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Genome-wide association mapping of starch granule size distribution in common wheat

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ABSTRACT

Starch is a crucial component in wheat endosperm and plays an important role in processing quality. Endosperm of matured wheat grains contains two distinct starch granules (SG), referred to as larger A- and smaller B-granules. In the present study, 166 Chinese bread wheat cultivars planted in four environments were characterized for variation in SG size. A genome-wide association study (GWAS) using the 90 K SNP assay identified 23 loci for percentage volumes of A- and B-granules, and 25 loci for the ratio of A-/B-granules volumes, distributing on 15 chromosomes. Fifteen MTAs were associated with both the percentage volumes of A-, B-granules and the ratio of A-/B-granules volumes. MTAs *IWB34623* and *IWA3693* on chromosome 7A and *IWB22624* and *IWA4574* on chromosome 7B associated with the percentage volumes of A- and B-granules consistently identified in multiple environments were considered to be stable. Linear regression analysis showed a significantly negative correlation of the number of favorable alleles with the percentage volumes of A-granules and a significantly positive correlation between the number of favorable alleles and the percentage volumes of B-granules, respectively. The loci identified in this study and associated markers could provide basis for manipulating SG size to obtain superior noodle quality in wheat.

1. Introduction

Starch is a main component of wheat endosperm, accounting for 65–75% of dry weight and serving as a multifunctional ingredient for the food industries (Zhang et al., 2010). Starch granules (SG) are usually classified into two classes according to size, viz. larger A-granules (10–35 μm) and smaller B-granules (<10 μm) (Stoddard, 2003). Bechtel et al. (1990) proposed a third type, C-granules (<5 μm), which was controversially classified as B-granules because of the difficulty in defining a boundary between them. A-granules are disk-shaped, accounting for 70% of the wheat endosperm starch by weight and less than 10% by number, whereas B-granules are spherical or irregular, making up 30% by weight and up to 90% by number (Peng et al., 1999).

Different SGs have different physical, chemical, and functional properties. Starch amylose (Peterson and Fulcher, 2001; Li et al., 2008), gelatinization and swelling properties (Soh et al., 2006; Balmeet et al., 2007), rapid visco-anaylzer parameters (Geera et al., 2006; Kim and Huber, 2010) and rheological properties (Tang et al., 2001; Barrera et al., 2013) were all affected by granules size. B-granules have higher water adsorption than A-granules due to a less ordered arrangement of the polysaccharide chains in the smaller granules (Chiotelli and Meste, 2002). The differences of SG affect the quality of many final products. Park et al. (2005) showed that the bread with 30% small granules and 70% large granules had the highest crumb grain score and peak fineness value through reconstituted flour. Guo et al. (2014) reported that the content of B-granules was positively related to color, elasticity and smoothness of raw white noodles.

Abbreviations: GWAS, genome-wide association study; MAF, minor allele frequency; MAS, marker-assisted selection; MTA, marker-trait association; PIC, polymorphism information content; QTL, quantitative trait loci; SG, starch granule; SGP, starch granule bound protein; SNP, single nucleotide polymorphism.

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SG size is largely controlled by genetic factors (Peterson and Fulcher, 2001; Li et al., 2008), and to some extent, it is also influenced by environment (Xiong et al., 2014). Stoddard (2003) reported that percentage volumes of A- and B-granules in wheat starch are controlled by major and minor genes, respectively. Borém and Mather (1999) found three QTL on barley chromosomes 2H, 4H and 5H affecting SG. Batey et al. (2001) identified a QTL for percentage volume of B-granules on wheat chromosome 4B. Igrejas et al. (2002) confirmed one QTL on wheat chromosome 1B explaining 34% of the variation in B-granules volume percentage and two QTL on chromosomes 7A and 4D explaining 20% and 27% of variation in A-granule volume percentage, respectively. Howard et al. (2011) mapped a major QTL on chromosome 4S in *Ae. peregrina*, accounting for 44.4% of the variation in percentage volume of B-granules. Feng et al. (2013) detected three QTL on chromosomes 1D, 4A and 7B for percentage volume of A-granules.

All instances of QTL mapping for SG size were performed in biparental mapping populations where only two allelic effects can be evaluated in any single population. In contrast, genome-wide association (GWAS) is an efficient method to discover significant association between genotypes and phenotypes in germplasm (Hamblin et al., 2011). Such studies have been carried out in agronomic traits in wheat and were successful in identifying loci determining genetic factors affecting complex traits (Li et al., 2015; Rasheed et al., 2015). To date, no GWAS on wheat SG size has been reported. In the present study, a GWAS analysis of SG size was performed using a panel of 166 Chinese bread wheat cultivars and 18,207 mapped SNP markers present in the wheat 90 K iSelect chip. The aim was to identify loci associated with SG size and molecular markers for noodle quality improvement in bread wheat.

