

Determination of ion content and distribution in leaf and root in adult plants of “lluteño” maize (*Zea mays* L.) of the XV Región of Arica and Parinacota, Chile

Determinación del contenido y distribución de iones en hoja y raíz en plantas adultas del maíz (Zea mays L.) “lluteño” de la XV Región de Arica y Parinacota, Chile

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ABSTRACT

The current study presents the way in which ions are distributed in an adult maize plant after flowering, and as the pressure of permanent ion stress throughout approximately six months; including all the phenological periods. The research was conducted under field conditions with the climatic characteristics of an arid or hyper-arid zone. Maize is a sensitive crop regarding the salinity of soil and water, but “lluteño” maize has a higher degree of tolerance to salinity, excess of boron (B) and other ions, this suggests that the distribution plays an important role in the tolerance mechanisms of this ecotype. The variety accumulates twice as much sodium (Na⁺) in the root than in the leaf, and 25 times of B more in the leaf than in the root; calcium (Ca²⁺), magnesium (Mg²⁺) and potassium (K⁺) were also significantly accumulated in the leaf. High concentrations of B, Ca²⁺, and Mg²⁺ were found in the thick root and K⁺ in the fine roots. Sodium accumulated similarly in thick and fine roots, suggesting a very active mechanism of Na⁺ accumulation in roots, independent of their thickness or age.

Keywords: maize (*Zea mays* L.) “lluteño”, root, leaf, salinity.

RESUMEN

El presente estudio muestra cómo se distribuyen los iones en una planta de maíz adulta después de la floración, y como la presión del estrés iónico es permanente durante un largo período, aproximadamente seis meses; incluye la totalidad de los períodos fenológicos. La investigación se realizó en condiciones de campo con las características climáticas de una zona árida o hiperárida. El maíz es un cultivo sensible a la salinidad del suelo y el agua, pero el maíz “lluteño” presenta un alto grado de tolerancia a la salinidad, el exceso de boro (B) y otros iones, lo que sugiere que la distribución de estos juega un papel importante en sus mecanismos de tolerancias en este ecotipo. Acumula el doble de sodio (Na⁺) en la raíz que en la hoja, 25 veces más que el B en la hoja, también el calcio (Ca²⁺), el magnesio (Mg²⁺) y el potasio (K⁺) se acumularon significativamente en la hoja. Con respecto al tipo de raíz, en las gruesas se observó una alta concentración de B, Ca²⁺, Mg²⁺ y en las raíces finas se encontró el K⁺. El sodio se acumula de manera similar en las raíces gruesas y finas, lo que sugiere un mecanismo muy activo de acumulación de Na⁺ en la raíz, independientemente del grosor o la edad de las mismas.

Palabras clave: maíz (*Zea mays* L.) “lluteño”, raíz, hoja, salinidad.

Introduction

Plants are frequently exposed to multiple abiotic and biotic stress factors at the same time in nature. For instance, they may need to grow and reproduce in soil affected by drought, heavy

metal or salinity (Forieri *et al.*, 2016). About 1/5 of the irrigated land used for agriculture is affected by salt, currently causing huge annual economic losses of 10 billion USD (Qadir *et al.*, 2014). An approach using halophytes or salt-tolerant crops was proposed to combat the salinity in agricultural

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soils, which is the most substantial environmental constraint leading to the perturbation of all or some of the physiological and biochemical process and consequently limits the production and quality of agriculture crops worldwide (Duarte *et al.*, 2013). Lluta Valley in northern Chile is an agricultural area affected by salinity, excess B and other ions (Bastías *et al.*, 2004a), with elevated ionic levels in soils and irrigation water that limit local agricultural production to a few landrace crops, which has an annual precipitation less than 1 mm (Novoa *et al.*, 1989). *Zea mays* L. *amylacea* is a sweet maize variety well adapted to the agro-ecological characteristics of this valley. This maize variety is considered a source of germplasm to improve salt and B tolerance in other maize (Bastías *et al.*, 2004a). Maize is classified as a salt-sensitive crop plant, which growth and productivity are reduced when salinity is above 2.5 mS cm⁻¹, although this sensitivity depends on the crop (Cramer *et al.*, 1994).

