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.

Keywords: *Waxy* gene; dosage effects; branch-chain length distribution; starch properties

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 (Ferguson, 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 & 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 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:

$$\text{Amylose (\%)} = 100\% \text{ IA}_s / 0.2, \text{ where IA}_s \text{ was the iodine affinity of the starch.}$$

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:

$$\text{Lipid (\%)} = \text{Weight of extracted lipids} / \text{Weight of the starch (db)} \times 100\%.$$

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 hr. The mixture in a sealed flask was placed 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 hr. 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). The HPSEC system consisted of a Prostar 210 pump (Varian, Walnut Creek, CA), a refractive-index detector (Prostar 355, Varian, Walnut Creek, CA), and a Shodex SB-803 analytical column (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.

2.9 Thermal Properties of the Starch

Thermal properties of the starch were analyzed using a differential scanning calorimeter (DSC, Diamond, Perkin-Elmer, Norwalk, CT). Starch samples in excess water (starch : water = 1: 3, w/v) were heated in a DSC from 20 to 110 °C, at a heating rate of 10 °C/min. The gelatinization onset (T_o), peak (T_p), and conclusion temperatures (T_c) and enthalpy change (ΔH) of the starch were determined using a Pyris software (Perkin-Elmer).

2.10 Pasting Properties of the Starch

Pasting properties of the starch in an aqueous suspension (8%, w/w, db) were analyzed using a rapid visco-analyzer (Newport Scientific, Sydney, Australia) following the method of Jane, et al. (1999). The pasting temperature, and the peak, breakdown, and final viscosities were determined using the Thermocline software (Newport Scientific).

2.11 Statistical Analysis

Data were subjected to ANOVA and Tukey's multiple comparison analysis using PROC ANOVA procedure of SAS 9.4 (SAS Institute, Inc., Cary, NC). The normality of data for each variable was investigated using the PROC UNIVARIATE procedure. Because the dosage of *waxy* gene and most of the other variables were not normally distributed (data not shown), a Spearman correlation test was used to analyze correlations between the *waxy* gene dosage and

the physicochemical properties of the starch. Statistical significance was declared at $p < 0.05$ if without further indication.

3. Results and Discussion

3.1 Content and Average Molecular-Weight of Amylose

Amylose contents and amylose molecular-weights of starches from corn with different dosages of *Waxy* gene are shown in **Table 2**. The amylose contents ranged from 1.6 to 27.9% for the Set 1 samples and from 0.0 to 26.6% for the Set 2 samples. The amylose content was positively correlated with the dosage of *Waxy* gene ($r = 0.98$, $p < 0.01$), which was in line with the effects of *Waxy*-gene dosages on wheat starch (Miura et al. 2002; Wichramasinghe & Miura, 2003). The increase in the amylose content, however, was not proportional to the increase in the dosage of *Waxy* gene in the endosperm. For example, the relative amylose content increased by 69 or 75%, using the amylose content of the isogenic normal corn starch as the reference (100%), when the *Waxy*-gene dosage increased from 0 to 1 in the endosperm, whereas further increase in the *Waxy*-gene dosage from 1 to 2 only showed small increase in the relative amylose content by 5 or 6% (**Table 2**). These results were in line with that previously reported (Tsai, 1974).

Average molecular-weight of the isolated amylose ranged from DP 629.8 to 651.3 for the Set 1 samples, and from DP 637.8 to 646.1 for the Set 2 samples (**Table 2**). There were no significant differences in the average molecular-weight of amylose between the starch samples with different dosages of the *Waxy* gene in the endosperm. The molecular-weight distribution of amylose as indicated by the HPSEC chromatograms (data not shown) displayed no differences with different dosages of the *Waxy* gene in the endosperm. These results indicated that the *Waxy* gene had little effects on the molecular weight of amylose.

