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Full Length Article

Medium Supplementation of Ascorbic Acid Partially Recuperates the Salt Induced Impairments in Maize Radicles

Asma Saleem, Abdul Wahid* and Muhammad Arfan
Department of Botany, University of Agriculture Faisalabad 38040, Pakistan
*For correspondence: drawahid@uaf.edu.pk

Abstract

Salinity stress impairs the plant development and physiology primarily due to ion-toxicity. Initial growth stages are more prone to salinity and therefore offer a better system for studying mechanisms of salinity responses in plants. The toxic effects of salinity can be partially reversed with the exogenous use of stress alleviating compounds. In this study, effect of selected levels of salinity (NaCl; 120 mM) and medium supplemental ascorbic acid (AsA; 2.5 mM) and their interactive effects were determined on the changes in ion accumulation and cells and tissue architectural changes in the radicle stele and sleeve of a salt-tolerant (32B33) and a salt-sensitive (30Y87) maize hybrids. Results revealed that salt stress led to a reduction in dry weight and diameter of radicle due to buildup of Na^+ and a concomitant reduction in K^+ and K^+/Na^+ ratio in the stele and sleeve tissues, while the medium supplied AsA was quite more effective in reducing the Na^+ content and improving the K^+ contents and K^+/Na^+ ratio more in the sensitive hybrid. Applied salinity reduced the stele diameter, stele area, metaxylem area and phloem area but increased the number of metaxylem in the stele. Salt stress reduced the sleeve area, area of cortical cells but increased the cortex lysed area. Medium supplementation of AsA was effective under no salinity stress but more effectively recuperated the salinity induced damage on both the stele and sleeve tissues. Establishment of correlations of stele and sleeve ionic contents with different cells and tissues in these parts revealed that salinity tolerance in the maize hybrids was tightly correlated with the AsA triggered retention of greater K^+ contents and K^+/Na^+ ratio and reduced Na^+ contents especially in the sensitive maize hybrid. It is concluded that AsA partially repairs the tissue architecture by exercising effective control over the regulation of toxic ions.

Keywords: Ion-toxicity; Root architecture; Correlations; Phloem; Cortex damage; Maize

Introduction

Soil salinity is a noxious factor in agricultural practices, which hampers the plant growth and development by virtue of its multifarious effects (Munns and Tester, 2008; Morgan and Connolly, 2013). Plant growth and development under salinity stress is strongly dependent upon the effective regulation of ions in various parts of the plant (Epstein and Bloom, 2005). Since root is directly concerned with the absorption of water and nutrient from the soil solution, effective regulation of ions has a pivotal role in achieving the plant survival and performance in given soil conditions.

High toxic ions concentrations especially chloride and sulfate of sodium affect the plant growth by modifying their morphological, anatomical and physiological traits (Szepesi *et al*., 2009; Maqbool *et al*., 2016). High salinity reduced stomata number (Çavişoglu *et al*., 2007), decrease bundle length, xylem rows, vessels number and increase in both palisade and spongy tissue (Hussein *et al*., 2012). Cortical parenchyma and vascular cylinder diameter were reduced under salt stress condition in *Chloris gayana* (Ceccoli *et al*., 2011). Working on maize under salt stress, Farhana *et al.* (2014) recorded a reduction in root pith, metaxylem number, size of vascular cylinder and cortical parenchyma. Akcin *et al.* (2017) reported that in *Salicornia freitagii*, application of high salt stress (46.3 dS/m) reduced the cortical parenchyma, which was crucial for enhancing the water uptake by the plant roots.

The deleterious effects of salinity can be successfully lessened with the exogenous use of stress alleviating chemicals. For instance, in a comparative study using defined levels of different growth promoting agents, hydrogen peroxide, ascorbic acid, thiourea and gibberellic acid were quite effective in producing profound metabolic changes and improving germination and seedling growth attributes in sunflower (Wahid *et al*., 2008). In maize salicylic acid and 24-epibrassinolide have been successfully used to reduce the effect of salinity on the anatomical characteristics of maize (Agami, 2013). Rasheed *et al.* (2016) reported that pretreatment of sugarcane setts with proline and glycine betaine was of high significance in improving the nodal primordial activation thereby improving the sprouting of buds. Seed pretreatment, medium supplementation and

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foliar spray of thiourea proved of high value in improving, germination, growth and final yield in a number of crop species (Wahid *et al.*, 2017).

