

Grain Quality Characteristics and Milling Performance of Full and Partial Waxy Durum Lines

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ABSTRACT

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Mutation of the gene coding for the granule bound starch synthase (waxy protein) leads to reduced amylose content in cereal endosperm. Durum wheat (*Triticum turgidum* L. var. *durum*) has one waxy locus in each of its two genomes. Full waxy durum wheat is produced when both genomes carry the waxy null alleles. When only one locus is mutated, partial waxy durum wheat is obtained. Partial and full waxy near-isogenic lines of durum wheat developed by a breeding program were analyzed as to their quality characteristics. Amylose was largely eliminated in full waxy lines; however, no reduction in amylose content was detected in partial waxy lines. The waxy mutation did not affect grain yield, kernel size, or kernel hardness. Full waxy durum lines had higher kernel ash

content, α -amylase activity, and a unique nonvitreous kernel appearance. Protein quality, as evaluated by SDS microsedimentation value, gluten index, and wet gluten was slightly lower in the full waxy lines than in the other genotypes. However, comparisons with current cultivars indicated that protein quality of all derived lines remained in the range of strong gluten cultivars. Semolina yield was lowered by the waxy mutations due to lower friability that resulted in less complete separation of the endosperm from the bran. Waxy semolina was more sensitive to mechanical damage during milling, but modified tempering and milling conditions may limit the damage. Overall, quality characteristics of waxy durum grain were satisfactory and suitable for application testing.

Starch is composed of two types of glucose polymers: amylose (10–37% of total starch), a mostly linear molecule consisting of α -(1-4)-linked D-glucan chains, and amylopectin (63–90% of total starch), a highly branched molecule containing α -(1-4)-linked D-glucan chains with α -(1-6)-linked branches (Galliard and Bowler 1987; Lineback and Rasper 1988). In cereal endosperm, amylose is synthesized in the amyloplast by granule bound starch synthase (GBSS) known as the waxy protein (Nelson and Rines 1962; Murata et al 1965; Shannon and Garwood 1984; Shure et al 1983; Sano 1984; MacDonald and Preiss 1985). Elimination or reduction of GBSS activity can result in plants with no or reduced amylose content. Waxy proteins are encoded by *Wx* genes located at homeologous loci on the chromosome 7 (Tsai 1974; Hovenkamp-Hermelink et al 1987; Hseih 1988; Chao et al 1989; Ainsworth et al 1993; Nakamura et al 1993; Yamamori et al 1994). In tetraploid wheat, the two homeologous waxy genes are located on the short arms of chromosome 7A (*Wx-A1*) and on the long arms of chromosome 4A (*Wx-B1*). *Wx-B1* was translocated from 7B chromosome to 4A chromosome during the evolution of wheat. Identification of the two GBSS isozymes in durum wheat is possible using a simple one-dimensional SDS-PAGE method (Zhao and Sharp 1996). The presence of homozygous waxy null alleles in one or both genomes results in partial or full waxy durum wheat, respectively, and is expected to change the ratio of amylose to amylopectin and, consequently, end-use quality of wheat. Development of waxy durum lines at North Dakota State University in collaboration with USDA-ARS started in 1997 with the long-term objective of releasing waxy and partial waxy durum cultivars adapted to North Dakota.

Milling performance of waxy hexaploid wheat (*Triticum aestivum*) was studied by Yasui et al (1999). Flour yield was \approx 20% lower for waxy mutant lines compared with the nonwaxy parent. The re-

duced yield was attributed to either reduced starch content, higher fat concentration, or higher β -glucan concentration. They suggested that higher fat content in waxy lines reduced flour flowability and yielded less flour. Hardness also was tested as a potential factor for reducing milling yield, but results showed that waxy kernels were not softer than nonwaxy kernels as measured by flour mean particle size. To identify the significant sources of variation of waxy flour properties, Graybosch et al (2003) used waxy hexaploid wheats with diverse genetic backgrounds. SDS sedimentation volume, flour yield, flour ash, and starch pasting properties were significantly affected by the waxy mutation regardless of the genetic background and growing environment. Other properties such as grain yield, grain hardness, and grain ash content were primarily affected by the environment and genetic background. Therefore, a common genetic background was preferred to identify the single effect of the waxy genotype on wheat product quality.

Semolina derived from durum wheat is composed of coarser particles than common wheat flour. Other products from the milling of durum wheat are flour, bran, and shorts. Effects of waxy mutations on the separation of the durum kernel into these fractions remain to be established.

In this study, partial and full waxy durum lines derived from a cross between the partial waxy hard red winter wheat cultivar Ike and durum cultivar Ben (Hegstad et al 1998) were analyzed for agronomic and milling properties. The goal of this study was to understand the influence of combinations of waxy null alleles on kernel characteristics and milling yield in a similar durum wheat genetic background. Results might provide insight into the feasibility of developing waxy durum lines with practical end-use applications.

MATERIALS AND METHODS

Waxy and partial waxy durum lines were cooperatively developed by North Dakota State University and the USDA-ARS (Hegstad et al 1998). The *wx-A1* and *wx-B1* alleles were introgressed from the partial waxy hard red winter wheat cultivar, Ike (Martin et al 1994) into the strong gluten durum cultivar, Ben (Elias and Miller 1998). Ike is a semi-dwarf cultivar well adapted in western Kansas and occupying 2.6% of the wheat acreage in 2003. Ben is widely grown in the northern plains of the United States. Ben exhibits medium height, high test weight, large kernels, and strong gluten. After the initial cross of Ike and Ben, Ben was used as the recurrent parent to develop BC₄ waxy and partial waxy durum lines.

