

SCREENING OF SALINITY TOLERANT JUTE (*CORCHORUS CAPSULARIS* & *C. OLITORIUS*) GENOTYPES VIA PHENOTYPIC AND PHSIOLOGY-ASSISTED PROCEDURES

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Abstract

To obtain salt tolerant genotypes, salt tolerance of 10 jute genotypes of different origins was evaluated by relative salt harm rate at germination stage and by index of salt harm at seedling stage, respectively. The results indicated that salt tolerance of germination stage of jute was consistent with that of seedling stage, with a markedly significant ($P < 0.01$) correlation of 0.8432 ($n = 10$). Two high salt tolerant genotypes (Huang No.1 and 9511) and two salt sensitive genotypes (Mengyuan and 07-21) were screened out by these methods. Further activity analysis of POD, SOD and CAT and determination of MDA content at seedling stage validated that genotypes Huang No.1 and 9511 were more salt tolerant than genotypes Mengyuan and 07-21. Our results indicated that the combination of relative salt harm rate at germination stage and index of salt harm at seedling stage can be used to evaluate salt tolerance of jute genotypes.

Introduction

Salt stress is a worldwide major abiotic stress in agriculture. It is estimated that about 20% of the earth's land mass and nearly half of all irrigated land are affected by salinity. Increased salinization of arable land is expected to have devastating global effects, with prediction of 30% land loss within the next 25 years, and up to 50% by the year 2050 (Wang *et al.*, 2008). Salt stress is a complex physical-chemical process, in which many biological macromolecules and small molecules are involved, such as nucleic acids (DNA, RNA, microRNA), proteins, carbohydrates, lipids, hormones, ions, free radicals, and mineral elements (Munns, 2002; Liu & Baird, 2004). In addition, salt stress is also related to drought, cold, high temperature, acid, alkaline, pathological reactions, senescence, growth, development, cell cycle, UV-B damage, wounding, embryogenesis, flowering, signal transduction, etc (Anand *et al.*, 2003; Wei *et al.*, 2004; Shao *et al.*, 2005). Due to increasing land salinization problems in the world, breeding for salinity tolerance in many crops needs to be paid more attention.

Jute (*Corchorus capsularis* & *olitorius* L.) is an herbaceous annual plant from the Tiliaceae family, mostly grown in Southeast Asian countries (José *et al.*, 2009). Jute fibre can be separated from the bast or outer region of the stem after retting of the whole plant and is mainly used for the manufacture of cloth, cordage, carpets, bagging, wrapping materials, etc., with an annual production of 2.65 million tons around the world. In addition, jute fibre is a good source of different grades of paper pulp (Akhtaruzzaman & Shafi, 1995; Jahan *et al.*, 2001). In recent years, requirement for jute fibre has tremendously increased in China. Jute can grow readily in saline soils (Ma *et al.*, 2009). Considering its tolerance especially to the chlorine salinity, jute has been recently suggested as a promising candidate for planting in wetlands and saline soils in China. China has more than 33 million hectare saline land, which is tremendous

potential for developing the production of jute fibre. As different jute genotypes would possess different tolerances to salinity, it is necessary to screen out high salt-tolerant jute genotypes for utilization.

In the present study, to obtain salt tolerant genotypes, salt tolerance of 10 jute genotypes of different origins was evaluated by relative salt harm rate at germination stage and by index of salt harm at seedling stage, respectively. To further validate their salt tolerance, POD, SOD and CAT activities and MDA content of two high salt tolerant genotypes and two salt sensitive genotypes screened out were investigated at seedling stage.

Materials and Methods

Plant materials: Ten jute genotypes (Huang No.1, 9511, Mengyuan, 179, 95-12, 93-13, 93fan-12, 93fan-18, 93fan-13, and 07-21) of different origins were used in this study. These genotypes were supplied by Jiangsu Redbud Textile Science and Technology Corp. Ltd. and Institute of Bast Fibre Crops, Chinese Academy of Agriculture Sciences, respectively.

