

NOTE

Evaluation of Canadian and Other Wheats for Waxy Proteins¹T. DEMEKE,^{2,3} P. HUCL,³ R. B. NAIR,² T. NAKAMURA,⁴ and R. N. CHIBBAR,^{2,5}

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Starch consists of amylose (a linear $\alpha(1-4)$ -D-glucan polymer) and amylopectin (a branched $\alpha(1-4$ and $1-6)$ -D-glucan polymer). Wheat endosperm starch normally contains ~21–30% amylose, the rest being amylopectin. On the other hand, waxy starch consists of >95% amylopectin. Amylose content has been reported to affect starch gelatinization, pasting, and gelation properties in wheat (Zeng et al 1997). Lower amylose content was associated with higher peak viscosity. Wheat grain with relatively high amylopectin (partially waxy) is preferred for the production of *udon* noodles (Yamamori et al 1992, Miura and Tanii 1994). In addition, waxy starch is used in thickeners, soup mixes, cookies, syrup, etc. in the food and beverage industries, and as an adhesive in the pulp and paper industry.

Granule-bound starch synthase (GBSS, E.C.2.4.1.21) is the key enzyme involved in the production of amylose (Preiss and Sivak 1996). In cereals, GBSS is encoded by the waxy locus (*wx*) and is termed waxy protein (Wx). The waxy loci in wheat are located on chromosomes 7AS, 4AL (a part of chromosome 7BS is translocated to chromosome 4), and 7DS (Chao et al 1989, Yamamori et al 1994). Mutations at all three loci result in fully waxy or amylose-free wheat (Nakamura et al 1995). The waxy protein (GBSS) has a M_r of 60 kDa as analyzed by SDS-PAGE. The GBSS A isoprotein has a slightly higher M_r than the GBSS B and D isoproteins. The GBSS A isoprotein can be separated from the GBSS B and D isoproteins by modification of the SDS-PAGE conditions (Nakamura et al 1993c). The GBSS B and D isoproteins can be separated from each other by a combination of isoelectric focusing and SDS-PAGE (two-dimensional gel electrophoresis [2D-PAGE]).

Mutations at the waxy loci have been reported in wheat genotypes from Japan, Australia, and China (Yamamori et al 1994), and recently in U.S. cultivars (Graybosch 1996, Zeng et al 1997). To date, a limited number of Canadian and U.S. spring wheat cultivars have been evaluated for the presence of *wx* mutations. The objective of the present study was to identify Canadian and U.S. wheat cultivars and wheat accessions from Japan and Australia with mutations at the waxy loci.

MATERIALS AND METHODS

Seed Sources

Australian and Japanese wheat accessions were obtained from the USDA germplasm collection at Aberdeen, ID. Seeds of Cana-

dian and U.S. cultivars were supplied by the Canadian Grain Commission, Winnipeg, MB. Spring wheat cultivars registered in Canada and used for the study were: AC Baltic, AC Barrie, AC Domain, AC Cora, AC Foremost, AC Michael, AC Mimi, AC Minto, AC Reed, AC Pollet, AC Taber, AC Voyageur, Algot, Aquino, Belvedere, Benito, Biggar, Bluesky, CDC Bavaria Spring, CDC Makwa, CDC Merlin, CDC Teal, Casavant, Celtic, Columbus, Consens, Conway, Cutler, Fielder, Genesis, Glenlea, HY 320, Invader, Katepwa, Kenyon, Laura, Laval 19, Leader, Manitou, Messier, Neepawa, Opal, Oslo, Park, Pasqua, Pitic 62, Prelude, Reward, Roblin, Wildcat, and Hard Red Calcutta (parental cultivar from India). The Canadian winter wheat cultivars used were: AC Readymade, CDC Kestrel, Harus, Norstar, Norwin, and Winalta. The U.S. spring wheat cultivars used were: Era, Grandin, Marshall, and McNeal; the winter wheat cultivars used were: Augusta and Yorkstar. The five durum wheat cultivars used were: Arcola, Kyle, Medora, Plenty, and Sceptre. The triticale cultivar used in this study was Frank.

