

Characterization of Starch in *Aegilops* SpeciesF. L. Stoddard<sup>1-3</sup> and R. Sarker<sup>1</sup>

## ABSTRACT

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Starch was extracted and cleaned from 99 accessions of 20 species of *Aegilops* and also from 200 accessions of hexaploid wheat. Amylose content was determined by iodine staining and absorbance at 535 and 620 nm. Particle-size distribution was determined by laser scattering. The amylose content of the *Aegilops* accessions did not exceed the extremes found in domesticated wheat. *Aegilops* species, on the whole, had a lower content

of small particles than the hexaploid wheats. There was no correlation between amylose content and particle-size distribution. Some species of *Aegilops* may be useful sources of low-starch B-type granules for hexaploid wheat, if the trait can be transferred, but they are unlikely to contribute to further variation in amylose content.

Starch constitutes 60–70% of the wheat grain and 75–82% of white flour on a dry matter basis (Åman and Hesselman 1984, Davis 1994). It consists of two classes of  $\alpha$ -1,4-linked glucans, amylose and amylopectin. Amylose has few branches and long chains, while amylopectin has much greater branching and shorter individual chains (Morell et al 1995). Slightly reduced amylose content has been associated with improved eating quality for Japanese noodles (Oda et al 1980). Increased amylose content may be desirable for a number of end uses. It is more slowly digested by humans and other monogastric animals, leading to a more sustained release of glucose into the blood and a reduced release of insulin (Heijnen et al 1995). It can also be used as a raw material for production of biodegradable plastics (Nawrath et al 1995).

Starch granules in wheat fall into two major size classes: A-type with a mean diameter of  $\approx 20 \mu\text{m}$ , and B-type with a mean diameter of  $\approx 5 \mu\text{m}$ . B-type granules do not precipitate from suspension in some procedures for the industrial preparation of starch and gluten from flour, so they represent both a wasted resource and additional cost in terms of wastewater treatment. A broad range of B-type granules of  $\approx 17$ –62% was found in wheat and related species (Stoddard 1999). Lower values would be desirable for the starch-gluten industry.

The genus *Aegilops* contains many species that have been or could be used in improvement of cultivated wheats. For example, *Aegilops tauschii* is the progenitor of the D genome, and *A. speltoides* is the nearest relative to the progenitor of the B genome. *A. ventricosa* has contributed valuable disease-resistance genes to bread wheat, and numerous cultivars carry these genes (Bariana and McIntosh 1994). The American winter wheat Plainsman V is believed to have been derived from a cross with *A. geniculata* (Costa and Kronstad 1994), and the leaf rust resistance gene Lr9 was derived from *A. umbellulata* (Young and Smith 1981). For these reasons, the variation available in starch qualities in the genus *Aegilops* was assessed and compared with variation in domesticated hexaploid wheat.

## MATERIALS AND METHODS

## Source Materials

Ninety-nine accessions, representing 20 *Aegilops* species, were obtained from the Australian Winter Cereal Collection, Tamworth,

NSW. Two hundred hexaploid wheat accessions, representing *compactum*, *macha*, *spelta*, *sphaerococcum*, and *vavilovii* types; landraces from the fertile crescent, India, and China; and a range of historical Australian cultivars were obtained from the same source. The supplied samples were used for starch extraction.

Subsequently, 70 of the *Aegilops* accessions were grown in controlled-environment growth chambers with 18°C days, 13°C nights, and a 14-hr photo-thermo period. A soil-free potting mix (21 L of washed sand, 14 L of peat, 1 kg of dolomite, 400 g of lime, 300 g of fertilizer mix) was used to fill 15-cm pots, and one seed was planted per pot. Plants were watered daily and fed with half the recommended concentration of a commercial water-soluble fertilizer (N:P:K 23:4:18, Aquasol, Hortico Ltd., Laverton North, Vic., Australia) once per week.

Species nomenclature follows van Slageren (1994) and genome symbols follow Dvorak (1998).

## Starch Extraction

Starch extraction and cleaning were as described previously (Stoddard 1999). Samples of grain material (60–100 mg) were cracked into 2-mL Eppendorf tubes and soaked in 0.5 mL of 0.5M NaCl overnight. A plastic Eppendorf pestle attached to an electric drill press was used to grind the samples in the soaking medium until the gluten formed a ball and the bran was broken into large flakes. The slurry was decanted through a 200- $\mu\text{m}$  sieve into a fresh 2-mL microcentrifuge tube. The solids were ground again in a further 0.5 mL of 0.5M NaCl. The slurry was decanted into the same tube as before, and the solids were ground and the slurry was decanted for a third time. The starch slurry was centrifuged at  $5,500 \times g$  for 2 min, and the supernatant discarded. The starch was further washed by centrifugation through 2M NaCl, 2% SDS, and two changes of water. Three samples of each accession were resuspended in water and frozen at  $-20^\circ\text{C}$  for particle-size analysis. The remaining samples were centrifuged through absolute ethanol twice and dried over silica gel for two days before amylose analysis.

