

MORPHO-PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF SALT-SENSITIVE AND SALT-TOLERANT POTATO VARIETIES TO SALINITY STRESS

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Abstract

A pot experiment was conducted with three potato varieties ['BARI Alu-72' (salt-tolerant), 'BARI Alu-25' (relatively salt-tolerant) and 'BARI Alu-13' (salt-sensitive)] under three levels of salinity stress (control; 0.2, moderate; 6-8 and severe; 10-12 dSm⁻¹) for evaluating their morphological, physiological and biochemical changes during *rabi* season of 2018-19. Salinity treatment was imposed from 10 days after emergence (DAE) to maturity stage by adding NaCl solution. Salinity stress caused higher reduction in chlorophyll (Chl), carotenoids (CAR), cell membrane stability index (CMSI), biomass and tuber yield plant⁻¹ but increase in contents of catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), glutathione peroxidase (GPX), glutathione S-transferase (GST) and glutathione reductase (GR) activities in all potato varieties and at all the salinity levels. The reduction of Chl, CAR, CMSI, biomass and yield plant⁻¹ were lower in 'BARI Alu-72' as compared to other two varieties. Moreover, 'BARI Alu-72' showed higher activities of CAT, POD, APX, GPX, GR and GST as well as contents of K⁺ and lower malondialdehyde (MDA), lipoxygenase (LOX) and Na⁺ contents in comparison with those of 'BARI Alu-25' and 'BARI Alu-13'. Activities of antioxidants were found moderate in 'BARI Alu-25'. 'BARI Alu-13' showed higher Na⁺ and Na⁺/K⁺ ratio. The results showed that salinity tolerance in potato was manifested by lower decrease in biomass and yield plant⁻¹ with higher antioxidant activities and K⁺ contents, and lower MDA, LOX and Na⁺ content than salinity sensitive genotype.

Keywords: Potato, salinity, ionic imbalance, physiological, antioxidants

Introduction

Salinity is one of the major abiotic stresses affecting plant growth, development and productivity. Plants exposed to salt stress, undergo changes in their metabolism in order to cope with the changes taking place in their environment (Gueta-Dahan *et al.* 1997). However, ion toxicity and biochemical changes occurring when plants are subjected to biotic or abiotic stresses is the production of reactive oxygen species (ROS). ROS are highly reactive and in the absence of any protective mechanism they can seriously disrupt normal metabolism through oxidative damage lipids, proteins and nucleic acids (Rohman *et al.*, 2019). Plants

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possess a number of antioxidant systems that protect them from these potential cytotoxic effects. Antioxidant enzymes are the most important components in the scavenging system of ROS (Molassiotis *et al.*, 2006; Rohman *et al.*, 2016). Catalase (CAT) is one of the most important enzymes of antioxidant systems having the highest turnover rates among all enzymes (Garg and Manchanda, 2009). Catalase (CAT), ascorbate peroxidase (APX), glutathione peroxidase (GPX) and a variety of general peroxidases (POD) catalyze the breakdown of H₂O₂ (Chen and Asada, 1989; Brigelius-Flohé and Flohé, 2003). Therefore, this enzyme system eliminates the damaging effects of toxic oxygen species. Several studies have shown that higher glutathione S-transferase (GST) activity can enhance abiotic stress tolerance of plants (Dixon *et al.*, 2010). Over expression of GST in plants increases antioxidant activity and improves tolerance to oxidative stress (Yadav *et al.*, 2005).

Potato (*Solanum tuberosum* L.) is one of the important vegetables as well as cash crop in Bangladesh. Recently it has become major food crop due to multiple uses as vegetable and delicious processed items. At present nearly 476 thousand hectares of cultivable land is under potato cultivation and the country produced 9725 thousand tons potato in the year 2016-2017 (BBS, 2018). Though the potato production increases, still there is a wide gap between national average yield and that of coastal areas of Bangladesh. The cultivable areas in coastal districts are affected with varying degrees of soil salinity ranging from 3.63-27.67 dS m⁻¹ (Akter *et al.*, 2008). Potato is considered moderately sensitive to salinity (Katerji *et al.*, 2000). Van Hoorn *et al.* (1993) reported that under irrigated potato with 5.9 dS m⁻¹ of salinity a yield loss of up to 37% was incurred. It has been reported that potato production is limited by high level of salinity greater than 50 mM NaCl (Rahman *et al.*, 2008). Increasing saline area demands salt tolerant cultivars for sustainable potato production in southern belt of Bangladesh. So far, Bangladesh Agricultural Research Institute (BARI) has released a salt tolerant potato variety and its tolerance mechanisms still unclear. Therefore, it is necessary to understand salinity tolerance mechanism in potato that will be helpful in developing stress tolerant potato varieties by using various modern techniques. Considering this scenario, the present investigation was undertaken to understanding insight into the tolerant mechanism of salt tolerance in potato.

Materials and Methods

Plant materials and Experimental Design

The pot experiment was conducted at the vinyl house and laboratory of Plant Physiology Division of BARI, Gazipur, during November 2018 to February 2019. Three potato varieties including one salt-tolerant variety ('BARI Alu-72'; as developed by TCRC, BARI) and one relatively salt-tolerant ('BARI Alu-25')

variety derived from in vitro assessment (Rahman *et al.*; 2018) and one salt-sensitive variety ('BARI Alu-13') derived from our previous field assessment under saline conditions were used in this study. Potato varieties were tested under three salinity levels (Control; 0.2 dS m⁻¹, Moderate; 6-8 and Severe; 10-12 dS m⁻¹). The seed tubers of varieties were collected from TCRC of BARI. Here, potato varieties represent Factor A and salinity levels represent Factor B. Pots were arranged in Randomized Complete Block design with four replications and each pot was considered as one replication.

