



Research article

Effect of salt stress on antioxidant enzyme activity and hydrogen peroxide content in chromosome segment substitution line of ‘Khao Dawk Mali 105’ rice

Panita Chutimanukul^a, Boonthida Kositsup^a, Kitiporn Plaimas^b, Meechai Siangliw^c, Theerayut Toojinda^c, Supachitra Chadchawan^{a,d,*}

^a Center of Excellence in Environment and Plant Physiology, Department of Botany, Faculty of Science, Chulalongkorn University, Bangkok 10300, Thailand

^b AVIC Research Center, Department of Mathematics and Computer Science, Faculty of Science, Chulalongkorn University, Bangkok 10300, Thailand

^c Rice Gene Discovery Unit, National Center for Genetic Engineering and Biotechnology, Kasetsart University, Kamphangsaeen Campus, Nakhonpathom 73140, Thailand

^d Omics Science and Bioinformatics Center, Faculty of Science, Chulalongkorn University, Bangkok 10300, Thailand

Article Info

Article history:

Received 17 November 2018

Revised 23 March 2019

Accepted 25 March 2019

Available online 31 October 2019

Keywords:

NaCl,

Oryza sativa,

Reactive oxygen species,

Salt tolerance

Abstract

Chromosome segment substitution lines (CSSLs) of Thai jasmine rice ‘Khao Dawk Mali 105’ (KDML105) have been developed containing drought-tolerance genes from DH212. One of these CSSLs (CSSL16) showed salt tolerance in physiological responses to salt stress at the seedling and vegetative stages. This study compared the antioxidant enzyme activity and hydrogen peroxide (H_2O_2) content at the seedling stage of CSSL16 with those of KDML105 rice and ‘Pokkali’, the standard check for salt-tolerant rice. CSSL16 had lower standard evaluation system (SES) scores under salt stress than KDML105. CSSL16, KDML105 and Pokkali under 75 mM NaCl treatment had differential responses in antioxidant enzyme activities, namely catalase (CAT), guaiacol peroxidase (GPX), superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR). Pokkali had the highest CAT activity after 3 d of salt stress (DSS), whereas CSSL16 and KDML105 showed low CAT activity. Thus, CAT might not be an important enzyme in the antioxidant system of CSSL16. In the ascorbate-glutathione cycle, APX and GPX activities in CSSL16 were higher than those of KDML105 after 2 DSS, which was consistent with the lowest SES phenotype in this line. The GR activity was similar in all three rice lines. The highest H_2O_2 content during salt stress of KDML105 was 1.9-fold higher than that of CSSL16. These data suggested that APX and GPX may play important roles in the reactive oxygen species scavenging system in CSSL16. Based on the comparison between CSSL16 and KDML105 genomic sequence, SNPs were found in *OsAPX7* and several peroxidase genes. These may contribute to the different in antioxidant enzyme activity levels and lead to the salt tolerant ability in CSSL16.

* Corresponding author.

E-mail address: s_chadchawan@hotmail.com, Supachitra.C@chula.ac.th (S. Chadchawan)

online 2452-316X print 2468-1458/Copyright © 2019. This is an open access article, production and hosting by Kasetsart University of Research and Development institute on behalf of Kasetsart University.

<https://doi.org/10.34044/j.anres.2019.53.5.04>

Introduction

High salinity in soil is a major abiotic stress that severely affects plant growth and development, plant survival, and crop production worldwide (Kumar et al., 2008; Munns and Tester, 2008). Osmotic stress reduces the soil water potential, leading to a limitation in water uptake (Flowers, 2004). Salt stress causes excessive uptake of ions, particularly Na^+ and Cl^- , that interfere with diverse metabolic processes during prolonged exposure to salt stress conditions (Maas, 1986; Cramer and Nowak, 1992). Ionic stress disrupts protein synthesis and interferes with enzyme activity, resulting in reduced energy production and other physiological changes (Larcher, 1980; Munns and Termaat, 1986).

During salt stress in plants, the overproduction and accumulation of reactive oxygen species (ROS), including superoxide (O_2^-), hydroxyl radicals (OH), singlet oxygen (O_2), and hydrogen peroxide (H_2O_2), are considered to play an important role in inhibiting plant growth and development; the ROS react with a variety of molecules, including DNA and proteins, resulting in membrane lipid peroxidation and ultimately leading to cell death (Blokhina et al., 2003; Miller et al., 2010). Therefore, ROS scavenging is essential for the maintenance of normal growth. The scavenging of ROS involves the promotion of a suite of antioxidant defense enzymes, such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), monodehydroascorbate reductase, ascorbate peroxidase (APX), guaiacol peroxidase (GPX), glutathione reductase (GR) and dehydroascorbate reductase (Gill and Tuteja, 2010; Aydin et al., 2013).

