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Activities of Antioxidant Enzymes in Six Rice (*Oryza sativa* L.) Varieties at Seedling Stage under Increasing Salinity Stress

Rajkumar D.[1*], Gangadhar Rao S.[1], Tirupathi B.[1] and Gonzalez Rodriguez, H.[2]

[1]Dept. of Botany, Osmania University, Hyderabad (500 007), India,
[2]Universidad Autonoma de Nuevo Leon, Facultadde CienciasForestales, Carr. Nac. No. 85, km 145, Linares, Nuevo Leon (67700), Mexico

Corresponding Author

Rajkumar D.
e-mail: rajviba@gmail.com

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Abstract

Present study deals with the activities of antioxidant enzymes in six rice (*Oryza sativa* L.) varieties, namely Sadamota, Patnai, Dhoodeshwar, Ghewas, Gontrabidan-2 and Malabati, which were subjected to increasing salinity stress (0.05 M, 0.1 M and 0.15 M NaCl) from germination to seedling stage along with control under laboratory conditions. The main objective of this study was to find out variations in the activities of antioxidant enzymes which can differentiate tolerance to salinity. Increasing salinity stress induced gradual increase in the activity of Superoxide dismutase (SOD), Peroxidase, Catalse (CAT), Glutathione reductase (GR), and Ascorbate peroxidase (APX). High activities of SOD, CAT, GR and APX were observed in Doodeshwar and Malabti under salinity stress. High levels of antioxidant enzymes (SOD, CAT, APX and GR) under salinity stress will contribute to salinity tolerance in rice varieties Doodeshwar and Malabathi. Significant genotype×salt treatment interaction suggested the differential effect of stress on genotype for antioxidant enzymes. Catalse activity showed significant (p<.001) positive correlation with SOD (r=.818), GR (r=.624), and APX (r=.593). High levels of Lipid peroxidation was noticed in Ghewas, Gonrabidan-2 and Sadamota, indicating higher membrane damage when compare to Doodeshwar and Malabathi under salt stress. Doodeshwar and Malabathi can be recommended as salt tolerant varieties for advance evaluation at field level. Analysis of antioxidant enzymes from rice seedlings exposed to salinity stress will provide rapid screening method and time saving. Mass screening will be conducted for preliminary selections which can be used in field conditions.

Keywords: Activities, antioxidant enzymes, rice, seedling, salinity, tolerance, varieties

1. Introduction

Drastic and adverse climate change associated with environmental stresses such as drought, salinity, extreme temperature, toxic metals, flooding, etc are prevailing common problem (Pereira et al., 2016). The aggravation of such diverse abiotic stresses has become a most important threat to sustainable crop production (Raza et al., 2019). Salt stress is one of the important and harsh abiotic stresses which reduces crop growth and productivity to the great extent (Wang and Huang, 2019). It is estimated that more than 6% of the world's total land area was affected by salinity (Munns et al., 2008). Approximately 20–50% of irrigated soils of the world are affected by salt stress (FAO, 2021).

Rice (*Oryza sativa* L.), belongs to the family Poaceae (Graminae). Rice is the vital global food crop that feeds over half of the world population and above 400 million people in rice producing areas expected to increase by another 38% within 30 years (Surridge, 2004; Joseph et al., 2010). Rice productivity is affected by salinity stress due to accumulation of underground salt and is intensified salt mining, deforestation and irrigation (Akbar, 1986).

Soil salinity directly affects biochemical, physiological, anatomical and morphological characteristics of plants (Hakim et al., 2014; James et al., 2011; Rahnama et al., 2010, Munns, 2005; Rozema and Flowers 2008). Rice plants are susceptible to soil salinity and accumulation of salts in soil or water results in abiotic stress, a main factor reducing crop production (Gao et al., 2007). The rice crop is relatively salt-tolerant at the germination, active tillering, and maturity stages, but it is highly sensitive at the early seedling and reproductive stages (Munns et al., 2008). Salt stress exerts both ionic and osmotic effects in plants which intern leads to membrane damage, metabolic toxicity, and generation of reactive oxygen species (ROS) which include hydrogen peroxide (H_2O_2) (Ahanger et al., 2020; Munns, 2008). Salinity stress enhances ROS generation which disturbs the equilibrium between antioxidant defense and ROS production (Hasanuzzaman et al., 2020; Bhattacharjee, 2019). ROS are major responsible molecules for oxidative damage (Halliwell, 1987; Chaparzadeh

