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Research Article
Effect of Salinity and Alleviating Role of Methyl Jasmonate in Some Rice Varieties

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Abstract

Background and Objective: Methyl jasmonate (MeJA) is involved in signal transduction pathway of plant development and responses to several environmental stress factors. This study was undertaken to explore the role of exogenous methyl jasmonate (MeJA) for mitigation of salt stress effect of six rice varieties. **Materials and Methods:** Two hybrid (BRRI dhan31 and BRRI dhan46) and four local (Gota, Kajalsail, Pokkali and Pengek) rice varieties were selected. Seedlings were grown hydroponically with normal conditions until 21 days and then subjected to 9 dS m^{-1} NaCl stress followed by application of 10 or 20 µM MeJA as foliar spray. Changes in biochemical components such as activity of antioxidant enzymes i.e., catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), chlorophyll content, Na^+ and K^+ contents of 28 days seedling stage were assayed. **Results:** The MeJA significantly increased plant growth, chlorophyll and K^+ content which were reduced by the application of NaCl while reduced Na^+ content in local varieties that was increased upon NaCl stress. Both NaCl stress as well as MeJA had varying trend in CAT activity while NaCl stress induced a significant increase in POD and APX activity but MeJA induced varying trend in different varieties. This study revealed that visual plant growth, chlorophyll, Na^+ and K^+ content support the positive effect of MeJA on mitigation of salt stress. **Conclusion:** The MeJA could be effectively sprayed on rice variety in saline belts as 10 µM MeJA adequately proved its salinity alleviating role.

Key words: Antioxidant enzymes, methyl jasmonate, rice, salinity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Salinity is one of the major abiotic stresses has become an important problem regarding agricultural production worldwide[1,2]. Over 800 million hectares of land is salt affected worldwide[3,4] and 1.06 Mha of land at coastal regions of Bangladesh were affected by salinity at different degrees[5]. It leads to a series of morphological, physiological, biochemical and molecular changes that adversely affects plant growth and productivity and disrupt the metabolic balance of cells resulting in enhanced production of Reactive Oxygen Species (ROS)[6]. As sessile organism, plants have developed complex antioxidant mechanisms to eliminate or limit ROS, which are effective at different levels of stress-induced deterioration[7].

Rice (*Oryza sativa* L.) is staple food crop in the world[8]. Though significant improvement in productivity has been achieved over the years but elevated salinity in soil along with other abiotic stresses limits its productivity in Bangladesh[9].

The role of different antioxidants as one of the key mechanisms in plants has already been reported[10,11]. The activity of catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX) is increased as a result of salt stress and they have the potential to eliminate H_2O_2 by converting it to water[12,13]. It has also been stated that genotypes with high chlorophyll content, high K/Na ratio and low Na^+ and Cl^- accumulation are more tolerant to salt[14].

Different Plant Growth Regulators (PGRs) have been potentially used for avoidance mechanisms of salt stress in plants[15,16]. Methyl jasmonate, a naturally occurring plant growth regulator that is widely used to regulate the morphological, physiological and biochemical processes in plants. It is effective in signal transduction pathway of plant responses to several environmental stress factors. Exogenous application of jasmonate amends several physiological responses so as to improved resistance against abiotic stresses[15,17].

It is alarming that affected areas of Bangladesh are increasing rapidly[5]. So we have looked forward to the mitigation of saline effect in those salt affected coastal regions. To the paramount of our knowledge, no study has been yet conducted regarding MeJA application on rice plant in Bangladesh. The aim of the present study was to examine the role of MeJA in different rice varieties by modulating enzymatic antioxidants enzymes, chlorophyll content, Na^+ and K^+ accumulation subjected to salt stress.

MATERIALS AND METHODS

The experiment was done at the laboratory of the Department of Biochemistry and Molecular Biology, Bangladesh Agricultural University during the period July, 2014-June, 2015.

Plant material: Two high yielding rice varieties (BRRI dhan31 and BRRI dhan46) and four local varieties (Pokkali, Pengek, Kajalsail, Gota) were used in the experiment.

Growing of rice seedling and treatments application: Properly imbibed seeds were placed on petri dishes and thereafter 2 days of placement for germination, sprouted seeds were transferred into the pot. Three days later, distilled water of each pot was replaced with Hoagland solution. After 21 days of transferring the sprouted seeds to the pot, treatments were applied. For salinization, NaCl was dissolved with nutrient solution to reach the desired salinity level and MeJA was applied as foliar spray.

