

Granule Bound Starch Synthase I (GBSSI) Gene Effects Related to Soft Wheat Flour/Starch Characteristics and Properties

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ABSTRACT

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Eight soft spring wheat (*Triticum aestivum* L.) genotypes representing the four granule bound starch synthase I (GBSSI) classes were evaluated with respect to flour/starch characteristics and pasting behaviors. Native starch was isolated from genotype straight-grade flours (94.8–98.1% of starch recovered) to approximate the starch populations of the parent flours. As anticipated, amylose characteristics varied among the genotypes according to GBSSI class and accounted for the primary compositional difference between genotypes. Total (TAM), apparent (AAM), and lipid-complexed (LAM) amylose contents ranged from 1.0–25.5 g, 0.7–20.4 g, and 0.3–5.6 g/100 g of native starch, respectively, and grad-

ually decreased with the progressive loss of active *Wx* alleles. In addition, genotype flour total starch (FTS) and A-type starch granule contents, which ranged from 81.7–87.6 g/100 g of flour (db) and 61.6–76.8 g/100 g of native starch (db), respectively, generally decreased with an increase in waxy character in parallel with amylose characteristics, as likely secondary effects of *Wx* gene dosage. Though amylose characteristics predominantly accounted for the majority of genotype flour pasting properties, FTS content and ratios of A- to B-type granules also exhibited significant influence. Thus, loss of one or more *Wx* genes appeared to induce measurable secondary effects on starch characteristics and properties.

Amylose content within wheat (*Triticum aestivum* L.) starch is controlled genetically and is synthesized by granule bound starch synthase I (GBSSI), also known as the “waxy” (*Wx*) protein (Yamamori et al 1992; Nakamura et al 1993). Due to its allohexaploid nature, bread wheat possesses three sets of nearly identical genomes (AABBDD), with the chromosome pairs of all three genomes being genetically similar. Thus, there are three structural genes located on each of the genomes that encode the three GBSSI isoforms that participate in amylose synthesis. These isoforms are encoded for on chromosome 7 of genome A (*Wx*-A1), chromosome 4 of genome A (*Wx*-B1), and chromosome 7 of genome D (*Wx*-D1) (Graybosch 1998). Through cross-breeding of mutant lines lacking one or more functional *Wx* genes, wheat genotypes with varying numbers of functional GBSSI isoforms can be generated. Depending on the number of functional isoforms present, wheat genotypes can be classified as wild-type (all three isoforms present), one gene null (any one isoform lacking), two gene null (any two isoforms lacking), or three gene null (all three isoforms lacking). The loss of one or more isoforms results in starches with reduced amylose content. Genotypes that possess a null allele at either one or two of the three *Wx* gene loci are termed partial waxy, while those that exhibit the complete absence of all three GBSSI isoforms are termed waxy (Graybosch 1998) and exhibit virtually amylose-free starch.

Though specific effects of *Wx* gene configuration on starch amylose content and behavior have been previously investigated and reported (Fujita et al 1998; Demeke et al 1999; Miura et al 1999; Yasui et al 1999; Araki et al 2000; Yamamori and Quynh 2000; Mangalika et al 2003), not all variability has been solely accounted for by fluctuations in amylose content. For example, some have described higher peak viscosities for waxy cultivars (relative to wild-type cultivars) (Kim et al 2003; Mangalika et al 2003), while others have either reported lower peak viscosities for waxy cultivars (Ishida et al 2003) or a lack of difference between waxy and nonwaxy lines (Graybosch et al 2003). Such observations are likely attributable to variability in starch composition or structure. In

characterizing a series of normal (wild-type), partial waxy, and waxy starches, Bertolini et al (2003) observed a trend toward an increased B-type starch granule content in genotypes possessing an increasing degree of waxy character. As the A- and B-type granules of wheat starch exhibit differential amylose characteristics, gelatinization behaviors, and pasting properties for both wild-type (Soulaka and Morrison 1985; Peng et al 1999; Shinde et al 2003) and, more recently, waxy genotypes (Geera et al 2005a), these phenomena along with other compositional factors could impact wheat flour rheological behavior. This study will seek to investigate fluctuations in flour/starch characteristics of wild-type, partial waxy, and waxy wheat genotypes in further explanation of wheat flour pasting behavior.

MATERIALS AND METHODS

Grain Source and Milling

Eight soft wheat lines derived from common parentage (excepting Penawawa) with coefficients of parentage similar to half sibs or closer were selected for study on the basis of GBSSI class. Each GBSSI class was represented by two genotypes (Table I). All genotypes were grown under both irrigated (Aberdeen, ID) and rainfed (Tetonia, ID) conditions over two successive crop years (2000, 2001) with the exception that genotypes A98054S-Gwxy-1 and Leona (waxy lines) from the 2000 crop year at Tetonia were unavailable and were not included in the study. After harvest, wheat representing each genotype/crop year/growing location combination was cleaned, tempered, and milled (Approved Method 26-10; AACC International 2000) using a Quadramat Sr. mill (C.W. Brabender Instruments, South Hackensack, NJ) to produce straight-grade flour.

