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A. INVESTIGACIONES

Combined effects of excess boron and salinity on root histology of *Zea mays* L. amylacea from the Lluta Valley (Arica, Chile)

Efectos combinados de exceso de boro y salinidad sobre la histología de la raíz de Zea mays *L. amylacea del Valle de Lluta (Arica, Chile)*

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ABSTRACT

Cell structure and alterations in tissue organization were analyzed for roots of *Zea mays* L. amylacea as a consequence of high salinity and boron (B) levels. Saline treatment concentrations were 100 mM NaCl (Low salinity, L) and 430 mM NaCl (High salinity, H). An excess of B was supplied as boric acid to obtain 20 (334 µM) and 40 (668 µM) mg B kg^{-1} in the nutrient solution for 20 days. Our results complement other studies on the amylacea ecotype and confirm the high degree of tolerance to salinity and excess B shown by this variety. The application of B under no salt and low salinity conditions did not result in structure changes in root cortex cells nor the vascular cylinder. Under high salinity conditions amylacea root cells showed slight alterations, such as an increase in the number of rows of cells. These high salinity conditions did not result in thickness of the stele.

Key words: Amylacea, boron, histological, root, salinity.

RESUMEN

La estructura celular y las alteraciones en la organización del tejido de raíz se analizaron en Zea mays *L. amylacea como consecuencia de altos niveles de boro (B) y de salinidad. Concentraciones de los tratamientos de salinidad fueron 100 mM NaCl (baja salinidad, L) y 430 mM NaCl (alta salinidad, H). El exceso de B se suministró como ácido bórico para obtener 20 (334 µM) y 40 (668 µM) B mg kg^{-1} en la solución de nutrientes durante 20 días. Nuestros resultados complementan otros estudios sobre el ecotipo amylacea y confirman el alto grado de tolerancia a la salinidad y el exceso de B mostrada por esta variedad. La aplicación de B bajo condiciones sin sal y baja salinidad no dio lugar a cambios en la estructura de las células de la corteza de la raíz ni el cilindro vascular. Bajo condiciones de alta salinidad células de la raíz amylacea mostraron alteraciones leves, como un aumento en el número de filas de células. Estas condiciones de alta salinidad no resultaron en el espesor de la estela.*

Palabras clave: *Amylacea, boro, histología, raíz, salinidad.*

Introduction

Root systems are particularly affected by unfavorable conditions because they are in direct contact with the soil environment. Studies root system response of maize to salinity has focused on physiological aspects, but few studies have dealt with the effects of salinity on anatomical and histological characteristics as in the present study.

According to FAO (2008), more than 6% of world land is affected by salinity, covering about 4 Mha. Salinity is rapidly increasing on a global scale and currently affects more than 10% of arable land, which results in a decline of greater than 50% in the average yield of major crops. A decline of up to 50-70% in major crop productivity has been attributed to abiotic stress on several occasions (Mittler 2006). The primary effects of salt stress are caused by the presence of ions in the rhizosphere, limiting the extraction of water and nutrients by roots, and therefore reducing plant growth. Secondary effects are caused by ionic imbalance, which causes alterations in the structure and function of plant cells (Hernández & Almanza 2002; Iqbal *et al*.

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2014). These structural changes occur at several levels of organization; root-specific processes are of particular importance. The anatomical-morphological changes at the whole plant level allow plants to counteract the effects of salts, mainly in maintaining the efficiency of water use (Shannon at al. 1994, Hale & Orcutt 1987). The homeostasis system is altered during the stress period when roots are forced to adopt several structural and functional modifications such as alterations of metabolism, membrane characteristics, cell wall hardening and reduction of root length (Atkinson & Urwin 2012). Greater inhibition of root growth rather than aerial growth due to salinity increases the root/shoot ratio. Most physiological and morphological studies on the effects of salinity have been conducted mainly in the aerial part of the plant. However, the root is the organ directly exposed to saline soil, and the stress effects are shown in a stronger manner (Hajibagheri *et al*. 1987). The tolerance of the plant crucially depends on root tolerance to salt stress (Jeschke & Wolf 1988). Many root studies related to anatomical changes induced by salt stress are based on research by Strogonov *et al*. (1964), who found no difference in the diameter of roots after 4 weeks of growth under saline conditions. Nevertheless, Neuman (1995) found an increased root diameter under saline conditions, suggesting that the reduction in cell size and increase in root diameter are adaptive advantages that enhance the survival rate of plants in saline and dry soils. Other studies (Kalaji & Pietkiewicz 1993; Shanon *et al*. 1994) have shown that salt stress increases suberization and thickening of the endodermis in the root, also resulting in an increase of root diameter and vascular cylinder. Changes have been also associated with an extensive root development of tyloses, with early formation of Casparian strip and processes that allow its lignification (Flowers *et al*. 1986). It has been demonstrated in numerous species that salinity reduces the size of the cortical root cells. In sorghum mesocotyl narrowing is produced in a structure which could act as an ion reservoir (Wahida *et al*. 1998). Furthermore, in the transition zone of the hypocotyl and the root base of cotton plants, salinity exodermis formation is induced by the Caspari strip (Reinhardt & Rost 1995). All these structures are essential to protect the root from water loss and/or from the release of solutes due to osmotic adjustment. Salinity also stimulates the development of secondary tissue lignification and increases the number of water-storing cells in the epidermis and in the cortical layer of the hypocotyl (Valenti *et al*. 1992). A recent study of maize seedlings grown under 100 mM NaCl conditions showed a reduction in the number of cell rows in the root cortex, a delay in the formation of the central stellar cylinder, slow division of meristematic cells, and consequently smaller elongation zone than in control plants (Belyavskaya *et al*. 2004). Other studies have shown that in varieties sensitive to salinity, cell damage in the root cortex is due to root cell collapse (Hakim *et al*. 2014). Given the importance of B as an essential element for larger plants, its deficiency and toxicity are a worldwide problem in food production because of reduced crop quality and yields, especially in arid areas (Nable *et al*. 1997). Salinity conditions are aggravated by the presence of B in soils and water in arid and semiarid environments. This is the case in the Lluta valley of northern Chile, where elevated levels of B in soils and irrigation water limit local agricultural production to a few landrace crops of this region, which has an annual precipitation less than 1 mm (Bastías *et al.* 2004b).