The treatments included: T_0-non-saline (0 dS m^{-1}), T_1-saline (9 dS m^{-1}), T_2-saline (9 dS m^{-1})+10 µM MeJA, T_3-saline (9 dS m^{-1})+20 µM MeJA

The salinity level was measured by using the EC meter and the pH was maintained at 5.25 by alkalinizing with sodium hydroxide eventually mixed with potassium hydroxide, to avoid sodium excess and measured using the pH meter containing a glass electrode. Twenty eight days old seedlings were used for sample collection (Fig. 1).

Determination of enzymatic activity: Leaf samples were subjected to assay for catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX) and the same extract was used for each analysis. Fifty milligrams of fresh leaf sample, collected from 28 days old seedlings was homogenized with 3 mL of 50 mM potassium phosphate buffer (pH 8.0) in a mortar and pestle. The buffer was made by adding 45 mL of 0.2 M KOH to 50 mL of 0.2 M KH_2PO_4 and finally volume was made up to 100 mL. The homogenate was centrifuged at 12000 rpm for 10 min. The clear supernatant was used for assaying the enzymatic activity.

Catalase activity was assayed by following the method of Aebi[18]. Peroxidase and ascorbate peroxidase activity was assayed by following the method of Nakano and Asada[19]. Changes in absorbance for CAT, POD and APX were recorded immediately at 240, 470 and 290 nm respectively at 30 sec interval for 2 min.

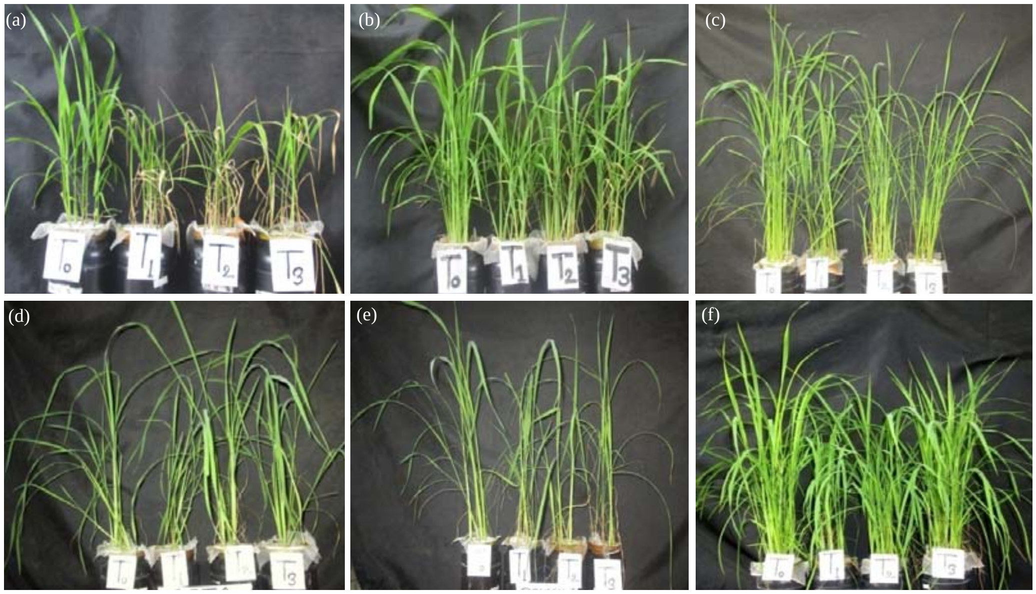

Fig. 1(a-f): Effect of different levels of MeJA on the growth of salt stressed rice varieties (a) BRRI dhan31, (b) BRRI dhan46, (c) Gota, (d) Kajalsail, (e) Pokkali and (f) Pengek. In each section (a-f), four treatment levels are indicated by T_0, T_1, T_2 and T_3 respectively

Determination of chlorophyll content: Chlorophyll content was determined according to the method developed by Coombs *et al.*[20]. An amount of 0.05 g fresh leaf samples was taken into a small vial containing 10 mL of 80% acetone and was covered by Aluminum foil and preserved in the dark for 7-10 days. Spectrophotometric reading was taken at 645 and 663 nm wavelengths. Data were assessed in formulae:

$$\text{Chlorophyll a} = (13.19\ A_{663}\text{-}2.57\ A_{645})\times\text{Dilution Factor}$$

$$\text{Chlorophyll b} = (22.10\ A_{645}\text{-}5.26\ A_{663})\times\text{Dilution Factor}$$

The Na^+ and K^+ contents: About10 mL of di-acid mixture was added to oven dried plant samples (200 mg) and heated gradually up to 180°C. The volume of digest was finally volumed to 100 mL. Sodium and potassium contents of the digest were determined by flame photometer. Percent emission was recorded following the method described by Ghosh *et al.*[21].