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Double-heterozygous waxy lines (lines heterozygous for genes in both genomes) were selected at each generation using a pollen iodine staining method. Anthers were collected at flowering time and isolated pollen grain were stained with a potassium iodide and iodine solution (0.2% KI, 0.04% I₂) and observed with a light microscope. The presence of a 3:1 ratio of blue to brown-red pollen indicated a double heterozygote *wx-A1/Wx-A1*; *wx-B1/Wx-B1* plant. Heterozygous plants were used as the male parents in the next backcross. Finally, BC₄F₁ were selfed to produce progeny representative of the four possible classes of homozygotes for the waxy alleles. The lines were selected using RFLP markers of the respective *wx* alleles. Specifically, an oat clone for GBSS1 was provided by Dave Somers, University of Minnesota (*unpublished data*). The clone was probed against a Southern blot of wheat genomic DNA. Polymorphism was observed between parental lines upon restriction with HindIII endonuclease. Aneuploid and parental analysis determined the association of RFLP bands. The *Wx-B1* allele was determined by the presence of an 18 kb or 12 kb band (size dependent on the background.) The alternative *wx-B1* allele was determined by the lack of the band associated with the *WT* allele. The *Wx-A1* allele was determined to associate with a 5 kb fragment; the alternative *wx-A1* allele was determined by the presence of a 15 kb fragment (Hegstad et al 1998; Hegstad 2001). Classes are 1) wild type (*Wx-A1Wx-A1 Wx-B1Wx-B1*), hereafter referred to as the WT genotype; 2) partial waxy mutated at the *Wx-A1* locus (*wx-A1wx-A1 Wx-B1Wx-B1*), hereafter referred to as the *wx-7A* genotype; 3) partial waxy mutated at the *Wx-B1* locus (*Wx-A1Wx-A1 wx-B1wx-B1*), hereafter referred to as the *wx-4A* genotype; and 4) full waxy (*wx-A1wx-A1 wx-B1wx-B1*), hereafter referred to as the *wx* genotype. Together, the developed material will be referred to as waxy isolines.

Experimental Design

Thirty-five BC₄F₄ lines of the four different genotypic classes were grown in replicated plots at two experimental stations (Langdon and Casselton, ND) in the summer of 2000. In addition, seven durum cultivars (Ben, Maier, Plaza, Mountrail, Rugby, Lebsock, and Belzer) were used as quality standards in the experiment. Cultivars used in the experiment were references of desired quality in a durum cultivar grown in North Dakota. Ben was included because it is the recurrent parent. Ike is a winter wheat and could not be part of the experiment because it is not adapted to the growing area. Rugby was included as a weak gluten check.

Planting included 10 lines of *wx*, four lines of *wx-4A*, nine lines of *wx-7A*, 12 lines of WT, and seven cultivars as checks for a total of 42 lines. Each line was planted in a 5.94 m² area, using a randomized complete block design (RCBD) with two replicates and two locations.

Grain Quality Evaluations

Agronomic data (heading date and height) were collected and seed harvested for quality tests. All grain samples were cleaned by passing them through a dockage tester (Carter-Day Co., Minneapolis, MN). Test weight (TW) was determined on cleaned grain using Approved Method 84-10 (AACC 2000). Thousand-kernel weight (TKW) was determined by counting the number of kernels in a 10-g sample of cleaned wheat using an electronic seed counter (Seedburo Equipment Co., Chicago, IL). Values are reported on 10% moisture basis. Moisture content of the cleaned grain was determined using a moisture meter (Motomoco, Dickey-John, Auburn, IL) according to the Approved Method 44-11 (AACC 2000). Grain protein concentration (GPC) on a 14% moisture basis was determined using a near-infrared (NIR) grain analyzer calibrated for durum. SDS micro-sedimentation values on ground whole meal were determined using the procedure developed by Dick and Quick (1983), modified from the AACC Approved Method 56-61. Falling number was determined according to the procedure based on Approved Method 56-81B (AACC 2000). Because of the variant reaction of waxy starch on the falling number test (Graybosch et al 2000), α -amylase activity was also measured directly according to the McCleary method (McCleary and Sheehan 1987) using a reagent kit (Ceralpha method, Megazyme Int., Wicklow, Ireland) and reported in international units per gram dry weight flour (IU). Ash concentration was determined by measuring the residue remaining after incinerating a 3-g sample for \approx 16 hr at 575°C according to Approved Method 08-01 (AACC 2000). Kernel hardness (hardness index) and kernel diameter were measured with the Single Kernel Characterization System (SKCS) (Perten Instruments) from about 300 valid measurements. Moisture of samples for the SKCS test was \approx 12%. The method was based on the procedure described by Martin et al (1993).

The high-performance size-exclusion chromatography (HPSEC) method was used for measuring amylose content according to the procedure of Grant et al (2002). A Hewlett Packard 1090 HPLC equipped with an autosampler, Hewlett Packard 1047A refractive index detector, and personal computer with Chemstation (HP ChemStation for LC Rev. A.04.01) for control and integration was used. Amylose (type III from potato), amylopectin (from potato) obtained from Sigma Chemical Co. (St. Louis, MO), as well as amylose and amylopectin isolated from HRS and durum were used as standards.

Milling Procedure

All samples were tempered to 17.5% moisture using a three-step tempering procedure (12.5, 14.5, and 17.5%) before milling into semolina on a pneumatic experimental mill (Buhler-Miag, Minneapolis, MN) fitted with two laboratory-scale purifiers (Ap-

TABLE I
Grain Characteristics of Waxy and Wild Type Genotypes Combined Across Locations

Genotype ^a	Observations	Test Wt (kg/hL)	TKW ^b (g)	Diameter (mm)	Kernel Ash (%)	Hardness Index
<i>wx</i>	40	71.8a ^c	31.5a	2.3ab	1.67c	79bc
<i>wx-4A</i>	16	71.8a	30.8a	2.3a	1.61ab	85d
<i>wx-7A</i>	36	72.2ab	33.4bc	2.4ab	1.56a	81c
WT	48	71.6a	32.5ab	2.3ab	1.57ac	80bc
Belzer	4	73.7b-d	42.1e	2.7d	1.57ab	72a
Ben	4	76.1e	41.9e	2.8d	1.67b	76ab
Lebsock	4	76.1e	38.4de	2.7cd	1.65a-c	80bc
Maier	4	76.3e	38.9de	2.7cd	1.61a-c	79bc
Mountrail	4	74.4c-e	38.2de	2.6cd	1.60a-c	77ab
Plaza	4	73.1a-c	35.1b-d	2.5bc	1.66a-c	76ab
Rugby	4	75.4de	36.7cd	2.6cd	1.60a-c	81bc