Rice (*Oryza sativa* L.) is the most important cereal crop worldwide, especially in Asia (Evans, 1988). The Thai jasmine rice cultivar 'Khao Dawk Mali 105' (KDML105) is a major crop grown in northeastern Thailand (Kanjoo et al., 2012). A chromosome segment substitution population consists of near-isogenic lines in which a particular genomic region is substituted in the same genetic background with different donor chromosomal segments (Ebitani et al., 2005; Takai et al., 2007). Previously, chromosome segment substitution lines (CSSLs) were introgressed with drought-tolerant quantitative trait loci (QTL) localized on chromosome 1 through marker-assisted backcross breeding between KDML105 and a drought-tolerant donor (DH212). The current study used CSSL lines that had been developed by Kanjoo et al. (2012) with a KDML105 genetic background that were generated by backcrossing with KDML105. The putative drought tolerance genetic region on chromosome 1 located between the markers RM1003 and RM3362 derived from the DH212 doubled haploid line was substituted in the CSSL16 line.

In a study to understand the physiological responses to salt stress of CSSL lines, certain CSSL lines containing fragments of different sizes of the salt tolerance QTL region on chromosome 1 showed a desirable salt-tolerance phenotype under salinity stress (Kanjoo

et al., 2011). The CSSL16 line showed significantly enhanced salt tolerance compared with the other CSSL lines and KDML105 at the vegetative stage (Chutimanukul et al., 2018a) and at the seedling stage (Chutimanukul et al., 2018b) under 75 mM NaCl treatment. However, information on the association of salt stress treatment, enzymatic antioxidant systems and the hydrogen peroxide content in CSSL16 has not been verified. Therefore, the aim of this work was to investigate the effect of salt stress on antioxidant enzyme activities, comprising SOD, CAT, APX, GPX and GR, as well as the H_2O_2 content of CSSL16 at the seedling stage compared to KDML105 and a standard salt-tolerant check, 'Pokkali'.

Materials and Methods

Plant materials and salt stress treatment

Seeds of the rice genotype CSSL16, which has a KDML105 genetic background, KDML105 and Pokkali were obtained from the Rice Gene Discovery Unit, National Center for Genetic Engineering and Biotechnology, Bangkok, Thailand. The CSSL16 line includes a chromosomal segment containing the putative drought-tolerance genes located between markers RM1003 and RM3362 on chromosome 1 of DH212 (Kanjoo et al., 2011).

Seeds were germinated in distilled water for 5 d in a plastic cup at room temperature, and then transplanted into clay soil for further growth. Bangsai nutrient solution (1:100) was supplied during the growing period for complete nutrition supply. Bangsai nutrient solution contains 50 g/L MgSO_4 , 80 g/L KNO_3 , 12.5 g/L $\text{NH}_4\text{H}_2\text{PO}_4$, 8.5 g/L KH_2PO_4 , 0.4 g/L Mn-ethylenediaminetetraacetic acid (EDTA), 0.8 g/L micronutrients, 100 g/L $\text{Ca}(\text{NO}_3)_2$ and 3 g/L Fe-EDTA. The experiment was conducted at the Faculty of Science, Chulalongkorn University, Bangkok, Thailand, under natural conditions during May–June 2015. Salt treatment was started 20 d after germination by replacing the Bangsai nutrient solution with nutrient solution supplemented with 75 mM NaCl. Electrical conductivity was maintained at 8 dS/m for salt stress during the experimental period. Rice plants were evaluated and analyzed using a salt tolerance score and antioxidant enzyme activities in leaf tissues at 0, 1, 2, 3, 4, 5, and 6 d of salt stress (DSS).

Evaluation of salt tolerance scores

The visual symptoms of salt injury of individual rice seedlings were assessed. After a seed germination period of 20 d, the standard evaluation system (SES) for rice characters scored on the scale from 1 to 9 was applied at 0, 1, 2, 3, 4, 5, and 6 DSS, following the SES for salinity tolerance at the seedling stage in rice (Gregorio et al., 1997) as shown in Table 1.

Table 1 Standard evaluation system (SES) score of visual symptoms of salt injury at the seedling stage in rice (Gregorio et al., 1997).

Score	Observation	Tolerance
1	Normal growth, no leaf symptoms	Highly tolerant
3	Nearly normal growth, but leaf tips or few leaves whitish and rolled	Tolerant
5	Growth severely retarded, most leaves are rolled; few elongating	Moderately tolerant
7	Complete cessation of growth; most leaves dry; some plants dying	Sensitive
9	Almost all plants dead or dying	Highly sensitive

Enzyme extraction and antioxidant enzyme activity assay

Activities of five antioxidant enzymes (SOD, CAT, GR, APX and GPX) in leaves were measured. A sample (100 mg) of fresh leaves was homogenized in 50 mmol/L potassium phosphate buffer (pH 7.0) containing 4% (weight per volume) polyvinylpyrrolidone, 4 mmol/L dithiothreitol, and 1 mmol/L phenylmethylsulfonyl. The homogenate was centrifuged at $12,000\times g$ for 15 min at 4°C. The supernatant was collected and used to determine antioxidant enzyme activities.