Ascorbic acid (AsA) plays multifarious roles in the plant life and improves plant growth and development by inducing physiological and molecular changes and improving the plant architecture (Smirnoff and Wheeler, 2000; Kaviani, 2014). Its growth enhancing properties can be well conceived from the fact that it is found predominantly in the growing regions in leaves and flowers (Ebrahim, 2005; Smirnoff, 2011). At plants cells and tissue levels, AsA reduced thickness of cuticle, increased length and width of vascular bundles and also enhanced cell division, cell expansion and fluid uptake (Lee and Kader, 2000). It protects against reactive oxygen species (ROS) that are formed during respiration and photosynthesis (Asada, 1994; Smirnoff, 2011); donates electron for mitochondrial electron transport and photosynthetic processes (Miyake and Asada, 1996), oxalate and tartrate synthesis (Saito, 1996). However, the role of root applied AsA in the structural and physiological modification involved in root functions is not investigated at a greater depth.

Root has two main parts; sleeve and stele. Sleeve comprises epidermis, cortex and extends up to endodermis while the stele consists of conducting tissues i.e., xylem and phloem, cambium and pith (Dickison, 2000). In literature there is hardly any report regarding the structure and function of root sleeve and stele tissues under the influence of salinity and possible reversal of salinity toxicity effects on these tissues with the medium supplementation of AsA. It is surmised that AsA nullifies the salinity effects by reduced accumulation of toxic ions in a tissue specific manner The objective of this study was to determine the possible role of medium supplementation of AsA in recoupment of the salinity damage on the physiological and architectural properties of the radicle tissue of two maize hybrids.

Materials and Methods

The seeds to two maize hybrids, 32B33 and 30Y87 selected for this research were obtained from Pioneer Maize Seed Plant, Sahiwal, Pakistan. Both the hybrids were initially optimized for their comparative salt tolerance at seedling stage. Among five hybrids and six salinity levels, hybrid 32B33 indicated 35%, whereas 30Y87 showed 64% reduction in plant biomass over control at 160 mM NaCl level and were therefore ranked salt tolerant and salt sensitive, respectively. For this study, the selected healthy seeds were surface sterilized with mercuric chloride (0.1%) for two minutes and then repeatedly washed 4–5 times with sterile distilled water. Petri-dishes lined with double layered filter paper were autoclaved to avoid any contamination. Twenty seeds of two selected maize hybrid were placed on filter paper in sterilized Petri-dish on a double layer of wet filter papers. At the time of sowing of seed, the treatments applied were control (distilled water), salt stress (120 mM NaCl) and ascorbate (2.5 mM AsA), AsA and salt + AsA. The petri-dishes were placed in a growth chamber under continuous white fluorescent light (*PAR* 300 µmol m^{-2} s^{-1}) at 28/24 ± 2ºC with a photoperiod of 14 h. The experiment was set up in a completely randomized fashion with three replications.

With the emergence of radicle in about seven days under different treatments the radicle length was measured after excising from its attachment with the seed immediately after harvest. To determine the dry weight of stele and sleeve tissues, a cut was applied to the intact radicle and stele tissue was carefully separated from the sleeve. The dry weight of both these parts was taken after drying them in an oven at 70ºC for seven days.

For the determination of ionic concentration, the whole radicle and stele and sleeve tissues separately (0.25 g each) were digested in 5 mL mixture of concentrated nitric acid and perchloric acid (3:1 ratio) till the samples became clear. The digests were diluted to 20 mL and filtered. The values of Na^+ and K^+ were estimated using flame photometer (Sherwood 410, UK). The actual amounts of both these ions were computed after comparing with the standard curve constructed using a series of known standards separately for Na^+ and K^+ (Yoshida *et al.*, 1976).

To determine the histological changes in the stele and sleeve, tissues taken from mature zone of radicle were preserved in formaline, acetic acid, alcohol and water (in 10:5:1:4 ratio) and anatomical determinations made as described by Ruzin (1999). The killed and fixed samples were embedded in paraffin blocks by passing through the serial dehydration. Sections of 5–7 µm thickness were cut with the help of rotary microtome (Shandon, New Hampshire, USA) and placed on adhesive coated glass slides. The sections were deparaffinized in xylene and rehydrated in decreasing concentrations of ethanol (99–50%) followed by distilled water. These sections were stained with toluidine blue O stain, processed and fixed by coverslips using Canada balsam to make permanent slides. The photographs of the stained tissues were taken on a camera equipped microscope (DG3 LaboMed, USA) at various magnifications. The measurements of various cells and tissues were taken from the stained sections using an ocular micrometer and compared with stage micrometer to determine their exact sizes.

