

A Lectin Receptor Kinase Positively Regulates ABA Response During Seed Germination and Is Involved in Salt and Osmotic Stress Response

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Abstract Lectin receptor-like kinases (LecRK) are widespread in higher plants; however, little is known about their physiological roles. In this study, At1g70130 (designated *LecRK-b2*), an *Arabidopsis* LecRK gene, has been investigated. *LecRK-b2* was predominantly expressed during seed germination, and its expression was ceased following germination. The expression of *LecRK-b2* was induced by abscisic acid (ABA), salt, and osmotic stress. *LecRK-b2* loss-of-function mutation slightly reduced the ABA sensitivity during seed germination, and this reduced sensitivity was demonstrated not due to lower ABA accumulation level in the seeds. Dual-luciferase transient expression assay confirmed that the transcription factor ABSCISIC ACID INSENSITIVE3 (ABI3) could activate the luciferase under driving of *LecRK-b2* promoter. *LecRK-b2* transcription level was found to be down-regulated in *abi3* during seed germination. Furthermore, *LecRK-b2* loss-of-function mutation reduced the salt and osmotic sensitivity during early development stage of *Arabidopsis*. Taken together, these results suggest that *LecRK-b2* functions as a positive regulator of the ABA response during the seed germination and is involved in salt and osmotic stress response in the early development stage.

Keywords Abscisic acid · Germination · Lectin receptor kinases · Osmotic stress

Lectin receptor-like kinases (LecRK) are a class of proteins, which include an extracellular legume lectin-like domain, a transmembrane domain, and an intracellular kinase domain (Herv et al. 1996). Despite their unique structures and wide spread in higher plants, little is known about the precise function of LecRK gene family. In the past decades, some studies suggested a variety of potential roles for these LecRK. Firstly, LecRK might take part in biotic and abiotic stress signal transduction. Poplar LecRK gene *PnLPK* and *Arabidopsis* LecRK gene *lecRK-a1* could be induced by wounding and senescence, and *Arabidopsis AtLecRK2* could be induced by salt stress (Nishiguchi et al. 2002; Riou et al. 2002; He et al. 2004). Furthermore, the extracellular legume lectin-like domain of LecRKs has led to suggestions that LecRK could be involved in legume-rhizobia symbiosis and pathogen resistance (Herv et al. 1996; Hirsch 1999), and recent studies have confirmed this hypothesis (Gouget et al. 2006; Chen et al. 2006). Moreover, LecRK also functions in plant development. SGC, an *Arabidopsis* LecRK gene, has been found to play an important role in pollen development (Wan et al. 2008).

Sequence analysis and molecular modeling of *Arabidopsis* LecRKs have revealed that some conserved residues are involved in binding hydrophobic molecular (Hervé et al. 1999; Barre et al. 2002). Thus they may be involved in the recognition of small hydrophobic hormones. Most recently, an *Arabidopsis* A4 subfamily of lectin receptor kinase has been found to negatively regulate ABA response in seed germination (Xin et al. 2009).

The phytohormone abscisic acid (ABA) regulates various aspects of physiological and developmental processes of

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plants, such as seed maturation, dormancy, and germination (Leung and Giraudat 1998; Kuhn et al. 2006; Yoshida et al. 2006). ABA also plays a critical role in response to biotic and abiotic stress, such as wounding, pathogen attack, drought, salt, and cold (Hetherington 2001; Finkelstein et al. 2002; Hetherington and Woodward 2003; Fan et al. 2004). ABSCISIC ACID INSENSITIVE3 (ABI3) is a transcription factor that mediates ABA responses in seeds (Parcy et al. 1994; Jones et al. 1997; Zeng et al. 2003). ABI3 acts in promoting seed maturation during the latter stages of seed development, and it may also play a role during seed germination (McCarty 1995; Li and Foley 1997; Bassel et al. 2006). ABI3 loss-of-function mutant *abi3* reduces embryo dormancy and exhibits precocious germination (Raz et al. 2001).

Here, we characterized the expression patterns of *LecRK-b2* gene in wild type and *abi3* mutant. More importantly, *LecRK-b2* was confirmed to play a role in the positive regulation of ABA response in seed germination. In addition, *LecRK-b2* loss-of-function mutation reduced the salt and osmotic sensitivity during the early development stage of Arabidopsis.

Materials and Methods

Plant Materials, Growth Conditions, and Stress Treatments

Columbia-0 ecotype *Arabidopsis* was used as wild type (WT). The *LecRK-b2* T-DNA insertion mutant line (SALK_020262) was obtained from Arabidopsis Biological Resource Center (<http://www.arabidopsis.org>). The homozygous was isolated and designate *lecrk-b2*. Seeds were cold-treated at 4°C for 3 days and then germinated and grown in the growth chamber for harvesting seeds. For stress treatment, the seedlings were grown on Murashige and Skoog (MS) agar for 10 days then transferred to MS liquid or MS liquid containing 100 μM ABA for 1, 2, 4, 6, 12 h, or 100 mM NaCl for 1, 2, 4, 6, 12 h, or 200 mM mannitol for 1, 2, 4, 6, 12 h, respectively.

Germination and Root Growth Assay

About 50 surface-sterilized seeds were sowed on 0.5× MS medium with various concentrations of ABA, NaCl, and mannitol. After being cold-treated at 4°C for 3 days, plates were transferred to a growth chamber (22°C, 80 μmol m⁻² s⁻¹ continuous white light). Germination (a clear appearance of radicle) rates and greening cotyledons rates were determined in three independent experiments (40 seeds per genotype and experiment).

