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ORIGINAL PAPER

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A salt-responsive receptor-like kinase gene regulated by the ethylene signaling pathway encodes a plasma membrane serine/threonine kinase

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Abstract *NTHK1* is a salt-inducible ethylene receptor gene in tobacco. Transgenic tobacco plants for this gene show reduced ethylene sensitivity. Using cDNA microarray analysis, we were able to identify those genes that have different expression levels between *NTHK1* transgenic plants and wild-type plants under salt stress conditions. One of these, *AtLecRK2*, which encodes a receptor-like kinase with an extracellular lectin-like domain, was characterized in detail in the present study. AtLecRK2 contains a signal peptide, an extracellular lectin-like domain, a single transmembrane domain and a cytoplasmic protein kinase domain. *AtLecRK2* is transcribed in the root, flower and leaf but not in the stem. In wild-type *Arabidopsis*, salt stress induced the transcription level of *AtLecRK2*, whereas in the transgenic *NTHK1 Arabidopsis* induction of the *AtLecRK2* transcript was inhibited and retarded. *AtLecRK2* was constitutively overexpressed in the ethylene-overproducer mutant, *eto1-1*, and could be induced by ethylene. However, in the ethylene-insensitive mutant, *ein2-1*, the salt-induced expression pattern of *AtLecRK2* was the same as that in wild-type plants. The results demonstrate that the induction of *AtLecRK2* in response to salt stress is regulated by the ethylene signaling pathway. The induction was inhibited by the ethylene receptor, NTHK1, while it was independent of EIN2. The kinase activity of AtLecRK2 was also studied. We found that that AtLecRK2 can be autophosphorylated and has serine/threonine kinase activities. The subcellular localization of AtLecRK2-GFP in onion epidermal cells indicates that AtLecRK2 is localized on the plasma membrane.

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Introduction

Receptor-like kinases (RLKs) belong to a large gene family with at least 610 members that represent nearly 2.5% of the *Arabidopsis* protein coding genes (Shiu and Bleecker 2001). Members of the plant RLK family share a highly conserved catalytic domain known to possess serine/threonine substrate specificity, yet the extracellular domain of these receptors is quite divergent, which enables the protein to selectively respond to diverse extracellular signals (Walker 1994). RLKs have been classified into several classes on the basis of the properties of the extracellular domains. Investigations on the functions of plant RLKs have shown that RLKs are involved in several aspects of plant signaling processes. SRKs in *Brassica* are expressed exclusively in the reproductive organs and are implicated in the control of sporophytic self-incompatibility (Stein et al. 1991; Nasrallah and Nasrallah 1993; Schopfer et al. 1999). CLV1, a receptor kinase, regulates shoot and floral meristem development in *Arabidopsis* (Clark et al. 1997). The phenotypes of the maize mutant *crinkly4* show that CRINKLY4, a TNFR-like receptor kinase, is involved in leaf epidermal differentiation (Becraft et al. 1996). The *Arabidopsis* cell-wall-associated RLK gene, *WAK4*, is regulated differentially by various biotic and abiotic factors and plays a vital role in cell elongation (Lally et al. 2001). The analysis of the brassinosteriod-response mutant shows that BRI1 is involved in the brassinosteroid signaling pathway in *Arabidopsis* (Li and Chory 1997; Wang et al. 2001). *Xa21* controls a bacterial disease resistance in rice (Song et al. 1995). The functions of the other members of the RLK family are still unknown.

The plant hormone ethylene has profound effects on development and growth responses including cell elongation, sex determination, leaf abscission, flower senescence, fruit ripening, defense against pathogens and responses to various abiotic stresses (Johnson and Ecker 1998). In *Arabidopsis*, the triple response phenotype is often used to screen for mutants that have altered their ethylene responses relative to plants with a normal morphology.

The phenotypes of triple response plants consist of a radical swelling of the hypocotyl, exaggeration of the apical hook and an inhibition of hypocotyl and root elongation (Bleecker et al. 1988). To date, many ethylene-related mutants have been identified using the triple response phenotype, such as ethylene-insensitive mutants *etr1*, *etr2*, *ein2*, *ein3* and *ein4*, ethylene-overproducer mutants *eto1*, *eto2*, *eto3* and the constitutive triple-response mutant *ctr1*. The cloning and characterization of the genes that have been disrupted in these mutants are leading to a complicated genetic model of the ethylene signal transduction pathway (Wang et al. 2002). However, the functional redundancy among the different genes in one family makes it difficult to identify all of the components of ethylene signaling pathway by only screening for ethylene-responsive mutants. Therefore, the whole genome-based DNA microarray may be an effective means to identify new components in the plant signal transduction pathway.

