OsMLP423 is a positive regulator of tolerance to drought and salt stress in rice

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Abstract

Drought and salinity is one of the important abiotic factors that adversely affects plant growth and production.We screened a drought-inducing gene containing about 158 amino acid residues from the transcriptome library of rice exposed to drought treatment, and found ABA-related cis-acting elements and multiple drought-stress-related cis-acting elements in its promoter sequence. The physiological and biochemical phenotype analysis of transgenic plants confirmed that overexpressed *OsMLP423* enhanced the tolerance of rice to drought and salt stress. The expression of the *OsMLP423*-GFP fusion protein showed that *OsMLP423* was localized in both the cell membrane and nucleus. Compared with the wild-type, the plants over-expressing *OsMLP423* showed the enhanced sensitivity to ABA. These results indicate that *OsMLP423* is a positive regulator of drought and salinity tolerance in rice, governing the tolerance of rice to abiotic stress through an ABA-dependent pathway. This paper provides basic knowledge on the drought- and salt-tolerant transgenic rice with a potential application value.

1. Introduction

Rice is one of the most important crops in the world, especially in Asia. One of the main environmental factors affecting rice yield is water deficit or drought stress. Drought tolerance is a complex quantitative trait. Although the genetic mapping of rice drought tolerance-related traits has been reported[1], and many rice drought stress-related genes have been identified, the molecular mechanisms of rice drought response and adaptation are still unclear.

Water limitation is the main reason for the loss of rice production. According to future climate prediction models, temperature increases and the severity of salinization will continue to increase [2, 3]. Therefore, increasing the yield of rice and maintaining its stability under limited water supply is a major challenge for improving food security[4]. As with other crops, the development of drought-tolerant rice varieties mainly depends on improving the capacity to capture and transport water. It is necessary to use the available water effectively in carbon assimilation and allocation of carbohydrates to grains. Therefore, it is very important to explore the genetics related to drought and salinity tolerance[5].

Plant hormones are synthesized only in certain cell types, but they are involved in regulating the physiological responses of the entire plant[6]. Plant hormones usually induce or prevent transcriptional regulators through the ubiquitin-proteasome system, allowing plant hormones to rapidly mediate gene expression through a wide range of adaptive responses[7–9]. Abscisic acid (ABA) is an important plant hormone involved in plant abiotic stress tolerance[10, 11]. ABA controls many stress adaptation responses, including activation of osmotic regulation genes, ion transport and changes in root hydraulic conductivity[12, 13].

There are no reports about *OsMLP423* (Os04g39150) in rice, but there are reports on homologous genes *At1G24020* (Arabidopsis), *Sb06g019320* (sorghum), *GRMZM02g102356* (maize), etc. Studies have found that the expression of *MLP43* gene in *Arabidopsis thaliana* is down-regulated by ABA and drought stress, and is expressed as a negative feedback regulatory loop in response to ABA and drought stress[14]. *MPL423* belongs to the *MLP* subfamily, whereas *MLP* (major latex protein) belongs to the disease-related protein family (*Bet v1*). The *Bet v 1* family proteins have been reported to participate in the response to biotic and abiotic stresses[15, 16]. Under osmotic stress conditions such as drought and high salinity, hypertonic signals lead to the accumulation of the plant hormone ABA, which in turn triggers many adaptive responses of plants[8, 17, 18] The expression of *PR10*, *MLP* and *CSBP* subfamily proteins is highly induced by pathogens as well as abiotic stresses such as salinity and drought.[19] The study presented here also confirmed that the overexpressed *OsMLP423* plants improved the tolerance of rice to drought and salinity, and enhanced the sensitivity to ABA. These results indicate that *OsMLP423* is a positive regulator of tolerance to drought and salinity in rice via an ABA-dependent pathway. This paper provides a theoretical basis for developing drought-resistant transgenic rice with a potential application value.

2. Materials And Methods

2.1 Plant materials and bacterial strains

The plant material used in this experiment was *Oryza sativa* L. subsp. *japonica* cv. Nipponbare. The strains used were *Escherichia coli* strain DH5a and *Agrobacterium* strain EHA105. The rice plants are grown at 22°C to 28°C in a cycle of 16 h light/8 h dark[20]. After 2 weeks of cultivation, uniform seedlings were selected and subjected to the following abiotic stress treatments: salinity (150 mM), drought (20% w/v polyethylene glycol) and abscisic acid (50 μM).

2.2. RNA extraction and cDNA synthesis

After the above abiotic stress treatment of rice, fresh leaves were cut and quick-frozen in liquid nitrogen. The total RNA was extracted using Trizol reagent according to the manufacturer's protocol[21]. The reverse transcription was done using a PrimeScript™ RT reagent Kit with a gDNA Eraser kit, and the cDNA was stored at -20°C.

2.3 RT-PCR

The extracted cDNA was used as a template to analyze the gene expression of *OsMLP423* under abiotic stress. The primers for *OsMLP423* and the reference gene ubiquitin were designed by using Primer Premier 5.0. The primers for *OsMLP423* gene were F 5'-AGC CGT ACA GTT CCA ACC AG-3' and R 5'-AAT GTC CTC GAC AGA GCA CC-3', and the primers for UBQ were F 5'-ACA ACT GGG ACG ACA TGG AG-3' and R 5'-GCC ACA TAC ATT GCT GGT GC-3'.