Zea mays L. amylacea is a sweet maize variety well adapted to the agro-ecological characteristics of the Lluta Valley. Physiological tolerance mechanisms to high levels of NaCl and B in amylacea maize have been studied previously with respect to salt accumulation capacity, photosynthetic assimilation and water relations in tissues (Bastías *et al.,* 2004b), as well as root hydraulic conductance (Lo), abundance of aquaporins and ATPase activity (Bastías *et al.* 2004a, Martínez-Ballesta *et al.* 2008). Studies are very few concerning anatomical or morphological changes that verify salt and/or boron tolerance in ecotypes which are agronomically-interesting. Currently only two studies at the leaf level have been published (Bastias *et al*. 2013a, Bastias *et al*. 2013b). The present study gives findings at the root level, and aimed to determine differences in histological and anatomical changes produced as a result of excess B and salinity in the roots of *Zea mays* L amylacea.

Materials and Methods

Growth conditions and experimental design

Maize germplasm native to Northern Chile, *Zea mays* amylacea ("lluteño" local variety), was

germinated in a mixture of perlite and vermiculite (1:1; v/v). Seedlings were grown in plastic pots (2 L) with four plants per pot, and irrigated every two or three days to maintain soil water at field capacity with Hoagland solution containing 20 mM NO_3-N/L (González-Moro *et al*. 1997) adjusted to pH 5.5. Placement of maize pots was done randomly in a greenhouse with average day/night temperature of 25/18 ºC and relative humidity of 60/70%. Light intensity was set at 350 mmol $m^{-2}s^{-1}$ and supplemented with warm-white lamps (Philips SON-T AGRO 400, Belgium), providing a 14 h-photoperiod. Nutrient solutions were prepared using deionized water. pH, osmotic potential and conductivity were monitored weekly. During the first ten days after germination, plants were irrigated with the basic nutrient solution to maintain non-saline growing conditions. Subsequently, when plants showed the third leaf fully expanded, they were exposed to an excess of boron and salt for 20 days. The basic nutrient solution without addition of salt (NaCl-0) or boron (B-0) was used as the control solution. This basic nutrient solution was supplemented in a factorial design with 100 mM NaCl (Low salinity, L) or 430 mM NaCl (High salinity, H), and with an excess of B supplied as boric acid to obtain 20 (334 µM) and 40 (668 µM) mg B kg^{-1} in the nutrient solution. Harvesting of fresh plant material was done between 10:00 and 11:00 A.M.

Preparation of tissue for histological studies

Small sections were cut from roots (4-5 mm) of ecotype amylacea and fixed in FAA (formaldehyde 5%: glacial acetic acid 5%: ethanol 90%) for 4-5 days at room temperature to favor the penetration of the fixative. Subsequently, 5 washes were carried out at intervals of 20 min with buffer (100 mM) at pH 7.2 phosphate. Then, two washes were done with distilled water for 1 h to remove the formaldehyde completely. Next, samples were dehydrated in an increasing series of ethanol: 70%, 80%, 90% and 100%, leaving the sample overnight in methyl benzoate. For paraffin embedding the samples were first suspended in benzene and then embedded in paraffin for 4 h. Semi-thin sections (7-10 mm thick) of root were cut transversely with a rotary microtome (Leitz 1512) and then subjected to a hydration process (dewaxing) in a decreasing alcohol series: xylene (10 min), ethanol 100% (10 min), 96% ethanol (2 min) and 70% ethanol (2 min). Finally, samples were stained with safranin and again subjected to a dehydration process (series of ethanol and xylol). Sections were observed with a photomicroscope (Olympus BX 51) connected to a camera (Photometric Cool Snap RS) and digitized with Cool Snap (Roper Scientific, Inc.) software version 1.2. The photographs were taken in three or more random fields in different sections in order to obtain representative photographs.

Results

Plant tolerance to salinity largely depends on the salinity tolerance of the roots (Hajibagheri *et al*. 1987). The internal structure of the root is relatively simple compared to the stem and leaf, mainly because of the absence of nodes and internodes. Thus the arrangement of the tissue shows few differences in different levels of the root. Three tissue systems in the root of the amylacea ecotype can be easily distinguished in a cross section of the root tissue of plants after 20 days of development: epidermis, stele (vascular cylinder) and vascular tissues. The epidermis (epidermal tissue system), cortex (basal tissue system) and vascular tissue can be clearly distinguished from each other in both control and treated plants with different levels of salinity in the absence or presence of B (Figures 1, 2, 3, 4 and 5). In maize, vascular cylinder size in the stele is very noticeable in proportion to the cortex. In our ecotype, vascular tissue corresponds to approximately 50% of all tissues in the control sample and in the different treatments with excess B and salinity (Figures 1a, 2a, 3a, 4a and 5a).

