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Original Paper

Overexpression of a rice long-chain base kinase gene *OsLCBK1* in tobacco improves oxidative stress tolerance

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Abstract Sphingolipids and their metabolites including long-chain bases (LCBs) and long-chain base 1-phosphates (LCBPs) have been shown to be involved in regulation of various aspects of biological processes in plants. However, little is known about the biological function of LCB kinases (LCBKs), which catalyze the phosphorylation of LCBs to form LCBPs in plant abiotic stress tolerance. In the present study, we performed a functional analysis in transgenic tobacco to explore the possible involvement of a rice LCBK gene *OsLCBK1* in abiotic stress tolerance. Root elongation of the transgenic tobacco seedlings with constitutive overexpression of *OsLCBK1* was less sensitive to exogenous abscisic acid as compared with the vector-transformed seedlings. The *OsLCBK1*-overexpressing transgenic tobacco plants showed increased tolerance against oxidative stress after treatment with methyl viologen or H_2O_2, and up-regulated expression of oxidative stress-related genes. However, the *OsLCBK1*-overexpressing transgenic tobacco plants showed similar phenotype as vector-transformed plants in response to salt stress and had no change in expression of salt stress-related genes. Our results suggest that OsLCBK1, an enzyme involved in synthesis of LCBPs, may be involved in ABA response and has functions in regulation of oxidative stress tolerance in plants.

Key words: Abscisic acid (ABA), long-chain base kinase (LCBK), long-chain base 1-phosphates (LCBPs), oxidative stress tolerance., transgenic tobacco

Plants suffer adverse conditions such as salt and oxidative stresses during their lives and have developed a plenty of complex and sophisticated signaling pathways to regulate their response to abiotic stresses (Mittler et al. 2004; Seki et al. 2007; Shinozaki and Yamaguchi-Shinozaki 2007; Zhu 2002). Extensive biochemical, molecular, genetic and genomics studies have identified a lot of stress-responsive genes that are activated during abiotic stress responses in a number of plant species, especially in Arabidopsis and some of such stress-responsive genes have been explored for their potential in improving stress tolerance in plants (Ashraf 2010; Bhatnagar-Mathur et al. 2008; Umezawa et al. 2006; Wang et al. 2003; Yamaguchi and Blumwald 2005).

Recent studies have demonstrated that lipid signaling is an integral part of the complex regulatory network in plant response to biotic and abiotic stresses (Munnik and Testerink 2009; Wang et al. 2006). Long-chain base (LCB) 1-phosphates (LCBPs) are widely conserved bioactive lipid molecules in eukaryotic cells and have been shown to be signaling and regulatory molecules in multiple pathways involved in cell proliferation, cell death and stress responses (Alden et al. 2011; Markham et al. 2011; Zäuner et al. 2010). LCB kinase (LCBK) catalyzes the phosphorylation of LCBs to form LCBPs (Funato et al. 2003). The first plant LCBK gene *AtLCBK1* was identified from Arabidopsis (Imai and Nishiura 2005). Bioinformatics analysis identified three LCBKs, AtLCBK1 (At5g23450), AtLCBK2/SPHK1 (At4g21540), and At2g46090 in Arabidopsis (Imai and Nishiura 2005; Worrall et al. 2008). AtLCBK1 can phosphorylate D-erythro-dihydrosphingosine and phytosphingosine (Coursol et al. 2005; Imai and Nishiura 2005), whereas AtLCBK2 (AtSPHK1) is a functional sphingosine kinase (SphK)(Worrall et al. 2008). The majority of leaf SphK activity in Arabidopsis plants is associated with membrane fractions (Coursol et al. 2005). Recently, it was found that SphK was involved in ABA-induced stomatal closure (Coursol et al. 2003; Guo et al. 2012; Worrall et al. 2008). On the other hand, degradation of LCBPs by LCBP phosphatase or LCBP lyase has also been shown to play roles in abiotic stress response. For

Abbreviations: ABA, abscisic acid; LCB, long-chain bases; LCBP, long-chain base 1-phosphate; LCBK, LCB kinase; MV, methyl viologen.
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examples, mutations in Arabidopsis *AtSPP1* (encoding a functional LCBP phosphatase) or *AtDPL1* (encoding a functional LCBP lyase) affect the ABA responsiveness and drought tolerance (Nishikawa et al. 2008; Nakagawa et al. 2011).

