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Máster en Producción Vegetal en Cultivos Protegidos

Trabajo Fin de Máster

“Behavior of *Sarcocornia fruticosa* under salt stress.”

**“Tendencias de la distribución de nutrientes en *Sarcocornia fruticosa*
bajo un gradiente salino”**

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1. Abstract

Soil salinity, one of the major abiotic stresses reducing agricultural productivity, affects large terrestrial areas of the world; the need to produce salt-tolerant crops is evident. Most crops in agricultural production are sensitive to salt stress. Consequently, salinity is an ever-present threat to agriculture, especially in areas where secondary salinisation has developed through irrigation or deforestation.

In turn, the research on salt-tolerant plants (known as halophytes plants) may provide the solution to this problem. Halophytes have evolved to grow in saline soils, developing a wide range of adaptations. In the present work we study the behavior of *Sarcocornia fruticosa* under salt stress. Two trials have carried out: Trial 1 with 2 saline treatments (3,51 and 58 mM NaCl) and trial 2 with three treatments (100 mM, 200 mM and 300 mM NaCl). The results indicate that *S. fruticosa* is capable of tolerating very high and continued exposure to salt, levels of 300 mM of NaCl presents similar biomass related 100 mM of NaCl. Nevertheless, the branches died increase significantly under high level of salinity.

2. Keywords: Salt, NaCl, native plant.

3. Introduction

Almost three quarters of the surface of the earth is covered by salt water and so it is not surprising that salts affect a significant proportion of the world's land surface (Flowers and Flowers, 2005). It is estimated that 20% of the irrigated land in the world is presently affected by salinity. This is exclusive of the regions classified as arid and desert lands (which comprise 25% of the total land of our planet) (Yamaguchi and Blumwald, 2005). Salinity is a major environmental factor limiting plant growth and productivity (Allakhverdiev *et al.*, 2000.)

The development and use of crops that can tolerate the high levels of salinity in the soils would be a practical contribution towards addressing the problem (Yamaguchi and Blumwald, 2005). Currently don't know exactly mechanisms of tolerance to salt stress. Be necessary to identify the major genetic determinants of salt stress tolerance. The existence of salt-tolerant plants (halophytes) and differences in salt tolerance between genotypes within salt-sensitive plant species (glycophytes) indicates that there is a genetic basis to salt response (Yamaguchi and Blumwald, 2005). Table 1 presents a classification of types of plants in saline environments is developed based on tolerance mechanisms.

Table1. Classification of plants from saline environments.

Denomination	Characteristics	Examples
<i>Euhalophytes</i>	Accumulate salt in tissues	<i>Arthrocnemum</i> , <i>Salicornia</i> , <i>Sarcocornia</i>
<i>Crinohalophytes</i>	Glands or hair excretory	<i>Atriplex spongiosa</i> , <i>Limonium</i> , <i>Tamarix</i>
<i>Glicohalophytes</i>	Selective absorption of salts	<i>Hordeum</i> , <i>Rhizophora</i>
<i>Locahalophytes</i>	Confined salt in special structures	<i>Atriplex halimus</i> , <i>Salsora oppositifolia</i>

3.1. Botanical classification

Sarcocornia fruticosa presents the following classification:

Kingdom: *Plantae*

Division: *Magnoliophyta*

Class: *Magnoliopsida*

Order: *Caryophyllales*

Family: *Amaranthaceae*

Genre: *Sarcocornia*

Species: *Sarcocornia fruticosa*

3.2. Morphological characteristics

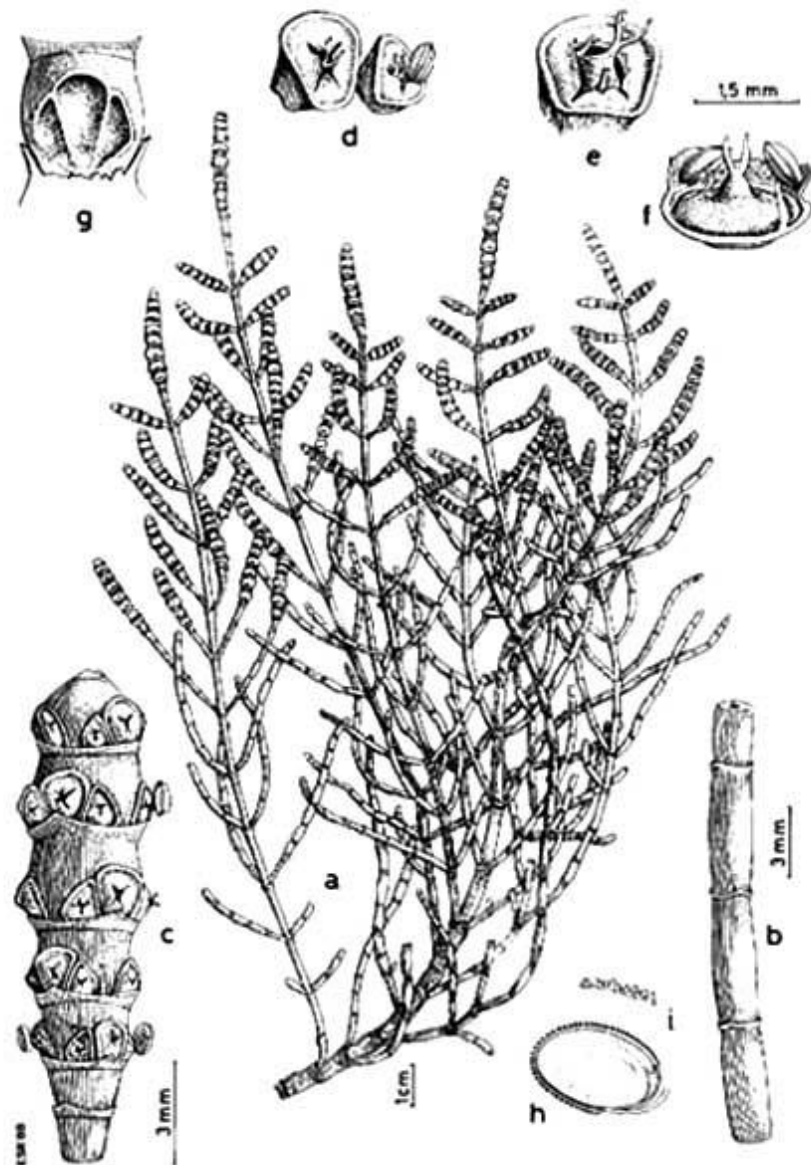


Fig. 1 *Sarcocornia fruticosa*, marshes Almonte, Huelva (MA 22/039), a) branch in flower and fruit; b) sterile twig; c) fertile branch or dowel floriferous; d) flowers at different stages of maturity; e) detail of a flower at anthesis, showing stigmas and filaments; f) showing dissection of a flower androecium and gynoecium; g) knuckle fertile in fruiting, showing the three holes left by the top triflora; h) section of a seed; i) details of the projections of the testa.

Figure 1 shows a picture of the organs which characterize *S. fruticosa*. Species of the genus *Sarcocornia* are shrubs (50-150 cm tall), erect, much branched, not radican in the case of *S. fruticosa*. The species of *Sarcococornia* has the stems are usually woody in the basal, fleshy-articulated in the rest. Sterile branches generally shorter than fertile. Opposing sheets welded together and forming a knuckle stem \pm cylindrical in which edge are observed the leaf blades reduced to flakes hyaline and acute. Bracts opposing, welded, similar to sheets, forming the knuckles fertile also gross. Bracts opposing, welded, similar to sheets, forming the knuckles fertile gross also. Inflorescence spiciform; tops 3 flowers in the axil of each bract. Hermaphrodite flowers ternades, each one embedded in the bottom of a knuckle bearing, and separated from each other by a partition which remains after the fruit has fallen off at maturity, then there appear three independent adjacent cavities. Central flower is at the highest point. Perianth fleshy and spongy fruiting. Stamens 2. Higher ovary, stigmas 2. Achene fruit is included in the periante fruitful; membranous pericarp. Seed vertical, brown or brownish-gray, covered with short conical hairs and bumps, not hooked, with short hairs or papillae; perisperma no or very little; embryo duplicate $2n = 54, 72$.

3.3. Environmental requirements

It is a plant that grows in the light, but supports the shade, and is continental, or supporting large temperature variations. Prefers moist or wet soils (in turn may be the humidity indicator), pH 4.5-7.5 some acidic and rich in nitrogen.

3.4. Distribution zone

The main distribution areas of *S. fruticosa* are saline marshes, mudflats and salt marshes with abundant moisture throughout the year in Europe, western Asia, northern and southern Africa, central and South America, Polynesia, Peninsula Coast and the Balears, exceptionally within some populations.

S. fruticosa (L.) A.J. Scott is distributed throughout the coastal systems of south and west Europe (Ball, 1993). In the marshes of the south-west of Spain, it is found growing in the middle and high elevations in the tidal frame, where it is subject to occasional tidal inundations and seasonal hypersalinity. Large variations in soil salinity have been measured in this zone, ranging from lower than 17 mM NaCl to extreme concentrations of more than 940 mM NaCl in the salt pans (Rubio-Casal *et al.*, 2001).

Figure 2 shows the typical zonation in a salt marsh of the Spanish coast east where *S. fruticosa* ranked No. 3 in the geobotany distribution.

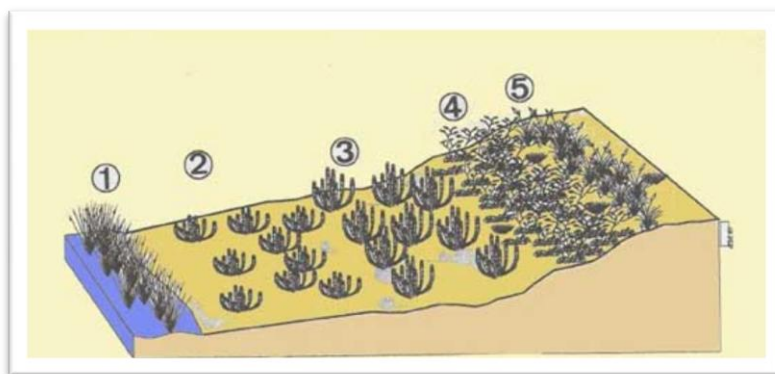


Fig.2 The typical zonation in a salt marsh of the Spanish coast east.: 1. *Juncus maritimus* (*Elymo elongati-Juncetum maritimi*), 2. *Arthrocnemum macrostachyum* (*Frankenio corymbosae-Arthrocnemetum macrostachyi*), 3. *Sarcocornia fruticosa* (*Cistancho phelipaeae-Arthrocnemetumfruticosi*), 4. *Limonium cossonianum* (*Limonietum angustebracteato-delicatuli*, 5. *Lygeum spartum* (*Senecio auriculae-Limonietum furfuracei*). Fuente: Alcaraz Ariza, F. 2008. La salinidad. Geobotánica: Tema 18. Universidad de Murcia.