2.4 Analysis and cloning of the OsMLP423 gene

In order to construct an overexpression vector of *OsMLP423*, its open reading frame was cloned into the *pCAMBIA1301S* vector (from Huazhong Agricultural University), and gene expression was initiated under the control of CaMV 35S promoter. The overexpression primers were F1 5’-cga acg ata gcc ggt acc c ATG GCG TCC AAG GTTGA GC-3’ and R1 5’- cgt acg aga tct ggg atc c GTT CTT GAG GAG GTA GTC GTC GA-3’. The connected clone vector was transferred to *E. coli*, screened and cultured on solid LB plates containing kanamycin. The white colony was selected for colony PCR and was cut with Hind III and BamH I and sent to sequencing.

2.5 Analysis and cloning of promoter

The sequence of about 1200 bp before the translation initiation codon (ATG) of rice *OsMLP423* gene was obtained by comparison with the National Center for Biotechnology Information (NCBI) database, and the collected data were analyzed for potential regulatory elements. The polymerase chain reaction was used to amplify the promoter sequence of about 1200 bp upstream of *OsMLP423*, and the target fragment was fused to the GUS reporter gene in *pCAMBIA1305*. The amplification primers used in this vector construction were: F 5'-acc tgc agg cat gca agc tt AAA TCA TGC CAT CCA CTT CTT CA-3' and R 5'-tta ccc tca gat cta ccc atg g AGC ACA CAC ACA GGA CGGGG-3'. Then, the constructed vector was transferred into callus from wild-type plant via Agrobacterium-mediated transformation to obtain transgenic plants.

2.6 Histochemical localization of GUS activity in plants

The method described by Jefferson was used to detect GUS activity histochemically. Different tissues of *OsMLP423pro:GUS* transgenic rice were placed in a buffer containing 50 mM $NaPO_4$ (pH 7.2), 5 mM $K_3Fe(CN)_6$, 5 mM $K_4Fe(CN)_6$, 0.1% w/w Triton-100 and 1 mm X-Gluc, and were incubated over-night at 37°C[22]. The tissues were then soaked in 70% v/v ethanol for 5 min to stop the staining; then, 95% v/v ethanol was added and boiled until the chlorophyll was completely removed. Finally, photos were taken with a ZEISS stereo microscope.

2.7 Subcellular localization of OsMLP423

The *OsMLP423* gene was linked to the *pCAMBIA2300-eGFP* vector (provided by the research group of Teacher Deng from the Rice Research Institute of Sichuan Agricultural University), and the reaction primers were: F 5’- gag ctc ggt acc cgc gga tcc gATG CGA AGG AGC AAG TGG TG-3’ and R 5’-ctt gct cac cat gga cta gtc CTT GGCCTC TCT CTC GTC G-3’. The successfully ligated vector was transformed into Agrobacterium EHA105 and stored at − 80°C. The 35:GFP served as the control group. Forty-eight hours after tobacco transformation, observations were made using a laser confocal microscope[23].

2.8 Transgenic plants treated with abiotic stress

Wild type and overexpression seeds were sterilized with 2% v/v NaClO solution for 30 minutes, and were then incubated at 28°C with a relative humidity of 70% and 14 h light/10 h dark cycle. The sterilized seeds were placed in 50 μM ABA solution for germination, and the germinated seeds were counted every day until the seventh day, and the control group was placed in the solution without ABA. The early seedlings (bud length about 2 mm) were placed in 50 μM ABA solution for 9 days, 20% w/v PEG and 150 mM NaCl solution for 7 days, and the height of each plant was measured. Finally, rice seedlings were cultured in sandy soil for 14 days, and then watering was stopped for 10 days to simulate field drought until the leaves curled, followed by recovery with normally watering for another 5 days to determine the survival rate.

2.9 Measurement of the physiological parameters

The two-week-old seedlings were used for two different treatments: drought for 5 days in the soil and 150 mM sodium chloride for 48 h. After completion of treatments, the plant physiological parameters were determined. We measured the activity of antioxidant enzymes superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT)[24]. The contents of MDA were measured by using a spectrophotometer as described elsewhere[25]. Leaves were placed in 1 mg/mL DAB and 6 mM NBT staining solution, incubated at 28°C

for 10 hours in light. Anhydrous ethanol was used to remove chlorophyll. The accumulation of hydrogen peroxide and superoxide anion O_2^- was observed under a stereo microscope.

2.10 Statistical analysis

The data were analyzed by analysis of variance using SPSS statistics program. The statistical difference was clarified through analysis of variance by t-test, with $P < 0.05$ (*) and $P < 0.01$ (**) to be significantly different.

3. Results

3.1. OsMPL423 sequence analysis

The *OsMLP423* gene is located on chromosome 4 of rice, with an open reading frame of 474 bp and encoding 157 amino acids. Some homologous genes of *OsMLP423* were analyzed, and their amino acid sequences were compared (Fig. 1a). Approximately 1500 bp upstream of the ATG was analyzed by PlantCARE and found to contain multiple elements associated with the stress response (Fig. 1b). These elements include CAAT-box (common cis-acting elements in promoter and enhancer regions), TATA-box response element, Box-4 (cis-acting element involved in light response), ABRE (ABA cis-acting element), CGTCA-motif (Jasmonic acid response cis element), MBS response element, MBS (MYB binding site involved in drought induction), etc. Through the analysis of the RiceGE (Gene Expression Map) website, it is found that *OsMLP423* is expressed in all tissues of rice, and its expression level in roots and young stems is significantly increased under drought and salt stresses.