et al., 2004). Besides, ROS are potentially injurious to cell, because they can increase the level of oxidative damage due to loss of cellular structure and changing cellular functions. ROS causes lipid peroxidation, protein oxidation, enzyme inactivation, destruction of nucleic acids and chlrophyll degradation (Hasanuzzaman et al., 2019). Balance between the detoxification and genesis of ROS is managed by both enzymatic and nonenzymatic antioxidant defense systems under abiotic stress conditions (Hasanuzzaman et al., 2020; Sachdev et al., 2021). Plants produced several antioxidant enzymes like, super oxide dismutase (Bowler et al., 1992), catalase (Mori, 1992), peroxidase (Ito et al., 1991), ascorbate peroxidase and gultathione reductase (Bowler et al., 1992), Ascorbate peroxidase and superoxide dismutase occupies vital roles in detoxification of ROS in cells. APX reduces H_2O_2 to water using ascorbic acid as a distinct electron donor (Asada, 1992, 1999; Foyer et al., 1994). Several researchers have suggested that the activities of these antioxidants mainly base on the salinity threshold, extent of salinity exposure and growth phases of plants (Cunha et al., 2016). SOD is the chief antioxidant enzyme and that triggers the defense mechanism by converting $02^{\bullet-}$ in to H_2O_2; this is more accumulated by CAT and APX, POD, GR (Liebthal, et al., 2018; Del Rio, et al; 2018, Ali et al., 2021). The role of these antioxidative enzymes at the commencement of oxidative stress in chloroplasts has been extensively characterized by Asada (1999). Lipid peroxidation is the result of oxidative damage of the lipid membrane of cells which measured by the content of malondialdehyde (MDA) (Alche, 2019). The present study was focused to determine the effect of different levels of salinity stress on activity of antioxidant enzymes and lipid peroxidation in six rice verities. Comparison of these responds will be useful in identifying variations among rice varieties for salinity tolerance at the seedling stage.

2. Materials and Methods

2.1. Plant materials used

The present study deals with the antioxidant enzyme activities in six rice (*Oryza sativa* L.) varieties namely Sadamota, Patnai, Dhoodeshwar, Ghewas, Gontrabidan-2, and Malabati. These varieties were subjected to salinity stress under laboratory conditions. The seed varieties were collected from Viswabarathi University, West Bengal. The present study was conducted at the plant physiology Laboratory, Department of Botany, Osmania University, Hyderabad, Telangana, India.

2.2. Standardization and preparation of NaCl concentrations

Sodium chloride (NaCl) was employed to impose salinity stress. The concentration of NaCl was calculated by using its molecular weight and accordingly 0.05 M NaCl, 0.1M NaCl, and 0.15 M NaCl concentrations were prepared and used along with control (0 M NaCl).

2.3. Imposing salinity stress

Rice seeds were surface sterilized with 5% (w/v) thirum solution. Twenty seeds were sown at a depth of 2 cm in a plastic pot (height 90 mm, diameter 90 mm) filled with coco peat (neutral delignified coir fibres) and then added water to control and different saline concentration to stress treatment up to two thirds of the pot. Each of the treatments was replicated four times for all varieties. The seeds in the upper coco peat layer at 2 cm depth which receive water or solution by capillarity. The temperature was about 27°C. Artificial light was provided for 12 hours during 25 days. This technique simulates a semi-hydroponic system where the upper layers of coco peat medium receive water/saline solution only by capillary movement, while the roots were immersed in saturated lower coco peat medium and during capillary movement there was free flow of oxygen to constant evapotranspiration. Biochemical studies were undertaken at 25 days old seedling stage.

2.4. Extraction and assay of enzymes

25 days old fresh seedlings (0.5 g of leaf tissue) were homogenized with 10 ml of 50 mM sodium phosphate buffer (pH 7) containing 0.2 mM EDTA, 1% (w/v) PVP (Polyvinylpyrrolidone), 1.0 mM PMSF (Phenylmethylsulfonyl fluoride) in a pre-chilled mortar with pestle. The homogenate was centrifuged at 4°C for 20 minutes at 12,000X*g* and the resultant supernatant was used for assaying the following enzyme assays. The amount of protein in the enzyme extract was calculated according to Lowry et al. (1951).

2.4.1. Catalase (CAT, E.C.1.11.1.6)

Activity was determined following Aebi (1974). The reaction mixture consisted of 50 mM phosphate buffer, 0.1 mM H_2O_2 and enzyme extract. The rate of H_2O_2 decomposition at 240 nm was measured spectrophotometrically and calculated using a molar extinction coefficient of 45.2 mM^{-1} cm^{-1}. One unit of catalase activity was assumed as the amount of enzyme that decomposed 1.0µmol of H_2O_2 per mg of soluble protein per minute at 30°C.