The measurement of various cells and tissues were taken using ocular micrometer at 10×and 40×magnifications. The parameters recorded were root diameter, number of cortical cell layers in sleeve, sleeve area, cortex lysed area and area of cortical cells in root sleeve while stele diameter, vascular cylinder area, number of metaxylem per stele, metaxylem area and phloem area for root stele. Salinity induced lysed area in the cortex was determined by importing the micrograph images into MS Paint (Windows 7 Professional, Microsoft Corporation 2009).

Analysis of variance of all parameters was performed using COSTAT computer package (CoHort Software, 2003, Monterey, California). The least significance difference between the mean values was calculated. The Duncan's New Multiple Range test (DMRT) at 5% level of probability was used to test the differences among the mean values. The correlations were established between different whole radicle and different anatomical attributes of the stele and sleeve tissues separately of both the hybrids (Steel *et al.*, 1996).

Results

Whole Radicle, Stele and Sleeve Dry Mass Yield

Results revealed significant ($P<0.01$) differences in the treatments with significant ($P<0.05$) interaction of hybrids and treatments for dry mass of whole radicle, stele and sleeve tissues. Applied salinity stress reduced the radicle dry mass by 25% in 32B33 and by 43% in 30Y87. Medium supplemental AsA increased the radicle dry weight by 3 and 7% in 32B33 and 30Y87, respectively while medium supply of AsA reduced the salinity damage by 26 and 44% in the respective hybrids (Table 1). As regards stele dry weight, salt stress reduced this parameter by 25 and 33% in hybrids 32B33 and 30Y87, respectively, while medium supplemented AsA did not improve the stele dry weight in 32B33 but improved it by 9% in 30Y87. Application of AsA to the salinity treated plants reduced the salinity stress effect on stele dry weight of 32B33 by 17%, while by 24% in 30Y87 (Table 1). As for sleeve dry weight, results showed that salt stress was more damaging to sleeve tissue in reducing its dry weight by 25 and 50% in 32B33 and 30Y87, respectively. On the contrary, AsA increased the sleeve dry mass by 10% in 32B33 and by 7% in 30Y87. However, medium supplementation of AsA to salinity stressed radicles reduced the salt stress effect by 48% in 32B33 and by 87% in 30Y87 (Table 1).

Ionic Relations of Stele and Sleeve Tissues

Data revealed significant differences ($P < 0.01$) in the hybrids and treatments for Na^+, K^+ and K^+/Na^+ ratio of the hybrids by the interaction of hybrids and treatments was significant ($P<0.01$) for Na^+ and K^+/Na^+ ratio but not K^+ contents of both stele and sleeve tissues. Results showed that both stele and sleeve tissues kept Na^+ contents to the lowest extent under control and the medium supply of AsA. Applied salinity substantially increased the Na^+ contents in both the tissues over respective controls being 126 and 133% in the stele but by 116 and 112% in sleeve of 32B33 and 30Y87, respectively. Medium supplementation of AsA to salt stressed tissues of both the hybrids indicated a pronounced reduction of Na^+ in the stele (43 and 36% in 32B33 and 30Y87, respectively) as compared to sleeve (8 and 5% reduction in32B33 and 30Y87, respectively) over respective salt-treated controls (Fig. 1a). Results regarding K^+ contents revealed that both the hybrids showed a similar K^+ content in stele tissue and sleeve tissue under control condition. Applied salinity decreased the stele and sleeve K^+ contents highly in 30Y87 (51 and 50%) than 32B33 (36 and 33%). Medium supplementation of AsA under no salt stress only minimally improved the K^+ content in the stele (3 and 6% in 32B33 and 30Y87) and sleeve (5% in both hybrids) tissues. However, medium supply of AsA to salt stressed plants effectively improved the K^+ contents in stele by 52 and 90% and in sleeve by 21 and 25% as compared to salinity stressed radicles of 32B33 and 30Y87, respectively (Fig. 1b). As regards K^+/Na^+ ratio, 32B33 showed a relatively higher stele K^+/Na^+ ratio than 30Y87 under control condition. Salt stress reduced stele K^+/Na^+ ratio by 72 and 79% in the hybrids. AsA treatment to non-stressed plants enhanced this ratio in stele by 2% in 32B33 but reduced by 12% in 30Y87 while under salt stress this ratio was increased by 167% in 32B33 and by 199% in 30Y87, respectively as compared to salt treated stele tissues. For root sleeve, both the hybrids exhibited a similar K^+/Na^+ ratio. Salt stress reduced K^+/Na^+ ratio more in 30Y87 than 32B33 (76 and 69%, respectively). Medium supply of AsA to no-salt treated sleeve enhanced this ratio relatively more in 32B33 than 30Y87 (by 14 and 10%, respectively). AsA supplied to salt treated sleeve improved this ratio by 64 and 52% in the respective hybrids (Fig. 1c).