TABLE I
Granule Bound Starch Synthase I (GBSSI) Allelic
Configuration of Genotypes

Genotype ^a	GBSSI Gene Classification
Whitebird, Jubilee	Wild-type (<i>Wx</i> -A1b, <i>Wx</i> -B1b, <i>Wx</i> -D1b)
Alturas, Penawawa	One gene null partial waxy (<i>Wx</i> -A1b, <i>Wx</i> -B1a, <i>Wx</i> -D1b)
IDO563, IDO565	Two gene null partial waxy (<i>Wx</i> -A1a, <i>Wx</i> -B1a, <i>Wx</i> -D1b)
Leona, A98054S-Gwxy-1	Waxy (<i>Wx</i> -A1a, <i>Wx</i> -B1a, <i>Wx</i> -D1a)

^a All genotypes, with the exception of Penawawa, were derived from common parentage.

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Native Starch Isolation from Flour

Native starch (defined as the population of starch granules present in a flour) was isolated from straight-grade flours of each genotype/crop year/growing location combination similar to the method of Shinde et al (2003) to maximize starch yield and purity. Flour (60.0 g) was suspended in 0.02M HCl (600 mL) with stirring (10 min). Sodium bisulfite (0.3 g) and thiomersal (0.006 g) were added to the slurry, which was adjusted to pH 7.6 using Tris-(hydroxymethyl)aminomethane. Protease solution, prepared by stirring enzyme (0.3 g, Sigma, P-5147) in 10 mL of 0.02M HCl (5 min), was added to the flour slurry. The entire slurry was incubated 24 hr at 4°C with continuous stirring. After incubation, the slurry was divided among four centrifuge bottles and centrifuged (2,500 × g, 20 min) to collect the starch pellet. The resulting supernatants were discarded, while the starch pellet in each bottle was suspended in aqueous cesium chloride solution (80%, w/v, 60 mL), and centrifuged (2,500 × g, 20 min) to further purify the starch fraction. Supernatants were again discarded. The purification step, beginning with addition of cesium chloride solution (30 mL/bottle) was repeated one more time, after which each resulting starch pellet was washed with deionized water (3×, 100 mL) to remove residual cesium chloride. The starch slurry of the final wash was passed through a 75-µm sieve to remove remaining cell wall material and insoluble fiber. After stirring thoroughly, one drop of the homogenous starch slurry was transferred to a 25-mL beaker and set aside for subsequent particle size analysis. The remainder of the starch was collected by centrifugation, suspended in absolute alcohol (140 mL), recovered on a Büchner funnel, and allowed to air-dry.

Measurement of Starch Granule Size Distribution

Granule size distributions for each native starch isolation of each genotype/crop year/growing location combination were determined using an Accusizer model 780 with SW 788 Windows software (Particle Sizing Systems, Santa Barbara, CA). The Accusizer instrument detects individual particles in the range of 0.5–400 µm as the sample passes through a laser-illuminated sensing zone, and calculates the size of the granules as a function of the degree of light scattering or blockage (Nicoli et al 1992). The instrument software calculates granule size distributions on a volume basis, which is interchangeable with weight basis (Soulaka and Morrison 1985). A 10-µm cutoff was used to differentiate A- and B-type granules. Before analysis, the instrument was calibrated using standard latex particles of known particle diameter (0.49–200 µm). One drop of the purified native starch slurry (previously set aside during starch isolation) was suspended in deionized water (25 mL) with stirring (10 min), after which 100 µL of the resulting suspension was further diluted in deionized water (400 mL) to achieve the appropriate starch concentration for injection into the instrument. Before injecting the sample (25 mL), the instrument was flushed with three volumes of deionized water (25 mL). Duplicate readings were taken for each prepared starch slurry and averaged to yield single A- and B-type granule contents for each starch isolation. Deionized water was run as a blank and the background was effectively subtracted from the obtained starch granule size distribution profiles. Between readings, the instrument was flushed with two volumes of deionized water (25 mL) to prevent cross-contamination of samples.

Determination of Amylose

Apparent (AAM), total (TAM), and lipid-complexed (LAM) amylose contents of the isolated native starches representing each genotype/crop year/growing location were determined using the colorimetric method of Morrison and Laignelet (1983). The AAM content was measured before the removal of starch lipids, while TAM was determined on defatted starch. The difference between TAM and AAM provided a measure of LAM.

Evaluation of Flour/Starch Pasting Properties

Pasting properties of straight-grade flours representing each genotype/crop year/growing location and their respective isolated native starches were determined as outlined by Batey et al (1997) using the Rapid Visco Analyser (RVA) (Newport Scientific, NSW, Australia). For analysis of straight-grade flours, 3.5 g (db) of flour was weighed into an RVA canister followed by addition of silver nitrate solution (0.012M) to achieve a final net weight of 29.0 g. For isolated native starches, the quantity of starch was set at 3.0 g (db), while deionized water was used in place of silver nitrate solution. Flour and starch suspensions were analyzed under continuous shear (160 rpm) beginning with an initial hold at 60°C (2 min), linear heating to 95°C (5 min), an intermediate hold at 95°C (4 min), linear cooling to 50°C (5 min), and a final hold at 50°C (4 min) to yield a total test time of 20 min.