Salt stress adversely affects plant growth and crop production. Consequently, an understanding of the mechanisms by which plants perceive salt stress and then transmit the signals to the plant's corresponding cellular machinery to activate adaptive responses is very important. The cellular and molecular responses of plants to environmental stresses have been studied extensively (Hasegawa et al. 2000; Zhu 2001), but the function of the ethylene signaling pathway in the salt stress response is still unclear. We previously characterized an ethylene receptor gene, *NTHK1*, from tobacco and found that this gene is salt-inducible (Zhang et al. 2001). Overexpression of this gene in *Arabidopsis* reduced ethylene sensitivity (Xie et al. 2002). Using microarray analysis of *NTHK1* transgenic *Arabidopsis*, He et al. (unpublished results) found that many genes are regulated by *NTHK1*. We report here the characterization of one of the genes they identified—an RLK gene, *AtLecRK2*. Our results indicate that the transcript level of *AtLecRK2* is induced by salt stress and that the induction depends on the functioning of ethylene signaling pathway. These results may suggest that there is an AtLecRK2-mediated signaling pathway in response to salt stress, which is in turn influenced by the ethylene signaling pathway in plants. The kinase activity and localization of the protein were also investigated.

Materials and methods

Plant materials

Homozygous *NTHK1* transgenic *Arabidopsis thaliana* (C.L.) Heynh. (Columbia) plants under the control of cauliflower mosaic virus (CaMV) 35S promoter and ethylene-related mutants *eto1–1* and *ein2–1* were grown on solid MS medium(Murashige and Skoog 1962). Following culture at 23°C under continuous light for 10 days, the seedlings were carefully removed from the plates and subjected to two treatments for various lengths of time: immersion in 200 m*M* NaCl for the salt stress treatment and immersion in a 200 μ*M* 1-aminocyclopropane-1-carboxylic acid (ACC) solution for the ethylene treatment. After these treatments, the plants were harvested and frozen in liquid nitrogen for total RNA isolation. The total RNA for organ-specific expression analysis was isolated from wild-type Columbia plants grown in soil for 2 months.

RNA isolation and quantitative reverse transcription (RT)-PCR

Total RNA was isolated following the protocol of Zhang et al. (1996). The samples were powered in liquid nitrogen, extracted with guandinium-thiocyanate-chloroform, precipitated by ethanol and purified with LiCl. Total RNA was suspended in RNase-free water and stored at −20°C following quantification. Total RNA (5 μg) from each sample of treated material was reverse-transcribed with olig(dT) (Promega, Madison, Wis.), and the cDNA product served as templates for RT-PCR. The constitutively expressed gene in *Arabidopsis*, *Actin7*, was also subjected to RT-PCR at the same time as an internal standard. The *AtLecRK2* sequence was amplified with *AtLecRK2*-specific primers 5′-TGATGAGTTCCTTACACTGG and 5′-AGAGTTGCAAATGGGTCATG, and the *Actin7* sequence was amplified with*Actin7*-specific primers 5′-GATGCTTACGTTGGTGATGA and 5′-CTGACTCATCGTACTCACT. The RT-PCR products were resolved on a 1% agarose gel.

Subcellular localization of AtLecRK2-GFP in onion epidermal cells

A transient expression vector with *AtLecRK2-GFP* (green fluorescent protein) was constructed to investigate the subcellular localization of AtLecRK2 in onion epidermal cells. The *AtLecRK2* gene-specific primers 5′-ACAGGATCCTTAGCAATGGCTTGTAGAC and 5′-CAGGTCGACTCTTCCATGCCCGTCCAAC, containing the *Bam*HI and *Sal*I sites, respectively, were used to amplify the full-length *AtLecRK2* cDNA. Following digestion with *Bam*HI and *Sal*I, the cDNA fragment was fused to the 3′-terminal end of GFP in the PUC18 vector, which can be expressed under the control of CaMV 35S promoter. The construction was confirmed by sequencing. Transformation of AtLecRK2-GFP in onion epidermal cells was performed using a Bio-Rad (Hercules, Calif.) biolistic particle delivery with 5 μg of each plasmid DNA precipitated onto M-17 tungsten particles. The PUC18 vector containing the *GFP* gene was used as a control for the localization study (Xie et al. 2003). Onion peels were incubated at 25°C on solid MS medium supplemented with sucrose for 48 h following bombardment. GFP fluorescence in onion epidermal cells was visualized with a confocal microscope. Plasmolysis of the onion epidermal cell was induced by the addition of 0.8 *M* mannitol as described by Friedrichsen et al. (2000).

