J. Plant Biol. (2011) 54:180–189
DOI 10.1007/s12374-011-9154-y

ORIGINAL RESEARCH

# Rice Gene *OsDSR-1* Promotes Lateral Root Development in *Arabidopsis* Under High-Potassium Conditions

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Received: 12 July 2010 / Revised: 27 January 2011 / Accepted: 27 January 2011 / Published online: 18 February 2011


**Abstract** Rice gene *Oryza sativa* Drought Stress Response-1 (*OsDSR-1*) was one of the genes identified to be responsive to drought stress in the panicle of rice at booting and heading stages by both microarray and quantitative real-time PCR analyses. *OsDSR-1* encodes a putative calcium-binding protein, and its overexpression in *Arabidopsis* rendered transgenic plants to produce much shorter lateral roots (LRs) than wild-type (WT) plants in the medium supplemented with abscisic acid (ABA), suggesting that *OsDSR-1* may act as a positive regulator during the process of ABA inhibition of LR development. No significant difference was observed in the total LR length between WT and transgenic plants in the media with the increase of only osmotic stress caused by NaCl, LiCl, and mannitol, while transgenic *Arabidopsis* seedlings appeared to produce larger root systems with longer total LR lengths under high-potassium conditions than WT seedlings. Further analysis showed that external $Ca^{2+}$ was required for the production of larger root systems, indicating that the promotion by *OsDSR-1* of the LR development of transgenic *Arabidopsis* seemed to occur in a $Ca^{2+}$-dependent manner under high-potassium conditions. We propose that *OsDSR-1* may function as a calcium sensor of the signal transduction pathway controlling the LR development under high-potassium conditions.

**Keywords** *Arabidopsis* · Lateral root (LR) · Potassium · Stress

**Electronic supplementary material** The online version of this article (doi:10.1007/s12374-011-9154-y) contains supplementary material, which is available to authorized users.

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## Introduction

Abiotic stress response in plants is under the control of numerous signaling pathways that function as an integrated network. Among them, calcium signaling was found to be involved in different signaling pathways mediating responses to heat, cold, drought, and salt (Shi 2007). Calcium signals generated upon stimulation are transmitted by three major types of $Ca^{2+}$ sensor proteins including calmodulin (CaM) proteins, calcium-dependent protein kinases, and calcineurin B-like proteins. The CaM is one of the best characterized $Ca^{2+}$-responsive proteins in eukaryotic cells (Snedden and Fromm 2001; Yang and Poovaiah 2003; Cheng et al. 2002; Luan et al. 2002; Zhu 2002). Previous studies have revealed that CaM genes from rice exhibit different expression levels in response to the presence of NaCl and mannitol and to wounding (Phean-o-pas et al. 2005). Most calmodulin-like (CML) genes display distinct abiotic stress response in *Arabidopsis*. For example, the expression levels of CML37 and CML39 were highly increased under salt stress condition (McCormack et al. 2005). Delk et al. (2005) have demonstrated that CML24 was involved in multiple stresses and hormonal responses.

The root system is an important agronomic trait, and the response of the root system architecture to environmental

stresses is regulated by different signal transduction pathways. The growth and development of the root system reflect the stress tolerance ability of plants under unfavorable growth conditions, and it has served as a model for studies of the mechanisms by which plants respond to environmental stresses. The inhibition of lateral root (LR) development of *Arabidopsis* by drought stress has been identified as an adaptive response to drought, and plants produce less LRs in response to drought stress (Xiong et al. 2006). The bioavailability of nutrients such as nitrate, phosphate, sulfate, and iron in the soil may have a profound impact on root system architecture (López-Bucio et al. 2003). Plants developed longer roots with a larger number of LRs under the sulfur-deprivation conditions (Kutz et al. 2002). The primary root length decreased with increasing nitrate availability, and the LR elongation was suppressed by high nitrate supplies, but the LR density was not affected across a range of nitrate supplies (Linkohr et al. 2002). Bao et al. (2007) have demonstrated that LR development was affected under high nitrate (50 mM) and remained normal under low nitrate (from 0.1 to 10 mM). The availability of phosphate also has an important impact on root system architecture in terms of LR number, LR density, and primary root length (Williamson et al. 2001; Sánchez-Calderón et al. 2006). Genetic and physiological evidence suggests that auxin is required at several specific developmental stages to facilitate LR formation. For example, young LR primordia are unable to continue to divide when excised from the primary root without indole-3-acetic acid supplementation (Casimiro et al. 2003).

ABA plays major roles in the regulation of numerous developmental processes, such as seed germination and stomatal activity, and is a central regulator of plant adaptation to environmental stresses, such as drought and high salinity (Kariola et al. 2006; Finkelstein et al. 2008; Cutler et al. 2010; Kim et al. 2010). Previous studies in *Arabidopsis* have identified a calcium-binding protein, ScaBP5, and its interacting protein kinase, PKS3, as global regulators of ABA response in this species (Guo et al. 2002).

To identify abiotic stress-responsive genes, we conducted genome expression profiles of rice under multiple stresses by Affymetrix microarray analysis (unpublished data). Among the genes identified as responsive to stresses, Drought Stress Response-1 (*OsDSR-1*) was highly induced by drought stress in the panicles of rice at booting and heading stages and was chosen for further study. Here, we present data on *OsDSR-1*, a novel rice gene encoding a putative calcium-binding protein, and the promotional effect on the LR development in transgenic *Arabidopsis* plants overexpressing *OsDSR-1* under high-potassium conditions.

