Characterization of ABA sensitivity in mutants with altered abiotic stress tolerance in common wheat

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INTRODUCTION

ABA, a phytohormone, regulates many agronomically important aspects of plant growth and development, including seed maturation, dormancy and stress tolerance (Finkelstein et al. 2002). During vegetative growth, endogenous ABA levels increase upon conditions of water stress, and ABA acts as an essential mediator in triggering the plant response to these adverse environmental stresses (Finkelstein et al. 2002). Under low temperature conditions, although a non-ABA-regulated pathway acts as a major stress signal transduction mechanism (Shinozaki and Yamaguchi-Shinozaki 2000), cold/freezing and ABA regulatory pathways are not completely independent. In *Arabidopsis thaliana*, ABA-insensitive *abi1* mutant reduces the accumulation levels of drought/cold-induced and ABA-regulated *RAB18* transcripts, which resulted in its decreased freezing tolerance (Mäntylä et al. 1995). *firery1*, which is an ABA-hypersensitive mutant of *Arabidopsis*, showed higher freezing tolerance than wild-type without cold acclimation (Xiong et al. 2001).

In wheat, ABA-responsive *Wdreb2*, *Wlip19* and *Wabi5* encoding transcription factors are involved in cold acclimation and development of freezing tolerance through regulation of their downstream *Cor* (cold-responsive)/*Lea* (late-embryogenesis abundant) gene expression (Kobayashi et al. 2007, 2008a, b).

A mutant line EH47-1 was originally derived from ethylmethane sulfonate (EMS)-treated seeds of the common wheat cultivar 'Kitakei-1354 (Kitakei)' and selected as an ABA-insensitive mutant during seed maturation (Kawakami et al. 1997). EH47-1 was less-sensitive to exogenous ABA at the seedling stage, but the transcripts of ABA-responsive genes are more abundantly accumulated by ABA treatment than in Kitakei (Kobayashi et al. 2006). EH47-1 showed a significantly higher freezing tolerance than Kitakei at least in the seedlings without cold acclimation, although this mutation did not impair the cold acclimation ability *per se* of the mutant. EH47-1 mutant allele has no influences on the expression levels of cold-responsive transcription factors and *Cor/Lea* genes under low temperature conditions.

Other mutant lines, 'Mutant ABA 27 (ABA27)' and 'Mutant ABA 90 (ABA90)', were generated from the common wheat cultivar 'Chihoku-komugi (Chihoku)', and the ABA sensitivities of ABA27 and ABA90 are respectively higher and lower than that of Chihoku based on the inhibition rate of seedling growth by exogenous ABA (Kobayashi et al. 2008c; Kobayashi and Takumi 2007). ABA27 showed significantly increased freezing tolerance in seedlings with and without cold acclimation and activated gene expression of *Wabi5* and *Cor/Lea* such as *Wrab15* and *Wrab18* under normal temperature condition and early cold acclimation period (Kobayashi et al. 2008c). On the other hand, ABA90 showed lower freezing tolerance than Chihoku after cold acclimation, and slight differences were observed in gene expression at some time points of cold acclimation (Kobayashi and Takumi 2007). These results suggest that ABA sensitivity is associated with both determinations of the basal level of freezing tolerance and enhancement of freezing tolerance via cold acclimation including the activation of *Cor/Lea* genes in wheat.

To obtain further information on the role of ABA in cold acclimation and freezing tolerance in wheat, we analyzed other Chihoku-derived mutant lines, 'Mutant ABA 31 (ABA31)' and 'Mutant ABA 59 (ABA59)', besides ABA27 and ABA90. In addition to these mutants, 'Mutant ABA 122 (ABA122)' and 'Mutant ABA 126 (ABA126)' derived from common wheat cultivar 'Horoshiri-komugi (Horoshiri)' were also characterized.

