Changes in physiological and antioxidant activity of *indica* rice seedlings in response to mannitol-induced osmotic stress

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ABSTRACT

Morphological, physiological, and biochemical plant responses were studied in unstressed and stressed seedlings of *indica* rice (*Oryza sativa* L.) genotypes. The effect of NB medium supplemented with a 100 mM mannitol treatment, which induced drought stress conditions, was measured for relative growth rate, cell membrane stability, antioxidant enzyme activity (superoxide dismutase [SOD], catalase [CAT], and peroxidase [POD]), and total antioxidant capacity by DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2′-azino-bis[3-ethylbenzothiazoline-6-sulfonic acid] diammonium salt) assays. Results of morphological and physiological factors showed two contrasting rice groups, drought-sensitive and drought-tolerant genotypes. After drought stress, the increased rate of SOD activity was lower in drought-tolerant than in drought-sensitive genotypes, but the increased rate of CAT and POD activity and total antioxidant capacity was higher in drought-tolerant than in drought-sensitive genotypes. Increasing changes in activity levels of antioxidant enzymes and total antioxidant capacity indicated more rapid free radical scavenging compared with relative growth and cell membrane stability in drought-tolerant genotypes under short-term treatment. The trend toward change in CAT and POD antioxidant enzyme activity and total antioxidant capacity of stressed seedlings, as well as the correlation between these changes, and the morphological and physiological responses (0.96 correlation coefficient [R^2] between relative growth rate and CAT; 0.93 R^2 between relative growth rate and POD enzyme activity; 0.96 R^2 between relative growth rate and Trolox equivalent antioxidant capacity values) coincided with the degree of drought tolerance.

Key words: ABTS, antioxidant enzyme, DPPH, drought, *Oryza sativa*, rice.

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INTRODUCTION

Abiotic stresses, such as drought or water deficit, are the most important factors negatively affecting growth, production, and various physiological/biochemical plant processes worldwide (Cheng et al., 2013). Under drought stress, plants have a limited capacity to fix C. This condition restricts CO_2 assimilation due to stomatal closure that extends surplus electron flux to O_2 and overproduction of reactive oxygen species (ROS). In addition, hydrogen peroxide (H_2O_2) production is activated *via* the glycolate oxidase pathway during drought stress (Zhou et al., 2009). Therefore, drought conditions induce oxidative stress in plant cells. The ROS can damage chlorophyll pigments, proteins, DNA, and lipid membranes leading to cell death (Simova-Stoilova et al., 2008).

To protect themselves from this toxic condition, plants maintain a balance between ROS generation and consumption, which is strictly managed by the antioxidant defense system. This system is the accumulation of enzymatic and non-enzymatic components that defend cell membranes and other substances (Liu et al., 2013). Superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) are enzymatic components which play a central role in the defense system. The SOD decomposes superoxide radicals (O_2^-) to H_2O_2 by catalyzing the dismutation of the superoxide anion radical. The POD and CAT then reduce H_2O_2 to water using various substrates as electron donors (Carrasco-Ríos and Pinto, 2014). Rapid detoxification of O_2^- and H_2O_2 is essential to protect free radical damage in plant response.

The antioxidant metabolism is one of the plant metabolic pathways that can affect abiotic stress tolerance. The alteration and amount in antioxidant enzyme activity reveals a significant difference between plant species. Currently, numerous researchers have proven that plant stress tolerance is correlated with antioxidant enzyme activity, and they suggest an important tolerance mechanism to drought stress (Jaleel et al., 2009; Hameed et al., 2011). Rapid accumulation and maintenance of a high level of antioxidant enzyme activity can be a better protection mechanism under drought stress in plant cells. A correlation between the degree of drought stress tolerance and level of antioxidant enzyme activity was demonstrated in several plant species (Uzilday et al., 2012).

Rice (*Oryza sativa* L.) is a widely cultivated crop and one of the major staple foods for more than half of the world's population, and it provides approximately 30% to 40% of the calories consumed in Asia. These results are from a genetic and molecular study model in monocot plants. Rice is considered as

one of the cultivated species that is highly sensitive to water deprivation, and it is also one of the most water intensive for production yield. World rice production in the future will be reduced by approximately 50% due to drought (Shukla et al., 2012). Various mechanisms and behavioral responses to drought stress are complex and have different patterns in each plant species. Therefore, knowledge and understanding of morphological, physiological, and biochemical responses in drought-tolerant and drought-sensitive rice are required to reduce production loss (Fu et al., 2011).