Determination of relative salt harm rate and grade of salt tolerance during germination period: Measurement of relative salt harm rate and grade of salt tolerance was performed according to Bagci *et al.*, (2003) with some modification. Two groups of seeds of each jute genotype were laid on humid filter paper, respectively. One group was added ddH₂O as control, the other group was added 273 mM/L NaCl as treatment. Both two groups were kept at 30°C for 10 days. The filter papers were changed every day with 273 mM NaCl or ddH₂O added and then the germination rate of each group was investigated. This experiment was conducted in triplicate. The relative salt harm rate (RSHR) of each genotype was calculated by the following formula:

$$\text{RSHR}(\%) = \frac{\frac{(CK1 + CK2 + CK3)}{3} \times 100 - \frac{(T1 + T2 + T3)}{3} \times 100}{\frac{(CK1 + CK2 + CK3)}{3} \times 100} \times 100\%$$

where *CK* represents germination rate of control and *T* represents germination rate of treatment.

The grade of salt tolerance of each genotype at germination stage was determined according to Table 1.

Determination of index of salt harm and grade of salt tolerance during seedling period: The nutrient solution used for cultivation of jute seedlings was prepared according to Yoshida *et al.*, (1976). The nutrients contained in mg/L were 40 N, 10 P, 40 K, 40 Ca, 40 Mg, 0.05 Mo, 0.2 B, 0.5 Mn, and 0.01 Zn. The pH of nutrient solution was adjusted to 5.2 by adding HCl or NaOH.

Seeds of each jute genotype were sterilized with 0.1% sodium dodecyl sulphate (SDS) solution on a magnetic stirrer for 15 min and thoroughly washed with deionized water. The seeds were then soaked in sterile deionized water at 25 °C for 12 h and then transferred to two sheets of sterile filter paper moistened with deionized water for germination at 25 °C for 48 h in the dark. Germinated seeds were sown into holes of styrofoam boards in deionized water, and grown hydroponically in the growth room for 15 d under the standard condition of 10 h photoperiod at 800 $\mu\text{molm}^{-2}\text{s}^{-1}$, 25/20 °C (day/night temperature) and 70% relative humidity. Afterwards, the seedlings were cultivated in deionized water for 24 h, in solution of 1:1 ratio (v/v) of deionized water:full-strength

nutrient solution for 3 d, and then cultivated in full-strength nutrient solution. The nutrient solution was replaced twice a week. Water lost by evapotranspiration was compensated for by daily addition of deionized water.

Three weeks after transplant, the seedlings of one group of each genotype were imposed salt stress for six days by cultivation in the full-strength nutrient solution containing 160 mM/L NaCl. Nutrient solution without NaCl addition served as control group. 50 seedlings of each group were investigated for their appearance at 3rd and 6th day, respectively. This experiment was conducted in duplicate. All seedlings were classified into 5 grades (0, 1, 2, 3, and 4) according to their appearance:

- 0: Seedling grows normally without any injury.
- 1: The edge of one or two leaves of seedling turns yellow and presents some black spots or withers.
- 2: One whole leaf of seedling turning yellow withers, bestrewing with black spots, or falls off.
- 3: Seedling growth is restrained with 2 or 3 leaves severely withering, turning yellow or falling off.
- 4: Seedling growth is severely restrained with many leaves severely withering and falling off or the whole seedling on the verge of death.

Table 1. Grade standard of salt tolerance of jute.

Grade	Salt tolerance	Relative salt harm rate (%)	Average index of salt harm (%)
1.	High salt tolerance	0-20.0	0-20.0
2.	Salt tolerance	20.1-40.0	20.1-40.0
3.	Middle tolerance	40.1-60.0	40.1-60.0
4.	Salt sensitivity	60.1-80.0	60.1-80.0
5.	High salt sensitivity	80.1-100.0	80.1-100.0

Then, the index of salt harm (ISH) of each genotype was calculated by the following formula:

$$\text{AISH}(\%) = \frac{\sum[(\text{number of '0'} \times 0) + (\text{number of '1'} \times 1) + (\text{number of '2'} \times 2) + (\text{number of '3'} \times 3) + (\text{number of '4'} \times 4)]}{4 \times 50} \times 100$$

where “number of ‘0’ ” represents the seedling number of Grade 0, and so forth.

