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M. K. Yerka
USDA–ARS, melinda.yerka@ars.usda.gov

J. J. Toy
USDA-ARS, John.Toy@ars.usda.gov

Deanna L. Funnell-Harris
USDA-ARS, Deanna.Funnell-Harris@ars.usda.gov

Scott E. Sattler
USDA-ARS, Scott.Sattler@ars.usda.gov

J. F. Pedersen
USDA–ARS, jpedersen1@unl.edu

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GENETIC STOCK

Registration of N619 to N640 Grain Sorghum Lines with Waxy or Wild-Type Endosperm

M. K. Yerka,* J. J. Toy, D. L. Funnell-Harris, S. E. Sattler, and J. F. Pedersen

ABSTRACT

Sorghum [*Sorghum bicolor* (L.) Moench] lines N619 to N636 (A lines; Reg. No. GS-699 to GS-716, PI 670134 to PI 670151); N619 to N636 (B lines; Reg. No. GS-721 to GS-738, PI 671777 to PI 671794); and N637 to N640 (R lines; Reg. No. GS-717 to GS-720, PI 670152 to PI 670155) comprise nine pairs of seed parent (A/B) lines, and two pairs of pollinator (R) lines (11 pairs total) that are near-isogenic for waxy (low-amylose) or wild-type endosperm. Breeding work was conducted jointly by the USDA–ARS and the Agricultural Research Division, Institute of Agriculture and Natural Resources, University of Nebraska, and the lines were released in May 2014. Release of these lines makes available two different *waxy* (*wx*) alleles (wx^a and wx^b) for development of grain sorghum as a source of low-amylose starch, whose end use is targeted to the ethanol and food industries. In particular, the release of *wx* and wild-type near-isogenic pairs facilitates the evaluation of agronomic performance of *wx* genotypes, and the release of both A/B and R lines facilitates the production of waxy grain hybrids.

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5585 Guilford Rd., Madison, WI 53711 USA
*Corresponding author (melinda.yerka@ars.usda.gov)

Amylose content ranges from 20 to 30% of the starch within wild-type sorghum [*Sorghum bicolor* (L.) Moench] grain (Rooney and Serna-Saldivar, 2000). The *Waxy* (*Wx*) gene encodes granule-bound starch synthase (GBSS), which synthesizes the long chain starch amylose (Denyer et al., 2001). Loss-of-function mutations in *Wx* result in the endosperm having a waxy appearance and starch almost entirely composed of amylopectin (Denyer et al., 2001). The waxy phenotype has been recognized in sorghum since 1933 (Karper, 1933). Low-amylose starch is preferable to wild-type starch in the ethanol industry because amylose requires higher temperatures to paste than amylopectin; it increases viscosity and forms complexes with lipids and with itself, restricting access of hydrolytic enzymes to starch molecules and lengthening fermentation times (Sharma et al., 2007; Wang et al., 2008; Wang and Copeland, 2013). Low-amylose starch is further utilized in the food industry to improve the shelf life of breads, cakes, and pastes due to its stickiness and slower retrogradation (Wang and Copeland, 2013). Predominant crop sources of low-amylose starch include waxy corn (*Zea mays* L.), wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), and potato (*Solanum tuberosum* L.) varieties. Sorghum requires less water than these crops except for wheat, so the opportunity exists to increase the supply of low-amylose starch to the ethanol and food industries through deployment of improved waxy varieties in drier regions of the world.

In sorghum, two *waxy* (*wx*) mutations were identified in the *Wx* gene and characterized for the presence or absence of GBSS protein (Pedersen et al., 2005; Sattler et al., 2009). The wx^a allele contains a large DNA insertion within the third exon of the gene, and both the GBSS protein and enzyme activity were undetectable in wx^a grain (Pedersen et al., 2005; Sattler et al., 2009). The wx^b allele contains a missense mutation that changes amino acid 268 from glutamine to histidine; although GBSS protein was detectable, GBSS enzyme activity was reduced

USDA–ARS, Grain, Forage, and Bioenergy Research Unit, 137 Keim Hall, East Campus, Univ. of Nebraska-Lincoln, Lincoln, NE 68583-0937. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.