MATERIALS AND METHODS

The mutant lines, ABA27, ABA31, ABA59, ABA90, ABA122 and ABA126, were registered in database of National BioResource Project (NBRP)-Wheat (http://www.shigen.nig.ac.jp/wheat/komugi/top/top.jsp). ABA27, ABA31, ABA59 and ABA90 were derived from Chihoku, and ABA122 and ABA126 were from Horoshiri after EMS mutagenesis in 1986 (Dr. Noda, personal communication). The mutants and their parental lines were used for bioassays for ABA sensitivity during germination and post-germination growth. Conditions for the bioassays were according to Kobayashi et al. (2008c). Transcript levels of nine wheat ABA-responsive genes were studied by RT-PCR using the corresponding gene specific primer sets. Seven-day-old seedlings of the mutants and their parental lines grown under standard conditions (20˚C) according to Kobayashi et al. (2006) were treated with a solution containing 20 μM ABA by a foliar spray. Total RNA was extracted from seedlings as previously reported (Kobayashi et al. 2006).

RESULTS AND DISCUSSION

Morphological differences between Chihoku and its derived mutants were found in their spikes (Table 1). ABA27 and ABA31 have longer and ABA59 has shorter spikes than Chihoku, although no significant difference was observed in spike length of ABA90, ABA122 and

Table 1. ABA-sensitivity mutant lines registered in NBRP-Wheat and their morphological and agricultural characteristics

Strain name	Strain ID	Spike length (cm)	Seed length (cm)	Kernel shape (% width/length)	1000 kernel weight (g)	Days to heading (2008, Tsukuba)	Days to flowering (2008, Tsukuba)
Chihoku-komugi (Chihoku)	KT020-124	9.13±0.94	0.65±0.015	56.2±1.98	53.03	138.75±0.96	142.75±0.96
Mutant ABA 27 (ABA27)	KT020-133	11.3±0.64**	0.74±0.017**	42.88±1.46**	52.35	138.25±0.5	143±0
Mutant ABA 31 (ABA31)	KT020-134	10.95±0.7**	0.69±0.022**	43.39±1.73**	46.55	138±0	143±0
Mutant ABA 59 (ABA59)	KT020-135	7.93±0.65**	0.6±0.019**	57.3±2.31	43.32	142±2.83	145±1.41
Mutant ABA 90 (ABA90)	KT020-136	8.78±0.81	0.62±0.021**	56.62±2.5	47.85	140.25±2.5	143±2
Horoshiri-komugi (Horoshiri)	KT020-063	10.65±0.62	0.67±0.02	33±2.28	24.59	142.25±4.72	144.75±3.4
Mutant ABA 122 (ABA122)	KT020-137	10.33±0.65	0.69±0.017*	43.37±1.21**	37.68	152	153
Mutant ABA 126 (ABA126)	KT020-138	10.06±0.73	0.68±0.021	41.98±1.3**	38.68	147	151

Student's *t*-test was used to test the statistical significance (*P<0.05, **P<0.01).

ABA126 compared with their parental lines. Length and shape of the mutant kernels were also altered (Table 1); ABA27 and ABA31 have longer kernels than other lines, kernel shapes of ABA59 and ABA90 are spherical as well as Chihoku distinct from ABA27 and ABA31, and ABA122 and ABA126 are more spherical than Horoshiri. Several agricultural characteristics such as 1000 kernel weight, and heading and flowering dates of mutant lines were different from their parental lines (Table 1).

Germination rates of mature seeds were compared under both ABA and non-ABA conditions among the mutants and their parental lines. ABA27 showed delayed germination as compared with Chihoku in the absence of ABA (Kobayashi et al. 2008c; Fig. 1A, B). Exogenous ABA further delayed germination of ABA27, whereas that of Chihoku was not significantly affected, indicating that ABA27 is hypersensitive to ABA during germination (Kobayashi et al. 2008c; Fig. 1A, B). Germination of ABA31, ABA59 and ABA90 was faster than that of Chihoku under non-ABA conditions (Fig. 1A). Exogenous ABA delayed germination of ABA31 and ABA59, whereas germination of ABA90 was scarcely altered (Fig. 1B). These results indicate that ABA31 and ABA59 are sensitive to exogenous ABA but ABA90 is ABA-insensitive during germination. Both ABA122 and ABA126 delayed germination as compared with Horoshiri, and germination rate of ABA126 was lowest among three lines under non-ABA condition (Fig. 1C). Exogenous ABA delayed germination of Horoshiri and ABA122 but not ABA126 significantly (Fig. 1D). These results indicate that ABA122 and ABA126 are ABA-hypersensitive and less-sensitive mutants to exogenous ABA during germination, respectively.