Root epidermis is formed by parenchymal cells (Figures 1b, 2b, 3a, 4a and 5a) whose function is related to the absorption of water, ions and small molecules. Cortical cells are arranged in a variable number of rows (10, 14) during the entire lifecycle of monocotyledonous plants, while dicotyledonous plants lose cortex in the early stages of growth. The cells are large and compact with thinner walls (Figure 1e) due to the presence of plasmodesmata, which allow movement of substances between cells through the cortex. These cells are separated by small intercellular spaces. Immediately following the inward cortex is the cell layer is located called endodermis (ED), as seen in the Figures corresponding to the control plants (Figures 1c and 1e). This cell

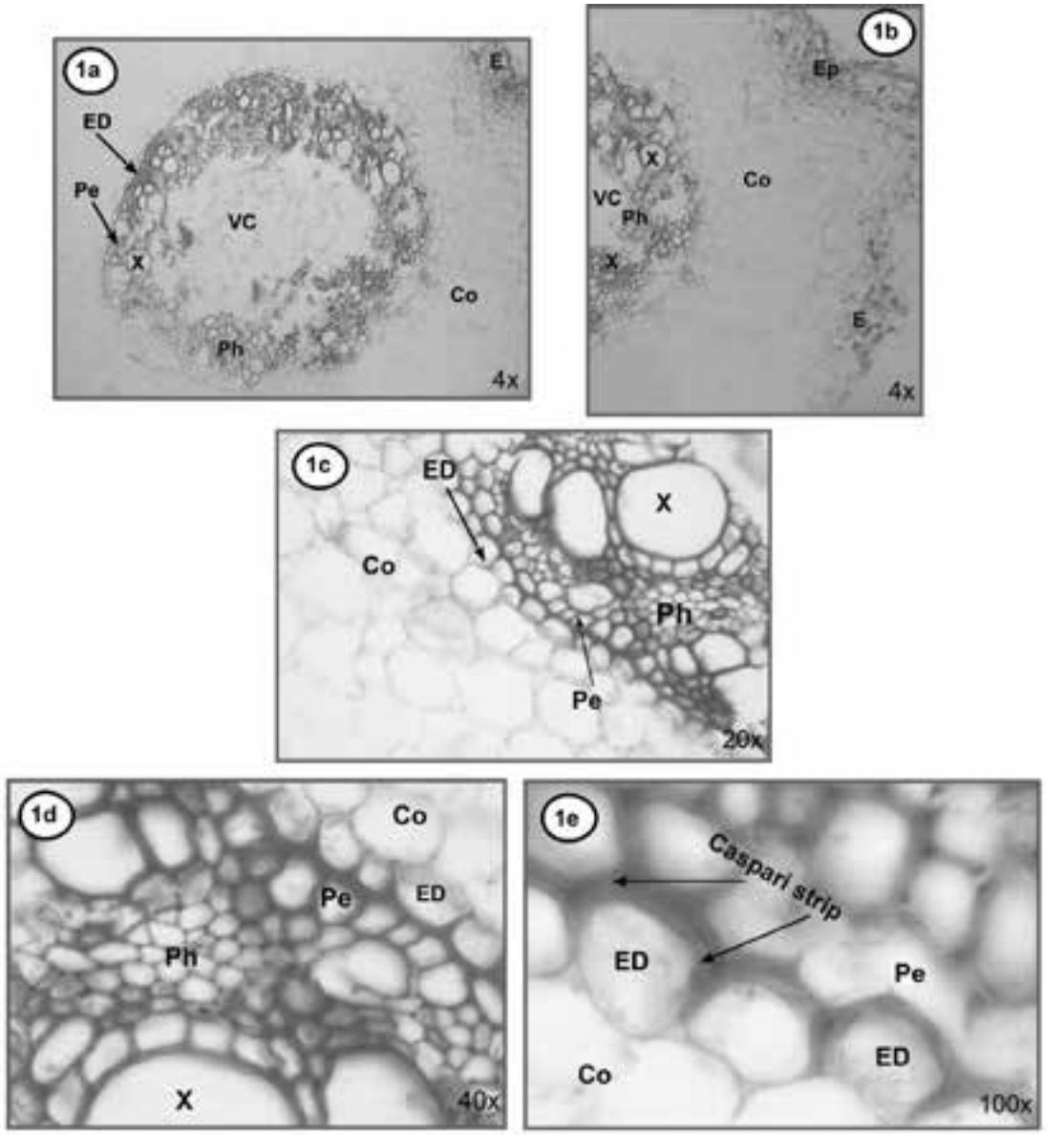

Figure 1. Light micrographs of cross sections of root in **control plants** of *Zea mays* L. amylacea. Showing the organization of the root tissue in control plants at different magnification. Samples stained with Safranin 50%. Labels: E, epidermis, X, xylem; Pe, pericicle, ED, endodermis; Co, cortex cells; Ph, Phloem; VC, vascular cylinder.

monolayer is compact and lacks intercellular spaces. The endoderm cells are of particular interest and importance from the point of view of the movement of water and ions in the plant, as their transverse and radial cell walls include a thickening called the Casparian strip (Figure 1e). The Casparian strip is a portion of the primary wall in strip form that is infused with a lipid substance called suberin, which is highly hydrophobic and may present some lignification. The Casparian strip cell endodermis is an impenetrable barrier to the apoplastic pathway, which must necessarily cross the plasmalemma of endodermal cells. Permeability, selectivity and affinity of the channels and transporters localized in the plasmalemma of the endodermal cells ultimately determine how fast solutes are incorporated or released into the xylem (Figures 1d and 1e).