Starch-Granule Isolation

Starch granules were isolated from two to three embryoless half-seeds of each wheat genotype according to the procedure

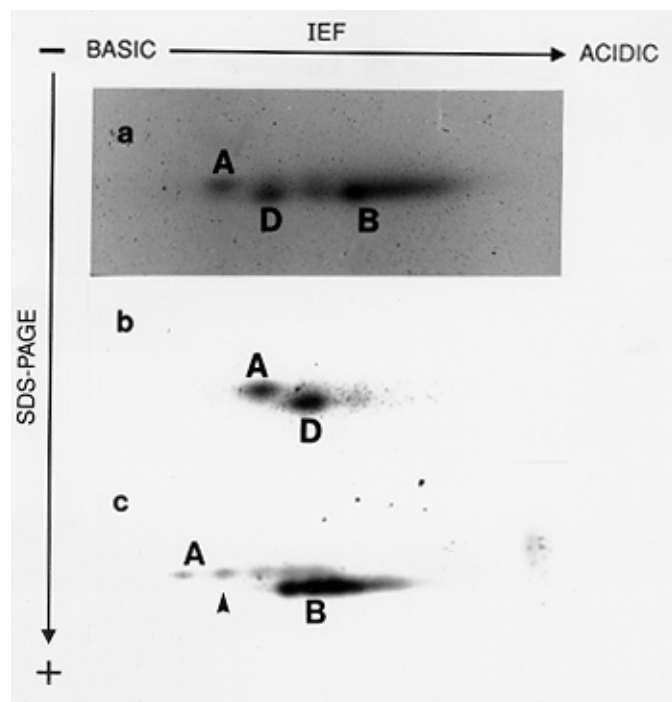


Fig. 1. Separation of A, B, and D granule-bound starch synthase isoproteins by two-dimensional PAGE for AC Reed (a), Cadoux (b), and AC Melita (durum) (c). A, B, and D show the positions of the three GBSS isoproteins.

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reported by Zhao and Sharp (1996). The half-seeds were crushed and soaked overnight in 1 mL of water at 4°C. The supernatant was removed by centrifugation, and the pellet was crushed in 300 µL of water. The slurry was overlaid on 1 mL of 80% cesium chloride and centrifuged at 15,000 × g for 5 min. The starch granule pellet was washed twice with 1 mL of wash buffer (55 mM tris-HCl, pH 6.8; 2.3% w/v, SDS; 5% v/v, β-mercaptoethanol; and 10% v/v, glycerol), and washed three times with water and once with acetone. Starch granules (3–5 mg) obtained from two half-seeds, were stored in a desiccator at –20°C. Proteins from the starch granules were extracted with 100 µL of denaturing electrophoresis buffer (62.5 mM tris-HCl, pH 6.8; 4% w/v, SDS; 5% v/v, β-mercaptoethanol; and 10% v/v, glycerol) boiled for 5 min, then cooled on ice. The cooled starch suspension was centrifuged, and the supernatant was analyzed by gel electrophoresis after addition of 0.005% (w/v) bromophenol blue.

One-Dimensional Gel Electrophoresis

Proteins isolated from starch granules were separated by SDS-PAGE (Nakamura et al 1993c) with an acrylamide-bisacrylamide concentration of 30:0.135%. The proteins were electrophoresed in a homogeneous polyacrylamide (10%) or gradient gel (5–12, 8–12, or 10–14%) using electrophoresis apparatus (Protean II, BioRad). For gradient gels, heavy acrylamide solution with 14% sucrose and light acrylamide solution with no sucrose were cast with a gradient mixer. Proteins were electrophoresed under a constant current of 9–11 mA per gel for 15–20 hr at 14 ± 1°C. Gels were silver-stained (Silver Stain Plus, BioRad) and photographed.