ABA sensitivity during post-germination growth was studied based on the magnitude of inhibition of shoot and root growth by 20 μM exogenous ABA. Shoot and root growth were greatly inhibited by ABA in both the mutants and their parental lines. Previously, the magnitude of inhibition estimated by the relative growth rate (% growth in the presence of ABA relative to growth in the absence of ABA) was greater in ABA27 than in Chihoku (Kobayashi et al. 2008c). In the present study, the inhibition of shoot and root growth in ABA27 was the greatest among the Chihoku-derived mutant lines (Fig. 2A, B). ABA31 also showed greater inhibition rate of seedling growth than Chihoku (Fig. 2A, B). The growth inhibition in ABA59 was slightly less than that in Chihoku (Fig. 2A, B). The ABA inhibition in ABA90 was slightly less than that in Chihoku previously (Kobayashi and Takumi 2007), whereas it was similar to Chihoku in the present study (Fig. 2A, B). As to Horoshiri-derived mutants, the magnitude of ABA inhibition of shoot and root growth in ABA122 was significantly less than that in Horoshiri, while the inhibition of root growth in ABA126 was significantly greater than that in its parent (Fig. 2C, D). These results indicate that ABA31 and ABA126 are ABA-hypersensitive, and ABA59 and ABA122 are less-sensitive mutant lines during seedling growth.

Transcript accumulation of ABA-responsive genes such as transcription factor genes (*Wdreb2*, *Wlip19* and *Wabi5*; Kobayashi et al. 2006, 2008a-c), *Cor/Lea* genes (*Wdhn13*, *Wrab15*, *Wrab17* and *Wrab18*; Kobayashi et al. 2006, 2008c) and *TaGA1* encoding G-protein α subunit (Hossain et al. 2003) was compared among the mutants and their parental lines using non-ABA and ABA-treated seedlings. Our previous study showed that ABA-hypersensitive ABA27 accumulated more transcripts of several ABA-responsive genes than Chihoku under ABA- and non-ABA-treated conditions (Kobayashi et al. 2008c). In the present study, transcript levels of most of the examined genes were more accumulated in ABA31 than in Chihoku as well as in ABA27. Transcripts of *Wlip19*, *Wdreb2*, *Wrab15*, *Wrab17*, *Wrab18* and *TaGA1* were more abundant in both ABA27 and ABA31 after ABA treatment. The results confirmed that the ABA sensitivity of ABA27 and ABA31 was higher than that of Chihoku. In ABA-less-sensitive ABA59, *TaGA1* was lower than in Chihoku, whereas other genes were abundantly accumulated under ABA-treated condition. Although ABA90 showed no significant difference in ABA sensitivity compared with Chihoku (Fig. 2A, B), expression of genes in ABA90 was increased after ABA treatment as in our previous report (Kobayashi and Takumi 2007). Transcripts of *Wabi5*, *Wrab18* and *TaGA1* in ABA-less-sensitive ABA122 were lower than in Horoshiri under normal conditions. However, no notable differences were observed in the expression of these genes between ABA122 and its parent after ABA treatment. In ABA-hypersensitive ABA126, expression of *Wabi5*, *Wrab18* and *TaGA1* was lower than in Horoshiri under non-ABA and ABA-treated condition.

