DOI: 10.1007/s10535-014-0454-1 BIOLOGIA PLANTARUM 58 (4): 649-658, 2014

Over-expression of *ApKUP3* enhances potassium nutrition and drought tolerance in transgenic rice

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Abstract

ApKUPs are typical high-affinity potassium (K^+) transporters of *Alternanthera philoxeroides* which are involved in its response to K^+ starvation and abiotic stresses. In this study, the overexpression of *ApKUP3* gene in rice resulted in enhanced K^+ nutrition and drought tolerance of transgenic plants. Compared with wild-type (WT) plants, the transgenic plants showed a better growth performance and a strengthened K^+ accumulation under different K^+ supplies. The *ApKUP3* overexpression in the rice plants also enhanced tolerance to a drought stress, as evidenced by a reduced leaf water loss and an increased total leaf chlorophyll content, stomatal conductance, net photosynthetic rate, and activities of superoxide dismutase, peroxidase, and ascorbate peroxidase (APX). Moreover, the transcription of genes involved in the antioxidation defense system were higher in the transgenic plants than in the WT plants upon the drought stress.

Additional key words: *Alternanthera philoxeroides*, antioxidant enzymes, chlorophyll, K^+ transporter, *Oryza sativa*, photosynthesis, stomatal conductance, transgenic plant.

Introduction

Cultivated rice (*Oryza sativa* L.) demands vast quantities of water during growth. Drought stress is one of the major causes of reduced crop yield (Ozturk *et al*. 2002). Thus, great efforts have been made to breed drought-tolerant cultivars. Plants subjected to adverse abiotic stresses, including drought, produce excess of reactive oxygen species (ROS), such as superoxide, hydrogen peroxide, and hydroxyl radical, which elicit oxidative stress (Foyer and Noctor 2005, Wang *et al*. 2009). Plants cope with this stress by enzymatic and non-enzymatic antioxidants and the activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), *etc.*, are correlated with plant tolerance to abiotic stresses (Turkan *et al*. 2005, Wang *et al*. 2009, Huang *et al*. 2013, Mishra *et al*. 2013).

Potassium ion (K^+), one of the most abundant cations in cells, improves plant tolerance to various abiotic stresses, such as drought (Tiwari *et al*. 1998, Benlloch-González *et al*. 2008, Li *et al*. 2011, Song and Su 2013), NaCl (Maathuis and Amtmann 1999, Mian *et al*. 2011, Upadhyay *et al*. 2012), and cold (Rai *et al*. 2008, Ramalho *et al*. 2013). To meet growth demands, a sufficient amount of K^+ must be absorbed from soil *via* plant roots and then distributed to different parts of the plant (Gaymard *et al*. 1998, Gierth and Mäser 2007, Lebaudy 2007, Ward *et al*. 2009). The KT/HAK/KUP family as part of the high-affinity K^+ transport system has attracted comprehensive research interest because of its

Submitted 22 May 2013, *last revision* 1 February 2014, *accepted* 21 March 20014.
Abbreviations: APX - ascorbate peroxidase; CAT - catalase; g_s - stomatal conductance; KT/HAK/KUP - K^+ transporter/high-affinity K^+ transporter/K^+ uptake permease; P_N - net photosynthetic rate; POD - peroxidase; RT-qPCR - real time quantitative polymerase chain reaction; SOD - superoxide dismutase; WT - wild type.
Acknowledgments: This work was financially supported by grants from the National Natural Science Foundations of China (91125028), Jiangsu Postdoctoral Science Foundation (1401080B), and Jiangsu Agriculture Science and Technology Innovation Fund [CX(15)2014]. The first two authors contributed equally to this work.
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effectiveness in transporting K^+ at submillimolar concentrations. Since the first member of this family was identified in barley (Santa-Maria *et al*. 1997), numerous studies have provided extensive insights into high-affinity K^+ transport in various plant species (for detail see Song and Su 2013).

Initial studies of complementation of prokaryote K^+ transporter mutants and K^+ uptake kinetics showed that the identified KT/HAK/KUP members mediate K^+ transport (Gierth and Mäser 2007, Alemán *et al*. 2011, Coskun *et al*. 2013). Several members of the KT/HAK/KUP family have been characterized *in planta*, either by overexpression in *Arabidopsis* suspension cells (Fu and Luan 1998) or by analyses of T-DNA insertion mutants (Rigas *et al*. 2001, Gierth *et al*. 2005), and mainly mediated high-affinity K^+ uptake (Alemán *et al*. 2011, Coskun *et al*. 2013). In particular, *Alternanthera philoxeroides* is well known for its strong capacity for K^+ accumulation in response to external K^+ concentrations ranging from micromolar to tens of millimolar. The distinctive K^+ accumulation capability of *A. philoxeroides* is linked to a high-affinity K^+ transport facilitated by K^+-uptake transporters (ApKUPs) (Song and Su 2013).