3.2 Lipid Content of Starch

Lipid contents of the starch samples are shown in **Table 2**. The waxy corn starches consisted of little lipids (0.24% for the Set 1 waxy corn and 0.10% for the Set 2 waxy corn). Lipid contents of the partial-waxy and normal corn starches ranged from 0.71 to 0.84% for the Set 1 samples and from 0.55 to 0.84% for the Set 2 samples. The correlation coefficient between the amylose and lipid contents of the starch samples was 0.75 ($p = 0.086$).

3.3 Molecular Weight of Amylopectin

The molecular-weight and gyration radii of amylopectin with different dosages of *Waxy* gene are shown in **Table 3**. The amylopectin molecules of the waxy corn starch displayed larger molecular weights and gyration radii than the partial-waxy and normal isogenic corn lines containing 1 to 3 *Waxy*-gene dosage(s). These results were consistent with that reported on waxy and normal corn, wheat, and potato starches (Bello-Perez, Roger, Baud, & Colonna, 1998; Yoo & Jane, 2002b). The correlation coefficient between the molecular weights of amylopectin and amylose contents of the starch samples was -0.66, and the correlation was **moderately significant** ($p = 0.063$). For the partial-waxy and normal corn, biosynthesis of amylose competed with that of amylopectin for the substrate of adenosine-5'-diphosphate glucose (ADP-Glu), which resulted in a smaller molecular weight of amylopectin than that of the waxy corn starch (Yoo & Jane, 2002a).

3.4 Branch-Chain Length Distribution of Amylopectin

Branch-chain length distributions of the debranched amylopectin-molecules determined using a HPSEC are shown in **Figure 1**, and the results are summarized in **Table 4**. Both sets of the samples showed the same trends in the branch-chain length distribution of amylopectin with different dosages of *Waxy* gene. The chromatograms showed a major peak at DP ~22 and two minor peaks at DP ~13 and DP ~40 for both sets of samples. For the Set 1 samples, percentages

of the extra-long branch chains (DP 100-287) increased from 0.2% to 2.0%, and for the Set 2 samples, percentages of the extra-long branch chains (DP 100-256) increased from 0.3% to 1.8% with the increase in the *Waxy*-gene dosage from 0 to 3 (Table 4). Percentages of the extra-long branch chains were **positively** correlated with the *Waxy*-gene dosage ($r = 0.93$, $p < 0.01$). It has been reported that extra-long branch chains are present in partial-waxy and normal starches from various plants, including wheat, rice, and sweet potato (Yoo & Jane, 2002a; Kitahara, et al. 2007; Hanashiro, et al. 2008), and GBSSI is responsible for the biosynthesis of extra-long branch chains (Denyer, Clarke, Hylton, Tatge, & Smith, 1996; Hanashiro, et al. 2008). Yasui et al. (2009) postulated that amylose could have been eluted with amylopectin during the GPC separation and the presence of extra-long branch chains could be a result of contamination of the amylose. In this study, the largest detectable chain lengths of extra-long branch chains were DP 287 for the Set 1 samples and DP 256 for the Set 2 samples, which were substantially smaller than the average molecular weight of amylose (DP 629.8-651.3 for the Set 1 samples and DP 637.8-646.1 for the Set 2 samples, respectively) (**Table 2**). Therefore, the presence of extra-long branch chains was unlikely the contamination of amylose molecules. The **positive** correlation between the *Waxy*-gene dosage and the proportion of extra-long branch chains further supported that GBSSI was responsible for the biosynthesis of extra-long branch chains of amylopectin.

The HPSEC chromatograms showed that, for both sets of the samples, percentages of the short chains (DP < 17) (47.3-51.0% for the Set 1 samples and 46.1-49.9% for the Set 2 samples) (**Table 4**) were **negatively** correlated with the *Waxy*-gene dosage ($r = -0.94$, $p < 0.01$). Percentages of the intermediate chains of DP 17-30 (30.0-33.5% for the Set 1 samples and 31.2-37.4% for the Set 2 samples) were **positively** correlated with the *Waxy*-gene dosage ($r = 0.86$, $p < 0.01$). Percentages of the long branch-chains (DP 30-80) (16.2-17.9% for the Set 1 samples and