Miscellaneous Analyses

Moisture contents of straight-grade flours and their respective native starches were determined according to AOAC Method 925.09 (AOAC 1990). Flour total starch contents were determined using the Megazyme Total Starch Assay (Approved Method 76-13; AACC International 2000). Flour protein contents were determined with a near-infrared (NIR) analyzer (Instalab 600, Dickey-John Corp., Auburn, IL) (Approved Method 39-10A) calibrated by automated combustion analysis of total nitrogen (LECO model NFP-428, LECO Corp., St Joseph, MO), and presented on a 12% moisture basis. Isolated native starch yields from straight-grade flour were reported as grams of starch (db) recovered from 100 g of flour (db). Isolated native starch recoveries (from straight-grade flour) were calculated on a dry weight basis according to the equation

$$\text{Starch Recovery} = \left(\frac{\text{Starch Yield (db)}}{\text{Flour Total Starch content (db)}} \right) \times 100$$

Experimental Design

Wheat samples from the field were bulked for the study to generate a single source of grain for each genotype/crop year/growing location combination. The entire experiment was replicated 2× beginning with the isolation of native starch from each bulked flour sample. Flour total starch, native starch yield from flour, starch recovery from flour, native starch particle size distribution, and RVA flour/starch pasting experiments were conducted for each experimental replicate as described above.

To facilitate comparison, genotype mean values for flour/starch attributes were averaged across all crop years and growing locations (each crop year/growing location combination was considered a replicate within a genotype). A more comprehensive analysis of environmental effects and their associations with genotype is addressed elsewhere (Geera et al 2005b). Because the 2000 crop for both waxy lines at Tetonia were not available for study, mean flour/starch characteristics and properties for each of these two lines were pooled from three instead of four crop year/growing location combinations. Analysis of variance (ANOVA) was used to test for significant differences among genotypes, while a least significant difference test (LSD, $P < 0.05$) was used to identify differences in genotype mean values for the various flour/starch characteristics and properties. Correlation analysis was conducted to assess associations among the measured flour/starch characteristics and flour pasting properties to better understand the factors influencing genotype flour pasting behavior. All statistical analyses were computed using SAS v. 8.2 for Windows (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Mean values for flour/starch characteristics and flour pasting attributes were calculated across both growing locations and crop

years to generate single representative values for each genotype. The two locations (Tetonia and Aberdeen, ID) contributed diverse growing conditions and management schemes and were intentionally selected to introduce environmental variability. Thus, genotype mean values for flour/starch characteristics in this study reflect anticipated and inherent environmental fluctuations. The ANOVA and correlation analysis were conducted to assess associations among the measured flour/starch characteristics and pasting properties to better understand the factors influencing genotype flour pasting behavior. With the exception of Penawawa, all lines were closely related. For this reason, the data of Penawawa may not always fit precisely with other genotype trends. Differences observed among genotypes with respect to both flour/starch characteristics and pasting properties are discussed below.

Starch Amylose Content

As anticipated, amylose content was the primary starch characteristic that was influenced in accordance with genotype *Wx* gene dosage. Mean total amylose (TAM) contents of the genotypes had a range of 1.0–25.5 g/100 g of starch (db), while apparent amylose (AAM) contents had a range of 0.7–20.4 g/100 g of starch (db) (Table II). Both TAM and AAM contents, which were positively correlated (Table III), gradually decreased with the loss of GBSSI isoforms in agreement with previous reports (Miura et al 1999; Ishida et al 2003; Kim et al 2003; Mangalika et al 2003). From other studies involving wheat lines of varying *Wx* gene configu-

ration, it is known that the contribution of each of the three homologous *Wx* genes to amylose biosynthesis is not equal. Starch amylose content is not simply linearly proportional to the number of active *Wx* genes. Rather, it is the interaction among the three genes that determines the amylose content of wheat starch (Miura et al 1999; Araki et al 2000; Wickramasinghe and Miura 2003). The gradual reduction in amylose content over the range of wild-type to two-gene null genotypes (Table II) has been explained by the ability of the remaining functional genes to partially, but not fully, compensate for GBSSI null alleles. Thus the decline in amylose levels over this range is more gradual than would be anticipated in the absence of a compensating effect. Furthermore, low levels of amylose detected in the waxy lines by the colorimetric assay (Table II) are similar to those previously reported for other waxy wheats (0.4–2.1%) (Yasui et al 1996; Kiribuchi-Otobe et al 1997; Mangalika et al 2003). As waxy wheat starches have been reported to have a higher proportion of long branch chains on amylopectin molecules compared with normal wheat starch (Yoo and Jane 2002), these long chains have the potential to complex with iodine and inflate amylose values determined by iodometric means (Jane et al 1999). In summary, the amylose levels among the normal, partial waxy, and waxy soft wheat starches are very comparable to those reported for other wheat market classes.