## Material and Methods

### Rice Materials and Stress Treatments for Microarray Analysis

Leaf and panicle of Pei'ai 64S (*Oryza sativa* L. ssp. *indica* cv.) plants treated under drought, cold, and heat conditions at three development stages, seedling, booting, and heading stages, were used as the experimental materials. The seeds of Pei'ai 64S were surface-sterilized with 0.1% (*w*/*v*) $HgCl_2$ for 10 min and washed three times with distilled water. The sterilized seeds were soaked in distilled water at 25°C in darkness for 72 h for imbibition. Fully imbibed seeds were germinated at 37°C in darkness for 48–72 h, and germinated seeds were sown on plastic pots (20 cm in height and 10 cm in diameter) filled with soil in the greenhouse at 28°C/22°C (day/night) with a 16-h photoperiod. Rice plants at five-leaf, booting, and heading stages were subjected to environmental stress treatments. The drought treatment was performed by spilling the water out of the pots and keeping the plants growing without further watering. The sample materials were collected 16 h after leaf curling initiation. For cold stress treatment, rice plants at the five-leaf stage and at booting and heading stages were cold-treated at 4°C in darkness for 12 and 16 h, respectively, in a PGC15.5 Percival growth chamber (USA). For heat stress treatment, rice plants at booting and heading stages were placed in a Percival growth chamber at 45°C in darkness for 2 h. The control plants were kept growing under normal conditions under the same illumination conditions as for the treated plants.

### Rice Materials and Stress Treatments for Expression Analysis in Root

Germinated seeds were placed on a mesh above a plastic pot (15 cm in height and 10 cm in diameter) filled with 1/2 strength Murashige and Skoog (1/2 MS) liquid medium and grown in a PGC15.5 Percival growth chamber (USA) at 28°C/22°C (day/night) and 75% relative humidity with a 12-h photoperiod. The 1/2 MS liquid medium in the plastic pot was replaced every 3 days during growth. Rice plants at the five-leaf stage were used as the treatment material. For polyethylene glycol (PEG), salt, and ABA treatments, rice seedlings were irrigated with 1/2 MS liquid medium containing 10% (*w*/*v*) PEG, 100 mM NaCl, and 100 μM ABA, respectively. For cold and heat treatments, the seedlings were kept in a PGC15.5 Percival chamber at 4°C for 6 h and 45°C for 4 h, respectively. The liquid medium was kept unchanged in the plastic pot during the stress treatment. The roots from three seedlings were collected as one biological replicate, and each treatment was repeated three times.

### RNA Extraction

Total RNAs were extracted by using TRIzol reagent (Invitrogen, Corp.) according to the manufacturer's instructions. The concentrations of the RNA samples were spectrophotometrically determined based on absorbance at 260 nm and their quality examined by gel electrophoresis.

### Microarray Analysis

Microarray analysis was performed with the GeneChip Rice Genome Array according to the GeneChip® Expression Analysis Technical Manual (2005 version) provided by Affymetrix. The main operation procedures were as follows: (1) extraction and purification of total RNA, (2) synthesis and purification of cDNA, (3) synthesis and segmentation of cRNA, (4) array hybridization and washing, (5) array scanning, and (6) data analysis.

### qRT-PCR Analysis

Total RNAs were further treated with DNAase (Fermentas) to remove the co-extracted DNA, according to the manufacturer's protocol. Quantitative real-time PCR (qRT-PCR) was performed by using an ABI 7900HT Sequence Detection System (Applied Biosystems, USA) with the SYBR Green qRT-PCR One-Step Kit (QIAGEN cat. no.204243). The *OsDSR-1*-specific primers used for qRT-PCR experiments were 5′-AAG GCG GTG GAT GAA ACG G-3′ (forward) and 5′-AAG CAG GGT GGA AGA AGT GG-3′ (reverse). The specific primers for the 18S rDNA gene internal control used were 5′-CGT CCC TGC CCT TTG TAC AC-3′ (forward) and 5′-CGA ACA CTT CAC CGG ATC ATT-3′ (reverse). The PCR amplification was performed with an initial 10-min incubation at 95°C for Taq polymerase activation, followed by 40 cycles consisting of 15 s at 94°C, 40 s at 58°C, and 20 s at 72°C. At the end of the PCR reactions, a dissociation curve analysis (60–95°C) was performed to verify the fidelity of the amplification. Each sample reaction was run in duplicate.

### Cloning of the *OsDSR-1* cDNA

To obtain the cDNA of *OsDSR-1* with the full-length open reading frame (ORF), the total RNA extracted from the panicle of the drought-treated rice Pei'ai 64S at the heading stage was heated to 70°C for 5 min and then subjected to a reverse-transcription reaction using SuperScript II reverse transcriptase (Invitrogen, CA) with the oligo (dT) primer for 60 min at 42°C. The PCR amplification of the cDNA was carried out using the primers 5′-TCT AGA GCT TGG TGC CAT TGC TGC-3′ (forward) and 5′- CAC GTG CTT CTA TTC ACT CGG TGA TGA CAC-3′ (reverse) at 95°C for 3 min, followed by 30 cycles at 94°C for 30 s, 56°C for 1 min, and 72°C for 2 min. Purified cDNA fragments were ligated into vector pMD18-T (Takara) and cloned into *Escherichia coli*. The plasmid insert of a selected clone was then sequenced.