In the present study, *ApKUP3* was introduced into rice to elucidate its function in plant response to K^+ deficiency and drought, as well as to analyze the resultant phenotypic and physiological changes. The behavior of the transgene and putative stress responsive antioxidation genes was analyzed using the Northern blot or real-time quantitative polymerase chain reaction (RT-qPCR). The present study aims to provide direct evidence that *ApKUP3* contributes to plant tolerance to abiotic stresses, specifically K^+ deficiency and drought.

Materials and methods

Rice (*Oryza sativa* L. subsp. *japonica*) cv. ZH11 was used in this study. Seeds were washed with distilled water and germinated in the dark at 28 °C for 3 d. Germinated seeds were cultured in a nutrient solution based on a half-strength Murashige and Skoog (MS) medium with added KCl to the desired 1 mM K^+ under day/night temperatures of 26/24 °C, a 12-h photoperiod, an irradiance of 1 000 μmol m^{-2} s^{-1}, and a relative humidity of 75 %. Then, 14-d-old seedlings with similar sizes were subjected to different treatments. For K^+ treatments, the final K^+ concentration in a nutrient solution was adjusted to 0.05 mM (K^+ deficiency), 1 mM (control), or 30 mM (K^+ excess). Drought was simulated by the supplementation of PEG 6000 to a final concentration of 15 % (m/v). The seedlings were treated for 48 h before quantitative RT-PCR determination, and for 28 d before physiological analyses.

The seedlings were rinsed in distilled water, soaked, and then weighed to obtain the fresh mass. Roots were scanned with an *Epson Rhizo* scanner (*Epson*, Long Beach, CA, USA), and the total root length and surface area were acquired using the *Epson WinRhizo* software. The seedlings were separated into roots, stems, and leaves, dried in an oven at 105 °C for 30 min, and then at 70 °C for 48 h to obtain the dry mass. The dried samples were ground into a fine powder, then fully digested with HNO_3-$HClO_4$, and the K^+ content was measured using flame atomic absorption spectrometry (*ZCA-1000SF*, *Jinan ShangDi Electronic Technology Co.*, Jinan, China).

The water loss rate was measured as previously described (Wu *et al*. 2009). Plant leaves were separated, weighed immediately, and then the leaf mass was measured under a temperature of 25 °C at a 70 % relative humidity at designated time intervals. The water loss rate was standardized as percentage relative to the initial water content.

The content of chlorophylls was measured according to Li *et al*. (2011). Fresh leaves were immersed in 96 % (v/v) ethanol, kept at 4 °C in darkness for 12 h and then centrifuged at 1 000 *g* and 4 °C for 10 min. The absorbances of the supernatant were measured at 664 and 648 nm using a *BioRad SmartSpec 3000* spectrophotometer (Wadsworth, IL, USA).

The free proline content was determined according to Zhao *et al*. (2009). Fresh leaf tissue was extracted in 3 % (m/v) sulphosalicylic acid at 95 °C for 15 min. After centrifugation at 12 000 *g*, 2 cm^3 of the supernatant was transferred into a new tube containing 2 cm^3 of acetic acid and 2 cm^3 of an acidified ninhydrin reagent, incubated at 95 °C for 30 min, and 5 cm^3 of toluene was added and then shaked. The absorbance of the toluene layer was determined at 532 nm.

For determinations of antioxidant enzyme activities, seedlings were homogenized on ice with a mortar and pestle in a 0.1 M potassium phosphate buffer (PBS, pH 7.0) containing 0.1 mM EDTA, 0.5 % (v/v) *Triton-X 100*, and 1 % (m/v) polyvinylpyrrolidone (PVP), then centrifuged at 12 000 *g* and 4 °C for 15 min. The supernatant (crude protein) was used immediately for antioxidant enzyme assays. The total protein content was determined according to Bradford (1976) using *Bio-Rad* (Hercules, USA) protein assay reagent. The activity of SOD was estimated as blue tetrazolium (NBT) reduction following the method of Beyer and Fridovich (1987). One unit of SOD activity was defined as the amount of protein that inhibits the rate of NBT reduction by 50 %. POD and CAT activities, and the H_2O_2 content were determined using relevant detection kits (*Nanjing Jiancheng Bioengineering Institute*, Nanjing, China), according to the manufacturer's description. One unit of POD activity was defined as the increase of 1 per min in absorbance recorded at 470 nm, and one unit of CAT,

Table 1. Primer pairs and amplicon sizes used for RT-qPCR experiments.

