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DIFFERENCES IN STARCH COMPOSITION AND PHYSIOCHEMICAL PROPERTIES ARE INFLUENCED BY GRANULE TYPES IN WHEAT AND ITS RELATIVES

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Abstract

Starch morphology, composition, and physiochemical properties were characterized from wild wheat species and wheat cultivars with diploid (AA, BB, and DD genome), tetraploid wheat (AABB genome), and hexaploid (AABBDD) genomes. The A-type and B-type granules were separated and purified from each wheat genotype. Starch size, distribution, amylose content, distribution of amylopectin chain lengths, gelatinization, and retrogradation were analyzed in different wheat genotypes. Our results indicate that *Aegilops speltoides* (BB genome) has a significantly higher percentage of large A-type granules. The A-type granules contained significantly higher amylose content than the B-type granules in all accessions. Amylopectin exhibited more B2 and B3 chains (DP 25 and up) but less A chains (DP 6-12) in the A-type than the B-type granules. The extent of enthalpy changes during starch gelatinization was greater and retrogradation rates were higher in the A-type than the B-type granules. However, the B-type granules have broader ranges of gelatinization temperatures (Tc–To) than the A-type granules. Additionally, the B-type granules of wild diploid species (AA and BB genome) consistently exhibit lower onset (To) and higher peak (Tp) and conclusion (Tc) temperatures. Thus, starch structure is closely related to functionality, and granule size and distribution are significantly correlated to starch thermal properties.

Key words: *Triticum urartu, Aegilops speltoides, Aegilops tauschii,* Hybridization, Starch, granules.

Introduction

Wheat (*Triticum aestivum* L.) is one of the most consumed cereals worldwide. It is an allohexaploid species, consisting of 3 sets of highly related genomes (A, B, and D). Hexaploid wheat originated from 2 independent hybridization events. The first event involved the hybridization between an ancestor of *Triticum urartu* (2n = 2x = 14, genome AA) and possibly *Aegilops speltoides* (2n = 2x = 14, genome BB), resulting in the cultivated tetraploid emmer wheat (*Triticum turgidum* ssp. *dicoccum*, genome AABB). In the second event, the diploid *Aegilops tauschii* (2n = 2x = 14, genome DD) hybridized with the tetraploid emmer wheat to make the hexaploid wheat (genomic constitution AABBDD), in which spelt wheat (*T. speltoides*) and common wheat (*T. aestivum*) are 2 major species with an AABBDD genome (Breiman & Graur, 1995; Dvorak *et al*., 1998).

As the major component in cereal grains, starch provides nutrition and energy for humans and serves as a functional industrial material (Hannah & James, 2008). Starch is mainly composed of 2 types of glucose polymers, the essentially linear amylose and the highly branched amylopectin (Tetlow, 2006; Hannah & James, 2008). Starch granules from wheat, triticale and barley show a bimodal of size distribution and can be divided into two classes, the large A-type granule (>10μm) and the small B-type granule (≤10μm) (Salman *et al*., 2008). The number, shape, and size distribution of starch granules significantly vary among different botanical sources (Ao & Jane, 2007; Salman *et al*., 2008), enabling use of different types of starch granules in different food and industrial applications (Lim *et al*., 1992).

Owing to the difficulty in isolating and purifying starch from small seeds of Triticeae wild species, little information is available on starch morphology, composition and physiochemical properties of wheat diploid species, such as *T. urartu*, *Ae. speltoides*, and *Ae. tauschii.* In this study, we focused on diploid wheat (AA, BB, and DD genome), tetraploid wheat (AABB genome), and hexaploid wheat (AABBDD genome) to characterize starch morphology, composition, and physiochemical properties. We determined the parameters of granule size and distribution, total starch and amylose content, distribution of amylopectin chain lengths, and thermal properties of the A-type and B-type granules from different wheat genotypes. We also investigated starch structure and physiochemical properties in order to enhance our understanding of starch evolution and differentiation in cereal crops.

Materials and Methods

Plant materials: Six wild diploid species lines (2 from AA genome, 2 from BB genome, and 2 from DD genome), 2 tetraploid wheat lines (AABB), and 3 hexaploid wheat lines (AABBDD) were used in this study (Table 1). All 6 accessions of wild wheat species (*T. urartu*, *Ae. speltoides*, and *Ae. tauschii*) were kindly provided by the Wheat Genetic and Genomic Resource Center, Kansas State University, MO, USA. The plants were grown during the same time period under controlled environmental conditions (24°C day/20°C night with a photoperiod of 16 h) in a growth cabinet. The seeds were harvested separately, dried to less than 10% humidity, and then ground into flour on a laboratory mill fitted with a 0.5-mm screen (Retsch, ZM-100, Germany).

Table 1. Size and distribution of starch granules from wild species and cultivars of wheat*.

Species	Accession	A-type granules		B-type granules		Average diameter of all starches (µm)
		Average diameter (µm)	Proportion (%)	Average diameter (µm)	Proportion (%)	
T. urartu	TA744	13.6 ± 0.3c	10.6 ± 0.2cd	3.8 ± 0.1e	89.4 ± 0.4ab	4.9 ± 0.4c
	TA783	12.1 ± 0.1d	22.4 ± 0.4b	5.5 ± 0.2a	77.6 ± 0.3cd	7.0 ± 0.3ab
	Mean	12.9	16.5	4.7	83.5	6.0
Ae. speltoides	TA1790	14.3 ± 0.4ab	44.2 ± 0.5a	4.6 ± 0.5bc	55.8 ± 0.5e	6.7 ± 0.5ab
	TA2774	13.9 ± 0.3c	45.1 ± 0.4a	5.2 ± 0.6a	54.9 ± 0.4e	7.6 ± 0.4a
	Mean	14.1	44.7	4.9	55.4	7.2
Ae. tauschii	TA1624	14.6 ± 0.6a	22.2 ± 0.3b	4.8 ± 0.4b	77.8 ± 0.4cd	6.9 ± 0.4ab
	TA2402	14.3 ± 0.2a	14.9 ± 0.4c	5.2 ± 0.2a	85.2 ± 0.6bc	6.5 ± 0.5b
	Mean	14.5	18.6	5	81.5	6.7
T. durum	AC Avonlea	14.3 ± 0.5a	9.2 ± 0.5cd	4.0 ± 0.2e	90.8 ± 0.5ab	5.0 ± 0.5c
	AC Navigator	14.4 ± 0.4a	5.7 ± 0.4d	4.1 ± 0.2de	94.3 ± 0.4a	4.7 ± 0.4c
	Mean	14.4	7.5	4.1	92.6	4.9
T. speltoides	Spelt	13.9 ± 0.3c	4.4 ± 0.5d	4.6 ± 0.1bc	95.6 ± 0.5a	5.0 ± 0.3c
T. aestivum	Chinese Spring	13.6 ± 0.3c	5.0 ± 0.6d	4.4 ± 0.2cd	94.9 ± 0.3a	5.0 ± 0.2c
	Chuannong 16	13.9 ± 0.5c	3.9 ± 0.3e	3.9 ± 0.2e	96.1 ± 0.4a	4.9 ± 0.3c
	Mean	13.8	4.4	4.3	95.5	5.0

