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Variation in abscisic acid responsiveness at the early seedling stage is related to line differences in seed dormancy and in expression of genes involved in abscisic acid responses in common wheat

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ABSTRACT

Abiotic stresses and pre-harvest sprouting reduce grain yield and quality. ABA plays important roles in regulation of abiotic stress tolerance and seed dormancy. We show wide genetic variation in ABA responsiveness evaluated as root growth inhibition under exogenous ABA treatment using a core collection of hexaploid wheat with 186 accessions. Significantly higher ABA responsiveness was observed in Japanese wheat cultivars, which included some dormant seed-producing cultivars. Transcript accumulation of a *WABI5* transcription factor and its downstream *Cor/Lea* genes was more abundant in the highly ABA-responsive lines than in the less responsive lines after ABA treatment. The germination index and α-amylase activity tended to be higher in seeds of the low ABA-responsive lines than in those of highly ABA-responsive lines for 30–50 days post-anthesis. Significant correlations among ABA responsiveness, germination index and α-amylase activity were confirmed for the KU-3162/Chinese Spring mapping population. Two QTLs for ABA responsiveness were found on chromosomes 2A and 4A in this population. The 2A QTL could regulate *WABI5* expression and accumulation of its downstream COR/LEA proteins through *TaAFP*, a putative negative regulator of *WABI5*. The core collection of hexaploid wheat is thus useful for identification and functional characterization of QTLs for ABA responsiveness.

1. Introduction

The phytohormone abscisic acid (ABA) plays important roles in various signal transduction pathways related to environmental stress responses and to seed maturation and dormancy (Finkelstein et al., 2002). Under abiotic stress conditions, ABA induces expression of a variety of genes, including the *cold-responsive* (*Cor*)/*late embryogenesis abundant* (*Lea*) genes, that function in stress tolerance; the COR/LEA proteins promote abiotic stress tolerance by protecting cellular components from environmental stresses (Yamaguchi-Shinozaki and Shinozaki, 2006). Expression of these *Cor/Lea* genes is regulated by major transcription factors in the C-repeat binding factor (CBF)/dehydration responsive element binding protein (DREB) and ABA-responsive element binding protein (AREB)/ABA-responsive element binding factor (ABF) families (Yamaguchi-Shinozaki and Shinozaki, 2006).

In common wheat (*Triticum aestivum* L.), C-repeat/dehydration-responsive element (DRE), ABA-responsive element (ABRE) and other cold-responsive motifs have been identified in the promoter regions of several wheat *Cor/Lea* genes (Kobayashi et al., 2008a). These *Cor/Lea* genes are commonly responsive to low temperature, drought and ABA, and good correlations are observed between the levels of their transcripts and the levels of freezing tolerance during cold acclimation (Kobayashi et al., 2006). Wheat bZIP-type transcription factors WABI5 and WLIP19 and ethylene-responsive factor/APETALA2 domain-containing transcription factor WDREB2 activate the *Cor/Lea* genes (Kobayashi et al., 2008a, 2008b). *Cor/Lea* expression patterns and basal levels of freezing tolerance are

Abbreviations: ABA, abscisic acid; ABA8′OH, ABA 8′-hydroxylase; ABI, ABA-insensitive; AFP, ABI five binding protein; bZIP, basic region leucine zipper; Cor, cold responsive; CS, Chinese Spring; DOG, Delay Of Germination; GI, germination index; Lea, late embryogenesis abundant; LOD, log-likelihood; QTL, quantitative trait locus; RAB, responsive to ABA; SSR, simple sequence repeat.

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altered in wheat mutants for ABA sensitivity (Kobayashi et al., 2006). Other ABA-signaling components including two *SnRK2* genes, *PKABA1* (Johnson et al., 2002) and *TaSnRK2.4* (Mao et al., 2010), functioning in abiotic stress responses of common wheat have been cloned. Thus, ABA sensitivity is at least partly related to ABA-responsive gene expression patterns and abiotic stress tolerance in wheat.

Pre-harvest sprouting, or premature germination of seeds in the spikes before harvest, is one of the important targets for wheat breeding. Alteration of seed dormancy levels is one efficient way to avoid pre-harvest sprouting, and induction and maintenance of seed dormancy are related to the ABA level and ABA signaling in seeds (Gubler et al., 2005). Reduced seed dormancy mutants have decreased ABA sensitivity in common wheat, and levels of seed dormancy and pre-harvest sprouting are altered in ABA-responsive mutants (Rikiishi and Maekawa, 2010). Thus, ABA-responsive pathways might participate in the processes of seed dormancy and pre-harvest sprouting. Recently, common wheat homologs of several ABA-signaling components identified in *Arabidopsis* and rice seeds have been confirmed to function in seed dormancy and pre-harvest sprouting (Ashikawa et al., 2010; Rikiishi and Maekawa, 2014). *DELAY OF GERMINATION1* (*DOG1*) has been identified as explaining the natural variation in seed dormancy of *Arabidopsis*, and *DOG1* is involved in the B3 transcription factor-regulated pathway controlling seed maturation and dormancy (Bentsink et al., 2006; Nakabayashi et al., 2012). Wheat *DOG1* homologs are also related to the regulation of seed dormancy and germination (Ashikawa et al., 2010), and their transcript level in seeds is a good marker for evaluating the degree of seed dormancy of wheat cultivars (Rikiishi and Maekawa, 2014). *Arabidopsis* ABI3 B3 transcription factor regulates establishment of seed dormancy and is required for appropriate *ABI5* expression (Finkelstein and Lynch, 2000), and ABI5 transcription factor is related to post-germination ABA-dependent growth (Lopez-Molina et al., 2002). Therefore, the wheat *ABI5* homolog, *WABI5*, could be a good marker for estimating the strength of seed dormancy as well as tolerance to dehydration stress. In *Arabidopsis*, an ABI5-interacting protein, ABI five binding protein (AFP), attenuates *ABI5*-mediated ABA signaling (Lopez-Molina et al., 2003). Transcripts of wheat *AFP* homologs assigned to homoeologous group 2 chromosomes are accumulated in maturing seeds of common wheat (Ohnishi et al., 2008).

