Journal of Cereal Science 75 (2017) 165–174

Contents lists available at ScienceDirect

Journal of Cereal Science

journal homepage: www.elsevier.com/locate/jcs

# Combined mutations in five wheat *STARCH BRANCHING ENZYME II* genes improve resistant starch but affect grain yield and bread-making quality

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ARTICLE INFO

*Article history:*
Received 10 November 2016
Received in revised form
10 January 2017
Accepted 28 March 2017
Available online 8 April 2017

ABSTRACT

Increases in the proportion of amylose in the starch of wheat grains result in higher levels of resistant starch, a fermentable dietary fiber associated with human health benefits. The objective of this study was to assess the effect of combined mutations in five *STARCH BRANCHING ENZYME II* (*SBEII*) genes on starch composition, grain yield and bread-making quality in two hexaploid wheat varieties. Significantly higher amylose (~60%) and resistant starch content (10-fold) was detected in the *SBEII* mutants than in the wild-type controls. Mutant lines showed a significant decrease in total starch (6%), kernel weight (3%) and total grain yield (6%). Effects of the mutations in bread-making quality included increases in grain hardness, starch damage, water absorption and flour protein content; and reductions in flour extraction, farinograph development and stability times, starch viscosity, and loaf volume. Several traits showed significant interactions between genotypes, varieties, and environments, suggesting that some of the negative impacts of the combined *SBEII* mutations can be ameliorated by adequate selection of genetic background and growing location. The deployment of wheat varieties with increased resistant starch will likely require economic incentives to compensate growers and millers for the significant reductions detected in grain and flour yields.



## 1. Introduction

Wheat (*Triticum* spp.) is a major source of carbohydrates for human nutrition, representing nearly 20% of the ingestion of calories worldwide (FAOSTAT, 2015). The starch in the grain endosperm accounts for most of this intake and comprises two different types of polysaccharides. Amylose is a polymer of α-D-(1–4) linked D-glucose molecules with limited branching (20–30% of the grain starch), whereas amylopectin consists of chains of D-glucose that are highly branched through α-D-(1–6) linkages (70–80% of the grain starch) (Sharma et al., 2008).

The linear helical chains of amylose form complexes that limit access and digestion by amylases. Thus, high levels of amylose in the starch are associated with increased levels of resistant starch (RS), which is defined as the starch that resists digestion in the small intestine of healthy human individuals. Resistant starch acts as a prebiotic dietary fiber, and plays a beneficial role in human digestive physiology (Sharma et al., 2008). Given the importance of wheat in human nutrition, higher levels of RS in wheat products have the potential to deliver health benefits to a considerable fraction of the human population (Regina et al., 2006; Hazard et al., 2015). With increased awareness of the impact of diet on human health, many consumers are showing a growing interest in functional foods (Homayouni et al., 2014).

As RS reaches the colon, it is fermented by gut bacteria and yields short-chain fatty acids, in particular butyrate (Raigond et al., 2015). These substances are a major energy source for colonocytes and can thus improve mucosal integrity. Short-chain fatty acids also improve the lumen environment, making it less conducive to the formation of cancerous tumors (Chapman, 2003). As a result, long-term consumption of RS has the potential to prevent colorectal

*Abbreviations:* AACCI, American Association of Cereal Chemists International; ANOVA, analysis of variance; ANCOVA, analysis of covariance; BU, Brabender units; FN, falling number; MTI, mixing tolerance index; RS, resistant starch; RVA, rapid visco analyzer; RVU, rapid visco units; SKCS, single kernel characterization system; SRC, solvent retention capacity.

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http://dx.doi.org/10.1016/j.jcs.2017.03.028

cancer, the third most common cause of cancer-related mortality worldwide (Amini et al., 2016).

Resistant starch may also be implicated in the prevention of type 2 diabetes by an increase in insulin sensitivity (Robertson et al., 2005). Foods containing RS have lower glycemic index and rate of digestion, which leads to sustained and lower levels of glucose release into the bloodstream (Fuentes-Zaragoza et al., 2010; Wong and Louie, 2016). RS has also been reported to promote satiety and increase gut hormones that are effective in reducing energy intake, thus increased levels of RS in wheat products can contribute to reduce the occurrence of obesity (Keenan et al., 2006; Willis et al., 2009).

Down regulation of genes involved in amylopectin biosynthesis has been a useful strategy to increase the amount of amylose in the wheat starch (Regina et al., 2006; Sestili et al., 2010; Slade et al., 2012; Hazard et al., 2015; Schönhofen et al., 2016). We previously demonstrated that down regulation of five *STARCH BRANCHING ENZYME II* (*SBEII*) genes in common wheat led to an increase in the amount of grain amylose (63%) and resistant starch (1057%) (Schönhofen et al., 2016). However, a significant decrease in total starch (7.8%) and altered starch viscosity parameters suggested that further studies were necessary to understand the impact of the combined *SBEII* mutations on grain yield and bread-making quality.

The aim of this study was to quantify the impacts of the *SBEII* mutant alleles on common wheat grain yield and grain yield components as well as on grain, flour, starch and bread quality properties. The findings presented in this study provide valuable information for the deployment of common wheat varieties and products with increased resistant starch.

