International Journal on Emerging Technologies **10**(4): 131-136(2019)

ISSN No. (Print): 0975-8364
ISSN No. (Online): 2249-3255

Salt (NaCl) Stress-induced Antioxidant Enzyme activities, Osmolytes, ABA, Lipid Peroxidation and Electrolytic leakage in Two Ragi (*Eleusine coracana* (L.) Gaertn) varieties

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(Received 29 August 2019, Revised 25 October 2019, Accepted 02 November 2019)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: In the present study, the effect of salt (NaCl) on antioxidant enzymes, osmolytes (proline and glycine betaine), abscisic acid (ABA), lipid peroxidation and electrolytic leakage were investigated in two ragi varieties (Indaf-7 and PAIYUR-1). Salinity was given as a basal dose of different concentrations (0, 40, 80, 120mM) and sampling was done in leaves after 30 days of treatments. The activities of superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (POD), ascorbate peroxidase (APX) and glutathione reductase (GR) were assayed in the leaf extracts of control and salt treated plants. Antioxidant enzyme activities were enhanced in two ragi varieties in response to salinity treatment. However, Indaf-7 showed significantly higher activities of all the five antioxidant enzymes in response to salinity compared to leaves of PAIYUR-1. Foliar content of osmolytes and phytohormone (abscisic acid) showed variation in two ragi varieties under salt stressed conditions. The leaves of Indaf-7 accumulated more proline, glycine betaine and abscisic acid under salt stress compared to PAIYUR-1. Membrane lipid peroxidation and electrolytic leakage were lower in the leaves of Indaf-7 than PAIYUR-1. The results show that variety Indaf-7 exhibits an efficient antioxidant regulatory mechanism and osmoprotectants, which could prevent the oxidative damage in the leaves caused by salt stress.

Keywords: Abscisic acid, antioxidant enzymes, electrolytic leakage, lipid peroxidation, osmolytes, ragi, salt.

I. INTRODUCTION

Salinity is an environmental stress that limits growth and development in plants and is a major constraint for the global agricultural production [1, 2]. Finger millet (*Eleusine coracana* (L.) Gaertn) is an important minor cereal in India, rich in calcium, dietary fiber and known for its health benefits. The stresses most commonly associated with water deficits are drought, high salinity and low temperature [3]. 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 ($^1O_2$) [4, 5]. 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 [6]. Plants are endowed with a complex antioxidant system to cope with ROS [7], which includes three general classes: (1) lipid-soluble, membrane-associated antioxidants (e.g. ∝-tocopherol, β-carotene); (2) small, water-soluble antioxidant molecules (e.g. ascorbate, glutathione); and 3. enzymatic antioxidants. 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 [8]. Melondialdehyde (MDA) is decomposition product of polyunsaturated fatty acids of membranes under stress and the rate of lipid peroxidation level in terms of MDA can therefore be used as an indication to evaluate the tolerance of plants to oxidative stress as well as the sensitivity of plants to salt stress [9]. Cell membrane stability is frequently related to salt tolerance in plants [10] and electrolytic leakage is usually used as an indicator of membrane injuries in salt treated plants [11]. Proline and glycine betaine are two major organic osmolytes that accumulate in a variety of plant species in response to environmental stresses such as drought, salinity, extreme temperatures, UV radiation and heavy metals [12]. Abscisic acid (ABA), the phytohormone plays prominent role in various physiological and biochemical processes related to environmental stresses [13]. In this study, we investigated the relationship between salinity stress and the antioxidant, osmolytes and ABA responses in two ragi varieties. To find out the severity of salinity, also measured the membrane lipid peroxidation and electrolytic leakage.