^a WT, wild type (*Wx-A1Wx-A1 Wx-B1Wx-B1*); *wx-7A*, partial waxy mutated at the *Wx-A1* locus (*wx-A1wx-A1 Wx-B1Wx-B1*); *wx-4A*, partial waxy mutated at the *Wx-B1* locus (*Wx-A1Wx-A1 wx-B1wx-B1*); *wx*, full waxy (*wx-A1wx-A1 wx-B1wx-B1*).

^b TKW, 1,000 kernel weight, 10% mb.

^c Values with the same letter in the same column do not differ significantly at $P < 0.05$ (means separation, LSD). Means separation was performed using different LSD for each comparison due to different number of observation.

proved Methods 26-10A and 26-41, AACC 2000). Flour, semolina and bran fractions were collected and weighted, and total extraction (TE) and semolina extraction (SE) (% db) were calculated as SE = semolina × 100/total (bran+shorts+semolina +dust+flour) and TE = (semolina + flour)×100/ total (bran+ shorts+semolina +dust+flour).

Semolina Quality

Semolina was characterized for moisture, ash content, and protein content according to Approved Methods 44-15A, 08-01 and 46-30, respectively (AACC 2000). The enzymatic digestion assay kit (Megazyme) was used to determine semolina starch damage. Approved Method 76-31 (AACC 2000) is based on the procedure described by Gibson et al (1992). The gluten index (GI) and wet gluten (WG) values were determined according to Approved Method 38-12 (AACC 2000). The WG content was determined by washing 10 g (14% mb) of semolina with 300 mL of buffered NaCl solution.

Semolina color was measured with a Minolta colorimeter equipped with a DP-301 data processor and CR-300 series chromameter. *L** (brightness) values range from 0 (black) to 100 (white). The *a** (redness) range is negative values (green) to positive values (red), whereas the *b** (yellowness) range is from negative values (blue) to positive values (yellow). Values reported are the means of triplicate determinations.

Statistical Analysis

For each quality test, an analysis of variance (ANOVA) was performed by following the general linear model (GLM) proce-

dures of SAS v. 6.10 (SAS Institute, Cary, NC.). Location was considered a random effect, and genotype and lines were considered fixed effects. Results were analyzed separately per location and combined when variance was homogeneous across location using Bartlett's test of homogeneity. Fisher's protected least significant differences (LSD) test was used to differentiate treatment means at the 5% significance level. Because of unbalanced data, different LSD values were used to compare different means (not shown).

RESULTS

Field results indicated that waxy isolines and cultivars all headed between 58 and 60 days after planting. The developed lines were much shorter than the cultivars and could be classified as semi-dwarf, like Ike. This was due to selection for the semi-dwarf alleles from Ike during the back-crossing of the lines. Grain yield was not significantly different among lines and cultivars (data not shown).

Grain Characteristics

No significant difference in test weight or kernel diameter was found among the waxy isolines (Table I). The TKW for the wx-7A genotype was higher compared with the wx or wx-4A genotype but was not significantly different from the TKW in the WT genotype (Table I). Therefore, TKW differences did not appear to be affected by the waxy mutations. Test weight, height, and kernel mass means were lower for the waxy isolines compared with values obtained from the cultivars, including Ben. This might be a reflection that not all of the recurrent parent (Ben) characteristics were recovered. Possible explanations are that these genes

TABLE II
Protein Content and Protein Quality of Waxy and Wild Type Genotypes Combined Across Locations

Genotype ^a	Observations	Protein ^b (%)	SDS Micro-Sedimentation ^b (mm)	Amylose Content
wx	40	12.9ab ^c	68d	1a
wx-4A	16	13.3b-e	73e	24b
wx-7A	36	13.0a-c	71de	24b
WT	48	12.7a	74e	25b
Belzer	4	14.1d-f	74de	25b
Ben	4	14.6f	62bc	24b
Lebsock	4	14.1c-f	55b	26b
Maier	4	14.1c-f	70de	28b
Mountrail	4	14.4ef	57bc	28b
Plaza	4	12.9a-d	65cd	25b
Rugby	4	14.1def	25a	23b

^a WT, wild type (*Wx-A1Wx-A1 Wx-B1Wx-B1*); wx-7A, partial waxy mutated at the *Wx-A1* locus (*wx-A1wx-A1 Wx-B1Wx-B1*); wx-4A, partial waxy mutated at the *Wx-B1* locus (*Wx-A1Wx-A1 wx-B1wx-B1*); wx, full waxy (*wx-A1wx-A1 wx-B1wx-B1*).

^b 14% mb.

^c Values with the same letter in the same column do not differ significantly at *P* < 0.05 (means separation, LSD). Means separation was performed using different LSD for each comparison due to different number of observation.

TABLE III
Milling Performance of Waxy and Wild Type Genotypes Combined Across Locations

Genotype ^a	Semolina Extraction (%)	Total Extraction (%)	Friability ^b
wx	59.0a ^c	64.2a	8a
wx-4A	61.7cd	67.7b	9ac
wx-7A	61.4cd	68.3b	10b
WT	60.5b	68.1b	11d
Belzer	60.6bc	67.8b	11b-d
Ben	62.5cd	68.7bc	9a-c
Lebsock	63.6d	69.9c	9a-c
Maier	63.4d	70.3c	10a-d
Mountrail	62.4cd	68.5bc	9ab
Plaza	61.3b-d	68.6bc	11b-d
Rugby	62.7cd	69.3c	10a-d

^b WT, wild type (*Wx-A1Wx-A1 Wx-B1Wx-B1*); wx-7A, partial waxy mutated at the *Wx-A1* locus (*wx-A1wx-A1 Wx-B1Wx-B1*); wx-4A, partial waxy mutated at the *Wx-B1* locus (*Wx-A1Wx-A1 wx-B1wx-B1*); wx, full waxy (*wx-A1wx-A1 wx-B1wx-B1*).