Figure 1. OsMLP423 **bioinformatics and expression level analysis.** (a) Analysis of homologous amino acid sequence of *OsMLP423*. (b) Analysis of the relevant elements of the promoter region.

3.2 OsMLP423 localizes to the nucleus and the membrane system

In order to detect the subcellular localization of *OsMLP423* in plant cells, *OsMLP423* was fused with a GFP reporter gene and transiently expressed in tobacco tissues under the initiation of a strong 35S promoter. The results showed that the GFP signal of the *OsMLP423*-GFP fusion protein was mainly localized in the nucleus. *OsMLP423* was also distributed in the membrane system, and the GFP signal of some *OsMLP423*-GFP fusion proteins was fused with the membrane localization signal (GFP) (Fig. 2b).

3.3 GUS staining of promoter transgenic plants

To confirm the tissue-specific expression pattern of *OsMLP423*, we analyzed the activity of β-glucuronidase (GUS) in transgenic plants controlled by the *OsMLP423* promoter. Consistent with the RT-PCR results, we found that the *OsMLP423* gene was expressed in stems, leaves and spikes (Fig. 2a). After exposing *OsMLP423* to different stress treatments, the GUS signal in the treatment group was significantly stronger than that in the control group (Fig. 2c), further indicating that *OsMLP423* may be involved in multiple stress responses. The GUS signal was particularly strongly expressed in roots (Fig. 2d), which was consistent with the prediction results of the website, indicating that *OsMLP423* may improve osmotic stress by regulating the root water uptake.

Figure 2. Analysis of OsMLP423 **expression pattern.** (a) *OsMLP423pro:GUS* transgenic plants showed staining of different tissues. (I: glumes, II: stems, III: mature leaf). (b) Subcellular localization of *OsMLP423* in tobacco. Scale bars are 20 mm. (c) GUS staining of *OsMLP423pro:GUS* transgenic plants under different stress treatments. (□: CK; □: ABA; □: PEG-600; □: NaCl ; □: 42°C; □: 4°C). (d) Detection of GUS activity in roots of *OsMLP423* promoter under abiotic stress.

3.4 OsMLP423 is highly induced by various stresses

The relative expression levels of *OsMLP423* in overexpression lines were verified by qRT-PCR. Compared with the gene expression data on the RiceGE (gene expression map) website, it was found that both drought and saline-alkali stress treatments induced the expression of *OsMLP423*. The expression of *OsMLP423* under stress treatment was further analyzed by RT-PCR, and it was found that the gene had a higher expression level under salt stress and drought stress compared with control (Fig. 3a, b, c).

Figure 3. Expression analysis of OsMLP423 **under different stresses.** (a) RT-PCR analysis of expression levels of *OsMLP423* over-expressing plants. (b) and (d) RT-PCR analysis of *OsMLP423* (b: 50 μM ABA; (c)20% w/v PEG; d: 150 mM NaCl).

3.5 Enhanced tolerance of OsMLP423-overexpressing transgenic plants to drought stress

To verify the importance of *OsMLP423* overexpression to drought stress tolerance, wild-type and overexpressed young seedlings were treated with 20% w/v PEG solution, and a few overexpressed plants were randomly selected to measure the plant height after one week of treatment. Plant height of transgenic and wild-type plants was not different under control conditions However, after 7 days of 20% w/v PEG stress, the heights of transgenic lines OE7-4, OE8-3 and OE11-1 were significantly higher than the wild type (Fig. 4a, b). The above-mentioned young seedlings were transferred to sandy soil for two weeks, and then watering was stopped for 5 days (leaves started curling), and then returned to normal watering for 12 days. Compared with wild-type seedlings, *OsMLP423*-overexpressing plants showed less severe symptoms of drought stress, with delayed and less leaf curling. After restoration of watering, wild-type plant survival was 32%, and overexpression lines had an average survival rate of 60% (Fig. 4c, d, e).

Figure 4. Drought stress treatment of OsMLP423 **transgenic lines.** (a) *OsMLP423* transgenic plants grown at 20% w/v PEG for 7 days. (b) Relative plant height. (c) and (d) Phenotypic differences between *OsMLP423* over-expressing and wild-type plants during recovery after drought treatment. (e) Survival rate. Values are mean ± SE (n = 3). Asterisks indicate significant differences between transgenic lines and WT (*P < 0.05, **P < 0.01). n = 20 plants per treatment.

3.6 Enhanced tolerance of OsMLP423-overexpressing transgenic plants to salt stress

To verify the effect of *OsMLP423* overexpression on tolerance to salt stress, the overexpressed seedlings were treated with 150 mM sodium chloride solution, and the control group was treated with standard solution without NaCl. After 7 days of treatment, we observed that the height of overexpressing plants was similar to wild type under control conditions. However, after 150 mM NaCl treatment, the plant heights of the overexpression lines OE7-4, OE8-3 and OE11-1 was significantly higher than

that of the wild-type plants (Fig. 5a, b). After NaCl treatment, plants were transferred to the standard solutions without NaCl for 10 days, and average survival rate (63%) of the overexpressing lines was significantly higher than that of the wild type (40%) (Fig. 5c, d, e).