II. MATERIALS AND METHODS

The certified Finger millet seeds (varieties Indaf-7 and PAIYUR-1) 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, 400-700 nm) 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 [14]. 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 [15] 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 [16]. The activity of CAT (EC 1.11.1.6) was estimated by measuring the rate of decomposition of H_2O_2 [17]. GR (EC 1.6.4.2) activity was measured by oxidized GSH–dependent oxidation of NADPH [18]. APX (EC 1.11.1.11) activity was estimated by monitoring the decline in absorbance at 240nm [19]. POD (EC 1.11.1.7) activities were determined with guaiacol at 470nm (extinction coefficient 25.2mM cm^{-1}) [20].

Lipid peroxidation rates were determined by measuring the malondialdehyde (MDA) equivalents [21]. Electrolytic leakage was recorded by measuring the total inorganic ions leaked out in the leaves during salinity stress [22]. Proline and glycine betaine contents were estimated in leaf extracts under salinity stress [23, 24].

Extraction and quantification of ABA was done in leaves by ELISA method [25]. One gram of the leaf tissue was homogenized with 15ml of extraction medium containing 80% methanol, 100mg/1 butylated hydroxy toluene (BHT) and 0.5g/l citric acid monohydrate. These suspension was centrifuged at 1000xg for 20min at 4^0C. The supernatant was passed through Sep-Pak-C-18 cartridge and the pooled washings were evaporated under vacuum. The residue was partitioned three times against equal volume of ethyl acetate (pH 3.0). Resulting organic phase was evaporated and the residue was re-dissolved in 2ml of TBS buffer (pH 7.5). The aliquots were conjugated with bovine serum albumin (BSA) and the conjugate was administered subcutaneously to albino rabbits (1kg body weight). The antibodies were extracted from rabbit's blood and were used for the estimation of ABA.

ELISA was performed on a 96 well micro titration plate and each well on the plate was coated with 100µl coating buffer (1.5gl^{-1} Na_2CO_3, 2.93gl^{-1} $NaHCO_3$ and 0.02gl^{-1} NaN_3, pH 9.6) containing 0.25µgml^{-1} antigen of the hormone. The coated plates were incubated for overnight at 4℃ and then kept at room temperature for 32-40min. After washing four times with PBS-tween 20 (0.1%, v/v) buffer (pH 7.4), each well was filled with 50µl of 20µgml^{-1} antibody raised against ABA antigens, respectively. The plate was incubated for three hours at 28℃ and then washed as given before. One hundred microliters of 1.25µgml^{-1} IgG horse radish peroxidase substrate was added to each well and incubated for 1h at 30℃. The plate was rinsed five times with above PBS-tween 20 buffer and 100µl of solution containing 1.5mgml^{-1} O-phenylenediamine and 0.008% (v/v) H_2O_2 was added to each well. The reaction was stopped by adding 50µl of 6N H_2SO_4. The colour development in each well was detected using ELISA reader (Multi-scope, Lab systems, Finland) at optical density A_{490}.

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 ($P<0.05$)

III. RESULTS AND DISCUSSION

Oxidative stress is a complex chemical and physiological phenomenon that accompanies virtually all biotic and abiotic stresses in higher plants and develops as a result of overproduction and accumulation of reactive oxygen species (ROS) [26]. Antioxidant levels and the activities of ROS scavenging enzymes have been correlated with tolerance to several different environmental stresses [27, 28]. Superoxide dismutase, since discovered by McCord and Fridovich, [29] attracted the attention of many researchers because they are essential component in an organism's defense mechanism and is the first enzyme involved in the antioxidative process and this enzyme converts superoxide radical to hydrogen peroxide (H_2O_2) and molecular oxygen (O_2) [30]. In this study, both the ragi varieties treated with salinity showed increased SOD activity compared to control plants (Table 1). For instance, SOD activity was enhanced to the tune of 69% in Indaf-7 and 35% in PAIYUR-1 as compared to respective control plants under higher (120mM) salinity treatment. In response to salinity, SOD activity in Indaf-7 was relatively high compared to PAIYUR-1 at all the treatments. Although activity of SOD in two ragi varieties in response to high salinity treatment may suffice to withstand the amount of oxidative stress, our results clearly show that Indaf-7 is more tolerant than PAIYUR-1.