### Sequence Analysis of *OsDSR-1*

The genomic sequence and chromosome location of *OsDSR-1* were determined by comparison of the cloned cDNA with the genomic DNA sequences in GenBank. PlantCARE software (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) was used to identify *cis*-elements related to stress responses in the putative promoter region of *OsDSR-1*. The SMART database was used to identify conserved domains (http://smart.embl-heidelberg.de/) in *OsDSR-1*. To reveal possible post-translational modifications and its possible cellular localization, the protein sequence was analyzed with the computer programs Myristoylator (Bologna et al. 2004) and TargetP (Emanuelsson et al. 2000). Expression characteristics were predicted by searching UniGene entries corresponding to *OsDSR-1* in GenBank.

### Vector Construction for *Arabidopsis* Transformation

The cloned full-length cDNA was ligated into the binary vector pCAMBIA1300 under the control of the double 35S promoter of the *Cauliflower mosaic virus*. The pMD18-T plasmid harboring the cloned cDNA was digested with BamH I and Hind III, and the target band was isolated and ligated into the vector pJIT163 containing the double 35S promoter. The resulting plasmid containing the cloned cDNA was digested with Kpn I and Xho I, and the purified DNA fragment was ligated into the binary vector pCAMBIA1300.

### Generation of Transgenic Plants

*Arabidopsis* (ecotype Columbia-0) seeds were surface-sterilized with 70% ethanol for 2 min and 10% (*v*/*v*) bleach plus 0.2% Triton X-100 for 25 min and washed five times with sterilized water. The seeds were sown on agar plates containing 1/2 MS salts with 1% (*w*/*v*) sucrose and 0.8% (*w*/*v*) agar, pH 5.8 (1/2 $MS_{10}$), and then incubated at 4°C for 48 h to synchronize germination. Seedlings with four true leaves were transplanted to soil and grown in a growth room (24°C/16-h photoperiod).

*Agrobacterium tumefaciens* GV3101 carrying the *OsDSR-1* binary plasmid construct was used to transform *Arabidopsis* by the floral dip method (Clough and Bent 1998). Transgenic seedlings were selected as described by Harrison et al. (2006) with some modifications. Hygromycin (25 μg/mL) was used to screen for transformants in 1/2 $MS_{10}$ plates, and 10 transgenic *Arabidopsis* lines were

selected following verification by PCR detection of *OsDSR-1* (Fig. 1 supplementary data).

### Measurement of LR Length

Seeds of 10 transgenic *Arabidopsis* lines were sown on agar plates and grown for 4 days with the plates in a vertical orientation. Seedlings were then transferred to plates containing salt or osmoticum supplements. Plates were maintained in a vertical position, and the total LR length was measured after 5 days as described by Verslues and co-workers (Verslues et al. 2006). Seedlings of *Arabidopsis* were photographed with a digital camera. The images were analyzed using the National Institutes of Health image software (http://rsb.info.nih.gov/nih-image). The length of primary roots and the number and length of LRs were measured using the software. The total LR length of each individual plant was calculated. The average±S.E. of 10 plants was used as an index to measure LR growth after 5 days growth on each treatment media (Xiong et al. 2006). A total of seven transgenic lines (2–8) showed a similar phenotype in terms of LR development, and three homozygous transgenic lines (2–4) overexpressing *OsDSR-1* (Fig. 2 supplementary data) were used for the phenotypic characterization. Data were analyzed by ANOVA with Fisher's LSD test ($P$=0.05) using the SPSS program.

## Results

### Expression Analysis of *OsDSR-1*

The data from both microarray and qRT-PCR analyses showed that the expression levels of *OsDSR-1* (AK064016.1 accession number) were up-regulated by 60- and 5-fold in the panicle of rice at booting and heading stages, respectively, under drought stress condition relative to panicles under normal growth conditions (Fig. 1). To further study the gene activity, expression levels of *OsDSR-1* were measured in rice roots treated with heat, cold, PEG, ABA, and NaCl by using qRT-PCR. The results showed that *OsDSR-1* transcripts increased by 6-, 10-, and 12-fold under heat, PEG, and ABA treatments (Fig. 2).

### Cloning and Sequence Analysis of *OsDSR-1*

To investigate possible functions of the *OsDSR-1* gene, a corresponding cDNA containing the complete ORF was first cloned from the *indica* rice cultivar Pei'ai 64S following RT-PCR and sequenced. Sequence analysis showed that the cDNA of *OsDSR-1* was 1,654 bp in length and shared 99% identity to the cDNA of AK064016.1 from *Nipponbare* (*O. sativa* L. ssp. *japonica* cv.) in GenBank (http://www.ncbi.nlm.nih.gov). The small difference between the *OsDSR-1* cDNA sequence and AK064016.1 was most probably due to the two different rice subspecies. Comparison of the cDNA and its corresponding genomic DNA sequence (AAAA02028602.1, ranging from positions 23,341 to 30,841) from GenBank showed that *OsDSR-1* contains five introns and is located at chromosome 10 of rice. Further search in GenBank showed that the cDNA of *OsDSR-1* shared high similarity (>70%) to several sequences corresponding to genes of unknown function, including sequences AK102990 from rice and AK332601 from *Triticum aestivum* and BT064334, BT034067, BT085938, and BT054526 from *Zea mays*. Several putative *cis*-elements related to stress responses were identified in the putative promoter region of *OsDSR-1* about 1 kb upstream of the putative TATA box, including ABRE (involved in the abscisic acid responsiveness), CE3 (ABA and VP1 responsiveness), HSE (heat stress responsiveness), and LTR (low-temperature responsiveness).

