Full Length Article

Effect of Salt Stress on Na Accumulation, Antioxidant Enzyme Activities and Activity of Cell Wall Peroxidase of Huckleberry (*Solanum scabrum*) and Eggplant (*Solanum melongena*)

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Abstract

Understanding the adaptive mechanisms to salinity stress is an important prerequisite for crop improvement and sustainable production. The objective of this study was to compare the adaptation of huckleberry and eggplant to salinity in terms of total dry weight, malondialdehyde (MDA) content, the activities of catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), soluble peroxidases (sPOD) and peroxidases ionically-bound to cell wall (cwPOD) and Na^+ and K^+concentrations. Growth was found to be more reduced in eggplant (63% reduction) than huckleberry (51% reduction). Na^+ concentration was 2.5-fold higher in leaf of eggplant than huckleberry. The MDA content significantly increased in root and leaf of eggplant, but remained unchanged in huckleberry. In the leaf of huckleberry, cwPOD alone was significantly enhanced by salt stress, whereas in the root all the enzymes except CAT were significantly elevated. In eggplant on the contrast, cwPOD activity tended to decrease in leaf and root, whereas CAT markedly increased in the leaf, but sharply declined in the root. APX activity in eggplant slightly increased in both root and leaf while sPOD significantly increased in the root but remained unaltered in the leaf. These results suggest that Na^+ exclusion in the leaf blade and greater antioxidant activity in the root constitute important adaptations to salt stress in huckleberry. Particularly, cwPOD activity in the leaf and root of huckleberry suggest a dual role in the adaptation; mainly reactive oxygen species detoxification in leaf and enhanced lignin deposition in root, which functions in controlling root-to-shoot Na^+ delivery. © 2015 Friends Science Publishers

Keywords: Na^+ distribution; Oxidative stress; Catalase; Ascorbate peroxidase; Glutathione reductase

Introduction

Under salinity stress conditions, one of the main factors of growth inhibition is oxidative stress damage (Munns and Tester, 2008). Oxidative stress is a secondary stress arising from either loss of electrons to oxygen during metabolic processes such as photosynthesis, respiration and photorespiration or reaction of activated oxygen with photosensitized chlorophyll, both of which lead to the generation of reactive oxygen species (ROS) (Mittler, 2002; Sharma *et al*., 2012). These ROS include singlet oxygen ($^1O_2$), the super anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (HO•) and are produced in various subcellular compartments including chloroplasts, mitochondria, peroxisomes, endoplasmic reticulum, plasma membranes, cell walls and apoplasts (Abogadallah, 2010; Sharma *et al*., 2012). If their production is not tightly controlled, they may affect plant metabolism by, denaturing proteins and nucleic acids, inducing lipid peroxidation and even cell death (Gill and Tuteja, 2010).

The detoxification of ROS constitutes an important trait in salt stress tolerance. This detoxification is achieved by the activity of antioxidants, which can be enzymatic, for example CAT, APX, GR and SOD, or non-enzymatic, for example ascorbic acid, flavonoids, total phenolics, carotenoids and tocopherol (Abogadallah, 2010; Gill and Tuteja, 2010; Sharma *et al*., 2012). Reduction in antioxidant activity will lead to increased oxidative damage, which is often seen as increased MDA content, a product of lipid peroxidation, whereas an increase in enzyme activity will correlate with low MDA, hence tolerance (Demiral and Türkan, 2005; Koca *et al*., 2007). In addition, Sairam and Tyagi (2004) observed an inverse correlation between high SOD, CAT and GR activities, and low MDA, membrane stability index and Na^+/K^+ ratio in the tolerant wheat cultivars under salinity stress. This suggests that, the maintenance of low Na^+/K^+ ratios, especially in the photosynthetic tissues, through shoot Na^+ exclusion mechanisms occurring in the lower parts of the plant, may minimize oxidative damage and increase tolerance to salinity (Dionisio-Sese and Tobita, 1998).