Determination of SOD activity

The activity of SOD was assayed in accordance with the method of McCord and Fridovich (1969). The reaction mixture (total volume 200 μ L) contained 50 mmol/L potassium phosphate buffer (pH 7.8), 0.02 mmol/L cytochrome C, 20 μ L, 0.2 mmol/L EDTA (pH 8.0) 20 μ L of the enzyme extract and 0.1 mmol/L Xanthine was added to the final solution. The reaction was determined spectrophotometrically by measuring the amount of enzyme required to result in a 50% inhibition of the rate of reduction at 550 nm and activity was calculated as units of enzyme per milligram of protein.

Determination of catalase activity

The CAT activity was analyzed using the modified method of Beers and Sizer (1952). The reaction mixture (total volume 200 μ L) was composed of 50 mmol/L potassium phosphate buffer (pH 7.0), 10 mmol/L hydrogen peroxide and 20 μ L of the enzyme extract. The enzyme activity was determined spectrophotometrically by measuring the absorbance at 240 nm and was expressed as units of enzyme per milligram of protein.

Determination of glutathione reductase activity

The GR activity was determined following the modified method of Smith et al. (1988). The reaction mixture was composed of 100 mmol/L potassium phosphate buffer (pH 7.5), 0.75 mmol/L 5,5'-dithiobis(2-nitrobenzoic acid), 0.5 mmol/L EDTA (pH 8.0), 0.1 mmol/L NADPH, 0.5 mmol/L GSSG and 20 μ L of the enzyme extract. The GR activity was recorded from the increase in absorbance at 412 nm and the activity was expressed as units of enzyme per milligram of protein.

Determination of ascorbate peroxidase activity

The APX activity was determined based on the method of Nakano and Asada (1981) with some modifications. A total volume of 200 μ L of the APX reaction solution contained 50 mmol/L potassium phosphate buffer (pH 7.0), 1 mmol/L EDTA (pH 8.0), 10 mmol/L H_2O_2 , 0.5 mmol/L ascorbic acid and 20 μ L of the enzyme extract. The APX activity was recorded as the decrease in absorbance at 290 nm and was expressed as units of enzyme per milligram of protein.

Determination of guaiacol peroxidase (GPX) activity

Activity of GPX was analyzed following the modified method of MacAdam et al. (1992). The reaction mixture (total volume

200 μ L) contained 50 mmol/L potassium phosphate buffer (pH 6.0), 2.5 mmol/L guaiacol, 0.2 mmol/L H_2O_2 and 20 μ L of the enzyme extract. The absorbance of the reaction mixture was measured at 470 nm and defined as units of enzyme per milligram of protein.

Protein content

The protein content in each leaf sample was estimated in accordance with the method of Bradford (1976) and was expressed as milligrams per gram fresh weight (FW). Bovine serum albumin (BSA) was used as the standard and absorbance was measured at 595 nm. The standard curve was linear between 0 μ g/mL BSA and 100 μ g/mL BSA (coefficient of determination (R^2) = 0.995).

Hydrogen peroxide content

The hydrogen peroxide content was determined in accordance with the method of Jana and Choudhuri (1982). Leaf tissue (100 mg) was homogenized with 100 mmol/L potassium phosphate buffer (pH 6.5) and 1 mmol/L hydroxylamine. After centrifugation at $12,000\times g$ for 15 min at 4°C, the supernatant was collected. The reaction mixture consisted of 300 μ L freshly prepared supernatant with 90 μ L of 0.3% (volume per volume; v/v) titanium sulfate in 20% H_2SO_4 (v/v) before use and then centrifuged at $12,000\times g$ for 10 min. The standard curves of H_2O_2 were constructed using a concentration ranging between 10 μ mol and 100 μ mol (R^2 = 0.991). The absorbance was measured at 410 nm and the amount of H_2O_2 was determined from the standard curve and expressed as micromoles per gram FW.

Statistical analysis

The experiment was designed according to a randomized complete block design (RCBD) with four replications (three seedlings of each line per replication). Significance was assessed using one-way analysis of variance. The differences between means were analyzed using Duncan's multiple range test with significance tested at the $p < 0.05$ level. IBM SPSS Statistics 22 (IBM Corporation; Armonk, NY, USA) was used for statistical analysis.

Single nucleotide polymorphism determination

Genomic DNA was extracted from leaves of the selected CSSL and KDML105 rice using a Genomic DNA Mini Kit (Plant) (Geneaid; Taiwan). Genomic DNA libraries were prepared for sequencing using the Illumina genome analyzer (Illumina; San Diego, CA USA) and the Illumina HiSeq200 protocol. The sequence reads were classified into the correct categories using the pipeline developed by Missirian et al. (2011). The genome of rice provided from MSU database (Kawahara et al., 2013) was used as a reference for mapping using BWA (Li and Durbin, 2009). Finally, CSSL16 and KDML105 genome data were compared using bioinformatics tools to identify loci containing different SNPs.