Expression and purification of the AtLecRK2 protein

The DNA fragment encoding the putative kinase domain of AtLecRK2 was amplified from the original *AtLecRK2* plasmid. The primers of the kinase domain were 5′-CGAGGATCCAGGAGAAAGAAATATGCAGAAC and 5′-CAGGCTAGCTCTTCCATGCCCGTCCAAC, which contain the *Bam*HI and *Nhe*I sites. The amplified PCR products were digested and cloned into the yeast expression vector pESP-2 (Stratagene, La Jolla, Calif.) and confirmed by sequencing. The constructed pESP-2 plasmid can express recombinant protein as a fusion of glutathione S-transferase (GST) in yeast. The recombinant plasmid was transformed into the *Schizosaccharomyces pombe* SP-Q01 yeast strain (Stratagene), and the positive colonies were identified by their ability to grow on Edinburgh minimal medium supplemented with thiamine. The fusion protein was induced by growing the cells in Edinburgh minimal medium without thiamine and purified using Glutathione Sepharose 4B (Amersham, UK) as described by Xie et al. 2003).

Autophosphorylation and phosphoamino acid analysis of AtLecRK2

The GST fusion protein AtLecRK2-KD was analyzed for its kinase activity. Phosphorylation was carried out in 25-µl volumes of assay buffer [50 m*M* Tris-HCl, pH 7.6, 50 m*M* KCl, 2 m*M* DTT, 10% (v/v) glycerol] each containing 0.5 µg GST fusion protein in the presence of 5 m*M* $MgCl_2$ and 5 m*M* $MnCl_2$. The phosphorylation was initiated by adding 4.625×10^5 Bq γ-[^{32}P]-ATP ($1.11\times10^{\wedge14}$ Bq); this was followed by a 45-min incubation at 22°C for 45 min at which time the reaction was terminated by the addition of EDTA to a final concentration of 10 m*M*. The phosphorylated protein was subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Bedford, Mass.). The incorporated phosphate was visualized by autoradiography. The stability of the incorporated phosphate was determined by treating the membranes with either water, 1 *N* HCl or 3 *N* NaOH for 4 h at room temperature. The treated membranes were then subjected to autoradiography.

For phosphoamino acid analysis, the region of the membrane corresponding to the labeled AtLecRK2-KD was excised and