^a Friability = total flour × 100/total flour + total semolina.

^c Values with the same letter in the same column do not differ significantly at *P* < 0.05 (means separation, LSD). Means separation was performed using different LSD for each comparison due to different number of observation.

had been selected along with the semi-dwarf alleles during the development of the lines or that reconstitution of the recurrent parent chromatin remains substantially incomplete at BC₄ generation.

Kernel ash content was significantly higher for wx lines compared with wx-4A and wx-7A lines (Table I). Using the SKCS to measure kernel hardness, wx lines did not score lower than WT lines or most of the cultivars tested. The wx-4A kernels were significantly harder than any other kernels derived from the waxy isolines and cultivars. Waxy kernels appearance was unique. Waxy kernels could not be classified as any of the known vitreous, starchy, and bleached kernel types. Kernel endosperms were nonvitreous, dull, and homogeneous.

Grain Protein Concentration and Quality

According to our results (Table II), grain protein concentration was significantly higher for wx-4A lines when compared with the WT lines. The waxy isolines are consistently lower in protein than the cultivars. This may be due to the retention of the lower protein alleles coming from Ike.

SDS micro-sedimentation was used on whole meal to measure gluten strength. Higher values indicate stronger gluten. When compared with other waxy isolines, wx genotype had slightly lower gluten strength as indicated by lower SDS value (Table II). However, when compared with the strong gluten cultivars included in the experiment (with the exception of Rugby), values for all waxy isolines fell into the same scale range (55–74). Therefore, derived lines could be considered strong gluten lines.

Amylose Content

Amylose content (Table II) was dramatically reduced in the wx lines but no differences could be detected among wx-4A, wx-7A, and WT lines. The line variation within genotypic classes was too large to detect small differences that may exist among those lines. There was no significant variation detected among lines of the same genotype but standard deviation of wx-7A lines and wx-4A amylose content were 3.2 and 3.7, respectively. These values indicate that a difference of 3% amylose content between partial waxy types and WT lines would not be detected.

Milling Performance

Because the mill settings were kept constant for all samples, results correspond to gross milling yield and do not take into account the purity of the products obtained.

From the results, it was possible to assess the friability of the starchy endosperm (Table III). The wx lines yielded significantly

less semolina than did WT or partial waxy lines. The average semolina yield of waxy lines was 59% and that of WT and partial waxy was 60.5 and ≈61.5%, respectively. Total extraction is the efficiency to recover semolina plus flour from the kernels. It also was significantly lower for waxy durum samples. This indicated that lower semolina yield for waxy lines were not due to higher flour production. Endosperm friability was lower for the waxy lines. Together, these results indicated that the waxy durum samples milled into more bran and shorts due to lower friability of the endosperm.

No difference in semolina ash content was found among the different genotypes from the two locations (Table IV). This result indicated that all semolina produced were of similar quality, even though less quantity was obtained when waxy durum was milled.

Wx semolina had higher levels of starch damage compared with the partial and WT semolina. Percent starch damage in wx durum semolina was double that of WT semolina (Table IV).

Falling Number and α -Amylase Activity

The falling number (FN) method was used to estimate α -amylase activity, an indicator of sprout damage. Falling number values for all wx lines were the same (63 sec), independent from α -amylase activity (data not shown). Therefore, the falling number test was not suitable for detecting sprout damage in full waxy durum wheat. This observation is consistent with that of Graybosch et al (2000) for hexaploid wheat.

The α -amylase activity was measured on semolina using an enzymatic assay. Higher values were obtained for the Casselton grown wheat than for the Langdon wheat (average of 0.193 vs. 0.108). These results were most likely due to the wet conditions in Casselton that favored sprouting. Waxy semolina had significantly higher α -amylase activity than partial waxy, WT, or the cultivars semolina samples (Table IV).

Semolina Color

Semolina color was assessed with three color attributes; brightness (L^*), redness (a^*), and yellowness (b^*) (Table IV) using a reflectance colorimeter. Semolina brightness (L^*) values were slightly lower for the wx, wx-4A, and wx-7A lines compared with the WT lines. The redness (a^*) values were the highest for the wx and wx-7A samples. The values for these lines were similar to the values obtained for Ben and Mountrail, which indicate that the wx and wx-7A kernels redness remain satisfactory. The third coordinate b^* (yellowness) was also affected by the genotype. Wx semolina appeared to be significantly less yellow than semolina from partial

TABLE IV
Semolina Characteristics of Waxy and Wild Type Genotypes Combined Across Locations

Genotype ^a	Ash %	α -Amylase Activity ^b (IU)	Starch Damage ^c (%)	Semolina Color ^d		
				L^*	a^*	b^*
wx	0.66	0.18a ^c	3.2a	83a	-2.1a	26a
wx-4A	0.65	0.15b	1.6b	83a	-2.5c	29b
wx-7A	0.62	0.14b	1.5b	83a	-2.1a	29b
WT	0.62	0.15b	1.5b	84b	-2.7bd	30c
Belzer	0.63	0.11bc	1.2b	84b	-2.5bcd	27a
Ben	0.67	0.10bc	1.3b	84b	-2.4abc	25d
Lebsock	0.65	0.10bc	1.4b	84b	-2.6bcd	26ad
Maier	0.66	0.10bc	1.3b	84b	-2.9d	30c
Mountrail	0.65	0.06c	1.3b	84b	-2.4abc	25d
Plaza	0.64	0.12bc	1.3b	84b	-2.8cd	29b
Rugby	0.66	0.08c	1.3b	84b	-2.6bcd	27a

^a WT, wild type (*Wx-A1Wx-A1 Wx-B1Wx-B1*); wx-7A, partial waxy mutated at the *Wx-A1* locus (*wx-A1wx-A1 Wx-B1Wx-B1*); wx-4A, partial waxy mutated at the *Wx-B1* locus (*Wx-A1Wx-A1 wx-B1wx-B1*); wx, full waxy (*wx-A1wx-A1 wx-B1wx-B1*).

