

EVALUATION OF SALT TOLERANT GARLIC CULTIVARS BASED ON PHYSIOLOGICAL AND YIELD RESPONSES

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ABSTRACT

An aggravated salt concentration in soil restricts to intensify the crop productivity. In a salinity tolerance test, five *Allium sativum* cultivars viz. BARI Rashun-1, BARI Rashun-2, BARI Rashun-3, BARI Rashun-4 and Local (Natore local) were compared subjecting four level of salt stress viz. 0, 4, 8 and 12 dS m⁻¹. Total dry matter (TDM) (g) plant⁻¹, bulb yield and yield supporting traits of all cultivars were depressed with successively induced salt. But, TDM productions, yield and yield supporting traits were less degraded in BARI Rashun-4 and BARI Rashun-3 at 12 dSm⁻¹ salinity and had a stronger ability to continue constant osmotic potential maintaining the uttermost K⁺/Na⁺ ratio. The variety BARI Rashun-4 showed higher activity of antioxidant enzyme and less cell membrane damage at 12 dSm⁻¹ salt level. BARI Rashun-3 and BARI Rashun-4 were considered as a relatively saline tolerant due to higher antioxidant enzyme synthesis, ion homeostasis and less degradation of yield.

Keywords: Ascorbate peroxidase, Catalase, Peroxidase, Reactive oxygen species

INTRODUCTION

Salinity stress is a major inanimate problem that harms the agriculture by deteriorating the productive capacity all over the earth (Arif et al., 2020). It was computed that, almost 20% soil and even about partly of the earth's total arable land have been damaged by salt (Velmurugan et al., 2020). An extrapolation revealed that, global annual cost would be 27.3 billion US\$ due to hamper of crop manufacture in salt induce soil (Qadir et al., 2014). Therefore, identifications of salt tolerant cultivars would be a great endeavor to feed rising population of the world. Salt has a

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detrimental influence on crop intensification and productivity, which is interconnected to metabolic and physiological activities. Owing to the onset of osmotic, ionic and dietary imbalances, metabolic and physiological behaviors of crops are still being disrupted (Gerona et al., 2019). Osmotic stress arises in high salt concentration areas due to Na^+ and Cl^- absorption, which lowers the osmotic potential and inhibits water flow (Hamouda et al., 2016) and thereby causes dehydration that accelerates the osmotic effect to the internal tissue and ions concentration. Plant physiological changes as a result of salinity, including the changes in photosynthetic pigment accumulation, diffusion rate, leaf water potential ability, K^+ , Ca^{2+} and Mg^{2+} content (Ferdous et al., 2018) and causes a chain of responses like the stomatal closure and therefore a restricted CO_2 fixation (Hirdi et al., 2016).

Salinity accelerates the manufacture of reactive oxygen species (ROS), which are highly toxic to the cell and disturb cell redox homeostasis, finally causes oxidative stress. Therefore, plant cells are equipped with well evolved to accumulate the proteolytic and non-proteolytic antioxidant enzymes like catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX) which is strong quencher and scavenger of ROS. Yield and its related traits are inversely related to abiotic stress during plant growth, such as salt (Zorb et al., 2019). Many previous extensive literatures have been expressed that extreme salt stress has a depressing effect on crop morphological performances such as root-shoot length, leaf area, biomass production and among other things. Salinity affects growth differently in different species and to a lesser extent, in different variants within a species. Garlic (*Allium sativum*) is a highly nutritive crop; it contains large number of important enzymes, antioxidants and vitamins (Mohamed and Akladios, 2014; El-Saadony et al., 2017). Further, Garlic is a valuable spice used in a variety of dishes all over the world for its pungent taste as a seasoning or condiment. Garlic is also used to treat several of diseases, including incurable stomach and intestinal dysentery, typhoid, cholera, and lung diseases according to Ayurvedic and Unani medicine. Garlic clove's aqueous extracts substantially lower cholesterol levels. Garlic cultivars have become more susceptible to abiotic stress as a result of the rapid loss of genetic diversity caused by cultivar substitution. So, the foremost objective of this research was to examine the escalation and physiochemical responses of native and high yielding *Allium sativum* cultivars to diverse salinity stress and mean while to explore the association among salt tolerance. By understanding physiological aspects of garlic's reaction to salinity stress, researchers may be able to create salt-tolerant cultivars.