The present study investigated the relative growth rate of seven *indica* rice genotypes under drought conditions induced by 100 mM mannitol. According to the relative growth rate and cell membrane stability, degrees of drought tolerance were selected in each rice genotype. The present study was also conducted to examine the pattern of antioxidant enzyme activity (superoxide dismutase, catalase, and peroxidase) and total antioxidant activity (ABTS and DPPH assays) on drought stress and associate the role of these enzymes in decreasing oxidative stress damage with the growth rate and cell membrane stability due to drought stress in sensitive and tolerant genotypes of *indica* rice seedlings.

MATERIALS AND METHODS

Plant materials and stress conditions

Seeds of seven rice (*Oryza sativa* L.) genotypes 'Khao Dawk Mali 105' (KDML105), 'Sangyod' (SY), 'Khaodang' (KD), 'Rice Department 15' (RD15), 'Klumsakolnakorn' (KS), 'Klumkhonkaen' (KK), and 'Pathumthani 1' (PT1) were dehusked and surface sterilized with 70% (v/v) ethanol for 2 min followed by 5% (v/v) commercial bleach (5.25% sodium hypochlorite) for 40 min and 30% (v/v) commercial bleach (5.25%) for 30 min. Seeds were thoroughly rinsed six times in sterilized distilled water. Sterilized seeds were blot dried with sterilized tissue paper and aseptically cultured in NB medium (a medium combining N_6 macroelement, B_5 microelement, and B_5 vitamin components; Li et al., 1993) and supplemented with 30 g L^{-1} sucrose and 8 g L^{-1} agar. Cultures were grown at 25 ± 2 °C, 80 ± 5% RH, and 60 ± 5 μmol m^{-2} s^{-1} photosynthetic proton flux density (PPFD) with a 16:8 h photoperiod provided by fluorescent lamps. After 7 d incubation, seedlings were germinated under photoautotrophic conditions using vermiculite as supporting material for 7 d. These conditions were applied to NB sugar-free liquid medium and the number of air exchanges was adjusted to 2.32 h^{-1} with CO_2 as a C source by punching a hole in the chamber (Ø 1 cm) and covering the hole with a microporous filter (0.20 μm pore size; Nihon Millipore, Tokyo, Japan) and grown at 25 ± 2 °C, 80 ± 5% RH, and 60 ± 5 μmol m^{-2} s^{-1} PPFD with a 16:8 h photoperiod provided by fluorescent lamps. For drought stress exposure, seedlings were grown in NB sugar-free liquid medium supplemented with 0 or 100 mM mannitol for 2 and 4 d under the previously described conditions. Subsequently, the unstressed and stressed seedlings were immediately collected and stored at -80 °C prior to analysis.

Growth measurements

The unstressed and stressed seedlings were randomly selected to estimate growth by the relative growth rate (RGR) assay following the method by Noggle and Friz (1976). Dry weight (DW) was determined by drying samples in an aerated oven at 70 °C for 72 h and calculating the increase in DW by the equation $RGR = (ln\ DW_f - ln\ DW_i)/(T_f - T_i)$ where DW is dry weight, t is time, and subscripts denote the initial (0 d) and final (2 and 4 d) measurements of the unstressed and stressed treatments.

Cell membrane stability assay

Cell membrane stability (CMS) was measured following the method by Yan et al. (1996). Samples were cut into 1-cm pieces and then kept in a capped vial containing 10 mL of deionized water and incubated in the dark for 3 h at 30 °C. Electrical conductivity (EC) was measured with a conductivity meter (Acorn series CON 6, Oakton Instruments, Vernon Hills, Illinois, USA). After the first measurement, vials were boiled for 2 min to release the electrolytes. Solutions were then cooled to 25 ± 2 °C room temperature and the second EC measurement was taken. The CMS was calculated as a measurement of ion leakage from EC data.

Enzyme extraction and antioxidant enzyme assays

The unstressed and stressed seedlings were crushed into a powder in a mortar with pestle with liquid nitrogen. The crude enzyme of the powder was extracted in 50 mM chilled sodium phosphate buffer (pH 7.8) and 1% (w/v) polyvinylpolypyrrolidone (PVPP) at 4 °C. The homogenate was centrifuged at 12 000 g at 4 °C for 15 min. In the supernatant, SOD, CAT, and POD enzyme activity was immediately determined.