Root growth analyses were performed by transferring 5-day-old seedlings onto 0.8% agar medium (0.5× MS, no sucrose) supplemented with the indicated ABA, NaCl, and mannitol, separately. Root growth was measured 6 days

after the transfer in three independent experiments with 30 individuals per genotype and experiment.

Semi-quantitative RT-PCR and Real-Time RT-PCR

Total RNAs were isolated from the treated or untreated Arabidopsis materials using a Qiagen RNeasy plant mini kit. The 3 μg RNA was used for first-strand cDNA synthesis by SuperScript RT-PCR system (Invitrogen). For semi-quantitative RT-PCR, the primers were 5'-ACTAGACTG TTGGGACAGTG GAGAC and 5'-CACTAGAG AGAAAC GATCCGTC. *Actin2* gene was used as an internal standard. The amplification primers were 5'-CACTGTGCC AATCTACGAGGGT and 5'-CACAAA CGAGGGCTG GAACAAG. PCR was performed with a 30-s denaturation at 95°C followed by 25 cycles with each cycle composed of 95°C for 30 s, 59°C for 30 s, and 72°C for 30 s. Real-time RT-PCR analyses were performed using Sybr Green PCR Master mix (Applied Biosystems) in Mx3000P thermal cycler (Stratagene), and data were analyzed with MxPro software (Stratagene). The primers used were 5'-ACACAAGGAG GAGCTGGTCAAGTT and 5'-TGGAGA AGGATGAAA CAGTGC CGT. *Actin2* was used as an internal control to normalize. The primers used for amplifying *Actin2* were 5'-CACTGTGCCAATCTACGAGGGT and 5'-CACAAACG AGGGCTGGAACAAG. RT-PCR reactions for each experiment were repeated at least three times, and the representative gel images were shown.

Measurement of Endogenous ABA Content

ABA content measurement was performed by using Plant hormone abscisic acid, ABA ELISA Kit (Uscnlife).

Construction of P_{LecRK-b2}:GUS and 2× 35S: LecRK-b2

LecRK-b2 promoter (from -904 to +19 bp relative to the translation start site) was amplified from genomic DNA template by PCR with forward 5'-GGGGACAAGTTTGTACAAAAAGCAGGCTTCGTCGGATTCTGATATGTGAGAGTC and reverse 5'-GGGGACCACTTTGTACAAGAAAGCTGGGTGA TCTTTAGAA GCAGAGACATG ATTC; *LecRK-b2* cDNA was amplified with forward 5'-GGGGACAAGTTTGTACAAAAAGCAGGCTTCATGTCTCTGCTTCTAAAGATGTTATTA and reverse 5'-GGG GACCACTTTGTACAAGAAAGCTGGGTGTTAGCGT CCACTAGAGAGAAACGA; italic sequences are enzyme sites or BP transferase recognition sites. The PCR fragment was cloned into entry vector by BP reaction and confirmed by sequencing analysis then subcloned into destination vector GW-GUS for P_{LecRK-b2}:GUS construction and PLele for 2× 35S: *LecRK-b2* construction through LR reaction. Gateway clone system was ordered from Invitrogen.

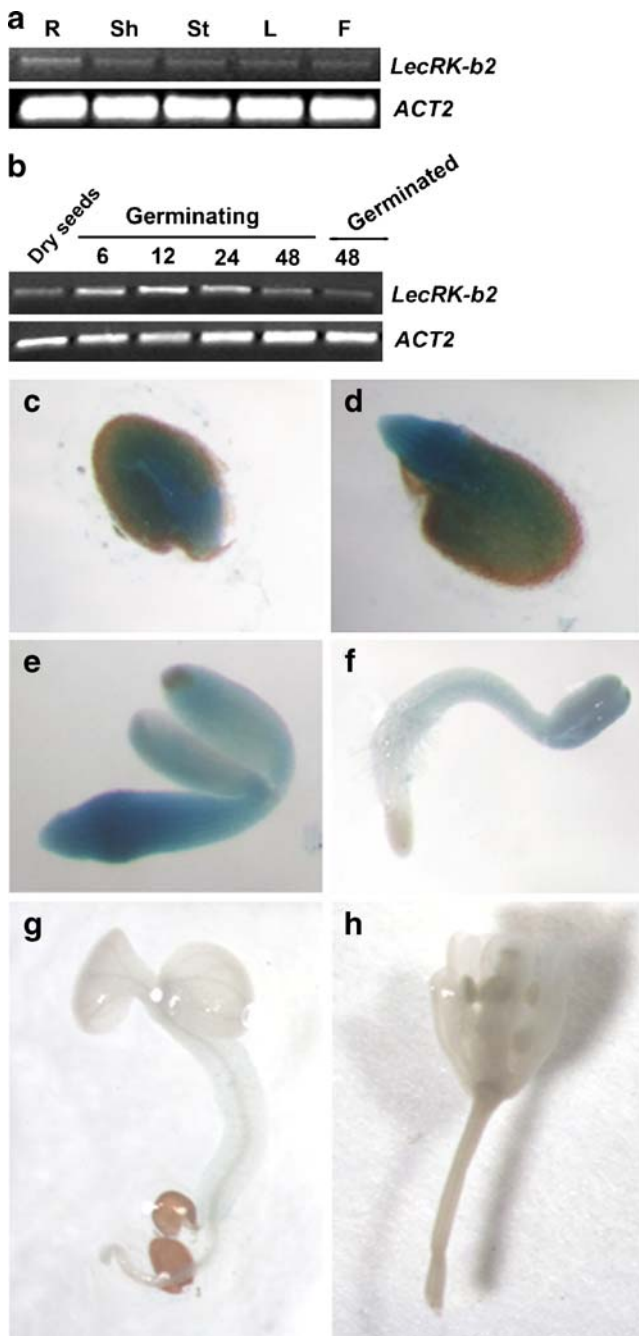


Fig. 1 Expression patterns of *LecRK-b2* in wild type. **a** Semi-quantitative RT-PCR analysis of *LecRK-b2* mRNA transcription levels in different organs. Shown are roots (*R*), shoots (*Sh*), stems (*St*), leaves (*L*), and flowers (*F*). *Actin2* (*ACT2*) gene was used as internal control. **b** Dry seeds or embryos dissected from intact seeds at 6–48 h after imbibition or germination. **c–h** Expression patterns of *LecRK-b2* revealed by *P_{LecRK-b2}:GUS*. Shown are germinating seed (**c** and **d**), germinating seed without seed coat (**e**), before cotyledon opening (**f**), germinated seedling (**g**), and flower (**h**)

