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Dry milling grain quality changes in Argentinean maize genotypes released from 1965 to 2016

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ABSTRACT

Argentina is one of the most important maize producers worldwide, and is internationally known for producing hard endosperm maize. The physicochemical characteristics of the maize grain directly affects the milling yield of large endosperm grits, the main dry milling product, and specific grain quality values are demanded by industry. Argentinean traditional maize grains used to have optimum hardness quality for dry milling, but higher yielding newer commercial genotypes slowly moved from hard endosperm flints to semi-dent or dent softer endosperm grain type. Our objective was to describe how grain hardness and composition changed in commercial maize genotypes released in Argentina from 1965 to 2016 as an indirect breeding effect when selecting for on-farm yield. Measured traits were yield, individual grain weight, dry milling quality (test weight, floaters, grain vitreousness, 8 mm screen retention), and composition (oil, protein, starch).

There were clear genotype differences in yield ($p < 0.001$), and they were positively correlated with release year at a rate of 113 kg ha^{-1} yr^{-1} (consistent with previous studies). Grain quality and composition traits also showed significant genotype effects ($p < 0.001$), and traits were also correlated with the genotype market release year. When estimating the average genetic gain across environments and stand density treatments, test weight decreased from 79.1 to 76.0 kg hL^{-1}, grain vitreousness decreased from 100 to 0%, screen retention decreased from 65 to 37%, oil concentration decreased from 5.1 to 4.7%, and protein concentration decreased from 11.6 to 8.7%, while floaters increased from 2 to 31% and starch concentration increased from 69.8 to 72.3%. As such, Argentinean grain hardness and protein concentration declined when selecting higher yielding genotypes. The largest grain hardness changes occurred between mid-1980 and 2000, and current commercial genotypes do not have optimum dry milling quality. This helps understand why the dry milling industry started selecting specific genotypes in the 1990s, and is solely relying on genotypes specially released for dry milling purposes since early 2000s. Consequences of the observed trade-offs between grain hardness and protein concentration with yield for the dry milling industry are discussed.

1. Introduction

Argentina is one of the most important maize producers worldwide (FAO, 2014), and is internationally known for its grain hardness. Today it is the single provider of hard endosperm maize to the European Union. Until the end of the 1980s, most maize grown in Argentina was considered hard endosperm flint maize (Gear, 2006). In the last decades the introduction of elite dent germplasm from the U.S. slowly replaced traditionally hard endosperm genotypes with higher-yielding and softer semi-dent ones (Brun and Dudley, 1989; Delucchi et al., 2012). However, the indirect effect of yield improvements over specific grain quality attributes relevant for the dry milling industry, or general grain composition traits, has never been reported. Because of the central role of Argentina as a relevant international supplier of hard endosperm maize, it is critical to describe and quantify how the traditional flint maize genotypes have evolved to current semi-dented ones as indirect breeding effects when selecting for yield improvement.

Genetic gain studies consist on evaluating under the same crop management and environmental conditions a range of genotypes released during different years (Bell et al., 1995). These studies help

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quantify the genetic progress of different traits as a result of breeding efforts (Masuka et al., 2017), and have been extensively documented in maize (Tollenaar, 1989; Eyhérabide et al., 1994; Duvick and Cassman, 1999; Duvick, 2005; Luque et al., 2006; Di Matteo et al., 2016; Masuka et al., 2017). Most studies have focused on genetic contributions to yield increases. In United States, Duvick (2005) showed yield gains ranging from 65 to 75 kg ha^{-1} yr^{-1} from 1934 to 2004. For Argentina, Luque et al. (2006) showed an overall genetic gain of 132 kg ha^{-1} yr^{-1} from 1965 to 1997, Eyhérabide et al. (1994) reported a genetic gain of 105 kg ha^{-1} yr^{-1} from 1979 to 1991, and Di Matteo et al. (2016) showed a genetic gain of 107 kg ha^{-1} yr^{-1} from 1965 to 2010. Under high input conditions, gains in Africa were 109.4 kg ha^{-1} yr^{-1} from 2000 to 2010 (Masuka et al., 2017).

Despite the large number of studies describing yield changes because of breeding efforts at different regions, studies describing changes in maize grain composition or specific quality traits relevant for the maize processing industry are limited. When considering genotypes released from 1920 to 2001, modern maize genotypes in the U.S. have lower protein, lower oil, and higher starch concentrations than older ones (Scott et al., 2006). A similar trend was observed in Chinese and American genotypes released from 1960 to 2001 in China (Li et al., 2015). For U.S. genotypes released from 1930 to 1991 and grown in Iowa, grain starch concentration increased 0.03% yr^{-1} while protein concentration decreased at a rate of 0.03% yr^{-1} (Duvick, 2005). Sun et al. (2014) showed lower rates of starch concentration increases (0.025% yr^{-1}) and a similar rate of protein concentration decrease (0.031% yr^{-1}) in Chinese maize genotypes. We hypothesize that a similar protein concentration decline happened in Argentina, with its concomitant effect on grain hardness as a result of the mechanistically related nature of endosperm hardness and endosperm protein concentration (Dombrink-Kurtzman and Knutson, 1997; Gerde et al., 2016). Today specially released hard endosperm commercial genotypes have grain yields 10 to 30% lower than normal regular dents (Tamagno et al., 2015, 2016; Abdala et al., 2018), evidencing the commonly observed tradeoff when selecting for yield and grain hardness.

Previous grain composition changes due to breeding efforts have not described physical grain quality variations over time. These traits are highly relevant for the maize dry milling industry because grain physical properties have large effects on milling yield (Paulsen and Hill, 1985; Lee et al., 2007; Macke et al., 2016). Argentinean hard endosperm flint maize yields 45–55% large flaking grits, which is considerably more than the milling yields commonly attained when using North American or European softer endosperm germplasm (25–35%).

The main objective of our study was to describe temporal changes in maize grain quality for dry milling and composition in Argentina as an indirect consequence of yield increases. Yield and grain quality or composition tradeoffs are evident in many species, and current regular semi-dent or dent maize genotypes are no longer suitable for optimum milling yields. Breeding consequences on maize grain quality when selecting for yield have been rarely described, especially for grain hardness and their consequence for dry milling. We tested 32 commercial maize genotypes released from one breeding company (Dekalb-Monsanto) from 1965 to 2016. Genotypes were selected for yield and agronomic improvement, without considering any grain quality effect. We focused on the specific traits currently used for exporting hard endosperm maize from Argentina to the European Union, approved by SENASA (MAGyP, 2015) and the European Commission for maize imports (European Commission, 1997). We also discuss consequences of described tradeoffs between grain quality and yield for the Argentinean dry milling supply chain.