Catalase (CAT) and peroxidase (POD and APX) appear to play an essential protective role in the scavenging processes when coordinated with SOD activity [27]. They are chloroplastic or cytosolic enzymes which scavenge H_2O_2 generated primarily through SOD action. 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 ragi varieties (Table 2). As observed in case of SOD, higher activities of CAT, POD and APX were observed in Indaf-7. Another factor that would contribute to counter the oxidative stress by maintaining reduced

glutathione content at the cellular level is the activity of the enzyme glutathione reductase (GR).

Table 1: Influence of salinity stress on superoxide dismutase, catalase and glutathione reductase activity in two ragi varieties.

Species	Salinity treatments (mM)			
	Control	40	80	120
Superoxide dismutase (units/mgprotein/min)				
Indaf-7	119.03	193.48	223.18	375.47
	±6.15	±6.93	±7.42	±7.98
PAIYUR-1	117.70	152.37	170.26	182.22
	±5.06	±6.72	±6.95	±7.20
Catalase (mmol/mgprotein/min)				
Indaf-7	16.97	26.75	34.50	44.62
	±2.02	±2.63	±2.85	±3.49
PAIYUR-1	15.52	20.84	23.62	26.14
	±1.06	±2.74	±2.98	±2.70
Glutathione reductase (µmol /mgprotein/min)				
Indaf-7	38.21	55.33	58.72	94.27
	±3.95	±3.09	±3.22	±4.31
PAIYUR-1	37.18	39.11	43.53	49.24
	±3.92	±3.01	±3.12	±3.88

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

Table 2. Changes of ascorbate peroxidase and peroxidase activity in two ragi varieties under salinity stress.

Species	Salinity treatments (mM)			
	Control	40	80	120
Ascorbate peroxidase (mmol/mgprotein/min)				
Indaf-7	28.72	35.02	45.18	59.47
	±1.95	±2.09	±2.22	±2.31
PAIYUR-1	26.97	30.35	32.84	37.22
	±1.92	±2.01	±2.12	±2.20
Peroxidase activity (µmol/mgprotein/min)				
Indaf-7	13.11	16.98	23.12	26.31
	±1.29	±1.38	±1.47	±1.54
PAIYUR-1	12.02	14.29	15.19	17.72
	±1.22	±1.32	±1.41	±1.45

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

An increase in the GR activity was also observed in both ragi varieties (Table 1) and ascribe this due to *de nove* synthesis [31]. In this study, 60% of GR activity was observed in Indaf-7, while 24% in PAIYUR-1 under high salinity treatments. The elevated levels of GR might be able to increase the ratio of $NADP^+$/NADPH, thereby ensuing the availability of $NADP^+$ to accept electrons from the photosynthetic electron transport chain [32].

It is conceivable that acquisition of the stress tolenrance in any plant is a multi–factorial function and amelioration of ROS–scavenging systems is an important index to assess the abilities of ragi varieties to tolerate the stressful conditions like salinity. We presume that the metabolism of the reactive oxygen species under stressful environment is dependent on different functionally interrelated antioxidant enzymes. The increased activities of ROS scavenging enzymes should have a greater significance as invaluable tools in the elucidation of plant metabolic regulation under stressful environment. SOD is an essential component of these defense mechanisms as it dismutase to produce hydrogen peroxide and oxygen [33]. Hydrogen peroxide has been implicated as an essential elicitor of several different genes related to both abiotic and biotic stress tolerance [34]. 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. Certain POD isomers utilize the phenolic compounds and H_2O_2 to initiate the biosynthesis of several secondary metabolites required for the plant growth, development and differentiation [35]. 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 ragi leaves which indicates that ragi is capable of effectively scavenging the ROS for the production of certain secondary metabolites to withstand during salinity stress. GR activity is believed to be an important factor, limiting the degree of photo–damage experienced by the ragi under salinity stress conditions. This enzyme has been suggested to play a pivotal role in the glutathione cycle in the eukaryotic cells [36]. GR over producing plants had a greater capacity to regenerate ascorbate during oxidative stress [37]. 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 Indaf-7 and there are certain variations in the activity of these antioxidant enzymes between two varieties to counteract the stresses.