Pot preparation

Soil was collected from the Kodda area of Gazipur and the soil texture was sandy loam. The soil was air dried (8-9% moisture) for 15 days followed by gently dispersing and mixing thoroughly. The pots used during the present study had five small holes at the bottom. The bottom of the pot was filled with a piece of mosquito net and some broken bricks. Treatment solution was applied in excess so that the extra solution dripped from the bottom of the pots.

Pot experiment management

The experiment was carried out by planting seed tubers of each variety in plastic pots (top dia: 120 cm, bottom dia: 90 cm, depth 42 cm) filled with 25 kg soil and cowdung in 4:1 ratio of collected soil and well decomposed cowdung (8-9% moisture). The recommended dose of chemical fertilizer for field grown potato (350, 225, 300, 115 kg ha⁻¹) from TCRC of BARI was taken into account in this study. However, we used double of the recommended dose for pot experiment following the protocol of pot experiment. According to fertilizer dose per hectare, the calculated amount of N-P-K-S for each pot was 4, 1.125, 3.75 and 0.5g, respectively as 1 ha land contains 2×10⁶ kg fresh soil (Hadis *et al.*, 2019). Full amount of triple super phosphate (TSP), muriate of potash (MOP), gypsum and 50% of urea were applied as basal during pot preparation and the remaining amount of urea was side dressed at 30 days after planting (DAP). Three healthy and equal-sized (50 ± 2g) seed tubers was planted on 27 November 2018 to a depth of 6 cm soil in each pot. All the seedlings were emerged within 6-7 DAP and no thin out was done. Salt solution was prepared artificially by dissolving calculated amount of lab grade NaCl (Mark, Germany) with pond water (0.2 dSm⁻¹) with the help of electrical conductivity (EC) meter (liquid probe; HI 993310, Hanna, Romania). After 9 days of emergence salinity treatment was imposed by adding NaCl solution and that was continued upto crop maturity. Treatment solution was applied in excess so that the extra solution dripped from the bottom of the pots. To avoid osmotic shock salinity was imposed gradually; 2 liter salt solution (5 dSm⁻¹) per pot was applied every alternate day until the respective level of salinity was attained. Salinity levels were maintained by

monitoring with the help of EC meter (soil probe; HI 993310, Hanna, Romania) and adding 1 liter salt solution (2 dSm^{-1}) in each pot as and when require up to crop maturity for maintaining adequate soil moisture (Table 1). However, pond water (0.2 dSm^{-1}) was used as a control and it also applied prior to salinity treatment imposed in the all pots and other agronomic managements were done as and when necessary.

Data collection

Morphological, physiological and biochemical parameters recorded in the study were as given below.

- i. **Morphological parameters:** One destructive sampling was performed at 60 DAP and plant height and tuber number were recorded average from the three plants.
- ii. **Physiological parameters:** After measurement of plant height, plants were separated into leaves and stem, and dried in an oven for 72 hours at 70°C and dry weight was recorded. At harvest (25 February 2019) yield data was recorded from three pots. Moreover, fully expanded 3rd leaf from top was collected from each treatment at 60 DAP and Chl*a*, Chl*b*, total chlorophyll, carotenoids, Na^+ and K^+ , Cell Membrane Stability Index (CMSI) were measured as given below.

Estimation of chlorophyll content

Fully developed 3rd leaves of each genotype were collected on 60 DAP and properly cut into small pieces and weighed and 0.5 g was taken for chlorophyll estimation. The absorbance of the solution was read at 663 and 645 nm for Chlorophyll a, Chlorophyll b and total chlorophyll, and at 470 nm for carotenoids with a spectrophotometer (UV-1800, Shimadzu, Japan) against 80% acetone as blank. Estimation of chlorophyll and carotenoids was done through the following formula.

Calculation:

$$\text{Chlorophyll a (mg g}^{-1}\text{)} = \{12.7 (\text{D}663) - 2.69 (\text{D}645)\} \times V / (1000 \times W)$$

$$\text{Chlorophyll b (mg g}^{-1}\text{)} = \{22.9 (\text{D}645) - 4.68 (\text{D}663)\} \times V / (1000 \times W)$$

$$\text{Total chlorophyll (mg g}^{-1}\text{)} = 20.2 (\text{D}645) + 8.02 (\text{D}663) \times V / (1000 \times W)$$

$$\text{Carotenoids} = \{(1000 \times (\text{D}470) \times V / W / 1000) - (1.9 \times \text{Chl a}) - (63.14 \times \text{Chl b})\} / 214$$

Where, D=optical density; V=final volume of 80% acetone (ml); W = fresh weight of sample taken (g) and Chl= chlorophyll.