The *OsDSR-1* cDNA sequence encodes a protein of 390 amino acid residues with PI≈4.66, and the encoded protein showed 99% amino acid identity with AK064016.1 from *Nipponbare*. No putative functional domain was found in the protein sequence except for the two $Ca^{2+}$-binding EF-hand motifs predicted by InterProScan (http://www.ebi.ac.uk/InterProScan/). Blast analysis showed that the predicted protein shared high similarity (>50%) to EF-hand containing

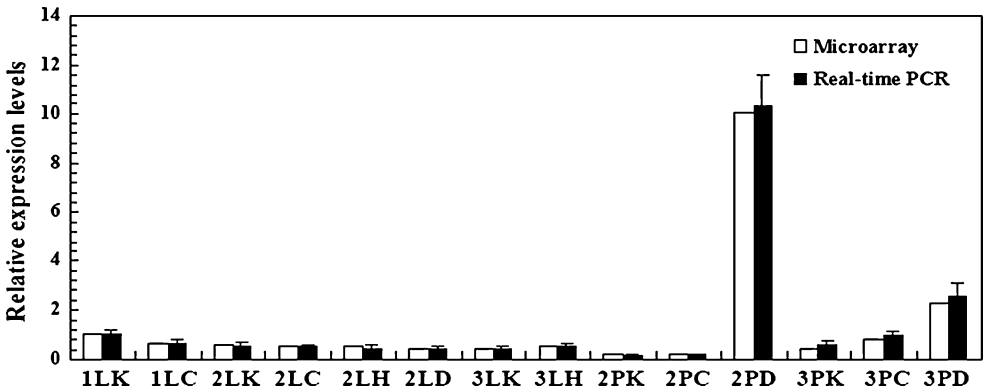


**Fig. 1** Relative expression levels of *OsDSR-1* in leaves and panicles of rice Pei'ai 64S at different developmental stages under the different stress and normal growth conditions. Relative expression levels were calculated relative to 1LK. *1*, seedling stage; *2*, booting stage; *3*, heading stage; *L*, leaf; *P*, panicle; *K*, control; *C*, cold; *H*, heat; *D*, drought

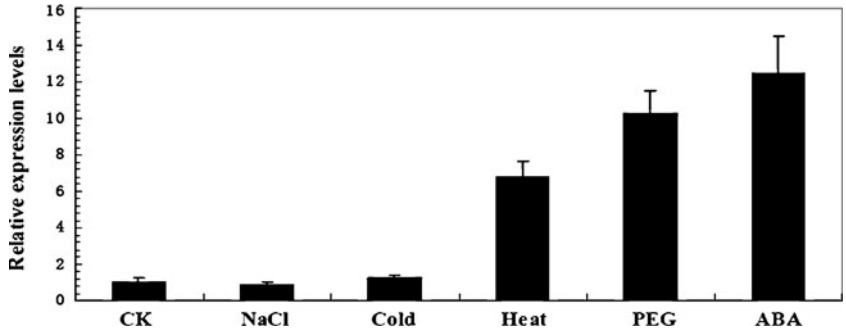


**Fig. 2** Relative expression levels of *OsDSR-1* in the root of rice Pei'ai 64S under the different stresses and normal growth conditions. *CK*, control. Relative expression levels were calculated relative to CK

proteins from rice (ABF94984), *Solanum tuberosum* (CAA04670), and *Arabidopsis* (CAB38615). However, the protein shared only 13.1% amino acid identity with OsCaM1-1, a protein classified as a true CaM by Boonburapong and Buaboocha (2007). No potential myristoylated glycine, either terminal or internal, was found in the protein sequence, as predicted by using the computer program Myristoylator (Bologna et al. 2004). The protein sequence was predicted to contain no targeting sequence by TargetP (Emanuelsson et al. 2000).

## Inhibition of the LR Elongation in Transgenic *Arabidopsis* by ABA

To determine whether or not the function of *OsDSR-1* will affect root system development in response to ABA, transgenic *Arabidopsis* plants were obtained with the vector harboring *OsDSR-1* driven by the double CaMV 35S promoter. The root system sizes of the transgenic *Arabidopsis* and wild-type (WT) controls were quantified by tracing and measuring the total LR length, the primary root length, and the LR number per individual plant (Fig. 3). We observed that seedlings of WT and transgenic *Arabidopsis* maintained on the control medium without ABA for 5 days produced similar root system architectures in terms of the total LR length, the primary root length, and the number of LRs. After the concentration of ABA was increased to 3 μM from 0.2 μM in the treatment media, the total LR length of WT seedlings was significantly longer than that of transgenic seedlings (Fig. 3a). However, no significant differences were observed in the primary root lengths (Fig. 3b) and the number of LRs (Fig. 3c) between WT and transgenic seedlings.