Statistical analysis: The experiment was arranged in a completely randomized design with three replications and the data collection and calculation were analyzed using MSTAT-C computer programs and comparison of means were tested for significance using Least Significant Difference (LSD) test, at 0.01 level of probability.

RESULTS

Catalase activity: Upon applying salt stress and exogenous MeJA on rice seedlings, the change in CAT activity didn't follow any regular trend (Fig. 2a). The BRRI dhan31, Gota and Pengek showed increase in catalase activity but in contrast, BRRI dhan46, Kajalsail and Pokkali had a decrease on NaCl stress. When 10 µM MeJA was applied on salt affected seedlings, increment in CAT activity was noticed in all the varieties except Pengek. Maximum activity was observed in Kajalsail (775.72%) followed by Pokkali (117.9%) compared to salt stress. Application of 20 µM MeJA reduced CAT activity when compared with those of 10 µM MeJA except in Pengek.

Peroxidase activity: On imposing salinity, there was a significant increase in POD activity and the rate of activity ranged from 9.07 to 66.64% (Fig. 2b). All the varieties had increment of POD activity upon applying 10 µM MeJA on saline affected plant, except BRRI dhan31 and Pengek. The increment rate was maximum (65.08%) in BRRI Dhan46. Upon applying 20 µM MeJA, POD activity showed different trend.

Ascorbate peroxidase activity: Under saline stress, there was significant increase in APX activity in all the varieties except in BRRI dhan31 (Fig. 2c). Maximum increment (43.55%) was observed in Pokkali compared to control. Upon applying

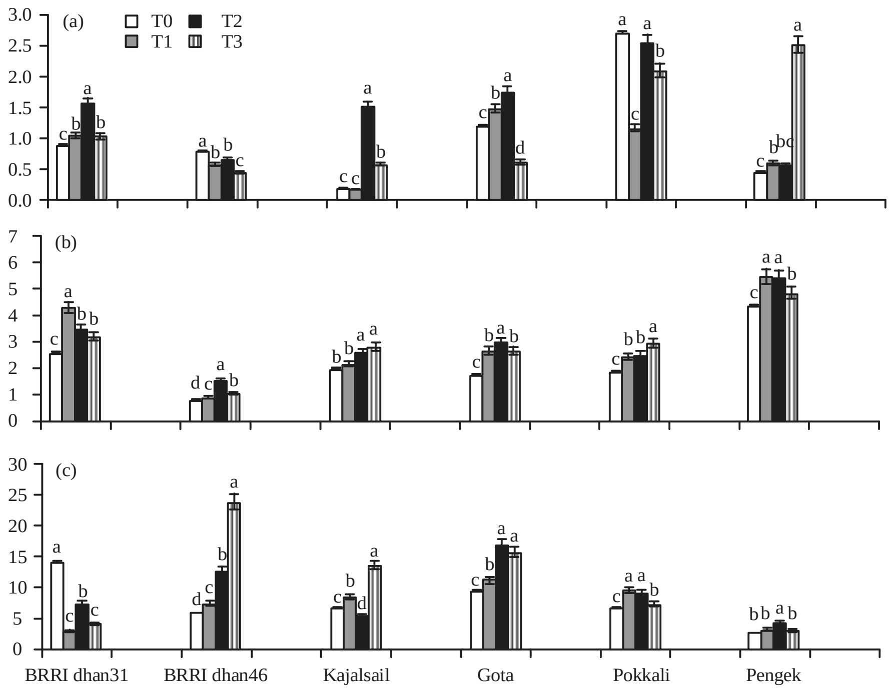

Fig. 2(a-c): The MeJA-induced changes in activity of antioxidant enzymes of salt stressed rice varieties, (a) Catalase, (b) Peroxidase and (c) Ascorbate peroxidase. For the same rice cultivar, bars with the same letters are not significantly different at p<0.01