2. Materials and methods

2.1. Sites and crop management

Two field experiments were conducted at Campo Experimental Villarino, Facultad de Ciencias Agrarias, Universidad Nacional de Rosario, in Zavalla, Santa Fe, Argentina (33° 1′ S, 60° 53′ W). The first experiment was planted on October 14, 2015 (early environment), and the second on December 19, 2015 (late environment). Within each experiment all genotypes were evaluated at two stand densities (6 and 10 plants m^{-2}). Thirty two commercial genotypes released by Dekalb-Monsanto in Argentina from 1965 to 2016 (Table 1) were used in both experiments. These genotypes can be considered a representative sample of the genetic commercial availability in Argentina during the last 51 years, and several old genotypes were used by the dry milling supply chain. Yield of genotypes grown under high stand density and in the earliest sowing date has been reported in Borrás and Vitantonio-Mazzini (2018). Sowing date and stand density treatment effects over dry milling quality were not the main objective of this study, but used as different growth environments. Our previous evidences have shown that reducing the stand density slightly increases grain quality for dry milling (Tamagno et al., 2016), while changes in the sowing date has minimum grain quality and composition effects for most genotypes in the region (Abdala et al., 2018).

Each field experiment was arranged following a completely randomized design with three replicates. Each plot had four rows 6 m long with 0.52 m of inter-row spacing. Plots were always overplanted and thinned at V3 to the target stand density. All measurements were done using the two central rows. Soil samples (0 to 60 cm) were taken before sowing and analyzed for N-NO3. At sowing, monoammonium phosphate (10-50-0, N-P-K) was applied at a rate of 160 kg ha^{-1} to all plots. The experimental area was fertilized with N using urea (46-0-0) at different rates for reaching 165 kg N ha^{-1} of N from soil sample plus added N. This urea was broadcasted manually over the plots at V4. Experiments were conducted under rain-fed conditions. The experimental area was kept free of weeds and pests throughout the growing season. Insect pressure was specifically monitored and controlled with recommended products throughout the season for minimizing any

Table 1
List of evaluated genotypes together with their market release year.

Genotype	Release year
DKF880	1965
DK4F33	1980
DK4F34	1980
DK2F10	1980
DK4F31	1980
DK4F32	1980
DK3F21	1982
DK3F22	1983
DK2F11	1984
DK4F37	1988
DK3F24	1988
DK3S41	1989
DK664VT3P	1993
DK752VT3P	1993
DK688MG	1997
DK696VT3P	1997
DK757MG	1997
DK765MG	1997
DK615MG	1999
DK682VT3P	2000
DK190VT3P	2002
DK690MG	2004
DK747VT3P	2004
DK699VT3P	2007
DK692VT3P	2010
DK70-10VT3P	2012
DK72-50VT3P	2012
DK70-20VT3P	2012
DK72-10VT3P	2012
DK73-10VT3P	2013
LT719VT3P	2014
DK73-20VT3P	2016

possible effect.

Rainfall from sowing to physiological maturity was 504 and 654 mm for early and late sowing dates, respectively. Average temperatures were 22.3 and 22.1 °C for early and late sowing dates, respectively. These values are within expected ones based on average historical data for the last 30 years. Historic rainfall data from sowing to physiological maturity are 522 and 514 mm for early and late sowing dates, respectively. Temperature historic averages are 21.7 and 22.4 °C for early and late sowing dates, respectively. Average days across genotypes from sowing to R1 were 74 and 57 days for our early and late sowing dates, respectively. Early sown maize reached R1 the 27th December 2015 while late sown maize the 14th February 2016. Average days from sowing to physiological maturity were 129 and 134 days for early and late sown maize, respectively. Early sown maize reached physiological maturity the 20th February 2016 while late sown maize the 30th April 2016. All crops reached physiological maturity before the first killing frost.

2.2. Grain yield

At commercial maturity the central two rows from each plot were manually 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.

2.3. Physical properties for grain quality and grain composition

Test weight, floaters percentage, and grain vitreousness were determined according to the methods approved by the European Commission for hard endosperm maize imports (European Commission, 1997) and SENASA (MAGyP, 2015).

Test weight was determined after grain sample homogenization (MAGyP, 2015) using a Schopper chondrometer (Cuenca, Rosario, Argentina). Results are expressed as kg hL^{-1}. The minimum test weight value for a maize lot to be considered hard endosperm flint is 76 kg hL^{-1} (MAGyP, 2015). Regular maize needs to achieve 75 kg hL^{-1} to reach the maximum internal quality, which is lower than the value imposed by the SENASA flint norm for hard endosperm maize. Test weight is the only physical trait demanded for regular maize.

Floaters percentage (%) was measured by adding a 100 grains aliquot in a $NaNO_3$ solution (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 grains were counted and reported as percentage. The test was done twice per field replicate, following Gerde et al. (2016). The maximum floaters percentage for a maize lot to be considered flint is 25% (MAGyP, 2015).

To determine vitreousness (%) 200 grains per plot were longitudinally dissected and visually inspected. The percentage of grains that were not indented in the crown, that had central floury endosperm completely surrounded by horny endosperm, and horny endosperm representing 50% or more of the endosperm were considered vitreous grains, and reported as percentage relative to the total number of inspected grains. For a particular maize lot to be considered as hard endosperm flint, percent grain vitreousness needs to be above 95%. However, there is a 3% tolerance that sets the limit value at 92% (MAGyP, 2015).

Screen retention (the proportion of grains sized over 8 mm) was measured 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 by the 8 mm sieve was determined after two minutes shaking and reported as percentage (%). This test was also done twice per field replicate (Tamagno et al., 2016). Dry milling processors prefer maize lots with screen retention values higher than 50%.

Grain starch, protein, and oil percentages were determined by near infrared spectroscopy using an Infratec 1241 instrument (Foss, Hillerød, Denmark) as in Borrás et al. (2002) and Abdala et al. (2018). Values were reported on a dry weight basis.

