Registration of Common Wheat Germplasm with Mutations in SBEII Genes Conferring Increased Grain Amylose and Resistant Starch Content

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Abstract

Starch present in the endosperm of common wheat (Triticum aestivum L.) grains is an important source of carbohydrates worldwide. Starches with a greater proportion of amylose have increased levels of resistant starch, a dietary fiber that can provide human health benefits. Induced mutations in STARCH BRANCHING ENZYME II (SBEII) genes in wheat are associated with increased amylose and resistant starch. Ethyl methane sulfonate mutations in SBEIIa and SBEIIb paralogs were combined in the hexaploid wheat cultivar Lassik. Four mutant combinations were generated: SBEIIa/b-AB (Reg. No. GP-997, PI 675644); SBEIIa/b-A, SBEIIa-D (Reg. No. GP-998, PI 675645); SBEIIa/b-B, SBEIIa-D (Reg. No. GP-999, PI 675646); and SBEIIa/b-AB, SBEIIa-D (Reg. No. GP-1000, PI 675647). The SBEII mutant lines were compared with a wild-type control in a greenhouse and field experiment. The quintuple mutant line (SBEIIa/b-AB, SBEIIa-D) presented significant increases in both amylose (51% greenhouse; 63% field) and resistant starch (947% greenhouse; 1057% field) relative to the control. A decrease in total starch content (7.8%) was observed in the field experiment. The quintuple mutant also differed in starch viscosity parameters. Registration of the hexaploid wheat SBEII-mutant lines by University of California, Davis can help expedite the development of common wheat cultivars with increased amylose and resistant starch content.

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Availability

The four mutant germplasm lines are available from the USDA–ARS National Center for Genetic Resources Preservation. The materials deposited were produced through a greenhouse increase.

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Wheat (*Triticum* spp.) is a major source of calories worldwide and provides on average more than 500 calories per capita per day (FAOSTAT, 2014). Starch is the main carbohydrate in the wheat kernel and is composed of amylose (20–30%), which exists as linear helical chains of D-glucose, and amyllopectin (70–80%), a highly branched D-glucose polysaccharide (Botticella et al., 2011). An increase in consumer awareness of healthy and nutritious foods has led manufacturers, researchers, and producers to aim to improve starch nutritional value in wheat grains by increasing fiber content (Slade et al., 2012; Hazard et al., 2012). An effective strategy toward this objective is to increase the amount of amylose in the starch, since high-amylose starch is associated with increased resistant starch (RS) (Raigond et al., 2015). Resistant starch is defined as the fraction of dietary starch that is not digested in the small intestine of healthy individuals, and it is recognized as a functional fiber and prebiotic that plays an important role in digestive physiology (Charalampopoulos et al., 2002; Sajilata et al., 2006).

In the colon, fermentation of RS by gut microbiota generates short-chain fatty acids, including butyrate, which nourishes colonocytes and improves mucosal integrity. As a consequence, long-term consumption of RS has the potential to prevent colonic diseases such as cancer and inflammatory bowel conditions (Cassidy et al., 1994; Nofrarías et al., 2007). It has also been suggested that intake of RS increases insulin sensitivity (Robertson et al., 2005), which can lower the risk of both Type 2 diabetes and cardiovascular diseases (Martin et al., 1992; Haffner et al., 1999). In a study comparing the intake of different types of fiber, Willis et al. (2009) found RS to be highly satiating. Foods containing RS also have a lower glycemic index, which can be used to prevent obesity and related diseases (Nugent, 2005; Sestili et al., 2014).

Resistant starch also has beneficial physicochemical properties that can be exploited in food products. In baked goods, it is implicated in improving crispness, texture, and organoleptic qualities without the negative attributes that are often associated with high-fiber breads (Sajilata et al., 2006).

Amylose levels in the grain can be increased by down regulating genes involved in amyllopectin biosynthesis since both pathways share adenosine diphosphoglucose as a substrate (Regina et al., 2006; Sestili et al., 2010; Slade et al., 2012; Hazard et al., 2014). In wheat, STARCH BRANCHING ENZYME II (SBEII) catalyzes the addition of branch points during amyllopectin biosynthesis (Tetlow and Emes, 2014). Two paralogous copies of the *SBEII* genes, designated *SBEIIa* and *SBEIIb*, are present in each of the genomes of polyploid wheat species. Therefore, durum wheat (*T. turgidum* L. subsp. *durum*; genomes AABB) has four *SBEII* copies, and common wheat (*T. aestivum* L.; genomes AABBDD) has six. The *SBEIIa* and *SBEIIb* isoforms are closely linked on chromosome arms 2AL, 2BL and 2DL (Rahman et al., 2001; Regina et al., 2005; Hazard et al., 2012).