During our studies on the molecular biology of rice disease resistance responses, we identified a rice LCBK gene *OsLCBK1*, encoding a putative LCBK in rice, and found that overexpression of *OsLCBK1* in transgenic tobacco plants affected disease resistance response. The objective of the present study was to explore the biological functions of *OsLCBK1* in plant abiotic stress response. Results from comparison of the phenotypes of the transgenic plants with vector-transformed plants in multiple abiotic stresses revealed that overexpression of the *OsLCBK1* gene in tobacco plants led to less abscisic acid (ABA) sensitivity, enhanced tolerance to oxidative stress and increased responsiveness of the stress-related gene expression. Our data suggest that OsLCBK1 plays an important role in plant abiotic stress response.

Materials and methods

Plant growth

The coding sequence of the *OsLCBK1* gene was cloned into plant binary vector CHF3pp2p212 under the control of the califlower mosaic virus (CaMV) 35S promoter and transformation of tobacco was performed using *Agrobacterium*-mediated leaf disc transformation as described previously (Luo et al. 2005). Two independent transgenic homozygous lines with single-copy of the *OsLCBK1* transgene and a CHF3pp2p212 vector-transformed line were used in the present study. All tobacco plants used were grown in a growth room under a 16 h/8 h day/night regime at 20–25°C and 8-week-old plants were used for all experiments.

ABA sensitivity assay

Seeds of the transgenic lines and vector-transformed plants were surface-sterilized and then placed on 1/2 MS medium containing indicated concentrations of ABA under 16 h/8 h day/night regime at 22–25°C for germination. Root lengths were recorded 2 weeks after germination and at least 40 individual seedlings were measured. The experiments were repeated independently for three times.

Abiotic stress tolerance assay

Fully expanded leaves from 8-week-old plants of the vector-transformed and *OsLCBK1*-overexpressing lines were rinsed with sterile distilled water and leaf discs (13 mm in diameter) were made by a hole puncher. Salt tolerance assay was done by floating the leaf discs in 10 ml NaCl solution (0, 0.4, and 0.8 M) for 3 days. Oxidative stress assay was performed by floating the leaf discs in methyl viologen (MV) or H_2O_2 solutions at different concentrations. In MV experiment, leaf discs were incubated in the dark for 1 h and then placed under illumination condition at moderate light intensity ($200\,\mu mol\,m^{-2}\,s^{-1}$) for 19 h at 25°C. In H_2O_2 experiment, leaf discs were incubated for 1 day under illumination condition at moderate light intensity ($200\,\mu mol\,m^{-2}\,s^{-1}$). At least 30 leaf discs from fully expanded leaves of 5 tobacco plants were included in each experiment and all experiments were repeated independently for three times.

Measurement of chlorophyll contents

Chlorophyll content was measured as described before (Veronese et al. 2003). The content of chlorophyll was tested by spectrophotometer, quantified according to the formula Chl (A+B) = $5.24A_{664}+22.24A_{648}$, where Chl is the chlorophyll concentration in μg per ml and A is the absorption (Lichtenthaler 1987). At least 30 leaf discs from fully expanded leaves of 5 tobacco plants were included in each experiment and the experiments were repeated independently for three times.

Gene expression analysis by RT-PCR

Leaf discs were harvested after treatments with salt or oxidative stresses and total RNA was extracted by TRIZOL reagent (Invitrogen, Shanghai, China) according to the manufacturer's instructions. The first strand cDNAs were synthesized using the SuperScript III Kit (Invitrogen, Shanghai, China) and 1 μg of the synthesized cDNAs were used for semi-quantitative RT-PCR in a total volume of 25 μl. PCR conditions were set as 94°C 30 s, 48–65°C 30 s and 72°C 30 s for 25–30 cycles based on the abundance of transcript for each gene, followed by 5 min of final extension at 72°C. PCR products were electrophoresed on a 1.2% agarose gel. Stress-related and internal reference genes and their gene-specific primers used are listed in Table 1. At least 30 leaf discs from fully expanded leaves of 5 tobacco plants were included in each experiment and the experiments were repeated independently for three times.