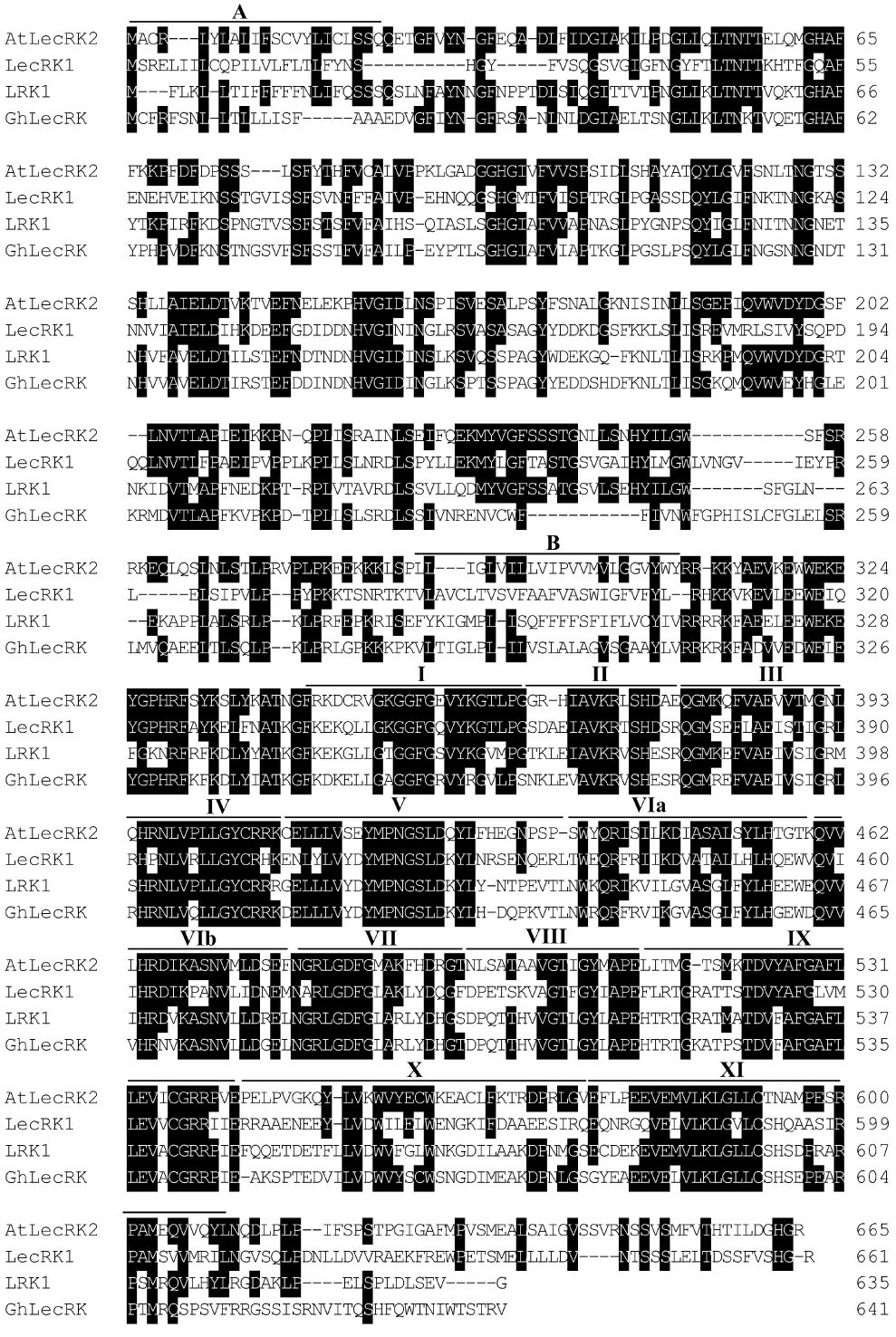

Fig. 1 Predicted amino acid sequence of AtLecRK2 and its comparison with related sequences. The sequence alignment was conducted with the MAGLIGN function of DNASTAR software. The identical residues among AtLecRK2, LecRK1, LRK1 and GhLecRK are *shaded*. *A* N-terminal signal peptide, *B* transmembrane domain, *I–XI* the twelve characteristic subdomains of the protein kinases (Hanks and Quinn 1991)

hydrolyzed in 6 *N* HCl at 110°C for 1 h. The supernatant was lyophilized and then dissolved in 10 µl of distilled water. The samples together with the phosphoamino acid standards (Sigma, St. Louis, Mo.) were spotted onto 0.1-mm-thick cellulose thin-layer chromatography (TLC) plates (Merck) and subjected to two-dimensional TLC electrophoresis as described (Xie et al. 2003). The positions of the three phosphoamino acid standards were visualized by spraying the plates with 0.25% ninhydrin in acetone followed by incubation in an oven at 65°C until the purple spots of the standards were visible. The plate was then autoradiographed to identify the labeled phosphoamino acids.

Sequencing and data analysis

The cloned DNA fragment was confirmed using an automated sequencing facility (ABI377; Applied Biosystems, Foster City, Calif.). The putative amino acid sequence encoded by the *AtLecRK2* gene was compared with the sequence deduced from the nucleotide sequence in the Genbank database using the GAPPED BLAST program. Amino acid sequence alignment and protein structure analysis were performed using DNASTAR software and the SMART program, respectively.

Results

Sequence analysis of *AtLecRK2*

By means of microarray analysis of salt-stressed *NTHK1* transgenic plants and wild type plants, we identified an RLK gene, *AtLecRK2*, being downregulated in *NTHK1* transgenic plants under salt stress. AtLecRK2 belongs to a subfamily of putative plant receptor serine/threonine kinases and is characterized by an extracellular legume lectin-like domain. In *Arabidopsis*, the lectin-like domain receptor protein kinases comprise a small multigenic family (Barre et al. 2002). *AtLecRK2* contains an open reading frame (ORF) that encodes a protein of 664 amino acids. The predicted protein has four putative distinct domains: a signal peptide domain, an extracellular lectin-like domain, a transmembrane domain and a kinase domain.