Table 1. Soil salinity levels in the pot during salinity stress period (from 10 days after emergence to crop maturity)

Variety	Treatment	Soil salinity levels (dS m ⁻¹)										
		3 rd week (11-17 Dec.)	4 th week (18-24 Dec.)	5 th week (25-31 Dec.)	6 th week (1-7 Jan.)	7 th week (8-14 Jan.)	8 th week (15-21 Jan.)	9 th week (22-28 Jan.)	10 th week (29-4 Feb.)	11 th week (5- 11 Feb.)	12 th week (12-18 Feb.)	13 th week (19-25 Feb.)
	Control	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
BARI Alu-25	Moderate	3.8	6.8	7.9	7.7	7.8	7.5	7.6	7.8	7.6	7.4	7.3
	Severe	4.0	6.9	10.3	11.7	11.8	11.4	11.7	12.0	11.8	11.4	11.3
	Control	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
BARI Alu-13	Moderate	3.7	6.6	7.8	7.3	7.4	7.6	7.2	7.4	7.5	7.4	7.1
	Severe	3.9	6.7	10.0	11.8	11.7	11.4	11.8	11.9	12.0	11.6	11.8
	Control	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
BARI Alu-72	Moderate	3.8	6.6	7.9	7.5	7.7	7.8	6.9	7.5	7.7	7.8	7.6
	Severe	3.9	6.8	10.2	11.9	11.6	11.5	12	11.8	11.9	11.7	11.6

Control; 0.2 dS m⁻¹, Moderate; 6-8 dS m⁻¹ and Severe; 10-12 dS m⁻¹

Determination of Na⁺ and K⁺ ions

At 60 DAP, fully expanded 3rd leaf from top was collected from each treatment for determination of Na⁺ and K⁺ ions content in leaf sap, which was extracted from leaves by pre-cooled mortar and pestle. LAQUA twin Na⁺ ion meter (Na-11, Horiba, Japan) and LAQUA twin K⁺ ion meter (K-11, Horiba, Japan) were used for determination of Na⁺ and K⁺ ion content, respectively. Na⁺/K⁺ ratio was measured from the estimated values.

Cell Membrane Stability Index (CMSI)

Leaf segments after washing with deionized water were placed in closed vials containing deionized water (10 ml) and incubated overnight at 25 °C. Electrical conductivity of the bathing solution (C1) was determined after 24 h by EC meter (HI 993310, Hanna, Romania). Samples were then put in a boiling water bath for 10–15 min and final conductivity reading (C2) was obtained upon equilibration at 25 °C (Sairam *et al.*, 2002). The cell membrane stability index (CMSI) was calculated using the following formula-

$$\text{CMSI (\%)} = 1 - (C^1/C^2) \times 100$$

Measurement of malondialdehyde (MDA)

The level of lipid peroxidation was measured by estimating malondialdehyde (MDA) following the method of Rohman *et al.*, 2016. Malondialdehyde content was measured by observing the difference in absorbance at 532 nm using an extinction coefficient of 155 mM⁻¹cm⁻¹ and expressed as nmol of MDA g⁻¹ FW. The concentration of MDA was calculated by using the extinction coefficient of 155 mM⁻¹ cm⁻¹ and expressed as nano mole of MDA per gram FW.

iii. Biochemical parameters:

Leaf samples (3rd leaf from apex) were collected on 60 DAP for determination of activity of enzymatic antioxidants like Catalase (CAT), Ascorbate peroxidase (APX) and Peroxidase (POD).

Extraction of soluble protein for activity assays

Using a pre-cooled mortar and pestle, 0.5 g of fresh leaf tissue of potato was homogenized in 1 ml of 50 mM ice-cold K-P buffer (pH 7.0) containing 100 mM KCl, 1 mM ascorbate, 5 mM β-mercaptoethanol and 10% (w/v) glycerol. The homogenates were centrifuged at 11,500×g for 10 min and the supernatants were used for determination of enzyme activity. All procedures were performed below 4°C.

Determination of protein

The protein concentration in the leaf extracts was determined following the method of Bradford (1976) using BSA as a protein standard where 5, 10, 15, 20, 25 $\mu\text{g } \mu\text{l}^{-1}$ protein concentrations were used to prepare standard curve.

Assay of enzymatic activities

Peroxidase (POD, EC 1.11.1.7): POD activity was estimated according to Hemeda and Klein (1990). The reaction mixture contained 25 mM K-P buffer (pH 7.0), 0.05% guaiacol, 10 mM H_2O_2 and the protein solution. Activity was determined by the increase in absorbance at 470 nm due to guaiacol oxidation for 1 min using extinction coefficient of $26.6 \text{ mM}^{-1} \text{ cm}^{-1}$.

Catalase (CAT, EC: 1.11.1.6): CAT activity was measured according to the method of Hossain *et al.* (2010) by monitoring the decrease of absorbance at 240 nm for 1 min caused by the decomposition or degradation of H_2O_2 . The reaction mixture contained 50 mM K-P buffer (pH 7.0), 15 mM H_2O_2 , and enzyme solution in a final volume of 0.7 ml. The reaction was initiated with enzyme extract, and the activity was calculated using the extinction coefficient of $39.4 \text{ M}^{-1} \text{ cm}^{-1}$.

Glutathione peroxidase (GPX, EC: 1.11.1.9): GPX activity was measured as described by Hasanuzzaman *et al.* (2014) using H_2O_2 as a substrate. The reaction mixture consisted of 100 mM Na-P buffer (pH 7.5), 1 mM EDTA, 1 mM NaN_3 , 0.12 mM NADPH, 2 mM GSH, 1 unit Glutathione reductase (GR), 0.6 mM H_2O_2 , and 20 μl of sample solution. The reaction was started by the addition of H_2O_2 . The oxidation of NADPH was recorded at 340 nm for 1 min, and the activity was calculated using the extinction coefficient of $6.62 \text{ mM}^{-1} \text{ cm}^{-1}$.

Ascorbate peroxidase (APX, EC: 1.11.1.11): APX activity was assayed following the method of Nakano and Asada (1981). The reaction buffer solution contained 50 mM K-P buffer (pH 7.0), 0.5 mM ascorbic acid (ASA), 0.1 mM H_2O_2 , 0.1 mM EDTA, and enzyme extract in a final volume of 0.7 ml. The reaction was started by the addition of H_2O_2 , and the activity was measured by observing the decrease in absorbance at 290 nm for 1 min using an extinction coefficient of $2.8 \text{ mM}^{-1} \text{ cm}^{-1}$.

