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Impact of Salt and Boron on Stem and Root Xylem Thickness of Corn

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Abstract

The high levels of naturally occurring salinity and B in the soil and the irrigation with water containing high levels of salts and B affect the growth and development of plants. This experiment aim to study the response of plants to the combination of B and salinity on plant growth and anatomy. The growth and yield of corn were measured at different B and salinity levels. The result from these experiments has revealed that salinity causes reduce in plants length without any effect of boron. Moreover, salinity increases xylem vessel thickness at the level of the root and the high levels of boron combined with high levels of salinity were able to mitigate salt effect on xylem thickness. Changes at the level of the stem were insignificant and the major effect of is on the root.

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Keywords

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Introduction

In the environment, plants are subjected to several stresses such as drought, salinity, mineral deficiency or toxicity and many others. At worldwide level, soil salinity is among the most devastating environmental stresses. It represents a major environmental constraint to crop production by affecting about 45 million hectares of irrigated land (Roy *et al.*, 2014).

In presence of stress, plant is unable to express its full genetic potential for growth, development and reproduction (Wallender and Tanji, 2011), but, they have developed several mechanisms to tolerate and overcome these stresses. Salt stress affect negatively the plants through water and ionic stress, nutritional disorders, oxidative stress, growth reduction, alteration in photosynthesis as well as in lipid and protein metabolism (Hasanuzzaman *et al.*, 2013; Munnus 2002; Munnus and Tester 2008; Carillo *et al.*, 2011).

High salinity affects the plants in two major phases: osmotic and ionic phases. The first one, start immediately when the concentration of salt around the roots reaches a threshold level (Munnus and Tester, 2008), and then the ability of roots to extract water from the soil was disturbed (Carillo *et al.*, 2011). This was resulted a significant decrease in shoot growth rate, leaf area and the emergence of new leaves and lateral buds (Munnus and Tester, 2008). Plants also close their stomata in order to reduce water loss (Carillo *et al.*, 2011). Moreover, salt decrease nutrients uptake, such as K^+ and Ca^{2+}, which reduces root cell growth and in particular, compromises root tips expansion (Carillo *et al.*, 2011). However, the ionic phases which takes days, weeks or months is the result of salt accumulation in leaves, leading to salt toxicity in the plant, primarily in the older leaves (*i.e.* salt-specific effect) (Lauchli and Grattan, 2007). As a result, there would be a reduction of the total leaf area which will in turn lead to a reduction in photosynthetic activities, and affecting then the overall carbon balance necessary to sustain growth (Lauchli and Grattan, 2007).

In the other side, the presence of some elements in the soil can affect the impact of salinity on plants. For example, plants may face a double stress: salt and boron (B) toxicity. This condition could be found in boron-rich soils with high contents of naturally occurring salinity, or in soils irrigated with groundwater containing high concentrations of salts and B (Bonilla and González-Fontes, 2011). Till now, there is a limited number of research works studying the combined effect of salinity and boron. These studies show that salt-boron interactions are complex and may be additive, synergistic, antagonistic or independent (Bonilla and González-Fontes 2011; Yermiyahu *et al.,* 2008). For example, (Hanson *et al.,* 1999) showed an independent effect of both stresses on barley, corn and alfalfa. Other studies showed an additive effect in which both stresses adversely affected the plant than each stress alone such as in wheat (Wimmer *et al.,* 2003) and hot pepper (Supanjani, 2006). However, it has been reported that the supply of B enhances crop salt tolerance and improve yield production in saline soils (Camacho-Cristóbal *et al.,* 2008). In fact, B reduces the uptake of Cl^- by plant and then decreases their accumulation in the leaves (Bonilla and González-Fontes 2011). Also, it has been proposed that, under simultaneous presence of B and salt stress, boric acid could affect the activity of specific membrane components regulating the functions of certain aquaporin isoforms and ATPase as possible components of the salinity tolerance mechanism (Camacho-Cristóbal *et al.,* 2008). Many studies have revealed that salt stress affects the histology of root and stem tissue. In particular, salinity affects different aspects of xylem vessels (Hameed *et al.*, 2010). Moreover, boron is well known for its role in cell wall structure and in lignification and differentiation of xylem.