Abbreviations: GBSS, granule-bound starch synthase.

by over 75% relative to wild-type in the grain (Pedersen et al., 2005; Sattler et al., 2009). While the effect of *wx*^{a} relative to *wx*^{b} on amylose content is unknown, the starch from both genotypes was identified as waxy following gelatinization and iodine staining, having a reddish-purple color compared with the deep blue color of starch containing amylose (Pedersen et al., 2004).

Interest in grain sorghum has increased since the USEPA announced in 2012 that ethanol produced from sorghum grain qualifies as an advanced biofuel (USEPA, 2012). However, *wx* genotypes often have reduced grain yield relative to their near-isogenic wild-type counterparts (Rooney et al., 2005). The objective of this study was to develop near-isogenic pairs of waxy and wild-type lines to facilitate plant breeding and research on the agronomic performance of waxy varieties, as well as their end uses in the ethanol and food industries. Each *wx* allele (*wx*^{a} and *wx*^{b}) was evaluated in multiple pedigrees and compared with their near-isogenic wild-type counterparts. Plant materials developed in this study with A and R fertility reactions facilitate future work evaluating waxy hybrids, including interallelic (*wx*^{a} × *wx*^{b}) hybrids.

Methods

Sorghum near-isogenic lines N619 to N636 (A lines; Reg. No. GS-699 to GS-716, PI 670134 to PI 670151); N619 to N636 (B lines; Reg. No. GS-721 to GS-738, PI 671777 to PI 671794); and N637 to N640 (R lines; Reg. No. GS-717 to GS-720, PI 670152 to PI 670155) were developed by crossing various lines with genetic male sterility (*ms3ms3*), previously developed by the USDA–ARS in Lincoln (Pedersen et al., 1997), with waxy lines developed at Texas A&M University (Table 1). F_1 plants from these original crosses were selfed to the F_2 generation, then backcrossed with one of the parents to generate the BC_1F_1 and then the BC_1F_2, hereafter referred to as F_1 and F_2, respectively. Grain from individual F_2 plants was subjected to iodine staining (Pedersen et al., 2004). Individuals segregating for *wx* were selfed to generate the F_3. A homozygous waxy and a homozygous wild-type plant were chosen among F_3 individuals as counterparts to form a pair. These were selfed to the F_7 generation for testing field agronomic performance. Nine pairs of B lines and two pairs of R lines were developed using these methods from eleven original crosses. When B lines were in the F_5 generation, A lines were developed by making crosses to the A_1 cytoplasm source ACK60, followed by backcrossing four times, to facilitate hybrid production. The result was nine pairs of near-isogenic A/B lines, one A/B line waxy and its counterpart A/B line wild-type, from each of the nine original B line crosses.

The F_7 B and R lines were tested for field agronomic performance at the University of Nebraska Field Laboratory at Lincoln, NE, and at the University of Nebraska Agricultural Research and Development Center near Mead, NE, in 2009. The wild-type cultivars B 'Wheatland' (Brown et al., 1936) and R 'Tx430' (Miller, 1984), common hybrid parents, were included for comparison. Each plot consisted of two 7.6-m rows spaced 76 cm apart with 120 seeds per row delivered by a precision vacuum planter in the third week of May. For both locations, nitrogen fertilizer was applied at 112 kg ha^{-1} before planting. Atrazine [6-chloro-*n*-ethyl-*N*′(1-methylethyl)-1,3,5-triazine-2,4,diamine] was applied at 1.12 kg ha^{-1} immediately after planting, followed by an application of quinclorac (3,7-dichloro-8-quinolinecarboxylic acid) and atrazine at 0.37 and 0.56 kg ha^{-1}, respectively, 5 wk after planting at Lincoln and 4 wk after planting at Mead. Supplemental irrigation (3.8 cm) was applied at Mead via overhead sprinklers on 6

Table 1. Pedigree, fertility reaction, endosperm type, and *waxy* source of the sorghum near-isogenic lines.