Glutathione reductase (GR, EC: 1.6.4.2): GR activity was measured by the method of Hossain *et al.* (2010). The reaction mixture contained 0.1 M K-P buffer (pH 7.8), 1 mM EDTA, 1 mM GSSG, 0.2 mM NADPH, and enzyme solution in a final volume of 1 ml. The reaction was initiated with GSSG, and the decrease in absorbance at 340 nm due to NADPH oxidation was recorded for 1

min. The activity was calculated using an extinction coefficient of $6.2 \text{ mM}^{-1} \text{ cm}^{-1}$.

Glutathione S-transferase (GST, EC: 2.5.1.18): GST activity was determined spectrophotometrically by the method of Rohman *et al.* (2010). The reaction mixture contained 100 mM Tris-HCl buffer (pH 6.5), 1.5 mM GSH, 1 mM 1-chloro-2, 4-dinitrobenzene (CDNB), and enzyme solution in a final volume of 0.7 ml. The enzyme reaction was initiated by the addition of CDNB, and the increase in absorbance was measured at 340 nm for 1 min. The activity was calculated using the extinction coefficient of $9.6 \text{ mM}^{-1} \text{ cm}^{-1}$.

Lipoxygenase (LOX, EC: 1.13.11.12): LOX activity was measured following Rohman *et al.* (2016). The substrate solution was prepared by adding 35 μl linoleic acid to 5 ml distilled water containing 50 μl Tween-20. The solution was kept at pH 9.0 by adding 0.2 M NaOH until all the linoleic acid was dissolved and the pH remained stable. After adjusting the pH to 6.5 by adding 0.2 M HCl, 0.1 M phosphate buffer (pH 6.5) was added to a total volume of 100 ml. The substrate solution was flushed with and kept under a nitrogen atmosphere. LOX activity was determined spectrophotometrically by adding 10 μl of sample to 590 μl substrate solution. The increase in absorbance at 234 nm was measured for 1 min at 25°C. The activity was expressed as μmol hydroperoxide formed $\text{min}^{-1} \text{ mg}^{-1}$ protein using a molar extinction coefficient of $25,000 \text{ M}^{-1} \text{ cm}^{-1}$.

Statistical analysis

Data on different parameters were analyzed statistically by using Statistix 10 program. The mean separation was done by the least significant difference (LSD) at 5% level of probability.

Results and Discussion

Plant height and tubers number per plant

Plant height and tubers number plant^{-1} of three potato varieties ('BARI Alu-25', 'BARI Alu-13' and 'BARI Alu-72') decreased with increasing intensity of salinity (Fig. 1 A-B). Results revealed that plant heights ranged from 43-53 cm at 60 DAP in control treatment, where 'BARI Alu-25' produced the tallest plant followed by 'BARI Alu-13' and 'BARI Alu-72' being the smallest. Similar trend was observed by the moderate and severe stress conditions. Moreover, at severe salinity stress, the lowest reduction of plant height was recorded in 'BARI Alu-72' (46.69%), which was statistically identical with 'BARI Alu-13' (47.78%) and the highest reduction was recorded in 'BARI Alu-25' (52.01%) compared to control, which was also similar with others. Plants growth in saline soil depends on the

salt tolerance of the plants species. The reduction in plant height is a consequence of several physiological responses including modification of ion balance, water status, mineral nutrition, photosynthetic efficiency (Koyro, 2006). Reduced plant height under salinity is also associated with reactive oxygen species (Akter *et al.*, 2008). Our findings agree with previous studies which indicated the adverse effects of salinity on plant height (Mohamed *et al.*, 2010). Moreover, all the potato varieties produced the higher number of tubers plant⁻¹ (14.22-18.89) at control treatment. But the tubers number plant⁻¹ of all tested varieties decreased gradually with the increasing of salinity levels. However, at severe salinity condition maximum reduction of tubers number plant⁻¹ was 37% found in 'BARI Alu-13' and minimum 11% in 'BARI Alu-72' compared to control condition.

Effect of salinity levels on pigment contents

Photosynthetic pigment contents decreased sharply with increasing salinity levels in all the varieties (Fig. 2A-D). The present study showed that pigment contents were more in 'BARI Alu-72' in all the salinity levels and less in 'BARI Alu-13'. As compared to control, Chl*a* content reduced by 21 and 46%, in 'BARI Alu-72' leaves at moderate and severe salinity stresses, respectively. However, the highest reduction of Chl*a* content was 37 and 65%, in the leaves of 'BARI Alu-13' at moderate and severe salinity stresses, respectively. Similar trend was followed by Chl*b* contents at all the potato varieties in all salinity levels. Moreover, total chlorophyll content of 'BARI Alu-72' decreased by 16 and 25%, in moderate and severe salinity stresses, respectively. Decreasing the amount of photosynthetic pigments was one of the effects of salinity in plants and had already been reported in many crop species including tomato (Juan *et al.*, 2005) and apple rootstock (Molassiotis *et al.*, 2006). The decrease in chlorophylls in salinized plants could be attributed to the increased activity of the chlorophyll-degrading enzyme, chlorophyllase, and ion accumulation in leaves. The carotenoids content also decreased by 36 and 37% in moderate and severe salinity stresses, respectively. The decrease in carotenoids under salt stress leads to degradation of β -carotene and formation of xanthins, which are apparently involved in protection against photoinhibition (Sultana *et al.*, 1999).