Lipid-complexed amylose (LAM) mean values for all genotypes had a range of 0.3–5.6 g/100 g of starch (db) (Table II). Both AAM and TAM contents showed positive correlations with

TABLE II
Mean Flour/Starch Characteristics by Genotype^{a,b}

Genotype	TAM	AAM	LAM	FTS	NSY	STR	FLPRC	A-Type	B-Type
Wild-type									
Whitebird	25.5 ± 0.7a	20.0 ± 0.6a	5.6 ± 1.0a	87.6 ± 1.1a	84.2 ± 0.6a	96.1 ± 1.0b	10.2 ± 0.9a	75.6 ± 2.8ab	24.4 ± 2.8cd
Jubilee	25.5 ± 0.6a	20.4 ± 0.4a	5.2 ± 0.7a	86.2 ± 1.7ab	83.9 ± 1.0ab	97.4 ± 1.2ab	10.3 ± 1.4a	75.3 ± 1.8ab	24.7 ± 1.8cd
One Gene Null									
Alturas	23.6 ± 0.7b	18.3 ± 0.6b	5.3 ± 0.6a	86.1 ± 0.9ab	83.5 ± 1.4ab	97.0 ± 1.8ab	10.1 ± 1.1a	71.7 ± 3.1c	28.3 ± 3.1b
Penawawa	22.2 ± 0.7c	16.8 ± 0.4c	5.4 ± 0.4a	86.0 ± 0.9ab	82.5 ± 1.1bc	95.9 ± 1.5b	10.3 ± 1.0a	76.8 ± 1.5a	23.2 ± 1.5d
Two Gene Null									
IDO563	18.5 ± 0.8d	14.1 ± 0.6d	4.4 ± 0.4b	85.6 ± 3.1b	82.8 ± 1.9abc	96.9 ± 1.8ab	9.8 ± 1.0a	72.4 ± 2.1c	27.6 ± 2.1b
IDO565	17.0 ± 1.4e	12.8 ± 1.5e	4.2 ± 0.9b	83.4 ± 2.3b	81.9 ± 2.0c	98.1 ± 2.3a	10.5 ± 1.0a	73.5 ± 2.1bc	26.5 ± 2.1bc
Waxy									
Leona	2.5 ± 1.6f	1.9 ± 1.3f	0.6 ± 0.4c	81.7 ± 1.0c	78.4 ± 1.2d	96.0 ± 0.8b	10.4 ± 0.9a	63.6 ± 1.5d	36.4 ± 1.5a
A98054S-Gwxy-1	1.0 ± 0.4g	0.7 ± 0.4g	0.3 ± 0.3c	81.8 ± 1.1c	77.6 ± 1.3d	94.8 ± 1.5bc	10.8 ± 1.3a	61.6 ± 2.3d	38.4 ± 2.3a
<i>P</i> value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0083	0.8509	<0.0001	<0.0001

^a Mean values determined across four crop year/growing location combinations. Values within a column followed by the same letter are not significantly different ($P < 0.05$).

^b TAM, total amylose (g/100 g of native starch); AAM, apparent amylose (g/100 g of native starch); LAM, lipid-complexed amylose (g/100 g of native starch); FTS, flour total starch (g/100 g of flour); NSY, native starch yield (isolated from flour) (g/100 g of flour); STR, starch recoveries (%); FLPRC, flour protein content (12% moisture basis); A-type granules by weight (g/100 g of native starch); B-type granules by weight (g/100 g of native starch).

TABLE III
Correlation Coefficients (*r*) Among Flour/Starch Characteristics and Flour Pasting Attributes^{a,b}

	Trough	Breakdown	Setback	Final	FLPRC	FTS	NSY	A-Type	B-Type	TAM	AAM	LAM
Peak	0.04	0.79*	0.06	0.05	-0.20	0.34*	0.41*	0.36*	-0.37*	0.15	0.14	0.2
Trough		-0.57*	0.98*	0.99*	-0.04	0.69*	0.76*	0.81*	-0.82*	0.91*	0.92*	0.84*
Breakdown			-0.55*	-0.56*	-0.14	-0.14	-0.12	-0.19	0.19	-0.43*	-0.45*	-0.34*
Setback				0.99*	-0.09	0.73*	0.82*	0.83*	-0.84*	0.95*	0.95*	0.86*
Final					-0.07	0.72*	0.80*	0.82*	-0.83*	0.94*	0.95*	0.86*
FLPRC						-0.25	-0.30*	-0.04	-0.13	-0.13	-0.15	-0.05
FTS							0.82*	0.69*	-0.70*	0.71*	0.72*	0.62*
NSY								0.83*	-0.83*	0.83*	0.83*	0.75*
A-type									-1.00*	0.85*	0.84*	0.83*
B-type										-0.85*	-0.84*	-0.83*
TAM											0.99*	0.95*
AAM												0.92*

^a * Significant at $P < 0.05$; $n = 60$.

^b Peak, flour peak viscosity; Trough, flour trough viscosity; Breakdown, flour breakdown viscosity; Setback, flour setback viscosity; Final, flour final viscosity; FLPRC, flour protein content (12% moisture basis); FTS, flour total starch (g/100 g of flour); NSY, native starch yield from flour (g/100 g of flour); A-type granule content (g/100 g of native starch); B-type granule content (g/100 g of native starch); TAM, total amylose (g/100 g of native starch); AAM, apparent amylose (g/100 g of native starch); LAM, lipid-complexed amylose (g/100 g of native starch).

LAM, indicating a higher LAM content for starches with increasing amylose levels (Table III). LAM content was highest for wild-type and one gene null partial waxy genotypes, slightly reduced for two gene null partial waxy genotypes, and lowest for the full waxy starches. For cereal starches, the amount of LAM has been generally demonstrated to parallel amylose content (Morrison et al 1984).