The SOD enzyme activity was estimated according to Beauchamp and Fridovich (1971). The reaction mixture contained 390 mM methionine, 2.25 mM nitrotetrazolium blue chloride (NBT), 3 mM EDTA, 1.5 M Na_2CO_3, and 150 μL crude enzyme extract. The reaction pH was adjusted to 10.2 and it was initiated by adding 1 mL 60 µM riboflavin. The solution was placed below a light source of 18 W fluorescent lamps for 10 min. Absorbance was recorded at 560 nm with a UV-visible spectrophotometer (Model Bio Mate 3, Thermo Electron, Waltham, Massachusetts, USA). The quantity of SOD generated the 50% reduction of NBT in the reaction and was defined as one unit of enzyme activity. Specific enzyme activity was expressed as units per milligram of protein.

The CAT enzyme activity was estimated according to Aebi (1984). The reaction mixture contained 19 mM H_2O_2 and 50 mM sodium phosphate buffer (pH 7.0). It was initiated by adding 200 µL crude enzyme extract to the

reaction buffer solution. The reaction mixture was incubated for 30 s at 25 ± 2 °C room temperature. Absorbance was recorded at 240 nm with a UV-visible spectrophotometer. The decomposition of H_2O_2 was determined after absorbance decreased at 240 nm. Activity was calculated as the H_2O_2 extinction coefficient of 36 μM^{-1} cm^{-1} and expressed as µmol H_2O_2 mg^{-1} protein.

The POD enzyme activity was estimated according to Beffa et al. (1990). The reaction mixture contained 0.1 M sodium phosphate buffer (pH 6.0), 9 mM guaiacol, and 2 mM H_2O_2. The reaction was started by adding 50 µL crude enzyme extract. Absorbance was measured at 460 nm with a UV-visible spectrophotometer. Activity was calculated as the H_2O_2 extinction coefficient of 6.39 μM^{-1} cm^{-1} and expressed as µmol H_2O_2 mg^{-1} protein.

Total protein was assayed according to the Bradford (1976) method using bovine serum albumin as a standard.

Total antioxidant capacity assays (DPPH and ABTS radical scavenging capacity)

The unstressed and stressed seedlings (1 g) were ground in a mortar with pestle with liquid nitrogen. The fresh powder was used for antioxidant extraction with 4 mL of 60% (v/v) acidic methanol (methanol:HCl; 99:1) at 25 ± 2 °C room temperature for 2 h. After extraction, the chlorophyll was removed with chloroform at 25 ± 2 °C room temperature and centrifuged at 10000 rpm at 4 °C for 10 min. The supernatant solution was used to determine the total antioxidant capacity assay.

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging capacity was determined according to Brand-Williams et al. (1995). The 0.8 mM $DPPH^{\bullet}$ solution in 95% (v/v) methanol was prepared daily. A total of 150 μL of this solution was mixed with 0-150 μL plant extracts (250 mg mL^{-1}) and the volume was adjusted to 1.5 mL by adding 95% (v/v) methanol. Samples were mixed and incubated in the dark at room temperature for 30 min. After incubation, absorbance was detected at 517 nm with a UV-visible spectrophotometer. The level of $DPPH^{\bullet}$ remaining from each reaction was calculated as the percentage of inhibition (% inhibition) and expressed as half of the maximal effective concentration (EC_{50}). The EC_{50} parameter, which reflects 50% depletion of the DPPH free radicals, was expressed as μL plant extract g^{-1} FW.

The ABTS (2,2′-azino-bis[3-ethylbenzothiazoline-6-sulfonic acid] diammonium salt) radical scavenging capacity was determined according to Re et al. (1999). The ABTS radical cation ($ABTS^{+\bullet}$) solution was produced by a reaction between 7 mM ABTS solution and 2.45 mM potassium persulfate. The reaction mixture was incubated in the dark at room temperature for 12-16 h before use. The $ABTS^{+\bullet}$ solution was diluted with ethanol to an absorbance of 0.7 ± 0.02 at 734 nm. For the initial assay, 1 mL diluted $ABTS^{+\bullet}$ solution and 10 μL plant extract were mixed and measured spectrophotometrically at 734 nm until the reaction reached a plateau. These measurements were compared to a Trolox standard curve, Total antioxidant capacity was expressed as the μM Trolox equivalent antioxidant capacity (TEAC).