2.4.2. Peroxidase (POD, E.C.1.11.1.7)

Activity was assayed by employing the procedure of Kar and Mishra (1976). To 0.5 ml of enzyme extract, 2.5 ml of 0.1 M phosphate buffer (pH 7), 1.0 ml of 0.01 M pyrogallol and 1.0 ml of 0.005 M H_2O_2 were added. A blank was prepared with 0.5 ml of enzyme extract, 3.5 ml of 0.1 M phosphate buffer and 1 ml of 0.005 M H_2O_2. After 5 minutes of incubation at 25°C, the reaction was stopped by adding 1 ml of 2.5 N H_2SO_4. The amount of purpurogallin formed was estimated by measuring the absorbance at 420 nm against a blank. The enzyme activity was expressed as change in absorbance Units mg^{-1} protein min^{-1}.

2.4.3. Superoxide dismutase (SOD, E.C 1.15.1.1)

SOD activity was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) according to Beauchamp and Fridovich (1971). Three ml of reaction mixture contained 40 mM phosphate buffer (PH

7.8), 13 mM methionine, 75 µM nitroblue tetrazolium, 0.1 mM EDTA, 0.1 ml of enzyme extract and 2 µM riboflavin. Riboflavin was added at the end. After mixing the contents, test tubes were shaken and placed 30 cm below light source consisting of two 15-watt fluorescent tubes. The reaction was started by switching on the lights. The reaction was allowed to take place for 30 minutes and was stopped by switching off the lights. A tube with protein kept in the dark served as blank, while the control tube was without the enzyme and kept in the light. The absorbance was measured at 540 nm. The activity of superoxide dismutase is the measure of NBT reduction in light without protein minus NBT reduction in light with protein. One unit of activity is the amount of protein required to inhibit 50% initial reduction of NBT under light.

2.4.4. Ascorbate peroxidase (APX; E.C 1.11.1.11)

APX was assayed by the method of Nakano and Asada (1981). The reaction mixture contained 1.5 ml of 50 mM sodium phosphate buffer (pH 7), 0.2 mM EDTA, 0.5 ml of 0.5 mM ascorbic acid, 0.5 ml 0.5 mM H_2O_2 and 0.5 ml of enzyme sample. The activity was recorded as the decrease in absorbance at 290 nm for 1 minute and the amount of ascorbate oxidized was calculated from the extinction coefficient of 2.6 mM^{-1} cm^{-1}.

2.4.5. Glutathione reductase (GR; EC 1.6.4.2)

GR activity was performed according to Jiang and Zhang (2001). The reaction mixture contained 500 µl of sodium phosphate buffer (pH 7.0), 100 µl each of 10 mM GSSG, 1 mM NADPH and 180 µl of distilled water. The reaction was started by addition of enzyme extract and NADPH oxidation was recorded as the decrease in absorbance at 340 nm for 1 min. The activity was calculated using the extinction coefficient of NADPH; 6.22 mM^{-1} cm^{-1}.

2.4.6. Lipid peroxidation

Lipid peroxidation was determined by estimating the malondialdehyde (MDA) content following the method of Heath and Packer (1968). One gram plant material was macerated in 5 ml of 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 10,000X*g* for 5 minutes. For 1.0 ml of the aliquot of the supernatant, 4 ml of 20% TCA containing 0.5% TBA was added. The mixture was heated at 95°C for 30 minutes and cooled quickly in an ice bath. The contents were centrifuged at 10,000X*g* for 10 minutes and the absorbance was measured at 532 nm and the value for the non-specific absorbance at 600 nm was subtracted. The concentration of malondialdehyde (MDA) was calculated by using extinction coefficient of 155 mM^{-1} cm^{-1}. MDA content was expressed as mg g^{-1} fresh weight.

2.5. Statistical analysis

Data was statistically analyzed according to a completely randomized design (one-way analysis of variance) with a factorial arrangement. Being the Genotypes (varieties) and treatments (NaCl Concentration) the main factors and their interaction was calculated. The correlation coefficients were calculated to determine the degree of association among parameters studied (Steel and Torrie, 1980).

3. Results and Discussion

The values of each parameter in control and NaCl treatments were provided in the form of graphs and described in text in the form of % of increase or decrease over control of relevant variety studied.Analysis of variance (ANOVA), mean square and CV (%) vales are provided in Table 1. Highly significant differences were observed among main effects (genotypes and NaCl concentrations) as well as in their interaction for all the parameters analyzed. High r^2 and low CV% values were indicating reliability of the techniques used.