P_{LecRK-b2}:GUS and *2× 35S:LecRK-b2* were transformed into the wild-type *Arabidopsis* and the *LecRK-b2* knockout mutant (*lecrk-b2*), respectively, using floral dip method (Steven and Clough 1998). Independently, *P_{LecRK-b2}:GUS*

transformed lines were used for GUS activity assay **a**. Three representative *2× 35S:LecRK-b2* lines, 3–1, 6–4, and 14–6 were chosen for ABA response assays.

Transient Transcription Dual-luciferase (Dual-LUC) Assays

Dual-LUC assays were performed as previously described (Liu et al. 2008). *ABI3* cDNA was amplified with forward 5'-AAGGAAAAAAGCGGCCGCATGAAAAGC TTGCATG TGGC and reverse 5'-ACGCGTCTGACTCATTTAACAG TTTGAGAAGT TGG; the effector plasmid, pGreenII62-K-*ABI3*, was constructed by inserting the *ABI3* cDNA to pGreenII62-SK between the multicloning sites *NotI/SalI*. For the reporter plasmid construction, the promoter region of *LecRK-b* from -886 to +8 bp relative to the translation start site was cloned by PCR and inserted into the *SalI/PstI* sites of the vector pGreen-0800-LUC. The primer, forward 5'-ACGCGTC GACGAGTCAAGATTTGTTATATGTAAAT and reverse 5'-AAAACCTGCAGCAGA GACATGATTCAT TAAATTTTG, was used for reporter plasmid construction. Italic sequences are enzyme site. Three biological repeats were measured for each sample.

Results and Discussion

LecRK-b2 Expression Patterns

To determine the expression patterns of *LecRK-b2*, both semi-quantitative RT-PCR and promoter-GUS reporter analysis were performed. Semi-quantitative RT-PCR analysis showed that *LecRK-b2* is weakly expressed in all

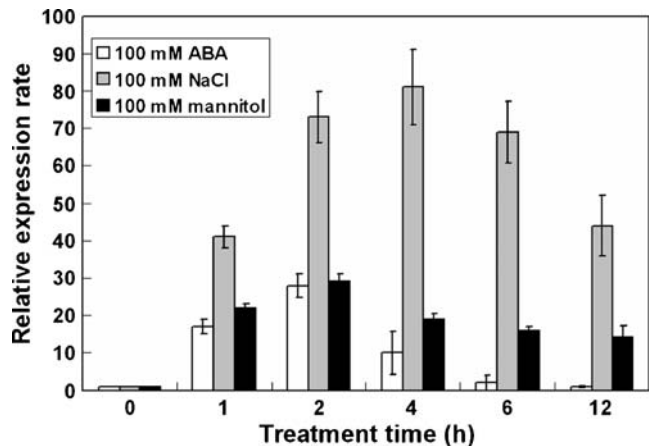
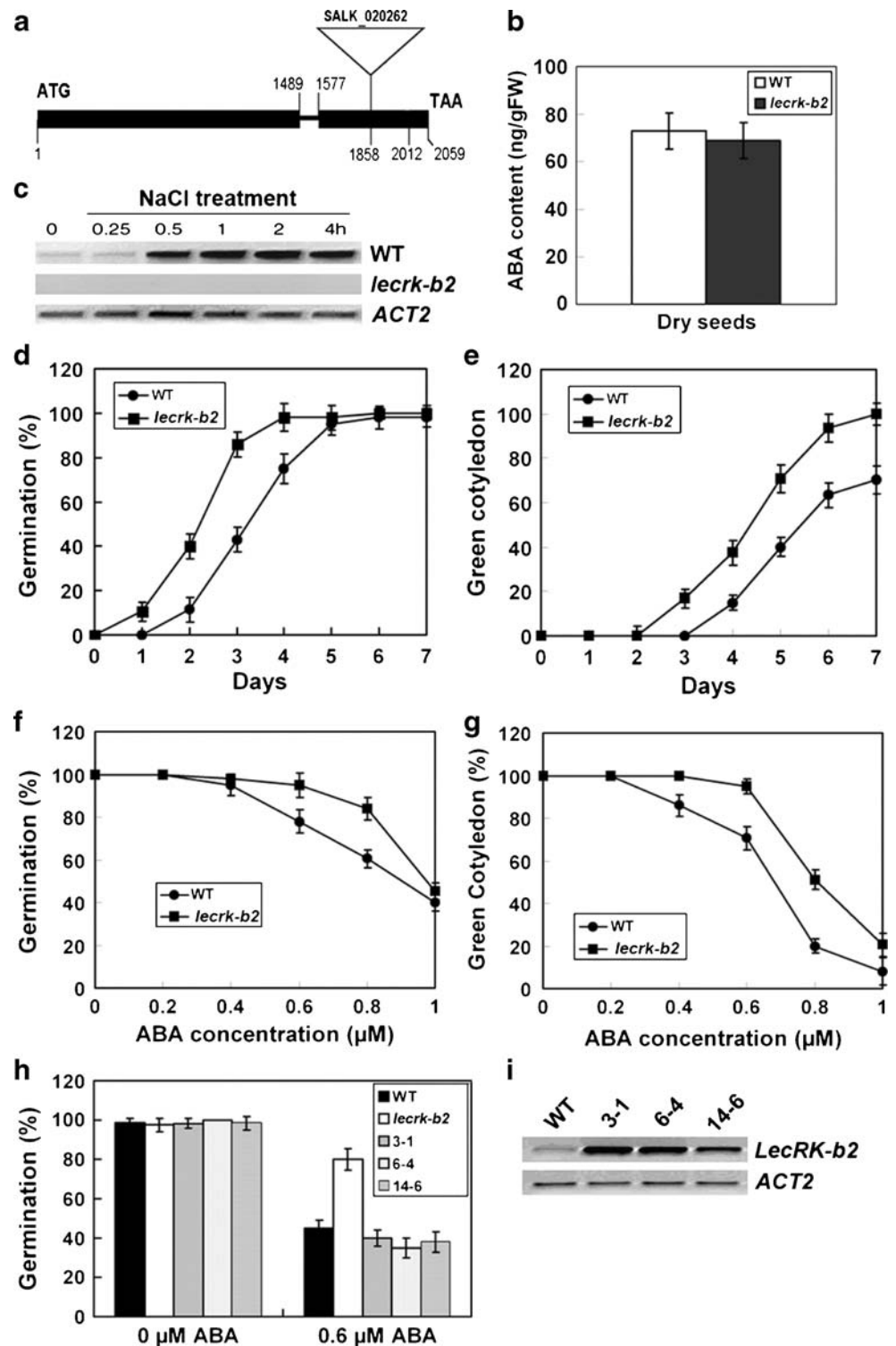