## Effects of Hormones on LR Development

To investigate whether the LR development of *Arabidopsis* was altered in response to hormones, seedlings of WT and transgenic *Arabidopsis* were transferred to media supplemented with 0.1 μM 6-benzyladenine (6-BA) or 0.5 μM 1-naphthalene acid (NAA). The primary root length (Fig. 4b) and the total LR length (Fig. 4a) of both WT and transgenic lines were found to be reduced in the presence of 6-BA and NAA, but the number of LRs in individual plants (Fig. 4c) appeared to have increased. No obvious differences in the total LR length, number of LRs, and primary root length of individual plants were found between them, indicating that the sensitivity of transgenic *Arabidopsis* to these hormones was not altered by overexpression of *OsDSR-1*.

## Repression of the Root Development of *Arabidopsis* by Osmotic Stress

To study the possible functions of *OsDSR-1* in response to osmotic stress, we compared the total LR length of *Arabidopsis* seedlings grown in the control medium (1/2 MS salts with 40 g/L sucrose and 8 g/L agar) and the treatment media supplemented with different concentrations of NaCl, LiCl, and mannitol as the osmotica. The root system sizes of WT and transgenic plants grown on the normal medium for 5 days were similar to each other. The total LR lengths of 5-day-old seedlings of both WT and transgenic plants were observed to be reduced in the media containing increased LiCl, NaCl, and mannitol (Figs. 3 and 4 and supplementary data), and the phenotypes of WT and transgenic *Arabidopsis* seedlings were similar to each other in the treatment media. The obtained results suggested that overexpression of *OsDSR-1* in *Arabidopsis* did not confer the plants with the ability to tolerate to LiCl, NaCl, or mannitol stresses.

## The Effect of Potassium on LR Development

High concentrations of salt and mannitol in the soil decreased its osmotic potential and profoundly affected the root system architecture of *Arabidopsis* (Deak and Malamy 2005). *Arabidopsis* plants grown under high KCl or mannitol were shown to develop small root systems and lacked LRs (Deak and Malamy 2005). To investigate the response of the LR development of transgenic *Arabidopsis* to KCl, seedlings were also tested in media supplemented with different concentrations (0, 15, 30, 50, 80, and

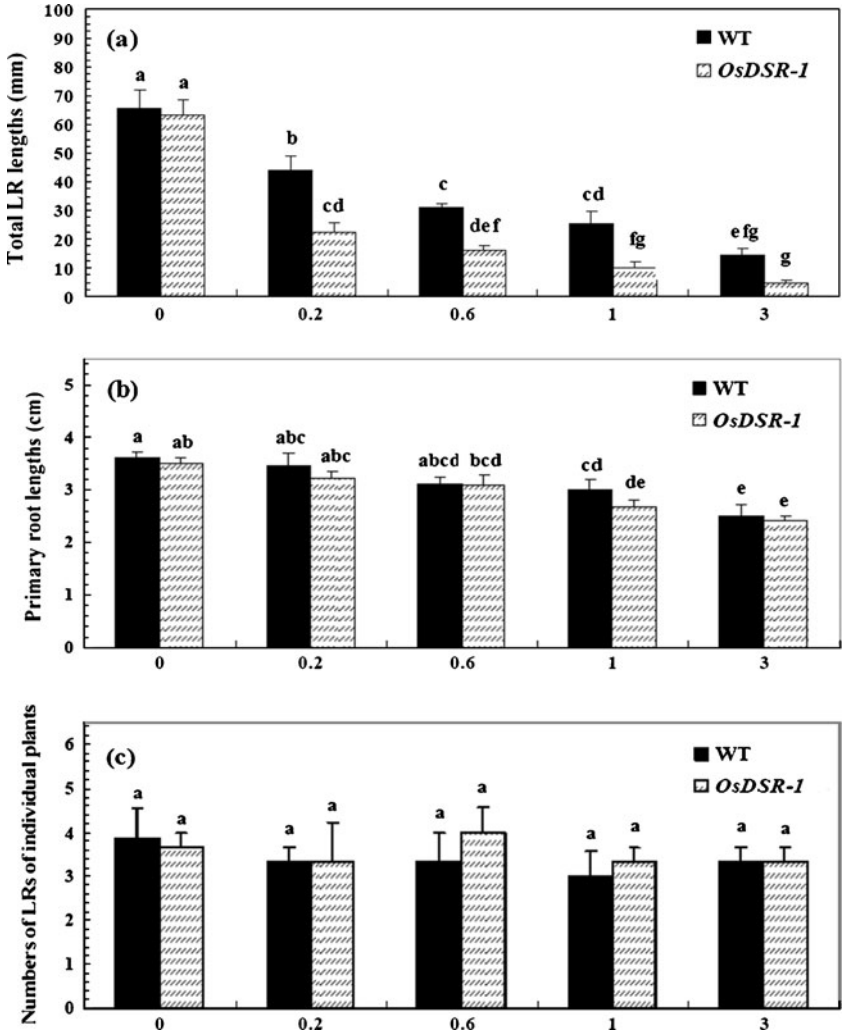


**Fig. 3** Effect of ABA on root system development. *Arabidopsis* seedlings after 4 days on the germination medium were transferred to a basal medium (1/2 MS salts with 40 g/L sucrose and 8 g/L agar) supplemented with different concentrations of ABA (0, 0.2, 0.6, 1.0, and 3.0 μM). The total LR lengths (**a**), the length of primary roots (**b**), and the LR numbers (**c**) of individual plants were presented. Data with *different letters* are significantly different at $P$=0.05

100 mM) of KCl. The LR development of 5-day-old seedlings of both WT and transgenic plants were found to be inhibited by the increased potassium concentrations in the treatment media (Fig. 5a). Thus, the supplementation of KCl in the medium affected the root system development of *Arabidopsis* as effectively as LiCl, NaCl, and mannitol. Different from the results obtained by including NaCl, LiCl, and mannitol as the osmotica in the media, the root systems of transgenic *Arabidopsis* grown in the medium supplemented with KCl were much larger than that of WT grown in the same treatment medium. Further analysis showed that such a difference was due to the longer LRs of transgenic plants (Fig. 5a), and no difference was observed in the primary root length between them (Fig. 5b). In terms of the LR number, transgenic plants produced more LRs than WT on the control medium and the treatment media containing 15 and 30 mM potassium. However, the difference was not significant in the LR numbers between them in the media containing higher levels (50, 80, and 100 mM) of potassium (Fig. 5c).