3.1. Catalase (CAT)

The effect of different concentrations of salinity stress (0.05, 0.1, and 0.15 M NaCl) on catalase levels on six rice varieties is shown in Figure 1. Catalase levels were progressively increased under increasing saline concentrations in case of all the six rice varieties as compared to the relevant control treatments. Highest increase in catalase levels were noticed in Doosehwar (1.64% at 0.05 M NaCl, 24.3% at 0.1 M NaCl and 66.4% at 0.15 M NaCl) and Malabati (1.47% at 0.05 M NaCl, 24.1% at 0.1 M NaCl and 62.5% at 0.15 M NaCl) compared to respective controls. Patnai showed moderate level of catalase increase (2.7% at 0.05 M NaCl, 20.9% at 0.1 M NaCl 32% over control at 0.15 M NaCl. Less increase of catalase was observed in Gontrabidan-2 1 (1% at 0.05 M NaCl, 4.8% at 0.1 M NaCl and 5.7% at 0.15 M NaCl), Ghewas(1.2% at 0.05 M NaCl, 5.7% at 0.1 M NaCl and 8.7% at 0.15 M NaCl) and Sadamota (0.8% at 0.05 M NaCl, 5.6% at 0.1 M NaCl and 10% at 0.15 M NaCl) over respective controls.

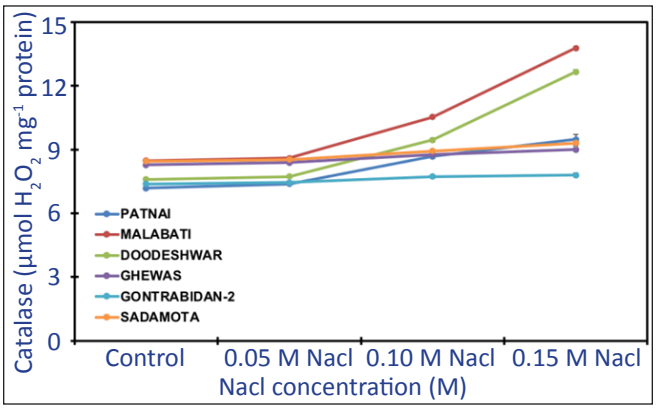

Figure 1: Effect of different NaCl concentrations on Catalase activity in six rice varieties

Catalse activity showed a significant (p<0.001) positive correlation with SOD (r=.818), GR (r=.624), and APX (r=.593). Under salinity conditions, high activity of catalase observed in the cultivars of saline rice areas (Joseph et al., 2015), Chunthaburee et al. (2016). Safeena and Bandara (2006) observed similar results in Pokkali (salinity tolerant variety)

Table 1: Summary of the analysis of variance of a completely randomized design (one-way analysis of variance) with a factorial arrangement, being the Genotypes (varieties) and treatments (NaCl concentration) the main factors and their interaction

Variable	Source of variation	Statistics				
		Mean square	F-value	P-value	CV (%)	R^2 adj.
Catalase	Genotype (G)	14.682	573.835	<.001	1.83	.99
	Treatment (T)	30.771	1202.663	<.001		
	G×T	4.367	170.685	<.001		
	Error	0.026				
Peroxidase	Genotype (G)	0.198	7691.708	<.001	3.57	.999
	Treatment (T)	0.534	20714.556	<.001		
	G×T	0.095	3664.129	<.001		
	Error	<.001				
Superoxide dismutase	Genotype (G)	25.211	1552.034	<.001	1.52	.993
	Treatment (T)	22.121	1361.776	<.001		
	G×T	2.628	161.765	<.001		
	Error	0.016				
Ascorbate peroxidase	Genotype (G)	0.423	626.999	<.001	4.07	.98
	Treatment (T)	0.208	307.656	<.001		
	G×T	0.031	46.01	<.001		
	Error	0.001				
Glutathione reductase	Genotype (G)	8.506	453.4	<.001	1.65	.97
	Treatment (T)	4.818	256.805	<.001		
	G×T	0.13	6.941	<.001		
	Error	0.019				
Lipid peroxidation	Genotype (G)	17.421	788.066	<.001	1.99	.992
	Treatment (T)	40.564	1834.922	<.001		
	G×T	3.557	160.923	<.001		
	Error	0.022				

compared to their counter parts (IR 29; salinity susceptible variety). CAT enzyme is the key in neutralizing toxic H_2O_2 molecules (Willekens et al., 1995). Similar results were observed in mustard (Ahmad et al., 2012).