Statistical analysis

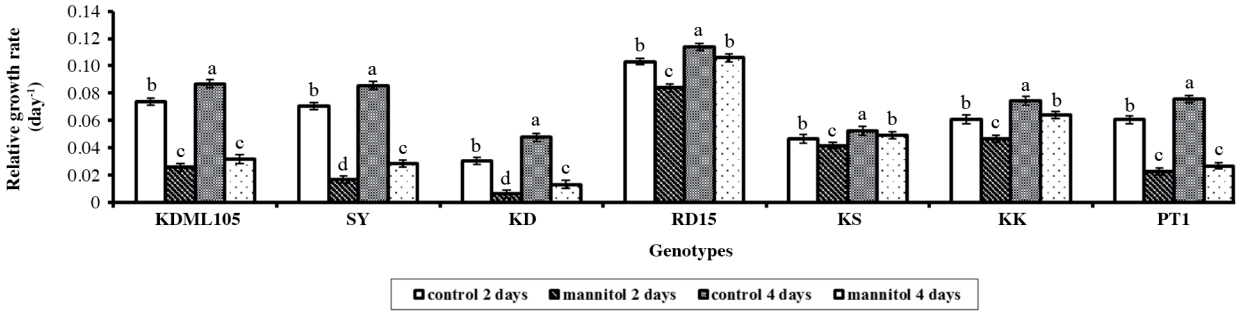
Results were shown as mean values ± standard deviation (SD) of five independent experiments (n = 5) and arranged in a completely randomized design. The data for various parameters were analyzed by the *t*-test and Duncan's multiple range test ($P \leq 0.05$) (SPSS software package, SPSS for Windows, V. 11.0; IBM, Armonk, New York, USA).

RESULTS

The growth of unstressed and stressed seedlings in seven rice genotypes, estimated as the relative growth rate, was affected by 100 mM mannitol in the NB medium. The second and fourth days after initiating the drought treatment, the relative growth rate decreased between 7% and 80% in stressed seedlings of all the rice genotypes compared with the control plants when exposed to 100 mM mannitol (Figure 1). The RD15, KS, and KK genotypes maintained a high relative growth rate under drought stress and also showed lower reduction percentages (7% to 23%) as compared with the KDML105, SY, KD, and PT1 genotypes (63% to 80%).

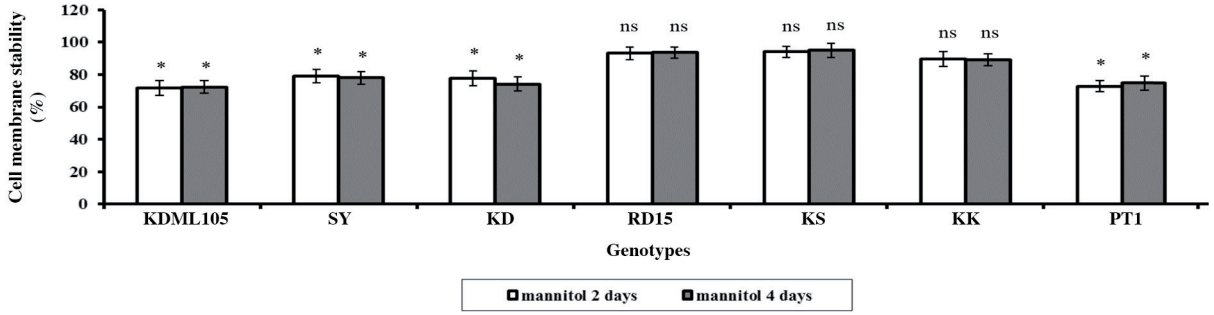
Cell membrane stability, estimated as electrolyte leakage, decreased under drought stress in all the genotypes (Figure 2). The RD15, KS, and KK genotypes showed a slight decrease in cell membrane stability (6.50% to 10.50%) whereas the decrease in the KDML105, SY, KD, and PT1

Figure 1. Relative growth rate of unstressed and stressed seedlings of *indica* rice grown in a photoautotrophic system under drought stress (100 mM mannitol).



Values are means ± SD.
Different letters for the same genotype are significantly different ($P \leq 0.05$).

Figure 2. Cell membrane stability of stressed seedlings grown in a photoautotrophic system under drought stress (100 mM mannitol) for 2 and 4 d compared to control treatment (0 mM mannitol) set as 100%.