### The LR Development of Transgenic *Arabidopsis* is $Ca^{2+}$-Dependent in the Presence of Potassium

To determine whether external $Ca^{2+}$ might affect LR development in the presence of NaCl or mannitol, seedlings of the WT and transgenic *Arabidopsis* were transferred into the treatment media with or without $Ca^{2+}$. The result showed that both WT and transgenic *Arabidopsis* produced larger root systems with longer total LR lengths in the treatment and control media with $Ca^{2+}$ than those in the media without $Ca^{2+}$. The root system sizes of WT and transgenic *Arabidopsis* produced in the treatment media without $Ca^{2+}$ were similar to each other (Fig. 4 supplementary data). This result suggested that calcium mainly acts as a nutrition component affecting LR development of both WT and transgenic *Arabidopsis* in response to NaCl and mannitol stresses. To further investigate whether supplementation or displacement of $Ca^{2+}$ will impact the response of root system architecture of WT and transgenic *Arabidopsis* to potassium stress, similarly, seedlings of the WT and transgenic *Arabidopsis* were transferred into the

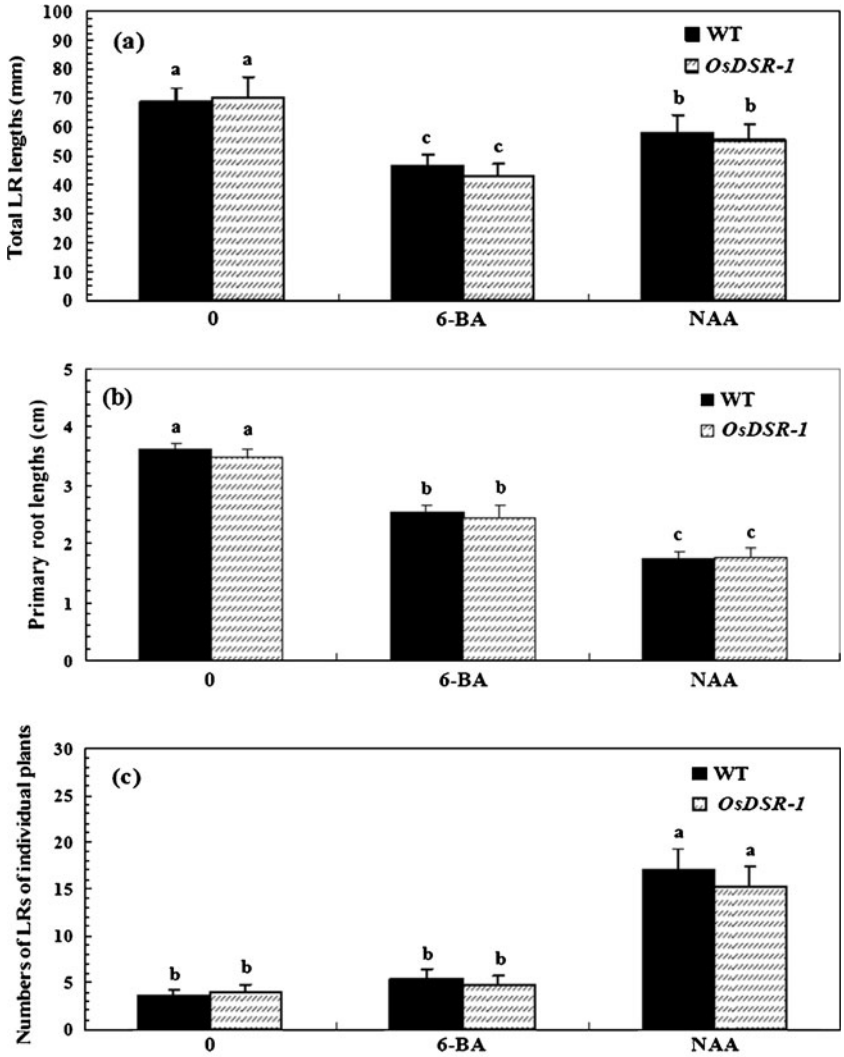


**Fig. 4** Effects of 6-BA and NAA on root system development. The total LR lengths (**a**), the lengths of the primary roots (**b**), and the numbers of LRs (**c**) of *Arabidopsis* seedlings treated with 6-BA (0.1 μM) and NAA (0.5 μM) are shown. Data with *different letters* are significantly different at $P=0.05$

treatment media with or without $Ca^{2+}$. The obtained results showed that the total LR length of transgenic *Arabidopsis* in the media supplemented with $Ca^{2+}$ was longer than that of transgenic *Arabidopsis* in the media without $Ca^{2+}$ (Fig. 5a). The total LR lengths and the LR numbers among WT plants were close to each other in the media with or without $Ca^{2+}$ (Fig. 5a, c). The primary root development of both WT and transgenic plants showed similar sensitivity to $Ca^{2+}$ supply (Fig. 5b), and the difference in the primary root length produced in the treatment media with $Ca^{2+}$ or without $Ca^{2+}$ was very small (Fig. 5b). Thus, $Ca^{2+}$ in the media made a significant contribution to the establishment of the root system architecture of transgenic *Arabidopsis* in the presence of increased potassium.

