Evaluation of Quality Factors in Argentine Maize Races[1]

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ABSTRACT

Samples of Argentine maize from 12 landraces were analyzed for starch and amylose content and were evaluated for hardness parameters. Amylose contents of these Argentine landraces were generally higher than for typical dent hybrids grown in the United States. Hardness, as estimated by near-infrared reflectance; from wavelength shift of the near-infrared transmittance spectrum absorbance maximum (λ_{max}) in the 610–680 nm region; and by percentage of γ-zein in zein-2 (determined by reversed-phase HPLC), correlated well with classical definitions of endosperm type and with amylose content. Starch content correlated negatively with hardness. Flint landraces varied substantially in amylose and starch content. The strong correlation between amylose content and maize endosperm hardness confirms and strengthens previous observations, and may provide a compositional basis for endosperm quality.

Scientists at Instituto Nacional de Tecnologia Agropecuaria (INTA) are working to improve quality of Argentine maize and to develop better products for food and nonfood industrial uses, thereby increasing the economic viability of Argentine maize on both domestic and export markets. Quality requirements of maize processors are increasingly diversified and exacting, so these efforts are directed toward breeding commercial materials of suitable quality. Native landraces of Argentine maize are a most valuable resource for plant breeding. Since Argentina varies greatly in latitude and altitude, much diversity has developed in native maize during its long history of cultivation there. To properly use these native maize landraces, comprehensive assessments of their characteristics are necessary.

The Maize Active Germplasm Bank at Pergamino has, since the early 1960s, collected throughout Argentina more than 2,500 accessions of different landraces that are kept in that Bank and were classified into 44 races. These races are distributed among classical maize groupings based on relative endosperm hardness, e.g., floury, dent, semiflint, flint, and popcorn (which is comparable to flints in its hardness). All these materials are potential germ plasm sources to improve end-use quality of newly developed cultivars. A cooperative research effort between Proyecto de Calidad Industrial de Maíz (PROCIM) (Maize Industrial Quality Research Project) at INTA and USDA-ARS has been established to more thoroughly characterize differences among races to expand the potential of Argentine maize for domestic and export uses.

Endosperm hardness is perhaps the most important characteristic for dry milling, as well as durability during transportation, and handling. Endosperm protein composition is an important determinant of hardness; several investigators have associated endosperm hardness with certain zeins (Lopes and Larkins 1991, Paiva et al 1991, Paulis et al 1991, Dombrink-Kurtzman and Bietz 1993, Moro et al 1997). Also, Dombrink-Kurtzman and Knutson (1997) reported an association between endosperm type and percentage of amylose in starch. This proposed relationship is supported by unpublished data from our laboratory at INTA-Pergamino that associates percentage of amylose with certain hardness estimators among 12 commercial and experimental maize hybrids grown in two environments.

In addition to the apparent contribution of amylose to endosperm hardness, starch is the most important component of maize that affects its utilization. The functional properties of starch are strongly influenced by its relative percentages of amylose and amylopectin (Mua and Jackson 1998).

A major goal of this study has been to evaluate the variability of starch and amylose content among native Argentine maize races and to relate this variation to commercial value. In addition, we wished to investigate the association of amylose content with hardness parameters. This study, therefore, related end-use quality parameters of accessions from the Pergamino Maize Active Germplasm Bank to starch composition and looked for race-specific patterns useful in breeding for improved quality.

MATERIALS AND METHODS

Samples

Samples from 239 accessions from 12 landraces stored in the Pergamino Maize Germplasm Bank were studied. These races are further categorized by the classical definitions of maize endosperm types (i.e., floury, dent, semiflint, flint, and popcorn). Each endosperm type was represented by races included in this study. Samples were collected according to standard procedures (Solari and Gómez 1997). Races, endosperm type, and number of samples evaluated for each race are shown in Table I.

Analytical Procedures

Hardness estimators. Three methods were used to evaluate hardness. Near-infrared reflectance (NIR) hardness was determined by grinding 50-g samples in a model 3360 Falling Number laboratory mill at a machine setting of 0, and measuring reflectance at 1,680 nm in a Trebor 7700s instrument (Pomeranz et al 1984). Hardness was also estimated from the wavelength shift of the absorbance maximum (λ_{max}) in the 610–680 nm region of the near-infrared transmittance spectrum (Robutti 1995). This peak shifts toward longer wavelengths for softer maize types. This parameter is highly and negatively associated with other maize kernel hardness estimators (Robutti 1995).

Hardness was also estimated by analyzing zein composition by reversed-phase high-performance liquid chromatography (RP-HPLC) (Eyherabide et al 1996). Zeins were assayed on a Vydac C_{18} column (4.6 × 250 mm, 5-µm particles, 300 Å pores). Flow rate was 1 mL/min, and proteins were eluted using a 50-min linear gradient from 28 to 60% solvent B (solvent A, water +0.1% trifluoroacetic acid [TFA]; solvent B, acetonitrile + 0.1% TFA). RP-HPLC analyses typically resolve zein into an early-eluting group of peaks, termed zein-2, followed by a major group of α-zeins (zein-1). Peak 2 (eluting at ≈26.1 min in the zein-2 region)

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contains γ-zein associated with maize endosperm hardness (Lopes and Larkins 1991, Paiva et al 1991, Paulis et al 1991, Dombrink-Kurtzman and Bietz 1993, Moro et al 1995, Robutti 1997). Amounts of peak 2 were expressed as percentage of zein-2 (p2/z2).