^b IU, international unit.

^c 14% mb.

^d L^* , brightness; a^* , redness; and b^* , yellowness.

^e Values with the same letter in the same column do not differ significantly at $P < 0.05$ (means separation, LSD). Means separation was performed using different LSD for each comparison due to different number of observation.

and WT lines. Also, semolina produced with the two partial waxy genotypes showed an intermediate value for this characteristic.

Semolina Protein Quality

Protein concentration was slightly higher for wx and wx-4A lines compared with wx-7A and WT lines. However, all waxy isolines were in the same range found for the durum cultivars. Gluten quality was studied using gluten index (Table V). High values indicate strong gluten. Results were not influenced by location. Variation as determined by ANOVA was primarily due to genotypic differences, particularly relating to the waxy genotype (data not shown). Waxy durum lines gave the lowest gluten index among the four genotypic classes (Table V). Gluten index values for wx lines were equivalent to that of wx-4A lines. Thus, the mutation in wx-4A gene lowered the gluten index. Wet gluten also was measured and was only lower when both wx loci were homozygous for the null allele. Results obtained for the derived lines were in the range of the values obtained for the cultivars (except Rugby), which indicate that all the lines developed had strong gluten and could be considered well suited for further processing.

Environment Stability

Significant differences in all grain and semolina quality traits were observed among genotypes except for grain yield and semolina ash (data not shown). However, there was no significant genotype × line interaction (within genotype variation) except for TKW, kernel diameter, and gluten index. Lastly, significant environmental variation was observed only for kernel diameter.

DISCUSSION

A goal of this study was to determine whether the mutations responsible for the reduction of amylose content in durum also affected grain yield and kernel characteristics. Although the environment replication was limited, the field data indicated that differences among genotypic classes were small. This result agrees with data reported by Miura et al (2002) and Graybosch et al (2003) on hexaploid wheat. Also, other waxy durum lines derived from a different Ike/Ben cross have exhibited yield and test weight identical to Ben (not shown). Thus, we suggest that high quality, high yielding waxy durum wheat can be developed.

Data on amylose content variation indicated that, as expected, inclusion of wx null genes in both waxy loci of durum wheat reduced endosperm amylose content to trace values. According to the method used, waxy wheat starches have been reported to contain 0–6% amylose, waxy maize 0.6–2.9%, waxy barley 4–8.4%, and waxy rice 0–2.3% (Nakamura et al 1995; Hayakawa et al 1997; Demeke et al 1999). Our results (Table II) using the HPLC method are consistent with previously reported results. However,

when only one gene was mutated, no significant amylose content reduction could be detected. The dosage effect of waxy null alleles on amylose content has been extensively studied in hexaploid wheat and in all cases partial waxy 4A demonstrated significantly lower amylose content (Miura and Sugawara 1996; Miura et al 1999; Araki et al 2000; Yamamori and Quynh 2000; Miura et al 2002). In durum wheat, a recent study also reported a stronger contribution of 4A gene than 7A gene on amylose content (Sharma et al 2002). Amylose content absolute values depend on the method used for their determination. For instance, the wheat line K107 (mutated in the 4A and 7A waxy loci) was reported to contain 16% (Miura and Tanii 1994) and 24% (Hayakawa et al 1997) amylose according to the method of determination. Using HPLC, our results indicate that a single pair of null alleles did not reduce GBSS activity to an extent whereby a concomitant reduction in amylose content was detected. There are three possible explanations for this result. 1) The functional Wx-7A gene compensated for the mutation at the other locus by producing more enzyme. 2) There is a plateau in amylose synthesis already reached with GBSS 7A activity. 3) The standard deviation of the HPLC method was too large to detect small differences. In diploid wheat (*Triticum monococcum* L), the number of waxy genes present in the endosperm tissue was proportional to GBSS activity but not to the amylose content and amount of waxy protein (Fujita et al 2001).

A negative relationship is often reported between grain yield and protein concentration. Thus, it was plausible that modifying the starch synthetic pathway in waxy wheat was going to be accompanied by an altered pattern of storage protein accumulation, revealed by a change in protein content or quality. Our study indicated no effect of waxy mutation on protein concentration in whole grain (Table IV). Sharma et al (2002) found the same result when comparing wx-4A and WT lines of durum wheat. However, in hexaploid wheat, the effect of waxy mutation on protein concentration was different, as reported by Ross et al (1996), who found lower protein concentration in wx-4A lines compared with WT lines. Our data show that protein quality as measured by SDS micro-sedimentation and gluten index was affected by the waxy mutation to some extent. The effect was small and all lines could be considered strong protein lines. These results indicate that the waxy mutation and strong grain protein are not mutually exclusive.

Higher ash content in wx kernels was found in our study (Table I) and in the recent work of Grant et al (2004) using independently bred waxy durum lines. Abdel-Aal et al (2002) found lower starch ash content in waxy wheat lines, although they did not report on flour or kernel ash content. The reason for the increased ash in waxy mutants is not known.

