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ORIGINAL PAPER

Effect of salt stress on growth, physiological and biochemical parameters and activities of antioxidative enzymes of rice cultivars

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Abstract

Salinity is a key abiotic stress that limits the plant growth and productivity of crops worldwide. In this study, variations in the growth, physiological and biochemical parameters, and antioxidant defense systems under saline soil conditions among three rice cultivars (Ahlami-Tarom, Salari, and Binam) were investigated. Our results indicated that the all rice cultivars had normal growth and yield under control conditions. All the parameters tested were significantly different in the environments studied (stress vs. control). For all three genotypes, salinity induced a significant decrease in growth characteristics, chlorophyll content and grain yield. By contrast an increase in lipid peroxidation and activity of antioxidant enzymes were observed in all rice cultivars. Among the cultivars studied, Binam genotype with the highest increase in the activity of antioxidant enzymes such as catalase, ascorbate peroxidase, and peroxidase, the lowest increase in malondialdehyde content and smaller drop in chlorophyll content showed better protection against salt stress, which made it possible to maintain a higher yield even under stress conditions. The findings of the present study suggested that the activity of antioxidant enzymes can act as an efficient defense mechanism to deal with salinity stress, and accordingly, the potential tolerance of Binam cultivar with a good antioxidant defense system increases its yield compared to other cultivars under salinity stress conditions.

Keywords Antioxidant enzymes · Chlorophyll content · Grain yield · Lipid peroxidation · Rice · Salinity

Introduction

Rice (*Oryza sativa* L.) is the most important source of food and energy for half of the world's population (Manjappa Uday and Hittalmani 2014). After drought, salinity is the second destructive phenomenon and hinders rice production in the world (Ghosh et al. 2016). Salinity is one of the major abiotic stresses that severely limits crop growth and metabolism, and reduces plant productivity (Taibi et al. 2016). It is reported that more than 800 million hectares of cultivated land in the world are affected by different levels of salinity (Reddy et al. 2017). According to FAO statistics (2010), approximately 25.5 million hectares of Iran's agricultural lands are saline and about 8.5 million hectares are extremely saline.

Soil salinity occurs when excessive accumulation of sodium ions with dominant chloride and sulfate anions, which ultimately increases electrical conductivity (> 4 dS m^{-1}) (Ali et al. 2013). Soil salinity affects plants through an initial osmotic stress followed by ions accumulation (Chunthaburee et al. 2016). Salinity associated with excess NaCl adversely affects the morphology, biomass, and biochemical processes of the plants (Rahneshan et al. 2018). Barus et al. (2019) found that the salinity stress affected the growth and biochemical parameters of the all rice cultivars studied. Salt stress induces the reactive oxygen species (ROS) production in plants cells, which results in oxidative stress and ultimately leading to cell and plant death (Sahin et al. 2018). Salinity has a negative effect on crop yield through membrane stability distraction, increasing the lipid peroxidation, and decreasing the chlorophyll content and the yield components (Forough et al. 2018). Plants have various mechanisms to respond to salinity stress, which varies

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depending on different factors such as the type and amount of solutes, growth time and genetic potential of the plant, as well as environmental factors (Kordrostami et al. 2017). Jankangram et al. (2011) reported that most of the proteins identified at increased levels in the saline growth environment in the salinity-tolerant genotype Pokkali were related to photorespiration, photosynthesis and defense against oxidative stress. Kong et al. (2021) found that the difference in tolerance to salt stress between indica and japonica rice cultivars was due to the difference in the dataset of expressed genes related to salinity stress. Li et al. (2018) confirmed that there were clear differences in the level of transcription between salt-tolerant genotype (Pokkali) and salt-sensitive genotype (IR29) in both salt and control conditions. The plants protect the plant cells against the oxidative damage through activation of antioxidants enzymes (Kumar et al. 2021) such as catalase (CAT; EC 1.11.1.6), ascorbate peroxidase (APX; EC 1.11.1.1), and peroxidase (POX; EC 1.11.1.7) (Soares et al. 2019). These enzymes are the main ROS-scavenging antioxidant enzymes, which contribute to the tolerance of plants against salinity (Kibria et al. 2017). Rossatto et al. (2017) demonstrated that there was a positive correlation between antioxidant enzymes and plant tolerance to salt stress. The positive role of antioxidant enzymes such as CAT, APX (Bagheri and Khosravinejad 2016; Forough et al. 2018), and POX (Yaghubi et al. 2014) in rice tolerance to salt stress has been reported in previous studies. Moraditelavat et al. (2018) found that the rice cultivars with higher antioxidant enzyme activity and lower lipid peroxidation indicated a higher ability to tolerate salt stress.