Results and Discussion

CSSL16 was more salt tolerant than KDML105 at the seedling stage

There was no significant difference between seedlings of CSSL16 and KDM105 compared with the standard check Pokkali for salt tolerance under the non-stress condition (0 mM NaCl) as shown in Fig. 1A. Of the seedlings treated with the salt stress condition (75 mM NaCl), CSSL16 had a lower SES than KDML105 after 4 DSS, which was indicative of the higher salt susceptibility of KDML105, whereas Pokkali had the lowest SES under the salt stress condition (Fig. 1B). In agreement with the present research, Kanjoo et al. (2011) reported that the mean SES of some CSSL lines, which contained the putative drought tolerance gene region on chromosome 1, was lower than that of KDML105 (susceptible phenotype) but higher than those of Pokkali and DH212 (tolerant phenotype) after treatment with 150 mM NaCl for 16 DSS and 21 DSS. Chutimanukul et al. (2018a) observed that CSSL16 had a lower SES compared with that of the recipient parent (KDML105), but higher than that of the donor parent (DH212) under 75 mM NaCl treatment for 6 DSS, 12 DSS and 18 DSS in the vegetative stage. These results suggested that CSSL16 had higher salt tolerance than KDML105 at the seedling stage.

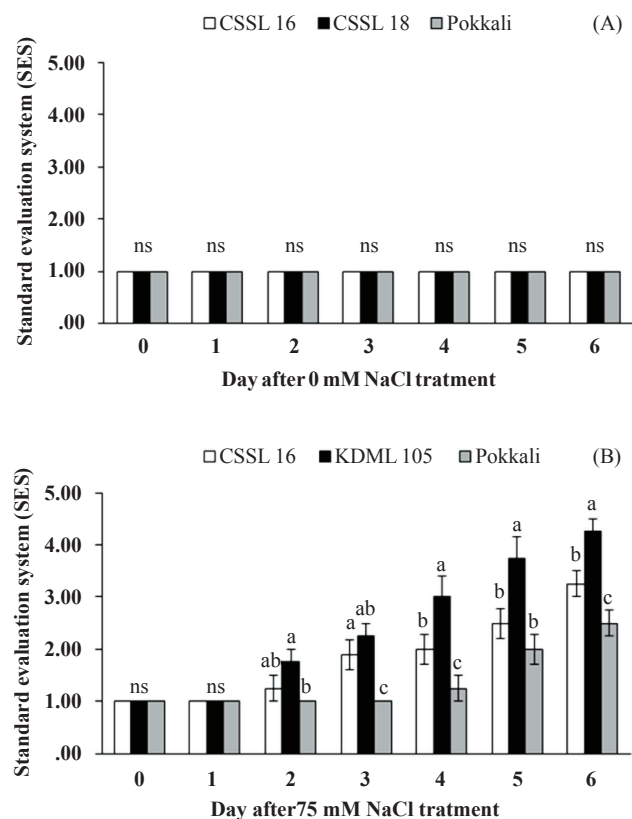


Fig. 1 Standard evaluation system values of CSSL16, KDML105 and Pokkali at the seedling stage: (A) after 0 d, 1 d, 2 d, 3 d, 4 d, 5 d, and 6 d under no stress (0 mM NaCl) conditions; (B) salt stress (75 mM NaCl) conditions, where all data presented are the mean of four biological replicates, error bar = $\pm$ SE, different letters above the bars indicate a statistically significant difference between means within each time point ($p < 0.05$) and ns = not significant different

Effect of salt stress on H_2O_2 content

Hydrogen peroxide may play an important role in the mechanism of salt stress and drought-stress damage in rice (Singha and Choudhuri, 1990). A low SES may result from low oxidative stress in plants under the salt stress condition. Therefore, the H_2O_2 content of leaves was evaluated.

During the first 5 d under salt stress, the H_2O_2 content in CSSL16 was lower than those observed in KDML105 and Pokkali (Fig. 2A). The H_2O_2 content in CSSL16 was 2–4 $\mu\text{mol/g}$ FW, whereas that of KDML105 was 2.5–8 $\mu\text{mol.g}^{-1}$ FW. This finding suggested that oxidative stress as a result of salt stress was greater in KDML105 than CSSL16. However, at the end of the experiment (6 DSS), CSSL16 had a significantly higher H_2O_2 content.

Effects of salt stress on activities of reactive oxygen species scavenging enzymes

Under salt stress, the ROS generated can cause oxidative stress to proteins, nucleic acids and lipid membranes (Scandalios, 1993; Chawla et al., 2013). Plants have defensive mechanisms for scavenging ROS by the enzymes SOD, CAT, APX, GPX, POX and GR, as well as the non-enzymatic antioxidants glutathione, beta-carotene, and ascorbic acid (Gill and Tuteja, 2010). Hydrogen peroxide is a product of SOD activity that detoxifies the superoxide molecule into H_2O_2 (Aydin et al., 2013). Twenty-day-old seedlings of Pokkali rice had significantly higher SOD activity than that of KDML105 and CSSL16 (Fig. 2B). This may have been the cause of the higher H_2O_2 content of Pokkali at the beginning of the experiment. After 2 d of salt stress, CSSL16 had significantly lower SOD activity, which may have led to the lower H_2O_2 content in CSSL16 after exposure to salt stress. However, CSSL16 subsequently had the highest SOD activity after 6 DSS, which may have resulted in the higher H_2O_2 content at that time (Fig. 2A).