2.4. Statistical analysis

Data were analyzed using a general linear ANOVA model in R software with agricolae package (R Core Team, 2016). Evaluated fixed effects were genotype (G), stand density (SD), environment (E), G x SD, G x E, SD x E, and G x SD x E interactions. Variance components and least significant difference (LSD) of all evaluated traits were estimated from the ANOVA analysis.

The genetic gain for the evaluated traits was expressed as the slope of the linear relationship between the specific trait and genotype release year. Genotype release years are available at INASE (https://www.inase.gov.ar/; Accessed 12 Feb. 2018). Slope differences among environments were tested by an analysis of covariance as implemented in Graph Pad Prism version 5.00 for Windows (GraphPad, 2011).

3. Results

3.1. Grain yield

Crop grain yields showed significant genotype, stand density, and environment main effects ($p < 0.001$; Table 2). Significant interactions were genotype x stand density ($p < 0.001$), genotype x environment ($p < 0.05$), and genotype x stand density x environment ($p < 0.05$; Table 2). However, each interaction explained less than 5% of the total explored variation (Table 2). The genotype component explained the largest portion of the model variation for grain yield (66%, Table 2), suggesting that their behavior was consistent across environments and stand densities. When averaged across environments and stand densities, genotypes grain yield ranged from 7,981 to 13,973 kg ha^{-1} (Table 3). Higher yields were obtained in the earlier environment. When averaged across stand densities and genotypes, early and late environments led to average yields of 11,577 and 10,591 kg ha^{-1}, respectively (Table 3). When comparing stand densities, grain yield was 10,811 and 11,365 kg ha^{-1} when genotypes were grown at the low and high stand densities, respectively (Table 3).

Grain yield was positively correlated with genotype release year. When averaging across environments and stand densities, mainly because grain yield was mostly related to genotypes differences, the slope of the linear relationship between grain yield and genotype release year was 113 kg ha^{-1} yr^{-1} (Fig. 1A). This totals an increase in grain yield of 5,763 kg ha^{-1} from 1965 to 2016. When analyzing the earlier environment, genotypes sown at the high stand density always yielded more than sown at the lower one, and the genetic gain in the earlier environment was 113 kg ha^{-1} yr^{-1} for both high and low stand density treatments. When analyzing the later environment older genotypes yielded more when sown at the lower stand density, while the newest genotypes showed higher yields when sown at higher stand densities (Supplemental Information). The genetic gains in the later environments were 135 and 94 kg ha^{-1} yr^{-1} for high and low stand density treatments, respectively, averaging 114 kg ha^{-1} yr^{-1}.

Individual grain weight showed genotype and stand density effects ($p < 0.001$; Table 2), and significant interactions were genotype x stand density ($p < 0.05$) and genotype x environment ($p < 0.001$). Genotype was the effect that explained the largest portion of individual grain weight variation (46%; Table 2), followed by stand density (10%) and genotype x environment interaction (10%) effects. The significant genotype x stand density interaction only explained 5% of total individual grain weight explored variability (Table 2). Genotype grain weight differences ranged between 195 and 290 mg $grain^{-1}$ when averaged across environments and stand densities (Table 3), and there was no clear trend for changes in individual grain weight due to genotype release year (Fig. 1B). Two genotypes, DK752VT3P and

Table 2
Genotype, stand density, and environment effects over yield, individual grain weight, test weight, floaters, vitreousness, 8 mm screen retention, oil, protein, and starch for thirty two genotypes. Variance components (in percentage) associated to genotype (G), stand density (SD), environment (E), residual, and all possible interactions.

Effect	Yield kg ha^{-1}	Grain weight mg grain^{-1}	Test weight kg hL^{-1}	Floaters %	Vitreousness %	Screen retention %	Oil %	Protein %	Starch %
Genotype (G)	***	***	***	***	***	***	***	***	***
Stand Density (SD)	***	***	ns	* (2)	***	*** (1)	***	***	***
Environment (E)	***	ns	***	ns	***	***	***	***	***
G x SD	***	* (12)	ns	ns	*** (5)	ns	** (0.2)	* (0.5)	***
G x E	*	*** (12)	* (0.9)	*** (10)	*** (5)	*** (8)	*** (0.2)	** (0.5)	***
SD x E	ns	ns	ns	ns	ns	ns	ns	ns	ns
G x SD x E	* (1,546)[a]	ns	ns	ns	ns	ns	ns	ns	* (0.9)
% Variance									
G	66	46	74	71	97	81	57	57	57
SD	2	10	0	2	0	3	1	5	8
E	6	0	3	0	0	0	9	6	3
G x SD	5	5	2	0	0	1	4	4	5
G x E	3	10	7	8	1	4	11	5	7
SD x E	0	0	0	0	0	0	0	0	0
G x SD x E	3	4	2	2	0	1	2	3	3
Residual	15	25	12	17	1	8	16	19	16

ns: non significant at $P < 0.05$.
* Significant at $P < 0.05$.
** Significant at $P < 0.01$.
*** Significant at $P < 0.001$.
[a] Numbers in parentheses represent the least significant differences (LSD) of the means presented in Table 3.

Table 3
Mean values of yield, individual grain weight, grain hardness and composition attributes for the main effects of thirty two genotypes tested at two stand densities (low and high) in two environments (early and late). Statistical significance for the main effects and their interactions are described in Table 2, and a full description of each genotype at each combination of environment and stand density is described as supplemental information (SI Table). Genotypes were arranged by year of release, from the oldest (release year 1965) to the newest (release year 2016).