Results and discussion

In our previous studies aiming at elucidating the molecular basis of rice disease resistance responses, we identified a rice LCBK gene *OsLCBK1* that encodes a putative LCBK in rice. Bioinformatics analysis revealed that there are two putative LCBKs in rice genome, Os10g37280 (*OsLCBK1*) and Os04g45800. *OsLCBK1* encodes a 757 aa protein, which contains a conserved diacylglycerol kinase catalytic domain and conserved C1–C5 domains, similar to the characteristic structures in AtLCBK1. OsLCBK1 and Os04g45800 show an identity of 61.4% to each other and shows identity of 58% to AtLCBK1, indicating that OsLCBK1 is likely to be a LCBK in rice. To explore the biological function of *OsLCBK1* in disease resistance response, we generated transgenic tobacco lines that overexpress the *OsLCBK1* gene. The transgenic lines were allowed to grow for 3 generations and homozygous and single-copy lines were screened and confirmed by 3 : 1 segregation ratio

Table 1. Primers used in this study.

Genes (Accession ID)	Primers	Sequences (5′-3′)
NtGST (D10524)	NtGST-1F	GGC GAT CAA AGT CCA TGG TAG
	NtGST-1R	GCT TCT CCA ATC CCT TAA CCC
NtCAT1 (U93244)	NtCAT-1F	GGA TCC ATA CAA GTA CCG TCC
	NtCAT-1R	CAA GGA CCC TCC AAT TCT CCT G
NtAPX (AF443182)	NtAPX-1F	GCA TGG CAC TCT GCT GGT ACC
	NtAPX-1R	GGG GAT TGG TAG TCC AAG GTC
NtSAM1 (AF127243)	NtSAM-1F	CAG ACC AAT AAA CAA GCT TCA
	NtSAM-1R	ATT CCC TGA AGG ACT CTT TCA
NtTOBLT (D13952)	NtTOBLT-1F	CTG ACC GGA AGA CTG CAT GCA
	NtTOBLT-1R	AAC CAT CCA CCA AAG TTT CA
NtLEA5 (AF053076)	NtLEA5-1F	TGC TTT CGT CGT TGA TAC TGT
	NtLEA5-1R	GAT TGC GCT ATG GGA CGT GGT
NtACT9 (X69885)	NtActin-2F	CTA TTC TCC GCT TTG GAC TTG GCA
	NtActin-2R	ACC TGC TGG AAG GTG CTG AGG GAA

of the antibiotic resistance marker on 1/2 MS medium containing 200 μg ml^{-1} kanamycin. Two independent T3 generation homozygous lines (K1–5 and K1–44) that contain single copy of the *OsLCBK1* gene were chosen for this study. In the present study, we examined whether *OsLCBK1* has functions in plant abiotic stress response by comparison of the phenotypes of the *OsLCBK1*-overexpressing transgenic plants with the vector-transformed plants.

Decreased ABA sensitivity in the OsLCBK1-overexpressing plants

Previous studies have shown that *AtLCBK1* was slightly induced by low humidity or ABA (Imai and Nishiura 2005; Nishiura et al. 2000). Therefore, we first compared the sensitivity of the *OsLCBK1*-overexpressing transgenic and the vector-transformed plants to exogenous ABA. Without the treatment of ABA, the root growth showed no difference between the *OsLCBK1*-overexpressing seedlings and the vector-transformed seedlings (Figure 1A and B). After the treatment of ABA, the root growth of the vector-transformed seedlings was inhibited and this inhibition was more obvious when the concentration increased. Compared with the vector-transformed seedlings, root growth of the *OsLCBK1*-overexpressing seedlings was less inhibited by exogenous ABA and the roots were much longer than the vector-transformed seedlings at high concentrations of ABA (2 μM and 4 μM) (Figure 1A). Grown on 1/2 MS containing 4 μM ABA, the root length was only ca. 15% of those grown on 1/2 MS without ABA in the vector-transformed seedlings, while the root length was ca. 30% of those grown on 1/2 MS without ABA in seedlings of the transgenic lines (Figure 1B). These results suggest that overexpression of *OsLCBK1* in transgenic tobacco resulted in a decreased ABA sensitivity. Our observation differs from a previous finding that the stomata of the *AtLCBK2*/*AtSPHK2*-overexpressing Arabidopsis plants were more sensitive,

