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Dosage effects of *Waxy* gene on the structures and properties of corn starch

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ABSTRACT

The objective of this study was to understand dosage effects of the *Waxy* gene on the structures of amylose and amylopectin and on the properties of corn starch. Reciprocal crossing of isogenic normal and waxy corn lines was conducted to develop hybrids with different dosages (0, 1, 2, 3) of *Waxy* gene in the endosperm. The amylose content of starch and proportions of branch chains of DP 17–30 and extra-long branch chains (DP > 100) of amylopectin were positively correlated with the *Waxy*-gene dosage. Proportions of short (DP < 17) and long branch-chains (DP 30–80), however, were negatively correlated with the *Waxy*-gene dosage. The gelatinization conclusion-temperature and temperature-range of the starch were negatively correlated with the *Waxy*-gene dosage, indicating that amylose facilitated dissociation of the surrounding crystalline regions. These results helped us understand the function of granule-bound starch synthase I in the biosynthesis of amylose and amylopectin and impacts of *Waxy*-gene dosages on the properties of corn starch.

1. Introduction

Corn (*Zea mays* L.) is the largest crop produced worldwide, followed by rice and wheat (FAOSTAT, 2012). Most of the corn is used for livestock feed, and the remaining is processed for many food and industrial applications, including corn meal, corn starch, corn syrup and high-fructose corn syrup, alcoholic beverage, and fuel ethanol, etc. (USDA ERS, 2015). Starch is the major component of corn kernels (~72% by dry mass) and the energy source for food, feed, and ethanol fuel. Starch of normal (wild type) corn consists of two glucans: amylose and amylopectin. Amylose is primarily a linear polysaccharide of D-glucopyranose units connected by α-1,4 glycosidic bonds with few α-1,6 linked branches (Takeda, Tomooka, & Hizukuri, 1993). Amylopectin is a highly branched polysaccharide, in which the short linear-chains are connected by α-1,6 glycosidic branch linkages (Tester, Karkalas, & Qi, 2004).

Waxy corn is a naturally occurring corn mutant, and the starch of waxy corn consists of almost exclusively amylopectin. Compared with normal corn starch, waxy corn starch provides unique functions for many food and non-food applications. For example, waxy corn starch is preferably used in frozen foods to improve the freeze-thaw stability, and in textile, corrugating, and adhesive industries because of its clear film-forming property (Fergason, 1994). Waxy corn can be preferred as livestock feed because of its good feed-conversion efficiency resulting from the greater digestibility of the waxy starch (Collins, Moran, & Stillborn, 2003).

The endosperm of waxy corn kernels lacks the enzyme activity of granule-bound starch synthase I (GBSSI) encoded by the *Waxy* gene (Denyer, Johnson, Zeeman, & Smith, 2001). The GBSSI, with a molecular weight of 58–60 kDa, is the only known enzyme responsible and required for the biosynthesis of amylose molecules in corn (Denyer et al., 2001). It has been reported that GBSSI is also involved in the biosynthesis of extra-long branch-chains of amylopectin in various plants, such as wheat (Yoo and Jane, 2002a), rice (Hanashiro et al., 2008), and sweet potato (Kitahara et al., 2007). Some other studies using near-isogenic waxy and non-waxy wheat, however, showed contradictory results: non-waxy wheat starch consisted of no extra-long branch-chains and GBSSI showed no effects on the branch-chain length distribution of amylopectin (Miura, Wickramasinghe, Subasinghe, Araki, & Komae, 2002; Yasui, Ashida, & Sasaki, 2009). Yasui et al. (2009) argued that the extra-long branch-chains of amylopectin in normal or non-waxy starches were possibly contamination of amylose molecules resulting from incomplete separation of the amylose from amylopectin molecules.

The endosperm tissue of corn has three sets of chromosomes, two sets from maternal parent and one set from pollen parent (Darrah, McMullen, & Zuber, 2003). Therefore, for a single gene, there could be four different dosages of the gene (0, 1, 2, 3) in the

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endosperm. The dosage effects of *Waxy* gene on the structures and properties of corn starch are not fully understood, and the effects of GBSSI on the biosynthesis of extra-long branch-chains are still being debated.

The objective of this study was to understand the dosage effects of *Waxy* gene on the structures of amylose and amylopectin of corn starch and on starch properties. Results obtained from this study will add to the understanding of the physiological function of GBSSI in the biosynthesis of starch molecules and the properties of starch with different dosages of *Waxy* gene. Understanding properties of starch with different *Waxy*-gene dosages can facilitate developments of value-added utilizations of waxy and partial-waxy corn starch.