Gene	Description	Primer (5' to 3')	Amplicon size [bp]
OsActin (NCBI No. JN635519)	actin 1	[F]:GGAAGCTGCGGGTATCCAT [R]:CAGGAGGGAGCGACCACCTTG	191
ApKUP3 (NCBI No. JN635519)	K+ transporter KUP3 of *A. philoxeroides*	[F]:GGGTCCCACTTGTTATCGCA [R]:GACCCCACTGGCTAGTTCTG	195
OsFSOD1 (LOC_Os06g02500)	Fe-SOD	[F]: TCACGTGTACTCCAGTGTGC [R]:GAGGCTGCTGCTCTATCCAG	181
OsFSOD2 (LOC_Os06g05110)	Fe-SOD	[F]:TGCAGCACAGGTATGGAACC [R]:CAAGACAAGCCAAACCCAGC	184
OsCZSOD1 (LOC_Os07g46990)	Cu/Zn-SOD	[F]:TGGGGCACCACAAGATGAG [R]:GTCCACCCTTGCCAAGATCA	179
OsCZSOD2 (LOC_Os03g22810)	Cu/Zn-SOD	[F]:CTACCTGGACACCGGATCT [R]:GAGCTTCTGGAAGGTTCCCC	173
OsCZSOD3 (LOC_Os08g44770)	Cu/Zn-SOD	[F]:GGTGTAGCTGAGGCAACCAT [R]:ACAACACCGCATGCAAGTC	170
OsCZSOD4 (LOC_Os03g11960)	Cu/Zn-SOD	[F]:CCGGGCCCCATTTTAATCCT [R]:CAACAACTGCCCTTCCCAA	177
OsMSOD1 (LOC_Os05g25850)	Mn-SOD	[F]:GCAGCACAGGTATGGAACC [R]:CAAGACAAGCCAAACCCAGC	183
OsPOD1 (LOC_Os07g02440)	POD precursor	[F]:GTTCGCACACTGCAACACAT [R]:GTTCGCACACTGCAACACAT	193
OsPOD2 (LOC_Os03g55420)	POD precursor	[F]:GGGATTCGGATTCTGGTGG [R]:GACGAAGCAGTCGTGGAAGA	198
OsPOD3 (LOC_Os08g42030)	POD precursor	[F]:CTCCAACCTCGTCAATGGC [R]:GTGCAGTCTCTCCTGACCT	195
OsCAT1 (LOC_Os03g03910)	CAT isozyme A	[F]:GAAGTTCCCGGACATGGTG [R]:GACGAGCGTGTAGGTGTTGA	193
OsCAT2 (LOC_Os02g02400)	CAT isozyme B	[F]:GAAGCTGTTCGTCCAGGTG [R]:GCTGCTCGTTCTCGTTGAA	164
OsCAT3 (LOC_Os06g51150)	cytosolic APX	[F]:GCTTGCACAGTTTGACAGGG [R]:CGACTGTGGAGAACCGAACA	170
OsAPx1 (LOC_Os03g17690)	cytosolic APX peroxidase	[F]:GGGTTCTGACCACCTAAGGC [R]:CCCTCCTTGTCACCACTCAG	194
OsAPx2 (LOC_Os07g49400)	peroxisomal APX	[F]:TCCTCTCCTACGCCGACTT [R]:ACCTGCCTTAGGTGGTCAGA	163
OsAPx3 (LOC_Os04g14680)	peroxisomal APX	[F]:GGAAAGCTCGTCCCGAAAGA [R]:CATAGCGGCGGAATGTAGG	165
OsAPx4 (LOC_Os08g43560)		[F]:CGTGATTCGTCAGTTTGCCC [R]:GTCCATGCGCCTTCAAATCC	179

activity was defined as the amount of the enzyme that disproportionates 1 % of the H_2O_2 in 1 min. The activity of APX was quantified according to Nakano and Asada (1981) using ascorbic acid as substrate. The oxidation of ascorbate was initiated by H_2O_2, and the decrease of absorbance at 290 nm was monitored for 1.5 min. One unit of APX activity was defined as the amount of the enzyme required to oxidize 1 μmol of ascorbate.

A portable photosynthetic system *LI-6400* (*Li-COR, Lincoln*, NE, USA), was used to determine the net photosynthetic rate (P_N) and stomatal conductance (g_s) in terminal leaflets from fully mature second leaves at an irradiance of 1 000 μmol m^{-2} s^{-1} and a CO_2 concentration of 400 cm^3 m^{-3} as described by Kumar *et al*. (2006).

The coding region of *ApKUP3* was subcloned into a binary vector pBI121 (*Clontech*, Kyoto, Japan) under the control of the CaMV 35S promoter with *nptII* as selectable marker for kanamycin resistance (Fig. 1*A*). The constructs were introduced into *Agrobacterium tumefaciens* strain EHA 105. Rice cv. ZH11 was used as transformation host, and transformation was performed according to Hiei *et al*. (1994). Independent T0 transgenic lines were obtained by screening kanamycin-resistant regenerated rice plants and verified by genomic DNA-PCR of a 900 bp product including 760 bp of the pBI121 vector and 240 bp of *ApKUP3*-specific nucleotides (primer pairs of 5'-GATCTACCCGAG CAATAATCTCCAGG-3' and 5'-GAGATGTGCCCA AGTCCCCATACAC-3'). Three independent T1 generation transgenic lines, #1, #3, and #6, were re-examined

by the Northern blot analysis using the coding DNA sequence of the *ApKUP3* gene as probe.