The grade of salt tolerance of each jute genotype at seedling stage was determined according to Table 1.

Determination of physiological indexes: Two salt tolerant genotypes (Huang No.1, 9511) and two salt sensitive genotypes (Mengyuan, 07-21) were chosen for further measurement of physiological indexes. After 7 days of salt treatment, seedling leaves of each genotype were quickly cut and stored at -20°C for measurement of physiological indexes. Salt treatment for each genotype was conducted under 3 level salt concentrations (0 mmol/L, 70 mmol/L, and 140 mmol/L NaCl).

Enzyme extraction and assay: For protein and enzyme extraction, fresh jute leaves (0.5 g) were well homogenized with 50 mM sodium phosphate buffer (pH 6.8) containing 1 mM EDTA, $\text{Na}_2\text{S}_2\text{O}_5$ and 2% (w/v) polyvinylpyrrolidone (PVPP). The whole extraction procedure was carried out at 4 °C. Homogenates were then centrifuged at 4 °C for 40 min at 13,000 \times g, and supernatants were used for determination of enzyme activity. Protein concentration was determined according to Bradford (1976), using bovine serum albumin as a standard.

Peroxidase (POD) activity was determined according to Herzog & Fahimi (1973). The reaction mixture was 3,3'-diaminobenzidine-tetrahydrochloride dihydrate (DAB) solution containing 50% (w/v) gelatine, 0.15 M Na-phosphate-citrate buffer (pH 4.4) and 0.6% H_2O_2 . The increase in absorbance was recorded at 465 nm through 3 min. A unit of peroxidase activity was defined as $\mu\text{mol mL}^{-1} \text{H}_2\text{O}_2$ decomposed per minute.

Superoxide dismutase (SOD) activity was assayed spectrophotometrically as the inhibition of photochemical reduction of nitro-blue tetrazolium (NBT) at 560 nm (Beauchamp & Fridovich, 1971). The reaction mixture contained 33 μM NBT, 10 mM L-methionine, 0.66 mM EDTA- Na_2 , and 0.0033 mM riboflavin in 0.05 M Na-phosphate buffer (pH 7.8). Lastly, the riboflavin was added. The test tubes containing reaction mixture were shaken and waited for 10 min under 300 $\mu\text{molm}^{-2}\text{s}^{-1}$ irradiance at room temperature. The reaction mixture with no enzyme developed maximum color due to maximum reduction of NBT. A non-irradiated reaction mixture did not develop color and served as the control. The reduction of NBT was inversely proportional to the SOD activity. One unit of SOD was defined as the amount of enzyme inhibiting 50% NBT photoreduction.

Catalase (CAT) activity was estimated according to Bergmeyer (1970), which measures the initial rate of disappearance of H₂O₂ at 240 nm. The reaction mixture contained 0.05 M Na-phosphate buffer (pH 7.0) with 0.1 mM EDTA and 3% H₂O₂. The decrease in the absorption was followed for 3 min and $\mu\text{mol H}_2\text{O}_2$ destroyed per minute was defined as one unit of CAT. The specific enzyme activity for all enzymes determined was expressed as unit of mg^{-1} protein g^{-1} FW.

Determination of lipid peroxidation (MDA): The level of lipid peroxidation in leaf samples was determined in terms of malondialdehyde (MDA) content according to the method of Rao & Sresty (2000). Content of MDA, which is an end product of lipid peroxidation, was determined by the thiobarbituric acid reaction. MDA concentration was calculated from the absorbance at 532 nm and measurements were corrected for non-specific turbidity by subtracting the absorbance at 600 nm. The concentration of MDA was calculated using an extinction coefficient of $155 \text{ mM}^{-1}\text{cm}^{-1}$.

Statistical analysis: All analyses were done on a completely randomized design. All data obtained was subjected to one-way analyses of variance (ANOVA) and mean differences were compared by lowest standard deviations (L.S.D.) test. Experiment for determination of physiological indexes was conducted twice for each genotype with 3 repeated measurements ($n=6$) and comparisons with $p<0.05$ were considered significantly different.