## Discussion

Calcium-binding proteins participate in calcium cell signaling pathways by binding to $Ca^{2+}$. It has been demonstrated that calcium-binding proteins play important roles in multiple stress responses, such as salt tolerance (Xiong et al. 2002). Although many rice genes encoding CaM and CaM-like (CML) proteins have been identified (Boonburapong and Buaboocha 2007), their roles in plants are still largely unknown and remain to be elucidated. *OsDSR-1* was identified and cloned from rice and found to encode a putative calcium-binding protein involved in mediating LR development in response to ABA and potassium.

ABA regulates the expression of some *OsCML* genes (White and Broadley 2003). Chen et al. (2006) have identified an ABA-induced calcium-binding protein from rice root through investigating the effects of ABA on rice root growth and development. This calcium-binding protein (CAED1785.1 accession number) contains two EF-hand motifs and shares 90% similarity to the amino acid sequence of *OsDSR-1*, suggesting that the two corresponding genes are homologous, belonging to the CML gene family of rice. The induction of *OsDSR-1* expression by ABA in the rice root and the presence of matches to ABRE sequence in the

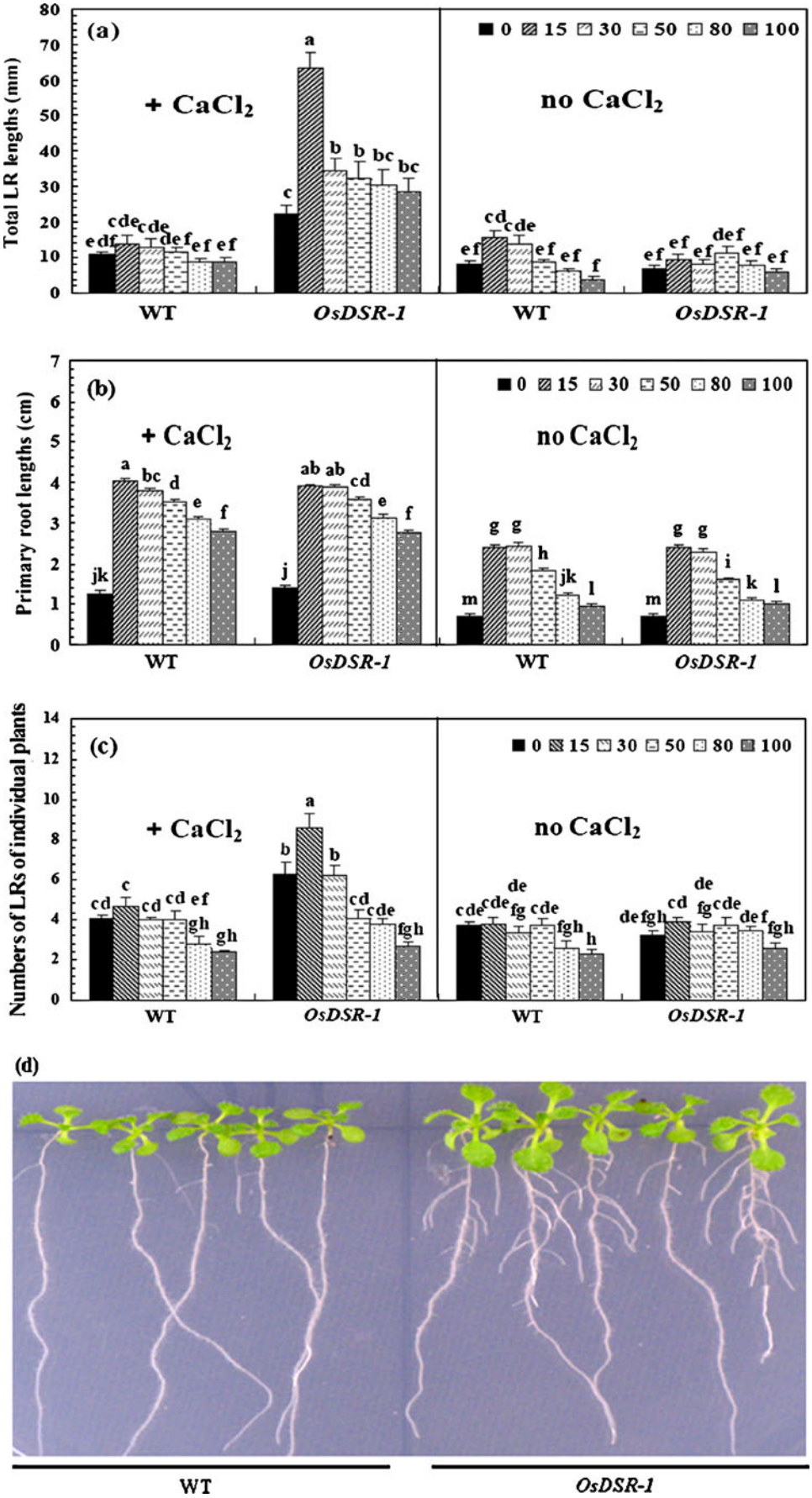


**Fig. 5** Effect of potassium on root system development. The total LR lengths (**a**), the lengths of the primary roots (**b**), and the numbers of LRs (**c**) were measured after plants treated with different concentrations of KCl (0, 15, 30, 50, 80, and 100 mM) for 5 days. In **d**, representative seedlings were transferred to the treatment medium containing 15 mM potassium for 5 days. Data with *different letters* are significantly different at $P=0.05$. The *panels on the left* of the **a**, **b**, and **c** show the data obtained from WT and transgenic *Arabidopsis* in the treatment medium with $CaCl_2$ (1.5 mM); the panels on the right of the figure show data obtained in the treatment medium without $CaCl_2$

putative promoter region of *OsDSR-1* suggest that the gene may be involved in regulating the response of root growth and development to ABA.

The process of LR formation in *Arabidopsis* can be divided into the following major stages: (1) stimulation and de-differentiation of pericycle cells, (2) ordered cell divisions and cell differentiation to generate a highly organized lateral root primordium (LRP), (3) emergence via cell expansion, and (4) activation of the LRP meristem to allow continued growth of the organized LR (Bao et al. 2007). Previous studies have indicated that the inhibition effect of ABA on LR development occurs immediately after the emergence of the LRP from the parent root and prior to the activation of the LR meristem (Smet et al. 2003). Based on the above information, *OsDSR-1* seemed to reduce the total LR length of transgenic plants mainly by inhibiting the elongation of LR in the presence of ABA and to promote the ABA inhibition of LR development at the stage of activation of LRP meristem. Thus, we propose that *OsDSR-1* may play a direct role in mediating the inhibitory effect of ABA on LR elongation by functioning as a positive regulator in the plant response to ABA. Many studies support a role for auxin during LR formation at several specific developmental stages (Casimiro et al. 2003). In this study, the WT and transgenic plants showed a similar phenotype in response to auxins, suggesting that *OsDSR-1* did not affect auxin-related signal transduction pathways regulating the LR development in *Arabidopsis*.