Fig. 2 Real-time PCR analysis of *LecRK-b2* gene expression in response to ABA, NaCl, and mannitol. Seven-day-old seedlings grown in MS agar medium were treated with 100 μM ABA, 100 mM NaCl, or 100 mM mannitol for 0, 1, 2, 4, 6, and 12 h, respectively. The mRNA levels were normalized with *ACT2*. Relative mRNA expression rate in untreated seedlings were set at 1. Data represent the means (±SD) of three independent assays

organs of adult *Arabidopsis* (Fig. 1a), while its transcription level is relatively high in the dry seeds, especially in the seeds during germinating (Fig. 1b). Consistent with the semi-quantitative RT-PCR result, a 904-bp fragment of *LecRK-b2* promoter directed GUS reporter also express in the germinating seeds but not in the adult leaves or

flowers (Fig. 1c–f). It indicates that the 904 bp fragment of *LecRK-b2* promoter likely contains all of the necessary *cis* elements for proper expression of the *LecRK-b2* gene. Four homozygous $P_{LecRK-b2}:GUS$ lines were used for GUS staining assay. We found that the promoter is very active during the seed germinating (Fig. 1), while after the cotyledon

Fig. 3 ABA insensitive phenotype of *LecRK-b2*. **a** T-NDA insertion site of SALK_020232, **b** endogenous ABA content of dry seeds of wild type (*white bar*) and *lecrk-b2* (*black bar*). **c** Disrupted mRNA expression of *LecRK-b2* in the seedlings of *lecrk-b2*. **d** and **f** Germination rates (radicle emergence); **e** and **g** greening cotyledon rates of wild-type (*black circles*) and *lecrk-b2* (*black squares*); seeds were in the presence of 0.6 μ M ABA for 7 days after cold treatment (**d** and **e**) or in the presence of various concentrations of ABA at 7 days after cold treatment (**f** and **g**). **h**, **i**, and **j** Functional complementation of *lecrk-b2* by $P_{Leela-LecRK-b2}$. The seeds of transgenic lines 3–1, 6–4, 14–6, and wild-type *lecrk-b2* were germinated in the 0 or 0.6 μ M ABA for 3 days after cold treatment (**h**) or 7 days (**j**). **i** RT-PCR analysis of *LecRK-b2* gene expression levels in $P_{Leela-LecRK-b2}$ transgenic lines. In **b** and **d–h**, data represent the means (\pm SD) of three independent assays