14.0-17.8% for the Set 2 samples) were **negatively** correlated with the **Waxy**-gene dosage ($r = -0.76$, $p < 0.05$). It was plausible that, with the presence of GBSSI in the partial-waxy and normal corn, the short chains ($DP < 17$) were elongated to the intermediate chains ($DP 17-30$). These results also suggested that GBSSI could synthesize the extra-long branch-chains by elongating the long branch-chains ($DP 30-80$), resulting in decreases in the proportion of the long branch-chains in the partial-waxy and normal corn starches. More studies are needed to understand the mechanisms of the effects of GBSSI on the elongation of amylopectin branch-chains.

3.5 Thermal Properties of Starch

Starch thermal properties are shown in **Table 5**. The gelatinization conclusion-temperature ($74.4-77.0\text{ }^{\circ}\text{C}$ for the Set 1 samples and $72.0-74.4\text{ }^{\circ}\text{C}$ for the Set 2 samples) and temperature-range ($8.8-11.2\text{ }^{\circ}\text{C}$ for the Set 1 samples and $9.5-12.1\text{ }^{\circ}\text{C}$ for the Set 2 samples) were negatively correlated with the amylose content of starch (**Table 2**) ($r = -0.91$, $p < 0.05$ for gelatinization conclusion-temperature; $r = -0.83$, $p < 0.01$ for gelatinization temperature-range). These results were in line with that reported on **waxy, partial-waxy, and normal** wheat starch (Sasaki, Yasui, & Matsuki, 2000). It is known that gelatinization of starch begins at the hilum of the granule and proceeds radially (Hoseney, Zeleznak, & Yost, 1986). Amylose is interspersed among amylopectin and is oriented from the hilum to the periphery, extending through multiple clusters of amylopectin molecules (Jane, 2006). During gelatinization of starch, amylose molecules facilitate dissociation of surrounding crystalline clusters of amylopectin, proceeding from the hilum to the periphery, resulting in a lower gelatinization conclusion-temperature and a narrower gelatinization temperature-range (Hoseney, et al. 1986).

3.6 Pasting Properties of Starch

Starch pasting properties are shown in **Figure 2**, and the results are summarized in **Table 6**. Pasting temperatures of the starch (71.0-73.8 °C for the Set 1 samples and 69.0-72.5 °C for the Set 2 samples) had no correlation with the *Waxy*-gene dosage but were positively correlated ($r = 0.81$, $p < 0.01$) with the lipid contents of the starch (**Table 2**). Although there was no correlation between the peak viscosity and the *Waxy*-gene dosage, the breakdown viscosity was **negatively** correlated with the *Waxy*-gene dosage ($r = -0.83$, $p < 0.01$) and the setback viscosity was **positively** correlated with the *Waxy*-gene dosage ($r = 0.97$, $p < 0.01$). It has been reported that amylose intertwines with amylopectin and maintains the integrity of the starch granule (Hermansson & Svegmarm, 1996; Jane, 2007), which reduces the breakdown of swollen starch-granules. Amylose participates in gel-network formation during the cooling of the starch paste, resulting in a higher setback viscosity (Deffenbaugh & Walker, 1989; Jane, et al. 1999; Sasaki, et al. 2000).

4. Conclusions

To our knowledge this is the first study to report dosage effects of the *Waxy* gene on amylopectin branch-chain length distribution in isogenic corn starch. The **positive** correlation between the *Waxy*-gene dosage and the percentage of extra-long branch chains supported that GBSSI was responsible for the biosynthesis of extra-long branch chains. GBSSI also increased the proportion of intermediate chains with DP 17-30 and decreased the proportion of short chains with $DP < 17$. The *Waxy* gene showed dosage effects on the amylose content, but no effects on the molecular weight of amylose. The conclusion gelatinization-temperature and gelatinization temperature-range of the starch were **negatively** correlated with the *Waxy*-gene dosage, indicating that amylose facilitates dynamic gelatinization of the crystalline structures of

amylopectin. The breakdown viscosity of starch was negatively correlated but the setback viscosity was positively correlated with the *Waxy*-gene dosage. These results showed that GBSSI was responsible for the biosynthesis of both amylose and amylopectin, which in turn affected the thermal and pasting properties of the starch.