Calcium not only has a nutritional role in plant growth but also acts as a second messenger in the calcium signaling pathways controlling plant growth and development. In this study, no obvious difference was observed in phenotype between WT and transgenic *Arabidopsis* under the normal growth condition. Thus, the phenotype of *Arabidopsis* was unaffected by overexpression of *OsDSR-1*. The obtained results showed that the total LR length of transgenic *Arabidopsis* is longer than that of WT in the control medium without potassium (Fig. 5a), and the sizes of the leaf and root system of transgenic *Arabidopsis* were considerably larger than those of WT plants growing under high potassium conditions (Fig. 5d). The overexpression of *OsDSR-1* might improve the stress-resistance ability of transgenic *Arabidopsis* under limited potassium conditions, and consequently the transgenic plants grew larger root systems and uptook more nutrient than WT from the media supplemented with high concentration of potassium. The results showed that the expression of *OsDSR-1* in rice roots was affected greatly by PEG stress, but unaffected by NaCl stress (Fig. 2), and that its overexpression in transgenic *Arabidopsis* promoted the growth and development of LRs under high-potassium conditions. At the same time, calcium is essential for the promotion effect of *OsDSR-1* on the LR development in transgenic plants under high-potassium conditions, and the promotion effect seemed to occur at the emergence stage via cell expansion and the activation of the LRP meristem. With all the information obtained from this study, we propose that *OsDSR-1* may participate in regulating the root system architecture under osmotic stress caused only by KCl and that the LR development in transgenic plants is $Ca^{2+}$-dependent under high potassium conditions. *OsDSR-1* may function in the LR development, at the activation of the LRP meristem stage, in the presence of ABA or high potassium. Previous studies have revealed the ABRE-related sequence as $Ca^{2+}$-responsive *cis*-element in *Arabidopsis* (Kaplan et al. 2006). The presence of ABRE-related sequence in the putative promoter region of *OsDSR-1* indicates that the gene may act as a calcium sensor and function as a relay at the crossroad of the ABA signal transduction pathway and the pathway controlling the potassium-related LR development.

## Conclusions

LR development is affected strongly by its environment. The regulation of LR development in response to a changing environment is complicated by the involvement of different developmental signal transduction pathways. Understanding how these signals interact to regulate LR development is of great importance for studying the mechanisms by which plants respond to multiple stresses. In this study, we characterized *OsDSR-1*, a novel calmodulin-like protein gene from rice. Overexpression of *OsDSR-1* in *Arabidopsis* enhanced the inhibition effect of ABA on the LR development and promoted the LR development under high-potassium conditions. Thus, the *OsDSR-1* protein may participate in signal transduction, regulating the LR development of plants in response to ABA and potassium. It is evident that further studies, at both molecular and protein levels, will identify downstream components of the signal transduction pathway and unravel the mechanism by which plants respond to high potassium conditions and ABA.

**Acknowledgments** This research was supported by One-Hundred Person Project of the Chinese Academy of Sciences (02200420062903) and the project of Nitrogen and Phosphorus Cycling and Manipulation for Agro-Ecosystems, the Knowledge Innovation Program of the Chinese Academy of Sciences (KZCX2-YW-T07)

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