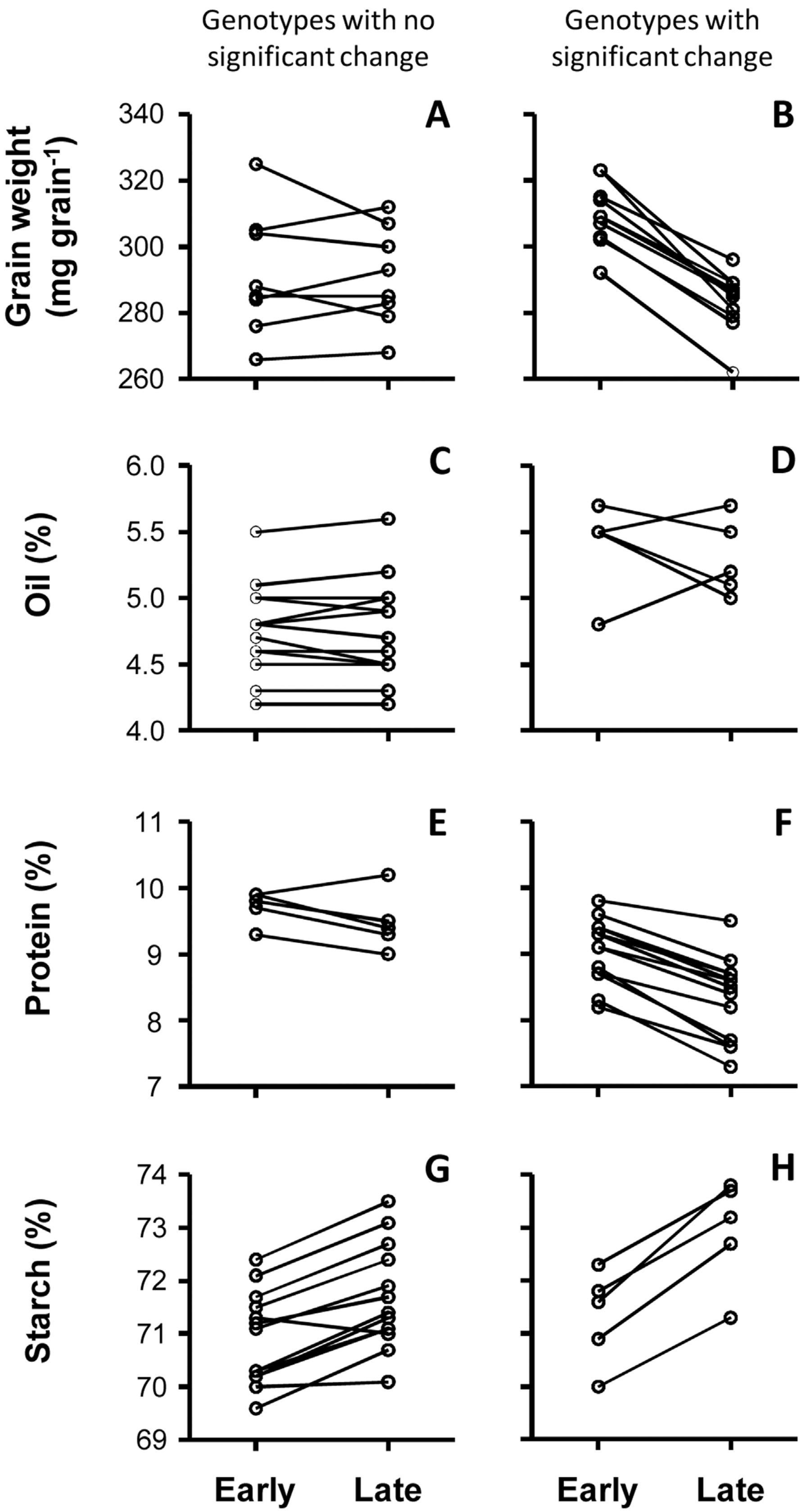

Fig. 3. Relationship between grain weight and composition traits (oil, protein, and starch) and sowing date for genotypes not showing a significant change with sowing date (Fig. 3A, C, E, and F) and for those that did have a significant change due to sowing date (Fig. 3B, D, F, and H) ($p < 0.05$). Symbols indicate the average of each genotype at each sowing date during two growing seasons.