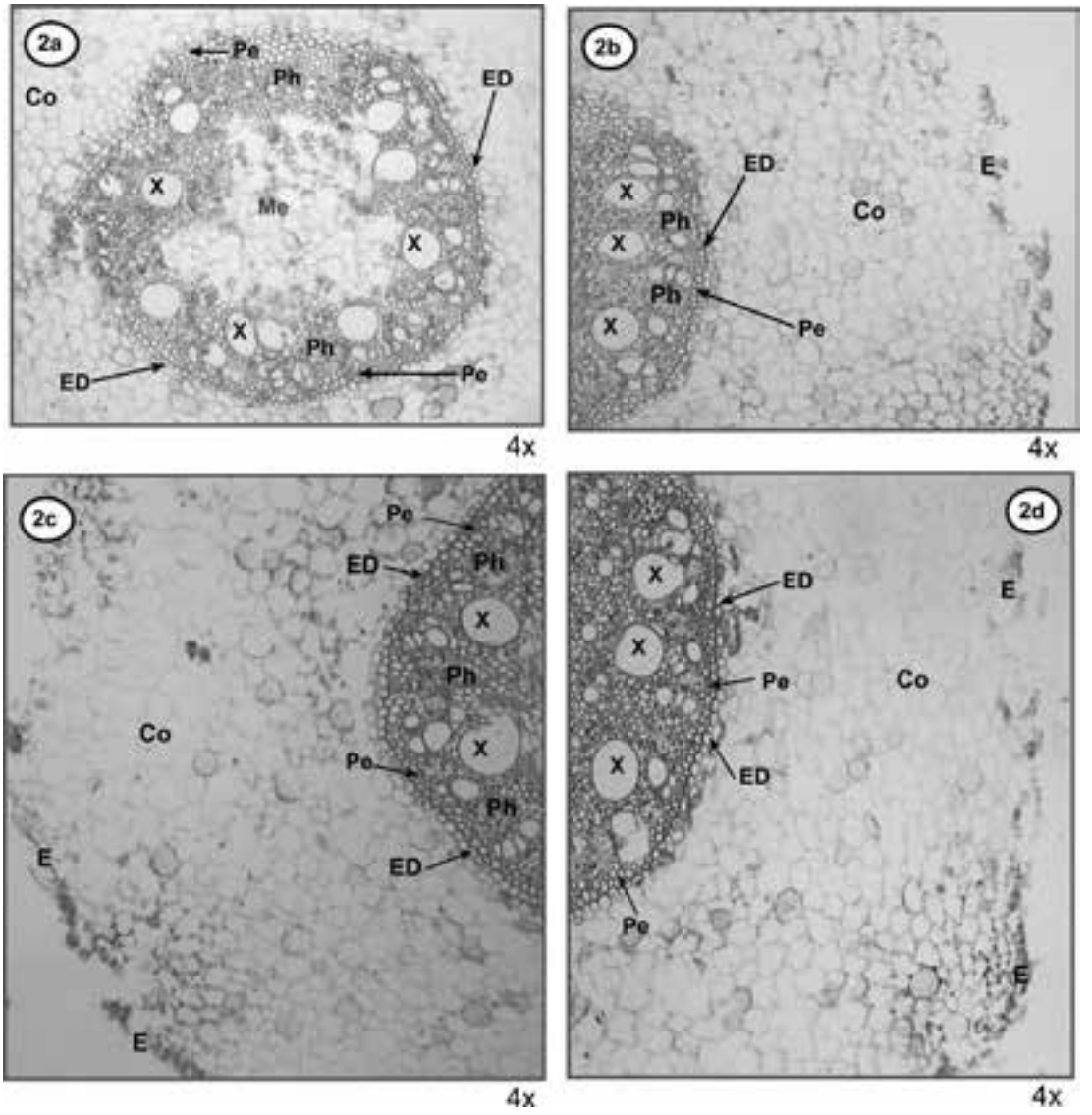

Figure 2. Light micrographs of cross sections of root in plants of *Zea mays* L. amylacea. Showing the organization of the root tissue in **B20 (20 mg kg^{-1} of B**) at different magnification. Samples stained with Safranin 50%. Labels: E, epidermis, X, xylem; Pe, pericicle, ED, endodermis; Co, cortex cells; Ph, Phloem; VC, vascular cylinder.

Xylem (X) and phloem (Ph) elements in the amylacea ecotype under control conditions are surrounded by a layer of living cells, composed of one or three rows of cells, known as the pericycle (Pe) (Figures 1c, 1d and 1e). The pericycle plays various important roles at the time of lateral root formation and development of the vascular cambium. The vascular cambium is a meristematic region which is towards the inside of the xylem and phloem (to the outside in the case of the dicots), but in the case of monocotyledonous plants such as maize, conducting vessels are arranged alternately in the vascular cylinder (Figure 1c). Salinity treatments in the presence or absence of B did not cause major changes in the ecotype amylacea regarding the organization of root tissue after 20 days under stress. As may be seen in the cross sections of the roots from plants treated with B (20 and 40 mg kg^{-1}) and NaCl (100 mM and 430 mM) (Figures 3, 4 and 5), there were significant changes in the root structure.

No increase in the number of cell layers was observed in the area of the cortex and stele, nor