* Values are means of at least 3 biological replicates. Different letters indicate statistical significance at $p<0.05$

Isolation and separation of starch granules: Starch granules were isolated from mature seeds according to the method reported by Peng *et al.*, (1999) with some modifications. Wheat grains (6–8) were steeped in 1 ml double-distilled water at 4°C overnight. The softened seeds were ground with a mortar and pestle in 1 ml of fresh double-distilled water. The slurry was transferred into a 1.5-ml tube and centrifuged at 4000 × *g* for 5 min to remove the yellow gel-like layer on top of the starch granule pellet. The starch granule pellet was then suspended in 0.5 ml water and overlaid with 1.5 ml of 80% (w/v) cesium chloride before it was centrifuged at 4000 × *g* for 5 min. The supernatant containing cesium chloride and debris was discarded. This purification procedure was then repeated twice. Finally, the starch granule pellet was washed once with 0.8 ml wash buffer (62.5 mM Tris-HCl, pH 6.8, 10 mM EDTA, 4% SDS, and 5% beta-mercaptoethanol), twice with water, and once with acetone, and then dried at room temperature and stored at -20ºC.

The starch preparation containing both A-type and B-type granules was separated by centrifugation through Percoll as described by Peng *et al.*, (1999). Briefly, 5 ml of starch suspension (0.1 g/ml) was laid on top of 10 ml of 70% (v/v) Percoll solution in a 15-ml tube and centrifuged at 10 × *g* for 10 min. The supernatant containing B-type starch granules was collected and transferred to a new 15-ml tube. The pellet was washed twice in water, suspended in 5 ml of deionized water, and centrifuged 3 times in 70% (v/v) Percoll solution at 10 × *g* for 10 min. The precipitation pellet, containing predominantly A-type starch granules, was dissolved and purified by 3 cycles of Percoll (100%) centrifugation (10 × *g*, 10 min for each cycle). The supernatant were pooled and centrifuged at 3500 × *g* for 5 min, and the resulting starch pellets were collected as the B-type starch granules. A-type and B-type starch granules were washed 3 times in deionized water and once in acetone, and then dried at room temperature.

Microscopic observation of starch granules; Granule morphology of native starch was examined by scanning electron microscopy (SEM). The starch samples were placed on aluminum stubs with double-sided sticky tape and coated with gold. Pictures were then taken under SEM (Hitachi, S-570, Japan) at a voltage of 10 KV. The granule sizes were determined with a flow image analyzer (Sysmex FPIA-3000, Malvern Instruments, UK), and 5 mg of starch granules was suspended in 1 ml of deionized water and analyzed. The granule sizes and distributions were statistically analyzed via the FPIA-3000 Sysmex software.

Determination of starch and amylase content: Total starch from each sample was analyzed using the Total Starch Assay Kit (Megazyme, Ireland, Cat. no. K-TSTA). The amylose content in starch was determined following the modified iodine-binding method by Zhu *et al.*, (2008). Ten milligrams of dried starch granules was mixed with 20 µl of 95% ethanol and dissolved in 1 ml of 1N NaOH in a 15-ml tube, before dilution to 10 ml with deionized water. The solution was further diluted to 0.1 N NaOH, and 0.5 ml of the resulting solution was transferred into a 2-ml tube. An aliquot of the starch solution was neutralized with 0.1 N HCl and diluted with water to make a 0.25 mg/ml final stock solution. A reaction mixture (2 ml) consisting of 0.2 ml of the stock solution, 1.7 ml H_2O, and 0.1 ml of 0.2% iodine solution containing 2.0 g potassium iodide and 0.2 g iodine in 100 ml H_2O was then made. The tubes were incubated for 30 min before the mixed solution was transferred into a disposable cuvette and scanned at 400–750 nm using a spectrophotometer. Amylose content was calculated using the following formula:

$$\text{Amylose \%} = \frac{\text{A620 - A510 + 0.0542}}{0.3995} \times 100.$$

Distribution of amylopectin chain lengths: Amylopectin was debranched using isoamylase (Jane *et al.*, 1999). Branch chain-length distribution of amylopectin was than analyzed using capillary electrophoresis (CE, PA800plus; Beckman Coulter Canada, Ontario, Canada) as follows: 5 mg of starch was suspended in 5 ml H_2O in a 50-ml glass test tube and heated at 130°C for 30 min with intermittent vortexing. One ml of the solution was transferred into a 2-ml tube, and then 55 µl of 1 M sodium acetate (pH 4.0) and 4 units of isoamylase (Megazyme, UK) were added. The reaction mixture was mixed and incubated at 40°C for 4 h before the reaction was stopped by heating at 95°C for 20 min. The digested mixture was then freeze-dried and re-dissolved in 1 ml H_2O by heating at 95°C for 5 min. Ten microliters of re-dissolved solution was vacuum dried and labeled with 8-amino-(1, 3, 6)-pyrenetrisulfonic acid (APTS) using the Carbohydrate Labeling Kit (Beckman Coulter Canada Inc., Ontario, Canada). The labeled carbohydrate chains were separated by CE and detected through an laser induced fluorescent (LIF) quipped detector and analyzed for the degree of polymerization (DP) values with the 32 Karat software (Beckman Coulter, Canada).