The responses of *S. fruticosa* to salinity are of particular interest, as it is one of the few species that is found growing across this range of heterogeneous soil salt concentrations (Redondo-Gómez *et al.*, 2006). Figure 3 shows distribution map of *S. fruticosa* in Spain.



Fig.3 Distribution of *Sacocornia fruticosa* in Spain.

3.5. Salinity effects on plants

Salinity of soil and water is caused by the presence of excessive amounts of salts. Most commonly, high Na^+ and Cl^- cause the salt stress. Salt stress has threefold effects; it reduces water potential and causes ion imbalance or disturbances in ion homeostasis and toxicity. This altered water status leads to initial growth reduction and limitation of plant productivity. Since salt stress involves both osmotic and ionic stress (Hagemann and Erdmann, 1997; Hayashi and Murata, 1998), growth suppression is directly related to total concentration of soluble salts or osmotic potential of soil water (Flowers *et al.*, 1977; Greenway and Munns, 1980). The detrimental effect is observed at the whole-plant level as death of plants or decrease in productivity (Parida and Das, 2004).

Although the change in water status is the cause of growth suppression, the contribution of subsequent processes to inhibition of cell division and expansion and acceleration of cell death has not been well documented (Hasegawa *et al.*, 2000). Salt stress affects all the major processes such as growth, photosynthesis, protein synthesis, and energy and lipid metabolism (Parida and Das, 2004).

3.6. Salt tolerance of plants

Salt tolerance is the ability of plants to grow and complete their life cycle on a substrate that contains high concentrations of soluble salt (Parida and Das, 2004).

Plants develop a plethora of biochemical and molecular mechanisms to cope with salt stress. Biochemical pathways leading to products and processes that improve salt tolerance are likely to act additively and probably synergistically (Iyengar and Reddy, 1996). Biochemical strategies include according to Parida and Das (2004):

- 1) Selective accumulation or exclusion of ions.
- 2) Control of ion uptake by roots and transport into leaves.
- 3) Compartmentalization of ions at the cellular and whole-plant levels.
- 4) Synthesis of compatible solutes.
- 5) Change in photosynthetic pathway.
- 6) Alteration in membrane structure.
- 7) Induction of antioxidative enzymes.
- 8) Induction of plant hormones.

Salt tolerance mechanisms are either low-complexity or high-complexity mechanisms. Low-complexity mechanisms appear to involve changes in many biochemical pathways. High-complexity mechanisms involve changes that protect major processes such as photosynthesis and respiration, water use efficiency, and those that preserve such important features as cytoskeleton, cell wall, or plasma membrane–cell wall interactions (Botella *et al.*, 1994) and chromosome and chromatin structure changes, DNA methylation, polyploidization, amplification of specific sequences, or DNA elimination (Walbot and Cullis, 1985). It is believed that for the protection of higher-order processes, low-complexity mechanisms are induced coordinately (Bohnert *et al.*, 1995).

Respect to salinity of plants have been divided into two groups: the salt-sensitive (glycophytes) and the salt-tolerant plants that can survive on high concentrations of salt in the rhizosphere and grow well (halophytes) (Flowers and Flowers, 2005), although in reality one group merges into the other. Nonetheless, the division is useful in that the true halophytes can be used as models in which the range of their adaptations and their importance can be evaluated. Salt-tolerant plants occupying terrestrial habitats come from a wide spectrum of families (Flowers *et al.*, 1977).

The primary environmental factor faced by plants growing in salt marshes and salt deserts is the high concentration of salts that they encounter; soil water potentials can be lowered by the equivalent of about 1 to -2.5 MPa (-5 MPa in extreme conditions) by the salts present in the soil. For these saline environments the plant water potential must then be lowered by an equivalent of up to about 500 mM NaCl (Flowers, 1985). This is achieved through adjustment of plant water and solute content.

In turn, the very salts necessary for osmotic adjustment are potentially toxic and so must be separated from the metabolic machinery of the cells. This is achieved by compartmentation: salt-sensitive metabolic processes take place in the cytoplasm, while the salt necessary for osmotic adjustment is stored in vacuoles (Flowers *et al.*, 1986).

Within the cytoplasm, osmotic adjustment is effected by compatible solutes. These are organic compounds, such as glycinebetaine, mannitol and proline, which do not damage (and probably protect) the metabolism (Pollard and Wyn, 1979; Rathinasabapathi, 2000).

The process of compartmentation requires that halophytes have a mechanism to maintain differences in ion concentration across the membrane that surrounds their vacuoles; this mechanism depends on membrane structure (Leach *et al.*, 1990) and on the proteins that transport ions across membranes.

Ions enter plant cells through proteins that form an integral part of cell membranes. These proteins can either form channels through which ions diffuse down electrochemical potential gradients or carriers, where the protein binds an ion on one side of the membrane and releases it on the other side. Both processes are driven by energy-consuming ion pumps. These proteins (ion pumps) use the energy stored in ATP (or in the case of the vacuolar membrane, ATP and pyrophosphate) to move protons across the membrane generating a difference of hydrogen ion concentration (pH) and electrical potential (ΔE). It is the difference in electrical potential that drives the inward movement of cations through channels and the difference in hydrogen ion concentration that drives the movement of ions through carriers to which protons and ions bind (Flowers and Flowers, 2005).

In spite of considerable knowledge of the way in which K^+ ions cross membranes, it is not clear how Na^+ enters into plant cells. Although it is generally thought that Na^+ is 'mistaken' for by K^+ carriers or channels, it is also possible that Na^+ enters cells through non selective cation channels, particularly those activated by glutamate (Demidchik *et al.*, 2002; Maathuis and Amtmann, 1999; Maser *et al.*, 2002). It is also apparent that in some plants, ions can reach the leaves through pathways that bypass the controls that normally force ions through a specialised layer of cells in the roots known as the endodermis. If there are breaks

in the endodermis, water and dissolved solutes can flow to the leaves without encountering the selective barriers of cell membranes. This so-called 'bypass flow' is potentially dangerous and normally contributes little to the net transport of ions to the shoots (Perry and Greenway, 1973).

However, if external concentrations are high salinity in the case of even a small fraction of the flow of perspiration are not controlled by the endodermis will be assumed that the amounts of NaCl major outbreaks. This pathway appears to be particularly important in rice (Garcia *et al.*, 1997; Yeo *et al.*, 1987).

Halophytes must balance their requirement for the salts needed for osmotic adjustment with their growth rate (Flowers and Yeo, 1986). Regulating transpiration plays an important part in this process, as it is the transpiration stream that carries ions between root and shoot. Consequently, factors that influence the rate of water loss by plants are important in salt tolerance. Many halophytes show morphological adaptations associated with limiting transpiration, such as reduced leaves (which may be 'fleshy' or 'succulent'). In some species, an additional feature has evolved to regulate leaf ion concentrations. The leaf epidermis carries modified cells (salt glands) that secrete excess salt from the leaves (Thomson *et al.*, 1988).

Although all halophytes are able to complete their life cycles under conditions of elevated salinity, halophytic species differ greatly in their tolerance of salt. Some halophytes grow best at relatively low salinity (Wang *et al.*, 1997), whereas others are able to perform well at Na⁺ concentrations greater than 500 mM (Inan *et al.*, 2004). In some species, long-term exposure to salinity affects growth by closing stomata in order to limit transpiration and thus transport of salts (Véry *et al.*, 1998). The resulting lower CO₂ diffusion rates limit photosynthetic capacity. This can lead to exposure to excess energy, causing over-reduction of the reaction centers of photosystem, if the plant is unable to dissipate the excess of energy (Demmig-Adams and Adams, 1992). However other halophytes show no evidence of photoinhibition provoked by salinity stress (Qiu *et al.*, 2003).

3.7. Salt tolerance of *S. fruticosa*

According to Redondo-Gómez *et al.*, (2006) *S. fruticosa* demonstrated remarkable tolerance of salinity, even in comparison with many other halophytes. It showed the greatest growth when treated with 510 mM NaCl, a salinity similar to that of seawater, and maintained some growth even in 1030 mM NaCl.

The photosynthetic apparatus of *S. fruticosa* appears to be capable of accommodating prolonged exposure to high external salt concentrations, indicating considerable physiologic plasticity (Redondo-Gómez *et al.*, 2006).

According to Redondo-Gómez *et al.* (2006) growth rate increased with the photosynthetic area and was independent of the net photosynthetic rate. Hence, lower rates of CO₂ assimilation could be more than compensated for by a greater photosynthetic area. This response might provide positive feedback, since larger photosynthetic areas would induce higher growth rates and therefore more photosynthetic area, amplifying the difference between plants at different salinities over time. On the other hand, Eckardt (1972) showed that respiration in lignified stems of *S. fruticosa* consumes a considerable part of the energy fixed in photosynthesis, especially during summer drought at high temperature.

Also observed, an increase in atrophied distal ends of photosynthesizing branches was recorded under hypersaline conditions in *S. fruticosa* (1030 mM); this may indicate that the plants were unable to sequester all of the incoming Na⁺ in the cell vacuoles, even though stomatal conductance decreased, reducing the transport of salts to the growing tips (Véry *et al.*, 1998). Once the build-up of salt in cells reaches a point where Na⁺ can no longer be sequestered in vacuoles, the concentration in the cytoplasm rapidly reaches toxic levels and

causes cell death through dehydration or salt poisoning (Munns, 2002). The death of the apical meristems would have limited the production of new internodes, reflected in the decrease in photosynthetic length of branches, and consequently a decrease in photosynthetic area at the highest salinity (Redondo-Gómez *et al.*, 2006). It has been suggested that salinity can increase photorespiration (Parida and Das, 2005) and cyclic electron transport (Bukhov and Carpentier, 2004). These two physiologic processes could be relevant mechanisms to protect *S. fruticosa* against excess radiation under conditions of high salinity. The lower assimilation of CO₂ at higher salinities appeared to be due to a reduction in intercellular CO₂ concentration which can be explained by the decrease in stomatal conductance in response to water stress. Nevertheless, *S. fruticosa* showed no decline in relative water content even though water potential fell sharply at the highest salinity (Redondo-Gómez *et al.*, 2006).

S. fruticosa is able to adapt physiologically to a wide range of salinities and grow under extremely hypersaline conditions (Redondo-Gómez *et al.*, 2006).

3.8. Employ of native plants in gardening.

During the XVIII century began scientific expeditions to the Americas, Africa and Asia, and began the cultivation of exotic ornamentals purposes, mainly from China and South America. The current historic parks and gardens are largely inherited from that time, and contain copies of great interest. What at the time was a consequence of scientific interest and taste for botanical collections, has persisted over time because of the advantages offered in many ways these species. Exotic species that best acclimate to each area have native tax, and are dominant in most current parks and gardens.