Compositional analysis. Protein content (N × 6.25) ([Watson 1987) was determined by micro-Kjeldahl procedure (Approved Method 46-13, AACC 1995) and expressed on a dry basis.

Amylose was determined by the method of Knutson (1986) with slight modification of the dissolution procedure. Starch from very flinty maize did not completely dissolve in dimethylsulfoxide (DMSO) under normal heating conditions (50°C for 16 hr), so 70°C and 48 hr were used to solubilize all samples.

Starch was analyzed by the method of Dubois et al (1956). Starch and amylose contents were determined for all samples (Table I), but due to lack of sufficient sample for all tests, Canario Formosa was omitted from all other measurements, and Cristalino Amarillo, Cristalino Blanco, and Cristalino Anaranjado were omitted from tests for NIR hardness and λ_{max}.

Regression analyses among all parameters were performed on most samples. Differences of means among races were tested by the Student's *t*-test.

RESULTS AND DISCUSSION

Quality parameters of all endosperm types are summarized in Table II, with samples arranged in order of increasing hardness according to classical endosperm definitions. Statistically significant variations are defined at $P < 0.001$ for hardness parameters and $P < 0.01$ for starch and amylose content.

Kernel Hardness and Zein Composition

NIR hardness measurements divided the races into three well-defined groups, coinciding with the endosperm types floury, dent-semiflint, and flint. Avatí Morotí and Capia constituted the softest group; Dentado Blanco, Dentado Amarillo, and Complejo Tropical were intermediate; and Camelia, Pisingallo, and Cristalino Colorado formed the hardest group. The range of hardness values was comparable to those reported by Pomeranz et al (1984, 1986) for U.S. hybrids (292–556 reflectance units for dents and 540–626 for flints).

Values for zein RP-HPLC peak 2 expressed as % of zein-2 (p2/z2) were coincident with NIR values, confirming the association between these parameters. In this instance Camelia, Cristalino Colorado, and Cristalino Anaranjado formed the highest group, followed by the intermediate races Pisingallo, Dentado Blanco, Cristalino Amarillo, Cristalino Blanco, Dentado Amarillo, and Complejo Tropical. Here again, Avatí Morotí and Capia were the lowest. Pisingallo was an exception, it had an intermediate p2/z2 value but was categorized as part of the hardest group by NIR.

The λ_{max} parameter (wavelength shift of the near-infrared transmittance absorbance maximum) was highly and negatively associated with the other maize kernel hardness estimators (Table II), in agreement with previous findings by Robutti (1995). By this definition, Avatí Morotí and Capia appeared softest; Pisingallo, Dentado Blanco, Cristalino Colorado, and Dentado Amarillo were intermediate; and Camelia and Complejo Tropical were hardest. This ranking of Complejo Tropical was surprising as this race is classified as semiflint and was ranked intermediate by both NIR and p2/z2.

TABLE I
Maize Landraces Evaluated

Endosperm Type	Landrace	*n*
Floury	Avatí Morotí	19
	Capia	12
Dent	Dentado Amarillo	31
	Dentado Blanco	23
Semiflint	Complejo Tropical	11
	Canario Formosa	10
Flint	Camelia	10
	Cristalino Colorado	39
	Cristalino Blanco	19
	Cristalino Amarillo	44
	Cristalino Anaranjado	10
Popcorn	Pisingallo	11

TABLE III
Correlation Coefficients and Significance Levels[a] Among Hardness Parameters and Composition

	NIR[b]	p2/z2[c]	λ_{max}[d]	Starch
Amylose	0.281***	0.172**	–0.403***	ns
Starch	–0.273**	–0.332***	ns	
λ_{max}	–0.701***	ns		
p2/z2	0.334**			

[a] *, **, *** = significant at $P < 0.05$, 0.01, and 0.001, respectively; ns = not significant.
[b] Near-infrared reflectance expressed in arbitrary units.
[c] Amounts of peak 2 were expressed as percentage of zein-2
[d] Absorbance maximum.