4. Discussion

The present manuscript reports on how yield, grain hardness, and composition respond to sowing date and genotype changes in the central Argentinean region. In our experiments semi-dented genotypes yielded, on average, more than flint genotypes. Yield differences between both grain types are in general agreement with our earlier reports (Tamagno et al., 2015, 2016), showing flint grain type genotypes yielding approximately 75–90% of semi-dents yield. When evaluating across sowing dates, the relative yield difference between flint and dent grain type remained constant (flints yielding 87% of semi-dent grain type genotypes), indicating that both grain types reduced their grain yield similarly in later sowings. As such, hard endosperm non-gmo flint producers should not expect higher yield reductions than regular semi-dent producers in later sowings if insect control is adequate. It is important to recall flint genotypes have no Bt technology, and are more susceptible to insects when compared to regular semi-dent genotypes having different Bt technologies. It is relevant to understand that both sowing dates permitted all genotypes to reach physiological maturity before the first killing frost was evident.

Cirilo et al. (2011) described reductions in grain weight and screen retention values due to sowing date delays at some environments. And Cerrudo et al. (2017) described reductions in grain hardness, measured as grain coarse to fine ratio, at higher latitude environments with delayed sowing date. When testing a larger set of hybrids our results agree with these previous studies partially, because only some genotypes did show changes in grain hardness and composition at delayed sowings (Figs. 2 and 3). Sowing date showed to significantly affect grain quality traits only in some genotypes, and it is evident that its effect was really minor when compared to genotype to genotype differences. Only genotypes ACA2002 and ACA514 significantly reduced their screen retention with delayed sowing date (Fig. 2G and H). A decrease in grain vitreousness was evident in only three genotypes (ACA2002, NT525, and NT525BT) when both sowing dates are compared (Fig. 2E and F). As such, grain quality traits relevant for dry milling (test weight, flotation index, vitreousness and screen retention) were largely determined by the specific genotype, the sowing date effect was really minor.

Maize in Argentinean central temperate region has significantly shifted its sowing date more than three months, from September-October to December (Mercau and Otegui, 2014; Bolsa de Cereales, 2015; Gambin et al., 2016), and it is evident that high quality grain for dry milling can be produced at later sowings. Our results reinforce the importance of genotype selection as a critical crop management option for producing high quality grain for dry milling, and for minimizing the risk of not reaching the minimum grain quality standards for hard endosperm maize (MAGyP, 2015).

Late sowings are known to present higher disease pressure during grain filling, generating a higher mycotoxins contamination risk. Genotype differences in grain hardness are correlated to differences in mycotoxins contamination (Ramirez et al., 1996; Presello et al., 2007), and several authors (Blandino et al., 2009; Cao et al., 2014) reported higher fumonisins contamination in maize with delayed sowing dates. Our study focused in describing grain quality changes in relation to gran hardness, but the sowing date effect over micotoxins contamination will also need to be considered in future studies.

Grain hardness has been traditionally associated with specific endosperm proteins, known as zeins (Robutti et al., 1997; Pratt et al., 1995; Fox and Manley, 2009). Grain hardness expression would not seem to be expensive in terms of energy utilization, as only a few specific endosperm proteins are responsible of grain hardness variations (Dombrink-Kurtzman and Bietz, 1993; Robutti et al., 1997; Gerde et al., 2016). This might help explain why changes in sowing date reduced grain protein concentration for most genotypes, but had negligible effects on test weight, flotation index, vitreousness, and screen retention traits. Gerde et al. (2017) showed that the relative change in the different zein fractions due to sowing date changes was minor when compared to the genotype effect. Together, these results are reinforcing the importance of considering the changes of only some very specific protein fractions with crop management changes for studying grain hardness.

It is widely known that maize grain hardness is negatively correlated with high physical yields on farmer fields (Eyhérabide et al., 2004). In the present study, although genotypes showed significant differences in yield and in several physical grain quality traits, only two genotypes reached the highest quality for optimum dry milling. These two genotypes (Mil522 and ACA530) were not the highest yielding ones (Table 3). Macke et al. (2016) reported similar results, showing negative correlations between genotype dry milling efficiency and grain field yield. It is important to evaluate different ways for preserving yield while improving grain hardness attributes. Flint maize in Argentina is mostly intended for Europe human consumption, and many plant breeders are becoming more interested in improving crop quality traits for human nutrition and health (Diepenbrock and Gore, 2015). High throughput phenotyping tools as near-infrared spectroscopy (NIR) (Araus and Cairns, 2014) are commonly used by breeders for testing several grain quality and composition traits in cereal crops (Osborne, 2006). Fox and Manley (2009) stated that NIR spectroscopy has shown to be an accurate tool for rapidly estimating maize grain hardness. This can be used in breeding programs for rough screening for high yield and grain quality for dry milling.

5. Conclusions

As expected from previous studies, semi-dent grain type genotypes showed higher yields when compared to hard endosperm flints (13 110 and 11 463 kg ha^{-1}, respectively). Delaying the sowing date reduced yield, 12 737 and 11 003 kg ha^{-1} for early and late sowings, respectively, but yield differences between flints and dents were similar (12–13%).