The aim of this work is to investigate the effect of different salt and boron concentrations on sweet corn grown on Murashige and Skoog medium. Plant length and different aspects of stem and root metaxylem vessels including their thickness, diameter and number are studied. In other words, we would like to test if the interaction of boron and salts on the studied parameters are independent, antagonistic, synergistic or additive.

Materials and Methods

Seeds sterilization

First of all, the sweet corn seeds were washed with water and soap. Then they were washed with 70% ethanol for 30 seconds. Next, seeds were washed with 20% commercial bleach (1% NaOCl) with two drops of tween 20 and while shaking for 15 minutes. Finally, they were washed for three times with sterile distilled water for 2, 5 and 10 minutes respectively.

Sterile seeds were aseptically transferred to tubes containing MS medium alone (control), or with different concentrations of either salt (NaCl), boron {boric acid (B $[OH]_3$)} or a combination of both elements. The concentrations used in this experiment are listed in table 1.

Plants analysis

Four weeks after seeds germination, corn plants were removed from the tubes and transverse sections were cut from their roots and stems using a razor blade. The sections were immediately placed in water after cutting to prevent them from drying out. Then, the sections were stained using the double staining protocol. Briefly, plant sections were soaked with javel water (commercial bleach) for 15 minutes, and then the sections were washed several times with water in order to remove any traces of the bleach. The washed sections were then soaked with concentrated acetic acid for 5 minutes. One drop of iodide green (1%) and 8 drops of carmine stain (1%) were added and allowed to stain the sections for 3 minutes. The sections were washed with water. Finally, slides of root and stem sections of the different plants were prepared and observed under a light microscope in order to observe changes at the level of xylem vessels.

Studied Parameters

Metaxylem thickness and inner diameter were measured from snapshots taken at 100X magnification. The number of metaxylem vessels per vascular bundle was counted. Three replicates were done for each parameter.

Statistical Analysis

Statistical analyses were performed using XLSTAT software (Addinsoft TM). When necessary, data were log-transformed before analysis in order to improve normality and variance homogeneity of residues.

Variance analyses were performed by a one-way ANOVA with fixed effect (type l F statistics). Post hoc tests were performed using the Duncan method at the 5% level.

Results and Discussion

Plant length

Salinity caused a significant decrease in the length of corn plants compared to control ones (Figure 1A). There was a 25.6 % reduction at S1, 18.1 % at S2, 22.8% at S3 and 40.0% at the highest salinity level, S4. However, the change in length between different salinity levels was insignificant. Salinity has been shown to cause plant height reduction in corn (Maas *et al.*,, 1983) as well as in several plants including tomato and cucumber (Al-Harbi, 1995), soybean (Dolatabadian *et al.,* 2011) and pepper (Yermiyahu *et al.,* 2008). Plant height reduction under salt stress maybe due to changes in plant- water relationships which suppress meris1qtem activity as well as cell elongation (Dolatabadian *et al.*, 2011). Moreover, the change in length at different boron concentrations was insignificant compared to control plants (Figure 1B). This may be explained by the fact that boron in high concentrations is not toxic to all plants and that it may suit some plants (Bastías *et al.,* 2004).

When combinations of the different levels of salinity and boron were applied, different results were obtained. Plants subjected to S1 in combination with the different boron concentration (S1B1, S1B2 and S1B3) showed insignificant increase in length compared to plants subjected to S1 only (Figure 1C). Similarly, plants subjected to S2 in combination with either B1 or B2 (S2B1 and S2B2) showed insignificant increase in length compared to S2 plants; whereas, at the highest boron level (S2B3), a 15% decrease was obtained but it was insignificant. However, this decrease was significant compared to control plants (30.3%) (Figure 1D). As for S3B1, S3B2 and S3B3 plants, the changes in length were all insignificant compared to both S3 and control plants (Figure 1E). On the other hand, S4B1 and S4B2 plants showed a slight increase while S4B3 showed a slight decrease (27.3%) compared to S4 but these changes were statistically insignificant. However, S4B1, S4B2 and S4B3 showed significant decrease in length compared to control plants (22.8%, 32.0 % and 56.4 % respectively) (Figure 1F). These results indicate that salinity had the major negative impact on plant length and that boron caused a slight increase in length when combined with low salinity levels, however it was in significant. At high salt concentration (S4), the highest boron concentration (B3) caused a further, but rather insignificant, decrease in length compared to S4. This shows that boron and salt effects are independent with regard to plant length in sweet corn. Study done on wheat, alfalfa and eucalyptus showed similar results (Yermiyahu *et al.,* 2008).