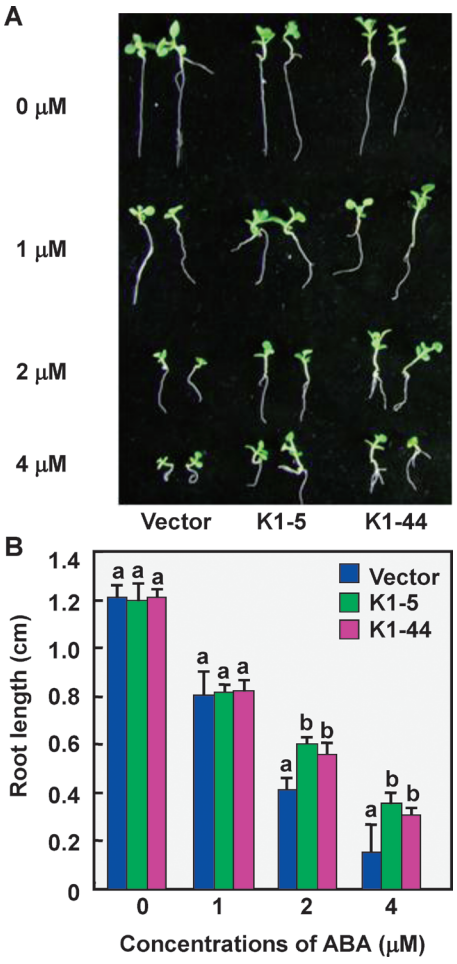

Figure 1. ABA sensitivity of the *OsLCBK1*-overexpressing transgenic seedlings. A) Growth phenotype of the *OsLCBK1*-overexpressing transgenic and vector-transformed (vector) seedlings on 1/2 MS with different concentrations of ABA. Photos were taken 2 weeks after germination. B) Root lengths of two-week-old *OsLCBK1*-overexpressing transgenic and vector-transformed seedlings grown on 1/2 MS with different concentrations of ABA. Data presented are the means±SD from three independent experiments and different letters above the columns indicate significant differences at $p<0.05$ level. vector, vector-transformed plants; K1–5 and K1–44, *OsLCBK1*-overexpressing transgenic lines #5 and #44.

than wild type to exogenous ABA (Worrall et al. 2008). The difference in ABA responsiveness between the *OsLCBK1*-overexpressing tobacco seedlings and the *AtLCBK2*/*AtSPHK2*-overexpressing Arabidopsis plants may be due to the facts that *OsLCBK1* encodes a putative LCBK while AtSPHK1 is a functional SphK (Worrall et al. 2008), and that the root elongation assays were used for examining ABA sensitivity of the *OsLCBK1*-overexpressing tobacco plants in our experiments while stomatal response assays were carried out for assessing ABA sensitivity of the *AtSPHK1*-overexpressing Arabidopsis plants (Worrall et al. 2008). It was found that sphingolipids including S1P can function as intracellular messengers in response to ABA and that AtSPHK1 and phyto-S1P play important roles in mediating the ABA response (Coursol et al. 2003; Guo et al. 2012; Ng et al. 2001). For example, LCBPs were shown to be involved in ABA inhibition of stomatal opening and promotion of stomatal closure in Arabidopsis (Coursol et al. 2003, 2005). Therefore, it is likely that overexpression of *OsLCBK1* in transgenic tobacco plants may promote synthesis of LCBPs and thus affect the balance of LCBPs, which in turn interfere with the ABA signaling leading to changes in ABA sensitivity. However, mutations in Arabidopsis LCBP phosphatase gene *AtSPP1*, which is likely to suppress the degradation of LCBPs, did not affect the ABA-mediated inhibition of root elongation (Nakagawa et al. 2011). Further investigations with combined biochemical and genetic approaches are required to elucidate the details of the functions for OsLCBK1 in ABA signaling and possible involvement of LCBPs in ABA-mediated root elongation.

Enhanced oxidative stress tolerance in the OsLCBK1-overexpressing plants

Possible involvement of OsLCBK1 in oxidative stress tolerance was studied using H_2O_2 and MV as artificial stress conditions. During our experiment time, no significant phenotype appeared on the leaf discs from the transgenic lines and the vector-transformed plants without H_2O_2 or MV treatment (Figures 2A, 3A). With the treatment of H_2O_2 or MV, bleaching or chlorosis were observed in leaf discs from *OsLCBK1*-overexpressing and the vector-transformed plants (Figures 2A, 3A). Bleaching or cholorosis symptom in leaf discs from the *OsLCBK1*-overexpressing plants was less severe than those of the vector-transformed plants (Figures 2A, 3A). These observations were further confirmed by measuring chlorophyll contents in leaf discs from the transgenic and the vector-transformed plants after MV or H_2O_2 treatments (Figures 2A, 3A). After treatments with MV or H_2O_2, relative chlorophyll contents in leaf discs of the *OsLCBK1*-overexpressing plants were markedly higher than those from the vector-transformed plants (Figures 2A, 3A). Notably, the difference in relative chlorophyll