Characterization of starch thermal properties: Thermal properties of the native, gelatinized, and retrograded starch were analyzed using a differential scanning calorimeter (DSC 2920; TA Instruments, Delaware, USA) equipped with a refrigerated cooling system (RCS). The starch samples (10 mg) were precisely weighed into the aluminum Tzero pan (TA Instruments) and mixed with deionized water (20 µl) at a starch:water ratio of 1:2. The pan was sealed and equilibrated at room temperature for 1 h. The heating rate was at 10°C/min over the temperature range of 30–100°C. The instrument was calibrated using indium and an empty pan as reference standards. Enthalpy change (ΔH), gelatinization onset temperature (To), peak temperature (Tp), and conclusion temperature (Tc) were measured using the Universal Analysis 2000 v 4.7A software (TA Instruments, USA). The gelatinized starch sample was store at 4°C for 1 month. The properties of retrograded starch and percentage retrogradation were analyzed using the same method as that used to measure gelatinization using the same starch samples.

Statistical analysis: All chemical analyses were independently performed using duplicate samples. Statistical analysis of all the data was performed using the SPSS statistics 17.0 for Windows statistical software package (SPSS, Chicago, IL, USA). Means were compared using Fisher's Protected LSD text at the 0.05 probability level of significance. The appropriate error term from the SAS output was used to calculate the LSD values.

Results

Morphology and size distribution of starch granules: The starch purified from different wild species and cultivars of wheat was found to be a mixture of large and small granules. Large starch granules displayed a disc or lenticular shape with a diameter of 10–35 µm, while the small granules were roughly spherical or polygonal, ranging from 1 to 10 µm (Fig. 1). In this study, the bimodal distribution of starch granules in different wheat genomes was further verified, because the large granules (A-type) and small granules (B-type) had a clear cut-off point at diameter 10.0 µm (Fig. 2). In all accessions of diploid, tetraploid, and hexaploid species, A-type granules possessed average diameters of 12.1–14.6 µm, whereas B-type starch granules exhibited average diameters of 3.8–5.5 µm (Table 1). Although the average diameters of each granule type did not differ significantly among wild species and cultivars of wheat, the proportion of A-type or B-type granules significantly differed among all accessions analyzed in this study. The proportions of A-type granules were significantly higher in accessions of diploid species (except TA744) than in those of tetraploid and hexaploid species. For example, the proportions of A-type granules in 2 accessions of *Ae. speltoides* were up to 44.2% and 45.1%.

Total starch and amylose content: Total starch from different wheat genotypes and amylose contents from A-type and B-type granules were analyzed (Table 2). Compared to tetraploid wheat (AABB genome) and hexaploid wheat (AABBDD genome), 3 wild diploid species (AA, BB, and DD genomes) had significantly less total starch content; among these, 2 accessions of *Ae. speltoides* had only 29.6% and 25.7% of total starch. The amylose content of A-type granules was higher than that of B-type granules in accessions of *T. urartu*, *Ae. tauschii, T. durum*, and hexaploid wheat. However, the amylose content did not differ significantly between A-type and B-type granules in 2 accessions of *Ae. speltoides*.

Distribution of amylopectin chain lengths: There were significant variations in amylopectin chain lengths among various wheat genotypes analyzed in this study (Table 3; Fig. 3). The DP values of oligosaccharide chains varied from 6 to 60 glucose molecules, and the chain length of AP oligosaccharides, whose proportion was the highest, was approximately 10 or 11 glucose molecules. In general, amylopectin chain lengths can be classified into 4 types: short A chains (DP = 6–12), intermediate B1 chains (DP = 13–24), long B2 chains (DP = 25–36), and very long chains (DP > 36). Our results indicate that the A-type granules exhibited more number of B2 and B3 chains (DP 25 and up) but lesser number of A chain (DP 6-12) than did the B-type granules, in all accessions (Table 3). However, the distributions of chain lengths between A-type and B-type granules in the differential histogram were not consistent among different wheat genomes (Fig. 4 A-E). The B-type granules in accessions of diploid species (AA, BB, and DD genome) consisted of more DP 6-23 branch chains but fewer DP > 23 branch chains than the A-type granules did. The B-type granules in tetraploid wheat (AABB genome) had more DP 6-17 branch chains but fewer DP >17 branch chains than the A-type granules. The B-type granules of hexaploid wheat (AABBDD genome) had more DP 6-25 branch chains but fewer DP >25 branch chains than the A-type granules (Table 3).