This is the first study on field conditions of this variety of sweet corn originated in Lluta valley, *Zea mays* L. *amylacea* (Bastías *et al.*, 2004a), all the results that have been reported indicate a great tolerance to elevated salt and B levels. Our results showed that the availability of B mitigated in part the negative effects of salinity on this ecotype, through the recovery of K⁺ levels (homeostasis) and the maintenance of Na⁺ homeostasis for action of Na⁺ transport, which limits the partition of Na⁺ between root and shoot, suggesting that *amylacea* maize behaves as a salt excluder, which overcomes salt stress by the restriction of salt to root tissues, preventing Na⁺ from being transported to leaves and accumulating in photosynthetic tissues. By contrast, B accumulation takes place in leaves and is enhanced under saline conditions (Bastías *et al.*, 2004a). Recently we showed that under salt stress, the activity of specific membrane components can be influenced directly by boric acid, regulating the function of certain aquaporins and ATPases as possible components of the salinity-tolerance mechanism (Martinez-Ballesta *et al.*, 2008). Also, the histological study showed that only high salinity conditions of the leaf cells of *amylacea* had some alterations in mesophyll cells chloroplasts, which appeared swollen and rounded. The bundle chloroplasts sheath cells change their perpendicular disposition to the cell wall and appear horizontally. Moreover, absolute absence the plastoglobuli, could indicate a greater resistance to oxidative damage

(Bastías *et al.*, 2013). The studies cited above have been carried out under controlled conditions of growth and inert substrates and pots, however, the results presented in this study were collected in real conditions of growth in the field. You can observe if possible mechanisms of stress tolerance “lluteño” remain independent of the conditions of plant growth.

There are few studies on the measurement of the content of vertical distribution of ions in the leaf and in the root: thick and thin roots in adult plants as “lluteño” maize grow and produce under field conditions in salinity stress that provide new insights on the underlying tolerance mechanisms.

Materials and methods

Experimental site

The experiment was conducted in the locality of Molino, located at Km 52 in Lluta Valley. The valley is located in the north of Chile, in the Region of Arica and Parinacota, which is located at 17° 40' south latitude and between meridians 69° 22' and 70° 20' west latitude. This valley is characterized by its high concentration of salts, both in the soil and in the irrigation water. The research work was carried out under field conditions, the physiological analysis of the plants, the soil chemistry and irrigation water were carried out in the laboratory. The field work is basically oriented to the establishment and monitoring of the crop during its phenological stages.

Plant material and culture conditions

The seeds of “lluteño” maize ecotypes were sown at densities of 1m x 1m. The irrigation was carried out by traditional furrowing with an interval of 7 days. The soil texture is franc-sandy, with a pH of 6 and an electrical conductivity of soil saturation extract of 3 mS/cm⁻¹, the Na⁺ concentration in soil is 300 mg L⁻¹, and 13.26 mg L⁻¹ B. Different phenological stages of “lluteño” maize lasted 140 days total from germination to production. The average temperature and humidity maintained under field conditions during the study was a maximal temperature of 27 °C and the minimum temperature of 6 °C, and 72% relative humidity. Additionally, the prevalent type of chemical fertilizers used the proportional 200:90:20 (N:P₂O₅:K₂O) units per hectare.

Methodologies

There was harvested eight “luteño” maize plants randomly sampled, during the phenological stage after flowering, taking one plant for each sowing stroke. Also, another plant was taken from the same sowing stroke, to determine its fresh and dry weight (leaves and roots), this is relevant data to calculate the concentration of the element under study (Na^+ , Cl^- and B) in the leaf or root using the following equation:

$$\text{Na}^+ \text{ mg kg}^{-1} = \frac{\text{concentration} (\text{mg L}^{-1}) * \text{dilution} * 50 (\text{gauge})}{\text{Sample mass} (\text{g})}$$

The sampling of each structure was initiated from top to base of the stem. For each plant sampled, pair of leaves was harvested, leaving the next pair of leaves and continuing the sampling of the plant in the same way, until reaching the base of the plant (Figure 1). In addition, the roots of the selected plant were sampled. However, a separation of the new (absorbent or radical hairs), and of the old roots (suberized or anchoring/support) was carried out, due to the difference of functions of these (Figure 2) these two groups were analyzed separately.