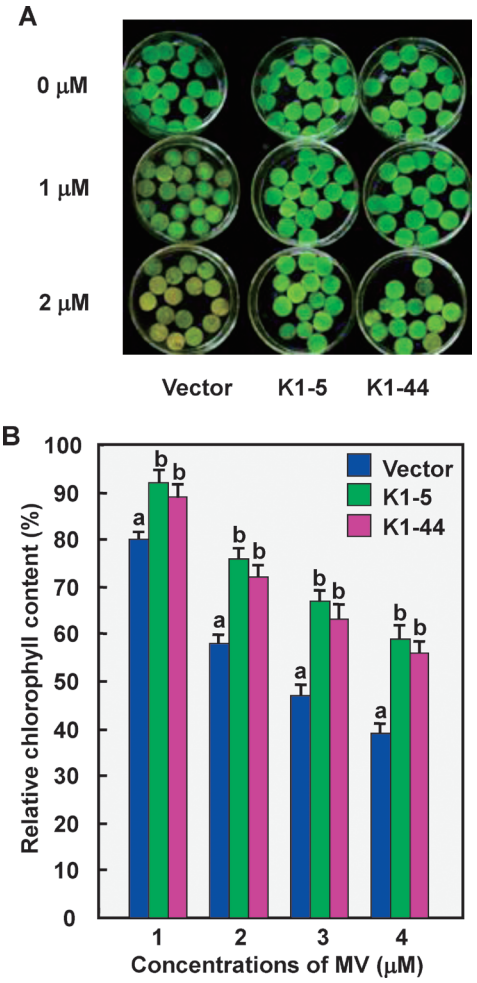

Figure 2. Tolerance of the *OsLCBK1*-overexpressing transgenic plants to exogenous methyl viologen. A) Phenotype of leaf discs from eight-week-old *OsLCBK1*-overexpressing transgenic and vector-transformed plants in MES buffer supplemented with different concentrations of methyl viologen. Photos were taken at 19 h after treatment. B) Relative chlorophyll contents in leaf discs from the *OsLCBK1*-overexpressing transgenic and vector-transformed plants after treatment with different concentrations of methyl viologen. Data presented are the means±SD from three independent experiments and different letters above the columns indicate significant differences at $p<0.05$ level. Vector, vector-transformed plants; K1–5 and K1–44, *OsLCBK1*-overexpressing transgenic lines #5 and #44.

contents in leaf discs of the *OsLCBK1*-overexpressing and the vector-transformed plants was increased along with the increase of the concentrations of MV or H_2O_2 (Figures 2A, 3A). These results indicate that overexpression of *OsLCBK1* in transgenic tobacco plants can improve oxidative stress tolerance. It was recently found that mutations in Arabidopsis *FBR11* (encoding a subunit of serine palmitoyltransferase that catalyzes *de novo* synthesis of LCBs) led to attenuated formation of LCBs in response to Fumonisin B1 and that exogenous LCBs efficiently induced generation of reactive oxygen species (ROS) while exogenous LCBPs specifically blocked LCB-induced ROS generation in *fbr1* mutant plants (Shi et al. 2007). These observations demonstrated