Entry	Pair	Pedigree†	Fertility reaction‡	Endosperm	*waxy* source
N619	1	F_8 [(F_2 BN226*ms3ms3* × B9307)/B9307 BC_1]	A/B	waxy	*wx*^{b} B9307
N620	1	F_8 [(F_2 BN226*ms3ms3* × B9307)/B9307 BC_1]	A/B	wild-type	
N621	2	F_8 [(F_2 BN230*ms3ms3* × B94C274)/B94C274 BC_1]	A/B	waxy	*wx*^{a} B94C274
N622	2	F_8 [(F_2 BN230*ms3ms3* × B94C274)/B94C274 BC_1]	A/B	wild-type	
N623	3	F_8 [(F_2 BN230*ms3ms3* × B94C274)/BTx631 BC_1]	A/B	waxy	*wx*^{a} B94C274
N624	3	F_8 [(F_2 BN230*ms3ms3* × B94C274)/BTx631 BC_1]	A/B	wild-type	
N625	4	F_8 [(F_2 BN241*ms3ms3* × B9307)/B9307 BC_1]	A/B	waxy	*wx*^{b} B9307
N626	4	F_8 [(F_2 BN241*ms3ms3* × B9307)/B9307 BC_1]	A/B	wild-type	
N627	5	F_8 [(F_2 BN224*ms3ms3* × BTxARG-1)/BTxARG-1 BC_1]	A/B	waxy	*wx*^{b} BTxARG-1
N628	5	F_8 [(F_2 BN224*ms3ms3* × BTxARG-1)/BTxARG-1 BC_1]	A/B	wild-type	
N629	6	F_8 [(F_2 BN224*ms3ms3* × B94C274)/B94C274 BC_1]	A/B	waxy	*wx*^{a} B94C274
N630	6	F_8 [(F_2 BN224*ms3ms3* × B94C274)/B94C274 BC_1]	A/B	wild-type	
N631	7	F_8 [(F_2 BN227*ms3ms3* × BTxARG-1)/BTxARG-1 BC_1]	A/B	waxy	*wx*^{b} BTxARG-1
N632	7	F_8 [(F_2 BN227*ms3ms3* × BTxARG-1)/BTxARG-1 BC_1]	A/B	wild-type	
N633	8	F_8 [(F_2 BN231*ms3ms3* × BTxARG-1)/BTxARG-1 BC_1]	A/B	waxy	*wx*^{b} BTxARG-1
N634	8	F_8 [(F_2 BN231*ms3ms3* × BTxARG-1)/BTxARG-1 BC_1]	A/B	wild-type	
N635	9	F_8 [(F_2 BN232*ms3ms3* × B9307)/B9307 BC_1]	A/B	waxy	*wx*^{b} B9307
N636	9	F_8 [(F_2 BN232*ms3ms3* × B9307)/B9307 BC_1]	A/B	wild-type	
N637	10	F_8 [(F_2 RN228*ms3ms3* × R94C289)/RSC110-9 BC_1]	R	waxy	*wx*^{a} R94C289
N638	10	F_8 [(F_2 RN228*ms3ms3* × R94C289)/RSC110-9 BC_1]	R	wild-type	
N639	11	F_8 [(F_2 RN229*ms3ms3* × R94C289)/R94C289 BC_1]	R	waxy	*wx*^{a} R94C289
N640	11	F_8 [(F_2 RN229*ms3ms3* × R94C289)/R94C289 BC_1]	R	wild-type	

† *ms3ms3* lines are from Pedersen et al. (1997).

‡ Fertility reaction to A_1 cytoplasmic male-sterile cytoplasm: A/B = male-sterile/maintainer pair, R = fertility restorer.

and 29 August. No supplemental irrigation was applied at Lincoln. Field emergence was recorded 4 wk after planting at Lincoln and 5 wk after planting at Mead. Days to 50% anthesis was recorded. Height was measured at maturity. Grain yield was adjusted to 145 g kg^{-1} water content before analysis. Test weight was recorded. The experimental design was a randomized complete block with four replicates at each location. Entry, endosperm type (waxy vs. wild-type), *Waxy* allele (*Wx, wx^a, wx^b*), and environment (field location) were treated as fixed effects and replicate was a random effect. Data were subjected to ANOVA in the PROC MIXED procedure of SAS software, version 9.3 (SAS Institute, 2012). Within-pair least-squares means of waxy and wild-type counterparts were separated with a two-sample *t* test. Two-sample *t* tests were also used to compare least-squares means from B line entries to BWheatland, and R line entries to RTx430. The homogeneity of variance assumption of ANOVA was tested in PROC GLM with a Levene's test. The normality assumption of ANOVA was tested in PROC UNIVARIATE with a Shapiro-Wilke test and analysis of Q-Q plots. All tests were performed at the $P = 0.05$ level of significance. Location × treatment interactions were insignificant for all statistical tests so that data were pooled across both environments.