Figure 5. OsMLP423 **overexpression in the response to salt stress.** (a) *OsMLP423* transgenic plants after 150 mM NaCl for 7 days. (b) Relative plant height. (c) and (d) Phenotypic differences in *OsMLP423* over-expression and wild-type recovery after 150 mM NaCl treatment. (e) Survival rate. Values are mean ± SE (n = 3). Asterisks indicate significant differences between transgenic lines and WT (*P < 0.05, **P < 0.01). N = 20 plants per treatment.

3.7 OsMLP423-overexpressing lines are sensitive to ABA

To further confirm the involvement of *OsMLP423* in ABA-dependent stress responses, the ABA sensitivity of *OsMLP423* lines was examined by analyzing seed germination and seedling growth. The germination rate of wild-type plants was 90%, while the germination of seeds of overexpressed lines was delayed by 3 days and the germination rate was significantly lower (Fig. 6a, b, c). Early-stage overexpression seedlings (bud length about 2–3 mm) were treated with 50 μM ABA, and standard nutrient solution without ABA was used in the control group. After 9 days, we randomly selected several overexpressing seedlings to measure plant height. Under control conditions, the height of overexpressing plants was similar to that of wild type. However, after treatment with 50 μm ABA, the plant height of WT was 1.6-2 times that of overexpressing plants (Fig. 6d, e). This result indicates that overexpression of *OsMLP423* can enhance the sensitivity of rice to ABA. Exogenous ABA resulted in slower seed germination. The assumed increase of endogenous ABA concentration activated the expression of downstream stress-related genes, and plants enhanced their stress resistance by reducing plant height.

We analyzed the changes in the expression levels of the ABA synthesis and response genes under drought and salt stress treatments by RT-PCR. The results showed that under drought treatment conditions, the expression of ABA synthesis gene *OsNCED3*; *OsNCED4* and ABA response genes *OsRAB21* and *OsLEA3* was significantly increased in overexpressed plants and was higher than in wild-type plants (Fig. 6f, g, h, i).

Figure 6. ABA treatment of OsMLP423-overexpressing **transgenic plants.** (a) Germination of *OsMLP423* over-expressing plants treated with 50 μM ABA for 7 days. (b) Line graph of germination rate of *OsMLP423* over-expressing plants treated with 50 μM ABA for 7 days. (c) *OsMLP423* transgenic plants treated with 50 μM ABA for 9 days. (d)and(e) Relative plant height. (f), (g),(h) and (i) The analysis of the expression levels of, respectively, *OsNCED3*, *OsNCED3*,*OsRAB21* and *OsLEA3* under 20% w/v PEG stress.

3.8 OsMLP423 overexpression affects the ROS accumulation and scavenging under different stresses

To detect the activities of enzymes that scavenge reactive oxygen species under stress conditions, we examined the accumulation of reactive oxygen species (ROS) in *OsMLP423*-overexpressing lines under drought and salt treatments, and assessed the accumulation of

superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) by DAB and NBT staining, respectively. Under control conditions, there was no obvious color difference between Nipponbare and transgenic plants. After drought and salt treatments, DAB-stained transgenic leaves had lighter surface browning compared to the control (Fig. 7a), and NBT-stained leaves had fewer surface spots than Nipponbare (Fig. 7b). This further indicated that the overexpressed transgenic plants produced less reactive oxygen species than wild-type plants under drought and salt stress.

Figure 7. Accumulation of reactive oxygen species in OsMLP423 **transgenic plants.** (a) DAB staining was used to detect superoxide anion (O_2^-) in the plants overexpressing *OsMLP423* before and after drought and salt treatments; (b). Hydrogen peroxide accumulation in *OsMLP423* over-expressing plants before and after drought and salt treatments was detected by NBT staining.

In addition, the activities of SOD, POD and CAT under salt and drought stress treatments were determined. The results showed that there was no significant difference in the activities of superoxide dismutase, peroxidase and catalase between the overexpression lines and the wild type under the control conditions, whereas the activities of the three enzymes were significantly increased under the salt and drought stress conditions. The *OsMLP423*-OE line exhibited significantly higher enzymatic activity than WT (Fig. 8a, b, c). We also found that the content of malondialdehyde in the overexpression line was significantly lower than that in the wild type, indicating that the *OsMLP423*-OE line had less intracellular oxidative damage under stress conditions (Fig. 8d).

Figure 8. Physiological activity of WT and transgenic plants in response to drought and 150 mM NaCl. (a) SOD activity. (b) POD activity. (c) CAT activity. (d) MDA content. WT: wild type rice; OE7-4, OE8-3 and OE11-1 are three independent transgenic lines.

4. Discussion

Rice is the main food source for more than half of the world's population. Most rice varieties are severely damaged by abiotic stress, which has severe social and economic impacts[26]. Drought, as one of the main factors limiting rice productivity, poses a challenge for researchers to improve water management systems and rice tolerance[27]. Therefore, to understand the response of rice to stress, it is particularly important to characterize potential candidate genes for improved drought-tolerant transgenic rice varieties.