The use of native plants has many advantages:

- 1) These are species that are well adapted to adverse conditions and self-regenerating once planted.
- 2) The market demands new species better adapted to certain environmental conditions, and between native, there is a great variety to choose the most suitable for a variety of uses.
- 3) These are species that are environmentally friendly as they avoid genetic contamination and maintain biodiversity.
- 4) Overall, mean lower water consumption.
- 5) Because of its hardiness less have lower nutrient requirements.
- 6) Native plants generally have greater resistance to disease.
- 7) The use of these species is reduced compared to other traditional investment plus low maintenance cost.
- 8) The production is simple, because installation and culture techniques are simple.
- 9) There is a growing social demand for the use of native plant, the result of environmental awareness.

Using of native plants in public and private gardening has increased greatly in recent years. In gardening public use in the remodeling and construction of new gardens presents certain obstacles that must be overcome as the slow-growing plants are generally a showy bloom that is only in some genera (*Cistus*, *Nerium*, ...) , lack of cultivation techniques, adaptation to artificial culture media, the lack of variety and plant reduced format, use the part of the population, etc.. Similarly, private gardening also detected an increased demand for native plants, sometimes for ease of maintenance, and sometimes by an intention to approach nature.

3.9. Possibilities to increase tolerance to salt stress

Two basic genetic approaches that are currently being used to improve stress tolerance (Yamaguchi *et al.*, 2005):

- 1) Exploitation of natural genetic variations, either through direct selection in stressful environments and subsequent marker-assisted selection.
- 2) Generation of transgenic plants to introduce novel genes or to alter expression levels of the existing genes to affect the degree of salt stress tolerance.

Transgenic technology will undoubtedly continue to aid our search for the cellular mechanisms that underlie salt tolerance, but the complexity of the trait is likely to mean that the road to engineering tolerance into sensitive species will be long (Flowers *et al.*, 2005).

In the meantime, it would be expedient to continue to invest in other avenues such as the manipulation of ion excretion from leaves through salt glands, the use of physiological traits in breeding programs and the domestication of halophytes (Flowers *et al.*, 2005).

The aim of this study is to know the effect of NaCl salt increase in water irrigation on *S. fruticosa* biomass and nutrient uptake and their organs distribution.

My experimental contribution of this work has been the following: To process the data of fresh and dry weight, to pulverize samples of dry matter, to make the digestion of these samples, to determine organic N and Total P of digested samples. To solubilize and analyze NO_3^- and Cl^- by ionic chromatography.

4. Material and methods

4.1. Environmental Conditions and Irrigation System

The trial was carried out in a tunnel greenhouse of 150 m², with zenith ventilation and relative humidity and temperature control. The specie studied was *S. fruticosa*.

Single plants were grown in 1.5 L pots and the substrate used was a mixture of peat and perlite 80:20 (v/v). Fertigation was applied by drip system, being the fertigation dose 70 mL per plant and day.

4.2. Treatments

Were performed 2 tests, in both the concentration of nutrients in solution nutritive was: H_2PO_4^- , NO_3^- , SO_4^{2-} , K^+ , Ca^{2+} and Mg^{2+} were 0.70, 6.00, 2.00, 3.00, 2.00 and 1.40 mmol L⁻¹, respectively. In first trial the electrical conductivity of the nutritive solution was incremented by application of NaCl to obtain the following values: T₁₁ (2 dS m⁻¹) and T₁₂ (7.5 dS m⁻¹) which corresponds to NaCl concentrations of 3,51 and 58 mM. In the second trial were applied 100 mM, 200 mM and 300 mM NaCl to that nutritive solution.

4.3. Sampling

Temperature, relative humidity and radiation were registered every 15 minutes with a sensor HOBO Onset U15 connected to a pyranometer, placed in the central part of the greenhouse, at the canopy height. Average temperature, relative humidity and photosynthetically active radiation were 25.4°C, 65.6% and 19.5 E m⁻²day⁻¹, respectively. Growth parameters were registered at the end of the cultivation, 60 days after the beginning of the trial. After removing the substrate, roots, branches and dead branches they were dried in a NüveE FN500 oven (range 30 to 300 °C) at 60°C for 48 h were weighted separately on a COBOS series CSC scale (precision 0.01 g) to determine dry weight.

The soluble ionic forms (NO_3^- and total Cl^-) were extracted with water to determine N- NO_3^- by ionic chromatography. Dry matter was digested with 96% sulfuric acid (H_2SO_4) in the presence of hydrogen peroxide (H_2O_2) to analyze total P (Hogue wilcow and Cantliffe, 1970) and organix N (Krom 1980). Total N was calculated as the sum of organic N and NO_3^- .

4.4. Experimental Design and Statistical Analysis

The experimental design was completely randomized, with 2 and 3 treatments (different concentrations of NaCl), and 4 replications per treatment in first and second trial,

respectively. An analysis of variance (ANOVA) and a Least Significant Difference (LSD) by $p < 0.05$ were conducted to determinate the influence of salinity on vegetative growth.

5. Results and discussion

5.1. Distribution of biomass

The results obtained in trial 1, are show in figures 4, 5, 6, 7, 8 and 9, where are presented of both treatments essayed (3,51 and 58mM NaCl applied in T₁₁ and T₁₂ respectively):

Figure 4 shows the root dry weight. No significant differences were found. These results are agreed with Albacete *et al.* (2008) that consider tomato root grown is maintained under salinity. But there are other authors who described a slight decrease in root of tomato plant under salinity (Tal, 1971; Abrisqueta *et al.*, 1991). Nevertheless, *Raphanus sativus* tuber size increases under salinity conditions (Marcelis y Hooijdonk, 1999).

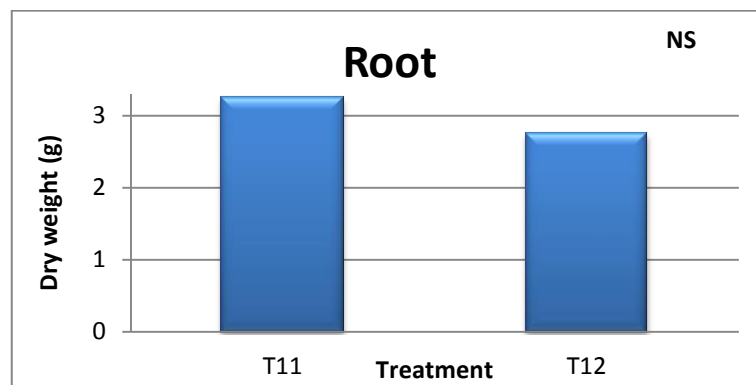


Fig. 4 Root dry weight.

Figure 5 shows the branches dry weight. Significant differences were found between treatments. When increase salinity branches biomass increases, this results are agree with Kham *et al.* (2005) that found a significant increase of shoot dry weight from 0 to 200 mM in *Arthrocnemum macrostachyum*. These fundings are opposites to those found by Rajaona *et al.* (2012) that indicated a decrease of foliar surface in crop of *Jatropha curcas* L., in response the increase of salinity from 0 to 300 mM NaCl.

Figure 6 shows the branches dead dry weight. Significant differences were found between treatments. With low salinity level dead branches are higher.

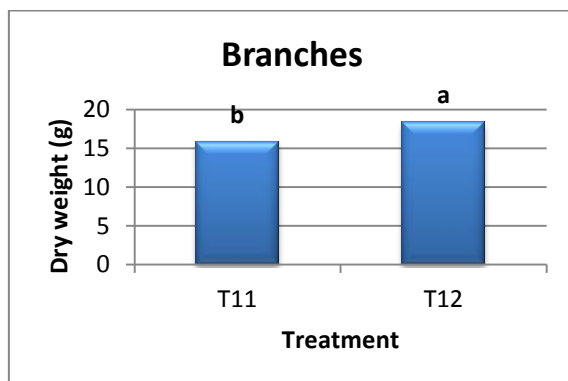


Fig. 5 Weight of branches with treatment T₁₁ and T₁₂.

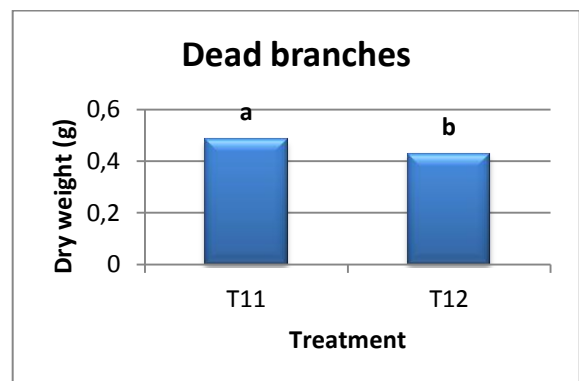


Fig. 6 Weight of dead branches with treatment T₁₁ and T₁₂.

In Figure 7 present the distribution of the total plant biomass. Although no significant differences between treatments were found, is a slight increase in the mass of branches under T₁₂ treatment, which constitutes 85% of the total mass versus to 81% under T₁₁ treatment.

These results are disagree with their obtained by Mohammad *et al.* (1998) that found that salinity is accompanied by significant reductions in shoot weight, plant height, number of leaves per plant, root length, and root surface area per plant in tomato crop. Meloni *et al.* (2001) found that increased NaCl levels results in a significant decrease in root, shoot, and leaf growth biomass and increase in root/shoot ratio in cotton. Kurban *et al.* (1999) have reported that in *Alhagi pseudoalhagi* (a leguminous plant), increases the total weight of the plant at low salinity (50 mM NaCl), but decreases under high salinity (100 and 200 mM NaCl).

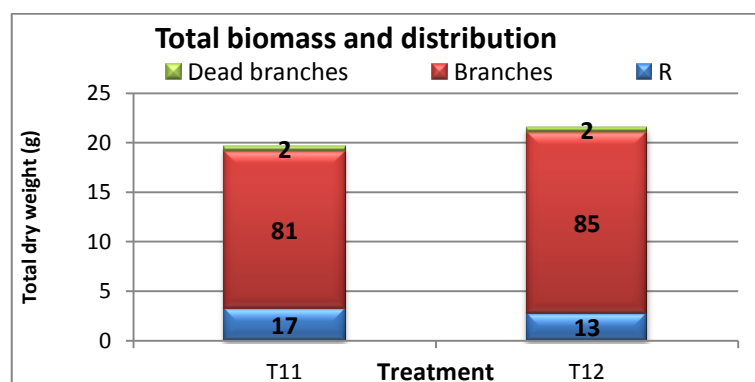


Fig. 7 Distribution of the total plant biomass with treatment T₁₁ and T₁₂.

Figure 8 shows the ratio roots/branches. Significant differences between treatments were found. Under salinity the ratio is lower. These results are disagreeing with Albacete *et al.* (2008) that found the opposite response.

