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Contribution of zein content and starch characteristics to vitreousness of commercial maize hybrids

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ABSTRACT

Fast, simple laboratory methods were used to analyze 22 maize samples varying in kernel vitreousness from 50.23% to 76.41%. Samples were analyzed in terms of zein content (53.86–86.37 g/kg endosperm DM), amylose content (190.76–259.77 g/kg endosperm DM), amylose to amylopectin ratio in starch (0.28–0.43), as well as starch granule size (10.95–14.89 μm in equivalent diameter) and starch granule shape (circularity, 0.85–0.94). More vitreous samples had higher zein and amylose content, as well as smaller and less circular starch granules. Nearly all grain traits on their own significantly affected vitreousness, and a multiple regression model to account for their combined effects was able to explain 61.8% of variability in kernel vitreousness. Zein content contributed most to the model, followed by starch granule projected area and circularity. In contrast, the amylose content contributed only 5.1% to the model. These results suggest that starch-protein interactions influence maize kernel vitreousness more strongly than starch molecular properties do.

1. Introduction

Vitreousness and hardness-associated properties are significantly correlated with end usage of maize and are strongly determined by genotype. Grain vitreousness, which refers to the ratio of vitreous (hard) to floury (soft) endosperm, is a key agronomic trait that influences hardness, post-harvest resistance to insects and fungi, rate of starch digestibility, and semolina yield for food production (Gayral et al., 2015).[1]

Maize grain is a important source of energy for humans in Africa and South America, and the primary source of energy for domestic animal nutrition. Starch is its key energetic component. Vitreousness strongly affects the energy released when starch is digested by ruminants (Philippeau et al., 2000) and monogastric animals (Giuberti et al., 2013); these studies indicate that lower vitreousness is associated with greater starch digestibility. However, improved starch digestibility does not necessarily imply better energy utilization; in fact, more vitreous maize hybrids have been linked to better feed conversion ratios (Zhao et al., 2016). Higher vitreousness may also be associated with superior food quality, leading to slower cooking rates and higher content of resistant starch, which may offer health benefits (Osorio-Díaz et al., 2011).

Maize is usually subdefined according to the kernel characteristic of grain vitreousness. Flint maize features a large, continuous volume of vitreous endosperm, while floury maize contains floury endosperm nearly exclusively (Watson, 2003). Dent maize hybrids, which are derivatives of flint-flour classes, differ in their ratio of vitreous to floury endosperm. Vitreousness also varies with the position of kernels on the ear as well as with environmental conditions (Watson, 2003). The texture of vitreous and floury endosperm differs due to the interaction between starch and proteins. In both types of endosperm, protein matrix surrounds starch granules. In vitreous endosperm, the granules are tightly packed, while in floury endosperm, the protein matrix is thinner and features numerous air-filled spaces. The protein matrix itself comprises abundant zein proteins embedded in a matrix of glutelin proteins (Philippeau et al., 2000). The texture differences between vitreous and floury endosperm, therefore, lead to differences in physical properties in maize hybrids varying in vitreousness (Kljak et al., 2011).

The potentially substantial effect of vitreousness on maize grain properties means it should be taken into account when selecting hybrids for targeted production. The most important factors affecting maize grain vitreousness are related to starch, the

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[1] A/AP, amylose to amylopectin ratio; DM, dry matter; EqDi, equivalent diameter of starch granule; MinFeret and MaxFeret, minimal and maximal Feret's diameters; TS, total starch.

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dominant constituent in maize endosperm. Starch molecular architecture can be categorized into six levels (Dona et al., 2010) and each of these levels – from individual branches of amylose and amylopectin fractions to endosperm – could influence grain vitreousness. Thus, amylose content and starch granule size and shape should be taken into account, as well as the content of zein, which is the major storage protein in endosperm. Vitreous endosperm has higher zein and amylose content than floury endosperm, as well as smaller starch granules (Cagampang and Kirleis, 1985; Dombrink-Kurtzman and Bietz, 1993; Gayral et al., 2015; Landry et al., 2004). One would therefore predict similar characteristics in more vitreous hybrids. However, amylose content, zein content and starch granule size do not influence vitreousness independently of one another (Gayral et al., 2016), so they should be analyzed jointly. Previous studies have focused on only amylose or zein content separately (Dombrink-Kurtzman and Bietz, 1993; Dombrink-Kurtzman and Knutson, 1997; Landry et al., 2004; Mestres and Matencio, 1996).

The aim of the present study, therefore, was to explore the combined effect of starch properties and zein content, determined using fast and simple laboratory methods, on kernel vitreousness of commercial maize hybrids. The joint contribution of individual properties to kernel vitreousness was assessed using multiple linear regression.