Lipid peroxidation is a destructive chain reaction and it can directly damage the structure of membrane [28] [38]. Salt stress affected the activity of plasma membrane ATPase activity and peroxidation of memebrane lipids is an indication of membrane damage and leakage under salt stress conditions [39]. Lipid peroxidation and electrolytic leakage were more in variety PAIYUR-1 compared to Indaf-7 under salt stress (Table 3). At higher salinity treatment, Indaf-7 showed 29% of membrane lipid peroxidation, while in PAIYUR-1 by 58% when compared to respective control plants. Variation in MDA contents were found in rice [40], cotton [41] and *Alfalfa* [42] cultivars differing in salt tolerance. Lower levels of lipid peroxidation are associated with higher APX activity in drought or salt tolerant tomato [43], sugar beet [44] and rice [45] plants. In the present study, higher percentage of electrolytic leakage was observed in PAIYUR-1 under salinity stressed conditions (Table 3). An undamaged plasma membrane is crucial to the survival of the whole cell [44, 46] and electrolytic leakage can indicate plasma membrane injury induced by salt stress [47].

To counteract the salinity stress, plants developed different adaptive mechanism and one of them is the synthesis and accumulation of low molecular weight organic compounds such as proline and glycine betaine in cytosol and organells and these compounds are collectively called compatible osmolytes because they accumulate and act without perturbing intracellular biochemistry in the cell [48]. Osmolytes have positive effects on enzyme and membrane integrity along with adaptive roles in mediating osmotic adjustment in plants grown under stress conditions [12].

Table 3. Effect of salinity stress on lipid peroxidation and electrolytic leakage in two ragi varieties.

Species	Salinity treatments (mM)			
	Control	40	80	120
Lipid peroxidation (nmol/ml)				
Indaf-7	1.42	1.61	1.76	1.99
	±0.062	±0.071	±0.085	±0.097
PAIYUR-1	1.65	1.88	2.47	3.89
	±0.078	±0.089	±0.10	±0.25
Electrolytic leakage (%)				
Indaf-7	1.73	2.07	2.21	2.49
	±0.079	±0.081	±0.092	±0.12
PAIYUR-1	2.11	2.69	3.82	5.11
	±0.098	±0.11	±0.28	±0.57

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

Quantitative estimation of proline and glycine betaine, between the two ragi varieties were depicted in Table 4. Sigificantly higher amount of proline and glycine betaine were accumulated in the variety Indaf-7 at all salnity treatments when compared to PAIYUR-1. Indaf-7 accumulated proline by 59% and glycine betaine by 62% when compared to respective control plants at 120 mM salinity treatments. Proline and glycine betaine are known to serve as nitrogen and carbon source which can be used as during recovery from the stress [48]. 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 [49]. Enhanced synthesis of proline under drought or salt stress has been implicated as a mechanism to alleviate cytoplasmic acidosis and maintain $NADP^+$: NADPH at values compatible with metabolism [50]. Glycine betaine accumulates in response to stress in many crops, including spinach, barley, tomato, potato, rice, carrot, wheat and sorghum [51].

Table 4. Accumulation of proline, glycine betaine and abscisic acid content in two ragi varieties under different salinity concentrations.