Anatomical Attributes of Stele and Sleeve Tissues

As can be visualized from the images of anatomical attributes of stele and sleeve tissues of both the hybrids under the effect of different treatments, it is clear salinity stress dislodged the pith and cortical tissue, reduced the cortical cell layers, reduced the phloem area and the cortical cell size (Fig. 2). Measurements taken from the stele and sleeve sections under the effects of various treatments are given in Fig. 3.

Data recorded for various structures in stele tissue revealed that under control condition the stele diameter was relatively more in hybrid 32B33 than hybrid 30Y87. Applied salinity reduced this diameter by 27 and 30%, respectively in both the hybrids. AsA treatment under no stress conditions marginally improved the stele diameter (by 6 and 8% in 32B33 and 30Y87) but medium supply of AsA increased this parameter by 36 and 33% over the salinity treated plants in the respective hybrids (Fig. 3a). The area of stele was greater in 32B33 than 30Y87 under control condition. Under salinity stress stele area was reduced by 15 and 25% in both the hybrids. Treatment with AsA under no salt stress nominally (~3%) improved this area in both hybrids, although AsA supplementation to salt stressed radicles was quite more effective in improving this area in 30Y87 (26%) than 32B33 (15%) as compared to salinity treated radicles (Fig. 3b).

Table 1: Changes in the dry weight of radicle diameter, stele and sleeve tissues of two maize hybrids as affected by individual and combined application of salinity and AsA

Hybrids	Treatments	Radicle dry weight	Stele dry weight	Sleeve dry weight
32B33	Control	72.67±2.31b	30.93±2.81a	41.73±1.22b
	Salinity	54.33±3.21d	23.13±2.30d	31.20±2.09c
	AsA	75.00±3.00a	30.93±2.20ab	46.07±2.58a
	Salinity + AsA	73.33±3.51b	28.53±1.80b	44.80±2.78ab
30Y87	Control	71.33±3.51b	28.53±1.47b	42.80±2.11b
	Salinity	40.33±1.53e	19.00±1.97e	21.33±3.22d
	AsA	76.67±3.06a	31.00±2.31ab	45.67±2.16a
	Salinity + AsA	69.67±3.79c	25.87±2.31c	43.80±3.61b

Means sharing same letter differ non-significantly (P>0.05)

Table 2: Correlation coefficients (r) of root characteristics with Na^+, K^+ and K^+/Na^+ ratio across different treatments in two maize hybrids differing in salinity tolerance (n = 4)