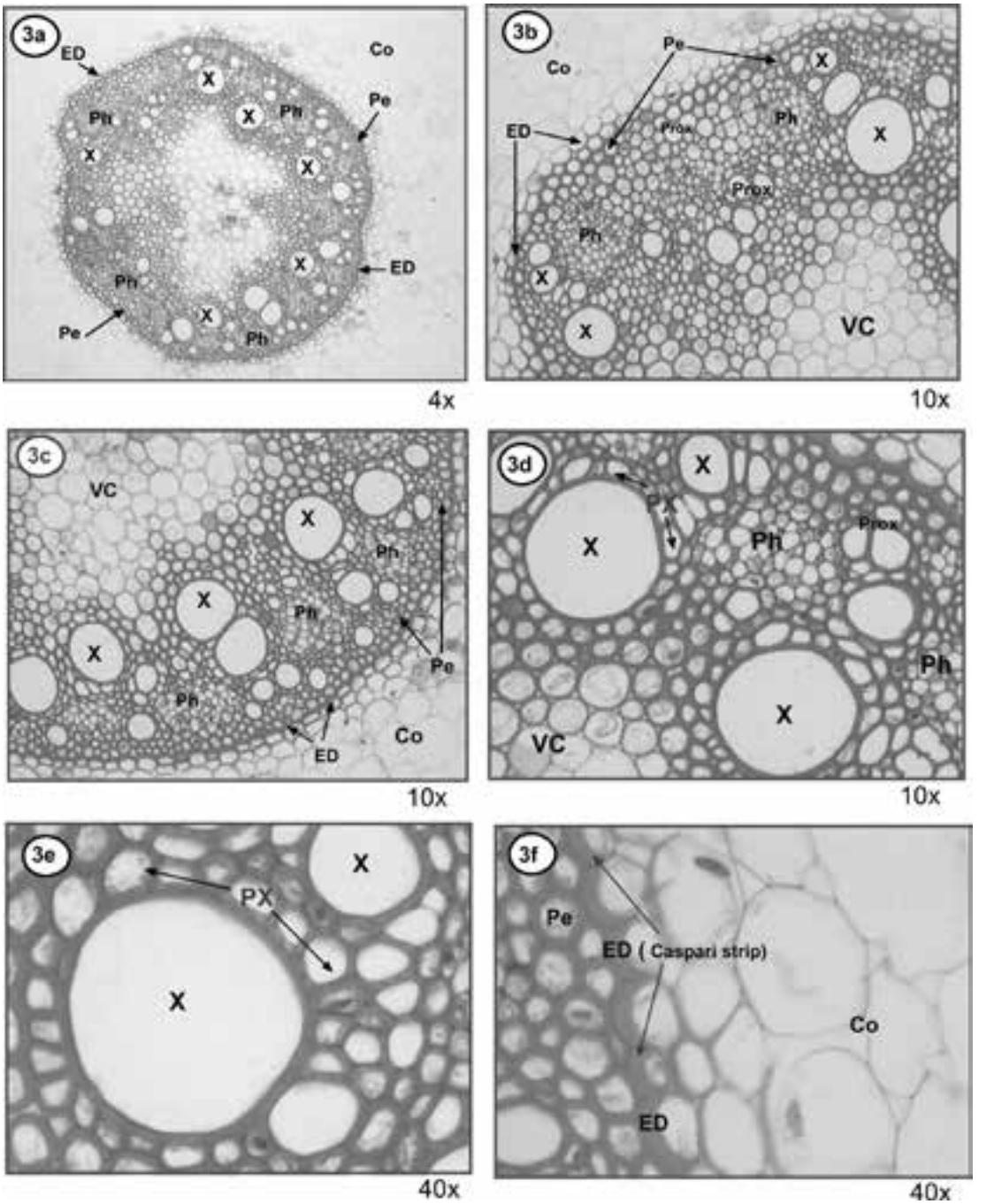

Figure 3. Light micrographs of cross sections of root in plants of *Zea mays* L. amylacea. Showing the organization of the root tissue in **B20 (20 mg kg^{-1} of B) + 100 mM NaCl** at different magnification. Samples stained with Safranin 50%. Labels: E, epidermis, X, xylem; Pe, pericicle, ED, endodermis; Co, cortex cells; Ph, Phloem; VC, vascular cylinder.

was there variation in the size of the cortical cells; similar to that observed in control plants. However, in high salt conditions, and in the presence of B, the appearance of some cortical cells was slightly distorted; they were collapsed, (Figures 4d, 5b and 5c). Those specific areas where alterations or changes in the structure are observed are indicated with asterisks (*). Cell walls were thicker