Total RNA was extracted from leaves or roots using a plant RNA kit (*Omega*, Norcross, USA) and was reverse transcribed into cDNA using a reverse transcription kit (*TaKaRa*, Tokyo, Japan). Primer sequences of genes encoding putative antioxidant enzymes used (Table 1) were obtained using the query protein sequence of *Arabidopsis* SOD (AT4G25100 and AT1G08830), POD (AT4G30170), CAT (AT1G20630), or APX (AT4G35970) to the *BLAST O. sativa* genome database at *Phytozome* (http://www.phytozome.net). RT-qPCR was carried out on *Light Cycler 480II* (*Roche*, Pleasanton, CA, USA). The *actin* gene of rice was also amplified as internal control. The relative expressions of the target genes were presented after normalization to the internal control from three independent biological replicates.

For the Northern blot, total RNA was extracted from leaves of 3-week-old rice plants. RNA (5 μg) was separated on formaldehyde-containing agarose gels and then transferred onto nylon membranes. Equal loading RNA was verified by visualizing rRNA stained with ethidium bromide. The probe of the coding region of *ApKUP3* was labeled with [^{32}P]dCTP according to the protocol of a random primer kit (*Promega*, Madison, USA). Northern hybridization was carried out in an *Hybaid Shake 'n' Stack* hybridization oven (*Thermo*, Waltham, USA) as previously described by Alwine *et al*. (1977).

For all experiments, data were statistically analyzed using the *SPSS 13.0* software (*SPSS*, Chicago, IL, USA). The WT and transgenic plants were compared and details are described in figure legends. Graphs were produced using the *Origin 8.0* software.

Results

The high-affinity K^+ transporter, ApKUP3 (acc. No. JN635519) is predominately localized in the leaves of *A. philoxeroides* and is involved in promoting K^+ accumulation under abiotic stresses (Song and Su 2013). We introduced the *ApKUP3* gene into the vector pBI121 (Fig. 1*A*) and then transferred it into rice cv. ZH11 to further elucidate the function of this K^+ transporter. Putative T0 generation transgenic lines were tested for the presence of the binary vector using semi-quantitative RT-PCR with primers that amplified 900 bp and 2.4 kb fragments of *ApKUP3*. Ten out of 14 transformed lines yielded the expected band (data not shown). Three independent T1 generation transgenic lines #1, #3, and #6, were chosen for the subsequent studies. We used the CaMV 35S promoter and therefore we could observed the expression of *ApKUP3* in tissues where it is not normally expressed. Although predominately localized in the leaves of *A. philoxeroides* (Song and Su 2013), the Northern blot analysis shows that *ApKUP3* was constitutively expressed in both shoots and roots of all the three T1 generation rice lines (Fig. 1*B*).

The transgenic and WT plants were grown in liquid culture media supplied with 0.05, 1, or 30 mM K^+ to evaluate whether the *ApKUP3* overexpression affected overall plant growth and development. The lines #1, #3, and #6 exhibited similar plant performance and physiological responses. Thus, we designated data from line #1 as representative for the three lines.

Compared with control conditions, the K^+ deficiency significantly suppressed WT plant growth (a ~29 % loss of total fresh biomass) (Fig. 2*A*), accompanied with a decreased total root length (Fig. 2*B*) and a remarkably reduced tissue K^+ content (Fig. 2*D*). In addition, K^+ excess dramatically inhibited WT plant growth

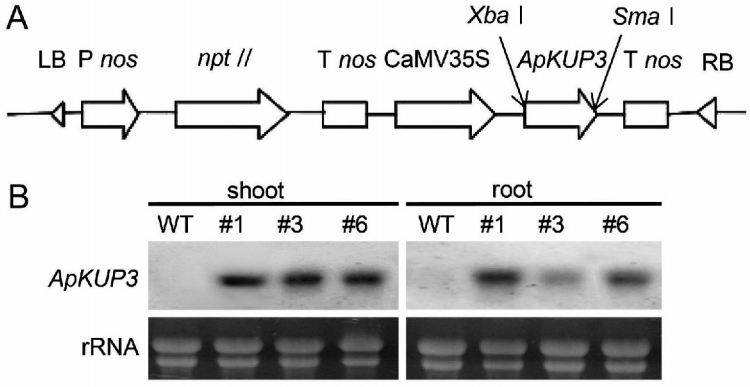

Fig. 1 *A* - A plasmid construct pBI121 that contains an *ApKUP3* open reading frame driven by the CaMV 35S promoter and *npt II*. LB - left border, P *nos* - nopaline synthase promoter, *npt II* - neomycin phosphotransferase, T *nos* - nopaline synthase terminator, CaMV 35S - cauliflower mosaic virus 35S promoter, RB - right border, *Xba* I and *Sma* I indicate restriction enzyme cutting sites. *B* - The expression of the *ApKUP3* gene in a wild-type (WT) plant and in T1 generation transgenic lines #1, #3, and #6. Total RNA (5 μg) extracted from 3-week-old seedlings was analyzed by the RNA gel blot.