Flour Total Starch (FTS) Content and NSY from Flour

Mean flour total starch (FTS) contents by genotype had a range of 81.7–87.6 g/100 g of flour (db), while native starch yields (NSY) from the same straight-grade flours had a range of 77.6–84.2 g/100 g of flour (db) (Table II). Starch recoveries, which indicate the efficiency of starch extraction from straight-grade flours, had a range of 94.8–98.1% (Table II). An increasing FTS content generally was accompanied by an increasing NSY from flour (Tables II and III). As a high percentage of starch was recovered from flours, it is likely that the isolated native starches represented a reasonable approximation of the starch populations present within the parent flours.

As the number of functional *Wx* genes decreased over the range of wild-type to waxy, both FTS content and NSY from flour generally decreased (Table II). Though there was not always a clear distinction between wild-type, one gene null partial waxy, and two gene null partial waxy genotypes for both FTS content and NSY from flour, an observable trend was clearly evident. Waxy genotypes, which generally exhibited both the lowest FTS contents and NSY from flour (Table II), experienced a 6–7% reduction in FTS content compared with wild-type genotypes.

Several studies have reported a decreased starch content for waxy wheat flour compared with that of wild-type wheat. Yasui et al (1999) reported that the endosperm starch content of waxy mutant lines was ≈95% of the nonwaxy parent (two gene null); this finding is similar to the results of this study. Similarly, Abdel-Aal et al (2002) confirmed that waxy wheat lines yielded lower amounts of starch compared with wild-type lines of hard red spring wheat. Traditionally for wheat, starch content has been negatively correlated with flour protein content (Raeker et al 1998; Kim et al 2003) with fluctuations in FTS levels generally accompanied by corresponding and compensating mass fluctuations in protein levels. However, in this study, flour protein contents among genotypes were not statistically different (Table II). On a weight basis, decreases in FTS content for the waxy genotypes occurred independently of any compensating increase in flour protein content.

Conversely, reductions in FTS content were correlated to changes in amylose characteristics (Tables III). It is plausible that the gradual loss of GBSSI isoforms disrupted the efficiency of starch biosynthesis, and contributed to genotype reductions in FTS content. The observation that only very minimal reductions in FTS were noted for the partial waxy (relative to the wild-type) genotypes might be somewhat explained by the compensating effects of the remaining functional GBSSI genes previously used to explain the gradual reduction of amylose levels within the same genotypes. As would be expected in such a scenario, the waxy genotypes generally experienced the greatest step-wise reduction in FTS content with the loss of all three GBSSI isoforms (complete lack of GBSSI compensating effect) (Table II). The fact that this gradual decrease in FTS content occurred independently of any corresponding change in protein content and in conjunction with a known change in GBSSI gene configuration implies a starch biosynthetic basis. Thus, the trend toward a decreasing flour starch content in the transition from wild-type to waxy wheat could occur as a secondary effect of GBSSI gene dosage.

Granule Size Distributions of Isolated Native Starches

On the basis of granule number, isolated native starches exhibited a trimodal granule size distribution representing A-type, B-

type, and C-type granule fractions (data not shown) similar to previous reports (Bechtel et al 1990; Raeker et al 1998). However, on a weight basis, the C-type granule fraction did not register an obvious peak (Fig. 1) and constituted only a negligible percentage (<1%) of the total starch weight. For practical purposes, discussion of starch granule populations in this study will be limited to A- and B-types, with C-type granules considered a subpopulation of the B-type granule fraction. Also, all further comparison of A- and B-type starch granule populations among genotypes of this study will be based on proportions of starch by weight (as opposed by starch granule number) unless specifically noted otherwise.

Limited information exists regarding starch granule size distributions for genotypes with varying *Wx* gene deficiencies. Fujita et al (1998) and Mangalika et al (2003) reported no differences in the mean granule diameters for waxy and nonwaxy wheat starches, though specific details regarding the nature of the analyses were not described. In this study, mean granule diameters for the waxy native starches were significantly lower than those of the normal native starches (data not shown). These initial differences were investigated further in regard to genotype A- and B-type granule contents.

Based on a 10- μ m cutoff, weight-based proportions of A- and B-type granules were calculated for each genotype. Mean values of native starch A- and B-type granule contents for all genotypes had a range of 61.6–76.8 g/100 g of starch (db) and 23.2–38.4 g/100 g of starch (db), respectively (Table II). They were used to approximate the native proportions of A- and B-type granules within the parent flours. As the number of functional *Wx* alleles decreased from wild-type to waxy, there was a gradual decrease in A-type and a corresponding increase in B-type granule contents. This observation was substantiated by significant correlations between amylose characteristics and granule types (Table