Above findings supports that high levels of CAT in Doosehwar and Malabati will contribute salinity tolerance for better performance and growth in saline stress when compared to Gontrabidan-2 and Ghewas, Patnai and Sadamota.

3.2. Peroxidase (POD)

The effect of different NaCl concentrations (0.05M, 0.1M, and 0.15M) on POD levels on six rice varieties is illustrated in Figure 2. POD levels were progressively increased under increasing saline concentrations in case of all the six rice varieties as compared to the relevant control treatments. High increase in POD levels were noticed in Ghewas (0.26% at 0.05 M NaCl, 30.1% at 0.1 M NaCl and 64.5% at 0.15 M NaCl), Gontrabidan-2 (0.20% at 0.05 M NaCl, 23.4% at 0.1 M NaCl and 60.9% at 0.15 M NaCl) and Sadamota (0.35% at 0.05 M NaCl, 21.2% at 0.1 M NaCl and 57.8% at 0.15 M NaCl) compared to respective controls. Low levels of POD increase

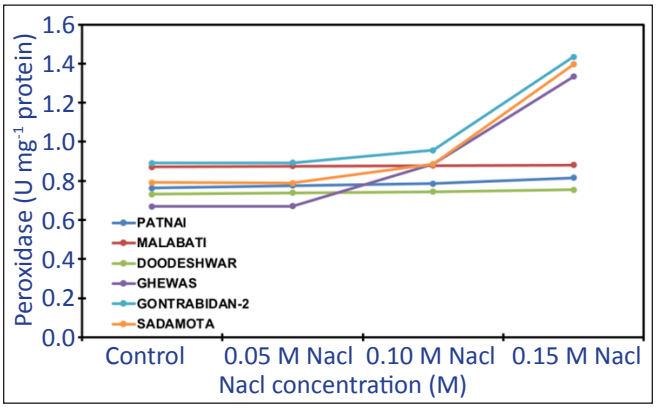

Figure 2: Effect of different NaCl Concentrations on POD activity in six rice varieties

was observed in case of Malabati (0.52% at 0.05 M NaCl, 0.81% at 0.1 M NaCl and 1.12% at 0.15 M NaCl), Doodeshwar (0.89% at 0.05 M NaCl, 1.64% at 0.1 M NaCl and 3.11% at 0.15 M NaCl) and Patnai (1.70% at M NaCl, 2.95% at 0.1 M NaCl and 6.78% at 0.15 M NaCl).

POD activity showed non significant correlation with GR (r=.068) and SOD (r=.065). POD activity was negatively correlated with APX (r=-.165). Safeena and Bandara 2006) reported that POD activity increased in sensitive rice variety (IR 29) as compared to highly tolerant rice variety (Pokkali) with increasing salinity level. Higher activity of POD under high salt stress may be an early trait marker of salt injury (Peiris et al., 1991). Similar observations were seen in the present study. Chunthaburee et al. (2016), observed salt stress caused an increase in the activities of POX and CAT in all rice cultivars, however higher POX activity was reported in IR29 (sensitive to salt) when compared to Pokali cultivar (salt tolerant) and a reverse effect was noticed in CAT activity. Salinity caused POD activity in *Catharanthus roseus* increased in lower levels of salinity and decreased in higher level of salinity as compared to the control (Abdul et al., 2007). The findings of present study supports that high increase in POD level in Ghewas, Gontrabidan-2 and Sadamota are considered to be susceptible to salinity stress as compared to Malabati, Doodeshwar and Patani.