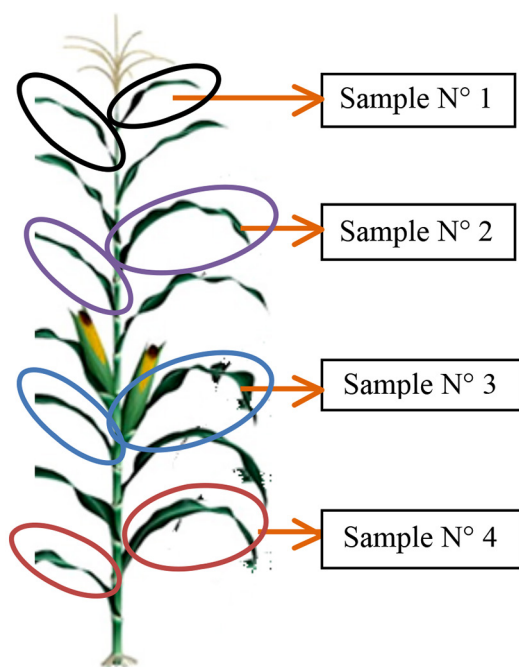


Figure 1. Scheme of the leaves samples harvested of “luteño” maize plant.

The samples of each pair of leaves and roots were deposited in aluminum foil in field, previous identification, refrigerated and taken to the laboratory of Facultad de Ciencias Agronómicas of Universidad de Tarapacá, where each pair of leaves were weighed and placed in the ultra-freezer ($-80\text{ }^{\circ}\text{C}$) in plastic bags with zipper closure for around 24 hours. Then each pair of leaves and roots, were placed in a mortar and “pistil” with liquid nitrogen and grinded. After homogenizing the samples were sent to the soil and water laboratory for a chemical analysis. Once in the laboratory, weighs 1 g of sample in porcelain crucibles previously labeled to be placed in a muffle at a temperature of $600\text{ }^{\circ}\text{C}$ for 150 min. Subsequently, the acid digestion was carried out with 2 M nitric acid and 50 ml with distilled water. Later, the corresponding dilutions were made to determine the elements under study. Their K^+ , Ca^{2+} , Mg^{2+} and Na^+ contents were determined with a flame photometer (model PFP7, Jenway, Stone, UK). Boron was determined in the dry ash using the Azomethine-H method (Matt *et al.*, 1975).



Figure 2. Scheme of sample taking in new (absorbent or radical hair) and old roots (suberized or anchoring/ support) of “luteño” maize plants.

Data Analysis

All experiments described were repeated twice independently with eight samples each time. A completely randomized design was used in the study. The experimental data were analysed by ANOVA, and the differences were compared by employing the Tukey test with a significance of $p < 0.05$ using the SPSS software (SPSS version 15.0, 2006).

Results

Saline conditions and B excess in soil and irrigation water significantly change the distribution of Na^+ , B, K^+ , Mg^{2+} and Ca^{2+} under field condition in adult plant “Iluteño”. The content of these ions in roots and leaves is different. Also, the information generated is a starting point to understand the mechanism of ionic tolerance in the adult plant that should be taken into account when developing management practices of this maize.

In the root, a separation of the fine roots (absorbent or radical hairs) and the thick roots (suberized or anchoring/support) is carried out, due to the difference of functions of these (Figure 2) these two groups were analyzed separately. In Figure 3a, the B content notoriously decreased around 40% in the fine roots (29.88 mg kg^{-1}) when compared with the thick roots (49.75 mg kg^{-1}). In contrast, the Na^+ content was similar in both types of roots with high mean values of $5429.60 \text{ mg kg}^{-1}$ (Figure 3b). The Ca^{2+} and Mg^{2+} content were higher in the thick roots with mean values of $6269.8 \text{ mg kg}^{-1}$ and 1322 mg kg^{-1} , respectively, over 50%. Nevertheless, the K^+ content was significantly higher in fine roots (about 40%) when compared with thick roots, 14923 mg kg^{-1} , and 8635 mg kg^{-1} , respectively. The saline condition and excess of B altered the ions status in leaf tissue (Figure 4). Thus, the levels of Na^+ ions (Figure 4b) significantly decreased in the maize leaf (time five) 967.6 mg kg^{-1} , while a larger increase of B (time eleven) was measured (Figure 4a) 390 mg kg^{-1} .