(a ~33 % loss of total fresh biomass; Fig. 2*A*) accompanied with a decreased total surface area (Fig. 2*C*). The total fresh masses of the *ApKUP3* overexpressing transgenic plants were ~34 % (K^+ deficiency), ~37 % (control), and ~30 % (K^+ excess) higher than those of the WT plants (Fig. 2*A*). In particular, the root biomass of the transgenic lines was obviously increased together with an enhanced total root length under the K^+ deficiency compared to that in the WT plants (Fig. 2*B*). The tissue K^+ content was also increased in the transgenic lines especially under the K^+ deficiency (with a ~67 % increase in shoots and ~40 % in roots) (Fig. 2*D*). These findings suggest that the *ApKUP3* overexpression improved plant performance and a K^+ accumulation, especially under unfavorable K^+ nutrient conditions.

Further, we examined the performance of transgenic line #1 under 15 % PEG 6000 to investigate how the *ApKUP3* overexpression affected the plant response to the drought stress. The PEG treatment significantly decreased the total fresh mass accompanied by curling and chlorotic leaves in the WT plants (Fig. 3*A*). Compared with the WT plants, the transgenic plants showed a higher total fresh mass and non-chlorotic leaves accompanied by significantly higher amounts of total chlorophyll and

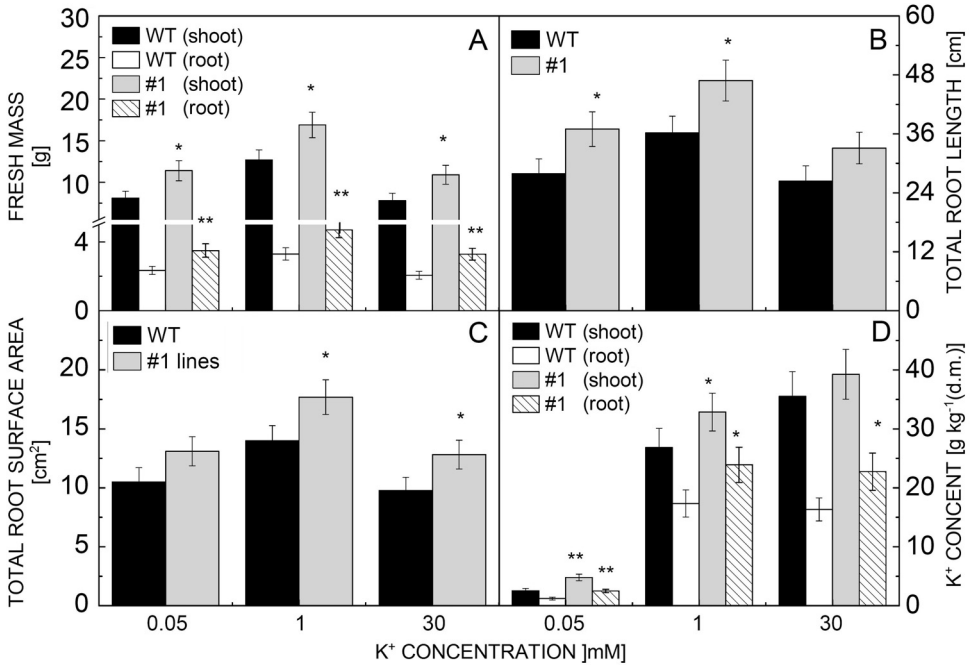

Fig. 2. The responses of 14-d-old seedlings of WT and transgenic plants to various external K^+ concentrations (0.05, 1, or 30 mM KCl in a 1/2 MS solution) after 21 d. Data are means ± SE (n = 21). Each treatment was performed in triplicate, with seven seedlings each. *Asterisks* indicate a significant difference between transgenic lines and WT plants (* - $P < 0.05$, **- $P < 0.01$, t-test).

Table 2. The responses of 14-d-old seedlings of WT and transgenic rice line #1 to the drought stress induced with 15 % (m/v) PEG 6 000 for 21 d. Data are means ± SE (n = 21). Each treatment was performed in triplicate, with seven seedlings each. *Asterisks* indicate statistical differences between transgenic lines and WT plants (* - $P < 0.05$, ** - $P < 0.01$, t-test).

