Plant Archives Volume 20 No. 2, 2020 pp. 3742-3746 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EFFECT OF SALT (NACL) STRESS ON ANTIOXIDATIVE CHARACTERISTICS, OSMOLYTES AND ABA IN TWO SESAME (*SESAMUM INDICUM* L.) VARIETIES

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Abstract

Two sesame varieties (TMV-6 and VRI-3) subjected to salt stress of different concentrations (0, 40, 80 and 120mM) as a basal dose and sampling was done in leaves on 30th Days After Treatment (DAT), Higher antioxidant enzyme activities (superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), ascorbate peroxidase (APX), peroxidase (POD) were observed in the leaf extracts of TMV-6, while the lowest activities were recorded with VRI-3. The leaves of TMV-6 accumulated more proline, glycine betaine and abscisic acid under salt stress while lower content in VRI-3. Our data demonstrated that TMV-6 have efficient antioxidative characteristics with osmoregulation which could provide better protection against oxidative stress in leaves under salt stressed conditions.

Key words : Abscisic acid, antioxidative enzymes, osmolytes, salt stress, *Sesamum indicum*.

Introduction

Plants experience a multiple of stress, of which salt stress is important one which affects tremendously the physiology of plants (Causin *et al.,* 2020). The stresses most commonly associated with water deficits are drought, high salinity and low temperature (Bohnert *et al.,* 1994). When CO_2 fixation is limited because of stomata closure caused by water deficit, the rate of active oxygen formation increases in chloroplasts because an excess of excitation energy that is not dissipated by the protective mechanisms, is used to form reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide ($.O_2^-$), hydroxyl radicals (.OH) and singlet oxygen ($^1O_2$) (Mallik *et al.,* 2011). Plants possess defense antioxidant mechanisms, which can overcome this oxygen toxicity and delay the deleterious effects of free radicals and these ROS attack lipids, proteins and nucleic acids, causing lipid peroxidation, protein denaturation and DNA mutation (Shao *et al.,* 2008). The enzymatic system in turn includes superoxide dismutase (SOD), which catalyze the reaction from superoxide ($.O_2^-$) to H_2O_2 and catalase (CAT), guaiacol-type peroxidases and enzymes of the ascorbate-glutathione cycle, *e.g.* ascorbate peroxidase (APX), peroxidase (POD) and glutathione reductase (GR), which function to detoxify the H_2O_2 produced (Foyer, 2018). Accumulation of protective solutes like proline and glycine betaine is an unique plant response to environmental stresses, particularly to salt stress (Wutipraditkul *et al.,* 2015). Abscisic acid (ABA), the phytohormone plays prominent role in various physiological and biochemical processes related to environmental stresses (Khadri *et al.,* 2007). Sesame (*Sesamum indicum* L.) is one of the world's oldest spice and oil seed crop grown mainly for its seeds that contain approximately 50% oil and 25% protein. In this study, we investigated the relationship between salinity stress with antioxidant, osmolytes and ABA responses in two sesame varieties.

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Materials and Methods

The certified sesame seeds (varieties: TMV-6 and VRI-3) were procured from PASIC, Pondicherry. Seeds with uniform size were selected and the plants were raised in pots containing red and clay soil. After 20 days, seedings were thinned and plants of uniform vigor were maintained in each pot. The maximum irradiance (PAR,

400-700nm) available during growth was 1800-2000 µmol m^{-2} s^{-1} on clear day. Daily maximum and minimum temperatures were 29-33ºC and 20-22ºC, respectively. After germination, plants were watered for the first 20 days.

The seedlings were divided into four groups. One group of seedlings was maintained under non-salinized condition which served as control. The watering solution for control plants consists of tap water and one-fourth strength of Hoagland nutrients (Hoagland and Arnon, 1950). Other three group were salinized by irrigation daily to soil capacity (500 ml d^{-1}) with the nutrient medium containing 40mM, 80mM and 120mM NaCl. All the plants used in this study were of comparable size. Young and fully matured leaves were taken at 30 days after salinity treatments for all the experiments described below.

Enzymes are extracted from leaf tissues using an ice-cold mortar and pestle, 60 mg polyvinyl polypyrrolidone and 1ml of following optimized extraction media: SOD (100mM K-phosphate buffer, pH 7.8, 0.1mM EDTA and 0.1% Triton X-100); CAT, GR (100mM K-phosphate buffer, pH 7.0 and 0.1mM EDTA); APX (50mM K-phosphate buffer, pH 7.0 and 1mM ascorbate) and Peroxidase (POD) (50mM K-phosphate buffer, pH 7.0). The resulting slurry was centrifuged at 15000Xg for 15min at 4ºC. The supernatants were collected and used for the assays of protein content by the method of Bradford, (1976) and enzyme activities.