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FIGURE CAPTIONS

Figure 1. Amylopectin branch-chain length distribution of the corn starches with different dosages of the *Waxy* gene. The chromatograms indicate the changes of RI signal intensities over time. The RI signals have been normalized using the total peak area of the curves. The Set 1 samples included the pedigree DKXL370:N11a20-036-002, and the Set 2 samples included the pedigree AR16035:S02-615-001. Dosages of the *Waxy* gene for samples *wx*, $wx \times N$, $N \times wx$, and *N* were 0, 1, 2, and 3, respectively.

Figure 2. Starch pasting properties of the corn starches with different dosages of the *Waxy* gene. The Set 1 samples included the pedigree DKXL370:N11a20-036-002, and the Set 2 samples included the pedigree AR16035:S02-615-001. Dosages of the *Waxy* gene for samples *wx*, $wx \times N$, $N \times wx$, and *N* were 0, 1, 2, and 3, respectively.

TABLE CAPTIONS

Table 1. Pedigree, genotype, and *Waxy*-gene dosage of the endosperm of the GEM corn samples

Table 2. Amylose and lipid contents of starch and amylose molecular-weight of the corn starches with different dosages of the *Waxy* gene

Table 3. Amylopectin molecular-weight and gyration radii of the corn starches with different dosages of the *Waxy* gene

Table 4. Amylopectin branch-chain length distribution of the corn starches with different dosages of the *Waxy* gene

Table 5. Starch thermal-properties of the corn starches with different dosages of the *Waxy* gene

Table 6. Starch pasting-properties of the corn starches with different dosages of the *Waxy* gene

Table 1. Pedigree, genotype, and *Waxy*-gene dosage of the endosperm of the GEM corn samples

	Sample ^a	Pedigree	Genotype of endosperm	<i>Waxy</i> -gene dosage
Set 1	wx	DKXL370:N11a20-036-002-B wx	wxwxwx	0
	wx × N	DKXL370:N11a20-036-002-B wx × DKXL370:N11a20-036-002-B-B-B-B	wxwxWx	1
	N × wx	DKXL370:N11a20-036-002-B-B-B-B × DKXL370:N11a20-036-002-B wx	wxWxWx	2
	N	DKXL370:N11a20-036-002-B-B-B-B	WxWxWx	3
Set 2	wx	AR16035:S02-615-001-B wx	wxwxwx	0
	wx × N	AR16035:S02-615-001-B wx × AR16035:S02-615-001-B-B-B-B	wxwxWx	1
	N × wx	AR16035:S02-615-001-B-B-B-B × AR16035:S02-615-001-B wx	wxWxWx	2
	N	AR16035:S02-615-001-B-B-B-B	WxWxWx	3

^a wx = waxy corn ; N = normal corn. In each case the female parent line appears first and has the endosperm genotype.

Table 2. Amylose and lipid contents of starch and average molecular-weight of amylose of the corn starches with different dosages of the *Waxy* gene ^a

	Sample	<i>Waxy</i> gene dosage	Amylose (%)	Relative amylose content	Average molecular- weight of amylose (DP)	Lipid (%)
Set 1	wx	0	1.6 d ±0.0	6%	N/A ^b	0.24 c ±0.06
	wx × N	1	20.9 c ±0.1	75%	640.5 a ±10.3	0.75 ab ±0.02
	N × wx	2	22.4 b ±0.4	80%	651.3 a ±16.4	0.84 a ±0.05
	N	3	27.9 a ±0.5	100%	629.8 a ±21.4	0.71 b ±0.03
Set 2	wx	0	0.0 d ±0.0	0%	N/A	0.10 c ±0.00
	wx × N	1	20.0 c ±0.1	75%	644.2 a ±8.2	0.74 a ±0.04
	N × wx	2	21.5 b ±0.1	81%	646.1 a ±9.7	0.84 a ±0.07
	N	3	26.6 a ±0.2	100%	637.8 a ±1.8	0.55 b ±0.03