Species	Salinity treatments (mM)			
	Control	40	80	120
Prolene (mg/gfw)				
Indaf-7	2.20	3.11	3.93	5.37
	±0.23	±0.26	±0.32	±0.46
PAIYUR-1	1.90	2.06	2.22	2.40
	±0.072	±0.089	±0.13	±0.16
Glycine betaine (mg/gfw)				
Indaf-7	4.87	7.80	9.21	12.25
	±0.20	±0.32	±0.44	±0.52
PAIYUR-1	4.03	5.27	5.84	6.14
	±0.085	±0.096	±0.27	±0.30
Abscisic acid (µg/gfw)				
Indaf-7	8.11	12.23	14.12	18.85
	±0.95	±1.09	±2.11	±2.31
PAIYUR-1	6.92	8.24	9.09	10.13
	±0.71	±0.98	±1.01	±1.23

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

Abscisic acid is found in a wide variety of organisms and in the plant kingdom, ABA's role in mediating responses to abiotic stress has been conserved and enhanced throughout evolution [28, 52]. ABA accumulation was investigated in the leaves of Indaf-7 and PAIYUR-1 under control and salt-stressed conditions (Table 4). There was approximately two-three fold increase of ABA content in salt-stressed leaves compared to well watered plants. However, Indaf-7 possessed nearly 1.5-2 fold higher amount of ABA compared to PAIYUR-1. Accumulation of ABA in higher plants is well known to limit the transpirational losses particularly under water deficit conditions [53]. Our results clearly suggest that accumulation of ABA is a sensitive indicator to changes in soil water availability to the ragi varieties. ABA accumulation in higher plants was reported to be related to oxidative stress tolerance in plants [54]. Further ABA is also known to induce the expression of antioxidant genes encoding SOD, CAT, GR APX and POD in plant tissues [55].

IV. CONCLUSION

Two ragi varieties used in the present study have a different relative tolerance to high salinity and this tolerance was larger in Indaf-7 compared to PAIYUR-1. The present study clearly shows that Indaf-7 is superior with respect to its antioxidant defense systems, osmoprotectant and ABA accumulation than PAIYUR-1. Variety Indaf-7 also showed lower rates of lipid peroxidation and electrolytic leakage under salinity stressed conditions. Such studies can be used in ragi breeding programmes or transgenic ragi 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 and PASIC, Pondicherry Agricultural Unit for providing seeds of ragi varieties.

Conflict of interest. No conflict of interest.

REFERENCES

[1]. Zhang, P., Senge, M., & Dai, Y. (2017). Effects of salinity stress at different growth stages on tomato growth, yield, and water-use efficiency. *Communications in soil science and plant analysis*, *48*(6), 624-634.

[2]. Zörb, C., Geilfus, C. M. & Dietz, K. J., (2019). Salinity and crop yield. *Plant Biology*, *21*: 31-38.

[3]. Bohnert, H.J., Thomas, J.C., DeRocher, J., Michalowski, C.B., Breiteneder, H., Vernon, D.M., Deng, W., Yamada, S. and Jensen, R.G., (1994). Responses to salt stress in the halophyte *Mesembryanthemum crystallinum*. In: J.H. Herry(eds.). Biochemical and Cellular Mechanism of Stress Tolerance in Plants. NATO ASI series. Vol. H 86: PP. 415-428. Springer Verlag. Berlin, Heidelberg.

Bohnert, H. J., Thomas, J. C., DeRocher, E. J., Michalowski, C. B., Breiteneder, H., Vernon, D. M., Deng, W., Yamada, S., & Jensen, R. G. (1994). Responses to salt stress in the halophyte *Mesembryanthemum crystallinum*. In *Biochemical and Cellular Mechanisms of Stress Tolerance in Plants,* NATO ASI series. *86*, 415-428. Springer, Berlin, Heidelberg.

[4]. Scandalios, J. G. (1993). Oxygen stress and superoxide dismutases. *Plant Physiology, 101*: 7-12.