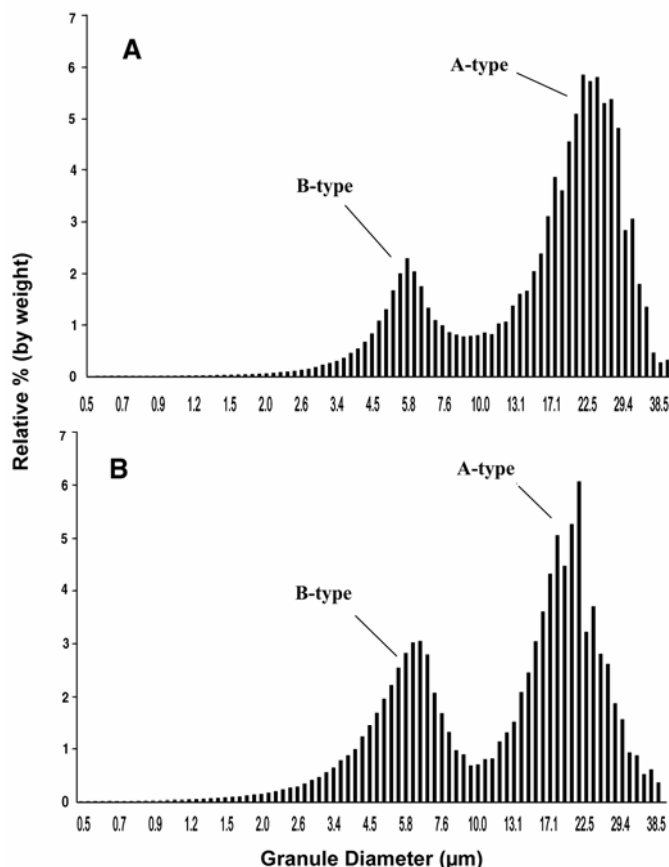


Fig. 1. Starch granule size distribution for (A) Whitebird (wild-type) and (B) A98054-Gwxy-1 (waxy) genotypes illustrating proportions of A- and B-type granules on a weight basis.

III). The lowest A-type and the highest B-type granule contents were exhibited by waxy starches (Fig. 1B), while the reverse was observed for normal starches (Fig. 1A). Partial waxy starches possessed A- and B-type granule contents intermediate of those of waxy and wild-type genotypes (excepting Penawawa, as previously noted). This finding confirms an earlier report of Bertolini et al (2003), who analyzed some of the same genotypes characterized in this study, though in a more preliminary fashion (data represented only single crop year and growing location). The fact that Penawawa (with a different genetic background) did not fit within the trend exhibited by the other more closely related genotypes provides further evidence that granule size distribution is a genetic trait (Stoddard 2000).

Though the precise molecular events that control starch granule size distribution are not completely understood, there are reports suggesting that starch debranching enzymes play a key role. Burton et al (2002) suggested that the number of starch granules synthesized within a plastid is determined by the concentration of soluble α -glucans. The action of starch debranching enzymes, which would tend to deplete or reduce the number of soluble α -glucans, was proposed to decrease the number of nucleation events and thus the number of initiated granules. Bustos et al (2004) reported that transgenic potato tubers, in which the activity of isoamylase was reduced by expression of antisense RNA, produced larger numbers of small granules compared with normal tubers. Conversely, Stahl et al (2004) reported that an increased activity

of the starch debranching enzyme, limit dextrinase, produced a transgenic barley starch with reduced numbers of small B-type starch granules compared with normal barley starch. In the latter report, unpredicted pleiotropic effects were also observed on numerous other biosynthetic enzymes. In waxy wheat genotypes, the trend toward a higher percentage of smaller granules could be explained in part by the absence of functional GBSSI genes, which could reduce starch debranching enzyme efficiency (by altering its starch substrate) or induce unpredicted pleiotropic effects on other starch biosynthesizing enzymes to alter proportions of large and small granules. Thus the observed shift toward an increased B-type granule content for starches with increasing waxy character is likely another secondary effect (in addition to the shift in FTS content already noted) of the gradual loss of functional GBSSI genes.

Flour Pasting Properties

Considerable differences were observed among genotypes with respect to flour pasting properties. Genotype mean ranges for flour RVA pasting attributes (displayed in Rapid Visco Analyser units [RVU]) were peak viscosity 359.0–432.3; breakdown viscosity 182.4–270.5; trough viscosity 110.1–177.9; final viscosity 180.9–372.9; and setback viscosity 70.1–195.0 (Table IV). A definite pattern was evident for most flour pasting attributes, which generally aligned according to GBSSI class (Fig. 2).

Flour peak and breakdown viscosities, which were positively correlated (Table III), exhibited similar patterns over the range of wild-type to waxy genotypes (Fig. 2). Flour peak viscosities generally increased for one gene null partial waxy genotypes relative to wild-type genotypes and experienced a gradual downward trend from partial waxy toward waxy genotypes. Flour peak viscosities of waxy and wild-type genotypes were not statistically different (Table IV). Partial waxy and waxy genotypes exhibited higher flour breakdown viscosities than wild-type genotypes, though no distinction was observed between partial waxy and waxy genotypes.

Flour trough, setback, and final viscosities, which were highly intercorrelated (Table III), gradually decreased from wild-type to waxy. Trough, setback, and final viscosities of the flours clearly aligned into distinct groupings according to GBSSI class (Table IV) and exhibited similar patterns across genotypes (Fig. 2). Wild-type genotypes exhibited the highest flour trough, final and setback viscosities, while waxy genotypes exhibited lowest values for the same three measurements (Table IV).