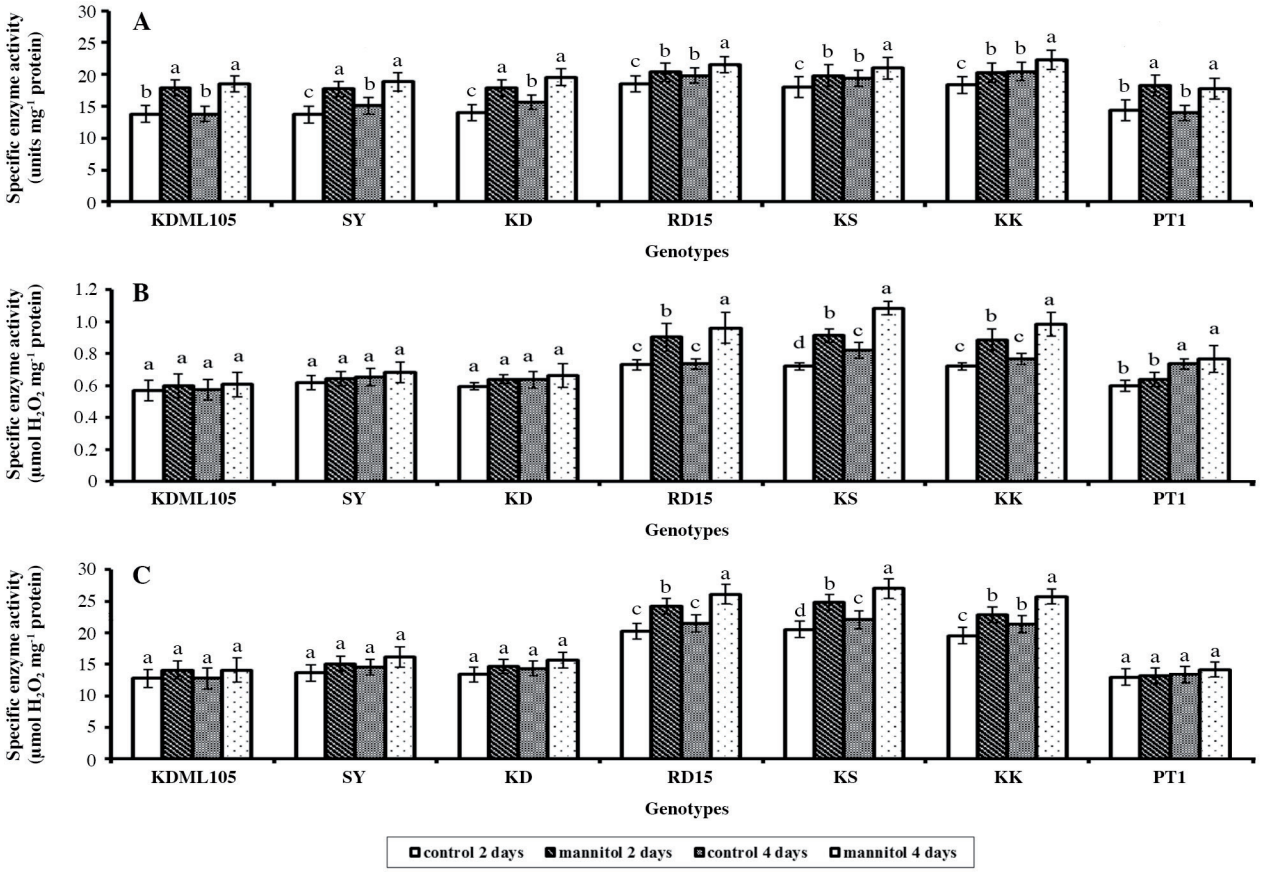
Values are percentage means ± SD.
*Significant at the 0.05 probability level.
ns: Nonsignificant difference.

genotypes under drought stress was 20.86% to 28.14% compared with control plants, indicating that stressed seedlings from the RD15, KS, and KK genotypes have a greater ability to endure drought stress.

Drought stress significantly decreased the relative growth rate and cell membrane stability of drought-stressed plants. These results showed that a decrease in the relative growth rate was associated with impaired cell membrane stability in stressed seedlings (Figures 1 and 2). *Indica* rice genotypes RD15, KS, and KK showed a significantly lower decrease in the relative growth rate and cell membrane stability than genotypes KDML105, SY, KD, and PT1 under drought stress, which reflects the tolerance trait of the RD15, KS, and KK genotypes and sensitivity trait in the KDML105, SY, KD, and PT1 genotypes.

