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# Identification of quantitative trait locus for abscisic acid responsiveness on chromosome 5A and association with dehydration tolerance in common wheat seedlings

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ABSTRACT

The phytohormone abscisic acid (ABA) plays important roles in response to environmental stress as well as in seed maturation and dormancy. In common wheat, quantitative trait loci (QTLs) for ABA responsiveness at the seedling stage have been reported on chromosomes 1B, 2A, 3A, 6D and 7B. In this study, we identified a novel QTL for ABA responsiveness on chromosome 5A using an $F_2$ population derived from a cross between the common wheat cultivar Chinese Spring (CS) and a chromosome substitution line of CS with chromosome 5A of cultivar Hope (Hope5A). This QTL was found in a similar chromosomal region to previously reported QTLs for drought tolerance and seed dormancy. Physiological characterization of the QTL revealed a small effect on dehydration tolerance and seed dormancy. The rate of water loss from leaves during dehydration was lower, and transcript accumulation of the cold responsive (COR)/late embryogenesis abundant (LEA) genes Wrab18 and Wdhn13 tended to be higher under dehydration stress in $F_2$ individuals carrying the Hope allele of the QTL, which also showed higher ABA responsiveness than the CS allele-carrying individuals. Seed dormancy of individuals carrying the Hope allele also tended to be lower than those carrying the CS allele. Our results suggest that variation in ABA responsiveness among common wheat cultivars is at least partly determined by the 5A QTL, and that this QTL contributes to development of dehydration and preharvest sprouting tolerance.



## Introduction

The phytohormone abscisic acid (ABA) serves as an endogenous messenger to mediate responses to environmental stress; it is also involved in plant growth and development, including seed maturation and dormancy (Finkelstein et al., 2002). During abiotic stress, ABA induces expression of a variety of genes that function in the regulation of gene expression, signal transduction and stress tolerance (Yamaguchi-Shinozaki and Shinozaki, 2006). One family of activated genes encodes the cold responsive (COR)/late embryogenesis abundant (LEA) proteins, which promote stress tolerance by protecting cellular components from environmental stress (Thomashow, 1999).

In common wheat, cold-responsive motifs such as C-repeat/dehydration-responsive element (DRE), ABA-responsive element and others have been identified in the promoter regions of four *Cor/Lea* genes, *Wdhn13*, *Wrab17*, *Wrab18* and *Wrab19* (Kobayashi et al., 2008a). These *Cor/Lea* genes are responsive to low temperature, drought and ABA (Ohno et al., 2003; Kobayashi et al., 2004, 2006; Egawa et al., 2006). Some of the transcription factors acting in ABA signaling are bZIP-type transcription factors WLIP19 (Kobayashi et al., 2008b) and WABI5 (Kobayashi et al., 2008c), and ethylene-responsive factor/APETALA2 domain-containing transcription factors WDREB2 (Egawa et al., 2006) and TaDREB1 (Shen et al., 2003). *Trans*-activation analysis of *Cor/Lea* gene promoters revealed that *Wdhn13*, *Wrab18* and *Wrab19* expression is induced by WABI5, WLIP19 and WDREB2, whereas *Wrab17* expression is activated by WLIP19 and WDREB2 but not by WABI5 (Kobayashi et al., 2008a,b,c).

In crop species, such as wheat, seed dormancy must be high enough to prevent preharvest sprouting and low enough to promote uniform germination. The catabolism of ABA is a crucial step in dormancy release in several plant species (Gubler et al.,

*Abbreviations:* ABA, abscisic acid; ABA8′OH, ABA 8′-hydroxylase activity; Cor, cold responsive; CS, Chinese Spring; DRE, dehydration-responsive element; DPA, days post-anthesis; GI, germination index; Hope5A, a CS chromosome substitution line with chromosome 5A of Hope; LEA, late embryogenesis abundant; LOD, log-likelihood; QTL, quantitative trait locus; RL, root length; RRGI, relative root growth inhibition; RSGI, relative shoot growth inhibition; SL, shoot length; SSR, simple sequence repeat.

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2005). The hydroxylation of ABA at the 8′-position, catalyzed by a cytochrome P450 with ABA 8′-hydroxylase activity (ABA8′OH), is a key step of ABA inactivation. In both wheat and barley, two genes encoding ABA8′OH have been reported (Millar et al., 2006; Nakamura et al., 2010; Chono et al., 2013). *TaABA8′OH1* is on the long arm of homoeologous group 6 chromosomes and an important role has been suggested for it in wheat seed dormancy (Chono et al., 2013). On the other hand, an einkorn wheat ABA8′OH gene, *TmABA8′OH2*, has been mapped to the centromeric region of chromosome $5A^m$. Using a mapping population of recombinant inbred lines derived from a cross between two einkorn wheat accessions of *Triticum monococcum* L. and *Triticum boeoticum* Boiss., *TmABA8′OH2* was found to be closely linked to the chromosomal region of quantitative trait locus (QTL) for seed dormancy (Nakamura et al., 2010).