Radicle tissue	Ions	Anatomical characters	Hybrid 32B33	Hybrid 30Y87
Dry weight	Na^+	-	-0.927ns	-0.943ns
	K^+	-	0.884ns	0.900ns
	K^+/Na^+ ratio	-	0.815ns	0.797ns
Diameter	Na^+	-	-0.994**	-0.955*
	K^+	-	0.998**	0.929ns
	K^+/Na^+ ratio	-	0.984**	0.834ns
Stele	Na^+	Stele dry weight	-0.996**	-0.976*
		Stele diameter	-0.980*	-0.939ns
		Stele area	-0.990**	-0.964*
		No. of metaxylem/stele	0.965*	0.929ns
		Metaxylem area/stele	-0.994**	-0.912ns
		Phloem area	-0.955*	-0.919ns
	K^+	Stele dry weight	0.975*	-0.903ns
		Stele diameter	0.993**	0.986*
		Stele area	0.988*	0.997**
		No. of metaxylem/stele	-0.942ns	-0.794ns
		Metaxylem area/stele	0.0.975*	0.876ns
		Phloem area	0.976*	0.925ns
	K^+/Na^+ ratio	Stele dry weight	0.998**	0.912ns
		Stele diameter	0.964*	0.936ns
		Stele area	0.986*	0.952*
		No. of metaxylem/stele	-0.947ns	-0.955*
		Metaxylem area/stele	0.998**	0.947ns
		Phloem area	0.941ns	0.941ns
Sleeve	Na^+	Sleeve dry weight	-0.954*	-0.867ns
		Cortical cell layers	-0.963*	-0.892ns
		Sleeve area	-0.998**	-0.977*
		Cortex lysed area	0.987*	0.864ns
		Cortex cell area	-0.997**	-0.917ns
	K^+	Sleeve dry weight	0.966*	0.820ns
		Cortical cell layers	0.972*	0.879ns
		Sleeve area	0.993**	0.963*
		Cortex lysed area	-0.995**	-0.833ns
		Cortex cell area	0.983*	0.887ns
	K^+/Na^+ ratio	Sleeve dry weight	0.888ns	0.749ns
		Cortical cell layers	0.964*	0.851ns
		Sleeve area	0.971*	0.938ns
		Cortex lysed area	-0.993**	-0.788ns
		Cortex cell area	0.955*	0.847ns

Significant at: *, P<0.05; **, P<0.01 and ns, P>0.05

With no significant (P>0.05) difference among treatments and hybrids with no (P>0.05) interaction of both these factors, the number of metaxylem per stele was not very different among the treatments (Fig. 3c). For metaxylem area, there was significant difference in the hybrids, treatments with a significant (P<0.01) interaction of these factors. Applied salinity reduced the metaxylem area by 23% in 32B33 and 31% in 30Y87. Medium supply of AsA to no salt stress condition was least effective in improving the metaxylem area (2 and 3% in 32B33 and 30Y87, respectively), while AsA supplementation to salinity stressed tissue improved

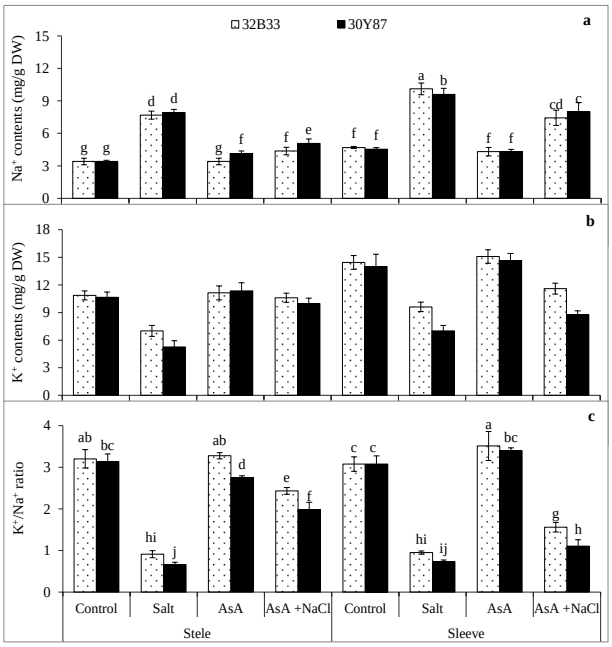

Fig. 1: Effect of salinity, AsA and combined salinity and AsA treatments on changes in Na^+, K^+ and K^+/Na^+ ratio in root sleeve and stele diameter of salt-stressed maize hybrids. Data bars sharing same letters differ non-significantly (P>0.05)