Due to the lack of sufficient information on the physiological and biochemical responses of rice genotypes to salinity, the present study aimed to evaluate the effects of salinity stress on growth, yield, chlorophyll content, lipid peroxidation and antioxidant enzymes activities in three rice cultivars.

Materials and methods

Experimental site

The experiments were conducted in two farms with different soils (saline and control conditions) in Sari, Mazandaran Province, Iran (36°39′ N, 53°4′ E; − 11 m above sea level), during the two crop years of 2016 and 2017. Physical and chemical analyses of experimental soils included analysis of soil texture by hydrometric method (Bouyoucos 1962), soil electrical conductivity (EC) and pH by preparation of soil suspension (soil/water 1:5), total N by Kjeldahl method (Bremner and Mulvaney 1982), extractable P by sodium bicarbonate extraction (Olsen et al. 1954), and extractable K by flame photometer techniques (Toth and Prince 1949). The physical and chemical properties of the experimental soils are presented in Table 1.

Experimental design and treatments

The experiment was conducted in a randomized complete block design (RCBD) with three replications. The experimental treatments included rice genotypes (Ahlami-Tarom, Salari, and Binam) that were evaluated in two separate experiments in control conditions and saline soil.

Field experiment

The rice genotypes used in the experiment were local cultivars. The rice seeds were pregerminated by soaking in water for 24 h and incubated for 48 h in the dark. Germinated seeds were sown in nursery beds. The nursery land was prepared one week before sowing. Land preparation was performed by puddling, harrowing and leveling the soil in the fields. The experiments were conducted in plots with dimensions of 18 m^2 (3 m × 6 m). Three 30 days old rice seedlings were transplanted per hill with a constant spacing of 20 × 20 cm. All the experimental plots received 100 kg ha^{-1} of P_2O_5 and K_2O as triple superphosphate and potassium sulfate, respectively, before the seedlings were transplanted. Each plot received urea-N 150 kg ha^{-1}, (½ as a basal fertilizer, ¼ as topdressing at tillering stage, and ¼ as topdressing at heading stage. To control weeds, Butachlor herbicide was applied at a concentration of 3.5 L ha^{-1} 1 week after transplanting, and the manual weeding was performed at 2 and 4 weeks after transplanting. In order to control *Chilo suppressalis*, diazinon insecticide (5% Granule) was used at a rate of 30 kg ha^{-1} at the maximum tillering and heading stages.

Table 1 Physical and chemical properties of the experimental soils (saline and control conditions)

Soil	Control conditions	Saline
Soil texture	Silty clay	Silty clay
Sand (%)	22	18
Silt (%)	43	45
Clay (%)	35	37
PH	6.3	6.7
EC (ds.m^{-1})	0.42	4.92
Organic matter (%)	2.5	2.1
Total N (%)	0.22	0.18
Available P (mg kg^{-1})	12	15
Available K (mg kg^{-1})	138	155

Measurement of photosynthetic pigments

The photosynthetic pigments (chlorophyll a and b) were determined according to Arnon (1949). Chlorophyll extract was prepared from 100 mg fresh leaves by grinding in a tissue homogenizer together with 10 mL of ice cold 80% acetone. The homogenate was centrifuged at 3000 rpm for 2 min. The supernatant was saved and pellet was re-extracted twice with 5 mL of 80% acetone. All the supernatants were pooled and saved. The absorbance of the extract was read at 663.2 and 646.8 nm by spectrophotometer, and pigment concentrations were calculated using Arnon's equations.