MATERIALS AND METHODS

Pot house research was carried out following Randomized Complete Block Design (RCBD) with six replications. Clove of Garlic (*Allium sativum*) varieties viz. BARI Rashun-1, BARI Rashun-2, BARI Rashun-3, BARI Rashun-4 and Natore local were planted in pots on 26 November, 2020. Five cloves of each variety were planted in plastic pots filled with soil and well decomposed farm yard maure at 4:1 volume ratio with a small five perforation system at the pots base for facilitating drainage. Each pot contained approximately 12 kg soil. Pots soils were nourished by fertilizer according to (FRG, 2018). Before the salt treatments, water and fertilizers were provided to all pots to achieve uniform soil conditions. The tested crops were subjected to different level of salinity stresses viz. 0 (control), 4, 8 and 12 dSm⁻¹ respectively by adding unpurified sea salt solution. The application of salt solution was started from 30 Days after emergence (DAE) with an increment of 5, 10 and 15 dSm⁻¹ for 4, 8 and 12 dSm⁻¹ salinity level respectively in every 7-10 days intervals and continued up to reach a desire concentration of salinity level. During the crop growing and harvesting period physiological and agronomic parameters were measured.

Physiological parameters

Chlorophyll synthesis (mg g⁻¹) in leaf was measured according to Arnon (1994). In brief, 0.5 g leaf sample was ruined in 80% acetone for 48 hours under dark condition. The pigment density was measured with UV-1800 spectrophotometer using wavelength 663nm for Chlorophyll *a* and 645 nm for Chlorophyll *b*. At 60 days after emergence, Na⁺ and K⁺ ion was measured following the protocol of Rohman et al. (2019). Relative water content was measured with protocol of Ben Ahmed et al. (2011) by using following formula:

$$\text{Relative water content (RWC)\%} = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100$$

Where, FW=Fresh weight (g), DW=Dry weight (g), TW=Turgid weight (g)

The level of lipid peroxidation (MDA) was expressed as nmol g⁻¹ fresh weight and assayed was performed according to Wu et al. (2003). In briefly, 1.5 ml leaf extract and 2.5 ml reaction solution (5% Trichloroacetic acid and 0.6% thiobarbituric acid) was mixed together and heated at 95°C for 15 minutes. The absorbance of the supernatant was recorded at 532 nm. The proline content was measured according to method of Bates et al. (1973).

Antioxidant enzyme

Antioxidant enzymes such as Catalase (CAT), Peroxidase (POD), and Ascorbate peroxidase (APX) were measured according to Wu et al. (2003). In brief, Catalase activity was measured out in a 3ml reaction volume containing 2.8 ml of 50 mM Tris-HCl buffer, 100 µl of enzyme extract and 100 µl of 300 mM H₂O₂ using UV spectrophotometer. Activity was determined at 240 nm wavelength, which measures

the decrease in absorbance for 30 second. POD activity was carried out in a 3ml reaction volume containing 2.7 ml of 50 mM Tris-HCl buffer, 100 μ l of enzyme extract and 100 μ l of 1.5% Guaicol, 100 μ l of 300 mM H_2O_2 using UV spectrophotometer. Activity was determined at 470 nm wavelength, which measures the decrease in absorbance for 60 second. APX activity was assayed using 50 mM Tris-HCl buffer and activity was determined at 290 nm for 60 second.

Agronomic observation

Agronomic parameters of Garlic cultivars like total dry matter weight ($g\ plant^{-1}$), yield contributing and bulb yield indices were measured, regardless of salinity level. Total dry matter weight ($g\ plant^{-1}$) measurement was started from 60 DAE, at 10 days intervals. Plants sample were divided into leaf and bulb categories. The separated parts were dried in an oven for 72 hours at $70^\circ C$ and dry weight was recorded.

Statistic analysis

Data analysis was performed using computer based software “R” developed by R core team (2019) for interpretation of results. Least significant different values were calculated at 0.05 probability level wherever the F-test was significant.