A lower H_2O_2 content may result from higher activities of the enzymes scavenging H_2O_2 , which comprise CAT, the enzymes in the ascorbate-glutathione cycle and other peroxidase enzymes. In the present investigation, activities of CAT, APX, GR and GPX were determined.

Similar changes in CAT activity were observed in all three rice lines studied, except that the highest activity of CAT after 6 DSS was observed in Pokkali (Fig. 2C). The pattern of CAT activity did not correspond well with the H_2O_2 content. Therefore, it was hypothesized that the lower content of H_2O_2 in CSSL16 was not due to the CAT activity. However, CAT may play an important role in scavenging H_2O_2 in Pokkali. Vaidyanathan et al. (2003) reported that Pokkali had higher activity of ROS scavenging enzymes (such as CAT, and antioxidants, including glutathione and ascorbate) compared to those of a salt-sensitive rice cultivar under salt stress.

The activities of two enzymes in the ascorbate-glutathione cycle (APX and GR) were investigated in the present study. The APX activity of CSSL16 increased more rapidly than those of KDML105 and Pokkali from 2 DSS to 5 DSS (Fig. 3A). After 2 DSS, CSSL16 had APX activity five-times higher than that of KDML105. This APX activity level was maintained in CSSL16 until 5 DSS. These

results were consistent with the observation of Vaidyanathan et al. (2003), who reported that APX activity plays a crucial role in ROS scavenging during salt stress in a salt-tolerant rice cultivar. A similar level of GR activity was observed among all three rice lines (Fig. 3B). A similar result was recorded for BRS Bojuru and BRS Pampa, which are salt-tolerant and -sensitive cultivars, respectively, as no significant difference in GR activity under treatments with 150 mM NaCl for 0 hr, 6 hr, 24 hr, 48 hr and 72 h was detected in these two cultivars (Vighi et al., 2017). These findings suggested that APX rather than GR may be important in ROS scavenging in rice and that APX may play an important role in the ROS scavenging system in CSSL16. The present data also suggested that in CSSL16, the GR activity might not be the

rate-limiting step in this detoxification pathway.

The enzyme GPX is involved in H_2O_2 scavenging (Gill and Tuteja, 2010). In the current study, CSSL16 had the significantly highest GPX activity compared with those of KDML105 and Pokkali from 2 DSS to 6 DSS. CSSL16 maintained a higher GPX activity after salt stress compared to the other two cultivars (Fig. 3C). Several studies have pointed out that salt-tolerant species had higher peroxidases activity than salt-sensitive species under salt stress treatment (Shalata and Tal, 1998; Mittova et al., 2002; Demiral and Türkan, 2005). Therefore, GPX may play an important role in the ROS scavenging system and contribute to the lower degree of H_2O_2 toxicity, whereas Pokkali rice utilizes other antioxidant enzymes for H_2O_2 scavenging.

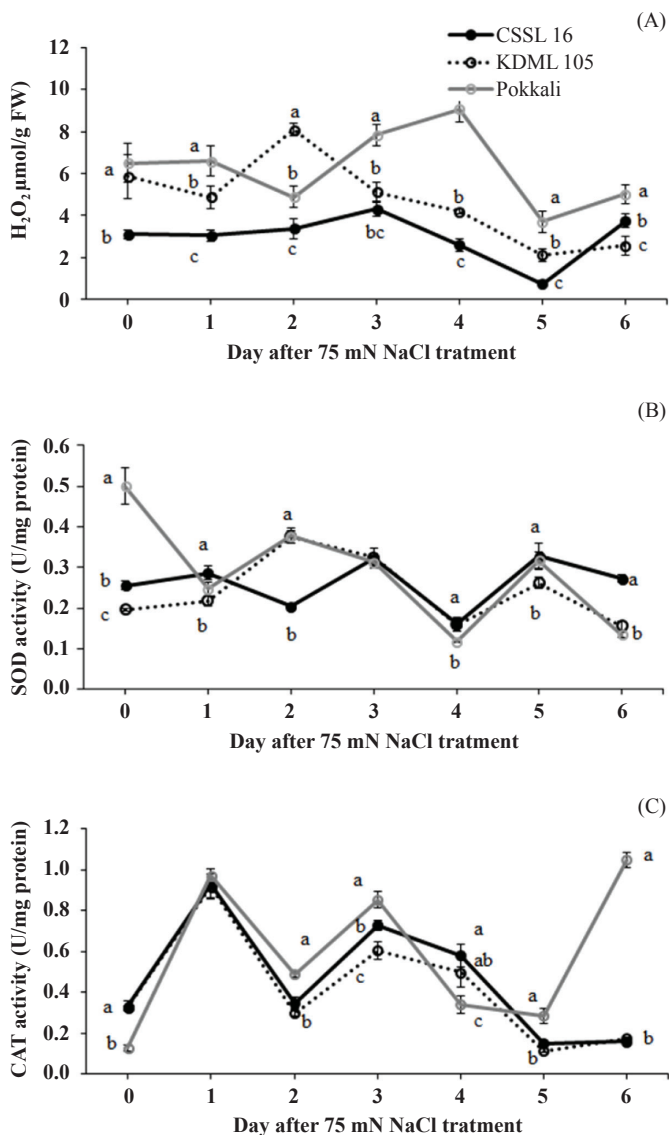


Fig. 2 Leaves of CSSL16, KDML105 and Pokkali plants grown under salt stress conditions showing: (A) hydrogen peroxide (H_2O_2) content; (B) superoxide dismutase (SOD) activity; (C) catalase (CAT) activity, for four biological replicates, FW = fresh weight, error bar = $\pm$ SE, different letters above values indicate a statistically significant difference between means at each time point ($p < 0.05$) and ns = not significant different.