3.3. Super oxide dismutase (SOD)

The effect of different concentrations of salinity stress (0.05M, 0.1M, and 0.15M) on SOD levels on six rice varieties is shown in Figure 3. SOD levels were progressively increased as increasing saline concentrations in case of all the six rice varieties as compared to the relevant control treatments. High increase in SOD levels were noticed in Doodeshwar (2.45% at 0.05 M NaCl, 35.4% at 0.1 M NaCl and 50.4% at 0.15 M NaCl), Malabati (1.42% at 0.05 M NaCl, 34.1% at 0.1 M NaCl and 47% at 0.15 M NaCl) and Patnai (1.83% at 0.05 M NaCl, 25.4% at 0.1 M NaCl and 34.4% at 0.15 M NaCl) compared to respective controls. Low levels of SOD increase in case of Gontrabidan-2 (1.39% at 0.05 M NaCl, 5.26% at 0.1 M NaCl and 6.7% at 0.15 M NaCl), Sadamota (1.43 at0.05 M NaCl, 5.69% 0.1 M NaCl and 7.2% at 0.15 M NaCl) and Ghewas (0.79% at 0.05 M NaCl, 7.7% at 0.1 M NaCl and 8.29% at 0.15 M NaCl). The SOD activity showed significant (p<.001) positive correlation with APX (r=.713) and GR (r=.408). SOD activity protects the plant against the superoxide radical. Some studies also suggested enhanced SOD activity against the potential oxidative damaged caused by salt stress (Khan et al., 2002; Panda and Khan, 2003; Ahmad and Umar, 2011). Wang et al., (2010) reported that high SOD activity enables the transgenic poplar plants to better control ROS homeostasis. In poplars and mangroves, similar effects were observed (Takemura et al., 2002; Parida et al., 2004; Wang et al., 2008). Lee et al. (2001) also observed that the salinity stress increased SOD activity in rice seedlings. Dionisio-Sese and Tobita (1998) and Bhattacharjee and Mukherjee (1997), noticed relatively high levels of SOD produced in salt-tolerant rice when compared to sensitive plants.

3.4. Ascorbate peroxidase (APX)

The effect of different concentrations of NaCl stress (0.05M, 0.1M, and 0.15M) on Ascorbate Peroxidase (APX) levels on six rice varieties is depicted in Figure 4. APX levels were progressively increased as increasing saline concentrations in case of all the six rice varieties as compared to the relevant control treatments. High increase in APX levels were noticed in Doodeshwar (2.56% at 0.05 M NaCl, 28.2% at 0.1 M NaCl and 46.5% at 0.15 M NaCl), Malabati (2.96% at 0.05 M NaCl, 25.2% at 0.1 M NaCl and 41.4% at 0.15 M NaCl) and Patnai (1.46% at 0.05 M NaCl, 26.7% at 0.1 M NaCl and 38.3% at 0.15 M NaCl) compared to respective controls. Low levels of APX increase in case of Ghewas (1.57% at 0.05 M NaCl, 2.36% at 0.1 M NaCl and 5.51% at 0.15 M NaCl), Gontrabidan-2 (1.0% at 0.05 M NaCl, 2% at 0.1 M NaCl and 8.7% at 0.15 M NaCl) and Sadamota (2.79% at 0.05 M NaCl, 4.1% at 0.1 M NaCl and 11.6% at 0.15 M NaCl).APX activity showed non-significant correlation (p>.05 level) with GR (r=.081). Antioxidant enzymes are very important for screening rice for salt tolerance (Ahmad and Umar, 2011; Chunthaburee et al., 2016). Antioxidant enzymes like POD, APX, and GR increase significantly. Enzymes can be activated in rice under oxidative stress induced by NaCl (Lin and Kao., 2004).

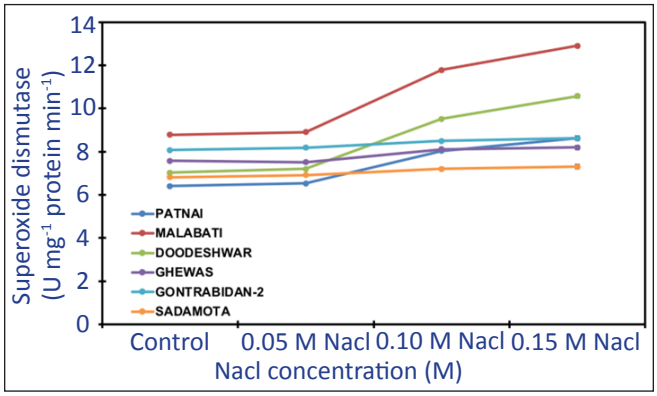

Figure 3: Effect of different NaCl Concentrations on SOD activity in six rice varieties

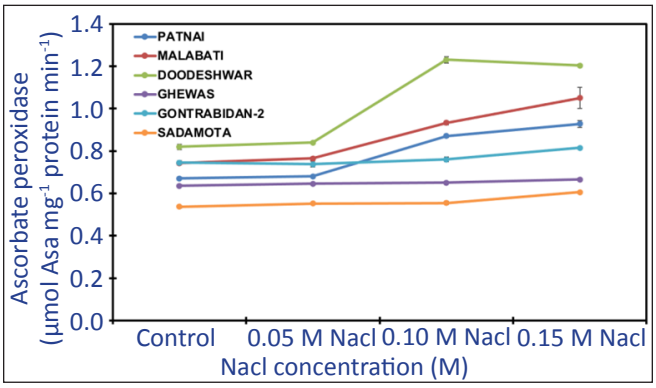

Figure 4: Effect of different NaCl Concentrations on APX activity in six rice varieties

Mohammad et al. (2017) documented that CAT and APX activities considerably decreased in salt-sensitive genotype whereas considerably increased in salt-tolerant rice varieties. Ascorbic acid peroxidase activity had an important role in response to salt stress. According to the above findings from several researchers, it is suggested that higher APX activity in Doosehwar, Malabati will contribute to salinity tolerance for better performance and growth in saline stress when compared to Gontrabidan-2, Ghewas, Patani and Sadamota.