Recently, results indicate the inadequacy of falling number test to measure α-amylase activity of waxy hexaploid wheat (Graybosch et al 2000; Abdel-Aal et al 2002). Our results on durum agree

TABLE V
Semolina Protein Content and Quality of Waxy and Wild Type Genotypes Combined Across Locations

Genotype ^a	Protein (%)	Gluten Index	Wet Gluten (%)
wx	12.1bc ^b	70b	27.9a
wx-4A	11.9bc	69b	33.1bc
wx-7A	11.4a	76a	31.1b
WT	11.3a	78a	30.9b
Belzer	12.5bc	62bc	34.2bc
Ben	12.8c	58c	36.8c
Lebsock	12.3bc	47c	36.8c
Maier	12.5bc	57c	37.6c
Mountrail	12.7bc	23d	34.5bc
Plaza	11.6ab	52c	32.5bc
Rugby	12.4bc	2e	30.4ab

^a WT, wild type (*Wx-A1Wx-A1 Wx-B1Wx-B1*); wx-7A, partial waxy mutated at the *Wx-A1* locus (*wx-A1wx-A1 Wx-B1Wx-B1*); wx-4A, partial waxy mutated at the *Wx-B1* locus (*Wx-A1Wx-A1 wx-B1wx-B1*); wx, full waxy (*wx-A1wx-A1 wx-B1wx-B1*).

^b Values with the same letter in the same column do not differ significantly at $P < 0.05$ (means separation, LSD). Means separation was performed using different LSD for each comparison due to different number of observation.

with the results on hexaploid wheat. Falling number values for waxy lines are consistently much lower than for normal wheat, independent of α -amylase activity. This observation can be explained by the unique rheologic properties of waxy starch. The principle of this method is that enzyme activity can be indirectly measured by the rheological properties of heated starch suspension. By the time falling number is recorded, the solution temperature is approaching the boiling point and waxy starch viscosity is lowest at this temperature, not because of enzyme activity but because of the inherent nature of this particular starch. A recent study of Grant et al (2004) indicated that stirring number test is effective in evaluating sprout damage in waxy durum.

Results on α -amylase activity of waxy crops are conflicting. We found higher activity in waxy durum semolina and recently, Grant et al (2001) also reported higher activity for waxy durum lines coming from a different breeding program. However, Yasui et al (1999) reported significantly lower α -amylase activity for two waxy hexaploid wheat flours compared with the parent. The relationship between the waxy trait and α -amylase activity remains to be understood.

Our milling results indicated that the separation of the endosperm and bran was less efficient for wx wheat. Kernel hardness was tested as a factor for the lower semolina yield but no differences were found between wx and WT kernels (Table I). Using the NIR spectroscopic method on ground grain, Grant et al (2004) found that waxy kernels were significantly softer than the parent, Ben. Indeed, wx durum kernels lack the vitreous character and apparently have a spectroscopic resemblance to softer wheats. Our results, which measured physical hardness, indicated that hardness was not responsible for the lower semolina yield in wx mutant lines. Yasui et al (1999) also reported lower flour yield in waxy bread wheat. They also found hardness did not differ among lines and suggested that lower starch, higher fat, and β -glucan content was responsible for lower flour yield. In our study, these traits were not measured but are likely factors in the lower semolina and total extraction yield obtained for wx lines. Grant et al (2004) also found higher fat content in durum waxy wheat. The influence of amylose on the granule architecture was demonstrated by use of enzyme gold labeling (Atkin et al 1999). In waxy starch, a framework of closely packed concentric layers of amylopectin exists in the granules and is likely to affect the behavior of the endosperm when crushed in the mill. Our results suggest that amylose content was a factor in milling yield. We have shown that using the traditional mill setting, WT durum wheat had better semolina extraction, as well as total extraction than wx durum. Semolina extraction from wx durum may be improved by changing the mill setting or adjusting the tempering protocol.

Starch damage results on wx durum wheat were consistent with previous reports on hexaploid (Bettge et al 2000; Abdel-Aal et al 2002) and durum wheat (Grant et al 2001). According to Meuser et al (1978), amylose is much less affected by mechanical impact than amylopectin. The existence of amylose in the starch reduces the proportion of starch molecules that could be damaged during milling. Also, the presence of amylose in the starch may help granules to retain integrity during milling stress. Even though the 4A partial waxy starch tends to have higher starch damage, it was not significantly different from the WT starch. This agrees with the recent work of Sharma et al (2002) on null-4A allele at the waxy locus in durum wheat. It is important to note that, in using the enzymatic method to determine starch damage, we may have encountered an aberration due to the particular nature of waxy starch. Waxy starch may be more susceptible to α -amylase digestion. However, because previous studies in common wheat using different starch damage determination methods showed that waxy starch is more susceptible to mechanical damage (Bettge et al 2000), we suggest that waxy durum starches are also more susceptible to the mechanical stress undergone during milling. The name waxy was originally given to waxy corn because of the

appearance of its endosperm. Wx durum wheat kernels possess that same unique appearance that can be defined neither as vitreous nor as floury. Thus, a concern was the consequence of this trait on semolina and pasta color. It was possible to distinguish wx semolina from all the other genotypic types with a naked eye. When semolina color was analyzed using the L^* , a^* , b^* scale, the differences were small but significant. Compared with other waxy isolines, full waxy semolina appeared to be redder and less yellow. However, values remained in the range of the values satisfactory for pasta manufacturing (as indicated by the cultivar results). Thus, it appears that the unique dull appearance of wx semolina observed with a naked eye was not entirely detected with color measurement. Even though wx kernels were not softer than normal kernels, it is possible that differences in particle size distribution among waxy isolines semolina existed and contributed to color differences.

To evaluate the effect of waxy null alleles only (as described by Graybosch et al [2003]), near-isogenic lines for waxy loci were developed in a common genetic background. Except for kernel size and gluten index parameters, this common background was confirmed by the nonsignificant genotype \times line (within genotype) interaction. The significant interaction found in kernel size and gluten index parameters is an indicator that developed lines were not fully isogenic and some genetic material from the donor parent Ike was dragged with the mutated waxy genes (linkage drag).