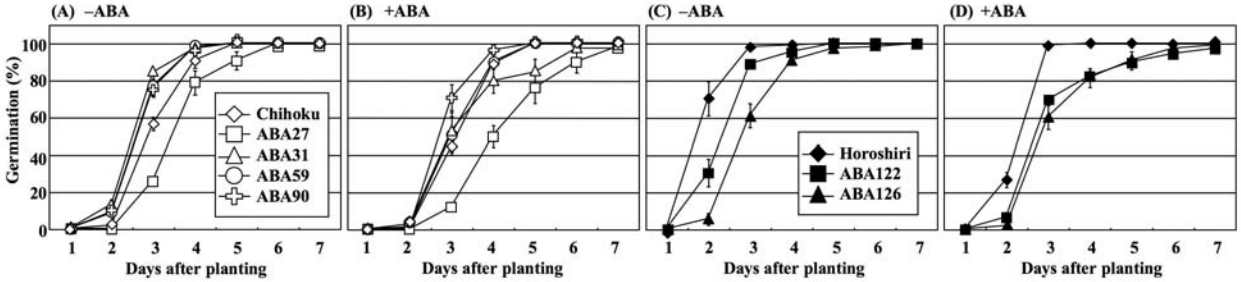

Figure 1. Inhibition of seed germination by ABA in Chihoku, Horoshiri and mutant lines. Seeds (n=36) were germinated with (B, D) and without (A, C) 20 μM ABA at 20˚C in the dark. Germinated seeds were counted daily after the start of imbibition. Comparison of germination rates between Chihoku and its derived mutants (A, B), and between Horoshiri and its derived mutants (C, D).

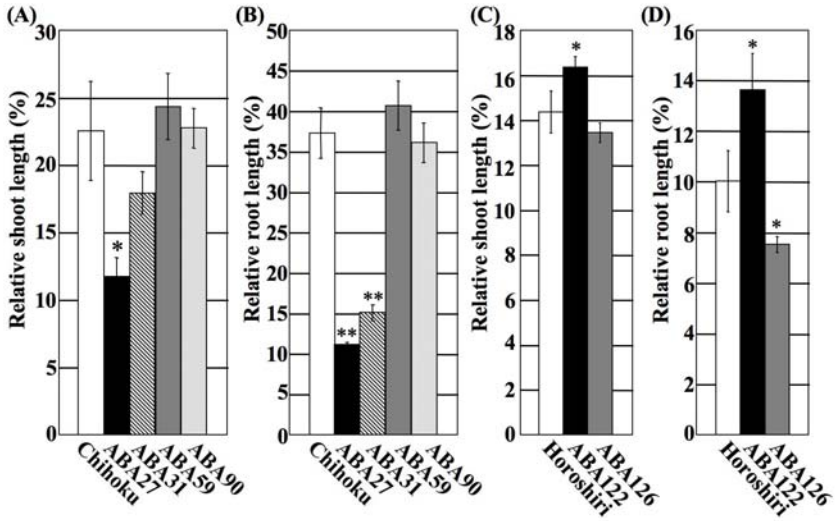

Figure 2. ABA sensitivity in Chihoku, Horoshiri and their mutant lines. (A, B) Comparison of magnitude of ABA inhibition of shoot (A) and root (B) growth between Chihoku and its derived mutant lines. (C, D) Comparison of magnitude of ABA inhibition of shoot (C) and root (D) growth between Horoshiri and its derived mutant lines. Student's *t*-test was used to test the statistical significance (**P*<0.05, ***P*<0.01) between mutant and its parent.

ABA27 showed significant increase in freezing tolerance at the seedling stage with and without cold acclimation, and transcripts of several ABA-responsive genes such as *Wabi5*, *Wrab15* and *Wrab18* were higher in ABA27 than in Chihoku under normal temperature condition and early cold acclimation periods, suggesting that the enhancement of these gene activities via the ABA27 mutation contributes to the improvement of freezing tolerance (Kobayashi et al. 2008c). Although the gene expression levels in other mutant lines do not necessarily reflect their ABA-sensitivity, ABA31 was hypersensitive to exogenous ABA during the germination and seedling stage (Figs. 1A, B and 2A, B) and abundantly accumulated transcripts of ABA-responsive genes like ABA27. The results suggest that freezing tolerance levels of ABA31 should be affected through the altered gene activities by the mutation allele. Therefore, the mutated loci in both ABA27 and ABA31 seem to be the factors regulating ABA-responsive *Cor/Lea* gene expression mediated by the transcription factors such as WDREB2, WLIP19 and WABI5 under cold/freezing stress condition. Further studies including molecular cloning of the mutated genes are required to understand the relationship between the ABA-responsive pathway and freezing tolerance.

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