Effect of salinity on sodium (Na⁺), potassium (K⁺) content and Na⁺/K⁺ ratio

The amount of Na⁺ and K⁺ ions accumulated in the leaves of potato varieties ('BARI Alu-25', 'BARI Alu-13' and 'BARI Alu-72') Na⁺/K⁺ ratio is shown in Fig. 3 A-C. Sodium (Na⁺) content increased in all the varieties with salinity intensity. Na⁺ accumulation was significantly higher in 'BARI Alu-13' leaves than the other varieties. In 'BARI Alu-13' leaves, Na⁺ content increased by 30

and 66%, in moderate and severe salinity stresses, respectively. However, accumulation of Na⁺ content was lower in 'BARI Alu-72', which was identical with 'BARI Alu-25'. Potassium (K⁺) content decreased with increasing salinity levels (Fig. 2B). On the other hand, the ratio of sodium and potassium increased significantly with salinity levels in all varieties (Fig. 3C). 'BARI Alu-13' showed higher sodium and potassium ratio than 'BARI Alu-25' and 'BARI Alu-72' in all the salinity treatments. The Na⁺ ion was increased and K⁺ ion decreased in all the varieties with increasing salinity stress, which usually happens in all plants under salinity stress. The accumulation of Na⁺ ion might be involved in the osmotic adjustment. Salinity increase in sodium and decrease in potassium contents in case of wheat had been reported earlier (Moustafa *et al.*, 1966). Joshi *et al.* (1979) reported that the plant's tolerance response was characterized by distinctly lower Na⁺/K⁺ ratio, which might be used to predict tolerance or sensitivity in potato varieties.

Effect of salinity levels on CMSI, MDA and LOX

The accumulation of lipid peroxidation (Malondialdehyde or MDA) and activities of lipoxigenase (LOX) remarkably increased and cell membrane stability index (CMSI) decreased in all the varieties with the increasing severity of salinity stress compared with control (Fig. 4 A, B & C). The CMSI decreased with the increase of salinity levels in all the varieties (Fig. 4A). Maximum reduction of CMSI was 15 and 27% in 'BARI Alu-13' (salt sensitive) and minimum decrease 9 and 13% in 'BARI Alu-72' (salt tolerant) under moderate and severe salinity, respectively. Similar results related to decreased CMSI were obtained in wheat (Sairam *et al.*, 2002) and *Amaranthus* (Bhattacharjee and Mukherjee, 1996). It had been suggested that decrease in cell membrane stability reflects the extent of lipid peroxidation caused by reactive oxygen species (Dhindsa *et al.*, 1981). CMSI and extent of lipid peroxidation had been used as indices of salt injury and salt tolerance in *Amaranthus* (Bhattacharjee and Mukherjee, 1996). Lower MDA and higher CMSI had been reported in drought tolerant genotypes of wheat (Sairam *et al.*, 1998).

The present study demonstrated that salt stress induced oxidative injury by the increase of MDA and LOX in all the potato varieties over control (Fig 4 B & C). Salinity stress led to a significant increase in the levels of MDA in all the potato varieties (Fig. 4B). Maximum MDA accumulation was observed in 'BARI Alu-13' which was significantly higher than other varieties. The lowest MDA accumulation was found in 'BARI Alu-72'. Accumulation of MDA was an indicator of lipid peroxidation level, which reflected the extent of tolerance to

salinity. It is reported that cultivars with higher salinity tolerance had lower MDA content when subjected to stress (Azevedo Neto *et al.*, 2006).

LOX production also increased in all potato varieties due to salinity stress. In 'BARI Alu-13' (Salt sensitive), LOX contents was increased about 120 and 206% at moderate and severe salt stress over the control, respectively. However, in 'BARI Alu-72' (Salt tolerant variety), it was increased about 68 and 99% at moderate and severe salt stress over the control, respectively. The increased LOX activity was responsible as a reason for oxidation of polyunsaturated fatty acids and thus enhanced lipid peroxidation under stress conditions as reported in many plants (Azooz *et al.*, 2009; Sánchez-Rodríguez *et al.*, 2012). In this study, salinity stress resulted in higher LOX activities in 'BARI Alu-13' and 'BARI Alu-25' and lower LOX content was observed in 'BARI Alu-72' under both control and stress condition (Fig. 4C) as compared to 'BARI Alu-13'. Similar relationship of increased LOX activity and oxidative stress was observed in previous research findings (Rohman *et al.*, 2016).

Effect of salinity levels on antioxidant activities

In the present study, CAT, POD, APX, GPX, GR and GST activities increased sharply in all the varieties with increasing salinity levels (Fig. 5 A-F). Similar results associated to increase the activity of these antioxidants were observed from experiments with salt-sensitive and salt-tolerant potato (Daneshmand *et al.*, 2010), wheat (Sairam *et al.*, 2002) under salt stress. However, in salinity stress condition activities of these antioxidants were more in 'BARI Alu-72' and less in 'BARI Alu-13'. Under moderate stress condition CAT activity increased by 53, 21 and 111% in 'BARI Alu-25', 'BARI Alu-13' and 'BARI Alu-72' compared to control, respectively. On the other hand, when plants were exposed to severe condition, CAT activities in 'BARI Alu-25', 'BARI Alu-13' and 'BARI Alu-72' were 87, 30 and 179% higher than in the controls, respectively. Moreover, POD activity increased by 1.65, 1.30 and 2.70 fold in 'BARI Alu-25', 'BARI Alu-13' and 'BARI Alu-72' compared to control at severe salinity stress, respectively. Similar trend was followed in activities of other studied antioxidants, at both moderate and severe salinity stress condition. Results revealed that, among the potato variety more antioxidants were found in 'BARI Alu-72' followed by 'BARI Alu-25' and 'BARI Alu-13' being the less in all the stress condition. These antioxidant enzymes were reported to increase under salinity stress (Gueta-Dahan *et al.*, 1997) as well as comparatively higher activity had been reported in tolerant cultivars than the susceptible ones (Sairam *et al.*, 1998; Sreenivasulu *et al.*, 2000), suggesting that higher antioxidant enzymes (CAT, POD, APX, GPX,

GST and GR) in ‘BARI Alu-72’ under increasing salinity stress signified its tolerance to salinity stress, while ‘BARI Alu-13’ was inferior on this account.