Development of ethyl methane sulfonate mutants for *SBEIIa* and *SBEIIb* genes in the tetraploid durum wheat cultivar Kronos using a TILLING (Targeted Induced Local Lesions in Genomes) population was previously described (Uauy et al., 2009; Hazard et al., 2012, 2014). Lines including combined mutations of the linked *SBEIIa* and *SBEIIb* genes in the A and B genomes showed dramatic increases in RS (753%) and amylose (66%) content. These
positive changes were also associated with a reduction of 7% in total starch (TS) and 8% in kernel weight (Hazard et al., 2014).

As common wheat represents approximately 95% of all wheat produced in the world (Taylor and Koo, 2015), the deployment of the SBEII mutations in the hexaploid background has potential to affect the health of a large number of people. Slade et al. (2012) developed a common wheat variety with combined mutations in the SBEIIa genes, which resulted in an increase in the contents of amylose (average 143%) and RS (average 1133%), but no public cultivars of common wheat with mutations in the SBEII genes are currently available.

The present study describes the registration of four mutant hexaploid wheat germplasm lines developed at the University of California, Davis, that carry mutations in linked SBEIIa and SBEIIb genes in the A and B genomes and the SBEIIa gene in the D genome. The lines were designated as SBEIIa/b-AB (Reg. No. GP-997, PI 675644), SBEIIa/b-A, SBEIIa-D (Reg. No. GP-998, PI 675645), SBEIIa/b-B, SBEIIa-D (Reg. No. GP-999, PI 675646) and SBEIIa/b-AB, SBEIIa-D (Reg. No. GP-1000, PI 675647). The effects of the mutations relative to a control with no SBEII mutations were determined for grain amylose, RS, and TS content and for flour viscosity parameters.

**Methods**

**Development of SBEII Germplasm Lines**

A TILLING population of the Desert durum cultivar Kronos (Arizona Plant Breeders Inc.) was screened for mutations in the SBEII genes (Uauy et al., 2009). Tetraploid wheat germplasm lines were previously developed that carried mutations in SBEIIa/b-A (mutated SBEIIa and SBEIIb genes in the A genome) and SBEIIa/b-B (mutated SBEIIa and SBEIIb genes in the B genome) (Hazard et al., 2012). These lines were backcrossed twice to ‘Lassik’ (PVP No. 200800176), a hard red spring cultivar developed by the University of California, Davis, from the cultivar Anza. Lassik was chosen for its broad adaptability, consistently high yield potential, and disease resistance. It carries three different resistant genes for stripe rust (Yr17, Yr18, and Yr36), two for leaf rust (Lr34 and Lr37), and one for stem rust resistance (Sr38). Lassik also exhibits good levels of resistance to fungi Mycosphaerella graminicola (Septoria tritici blotch) and Magnaporthe oryzae (Triticum pathotype, Cruz et al., 2016), Barley yellow dwarf virus, and root-knot nematodes Meloidogyne incognita and M. javanica (Williamson et al., 2013) Additionally, Lassik has higher grain protein content than Anza due to the presence of the high grain protein content gene (Gpc-B1) (Uauy et al., 2006) and stronger gluten due to the introgression of positive high molecular weight subunit alleles in the Glu-A1 and Glu-D1 loci.