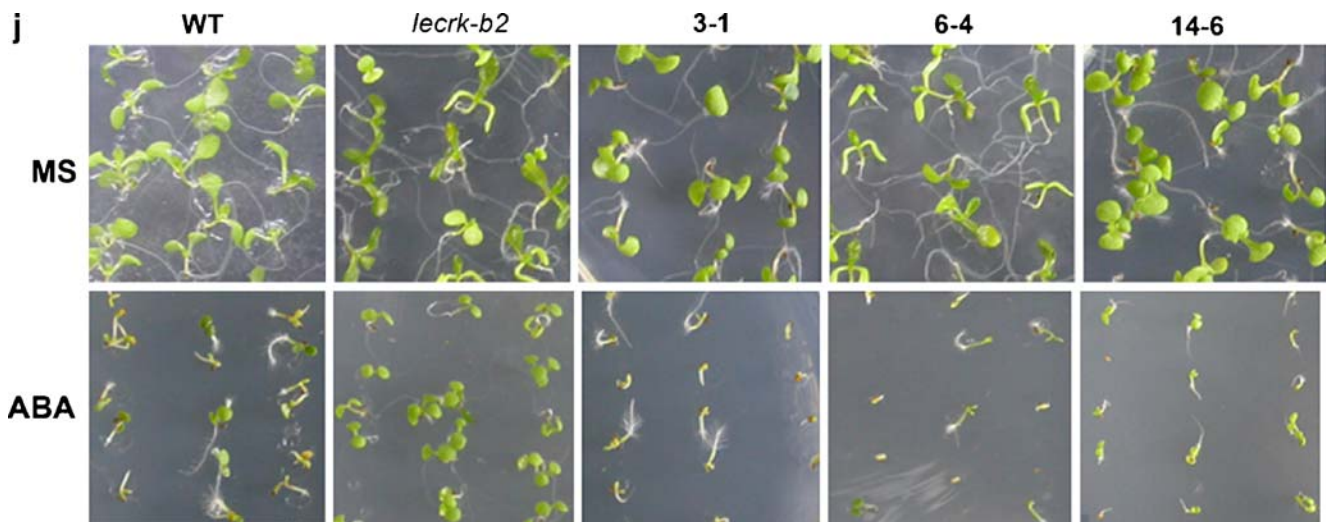


Fig. 3 (continued)

turned green and opened, the GUS activity fades away (Fig. 1). These results reveal that *LecRK-b2* expression is activated during germination, but declines following the completion of germination.

LecRK-b2 Transcription Is Induced by ABA and Osmotic Stress

To analyze the gene transcription of *LecRK-b2* after ABA, salt, and osmotic treatment, real-time PCR was employed. The results showed that the *LecRK-b2* transcripts quickly accumulated to high levels in the Arabidopsis seedlings after 1-h exposure to 50 μ M ABA, but the transcripts came back to the original level 6 h later (Fig. 2). In contrast, treatment with 100 mM NaCl or 200 mM mannitol resulted in an accumulation of *LecRK-b2* transcripts between 2 and 12 h after treatment (Fig. 2). These results indicate that gene transcription of *LecRK-b2* can be transiently induced by ABA treatment, while it can be strongly induced after NaCl or mannitol treatment, and its transcription can preserve on a high level. This differential expression patterns imply that *LecRK-b2* plays different roles in ABA signaling and NaCl or osmotic stress response. Furthermore, three ACGT-core motifs (Fig. S1A, D, and E), which were related to ABA inducibility (Kamisugi and Cuming 2005), had been characterized in the promoter sequence of *LecRK-b2*. These ACGT-core motifs may be responsible for the ABA-inducible gene expression of *LecRK-b2*.

LecRK-b2 Mutant Slightly Reduced ABA Response in Seed Germination Inhibition

To elucidate the function of *LecRK-b2* in the ABA response, we characterized a T-DNA line of *LecRK-b2* (SALK_020262), which was designated *lecrk-b2* (Fig. 3a).

RT-PCR analysis showed that *LecRK-b2* transcript was absent in this SALK line (Fig. 3c). It indicates that *lecrk-b2* is a *LecRK-b2* knockout mutant. Then, we investigated the efficiency of radicle emergence and early growth of *lecrk-b2* in the presence of various concentrations of ABA. The germination kinetic profile in the presence of 0.6 μ M ABA showed that more *lecrk-b2* seeds germinated than wild-type seeds before 5 days after cold treatment, and after 5 days, they all germinated (Fig. 3d). A dose response curve showed that after 4 days at both 0.6 and 0.8 μ M ABA, *lecrk-b2* had higher germination rates than the wild type (Fig. 3f). We also investigated the postgerminative seedling growth by scoring the cotyledon-greening phenotype. The results showed that *lecrk-b2* had 30% more seedling showing green cotyledons than that of *lecrk-b2* after 6 days in the presence of 0.6 μ M ABA (Fig. 3e) or after 7 days in the presence of 0.8 μ M ABA (Fig. 3g).

To confirm that this ABA insensitive germination phenotype of *LecRK-b2-1* is due to *LecRK-b2* loss-of-function mutation, a functional complementation test was performed. The full-length *LecRK-b2* CDS was cloned to PLeela vector for overexpression. Then PLeela-*LecRK-b2* was transformed into *lecrk-b2*, and three independently transformed lines, 3–1, 6–4, and 14–6, which have high levels of *LecRK-b2* transcript (Fig. 3i) were used for germination assay. The result showed that three *LecRK-b2* overexpression lines had 100% germination rate, similar to *LecRK-b2* and wild type (Fig. 3h, j). In the presence of 0.6 μ M ABA, overexpression lines had a reduced germination rate close to that for the WT compared with a relative higher germination rate for *lecrk-b2* (Fig. 3i, j). These results confirm that *LecRK-b2* loss-of-function mutation is responsible for the ABA insensitive germination phenotype.

To clarify that whether this ABA insensitive germination phenotype of *LecRK-b2* was caused by the lower endogenous

ABA accumulation, we examined the ABA levels in matured dry seeds. The result shows that there is no significant difference between *LecRK-b2* and wild type on the ABA accumulation level in the matured dry seeds (Fig. 3b).

To further investigate whether the faster cotyledon greening is caused by reduced ABA sensitivity, we tested the cotyledon-greening rate and root-elongation rate of germinated seeds in different ABA concentration. However, we did not observe any difference in cotyledon-greening rate and root-elongation rate of germinated seeds between *lecrk-b2* and wild type (data not shown). Therefore, the postgerminate growth enhancement of *lecrk-b2* is not due to altered ABA sensitivity in later growth stages but presumably due to faster germination.

Taken together, these results suggest that *LecRK-b2* loss-of-function mutation reduces the ABA sensitivity during seed germination and then retards the cotyledon greening, and this reduced sensitivity during seed germination is not due to lower ABA accumulation level in the seeds; it more likely relates to ABA or other signaling pathway.