The signal peptide domain, located at the N-terminal of AtLecRK2, consists of 20 amino acids. Following the signal peptide is the lectin-like domain, which is similar to that of legume lectins; this domain binds to extracellular signals. The kinase domain is located at the C-terminal of AtLecRK2, which is the general location of RLKs in plants. There are 12 conserved subdomains in the kinase domain, and most of the subdomains of the AtLecRK2 kinase domain have conserved motifs that are identical to those of other members of the protein kinase superfamily (Hanks and Quinn 1991). A hydrophobic transmembrane domain was also identified between the kinase domain and the lectin-like domain, suggesting that AtLecRK2 is a putative transmembrane protein (Fig. 1).

The amino acid sequence of AtLecRK2 was compared with those of three other lectin-like RLKs: AtLecRK1, AtLRK1 and GhLecRK. The degree of similarity of identity of AtLecRK2 with AtLecRK1, AtLRK1 and GhLecRK1 was 36.5%, 42.2% and 40.7% for the full-length amino acid sequence and 47.2%, 51.7% and 53.2% for kinase domain sequence, respectively. The degree of sequence identity among the lectin-like domains was relatively lower—32.7% (AtLecRK2 and AtLecRK1), 38.8% (AtLecRK2 and AtLRK1) and 35.2% (AtLecRK2 and GhLecRK1). Therefore, the lectin-like domains of these proteins are more divergent than the kinase domains.

Analysis of *AtLecRK2* expression

The transcription levels of *AtLecRK2* were examined in the root, stem, leaf and flower by RT-PCR. The same cDNA was also used to amplify *Actin7* as an internal control. The results show that the strongest expression of *AtLecRK2* was found in the root and flower; lower expression was observed in the leaf, and almost no expression was detected in the stem (Fig. 2A).

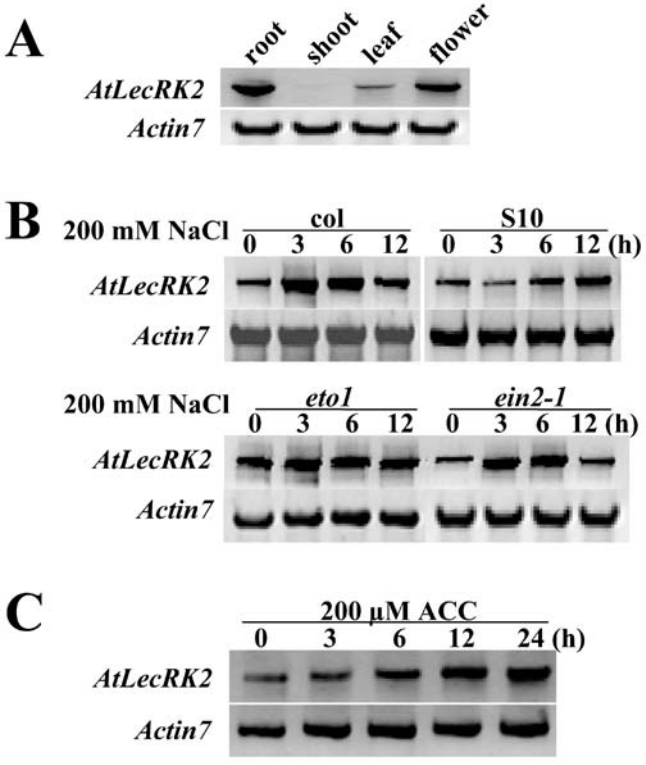

Fig. 2A–C Analysis of *AtLecRK2* expression by RT-PCR. The *Arabidopsis Actin7* gene was amplified at the same time as an internal control. **A** The expression of *AtLecRK2* in different organs of *Arabidopsis*. Total RNA isolated from the root, stem, leaf and flower of mature *Arabidopsis* plants was subjected to RT-PCR analysis. **B** The expression of the *AtLecRK2* gene in different *Arabidopsis* materials. Wild-type *Arabidopsis* Columbia (*col*), *NTHK1* transgenic *Arabidopsis* S10 (*S10*), ethylene-overproducer mutant *eto1-1* (*eto1*) and ethylene-insensitive mutant *ein2-1* (*ein2-1*) were treated with 200 m*M* NaCl. RNA was isolated at the times indicated and subjected to RT-PCR. **C** Accumulation of *AtLecRK2* mRNA in response to ACC. Wild-type *Arabidopsis* seedlings were removed from solid MS medium and soaked in 200 µ*M* ACC. The materials were harvested at the times indicated, and RNA was isolated for RT-PCR analysis