OsMLP423 is a functional gene that has not been reported in the literature. The results of this study indicate that plants overexpressing *OsMLP423* are extremely sensitive to ABA, and exhibit high tolerance to salt and drought[28, 29]. Abscisic acid participates in the plant development and regulates the adaptive response of plants to drought and high salinity. Current models indicate that the abscisic acid-dependent pathway operates through ABRE, MYCRS, MYBRS, or NACRS cis-acting elements, while the abscisic acid-independent pathway operates using DRE/CRT or NACRS elements[30]. Promoter analysis showed that the promoter regions of all ABA-regulated genes contain the ABREcis motif[29]. Our analysis found that ABA-inducing elements also exist in the upstream promoter region of *OsMLP423* gene; in addition, it also contains MBS (MYB binding site involved in drought response induction). In the presence of ABA, the germination and seedling growth of *OsMLP423*-overexpressing plants were severely inhibited, indicating that *OsMLP423* is an ABA-dependent gene. We speculate that *OsMLP423* is involved in the ABA signal transduction pathway during rice germination and early growth.

We also found that overexpression of *OsMLP423* in transgenic rice enhanced its tolerance to drought stress compared with the wild type. It has been reported that overexpression of the *AtMLP43* gene can mediate ABA signaling to improve drought tolerance [31]. Overexpression in transgenic tobacco plants of the major latex-like protein gene *NtMLP423* resulted in greater sensitivity to abscisic acid (ABA)-mediated seed germination and ABA-induced stomatal closure, and enhanced drought tolerance in tobacco by increasing ABA levels[32]. Based on this, we speculated that the expression of ABA-responsive genes might be altered in overexpressed plants. To test this hypothesis, we analyzed the expression of stress-inducible marker genes that function in the ABA-dependent pathway. The expression of the ABA biosynthesis gene *OsNCED3, OsNCED4* and ABA-inducible genes *OsRAB21* and *OsLEA3* in the overexpression line was significantly higher than that in the wild type under drought conditions. However, due to insufficient materials, the content of ABA in transgenic plants under drought and salt stress could not be measured to further confirm this conclusion.

In the present study, ABA concentrations were strongly increased in all plants in response to drought despite varying levels of gene induction, consistent with the well-known function of this hormone in response to stress signals such as drought and salinity. Increased ABA levels are due to induction of ABA biosynthetic genes, which in turn reprogram plant cells to resist and survive adverse environmental conditions[33, 34]. Studies have shown that ectopic overexpression of cotton *GhMLP28* gene in Arabidopsis enhances tolerance to salt stress[35]. These results suggest that *OsMLP423* at least partially regulates drought and salt tolerance in plants through an ABA-dependent pathway.

Under salt stress conditions, the sodium ion transporter HKT plays an important role in sodium absorption. Tomoaki et al. found that *OsHKT2* is localized in the cell membrane and its expression in roots is very high[36]. In the study presented here, we found that *OsMLP423* was also localized in the membrane system, and GUS test results showed that its expression in roots was also high under salt stress, similar to *OsHKT2*; therefore, it was speculated that *OsMLP423* might be involved in Na^+ transport. The water deficits of drought- or salt-stressed plants are very similar in their early physiology, indicating a crosslink between drought and salt stress[37].

The expression of genes encoding osmoprotectant synthase under drought stress is one of the mechanisms of plant defense against drought by maintaining water content by accumulating various solutes and helping stabilize proteins and protect cell membranes from the denaturing effects caused by stress[38, 39].

Under stress conditions, the SOD, POD and CAT activities of transgenic plants can reflect the impact of stress on plants to a certain extent. In the present study, there were no significant differences in physiological indicators between transgenic and wild-type seedlings under normal growth conditions. However, under drought and salt stress, the activities of SOD, POD and CAT in *OsMLP423*-overexpressing plants were significantly increased, and the accumulation of MDA was significantly lower than that of the wild-type plants. After drought and salt stress treatments, the overexpression lines accumulated less H_2O_2 and O_2^-. Hence, we speculate that overexpressed *OsMLP423* plays a role in the alleviation of osmotic stress in rice.

Declarations

Ethics approval and consent to participate Consent for publication (Not applicale)

Ethics approval and consent to participate (Not applicale)

Availability of data and material

All the data supporting the findings of this study are available within the article.

Declaration of Competing Interest

The authors declare that they do not have any competing financial or commercial interest that represents a conflict of interest in connection with this paper.

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Author contributions

Zhengjun Xu[ES] conceived and designed the experiments; Zhouzhan mei[AB] and Jiangbo Fan[AB] performed the experiments, and wrote the article; Jia Zhang[ES], Yanmei Yang[ES], Yifan Zhang[ES], Xiaofei Zan[ES], xiaohong Li[ES], jiale Wan[ES] analyzed the data, produce the figures; Lihua Li[FG], Rongjun Chen[FG], Xiaoling Gao[FG], Zhengjian Huang[FG], provided support and experimental guidance for this study. All authors read and approved the final manuscript.