Results

Evaluation of salt tolerance of 10 jute genotypes at germination stage: The relative salt harm rate and grade

of salt tolerance of 10 jute genotypes were determined under 273 mM/L NaCl stress during germination period, and the results are listed in Table 2. The relative salt harm rate of genotypes 9511, Huang NO.1, and 179 was lower than 20%, and according to Table 1, their grade of salt tolerance was classified into Grade 1, the type of high salt-tolerance. Genotype 95-12 had the relative salt harm rate between 20.1% and 40.0% and was grouped into Grade 2, the type of salt tolerance. Genotypes 93fan-12, 93fan-13 and 93-13 with the relative salt harm rate between 40.1% and 60.0% were classified into Grade 3, the type of middle tolerance, while genotype 93fan-18 with the relative salt harm rate between 60.1% and 80.0% was grouped into Grade 4, the type of salt sensitivity. Genotypes 07-21 and Mengyuan had the highest relative salt harm rate between 80.1% and 100.0% and were grouped into Grade 5, the type of high salt sensitivity.

Evaluation of salt tolerance of 10 jute genotypes at seedling stage: Index of salt harm and grade of salt tolerance of 10 jute genotypes during seedling period under 160 mM/L NaCl stress were investigated at 3rd and 6th day, respectively, and the results are listed in Table 3. The average index of salt harm of 3 jute genotypes (9511, 93fan-13, and Huang No.1) was found to be lower than 20%. According to Table 1, they were grouped into Grade 1, the type of high salt tolerance. The average index of salt harm of 2 jute genotypes (07-21 and Mengyuan) was around 63.0%, and they were classified into Grade 4, the type of salt sensitivity. The rest had the average index of salt harm between that of the two types, with genotypes 179 and 95012 falling into Grade 2 (salt tolerance type), and genotypes 93-13, 93fan-12 and 93fan-18 into Grade 3 (middle tolerance type).

Table 2. Relative salt harm rate and grade of salt tolerance of jute genotypes during germination period.

Materials	Relative salt harm rate (%)	Grade
9511	9.2	1
Huang No.1	11.1	1
179	19.5	1
95-12	20.7	2
93fan-12	43.5	3
93fan-13	53.9	3
93-13	58.6	3
93fan-18	67.8	4
07-21	81.3	5
Mengyuan	93.4	5

Table 3. Index of salt harm and grade of salt tolerance of jute genotypes during seedling stage.

Material	Index of salt harm (%)			Grade
	At 3 th day	At 6 th day	Average	
9511	8.00	16.00	12.00	1
93fan-13	7.50	17.00	12.25	1
Huang No.1	12.00	27.00	19.50	1
179	13.50	30.00	21.75	2
95-12	16.00	37.50	26.75	2
93-13	30.50	57.00	43.75	3
93fan-12	35.50	66.00	50.75	3
93fan-18	32.50	63.50	48.00	3
07-21	51.50	74.50	63.00	4
Mengyuan	49.00	78.50	63.75	4

According to the above results (Tables 2 and 3), genotypes 9511 and Huang NO.1 showed high salt tolerant during both germination and seedling stages, while genotypes 179 and 93fan-13 represent high salt tolerant only in one stage. It might imply that for some high salt tolerant genotypes, there existed inconsistency of salt tolerance between two stages. Genotypes 07-21 and Mengyuan were found to fall into either high salt sensitivity type or salt sensitivity type in both stages. Taken together, we obtained 2 high salt tolerant genotypes (9511 and Huang NO.1) and 2 salt sensitive genotypes (07-21 and Mengyuan) through germplasm screening.

Further evaluation of salt tolerance of two high salt-tolerant and two salt-sensitive genotypes by physiological indexes: To further validate their salt tolerant levels, salt tolerance of two high salt tolerant genotypes (9511 and Huang NO.1) and two salt sensitive genotypes (07-21 and Mengyuan) screened out were evaluated by some physiological indexes at seedling stage under three salt concentration stresses (0 mmol/L, 70 mmol/L, and 140 mmol/L NaCl).