Main effect		Yield kg ha^{-1}	Grain weight mg grain^{-1}	Test weight kg hL^{-1}	Floaters %	Vitreousness %	Screen retention %	Oil %	Protein %	Starch %
Genotype	DKF880	7,981	284	80.7	3	100	84	5.0	11.9	69.4
	DK4F33	8,880	270	80.6	3	98	71	5.1	10.3	70.5
	DK4F34	8,654	271	80.4	6	92	63	4.6	10.5	71.4
	DK2F10	9,883	271	79.7	11	92	74	4.7	10.0	71.5
	DK4F31	9,163	278	80.6	7	97	79	4.7	11.1	70.4
	DK4F32	9,203	267	79.8	8	86	64	5.1	10.6	70.5
	DK3F21	9,450	258	79.2	14	63	62	4.9	9.6	71.2
	DK3F22	8,014	257	80.5	7	98	71	4.8	10.7	70.6
	DK2F11	9,639	281	81.0	5	99	85	4.9	11.0	70.1
	DK4F37	10,329	247	81.8	8	84	57	5.5	10.7	69.8
	DK3F24	10,549	264	81.3	7	70	58	5.4	10.3	70.1
	DK3S41	10,586	256	81.2	4	52	42	5.1	11.0	70.3
	DK664VT3P	11,955	276	82.2	2	68	64	4.6	10.1	71.5
	DK752VT3P	11,212	231	79.6	3	48	21	5.0	10.5	71.2
	DK688MG	10,755	278	81.6	1	78	80	4.9	10.2	70.7
	DK696VT3P	10,960	276	79.5	6	13	38	5.0	10.6	70.5
	DK757MG	11,221	195	79.5	9	33	10	5.1	9.4	72.5
	DK765MG	12,013	267	77.7	26	1	27	4.5	9.7	71.9
	DK615MG	11,004	286	75.3	53	5	58	4.8	9.7	71.3
	DK682VT3P	12,923	275	79.9	12	18	54	4.7	9.7	71.8
	DK190VT3P	12,211	254	79.0	16	5	39	4.7	8.8	72.3
	DK690MG	10,450	281	77.3	30	7	70	4.9	10.6	70.5
	DK747VT3P	12,045	274	80.4	8	6	43	4.5	9.1	72.3
	DK699VT3P	12,889	288	79.8	9	16	70	5.2	8.7	71.0
	DK692VT3P	12,870	278	78.9	33	7	51	4.6	8.2	73.0
	DK70-10VT3P	12,622	278	78.9	33	7	51	4.6	8.2	73.0
	DK72-50VT3P	12,824	276	74.8	64	2	61	4.6	8.7	72.2
	DK70-20VT3P	13,368	272	79.5	7	21	50	4.8	9.4	71.8
	DK72-10VT3P	12,253	286	79.1	24	2	50	4.6	8.5	72.2
	DK73-10VT3P	12,769	284	78.5	26	6	54	4.7	9.0	71.6
	LT719VT3P	12,444	268	79.4	17	13	54	4.6	9.3	71.8
	DK73-20VT3P	13,973	290	79.0	15	6	52	4.7	9.1	71.6
Environment	Early	11,577	269	79.9	14	42	55	4.9	10.2	71.0
	Late	10,591	267	79.3	15	47	58	4.7	9.5	71.5
Stand density	Low	10,811	276	79.5	13	43	60	4.9	10.1	70.9
	High	11,365	260	79.7	15	46	53	4.8	9.6	71.6

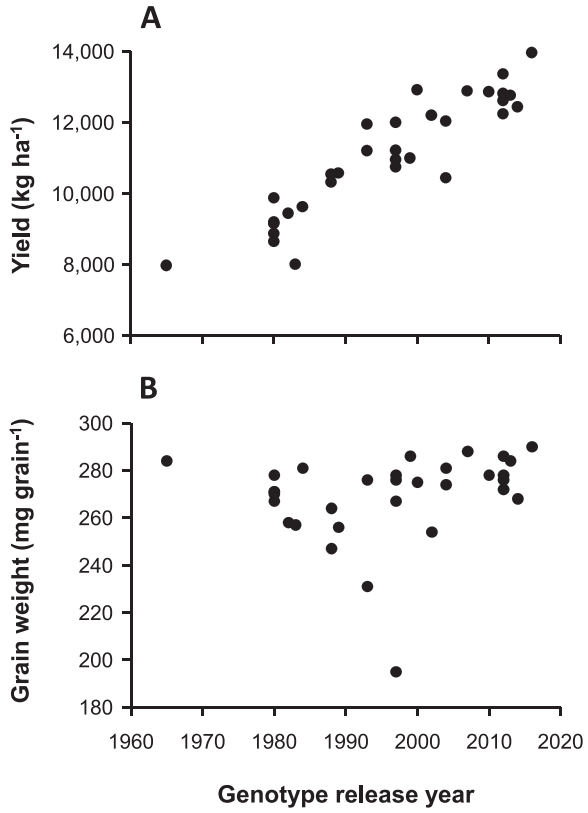

Fig. 1. Changes in grain yield (Fig. 1A) and individual grain weight (Fig. 1B) as a function of genotype release year for 32 genotypes released from 1965 to 2016. Symbols describe the average of two environments and two stand densities. The equation of the linear regression in Fig. 1A is: Y = 113.2 * X – 214,900 (r^2: 0.84; P < 0.001; N: 32). The relationship between individual grain weight and release year in Fig. 1B was not significant (p > 0.05).

DK757MG released in 1993 and 1997 respectively, showed significantly lower grain weights when compared to all other genotypes (Table 3). Reducing stand density had a positive effect on individual grain weight. Growing plants at the low and high stand densities resulted in average grain weights of 276 and 260 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, floaters percentage, grain vitreousness, and screen retention. Grain composition was evaluated by measuring grain oil, protein, and starch concentration.

Test weight showed significant genotype and environment main effects (p < 0.001; Table 2). Genotype accounted for most test weight variation (74%; Table 2), and although there was a significant genotype x environment interaction (p < 0.05) it only explained 7% of the total explored variation (Table 2). When averaged across environments and stand densities, genotypes ranged from 74.8 to 82.2 kg hL^{-1} (Table 3). The late environment had lower test weight than the earlier one (79.3 and 79.9 kg hL^{-1}, respectively), but the environment main effect only explained 3% of the total explored variation (Table 2).

Test weight values were significantly (p < 0.01) reduced with increasing genotype release year at an average rate across environments and stand densities of 0.059 kg hL^{-1} yr^{-1} (Fig. 2A). However, only 23% of test weight variation was explained by genotype release year (Fig. 2A). Test weight values lower than 76 kg hL^{-1} were only evident for two genotypes (DK72-50VT3P and DK615MG), which were released after 1990.

Floaters percentage showed significant genotype (p < 0.001) and stand density main effects (p < 0.05), and there was a significant genotype x environment interaction (p < 0.001; Table 2). Genotype differences accounted for the majority of model variation (71%; Table 2), and their mean values ranged from 1 to 64% (Table 3). Floaters percentage was lower at the lowest stand density, but the effect was minor (13 and 15% for low and high stand densities, respectively, Table 3). Significant stand density and genotype x environment interaction effects only explained 2 and 8% of the total explored variation, respectively (Table 2).