Despite the limited number of locations used in our experiment, the environment \times genotype interaction provided some insight as to the stability of waxy gene expression across environments. Except for kernel diameter, the growing location of the lines had no effect on the quality attributes of waxy durum wheat. This result agrees with the recent report by Graybosch et al (2003) that showed stability of quality attributes of waxy hexaploid wheat across environment.

CONCLUSIONS

The full and partial waxy durum wheat lines were evaluated for agronomic performances in a one-year field study over two locations in North Dakota. Waxy alleles affected amylose content, kernel ash percentage, α -amylase activity, and the falling number test. Falling number, amylose content, and ash content results are similar to previous results with other waxy crops. Protein concentration and strength did not appear to be affected by the waxy null alleles. This suggests that waxy and partial waxy durum lines with high protein quality can be developed.

Partial waxy lines did not exhibit lower amylose content. All the measurements grouped partial waxy lines with wild type lines. A single mutation in partial waxy lines did not modify durum wheat strongly enough to be detected with the tests reported here.

Endosperm/bran separation was less efficient for full waxy samples, resulting in lower semolina extraction and total extraction. This difference could not be attributed to hardness differences but may be due to altered starch granule structure. Semolina purity (as measured by ash content) was similar for each genotype but color appeared duller with a naked eye. As previously described, full waxy starch was more susceptible to mechanical damage than partial or wild type starch, as determined by starch damage. Protein concentration and quality was not affected by the waxy allelic state. Our agronomic and milling evaluation of waxy durum wheat provided an encouraging basis to researchers for finding beneficial applications that will bring new markets to the durum wheat growers.