Parameter	Control		PEG treatment	
	WT	#1	WT	#1
Total plant fresh mass [g]	14.57 ± 1.08	18.52 ± 1.39*	6.75 ± 0.79	10.58 ± 1.29 **
Total root length [cm]	35.71 ± 2.79	45.82 ± 3.14*	18.26 ± 0.79	32.58 ± 4.29 **
Total root surface area [cm^2]	13.14 ± 0.88	17.23 ± 1.17*	4.18 ± 1.29	8.32 ± 1.29 **
K^+ content [g kg^{-1}(d.m.)]	22.25 ± 1.64	28.16 ± 2.18*	31.32 ± 4.18	37.49 ± 0.15 *
Chlorophyll content [g kg^{-1}(f.m.)]	1.83 ± 0.16	2.01 ± 0.22	0.77 ± 0.10	1.29 ± 0.13 **
P_N [µmol(CO_2) m^{-2} s^{-1}]	17.44 ± 0.62	17.87 ± 0.56	9.83 ± 0.42	13.33 ± 0.39 **
g_s [mol m^{-2} s^{-1}]	0.39 ± 0.02	0.42 ± 0.03	0.21 ± 0.01	0.31 ± 0.03 **
Proline content [g kg^{-1}(f.m.)]	7.67 ± 0.83	8.04 ± 1.02	22.34 ± 3.43	38.12 ± 4.26 **

proline, enhanced g_s and P_N (Table 2), and a slower rate of water loss (Fig. 3*A*). The transgenic plants showed dramatically enhanced root formation under the PEG treatment, as evidenced by an increased total root length and surface area (Table 2). In addition, the transgenic lines had a higher K^+ content than the WT plants at the end of the drought stress treatment (Table 2). We also evaluated the H_2O_2 content and SOD, POD, CAT, and APX activities in the WT and transgenic plants under the PEG treatment. The content of H_2O_2 was lower in the shoots of the transgenic plants than in the WT plants (Fig. 3*B*). Correspondingly, significantly higher activities of SOD, POD, and APX were observed in the leaves of the transgenic plants than in the WT plants from day 15 to day 21 (Fig. 3*C*,*D*,*E*). However, no difference in CAT activity was found between the WT and transgenic plants (Fig. *3F*).

The transcriptional profiles of candidate antioxidation -related genes in WT and transgenic plant leaves were analyzed and compared to elucidate the molecular mechanisms underlying the relation between antioxidant enzyme activities and drought tolerance. The genes encoding SOD, POD, and APX had a higher expression in the transgenic plants than in the WT plants with different dynamics under the PEG treatment. Specifically, the expression of the *OsFSOD1* and *OsFSOD2* genes (encoding putative Fe-SOD) were consistently higher in the transgenic plant than in WT under the PEG treatment at all tested time points (Fig. 4*A*,*B*). The *OsMSOD1* gene expression (encoding Mn-SOD) in the WT leaves was upregulated at the early stage and did not change after 12 h, whereas that in the transgenic plant leaves steadily increased (Fig. 4*C*). The expression of the POD precursor gene *OsPOD2* was rapidly and transiently induced after 4 h in the WT plants but was progressively activated in the transgenic plant leaves upon drought (Fig. 4*D*). The transcripts of *OsAPx1* and *OsAPx2*, which encode cytosolic APX, were continuously increased in both WT and transgenic plant leaves under the PEG treatment (Fig. 4*E*,*F*). No difference in the transcription of other genes (Table 2), including three *OsCAT* genes, was found between the WT and transgenic plants (data not shown)