Lines with linked mutations in the SBEIIa and SBEIIb genes in both the A and B genomes of tetraploid wheat developed previously (Hazard et al., 2012) were crossed with Lassik and then backcrossed to the same cultivar for two additional generations. Genotypes carrying linked SBEIIa and SBEIIb mutations in each genome (SBEIIa/b-A and SBEIIa/b-B) and in both genomes combined (SBEIIa/b-AB) were selected and backcrossed twice to Lassik.
The *SBEIIa* gene mutation in the D genome (*SBEIIa-D*) was selected from a TILLING population of the hard red spring common wheat breeding line UC1041+Gpc-B1/Yr36, developed by the University of California, Davis (Uauy et al., 2009). The *SBEIIa-D* mutant line was backcrossed to Lassik for five generations and the selected progeny crossed to the hexaploid lines described above. Plants with mutations in the following genomes were selected: A and B (*SBEIIa/b-AB*), A and D (*SBEIIa/b-A, SBEIIa-D*), B and D (*SBEIIa/b-B, SBEIIa-D*) and A, B, and D genomes combined (*SBEIIa/b-AB, SBEIIa-D*). The selected plants were self-pollinated and originated the germplasm lines registered herein. The crossing scheme used to develop the hexaploid Lassik line with five *SBEII* mutations (*SBEIIa/b-AB, SBEIIa-D*) is presented in Fig. 1; a summary of the mutations is presented in Table 1.

**Phenotypic Characterization of SBEII Germplasm Lines**

Mutant lines and a wild-type Lassik control were grown in a greenhouse and a field experiment. The greenhouse experiment was performed at the University of California Orchard Park Facility in Davis, CA. Seeds were sown in October 2014 in 2.4-L pots, and the experiment was set up using a randomized complete block design with six blocks (replications) and two plants per pot (experimental unit). The field experiment was grown in the University of California Experimental Field Station in Davis (38°32′ N, 121°46′ W). Seed was sown in November 2014 in a Yolo loam soil (fine-silty, mixed, superactive, nonacid, thermic Mollic Xerofluvents). The fertilization regime consisted of a preplanting application of 80 kg ha⁻¹ N and a top-dress application of 60 kg ha⁻¹ N at tillering. The experiment was arranged in a randomized complete block design with three blocks. In each block, 20 seeds per genotype were sown in 1-m rows (experimental unit).

Grains were manually harvested at maturity, and each sample was milled to produce whole grain flour in a UDY Cyclone Mill (UDY Corporation) using a 0.5-mm screen. Grain moisture was determined at the California Wheat Commission Milling and Baking Laboratory using the AOAC Official Method 925.10 (AOAC, 2000).

Analyses of the relative amylose content (amylose as a percentage of total starch) for 25-mg whole grain flour samples were conducted using the AMYLOSE/AMYLOPECTIN kit manufactured by Megazyme International (2014a), according to the protocol instructions. Resistant starch content was measured for 100-mg samples of whole grain flour samples using the RESISTANT STARCH kit developed by Megazyme International (2014b), following the manufacturer’s protocol. Total starch content was determined for 100-mg whole-grain flour samples using the TOTAL STARCH kit manufactured by Megazyme International (2014c), according to the protocol directions for the recommended KOH format. Rapid visco analyzer (RVA) parameters for flour samples from field-harvested grains were conducted according to AACC Method 76-21.01 (AACC International, 1999).

To consider the potential variation among assays performed on different days, one or two complete sets of samples representing all genotypes were analyzed on each day. Means of the individual mutant lines were compared with the Lassik control using Dunnett tests. For each experiment, the ANOVA assumption of normality of residuals was tested using...
Shapiro-Wilk’s test and the homogeneity of variances with Levene’s test. All statistical analyses were performed using SAS 9.4 (SAS Institute, 2015).

Characteristics

Amylose

We observed significant increases in relative amylose content for the quintuple mutant line (\textit{SBEIIa/b-AB, SBEIIa-D}) compared with the Lassik control (\(P < 0.01\)), both in the greenhouse (51%) and in the field (63%) (Table 2). A significant increase in amylose was also observed for the \textit{SBEIIa/b-AB} (16%) and \textit{SBEIIa/b-A, SBEIIa-D} (14%) lines in the field experiment. The same trend was observed for these lines in the greenhouse experiment, although without significant difference from the control (Table 2).

Resistant Starch

The quintuple mutant line (\textit{SBEIIa/b-AB, SBEIIa-D}) was the only genotype with a significant increase in RS content relative to the control; 947% in the greenhouse and 1057% in the field experiment (\(P < 0.01\)) (Table 2). The \textit{SBEIIa/b-AB} and \textit{SBEIIa/b-A, SBEIIa-D} lines showed increased values relative to the control, but the differences were not significant (Table 2).

Total Starch

Total starch content decreased by 7.8% (\(P < 0.01\)) for the quintuple mutant line (\textit{SBEIIa/b-AB, SBEIIa-D}) compared with the control in the field experiment (Table 2). The same trend was observed for this line in the greenhouse experiment (6.9% decrease) but without significant difference from the control.