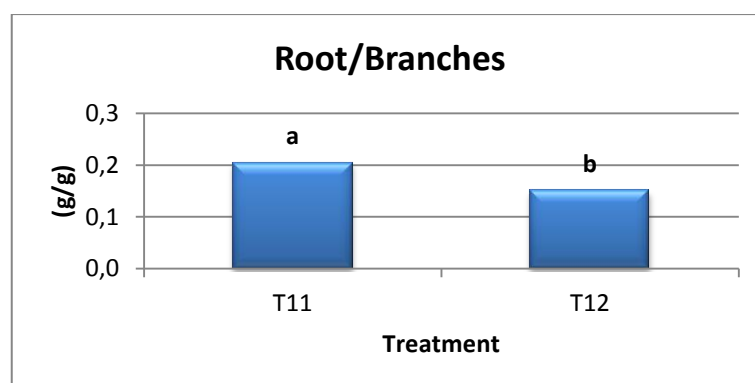


Fig. 8 Ratio roots/branches dry weight.

The results obtained in trial 2, are show in figures 9, 10, 11, 12 and 13 where are presented results of three treatments essayed (100, 200 and 300 mM NaCl applied in T₂₁, T₂₂ and T₂₃ respectively):

Figure 9 shows the roots dry weight. No significant differences were found. Similar results to trial 1 are found even under higher salinity levels. Witzel *et al.* (2009) found a decrease of root dry weight in barley to 40-50% under concentrations between 100 and 150 mM NaCl. Also, Nanawati y Maliwal (1974) found a negative effects on root biomass under salinity. The results match with Gururani *et al.* (2013) that observed average tuber yield per plant in 68.5 g under salinity stress comparing with 80 g under normal conditions.

Quite the opposite to the results obtained by por Hajihashemi *et al.* (2007) that indicates increased of the root length in wheat plants with higher salinity of treatment applied (75, 150 y 250 mM NaCl solution).

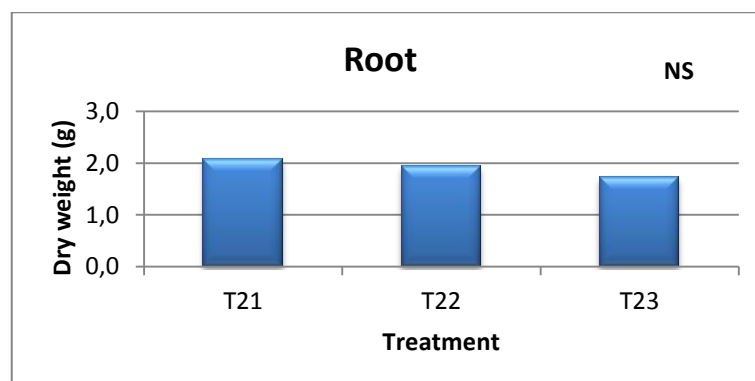


Fig. 9 Root weight with treatment T₂₁, T₂₂ and T₂₃.

Figure 10 shows branches dry weight. No significant differences were found between treatments. These results are opposite to found by Albacete *et al.* (2008) in tomato crop.

Khan *et al.* (1999) have reported that when *Halopyrum mucronatum* (a perennial grass found on the coastal dunes of Karachi, Pakistan) is treated with 0, 90, 180, and 360 mM NaCl in sand culture, fresh and dry mass of roots and shoots peaks at 90mM NaCl, a further increase of salinity inhibits plant growth, ultimately resulting in plant death at 360mM NaCl, and maximum succulence is also noted at 90mM NaCl. Experimental evidence shows that in a salt non secretor mangrove *B. parviflora* the plant growth is optimal at 100mM NaCl under hydroponic culture, whereas further increase in NaCl concentration retards plant growth and 500 mM NaCl is found to be lethal in this species (Parida *et al.*, 2004). On the other hand a salt secretor mangrove *Aegiceras corniculatum* can tolerate up to 250mM NaCl and 300mM is found lethal in this case (Mishra and Das, 2003).

In barley, higher NaCl concentration to 250 mM NaCl induced a leaf area decreasing about 40-50% (Witzel *et al.*, 2009).

In cultivation of transgenic potato plants wich trateid with 200 mM NaCl average plant height was significantly higher, but average number of leaves per plant was not significantly different compared with control plant (Gururani *et al.*, 2013).

Xue *et al.* (2001) observed that transgenic wheat lines exhibited improved biomass production at the vegetative growth stage and germination rates in severe saline conditions (100 and 150 mM NaCl).

These results are opposites to those found by Jeong-Ho *et al.* (2012), that observed decreased of the fresh weight of sprouts of the buckwheat by NaCl treatment (200 mM). Noreen y Ashraf, (2009) has reported that the fresh weight of various pea cultivars decreased by approximately 50–70% compared with the control group at treatment concentrations of 120 mM NaCl or higher. Hajihashemi *et al.* 2007 indicates a decrease in plant height and leaf surface in wheat by increasing salt concentrations in the treatment applied up to 200 mM NaCl.

Hussin *et al.* (2012) observed increased shoot (stem and leaves) rather than root fresh weights en plant halophyte *Atriplex nummularia* L. with moderate salinity level (250 mMol NaCl). According to Araújo *et al.* (2006) the leaf of dry mass gain of plants was stimulated up to 300 mM NaCl then reduced significantly in the highest NaCl treatment. In 600 mM NaCl leaf of dry mass decreased 26%, while root growth was not affected by salinity. The optimal growth for this species is stimulated in the range from 150 to 300 mMol NaCl when the upper limit of growth is 600 mMol NaCl (Araújo *et al.*, 2006)

Figure 11 shows the branches dead dry weight. Significant differences were found between treatments, being higher with salinity increase. These results are similar to obtain by Redondo-Gómez *et al.* (2006) that found the death of the apical meristems.

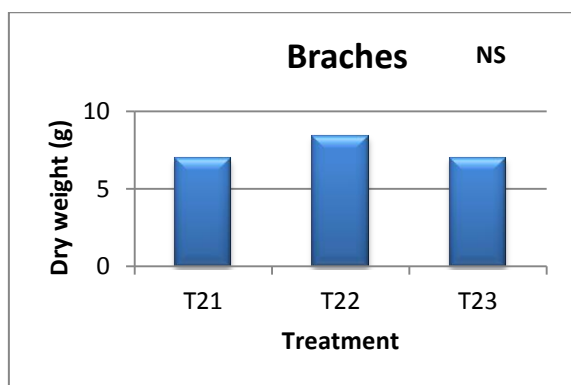


Fig. 10 Weight of branches with treatment T₂₁, T₂₂ and T₂₃.

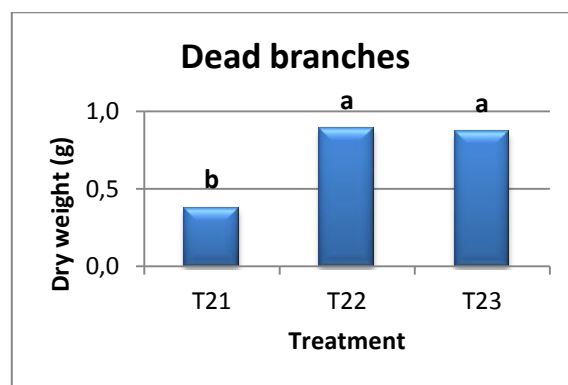


Fig. 11 Weight of dead branches with treatment T₂₁, T₂₂ and T₂₃.

Figure 12 and shows total biomass and perceptual biomass distribution between organs. Significant differences between treatments were found. Plants under 200 mM NaCl (T₂₂) present the higher growth. These results are disagree with Redondo-Gómez *et al.* (2006), that found the higher growth under 510 mM NaCl. On the other hand, the percentual biomass distribution changes in function of salinity, decreasing root biomass and increasing dead branches.

Sui *et al.* (2010) show that the growth of *Sueda salsa* L. was significantly increased by 300 mM NaCl treatment compared to that of 1 mM NaCl.

In the case of *Atriplex nummularia* L., a potential fodder halophyte plant for saline agriculture, maximum growth occurred at moderate salinity level (250 mM NaCl) (Hussin *et al.*, 2012). Results of the present study indicate that *A. nummularia* is highly salt-tolerant species in terms of biomass production under saline conditions, especially in arid climates (Hussin *et al.*, 2012). Similar results have been reported for *A. nummularia* (Silveira *et al.*, 2009), *A. triangularis* (Karimi and Ungar 1984), *A. semibaccata* (De Villiers *et al.*, 1996), *A. prostrate* (Wang *et al.*, 1997) and *A. griffithii* (Khan *et al.*, 2000).

These fundings are opposites to those found by Jiang *et al.* (2006) that observed that cotton seedling growth, indicated by height, leaf area, and fresh and dry weights, was reduced by NaCl with high salt concentration (salinity stress were studied under 0, 50, 100 and 200 mM NaCl treatments).

Hajihashemi *et al.* 2007 observed a decrease in the overall biomass production of wheat under salt stress (200 mM NaCl).

Cakile maritima (*Brassicaceae*) or sea rocket is an annual succulent halophyte that thrives on dunes along the Tunisian seashore. *Cakile maritima* showed a maximal growth potential at 100 mM NaCl while the growth was reduced at 200 and 400 mM NaCl (Amor *et al.*, 2010). This result coincides with Megdiche *et al.* (2007).

Hordeum maritimum (*Poaceae*) is an annual facultative halophyte species frequent in the Mediterranean basin and the European littoral (Cuénod *et al.*, 1954). In Tunisia, this plant is often observed at the close vicinity of strict halophytes in saline depressions, where it is significantly contributes to the annual biomass production (Abdelly *et al.*, 1995). Hafsi *et al.* (2007) showed that this plant appears to be a promising species for fodder production in saline soils. Recently, Hafsi *et al.* (2010) showed that adding 100 mM NaCl salinity was beneficial for *H. maritimum*. While Zribi *et al.* (2012) observed growth reduction with moderate levels

of salinity (100 mM NaCl). These results are in agreement with those of Yousfi *et al.* (2010) who reported better salt tolerance of this species as compared to *H. vulgare*.

Nitraria tangutorum Bobr. is a typical desert halophyte. Studies performed by Yang *et al.* (2010) indicate decreased in biomass production with moderate levels of salinity 200 mM NaCl. Similar result was obtained in rice (Ahmad *et al.*, 2009).

The plants of *Sesuvium portulacastrum* showed no significant change in the growth parameters (shoot length, dry weight, and water content) at 250 mM NaCl. However, growth of plants was significantly affected at 1000 mM NaCl (Lokhande *et al.*, 2011).

Suaeda fruticosa showed optimal growth at 300 mM NaCl (Hameed *et al.*, 2012) which is in accordance with a previous report on same species in a growth chamber (Khan *et al.*, 2000). The plants grew slowly at 600 mM NaCl. Species of genus *Suaeda* are reported to be obligate halophyte because their optimal growth is obtained in the presence of salinity like *S. salsa* (100–200 mM NaCl, Song *et al.*, 2009) and *S. maritime* (170–340 mM NaCl) (Flowers, 1972).