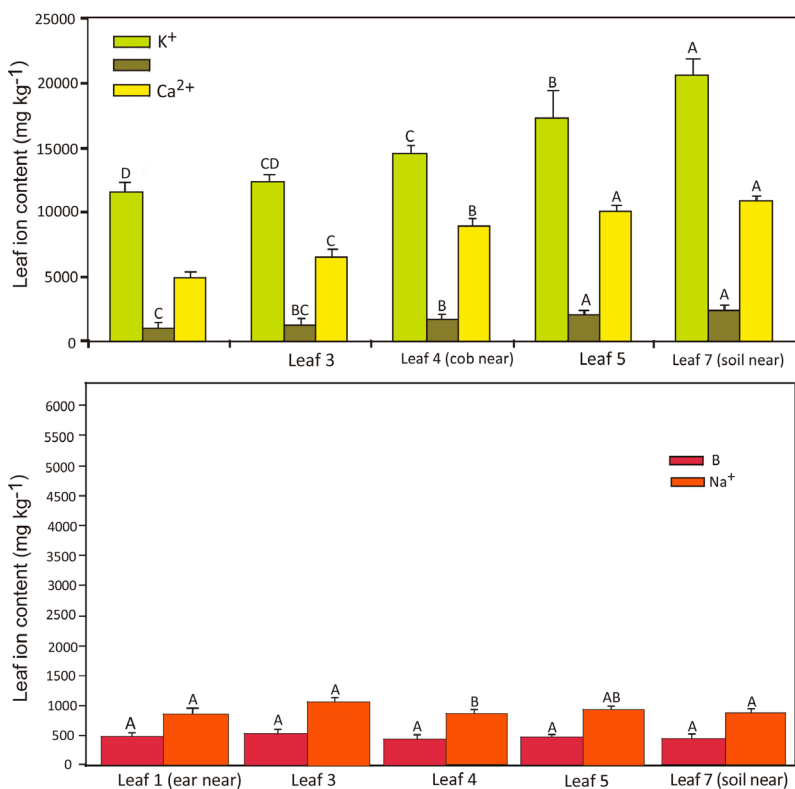


Figure 3. The ion content in thick root and fine root in adult plants of *Zea mays* L. amylacea. Values are means ($n=8$) \pm SE; significant differences between the means at least $P \leq 0.05$ appear with differences letters.

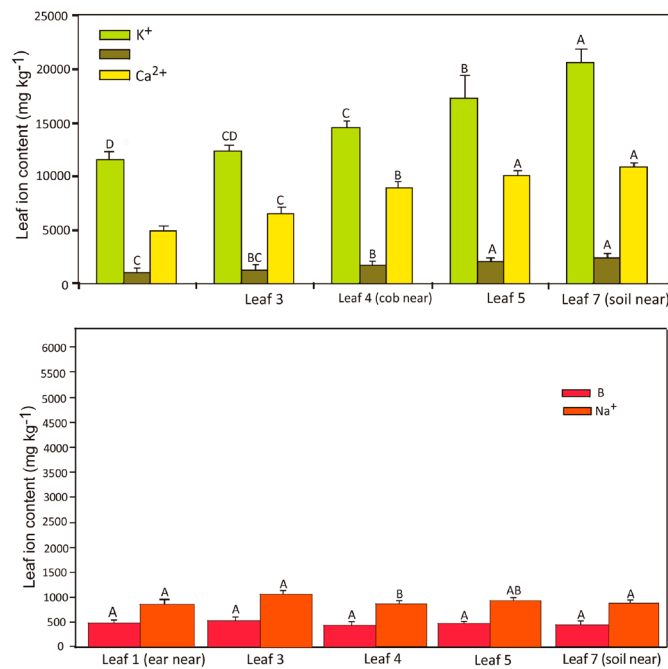


Figure 4. Ion Fluxes along the adult plants (Leaf) *Zea mays* L. amylacea in the four regions.

Values are means ($n = 8$) \pm SE; significant differences between the means at least $P \leq 0.05$ appear with differences letters.

The Ca^{2+} and Mg^{2+} concentrations tended to values similar both in roots and leaves (Figure 3cd and 4cd) about 1300 mg kg^{-1} . Under field conditions, the K^+ content in leaf was markedly increased with mean values between $20576.40 \text{ mg kg}^{-1}$ and $11551.8 \text{ mg kg}^{-1}$.

Ion fluxes were determined along the plant (leaf) from base to top in the four regions (Figure 1). To evaluate where the more significant accumulation of ions occurred, specifically, Na^+ and B. In the top region of the plant, B concentration was lower (30%) compared to the basal region (Figure 4a), did not show significant differences between them. By contrast, in the top region of the plant, Na^+ concentration was higher (30%) compared to the basal region (Figure 4b), did not show significant differences between them. Furthermore, a significantly enhanced Mg^{2+} , Ca^{2+} and K^+ concentrations were found in the basal region (near soil; Figures 4c, d and e), this increase was around 50% concerning the top region of the plant. However, a more pronounced K^+ concentration was observed in comparison to the other ions; this result indicated a physiological role of K^+ in salt tolerance and K^+/Na^+ ratio in the tissue.