Root metaxylem

Thickness

Increasing salinity level from S1 to S4 caused increase in metaxylem wall thickness (Figure 2). This increase was insignificant at S1 (15.9 %), but it was significant at S2 (66.3%), S3 (117 %; 2.16 fold) and S4 (171%; 2.71 fold) (Figure 3A). The increased thickness is probably due to the greater deposition of lignin in vascular tissues which was suggested to aid in inhibiting root growth as a mechanism to tolerate salt stress (Dolatabadian *et al.,* 2011).

At different boron concentrations, there was a significant increase in xylem thickness but it was slight compared to the increase obtained under high salinity levels. The increase ranged between 12.3 % and 48.3% (Figure 3B). This result is expected since boron is known for its role in enhancing lignification (Jameson and Schmidt, 1939).

In S1B1, S1B2 and S1B3 plants, no significant changes in thickness were observed compared to S1 plants (Figure 3C). Similarly, in S2B1, S2B2 and S2B3 plants, no significant changes in thickness were observed compared to S2 plants (Figure 3D). However, in plants subjected to different concentrations of boron in combination with S3 (S3B1, S3B2 and S3B3), a significant decrease in thickness was observed compared to S3 plants. The decrease ranged between 29.8 % and 33.9 %. However, the thickness was still significantly higher than the thickness in control plants (Figure 3E). In S4B1, S4B2 and S4B3 plants, significant decrease of 23.7 %, 41.8 % and 57.5 % respectively was obtained compared to S4 plants (Figure 3F). The thickness in S4B1 and S4B2 plants was still significantly higher than in control plants. However, interestingly, the thickness in S4B3 plants was almost similar to control plants (Figure 3F). These results show that at the lower salt concentration (S1 and S2), boron was unable to oppose salt effect on xylem thickness, whereas at higher salt concentrations (S3 and S4), it was able to revert salt effect by decreasing xylem thickness.

Table.1 the concentrations of salts and boron in different treatments

			NaCl (g/L)			
			S_1	**S_2**	**S_3**	**S_4**
			3	4.5	6	9
	B_1	9	S_1B_1	S_2B_1	S_3B_1	S_4B_1
$B[OH]_3$(mg/L)	**B_2**	12	S_1B_2	S_2B_2	S_3B_2	S_4B_2
	B_3	15	S_1B_3	S_2B_3	S_3B_3	S_4B_3

Fig.1 The variation of plant length (cm) in response to different concentrations of salt (NaCl) and boron. Bars carrying common letters are insignificantly different