2. Materials and methods

2.1. Materials

Isogenic normal and waxy corn lines were used in this study to minimize the interference of different genetic background. Two sets of isogenic normal and waxy corn lines were used as parent lines. The Set 1 samples included the pedigree DKXL370:N11a20-036-002, and the Set 2 samples included the pedigree AR16035:S02-615-001. The normal corn used in this study was at the S6 level of inbreeding (6 generations of self-pollination). The isogenic waxy corn was developed by crossing the normal corn to a waxy corn and backcrossing to the normal inbred for five generations (BC5). Selection for the waxy phenotype was made at each generation. Reciprocal crossing between the isogenic normal and waxy corn were conducted to develop corn lines with different dosages of the *Waxy* gene in the endosperm: waxy × waxy (0 dosage), waxy × normal (1 dosage), normal × waxy (2 dosages), and normal × normal (3 dosages). All the corn lines were developed within the USDA-ARS Germplasm Enhancement of Maize (GEM) Project and grown at the North Central Regional Plant Introduction Station farm (Ames, IA) in 2014. For each genotype, two rows of corn plants (~10 plants each row) were grown and all the corn ears with the same genotype were harvested and pulled together in bulk. Corn ears were dried to approximately 12% moisture and shelled. The pedigree, genotype, and the dosage of *Waxy* gene in the endosperm of the corn lines are listed in Table 1.

Pseudomonas isoamylase (EC 3.2.1.68, 280 U/mg) was purchased from Megazyme International Ireland (Wicklow, Ireland). All other chemicals were reagent grade and were purchased from either Sigma-Aldrich Co. (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA) and used without further purification.

2.2. Starch isolation by wet-milling

Starches were isolated from the corn kernels using a wet-milling method reported by Li, Jiang, Campbell, Blanco, & Jane (2008).

2.3. Amylose content of starch

The amylose content of the corn starch was determined using an iodine potentiometric- autotitrator (702 SM Titrino, Brinkmann Instrument, Westbury, NY) using a method reported by Song & Jane (2000). Lipids of the starch were removed using 85% methanol in a Soxhlet extractor for 16 h prior to the analysis. The iodine affinity of amylose used for the calculation was 0.2 (Takeda & Hizukuri, 1987). The amylose content was calculated using the equation:

$$\text{Amylose}(\%) = 100\%\text{IA}_s/0.2,$$

where IA_s is the iodine affinity of the starch.

2.4. Lipid content of starch

Lipids of the starch were extracted following the AOAC method 996.06 (2000). The lipid content of the starch was determined gravimetrically after removal of the solvent and calculated using the equation:

$$\text{Lipid}(\%) = \frac{\text{Weightofextractedlipids}}{\text{Weightofthestarch(db)}} \times 100\%.$$

2.5. Fractionation of amylose

Amylose of the starch was separated from amylopectin using 1-butanol, following the method reported by Jane & Chen (1992) and Schoch (1942). Dispersed starch in an aqueous medium (1%, w/v) was mixed with 1-butanol (20%, v/v) and refluxed under mechanical stirring in a boiling-water bath for 1 h. The mixture in a sealed flask was place in a Duwar flask filled with boiling water, and the Duwar flask was sealed and allowed to slowly cool down to room temperature over 24–30 h. During the cooling process, amylose formed helical complex with 1-butanol, which crystallized and precipitated from the starch dispersion. Amylopectin molecules, however, remained in the supernatant.

2.6. Average molecular-weight of amylose

Molecular-weight distribution of the isolated amylose was determined using a high-performance size-exclusion chromatography (HPSEC) following the method of Jiang, Campbell, Blanco, & Jane (2010). The HPSEC system consisted of a Prostar 210 pump (Varian, Walnut Creek, CA), a refractive-index detector (Prostar 355, Varian, Walnut Creek, CA), and Shodex SB-804 and SB-803 analytical columns (Showa Denko K.K., Tokyo, Japan). The temperature of the columns was maintained at 50 °C in a column oven (Prostar 510, Varian, Walnut Creek, CA). The mobile phase was degassed and distilled-deionized water at a flow rate of 0.5 ml/min. Maltose, maltotriose, maltotetraose, maltohepaose, and pullulan standards (P10, P20, P100, Showa Denko K.K., Tokyo, Japan) were used as references to make a standard curve between the elution time and molecular weight. The weighted mean of the molecular weight of amylose was calculated by using the refractive-index value as the weight.

2.7. Molecular weight and gyration radii of amylopectin

Molecular weight and gyration radii of the amylopectin were determined using a HPSEC equipped with a multi-angle laser-light scattering detector (Dawn DSP-F, Wyatt Tech. Corp., Santa Barbara, CA) and a refractive-index detector (HP 1047A, Hewlett Packard, Valley Forge, PA) following the method of Yoo & Jane (2002b). Shodex SB-806 and SB-804 analytical columns (Showa Denko K.K., Tokyo, Japan) were used to separate amylopectin from amylose. The temperature of the columns was maintained at 50 °C using a CH-460 column heater and a TC-50 controller (Eppendorf, Madison, WI). The mobile phase was degassed and distilled-deionized water at a flow rate of 0.5 ml/min.

2.8. Amylopectin branch-chain length distribution

Amylopectin of the corn starch was separated from amylose using a gel-permeation chromatographic column packed with Sepharose CL-2B gel. The isolated amylopectin was debranched using *Pseudomonas* isoamylase (Megazyme International Irelands, Wicklow, Ireland) following the method of Li et al. (2008). Branch-chain length distribution of the debranched amylopectin was analyzed using a HPSEC following the method of Jiang et al. (2010).