[5]. Mallik, S., Nayak, M., Sahu, B. B., Panigrahi, A. K., & Shaw, B. P. (2011). Response of antioxidant enzymes to high NaCl concentration in different salt-tolerant plants. *Biologia plantarum*, *55*(1), 191-195.
[6]. Shao, H. B., Chu, L. Y., Lu, Z. H., & Kang, C. M. (2008). Primary antioxidant free radical scavenging and redox signaling pathways in higher plant cells. *International Journal of Biological Sciences*, *4*(1), 8-14.
[7]. Gupta, B., & Huang, B. (2014). Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization. *International journal of genomics*, *115*: 1-18.
[8]. Lin, K. C., Jwo, W. S., Chandrika, N. N. P., Wu, T. M., Lai, M. H., Wang, C. S., & Hong, C. Y. (2016). A rice mutant defective in antioxidant-defense system and sodium homeostasis possesses increased sensitivity to salt stress. *Biologia plantarum*, *60*(1), 86-94.
[9]. Jain, M., Mathur, G., Koul, S., & Sarin, N. (2001). Ameliorative effects of proline on salt stress-induced lipid peroxidation in cell lines of groundnut (*Arachis hypogaea* L.). *Plant Cell Reports*, *20*(5), 463-468.
[10]. Dionisio-Sese, M. L., & Tobita, S. (1998). Antioxidant responses of rice seedlings to salinity stress. *Plant Science*, *135*(1), 1-9.
[11]. Mandhania, S., Madan, S., & Sawhney, V. (2006). Antioxidant defense mechanism under salt stress in wheat seedlings. *Biologia Plantarum*, *50*(2), 227-231.
[12]. Wutipraditkul, N., Wongwean, P., & Buaboocha, T. (2015). Alleviation of salt-induced oxidative stress in rice seedlings by proline and/or glycinebetaine. *Biologia Plantarum, 59*(3), 547-553.
[13]. Khadri, M., Tejera, N. A., & Lluch, C. (2007). Sodium chloride–ABA interaction in two common bean (*Phaseolus vulgaris*) cultivars differing in salinity tolerance. *Environmental and experimental botany*, *60*(2), 211-218.
[14]. Hoagland, D. R., & Arnon, D. I. (1950). The water-culture method for growing plants without soil. *Circular. California agricultural experiment station*, *347*(2nd edit).
[15]. Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry*, *72*(1-2), 248-254.
[16]. Beauchamp, C., & Fridovich, I. (1971). Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Analytical biochemistry*, *44*(1), 276-287.
[17]. Havir, E. A., & McHale, N. A. (1987). Biochemical and developmental characterization of multiple forms of catalase in tobacco leaves. *Plant Physiology*, *84*(2), 450-455.
[18]. Foyer, C. H., & Halliwell, B. (1976). The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. *Planta*, *133*(1), 21-25.
[19]. Nakano, Y., & Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant and cell physiology*, *22*(5), 867-880.
[20]. Polle, A., Otter, T., & Seifert, F. (1994). Apoplastic peroxidases and lignification in needles of Norway spruce (*Picea abies* L.). *Plant Physiology*, *106*(1), 53-60.