^a Values are means ± standard deviations of two replicates. Different letters following the mean values within the same columns indicate statistically different mean values within each set of the corn starch samples (p<0.05). The Set 1 samples included the pedigree DKXL370:N11a20-036-002, and the Set 2 samples included the pedigree AR16035:S02-615-001. ^b Not available.

Table 3. Amylopectin molecular-weight and gyration radii of the corn starches with different dosages of the *Waxy* gene ^a

	Sample	M _w ($\times 10^8$ g/mol)	R _z (nm)
Set 1	wx	11.8 a ± 0.2	380.0 ± 1.4
	wx $\times$ N	10.3 b ± 0.6	362.3 ± 5.9
	N $\times$ wx	9.5 b ± 0.2	355.4 ± 1.3
	N	10.1 b ± 0.0	363.0 ± 0.8
Set 2	wx	12.4 a ± 0.4	387.4 ± 5.6
	wx $\times$ N	10.5 a ± 0.7	368.2 ± 7.9
	N $\times$ wx	11.7 a ± 0.6	380.2 ± 15.8
	N	11.1 a ± 0.4	376.3 ± 4.9

^a Values are means $\pm$ standard deviations of two replicates. Different letters following the mean values within the same columns indicate statistically different mean values within each set of the corn starch samples ($p < 0.05$). The Set 1 samples included the pedigree DKXL370:N11a20-036-002, and the Set 2 samples included the pedigree AR16035:S02-615-001. Dosages of the *Waxy* gene for samples wx, wx $\times$ N, N $\times$ wx, and N were 0, 1, 2, and 3, respectively.

Table 4. Amylopectin branch-chain length distribution of the corn starches with different dosages of the *Waxy* gene ^a

	Sample	DP<17	DP17-30	DP30-80	DP80-100	DP>100
Set 1	wx	51.0 a ±0.5	30.0 d ±0.2	17.9 a ±0.2	0.9 a ±0.1	0.2 d ±0.0
	wx × N	50.4 ab ±0.3	30.8 c ±0.1	17.3 b ±0.1	0.9 a ±0.1	0.6 c ±0.0
	N × wx	49.8 b ±0.4	31.6 b ±0.2	16.7 c ±0.2	0.9 a ±0.0	1.0 b ±0.1
	N	47.3 c ±0.2	33.5 a ±0.0	16.2 c ±0.2	1.0 a ±0.0	2.0 a ±0.0
Set 2	wx	49.9 a ±0.3	31.2 d ±0.1	17.8 a ±0.1	0.8 a ±0.1	0.3 c ±0.0
	wx × N	49.3 a ±0.3	33.0 c ±0.1	15.6 b ±0.0	0.9 a ±0.1	1.2 b ±0.0
	N × wx	47.3 b ±0.5	35.6 b ±0.4	14.8 c ±0.0	0.9 a ±0.1	1.4 b ±0.0
	N	46.1 c ±0.3	37.4 a ±0.3	14.0 d ±0.1	0.7 a ±0.1	1.8 a ±0.0

^a Weight averaged. Values are means ± standard deviations of two replicates. Different letters following the mean values within the same columns indicate statistically different mean values within each set of the corn starch samples (p<0.05). The Set 1 samples included the pedigree DKXL370:N11a20-036-002, and the Set 2 samples included the pedigree AR16035:S02-615-001. Dosages of the *Waxy* gene for samples wx, wx × N, N × wx, and N were 0, 1, 2, and 3, respectively.