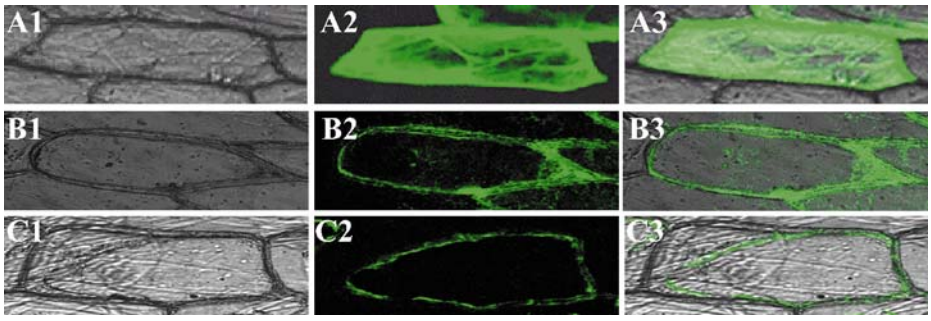

Fig. 3A–C Plasma membrane localization of AtLecRK2-GFP. The AtLecRK2-GFP fusion and GFP alone were each expressed transiently under the control of the CaMV 35S promoter in onion epidermal cells and observed under a confocal microscope. The photographs were taken in a dark field for the localization of the green fluorescence (**A1**, **B1**, **C1**), in a bright field for the examining the morphology of the cell (**A2**, **B2**, **C2**) and in combination (**A3**, **B3**, **C3**). Green fluorescence indicates the presence of AtLecRK2-GFP or GFP. **A1**, **A2**, **A3** The cell is transiently expressing the GFP control. **B1**, **B2**, **B3** The cell is expressing the AtLecRK2-GFP fusion. **C1**, **C2**, **C3** Plasmolysis of the cell expressing AtLecRK2-GFP

In order to investigate the different expression of *AtLecRK2* in *NTHK1* transgenic and wild-type plants, we performed RT-PCR using RNA obtained from plant material exposed to 200 m*M* NaCl. Our results show that in the wild-type plants *AtLecRK2* transcription was induced upon salt treatment, reaching its highest level within 3 h to 6 h of initiation of the stress and falling thereafter to a normal level (Fig. 2B). In *NTHK1* transgenic plants, the expression pattern was different (Fig. 2B). The transcription level of *AtLecRK2* in *NTHK1* transgenic plants was not significantly affected during the first 6 h of salt stress. Subsequently, however, *AtLecRK2* was apparently induced. The induction was weaken and late in comparison to that in the wild-type plants. Ethylene-overproducer mutant *eto1-1* and ethylene-insensitive mutant *ein2-1* were also used to investigate the dynamic expression of *AtLecRK2* in response to salt treatment. The results indicate that *AtLecRK2* in *eto1-1* was constitutively expressed at a relatively higher level and hardly at all influenced by salt stress. However, the expression level of *AtLecRK2* in ethylene-insensitive mutant *ein2-1* was almost the same as that of the wide-type plants in response to salt stress (Fig. 2B). Wild-type *Arabidopsis* plants were also exposed to 200 μ*M* ACC, and the results show that the transcription level of *AtLecRK2* increased steadily upon this exposure to 200 μ*M* ACC (Fig. 2C).