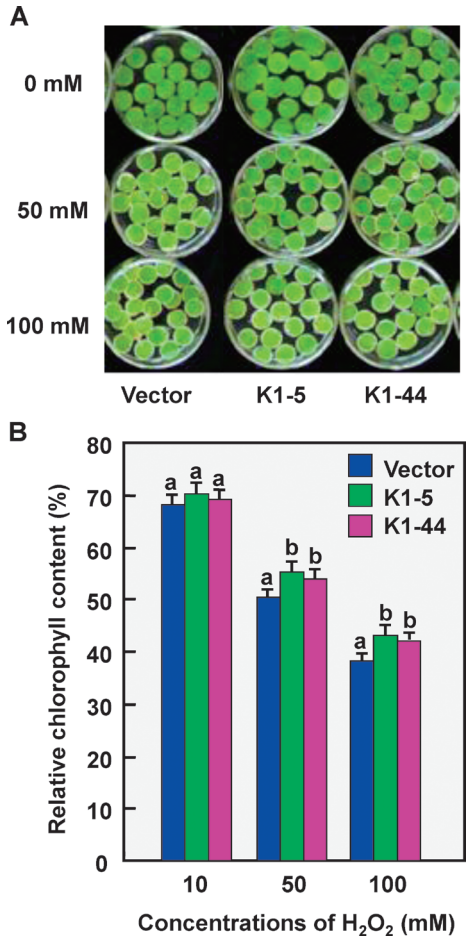

Figure 3. Tolerance of the *OsLCBK1*-overexpressing transgenic plants to exogenous hydrogen peroxide. A) Phenotype of leaf discs from eight-week-old *OsLCBK1*-overexpressing transgenic and vector-transformed plants in MES buffer supplemented with different concentrations of H_2O_2. Photos were taken at 1 day after treatment. B) Relative chlorophyll contents in leaf discs from the *OsLCBK1*-overexpressing transgenic and vector-transformed plants after treatment with different concentrations of H_2O_2. Data presented are the means±SD from three independent experiments and different letters above the columns indicate significant differences at $p<0.05$ level. Vector, vector-transformed plants; K1–5 and K1–44, *OsLCBK1*-overexpressing transgenic lines #5 and #44.

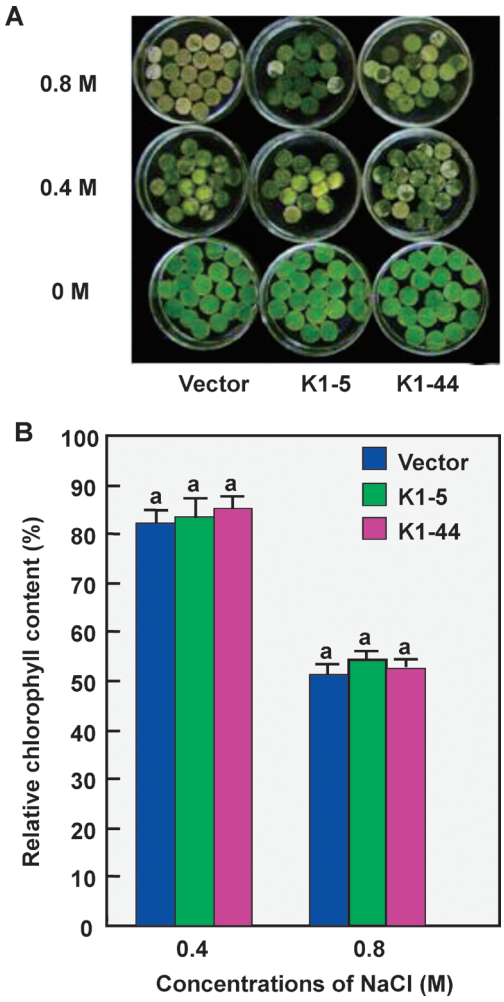

Figure 4. Salt tolerance of the *OsLCBK1*-overexpressing transgenic plants. A) Phenotype of leaf discs from eight-week-old *OsLCBK1*-overexpressing transgenic and vector-transformed plants under salt stress condition. Photos were taken at 3 days after treatment. B) Relative chlorophyll contents in leaf discs from the *OsLCBK1*-overexpressing transgenic and vector-transformed plants after treatment with different concentrations of NaCl. Data presented are the means±SD from three independent experiments and different letters above the columns indicate significant differences at $p<0.05$ level. Vector, vector-transformed plants; K1–5 and K1–44, *OsLCBK1*-overexpressing transgenic lines #5 and #44.

that the homeostatic balance between LCBs and LCBPs plays important roles in regulating of ROS level in cells. In our study, when overexpressed, the OsLCBK1 may accelerate the rate of LCBP synthesis, resulting in reduced levels of LCBs and increased levels of LCBPs, and thus affect the homeostatic balance between LCBs and LCBPs, which favors, probably through regulating cellular ROS level, to improve oxidative stress tolerance in the *OsLCBK1*-overexpressing transgenic tobacco plants. However, whether overexpression of *OsLCBK1* in transgenic tobacco plants affects ROS generation and the physiological mechanisms of enhanced oxidative stress tolerance regulated by OsLCBK1 in transgenic plants need further examined.