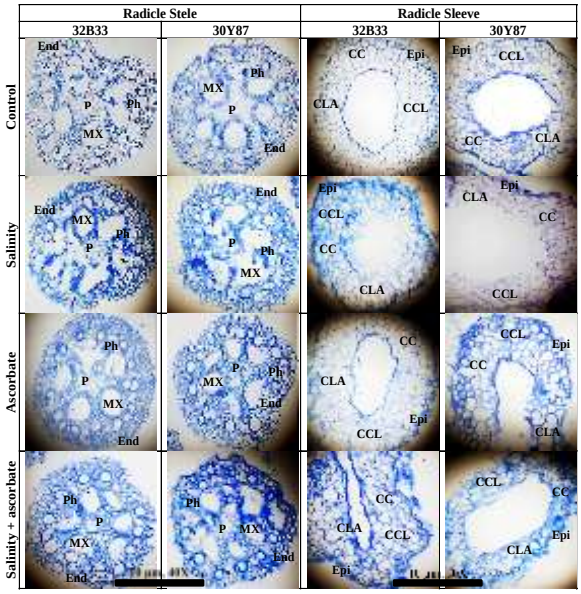

Fig. 2: Changes in root anatomical structure of differentially salt resistant maize varieties by medium supplementation of selected level of ascorbate under salt stress: **End,** endodermis; **MX,** metaxylem; **Ph,** phloem; **P,** pith; **Epi,** epidermis; **CCL,** cortical cell layers; **CLA;** cortex lysed area; **CC,** cortical cells

this attribute by 22 and 16% in the respective hybrids (Fig. 3d). Phloem area was relatively more reduced (36%) in 30Y87 than 32B33 (26%) under salt stress. Medium supply of AsA under no-stress condition was capable of improving phloem area by 11 and 8% in the respective hybrids, whereas AsA supplementation to salt-stressed maize stele improved this attribute by 28% in 32B33 and by 29% in 30Y87 (Fig. 3e).

Measurements taken for different root sleeve attributes showed that irrespective of the treatments the number of cortical cell layers (CCL) were higher in 32B33 than 30Y87. Applied salt stress reduced this feature in both the hybrids although more in 32B33 (13%) than 30Y87 (20%). Treatment with AsA improved the CCL by 6 and 9% in 32B33 and 30Y87 while AsA supplementation to salt stressed plants improved this attribute by 20 and 21% in the respective hybrids (Fig. 3f). Sleeve area was similar in both the hybrids under control condition but was substantially reduced under salt stress by 27% in 32B33 and by 51% in 30Y87. Medium supply of AsA to control radicles produced 2–3% change in the sleeve area of the respective hybrids while AsA supplementation to salt treated plants produced an increase in the sleeve area by 19% in 32B33 and by 57% in 30Y87 (Fig. 3g). There was statistically no difference in the lysing of cortical area in the control and AsA supplemented sleeves. However, applied salinity increased the lysed area in the cortex up to 96% in 32B33 and 138% in 30Y87, while medium supply of AsA to salinity treated radicles nullified the salinity stress effects by 16% in 32B33 and by 50% in 30Y87 (Fig. 3h). Concerning area of cortical cells, there was no difference in the control and AsA treated sleeves. Applied salinity stress reduced the area of cortical cells by 47 and 62% in hybrids 32B33 and 30Y87, respectively, while AsA supplied to salinity treated radicles improved this parameter by 64 and 76% in respective hybrids (Fig. 3i).

Data revealed that irrespective of the treatments applied, hybrid 32B33 had higher radicle diameter than 30Y87.The radicle diameter was significantly reduced by salinity stress in both hybrids but 30Y87 was more affected than 32B33 (37 and 32%, respectively). Medium supply of AsA to control radicles improved radicle diameter by 5 and 14% in the respective hybrids. Medium supply of AsA to salinity stressed plants enhanced the radicle diameter by 27 and 44% in 32B33 and 30Y87, respectively as compared to salt stressed control (Fig. 3j).

Correlations

Radicle dry weight was correlated with none of the ions in both the hybrids. For stele tissue, stele dry weight and stele area were negatively correlated with stele Na^+ in both the hybrids while stele diameter was negatively correlated in 32B33 only. The number of metaxylem per stele was positively correlated but metaxylem area and phloem area were negatively correlated with stele Na^+ contents