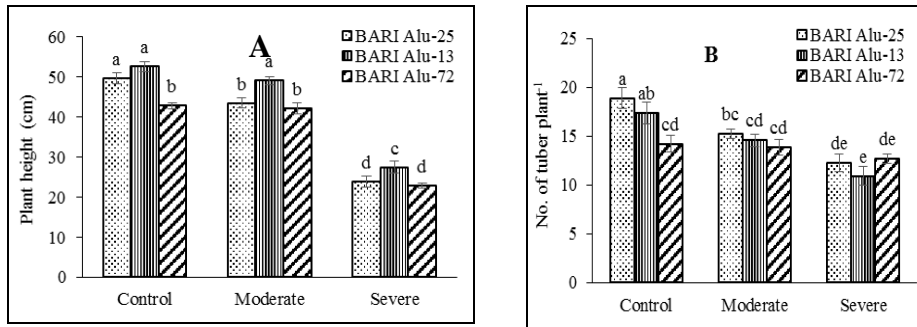


Fig 1. Effect of salinity levels on (A) Plant height, (B) Number of tubers plant⁻¹ in potato varieties at 60 DAP. Vertical bars represent SE; Control = 0.2, Moderate = 6-8 and Severe = 10-12 dSm⁻¹.

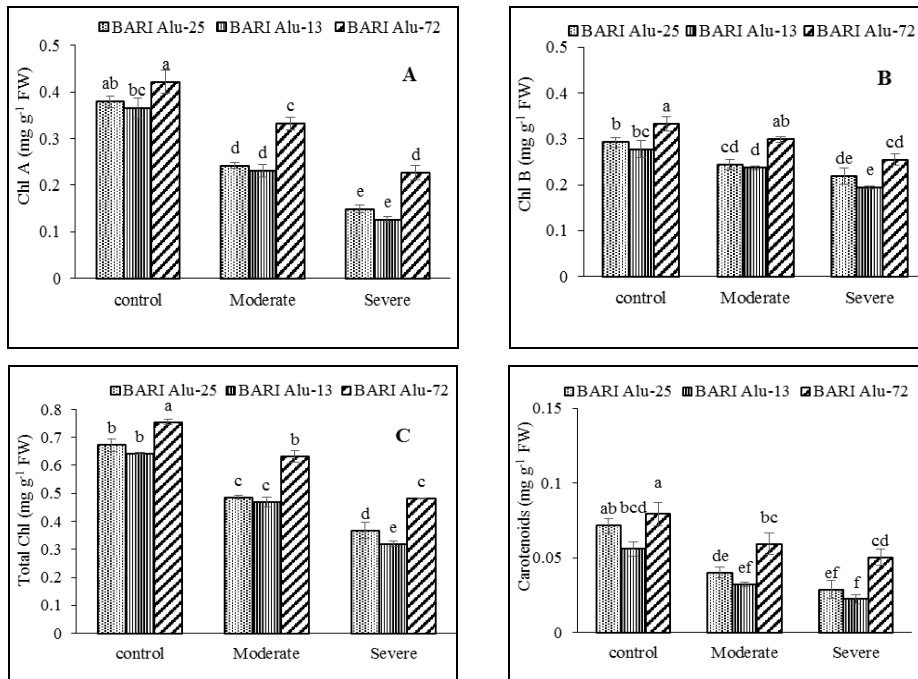


Fig. 2. Effect of salinity levels on (A) Chlorophyll a, (B) Chlorophyll b (C) Total Chlorophyll and (D) Carotenoids contents in potato varieties at 60 DAP. Vertical bars indicate SE; Control = 0.2, Moderate = 6-8 and Severe = 10-12 dSm⁻¹.

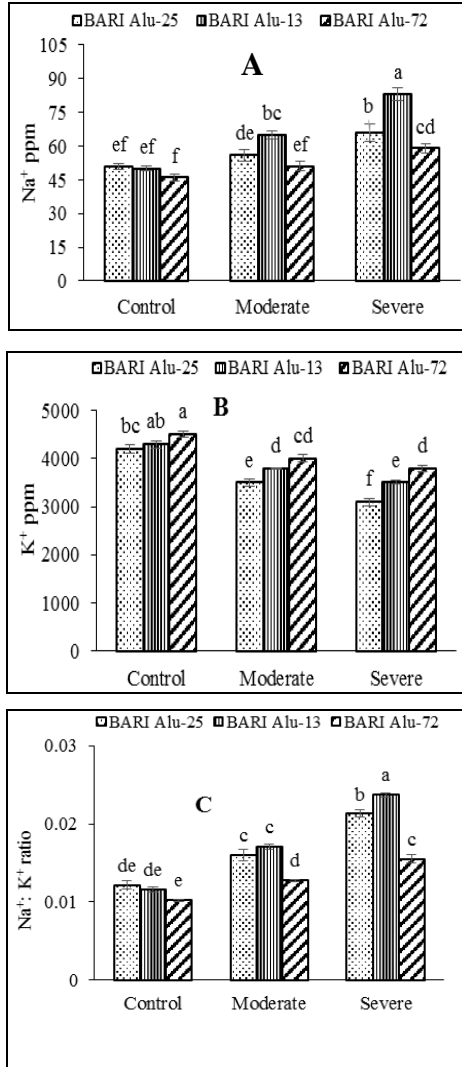


Fig. 3. Effect of salinity levels on (A) Na⁺, (B) K⁺ and (C) Na⁺: K⁺ ratio in the leaves of potato varieties at 60 DAP. Vertical bars indicate SE; Control = 0.2, Moderate = 6-8 and Severe = 10-12 dSm⁻¹.