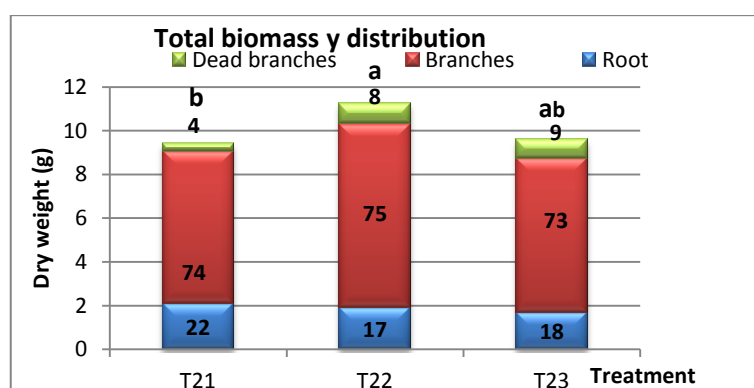


Fig. 12 Distribution of the total plant biomass with treatment T₂₁, T₂₂ and T₂₃.

Figure 13 shows the ratio roots/branches. Significant differences between treatments were found. Under salinity the ratio is lower. These results are disagreeing with Albacete *et al.* (2008) that found the opposite response.

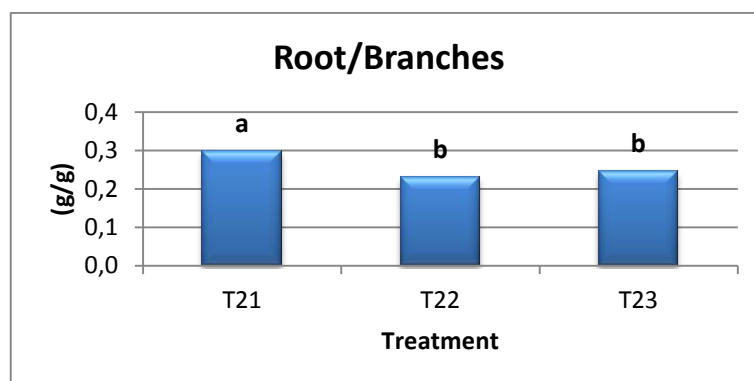


Fig. 13 Ratio roots/branches dry weight.

5.2. Distribution of organic nitrogen

The results obtained in trial 1, are show in figures 14, 15, 16, 17 and 18, where are presented of both treatments essayed (3,51 and 58mM NaCl applied in T₁₁ and T₁₂ respectively):

Figures 14 and 15 show significant differences between two treatments: with low salinity is observed greater amount of organic nitrogen in the plant roots.

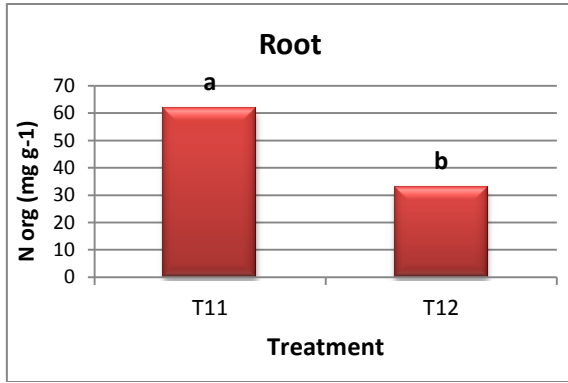


Fig. 14 Concentration of organic Nitrogen in the root with treatment T₁₁ and T₁₂.

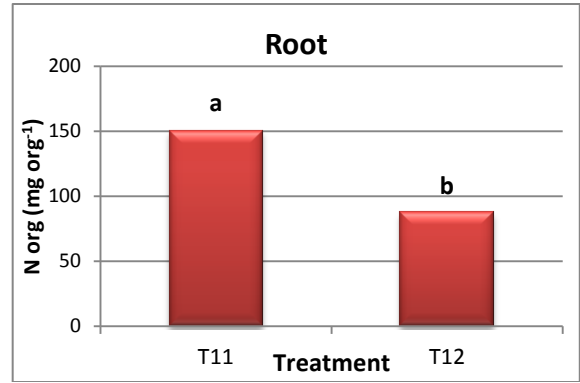


Fig. 15 Content of organic Nitrogen extracted from the root with treatment T₁₁ and T₁₂.

Figures 16 and 17 show the concentration and the content of organic nitrogen in the branches of the plant. Significant differences were found between treatments, is observed increase of organic nitrogen with increasing water salinity.

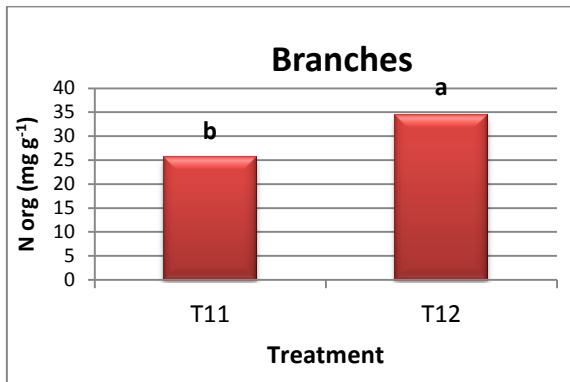


Fig. 16 Concentration of organic Nitrogen in the braches with treatment T₁₁ and T₁₂.

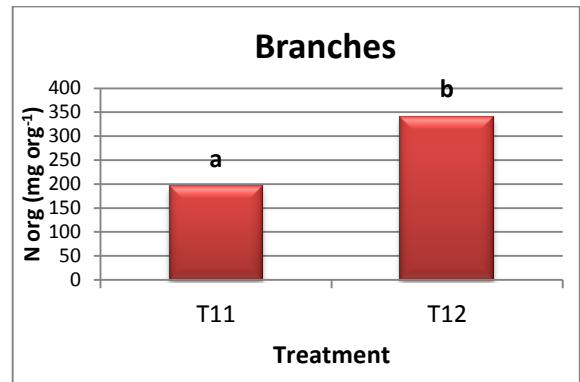


Fig. 17 Content of organic Nitrogen extracted from the branches with treatment T₁₁ and T₁₂.

Figure 18 shows distribution total of organic nitrogen per plant with both treatments. Is observed increase of organic nitrogen with increasing salinity, but change the distribution within the plant organs: increases in the branches, at a time, which decreases in the roots.

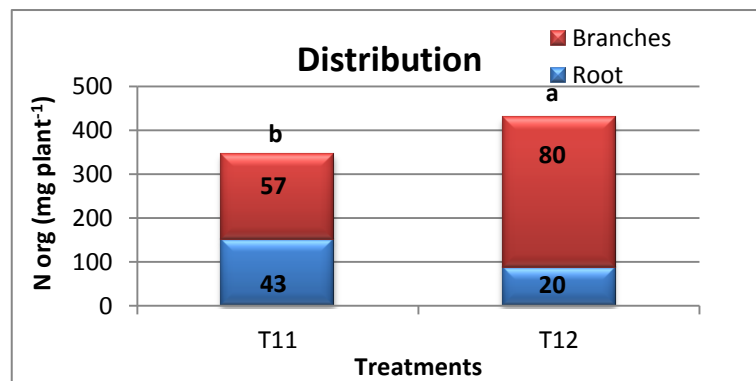


Fig.18 Distribution total of organic Nitrogen per plant with treatment T₁₁ and T₁₂.

The results obtained in trial 2, are show in figures 19, 20, 21, 22 and 23 where are presented results of three treatments essayed (100, 200 and 300 mM NaCl applied in T₂₁, T₂₂ and T₂₃ respectively):

Figures 19 and 20 show concentration and content of organic Nitrogen in the root of the plant. Significant differences between treatments T21, T22 y T23 were found. With higher salt levels content of organic nitrogen in the root of the plant increase.

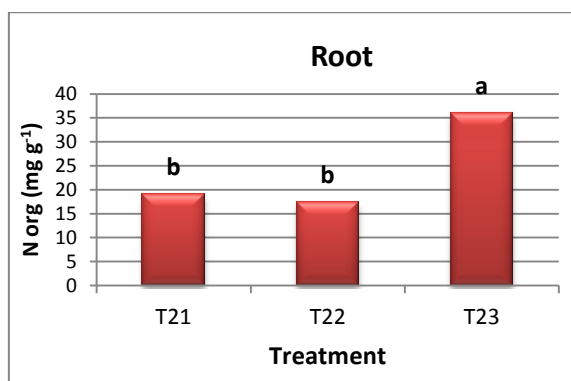


Fig. 19 Concentration of organic Nitrogen in the root with treatment T₂₁, T₂₁ and T₂₃.

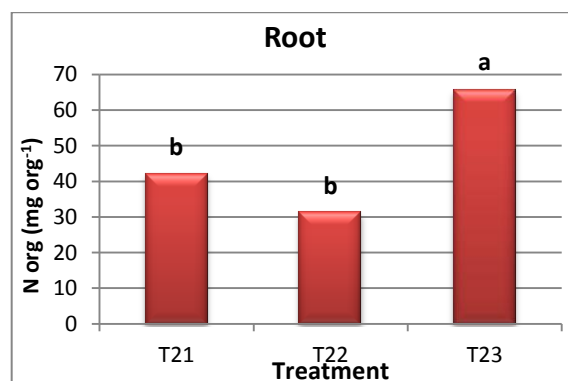


Fig. 20 Content of organic Nitrogen extracted from the root with treatment T₂₁, T₂₁ and T₂₃.

Figures 21 and 22 show concentration and content of organic nitrogen in the branches of the plant. Significant differences between treatments were found, is observed increase of organic nitrogen in the branches with increasing salinity.

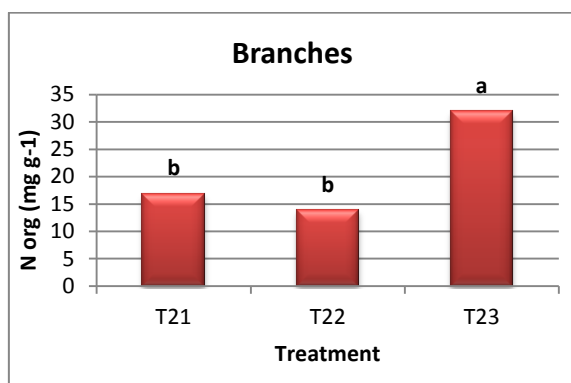


Fig. 21 Concentration of organic Nitrogen in the branches with treatment T₂₁, T₂₁ and T₂₃.

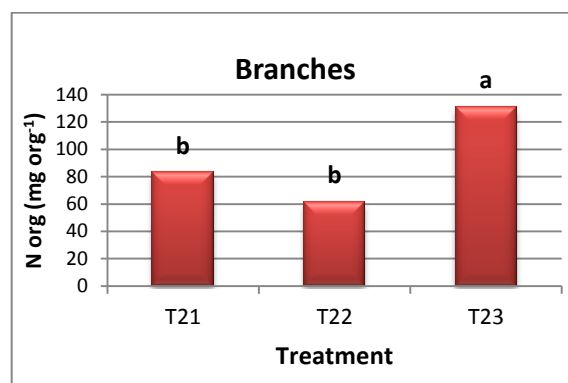


Fig. 22 Content of organic Nitrogen extracted from the branches with treatment T₂₁, T₂₁ and T₂₃.