The roots are always in direct contact with saline

environments and have been shown to serve as control points for root-to-shoot Na^+ delivery. For example, many transporters responsible for regulating Na^+ uptake and transport to the leaf have been identified in the root, including the *salt overly sensitive* (SOS1) transporter and the *high affinity K^+ transporter* (HKT) (Tester and Davenport, 2003; Munns and Tester, 2008; Møller *et al.*, 2009). Furthermore, antioxidant enzyme activities in roots have been found to play crucial roles in salinity tolerance, by scavenging ROS (Demiral and Türkan, 2005; Jaleel *et al.*, 2007). In addition, peroxidases ionically-bound to cell wall are involved in the synthesis of lignin, a constituent of the Casparian strip in the root, which functions in restricting the passage of solutes including Na^+ to the stele (Lee *et al.*, 2013). Enhanced cwPOD under salinity has been found to protect plants by scavenging ROS (Maia *et al.*, 2013), as well as correlates with enhanced deposition of lignin, which has been suggested to control the transport of Na^+ to the shoot (Jbir *et al.*, 2001). Thus, cwPOD activity may be an important attribute in salinity stress tolerance. These observations suggest that, changes occurring in root in response to salt stress are important for stress tolerance and thus may define the overall adaptation of a plant under such environments. Understanding these changes may offer new targets for molecular engineering of salinity tolerance in crops.

Huckleberry (*Solanum scabrum* Mill.) and eggplant (*Solanum melongena* L.) are two solanaceous vegetables that differ in the pattern of Na^+ accumulation (Assaha *et al.*, 2013). Huckleberry accumulates very low Na^+ in leaf and more in root and stem, while eggplant accumulates more in leaf and less in root and stem. It is this uncontrolled accumulation of Na^+ in eggplant that is thought to be responsible for its growth reduction under salt stress (Shaheen *et al.*, 2012; Assaha *et al.*, 2013). Excess accumulation of Na^+ in the leaf of the eggplant cultivar, New Nobel, was shown to correlate with increased H_2O_2 and MDA production, indicating Na^+-induced oxidative damage (Shaheen *et al.*, 2012). In this study, the excess Na^+ accumulation was also accompanied by an increase in SOD, POD and CAT activities, but this increased enzyme activity did not correlate with tolerance in the eggplant cultivar studied, indicating the overwhelming effects of high Na^+ accumulation on antioxidant defense system in the leaf of eggplant.

No information on the injury and enzyme activity at the level of the root has been established in eggplant, while antioxidant activity in huckleberry is non-existent. Since high Na accumulation induces oxidative stress damage it is expected that high salt accumulation in root of huckleberry would be deleterious, whereas this damage would be reduced in the leaf, where less Na is accumulated. In eggplant on the contrast with lower Na accumulation in the root, oxidative stress should be more pronounced in the leaf. Thus, the objective of this study was to compare Na^+ accumulation in different organs and evaluate the antioxidant enzyme activity in the leaf and root of huckleberry and eggplant under high salt stress.

Materials and Methods

Plants and Growth Conditions

The huckleberry and eggplant used in this study were the same as described in Assaha *et al.* (2013). Seeds of both plants were sown in seed beds, transferred 4 weeks after germination to 2 L pots, filled with a mixture of regosol soil, perlite and peat moss (2: 1: 1, v/v) and irrigated with ¼ nutrient solution for 10 d. Thereafter, the plants were arranged in a randomized complete block design with 4 groups of 4 replicates each and irrigated with ½ strength nutrient solution, supplemented with 0 (for control) and 150 (stressed) mM NaCl to induce salinity stress. The composition of the nutrient solution was as described by Assaha *et al.* (2013). The NaCl solution was applied at 50 mM increments every day until the maximum concentration (150 mM) was reached, at which point start of treatment was considered.

Determination of Growth, Na^+ and K^+ Concentrations, and Enzyme Activities

After 10 d of salt treatment, samples were collected for analysis. The plants were separated into root, stem, petiole and leaf blade and their fresh weights (FW) recorded. Then, portions of these samples were frozen in liquid nitrogen stored at -80°C, while the rest were oven-dried and their dry weights (DW) recorded.

Na^+ and K^+ concentrations were determine using freeze-dried samples of leaf blade, petiole, stem and root. The samples were powdered, digested with HNO_3/H_2O_2 (1: 1, v/v) and measured using a flame photometer (ANA 135, Tokyo Photoelectric, Tokyo, Japan).