Floaters percentage significantly (p < 0.001) increased with increasing genotype release year (Fig. 2B). Despite this significant trend, only 27% of the floaters percentage variation was explained by genotypes release year (Fig. 2B). Larger genotype to genotype variability was observed among genotypes within the most recent years (Fig. 2B). Older genotypes had very similar floaters percentages, while more modern ones showed larger differences (Table 3; Fig. 2B). Flotation index values higher than 25% were evident after 1997.

Vitreousness was significantly affected by genotype, stand density, and environment main effects (p < 0.001; Table 2). However, the genotype explained 97% of the model variation, and environment, stand density, and significant interactions genotype x stand density and genotype x environment (p < 0.001) explained less than 1% of total variability each (Table 2). As such, although grain vitreousness showed significant changes across environments and stand densities, it is evident that variations in this specific trait are highly related to genotype to genotype differences (Table 2).

Grain vitreousness has significantly decreased (p < 0.001) from 1965 to 2016, with a clear change between years 1990 and 2000 (Fig. 2C), although a slight decrease can also be detected starting mid-1980s. Older and modern genotypes showed higher and lower grain vitreousness values, respectively (Fig. 2C; Table 3). Overall lineal genetic grain vitreousness decline was 2.58% yr^{-1}, but a clear vitreousness decline was evident in genotypes released after late 1990s.

Screen retention showed significant genotype, stand density, and environment main effects, and a significant genotype x environment interaction (p < 0.001; Table 2). Genotype to genotype differences accounted for most of the model variation for screen retention (81%; Table 2) with mean values ranging from 10 to 85% (Table 3). When averaged across stand densities, early and late environments led to different screen retention values (55 and 58% respectively; Table 3). And when averaged across environments, screen retention values were 60 and 53% when genotypes were grown at low and high stand densities, respectively (Table 3). However, stand density, environment, and genotype x environment interaction effects explained less than 5% of the total explored variation (Table 2). Genotypes with the lowest screen retention values (10 and 21%, DK757MG and DK752VT3P, respectively) were also the genotypes with the lowest individual grain weights.

Screen retention significantly (p < 0.001) decreased from 1965 to 2016 at a rate of 0.53% yr^{-1} (Fig. 2D). However, only 17% of screen retention variation was explained by genotypes release year (Fig. 2C). Also, the largest spread in screen retention values was observed in genotypes released between 1988 and 2000 (Fig. 2D; Table 3). Except for particular genotypes (DK757MG, DK752VT3P, and DK765MG) screen retention values were always close to 50%, meeting the expectations of dry milling processors for this parameter.

Significant grain oil concentration differences were observed among genotypes, stand densities, and environments (p < 0.001; Table 2), and significant interactions were genotype x stand density (p < 0.01) and genotype x environment (p < 0.001; Table 2). Genotype was the effect that accounted for most variation for grain oil concentration (57%; Table 2), followed by the genotype x environment interaction (11%) and environment main effect (9%). Grain oil concentration ranged from 3.3 to 5.6%. For most genotypes, the early environment and low stand density favored higher grain oil concentration (see Supplemental Information for interaction descriptions).

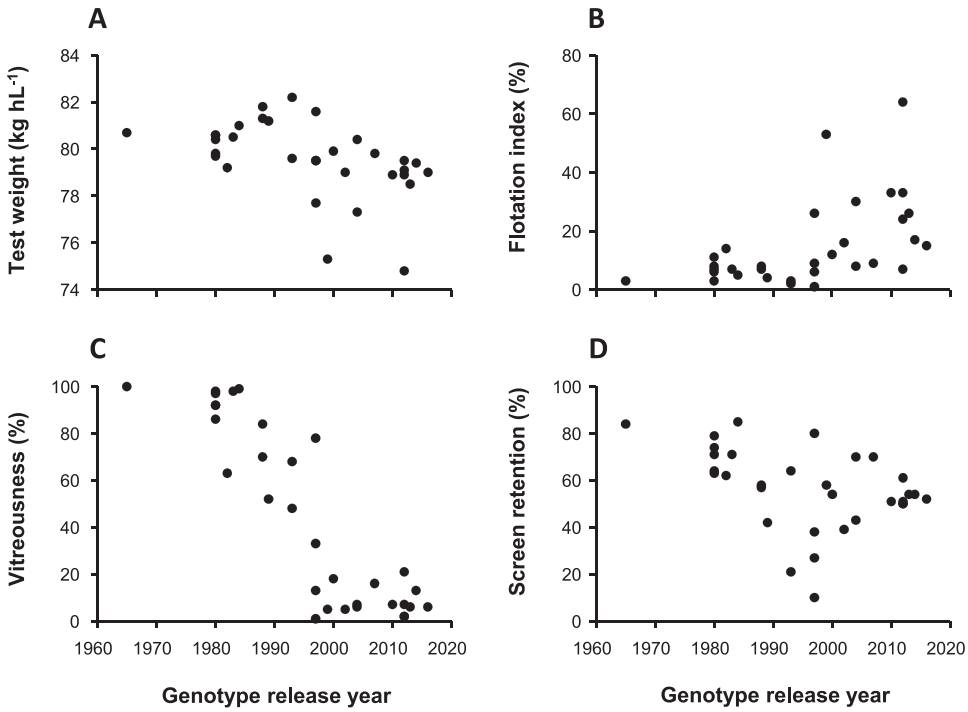

Fig. 2. Relationship between test weight (Fig. 2A), floaters (Fig. 2B), grain vitreousness (Fig. 2C), and screen retention (Fig. 2D) with genotype release year for a total of thirty two genotypes released from 1965 to 2016. Symbols describe the average of each genotype during two sowing dates and two stand densities. The equations of the linear regressions for significant traits are: $Y = -0.06 * X + 197$ (r^2: 0.23; $P < 0.01$; N: 32; Fig. 2A); $Y = 0.58 * X - 1138$ (r^2: 0.27; $P < 0.01$; N: 32; Fig. 2B); $Y = -2.58 * X + 5187$ (r^2: 0.78; $P < 0.001$; N: 32; Fig. 2C); $Y = -0.532 * X + 1110$ (r^2: 0.16; $P < 0.05$; N: 32; Fig. 2D).