Starch Viscosity

The quintuple mutant line (\textit{SBEIIa/b-AB, SBEIIa-D}) differed significantly from the control (\(P < 0.01\)) for all viscosity parameters except pasting temperature, for which a nonsignificant increase of 8.6% was detected (Table 3), and is easily distinguished from the other genotypes on the RVA viscograph (Fig. 2). The increased amylose content of the quintuple mutant line likely reduced the swelling of the starch granules, which may have caused the observed lower peak viscosity (−59%) (Tester and Morrison, 1990). We also observed a significantly lower breakdown value (−89%), which suggests an enhanced ability of the starch to withstand high temperatures and shear stress rates. Additionally, significant decreases in final viscosity (−50%) and setback values (−52%) were observed.

Discussion

Beneficial Effects Associated with the \textit{SBEII} Mutations

The quintuple mutant line with combined \textit{SBEIIa/b} mutations in the A and B genomes and a \textit{SBEIIa} mutation in the D genome (\textit{SBEIIa/b-AB, SBEIIa-D}) showed dramatic increases in amylose and RS relative to the wild-type Lassik control and, therefore, can be a useful tool for common wheat breeding programs aiming to increase grain amylose and RS content. To expedite the utilization of the mutations in common wheat breeding programs, we used the
recurrent parent Lassik, a hard red spring cultivar with high yield potential and broad adaptation. The increases in amylose content (51% greenhouse; 63% field) and RS (947% greenhouse; 1057% field) relative to the wild-type Lassik control are similar to those previously reported for the same SBEIIa/b-AB mutations in durum wheat line Kronos (Hazard et al., 2014); and for a different set of proprietary SBEIIa mutations in the common wheat cultivar Express (Slade et al., 2012).

Interestingly, large increases in amylose and RS were observed in the quintuple mutant despite the presence of a wild-type SBEIIb allele in the D genome. This may be associated with the lower expression levels of the SBEIIb genes in wheat relative to SBEIIa (Regina et al., 2005; Table 4). A stronger effect of SBEIIa relative to SBEIIb was also evident in the study of Slade et al. (2012), in which a common wheat line with mutated SBEIIa had significant increases in amylose despite the presence of wild-type SBEIIb alleles. A reanalysis of the RNAseq data generated by Choulet et al. (2014) during grain development (using WheatExp; Pearce et al., 2015, http://wheat.pw.usda.gov/WheatExp/) shows that the transcript levels of SBEIIb contributed by the D genome are eightfold lower than those contributed by the A genome and 2.5-fold lower than those contributed by the B genome (Table 4). Taken together, these results may explain the dramatic increase in amylose and RS in the quintuple mutant despite the presence of a functional wild-type copy of the SBEIIb gene in the D genome. To explore this further, a recently identified nonsense mutation in the SBEIIb gene of the D genome of the hexaploid cultivar Cadenza will be combined with the other five SBEII mutations to generate a sextuple mutant, SBEIIa/b-ABD.

In addition, the field study showed greater increases in grain amylose content in the triple-mutant lines including mutations in the A genome (SBEIIa/b-A, SBEIIa-D) than in those including mutations in the B genome (SBEIIa/b-B, SBEIIa-D) (Table 2). This difference may be due to the presence of a more efficient mutation in the A genome copy of SBEIIb (a truncation mutation) than in the B genome copy (an amino acid change, Table 1), or by differences in expression between the A and B genome homeologs. Reanalysis of the RNAseq data for grain development (Choulet et al., 2014) showed no significant differences in expression between the A and B genome homeologs of SBEIIa (Table 4). However, the transcript levels of the A genome copy of SBEIIb were 3.3-fold higher than those of the B-genome copy at the peak of expression (Table 4). The previous two hypotheses are not mutually exclusive, so both can contribute to the strongest effects observed in the SBEIIa/b-A mutants than in the SBEIIa/b-B mutants when combined with the SBEIIa-D mutation (Table 2).