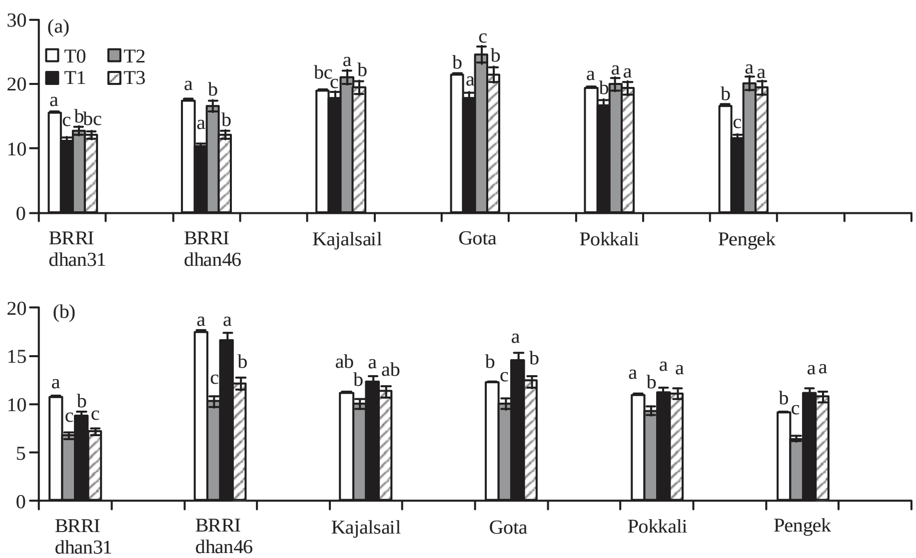

Fig. 3(a-b): The MeJA-induced changes in (a) Chlorophyll a and (b) Chlorophyll b content of salt stressed rice varieties. For the same rice cultivar, bars with the same letters are not significantly different at p<0.01

MeJA, 10 µM MeJA was found to increase APX activity was increased in all the varieties except in Kajalsail and Pokkali and it ranged from 44.42 to 221.74%. But in case of 20 µM MeJA, only BRRI dhan46 and Kajalsail got an increase.

Chlorophyll content: In saline condition, all the varieties had significant reduction of these pigments and the reduction rate ranges from 6.03 to 41.29% in case of chlorophyll a (Fig. 3a) while 9.82-41.29% in case of chlorophyll b compared to control (Fig. 3b).

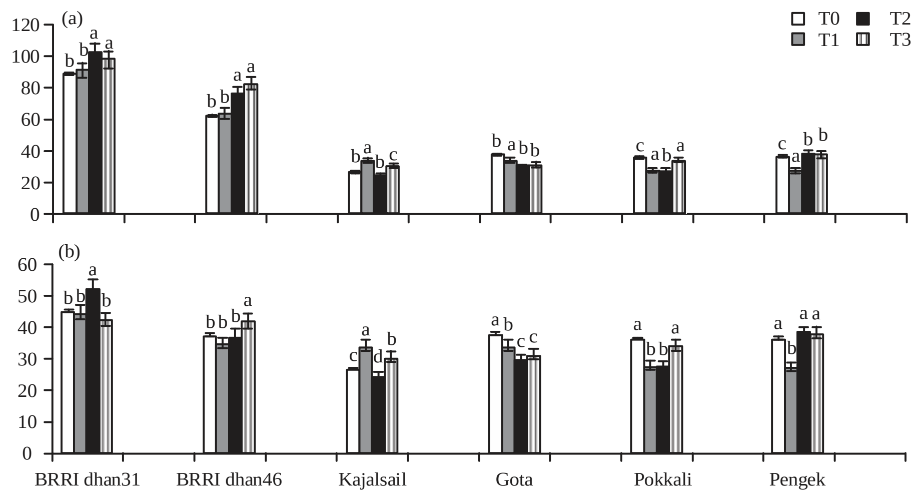

Fig. 4(a-b): The MeJA-induced changes in (a) Na^+ and (b) K^+ content of salt stressed rice varieties. For the same rice cultivar, bars with the same letters are not significantly different at $p \leq 0.01$

Upon applying 10 μM MeJA, chlorophyll a and chlorophyll b content increased in all varieties and it ranged from 14.51 to 74.15% in case of chlorophyll a while, 20.43 to 72.73% in case of chlorophyll b. On the other hand, 20 μM MeJA had a less increasing effect than the 10 μM MeJA treatment. Upon applying 20 μM MeJA, both chlorophyll a and chlorophyll b content also increased in all varieties.

The Na^+ and K^+ contents: The Na^+ accumulation was increased in hybrid varieties but except Kajalsail, while all the local varieties got a decrease due to saline stress (Fig. 4a). Upon applying MeJA, though Na^+ accumulation increased in hybrid varieties where it ranged from 7.91 to 29.07%. The decreases were observed in local varieties except Pengek. About 10 μM MeJA was found to decline Na^+ content more than the 20 μM MeJA.