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LITERATURE CITED

- Abdel-Aal, E.-S., Hucl, P., Chibbar, R. N., Han, H. L., and Demeke, T. 2002. Physicochemical and structural characteristics of flours and starches from waxy and nonwaxy wheats. *Cereal Chem.* 79:458-464.
- American Association of Cereal Chemists. 2000. Approved Methods of the AACC, 10th Ed. Methods 26-10A, 26-41, 76-31, 44-15A, 08-01, 44-11, 56-81B, 56-61, 38-12, 84-10, and 46-30. The Association: St. Paul, MN.
- Ainsworth, C. C., Clark, J., and Balsdon, J. 1993. Expression, organization and structure of the genes encoding the waxy protein (granule-bound starch synthase) in wheat. *Plant Mol. Biol.* 22:67-82.
- Araki, E., Miura, H., and Sawada, S. 2000. Differential effects of the null alleles at the three Wx loci on the starch properties of wheat. *Theor. Appl. Gen.* 100:1113-1120.
- Atkin, N. J., Cheng, S. L., Abeyssekera, R. M., and Robards, A. W. 1999. Localization of amylose and amylopectin in starch granules using enzyme-gold labeling. *Starch* 51:163-172.
- Bettge, A. D., Giroux, M. J., and Morris, C. F. 2000. Susceptibility of waxy starch granules to mechanical damage. *Cereal Chem.* 77:750-753.
- Chao, S., Sharp, P. J., Warland, A. J., Warham, E. J., and Koebner, R. M. D. 1989. RFLP-based genetic maps of wheat homoeologous group 7 chromosomes. *Theor. Appl. Gen.* 78:495-504.
- Demeke, T., Hucl, P., Abdel-Aal, E. S. M., Baga, M., and Chibbar, R. N. 1999. Biochemical characterization of the wheat waxy A protein and its effect on starch properties. *Cereal Chem.* 76:69-77.
- Dick, J. W., and Quick, J. S. 1983. A modified screening test for rapid estimation of gluten strength in early-generation of durum wheat breeding lines. *Cereal Chem.* 60:315-317.
- Elias, E. M., and Miller, J. D. 1998. Registration of 'Ben' durum. *Crop. Sci.* 38:895.
- Fujita, N., Hasegawa, H., and Taira, T. 2001. The isolation and characterization of a waxy mutant of diploid wheat (*Triticum monococcum* L.). *Plant Sci.* 160:595-602.
- Galliard, T., and Bowler, P. 1987. Morphology and composition of starch. Pages 55-78 in: *Starch: Properties and Potential*. T. Galliard, ed. John Wiley & Sons: Chichester, UK.
- Gibson, T. S., Qalla, A. J., and McCleary, B. V. 1992. An improved enzymatic method for the measurement of starch damage in wheat flour. *J. Cereal Sci.* 10:15-27.
- Grant, L. A., Vignaux, N., Doehlert, D. C., McMullen, M. S., Elias, E. M., and Kianian, S. 2001. Starch characteristics of waxy and nonwaxy tetraploid (*Triticum turgidum* L. var. *durum*) wheats. *Cereal Chem.* 78:590-595.
- Grant, L. A., Ostenson, A. M., and Rayas-Duarte, P. 2002. Determination of amylose and amylopectin of wheat starch using high performance size exclusion chromatography (HPSEC). *Cereal Chem.* 79:771-773.
- Grant, L. A., Doehlert, D. C., McMullen, M. S., and Vignaux, N. 2004. Spaghetti cooking quality of waxy and non-waxy durum wheats and blends. *J. Sci. Food Agric.* 84:190-196.
- Graybosch, R. A., Guo, G., and Shelton, D. R. 2000. Aberrant falling numbers of waxy wheats independent of α -amylase activity. *Cereal Chem.* 77:1-3.
- Graybosch, R. A., Souza, E., Berzonsky, W., Baenziger, P. S., and Chung, O. 2003. Functional properties of waxy wheat flours: Genotypic and environmental effects. *J. Cereal Sci.* 38:69-76.
- Hayakawa, K., Tanaka, K., Nakamura, T., Endo, S., and Hoshino, T. 1997. Quality characteristics of waxy hexaploid wheat (*Triticum aestivum* L.): Properties of starch gelatinization and retrogradation. *Cereal Chem.* 74:576-580.
- Hegstad, J. 2001. Development of Waxy Durum. M.S. thesis. North Dakota State University: Fargo, ND.
- Hegstad, J. B., Kianian, S. F., McMullen, M. S., and Doehlert, D. C. 1998. Development of waxy (low amylose) durum cultivars. in Proc. 9th Int. Wheat Genetics Symp. A. E. Slinkard, ed. University Extension Press: University of Saskatchewan, SK, Canada.
- Hovenkamp-Hermelink, J. H. M., Jacobsen, E., Ponstein, A. S., Visser, R. G. F., Vos-Scheperkeuter, G. H., Bijmolt, E. W., De Vries, J. N., Witholt, B., and Feenstra, W. J. 1987. Isolation of an amylose-free mutant of the potato (*Solanum tuberosum* L.) *Theor. Appl. Genet.* 75:217-221.
- Hsieh, J. S. 1988. Genetic studies in the Wx gene of sorghum (*Sorghum bicolor* L. Moench). 1. Examination of the protein product of the waxy locus. *Bot. Bull. Acad. Sinica.* 29:293-299.
- Lineback, D. R., and Rasper, V. F. 1988. Wheat carbohydrates. Pages 277-332 in: *Wheat Chemistry and Technology*, Vol. 1. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St Paul, MN.
- MacDonald, F. D., and Preiss J. 1985. Partial purification and characterization of granule-bound starch synthases from normal and waxy maize. *Plant Physiol.* 78:849-852.
- Martin, C. R., Rousser, R., and Brabec, D. L. 1993. Development of a single kernel wheat characterization system. *Trans. ASAE* 36:1399-1404.
- Martin, T. J., Harvey, T. L., Seifers, D. F., Cox, T. S., Sears, R. G., Bequette, R. K., Currar, S. P., Hatchett, J. H., Chung, O. K., and Witt, M. D. 1994. Registration of 'Ike' wheat. *Crop Sci.* 34:285.
- McCleary, B. V., and Sheehan, H. 1987. Measurement of cereal α -amylase: A new assay procedure. *Cereal Chem.* 6:237-251.
- Meuser, F., Klinger, R. W., and Niedeck, E. A. 1978. Characterization of mechanically modified starch. *Starch* 30:376-384.
- Miura, H., and Sugawara, A. 1996. Dosage effects of the three Waxy genes on amylose synthesis in wheat endosperm. *Theor. Appl. Genet.* 93:1066-1070.
- Miura, H., and Tanii, S. 1994. Endosperm starch properties in several wheat cultivars preferred for Japanese noodles. *Euphytica* 72:171-175.
- Miura, H., Araki, E., and Tanii, S. 1999. Amylose synthesis capacity of the three Wx genes of wheat cv. Chinese Spring. *Euphytica* 108:91-95.
- Miura, H., Wickramasinghe, M. H. A., Subasinghe, R. M., Araki, E., and Komae, K. 2002. Development of near-isogenic lines of wheat carrying different null Wx alleles and their starch properties. *Euphytica* 123:353-359.
- Murata, T., Sugiyama, T., and Akazawa, T. 1965. Enzyme mechanism of starch synthesis in glutinous rice grains. *Biochem. Biophys. Res. Commun.* 18:371-376.
- Nakamura, T., Yamamori, M., Hirano, H., and Hidaka, S. 1993. Identification of three Wx proteins in wheat (*Triticum aestivum* L.) *Biochem. Genet.* 31:75-86.
- Nakamura, T., Yamamori, M., Hirano, H., Hidaka, S., and Nagamine, T. 1995. Production of waxy (amylose-free) wheats. *Mol. Gen. Genet.* 248:253-259.
- Nelson, O. E., and Rines, H. W. 1962. The enzymatic deficiency in the waxy mutant of maize. *Biochem. Biophys. Res. Commun.* 9:297-300.
- Ross, A. S., Quail, K. J., and Crosbie, G. B. 1996. An insight into structural features leading to desirable alkaline noodle texture. Pages 115-119 in: *Proc. 46th Australian Cereal Chemistry Conference*. Sydney, Australia.
- Sano, Y. 1984. Differential regulation of waxy gene expression in rice endosperm. *Theor. Appl. Gen.* 68:467-473.
- Shannon, J. C., and Garwood, D. L. 1984. Genetics and physiology of starch development. Pages 26-86 in: *Starch: Chemistry and Technology*, 2nd Ed. R. L. Whistler, J. N. BeMiller, and E. F. Paschall, eds. Academic Press: New York.
- Sharma, R., Sisson, M. J., Rathjen, A. J., and Jenner, C. F. 2002. The null-4A allele at the waxy locus in durum wheat affects pasta cooking quality. *J. Cereal Sci.* 35:387-297.
- Shure, M., Wessler, S., and Federoff, N. 1983. Molecular identification and isolation of the waxy locus in maize. *Cell* 35:225-233.
- Tsai, C. Y. 1974. The function of the waxy locus in starch synthesis in maize endosperm. *Biochem. Genet.* 11:83-96.
- Yamamori, M., Nakamura, T., Endo, T. R., and Nagamine, T. 1994. Waxy protein deficiency and chromosomal location of coding genes in common wheat. *Theor. Appl. Genet.* 89:179-184.
- Yamamori, M., and Quynh, N. T. 2000. Differential effects of Wx-A1, -B1, and -D1 protein deficiencies on apparent amylose content and starch pasting properties in common wheat. *Theor. Appl. Genet.* 100:32-38.
- Yasui, T., Sasaki, T., and Matsuki, J. 1999. Milling and flour pasting properties of waxy endosperm mutant lines of bread wheat (*Triticum aestivum* L.). *J. Sci. Food Agric.* 79:687-692.
- Zhao, X. C., and Sharp, P. J. 1996. An improved 1-D SDS-PAGE method for the identification of three bread wheat 'waxy' proteins. *J. Cereal Sci.* 23:191-193.

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