Figure 23 shows distribution total of organic nitrogen per plant in trial 2 with three levels of salinity. A higher salinity is observed higher content of organic nitrogen, but does not change the distribution within the plant organs: are the same in the branches and in the roots with treatments applied.

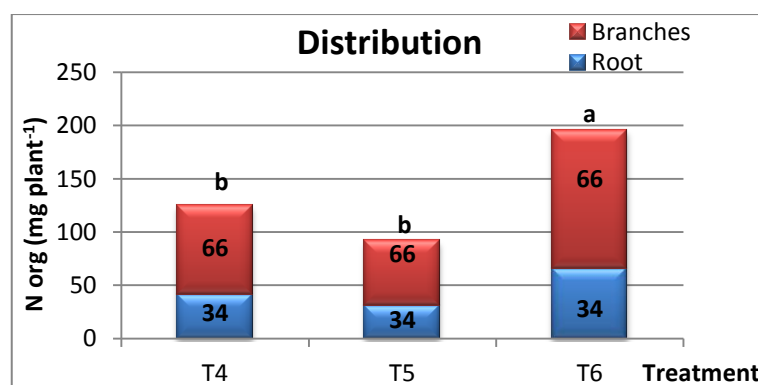


Fig. 23 Distribution total of organic Nitrogen per plant with treatment T₂₁, T₂₁ and T₂₃.

5.3. Nitrate distribution

The results obtained in trial 1, are show in figures 24, 25, 26, 27 and 28 where are presented of both treatments essayed (3,51 and 58mM NaCl applied in T₁₁ and T₁₂ respectively):

Figures 24 and 25 show concentration and content of nitrate in the root of the plant. No significant differences were found between treatments.

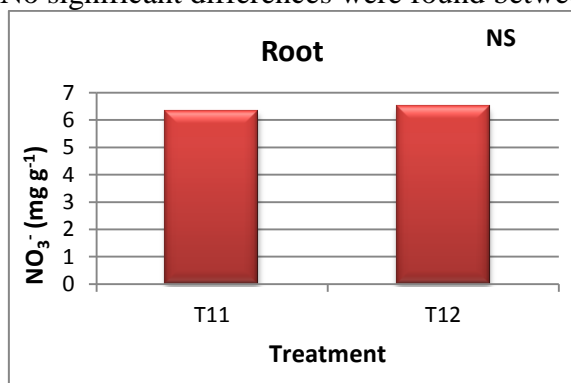


Fig. 24 Concentration of Nitrate in the root with treatment T₁₁ and T₁₂.

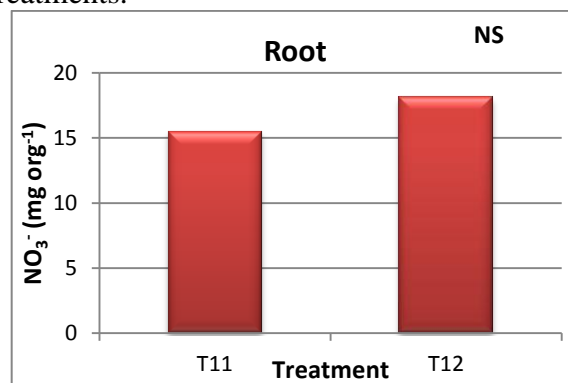


Fig. 25 Content of Nitrate extracted from the root with treatment T₁₁ and T₁₂.

Figures 26 and 27 show concentration and content of nitrate in the branches of the plant. Significant differences were found between treatments, is observed higher concentration to lower salinity levels.

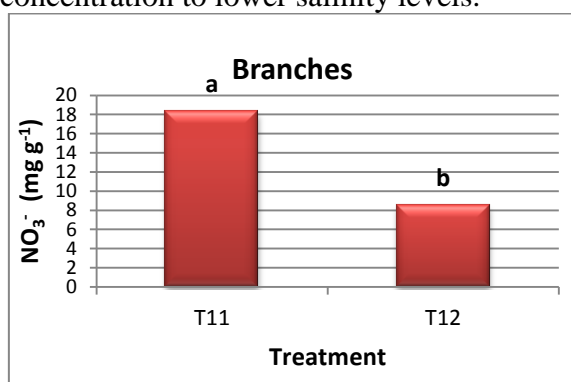


Fig.26 Concentration of Nitrate in the braches with treatment T₁₁ and T₁₂.

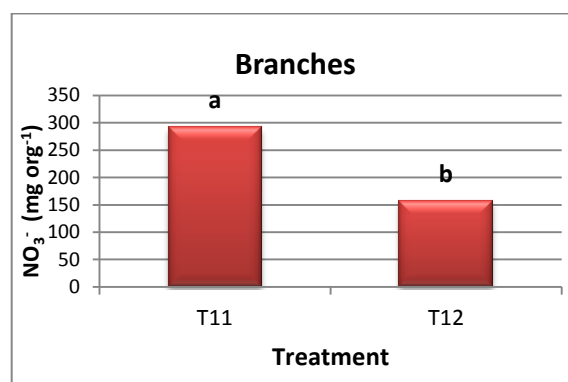


Fig. 27 Content of Nitrate extracted from the branches with treatment T₁₁ and T₁₂.

Figure 28 shows distribution total of nitrate per plant in trial 1. Significant differences were found between treatments. In treatment T₁₁with saline lowest level is observed higher content of nitrate in the plant, although the distribution within the plant organs is similar in both cases.

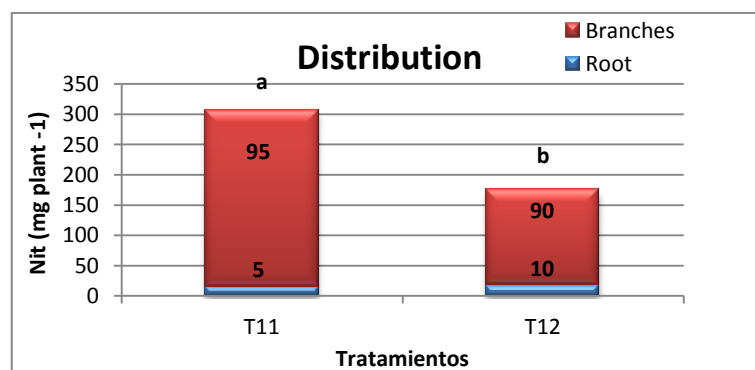


Fig.28 Distribution total of Nitrate per plant with treatment T₁₁ and T₁₂.

The results obtained in trial 2, are show in figures 29, 30, 31, 32 and 33 where are presented results of three treatments essayed (100, 200 and 300 mM NaCl applied in T₂₁, T₂₂ and T₂₃ respectively):

Figures 29 and 30 show the concentration and content of nitrate in the root of the plant. No significant differences were found.

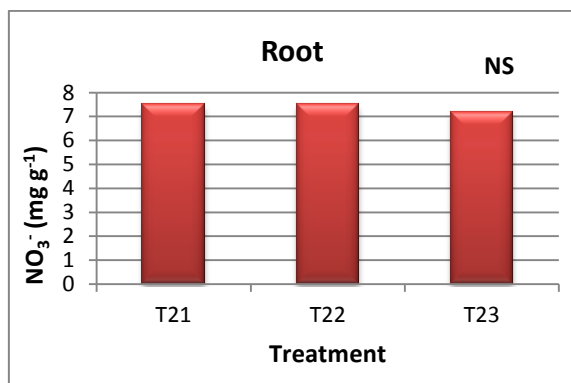


Fig. 29 Concentration of Nitrate in the root with treatment T₂₁, T₂₁ and T₂₃.

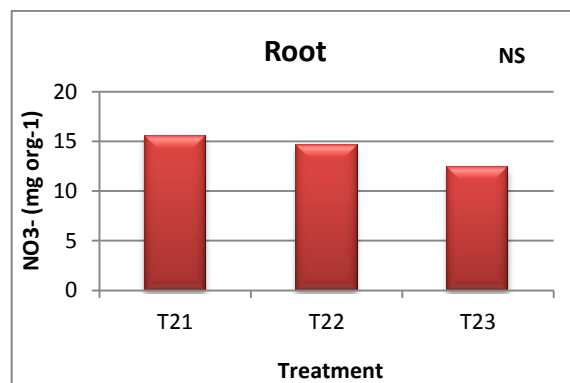


Fig.30 Content of Nitrate extracted from the root with treatment T₂₁, T₂₁ and T₂₃.

Figures 31 and 32 show the concentration and content of nitrate in the branches of the plant. No significant differences were found.

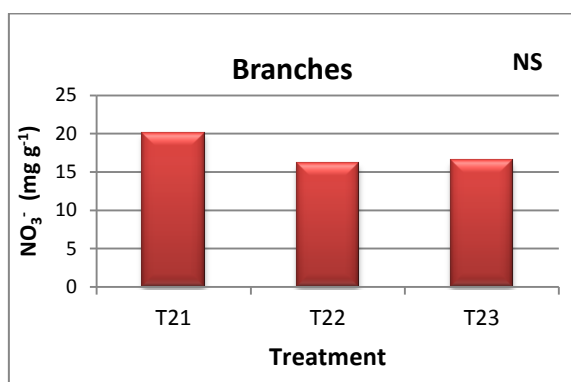


Fig. 31 Concentration of Nitrate in the braches with treatment T₂₁, T₂₁ and T₂₃.

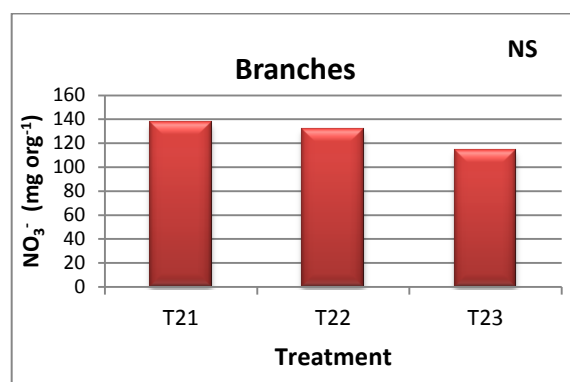


Fig. 32 Content of Nitrate extracted from the branches with treatment T₂₁, T₂₁ and T₂₃.

Figure 33 shows the distribution total of nitrate per plant in trial 2. No significant differences were found between treatments, also distribution within the plant organs is the same.