Subcellular localization of AtLecRK2

Because *AtLecRK2* has a transmembrane domain, we also looked at the subcellular localization of AtLecRK2. The fusion gene *AtLecRK2-GFP* and the control *GFP* in PUC18, driven by CaMV 35S promoter, were transformed into onion epidermal cells by particle bombardment. Gene expression was revealed by the green fluorescence of the GFP marker and observed under a confocal microscope. The AtLecRK2-GFP fusion protein was localized on the plasma membrane (Fig. 3B), whereas the GFP control protein was mainly present in cytoplasm (Fig. 3 A). In order to differentiate between the plasma membrane and the cell wall, the onion epidermal cells showing AtLecRK2-GFP expression were treated with 0.8 *M* mannitol. This treatment induces plasmolysis and caused an internalization of the plasma membrane while the cell wall remained unchanged. In plasmolyzed cells the AtLecRK2-GFP fluorescence on the plasma membrane was internalized and pulled away from the cell wall

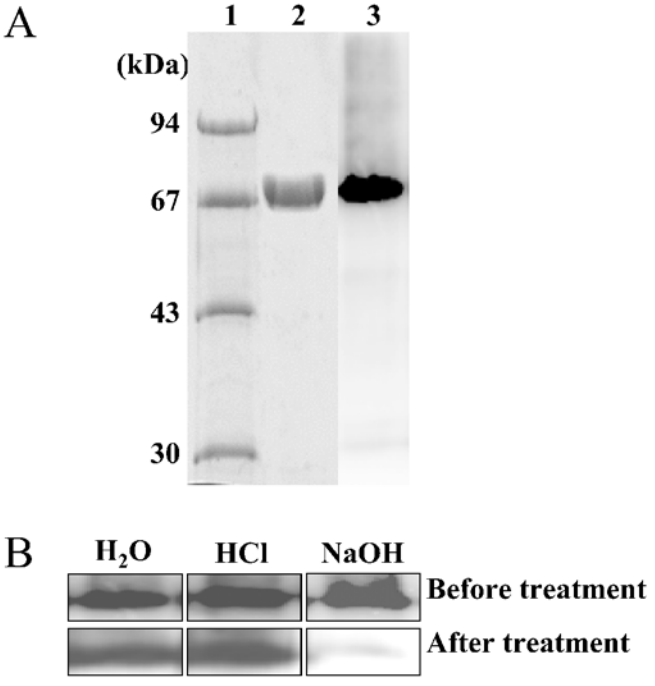

Fig. 4A, B Autophosphorylation property of the AtLecRK2 fusion protein. **A** Purified AtLecRK2-KD protein was subjected to SDS-PAGE and then stained with Coomassie brilliant blue (*lane 2*). The γ-[^{32}P]-ATP-incorporated protein was also subjected to SDS-PAGE and transferred onto PVDF membranes. Autoradiography of the PVPF membranes revealed a clear band at the region corresponding to AtLecRK2-KD (*lane 3*). *Lane 1* The molecular masses of the standard proteins. **B** The stability analysis of autophosphorylated AtLecRK2-KD. The stability of the incorporated phosphate was determined by treating the membranes with H_2O, 1 *N* HCl or 3 *N* NaOH for 4 h at room temperature. The treated membranes were then visualized by autoradiography

(Fig. 3C), demonstrating that AtLecRK2 is localized on the plasma membrane.

Autophosphorylation and phosphoamino acid analysis

Since AtLecRK2 protein possesses a putative kinase domain, we expressed this domain (AtLecRK2-KD) as a GST fusion protein in yeast cells (SP-Q01) and tested its kinase activity. The expressed protein was purified by affinity resin and resolved by SDS-PAGE. The fusion protein exhibited the predicted molecular mass of 67 kDa (Fig. 4A). The AtLecRK2-KD protein was incubated with γ-[^{32}P]-ATP, subjected to SDS-PAGE and then transferred onto PVDF membranes. It was apparent on the resulting autoradiograph (Fig. 4A) that [^{32}P] had been incorporated into the fusion protein and that there was only a single radioactive band. The position of this band matched that of the recombinant protein detected by Coomassie blue staining. Therefore, AtLecRK2 had the ability to autophosphorylate.

The phosphorylated AtLecRK2-KD protein was transferred onto PVDF membranes and the membranes treated with H_2O, 1 *N* HCl or 3 *N* NaOH to examine the stability of the phosphorylated residues. The phosphorylated residues were found to be stable under H_2O and acid treatment but sensitive to base treatment (Fig. 4B), indicating that the phosphorylated residues were phosphoserine, phosphothreonine and/or phosphotyrosine but not phosphohistidine.

Phosphoamino acid analysis was carried out using two-dimensional TLC-electrophoresis. The phosphorylated AtLecRK2-KD was hydrolyzed with 6 *N* HCl and analyzed together with the phosphoamino acid standards. Phosphoserine and phosphothreonine were detected, whereas phosphotyrosine was not detected (Fig. 5).