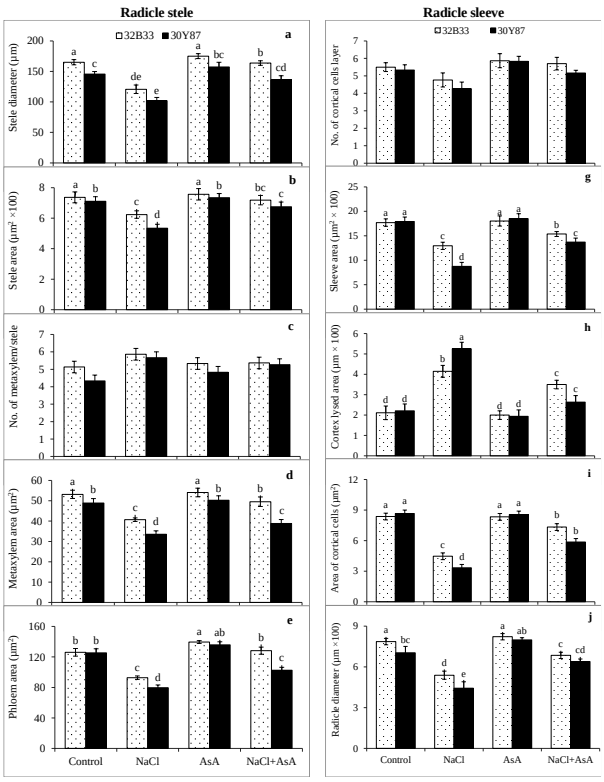

Fig. 3: Effect of salinity, AsA and combined salinity and AsA treatments on changes in root sleeve and stele diameter of salt-stressed maize hybrids. Data bars sharing same letters differ non-significantly (P>0.05)

in hybrid 32B33 only. In hybrid 32B33, the stele K^+ was positively correlated with stele dry weight, stele diameter, except number of metaxylem per stele and phloem area, while in hybrid 30Y87 only the stele diameter and stele area were positively correlated. In hybrid 32B33, stele K^+/Na^+ ratio indicated positive correlation with all the stele characters except number of metaxylem per stele and phloem area while in hybrid 30Y87 stele diameter and stele area were positively correlated and number of metaxylem per stele was negatively correlated with stele K^+/Na^+ ratio (Table 1). For sleeve tissue, in 32B33, sleeve Na^+ indicated negative correlations with all the sleeve attributes except a positive relationship with the cortex damage while in 30Y87 only a negative correlation of Na^+ was detected with sleeve area. As regards sleeve K^+, in the hybrid 32B33, all the sleeve attributes were positively correlated except a negative correlation of cortex lysed area. The sleeve K^+/Na^+ ratio showed no correlation with sleeve dry weight and negative correlation with cortex lysed area and rest of the correlation with other attributes were positive in hybrid 32B33.In hybrid 30Y87 none of the attributes indicated any correlation with K^+/Na^+ ratio (Table 1). In hybrid 32B33, the radicle diameter was negatively correlated with root Na^+ but positively with radicle K^+ and K^+/Na^+ in 32B33, while in 30Y87, a negative correlation of Na^+ but no correlation of K^+ and K^+/Na^+ ratio was noted (Table 1).

Discussion

In this study applied salinity markedly reduced dry mass production of the whole radicle and stele and sleeve tissues more of salt sensitive hybrid (30Y87). Furthermore, radicle sleeve was more affected than the stele tissue and sensitive maize hybrid was more damaged. Medium supplementation of AsA proved of immense significance in nullifying the salt damage to the maize radicles (Table 1). The roles of AsA as a coenzyme and a scavenger of reactive oxygen species, has gained considerable importance in plant biology (Smirnoff and Wheeler, 2000; Zhang, 2013). Exogenous supply of AsA improved multiple stress tolerance in sunflower with the induction of antioxidant system and reduction in the levels of malondialdehye (Kaya, 2017).

To probe into the possible reasons for enhanced dry mass yield of radicle, sleeve and stele tissues the determinations were made for Na^+, K^+ and K^+/Na^+ ratio in the stele and sleeve tissues separately in the salt-tolerant and salt-sensitive maize hybrids (Fig. 1). Enhanced salinity tolerance in plants has been ascribed to efficient absorption of K^+over Na^+ and maintenance of higher tissue K^+ in all plants especially the glycophytes (Wahid, 2004; Almeida *et al.*, 2017). Here the measurements were made separately in the stele and sleeve tissues of the two maize hybrids revealed that medium supplementation of AsA was quite effective especially in the sensitive maize in enhancing K^+ accumulation whilst reducing the Na^+ contents in the stele and sleeve tissues. This tendency further resulted in the enhanced K^+/Na^+ ratio especially in the sleeve tissue with the medium supplementation of AsA (Fig. 1). The plant salt tolerance has been correlated with enhanced K^+ contents and K^+/Na^+ ratio (Wahid *et al.*, 1997; Kao, 2011). The enhanced accumulation of Na^+ and a concomitant reduction in K^+ as well as K^+/Na^+ ratio is a well-known manifestation of salinity sensitivity in different plant species especially glycophytes (Alhasnawi *et al.*, 2015). Drawing the correlations of the parameters radicle dry mass and radicle diameter with Na^+, K^+ and K^+/Na^+ indicated that although there were no correlations in the radicle dry mass the of both the hybrids, the changes in radicle diameter was more tightly correlated with the radicle ionic contents than the sensitive maize (Table 2). This revealed that expansion and elongation of radicle was more important at initial growth stages.