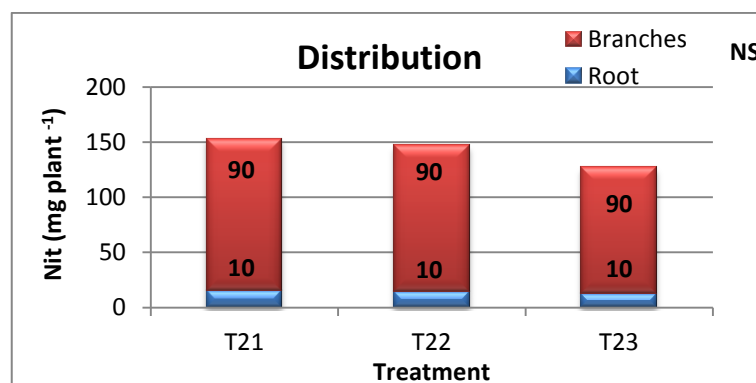


Fig. 33 Distribution total of Nitrate per plant with treatment T₂₁, T₂₁ and T₂₃.

5.4. Total nitrogen distribution ($N_{total} = N_{ogr} + N_{inorg}$)

The results obtained in trial 1, are show in figures 34, 35 and 36 where are presented of both treatments essayed (3,51 and 58mM NaCl applied in T₁₁ and T₁₂ respectively):

Figure 34 shows content of total nitrogen in the root of the plant. Significant differences were found between treatments, being more content with lower salinity levels (T₁₁).

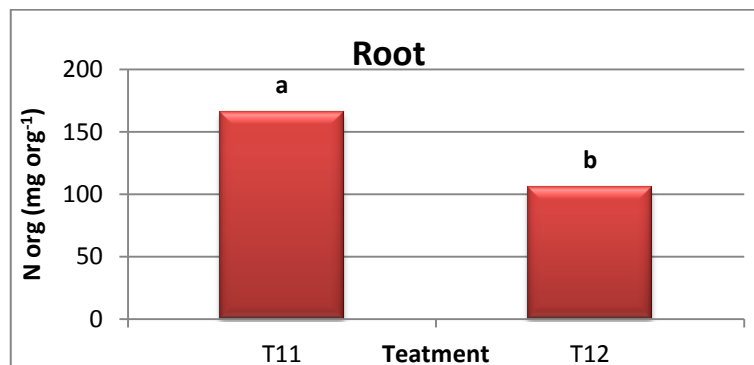


Fig. 34 Content of total Nitrogen in the root with treatment T₁₁ and T₁₂.

Figure 35 shows content of total nitrogen in the branches of the plant. No significant differences were found between treatments.

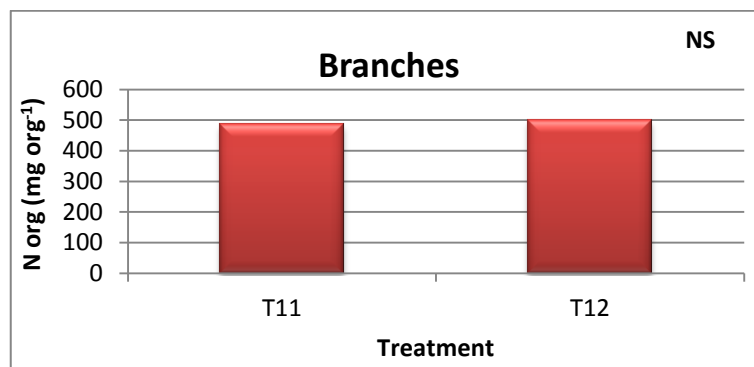


Fig. 35 Content of total Nitrogen extracted from the branches with treatment T₁₁ and T₁₂.

Figure 36 shows total nitrogen distribution within the plant organs. Significant differences were found between treatments, being higher total nitrogen content with lower salinity. And distribution within the plant organs: higher percentage of nitrogen in the root and lowest in the branches (25/75 with treatment T₁₁). According the salinity is increased the distribution changes decreases in the root while increases in the branches.

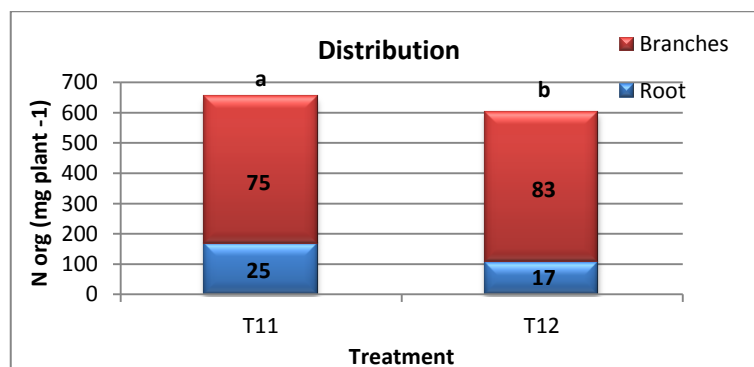


Fig. 36 Distribution of total Nitrogen per plant with treatment T₁₁ and T₁₂.

The results obtained in trial 2, are show in figures 37, 38 and 39 where are presented results of three treatments essayed (100, 200 and 300 mM NaCl applied in T₂₁, T₂₂ and T₂₃ respectively):

Figure 37 shows content of total nitrogen in the root of the plant. It is observed, that with higher salinity content is significantly higher.

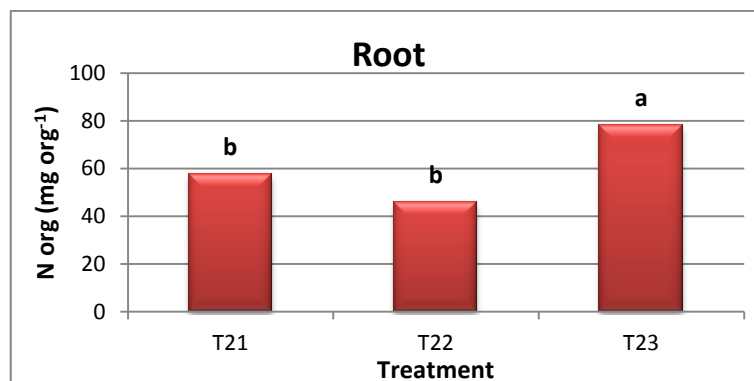


Fig. 37 Content of total Nitrogen in the root with treatment T₂₁, T₂₂ and T₂₃.

Figure 38 show content of total nitrogen in the branches. No significant differences between treatments were found.

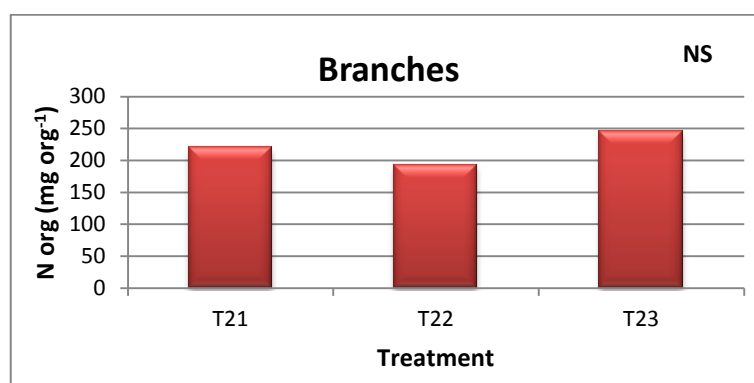


Fig. 38 Content of total Nitrogen in the branches with treatment T₂₁, T₂₂ and T₂₃.

Figure 39 shows distribution of total nitrogen within plant organs. Significant differences between treatments were found, when the total nitrogen content higher with higher levels of salinity. Although the distribution within the organs of the plant initially is similar to previous case (higher content in the roots and lower in the branches with lower salinity levels, is reduced in the roots and increases in the branches accordance is increased the NaCl present in the solution), to a certain level (200 mM) and then changes its initial distribution, increased content of nitrogen in the roots (300 mM). This phenomenon is due to the physiological processes of the plant.

This result coincides with that obtained in wheat, where higher salinity showed higher nitrogen content in plant tissues (Hajihashemi et al., 2007)

The opposite result is obtained in cotton transgenic cultivars (*Gossypium hirsutum* L), when the total nitrogen concentration decreased in response to 200 mMol NaCl treatment which was similar to observations in *Linum* (Singh and Singh, 1991), maize (Khan and Srivastava, 2000) and mung bean (Chakrabarti and Mukherji, 2003). These results indicated that N uptake might be inhibited by NaCl salinity stress in a similar pattern as conventional cotton and soybean (Gouia et al., 1994), (Jiang et al., 2006).

In *Atriplex nummularia* (L.), increasing water salinity transiently decreased N content of all organs, with significant reductions occurred at 50 % equivalent to 250 mMol NaCl (for root and adult leaves) and at 100 % equivalent to 750 mMol NaCl (for young leaves) (Hussin *et al.*, 2012).

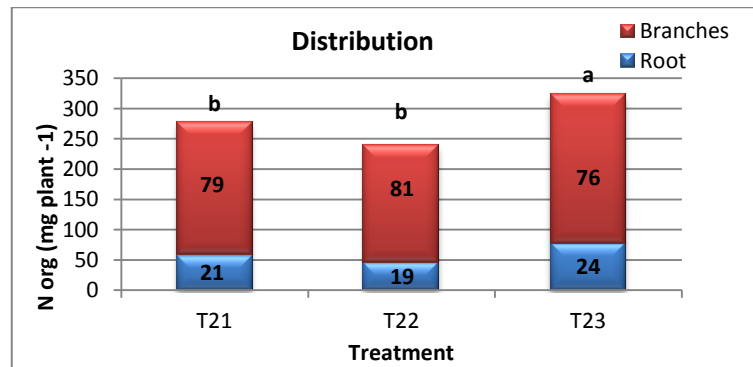


Fig. 39 Distribution of total Nitrogen per plant with treatment T₂₁, T₂₂ and T₂₃.

5.5. Distribution of phosphorus

The results obtained in trial 1, are show in figures 40, 41, 42, 43 and 44 where are presented of both treatments essayed (3,51 and 58mM NaCl applied in T₁₁ and T₁₂ respectively):

Figures 40 and 41 show total phosphorus concentration and content in the root of the plant. With higher concentrations of NaCl is observed higher content of phosphorus.

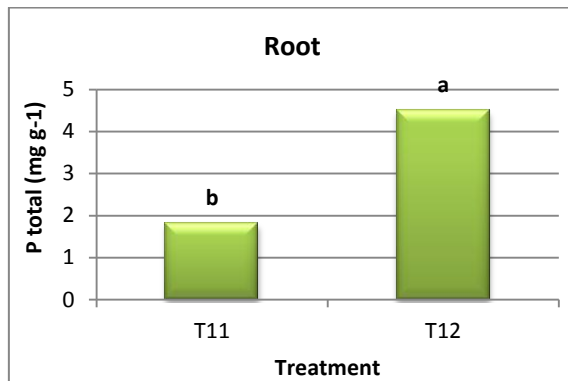


Fig. 40 Total Phosphorus concentration in the root with treatment T₁₁ and T₁₂.