Drought stress also induces the evident accumulation of free radicals and causes oxidative burst in plant cells. The antioxidant enzyme activity is simultaneously activated to eliminate the ROS. Results showed a significant increase in SOD, CAT, and POD activity (Figure 3). Overall, increased

Figure 3. Specific enzyme activity of superoxide dismutase enzyme (SOD) (A), catalase (CAT) (B) and peroxidase (POD) (C) of unstressed and stressed seedlings grown in a photoautotrophic system under drought stress (100 mM mannitol).



Values are means ± SD.
Different letters for the same genotype are significantly different ($P \leq 0.05$).

activity in all the antioxidant enzymes was induced in all the genotypes under drought stress compared with the unstressed seedlings.

Antioxidant enzyme activity, such as SOD, CAT, and POD, increased significantly in both genotype groups under stress. Drought tolerance or genotype sensitivity were correlated with antioxidant enzyme responses. In drought-sensitive genotypes, the drought treatment caused an approximate increase of 24.84% to 33.99% in total SOD activity compared with control plants; this was higher than the rate increase in drought-tolerant genotypes (7.90% to 10.74%) (Figure 3A). The CAT and POD activity of drought-tolerant seedlings, approximately 22.99% to 32.05% and 16.86% to 22.33%, respectively, had a higher rate increase than drought-sensitive seedlings compared with control seedlings (Figures 3B and 3C). The mean rate increase in CAT and POD activity of drought-sensitive seedlings was approximately 4.02% to 7.40% and 1.39% to 10.70%, respectively.

Figure 4 describes free radical scavenging in the unstressed and stressed seedling extracts. Scavenging of ABTS and DPPH radicals is a potent tool to establish the total antioxidant capacity of an extract. The extract contained 1.14-3.03 μM Trolox g^{-1} FW of the total antioxidant capacity expressed as the TEAC (Figure 4A). In these drought-tolerant genotypes, TEAC contents were found to be 1.92 to 2.63-fold higher than control seedlings; this increase was higher than the TEAC contents in drought-sensitive genotype extracts (1.20 to 1.26-fold).

The EC_{50} value is a widely used parameter to measure antioxidant activity and a lower value is correlated with higher antioxidant capacity of crude extract. The EC_{50} values of unstressed and unstressed seedling extracts are shown in Figure 4B. The EC_{50} value of the stressed seedling extracts ranged from 524.12 to 747.20 μL g^{-1} FW, and it had a lower capacity than the unstressed seedling extract from their genotypes (0.84 to 0.97-fold). For the group of drought-tolerant genotypes, the decrease in the EC_{50} value (0.84 to 0.87-fold) was higher than the decrease in the EC_{50} value (0.94 to 0.97-fold) of seedling extracts from drought-sensitive genotypes. These results indicated that the extract from drought-tolerant genotypes had greater antioxidant capacity than the extract from drought-sensitive genotypes.

The change in the relative growth rate and cell membrane stability was investigated in correlation with the change in antioxidant enzyme activity and total antioxidant capacity (Table 1). The correlation coefficient (R^2) between the relative growth rate with CAT (R^2 = 0.99) and POD (R^2 =

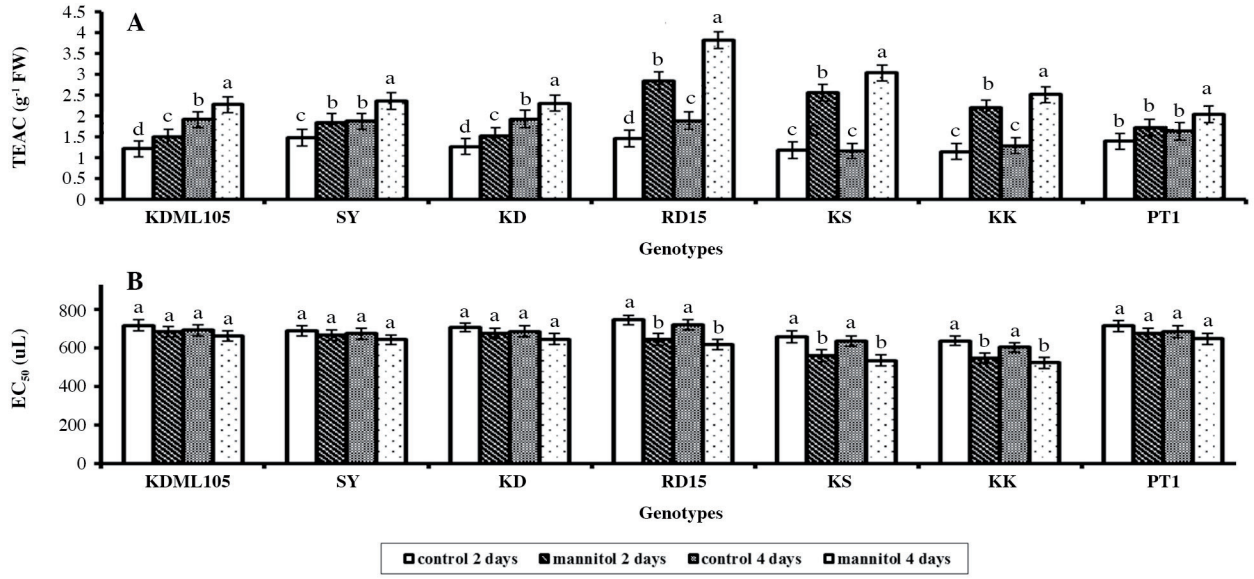
Table 1. Correlation coefficient (R^2) between percentages of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) enzyme activity, total antioxidant capacity using ABTS assay and total antioxidant capacity using DPPH assay and relative growth rate and cell membrane stability percentages from stressed seedlings grown in a photoautotrophic system under drought stress (100 mM mannitol).