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Figures

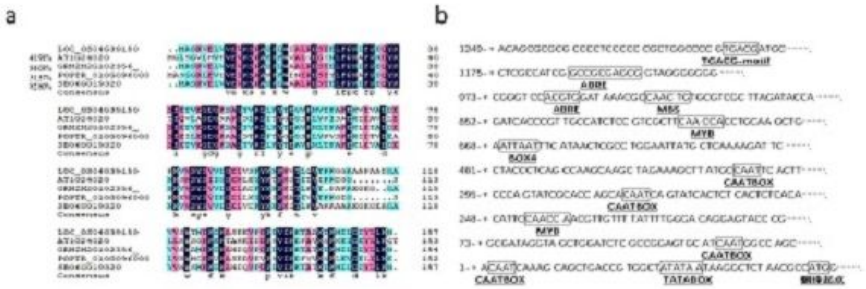

Figure 1

***OsMLP423* bioinformatics and expression level analysis.** (a) Analysis of homologous amino acid sequence of *OsMLP423*. (b) Analysis of the relevant elements of the promoter region.

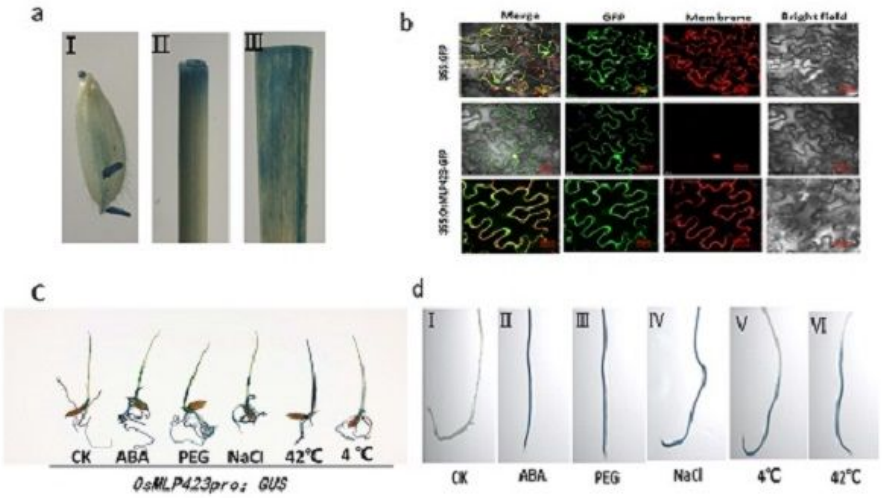

Figure 2

Analysis of *OsMLP423* expression pattern. (a) *OsMLP423pro:GUS* transgenic plants showed staining of different tissues. (I: glumes, II: stems, III: mature leaf). (b) Subcellular localization of *OsMLP423* in tobacco. Scale bars are 20 mm. (c) GUS staining of *OsMLP423pro:GUS* transgenic plants under different stress treatments. (□: CK; □: ABA; □: PEG-600; □: NaCl ; □: 42°C; □: 4°C). (d) Detection of GUS activity in roots of *OsMLP423* promoter under abiotic stress.

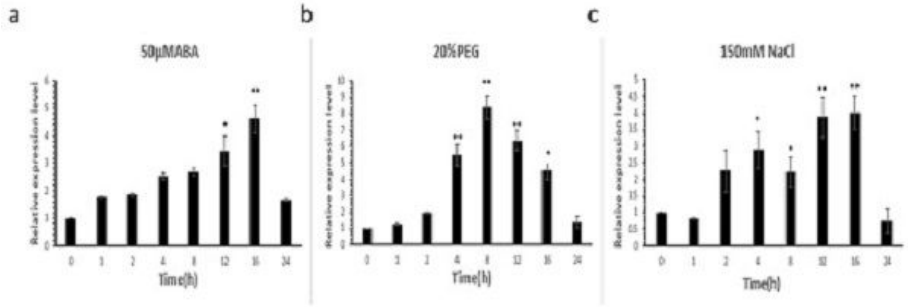

Figure 3

Expression analysis of *OsMLP423* under different stresses. (a) RT-PCR analysis of expression levels of *OsMLP423* over-expressing plants. (b) and (d) RT-PCR analysis of *OsMLP423* (b: 50 μM ABA; (c)20% w/v PEG; d: 150 mM NaCl).