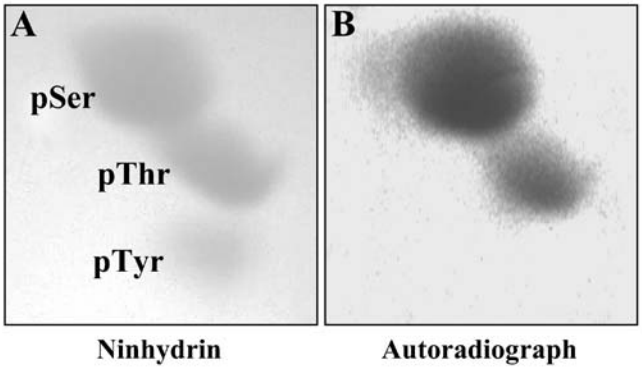

Fig. 5A, B Phosphoamino acid analysis of in vitro autophosphorylated AtLecRK2-KD. Phosphorylated AtLecRK2-KD was hydrolyzed with 6 *N* HCl, subjected to two-dimensional thin-layer electrophoresis and autoradiographed. **A** The positions of the phosphoamino acids were identified by spraying with ninhydrin. **B** The autoradiograph reveals that the phosphorylated amino acids were phosphoserine and phosphothreonine but not phosphotyrosine

Discussion

Receptor-like kinase genes compose a large gene family in plants. There are about 600 RLK genes in the *Arabidopsis* genome (Shiu and Bleecker 2001). These genes are involved in various aspects of plant life, and only a few of them have been characterized. The lectin-like RLKs have an extracellular domain sharing sequence similarity with the legume lectins. Previous studies have shown that legume lectins have a hydrophobic cavity that can bind hydrophobic molecules such as oligosaccharides and various plant hormones (Peumans and Van Damme 1995; John et al. 1997). This lectin-like domain may be important for lectin-like RLKs to perceive extracellular signals. Lectin-like RLKs may take part in various signal pathways through binding specific extracellular molecules. Alignment studies of the different lectin-like RLKs revealed that the lectin-like domain of different proteins is more divergent than the kinase domain. This phenomenon may imply that the lectin-like RLKs can perceive diverse extracellular signal molecules and transduce them to the downstream components through similar biochemical pathways. The detailed mechanisms need to be further investigated.

In the present study, *AtLecRK2* expression was induced by salt stress in wild-type *Arabidopsis* plants. In *NTHK1* transgenic plants the induced expression of *AtLecRK2* was inhibited and postponed, indicating an inhibitory effect of *NTHK1* on *AtLecRK2* expression. *AtLecRK2* expression was induced by ethylene and constitutively upregulated in the ethylene-overproducer mutant *eto1-1*. The induction by ethylene and inhibition by its receptor NTHK1 indicates a negative regulation between ethylene and NTHK1, which is consistent with the negative regulatory mechanism of the ethylene receptor (Hua et al. 1998; Schaller and Bleecker 1995). In the ethylene-insensitive mutant *ein2-1*, the expression pattern of *AtLecRK2* in response to salt stress is the same as that in the wild-type plant, which suggests that the induction of *AtLecRK2* by salt stress is independent of EIN2, which is a central component in the ethylene signaling pathway (Alonso et al. 1999). These facts also suggest that the salt-induced expression of *AtLecRK2* may need only the upstream components of the present ethylene signaling pathway and branch off before EIN2.

To date, several molecular mechanisms for the signal transduction pathway of salt stress in plants have been proposed (Zhu 2001). However, the receptors in response to salt stress have not yet been identified in plant cells. On the basis of our results, it is possible that AtLecRK2 may be a candidate salt stress receptor that transmits extracellular salt stress signals into intracellular reactions. We investigated the localization and biochemical properties of AtLecRK2 and found that AtLecRK2 is located in the plasma membrane and possesses serine/threonine kinase activity. These properties imply that AtLecRK2 represents a functional RLK involved in salt stress responses. Further research should focus on identifying the extracellular ligands and intracellular target molecules interacting with

AtLecRK2 along with a functional study of AtLecRK2 using a biochemical or transgenic approach. Such investigations would shed light on the AtLecRK2-mediated salt stress signaling pathway.

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