Salt stress caused reduction of K^+ accumulation in all varieties excluding Kajalsail and the reduction ranged from 1.27 to 24.70% (Fig. 4b). Upon applying MeJA, different K^+ content accumulation were observed in different varieties. BRRI Dhan46 and Pengek had the increase in K^+ accumulation in both MeJA level.

DISCUSSION

Methyl jasmonate evokes a wide variety of morphological, physiological and biochemical responses in plants under stress conditions[17,22]. This study indicates that salinity exposure led to severe decline in seedlings growth plausibly by oxidative stress (Fig. 1) but after application of MeJA, the growth seems to be increased in stressed plants. In order to repair the damage initiated by ROS, plants evolve complex antioxidant metabolism. The study also shown that the application of MeJA further enhanced the activities of all antioxidant enzymes, both in non stressed and stressed plants, by supplementing the ROS scavenging mechanism. In the present study, saline stress caused varying trend of catalase activity depending on the varieties. Ruan and Xue[23] reported that CAT activity in rice shoot differed at various salinity level depending on different varieties. Hossain *et al*.[24] reported that CAT activity declined by 12 and 22% in Pokkali, at 75 and 150 mM NaCl salinity respectively; which is in the range of the present findings. There is information that shows MeJA affects on the activity and/or pools of stress enzymes and causes the alleviation of oxidative stress. Abdelgawad *et al*.[17], Aftab *et al*.[25] and Jung[26] reported that the activities of CAT and POD were increased in the leaves on account of MeJA treatment. In this study, application of 10 μM MeJA found to increase further activity. Wang[27] reported while MeJA treatment significantly increased CAT of osmotic stressed plants. Exogenous application of MeJA might have contributed to this elevated activity of CAT.

The POD activity also increased in all the cultivars subjected to NaCl stress. Chawla *et al*.[28] and Hossain *et al*.[24] reported that peroxidase activity increased due to salinity that is resembled with the present study. On applying MeJA, 10 μM found to increase activity further in maximum varieties. Nojavan-Asghari and Norastehnia[29] opined that MeJA plays a role in signal transduction pathway in oxidative stress by the increase in POD activity. Abdelgawad *et al*.[17] also reported that pre-soaking plants with MeJA improved stress resistance by increase in POD activities as compared with corresponding drought stress level. So the finding of the study was in conformity with the reports of others. The APX activity in saline conditions continued to increase in most of the varieties which is also supported by Turan and Tripathy[12] and Weisany *et al*.[30] Reduction in APX activity was observed in BRRI dhan31 that was also supported by Hossain *et al*.[24] who reported that hybrid, BRRI dhan29 exhibited a significant decreased activity of APX. The MeJA application showed an irregular trend in spite of 10 μM had some positive trend. Under different abiotic stress conditions, MeJA proved to increase the activity of APX which are consistent with the finding of Li *et al*.[31]. But the irregular trend can't prove any definite findings.

The present study uncovered that under saline stress, chlorophyll a and chlorophyll b content of all the varieties of rice seedlings were reduced which was also reported by Hakim *et al*.[32]. Exogenously applied MeJA had a positive effect on alleviating chlorophyll reduction of salt stressed plants. Jamalomidi *et al*.[33] reported that exogenous MeJA on saline induced plants increased the chlorophyll content which is in conformity with the present work.

The lower Na^+/K^+ ratio would be beneficial for plants to tolerate salt stress. Potassium plays a key role in plant metabolism[34]. It activates a range of enzymes and plays a key role in plant osmotic stress and has been found to be the cationic solute which is responsible for stomatal movement in response to changes in bulk leaf water status[17,35]. The Na^+ and K^+ content in the leaves of crop plants could be used as an important indicator for salt tolerance. Hussain *et al*.[3] reported that salinity caused a substantial increase in Na^+ content and decrease in K^+ accumulation and the findings of the present study partially complied with those conclusion. Exogenous MeJA application reduces the Na^+ accumulation and increases K^+ accumulation in plant[34]. The present study also reveals that Na^+ accumulation reduced in all varieties except in hybrid varieties and K^+ accumulation increased in hybrid varieties upon applying MeJA though deviations were noticed in local varieties.

CONCLUSION

An important limitation of this study is that expected role of antioxidant enzymes were not established evidently. However, better visual growth, data on chlorophyll contents and Na^+ and K^+ accumulation support the positive effect of using 10 μM MeJA on partial alleviation of salt stress which might have been a different tolerance mechanism for rice plants. This finding could be helpful for exploring the application of exogenous MeJA for a short time recovery of rice plant in the tidal surge prone coastal region of Bangladesh. Further if MeJA over-expressed plants could be developed, they might have the possibility to be naturally tolerant to the salt stress.

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