Unaltered salt tolerance in OsLCBK1-overexpressing plants

Possible role of OsLCBK1 in salt stress tolerance was also studied by testing the tolerance of leaf discs from 8-week-old transgenic and the vector-transformed plants to different concentrations of NaCl solution. Without NaCl treatment, leaf discs remained green both in the *OsLCBK1*-overexpressing and vector-transformed plants. With the increasing of NaCl, the leaf discs showed slight bleaching symptom in the *OsLCBK1*-overexpressing and vector-transformed plants (Figure 4A). At the concentration of 0.4 M and 0.8 M, the relative chlorophyll contents were 81% and 52% of those without NaCl in the vector-transformed plants, respectively (Figure 4B). The

relative chlorophyll contents were 85% and 55% of those without NaCl treatment in the *OsLCBK1*-overexpressing plants (Figure 4B). Based on the bleaching symptom and reduction of chlorophyll contents, no significant difference was observed in salt tolerance between the *OsLCBK1*-overexpressing and vector-transformed plants. These results suggested that overexpression of *OsLCBK1* in transgenic tobacco does not affect the salt stress tolerance and thus OsLCBK1 has limited function in salt stress tolerance. This is contrary to the observations that, in Arabidopsis, AtLCBK2 (AtSPHK1) plays important roles in ABA signaling and defense response against drought and chilling stresses (Coursol et al. 2003; Dutilleul et al. 2012; Guo et al. 2011, 2012; Worrall et al. 2008). However, it is not clear whether or not AtLCBK2 has a function in salt stress response. Signaling pathways require for salt stress response are not identical to those required from drought stress response, although cross-talking between signaling pathways involved in salt and drought stress responses exist (Zhu 2002).

Differential expression of stress-related genes in OsLCBK1-overexpressing plants

Expression of some selected stress-related genes was analyzed to get insights into the possible mechanisms of the enhanced oxidative stress tolerance in the *OsLCBK1*-overexpressing transgenic tobacco plants. To this purpose, expression of oxidative stress-related genes, including genes encoding ascorbate peroxidase (APX), catalase (CAT) and glutathione *S*-transferases (GST), was first analyzed. In water-treated controls, expression of *GST* was not detected in leaf discs from the *OsLCBK1*-overexpressing and the vector-transformed plants, while expression of *APX* and *CAT* with different patterns was detected in leaf discs from the *OsLCBK1*-overexpressing and the vector-transformed plants (Figure 5A). When compared with those in the vector-transformed plants, increased expression of *APX* in leaf discs of the *OsLCBK1*-overexpressing plants was observed (Figure 5A). After incubation with H_2O_2 or MV, expression of *APX*, *GST* and *CAT* was up-regulated in leaf discs of the vector-transformed plants, indicating an oxidative stress was applied to leaf discs (Figure 5A). However, the expression levels of *APX*, *GST* and *CAT* in leaf discs of the *OsLCBK1*-overexpressing plants were higher than those in leaf discs from the vector-transformed plants (Figure 5A). Increased expression levels for *GST* and *CAT* were much evident in leaf discs from the *OsLCBK1*-overexpressing plants, as compared with those of the vector-transformed plants (Figure 5A). It is well established that enhanced oxidative stress tolerance is often associated with high levels of expression of genes that are involved in oxidative stress responses in plants (Mittler et al. 2004, 2006). Therefore, it is likely that the up-regulated expression of the oxidative stress-responsive genes after treatment with MV or H_2O_2 may be responsible for the increased oxidative stress tolerance observed in the *OsLCBK1*-overexpressing tobacco plants.