POD activity: In the results, POD activity of the 4 jute genotypes was determined, and the results are shown in Fig. 1. Genotype Huang No.1 significantly ($p<0.05$)

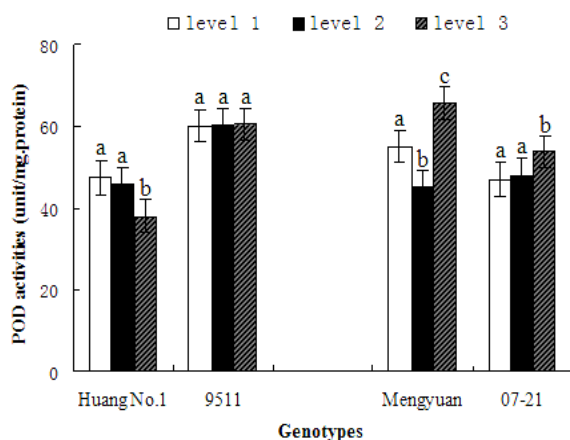


Fig. 1. POD activities of 4 jute genotypes at seedling stage. Vertical bars represent standard errors ($n=6$). Bars with different letters are significantly different at $p<0.05$.

CAT activity: In the study, CAT activity of the 4 jute genotypes was determined, and the results are shown in Fig. 3. Genotype Huang No.1 significantly ($p<0.05$) increased CAT activity at level 2 and markedly ($p<0.05$) decreased CAT activity at level 3, and genotype 9511 had a significant ($p<0.05$) decreased CAT activity with the increase of salt concentration. Both genotypes Mengyuan and 07-21 expressed similar trends, i.e., CAT activities decreased significantly ($p<0.05$) under the salt stress treatments (levels 2 and 3). Taken together, salt treatment reduced the CAT activity of 4 jute genotypes, with Mengyuan and 07-21 having more decreasing amplitude of activity.

MDA content: In this study, MDA content of the 4 jute genotypes was determined, and the results are shown in

decreased its POD activities at level 3, while genotype 9511 had the same or quite similar amount ($p>0.05$) of POD activity under the 3 salt stress levels, demonstrating that it more adapt to salt stress condition. This adapting mechanism is worth being further explored. Both genotypes Mengyuan and 07-21 expressed the same trend, having stronger ($p<0.05$) POD activities at level 3 than at levels 1 and 2. Taken together, POD activities significantly ($p<0.05$) decreased or remained unchangeable with increased NaCl concentration for genotypes Huang No.1 and 9511 while significantly ($p>0.05$) increased for genotypes Mengyuan and 07-21.

SOD activity: In present study, SOD activity of the 4 jute genotypes was determined under salt stress, and the results are shown in Fig. 2. Genotypes Huang No.1 and 9511 had the similar changing format, i.e. SOD activity at levels 2 and 3 was stronger ($p<0.05$) than that at level 1. SOD activity of genotypes Mengyuan and 07-21 tended to significantly ($p<0.05$) decrease with increased NaCl concentration. Taken together, SOD activities significantly ($p<0.05$) increased after the salt stress treatments for genotypes Huang No.1 and 9511 while significantly ($p<0.05$) decreased for genotypes Mengyuan and 07-21.

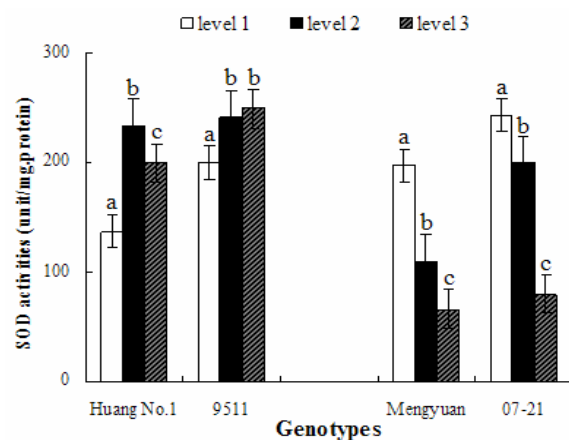


Fig. 2. SOD activities of 4 jute genotypes at seedling stage. Vertical bars represent standard errors ($n=6$). Bars with different letters are significantly different at $p<0.05$.