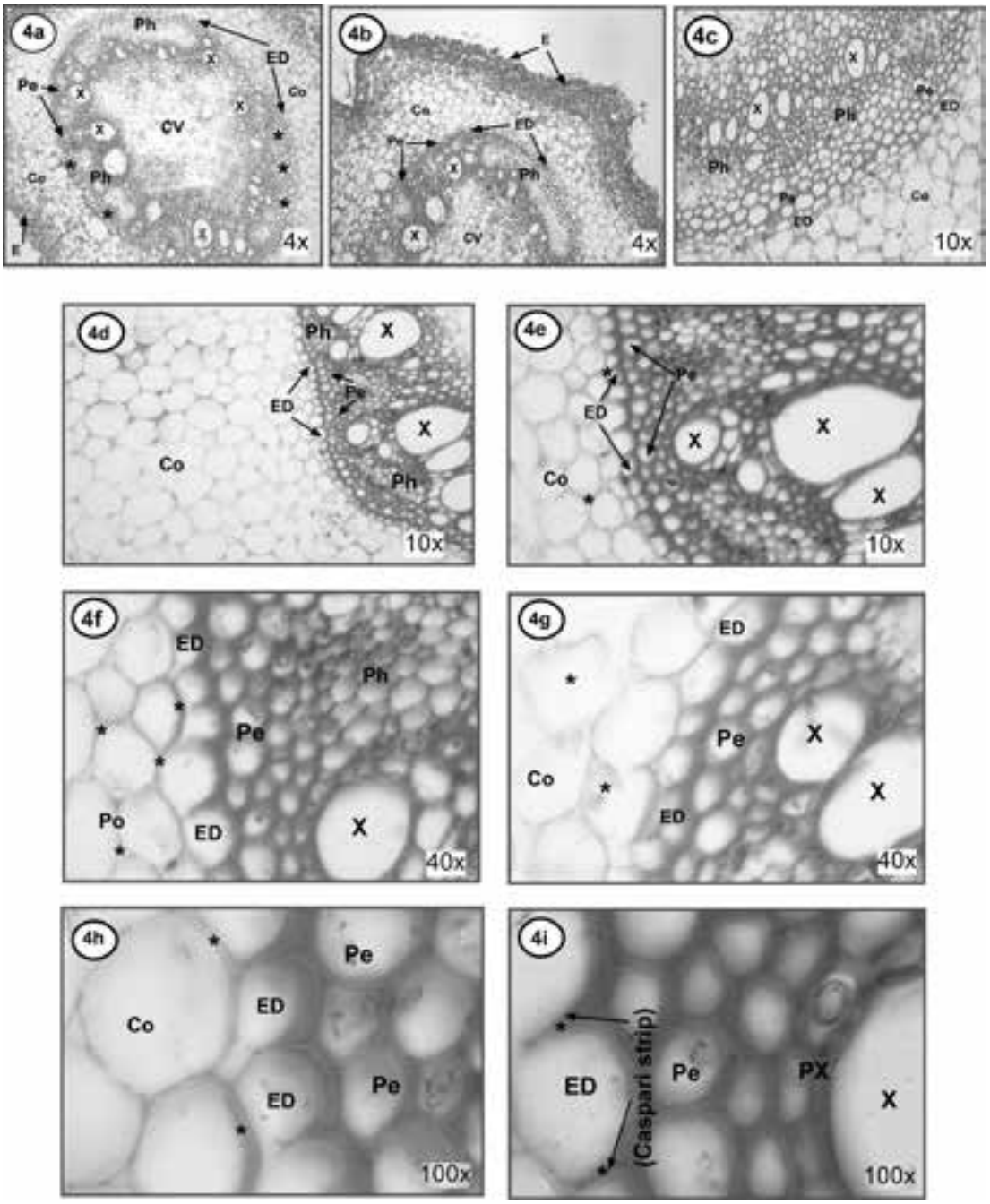

Figure 4. Light micrographs of cross sections of root in plants of *Zea mays* L. amylacea. Showing the organization of the root tissue in **430 mM NaCl** at different magnification. Samples stained with Safranin 50%. Labels: E, epidermis, X, xylem; Pe, pericicle, ED, endodermis; Co, cortex cells; Ph, Phloem; VC, vascular cylinder.

(Figures 4d, 4e and 5b); and in some cells there was a noticeable thickening (Figures 4d and 4e). In the anatomy and arrangement of xylem and phloem no apparent changes were produced by the levels of B and salinity studied (Figures 2c, 3c, 5c and 5h); the variety of internal diameters of vascular bundles was maintained. The cells of the endoderm in ecotype amylacea in saline conditions

and with excess B had a similar appearance to the control plants, even at high salt conditions (430 mM); in the absence of B, a slight or no significant increase was observed in the thickening of cell walls (Figures 4e and 5d). The pericycle was made up of one or two rows of cells with small intercellular spaces between them, and between pericycle and endodermis cells (Figures 2c, 3b, 4c and 5d).

In samples at high salinity in the presence of B, changes were also detected in pericycle thickness, mainly a marked increase in the number of rows of cells in parts of the stele (Figures 4b and 4a), which are transformed from 1 to 2 rows of cells in normal conditions to up to 7 rows in high salinity conditions. Increasing the number of rows of cells in plants under high salt did not result in an increase in thickness of the stele, possibly due to a lower number of cells in each row.

Discussion

Root anatomy of the amylacea ecotype did not appear to be severely affected by treatment of excess B, low or high salinity stress, showing similar morphological characteristics to control plants. This behavior confirms the observations made by Wang *et al*. (1991), who indicated that the mature root tissues of maize plants after more than 21 days are remarkably resistant to stressors such as drought and salinity. They may also have very low values of relative water content (RWC) in tissues, even below the minimum levels in leaves in herbaceous plants, without making major changes in the structure of tissues (Oppenheimer & Leshem 1966).

The tissues of the primary root of amylacea ecotype under excess B and interaction with salinity stress (Figures 2, 3, 4 and 5) showed few symptoms of dehydration, cortical cell collapse or increase in the number of rows. Only some slight alterations were observed at high salt conditions, such as irregular appearance of cortical cells, but this damage was not severe enough to dramatically affect root growth. This behavior is confirmed by a slight decrease in root growth of the amylacea ecotype under salt and excess B stress (Bastias *et al.* 2004b), confirming the high tolerance of this cultivar compared to the behavior described for other cultivars of maize and other crops.

In maize cultivar 225 Collectivnyi important anatomical alterations in the root were produced by salinity (100 mM NaCl), such as reduction in the number of rows of cells in the cortex and changes in the organization of the stele (Belyavskaya *et al.* 2004). Furthermore, similar responses were found in maize cultivar saccarata Koern spp. cv Bonanza, but due to the effect of other stresses such as the application of the herbicides chlorsulfuron or ethametsulfuron at low doses. This case reported a significant reduction in root growth, decreasing 55-72% due to a deformation of the cortex cell wall, possibly inhibiting root growth (Flaburiari & Kristen 1996). In rice plants (Samarajeewa *et al*. 1999) and barley (Shabala *et al*. 2003) induced root salinity has also been described, specifically the effect of osmotic stress and Na^+ content (Marschner 1995), which caused severe alterations in the root tissue, mainly in the cortical cells, even causing the death of cortical tissue. These damages significantly affected the growth of the primary root of barley, decreasing it by 70% (Shabala *et al*. 2003). It is important to consider the structural alterations in these cultivars and crops occurred after plant roots were subjected to salinity of 100 mM NaCl, which is a low salinity compared to that used in our study.