RESULTS AND DISCUSSION

Total dry matter (TDM)

TDM production varied significantly by Garlic cultivars at 70, 85, and 100 days after planting (DAP) and at harvest, regardless of salinity levels (Table 1). At 70 DAP; variety BARI Rashun-2 produced the maximum TDM ($4.76\ g\ plant^{-1}$) at control condition while the local variety produced the minimum ($2.60\ g\ plant^{-1}$) at $12\ dSm^{-1}$ salinity. At 85 DAP ($7.33\ g\ plant^{-1}$, 90 DAP ($7.56\ g\ plant^{-1}$) and at harvest ($9.40\ g\ plant^{-1}$) significantly maximum TDM was produced by the variety BARI Rashun-4 under control (no salinity) condition. TDM of the BARI Rashun-2, BARI Rashun-3 and BARI Rashun-4 was reduced by 43.7, 28.8 and 19.5%, respectively at harvest under $12\ dSm^{-1}$ salinity compared to $8\ dSm^{-1}$. Salinity is an ecological bottleneck to express genetic potentiality of crops. In this study, growths of garlic cultivars were significantly affected by an elevated level of salt stress. This reduction of plant growth and biomass potential under salt stress may be facilitated by reduction of water uptake by plant and excessive salt in plant body with transpiration stream causes wound to cells (Nassar et al., 2020), nutrient uptake (Parvin et al., 2019) and finally inhibiting cell division and elongation (Ahmad et al., 2018). In our study, BARI Rashun-4 showed the best growth at an elevated level of salinity ($12\ dSm^{-1}$) through the reduction of minimum dry matter over the control and considered as saline tolerant cultivars.

Table 1. Total dry matter (g plant⁻¹) production by Garlic cultivars under salinity levels

Salinity levels	Varieties	TDM (g plant ⁻¹)			
		70 DAP	85 DAP	100 DAP	At harvest
0 dSm ⁻¹	BARI Rashun-1	3.54 i	4.95 d	5.80 cd	6.35 ef
	BARI Rashun-2	4.76 a	4.65 e	5.70 c-e	8.97 a
	BARI Rashun-3	3.30 l	6.44 b	6.35 b	9.12 a
	BARI Rashun-4	3.81 h	7.33 a	7.56 a	9.40 a
	Natore Local	2.16 r	3.41 gh	5.41 de	6.74 c-e
4 dSm ⁻¹	BARI Rashun-1	3.35 j	4.14 f	5.48 c-e	6.96 cd
	BARI Rashun-2	4.63 c	3.46 gh	5.25 e	7.00 c
	BARI Rashun-3	3.28 m	5.69 c	5.97 bc	7.96 b
	BARI Rashun-4	4.36 d	6.51 b	5.31 de	8.12 b
	Natore Local	2.10 s	2.85 j	5.16 ef	6.00 fg
8 dSm ⁻¹	BARI Rashun-1	3.28 m	3.97 f	4.70 fg	5.00 h
	BARI Rashun-2	4.68 b	3.30 h	3.89 h	6.70 c-e
	BARI Rashun-3	3.33 k	5.00 d	4.69 fg	6.53 de
	BARI Rashun-4	4.21 e	5.51 c	4.67 fg	7.08 c
	Natore Local	2.86 p	1.90 m	1.98 k	2.93 j
12 dSm ⁻¹	BARI Rashun-1	3.00 o	2.10 l	2.62 j	2.33 k
	BARI Rashun-2	4.00 f	2.62 k	3.20 i	3.77 i
	BARI Rashun-3	3.10 n	3.11 i	3.82 h	4.65 h
	BARI Rashun-4	3.99 g	3.50 g	4.23 gh	5.70 g
	Natore Local	2.60 q	1.87 m	1.93 k	1.10 k
LSD _(0.05)		1.08	0.20	0.54	0.44
CV (%)		3.05	2.79	6.97	4.32