Enzymes	Relative growth rate	Cell membrane stability
	R^2	
SOD	- 0.90	- 0.92
POD	+ 0.96	+ 0.90
CAT	+ 0.80	+ 0.86
ABTS assay	+ 0.91	+ 0.89
DPPH assay	- 0.96	- 0.90

(+) positive correlation.
(-) negative correlation.

ABTS: 2,2′-Azino-bis[3-ethylbenzothiazoline-6-sulfonic acid] diammonium salt.
DPPH: 2,2-Diphenyl-1-picrylhydrazyl.

Figure 4. Total antioxidant capacity expressed as Trolox equivalent antioxidant capacity (TEAC) (A) and half of the maximal effective concentration (EC_{50}) (B) using the ABTS and DPPH assays of unstressed and stressed seedlings grown in a photoautotrophic system under drought stress (100 mM mannitol).



Values are means ± SD.
Different letters for the same genotype are significantly different ($P \leq 0.05$).
ABTS: 2,2′-Azino-bis[3-ethylbenzothiazoline-6-sulfonic acid] diammonium salt.
DPPH: 2,2-Diphenyl-1-picrylhydrazyl).

0.93) enzyme activity and TEAC values (R^2 = 0.96) was significantly positive. However, R^2 between the relative growth rate with SOD (R^2 = 0.93) enzyme activity and EC_{50} values (R^2 = 0.97) was significantly negative (Table 1).

The change in cell membrane stability with CAT and POD enzyme activity and TEAC values was positively correlated (Table 1). The R^2 values were 0.95, 0.92, and 0.91 for CAT and POD enzyme activity and TEAC values, respectively. In contrast, R^2 values for cell membrane stability with SOD activity and EC_{50} values were 0.94 and 0.96, respectively, which showed a negative correlation (Table 1). The results suggested that the accumulation of CAT and POD activity, as well as total antioxidant capacity of the seedling extracts significantly contributed to protecting stressed seedlings.

DISCUSSION

Plants have developed several mechanisms to manage the damaging effects of abiotic stresses. The initial plant morphological response to drought stress is the growth rate. Reduced growth may be an adaptive response to abiotic stress (Junjittakarn et al., 2014). Plant growth is one parameter in the drought-sensitive morphological processes caused by the decreased turgor pressure during drought stress (Misra et al., 2002; Inostroza et al., 2015). This growth response seems to indicate that the degree of tolerance to drought stress of the rice genotypes under study was compared with the growth response reported for other plants subjected to drought or water deficit conditions. In general, plant genotypes protect themselves from drought-induced oxidative stress by maintaining a higher growth rate and membrane stability, which indicate more tolerant genotypes (Yang et al., 2008).

The first organelle affected by various stresses is the cell membrane. Plant cell membranes are subjected to changes associated with ion leakage enhancement and cell integrity disadvantage under stress conditions. Drought stress that induces free radical formation causes lipid peroxidation and, therefore, membrane injury and degradation. Cell membrane stability is one of the main parameters for cellular responses under drought conditions and the abiotic tolerance factor in crop plants. Lower cell membrane stability in sensitive genotypes compared with tolerant genotypes has also been reported (Liu et al., 2008). Cell membrane stability, as a membrane integrity index, decreased under drought conditions. It was reported that membrane permeability in wheat (Huseynova, 2012), strawberry (Ghaderi and Siosemardeh, 2011), and marigold (Tian et al., 2012) increased under drought conditions with increasing levels of stress sensitivity.