The MDA content in leaf and root was determined by the thiobarbituric acid (TBA) reaction as described by Saneoka *et al.* (2004) with some modifications. Fresh leaf and root samples (0.1g) were homogenized in an extraction buffer (10 m*M* HEPES, pH 7, 15% tricarboxylic acid, 0.375% thiobarbituric acid, 0.25 N HCl, 0.04% butylated hydroxyl toluene and 2% ethanol), incubated at 95°C and then centrifuged. The absorbance of the supernatant was then read at 535 nm and 600 nm and the MDA content was calculated using the extinction coefficient (155 mM^{-1} cm^{-1}) (Dionisio-Sese and Tobita, 1998).

The protein concentration in enzyme extract was measured using the Protein Assay kit (Bio-Rad DC, California, USA) with bovine serum albumin as standard, according to manufacturer's instructions. For the activities of catalase (CAT, EC 1.11.1.6), as corbate peroxidase (APX, EC 1.11.1.11) and glutathione reductase (GR, EC 1.6.4.2), enzyme extraction was carried out using 0.5 g fresh sample according to the method of Takagi and Yamada (2013).

One mL CAT assay mixture contained 50 mM potassium phosphate buffer (pH 7.0), 10 mM H_2O_2 and 5% enzyme extract. A decrease in H_2O_2 was followed at 240 nm and activity calculated as mmol H_2O_2 consumed per minute. For APX activity, 1 mL assay mixture contained 25 mM phosphate buffer (pH 7.0), 0.25 mM ascorbic acid, 0.1 mM EDTA, 0.1 mM H_2O_2 and 10% enzyme extract. Oxidation of ascorbate was measured at 290 nm and its concentration calculated using the extinction coefficient of 2.8 m$M^{-1}cm^{-1}$. One unit APX was determined as 1 µmol as corbate oxidized per minute. For GR activity, 1 mL assay mixture contained 100 mM phosphate buffer (pH 7.5), 0.1 mM EDTA, 0.02 mM NADPH, 0.02 mM GSSG and 10% enzyme extract. Concentration of oxidized NADPH was determined using the extinction coefficient (6.22 m$M^{-1}cm^{-1}$) and 1 unit GR activity defined as µmol NADPH oxidized per min.

Extraction of soluble peroxidases (sPOD) and peroxidases ionically bound to cell wall (cwPOD) was carried out on fresh leaf and root samples. Samples (0.5 g) were ground in liquid nitrogen, to which phosphate buffer was added (pH 7.0). The homogenate was then centrifuged and the supernatant collected for the determination of sPOD. Then, cwPOD was subsequently extracted from the residual pellet as described by Lin and Kao (2001). For the measurement of sPOD activity, 1 mL reaction mixture contained 15 mM guaiacol, 73 mM phosphate buffer, 10 mM H_2O_2 and 2% enzyme extract. Increase in absorbance was monitored at 470 nm for 1 min and the enzyme activity calculated using the extinction coefficient (26.6 m$M^{-1}cm^{-1}$) for tetraguaiacol (Chance and Maehly, 1955). One unit sPOD activity was defined as mmol tetraguaiacol formed per min. For cwPOD activity, the reaction mixture contained 0.2 mM syringaldazine as substrate, 0.1 mM H_2O_2, 68 mM phosphate buffer and 2% enzyme extract. Increase in absorbance at 530 nm was monitored for 1 min and enzyme activity calculated using the extinction coefficient (27 mM^{-1} cm^{-1}) (Quiroga *et al.*, 2000). One unit cwPOD activity was defined as mmol syringaldazine oxidized per min. The protein concentration in both extracts was measured as described above.

Statistical Analysis

All data were analyzed by one way analysis of variance using the SPSS statistical package version 21 (IBM Inc. USA). All results are means ± SE (n = 4) and the means were separated using the Duncan Multiple Range Test at p = 0.05.

Results

Effect of Salinity on Plant Biomass

The DW of leaf blade, petiole, stem and root of both plants are presented in Table 1. The growth of both plants was
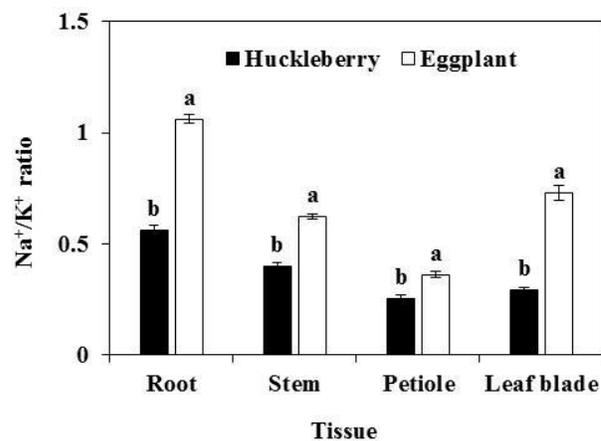