## 2. Materials and methods

### 2.1. Materials

The development of a hard red common wheat line carrying five loss-of-function mutations in the *STARCH BRANCHING ENZYME II* (*SBEII*) genes was previously described (Schönhofen et al., 2016). Mutant alleles of *SBEIIa* and *SBEIIb* genes in the A/B genomes and *SBEIIa* of the D genome (*sbeIIa/b*-AB, *sbeIIa*-D) were backcrossed five times (>98% identity) into the recurrent hard red spring variety Lassik (henceforth, Lassik *SBEII* quintuple mutant). This line has been deposited in the National Small Grain Collection (NSGC) under accession number PI 675647. For the present study, the same five mutations were backcrossed five times into Patwin-515HP, a hard white spring common wheat variety widely grown in California, carrying stripe rust resistance genes *Yr5* and *Yr15* and the high grain protein content gene *GPC-B1* (PVP No. 201600390). The resulting mutant line is >98% identical to the recurrent parent, and these two lines will be referred to hereafter as Patwin control and Patwin *SBEII* mutant for simplicity.

### 2.2. Experimental procedures

#### 2.2.1. Growth conditions

The Lassik and Patwin *SBEII* quintuple mutants were evaluated side by side their respective recurrent parents (wild-type alleles at all *SBEII* loci) in two locations in the 2015–2016 growing season. The first location was at the UC Experimental Field Station in Davis, CA (38° 32″ N, 121° 46″ W) in the Sacramento Valley. The second location was at the West Side Research and Extension Center (WSREC) in Five Points, CA (36° 20″ N, 120° 6″ W) in the San Joaquin Valley.

In the Sacramento Valley, plots were sown on November 7, 2015 at a density of 300 seeds/$m^2$ in a Yolo loam soil (fine-silty, mixed, superactive, nonacid, thermic Mollic Xerofluvents). Pre-planting fertilization consisted of 24 kg $ha^{-1}$ N followed by 24 kg $ha^{-1}$ N at tillering and irrigation consisted of two flood irrigations. In the San Joaquin Valley, seeds were sown on November 18, 2015 at 250 seeds/$m^2$ in a Panoche soil (fine-loamy, mixed, superactive, thermic Typic Haplocambid). The fertilization regime included four applications of 26 kg $ha^{-1}$ N (pre-planting, tillering, boot and flowering stages) and irrigation consisted of four sprinkler and four flood irrigations. The experimental units consisted of plots measuring 3.7 $m^2$ in the Sacramento Valley and 9.3 $m^2$ in the San Joaquin Valley.

#### 2.2.2. Yield and yield components

Spike density was estimated by selecting two 1 $m^2$ areas in each plot at random and counting the number of spikes before harvest. The number of spikelets per spike and the number of grains per spike were assessed by collecting ten spikes within each plot at random. Both locations were harvested in June 2016 and grain production per plot was determined and adjusted for plot length. Kernel weight was estimated based on a sample of 1000 grains.

#### 2.2.3. Quality evaluations

Grain evaluation, milling and quality determinations of flour and bread were performed at the California Wheat Commission Milling and Baking Lab (http://www.californiawheat.org/milling/). The analyses were conducted according to the standard American Association for Cereal Chemist International approved methods (AACCI Intl., Approved methods of analysis, 11th ed. AACC Intl., St. Paul) and are summarized in Supplementary Table 1.

Grain analyses included test weight, moisture, ash, protein, and kernel hardness. Wheat grains were tempered overnight to reach 14.5% moisture and were milled using AACCI Intl. approved method 26–21.02 in a Brabender Quadrumat Senior Mill. Total extraction and percent of white flour were recorded. Flour evaluations included moisture, ash, protein, wet gluten and gluten index, falling number and solvent retention capacity (SRC). Dough properties were assessed using a Brabender farinograph, and baking tests were performed using AACCI Intl. approved method 10–10.03. Two loaves per sample were produced using 200 g of flour and the water amount determined in the farinograph. Ascorbic acid, sugar and yeast solutions were added uniformly to all samples. After mixing to full development, dough fermentation and proofing time consisted of 90 and 42 min, respectively. Samples were baked for 23 min at 218 °C and were then removed from the oven and left to cool down to room temperature for 1 h. Loaf weights and volumes were recorded and crumb color determined using a Minolta Chroma Meter CR-310. Loaf symmetry, crumb structure and texture were scored using AACCI Intl. method 10–12.01 as a guideline. Bread blending tests were conducted by mixing flours from wild-type and mutant Patwin plants at 3:1, 1:1 and 1:3 ratios.

#### 2.2.4. Starch properties

Pasting curves for each sample were generated using a Rapid Visco Analyzer (RVA, AACCI Intl. Method 76–21.01, Supplementary Table 1). Relative amylose content, total starch, damaged starch and resistant starch were evaluated using kits developed by Megazyme International following the manufacturer's protocols (Supplementary Table 1). Relative amylose content (as a percent of total starch) was determined for 25 mg samples of white flour using the AMYLOSE/AMYLOPECTIN kit. Total starch content was evaluated in samples of 100 mg of white flour using the TOTAL STARCH kit (KOH format). Analyses of damaged starch in 100 mg white flour were conducted using the STARCH DAMAGE kit. Finally, resistant starch content was determined on samples of 100 mg white flour and 100 mg of ground bread crumb using the RESISTANT STARCH kit. For the bread crumbs, samples were freeze-dried using a