Delaying the sowing date in the central Argentinean temperate region from September-October to December had minimum effects over grain quality traits related to dry milling. Significant genotype differences were observed for physical grain quality attributes related to dry-milling and grain composition. Several genotypes reached optimum dry milling grain quality at early and late sowings. Grain hardness was strongly associated with genotype main effect, making genotype selection a critical crop management option for producing hard endosperm maize at early and late sowing dates.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.eja.2017.09.013.

References

Abendroth, L.J., Roger, W.E., Matthew, J.B., Marlay, S.K., 2011. Corn Growth and Development. Iowa State University Extension, Ames, Iowa.
Andrade, F.H., Vega, C., Uhart, S., Cirilo, A., Cantarero, M., Valentinuz, O., 1999. Kernel number determination inmaize. Crop Sci. 39, 453–459.
Araus, J.L., Cairns, J.E., 2014. Field high-throughput phenotyping: the new crop breeding frontier. Trends Plant Sci. 19, 52–61.
Blandino, M., Reyneri, A., Vanara, F., 2009. Effect of sowing time on toxigenic fungal infection and mycotoxin contamination of maize kernels. J. Phytopathol. 157, 7–14.

Blandino, M., Sacco, D., Reyneri, A., 2013. Prediction of the dry- milling performance of maize hybrids through hardness-associated properties. J. Sci. Food Agric. 93, 1356–1364.

Bolsa de Cereales, 2015. Panorama Agricola semanal. http://www.bolsadecereales.org (verified 26/11/2015).

Borrás, L., Curá, J.A., Otegui, M.E., 2002. Maize kernel composition and post-flowering source-sink ratio. Crop Sci. 42, 781–790.

Cao, A., Santiago, R., Ramos, A.J., Sout, X.C., Aguín, O., Malvar, R.A., Butrón, A., 2014. Critical environmental and genotypic factors for Fusarium verticillioides infection: fungal growth and fumonisin contamination in maize grown in northwestern Spain. Int. J. Food Microbiol. 177, 63–71.

Cerrudo, A., Martinez, D., Izquierdo, N., Cirilo, A., Laserna, P., Reinoso, L., Balbi, C., Valentinuz, O., Andrade, F., 2017. Environment, management, and genetic contributios to maize kernel hardness and grain yield. Crop Sci. 57, 2788–2798.

Cirilo, A.G., Andrade, F.H., 1994. Sowing date and maize productivity: I. Crop growth and dry matter partitioning. Crop Sci. 34, 1039–1043.

Cirilo, A.G., Actis, M., Andrade, F.H., Valentiluz, O.R., 2011. Crop management affects dry-milling quality of flint maize kernels. Field Crops Res. 122, 140–150.

Diepenbrock, C.H., Gore, M.A., 2015. Closing the divide between human nutrition and plant Breeding. Crop Sci. 55, 1437–1448.

Dombrink-Kurtzman, M.A., Bietz, J.A., 1993. Zein Composition in hard and soft endosperm of maize. Cereal Chem. 70, 105–108.

European Commission, 1997. Commission Regulation (EC) No 641/97 of 14 April 1997 amending Comission Regulation (EC) No 1249/96 of 28 June 1996 on rules of application (cereals sector import duties) for Council Regulation (EEC) No 1766/92. Off. J. Eur. Union L 98, 15.4.1997, p. 2–8.

Eyhérabide, G.H., Robutti, J.L., Percibaldi, N.M., Presello, D.A., Alvarez, M.P., 2004. Association between grain yield and endosperm hardness in maize cultivars. Maydica 49, 319–326.

FAO, Food and Agricultural Organization of the United Nations, 2014. Statistics of Farming Production. Maize. Available in. http://faostat3. fao.org/home/E.

Fox, G., Manley, M., 2009. Hardness methods for testing maize kernels. J. Agric. Food Chem. 57, 5647–5657.

Gambin, B.L., Coyos, T., Di Mauro, G., Borrás, L., Garibaldi, L.A., 2016. Exploring genotype, management, and environmental variables influencing grain yield of late-sown maize in central Argentina. Agric. Syst. 146, 11–19.

Gerde, J.A., Tamagno, S., Di Paola, J.C., Borrás, L., 2016. Genotype and nitrogen effects over maize kernel hardness and endosperm zein profiles. Crop Sci. 56, 1225–1233.

Gerde, J.A., Spinozzi, J., Borrás, L., 2017. Maize kernel hardness, endosperm zein profiles, and ethanol production. Bioenergy Res. 10, 760–771.

Gil, A., Vilariño, P., Lenardis, A.E., Guglielmini, A.C., 2010. Bases para el control y manejo de plagas. In: Satorre, H. (Ed.), Produccion de granos. Bases funcionales para su manejo. Editorial Facultad de Agronomía, UBA, Buenos Aires, Argentina, pp. 617–649.