Fig. 1: Effect of salinity on Na^+/K^+ratio in the different tissues of huckleberry and eggplant.The result represents the means ± SE (n = 4). Different letters for each tissue indicate significant difference (p≤0.05)
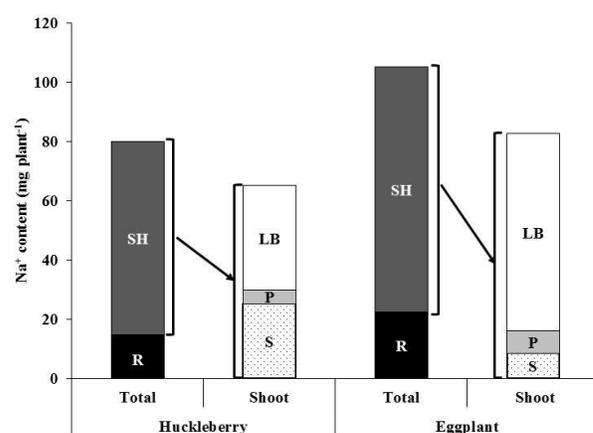

Fig. 2: The Distribution of Na^+ accumulated by huckleberry and eggplant under salt stress. LB = leaf blade, S = stem, P = petiole, R = root, SH = shoot

reduced by salinity stress. However, the decrease was more marked in eggplant (63% reduction) than huckleberry (51% reduction). Leaf DW reduction was lesser in huckleberry (47%) than in eggplant (68%). Reduction in petiole DW was also greater in eggplant (65%) than in huckleberry (51%). However, whereas stem DW reduction was similar for both plant species, whilst root DW reduction was more marked in huckleberry (49%) than eggplant (25%).

Effect of Salinity on Na^+ and K^+ Accumulation

The concentrations of Na^+ in the different tissues of both plants are presented in Table 2. Leaf Na^+ concentration was 2.5-fold higher in eggplant than in huckleberry, whereas stem Na^+ concentration was 53% higher in huckleberry than

Table 1: Effect of salinity stress on the dry weight of leaf blade (LB), petiole, stem and root of huckleberry and eggplant

Plant	Treatments (mM NaCl)	LB DW (g plant^{-1})	Petiole DW (g plant^{-1})	Stem DW (g plant^{-1})	Root DW (g plant^{-1})	Total DW (g plant^{-1})
Huckleberry	0	5.23 ± 0.24^b	0.43 ± 0.02^b	2.25 ± 0.11^a	1.45 ± 0.05^a	9.35 ± 0.29^a
	150	2.77 ± 0.08^c	0.21 ± 0.01^b	0.81 ± 0.03^c	0.74 ± 0.04^c	4.53 ± 0.11^b
Eggplant	0	6.15 ± 0.25^a	0.84 ± 0.14^a	1.20 ± 0.01^b	0.97 ± 0.16^{bc}	9.15 ± 0.19^a
	150	1.94 ± 0.04^d	0.29 ± 0.02^b	0.42 ± 0.04^d	0.73 ± 0.04^c	3.37 ± 0.09^c

Values are means ± SE(n = 4) at p≤0.05. For each column means followed by same letter are not significantly different

Table 2: Effect of salinity on Na$^+$ concentration in leaf blade, petiole, stem and root of huckleberry and eggplant

Plant	Treatment (mM NaCl)	Na$^+$ (mg/g DW)			
		Leaf blade	Petiole	Stem	Root
Huckleberry	0	0.22±0.01^d	0.37±0.02^d	0.50±0.04^d	1.12±0.04^d
	150	12.79±0.77^c	20.86±1.34^b	31.23±1.17^a	20.17±0.27^b
Eggplant	0	0.96±0.06^d	1.63±0.13^c	1.32±0.08^c	2.66±0.54^c
	150	34.53±1.80^a	26.01±0.90^a	20.44±0.36^b	31.01±0.37^a

Values are means ± SE (n=4) at p≤0.05. For each column means followed by same letter are not significantly different

Table 3: Effect of salinity on K$^+$ concentration in leaf blade, petiole, stem and root of huckleberry and eggplant