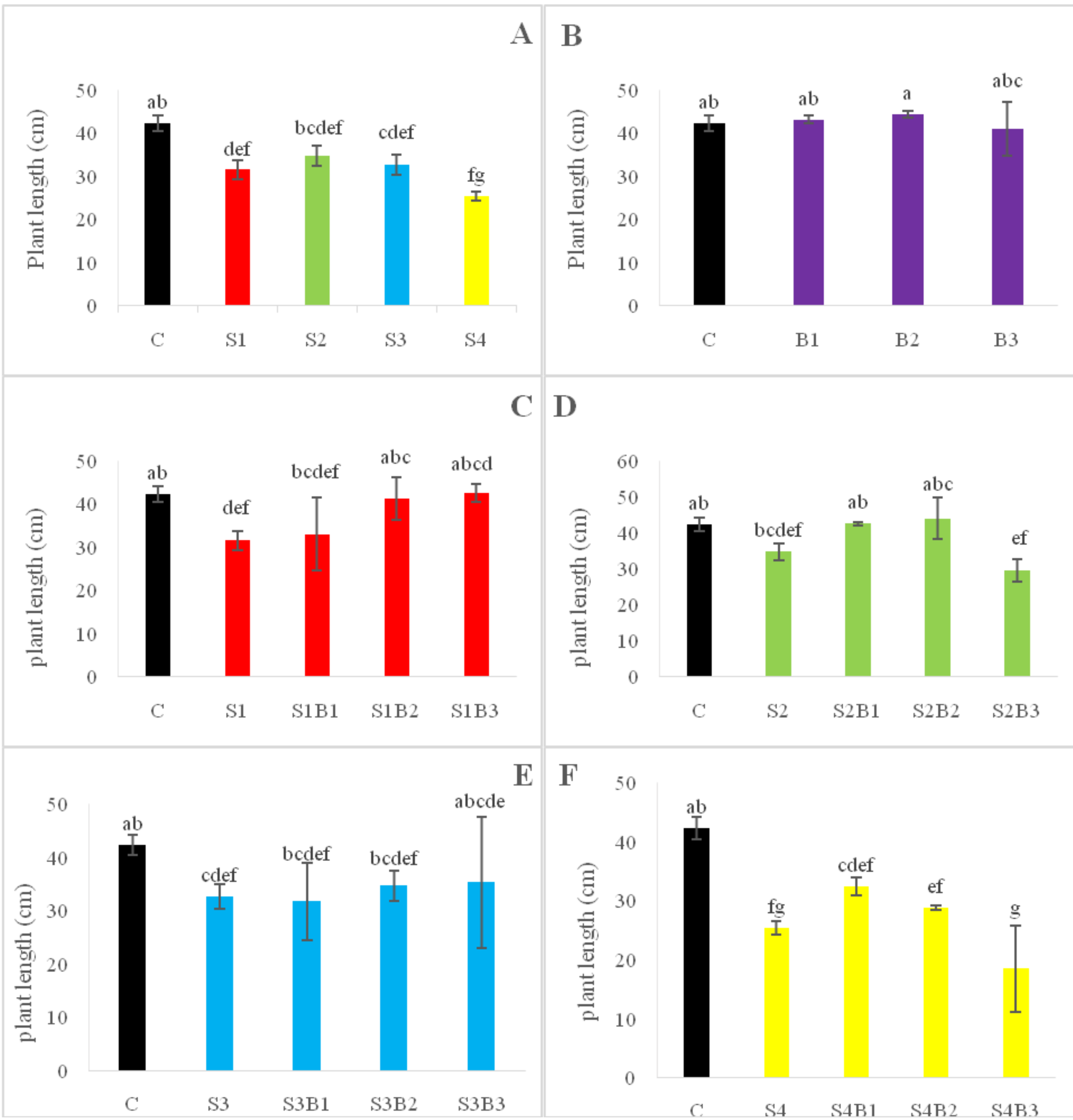


Fig.2 Cross sections of plant roots showing metaxylem vessels at different concentrations (a): Control- (b): S1- (c): S2- (d): S3- (e): S4- (f): S4B3

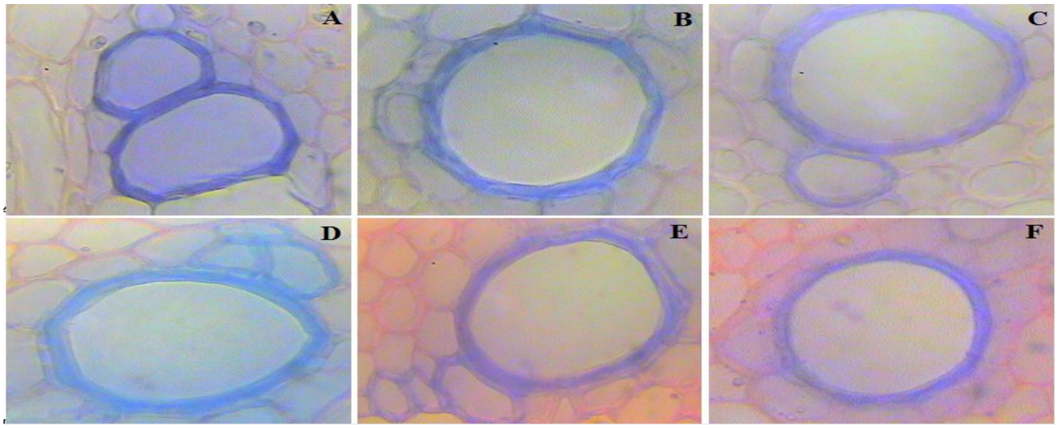


Fig.3 The variation of root metaxylem thickness (µm) in response to different concentrations of salt (NaCl) and boron. Bars carrying common letters are insignificantly different