**Detrimental Effects Associated with the SBEII Mutations**

Previous studies in tetraploid wheat have shown that large increases in amylose and RS are associated with a decrease in TS and potential yield penalties (Hazard et al., 2015). On average, the tetraploid SBEIIa/b-AB mutants showed a significant reduction of 5.2% in kernel weight and a nonsignificant decrease of 15.4% in grain yield relative to the wild-type control. We are currently performing similar field experiments for the hexaploid quintuple mutant; the results will be presented in a future study. If the hexaploid quintuple mutant...
shows similar reduction in grain yield, economic incentives may be required to motivate growers to grow high-RS common wheat cultivars.

The changes observed in the viscosity parameters of the quintuple mutant line can influence both the quality and functionality of the flour and should be considered for processing an end food product. Low values of setback, for instance, may indicate that the use of high amylose starch flour in frozen foods should be avoided due to a potential increase in syneresis (Nhan and Copeland, 2015).

**Future Directions**

More precise studies of the effect of the *SBEII* mutations on grain yield in different genetic backgrounds and in different environments are still required. This will be important to ameliorate some of the negative pleiotropic effects on grain yield associated with the *SBEIIa/b* mutations. Additional information is also required to determine the optimum levels of RS that can be incorporated without compromising the quality of the different wheat products (e.g., bread, tortillas, cookies, cakes). The public germplasm developed in this study will make these pending studies possible, and will likely stimulate the use of the *SBEIIa/b* mutations in common wheat breeding programs interested in the development of common wheat cultivars with increased RS.

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**Abbreviations**

- **RVA** rapid visco analyzer
- **RS** resistant starch
- **TILLING** Targeted Induced Local Lesions in Genomes
- **TS** total starch

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Fig. 1.
Crossing scheme used to introgress mutant SBEII alleles into the common wheat cultivar Lassik to develop the SBEIIa/b-AB, SBEIIa-D germplasm line. BC indicates backcross generations to Lassik; ⊗ indicates self-pollination. Mutations were selected in each cycle by sequencing the segregating lines.
Fig. 2.
Rapid visco analyzer (RVA) visco graph of whole wheat flour from the SBEII mutant lines and wild-type Lassik control. RVU, rapid visco units.
### Table 1
Summary of *SBEIIa* and *SBEIIb* mutations. Line ID indicates the mutant number in the TILLING population.

| Gene  | Genome | Line ID | DNA coordinates† | Type of mutation                        |
|-------|--------|---------|-------------------|-----------------------------------------|
| *SBEIIa* | A      | T4-2179 | G401A             | Premature stop codon                    |
| *SBEIIa* | B      | T4-1214 | G1347A            | Splice junction                         |
| *SBEIIb* | A      | T4-2574 | G308A             | Splice junction and stop                |
| *SBEIIb* | B      | T4-764  | C1290T            | Amino acid change (P283L)               |
| *SBEIIa* | D      | T6-630  | G850A             | Splice junction                         |

† DNA coordinates are based on partial genomic sequence used for TILLING (Uauy et al., 2009).
Table 2

Relative amylose, resistant starch (RS) and total starch (TS) contents of mutant SBEII lines and Lassik control. Untransformed arithmetic means and *P* values from Dunnett tests are presented separately for the greenhouse and field experiments.

| Line                  | Amylose | RS      | TS      |
|-----------------------|---------|---------|---------|
|                       | %       | —— g 100 g$^{-1}$ —— |
| **Greenhouse**        |         |         |         |
| Control               | 29.4 ± 0.8 | 0.17 ± 0.01 | 55.4 ± 0.5 |
| SBEIIa/b-AB           | 32.4 ± 1.0 ns | 0.22 ± 0.02 ns | 53.0 ± 0.7 ns |
| SBEIIa/b-A, SBEIIa-D  | 32.6 ± 0.8 ns | 0.23 ± 0.02 ns | 54.4 ± 1.4 ns |
| SBEIIa/b-B, SBEIIa-D  | 31.2 ± 1.2 ns | 0.16 ± 0.01 ns | 53.5 ± 0.7 ns |
| SBEIIa/b-AB, SBEIIa-D | 44.3 ± 1.4 ** | 1.78 ± 0.02 ** | 51.6 ± 1.4 ns |
| **Field**             |         |         |         |
| Control               | 30.7 ± 0.6 | 0.23 ± 0.03 | 60.1 ± 0.4 ns |
| SBEIIa/b-AB           | 35.6 ± 0.9 ** | 0.26 ± 0.01 ns | 58.9 ± 1.1 ns |
| SBEIIa/b-A, SBEIIa-D  | 34.9 ± 0.2 ** | 0.25 ± 0.01 ns | 59.3 ± 0.4 ns |
| SBEIIa/b-B, SBEIIa-D  | 31.6 ± 0.2 ns | 0.24 ± 0.03 ns | 60.2 ± 0.4 ns |
| SBEIIa/b-AB, SBEIIa-D | 50.0 ± 1.3 ** | 2.66 ± 0.07 ** | 55.4 ± 0.4 ** |