Relationship of Flour/Starch Characteristics to Flour Pasting Properties

Using a range of wild-type and one gene null partial waxy wheats, Zeng et al (1997) showed that an increasing amylose content was

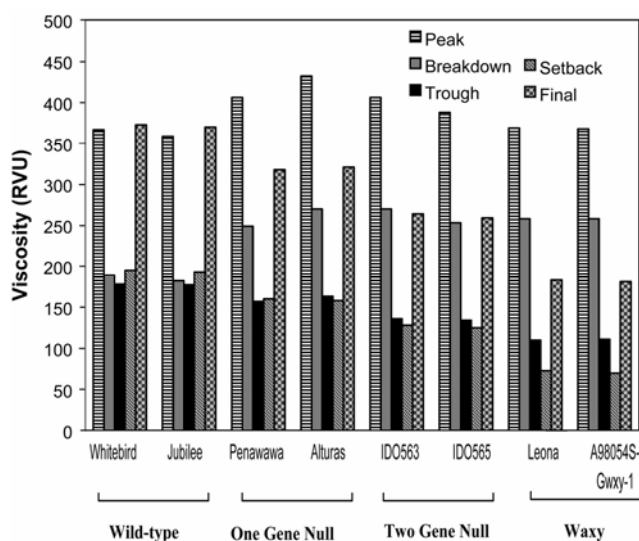


Fig. 2. Mean flour pasting trends of straight-grade flours across genotypes.

TABLE IV
Mean Flour Pasting Properties by Genotype^{a,b}

Genotype	Peak Viscosity	Breakdown Viscosity	Trough Viscosity	Final Viscosity	Setback Viscosity
Wild-type					
Whitebird	366.9 ± 24.7cd	189.0 ± 27.5b	177.9 ± 6.0a	372.9 ± 8.1a	195.0 ± 4.9a
Jubilee	359.0 ± 26.4d	182.4 ± 26.4b	176.7 ± 6.4a	370.0 ± 10.6a	193.3 ± 5.4a
One gene null					
Alturas	406.3 ± 13.5b	249.3 ± 15.0a	157.0 ± 6.3b	317.5 ± 14.8b	160.4 ± 8.6b
Penawawa	432.3 ± 24.5a	269.7 ± 21.1a	162.6 ± 5.6b	320.7 ± 11.8b	158.1 ± 6.6b
Two gene null					
IDO563	406.2 ± 24.2b	270.5 ± 25.9a	135.7 ± 3.7c	264.0 ± 7.1c	128.4 ± 4.0c
IDO565	388.2 ± 28.0bc	253.9 ± 26.2a	134.4 ± 3.1c	259.3 ± 10.3c	124.9 ± 7.4c
Waxy					
Leona	368.9 ± 30.9cd	258.8 ± 30.8a	110.1 ± 2.6d	183.4 ± 3.2d	73.2 ± 2.3d
A98054S-Gwxy-1	368.4 ± 23.5cd	257.6 ± 24.0a	110.8 ± 1.8d	180.9 ± 2.8d	70.1 ± 2.0d
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

^a Mean values determined across four crop year/growing location combinations. Values within a column followed by the same letter are not significantly different ($P < 0.05$).

^b Values shown in Rapid Visco Analyzer Units (RVU).

associated with both a low degree of granule swelling and a reduced paste peak viscosity. In contrast, our findings, which also included two gene null and waxy genotypes (in addition to wild-type and one gene null genotypes) exhibited no significant correlation between flour peak viscosity and amylose (TAM, AAM) contents (Table III). The lack of correlation was due to the fact that there was a nonlinear relationship (Fig. 2) among the genotypes for flour peak viscosity (wild-type and waxy genotypes at the extremes with regard to GBSSI gene dosage exhibited similar flour peak viscosities) (Table IV). The basis for weak positive correlations between flour peak viscosity and FTS, NSY from flour, and A-type granule contents (Table III) will be discussed later.

As FTS and A-type granule contents generally decreased over the range of wild-type to waxy (Table II), it was anticipated that both characteristics likely influenced flour pasting property patterns among the genotypes. Based on previous work, a decrease in FTS or the proportion of A-type granules within a starch would be expected to result in a peak viscosity reduction (Shinde et al 2003; Geera et al 2005a). For FTS, this hypothesis was substantiated by the fact that mean peak viscosities of isolated waxy native starches (308.4 and 321.7 RVU) were significantly higher than those of the wild-type genotypes (289.2 and 292.7 RVU) (Fig. 3), whereas mean flour peak viscosities for wild-type and waxy genotypes were not statistically different (Table IV, Fig. 2). The fact that waxy flours possessed lower FTS contents than wild-type flours likely accounts, in part, for these observed differences. In reexamining previous studies, it is interesting to note that those reporting lower or similar peak viscosities for waxy and wild-type genotypes utilized flours (Ishida et al 2003), while those reporting higher peak viscosities for waxy genotypes had analyzed isolated starches (Kim et al 2003; Mangalika et al 2003). Though amylose contents represented the most dominating genetic difference among the genotypes, it is likely that FTS and flour A- to B-type granule ratios contributed significant secondary effects to flour paste peak viscosities.