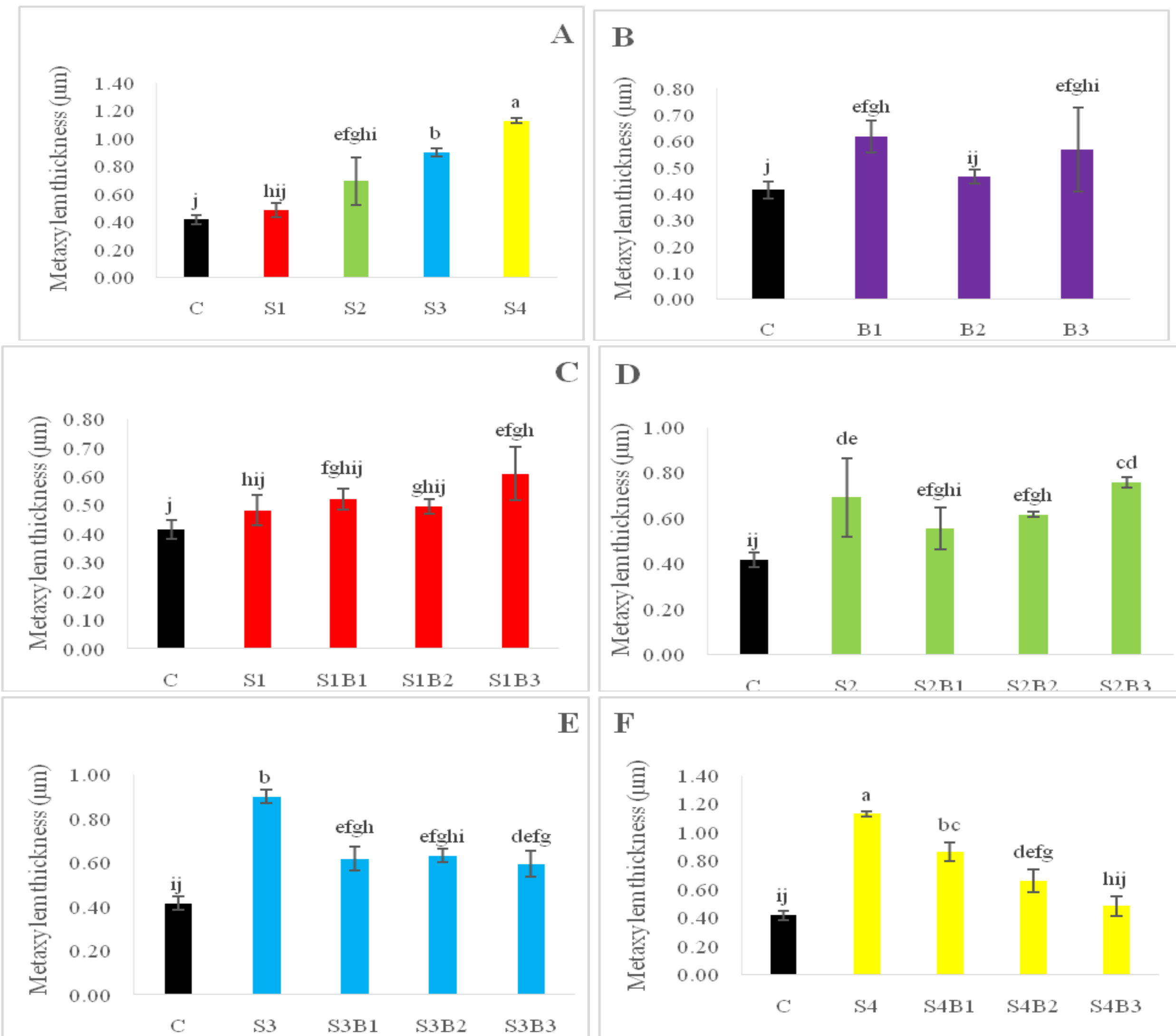


Fig.4 The variation of root metaxylem diameter (µm) in response to different concentrations of salt (NaCl) and boron. Bars carrying common letters are insignificantly different