The catabolism of ABA is a crucial step in dormancy release in several plant species (Gubler et al., 2005). The hydroxylation of ABA at the 8'-position, catalyzed by a cytochrome P450 with ABA 8'-hydroxylase activity (ABA8′OH), is the key step of ABA inactivation. In wheat, two genes encoding ABA8′OH have been reported (Nakamura et al., 2010; Chono et al., 2013; Son et al., 2016). *TaABA8′OH1* has been located on the long arm of homoeologous group 6 chromosomes, and a role in seed dormancy is suggested (Chono et al., 2013), whereas *TmABA8′OH2* has been mapped to the centromeric region of chromosome $5A^m$ (Nakamura et al., 2010). A deleterious mutation of the A genome copy of *TaABA8′OH1* results in reduced ABA catabolism and low germination as well as low *TaABA8′OH1* expression (Chono et al., 2013). In addition, *TaABA8′OH* transcript levels are correlated with ABA responsiveness in seedlings of various wheat lines (Iehisa et al., 2014), and ectopic expression of *TaABA8′OH* genes altered seed ABA levels and dormancy in *Arabidopsis* (Son et al., 2016).

Line differences in responses to exogenous ABA have been reported in common wheat (Kobayashi et al., 2010; Iehisa et al., 2011, 2014). The ABA responsiveness is conveniently assessed with monitoring the relative root growth inhibition rates under the ABA-treated condition, and the root growth inhibition rates are significantly correlated to the relative shoot growth inhibition rates (Iehisa et al., 2011, 2014). Quantitative trait locus (QTL) analysis for ABA responsiveness at the seedling stage was conducted in recombinant inbred lines derived from a cross between two common wheat cultivars, Chinese Spring (CS) (a low ABA-responsive variety) and Mironovskaya 808 (M808) (a highly ABA-responsive variety), and five significant QTLs were found, with 2A, 3A, 6D and 7B QTLs regulating both root-growth arrest and *Cor/Lea* gene expression in ABA-treated seedlings (Kobayashi et al., 2010). A QTL for ABA responsiveness was detected on chromosome 5A using an F_2 mapping population between another wheat cultivar, Hope (a highly ABA-responsive variety), and CS (Iehisa et al., 2014). At the 5A QTL, F_2 individuals with the high ABA-responsive allele tend to show high dehydration tolerance and low seed dormancy (Iehisa et al., 2014), implying that at least some of the QTLs for ABA responsiveness at the post-germination stage could control dehydration and pre-harvest sprouting tolerance. Here, we show variation in the ABA responsiveness among hexaploid wheat varieties. We selected varieties with much lower ABA responsiveness compared with that of CS, identifying a new QTL for ABA responsiveness. Based on these studies, we discuss the relationship among ABA responsiveness at the post-germination stage, ABA-responsive gene expression and seed dormancy.

2. Materials and methods

2.1. Plant materials

In total, 186 accessions from the hexaploid wheat (*Triticum aestivum* L.) core collection recently established by the National BioResource Project (NBRP)-KOMUGI (http://www.shigen.nig.ac.jp/wheat/komugi) (Takenaka et al., in preparation) were used (Suppl. Table S1), and seeds of these accessions were supplied from NBRP-KOMUGI. The core collection included 35 Japanese wheat varieties, and most of the other accessions (KU accessions) originating from various habitats were stocked in the Plant Germ-plasm Institute, Kyoto University. These accessions were grown in an experimental field under a plastic roof for protection from rainfall at Kobe University in the 2011–2012 season to obtain selfed seed. The seeds of the core collection were used for analyses of variation in ABA responsiveness. In the 2012–2013 season, 99 F_2 individuals from a cross between KU-3162 (a low ABA-responsive accession) and CS (a highly ABA-responsive accession), as well as the parental lines, were grown in a glasshouse at Kobe University, and the selfed seeds were used for QTL analysis. The F_2-derived F_3 ($F_{2:3}$) generation of the ten selected F_2 individuals from the KU-3162/CS population were also grown in the glasshouse in the 2013–2014 season.

2.2. Bioassay for ABA responsiveness

Bioassays were performed using the 186 wheat varieties and the $F_{2:3}$ generation as in our previous study (Iehisa et al., 2014). Mature seeds were stored for more than three months before bioassay. Relative growth inhibition was calculated as the difference between growth of the control group and growth of the ABA-treated group relative to the control. The average value for $F_{2:3}$ individuals was used for their respective F_2 individuals in QTL analysis. The data were statistically analyzed using JMP software version 5.1.2 (SAS Institute, Cary, NC, USA). The correlations among the examined traits were estimated based on Pearson's correlation coefficient values.

2.3. Measurement of flowering-related traits

Three flowering-related traits of the 99 F_2 individuals of the KU-3162/CS population were measured in the glasshouse. Heading and flowering times were recorded as days after sowing. Maturation