$$\begin{aligned}&\text{Chlorophyll a(mg/g FW)} = \left(12.25A_{663.2}\right) - \left(2.79A_{646.8}\right)\\&\text{Chlorophyll b(mg/g FW)} = \left(21.50A_{646.8}\right) - \left(5.1A_{663.2}\right).\end{aligned} \tag{1}$$

Determination of Lipid peroxidation

Lipid peroxidation was determined as described by Heath and Packer (1968). Approximately 0.5 g of leaves was homogenized with 5% (w/v) trichloroacetic acid (TCA). A 1.0 mL aliquot of the supernatant was added to 4 mL of thiobarbituric acid (TBA at 0.5% in TCA at 20%), prepared at the time of use. The mixture was incubated at 95 °C for 30 min and then quickly cooled in an ice bath, and centrifuged at 10,000 g for 10 min at 4 °C. Thereafter, the absorbance of the supernatant was recorded by a spectrophotometer at 532 nm, and the values corresponding to non-specific absorption (600 nm) were subtracted. Lipid peroxydation products were measured as the content of TBA-reactive substances. The malondialdehyde (MDA) content was calculated according to the molar extinction coefficient of 155 mM^{-1} cm^{-1}. The MDA content was measured as µmol g^{-1} FW.

Antioxidant enzyme extraction and assay

For extraction and assay of enzymes, the rice leaves (0.5 g) were homogenized in 1 mL of extraction buffer: 50 mM potassium phosphate (pH 7.0), 1 mM ethylenediaminetetraacetic acid (EDTA) in the presence of polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 13,000 g for 15 min at 4 °C. The supernatant was used for determine the activities of catalase (CAT), ascorbate peroxidase (APX) and peroxidase (POX). All steps in the preparation of the enzyme extract were carried out at 4 °C.

CAT (EC 1.11.1.6) activity was assayed according to the method described by Aebi (1984). The reaction mixture contained 50 mM potassium phosphate (pH 7.0), 10 mM H_2O_2 and the crude enzyme. The decomposition of H_2O_2 was monitored by the decrease in absorbance at 240 nm for 2 min. CAT activity was calculated by using the extinction coefficient of 39.4 mM^{-1} cm^{-1}, and expressed as µmol of H_2O_2 min^{-1} mg^{-1} protein.

APX (EC 1.11.1.11) activity was determined spectrophotometrically according to the method described by Nakano and Asada (1981), based on the decrease in absorbance at 290 nm as ascorbate (ASC) was oxidized. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 1.5 mM H_2O_2, 0.5 mM ascorbic acid, 0.1 mM EDTA and 0.1 mL enzyme extract in a total volume of 3 mL. APX activity was calculated by using the extinction coefficient of 2.8 mM^{-1} cm^{-1}, and expressed as µmol of ascorbate min^{-1} mg^{-1} protein.

POX (EC 1.11.1.7) activity was assayed spectrophotometrically by the method of Velikova et al. (2000). The reaction mixture contained 10 mM potassium phosphate buffer (pH 7.0), 3 mM H_2O_2, 0.2% guaiacol and 0.04 mL enzyme extract. The increase in absorbance was recorded at 470 nm. The activity of POX was calculated from the rate of formation of guaiacol dehydrogenation product (GDHP) by using the extinction coefficient of 26.6 mM^{-1} cm^{-1} and the activity was expressed as mol GDHP min^{-1} mg^{-1} protein.