Yield and yield contributing characters

All yield contributing characters were reduced significantly when the average root zone of crop varieties gradually exceeded to an augmented salinity level (Table 2). The variety BARI Rashun-3 generated the maximum single bulb weight (5.91 g) at the severe salinity (12 dSm⁻¹) followed by the variety BARI Rashun-4. Different salinity levels had an impact on bulb length and diameter. BARI Rashun-4 had the longest bulb length of 22.22 mm and the largest bulb diameter of 24.98 mm at 12 ds m⁻¹. With increased salinity, bulb development and yield reduced considerably (Table 1). At 12 ds m⁻¹ salinity level, the bulb yield (g) of five plants was severely reduced (71.6%) compared to the control. At 8 ds m⁻¹ of salinity, the reduction percentages

were 68.3, 63.3, 51.8 and 41.9% in case of BARI Rashun-1, BARI Rashun-2, BARI Rashun-3 and BARI Rashun-4, respectively over control. At highest salinity (12 ds m⁻¹); BARI Rashun-3 (60.8%) and BARI Rashun-4 (61.9%) showed good tolerance with $\geq 60\%$ yield reduction over control (Table 2). In this study, salinity stress had a significant impact on the agronomic traits of *Allium sativum*. Single bulb weight, bulb size was significantly affected at an elevated salt stress which may be accredited to inability of the plants to synthesis of photosynthetic pigments and ionic imbalance. In accordance with present study Ashraf (2009) found that, yield supporting parameters and yield reduction was accelerated due to reductions of leaf chlorophyll concentrations and ionic imbalance.

Table 2. Yield contributing parameters of Garlic varieties under different salinity levels

Salinity levels	Varieties	Single bulb weight (g)	Bulb length (mm)	Bulb diameter (mm)	Bulb yield of five plants (g)
0 dSm ⁻¹	BARI Rashun-1	14.96 a	28.81 a-c	33.45 a	74.8 a
	BARI Rashun-2	12.74 b	31.14 a	30.46 b	63.7 b
	BARI Rashun-3	13.33 b	30.16 ab	33.52 a	66.6 b
	BARI Rashun-4	15.56 a	27.96 b-d	34.02 a	77.7 a
	Natore Local	11.11 c	26.30 c-f	30.50 b	55.6 c
4 dSm ⁻¹	BARI Rashun-1	7.64 ef	26.45 c-e	26.72 cd	33.0 ef
	BARI Rashun-2	7.36 f	24.470 e-g	25.55 c-f	36.8 e
	BARI Rashun-3	9.54 d	26.53 c-e	30.65 b	38.1 e
	BARI Rashun-4	9.02 de	24.13 e-g	24.10 e-g	47.4 d
	Natore Local	5.61 gh	21.87 g-i	23.49 f-h	28.1 fg
8 dSm ⁻¹	BARI Rashun-1	4.74 hi	21.67 g-i	22.89 gh	23.7 gh
	BARI Rashun-2	4.68 h-j	25.17 d-f	25.10 j	23.4 g-i
	BARI Rashun-3	6.42 fg	25.44 d-f	27.72 c	32.1 ef
	BARI Rashun-4	6.60 fg	20.95 hi	26.25 c-e	45.1 d
	Natore Local	3.66 i-k	19.87 i	20.31 ij	18.3 h-j
12dSm ⁻¹	BARI Rashun-1	2.27 k	20.05 i	18.52 ij	11.4 j
	BARI Rashun-2	3.31 jk	16.33 j	20.96 hi	16.6 ij
	BARI Rashun-3	5.91 gh	23.59 f-h	22.90 gh	26.1 fg
	BARI Rashun-4	5.23 gh	22.22 g-i	24.98 d-g	29.6 fg
	Natore Local	2.46 k	15.95 j	14.68 k	12.3 j
LSD _{0.05}		1.39	2.80	2.55	6.91
CV (%)		5.50	7.07	6.06	11.00