Plant	Treatment (mM NaCl)	K$^+$ (mg/g DW)			
		Leaf blade	Petiole	Stem	Root
Huckleberry	0	46.83±0.51^b	86.06±0.49^a	82.59±1.22^a	47.16±0.91^a
	150	43.46±0.50^c	82.62±0.95^b	77.43±0.99^b	35.74±0.87^b
Eggplant	0	65.94±2.13^a	83.39±1.74^b	64.77±1.22^c	46.08±0.90^a
	150	47.53±0.57^b	73.04±1.08^c	33.21±0.29^d	29.28±0.30^c

Values are means ± SE (n = 4) at p≤0.05. For each column means followed by same letter are not significantly different
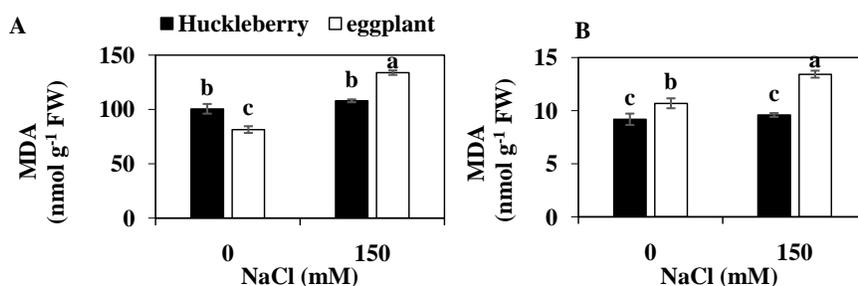

Fig. 3: Effect of salinity on MDA content in leaf (A) and root (B) of huckleberry and eggplant. The results represent the means ± SE (n = 4). The same letter indicates no significant difference (p≤0.05)

eggplant. In the root and petiole, Na$^+$ concentration was more elevated in eggplant. K$^+$ concentration decreased in all the studied tissues of both plants, but this reduction was more marked in eggplant than huckleberry, especially in the leaf, stem and root (28, 49 and 36%, respectively; Table 3). The Na$^+$/K$^+$ ratio was found to be more elevated for all the studied tissues in eggplant, especially in the root where it was above 1 (Fig. 1).

The total Na$^+$ content and its distribution in the root, stem, petiole and leaf blade of salt-treated plants are presented in Fig. 2. The total plant Na$^+$ content was found to be 32% higher in eggplant than huckleberry. Although, the shoot Na$^+$ content was more elevated in eggplant than huckleberry, their respective percentages, relative to total content, were not significantly different (81% for huckleberry and 79% for eggplant). Of the total shoot Na$^+$ content, about 50% accumulated in the leaf blade of huckleberry, whereas eggplant accumulated about 81%.

Root Na$^+$ content was 51% more elevated in eggplant than huckleberry.

Oxidative Stress Damage and Activities of Antioxidant Enzymes

The MDA content in the leaf did not significantly change in huckleberry, but was markedly elevated in eggplant (64%) (Fig. 3). In the root, MDA also increased in eggplant (26%), but was not significantly altered in huckleberry (Fig. 3).