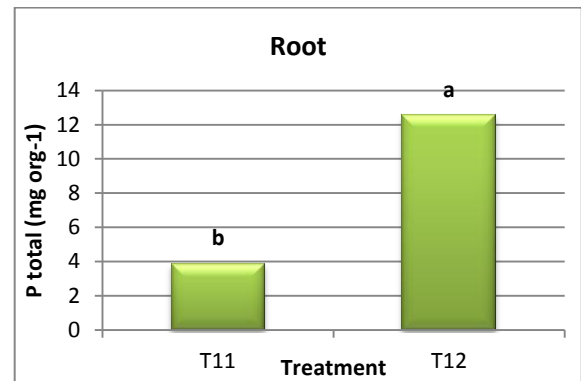


Fig. 41 The total Phosphorus content in the root with treatment T₁₁ and T₁₂.

Figures 42 and 43 show total phosphorus concentration and content in the branches of the plant. With higher concentrations of NaCl is observed higher content of phosphorus.

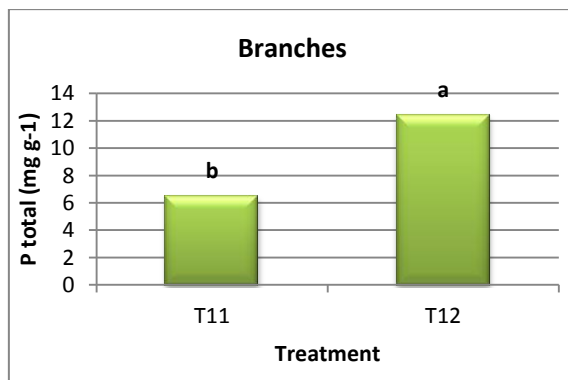


Fig. 42 Total Phosphorus concentration in the branches with treatment T₁₁ and T₁₂.

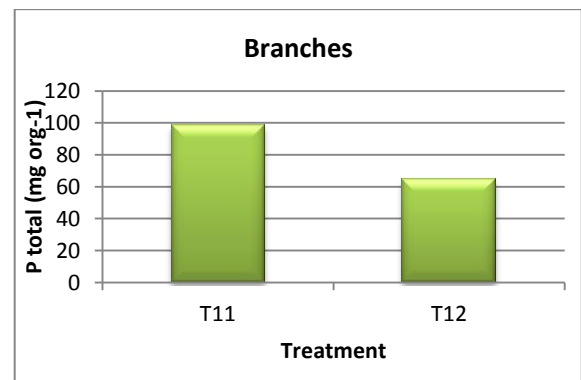


Fig. 43 The total Phosphorus content in the branches with treatment T₁₁ and T₁₂.

Figure 44 show total phosphorus distribution per plant. Significant differences were found between treatments, being higher the total content of phosphorus with low levels of NaCl, with the least percentage in the root and the highest in the branches of the plant. With increase the concentrations of NaCl increased the phosphorus content in the root, while decreased in the branches.

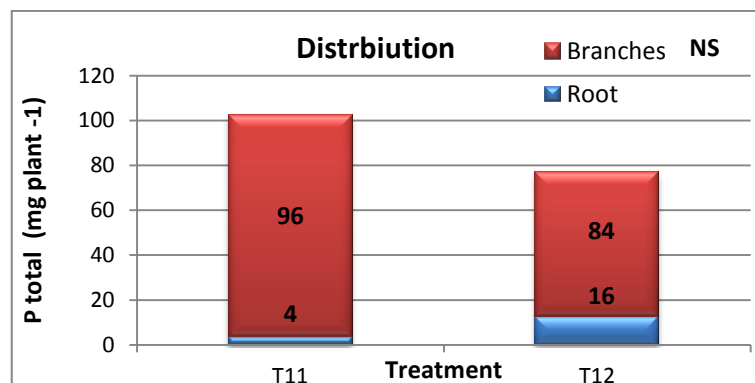


Fig.44 Total Phosphorus distribution per plant with treatment T₁₁ and T₁₂.

The results obtained in trial 2, are show in figures 45, 46, 47, 48 and 49 where are presented results of three treatments essayed (100, 200 and 300 mM NaCl applied in T₂₁, T₂₂ and T₂₃ respectively):

Figures 45 and 46 show total phosphorus concentration and content in the root of the plant, being significantly higher both NaCl concentrations higher as lower. The lowest result is obtained with the intermediate salinity level.

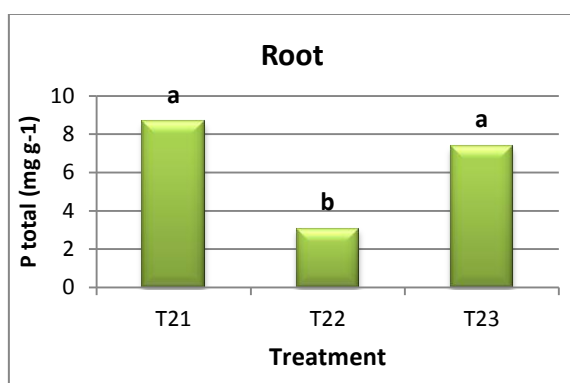


Fig. 45 Total Phosphorus concentration in the root with treatment T₂₁, T₂₂ and T₂₃.

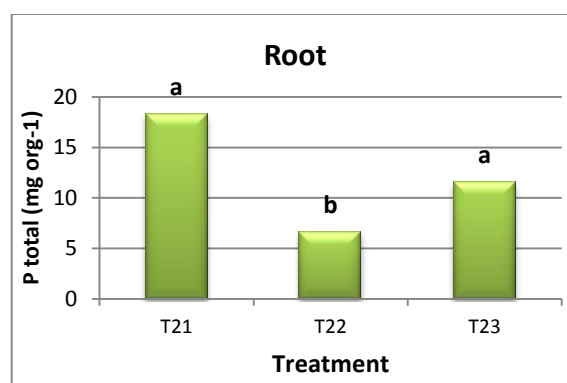


Fig. 46 Total Phosphorus content in the root with treatment T₂₁, T₂₂ and T₂₃.

Figures 47 and 48 show total phosphorus concentration and content in the branches of the plant. Significant differences between treatments were found, Similar to the concentration of total phosphorus content in the roots of the plant, similar to the concentration of total phosphorus content in the roots of the plant. The higher result is obtained with the lower concentration of NaCl, decreases with intermediate salinity level and increases with higher NaCl concentration.

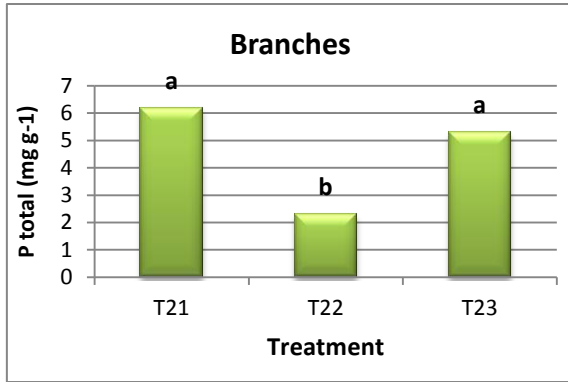


Fig. 47 Total Phosphorus concentration in the branches with treatment T₂₁, T₂₂ and T₂₃.

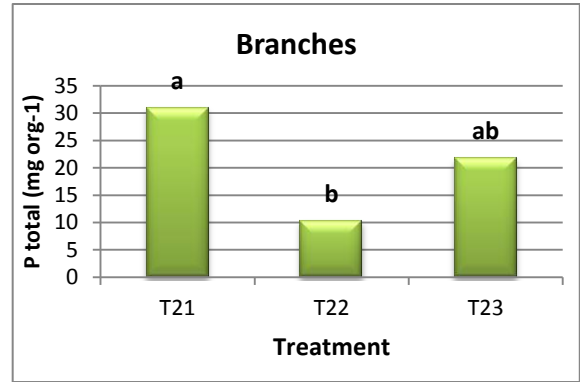


Fig. 48 Total Phosphorus content in the root with treatment T₂₁, T₂₂ and T₂₃.

Figure 49 shows total phosphorus distribution per plant. The higher content of phosphorus corresponds to treatment with lower salinity level, being the percentage distribution between the root and the branches similar in the three treatments.

The opposite result is obtained in the wheat plants in Ghods cultivar. Treatment with saline solution 225 mM NaCl increased the P. It is reasonable to suggest that this treatment may increase tolerance by diminishing nutritional imbalance in wheat caused by salt stress (Hajihashemi *et al.*, 2007).

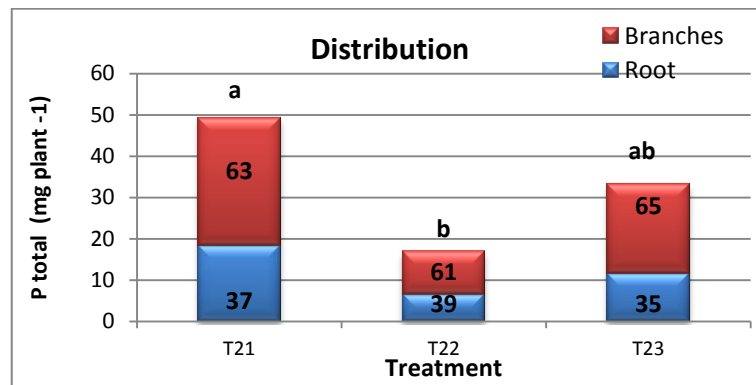


Fig. 49 Total Phosphorus distribution per plant with treatment T₂₁, T₂₂ and T₂₃.

6. Conclusions

From these studies, it can be concluded that *Sarcocornia fruticosa* respond well to an external concentration of 200 mM and 300 mM; as some halophytes such as *Suaeda fruticosa* (Khan *et al.*, 2000), *Suaeda maritima* (Moghaieb *et al.*, 2004), *Atriplex portulocoides* (Redondo-Gomez *et al.*, 2007) and *Salicornia europea* (Aghaleh *et al.*, 2009) with show optimal growth in saline conditions (Sekmen *et al.*, 2012). And that species has high possibilities to use it in coast Mediterranean area irrigated with low water quality.

In response to salt stress has observed an increase of content of N_{total} with the following distribution within the plant organs: content of N of the aerial part of plant increases, while it decreases in the roots with the highest levels of NaCl, which coincides with the results obtained by Medina *et al.* (2008) in the study of halophyte of the Carribbean coast of Venezuela and with results obtained by Parida *et al.* (2004).

At the same time, with high salinity is observed a decrease of content of P in *S. fruticosa* with the following distribution: decreases in photosynthetic tissues and increases in the roots. This result coincides with obtained by Medina *et al.* (2008) in the study of halophyte and with Plaza *et al.* (2009), (2012) in the study of *Cordyline fruticosa* var. Red Edge plant that tolerates certain levels of salinity (Plaza *et al.*, 2006), so it could be cultivated

in areas with poor water quality. Also according to the result obtained by Mills and Benton (1996) in *C. fruticosa* var. Firebrand.

7. References

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