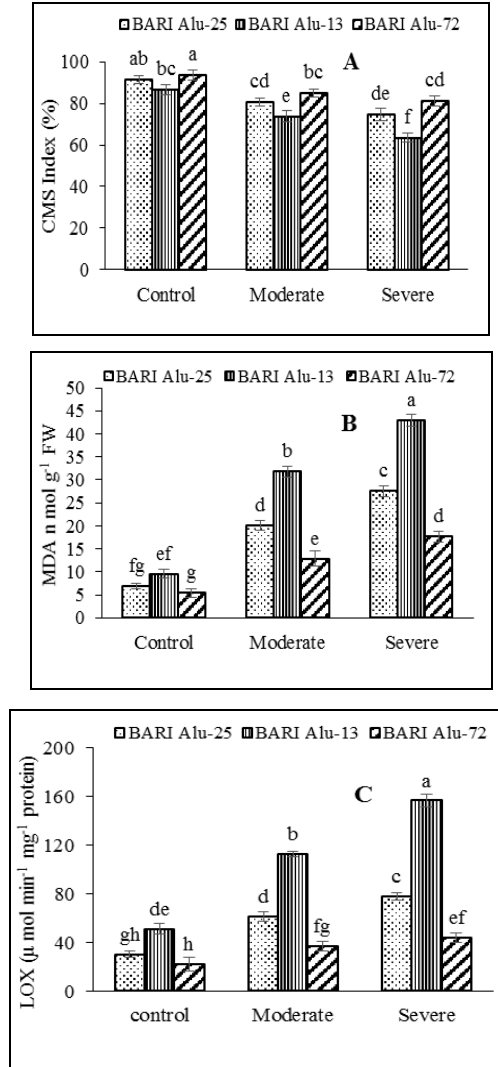


Fig. 4. Effect of salinity levels on (A) CMSI, (B) MDA and (C) LOX in potato varieties at 60 DAP. Vertical bars indicate SE; Control = 0.2, Moderate = 6-8 and Severe = 10-12 dSm⁻¹.