Potassium and Sodium ion in leaf tissue

With augmented salt stress from 4 to 12 ds m⁻¹, potassium (K⁺) concentration increased 10.5, 15.9, 17.5, 18.3 and 14.3% in BARI Rashun⁻¹, BARI Rashun-2, BARI Rashun-3, BARI Rashun-4, and Natore local, respectively (Fig. 1). Regardless of genotypes, the sodium (Na⁺) content in leaf tissue was considerably lower in the non-salt than the salt conditions. In salt stress, elevation was more pronounced in BARI Rashun-1 (158.5 and 34.0%) and BARI Rashun-2 (145.9 and 63.0%) at 12 dSm⁻¹ salinity than that of 4 dSm⁻¹ and 8 dSm⁻¹, respectively. The variety BARI Rashun-4 uptake the lowest Na⁺ (1090 ppm) under 12 dSm⁻¹ salinity which were 95.7 and 24.0% higher than 4 ds m⁻¹ and 8 ds m⁻¹ respectively. At 12 ds m⁻¹ salinity, the highest K⁺/Na⁺ was observed in BARI Rashun-4 (4.45) followed by the variety BARI Rashun-3 (4.20) but it was statistically similar with each other. Ion like Na⁺, K⁺ and Ca⁺ and their K⁺/Na⁺ and Ca⁺/Na⁺ ratios are considered an important indicator for tolerance screening (Khorshidi et al., 2009). Tolerant genotypes prevent salt from entering the roots transport less salt to the plant's aerial components. In our study, simultaneously increase Na⁺ and decrease K⁺ in shoots of BARI Rashun-1, BARI rashun-2 and local cultivar was found at 12 ds m⁻¹ salinity but the changes in Na⁺ and K⁺ were less accentuated in the latter two species that considered as more salt-tolerant. In earlier literature stated that high levels of K⁺ ion in young expanding tissue are associated with salt tolerance in many plant species (BandeH-Hagh et al., 2008). In this study, BARI Rashun-4 showed the considerable K⁺/Na⁺ ratio at 12 ds m⁻¹.

Effect of salt stress on physiological activity

Malondialdehyde increase indicates the rate of lipid peroxidation and oxidative damage. The MDA levels were elevated to 63.2, 64.9, 20.0, 14.0 and 85.2% in BARI Rashun-1, BARI Rashun-2, BARI Rashun-3, BARI Rashun-4 and Natore local cultivars, respectively, at 12 ds m⁻¹ salt, as compared to unstressed plants (Fig. 2).