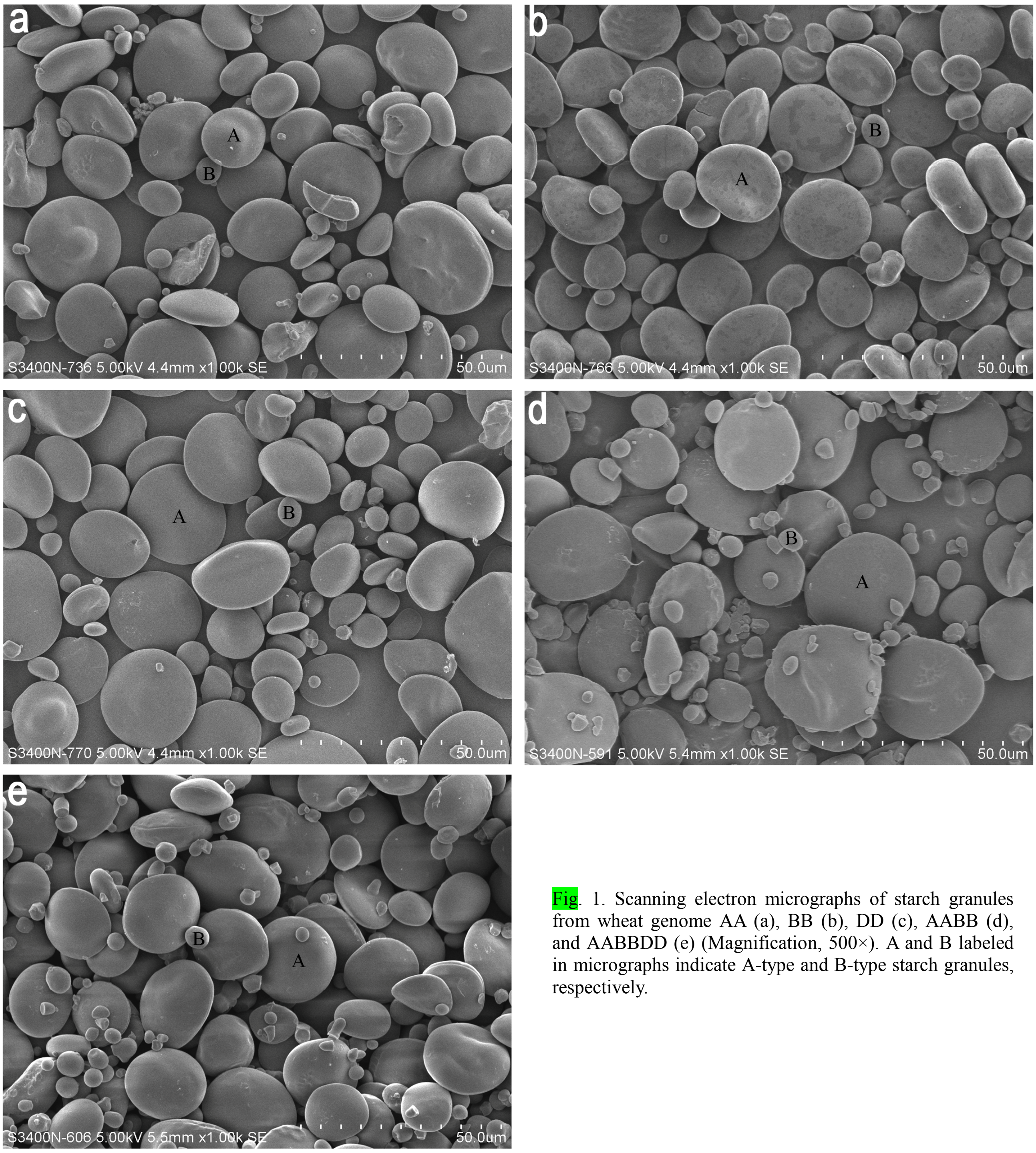

Fig. 1. Scanning electron micrographs of starch granules from wheat genome AA (a), BB (b), DD (c), AABB (d), and AABBDD (e) (Magnification, 500×). A and B labeled in micrographs indicate A-type and B-type starch granules, respectively.

Thermal properties: Gelatinization properties of A-type and B-type granules from wild species and cultivars of wheat were analyzed for To, Tp, Tc, and ΔH (Table 4). Our results indicate that the B-type granules had broader ranges of gelatinization Tc and (Tc–To) than the A-type granules did, in all accessions from diploid, tetraploid, and hexaploid genomes. The gelatinization Tp of the B-type granules was higher than that of the A-type granules in accessions of diploid and hexaploid wheat, but the results were the opposite in accessions of tetraploid wheat. The gelatinization To of the A-type granules was higher than that of the B-type granules in accessions of *Ae. speltoides*, *Ae. tauschii*, *T. durum*, and *T. aestivum*, whereas To of the A-type and B-type granules did not show significant differences among accessions of *T. urartu.* The B-type granules of *T. urartu* and *T. aestivum* showed larger gelatinization ΔH than the A-type granule counterparts did.

In this study, the gelatinized starch pastes underwent retrogradation after they were cooled. During starch retrogradation, the value of enthalpy provides a quantitative measure of the energy transformation that occurs during the melting of recrystallized amylopectin. We investigated the starch retrogradation rates of wheat wild species and cultivars and found that the peak of starch retrogradation appeared at a lower transition temperature after the gelatinized starch was stored at 4°C for 1 month. The A-type granules showed higher retrogradation rates than the B-type granules did, in all accessions analyzed in this study (Table 2).