Fig. 4. Genotype Huang No.1 produced higher ($p<0.05$) MDA amount under level 3 stress than under other 2 level stresses while no significant ($p>0.05$) difference of MDA content was found among 3 level stresses of genotype 9511. Both genotypes Mengyuan and 07-21 significantly ($p<0.05$) increased MDA content with increased NaCl concentration. Taken together, the growth rate of MDA content of genotypes Mengyuan and 07-21 under salt stress was higher ($p<0.05$) than that of genotypes Huang No.1 and 9511.

From above physiological results, we could conclude that genotypes Huang No.1 and 9511 were more salt tolerant than genotypes Mengyuan and 07-21. This result is consistent with their evaluation results of salt tolerance at germination and seedling stages.

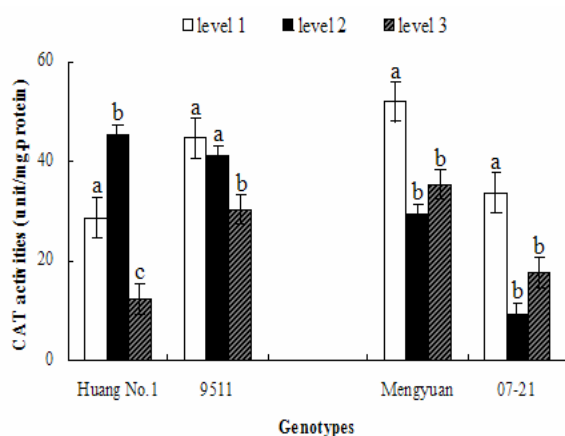


Fig. 3. CAT activities of 4 jute genotypes at seedling stage. Vertical bars represent standard errors ($n=6$). Bars with different letters are significantly different at $p<0.05$.

Discussion

Increased tolerance to salt stress in crop plants is necessary in order to increase productivity under cropping conditions with high salinity. Both germination and seedling stages of crops are sensitive to salinity. Some crop genotypes are found to be salt tolerant in germination stage but not in seedling stage, and vice versa (Foolad, 2004). As jute has been recently suggested as a promising candidate for planting in wetland and saline soils in China, identification and evaluation of salt tolerance of jute genotypes are both necessary and extremely urgent. In this study, salt tolerance of 10 jute genotypes was investigated by relative salt harm rate at germination stage and by index of salt harm at seedling stage, respectively. A markedly significant ($p<0.01$) correlation ($r=0.8432$, $n=10$) between salt tolerances of germination and seedling stages was found, indicating that salt tolerance of germination stage of jute was consistent with that of seedling stage. Out of them, two high salt tolerant genotypes (Huang No.1 and 9511) and two salt sensitive genotypes (Mengyuan and 07-21) were screened. These 4 genotypes showed high salt tolerant or salt sensitive during both germination and seedling stages. These results indicated that the combination of relative salt harm rate at germination stage and index of salt harm at seedling stage can be used to evaluate salt tolerance of jute genotypes. However, inconsistency between salt tolerances of germination and seedling stages was found in some genotypes, for example, genotype 93fan-13 in this study. This inconsistent phenomenon in other crops, which may be caused by genetic diversity between genotypes, was also found by some researchers (Foolad, 2004).

Reactive oxygen species (ROS) are free radicals that are atoms or groups of atoms having at least one unpaired electron. Although ROS in plants are produced under normal growth conditions and their concentration remains low (Polle, 2001), most environmental stresses induce enhanced production of ROS (Desikan *et al.*, 2001; Karpinski *et al.*, 2003; Laloi *et al.*, 2004). Salt stress is also known to trigger oxidative stress in plant tissues (Foyer & Noctor, 2003). Peroxidases (POD) are involved not only in scavenging of H_2O_2 produced in chloroplasts but also in many different physiological functions including the oxidation of toxic compounds, the biosynthesis of cell walls (lignin and suberin), growth and