2. Materials and methods

2.1. Plant materials

A collection of 166 cultivars and advanced lines from the Yellow and Huai Valley Facultative Wheat Region was used for this study (Table S1). Field trials were conducted in randomized complete blocks with three replicates in Anyang (Henan province) and Suixi (Anhui province) during the 2012–2013 (recorded as Anyang, 2013 and Suixi, 2013) and 2013–2014 (recorded as Anyang, 2014 and Suixi, 2014) cropping seasons, providing data for four environments. Each plot contained three 2 m rows spaced 20 cm apart. Details on the experimental layout and agronomic practices were described earlier (Dong et al., 2016).

2.2. Genotyping and quality control

Genomic DNA was extracted by a modified method according to Lagudah et al. (1991), then samples were sent to Capital Bio Corporation (Beijing, China; www.capitalbio.com) for genotyping with the high-density illumina 90 K infinium SNP array (Wang et al., 2014). PowerMarker V3.2.5 was used to calculate gene diversity, minor allele frequency (MAF) and polymorphism information content (PIC). Markers were removed if they either had no position information on chromosomes, exhibiting more than 30% missing values, showing MAF of less than 5% or containing more than 10% of heterozygosis.

2.3. Milling

Grain hardness and water content were measured on 300-kernel samples with a Perten 4100 Single Kernel Characterization System (SKCS, Perten Instruments, Springfield, IL, USA) and a Near Infrared Reflectance (Foss, Högänas, Sweden) instrument, respectively. Soft, medium and hard wheats were tempered to 14.5%, 15.5% and 16.5% moisture overnight. Selected 100 g samples were milled using a Brabender Quadrumat Junior Mill (Brabender Inc., Duisberg, Germany), following American Association of Cereal Chemists (AACC) approved method 26–50. The ground flour passed through a 60-mesh screen, cooled immediately and stored at −20 °C until analyzed.

2.4. Starch extraction and SG size determination

Starch was extracted following Liu et al. (2007) with minor modifications, in which the tailings were centrifuged twice and all the starch was pooled. Dough was made with 6 g flour and 4 g distilled water, and allowed to stand for 10 min before being washed and kneaded with 60 ml water. The liquid component containing the starch was collected. The gluten component was washed, kneaded twice with 20 ml of distilled water until no more starch was extracted, and the liquid component was pooled with the earlier liquid extract. This starch suspension was filtered through a nylon cloth (75 μm openings) to remove impurities, centrifuged at 2500 × g for 15 min; supernatant was discarded and the residue moved into a new centrifuge tube. Twenty ml water was added into the lower lighter-colored portions, and stirred to a uniform mixture. These steps were repeated until there were no gray-colored tailings on the top of the starch. The extract portions were combined, frozen, lyophilized and ground lightly with a mortar and pestle to pass a 100-mesh screen.

SG sizes were determined using a Laser Diffraction Particle Size Analyzer (HELOS and RODOS, Japan Laser Co, Ltd., Tokyo, Japan). Particles of 10.0–35.0 μm and <10.0 μm are defined as A- and B-granules, respectively (Peng et al., 1999), and those of >35.0 μm are considered to be aggregate fraction. Each sample was assayed twice, and further tested if differences between two repeats were more than 0.5%. The contents of A- and B-granules were calculated according to the formula $\%A = 100 * A/(A + B)$ and $\%B = 100 * B/(A + B)$, respectively.

2.5. Statistical analysis

Analysis of variance and correlation coefficients among the four environments were performed using PROC GLM and CORR in SAS software version 9.2 (SAS Institute Inc, Cary, NC, USA). Least square means were calculated for each parameter and used to test the significance of differences ($P < 0.001$) between samples. The broad-sense heritability (h^2) was calculated following Lin and Allaire (1977).

2.6. Population structure analysis

Population structure was described earlier (Dong et al., 2016). Briefly, Structure v. 2.3.4 was used to estimate population structure based on 5624 SNP markers distributed across the entire genome using Bayesian cluster analysis (Pritchard et al., 2000). To ensure the sampling variance of inferred population structure, each K value was run repeatedly and independently. A range of K from 1 to 10 was based on admixture and correlated allele frequencies models. Each run was carried out with 10,000 replicates for the burn-in period and 100,000 replicates during analysis. Then the optimum value of K was chosen by the highest ΔK (Evanno et al., 2005).

2.7. Association analysis

SG size, genotype, population structure (Q-matrix) and relative kinship matrix (K-matrix) were implemented in TASSEL software