CAT activity in huckleberry, remained unaltered in the leaf and root, whereas in eggplant, it significantly increased in the leaf (48%), but decreased in the root (50%) (Fig. 4). APX activity increased markedly in root and slightly in leaf of both plants under both treatments, although the activity was lower in eggplant root (16%) than huckleberry root (29%) (Fig. 5). GR activity significantly increased in root of
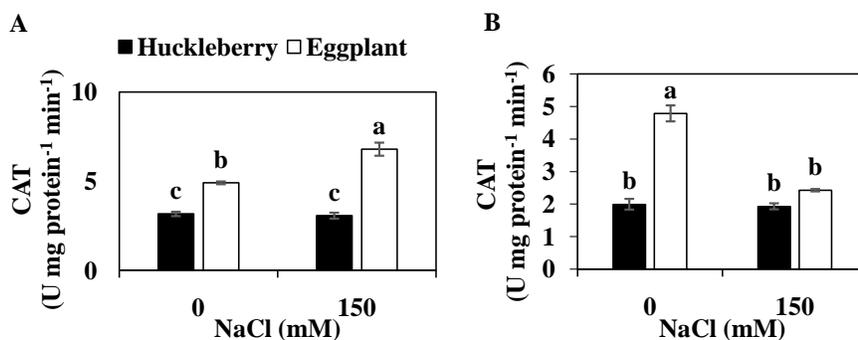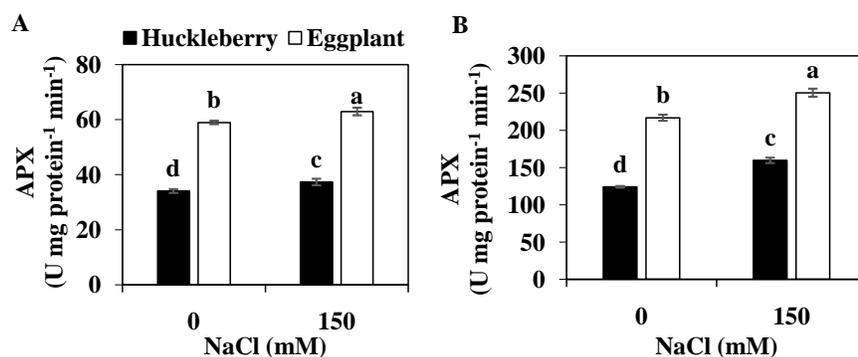
Fig. 4: Effect of salinity on CAT activity in the leaf (A) and root (B) of huckleberry and eggplant. The results represent the means ± SE (n = 4). The same letter indicates no significant difference (p≤0.05)

Fig. 5: Effect of salinity on APX activity in the leaf (A) and root (B) of huckleberry and eggplant. The results represent the means ± SE (n = 4). The same letter indicates no significant difference (p≤0.05)

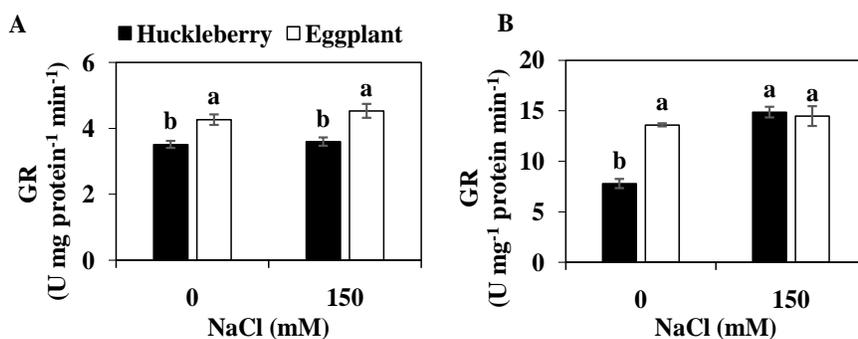**Fig. 6:** Effect of salinity on GR activity in the leaf (A) and root (B) of huckleberry and eggplant. The results represent the means ± SE (n = 4). The same letter indicates no significant difference (p≤0.05)

huckleberry (90%), but no significant increase was observed in eggplant, whereas in the leaf there was no significant change in GR activity of both plants (Fig. 6). Salt stress did not significantly change the activity of sPOD activity in leaf of both plants (Fig. 7). In the root however, sPOD activity significantly increased in both plants, but this increase was more marked in huckleberry (2.7 fold) than eggplant (65%) compared to the control plants (Fig. 7). The cwPOD activity in huckleberry showed a 2-fold and 34% increase in leaf and root, respectively, whereas in eggplant, it decreased (40%

and 23%, respectively), although this decrease was not statistically significant (Fig. 8).