To date, approximately 42 *LecRK* genes have been identified in the *Arabidopsis thaliana* genome (Barre et al. 2002). These *LecRK* genes share highly amino acid sequence identity. Redundant gene functions may result in the weak phenotype of *LecRK-b2* loss-of-function mutation.

P_{LecRK-b2}:LUC Can Be Activated by ABI3

To clarify the relationship between *LecRK-b2* and ABA signaling, the promoter sequence of *LecRK-b2* was analyzed with Softberry-NSITEP online program (<http://linux1.softberry.com/berry.phtml>). The result showed that a very similar RY/G motif, a binding site of ABI3 (Ezcurra et al. 2000), existed between -121 and -104 bp relative to the translation start site (Fig. S1A, B). In order to determine the transactivation activity of ABI3 to the *P_{LecRK-b2}*, a Dual-LUC transient expression system (Hellens et al. 2005) was used. We examined the effects of ABI3 transiently expressed on the activity of the recombinant *P_{LecRK-b2}:LUC* (Fig. 4a). As shown in Fig. 4b, transiently expressed effector plasmid or *P_{LecRK-b2}:LUC* solely did not alter the background LUC value, which was detected in the untreated leaves, while in transiently expressed effector plasmid and *P_{LecRK-b2}:LUC* together, the LUC value was elevated dramatically. It implies that ABI3 acts as the stimulator of the recombinant *P_{LecRK-b2}:LUC*. We further deduce that ABI3 as a transcript factor may also regulate the expression of *LecRK-b2* in the *Arabidopsis*. To confirm this hypothesis, the expression patterns of *LecRK-b2* in *abi3* were analyzed. The results showed that during the seed germination, the *LecRK-b2* transcription level was down-regulated in *abi3* compared with wild type (Fig. 4c). We also analyzed the expression pattern of *LecRK-b2* in *abi3* seedlings under the treatment of ABA

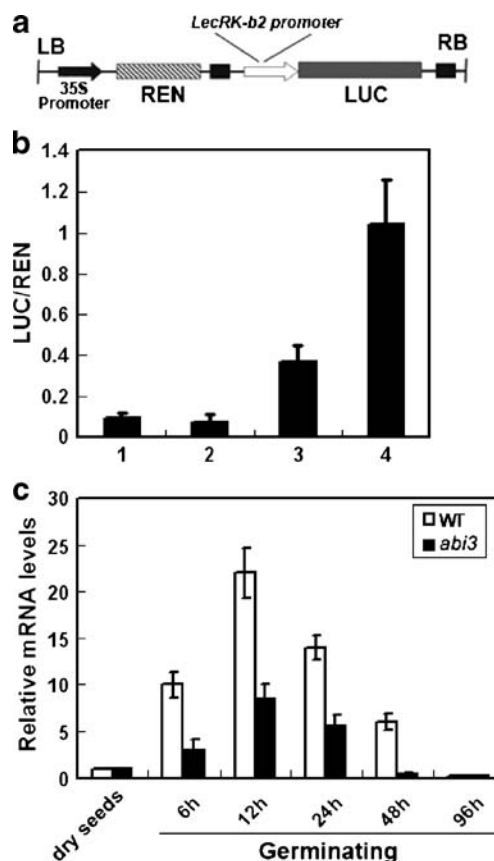


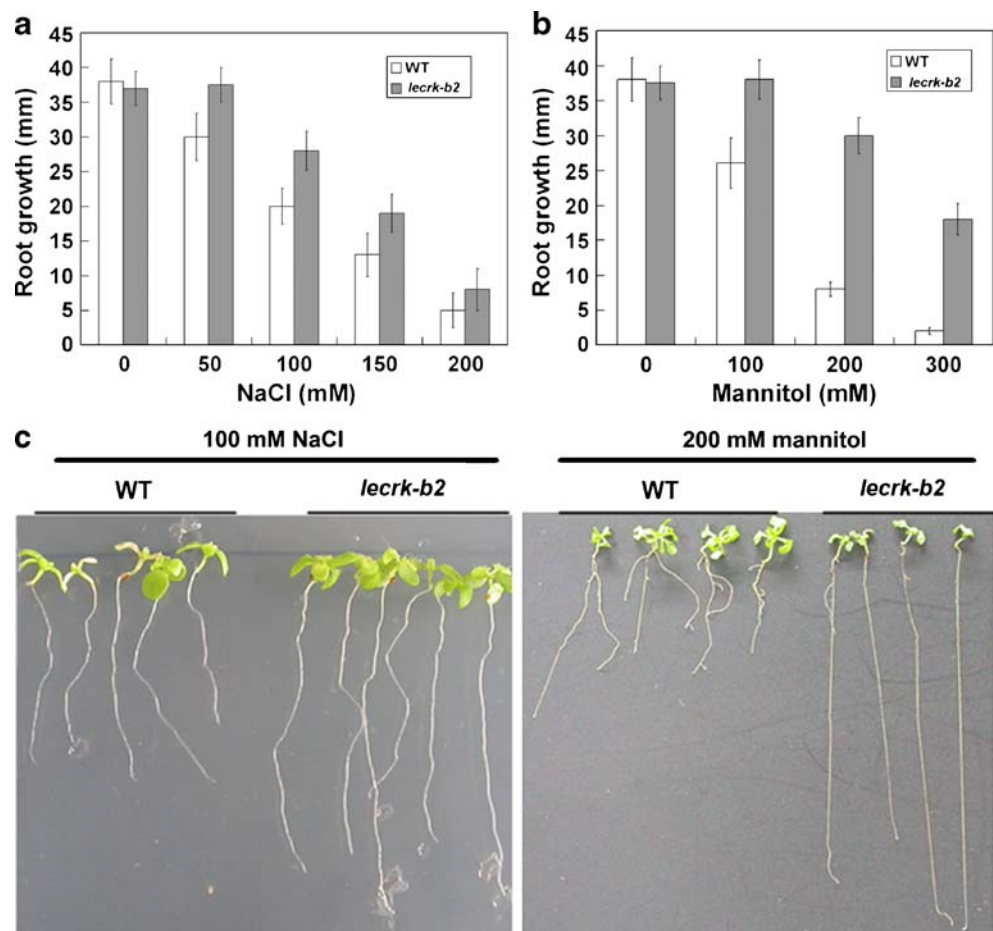
Fig. 4 ABI3 regulates the transcription of *LecRK-b2*. **a** Structure of the *P_{LecRK-b2}:LUC*. 35S promoter (black arrow), *Renilla luciferase* (*REN*), and firefly luciferase (*LUC*), *LecRK-b2* promoter (white arrow), T-DNA left border (*LB*), and right border (*RB*) were indicated. **b** The effects of ABI3 on the activity of *P_{LecRK-b2}:LUC* reporter; leaves of wild type were infiltrated with *Agrobacteria* harboring the *P_{LecRK-b2}:LUC* reporter with or without the effector (pGreenII62-SK-ABI3); (1) uninfiltrated leaves, (2) pGreenII62-SK-ABI3 with empty reporter vector (pGreen0800-LUC), (3) *P_{LecRK-b2}:LUC* reporter only, (4) *P_{LecRK-b2}:LUC* reporter with pGreenII62-SK-ABI3 effector. Leaves transfected with *Arabidopsis* were kept in white light for 3 days, and dual-luciferase assay was used to evaluate the *P_{LecRK-b2}:LUC* reporter activity (Liu et al. 2008). The relative LUC activities normalized to the REN activity are shown (LUC/REN, $n=3$). **c** *LecRK-b2* mRNA expression levels in wild type and *abi3* during germination; data represent the means (\pm SD) of three independent assays