Measurement of agronomical traits and grain yield

At the time of physiological maturity, after removing the marginal effects, plant samples were randomly selected from middle of each experimental plot. The number of tillers per hill was determined from 15 hills per plot. The number of grains per plant was recorded by counting from 15 plants per plot. The unfilled spikelets were calculated by counting the number of unfilled spikelet per spike. The 1000-grain weight was measured by counting and weighing ten samples of 100 seeds. The grain yield was recorded through harvesting an area of 4 m^2 (2 m × 2 m) in the middle of each plot and based on 14% moisture content.

Statistical analysis

The combined analysis of variance was performed using SAS 9.2 software. Mean values were compared using least significant difference (LSD) test at 5% probability level.

Results

Growth and grain yield

According to the results of combined analysis of variance (Table 2), the three rice cultivars were significantly different for all the agronomical traits and yield in both saline and control conditions, except for 1000-grain weight in control conditions. The influence of year was significant

Table 2 Combined analysis of variance for agronomical traits and grain yield in three rice cultivars in control conditions and saline soil

S.O.V	df	No. of tillers hill^{-1}	No. of grains plant^{-1}	Unfilled spikelets percent	1000-grain weight	Grain yield
Control conditions						
Year (Y)	1	14.04^{ns}	119.60^{ns}	0.020^{ns}	2.96^{ns}	336,309.34*
r(y)	4	4.08	80.13	0.53	5.63	126,703.71
Genotype (G)	2	267.05**	10,983.68**	158.25**	4.59^{ns}	6,913,980.06**
Y×G	2	1.80^{ns}	43.79^{ns}	10.29^{ns}	2.96^{ns}	25,963.05^{ns}
Error	8	5.64	98.67	8.21	5.46	45,722.44
C.V (%)	–	9.38	6.12	22.51	10.29	3.91
Saline soil						
S.O.V						
Year (Y)	1	18.40^{ns}	12.33^{ns}	0.12^{ns}	0.40^{ns}	190,262.24*
r(y)	4	5.30	147.63	7.28	1.59	155,915.18
Genotype (G)	2	246.53**	7171.36**	970.52**	5.63**	6,241,994.99**
Y×G	2	7.02^{ns}	43.80^{ns}	27.74^{ns}	0.99^{ns}	48,992.42^{ns}
Error	8	8.86	120.45	15.70	0.54	35,449.86
C.V (%)	–	14.75	8.63	17.26	3.44	4.20

ns, **, *: non-statistically significant and significant at 1% and 5%, respectively

only on grain yield, and the interaction between year and genotype was not significant on any of the studied traits.

In the present study, all the rice cultivars had normal growth and yield in the control conditions, as shown in Table 3. A decrease in growth and productivity was observed for all rice cultivars under saline soil conditions. The mean comparison of the genotype showed that the Salari cultivar produced higher tillers number per hill (32.21 tillers), grains number per plant (190.50 grains) and unfilled spikelet percent (18.58) among the genotypes under control conditions, although no significant difference was observed between Salari and Binam in terms of grains number per plant. Under saline soil conditions, the highest tillers No. hill^{-1} (26.50 tillers) was obtained for Salari genotype, while the highest grains No. plant^{-1} with an average of 153.85 belonged to Binam genotype. The percentage of unfilled spikelets in Salari cultivar was significantly higher than Ahlami-Tarom and Binam under both saline and control conditions. Although the 1000-grain weight of rice cultivars in control conditions did not have significant differences, the 1000-grain weight in saline soil showed a decrease of 7.3 and 7.8% for Salari genotype compared to genotypes of Ahlami-Tarom and Binam, respectively. The results suggested that salinity stress caused a decrease in grain yield for all rice cultivars. We found that under saline soil conditions, a slight decrease in grain yield (14.6%) was noted for the salt-tolerant Binam whereas the Salari exhibited a dramatic reduction in grain yield (22.2%), when compared to control conditions (Table 3).

Photosynthetic pigments

The results showed that there was a significant difference between the genotypes in terms of the chlorophyll a (Chl.a) and chlorophyll b (Chl.b) contents in both saline and control conditions. For the Chl.b content there was no statistical difference between the two years of the study, while for Chl.a, this difference was significant. The photosynthetic pigment content was not affected by interaction between year × genotype (Table 4).