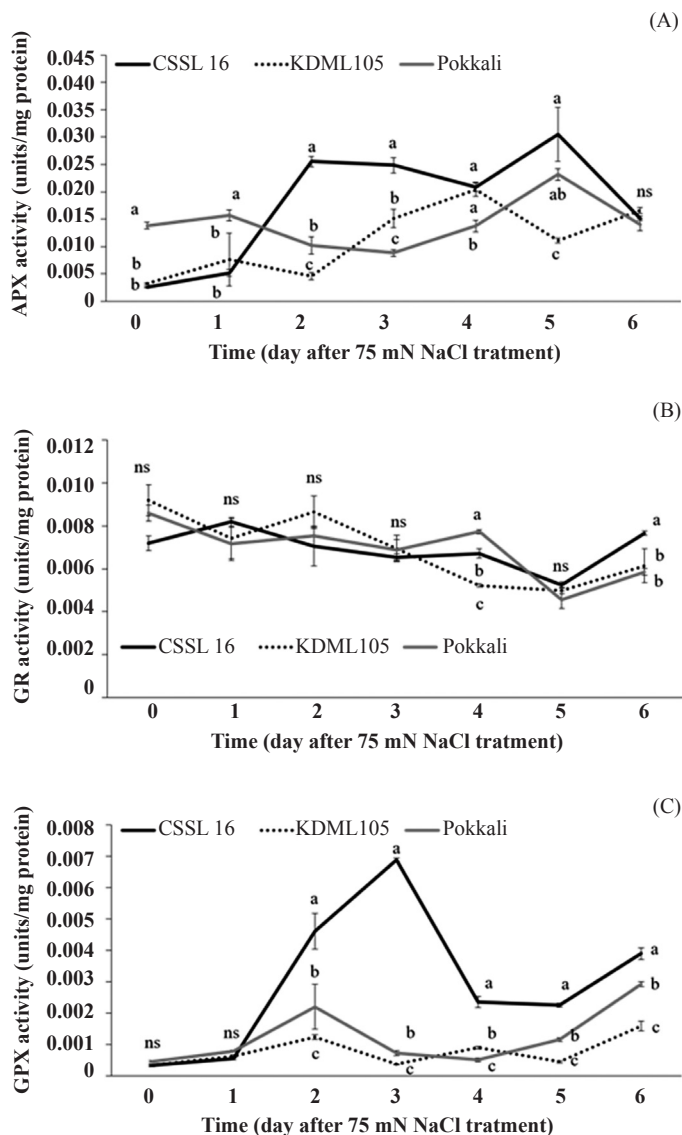


Fig. 3 Leaves of CSSL16, KDML105 and 'Pokkali' plants grown under the salt stress conditions: (A) ascorbate peroxidase (APX) activity; (B) glutathione reductase (GR) activity; (C) guaiacol peroxidase (GPX) activity, for four biological replicates, error bar = $\pm$ SE, different letters above values indicate a statistically significant difference between means at $p < 0.05$ and ns = not significant

In summary, CSSL16 utilized the ascorbate-glutathione pathway for ROS scavenging, and APX may be an important enzyme for initiation of this pathway. GPX is an additional ROS scavenging enzyme that was indicated to contribute to the enhanced salt-tolerance phenotype of CSSL16 rice.

Single nucleotide polymorphisms in the genes encoding for ascorbate peroxidase

To investigate if the differences in enzyme activities were due to gene sequence differences, the loci encoding the ROS scavenging enzymes were obtained from the Rice Genome Annotation Project database (Ouyang et al., 2007) and searched for the SNPs between CSSL16 and KDML105. Eight SOD loci were detected, none of which contained SNPs between the two genotypes. Thus, the small difference in the SOD activity during salt stress at the seedling stage was not due to gene sequence differences. An identical outcome was observed for the CAT and GR gene families.