Grain oil concentration significantly ($p < 0.001$) decreased with genotype release year at a rate of 0.008% yr^{-1} from 1965 to 2016 (Fig. 3A). However, only 16% of oil concentration variation was explained by genotype release year (Fig. 3A). The highest oil concentrations were evident in the oldest genotypes.

Grain protein concentration showed significant genotype, stand density, and environment main effects ($p < 0.001$; Table 2), and significant interactions were genotype x stand density ($p < 0.05$) and genotype x environment ($p < 0.01$). Genotype main effect explained 57% of the total model variation, and all other significant effects accounted for less than 7% each (Table 2). For most genotypes, higher protein concentrations were observed in genotypes when grown in the early environment and in the low stand density. Total variation observed in grain protein concentration ranged from 6.2 to 12.1% (see Supplemental information).

Grain protein concentration decreased ($p < 0.001$) at a rate of 0.057% yr^{-1} from 1965 to 2016. This decline represents a 2.9% reduction in protein concentration during the studied period (Fig. 3B). Higher grain protein values were always observed among older genotypes (Table 3).

Grain starch concentration showed significant genotype, stand density, and environment main effects ($p < 0.001$; Table 2). Significant interactions were genotype x stand density ($p < 0.001$), genotype x environment ($p < 0.001$), and genotype x stand density x environment ($p < 0.05$; Table 2). Variation was mostly explained by genotype (57%), and other significant effects explained less than 9% of the model variation each (Table 2). Starch concentrations observed as the result of the combination of genotype, stand density, and environment effects ranged from 68.7 to 74.3% with a large majority of starch percentages above 70% (Supplemental information).

Grain starch concentration increased with increasing genotype release year at a rate of 0.048% yr^{-1}. This represents a 2.4% increase after the evaluated 51 years of breeding (Fig. 3C). Overall, modern genotypes have higher grain starch concentrations (Table 3). Although the interaction genotype x stand density x environment was significant, genotypes, regardless of their release year, always had more grain starch concentration when sown at the higher stand densities. In Fig. 3 stand densities and environments are averaged because the genotype component explained most grain starch variations (Table 2; Fig. 3C).

In brief, grain physical and composition traits have significantly changed over the years. Test weight, screen retention, grain vitreousness, oil, and protein concentration decreased with increasing genotype release year, while floaters percentage and grain starch concentration increased. Although effects related to environment and stand density were statistically significant in many cases, most traits were predominantly related to genotypic differences.

Using the slope of each trait in their respective fitted functions (Figs. 1A, 2C, 3B, and C) we estimated a penalty of increasing grain yield over those traits that were strongly influenced by breeding. For every 44 kg ha^{-1} of grain yield increase there was a decrease of 1% in grain vitreousness. And for every 1,986 kg ha^{-1} of grain yield increase there was a 1% grain protein concentration decrease. Regarding starch concentration, for every 2,358 kg ha^{-1} of grain yield increase there was a 1% increase in grain starch.

The physical standards from SENASA for exporting high quality maize for dry milling from Argentina to the European Union are: minimum 76 kg hL^{-1} test weight and 92% grain vitreousness, and maximum 25% floaters. When evaluating genotypes for compliance with the SENASA requirements, the last genotype released to the market from Dekalb-Monsanto that reached the specific quality standard was in 1984 (DK2F11; Fig. 4). From this moment onwards genotypes started not meeting adequate vitreousness levels first, and then vitreousness and floaters adequate levels. From 1984 onwards the minimum vitreousness level was never reached again, and in 1997 the breeding program released the first genotype (DK765MG) having more floaters percentage than the maximum imposed by the SENASA flint norm. However, in rare cases genotypes did not reach the minimum test weight level of 76 kg hL^{-1}.

4. Discussion

4.1. Grain hardness and composition changes

Our research aimed to describe genetic changes as a consequence of breeding for yield in traits relevant for dry milling quality and composition in maize commercial genotypes released in Argentina. For this, we evaluated 32 genotypes released from 1965 to 2016 by the Dekalb-Monsanto breeding company. Average yield genetic gain was 113 kg