Drought stress can lead to water deficiency, which induces stomatal closure following the reduction and availability of CO_2 and inhibits C fixation by overly reducing the electron transport chain. This situation could increase the generation of ROS and influence oxidative damage. Increased ROS production in chloroplasts, mitochondria, and peroxisomes under drought stress has been described (Demirevska et al., 2008). The accumulation of ROS depends on the balance between ROS scavenging and ROS formation under stress conditions. The degree of antioxidant enzyme activity and the amount of non-enzymatic antioxidants have varied patterns among different plant species and stresses, stress duration, stress intensity, and plant development stage. An increase in antioxidant enzyme activity under abiotic stresses could indicate ROS production and the establishment of a protective mechanism to diminish exposure to oxidative damage in plant cells (Jaleel et al., 2009).

Tolerant plant genotypes usually have a better antioxidant system content to protect them from oxidative stress by maintaining high antioxidant enzyme and antioxidant molecule activity and contents under stress conditions (Chang-Quan and Rui-Chang, 2008). The SOD, CAT, and POD are key antioxidant enzymes in the plant cell. Many studies demonstrated that these enzymes had high activity during a water deficit stage and the defense mechanism competed against ROS formation (Tahi et al., 2008; Carrasco-Ríos and Pinto, 2014).

Among the enzymatic mechanisms, the first step in ROS scavenging catalyzes the rapid decrease of superoxide radicals into H_2O_2 by SOD. The CAT and POD then catalyze H_2O_2 into H_2O to protect the plant cell from H_2O_2 accumulation (Simova-Stoilova et al., 2008; Carrasco-Ríos and Pinto, 2014). The bulk of H_2O_2 from SOD catalyzation remained biologically toxic. Therefore, SOD can limit plant protection from ROS because the increased rate of SOD activity in drought-tolerant genotypes was lower than in drought-sensitive genotypes. The production of H_2O_2 due to activated SOD enzymes can function in oxidative stress signaling and play the role of a secondary messenger to protect reactions leading to induced CAT and POD activity in plants (Anjum et al., 2011). Results revealed that the low increase of CAT and POD activity in drought-sensitive genotypes could reduce plant cell efficiency to scavenge damaging free radicals. This research study suggested that most of the antioxidant activity had an important role in stress tolerance. Therefore, the high stability and increased rate of CAT and POD activity may be known to confer drought-induced oxidative stress tolerance (Tian et al., 2012).

Currently, applying ABTS and DPPH to measure a free radical for plant sample screening have been investigated in stress tolerance (Cui et al., 2010). The high increment in both ABTS and DPPH radical scavenging capacity also appeared to be correlated with the degree of plant seedling stress tolerance (Weidner et al., 2009; Dominguez-Perles et al., 2011). Evaluations of drought tolerance have been investigated as the reduction of the growth rate and cell membrane stability, which were differentiated among plant genotypes and correlated with enzymatic and non-enzymatic antioxidants (Sharifi et al., 2012).

Since antioxidant capacity can be directly correlated to drought tolerance, the level of TEAC and EC_{50} values

obtained in ABTS and DPPH assays indicated that it has high antioxidant capacity. Moreover, the observed correlation between CAT and POD activity and the relative growth rate and cell membrane stability emphasized the importance of maintaining an optimum content under the stress conditions to the degree of drought tolerance. Drought-tolerant plants generally have a great antioxidant system capacity that increased various times over the unstressed plants compared with drought-sensitive plants in response to stress conditions (Kolarovic et al., 2009). These were considered as significant physiological parameters of drought tolerance in plants. The above data demonstrated that the ROS scavenging system was a necessary component of the defense mechanism as opposed to drought stress in plant cells.

CONCLUSIONS

The higher relative growth rate level, cell membrane stability, constitutive catalase and peroxidase activity, and total antioxidant capacity in the drought-induced rice seedlings indicated that drought-tolerant genotypes had a high free radical degradation content. These results collectively suggested that the knowledge of these parameters in responses to drought stress may be further applied as criteria to drought-tolerant screening in *indica* rice genotypes or other plant species.

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