[21]. Hodges, D. M., DeLong, J. M., Forney, C. F., & Prange, R. K. (1999). Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta*, *207*(4), 604-611.
[22]. Sullivan, C. Y. & Ross, W. M. (1979). Selecting the drought and heat resistance in grain sorghum. In: M. Mussel and R.C. Stapes (eds). Stress Physiology in Crop Plants. pp. 263-281. *John Whiley and Sons*. New York.
[23]. Bates, L. S., Waldren, R. P., & Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. *Plant and soil, 39*(1), 205-208.
[24]. Storey, R., & Jones, R. W. (1977). Quaternary ammonium compounds in plants in relation to salt resistance. *Phytochemistry*, *16*(4), 447-453.
[25]. Daie, J., & Wyse, R. (1982). Adaptation of the enzyme-linked immunosorbent assay (ELISA) to the quantitative analysis of abscisic acid. *Analytical biochemistry*, *119*(2), 365-371.
[26]. Sharma, P., Jha, A. B., Dubey, R. S., & Pessarakli, M. (2012). Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *Journal of botany, 12*: 1-12.
[27]. Massacci, A., Iannelli, M. A., Pietrini, F., & Loreto, F. (1995). The effect of growth at low temperature on photosynthetic characteristics and mechanisms of photoprotection of maize leaves. *Journal of Experimental Botany*, *46*(1), 119-127.
[28]. Desingh, R. & Manikandan, K., (2019). Differential antioxidative and osmolytic responses to salt (NaCl) stress among seven bhendi (*Abelmoschus esculentus* (L.) Moench) varieties. *International Journal on Emerging Technologies*, *10*(3), 174-180.
[29]. McCord, J. M., & Fridovich, I. (1969). Superoxide dismutase, an enzymatic function for erythrocuprein (hemocuprein). *Journal Biological Chemistry*, *60*: 6049-6055.
[30]. Attia, H., Karray, N., Msilini, N., & Lachaâl, M. (2011). Effect of salt stress on gene expression of superoxide dismutases and copper chaperone in Arabidopsis thaliana. *Biologia Plantarum*, *55*(1), 159-163.
[31]. Kaymakanova, M., Lyubenova, L., Schröder, P., & Stoeva, N. (2010). Salt stress and glutathione-dependent enzyme activities in bean (Phaseolus vulgaris L.). *Gen. Appl. Plant Physiol.*, *36*(1-2), 55-59.
[32].Foyer, C. H. (2018). Reactive oxygen species, oxidative signaling and the regulation of photosynthesis. *Environmental and experimental botany, 154*, 134-142.
[33]. Hossain, M. S., El Sayed, A. I., Moore, M., & Dietz, K. J. (2017). Redox and reactive oxygen species network in acclimation for salinity tolerance in sugar beet. *Journal of experimental botany*, *68*(5), 1283-1298.
[34]. Prasad, T. K., Anderson, M. D., Martin, B. A., & Stewart, C. R. (1994). Evidence for chilling-induced oxidative stress in maize seedlings and a regulatory role for hydrogen peroxide. *The Plant Cell*, *6*(1), 65-74.
[35]. El-Bastawisy, Z. M. (2010). Variation in antioxidants among three wheat cultivars varying in tolerance to NaCl. *General Applied Plant Physiology*, *36*, 189-203.