Discussion

The present study sought to examine the pattern of Na^+ accumulation in different tissues of huckleberry and eggplant and to ascertain the effect of Na accumulation on antioxidant enzyme activity in leaf and root of both plants, under salinity stress. After subjecting both plants to high
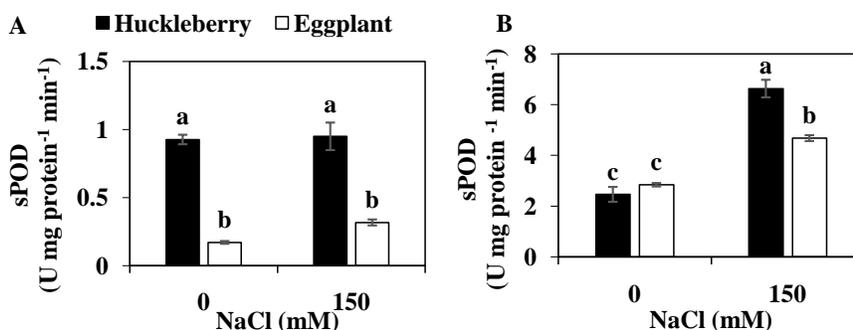
Fig. 7: Effect of salinity on sPOD activity in the leaf (A) and root (B) of huckleberry and eggplant. The results represent the means ± SE (n = 4). The same letter indicates no significant difference (p≤0.05)
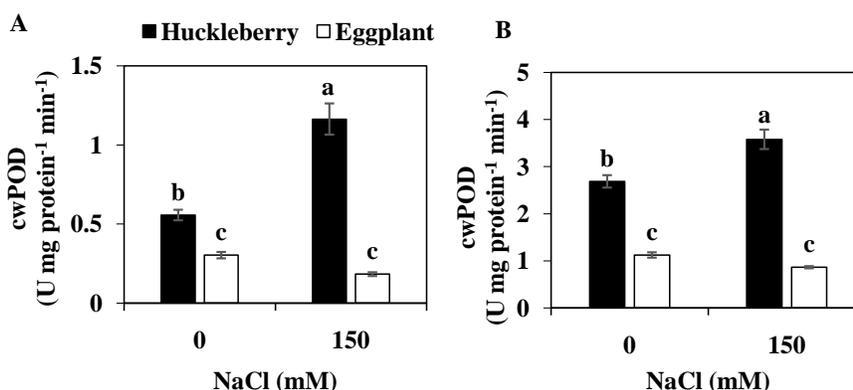

Fig. 8: Effect of salinity on cwPOD activity in the leaf (A) and root (B) of huckleberry and eggplant. The results represent the means ± SE (n = 4). The same letter indicates no significant difference (p≤0.05)

salinity (150 mM NaCl) for 10 d, the Na^+ concentration in huckleberry increased from the root to the stem and then progressively decreased through the petiole to the leaf blade (Table 1) yielding a more reduced Na^+ accumulation in its leaf blade (44% of total Na^+) than eggplant (63% of total Na^+) (Fig. 2). This pattern offers further support to the existence of a mechanism of Na^+ exclusion to the leaf blade in huckleberry (Assaha *et al.*, 2013). The present study further revealed that in addition to stem and root, the petiole also appears to be a potential control point for Na^+ delivery to the leaf blade. The leaf sheath has been shown to be a potential control point of Na^+ transport to the leaf blade in cereals under salinity. It contains the OsHKT1;4 transporter, which controls the delivery of Na^+ to the leaf blade by retrieving Na^+ from the transpiration stream in to xylem parenchyma cells (Hausser and Horie, 2010). Since the leaf sheath in the Poaceae is the equivalent of petiole in dicots, it is very likely that a similar control mechanism would exist in the petiole of huckleberry.

With reduced delivery of Na^+ to the leaf, the Na^+/K^+ ratio is usually kept low for normal metabolic processes to operate (Maathuis and Amtmann, 1999; Tester and Davenport, 2003). This lower Na^+/K^+ ratio, as shown for huckleberry in the present study, is important for plants under salinity stress, as K^+ is the most abundant element in plants, intervening in many biological processes including enzyme activation, transport of sugars, stomatal regulation and osmoregulation (Hu and Schmidhalter, 2005).

In the present study, oxidative stress damage was assessed by measuring the MDA content in leaf and root of both plants. The MDA content in root and leaf of eggplant was significantly elevated, but remained unaltered in huckleberry (Fig. 3). This is a clear sign that there is salt-induced oxidative stress damage in these tissues in eggplant. The increase in MDA associated with sensitivity and a decrease associated with tolerance, have been reported in many cultivars of plants including sesame (Koca *et al.*, 2007), maize (Azevedo-Neto *et al.*, 2006), sugar beet (Bor *et al.*, 2003) and rice (Demiral and Türkan, 2005). In these studies, low MDA corresponded to increased antioxidant enzyme activity, indicating a protective mechanism against oxidative damage. However, in the present study, evaluation of antioxidant enzyme activity in the leaf showed a marked increase in CAT activity in eggplant, where there was a corresponding Na^+ accumulation, but this enhanced activity apparently did not offer enough protection to the plants, similar to the observations of Shaheen *et al.* (2012). Although CATs are specific H_2O_2 scavengers, they operate

with very low affinity for it when compared other antioxidant enzymes such as APX (Sharma *et al*., 2012) indicating that the production of ROS in the leaf was above CAT scavenging capacity in eggplant and coupled with lower APX and GR activities, induction of oxidative stress was unavoidable resulting in growth reduction. Huckleberry, on the contrary, maintained almost unaltered activities of CAT, GR, APX and sPOD in the leaf, where Na accumulation was low, indicating reduced Na toxicity in the leaf.