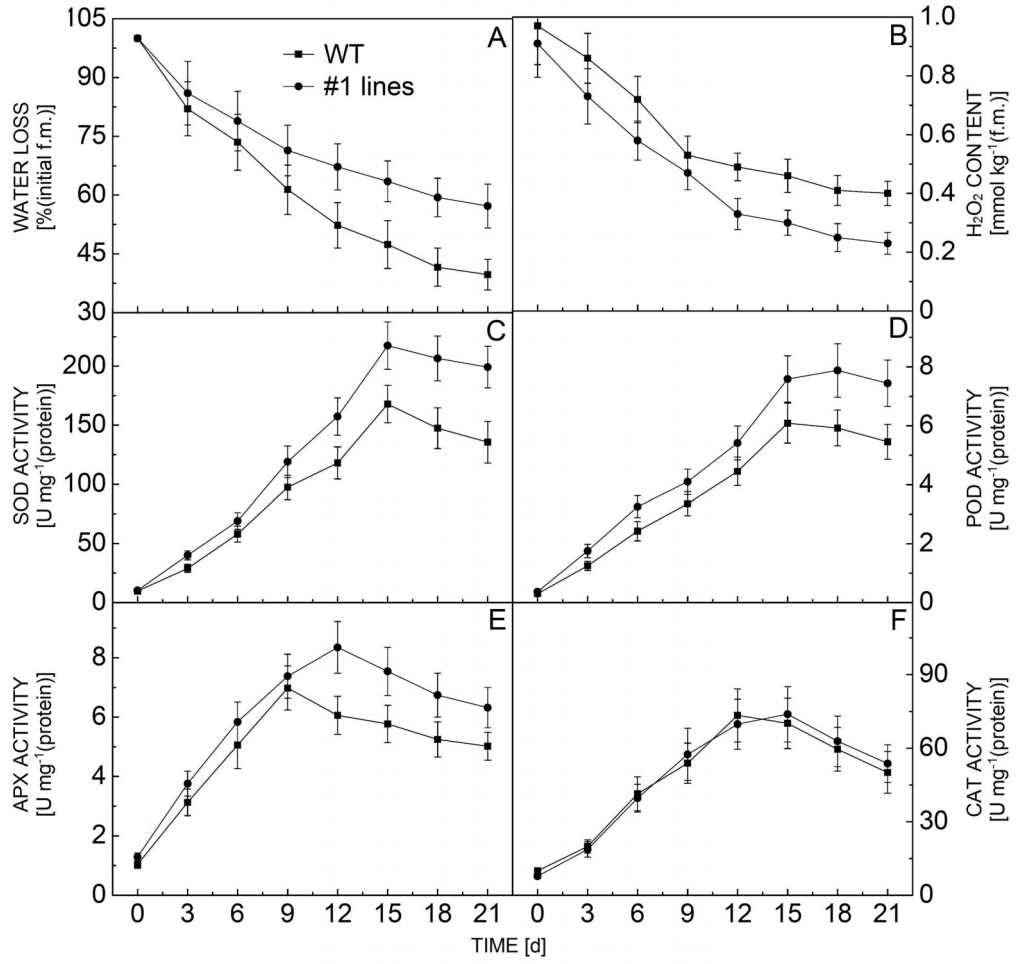

Fig. 3. The responses of 14-d-old seedlings of WT and *ApKUP3* overexpressing plants to the drought stress induced by 15 % (m/v) PEG 6000 for 21 d. Data are means ± SE (n = 21).

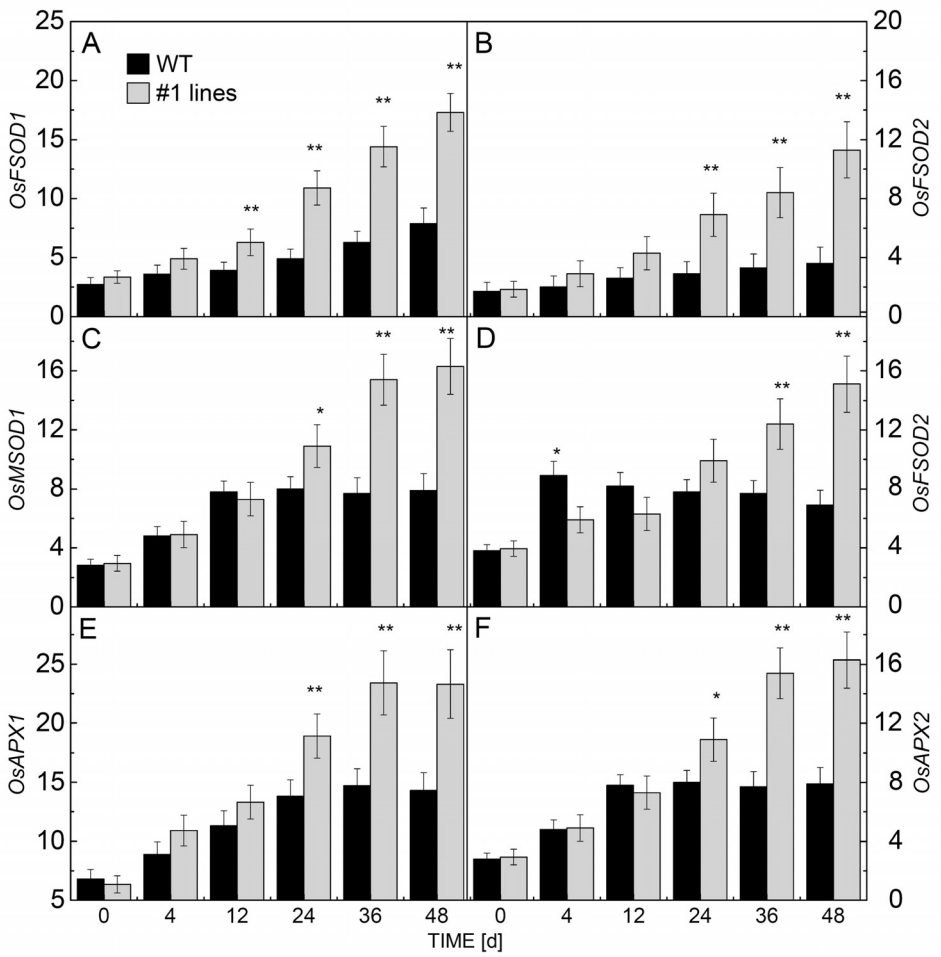

Fig. 4. The expression of genes encoding antioxidant enzymes in leaves of 14-d-old rice seedlings under the drought stress induced by 15 % (m/v) PEG 6000 and harvested at each time point for the RT-qPCR analysis. Means ± SE, n = 10. *Asterisks* indicate a significant difference between transgenic lines and WT plants (* - $P < 0.05$, ** - $P < 0.01$, t-test).