3.5. Glutathione reductase (GR)

The effect of different concentrations of salinity stress (0.05M, 0.1M, and 0.15M NaCl) on Glutathione Reductase (GR) levels on six rice varieties is shown in Figure 5. GR levels were progressively increased as increasing saline concentrations in case of all the six rice varieties as compared to the relevant control treatments. High increase in GR levels were noticed in Doodeshwar (2.73% at 0.05 M NaCl, 9.2% at 0.1 M NaCl and 18.4% at 0.15 M NaCl), Patnai (4.18% at 0.05 M NaCl, 9.6% at 0.1 M NaCl and 18.3% at 0.15 M NaCl) and Malabati (1.79% at 0.05 M NaCl, 7.4% at 0.1 M NaCl and 15.7% at 0.15 M NaCl) compared to respective controls. Low levels of GR increase in case of Ghewas (1.84% at 0.05 M NaCl, 3.5% at 0.1 M NaCl and 6.8% at 0.15 M NaCl), Sadamota (1.86% at 0.05 M NaCl, 3.4% at 0.1 M NaCl and 7.7% at 0.15 M NaCl) and Gontrabidan-2 (4.8% at 0.05 M NaCl, 8.8% at 0.1 M NaCl and 12.5% at 0.15 M NaCl). Joseph et al. (2015) reported that higher activities of GR noticed in rice cultivars from saline areas. Similar results were observed in tolerant pea variety under saline conditions (Hernandez et al., 2000). Higher levels of antioxidant enzymes in the protoplasm are an important parameter in determining salt tolerance in rice (Joseph et al., 2015). Chunthaburee et al. (2016) reported that categorization of rice cultivars based on antioxidant enzyme activities and physiological characters. According to the findings described from several studies suggest that higher GR activity in Doosehwar, Malabati will contribute to salinity tolerance for better performance and growth in saline stress when compared to Gontrabidan-2, Ghewas, Patani and Sadamota.

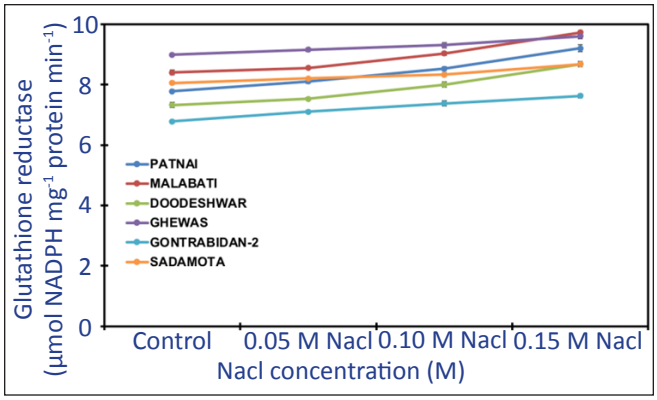

Figure 5: Effect of different NaCl Concentrations on GR activity in six rice varieties

3.6. Lipid peroxidation

The effect of different concentrations of salinity stress (0.05M, 0.1M, and 0.15M NaCl) on seedling Lipid Peroxidation (MDA) on six rice varieties is shown in Figure 6. MDA was progressively increased under increasing saline concentrations in case of all the six rice varieties as compared to the relevant control treatments. The adverse effect of salinity stress on Lipid Peroxidation was found to be much higher in case of rice varieties Ghewas, Gontrabidan-2 and Sadamota. The highest increase over control was observed in Ghewas(12.95% at 0.05M NaCl, 46.86% at 0.1 M NaCl, 78.74% at 0.15 M NaCl) followed by Gontrabidan-2 (7.0% at 0.05 M NaCl, 36.61.86% at 0.1 M NaCl, 74.80% at 0.15 M NaCl), and Sadamota (7.61% at 0.05 M NaCl, 32.18% at 0.1