and NaCl, but we found no significant changes (data not shown). These results suggest that ABI3 regulates the transcription of *LecRK-b2* during the seed germination, while in the adult seedlings, the expression of *LecRK-b2* may be controlled by other factors.

T-DNA Knockout Mutant of *LecRK-b2* Reduced Sensitivity to Osmotic Stress in the Early Development Stage of *Arabidopsis*

RT-PCR analysis has revealed that the gene expression of *LecRK-b2* was strongly induced by NaCl and mannitol. To

Fig. 5 Disruption of *LecRK-b2* causes NaCl and mannitol insensitive root elongation. **a** and **b** Comparison of root elongation of wild type and *lecrk-b2*; 5-day-old seedlings were transferred to 0.5× MS plate with 0, 50, 100, 150, and 200 mM NaCl or with 0, 100, 200, and 300 mM mannitol. Primary root elongation was measured 6 days after the transfer; data represent the means (\pm SD) of three independent assays. **c** Growth of wild type and *lecrk-b2* on 0.5× MS medium supplemented with 100 mM NaCl or 200 mM mannitol; 5-day-old seedlings were transferred to 0.5× MS plate with 100 mM NaCl or with 200 mM mannitol. Photographs were taken 6 days later



determine whether *LecRK-b2* plays a role during salt or osmotic stress response, the root growth assay was performed by transferring 5-day-old seedlings on 0.5× MS plate with 0, 50, 100, 150, and 200 mM NaCl or with 0, 100, 200, and 300 mM mannitol. Primary root elongation was measured 6 days after the transfer in three independent experiments (Fig. 4a, b). Disruption of *LecRK-b2* in *lecrk-b2* plant exhibits a moderate decline in NaCl stress sensitivity compared to wild type during root growth on 0.5 MS media supplemented with NaCl (Fig. 4a, c); by contrast, *LecRK-b2* loss-of-function mutant exhibited significant mannitol stress insensitivity compared with wild type during root growth (Fig. 4b, c). These results indicate that T-DNA knockout mutation of *LecRK-b2* reduced sensitivity to salt and osmotic stress in the early developmental stage of Arabidopsis (Fig. 5).

Conclusion

LecRK-b2 is predominantly expressed during seed germination, and its expression was ceased following

germination. Its transcription could be transiently induced by ABA, while the transcription could be constitutively induced under NaCl and mannitol treatment. Its transcript level was significantly declined in *abi3* during the seed germination. Loss-of-function mutation of *LecRK-b2* slightly reduced the ABA sensitivity during seed germination. The reduced sensitivity was demonstrated not due to lower ABA accumulation level in seeds. The mutation reduced salt and osmotic sensitivity during the early development stage of Arabidopsis, which was similar to the stress-induced expression pattern in adult plant. ABI3 could activate the luciferase under driving of the *LecRK-b2* promoter. Considering all these, these results offer conclusive evidence that *LecRK-b2* functions as a positive regulator of the ABA response during the seed germination and is involved in salt and osmotic stress response in the adult plant.