Characteristics

Descriptive characteristics of waxy and wild-type near-isogenic line pairs of grain sorghum are given in Table 2. All line pairs have a white caryopsis, no tannin-containing testa layer, no awns, and juicy culms. The line pairs have tan plant color, except that N627 and N628 (Pair 5) and N637 and N638 (Pair 10) have purple plant color. The line pairs are combine-height and photoperiod-insensitive, with A/B and R fertility reactions to A_1 cytoplasmic male-sterile cytoplasm that make them suitable for hybrid production and evaluation.

Least-squares means for field emergence, days to 50% anthesis, height, grain yield, and test weight are presented in Table 3. No overall *waxy* effect was observed in field emergence, days to 50% anthesis, height, or test weight among entries. However, the mean grain yield of all wild-type near-isogenic lines was greater than that of all waxy lines, 7825 ± 104 compared with 7096 ± 114 kg ha^{-1} ($P < 0.001$), respectively. Grain yield of four waxy B lines (N627, N629, N631, and N633) did not differ statistically from that of their wild-type counterparts. Waxy B line yield was generally lower than that of BWheatland (7936 ± 322 kg ha^{-1}), with the exception of N621 and N633 (7809 and 7376 ± 322 kg ha^{-1}, respectively), which did not significantly differ from BWheatland. No yield drag was observed in the two R line pairs evaluated. Grain yield of waxy R line N639 (7782 ± 322 kg ha^{-1}) was not statistically different from that of its wild-type counterpart R line N640 and RTx430 (7735 and 7045 ± 322 kg ha^{-1}, respectively). R line N637 had the highest yield (8856 ± 322 kg ha^{-1}) of all waxy lines evaluated, exceeding that of its wild-type counterpart, N638 (7747 ± 322 kg ha^{-1}) and RTx430. Thus, B lines N627, N629, N631, and N633 and R lines N637 and N639 did not demonstrate the yield drag often associated with *waxy* genotypes.

An allele effect was observed for grain yield, which was highest for WT lines (7777 ± 104 kg ha^{-1}), intermediate for *wx^a* lines (7591 ± 152 kg ha^{-1}), and lowest for *wx^b* lines (6580 ± 155 kg ha^{-1}). Average *wx^a* yield did not statistically differ from that of WT, but both *wx^a* and WT line yields were greater than that of *wx^b* lines. Yield differences were not associated with differences in test weight, as no allele effect on test weight was observed. These results are similar to our previous research (Pedersen and Toy, unpublished data), which showed a grain

Table 2. Descriptive characteristics of waxy and wild-type near-isogenic line pairs of grain sorghum.

Entry	Pair	Endosperm	Plant color	Caryopsis color	Testa	Awns	Culms
N619	1	waxy	tan	white	no	no	juicy
N620	1	wild-type	tan	white	no	no	juicy
N621	2	waxy	tan	white	no	no	juicy
N622	2	wild-type	tan	white	no	no	juicy
N623	3	waxy	tan	white	no	no	juicy
N624	3	wild-type	tan	white	no	no	juicy
N625	4	waxy	tan	white	no	no	juicy
N626	4	wild-type	tan	white	no	no	juicy
N627	5	waxy	purple	white	no	no	juicy
N628	5	wild-type	purple	white	no	no	juicy
N629	6	waxy	tan	white	no	no	juicy
N630	6	wild-type	tan	white	no	no	juicy
N631	7	waxy	tan	white	no	no	juicy
N632	7	wild-type	tan	white	no	no	juicy
N633	8	waxy	tan	white	no	no	juicy
N634	8	wild-type	tan	white	no	no	juicy
N635	9	waxy	tan	white	no	no	juicy
N636	9	wild-type	tan	white	no	no	juicy
N637	10	waxy	purple	white	no	no	juicy
N638	10	wild-type	purple	white	no	no	juicy
N639	11	waxy	tan	white	no	no	juicy
N640	11	wild-type	tan	white	no	no	juicy

Table 3. Grain yield and agronomic performance of waxy and wild-type near-isogenic line pairs of grain sorghum. BWheatland and RTx430 were included for comparison.