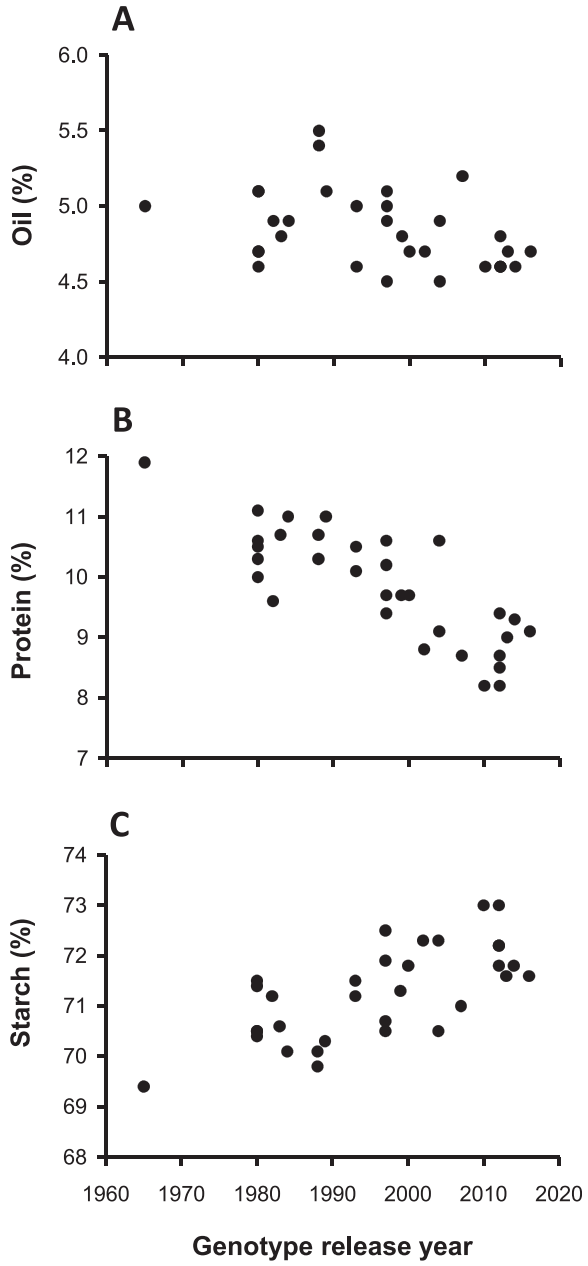

Fig. 3. Changes in grain oil (Fig. 3A), protein (Fig. 3B), and starch concentration (Fig. 3C) and genotype release years for a total of 32 genotypes released from 1965 to 2016. For each figure, symbols indicate the average of each genotype during two sowing dates and at two stand densities. The equations of the linear regressions for significant traits are: Y = −0.008 * X + 20.1 (r^2: 0.16; P < 0.05; N: 32; Fig. 3A); Y = −0.057 * X + 123.8 (r^2: 0.67; P < 0.001; N: 32; Fig. 3B); Y = 0.048 * X – 23.9 (r^2: 0.48; P < 0.001; N: 32; Fig. 3C).

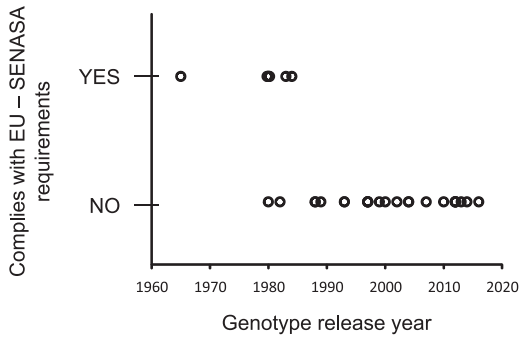

Fig. 4. Changes in genotype capacity to comply with the SENASA Flint Norm for exporting hard endosperm flint maize to the European Union over time. Grain lots need to reach a minimum of 76 kg hL^{-1} test weight, a maximum floaters percentage of 25%, and a minimum of 92% vitreousness. Individual genotype averages for each trait are described in Table 2.

ha^{-1} year^{-1} (Fig. 1A), similar to other values reported for our region (Eyhérabide et al., 1994; Luque et al., 2006; Di Matteo et al., 2016), and significant effects on grain hardness and composition were evident. To the best of our knowledge our study is the first to describe indirect breeding effects over traits directly relevant for the maize processing industry, like grain hardness attributes, highly relevant for the dry milling industry. Previous studies addressed grain composition traits only.

Negative correlations between grain protein concentration and yield potential or genotype release year are common, as reported in maize (Duvick and Cassman, 1999; Duvick, 2005; Scott et al., 2006), soybean (Rincker et al., 2014; de Felipe et al., 2016), and wheat (Laidig et al., 2016). Maize starch concentrations show the opposite trend, increasing over time (Duvick and Cassman, 1999; Scott et al., 2006). In our study, we showed that attributes related to grain hardness like vitreousness were also severely modified. Because grain hardness is mechanistically related to the concentration of specific endosperm proteins (Dombrink-Kurtzman and Knutson, 1997; Gerde et al., 2017), the result of grain hardness decreasing over time is not surprising. When selecting for yield, reductions in grain protein concentration have consequences other than only decreasing the value of the grain as animal feed.

When comparing our results with the study of Duvick and Cassman (1999), the rates of protein and starch concentration changes were higher in Argentina. Duvick and Cassman (1999) explored genotypes released from 1930 to 1991. In our study, the earliest release year was 1965. If we consider the overlapping time frame for both studies (1965–1991), protein concentration decreased at a rate of 0.035% yr^{-1} in Duvick and Cassman (1999) study while in our study it did at 0.043% yr^{-1}. In fact, this ~20% difference in protein decrease rate is in agreement with the also ~20% difference in yield gain rate observed between both studies. Thus, breeding for increasing grain yield had a direct and negative impact in protein concentration. For starch, the concentration increase rates during for the same timespan were very similar, 0.044 and 0.048% yr^{-1} for each study.

High throughput phenotyping and molecular tools (Bernardo, 2008; Araus and Cairns, 2014) are necessary to enhance hard endosperm maize genetic improvement to reduce the currently observed yield gap between flint and dent germplasm. Selecting for genotypes showing optimum both yield and grain quality is not an easy task considering the trait complexities. Yield and grain quality are both quantitative traits, usually controlled by many genes and highly influenced by the environment (Hallauer et al., 1988; Collard et al., 2005). However, previous studies show that only particular changes in endosperm proteins are involved in determining grain hardness (Dombrink-Kurtzman and Knutson, 1997; Gerde et al., 2016; Martínez et al., 2017; Gerde et al., 2017). Thus, describing the specific peptides and starch components determining grain hardness is necessary to unravel the genetic and biochemical mechanisms behind observed changes. This is the basis to try manipulate grain hardness in high yielding genotypes.

4.2. Consequences for the Argentinean dry milling supply chain

At present Argentina has a robust supply chain producing hard endosperm maize for sourcing internal and external dry milling markets. This high dry milling maize quality supply chain has evolved within the described grain protein and endosperm hardness tradeoff relationship with yield. This supply chain produces ca. 150.000 ha of hard endosperm GMO free flint maize, and specially released genotypes are needed to maintain the traditional grain quality observed until

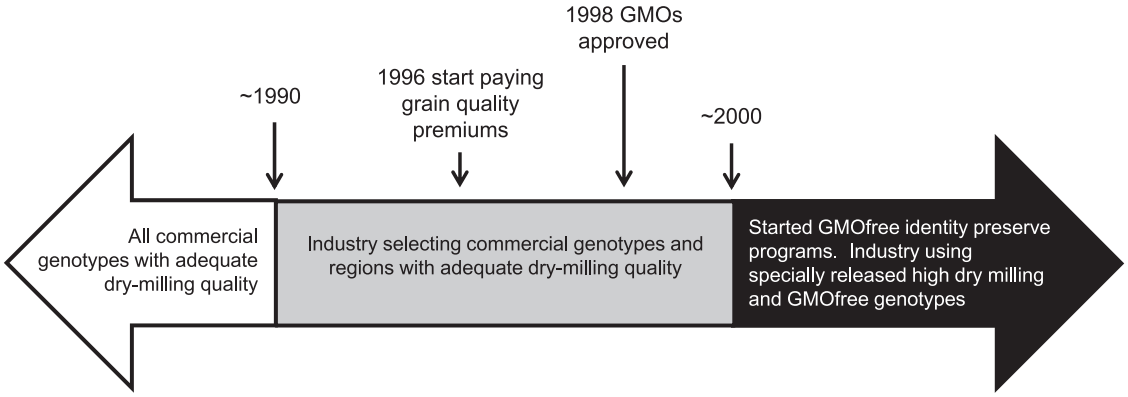

Fig. 5. Schematic diagram describing the changes over time that the Argentinean supply chain of hard endosperm GMO free maize had. Relevant moments for the supply chain are described.