In this study, the Chl.a content in both rice fields was higher in the first year of the experiment than in the second year. This value was 10.6% higher in control conditions and 6.8% higher in saline soil than the second year (Table 5).

Among the investigated cultivars, the highest Chl.a content in both saline and control conditions was observed in Ahlami-Tarom and Binam. Salari variety recorded the lowest Chl.a content with about 16.2% and 20.6% reduction in control conditions and saline soil, respectively. According to the results, Binam had the highest Chl.b content in both control conditions (0.030 mg.g^{-1} FW) and saline soil (0.026 mg.g^{-1} FW). However, no significant difference was observed between Ahlami-Tarom and Salari in terms of Chl.b content. When the rice plants were planted in the saline soil, the Chl.a and Chl.b contents decreased in all rice cultivars, especially in Salari (12.3% and 23.1%, respectively). The Chl.a and Chl.b contents decreased by 7.9% and 16.7% in Ahlami-Tarom genotype whereas Binam demonstrated a slight drop in the Chl.a and Chl.b contents (7.3% and 13.3%, respectively) (Table 5).

Table 3 Comparisons of agronomical traits and grain yield in three rice cultivars in control conditions and saline soil

Traits	No. of tillers hill^{-1}		No. of grains plant^{-1}		Unfilled spikelets percent		1000-grain weight (g)		Grain yield (kg.ha^{-1})	
Soil	Control conditions	Saline soil	Control conditions	Saline soil	Control conditions	Saline soil	Control conditions	Saline soil	Control conditions	Saline soil
Year (Y)										
2016	26.20a	21.18a	164.68a	127.93a	12.76a	23.03a	22.30a	21.56a	5595.5a	4576.9a
2017	24.43a	19.16a	159.53a	126.27a	12.70a	22.86a	23.11a	21.26a	5322.1b	4371.3b
Genotype (G)										
Ahlami-Tarom	18.90c	13.68c	112.90b	88.06b	8.96b	14.05b	23.58a	21.91a	4419.3c	3623.6c
Salari	32.21a	26.50a	190.50a	139.40a	18.58a	37.51a	21.83a	20.30b	5393.9b	4193.8b
Binam	24.83b	20.35b	182.93a	153.85a	10.65b	17.28b	22.70a	22.03a	6563.3a	5604.9a

Means followed by similar letters in each column are not significantly different according to LSD test ($P \leq 0.05$)

Table 4 Combined analysis of variance for Photosynthetic pigments and Lipid peroxidation in three rice cultivars in control conditions and saline soil

S.O.V	df	Chl.a	Chl.b	Lipid peroxidation
Control conditions				
Year (Y)	1	0.016**	0.000002^{ns}	0.00002^{ns}
r(y)	4	0.005	0.000006	0.000002
Genotype (G)	2	0.018**	0.00006**	0.00004*
Y×G	2	0.001^{ns}	0.000006^{ns}	0.000002^{ns}
Error	8	0.001	0.000005	0.000005
C.V (%)	–	5.65	9.03	9.56
Saline soil				
S.O.V				
Year (Y)	1	0.007*	0.00000002^{ns}	0.000061^{ns}
r(y)	4	0.003	0.0000004	0.000009
Genotype (G)	2	0.023**	0.000069**	0.00010*
Y×G	2	0.002^{ns}	0.000003^{ns}	0.000006^{ns}
Error	8	0.001	0.000002	0.00002
C.V (%)	–	6.10	7.38	15.18

ns, **, *: non-statistically significant and significant at 1% and 5%, respectively

Chl.a: Chlorophyll a, Chl.b: Chlorophyll b

Lipid peroxidation (malondialdehyde)

As shown in Table 4, there was a significant ($P \leq 0.05$) difference between genotypes in terms of lipid peroxidation in both saline and control conditions. However, the effects of year and year × genotype interactions were not significant on MDA content.