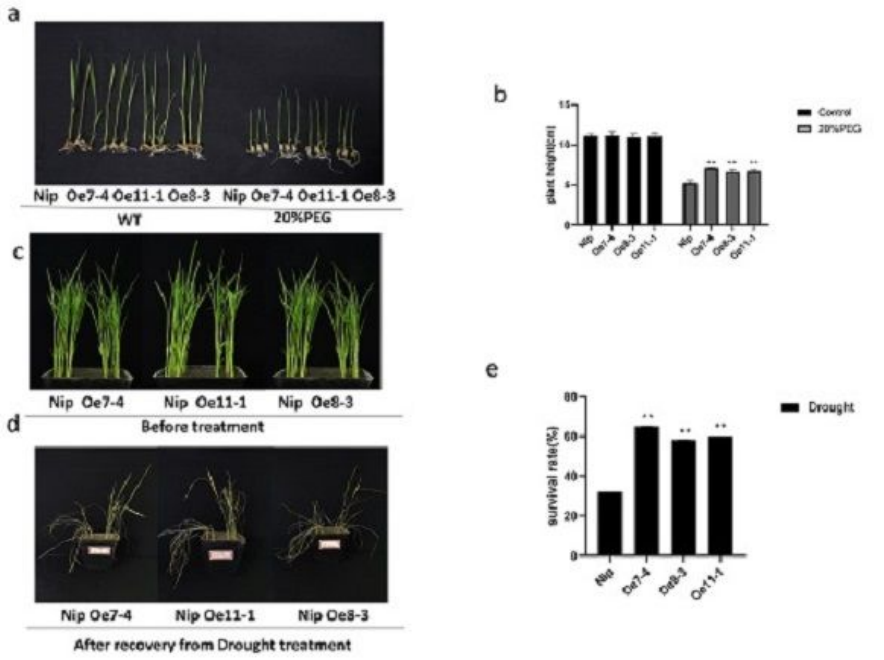

Figure 4

Drought stress treatment of *OsMLP423* transgenic lines. (a) *OsMLP423* transgenic plants grown at 20% w/v PEG for 7 days. (b) Relative plant height. (c) and (d) Phenotypic differences between *OsMLP423* over-expressing and wild-type plants during recovery after drought treatment. (e) Survival rate. Values are mean ±SE (n =3). Asterisks indicate significant differences between transgenic lines and WT (*P <0.05, **P <0.01). n =20 plants per treatment.

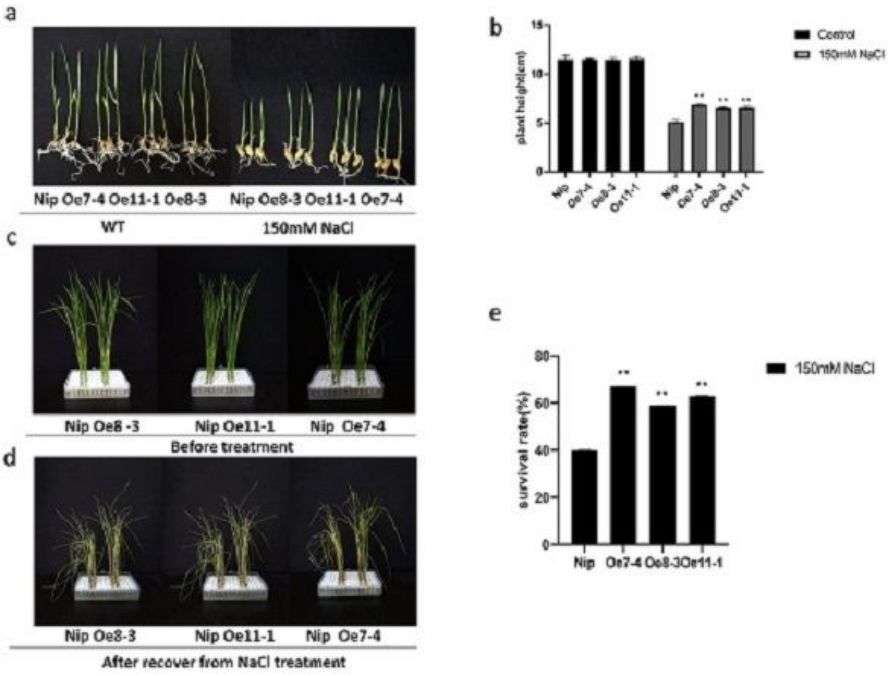

Figure 5

***OsMLP423* overexpression in the response to salt stress.** (a) *OsMLP423* transgenic plants after 150 mM NaCl for 7 days. (b) Relative plant height. (c) and (d) Phenotypic differences in *OsMLP423* over-expression and wild-type recovery after 150 mM NaCl treatment. (e) Survival rate. Values are mean ±SE (n =3). Asterisks indicate significant differences between transgenic lines and WT (*P <0.05, **P <0.01). N =20 plants per treatment.