The activity of SOD (EC 1.15.1.1) was assayed by measuring its ability to inhibit the photochemical reduction of nitro-blue tetrazolium (NBT) adopting the method of Beauchamp and Fridovich, (1971). The activity of CAT (EC 1.11.1.6) was estimated by measuring the rate of decomposition of H_2O_2 by the method of Havir and McHale, (1987). GR (EC 1.6.4.2) activity was measured by oxidized GSH – dependent oxidation of NADPH using the method of Foyer and Halliwell, (1976). APX (EC 1.11.1.11) activity was estimated by monitoring the decline in absorbance at 240nm following Nakano and Asada, (1981). POD (EC 1.11.1.7) activities were determined with guaiacol at 470nm (extinction coefficient 25.2mM cm^{-1}) following the method of Polle *et al.,* (1994).

The content of proline was estimated according to Bates *et al.,* (1973). Glycine betaine content in the leaf extracts was determined according to Storey and Wyn Jones, (1977). ABA content was determined by ELISA reader (Multiscope, Labsystems, Finland) at optical density $A_{490.}$ following Daie and Wyse, (1982).

For statistical analysis, five samples were taken for each treatment from five individual plants. Student's t-test and analysis of variance (ANOVA) were applied for analyzing significant differences between the control and treated plants ($P<0.05$).

Results and Discussion

Salinity is one of the most widespread environmental threats to global crop production, especially in arid and semi-arid climates, where land degradation, water shortage and population growth are already a major concern (Munns and Tester, 2008; Geissler *et al.,* 2010). Reactive oxygen species (ROS) are produced continuously as products of various metabolic pathways, even when plants are growing under non-stress conditions. These ROS are scavenged by a variety of antioxidant defense systems that prevent ROS from reaching toxic levels. Antioxidant levels and the activities of ROS scavenging enzymes have been correlated with tolerance to several different environmental stresses (Sharma *et al.,* 2012). SOD converts superoxide radical to hydrogen peroxide (H_2O_2) and molecular oxygen (O_2) (Naji and Devaraj, 2011). In this study, both the sesame varieties treated with salinity showed increased SOD activity compared to control plants (Table 1). For instance, SOD activity was enhanced to the tune of 64% in TNV-6 and 32% in VRI-3 as compared to respective control plants under higher (120mM) salinity treatment. Although activity of SOD in the two sesame varieties in response to high salinity treatment may suffice to withstand the amount of oxidative stress, our results clearly show that TMV-6 is more tolerant than VRI-3.

Catalase (CAT) and peroxidase (POD and APX) appear to play an essential protective role in the scavenging processes when coordinated with SOD activity (Hossain *et al.,* 2017). They are chloroplastic or cytosolic enzymes which scavenge H_2O_2 generated primarily through SOD action (Scandalios, 1993). An increase in the activity of CAT was observed in both varieties of salinity treatment (Table 1). The activities of guaiacol peroxidase (POD) and ascorbate peroxidase (APX) increased almost coordinately with SOD activity in both sesame varieties (Table 1). As observed in case of SOD, higher activities of CAT, POD and APX were observed in TMV-6. The reduction of H_2O_2 by ascorbate–glutathione cycle is an extremely efficient reaction sequence that dissipates energy and aids in the adjustment of ATP/NADPH ratios at times, when the severity of the salinity is more (Hossain *et al.,* 2017). A significant increase in the POD activity, using guaiacal as an artificial substrate under the stress conditions like salinity, indicates the formation of large amounts of H_2O_2 in sesame leaves which indicates that sesame is capable of effectively

Table 1: Influence of salinity stress on antioxidant enzyme activities activity in two Sesame Varieties.

Variety & Parameter	Salinity treatments (mM)			
	Control	40	80	120
Superoxide dismutase (units/mg protein/min)				
TMV-6	118.11	192.38	250.19	332.45
	±6.10	±6.73	±7.22	±7.85
VRI-3	116.74	152.53	167.21	172.53
	±5.17	±6.52	±6.75	±7.13
Catalase (mmol/mg protein/min)				
TMV-6	16.89	26.72	34.51	45.62
	±2.05	±2.53	±2.66	±3.52
VRI-3	15.48	18.39	21.40	22.53
	±1.97	±2.44	±2.85	±2.60
Glutathione reductase (mmol /mg protein/min)				
TMV-6	37.81	54.72	72.12	94.72
	±3.79	±3.12	±3.26	±4.44
VRI-3	36.69	38.92	43.05	50.51
	±3.71	±3.04	±3.15	±3.91
Ascorbate peroxidase (mmol/mg protein/min)				
TMV-6	27.69	34.17	49.19	67.51
	±2.08	±2.18	±2.83	±3.37
VRI-3	25.88	29.51	31.69	34.15
	±2.01	±2.11	±2.26	±3.05
Peroxidase (mmol/mg protein/min)				
TMV-6	12.65	16.29	27.66	35.76
	±1.31	±1.40	±1.49	±1.54
VRI-3	11.57	13.80	14.72	16.84
	±1.24	±1.37	±1.44	±1.48

The data are expressed as mean ±s.e. for five independent determinations (P<0.05).