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References

- Barre A, Herve C, Lescure B, Rouge P (2002) Lectin receptor kinases in plants. *Crit Rev Plant Sci* 21:379–399
- Bassel GW, Mullen RT, Bewley JD (2006) ABI3 expression ceases following, but not during, germination of tomato and Arabidopsis seeds. *J Exp Bot* 57:1291–1297
- Chen XW, Shang JJ, Chen DX (2006) A B-lectin receptor kinase gene conferring rice blast resistance. *Plant J* 46:794–804
- Ezcurra I, Wycliffe P, Nehlin L, Ellerström M, Rask L (2000) Transactivation of the *Brassica napus* napin promoter by ABI3 requires interaction of the conserved B2 and B3 domains of ABI3 with different *cis*-elements: B2 mediates activation through an ABRE, whereas B3 interacts with an RY/G-box. *Plant J* 24:57–66
- Fan L-M, Zhao Z, Assmann SM (2004) Guard cells: a dynamic signaling model. *Curr Opin Plant Biol* 7:537–546
- Finkelstein RR, Gampala SSL, Rock CD (2002) Abscisic acid signaling in seeds and seedlings. *Plant Cell* 14:S15–45
- Gouget A, Senchou V, Govers F, Sanson A, Barre A, Rouge P, Pont-Lezica R, Canut H (2006) Lectin receptor kinases participate in protein–protein interactions to mediate plasma membrane–cell wall adhesions in Arabidopsis. *Plant Physiol* 140:81–90
- He XJ, Zhang ZG, Yan DQ, Zhang JS, Chen SY (2004) A salt-responsive receptor-like kinase gene regulated by the ethylene signaling pathway encodes a plasma membrane serine/threonine kinase. *Theor. Appl. Genet* 109:377–383
- Hellens R, Allan A, Friel E, Bolitho K, Grafton K, Templeton M, Karunairetnam S, Gleave A, Laing W (2005) Transient expression vectors for functional genomics, quantification of promoter activity and RNA silencing in plants. *Plant Methods* 1:13
- Herv C, Dabos P, Galaud JP, Roug P, Lescure B (1996) Characterization of an Arabidopsis thaliana gene that defines a new class of putative plant receptor kinases with an extracellular lectin-like domain. *J Mol Biol* 258:778–788
- Hervé C, Serres J, Dabos P, Canut H, Barre A, Rougé P, Lescure B (1999) Characterization of the Arabidopsis lecRK-a genes: members of a superfamily encoding putative receptors with an extracellular domain homologous to legume lectins. *Plant Mol Biol* 39:671–682
- Hetherington AM (2001) Guard cell signaling. *Cell* 107:711–714
- Hetherington AM, Woodward FI (2003) The role of stomata in sensing and driving environmental change. *Nature* 424:901–908
- Hirsch AM (1999) Role of lectins (and rhizobial exopolysaccharides) in legume nodulation. *Curr Opin Plant Biol* 2:320–326
- Jones HD, Peters NC, Holdsworth MJ (1997) Genotype and environment interact to control dormancy and differential expression of the *VIVIPAROUS1* homologue in embryos of *Avena fatua*. *Plant J* 12:911–920
- Kamisugi Y, Cuming A (2005) The evolution of the abscisic acid-response in land plants: comparative analysis of group 1 LEA gene expression in moss and cereals. *Plant Mol Biol* 59:723–737
- Kuhn JM, Boisson-Dernier A, Dizon MB, Maktabi MH, Schroeder JI (2006) The protein phosphatase AtPP2CA negatively regulates abscisic acid signal transduction in Arabidopsis, and effects of *abh1* on AtPP2CA mRNA. *Plant Physiol* 140:127–139
- Leung J, Giraudat J (1998) Abscisic acid signal transduction. *Annu Rev Plant Physiol Plant Mol Biol* 49:199
- Li B, Foley ME (1997) Genetic and molecular control of seed dormancy. *Trends Plant Sci* 2:384–389
- Liu H, Yu X, Li K, Klejnot J, Yang H, Lisiero D, Lin C (2008) Photoexcited CRY2 interacts with CIB1 to regulate transcription and floral initiation in Arabidopsis. *Science* 322:1535–1539
- McCarty DR (1995) Genetic control and integration of maturation and germination pathways in seed development. *Annu Rev Plant Physiol Plant Mol Biol* 46:71–93
- Nishiguchi NM, Yoshida YK, Sumizono ST, Tazaki TK (2002) A receptor-like protein kinase with a lectin-like domain from lombardy poplar: gene expression in response to wounding and characterization of phosphorylation activity. *Mol Gen Genomics* 267:506–514
- Parcy F, Valon C, Raynal M, Gaubier-Comella P, Delseny M, Giraudat J (1994) Regulation of gene expression programs during Arabidopsis seed development: roles of the ABI3 locus and of endogenous abscisic acid. *Plant Cell* 6:1567–1582
- Raz V, Bergervoet JH, Koornneef M (2001) Sequential steps for developmental arrest in Arabidopsis seeds. *Development* 128:243–252
- Riou C, Herv C, Pacquit V, Dabos P, Lescure B (2002) Expression of an Arabidopsis lectin kinase receptor gene, *lecRK-a1*, is induced during senescence, wounding and in response to oligogalacturonic acids. *Plant Physiol. Biochem.* 40:431–438
- Steven J, Clough AFB (1998) Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J* 16:735–743
- Wan J, Patel A, Mathieu M, Kim SY, Xu D, Stacey G (2008) A lectin receptor-like kinase is required for pollen development in Arabidopsis. *Plant Mol Biol* 67:469–482
- Xin Z, Wang A, Yang G, Gao P, Zheng ZL (2009) The Arabidopsis A4 subfamily of Lectin receptor kinases negatively regulates abscisic acid response in seed germination. *Plant Physiol* 149:434–444
- Yoshida T, Nishimura N, Kitahata N, Kuromori T, Ito T, Asami T, Shinozaki K, Hirayama T (2006) ABA-hypersensitive germination3 encodes a protein phosphatase 2C (AtPP2CA) that strongly regulates abscisic acid signaling during germination among Arabidopsis protein phosphatase 2Cs. *Plant Physiol* 140:115–126
- Zeng Y, Raimondi N, Kermode AR (2003) Role of an ABI3 homologue in dormancy maintenance of yellow-cedar seeds and in the activation of storage protein and Em gene promoters. *Plant Mol Biol* 51:39–49