The amylacea ecotype showed a high capacity for Na^+ accumulation in the roots; able to accumulate up to 4% Na^+ in the root tissue, while the leaf values were around 1% Na^+ (Bastias *et al.* 2004b). This Na^+ may be preferentially localized in the cell cortex, referring to results described by Vetterlein et al. (2004) for maize cultivar Pioneer 3906, which also behaves as a Na^+ excluder, restricting the translocation of Na^+ from the root portion to the aerial part (Schubert & Läuchli 1986, 1990). Limiting the entry of Na^+ into the stele is performed at the level of the plasma membrane in cells and cortical cells of the epidermis (Vetterlein *et al*. 2004). Similar results have also been described in another cultivar of maize cv F1 3906 (Zorb *et al*. 2005) and barley plants (Wegner & Raschke 1994) grown in saline conditions, where an increase of Na^+ was observed in the vacuoles of the cell cortex. They further concluded that the storage capacity of Na^+ in the cortex is generally greater than that of the xylem parenchyma cells.

The changes in the fine structure of the root are promoted by suberization salinity induced in the hypodermis and endodermis (Shannon *et al.* 1994). The Casparian strip implies the presence of an apoplastic barrier between the cortex and

the stele in plant stems and roots. This barrier is formed by endodermal cells and contributes to form a highly organized network of primary walls. The cell wall of the Casparian strip is impregnated with hydrophobic molecules such as suberin, and the plasma membrane adheres strongly to the cell wall of the endodermis. As a result, apoplastic ion flow is restricted in the endoderm, so that this barrier appears to play an important role in preventing high salt entry into the stele under saline conditions (Karahara *et al*. 2004). It is therefore important to know whether a morphological change occurs in the structure of this barrier, which can act as a morphological strategy to limit the entry of salts to the vascular bundles of the stele. In the endodermis of amylacea ecotype (Figures 5d, 5e, 5f and 5g) some compaction of the cells that make up the Casparian strip was observed, although an increase in radical band width was observed (Zimmermann *et al.* 2000). This lack of effect on the Casparian strip in the amylacea ecotype is contradictory to the effect described by Karahara *et al*. (2004) in maize roots and ssp Sacchorata and by Reinhardt & Rost (1995) in cotton roots. These authors showed that in saline conditions increased thickness of Casparian strip cells in primary root endodermis occurred. Changes in the thickness of the Casparian strip may have an important function in strengthening the barrier of the apoplastic transport factor, enhancing the hydraulic resistance of the endodermis due to higher density of hydrophobic materials such as suberin and lignin (Karahara *et al.* 2004). However, no thickening of the Casparian strip in the root tissue of ecotype amylacea as an effect of salinity would not be a disadvantage for the passage of water. This is evidenced in part by the optimal values of RWC found in the leaf (about 91%) (Bastias *et al.* 2004b) even under severe salt stress. Barrieu *et al.* (1998) in corn root cv Oh43, found mRNA encoding aquaporins of the tonoplast, which were expressed at a high level in this region of the root, specifically in the endodermal cells and pericycle, confirming the transport of water by this pathway. Clearly the Casparian strip in the case of salinized plants would not further restrict the passage of water (Lehmann *et al.* 2000, Chen *et al*. 2011). In the case of the amylacea ecotype, decreased root hydraulic conductance was observed in saline conditions (Bastias *et al.* 2004a).

The pericycle, endodermis along represents the interface between the cortex and the stele of the root; the two layers of cells are an important site in the regulation of long-range transport of ions via xylem and phloem. In the amylacea ecotype, an increased number of rows of cells that form the pericycle in parts of the stele (Figures 4a and 4c) were observed, although this change was seen only in high salinity. This increase in the number of rows of cells in the pericycle is accompanied by the presence of smaller cell sizes, a behavior that probably increases the resistance of cells against collapsing under conditions of stress (Oertli 1990). This would also increase the ability of plants to maintain turgor, especially under conditions of water stress (Jones & Turner 1980). This resistance can be increased by around 20 times when the cells forming the pericycle are smaller compared to larger cells (Bosabalidis & Kofidis 2002). Thus the presence of a large number of smaller cell sizes for tissue surface remarkably improved water loss control under conditions of salt stress (Oertli 1990; Bosabalidis & Kofidis 2002). Moreover, the decrease in cell size could be due to the cells of the endodermis and pericycle being fully differentiated in the root, which allows a relatively large cytoplasmic region in both cell types where they can deposit significant levels of ions that are subsequently distributed between the cytoplasm and the cell vacuoles (Storey *et al.* 2003). Indeed, X-ray microanalysis performed in primary root vines and *Puccinellia tenuiflora* grown in saline conditions showed that Na^+ was mainly distributed in the vacuoles of the pericycle cells in vines and into the intercellular spaces of the endodermis in *P. tenuiflora* (Storey *et al.* 2003; Peng *et al*. 2004). In our case, we know from the data of Na^+ accumulated in the roots and in the aerial part that the amylacea ecotype handles the movement of Na^+ within the plant at the root level, so we suggest that Na^+ accumulation could occur mainly in cells of the cortex and secondly in the endodermis and pericycle. Thus the entry of Na^+ would be restricted to the aerial part of the plant, as shown by low levels of Na^+ found in leaf tissue.

Within the literature, it is shown that salinity causes increased lignification in the vascular bundles of the root, which increases the vascular cylinder and is manifested in a thickening of the cell wall of xylem vessels and consequently root diameter (Kalaji & Pietkiewicz 1993, Shannon *et al*. 1994).