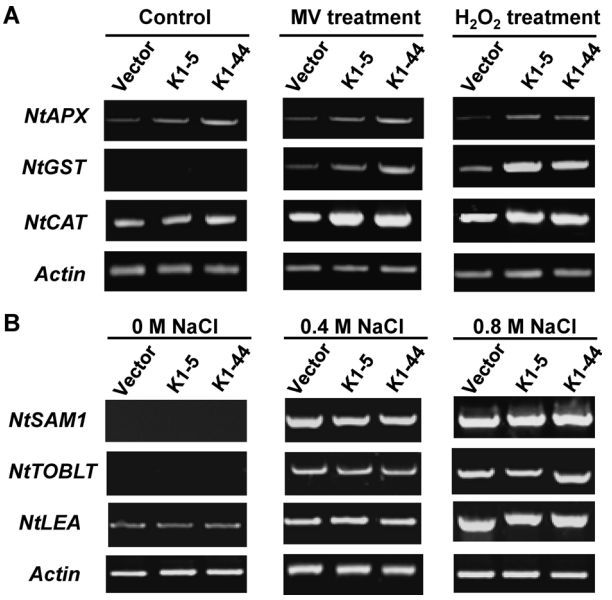

Figure 5. Expression of salt- and oxidative stress-responsive genes in *OsLCBK1*-overexpressing transgenic plants before and after treatment with NaCl or oxidative stress. A) Expression of oxidative stress-responsive genes. B) Expression of salt stress-responsive genes. Leaf discs collected from eight-week-old *OsLCBK1*-overexpressing transgenic and vector-transformed plants were treated with NaCl at different concentration, methyl viologen (MV, 2 μM), H_2O_2 (50 mM) or water as a control for 5 h. Expression of stress-responsive genes in leaf discs was analyzed by RT-PCR using gene-specific primers with actin as an internal control. Vector, vector-transformed plants; K1–5 and K1–44, *OsLCBK1*-overexpressing transgenic lines #5 and #44.

It is previously shown that *NtSAM1* (encoding *S*-adenosyl-L-Met synthetase), *NtTOBLT* (encoding a lipid transfer protein) and *NtLEA5* (encoding one of late embryogenesis abundant proteins) were induced during salt stress response (Espartero et al. 1994; Masuta et al. 1992; Torres-Schumann et al. 1992; Xu et al. 1996). When treated in water without NaCl, no significant expression of *NtSAM1* and *NtTOBLT* was detected in leaf discs from the vector-transformed plants, while no change in expression of *NtLEA5* was observed in leaf discs between the *OsLCBK1*-overexpressing and the vector-transformed plants (Figure 5B). After treatment with NaCl, expression levels of the *NtSAM1*, *NtTOBLT* and *NtLEA5* genes were increased in leaf discs from the vector-transformed plants and the *OsLCBK1*-overexpressing plants; however, expression levels of the *NtSAM1*, *NtTOBLT* and *NtLEA5* genes in leaf discs from the *OsLCBK1*-overexpressing plants were comparable to those in the vector-transformed plants (Figure 5B). The unchanged expression patterns of these salt stress-related genes in the *OsLCBK1*-overexpressing plants further support our observations that overexpression of *OsLCBK1* in transgenic tobacco plants does not affect the salt stress

tolerance.

In some previous studies, enhanced oxidative stress tolerance and increased disease resistance were simultaneously observed in transgenic tobacco overexpressing pepper peroxidase and ascorbate peroxidase genes (Choi et al. 2012; Sarowar et al. 2005). Similar results were also observed for the *OsLCBK1*-overexpressing transgenic tobacco as overexpression of *OsLCBK1* in transgenic plants resulted in enhanced oxidative stress tolerance and disease resistance (Zhang and Song unpublished data). These findings indicate a link between oxidative stress tolerance and disease resistance. It is well known that ROS homeostasis play important roles in oxidative stress and disease resistance responses (Miller et al. 2008; Mittler et al. 2011; Suzuki et al. 2012). Excessive ROS is generally cytotoxic and thus leads to oxidative stress; however, lower concentrations of ROS have been demonstrated to be important signal transduction molecules (Miller et al. 2008; Mittler et al. 2011; Suzuki et al. 2012). Up-regulated expression of the oxidative stress-responsive genes in the *OsLCBK1*-overexpressing transgenic plants may increase the ability to scavenger excessive ROS and thus maintain ROS to a level that can act as signaling molecules in regulating oxidative stress and disease resistance responses rather than act as cytotoxic factors to cause cellular damage.

Sphingolipids and their metabolites such as LCBPs are important regulators in animal cells (Hannun et al. 1986; Merrill et al. 1986; Wilson et al. 1986). Recently, it was demonstrated that LCBPs play important roles in regulating biotic and abiotic stress responses in plants (Alden et al. 2011; Chen et al. 2012; Dunn et al. 2004; Dutilleul et al. 2012; Lynch et al. 2009; Lynch and Dunn 2004; Michaelson et al. 2009; Ng and Hetherington 2001; Worrall et al. 2003). The present study explored the function of a rice LCBK gene *OsLCBK1* in abiotic stress and showed that overexpression of *OsLCBK1* in transgenic tobacco resulted in a decreased ABA sensitivity and improved oxidative stress tolerance but not salt stress tolerance. However, the involvement of *OsLCBK1* in other abiotic stresses (e.g. cold, heat and drought stresses) needs to be examined further by comparing the different phenotype of the *OsLCBK1*-overexpressing transgenic and the vector-transformed plants under different abiotic stress conditions. Most importantly, the physiological and biochemical mechanisms and the signaling pathway involved in OsLCBK1-regulated abiotic stress responses also need further investigations.

Acknowledgements

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