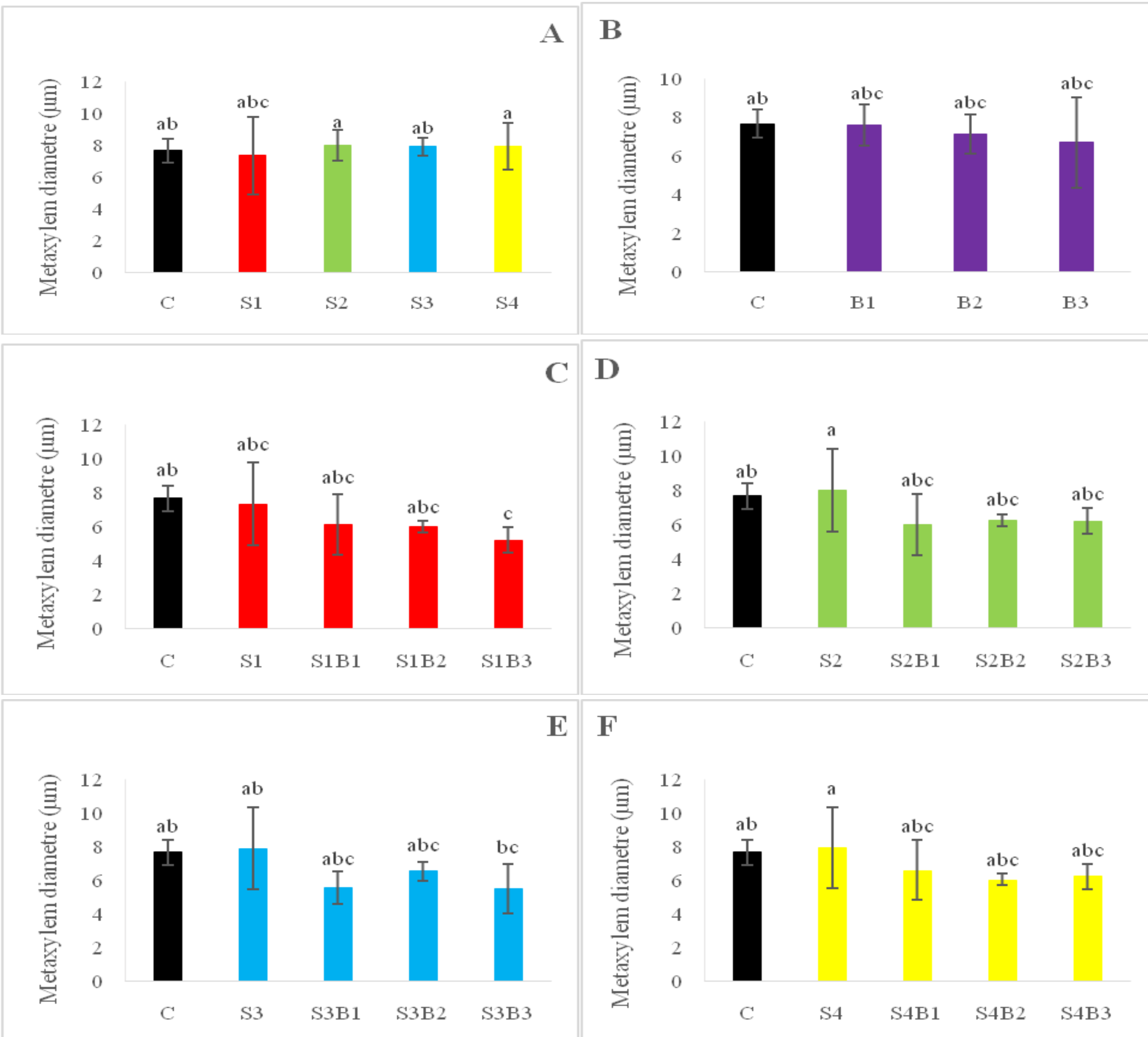


Fig.5 The variation of metaxylem vessels number/vascular bundle in sweet corn roots. Bars carrying common letters are insignificantly different