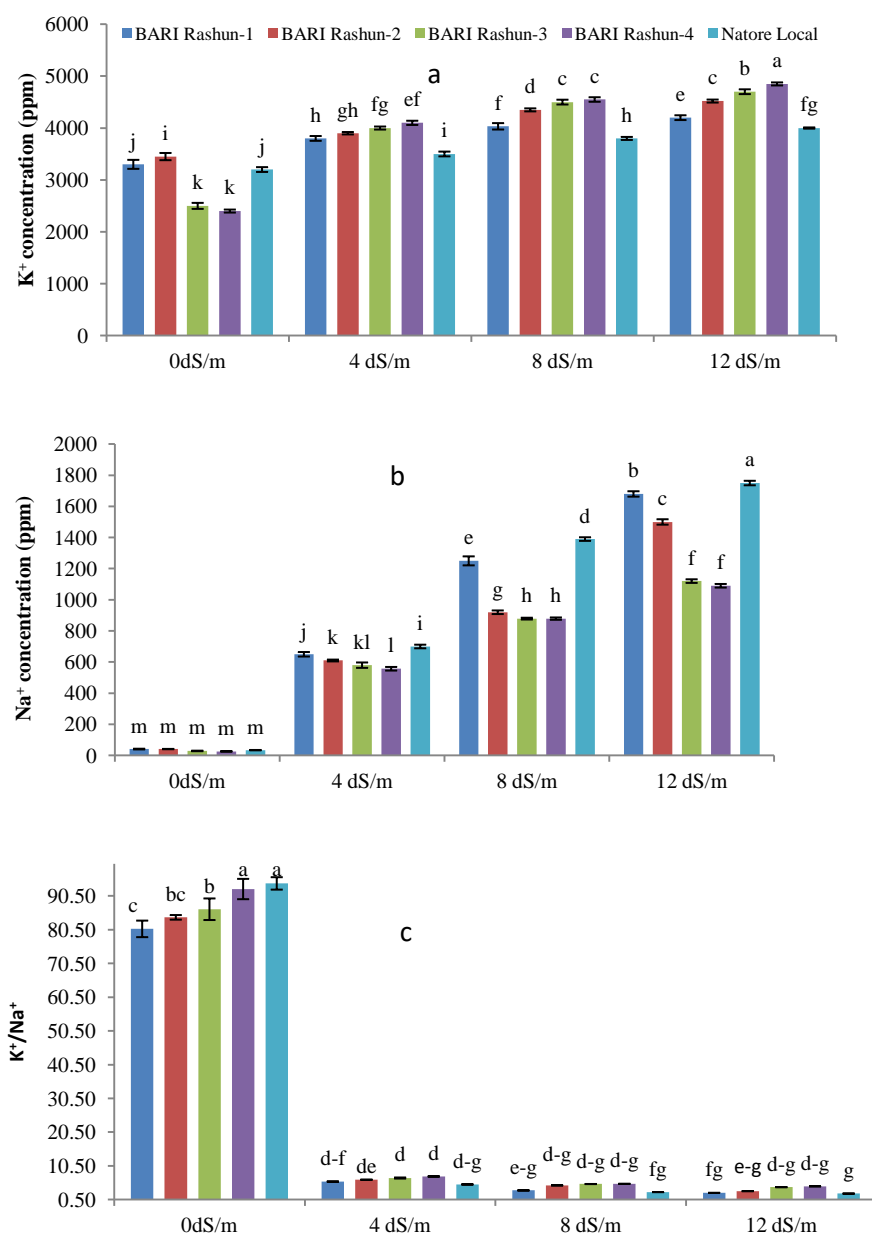


Figure 1. Accumulations of (a) Na^+ , (b) K^+ and (c) K^+/Na^+ ratio by Garlic cultivars under varying salinity level

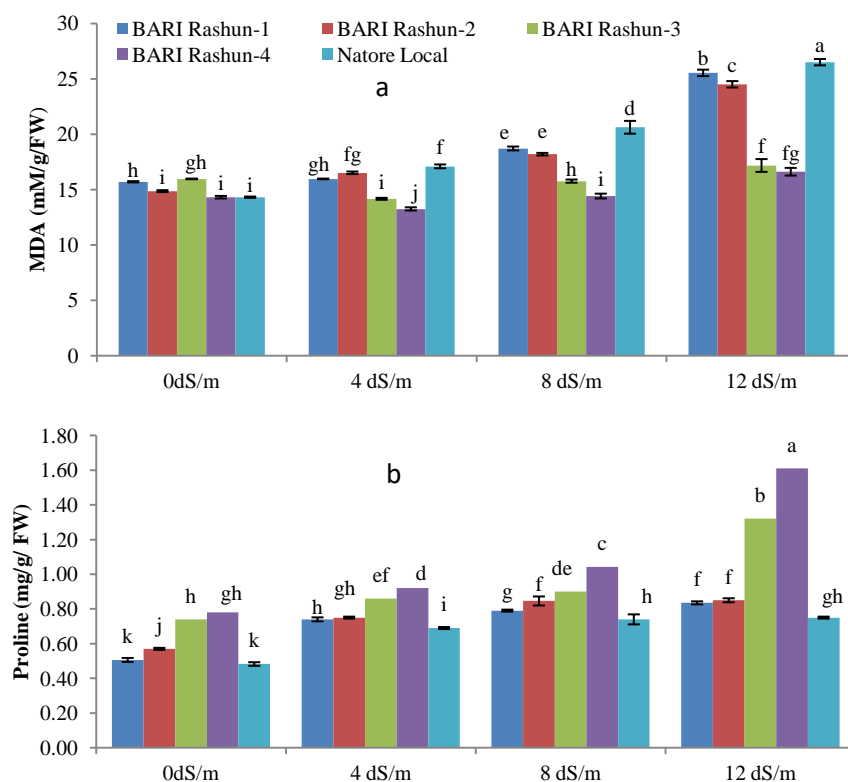


Figure 2. (a) Malondialdehyde (MDA) and (b) proline level of Garlic cultivars under varying salinity level

The variety BARI Rashun-4 accumulated maximum level of proline, which ranged from 0.78 mg g^{-1} in the control to 1.60 mg g^{-1} with 12 ds m^{-1} of salt. BARI Rashun-4 accumulated 92, 88, 21.2 and 113.3% higher proline than BARI Rashun-1, BARI Rashun-2, BARI Rashun-3 and Natore local, respectively, at 12 ds m^{-1} salt stress (Fig. 2). Lipid peroxidation indicated by MDA contents, happening during the oxidation of polyunsaturated fatty acids, can be categorized as an indicator for oxidative damage of stresses (Yin et al., 2011). Our results showed MDA content was increased remarkably under saline conditions. Among the cultivars, BARI Rashun-3 and BARI Rashun-4 had the lowest leaf MDA contents and highest synthesis of proline under 12 ds m^{-1} saline conditions. Under, 12 ds m^{-1} salinity, the variety BARI Rashun-1 and BARI Rashun-4 showed 7.40 and 3.70% higher chlorophyll *a* (Chl *a*) content compared to the control. Although, Chlorophyll *b* (Chl *b*) concentration decreased for all cultivars at 12 ds m^{-1} salinity over the control (no salinity) but total chlorophyll (Chl *a+b*) concentration was increased 6.75% in