Discussion

Plants require an appropriate amount of K^+, and K^+ deficiency or K^+ excess suppresses the growth of WT plants. The *ApKUP3* overexpression improved plant performance, especially under the unfavorable K^+ supply (Fig. 2*A*,*B*,*C*). The K^+ deficiency induced root formation and development especially in the transgenic plants which contributed to a better K^+ uptake and transport. When the plants were exposed to sufficient K^+ supply, *ApKUP3* might be involved in maintaining the luxury K^+ uptake and transport to places where it increased growth. It is worth mentioning that *ApKUP3* was constitutively expressed in both the shoots and roots of the transgenic lines (Fig. 1*B*) which might partically explain the favorable K^+ uptake and accumulation. Further studies about subcellular localization of ApKUP3 would help to clarify the possible mechanism. Correspondingly, the *ApKUP3* overexpressing plants showed the enhanced tissue K^+ content, and shoots accumulated more K^+ than roots (Fig. 2*D*), allowing the plants to maintain growth together with other K^+-dependent processes, such as photosynthesis, membrane polarization control, and osmoregulation under K^+ deficiency (Lebauty *et al*. 2007, Benlloch-González *et al*. 2008, Li *et al*. 2011, Ramalho *et al*. 2013, Sellin *et al*. 2013). Nonetheless, the observed K^+ acquisition in *A. philoxeroides* (Song and Su 2013) can be distinctly replicated in the *ApKUP3* over-expressing rice plants.

Generally, drought suppresses plant growth due to cellular dehydration and generally prompts stomata to close which restricts the net photosynthetic rate (Ort *et al*. 1994, Pal *et al*. 2013). In the present study, the transgenic plants overexpressing *ApKUP3* showed enhanced g_s, P_N, the total chlorophyll and proline content, and internal water-holding capacity under the drought stress (Table 2, Fig. 3*A*). These findings prove that the overexpression of *ApKUP3* contributed to maintaining K^+-dependent

cellular processes, especially photosynthesis, in the transgenic rice plants. Moreover, proline is an important stress-related metabolite that contributes to osmotic adjustment and enzyme stabilization enabling plants to tolerate better abiotic stresses (Nounjan *et al*. 2012, Huang *et al*. 2013). In the present study, the proline content was significantly higher in the transgenic plants than in the WT plants under the drought (Table 2).

Another consequence of exposure to stresses is the generation of ROS and the stimulation of the antioxidant defense system. In the present study, the SOD activity was higher in the transgenic plants than in the WT plants under the drought stress (Fig. 3*C*). This observation suggests that the *ApKUP3* overexpressing rice plants possessed a good superoxide-scavenging ability. Our findings support reports of previous studies that the SOD activity increase in drought-tolerant cultivars of maize, common bean, and alfalfa (Jagtap and Bhargava 1995, Turkan *et al*. 2005, Wang *et al*. 2009). H_2O_2 as byproduct of SOD activity must be eliminated in subsequent reactions involving POD, APX, and CAT (Foyer and Noctor 2005, Wang *et al*. 2009). The H_2O_2 content in the *ApKUP3* overexpressing plants was slightly lower than in the WT plants at all tested time points (Fig. 3*B*) suggesting an enhanced capacity for protection against oxidative damage caused by the drought stress. The POD and APX activities were higher in the *ApKUP3* overexpressing plants than in the WT plants under the PEG treatment (Fig. 3*D*,*E*). These results suggest that the *ApKUP3* overexpressing plants possessed an increased ROS-scavenging capacity. Such findings are consistent with previous studies of drought-tolerant plants, such as sunflower, sorghum, common bean, and alfalfa (Zhang and Kirkham 1996, Willeken *et al*. 1997, Turkan *et al*. 2005, Wang *et al*. 2009). In the present study, the transcription of *OsFSOD1*, *OsFSOD2*, *OsMSOD1*, *OsPOD2*, *OsAPx1*, and *OsAPx2* were significantly enhanced by the drought with different trends in the transgenic and WT plants (Fig. 4). The increase in transcription might partially explain the reduced H_2O_2 accumulation and the increased SOD, POD, and APX activities in the transgenic plants.

The manipulation of antioxidant capacities is a valuable method of obtaining stress-tolerant plants. Many studies have suggested that the overexpression of some stress-inducible transcription factors, such as CBF4, ABF3, AC, DST, NF-Y, and SGT, can increase plant tolerance to drought, salinity, heat, and cold stresses (Haake *et al*. 2002, Oh *et al*. 2005, Hu *et al*. 2006, Nelson *et al*. 2007, Mishra *et al*. 2013, Ni *et al*. 2013). In our study, the *ApKUP3* overexpressing rice plants showed the better growth, enhanced photosynthetic performance, increased antioxidant enzyme activity, and up-regulated expression of antioxidation-related genes, upon the drought stress. We speculate that *ApKUP3* might be involved in the stress-inducible signaling cascade contributing to plant photosynthetic performance and antioxidant metabolism under drought challenge. However, further studies need to be done to elucidate the molecular mechanisms behind the function of *ApKUP3* in plant antioxidant responses.

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