Two-Dimensional Gel Electrophoresis

The procedure was adapted from those of O'Farrel (1975) and Nakamura et al (1993a). Starch granules with the associated proteins were extracted from half-seeds by the procedure of Zhao and Sharp (1996) as described above. For isoelectric focusing, 10 mg of starch was dissolved in lysis buffer (8M urea; 2% v/v, Nonidet P-40 [NP-40]; 2% v/v, ampholine, pH 3.5–10; 5% v/v, β-mercaptoethanol; and 0.05–5% w/v, insoluble polyvinylpyrrolidone [PVPP]). The starch dissolved in lysis buffer was either heated at 40–60°C for 10 min or boiled for 2 min, cooled on ice, and centrifuged at 15,000 × g for 12 min. The supernatant (50–70 µL) was loaded on an isoelectric focusing gel tube (1.5 mm × 18 cm). The isoelectric focusing gel consisted of 3.5% (w/v) acrylamide-bisacrylamide, 2.5% (v/v) ampholines, pH 3.5–10 and pH 5–8, to give a range of pH 4.5–8; 8M urea; 2% (v/v) NP-40; 0.1% (v/v) TEMED; and 10% (w/v) ammonium persulphate. The proteins in isoelectric focusing gel were electrophoresed at 400 V for 15–17 hr and finally at 800 V for 1 hr. The temperature during electrophoresis was maintained at 14 ± 1°C. The acidic running buffer was 0.01M H₃PO₄, and the basic running buffer was 0.02M NaOH. The proteins separated by isoelectric focusing were then separated by SDS-PAGE as previously described.

RESULTS AND DISCUSSION

Electrophoretic Conditions

A 1.5-mm thick homogeneous polyacrylamide gel (10%) resolved the GBSS A isoprotein (with slightly higher *M_r*) from the GBSS B and D isoproteins. Polyacrylamide gradient gels of 5–12% and 8–12% showed better resolution than the 10% homogeneous gel or 10–14% gradient gel (*data not shown*).

In the 2D-PAGE procedure, the use of insoluble PVPP in the range of 0.05–5% (w/v) did not improve the resolution of the isoproteins. Nakamura et al (1993a) used 5% PVPP to separate the three wheat GBSS isoproteins by the 2D-PAGE procedure. Heating the starch samples dissolved in urea at 40–60°C for 10 min was as effective as boiling for 2 min. All three GBSS isoproteins were separated by 2D-PAGE analysis (Fig. 1). The higher *M_r* GBSS A isoprotein migrated more toward the cathode than both

the GBSS B and D isoproteins (Fig. 1a). The nonwaxy wheat cultivar AC Reed showed all three GBSS isoproteins. Fig. 1b shows a typical null B line from Australia with only GBSS A and D isoproteins. The GBSS D isoprotein was more basic than the GBSS B isoprotein. The durum wheat cultivars lacked the GBSS isoproteins encoded by the D genome (Fig. 1c, arrow).

Evaluation of Wheat Genotypes for Waxy Loci

Canadian and U.S. cultivars. Fifty-seven Canadian, six U.S., five durum wheat cultivars, and one triticale cultivar were screened by SDS-PAGE for the presence of GBSS isoproteins. The cultivars represented most of the currently registered spring wheats in Canada. The five durum wheat cultivars lacked the D isoprotein as expected (Fig. 1c, arrow). Of the 57 Canadian wheat cultivars, only Prelude and Reward had a reduced GBSS isoprotein encoded by the *wx* locus at the B genome. Three accessions of Reward (CN 1801, Citr 8182 TR, and PI351819 TR) showed a trace of the GBSS B isoprotein, as opposed to the dense and prominent GBSS B isoprotein band seen in other cultivars. Three accessions of Prelude (PGR 19523, Citr 7382 TR, and Citr 4323 TR93) also showed a reduced amount of GBSS B isoprotein, but the reduction was not as pronounced as in Reward. The cultivar Prelude is one of the parents of Reward. The six U.S. cultivars tested did not carry any null allele for GBSS. However, purification and separation of GBSS from more than 200 North American hexaploid wheat accessions has resulted in the identification of genotypes with *wx* mutations at A and B loci (Graybosch 1996). The lower amylose wheat cultivars, Penawawa and Klasic, have also been reported to have the *wx* mutation for the GBSS B isoprotein (Zeng et al 1997). In a related study, Penawawa and Klasic exhibited a high flour swelling volume (FSV) that was correlated with high paste peak viscosity (Morris et al 1997).