variety BARI Rashun-1 and 2.66% in BARI Rashun-4 (Fig. 3). Leaf water potential in stressed plant was comparatively lower than the control plants (Fig.4). Water potential of the cultivars was reduced from 1.78 to 6.13% due to the severity of salt stress. BARI Rashun-4 had the highest water potential, with the lowest (3.50%) dehydration, whereas Natore local had the lowest, with the highest (9.13%) dehydration at 12 dS m⁻¹ salt over control. Our results revealed that, salinity had a significant positive impact on photosynthetic pigment like Chl (a) and total chlorophyll accumulation in BARI Rashun-1 and BARI Rashun-4 at 12 ds m⁻¹ salinity over control. These variations were due to genetic potentiality towards the salt stress. Our results were also similar with previous conducted studies on crop plants like sesame and sunflower by Bazrafshan and Ehsanzadeh (2014) and Rivelli et al. (2010) respectively.

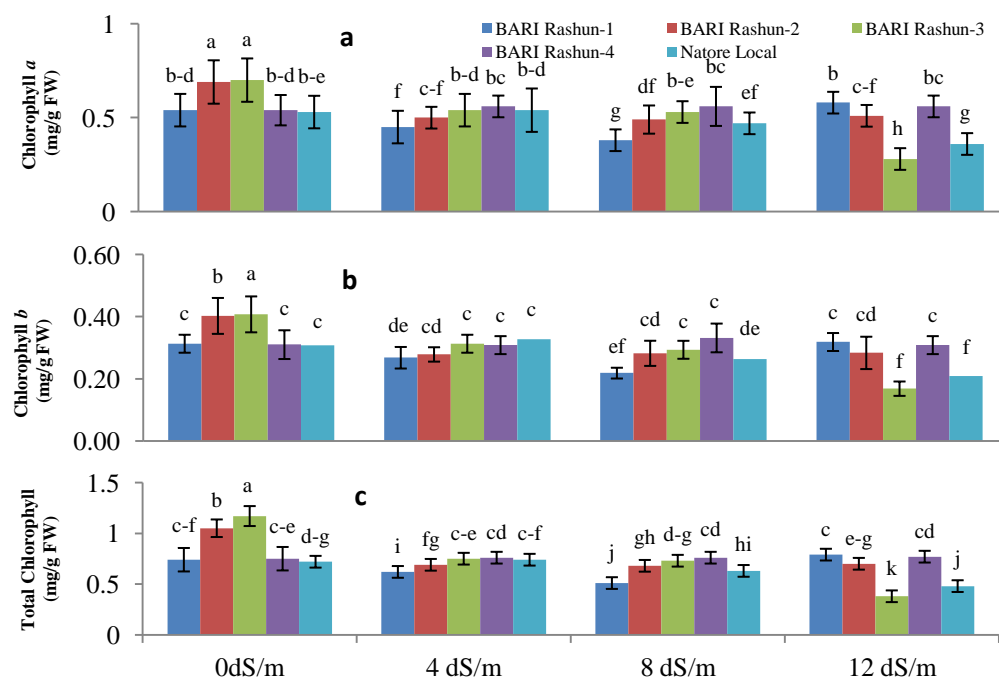


Figure 3. Photosynthetic pigments (a): Chl *a*; (b): Chl *b*; (c): Chl(*a*+*b*) accumulations by Garlic cultivars under varying salinity stress

Effects of salt stress on Antioxidant enzymes activity: In the present study, the activity of antioxidant enzymes like CAT, POD and APX were measured in all tested Garlic cultivars due to investigate the role of these enzymes to salinity tolerance mechanism (Fig.4).