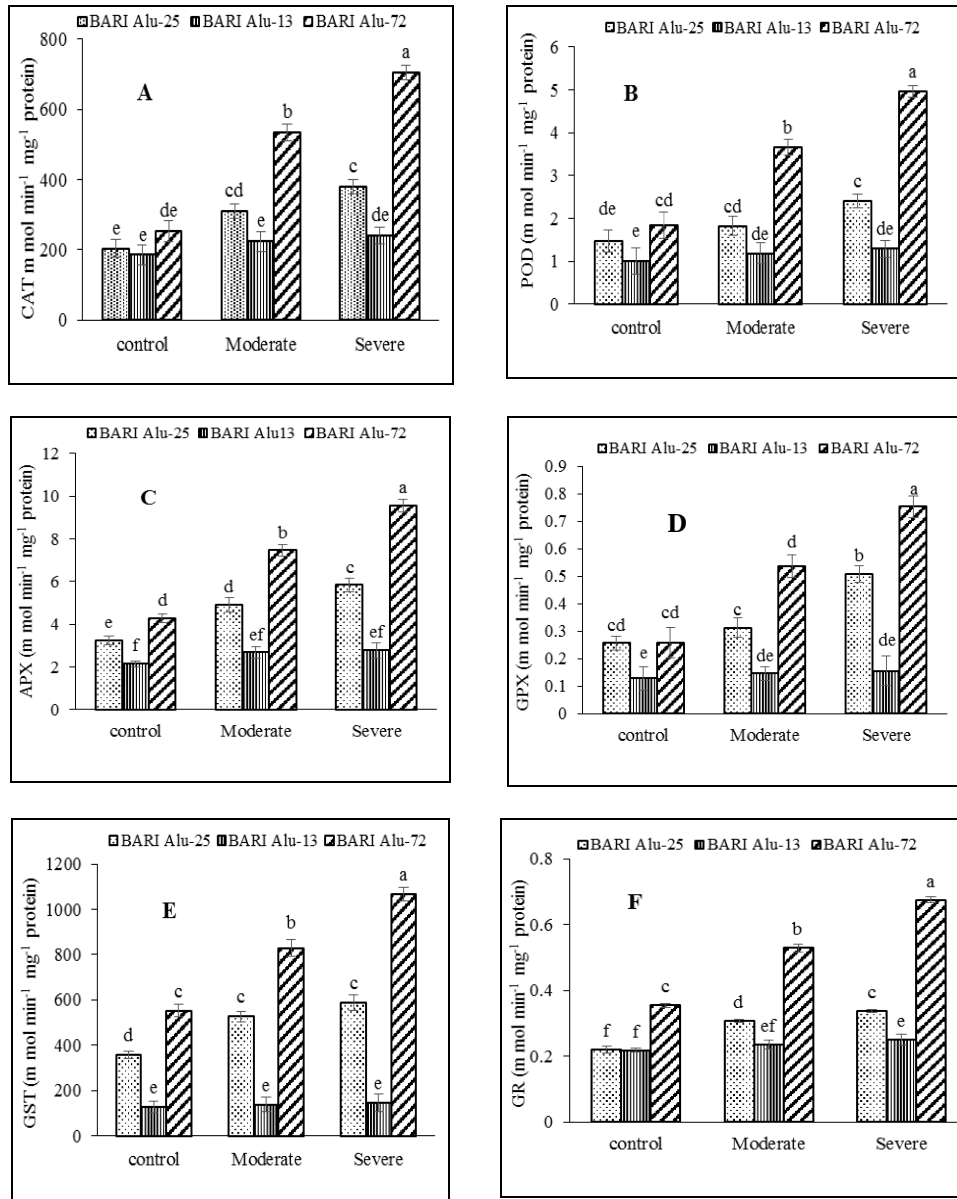


Fig. 5. Effect of salinity stress on antioxidant enzymes (A) CAT, (B) POD, (C) APX, (D) GPX (E) GST and (F) GR activities in the leaves of potato varieties at 60 DAP. Vertical bars indicate SE; Control = 0.2, Moderate = 6-8 and Severe = 10-12 dSm⁻¹.

Effect of salinity levels on biomass and yield plant⁻¹

Total dry matter (TDM)

In all the salinity treatments the total dry matter (TDM) decreased in all potato varieties (Fig. 6A). The TDM of 'BARI Alu-13' (sensitive to salt) decreased about 39 and 66% in moderate and severe salinity stress, respectively compared to control (no salinity). TDM reduction of 'BARI Alu-25' was moderate in both the stress conditions. However, the lowest TDM reduction was observed in 'BARI Alu-72' compared to others and maximum TDM production was also found in this variety under severe salinity condition. Based on the reduction of TDM, it can conclude that 'BARI Alu-72' was more tolerant and 'BARI Alu-25' relatively tolerant to salinity stress. Stem biomass accumulation was considered as an important trait to attain high tuber yield in potato. Salinity significantly affected partitioning of photosynthates between stems and tubers. Significant decrease in total biomass due to salinity had already been reported (Kondetti *et al.*, 2012).

Tuber yield

The yield of potato varieties was varied significantly among the treatment combinations. Reduced tuber yield plant⁻¹ was obtained at higher levels of salinity compared to control salinity level and gradual reduction in tuber yield was obtained with the increase of salinity (Fig 6B). Tuber yield at control (0.2 dSm⁻¹) salinity level ranged from 260-297 g plant⁻¹ whereas it ranged from 50-132 g at severe (10-12 dSm⁻¹) salinity level (Fig.5B). 'BARI Alu-25' (297 g) gave the highest yield, which was identical with other varieties at control. But yield reduction increased with increasing salinity levels. Under moderate salinity condition, 'BARI Alu-25', 'BARI Alu-13' and 'BARI Alu-72' showed 56, 66 and 30% of yield reduction, respectively over control. On the other hand, when plants were exposed to severe salinity stress, yield reduction was lowest in 'BARI Alu-72' (49%), moderate in 'BARI Alu-25' (67%) and highest in 'BARI Alu-13' (81%) compared to control. This finding agree with previous study that indicated yield reduction in potato varieties was upto 37% at 5.9 dSm⁻¹ salinity level (Van-Hoorn *et al.*, 1993). Moreover, Blom-Zandstra *et al.* (2004) reported 50% yield reduction in potato occurred under 7 dSm⁻¹ salinity stress.

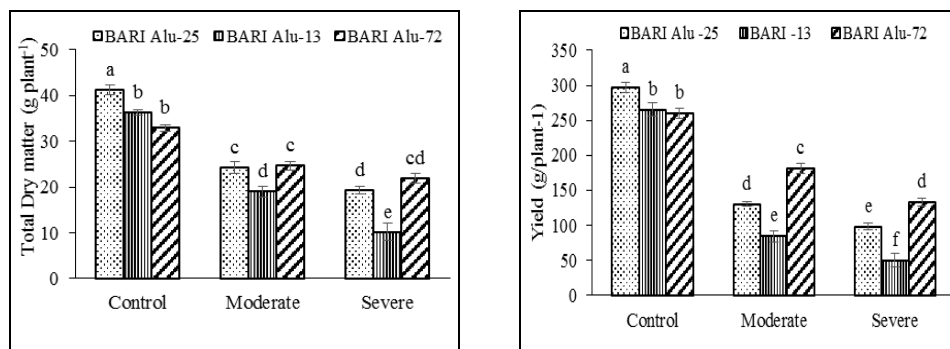


Fig. 6. Effect of salinity levels on (A) total dry matter (TDM) and (B) yield in potato varieties at 60 DAP. Vertical bars indicate SE; Control = 0.2, Moderate = 6-8 and Severe = 10-12 dSm⁻¹.

Conclusion

From the above discussion, it would be concluded that salinity impairs the production of plant biomass and yield in potato due to increase in Na⁺ concentration, MDA content and LOX activity. From our studies, salt tolerant potato variety was better performer or maintained superior physiological conditions by enhancing the activities of CAT, POD, APX, GPX, GR and GST antioxidant resulting in lower LOX and MDA production and better regulation of photosynthetic pigments. Higher K⁺ concentration in salt tolerant potato resulting in lower Na⁺ accumulation and Na⁺/K⁺ ratio, also contributed to its salinity stress tolerance.

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