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RESEARCH ARTICLE

A dCAPS marker developed from a stress associated protein gene *TaSAP7-B* governing grain size and plant height in wheat

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Abstract

Stress associated proteins (SAPs) are the A20/AN1 zinc-finger proteins which confer to abiotic stresses in plants. In this study, *TaSAP7-B*, including two AN1 domains, was isolated from B genome of wheat (*Triticum aestivum* L.). Sequencing analysis on *TaSAP7-B* illustrated one InDel (insertion-deletion) and one SNP (single nucleotide polymorphism) in the promoter region while no diversity was observed in the coding region. On the basis of SNP in the promoter region (–260 bp), a dCAPS (derived cleaved amplified polymorphic sequences) marker SNP-260 was developed for *TaSAP7-B*. Using a natural population consisting of 262 wheat accessions, significant associations were detected between the marker SNP-260 and agronomic traits, such as plant height (PH), peduncle length (PL), length of penultimate internode (LPI), number of spike per plant (NSP), and 1 000-grain weight (TGW). Two genotypes were identified using marker SNP-260 in the natural population. Among them, the genotypes possessing C allele exhibited a higher TGW and shorter PH than the T genotypes. Hence, base C was considered as the superior allele. The dCAPS marker of *TaSAP7-B* can be instrumental for marker-assisted selection for high grain size and short plant height.

Keywords: *Triticum aestivum* L., *TaSAP7-B*, single nucleotide polymorphism, association analysis, plant height, 1 000-grain weight

1. Introduction

Stress-associated proteins (SAPs) are a class of zinc-finger proteins composed of A20/AN1 domains. These proteins have recently been identified as novel stress regulatory proteins in plants (Giri *et al.* 2013). SAP genes are widely distributed in plant species. At present, 14 SAP genes in *Arabidopsis*, 18 in rice and 11 in maize have been reported (Vij and Tyagi 2006; Jin *et al.* 2007; Liu *et al.* 2011). Majority of SAP genes have been found to be stress inducible and their over-expression in plants conferred tolerance to abiotic stresses (Huang *et al.* 2008; Ben Saad *et al.* 2010; Dixit and Dhankher 2011; Charrier *et al.* 2013). *OsSAP1* was the first reported SAP gene. It affected the expression of 43 endogenous genes involved in stress response and enhanced stress tolerance in rice and tobacco (Mukhopadhyay *et al.* 2004; Dansana *et al.* 2014). In the

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same way, *OsSAP8* and *OsSAP11* enhanced tolerance to high salt, drought, and cold stresses in transgenic plants (Kanneganti and Gupta 2008; Giri *et al.* 2011).

Wheat is a staple food commodity for more than one third global population. Its growth and development are often impacted by abiotic stress which leads to yield reduction. Exploitation and utilization of superior genes and allelic variations can be helpful approaches for improving wheat production. Enormous amount of allelic variations are present in wheat germplasms. Developing functional markers using these allelic variations will provide the basis for marker-assisted selection breeding. Association analysis possesses the advantages of shorter research time and higher mapping resolution, therefore it is considered as a powerful approach for identifying superior allelic variations (Myles *et al.* 2009).

Previous studies on SAPs focused on the domains composed of A20 and AN1 domains, but SAPs composed of two AN1 domains are still unknown. In this study, the polymorphism of the *TaSAP7-B*, a member of SAP gene family from B genome of wheat including two AN1 domains was detected by sequencing 32 wheat accessions. A dCAPS marker was developed based on the polymorphic site in the promoter region. Furthermore, association analysis between the marker and agronomic traits using a natural population consisted of 262 accessions was implemented. Consequently, the marker conferred to plant height (PH) and 1 000-grain weight (TGW) was developed for the molecular marker-assisted selection in the breeding program.

2. Materials and methods

2.1. Plant materials and measurement of agronomic traits

Hanxuan 10 is a drought-tolerant cultivar and used as the plant material for cloning gene *TaSAP7-B*. The nulli-tetrasomic lines of Chinese Spring wheat were used for chromosomal location. A doubled haploid (DH) population was used for genetic mapping. Thirty-two highly diverse accessions were used to identify nucleotide polymorphism of *TaSAP7-B*. Population 1 (262 winter wheat accessions) was initially used for association analysis. These accessions were mainly from the Northern Winter Wheat Zone and Yellow and Huai River Valleys Facultative Wheat Zone. This population was sown at Shunyi (40°23′N, 116°56′E) and Changping (40°13′N, 116°13′E), Beijing, over three growth cycles (2010–2013). Two water treatments, i.e., well-watered (WW) and rain-fed (drought stress, DS), were supplied at each site. The WW plots were irrigated with 750 m^3 ha^{-1} (75 mm) at the pre-overwintering, booting, flowering, and grain filling phases, though, the DS plots were rain-fed. The rainfall in three growing seasons was recorded as 131, 180, and 158 mm, respectively. Another two populations were also used to verify the results of initial association analysis, geographic distribution, and frequencies analysis of allelic variation. Population 2 (348 modern cultivars) and population 3 (157 landraces) were taken from Chinese wheat core collection and Chinese wheat mini-core collection, respectively (Hao *et al.* 2011). Population 2 was sown at Luoyang (36°41′N; 112°45′E) in Henan Province in 2002 and 2005, and at Shunyi, Beijing in 2010. All plant materials were sown in the first week of October and harvested in the following mid-June. Each experimental unit consists of four 2 m rows having 40 plants in each row. Row-to-row distance was maintained at 0.3 m. Agronomic traits of populations 1 and 2 were measured by random selection of five plants to calculate the mean in each accession, including PH, peduncle length (PL), length of penultimate internode (LPI), number of spike per plant (NSP), and TGW.

2.2. Cloning *TaSAP7-B* gene in wheat

Based on conserved sequence of the AN1 domain, reference sequence of *TaSAP7-B* was obtained from URGI (Unité de Recherche Génomique Info) website (https://urgi.versailles.inra.fr/blast/). Specific primers for B genome (Sap7BF, 5′-TATAGGAGAAACTCCGCGAG-3′ and Sap7BR, 5′-TGACACGTTGTAGATGAGTTC-3′) were designed to amplify *TaSAP7-B* gene from Hanxuan 10, including the 5 and 3 flanking regions.

2.3. dCAPS marker development

A pair of primer was developed based on the SNP site at –260 (C/T) in the promoter region of *TaSAP7-B*. The primers were named as MF1 (5′-TCCGGAGCTGACCGG ATCGATCCAGGAGC-3′) and MR1 (5′-CTTGCGTTCGGG TGCGAAG-3′). MF1 was designed by one base mismatching at –264 bp, then a restriction enzyme *Sac*I recognition site was produced. By two rounds of PCR, the first round was to amplify fragments of *TaSAP7-B* with the genomic-specific primer pair of Sap7BF/Sap7BR; the second round was performed as follows: first round PCR products were diluted 100 times, followed by taking 1 μL as template for the second round PCR using the primer pair MF1/MR1 (annealing at 57°C for 30 s, and extension at 72°C for 30 s). The PCR products were digested by *Sac*I and separated in 4% agarose gels.

2.4. Genetic mapping

A DH population (150 lines) was established from the