Interestingly, 1 out of 9 *APX* loci and 5 out of 146 annotated peroxidase precursor genes contained SNPs between CSSL16 and KDML105. These SNPs may have contributed to the differences in enzyme activities under the salt stress condition and led to the salt-tolerant phenotype of CSSL16. The SNPs of CSSL16 observed in *OsAPx7* (LOC_Os04g35520) were located in the promoter region, exon and introns, whereas several SNPs were detected in the peroxidase loci LOC_Os01g57730, LOC_Os01g73170, LOC_Os01g73220, LOC_Os02g50770 and LOC_Os06g46799. The higher activity of APX may have been due to an elevated gene expression level in accordance with an increase in the transcription level, or the higher activity may have been caused by a missense mutation in the exon of *OsAPX7* in CSSL16 (Asp→Gly). The SNPs detected in peroxidase genes on chromosome 1 are located within the simple sequence repeat (SSR) markers for the abiotic stress phenotype (Kanjoo et al., 2011). The SNPs identified in these loci are in the promoter region, exons and intron. All of these SNPs might contribute to enhanced and more rapid expression of peroxidase genes in CSSL16 compared to KDML105. The SNPs detected in peroxidase genes on chromosomes 2 and 6 are located in exons, but are silent as the SNPs cause no amino acid residue changes. However, the possibility cannot be ignored of codon usage bias (Garcia et al., 2018) and RNA secondary structure (Sadia et al., 2018), which can contribute to gene expression regulation. The present observations suggested that the SSR markers used for abiotic stress selection may be markers for a group of genes that are involved in more than one mechanism that contributes to salt stress tolerance.

In conclusion, CSSL16 was shown to have a more effective oxidative scavenging system than its parent, KDML105, based on the significantly higher activities of APX and GPX. These results were consistent with the detection of SNPs in *OsAPx7* and a number of peroxidase genes in CSSL16. The contribution of these SNPs to the regulation of enzyme activity requires investigation in the future.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

This research was supported by the National Science and Technology Development Agency, Thailand (Project code P-12-01235) and Sci-Super IV funding, Faculty of Science Chulalongkorn University, Bangkok, Thailand. The first author was supported by Research Assistantship Funding from the Faculty of Science, Chulalongkorn University. The authors thank Professor Dr Luca Comai for the suggestion of genome sequence analysis, Miss Fonthip Noothong for assistance with antioxidant enzyme analyses and Dr Robert McKenzie from Edanz Group for editing a draft of this manuscript.

References

- Aydin, S.S., Büyük, I., Aras, S. 2013. Relationships among lipid peroxidation, SOD enzyme activity, and SOD gene expression profile in *Lycopersicon esculentum* L. exposed to cold stress. Genet. Mol. Res. 12: 3220–3229.
- Beers, R.F., Sizer, I.W. 1952. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J. Biol. Chem. 195: 133–140.
- Blokhina, O., Virolainen, E., Fagerstedt, K.V. 2003. Antioxidants, oxidative damage and oxygen deprivation stress: a review. Ann. Bot. 91: 179–194.
- Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principles of protein-dye binding. Anal. Biochem. 72: 248–254.
- Chawla, S., Jain, S., Jain, V. 2013. Salinity induced oxidative stress and antioxidant system in salt-tolerant and salt-sensitive cultivars of rice (*Oryza sativa* L.). J. Plant Biochem. Biot. 22: 27–34.
- Chutimanukul, P., Kositsup, B., Plaimas, K., Buaboocha, T., Siangliw, M., Toojinda, T., Comai, L., Chadchawan, S. 2018a. Photosynthetic responses and identification of salt tolerance genes in a chromosome segment substitution line of 'Khao Dawk Mali 105' rice. Environ. Exp. Bot. 155: 497–508.
- Chutimanukul, P., Kositsup, B., Plaimas, K., Buaboocha, T., Siangliw, M., Toojinda, T., Comai, L., Chadchawan, S. 2018b. Data in support of photosynthetic responses in a chromosome segment substitution line of 'Khao Dawk Mali 105' rice at seedling stage. Data Brief. 21: 307–312.
- Cramer, G.R., Nowak, R.S. 1992. Supplemental manganese improves the relative growth, net assimilation and photosynthetic rates of salt-stressed barley. Physiol. Plantarum. 84: 600–605.
- Demiral, T., Türkan, I. 2005. Comparative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance. Environ. Exp. Bot. 53: 247–257.
- Ebitani, T., Takeuchi, Y., Nonoue, Y., Yamamoto, T., Takeuchi, K., Yano, M. 2005. Construction and evaluation of chromosome segment substitution lines carrying overlapping chromosome segments of *indica* rice cultivar 'Kasalath' in a genetic background of *japonica* elite cultivar 'Koshihikari'. Breeding Sci. 55: 65–73.
- Evans, L.T. 1998. Feeding the ten billion: plants and population growth. Cambridge University Press, Cambridge, UK.
- Flowers, T.J. 2004. Improving crop salt tolerance. J. Exp. Bot. 55: 307–319.