Discussion

Soils with excess soluble salts or exchangeable sodium in the root zone are termed salt-affected soils. Owing to limited rainfall and high evapotranspiration demand, coupled with poor soil and water management practices, salt stress has become a serious threat to crop production in arid and semi-arid regions of the world (Munns, 2002). Although the general perception is that salinization only occurs in arid and semi-arid regions, no climatic zone is free from this problem (Rengasamy, 2006). High concentrations of salt in the soil disturb the capacity of the roots to extract water, and high concentrations of salt within the plant itself can be toxic, resulting in an inhibition of many physiological and biochemical process such as nutrient uptake and assimilation. Together, these effects reduce plant growth, development, and survival (Carrillo *et al.*, 2011). The foliar accumulation of Na^+ are characterized by the inhibition of plant growth and development (Zahoor *et al.*, 2011) and a significant decreasing effect by salinity were found in the shoot and root fresh and dry biomass in maize. “Luteño” maize showed significant variations in the ion content in

the shoot and root under salt and B excess. The concentration the Na⁺ accumulated more in the root; exclude Na⁺ in their shoots to prevent Na⁺ toxicity, which indicated the efficient capacity of this maize to restrict Na⁺ movement to the photosynthetic parts of the plant (Bastias *et al.*, 2004a).

Conversely, the B content was more elevated in leaf than in the root due to the transport of B from root to shoot driven by transpiration stream under combined stresses (Talaat *et al.*, 2015). Our result show excess B was also found to increase membrane permeability of root cells and enhance Na⁺ influx into the root cell (Alpaslan and Gunes, 2001). This well correlated with influences B in membrane-associated processes of the leaf, and it suggested that it play a structural role in the plasma membrane forming B complexes (Bastias *et al.*, 2004a) this may be one of the mechanisms underlying their superior tolerance. In this study, both the concentration of Na⁺ and B did not show considerable differences between the types of roots and different levels of the leaves (Figures 1 and 2).

Higher uptake and accumulation of K⁺ in the shoot and the roots (Figures 3 and 4) is regarded as better salt and B excess tolerance because that K⁺ plays an important role in the stomatal aperture and osmoregulation (Kusvuran *et al.*, 2012). Elevated K⁺ levels act osmotically, preventing a Na⁺ influx into the roots and shoots (Zahoor *et al.*, 2011), which shows that K⁺ nutrition is disturbed under salt stress due to a reduction in the deposition rate in growing cells. Moreover, others authors indicated that leaf ionic composition of all of the maize varieties showed a significant increase in Na⁺ concentrations and a decrease in K⁺ concentrations due to salinity (Ahmed *et al.*, 2012). In this study, in both leaf and root were found very high K⁺ concentrations in salinity conditions.

Especially, on the fine roots and the position of the seventh leaf near the soil. Maintenance of high K⁺ concentrations in the salt tolerant varieties may be one of the mechanisms underlying their superior salt tolerance (Kusvuran, 2012; Turan *et al.*, 2010). A high Ca²⁺ concentration can reduce permeability to Na⁺, because Ca²⁺ reduces the accumulation of Na⁺ (Cramer *et al.*, 1994). Our results showed that “lluteño” maize accumulate more their Ca²⁺ and limited Na⁺ uptake, especially, in the thick roots and the seventh leaf near of the soil. Similar behavior was observed in salt tolerant maize varieties (Kusvuran *et al.*, 2012). The maintenance of Ca²⁺ and transport under salt stress is also an important determinant of salinity tolerance (Shahzad *et al.*, 2012).

Conclusion

In conclusion, the present study on salt and excess B tolerance of “lluteño” maize showed a marked concentration of B, Mg²⁺, Ca²⁺ and K⁺ in the lower leaves of adult plants, conversely, only Na⁺ concentration on significant levels in the upper leaves were found, possibly due to having a role in the osmoregulation at a cheap energy cost for the plant. Whereas in the roots B, Mg²⁺ and Ca²⁺ show a higher concentration in the thick roots, except, the K⁺ that was found in the fine roots. The concentration of Na⁺ had an unexpected behavior; it was the ion with the highest concentration in the root, and these levels were similar in the thick and fine roots.

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