Greco, I.A., Martí Ribes, I., 2016. Actualidad en producción, exportación y tendencias en nuestro uso del maíz Plata/Flint Argentino para el mercado Europeo. In: Borrás, L. (Ed.), Optimizando el manejo del cultivo de maíz flint. Editorial Tecnigráfica 87 pgs.

Kirleis, A.W., Stroshine, R.L., 1990. Effects of hardness and drying air temperature on breakage susceptibility and dry-milling characteristics of yellow dent corn. Cereal Chem. 67, 523–528.

Kuiper, E., 2014. Usos del maíz flint. In: Borrás, L. (Ed.), Manejo eficiente del nitrógeno en maíz flint. Editorial Tecnigráfica 89 pgs.

Litchfield, J.B., Shove, G.C., 1990. Dry milling of U.S. hard- endosperm corn in Japan: product yield and corn properties. Appl. Eng. Agric. 6, 629–634.

Luo, D., Ganesh, S., Koolaard, J., 2014. predictmeans: Calculate predicted means for linear models. R package version 0.99. https://CRAN.R-project.org/package=predictmeans.

MAGyP, 2015. Ministerio de Agricultura, Ganadería y Pesca de la República Argentina. Norma XXIX from Resolución Número 757. Boletín Oficial, October 17th 1997, p. 17. http://www.infoleg.gov.ar (verified 20/11/16).

Macke, J.A., Bohn, M.O., Raush, K.D., Mumm, R.H., 2016. Genetic factors underlying dry milling efficiency and flaking grit yield examined in U.S. maize (*Zea mays* ssp. mays L.) germplasm. Crop Sci. 56, 2516–2526.

Mercau, J.L., Otegui, M.E., 2014. A modeling approach to explore water management strategies for late-sown maize and double-cropped wheat-maize in the rainfed Pampas region of Argentina. In: Ahuja, L.R., Ma, L., Lascano, R.J. (Eds.), Practical Applications of Agricultural System Models to Optimize the Use of Limited Water. Adv. Agric. Systems Model. 5. ASA, SSSA, CSSA, Madison, WI, pp. 351–374.

Osborne, B.G., 2006. Applications of near infrared spectroscopy in quality screening of early-generation material in cereal breeding programmes. J. Near Infrared Spectrosc. 14, 93–101.

PAS. Panorama Agrícola Semanal, 2015. Bolsa de Cereales, Argentina. URL http://www.bolsadecereales.org/.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., R Core Team, 2016. nlme: Linear and nonlinear mixed effects models. R package version 3. 1-127. http://CRAN.R-project.org/package=nlme.

Pratt, R.C., Paulis, J.W., Miller, K., Nelsen, T., Bietz, J.A., 1995. Association of zein classes with maize kernel hardness. Cereal Chem. 72, 162–167.

Presello, D.A., Iglesias, J., Botta, G., Eyhérabide, G.H., 2007. Severity of Fusarium ear rot and concentration of fumonisin in grain of Argentinian maize hybrids. Crop Prot. 26, 852–855.

R Core Team, 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Ramirez, M.L., Pascale, M., Chulze, S., Reynoso, M.M., March, G., Visconti, A., 1996. Natural occurrence of fumonisins associated to Fusarium contamination in commercial corn hybrids grown in Argentina. Mycopathologia 135, 29–34.

Robutti, J.L., Borrás, F.S., Eyhérabide, G.H., 1997. Zein composition of mechanically separated coarse and fine portions of maize kernels. Cereal Chem. 74, 75–78.

Rooney, L.W., Serna Saldívar, S.O., 2003. Food use of whole corn and dry-milled fractions. In: White, P.J., Johnson, L.A. (Eds.), Corn: Chemistry and Technology, 2nd ed. AACC, Saint Paul, MN p. 495–535.

Tamagno, S., Greco, I.A., Almeida, H., Borrás, L., 2015. Physiological differences in yield related traits between flint and dent Argentinean commercial maize genotypes. Eur. J. Agron. 68, 50–56.

Tamagno, S., Greco, I., Almeida, H., DiPaola, J.C., Marti Ribes, P., Borrás, L., 2016. Crop management options for maximizing maize kernel hardness. Agron. J. 108, 1561–1570.

Williams, P., Geladi, P., Fox, G., Manley, M., 2009. Maize kernel hardness classification by near infrared (NIR) hyperspectral imaging and multivariate data analysis. Anal. Chim. Acta 653, 121–130.

Zuur, A.F., Leno, E.N., Walker, N.J., Saveliev, A.A., Smith, G.M., 2009. Mixed Effect Models and Extentions in Ecology with R. Springer, New York.