- Garcia, V., Zoller, S., Anisimova, M. 2018. Accounting for programmed ribosomal frameshifting in the computation of codon usage bias indices. *G3: Genes Genom. Genet.* 8: 3173–3183.
- Gill, S.S., Tuteja, N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Bioch.* 48: 909–930.
- Gregorio, G.B., Dharmawansa, S., Mendoza, R.D. 1997. Screening rice for salinity tolerance, pp. 2–23. IRRI Discussion Paper Series No.22, International Rice Research Institute, Manila, the Philippines.
- Jana, S., Choudhuri, M.A. 1982. Glycolate metabolism of three submersed aquatic angiosperms during ageing. *Aquat. Bot.* 12: 345–354.
- Kanjoo, V., Jearakongman, S., Punyawaew, K., Siangliw, J.L., Siangliw, M., Vanavichit, A., Toojinda, T. 2011. Co-location of quantitative trait loci for drought and salinity tolerance in rice. *Thai J. Genet.* 4: 126–138.
- Kanjoo, V., Punyawaew, K., Siangliw, J.L., Jearakongman, S., Vanavichit, A., Toojinda, T. 2012. Evaluation of agronomic traits in chromosome segment substitution lines of KDML105 containing drought tolerance QTL under drought stress. *Rice Sci.* 19: 117–124.
- Kawahara, Y., de la Bastide M., Hamilton, J.P., et al. 2013. Improvement of the *Oryza sativa* Nipponbare reference genome using next generation sequence and optical map data. *Rice* 6: 4.
- Kumar, V., Shriram, V., Nikam, T.D., Jawali, N., Shitole, M.G. 2008. Sodium chloride-induced changes in mineral nutrients and proline accumulation in indica rice cultivars differing in salt tolerance. *J. Plant Nutr.* 31: 1999–2017.
- Larcher, W. 1980. *Physiological plant ecology*. Springer-Verlag, Berlin, Germany.
- Li, H., Durbin, R. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*. 25: 1754–1760.
- Maas, E.V. 1986. Salt tolerance of plants. *Applied Agricultural Research*. 1: 12–26.
- MacAdam, J.W., Nelson, C.J., Sharp, R.E. 1992. Peroxidase activity in the leaf elongation zone of tall fescue. *Plant Physiol.* 99: 872–878.
- McCord, J.M., Fridovich, I. 1969. Superoxide dismutase: an enzymic function for erythrocuprein (hemocuprein). *J. Biol. Chem.* 244: 6049–6055.
- Miller, G., Suzuki, N., Ciftci-Yilmaz, S., Mittler, R. 2010. Reactive oxygen species homeostasis and signalling during drought and salinity stresses. *Plant Cell. Environ.* 33: 453–637.
- Missirlian, V., Comai, L., Filkov, F. 2011. Statistical mutation calling from sequenced overlapping DNA pools in TILLING experiments. *BMC Bioinformatics* 12: 287.
- Mittova, V., Tal, M., Volokita, M., Guy, M. 2002. Salt stress induces upregulation of an efficient chloroplast antioxidant system in the salt-tolerant wild tomato species *Lycopersicon pennellii* but not in the cultivated species. *Plant Physiol.* 115: 393–400.
- Munns, R., Termaat, A. 1986. Whole-plant responses to salinity. *Funct. Plant. Biol.* 13: 143–160.
- Munns, R., Tester, M. 2008. Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.* 59: 651–681.
- Nakano, Y., Asada, K. 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol.* 22: 867–880.
- Ouyang, S., Zhu, W., Hamilton, J., et al. 2007. The TIGR Rice Genome Annotation Resource: improvements and new features. *Nucleic Acids Res.* 35: D883–D887.
- Sadia, B., Hamzaai, W.N., Baloch, I.A., Kazmi, S., Asyia, Jan, P.S., Irfan, S. 2018. Bioinformatics analysis of codon usage bias and RNA secondary structures for salt stress responsive genes in *Arabidopsis thaliana* and *Solanum lycopersicum*. *J. Anim. Plant Sci.* 28: 1532–1536.
- Scandalios, G.J. 1993. Oxygen stress and superoxide dismutases. *Plant Physiol.* 101: 7–12.
- Shalata, A., Tal, M. 1998. The effect of salt stress on lipid peroxidation and antioxidants in the leaf of the cultivated tomato and its wild salt-tolerant relative *Lycopersicon pennellii*. *Plant Physiol.* 104: 169–174.
- Singha, S., Choudhuri, M.A. 1990. Effect of salinity (NaCl) stress on H₂O₂ metabolism in *Vigna* and *Oryza* seedlings. *Biochem. Physiol. Pflanzen.* 186: 69–74.
- Smith, I.K., Vierheller, T.L., Thorne, C.A. 1988. Assay of glutathione reductase in crude tissue homogenates using 5,5'-dithiobis (2-nitrobenzoic acid). *Anal. Biochem.* 175: 408–413.
- Takai, T., Nonoue, Y., Yamamoto, S-i., et al. 2007. Development of chromosome segment substitution lines derived from backcross between *indica* donor rice cultivar 'Nona Bokra' and *japonica* recipient cultivar 'Koshihikari'. *Breeding Sci.* 57: 257–261.
- Vaidyanathan, H., Sivakumar, P., Chakrabarty, R., Thomas, G. 2003. Scavenging of reactive oxygen species in NaCl-stressed rice (*Oryza sativa* L.) - Differential response in salt-tolerant and sensitive varieties. *Plant Sci.* 165: 1411–1418.
- Vighi, I.L., Benitezi, L.C., Amaral, M.N., et al. 2017. Functional characterization of the antioxidant enzymes in rice plants exposed to salinity stress. *Biol. Plantarum.* 61: 540–550.