In our previous study, the chromosomes involved in the regulation of ABA responsiveness and *Cor/Lea* expression were identified using two sets of chromosome substitution lines in common wheat (Iehisa et al., 2011). In these lines, a chromosome pair of the common cultivar Chinese Spring (CS) was substituted with the corresponding homologous pair of a different cultivar, Cheyenne or Hope. Substitution of CS chromosomes 3A or 5A with the respective homologous pair from Cheyenne or Hope enhanced ABA responsiveness and *Cor/Lea* expression at the seedling stage (Iehisa et al., 2011). These two chromosomes were also found to be involved in the regulation of stomatal response during leaf dehydration. In a previous study using recombinant inbred lines of common wheat, QTLs for ABA responsiveness at the seedling stage were reported on chromosomes 1B, 2A, 3A, 6D and 7B (Kobayashi et al., 2010). The objective of the present study was to clarify the relationship between a genetic locus controlling ABA responsiveness and abiotic stress tolerance. Here, we identified a novel QTL for ABA responsiveness at the seedling stage using an $F_2$ population derived from a cross between CS and a chromosome substitution line derived from it carrying chromosome 5A of Hope (Hope5A), and studied the effect of this QTL on abiotic stress tolerance and seed dormancy.

## Materials and methods

### *Plant materials*

The mapping population of common wheat (*Triticum aestivum* L.) used in this study included 110 $F_2$ individuals derived from a cross between a cultivar Chinese Spring (CS) and its chromosome substitution line with chromosome 5A of cultivar Hope (Hope5A). The Hope5A substitution line consisted of the CS background (recipient), in which a chromosome 5A pair was replaced by the corresponding homologous pair of the donor cultivar Hope. The CS-Hope substitution lines were developed by E. R. Sears at the University of Missouri, as described in a previous study (Law and Worland, 1996). Seeds of the substitution line (KT733) were supplied by the National BioResource Project: Wheat, Japan (http://www.nbrp.jp/report/reportProject.jsp?project=wheat).

### *Bioassay for abscisic acid (ABA) responsiveness*

Bioassays were performed using the $F_3$ generation. After imbibition of seeds in tap water for 5 h, they were incubated overnight at 4 °C. Seeds were germinated for 24 h at 24 °C in darkness and then five to six synchronously germinating seeds were placed in plastic Petri dishes containing two overlapping sheets of filter paper wetted with 6 mL of distilled water (control) or 20 μM (±)-ABA (98% purity) (Sigma–Aldrich, St. Louis, MO, USA), then incubated at 24 °C in darkness. After 48 h, the length of shoots and primary roots was recorded in four to five independent experiments. At least 20 seeds each of control and ABA-treated groups were used. The average for $F_{2:3}$ individuals was used as the value for the respective $F_2$ individuals in QTL analysis. Relative growth inhibition was calculated as the difference in growth of the ABA-treated group relative to the control. The data were statistically analyzed using JMP software ver. 5.1.2 (SAS Institute, Cary, NC, USA). Correlations among the examined traits were estimated based on Pearson correlation coefficient values.

### *Genetic map construction and QTL analysis*

A total of 65 SSR markers was used to screen the parental lines for polymorphism. Total DNA was extracted from the parents and $F_2$ individuals using standard procedures. For SSR genotyping, 40 cycles of PCR were performed using 2× Quick Taq HS DyeMix (Toyobo, Osaka, Japan) and the following conditions: 10 s at 94 °C, 30 s at the annealing temperature, and 30 s at 68 °C. The last step was incubation for 1 min at 68 °C. Information on the SSR markers and their annealing temperatures was obtained from the National BioResource Project KOMUGI web site (http://www.shigen.nig.ac.jp/wheat/komugi/strains/aboutNbrpMarker.jsp) and the GrainGenes web site (http://wheat.pw.usda.gov/GG2/maps.shtml). The PCR products were separated by electrophoresis on 2% agarose or 13% nondenaturing polyacrylamide gels and stained with ethidium bromide. For polyacrylamide gel electrophoresis, the high efficiency genome scanning system (Nippon Eido, Tokyo, Japan) of Hori et al. (2003) was used. Genetic mapping was performed using MAPMAKER/EXP version 3.0b (Lander et al., 1987) and drawn in MapChart version 2.2 software (Voorrips, 2002). The threshold for log-likelihood (LOD) scores was set at 3.0, and genetic distances were calculated with the Kosambi function (Kosambi, 1944).

QTL analysis was carried out by composite interval mapping with Windows QTL Cartographer ver. 2.5 software (Wang et al., 2011) using the forward and backward method. An log-likelihood (LOD) score threshold for each trait was determined by computing a 1000 permutation test. The percentage of phenotypic variation explained by a QTL for a trait and any additive effects were also estimated using this software.

### *Expression analysis of genes involved in cell cycle regulation or inducible by ABA*

For expression analysis at the seedling stage, seeds ($F_3$ generation) were pregerminated and treated with ABA for bioassays. After 48 h of ABA treatment, total RNA was extracted from roots and shoots.

For analysis of expression during dehydration, $F_{2:3}$ seedlings were grown in soil at 24 °C under constant light. Seven-day-old seedlings were removed from soil and after washing their roots, they were kept on dry filter paper under standard conditions. Total RNA was extracted from leaves at various times after dehydration using Sepasol-RNA I (Nacalai Tesque, Kyoto, Japan). First-strand cDNA was synthesized from DNase I-treated mRNA samples with oligo-dT primers using high fidelity ReverTra Ace reverse transcriptase (Toyobo).

The transcript level of each gene was measured by quantitative RT-PCR using a LightCycler 480 Real-Time PCR System (Roche Diagnostics, Mannheim, Germany) with the gene-specific primer sets given in Table S1. The *Actin* gene was used as an internal control. The rate of amplification was monitored using Thunderbird SYBR qPCR mix (Toyobo) according to the manufacturer's protocol. The relative expression level was calculated as $2^{-\Delta\Delta Ct}$, where $\Delta Ct$ is the difference in number of PCR cycles required to reach the log phase of amplification of the target gene relative to *Actin*.