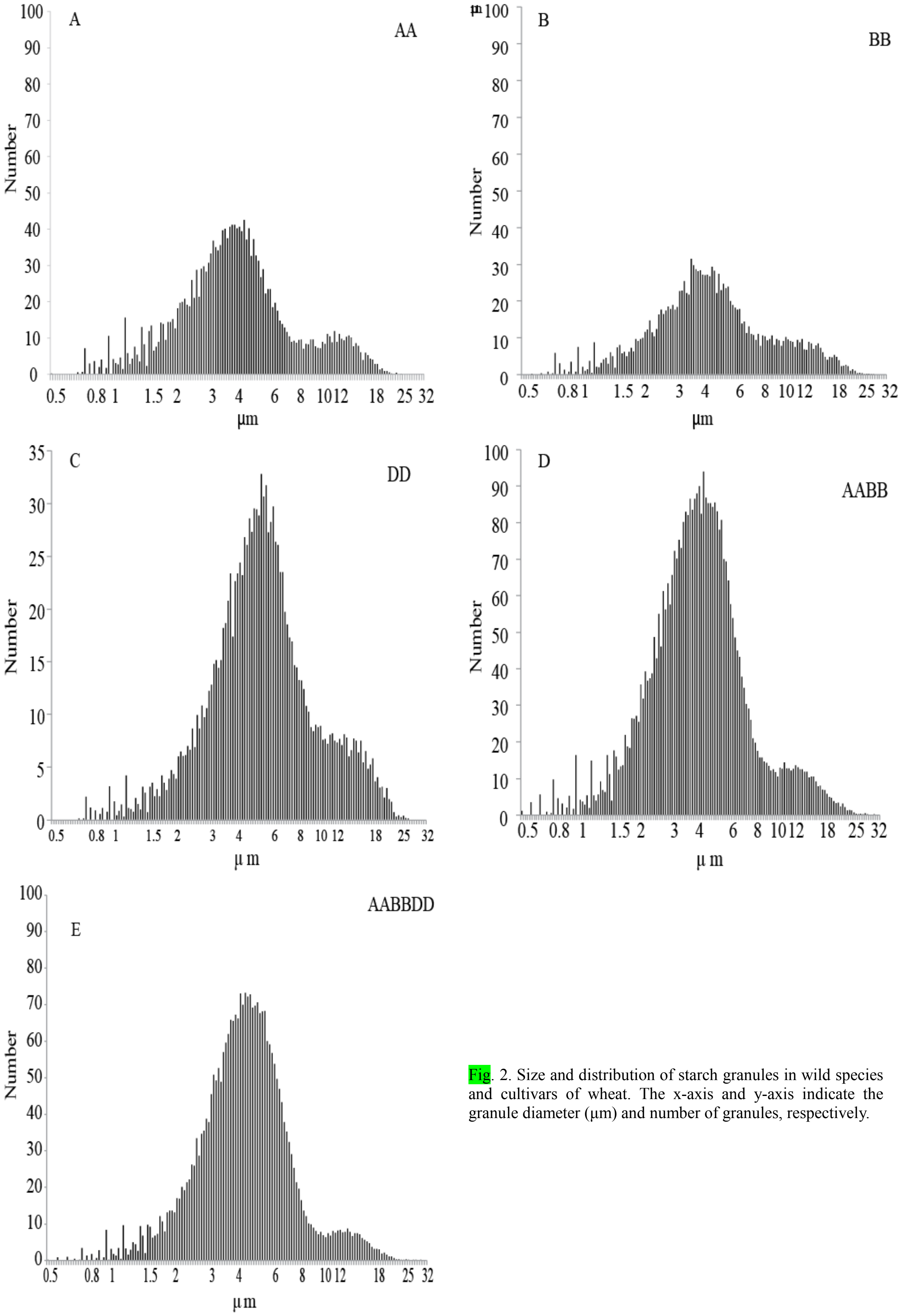

Fig. 2. Size and distribution of starch granules in wild species and cultivars of wheat. The x-axis and y-axis indicate the granule diameter (μm) and number of granules, respectively.

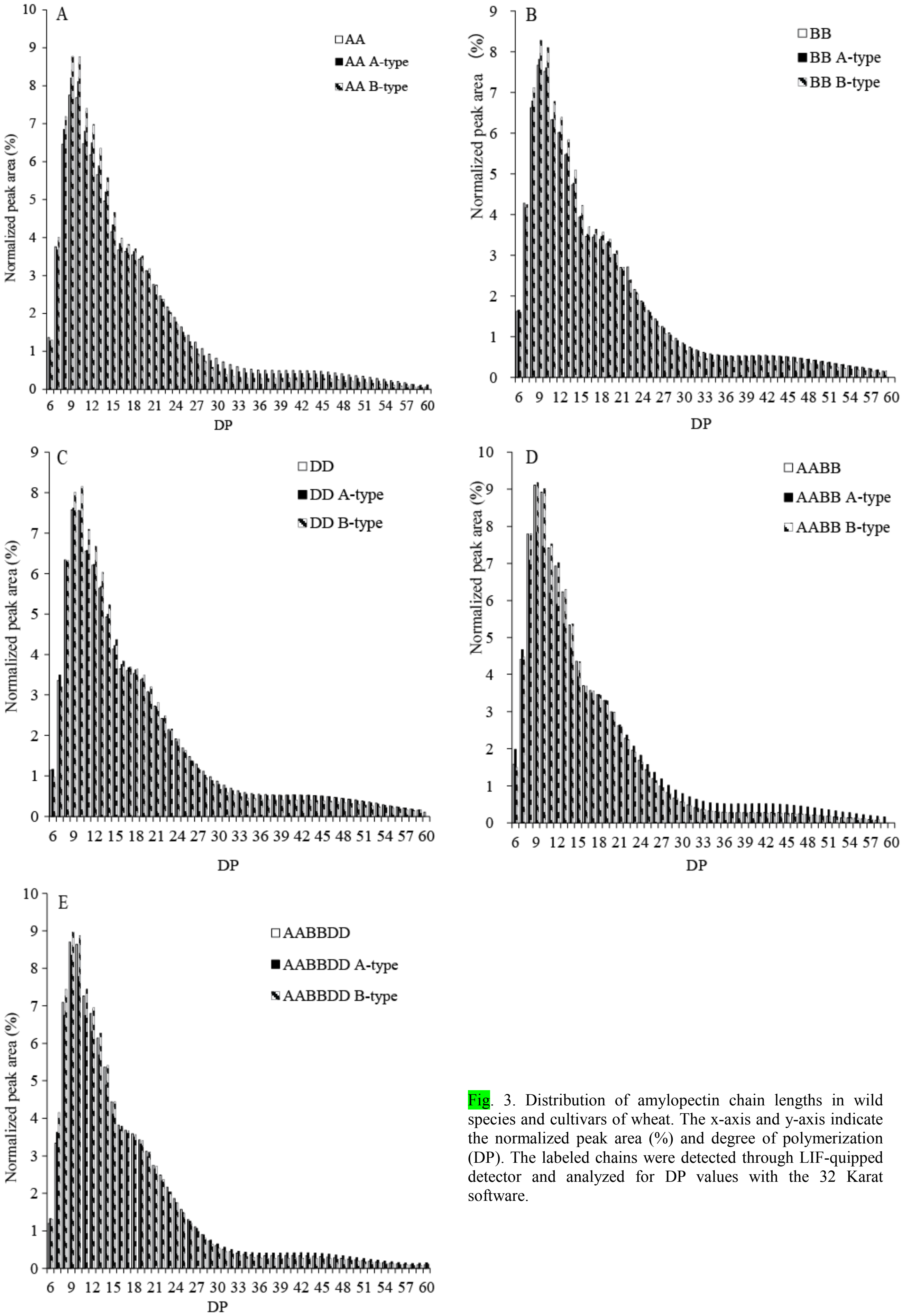

Fig. 3. Distribution of amylopectin chain lengths in wild species and cultivars of wheat. The x-axis and y-axis indicate the normalized peak area (%) and degree of polymerization (DP). The labeled chains were detected through LIF-quipped detector and analyzed for DP values with the 32 Karat software.

Table 2. Total starch and amylose content in granules of wild species and cultivars of wheat*.