[36]. Noctor, G., & Foyer, C. H. (1998). Ascorbate and glutathione: keeping active oxygen under control. *Annual review of plant biology*, *49*(1), 249-279.
[37]. Foyer, C. H., Souriau, N., Perret, S., Lelandais, M., Kunert, K. J., Pruvost, C., & Jouanin, L. (1995). Over expression of glutathione reductase but not glutathione synthetase leads to increases in antioxidant capacity and resistance to photoinhibition in poplar trees. *Plant physiology*, *109*(3), 1047-1057.
[38]. Koca, H., Ozdemir, F., & Turkan, I. (2006). Effect of salt stress on lipid peroxidation and superoxide dismutase and peroxidase activities of *Lycopersicon esculentum* and *L. pennellii*. *Biologia Plantarum*, *50*(4), 745-748.
[39]. Misra, N., Ansari, M. S., & Gupta, A. K. (2006). Differential response of scavenging of reactive oxygen species in green gram genotype grown under salinity stress. *American Journal of Plant Physiology, 1*: 41-53.
[40]. Demiral, T., & Türkan, I. (2005). Comparative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance. *Environmental and Experimental Botany*, *53*(3), 247-257.
[41]. Diego, A. M., Marco, A. O., Carlos, A. M., & Jose, C. (2003). Photosynthesis and activity of superoxide dismutase, peroxidases and glutathione reductase in cotton under salt stress. *Environmental and Experimental Botany*, *49*: 69-76.
[42]. Wang, X. S., & Han, J. G. (2009). Changes of proline content, activity, and active isoforms of antioxidative enzymes in two alfalfa cultivars under salt stress. *Agricultural Sciences in China*, *8*(4), 431-440.
[43]. Shalata, A., & Tal, M. (1998). The effect of salt stress on lipid peroxidation and antioxidants in the leaf of the cultivated tomato and its wild salt-tolerant relative Lycopersicon pennellii. *Physiologia Plantarum*, *104*(2), 169-174.
[44]. Bor, M., Özdemir, F., & Türkan, I. (2003). The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet Beta vulgaris L. and wild beet Beta maritima L. *Plant Science*, *164*(1), 77-84.
[45]. Demiral, T., & Türkan, I. (2005). Comparative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance. *Environmental and experimental Botany*, *53*(3), 247-257.
[46]. de Azevedo Neto, A. D., Prisco, J. T., Enéas-Filho, J., de Abreu, C. E. B., & Gomes-Filho, E. (2006). Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. *Environmental and Experimental Botany*, *56*(1), 87-94.
[47]. Koca, H., Bor, M., Özdemir, F., & Türkan, I. (2007). The effect of salt stress on lipid peroxidation, antioxidative enzymes and proline content of sesame cultivars. *Environmental and experimental botany*, *60*(3), 344-351.
[48]. De la Torre-Gonzalez, A., Montesinos-Pereira, D., Blasco, B., & Ruiz, J. M. (2018). Influence of the proline metabolism and glycine betaine on tolerance to salt stress in tomato (*Solanum lycopersicum* L.) commercial genotypes. *Journal of plant physiology*, *231*, 329-336.
[49]. Rontein, D., Basset, G., & Hanson, A. D. (2002). Metabolic engineering of osmoprotectant accumulation in plants. *Metabolic Engineering*, *4*(1), 49-56.
[50]. Hare, P. D., & Cress, W. A. (1997). Metabolic implications of stress-induced proline accumulation in plants. *Plant growth regulation*, *21*(2), 79-102.
[51]. Ashraf, M. F. M. R., & Foolad, M. (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and experimental botany*, *59*(2), 206-216.
[52]. Prerostova, S., Dobrev, P. I., Gaudinova, A., Hosek, P., Soudek, P., Knirsch, V., & Vankova, R. (2017). Hormonal dynamics during salt stress responses of salt-sensitive *Arabidopsis thaliana* and salt-tolerant *Thellungiella salsuginea*. *Plant Science*, *264*, 188-198.
[53]. Negin, B., & Moshelion, M. (2016). The evolution of the role of ABA in the regulation of water-use efficiency: From biochemical mechanisms to stomatal conductance. *Plant Science*, *251*, 82-89.
[54]. Bellaire, B. A., Carmody, J., Braud, J., Gossett, D. R., Banks, S. W., Cranlucas, M., & Fowler, T. E. (2000). Involvement of abscisic acid-dependent and—Independent pathways in the upregulation of antioxidant enzyme activity during NaCl stress in cotton callus tissue. *Free Radical Research*, *33*(5), 531-545.
[55]. Guan, L. M., Zhao, J., & Scandalios, J. G. (2000). *Cis*-elements and *trans*-factors that regulate expression of the maize Cat1 antioxidant gene in response to ABA and osmotic stress: H_2O_2 is the likely intermediary signaling molecule for the response. *The Plant Journal*, *22*(2), 87-95.

How to cite this article: Desingh, R. and Kanagaraj, G. (2019). Salt (NaCl) Stress-induced Antioxidant Enzyme activities, Osmolytes, ABA, Lipid Peroxidation and Electrolytic leakage in Two Ragi (*Eleusine coracana* (L.) Gaertn) varieties. *International Journal on Emerging Technologies*, *10*(4): 131–136.