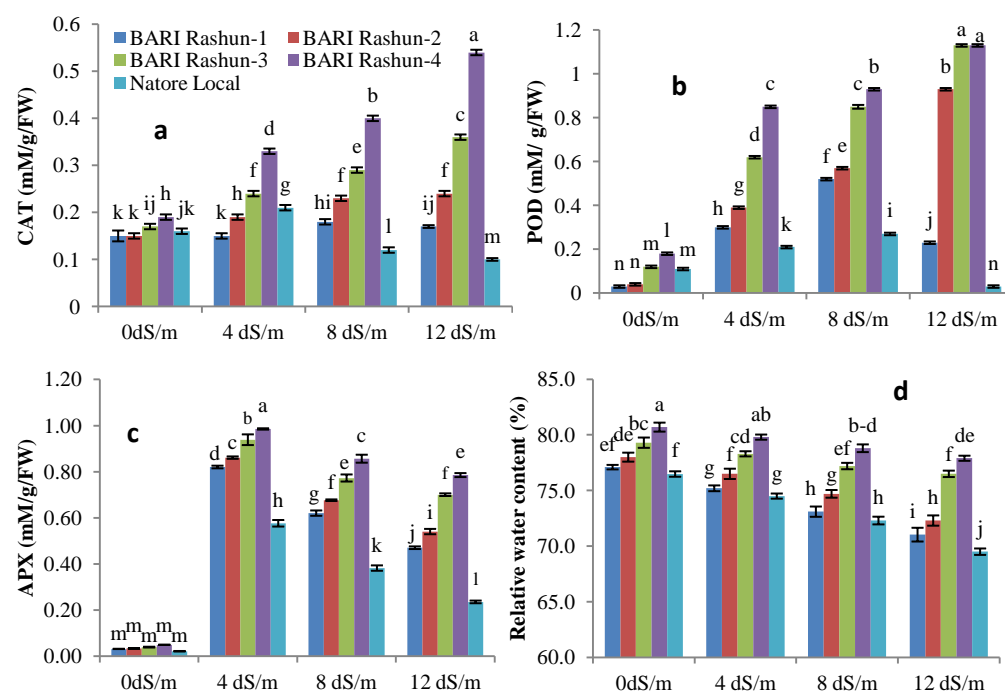


Figure 4. Antioxidant enzymes catalase (a), Peroxidase dismutase (b), Ascorbate peroxidase (c), Relative water content (d) of garlic cultivars under varying salinity stress

Activities of CAT in BARI Rashun-3 and BARI Rashun-4 had reached the maximum level, particularly treated with 12 ds m⁻¹ of salt and was significantly higher than that of other salt stressed, which had increased by 52.7 and 64.8% compared with control. The variety BARI Rashun-3 and BARI Rashun-4 accumulated statistically similar and maximum level (1.13 mM g⁻¹FW⁻¹) of POD at 12 ds m⁻¹ compared to other salt stresses. At 12 ds m⁻¹ salt, APX activities were decreased in BARI Rashun-1 (37.5%); BARI Rashun-2 (22.2%); BARI Rashun-3 (22.2%); BARI Rashun-4 (20.0%), Natore local (33.3%) in compared to 4 dS m⁻¹ salts. The activity of the APX was minimumly reduced in BARI Rashun-3 and BARI Rashun-4 at 12 dSm⁻¹ salt stress that indicates the higher protective mechanism against ROS than other cultivars. To manage with oxidative damage under stress condition, plants have developed an antioxidant defense mechanism includes the antioxidant enzymes SOD, APX, POD, and CAT (Ali et al., 2017; Polash et al., 2019). Antioxidant enzyme like CAT converts this H₂O₂ in water and oxygen and POD also helps to efficiently sift H₂O₂. The results of phenotypic and physiological studies and antioxidant defense system showed that BARI Rashun-4 & BARI Rashun-3 is more tolerant than other cultivars. Based on all phenotypic results of the current study, it is suggested that the tolerance of the Garlic may due to higher concentration of antioxidants enzymes activities viz. POD, CAT, APX and the lower lipid peroxidation level in the leaves.

CONCLUSION

Results revealed that higher antioxidant activity like CAT, POD, APX, proline and lower lipid peroxidation as well as higher yield was observed in BARI Rashun-4 and BARI rashun-3 at 12 ds m⁻¹ salinity level. Besides, these varieties also showed the maximum K⁺/Na⁺ ratios at higher salinity levels which indicated their tolerancy against salinity.

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