TABLE II
Comparison of Quality Parameters Among Endosperm Types

Landrace	Endosperm Type	NIR[a]	p2/z2[b] (%)	λ_{max}[c] (nm)	Starch (% of sample)	Amylose (% of starch)	Protein (% of sample)
Capia	Floury	101a[d]	23.5a	647ab	71.9fg	25.8a	8.8ab
Avatí Morotí	Floury	135a	32.6b	651a	71.7ef	26.8a–c	11.2b
Dentado Amarillo	Dent	422b	42.6cd	625c–e	68.9bc	27.2b–d	9.4ab
Dentado Blanco	Dent	437b	45.1ef	629cd	68.5b	27.6b–e	11.1bc
Complejo Tropical	Semiflint	421b	40.1c	619de	74.5gh	27.9c–e	10.6b
Canario Formosa	Semiflint	· · ·	· · ·	· · ·	75.6h	28.7e	· · ·
Cristalino Anaranjado	Flint	· · ·	50.6d–f	· · ·	71.2c–f	26.5ab	· · ·
Cristalino Amarillo	Flint	· · ·	44.9c–e	· · ·	71.3d–f	27.0bc	· · ·
Cristalino Blanco	Flint	· · ·	43.8c–e	· · ·	71.8fg	27.0a–d	· · ·
Cristalino Colorado	Flint	517c	56.4fg	626cd	69.9b–f	27.1b–d	11.7bc
Camelia	Flint	589c	60.9g	618e	66.2a	28.0de	10.4b
Pisingallo	Popcorn	578c	45.5c–f	634bc	69.2b–f	26.7a–c	11.9b

[a] Near-infrared reflectance expressed in arbitrary units.
[b] Amounts of peak 2 were expressed as percentage of zein-2.
[c] Absorbance maximum.
[d] Values followed by the same letter in the same column are not significantly different at $P < 0.001$ (for hardness parameters), $P < 0.01$ (for starch and amylose content), or $P < 0.05$ (protein content).

Starch and Amylose

Starch concentration varied from 66.2% for Camelia to 75.6% for Canario Formosa. Mean starch content of all samples analyzed was 70.7%. Only at extreme starch contents did races vary consistently and significantly from each other, with four races <69% and two >74%. No association between starch content and endosperm type was evident. For example, dent races had lowest starch content and semiflint races had the highest, in contrast to the usual expectation that the hardest endosperm contains highest protein (and hence the lowest starch) content.

Amylose contents of the maize races were generally higher than typical in U.S. dent hybrids. Mean values for amylose as percentage of starch ranged from 25.8% for Capia to 28.7% for Canario Formosa; the average for all samples was 27.1%. Variation in amylose content among hard and soft races was significantly higher than differences reported by Dombrink-Kurtzman and Knutson (1997) for hard and soft endosperm fractions. The trend to increased amylose content with increasing endosperm hardness was most evident among floury, dent, and semiflint races but less so among very hard races, which tended to have intermediate amylose contents. The range of amylose content, expressed as % of average, was 10.8%, which was slightly less than the range for starch (13.3%). Interestingly, the flint race Camelia containing 28.0% amylose had the lowest starch content (66.2%), whereas semiflints Canario Formosa and Complejo Tropical with equally high amylose contents (28.7 and 27.9%) had the highest starch contents (75.6 and 74.5%) of all races including floury ones.

Protein Content

Protein content of maize races estimated from Kjeldahl nitrogen determinations varied between 8.8 and 11.9%. Overall mean was 10.48%. No statistically significant correlation was found between protein content and hardness parameters or endosperm type.

Statistical Correlations

Correlations among hardness parameters and composition are listed in Table III. NIR was positively associated with amylose and p2/z2, and negatively associated with starch content and λ_{max}. Starch was also negatively correlated with p2/z2. The negative correlations between starch content and hardness parameters demonstrate the need for thorough systematic evaluation of populations inasmuch as no evident association between endosperm type (by classical definitions) and either starch content or protein content could be observed. Amylose was significantly correlated with all hardness parameters: positively with NIR and p2/z2, and negatively with λ_{max}. There was no significant correlation between amylose content and starch content or between λ_{max} and p2/z2.

CONCLUSIONS

The high correlation coefficients of NIR to λ_{max} and p2/z2 confirm previous findings about the association of λ_{max} and p2/z2 with maize kernel hardness (Robutti 1995, Eyherabide et al 1996, Robutti et al 1997). Since NIR is an estimator of kernel hardness and p2/z2 was associated with hardness, the negative correlation between these parameters and starch content suggests an inverse association between starch content and kernel hardness. The strong correlation between amylose content and all hardness parameters confirms previous findings of an association between amylose and kernel hardness in maize (Dombrink-Kurtzman and Knutson 1997) over a broader range of samples. However, the range of amylose content is quite narrow relative to the wide range of hardness parameters. We recognize that other, more subtle, factors must also affect hardness and that the question requires further study. Obvious factors such as thermal and storage history, moisture content, etc., are not likely explanations in this instance as they were the same for all samples used in this study. The high association between amylose and λ_{max} may suggest that peak shifting could be due to differences in amylose content among maizes differing in hardness. The correlation between amylose content and p2/z2 may provide a compositional basis for endosperm quality.

The unusual situation, wherein high amylose content was found among both high-starch and low-starch races, also requires further study. It may be desirable to characterize amylose and amylopectin fractions of these races by size-exclusion chromatography. Similarly, future studies need to address variability in nutritional quality of Argentine maize (i.e., lysine content or proportion of zein to total protein).

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