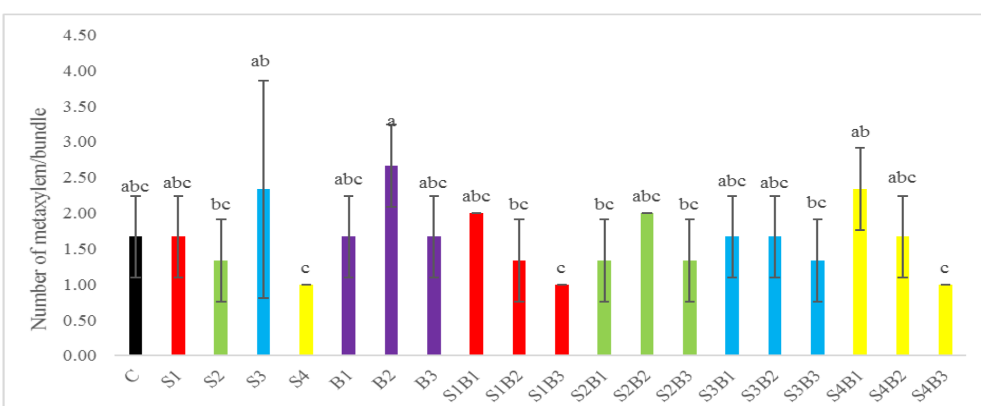


Fig.6 The variation of metaxylem thickness (µm) in response to different concentration of salt (NaCl) and boron. Bars carrying common letters are insignificantly different

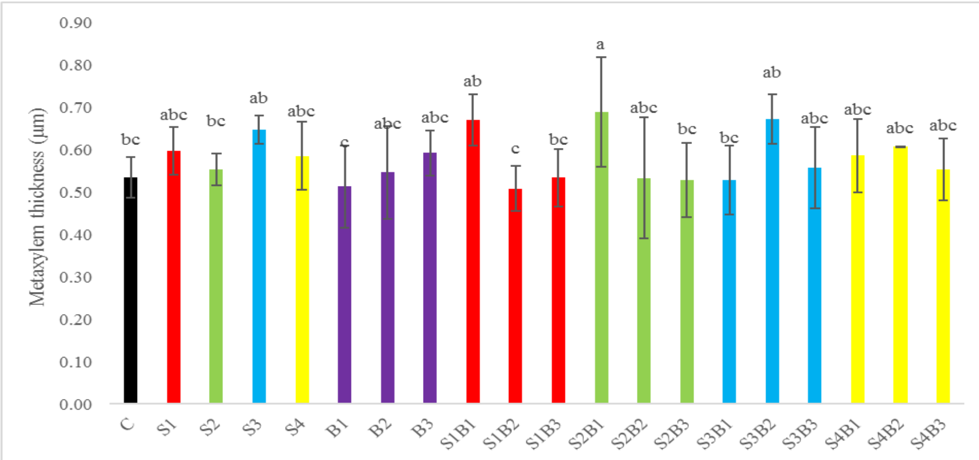
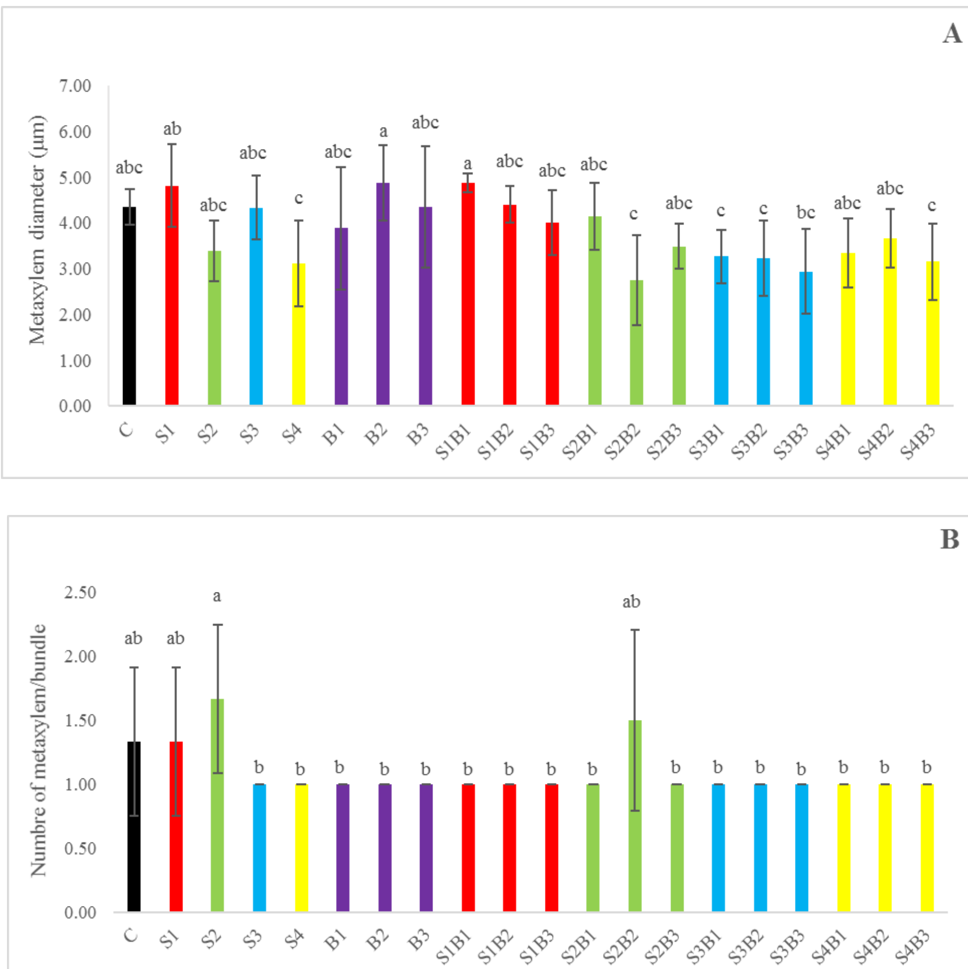