Samples from the material grown in the controlled-environment chambers were prepared as described above, except that 50% (w/w) CsCl was used instead of 2M NaCl, and the final step was a centrifugation through absolute ethanol, followed by drying over silica gel. This procedure left only clean white starch (Stoddard 1999) with a much lower content of large aggregate particles  $>100 \mu\text{m}$  and had no significant effect on B-type granule content.

## Particle-Size Analysis

Samples from the initial survey were evaluated with a laser-diffraction particle-size analyzer (Mastersizer 2600C, Malvern Instruments Ltd., Malvern, UK) connected to an 8086 desktop computer (Stoddard 1999). The flow-through module for particles in liquid

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was used with the 100-mm focal length lens. The sample was vigorously vortexed in the microfuge tube before being added to the distilled water in the recirculating system. Results were obtained as the volume percentage of material in each of the 32 size classes at 1.9–188  $\mu\text{m}$  diameter. These results were retyped into a spreadsheet for statistical analyses.

For samples from the material grown in the controlled-environment chambers, starch particle-size distribution was determined using a Malvern Mastersizer S laser-diffraction analyzer connected to a desktop computer. The stirred small-sample cell was used together with the 300-mm reverse Fourier lens, 2.4-mm beam length, and polydisperse function optimized for the refractive index of starch in water. This analyzer provided four times faster sample throughput than the older model, three times as many size classes, an extra digit of accuracy within each size class, and automated transfer of data to a useful spreadsheet format. When 616 samples were put through both analyzers, the correlation was 0.80 and the slope was 0.900 (Stoddard 2000).

Particles <9.8  $\mu\text{m}$  in diameter were considered B-type granules and particles 9.8–49.8  $\mu\text{m}$  were considered A-type granules (Blumenthal et al 1994, 1995; Stoddard 1999).

Selected grains were freeze-fractured across the middle of the endosperm, sputter-coated with 25 nm of gold, and examined with a Philips 505 scanning electron microscope at 10 kV.

### Amylose Determination

Amylose determination was based on the method of Mohammadkhani et al (1999). Three samples of each accession (5 mg) were weighed to the nearest 0.01 mg into preweighed 1.5-mL microfuge tubes. Samples were treated in batches of 20 with three reference standards: amylopectin (lot 38231, ICN Biomedicals Inc., Aurora, OH), amylose (lot 55172, ICN), and maize starch with 27% amylose content (courtesy of Starch Australasia Ltd., Lane Cove, NSW, Australia). For each 1 mg of starch, 15  $\mu\text{L}$  of 95% ethanol and 90  $\mu\text{L}$  of 1N NaOH were added. The tube was heated for 30 min in a block heater set to 105°C, cooled, and weighed. The solution was diluted with distilled water to give 1 mg of starch/100  $\mu\text{L}$ . Subsamples of 200  $\mu\text{L}$  were taken from each starch solution,

neutralized with 2.00 mL of 0.1N citric acid, stained with 800  $\mu\text{L}$  of iodine solution (0.2 g of  $\text{I}_2$  + 2 g of KI + 250 mL of distilled water) and diluted with 9.00 mL of water to give a final volume of 12 mL. The solutions were chilled in a refrigerator for 20 min and then two subsamples were read in a spectrophotometer at 535 and 620 nm. The apparent amylose content was calculated from both absorbance values according to the method of Haase (1993) and from the 620 nm absorbance value according to the method of Mohammadkhani et al (1999).

## RESULTS AND DISCUSSION

Use of two wavelengths instead of one for amylose determination reduced the standard errors by  $\approx 50\%$ . At 620 nm, the absorbance due to amylose has the greatest margin over that due to amylopectin, and at 535 nm the reverse holds true. Using both wavelengths, therefore, provides an internal correction for the dissolved starch content of the sample, and this method was used for all reported results.

The amylose content of the hexaploid wheats was 18–36%. Slightly lower minimum values were found in *Triticum monococcum* and rye, but the maximum value was higher than in either *A. tauschii* or *T. turgidum* (Mohammadkhani et al 1998). Of the five lowest values, two were from ssp. *macha* and three were landraces, two from India and one from Iran. The five highest values also included a *macha*, as well as a *spelta*, a *vavilovii*, a landrace from China, and an old Australian cultivar, Gallipoli.

Amylose contents of the *Aegilops* accessions were 20–35% (Table I). The highest values were found in accessions of *A. peregrina*, *bicornis*, *cylindrica*, and *sharonensis*, while the lowest were in *A. uniaristata*, *neglecta*, and *columnaris*. Because these values did not transcend those in hexaploid wheat or its nearest relatives, it appears that genus *Aegilops* has little to offer in terms of improved amylose content.