Table 5. Starch thermal-properties of the corn starches with different dosages of the *Waxy* gene ^a

	Sample	T _o ^b (°C)	T _p (°C)	T _c (°C)	ΔH (J/g)	T _c -T _o (°C)
Set 1	wx	65.7 ab ±0.0	71.8±0.1	77.0 a ±0.4	14.0 a ±0.1	11.2 a ±0.4
	wx × N	66.1 a ±0.1	71.3±0.1	75.9 b ±0.0	12.3 b ±0.1	9.7 b ±0.1
	N × wx	65.2 c ±0.1	69.9±0.1	74.7 c ±0.2	12.0 c ±0.1	9.5 b ±0.3
	N	65.6 b ±0.0	69.7±0.0	74.4 c ±0.1	11.9 c ±0.0	8.8 b ±0.0
Set 2	wx	62.3 a ±0.1	68.9±0.0	74.4 a ±0.0	13.2 a ±0.1	12.1 a ±0.2
	wx × N	62.2 a ±0.1	68.4±0.0	73.5 b ±0.1	11.6 b ±0.0	11.3 b ±0.1
	N × wx	62.3 a ±0.2	67.7±0.1	72.4 c ±0.0	11.6 b ±0.0	10.1 c ±0.3
	N	62.5 a ±0.0	67.4±0.1	72.0 d ±0.1	11.2 c ±0.1	9.5 c ±0.1

^a Values are means ± standard deviations of two replicates. Different letters following the mean values within the same columns indicate statistically different mean values within each set of the corn starch samples (p<0.05). **The Set 1 samples included the pedigree DKXL370:N11a20-036-002, and the Set 2 samples included the pedigree AR16035:S02-615-001. Dosages of the *Waxy* gene for samples wx, wx × N, N × wx, and N were 0, 1, 2, and 3, respectively.** ^b T_o= onset gelatinization temperature, T_p = peak temperature, T_c= conclusion temperature, ΔH= enthalpy change.

Table 6. Starch pasting-properties of the corn starches with different dosages of the *Waxy* gene ^a

	Sample	Pasting Temp.(°C)	Peak (RVU)	Hold (RVU)	Final (RVU)	Breakdown (RVU)	Setback (RVU)
Set 1	wx	71.0 b ±0.3	217.3 a ±4.0	78.3 b ±1.6	101.8 d ±2.9	138.9 a ±2.4	23.4 d ±1.3
	wx × N	73.8 a ±0.3	147.8 b ±2.2	73.6 c ±2.4	142.1 c ±2.2	74.2 b ±0.2	68.5 c ±0.2
	N × wx	72.3 ab ±0.6	150.2 b ±2.5	75.1 bc ±1.2	148.7 b ±1.5	75.1 b ±1.2	73.5 b ±0.3
	N	71.4 b ±0.3	157.6 b ±1.2	90.4 a ±0.8	182.8 a ±0.6	67.2 c ±1.9	92.5 a ±1.4
Set 2	wx	69.0 c ±0.3	221.0 a ±0.7	72.0 c ±1.7	102.1 d ±0.6	148.9 a ±1.1	30.1 d ±1.1
	wx × N	72.3 a ±0.0	149.9 b ±2.7	74.1 c ±0.6	146.8 c ±1.6	75.8 b ±2.1	72.7 c ±2.2
	N × wx	72.5 a ±0.2	143.6 b ±1.4	84.9 b ±1.7	169.8 b ±2.1	58.7 c ±0.3	84.9 b ±3.8
	N	71.3 b ±0.3	148.3 b ±0.7	95.6 a ±0.4	202.2 a ±2.0	52.5 d ±0.1	106.6 a ±2.4

^a Values are means ± standard deviations of two replicates. Different letters following the mean values within the same columns indicate statistically different mean values within each set of the corn starch samples (p<0.05). The Set 1 samples included the pedigree DKXL370:N11a20-036-002, and the Set 2 samples included the pedigree AR16035:S02-615-001. Dosages of the *Waxy* gene for samples wx, wx × N, N × wx, and N were 0, 1, 2, and 3, respectively. RVU = Rapid-visco unit(s).

Figure 1

Figure 2