Species	Accession	Total starch (%)	Granule type	Amylose in granules (%)
T. urartu	TA744	46.0 ± 0.2c	A	31.3 ± 0.2gh
			B	29.7 ± 0.5i
	TA783	45.3 ± 0.3c	A	29.7 ± 0.4i
			B	28.3 ± 0.3j
	Mean	45.7	A	30.5
			B	29
Ae. speltoides	TA1790	29.6 ± 0.2ef	A	33.1 ± 0.5cd
			B	33.4 ± 0.4cd
	TA2774	25.7 ± 0.3g	A	31.8 ± 0.2efg
			B	32.3 ± 0.3defg
	Mean	27.7	A	32.5
			B	32.9
Ae. tauschii	TA1624	33.4 ± 0.2d	A	30.5 ± 0.3hi
			B	28.5 ± 0.3j
	TA2402	32.1 ± 0.2de	A	32.5 ± 0.2def
			B	34.0 ± 0.1bc
	Mean	32.8	A	31.5
			B	31.3
T. durum	AC Avonlea	54.8 ± 0.4a	A	35.8 ± 0.5a
			B	36.0 ± 0.4a
	AC Navigator	54.9 ± 0.3a	A	34.2 ± 0.4bc
			B	32.8 ± 0.2de
	Mean	54.9	A	35
			B	34.4
T. speltoides	Spelt	56.6 ± 0.3a	A	35.0 ± 0.2ab
			B	32.6 ± 0.3def
T. aestivum	Chinese Spring	56.6 ± 0.4a	A	34.2 ± 0.4bc
			B	30.6 ± 0.1hi
	Chuannong 16	51.1 ± 0.3b	A	31.5 ± 0.2fgh
			B	31.9 ± 0.3efg
	Mean	54.8	A	33.6
			B	31.7

* Values are means of at least 3 biological replicates. Different letters indicate the statistical significance at $p<0.05$

Discussion

Starch is a major component in wheat grains, and different granule types have different functions on starch end-use quality. A-type granules constitute the majority of starch by weight, whereas B-type granules comprise over 90% of starch by number (Zhang *et al*., 2010). Starch with a high percentage of A-type granules has been reported to have special applications in the manufacture of biodegradable plastic film and carbonless copy paper (Nachtergaele & Van Nuffel, 1989). Small A-type granules (approximately 12 μm) can increase bread weight, whereas B-type granules can bind water more densely, which may increase dough stiffness and reduce the elasticity (Huang & Lai, 2010). By mixing A-type and B-type granules in various proportions, Soulaka & Morrison (1985) found that the optimum proportion of B-type granules for better bread quality is 25–35% by weight. Therefore, characterization of both granule types from wild species of wheat will be necessary to improve wheat-breeding efficiency by genetic modifications of starch. In this study, we found that *Ae. speltoides* (BB genome) has a significantly higher percentage of large A-type granules, which can be used as a unique germplasm to develop new wheat cultivars for starch with a high percentage of large granules. The species of *Aegilops* had a lower content of B-type granules than the hexaploid wheat did (Stoddard & Sarker, 2000). The content of B-type starch granules was determined by genetics (Stoddard, 2000) during the process of wheat domestication, when the B-type starch granule was decreased, because it was found to be unsuitable for human use (Hoseney *et al*., 1971).

Based on starch morphology, granule size and distribution, composition, and functional properties, we found that amylose contents in A-type granules are higher than those in the B-type starches in wheat diploid, tetraploid, and hexaploid species (AA, BB, DD, AABB, and AABBDD genome). This finding is consistent with that of previous studies on barley, triticale and wheat (Ao & Jane, 2007; Takeda *et al*., 1999). In accordance with previous reports, the differences in amylose content are attributed to the branch chain lengths of amylopectin (Jane & Shen, 1993). The B-type granules consist of amylopectin that have more short chains, which may possess a cone shape to fit in the spherical granules. In contrast, the amylopectin molecules of A-type granules have more long chains, which may play a key role in forming a lenticular shape (Tang *et al*., 2002; Ao & Jane, 2007). The studies on starch morphology and composition can greatly improve our understanding of the nature of developmental differentiation between large, lenticular A-type granules and small, spherical B-type granules.

Table 3. Distribution of amylopectin chain lengths in wild species and cultivars of wheat*