Entry†	Pair	Endosperm	Emergence	Days to 50% anthesis	Height	Grain yield‡	Test weight
			plants m^{-1}		cm	kg ha^{-1}	kg hL^{-1}
BN619	1	waxy	12	**79§**	**121**	**5969**	73
BN620	1	wild-type	12	**81**	**113**	**7850**	71
BN621	2	waxy	16	82	**141**	**7809**	**67**
BN622	2	wild-type	15	82	**149**	**8888**	**71**
BN623	3	waxy	14	81	150	**7038**	73
BN624	3	wild-type	16	80	154	**8873**	74
BN625	4	waxy	14	83	127	**6796**	72
BN626	4	wild-type	17	84	125	**7720**	74
BN627	5	waxy	16	80	128	6138	73
BN628	5	wild-type	15	79	129	6976	71
BN629	6	waxy	16	81	152	7028	71
BN630	6	wild-type	15	80	149	7531	72
BN631	7	waxy	14	82	116	7003	**72**
BN632	7	wild-type	14	81	119	7589	**76**
BN633	8	waxy	16	81	130	7376	**72**
BN634	8	wild-type	14	80	131	8293	**76**
BN635	9	waxy	15	**81**	**129**	**6229**	73
BN636	9	wild-type	17	**79**	**135**	**7304**	74
RN637	10	waxy	14	83	**119**	**8856**	77
RN638	10	wild-type	13	83	**131**	**7747**	76
RN639	11	waxy	16	83	**129**	7782	76
RN640	11	wild-type	14	84	**121**	7735	76
BWheatland		wild-type	17	81	106	7936	71
RTx430		wild-type	14	83	121	7045	66
$LSD_{0.05}$			3	2	5	1236	3.4

† B = B line; R = R line.

‡ Grain yield was adjusted to 145 g kg^{-1} moisture before analysis.

§ Boldface type denotes means within pair that differ, based on two-sample *t* tests at $P = 0.05$. Data were pooled across two environments: Lincoln and Mead, NE (2009).

yield reduction of 2.7% in wx^a BTx630 and 8.1% in wx^b B9307 relative to the wild-type (Sattler et al., 2009). The exact cause of greater yield reductions in wx^b genotypes remains unclear. Further work testing the effects of wx^a and wx^b alleles in near-isogenic backgrounds, as well as the new alleles recently reported in Kawahigashi et al. (2013) and Lu et al. (2013), will be of interest to researchers seeking to identify the best combinations of alleles and pedigrees for improved agronomic performance and end use of waxy sorghum varieties for the ethanol and food industries.

Conclusions

Release of lines N619 to N640 makes available two different *waxy* alleles of sorghum, wx^a and wx^b, for comparison to *Wx* counterparts in near-isogenic backgrounds, and for breeding of additional adapted waxy sorghum breeding lines and hybrids. The release provides potential sources of low-amylose starch genetic stocks for breeding applications in drier regions of the world. *Wx*, wx^a, and wx^b alleles had no overall effect on field emergence, days to 50% anthesis, height, or test weight among entries. Grain yields of B lines N627, N629, N631, and N633 and R line N639 were similar to those of their near-isogenic wild-type counterparts. R line N637 had the highest grain yield among all waxy lines evaluated.

Availability

Seed of these sorghum genetic stocks will be maintained and distributed by the USDA–ARS, Grain, Forage, and Bioenergy Research Unit, 137 Keim Hall, East Campus, University of Nebraska, Lincoln, NE 68583-0937, and will be provided without cost to each applicant on written request. Genetic materials of this release have been deposited in the National Plant Germplasm System, where it will be immediately available for research purposes, including development and commercialization of new cultivars. It is requested that appropriate recognition be made if these lines contribute to the development of new breeding materials.

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