The assay of MDA content revealed that all three cultivars were prone to lipid peroxidation significantly under salt stress when compared to plants were under control conditions. The content of MDA in saline soil was higher in Salari (25.7% and 17.1%, respectively) than Ahlami-Tarom and Binam, although no significant difference was observed between Salari and Binam in terms of MDA content. The lowest MDA content in both control conditions and saline soil with an averages value of 0.022 and 0.026 µmol g^{-1} FW, respectively, was observed in Ahlami-Tarom. Compared to the control conditions, Ahlami-Tarom, Salari and Binam showed an increase in MDA content by 15.4%, 22.8% and 10.3%, respectively, in saline soil. In general, the highest increase in lipid peroxidation was observed in the salinity-sensitive genotype Salari, while the salinity-tolerant genotype Binam had a lower increase in MDA content among the genotypes (Table 5).

Antioxidant enzyme activities

The results of combined analysis of variance for antioxidant enzymes activities in both saline and control conditions are

Table 5 Comparisons of Photosynthetic pigments, Lipid peroxidation and protein content in three rice cultivars in control conditions and saline soil

Traits	Chl.a (mg.g^{-1} FW)		Chl.b (mg.g^{-1} FW)		Lipid peroxidation (µmol g^{-1} FW)	
Soil	Control conditions	Saline soil	Control conditions	Saline soil	Control conditions	Saline soil
Year (Y)						
2016	0.66a	0.59a	0.027a	0.022a	0.026a	0.032a
2017	0.59b	0.55b	0.026a	0.022a	0.024a	0.028a
Genotype (G)						
Ahlami-Tarom	0.63a	0.58a	0.024b	0.020b	0.022b	0.026b
Salari	0.57b	0.50b	0.026b	0.020b	0.027a	0.035a
Binam	0.68a	0.63a	0.030a	0.026a	0.026a	0.029ab

Means followed by similar letters in each column are not significantly different according to LSD test ($P \leq 0.05$)

Chl.a: Chlorophyll a, Chl.b: Chlorophyll b

Table 6 Combined analysis of variance for antioxidant enzymes activities in three rice cultivars in control conditions and saline soil

S.O.V	df	CAT	APX	POX
Control conditions				
Year (Y)	1	0.0019^{ns}	0.0003^{ns}	0.0001^{ns}
r(y)	4	0.0034	0.0015	0.0024
Genotype (G)	2	0.022**	0.0079**	0.012*
Y × G	2	0.0005^{ns}	0.00008^{ns}	0.0008^{ns}
Error	8	0.0022	0.0008	0.0015
C.V (%)	–	9.33	4.29	6.88
Saline soil				
S.O.V				
Year (Y)	1	0.008^{ns}	0.0016^{ns}	0.0033^{ns}
r(y)	4	0.015	0.0019	0.0076
Genotype (G)	2	0.055**	0.0071*	0.044**
Y × G	2	0.0007^{ns}	0.0004^{ns}	0.0015^{ns}
Error	8	0.004	0.0013	0.0008
C.V (%)	–	11.05	5.16	4.42

ns, **, *: non-statistically significant and significant at 1% and 5%, respectively

CAT: catalase, APX: ascorbate peroxidase, POX: peroxidase

shown in Table 6. The results showed that the main effect of genotype was significant on activities of CAT, APX and POX. The activities of enzymes were not affected by the main effect of year and interactions between year × genotype.

Our findings indicated that the CAT and APX activities in Ahlami-Tarom and Binam were significantly higher than Salari in both saline and control conditions (Table 7). There were striking differences in the activity of CAT and APX enzymes between two genotypes of Ahlami-Tarom and Binam with Salari cultivar in both test soils, although no significant difference was observed between Ahlami-Tarom and Salari in terms of APX activity in saline soil. The Binam displayed the highest POX activity among the three cultivars in both studied soils. Mean values for POX activity in Binam genotype in both control conditions and saline soil (0.62 and 0.77) were significantly higher when compared to Ahlami-Tarom (0.56 and 0.64) and Salari (0.53 and 0.61), respectively (Table 7).