1980s (Greco and Martí Ribes, 2016). Today, all hard endosperm flint genotypes used in the supply chain are specifically released for this special market.

Current Argentinean maize genotypes are less suitable for optimum dry milling efficiency to produce large flaking grits, as evidenced by their test weight, floaters, and grain vitreousness values (Fig. 2). From 1965 to early 1980s most genotypes released to the market were above the minimum SENASA requirements for exporting high quality dry milling grain to the European Union (Fig. 4). Argentinean traditional hard endosperm started changing during the mid-1980s, and at present no regular commercial genotype reaches adequate requirements for optimum dry milling yields. Because of the evident changes in genotypes released between mid-1980s and 2000 (Fig. 2), together with the introduction of GMO maize to the Argentinean market in 1998, the dry milling industry started selecting specific available genotypes for maintaining their dry milling efficiency and GMO free. The GMO free requirement was specifically relevant for European Union exports. Fig. 5 describes how the supply chain adapted over time to grain quality changes and GMO adoptions in Argentina.

The supply chain of hard endosperm flint maize started paying premiums to farmers in 1996, and is coincident with our results showing that hard endosperm genotypes were more scarce after 1990 (Fig. 2). These premiums helped compensate the already evident yield gap between available flint germplasm and the more modern dented one, and increased in year 2000 when identity preserved GMO free programs had to be implemented. Today, this yield gap is estimated in 10–30% in the central Argentinean temperate region, depending on the specific genotype and environment (Tamagno et al., 2015, 2016; Abdala et al., 2018), and farmers contract-grow this specialty. Because large breeding companies are not offering GMO free or hard endosperm flint genotypes, seed is currently sourced by average to small breeding efforts.

An evident question for the dry milling supply chain is how the yield gap between regular GMO dents and hard endosperm GMO free maize will evolve. Using the relative yield gap estimated in our previous studies between hard endosperm flints and regular dents (Tamagno et al., 2015, 2016; Abdala et al., 2018), and using the release year of each genotype tested in each study, we estimated a relative genetic gain for hard endosperm maize compared to the one observed here. The estimated genetic gain for hard endosperm maize is about half the genetic gain of regular GMO maize (49.5%, Fig. 6). This reflects the difficulties breeders deal with when selecting for yield while simultaneously maintaining or improving grain quality (Eyhérabide et al., 2004; Diepenbrock and Gore, 2015). We realize that this estimate is highly speculative, but helps to visually understand how the tradeoff between grain hardness and yield affects the supply chain and how premiums to farmers will need to evolve in order to maintain the economic sustainability of this feedstock.

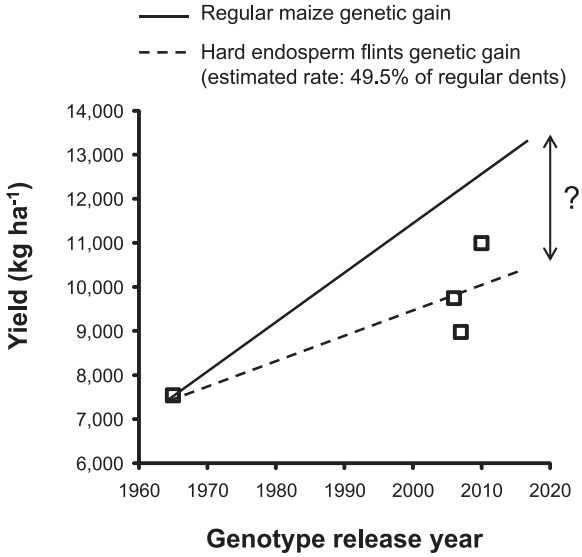

Fig. 6. Comparative estimated yield gains for regular Argentinean maize and hard endosperm flint germplasm. Regression for regular maize is based on Fig. 1A, and the relative comparative regression for hard endosperm flint maize is based on previous published evidences, where relative differences were 80% in Tamagno et al. (2015), 73% in Tamagno et al. (2016), and 87% in Abdala et al. (2018). Average release year for all genotypes reported in Tamagno et al. (2015) was 2006, in Tamagno et al. (2016) was 2007, and in Abdala et al. (2018) was 2010.

At present a relevant objective of the hard endosperm supply chain is to characterize environments and managements that help minimize this flint vs. dent yield gap while generating the highest grain quality. In this regard, the experiments by Tamagno et al (2016) helped determine that the best growing environments were those determining the smallest yield gaps in relative terms (% yield difference). This indicated the need for the supply chain to source hard endosperm flint maize from growing regions with minimum abiotic stresses and highest yield potentials. Concerning crop management, results from the present study also reinforce the concept that lower stand densities help achieve slightly better qualities (Tamagno et al., 2016), and that earlier sowings do not always generate higher grain hardness in our environments. Acceptable grain quality can be also obtained with late sowings if the genotype is adequately selected (Abdala et al., 2018).

5. Conclusions

Throughout the studied period (1965–2016) grain vitreousness and protein concentration were consistently reduced with increasing

genotype release year, while grain starch concentration showed the opposite trend. This was the result of introducing higher yielding, softer endosperm dented germplasm, and reinforces the common concept of tradeoffs between yield and grain protein concentration and endosperm hardness. Because grain hardness is mechanistically related to the concentration of endosperm proteins, the result of both traits decreasing is not surprising. When selecting for yield, reductions in grain protein concentration have consequences other than the value of the grain as animal feed.

The last genotypes with adequate grain hardness for dry milling released to the market by the Dekalb-Monsanto breeding company were in early 1980s, and the largest changes were evident between years 1990 and 2000. These genetic changes affected the Argentinean hard endosperm supply chain, and help understand why the supply chain started relying on genotypes specifically released for their particular needs since early 2000s.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.fcr.2018.07.008.

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