Japanese accessions. Of the 112 accessions screened, 10 carried the null allele for GBSS A isoprotein (Table I). Of the two acces-

TABLE I
List of Cultivars and Lines with Null Alleles
for Granule-Bound Starch Synthase

Inventory	Cultivar or Line	Source	Type of Mutation
PI235238	Norin 67	Japan	A and B
PI155261	Norin 5	Japan	A
PI155278	Sadabozu	Japan	A
Citr8428	Shirobunbu	Japan	A
PI182574	Shirosaya	Japan	A
PI182578	Tochigi Sekitori I	Japan	A
PI182592	Shonan	Japan	A
PI182594	Norin 1	Japan	A
PI197129	Shirasaya No. 1	Japan	A
PI197130	Sanshukomugi	Japan	A
...	Cadoux	Australia	B
PI511875	Rosella	Australia	B
PI268329	Gamenya	Australia	B
PI386165	Zenith	Australia	B
PI410845	Gabo	Australia	B
PI410846	Halberd	Australia	B
PI410874	Robin	Australia	B
PI428553	...	Australia	B
PI434643	Lance	Australia	B
PI434644	Warigal	Australia	B
PI483057	Gutha	Australia	B
PI483058	Takari	Australia	B
PI519327	...	Australia	B
PI519329	...	Australia	B
PI572695	Reeves	Australia	B
PI572700	Batavia	Australia	B
PI572702	Angas	Australia	B
...	Eradu	Australia	B
Citr 7382 TR ^a	Prelude	Canada	B
Citr 8182 TR ^b	Reward	Canada	B

^a The other two lines of prelude tested were PGR 19523 and Citr 4323TR 93.

^b The other two lines of Reward tested were CN 1801 and PI 351819 TR.

sions of Norin 67 evaluated, PI235238 had a strong vernalization requirement and was homogeneous for the GBSS isoprotein profile. 2D-PAGE confirmed the GBSS A and B isoprotein mutation in Norin 67 (PI235238). The seeds of PI235238 also had lower amylose content than the partially waxy Australian and nonwaxy Canadian cultivars (*data not shown*). PI155277, however, was a mixture of GBSS wild type and A isoprotein mutants and segregated for growth habit. Two spring growth habit sublines with GBSS A isoprotein mutations have been isolated from PI155277.

The line Kanto-107, of which Norin 67 is a parent (Nakamura et al 1993c), has been used in Japan to develop wheat with fully waxy endosperm starch (Nakamura et al 1995).

Australian accessions. Australian wheat genotypes are reported to have the null allele for the GBSS B isoprotein (Yamamori et al 1994; X. C. Zhao and P. J. Sharp, *unpublished data*). All the 126 genotypes tested by SDS-PAGE showed both the higher and lower molecular weight GBSS isoproteins. However, detailed analysis by 2D-PAGE showed the absence of GBSS B isoprotein in 18 wheat genotypes, thus inferring the mutation at the *wx* locus in the B genome (Table I). The GBSS B isoprotein was not detected by 2D-PAGE in lines such as Cadoux and Batavia, whereas, the other Australian lines showed reduced amount of subunits for the GBSS B isoprotein. The GBSS B isoprotein is more abundant than the GBSS A and D isoproteins (Nakamura et al 1993b). The most distinct isoform of the GBSS B isoprotein, referred to as WS4 by Nakamura et al (1993c), is present in very low amounts in the partially mutated lines. Yamamori et al (1994) and Zhao and Sharp (*unpublished data*) have classified the lines with the reduced acidic subunits of the B isoprotein as null alleles for the B genome. These lines with a GBSS B genome mutation at the *wx* locus have been used for the development of partially waxy wheat in Australia. For example, the cultivar Eradu, which has a reduced GBSS B isoprotein, is one of the Australian wheat cultivars used for making *udon* noodles.

CONCLUSIONS

We have identified wheat genotypes with mutations at the *wx* loci of GBSS for the A and B genomes of wheat. Ten percent of the Japanese lines had mutations at the *wx* locus of the A genome, whereas 14% of the Australian lines had the *wx* locus mutation in the B genome. Most of the Canadian and U.S. wheat cultivars lacked the null alleles for the waxy proteins. The wheat accessions with mutations at the *wx* loci are being used in a breeding program to develop partially waxy Canadian spring wheat.

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