Fig.7 The variation of stem metaxylem diameter (µm)(A) and number of metaxylem vessels/vascular bundle (B) in response to different concentration of salt (NaCl) and boron. Bars carrying common letters are insignificantly different



This effect was most obvious in S4B3 plants where the thickness returned to the normal state. Boron may have reduced the toxicity of salts on the plant by regulating ion uptake. This resulted in the inactivation of molecules induced by salinity which are responsible for lignification such as SAM (S- Adenosyl- L- methionine synthase) (Sánchez-Aguayo *et al.*, 2004). Therefore, combined boron and salinity effect is considered antagonistic as shown by the results.

Diameter and number

At the level of the metaxylem diameter, the changes obtained were not significant. The diameter showed a very slight increase at high salt concentrations (S2, S3 and S4) (Figure4A). In contrast, the diameter decreased slightly under different boron treatments (Figure 4B). When different salt concentrations were combined with different boron concentrations, a slight decrease in diameter was obtained compared to the plants subjected to salt stress only and to control plants (Figure 4C, D, E and F). The decrease in xylem diameter has been confirmed in other studies such as the one done on *Gazania harlequin* (Younis *et al.*, 2014). The decrease in xylem diameter decreases their area, and this may serve as an adaptive mechanism that reduces water transport and thus reduces water loss by aerial parts of the plant. Changes obtained at the level of the number of the metaxylem vessels under the different conditions were not significant and random (Figure 5).

Stem metaxylem

Thickness

The changes observed regarding stem metaxylem thickness were insignificant. However, a slight increase in thickness was obtained in the 4 salt concentrations and at the highest boron concentration (B3) compared to control plants (Figure 6). when combining the different salt levels with the three boron concentrations, a decrease in the thickness was obtained in which the thickness became almost similar to the thickness in control plants especially at the highest boron concentration (S1B3, S2B3, S3B3 and S4B3) (Figure 6). These results although insignificant, show an antagonistic effect of boron and salinity on stem metaxylem thickness, and confirm the role of salinity in enhancing lignification.

Diameter and number

Changes at the level of the stem in metaxylem thickness, diameter and number per vascular bundle were all insignificant at all concentrations of salinity, boron and their combinations. Thickness ranged between 0.509 and 0.69µm, the diameter between 2.94 and 4.90 µm, and the number ranged between 1-2 metaxylem vessels/ bundle.

Conclusion

This study has revealed that increasing salinity causes a decrease in sweet corn plants length which could not be ameliorated by high levels of boron. This implies an independent effect between salt and boron stress. The study also showed that increasing salinity increases xylem vessel thickness at the level of the root which may aid the plant tolerate salt stress. High levels of boron combined with high levels of salinity were able to mitigate salt effect on xylem thickness. This implies an antagonistic effect between the two stresses. Changes at the level of the stem were insignificant which may indicate that the major site of effect of these stresses is the root.

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