**P = 0.01 compared with control.
Table 3

Rapid visco analyzer parameters for the *SBEII* mutant lines and the wild-type Lassik control line in the field experiment. Untransformed arithmetic means and *P* values from Dunnett tests are reported.

| Parameter          | Unit | Control     | SBEIIa/b-AB | SBEIIa/b-a, SBEIIa-D | SBEIIa/b-B, SBEIIa-D | SBEIIa/b-AB, SBEIIa-D |
|--------------------|------|-------------|-------------|----------------------|----------------------|-----------------------|
| Peak               | RVU  | 77.8 ± 0.7  | 73.7 ± 0.6 ns | 77.8 ± 2.5 ns        | 86.6 ± 0.5 **        | 32.1 ± 0.9 **         |
| Trough             | RVU  | 57.6 ± 0.6  | 54.9 ± 0.7 ns | 58.8 ± 1.4 ns        | 59.2 ± 0.7 ns        | 29.9 ± 0.8 **         |
| Breakdown          | RVU  | 20.9 ± 0.5  | 18.8 ± 0.7 ns | 19.0 ± 1.2 ns        | 27.4 ± 0.4 **        | 2.2 ± 0.3 **          |
| Final viscosity    | RVU  | 118.1 ± 0.6 | 114.8 ± 1.6 ns | 127.0 ± 3.6 *       | 127.9 ± 0.8 *       | 59.1 ± 1.2 **         |
| Setback            | RVU  | 61.0 ± 0.8  | 59.9 ± 1.1 ns | 68.2 ± 2.3 *        | 68.7 ± 0.2 **        | 29.2 ± 0.9 **         |
| Peak time          | min  | 5.5 ± 0.04  | 5.5 ± 0.07 ns | 5.5 ± 0.04 ns       | 5.5 ± 0.02 ns       | 7.0 ± 0.02 **         |
| Pasting temp.      | °C   | 87.1 ± 0.7  | 79.7 ± 6.1 ns | 75.3 ± 6.0 ns       | 73.2 ± 1.5 ns       | 94.6 ± 0.01 ns        |

* *P* = 0.05.
** *P* = 0.01 compared with control.
† RVU, rapid visco units.
Table 4

Relative expression levels of *SBEIIa* and *SBEIIb* wheat homeologs in grain endosperm at three developmental stages. Reads per kilo base of transcript per million mapped reads (RPKM) data from RNA-seq ± SE of the mean (Choulet et al., 2014).

| Gene  | Genome | Ensembl ID          | RPKM Z71† | RPKM Z75‡ | RPKM Z85§ |
|-------|--------|---------------------|-----------|-----------|-----------|
| SBEIIa| A      | Traes_2AL_CC968FC52.2 | 124.7 ± 4.5 | 30.6 ± 1.3 | 17.2 ± 0.3 |
|       | B      | Traes_2BL_0E1E397A8.1 | 116.5 ± 3.2 | 28.8 ± 2.2 | 17.1 ± 0.3 |
|       | D      | Traes_2DL_647B61E84.1 | 71.4 ± 7.8  | 23.6 ± 1.0 | 7.6 ± 0.7  |
| SBEIIb| A      | Traes_2AL_FE11B55BA.1 | 0.34 ± 0.01 | 33.26 ± 0.90 | 0.10 ± 0.04 |
|       | B      | Traes_2BL_2C62185EE.1 | 0.80 ± 0.10 | 10.01 ± 0.12 | 0.05 ± 0.01 |
|       | D      | Traes_2DL_6CA449145.1 | 0.10 ± 0.00 | 4.07 ± 0.08  | 0.01 ± 0.01 |

† Zadok scale Z71 = kernel water ripe (no starch).
‡ Zadok scale Z75 = medium milk.
§ Zadok scale Z85 = soft dough.