Species	Accession	Type	DP6-12	DP13-24	DP25-36	DP>36
T. urartu	TA744	A	38.2 ± 0.2c	48.8 ± 0.3b	8.9 ± 0.2e	5.3 ± 0.6efg
		B	37.7 ± 0.3d	48.1 ± 0.2cd	8.0 ± 0.3e	4.9 ± 0.2gh
	TA783	A	32.7 ± 0.1l	45.7 ± 0.3gh	12.1 ± 0.1b	9.5 ± 0.2bc
		B	36.6 ± 0.1fg	49.8 ± 0.3a	9.3 ± 0.2e	4.4 ± 0.2hi
	Mean	A	35.5	47.3	10.5	7.4
		B	37.2	49.0	8.7	4.7
Ae. speltoides	TA1790	A	34.7 ± 0.2j	43.7 ± 0.1j	12.6 ± 0.1a	9.6 ± 0.3bc
		B	34.0 ± 0.3k	43.9 ± 0.3j	11.9 ± 0.1b	9.8 ± 0.1b
	TA2774	A	34.5 ± 0.2j	44.7 ± 0.2i	11.2 ± 0.1c	9.6 ± 0.3bc
		B	37.5 ± 0.4de	49.5 ± 0.1a	8.9 ± 0.4e	4.1 ± 0.2i
	Mean	A	34.6	44.2	11.9	9.6
		B	35.8	46.2	10.4	7.0
Ae. tauschii	TA1624	A	32.9 ± 0.2l	45.6 ± 0.2gh	11.8 ± 0.1b	10.5 ± 0.1a
		B	31.3 ± 0.2m	45.8 ± 0.2 h	12.7 ± 0.3a	9.8 ± 0.1b
	TA2402	A	32.5 ± 0.1l	46.1 g	11.7 ± 0.1b	9.7 ± 0.2bc
		B	36.2 ± 0.1gh	49.6 a	9.3 ± 0.4e	4.9 ± 0.2gh
	Mean	A	32.7	45.9	11.8	10.1
		B	33.8	47.7	11.0	7.4
T. durum	AC Avonlea	A	34.4 ± 0.3jk	44.0 j	11.7 ± 0.1b	9.9 ± 0.1b
		B	37.0 ± 0.5ef	47.6 ± 0.2e	10.3 ± 0.4d	5.1 ± 0.6fg
	AC Navigator	A	35.6 ± 0.3i	43.5 ± 0.1j	11.8 ± 0.1b	9.1 ± 0.2cd
		B	43.1 ± 0.4a	45.6 ± 0.5gh	7.4 ± 0.4g	3.9 ± 0.6ij
	Mean	A	35.0	43.8	11.8	9.5
		B	40.1	46.6	8.9	4.5
T. speltoides	Spelt	A	37.2 ± 0.5e	46.6 ± 0.5f	9.1 ± 0.3e	5.6 ± 0.2ef
		B	38.8 ± 0.4b	48.0 ± 0.1de	9.9 ± 0.5d	4.9 ± 0.4g
T. aestivum	Chinese Spring	A	34.6 ± 0.2j	44.0 ± 0.1j	11.9 ± 0.1b	8.9 ± 0.2d
		B	37.7 ± 0.4d	49.5 ± 0.4a	9.3 ± 0.5e	3.5 ± 0.5j
	Chuannong 16	A	32.9 ± 0.1l	45.7 ± 0.3gh	11.6 ± 0.2b	9.8 ± 0.2b
		B	35.8 ± 0.3hi	48.6 ± 0.3bc	9.8 ± 0.5d	5.7 ± 0.4e
	Mean	A	34.9	45.4	10.9	8.1
		B	37.4	48.7	9.7	4.7

* Values are means of at least 3 biological replicates. Different letters indicate the statistical significance at $p<0.05$

Different morphology and compositions in cereal starch can be the major factors responsible for the variations in physiochemical properties between A-type and B-type granules of native, gelatinized, and retrograded starch. The A-type granules may contain more double helices than the B-type granules, and the disruption of amylose-lipid complex can alter the thermal properties of phase transition (Shinde *et al*., 2003). We found that the enthalpy values of native, gelatinized, and retrograded starch from the A-type granules were lower than the enthalpy values of those from B-type granules, and this difference could be due to higher lipid content in the B-type granules of wheat starch. For B-type granules, the lower To and the higher Tc during starch gelatinization confer a wide range of crystallite perfection within small starch fractions. This is attributed to the fact that the A-type granules consist of more amylose and longer branch chains in the amylopectin than the B-type granules do. The retrogradation rate of wheat starch is inversely correlated with the proportion of short branch chains (DP 6-9) in amylopectin (Shi & Seib, 1992). As the B-type granules consist of less amylose, B-type granules retrograde more slowly than the A-type granules; moreover, the presence of lipids and phospholipids in the B-type granules retards their retrogradation. Further investigation on cereal lipid content and structure will be informative to explore the functionality on different types of starch granules.

Conclusion

Our results indicate that starch structure is closely related to functionality, and that granule size and distribution are significantly related to thermal properties. The A-type granules contained significantly higher amylose content than did the B-type granules in all accessions. Amylopectin exhibited more B2 and B3 chains (DP 25 and up) but less A chains (DP 6-12) in the A-type than the B-type granules. The extent of enthalpy changes during starch gelatinization was greater and retrogradation rates were higher in the A-type than the B-type granules. The crucial role of isoamylase in initiating starch granules introduces a new level of complexity in the relationship between starch morphology and biosynthesis enzymes. The genetic modification or enhancement of starch morphology and composition will be another way to achieve new functional applications of cereal starch.