In this research, all rice cultivars exhibited a significant increase in CAT, APX and POX activities when grown in saline soil. The CAT, APX and POX activities in saline soil in genotypes of Ahlami-Tarom (16.4, 2.8 and 12.5%, respectively), Salari (10.2, 5.9 and 13.1%, respectively) and Binam (17 6, 5.3 and 19.5%, respectively) were higher than when rice plants were grown in control conditions. However, the highest increase in the enzymes activities under saline soil was observed in the Binam genotype when compared to the plants that did not grow in a saline environment, while the lowest increase in the CAT and APX activities was seen in Salari and Ahlami-Tarom, respectively (Table 7).

Discussion

Our finding showed that the higher yield in Binam genotype under salinity conditions can be the result of greater tolerance to salinity due to the increased activity of the antioxidant enzyme defense system. Our results are confirmed by Kibria et al. (2017) who reported that the reduction in growth and accumulation of rice biomass in the salinity-sensitive varieties was higher than in salinity-tolerant cultivars. Salinity has a negative effect on plant growth and grain yield by reducing the activity of meristem cells and destroying important physiological and biochemical processes of the plant (Kordrostami et al. 2017). The findings in our study are in good agreement with one previous study in rice (Saeidzadeh et al. 2018) that the soil salinity led to a decrease in yield and yield components of all rice varieties compared

Table 7 Comparisons of antioxidant enzymes activities in three rice cultivars in control conditions and saline soil

Traits	CAT		APX		POX	
Soil	Control conditions	Saline soil	Control conditions	Saline soil	Control conditions	Saline soil
Year (Y)						
2016	0.51a	0.62a	0.68a	0.71a	0.57a	0.66a
2017	0.49a	0.57a	0.68a	0.73a	0.57a	0.69a
Genotype (G)						
Ahlami-Tarom	0.51a	0.61a	0.69a	0.71ab	0.56b	0.64b
Salari	0.44b	0.49b	0.64b	0.68b	0.53b	0.61b
Binam	0.56a	0.68a	0.71a	0.75a	0.62a	0.77a

Means followed by similar letters in each column are not significantly different according to LSD test ($P \leq 0.05$)

CAT: catalase (μmol H_2O_2 min^{-1} mg^{-1} protein), APX: ascorbate peroxidase (μmol ASC min^{-1} mg^{-1} protein), POX: peroxidase (mol GDHP min^{-1} mg^{-1} protein)

to control conditions. Kibria et al. (2017) also reported that the rice yield components such as number of tillers per hill decreased as concentration of NaCl increased. In a study related to salinity stress in rice cultivars, Moraditelavat et al. (2018) found that the all rice cultivars showed a decrease in the dry weight of seedlings under salinity stress. Barus et al. (2019) demonstrated that salt stress led to a decrease in growth in all three studied rice varieties. Likewise, Chunthaburee et al. (2016) and Ghadirnezhad Shiade et al. (2020) observed that salt stress caused a significant decrease in dry weight of all rice cultivars.

Our results show an inverse relationship between salinity and photosynthetic pigment content. A reduction in chlorophyll content was observed under salt stress conditions in all the genotypes, but this reduction was more pronounced in the salt-sensitive genotype than the salt-tolerant ones. We found that under saline soil conditions the highest and lowest reduction in chlorophyll content was observed in the genotypes of Salari and Binam, respectively. The chlorophyll accumulation was dissimilar between the rice cultivars, which are in-line with Cha-um et al. (2010). Kordrostami et al. (2017) supported that under salt stress the sensitive cultivars accumulated the lowest level of chlorophyll than the tolerant cultivars. The findings in our study that the chlorophyll content exhibited a significant reduction in the saline soil were consistent with Kibria (2017) who reported that the all rice genotypes showed a decrease in chlorophyll a and chlorophyll b content as the NaCl concentration was increased to 60 mmol/L. The decrease in the chlorophyll content of rice cultivars under salt stress may be due to disruption of the thylakoid membrane, degradation or inhibiting the synthesis of chlorophyll, increasing the chlorophyllase enzyme activity, and as a result the destruction of chloroplasts due to increase in the reactive oxygen species (ROS) (Hosseini et al. 2012; Ashraf and Harris 2013). Salinity stress can directly reduce carbon fixation and productivity in plants by disrupting photosynthetic processes (Kordrostami et al. 2017). Elsheery and Cao (2008) demonstrated that the photo-protection mechanism against salinity stress in plants leads to a decrease in light absorption and as a result, a decrease in chlorophyll content.