The hexaploid wheats had the usual bimodal starch granule size distribution, and the B-type granule content has already been published (Stoddard 1999). The B-type granule content of some of

TABLE I  
Genome Formulas, Number of Lines Examined, Maximum and Minimum B-Type Granules, and Amylose Contents for *Aegilops* Species

Genome	<i>Aegilops</i> Species	Lines	B-Type Granules (vol% of starch)	Amylose (% starch)
C	<i>caudata</i>	4	8–17	ne <sup>a</sup>
M	<i>comosa</i>	3	12–19	24–27
N	<i>uniaristata</i>	6	6–13	20–26
S <sup>b</sup>	<i>bicornis</i>	5	7–19	26–34
S <sup>l</sup>	<i>longissima</i> , <i>sharonensis</i>	10	10–18	22–33
S	<i>speltoides</i>	5	9–24	22–28
U	<i>umbellulata</i>	5	8–18	22–30
DC	<i>cylindrica</i>	5	18–27	25–33
UC	<i>truncialis</i>	6	13–25	24–26
DN	<i>ventricosa</i>	4	10–16	22–28
DX <sup>c</sup> ,		21	4–13	23–27
DX <sup>c</sup> D	<i>crassa</i>			
UM <sup>o</sup>	<i>biuncialis</i> , <i>geniculata</i>	10	6–20	24–28
UX <sup>i</sup>	<i>columnaris</i> , <i>neglecta</i>	6	8–19	21–29
US <sup>l</sup>	<i>kotschyi</i> , <i>peregrina</i>	6	5–13	23–35
UX <sup>i</sup> N	<i>neglecta</i>	2	5–14	23–24
DX <sup>c</sup> U	<i>juvenalis</i>	1	7	28
Standard error			0.8–1.2	0.7

<sup>a</sup> Not evaluated.

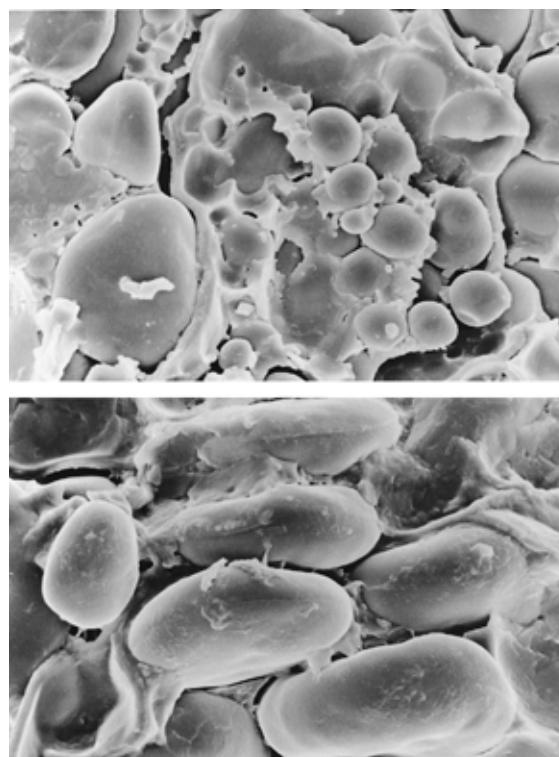


Fig. 1. Scanning electron micrographs ( $\times 1,340$ ) of fractured endosperm of *Aegilops ventricosa* (top) and *A. peregrina* (bottom).

## LITERATURE CITED

the *Aegilops* accessions showed much lower values, down to 4% of the starch, than any reported for hexaploid wheat. These accessions thus had a unimodal starch granule size distribution without a detectable peak or mode in the B-type granule size classes. *A. peregrina*, *kotschy*, *crassa*, and *juvenalis* had very low apparent B-type granule contents, and scanning electron microscopy showed that there were no small starch granules in these grains (e.g., *A. peregrina*) (Fig. 1). A peak in the B-type granule zone was found in all UM<sup>o</sup> and UX<sup>t</sup> accessions, so although some values were low, they were not as potentially valuable as the preceding group. *A. cylindrica*, *comosa*, *ventricosa*, and *triuncialis* had relatively high B-type granule contents, comparable to those in many wheats, and small granules were visible in scanning electron microscope preparations (e.g., *A. ventricosa*) (Fig. 1). Variation within each species was narrow (Table I), and in no species were unimodal and bimodal size distributions both represented.

B-type granule content is quantitatively affected by both environment (Blumenthal et al 1994, 1995) and genetics (Stoddard 2000). There is no evidence of any environmental condition that can qualitatively eliminate B-type granules, and the growing conditions described here were designed to maximize the expression of this trait. *A. peregrina*, *kotschy*, and *crassa* have now been grown in a variety of conditions, and their starch granules have remained unimodally distributed with large A-type granules and no apparent B-type granules.

The low B-type granule trait could not be attributed to a single genome. The US<sup>1</sup> and DX<sup>c</sup>-based accessions (DX<sup>c</sup>, DX<sup>c</sup>D, and DX<sup>c</sup>U genomes) all had much lower B-type granule contents than the parent genomes. Other polyploids derived from these diploids did not have exceptionally low B-type granule contents. This difference may be associated with the evolutionary modifications attributed to the various genomes in the polyploids (Kimber and Feldman 1987) or to other aspects of interactions between genomes.

Amylose and B-type granule contents were not significantly correlated ( $r = 0.156$ ,  $n = 99$  for the *Aegilops* accessions;  $r = 0.133$ ,  $n = 200$  for the wheats).

We are investigating the potential to transfer the low B-type granule trait from the US<sup>1</sup> and DX<sup>c</sup> genomes to hexaploid wheat.

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