2. Material and methods

2.1. Plant material

The selected maize samples are hybrids from different vegetation groups (FAO 200–700) and are known to range widely in kernel vitreousness. The following 11 commercial high-yield maize hybrids (*Zea Mays* L.) were provided by the Bc Institute (Zagreb, Croatia): Bc 244, Bc 282, Bc 354, Bc 394, Bc 408b, Bc 462, Bc 572, Bc 574, Bc 678, Bc 778 and Pajdaš. From them, Bc 354, Bc 394, Bc 408b, Bc 678 and Bc 778 were dent, Bc 244, Bc 282, Bc 572, Bc 574 and Pajdaš were dent × flint while Bc 462 was a flint hybrid. The samples were grown in-season in the years 2007 (Bc 244, Bc 408b, Bc 462, Bc 678, Bc 778, Pajdaš), 2008 (Bc 282, Bc 354, Bc 394, Bc 462, Bc 572, Bc 678, Bc 778, Pajdaš) and 2009 (Bc 244, Bc 354, Bc 394, Bc 462, Bc 572, Bc 574, Bc 678, Pajdaš). Hybrids grown in the same season were used in experiments conducted yearly in our laboratory. Maize hybrids were grown in a completely randomized design in test fields in central Croatia (2007, 2009) or eastern Croatia (2008) under the same agro-climate and production conditions. Each hybrid was planted on one 560-m^2 test lot; when the grain had reached physiological maturity, maize samples of each hybrid were collected from three places (2007) or five places (2008, 2009) across the lot (three or five replicates of each hybrid, respectively). Kernels were removed manually from cobs and stored at 4 °C. Maize samples were ground up in a laboratory mill (Cyclotec 1093, Foss Tocator, Hoganas, Sweden) equipped with a 0.3-mm screen for amylose analysis or 1-mm screen for all other analyses, then subjected to chemical analysis. All samples were also analyzed for dry matter (DM) content by drying at 103 °C until constant weight.

2.2. Maize kernel dissection and determination of vitreousness

Contents of mature maize kernel parts were determined by manual dissection as described (Dombrink-Kurtzman and Bietz, 1993). To achieve a representative sample, 100 kernels from each hybrid were randomly selected and divided into 10 visually homogeneous groups based on kernel size and form, and one kernel from each group was randomly selected for dissection. This process was done in duplicate for each sample, and repeated in case of deviations in the results. Kernels were soaked in boiling distilled water for 5 min then dried with a paper towel. The pericarp, germ and endosperm were separated with a scalpel. Floury endosperm was then manually removed from the rest of the endosperm using a scalpel. Endosperm fractions were dried for 24 h at 103 °C and weighed. Vitreousness was expressed as a weight proportion of vitreous endosperm in the total endosperm.

2.3. Amylose and zein determination

Ground maize samples were defatted by soaking in hexane, then apparent amylose content was determined as described (Knutson, 1986) based on a standard curve obtained using pure amylose (Sigma Aldrich, St. Louis, MO, USA). The apparent amylose was starch fraction dissolved in water-dimethylsulfoxide (1:9) containing 6×10^{-3} M iodine after incubation at 50 °C for 16 h. True amylose content was calculated by correcting the apparent content for amylopectin as described (Knutson, 1986) and expressed on a dry endosperm basis. The ratio of amylose to amylopectin (A/AP) was calculated based on amylopectin content, which was calculated based on amylose content in total starch. Total starch (TS) content was determined enzymatically (Total Starch Assay Procedure, Megazyme International Ireland, Wicklow, Ireland).

Total zeins in maize samples were extracted using 0.0125 M sodium borate (pH 10.0) containing 10 g/kg of sodium dodecyl sulphate and 20 g/kg of 2-mercaptoethanol as described by Wallace et al. (1990). After precipitation of non-zein proteins with ethanol, supernatant was dried and analyzed for nitrogen using Kjeldahl method with modification as described by Kljak et al. (2011). Total nitrogen content in maize grain samples was determined using the Kjeldahl method as described in ISO 5983-2:2009 (ISO, 2009). Protein content in grain samples and zein extracts was calculated using the conversion factor for maize 5.7. Zein was expressed as content in a dry endosperm and total starch.

2.4. Determination of maize starch granule size

Maize hybrid samples were subjected to non-starch extraction as described (Mistry and Eckhoff, 1992). The white starchy layer was separated with a spatula after incubation of suspension of ground maize sample and 1 g/kg NaOH solution with mild shaking at 55 °C for 90 min; after centrifugation, residue was washed three times with water to remove alkali and dried at room temperature. Starch granules were stained with KI-I_2 solution, immobilized in glycerol on a microscope slide, and images were acquired using a high-resolution color camera (ProgRes CT3 3.15 MPix 1/200 CMOS, 2048 × 1536, 10-bit; Jenoptic, Germany) placed on an adjustable stand. Images were analyzed using NIS–ELEMENTS 2.3 software (Laboratory Imaging, Prague, Czech Republic) as described (Šárka and Bubník, 2009) in order to determine starch particle size. Seven geometric parameters were measured: projected area, equivalent diameter (EqDi), perimeter, minimal and maximal Feret's diameters (MinFeret, MaxFeret), circularity and elongation. Parameter values were the arithmetic means of at least 1000 granules.

2.5. Statistical analysis

Measured traits of analyzed hybrids were subjected to one-way analysis of variance in SAS 9.3 software (SAS, 2011). Effect of maize hybrid and growing season on each measured trait was tested using PROC MIXED procedure; after determination of the effects on nearly every trait, each hybrid from each growing season was considered one sample ($n = 22 \times$ number of replications) for further assessments of trait relationships. The individual effects of