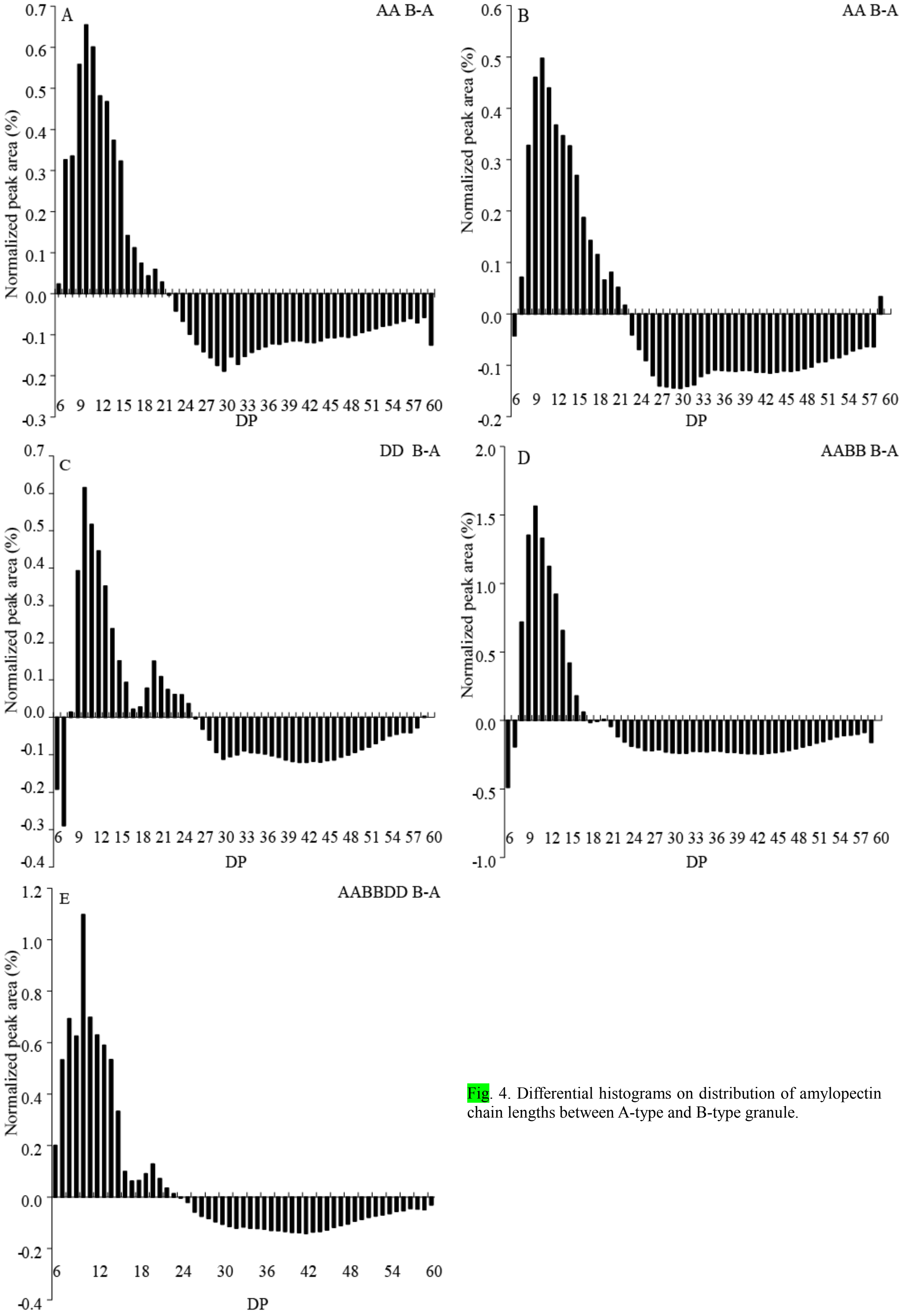

Fig. 4. Differential histograms on distribution of amylopectin chain lengths between A-type and B-type granule.

Table 4. Starch gelatinization properties of wild species and cultivars of wheat*.

Species	Accession	Type	To	Tp	Tc	Tc-To	ΔH
T. urartu	TA744	A	54.5 ± 0.2gh	60.2 ± 0.2gh	69.6 ± 0.2mn	15.1 hi	8.6 ± 0.2hi
		B	54.9 ± 1.0fgh	61.1 ± 0.7f	71.8 ± 0.7fgh	16.9 de	9.2 ± 0.4efg
	TA783	A	55.4 ± 0.2ef	62.1±0.2de	71.5 ± 0.2ghi	16.1 fg	9.3 ± 0.1def
		B	55.4 ± 0.2ef	63.3±0.2b	73.6 ± 0.2bc	18.2 bc	10.1 ± 0.1a
	Mean	A	55.0	61.2	70.6	15.6	9.0
		B	55.2	62.2	72.7	17.6	9.7
Ae. speltoides	TA1790	A	54.4 ± 0.3h	60.3±0.1gh	69.9 ± 0.1lm	15.5 fgh	9.8 ± 0.1abcde
		B	52.6 ± 0.1ij	60.4±0.1gh	72.6 ± 0.3def	20.0 a	9.4 ± 0.1cdef
	TA2774	A	56.1 ± 0.2cde	61.8±0.2e	70.5 ± 0.3jkl	14.5 i	9.9 ± 0.1abc
		B	55.3 ± 0.2efg	62.5±0.1cd	73.2 ± 0.3bcd	17.9 bc	9.6 ± 0.1abcdef
	Mean	A	55.3	61.1	70.2	15.0	9.9
		B	54.0	61.5	72.9	19.0	9.5
Ae. tauschii	TA1624	A	57.9 ± 0.2a	63.5±0.3b	73.2 ± 0.3bcd	15.3 ghi	9.9 ± 0.4abcd
		B	56.1 ± 0.2cde	64.5±0.2a	74.9 ± 0.3a	18.5 b	9.1 ± 0.1fg
	TA2402	A	57.2 ± 0.2ab	63.0±0.4bc	72.5 ± 0.4def	15.3 ghi	9.6 ± 0.1bcdef
		B	56.4 ± 0.3bcd	63.4±0.1b	73.9 ± 0.3ab	17.5 cd	9.7 ± 0.1abcdef
	Mean	A	57.6	63.3	72.9	15.3	9.8
		B	56.3	64.0	74.4	18.0	9.4
T. durum	AC Avonlea	A	54.4 ± 0.1h	59.9±0.4h	70.1 ± 0.1klm	15.7 fgh	8.2 ± 0.2ij
		B	51.9 ± 0.6j	60.6±0.1fg	72.2 ± 0.6efg	20.3 a	6.7 ± 0.7k
	AC Navigator	A	52.7 ± 0.2ij	58.5±0.2j	68.9 ± 0.2n	16.2 ef	8.0 ± 0.1j
		B	50.4 ± 0.2k	59.0±0.2i	70.7 ± 0.2jk	20.3 a	6.4 ± 0.1k
	Mean	A	53.6	59.2	69.5	16.0	8.1
		B	51.2	59.8	71.5	20.3	6.6
T. speltoides	Spelt	A	57.8 ± 0.2a	62.9±0.4bc	71.2 ± 0.2hij	13.4 j	9.7 abcdef
		B	56.7 ± 0.3bc	63.5±0.5b	72.8 ± 0.3de	16.1 fg	10.0 ab
T. aestivum	Chinese Spring	A	55.8 ± 0.8de	61.8±0.1e	70.9 ± 0.3ijk	15.1 hi	7.8 ± 0.3 j
		B	53.1 ± 0.7i	62.0±0.1de	72.9 ± 0.1bcd	20.0 a	8.1 ± 0.1ij
	Chuannong 16	A	56.8 ± 0.1bc	62.1±0.2de	72.0 ± 0.3fg	15.2 ghi	8.8 ± 0.3gh
		B	53.0 ± 0.1i	63.5±0.1b	72.9 ± 0.5cde	19.9 a	8.0 ± 0.2j
	Mean	A	56.8	62.3	71.4	14.6	8.8
		B	54.3	63.0	72.9	18.7	8.7

* Values are means of at least 3 biological replicates and different letters indicate the statistical significance at $p<0.05$. To, Tp, and Tc represent the onset, peak, and conclusion temperatures (°C) of starch gelatinization. ΔH represents the enthalpy change of dissociation during starch gelatinization

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