MDA is the main degradation product of unsaturated fatty acids in biological membranes, which enhances under salt stress (Meloni et al. 2003). MDA has been observed as a marker of membrane degradation under salt stress in rice and other plant species (Stepien and Klobus 2005). It has been reported that an increase in salinity favors an increase in oxidative stress due to ROS production and as a result lipid peroxidation caused membrane damage, especially in salinity-sensitive cultivars (Saha et al. 2010). The findings in our study that increased MDA under saline soil conditions were consistent with Singh et al. (2007) who reported that the all three rice varieties (Pokkali, CSR-30, and HBC-19) exhibited increase in MDA under salt stress. The increase of MDA in some rice cultivars indicates the sensitivity of these genotypes to salinity (Bagheri et al. 2020). Rice cultivars that produce less MDA under salinity conditions have higher tolerance to cell membrane damage (Chutipaijit et al. 2009). In similar results, Ghadirnezhad Shiade et al. (2020) reported that the amount of MDA produced in salt-tolerant rice cultivars was significantly lower than that of salt-sensitive cultivars.

In this study, all rice cultivars showed a significant increase in activities of CAT, APX and POX under salinity stress. However, the activity of antioxidant enzymes was higher in the Binam genotype than other cultivars. The higher activity of antioxidant enzymes in the Binam genotype shows the greater tolerance of this cultivar to salinity compared to the other two varieties of the study. The CAT and APX enzymes play a major role in the tolerance of rice to salinity stress due to their effect on removing ROS (Moraditelavat et al. 2018). According to Rossatto et al.

(2017), increase in the activity of CAT and the maintenance of APX levels during salinity stress were effective against oxidative stress. Forough et al. (2018) observed that salinity stress increased the CAT and APX activities in the leaves of mutant genotype and reduced the negative effects of salinity. In the present study, although POX activity increased in all three rice cultivars under salinity conditions, its activity was strongly higher in Binam genotype than other cultivars, which suggest that this enzyme plays a vital role in improving the stress tolerance. Previous studies showed that there is a strong correlation between increased POX activity and decreased ROS activity induced by salinity stress in rice plants (Chunthaburee et al. 2016; Kordrostami et al. 2017). Yaghubi et al. (2014) showed that the salt-tolerant plants have higher POX activity than salt-sensitive plants under salinity stress, which is consistent with the results of this study. Similar to the results of the present study, Kibria et al. (2017) stated that the increase in POX activity occurs under salt stress.

The results of this study indicated that all rice cultivars under salinity stress had a decreasing trend in yield components, yield and chlorophyll content, when compared with plants grown in control conditions. On the other hand, lipid peroxidation and the activity of antioxidant enzymes increased in all genotypes under soil salinity conditions. However, different cultivars had various responses to salinity stress in terms of percentage increase or decrease in studied traits. We found that among the rice genotypes, Binam was the salinity-tolerant cultivar, whereas Salari was the salinity-sensitive cultivar. The tolerance of Binam cultivar to salinity could be due to the higher activity of antioxidant enzymes (CAT, APX and POX) and the lower drop in chlorophyll content under salinity stress conditions. Therefore, the antioxidant enzymes can play an effective role as the most important defense factors in tolerance to salt stress in rice.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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