It is commonly observed that root tissue is directly exposed to the excess of ions present in the soil solution (Morte and Verma, 2014). These ions are inevitably taken up and reach the stele after passing through the cortex and endodermis (Maathuis *et al.*, 2014). Due to the excess of ions, there was an increased damage to the root structure and sensitive materials are more targeted than the tolerant

one (Zhu, 2007). Damage to the root structure may be in the form of lysing of the cortical tissue or reduction in cortical cell size, reduced size of the metaxylem (Rashid and Ahmed, 2011) and phloem tissues (Flowers *et al.*, 2014). This is likely to influence the vascular cambium activity which is involved in the production of xylem and phloem (Cavusoglu *et al.*, 2008). As depicted in Fig. 2, the applied salinity induced quite a few structural changes in the stele and radicle tissue of both the maize hybrids although the extent of changes was more distinct in the sensitive maize (30Y87). However, the medium supplementation of AsA under saline condition was quite more effective in recuperating the effect of salinity and sensitive maize was relatively on a greater advantage.

As mentioned above, of the two parts of root, the stele comprises, xylem and phloem and both these tissues are concerned with the long distance transport within the plant body. Salinity has been known to enhance the development of xylem with the enhanced expression of S-adenosyl-L-methionine (SAM) synthase enzyme leading to induce the lignification (Sanchez-Aguayo *et al.*, 2004). Considering the changes in stele part of the root, applied salinity reduced the stele diameter and its area, while medium supplemental AsA alone or in combination with salinity improved both these attributes. It was revealed that number of metaxylem elements was the highest under NaCl followed by AsA alone and combined application of NaCl and AsA, while, the metaxylem cellular area was the highest under control followed by salinity and combined application of salinity and AsA. The phloem area was greatly enhanced by AsA when applied singly or in combination with salinity (Fig. 2). Reduction in the phloem area of root and stem due to medium supplemented salinity stress has been reported to be one of the reasons for reduced growth of *Gazania harlequin* (Younis *et al.*, 2014). In this study, results revealed that the medium supplementation of AsA tended to reverse the salinity damage and partially revive especially the phloem area in the stele. It is plausible that being living tissue, the maintenance of structure of phloem is a crucial effect of AsA most probably with the reduced generation of ROS and sustained function of sieve element in providing the photoassimilates to root tissue (Thiomine, 2002). Taken together improved stele tissue structures and the positive correlation of K^+ and negative of Na^+ in the salt-tolerant hybrid, whilst their absence in the salt-sensitive maize, are plausible due to a clearer specificity of K^+ over Na^+(Table 2).

The results further showed that there was a well-marked reduction in the radicle diameter, sleeve width and cortical cell area and less-marked reduction in cortical cell layers and with salt treatment while medium supplement of AsA under saline or non-saline conditions was quite effectively alleviated the salt stress effects as determined from the final dimension of sleeve (Fig. 2). Salt-sensitive maize radicle exhibited overall reduction in sleeve area and radicle diameter especially due enhanced cortical lysed area and a reduction in the area of cortical cells due to salt stress, whilst the reversal of salt-damage was also more in this hybrid (Fig. 2). However, closer positive correlations of the K^+ and K^+/Na^+ ratio with the sleeve attributes except sleeve lysed area and negative ones of Na+ with sleeve characters except positive one of cortex lysed area in the tolerant maize hybrid indicate their significance in the salinity tolerance due to the medium supplementation of AsA (Table 2). The intactness of radicle sleeve tissue with AsA under salt stress is important in the steady water and mineral transport via apoplast and symplast pathways (Ola *et al.*, 2012; Gallie, 2013).

Conclusion

Although applied salinity variably modified the anatomical attributes of both the hybrids, nevertheless hybrid 32B33 was quite more tolerant and more responsive to AsA supplementation as evident from the structure of root sleeve and stele. The most conspicuous modifications associated to salinity tolerance were increased metaxylem elements, increased phloem area and reduced cortex lysed area with the medium supplementation of AsA especially under salt stress.

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