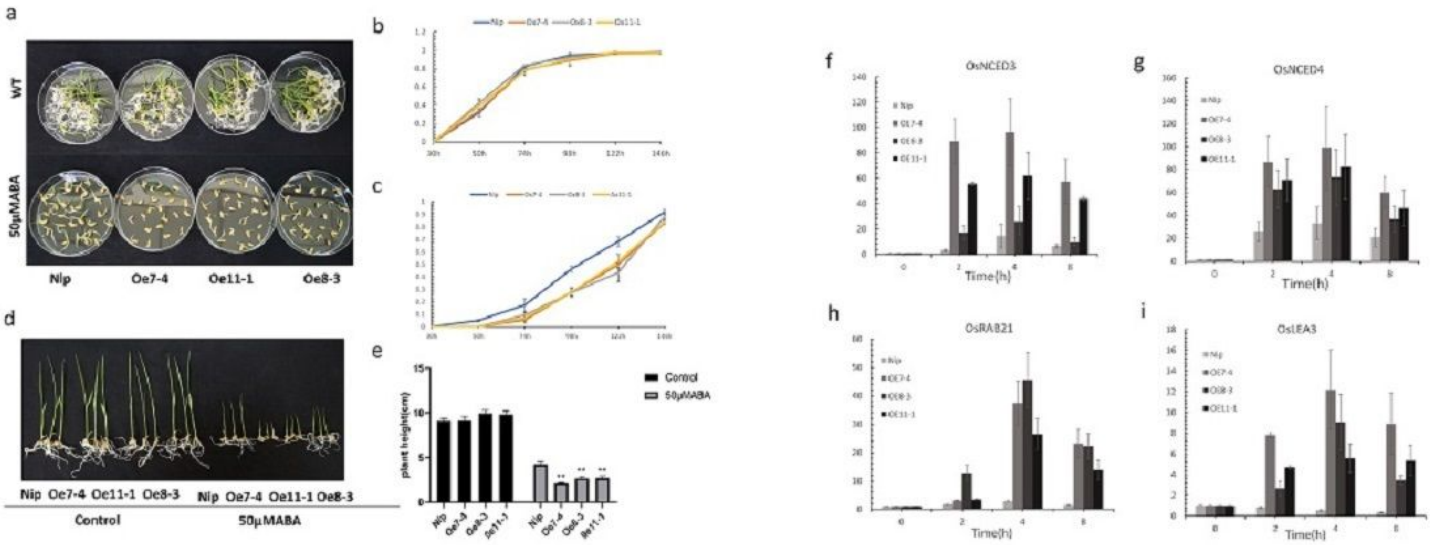

Figure 6

ABA treatment of *OsMLP423-overexpressing* transgenic plants. (a) Germination of *OsMLP423* over-expressing plants treated with 50 μM ABA for 7 days. (b) Line graph of germination rate of *OsMLP423* over-expressing plants treated with 50 μM ABA for 7 days. (c) *OsMLP423* transgenic plants treated with 50 μM ABA for 9 days. (d)and(e) Relative plant height. (f), (g),(h) and (i) The analysis of the expression levels of, respectively, *OsNCED3*, *OsNCED3*,*OsRAB21* and *OsLEA3* under 20% w/v PEG stress.

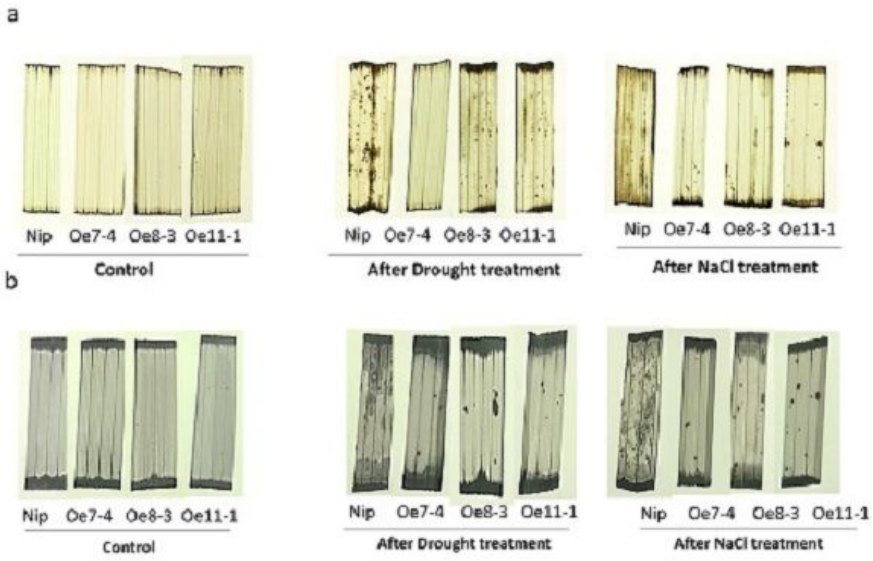

Figure 7

Accumulation of reactive oxygen species in *OsMLP423* transgenic plants. (a) DAB staining was used to detect superoxide anion (O_2^-) in the plants overexpressing *OsMLP423* before and after drought and salt treatments; (b). Hydrogen peroxide accumulation in *OsMLP423* over-expressing plants before and after drought and salt treatments was detected by NBT staining.

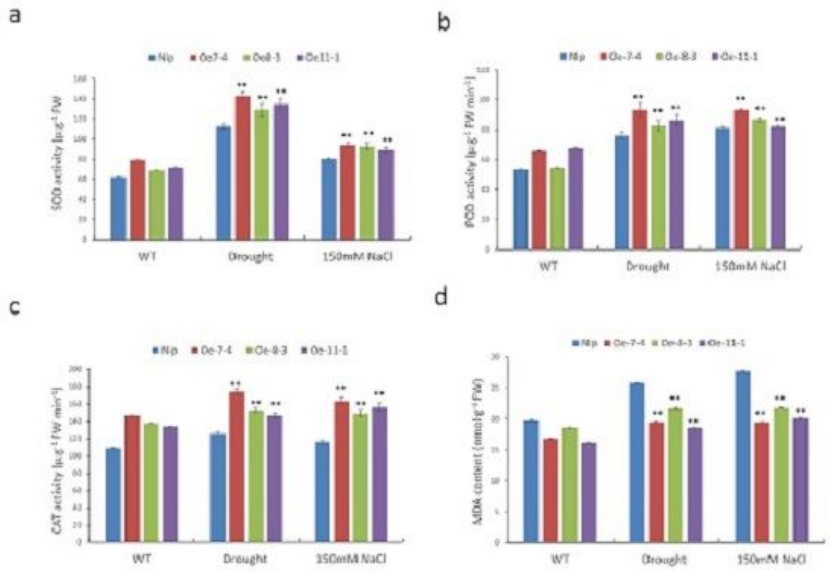

Figure 8

Physiological activity of WT and transgenic plants in response to drought and 150 mM NaCl. (a) SOD activity. (b) POD activity. (c) CAT activity. (d) MDA content. WT: wild type rice; OE7-4, OE8-3 and OE11-1 are three independent transgenic lines.

Supplementary Files

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