Flour breakdown viscosity exhibited negative correlations with TAM, AAM, and LAM contents (Table III). Both amylose and amylose-lipid complexes restrict granule swelling and contribute to granule integrity (Hermansson and Svegmak 1996; Yasui et al 1999). Partial waxy and waxy flours, which exhibited higher breakdown viscosities compared with wild-type genotypes, also possessed lower levels of TAM, AAM, and LAM (Table II). Though partial waxy and waxy flours possessed differing levels of starch TAM, AAM, and LAM, their breakdown viscosities were not significantly different (Table II). This observation could be due in part to the lower FTS levels and A-type granule contents of waxy compared with partial waxy flours (Table II). This hypothesis was supported by the fact that breakdown viscosity was substantially higher for the isolated starches of waxy genotypes (212.3 and 235.6 RVU) compared with those of one gene null (126.6 and 132.3 RVU) and two gene null partial waxy genotypes (161.8 and 173.1 RVU) (Fig. 3). Furthermore, B-type starch granules, irrespective of GBSSI class, possessed lower breakdown viscosities relative to their respective A-type granule counterparts within a genotype (Geera et al 2005a). This observation could also explain, in part, the lack of difference between breakdown viscosity values of partial waxy and waxy flours (waxy flours possessed higher proportions of B-type granules) (Table II).

Flour pasting attributes trough, setback, and final viscosities all gradually decreased over the range of wild-type to waxy (Fig. 2) and clearly aligned into four separate groups according to GBSSI class (Table IV). All three flour RVA attributes were significantly correlated with TAM, AAM, LAM, FTS, A-type, and B-type granule contents (Table III). Based strictly on correlation strength, it can be inferred that amylose characteristics exerted the primary effect on genotype flour pasting attributes. This finding was expected as the genotypes studied differed primarily according to *Wx* gene dosage and GBSSI class. Increases in TAM and AAM

contents over the range of waxy to wild-type have been related to higher trough (greater potential for amylose leaching), final, and setback (greater potential for reassociation of amylose to form a gel structure) viscosities (Mangalika et al 2003). However, differences in flour trough, setback, and final viscosities among the genotypes were also somewhat explained by variability in FTS and A- and B-type granule contents (Table III), which occurred in parallel to trends in TAM and AAM contents. Flours with high-amylose contents (wild-type genotypes) also possessed high FTS and A-type granule contents, while those with low-amylose contents (waxy genotypes) exhibited both reduced FTS and A-type granule levels. High trough, setback, and final viscosities would be expected for flours with high total starch and A-type granule contents (Shinde et al 2003; Geera et al 2005a). Though primary flour pasting attributes were predominantly a function of amylose characteristics, it is likely that fluctuations in FTS and A- and B-type granule contents contributed significantly to genotype pasting attributes as potential secondary effects of GBSSI gene configuration.

SUMMARY AND CONCLUSIONS

Eight soft spring wheat lines representing four different GBSSI classes were the focus of this study. In summary, three major differences were observed among genotypes with respect to flour/starch characteristics. As anticipated, starch amylose (TAM, AAM, and LAM) characteristics gradually decreased over the range of wild-type to waxy, with differences in amylose content among genotypes primarily explained by the number of functional *Wx* genes. In addition, FTS and A-type granule contents also decreased gradually over the range of wild-type to waxy, as possible secondary effects of *Wx* gene dosage.

With respect to flour pasting properties, the nonlinear trends for peak and breakdown viscosities across wild-type to waxy genotypes was likely attributable in part to observed decreases in FTS and A-type granule content over the same genotype range. The lack of correlation between amylose characteristics and flour peak and breakdown viscosities across genotypes was somewhat explained by the observed trends in FTS and A-type granule contents, which counteracted the effects of amylose characteristics on flour peak and breakdown viscosities. Flour trough, setback, and final viscosities gradually decreased from wild-type to waxy and were clearly differentiated into four separate groups based on GBSSI class. In contrast to flour peak and breakdown viscosities, the anticipated effects of FTS and A-type granule content on flour trough, setback, and final viscosities occurred in concert with those

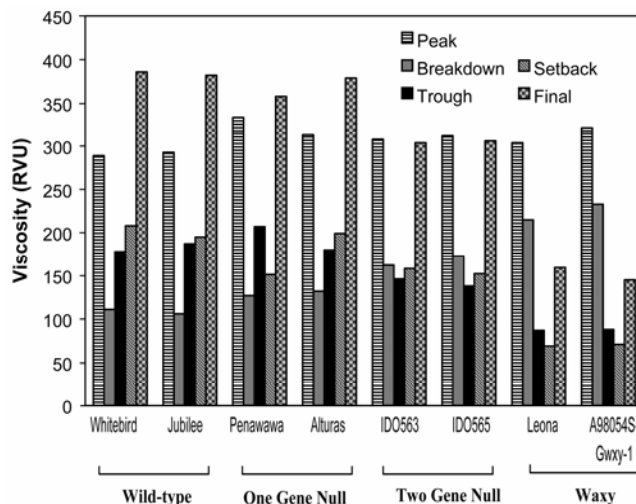


Fig. 3. Mean pasting trends of isolated starches across genotypes.

exerted by amylose characteristics. Highly significant correlations were observed between amylose characteristics, FTS, A-type granule content and trough, setback, and final viscosities. Nevertheless, the overall strength of correlations suggests that amylose characteristics exerted a primary effect on flour pasting properties, while FTS and A/B-type granule contents contributed secondary, yet significant, effects. In summary, it appears that the loss of one or more GBSSI isoforms within wheat has the potential to induce multiple secondary effects on starch characteristics aside from the previously documented effects on starch amylose content. These secondary effects most likely account for some of the documented differences in flour pasting behavior observed for wheat genotypes of differing GBSSI class.

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