Table 2: Effect of salinity stress on proline, glycine betaine and abscisic acid content in two sesame varieties.

Variety & Parameter	Salinity treatments (mM)			
	Control	40	80	120
Proline (mg/gfw)				
TMV-6	2.32	3.18	4.96	6.19
	±0.24	±0.26	±0.31	±0.46
VRI-3	1.95	2.12	2.29	2.95
	±0.074	±0.090	±0.15	±0.19
Glycine betaine (mg/gfw)				
TMV-6	5.74	8.62	10.22	13.79
	±0.22	±0.34	±0.40	±0.51
VRI-3	5.02	6.24	6.92	7.16
	±0.12	±0.25	±0.29	±0.35
Abscisic acid (mg/gfw)				
TMV-6	8.14	12.19	15.20	18.83
	±0.94	±1.10	±2.14	±2.36
VRI-3	7.47	8.11	9.12	10.15
	±0.73	±0.98	±1.05	±1.23

The data are expressed as mean ±s.e. for five independent determinations (P<0.05).

scavenging the ROS for the production of certain secondary metabolites to withstand during salinity stress. An increase in the GR activity was also observed in both sesame varieties table 1 and ascribe this due to *de nove* synthesis (Kaymakanova *et al.,* 2010). In this study, 60% of GR activity was observed in TMV-6, while 27% in VRI-3 under high salinity treatments. The elevated levels of GR might be able to increase the ratio of $NADP^+$/ NADPH, thereby ensuring the availability of $NADP^+$ to accept electrons from the photosynthetic electron transport chain (Foyer, 2018). From this study, it is clear that the damage which was inflicted by salinity can be ameliorated by over–expression of antioxidant enzymes as noticed in TMV-6 and there are certain variations in the activity of these antioxidant enzymes between two varieties to counteract the stresses.

Plants alter their metabolism in various ways to cope with stress induced altercations and these changes include production of compatible solutes to stabilize cellular structures and maintenance of cell turgor by osmotic adjustment (Kahlaoui *et al.,* 2018). In the present study, Proline and glycinebetaine are a compatible solute, which rapidly accumulates in order to distribute electrolytes between cytosolic and vacuolar compartments in response to salinity stress and contributes to stabilization of sub-cellular structures, scavenging free radicals and buffering cellular redox potential (Torre-Gonzalez *et al.,* 2018). In the present study, variety TMV-6 accumulated proline by 63% and glycine betaine by 58% when compared to respective control plants at 120mM salinity treatments (Table 2). Proline and glycine betaine are known to serve as nitrogen and carbon source which can be used as during recovery from the stress (Torre-Gonzalez *et al.,* 2018). These compatible solutes also involved in cell osmoregulations and protects the photosystem II (PS II) complex by stabilizing the association of the extrinsic PS II complex

proteins under salt stress (Rantein *et al.,* 2002). Chen and Murata, (2008) have proposed that in addition to other roles, proline and glycine betaine could be involved in inhibiting ROS accumulation, protection of photosynthetic machinery, activation of some stress related genes and membrane protection. Present investigation indicates that accumulation of more proline and glycine betaine in TMV-6 at all salinity treatments showed its tolerancy over VRI-3.

The phytohormone abscisic acid (ABA) is the main hormone known to regulate various stress responses in a variety of species from green algae to angiosperms (Yu *et al.,* 2019) and its levels increase significantly in plants under stress conditions and ABA is thought to serve as a key stress-response regulator (Prerostova *et al.,* 2017), In our study, TMV-6 possessed nearly 1.5-2 fold higher amount of ABA compared to VRI-3 (Table 2). For instance, at 120mM salinity, TMV-6 accumulated 18.83mg/gfw of ABA when compared to control plants (8.14mg/gfw). Accumulation of ABA in higher plants is well known to limit the transpirational losses particularly under water deficit conditions (Negin and Moshelion, 2016). ABA accumulation in higher plants was reported to be related to oxidative stress tolerance in plants (Bellaire *et al.,* 2000), protecting photosynthesis (Yang *et al.,* 2006) and by triggering other metabolic adjustments, including the induction of stress proteins and osmolytes (Finkelstein, 2013).

Conclusion

The present study clearly shows that TMV-6 is superior with respect to its antioxidant defense systems, osmoprotectant and ABA accumulation than VRI-3. Such studies can be used in sesame breeding programmes or transgenic sesame research to generable plants with elevated activities of antioxidant systems for improved tolerance to salinity.

Acknowledgement

The authors thankful to Dr. R. Panneerselvam, Prof. and Head, Department of Botany, Annamalai University for providing necessary Lab facilities.

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