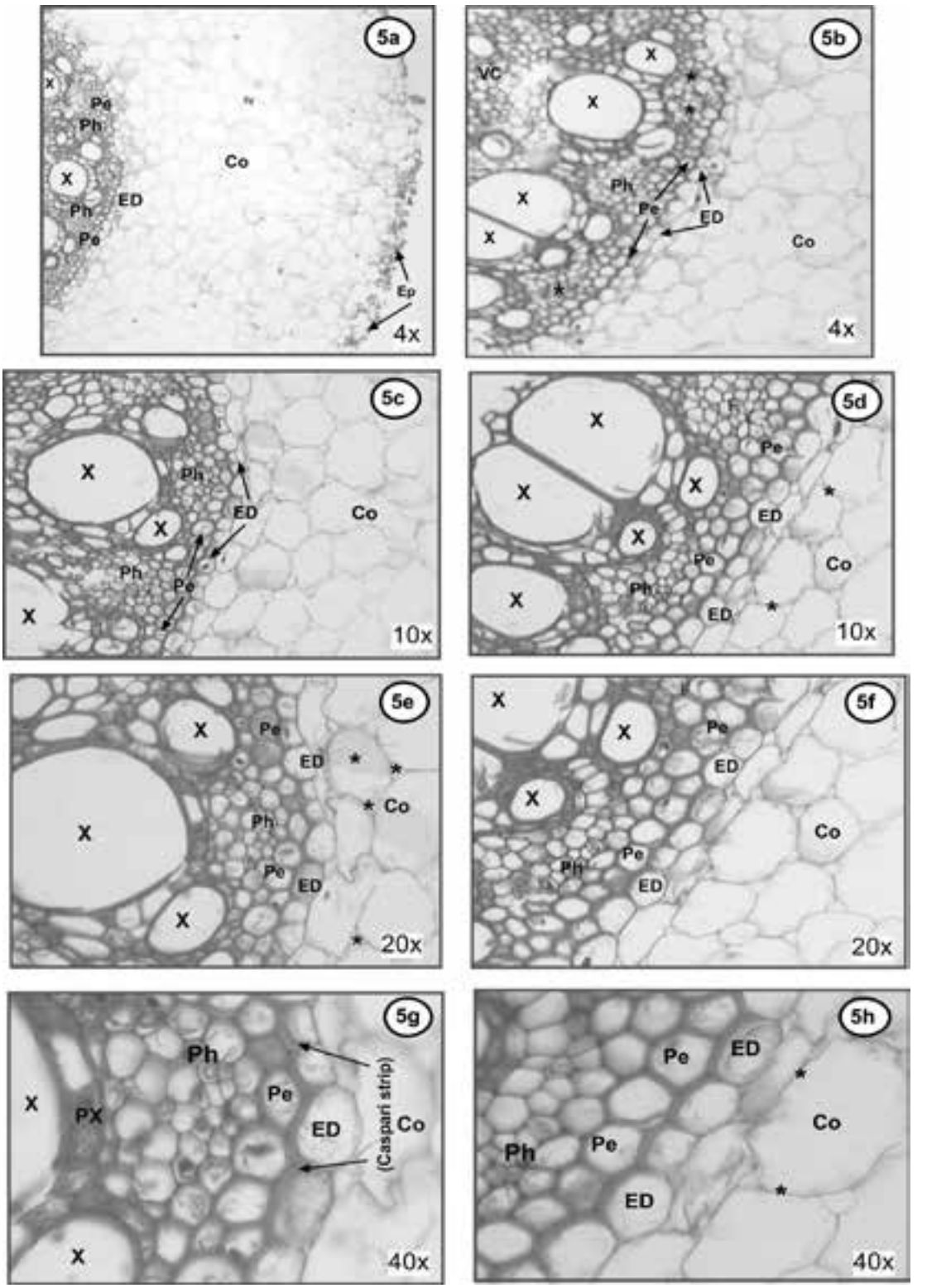

Figure 5. Light micrographs of cross sections of root in plants of *Zea mays* L. amylacea. Showing the organization of the root tissue in **B40 (40 mg kg^{-1} of B) + 430 mM NaCl** at different magnification. Samples stained with Safranin 50%. Labels: E, epidermis, X, xylem; Pe, pericicle, ED, endodermis; Co, cortex cells; Ph, Phloem; VC, vascular cylinder.

This effect was observed in cotton (Kurth *et al*. 1986) and bean plants (Cachorro *et al*. 1994). Conversely, Strogonov *et al.* (1964) found no difference in the diameter of the root in tomato and cotton plants under salinity for 4 weeks and Huang & Redmann (1995) also observed this behavior in barley plants under saline conditions. However, these authors did associate salinity with the presence of a large number of small diameter vessels in the root. According to our results, we suggest that the amylacea ecotype response to high salinity stress and excess B (Figures 1c , 2a , 3c , 4c and 5c) is essentially the maintenance of anatomic properties similar to those of control plants, not showing any of the disorders described above for other crops. Thus, the area occupied by the xylem vessels and surface distribution, the proportion occupied by the stele and cortex of the root and the number of rows of cortical cells were not altered.

Conclusions

In this study, roots of amylacea ecotype did not develop visible symptoms of B toxicity that could be reflected in any alteration of the anatomy of the root tissue. This result is likely because the concentration of B in the root remained low, with values of around 20 ppm B, compared to the levels found in leaf both in non-salt and salt conditions, with values of around 800 ppm B (Figures 2c and 2d) (Bastias *et al.* 2004b), even when there was high availability of B in the external medium (Oertli 1990; Nable *et al*. 1997). This characteristic may be attributed in part to the binding of B with some molecules of the cell wall of the root cells, which would reduce the amount of B in the cytoplasm, causing it not to accumulate to potentially toxic levels. The ability of plants to recognize and respond to specific stress combinations is particularly important when those individual stresses could elicit negative effects on plant growth and reproduction, whereas others indicate a positive role of the interaction of multiple stresses, compared to individual stresses applied separately (Sukuki *et al.* 2014), as in the case of "lluteño" maize (*Zea mays* amylacea).

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