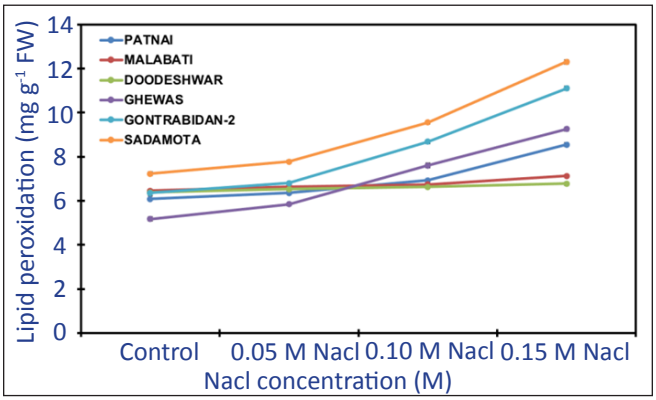

Figure 6: Effect of different NaCl concentrations on lipid peroxidation (MDA content) in six rice varieties

M NaCl, 70.24% at 0.15 M NaCl). Minimum effect of salinity stress on Lipid Peroxidation was observed in rice varieties i.e., less increase over control was observed in Doodeshwar (2.35% at 0.05 M NaCl, 4.0% at 0.1 M NaCl, 6.27% at 0.15 M NaCl), Malabati (2.91% at 0.05 M NaCl, 4.5% at 0.1 M NaCl, 10.47% at 0.15 M NaCl) and Patnai(4.44% at 0.05 M NaCl, 13.9% at 0.1 M NaCl, 40.63% at 0.15 M NaCl). Salt stress induces oxidative stress which leads to oxidative damage of lipids and cell membrane proteins (Mano, 2002). Lipid Peroxidation caused by salinity was reported by Ying et al. (1995), Keutgen and Pawelzik (2008) and Falleh et al. (2012). Peroxidation of membrane lipids and consequential electrolyte leakage are an indication of membrane damage under salinity stress (Katsuhara et al., 2005). Similarly, lower level of lipid peroxidation was observed in tolerant tomato (Shalata and Tal, 1998) and cotton (Meloni et al., 2003). Above the findings from several researchers supports that higher lipid peroxidation in Ghewas, Gontrabidan-2, Sadamota leads to high oxidative damage to membranes under salt stress when compared lower levels of Lipid Peroxidation in Doosehwar, Malabati and Sadamota.

Over all taken in to consideration, salt tolerance in rice seedlings is associated with higher levels of the various antioxidants and higher activity levels of the anti-oxidative enzymes SOD, CAT, POX, APX, and GR (Joseph et al.,2015).

Anti-oxidative enzymes in the protoplasm can serve as major determinants for developing salt tolerance in rice. Pallavi et al. (2013), indicate that higher activity of the enzymes include SOD, CAT, GPX, APX, and GR can serve as the main determinants in the model for depicting salt tolerance in indica rice seedlings.

3.7. Statistical Analysis

Significant (p<.001) differences were observed among six rice varieties for salinity tolerance. Salinity stress showed the significant differences within the varieties in the performance of all the traits studied. Significant (p<.001) genotype×salt treatment interaction suggested the differential effect of stress on genotype for all biochemical parameters. The degree of discrimination among the varieties for their performance differed highly under saline conditions compared to control conditions. Coefficients of correlations among studied parameters are provided in Table 2.

Table 2: Pearson (r) correlation analysis among studied variables

	CAT	POD	SOD	APX	GR
CAT					
POD	.001^{NS}				
SOD	.818***	.065^{NS}			
APX	.593***	-.165^{NS}	.713***		
GR	.624***	.068^{NS}	.408***	.081^{NS}	
MDA	.062^{NS}	.859***	-.045^{NS}	-.188^{NS}	.081^{NS}

*: Significant at p<.05;**Significant at p<.01; ***: Significant at p<.001; NS: Not Significant (p>.05).

4. Conclusion

High levels of antioxidant enzymes (SOD, CAT, APX and GR) under salinity stress contribute to salinity tolerance in rice varieties Doodeshwar and Malabathi. Analysis of antioxidant enzymes from rice seedlings exposed to salinity stress will provide rapid screening method and time saving. Results of the assay of antioxidants can be correlated with phenotypic characters at seedling and flowering stage to confirm their tolerance. Doodeshwar and Malabathi can be recommended as salt tolerant varieties for advanced evaluation studies at field level.

5. Further Research

Above selected lines may be needed for evaluation of biochemical from vegetative to maturity stage under salinity conditions and field screening for phenotypic observations for conformation for tolerance to salinity.

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