In the root on the other hand, the MDA content had the same trend like in the leaf, although the content was much lower (Fig. 3). Whereas huckleberry maintained an unaltered level of CAT, but enhanced sPOD, APX and GR activities, eggplant had a sharp decrease in CAT activity, and an increase in APX indicating that the latter alone was not sufficient to control the ROS produced in root of eggplant. APX and GR are key enzymes in the Halliwell-Asada pathway (Sharma *et al*., 2012), which is considered more effective in scavenging ROS hence enhanced protection against oxidative stress (Koca *et al*., 2007, Yazici *et al*., 2007). The enhanced activity of the two enzymes in the root of huckleberry in combination with sPOD indicated that huckleberry has a better capacity of containing ROS than eggplant. Since high root Na in eggplant corresponded to a reduction in CAT activity, it is possible that catalase in activation by salt stress occurred (Tejera *et al*., 2007).

Oxidative stress has also been shown to induce K^+ efflux from roots under salinity stress (Cuin and Shabala, 2007), a phenomenon that is thought to negatively affect growth (Shabala *et al*., 2005; Chen *et al*., 2007). In the present study, K^+ decreased in leaf and root of both plants, but the decrease was more marked in eggplant than in huckleberry, especially in the root (Table 2), where there was a corresponding elevated Na^+/K^+ ratio (Fig. 1) and MDA (Fig. 3). This suggests that besides selective inhibition of K^+ uptake under salinity (Tester and Davenport, 2003), oxidative stress-induced efflux from the root (Cuin and Shabala, 2007) could also contribute to the decrease in root K^+ of eggplant.

The cwPODs are generally known to be implicated in the process of lignification (Boerjan *et al*., 2003). Lignification has been shown to affect plant growth under salinity stress (Neves *et al*., 2010). Enhanced activity of this enzyme under salinity is an indication of enhanced lignin deposition and since it uses H_2O_2 as a substrate in the process, it could also indicate enhanced ROS detoxification, hence enhanced tolerance (Maia *et al*., 2013). In the present study, the activity of cwPOD increased markedly in the root and leaf of huckleberry but decreased in eggplant in both organs, although this decrease was not statistically significant (Fig. 8). The increased cwPOD in huckleberry coincided with an unaltered MDA content indicating that this enzyme could play a protective role against oxidative stress in both organs, especially in the leaf, where all the other enzymes were not significantly altered. Also, this

enhanced activity suggests enhanced deposition of lignin in these organs, which is in agreement with the findings of Sanchez-Aguayo *et al*. (2004). Lignin deposition inhibits cell elongation and eventually the growth of tissue or organ (Neves *et al*., 2010), indicating that the more reduced root DW in huckleberry compared with eggplant could be due to enhanced lignification in the root. However, under salt stress, this enhanced lignification could potentially contribute to the inhibition of Na influx into cells (Jbir *et al*. 2001). This inhibition of Na^+ influx has been suggested as an adaptive mechanism to control root-to-shoot Na^+ delivery under salinity (Karahara *et al*., 2004; Krishnamurthy *et al*. 2011), implying that the decreased activity of cwPOD in eggplant would correlate with reduced capacity for ROS detoxification as well as uncontrolled radial movement of solutes to the transpiration stream, thus allowing the free flow of Na^+ to the leaf, where it accumulates and causes oxidative stress damage.

Conclusion

The adaptation of huckleberry to salinity involves mechanisms for Na^+ exclusion in the leaf blade possibly located in the root, stem and petiole and enhanced antioxidant enzyme activity in the root. Although cwPOD is known to function primarily in lignin biosynthesis, especially in the root, its enhanced activity in the leaf of huckleberry, indicates a major role in ROS detoxification. Thus, in addition to conventional antioxidant enzymes, enhanced activity of cwPOD in leaf under salinity could constitute an important mechanism of stress adaptation.

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