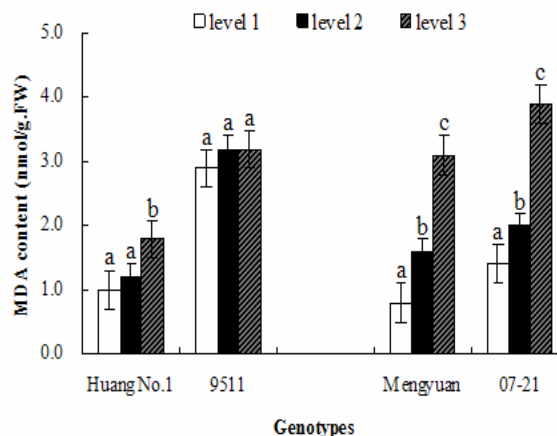


Fig. 4. MDA content of 4 jute genotypes at seedling stage. Vertical bars represent standard errors ($n=6$). Bars with different letters are significantly different at $p<0.05$.

developmental processes, etc. (Dionisio-Sese & Tobita, 1998). Peroxidases (POD) are involved in scavenging of H_2O_2 produced in chloroplasts (Dionisio-Sese & Tobita, 1998). Activity of POD has been reported to increase under salt stress and relatively higher activity has been reported in sensitive rice cultivars than in tolerant ones (Mittal & Dubey, 1991). Their result was somewhat different from ours. In this study, POD activities significantly ($p<0.05$) decreased or remained unchangeable with increased NaCl concentration for the high salt tolerance type while significantly ($p>0.05$) increased for the salt sensitivity type (Fig. 1). SOD (superoxide dismutase), which play a role in detoxification processes by catalyzing the conversion of free O_2^- to O_2 and H_2O_2 , is very often associated with stress situations (Davies & Dow, 1997). Dionisio-Sese & Tobita (1998) have reported that slightly increased and decreased activity of SOD was found in leaves of salt-tolerant rice and salt-sensitivity rice under increased salt concentrations, respectively. This result is almost the same as ours. Our result indicated that SOD activity significantly ($p<0.05$) increased after the salt stresses for the high salt tolerance type while significantly ($p<0.05$) decreased for the salt sensitivity type. Catalases (CAT) catalyses a redox reaction in which dismutation of hydrogen peroxide gives rise water and oxygen and plays a significant role in plant defense system against oxidative stress (Yazici *et al.*, 2007). Olmos *et al.*, (1994) reported that increase in the activity of CAT is related with the increase in stress tolerance. Yazici *et al.*, (2007) reported that CAT activity increased significantly after 18 days of exposure to 140 mM NaCl treatment. However, in this study, salt treatment was found reducing the CAT activity of the 4 jute genotypes, with salt sensitive type having more decreasing amplitude of activity. Our results are different from those of Olmos *et al.*, (1994) and Yazici *et al.*, (2007) Taken together, the diverse change patterns of POD, SOD, and CAT activities under different salt concentration stresses were found among crops, indicating that different crops and different tolerance types of the same crop may produce different types of antioxidants to scavenge/detoxify ROS. This case may be caused by genetic diversity among crops.

It is already known that free radical-induced peroxidation of lipid membranes is a reflection of stress-induced damage at the cellular level (Jain *et al.*, 2001).

Demiral & Türkan (2005) reported that the lower level of lipid peroxidation in roots of salt-tolerant rice cultivar (Pokkali) than of salt-sensitive rice cultivar (IR-28) is found under salt stress. Their results are consistent with ours. In this study, the growth rate of MDA content of salt sensitivity type under salt stress was higher ($p < 0.05$) than that of high salt tolerance type. This suggests that the salt-tolerant jute genotypes may have better protection against oxidative damage under salt stress.

Conclusions: Two high salt tolerant genotypes (Huang No.1 and 9511) and two salt sensitive genotypes (Mengyuan and 07-21) were screened out of 10 jute genotypes by both the relative salt harm rate at germination stage and salt harm index at seedling stage. The salt tolerance of germination stage of jute was consistent with that of seedling stage. Further physiological analysis also validated that genotypes Huang No.1 and 9511 belonged to high salt tolerance type while genotypes Mengyuan and 07-21 fell into salt sensitivity type.

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