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# Leaf, Stem and Root Content of Proline in *Atriplex Canescens* and *Suaeda Nigra*

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**Abstract**

Although the list of factors that can affect the environment can be even more extensive, it is considered that an increase of salts in the soil represents a significant threat to the survival of plants, and consequently its impact on food levels, thereby decreasing the production and harvesting capacity in agricultural soils, causing large losses, both economic for the productive sector, such as biological considering the growing areas. For these reasons and in order to contribute to understanding the mechanisms of plant adaptation to salt stress conditions, in this investigation was determined by spectrophotometry the proline content in leaves, stems and roots of *Suaeda nigra* and *Atriplex canescens*, species of the family Chenopodiaceae, drawn by different methods. The average values of this amino acid content for both species, regardless of location, ranged from 25.614 to 2.457 µmol $mg^{-1}$ for *S. nigra* and *A. canescens,* respectively. In *S. nigra*, the highest concentration of proline was 36.724 µmol$mg^{1}$ at the root, while in *A. canescens* the highest concentration was in the leaf with 3.951 µmol $mg^{-1}$. Extraction methods did not influence the proline content of *S. nigra*, while in *A. canescens*, when the extract was obtained by the macerated plus sonicated with prior dilution method, the results were statistically higher than those obtained when the sample was drawn only by the sonication and subsequent dilution method. The proline content in both species is contrasting, however, these results showed a different trend in the compartmentalization, as in *S. nigra*, proline accumulates in the root, while in *A. canescens*, it does in the leaf.

## 1. Introduction

The plant culture in excessive salty areas, can affect them with a lot of physiological, morphological and biochemical effects, such as the reduction of photosynthesis (Singh and Chatrath, 2001), reduction on the weight of fruits (Rosario et al., 1990; Perez et al., 1996), qualitative and quantitative changes in protein synthesis due to changes in salinity gene expression (Singh and Chatrath, 2001). According to Misra and Dwivedi (2004), high levels of salts in plants can result in an osmotic effect, ionic toxicity, or defects in the nutritional trafficking, which can affect the normal development on the plant. In several studies, hypersensitivity to salinity is more notable in the first stages of growth and development (Sivritepe et al., 2003). For all this we can tell that not all the plants have the same response to salt stress and this fact is related to different ranges of tolerance. Due to such fact the plants have been classified as glycophytes or halophytes according to their capacity to grow in a soil with high salt levels (Calu, 2006). Both kinds develop tolerance mechanisms to adapt to osmotic stress (Lee et al*.,* 2008) which allow osmotic adjustment through electrolytes and organic solutes (Driouich et al., 2001), sugars and amino acids like proline and glycinbetaine (Taji et al*.,* 2004; Denden et al*.*, 2005). Trying to tolerate the osmotic effect, a great number of plants have involved in their metabolism the capacity to synthesize and accumulate compatible solutes or osmoprotectors in their cytoplasm (Azevedo et al., 2004; Parida and Das, 2005). The benefit role of proline in increasing the tolerance to plant abiotic stress has been demonstrated with an osmotic agent protecting the plant of dehydration (Jimenez-Bremont et al., 2006; Tajdoost et al., 2007). However, in studies with transgenic plants, the increment of proline treatment looks very low to adjust the osmotic pressure in the whole plant (Zhu, 2001), despite of the existence of high levels of proline in some specific cells and subcellular compartments. Proline accumulation also depends on its degradation, catalyzed by the mitochondrial enzyme piruvate dehydrogenase (PDH), which transforms L-Proline in $\Delta^{1}$-pirroline-5 carboxylate (P5C) through consumption of $O_2$ and production of $H_2$ (Hare et al., 1999). It has been suggested that proline participates by multiple forms in plant stress tolerance, acting like a mediator of the osmotic adjustment (Delauney and Verma 1993; Kavi Kishor et al., 1995; Yoshiba

et al., 1997), protein and membrane stabilizer, osmotic gene activator (Iyer and Caplan, 1998), source of reduction power to support the oxidative phosphorylation and ATP regeneration during stress recovery (Hare y Cress, 1997; Hare et al., 1998). The Chenopodiaceae family members are plants from steppe environments like salt deserts of Central Asia, in our region are widespread, particularly adapted to environments with high salt concentration. The Chenopodiaceae are therefore halophilic and nitrophilous typical. This family includes important genus like *Chenopodium*, *Spinacia*, *Atriplex*, *Suaeda* and *Beta*. The proline content in *Atriplex canescens* and *Suaeda nigra* and their compartmentalization not been studied at all, probably because they are not economically important species, but we believe that the accumulation and compartmentation of proline in different vegetative organs, differ between these two species, suggesting that they have different mechanisms of salinity tolerance. The importance of this research is the need to identify and characterize new plant genetic resources tolerant to salt stress. The evaluation of proline content in different vegetative organs of *Suaeda nigra* and *Atriplex canescens*, help us to elucidate the adaptation strategy that perform these species, enriching the knowledge we have on plant ecophysiology these days.

## 2. Materials and Methods

### *2.1. Sample collection*

The plant material and soil samples were collected in an area with a high salt content in San Felipe, Dr. Arroyo, Nuevo Leon, Mexico, in the locality 100°17′57″ W 24°06′03″ N at a 1680 masl. The plant samples of the same age were taken for taxonomic identification and herbarium inclusion. *Atriplex canescens* is considered as a woody plant, while *Suaeda nigra* as a succulent plant. The collection was determined within 1 ha in which we took six samples of soil at different depths (0-30 y 30-60 cm). Samples were divided in a structure of leaves, stems and roots (100 g each).

### *2.2. Soil physicochemical analysis*

Three different relations of soil.-water (1:1, 1:5 y 1:10), in which 10 g of soil sample was taken and added 10 ml, 50 ml and 100 ml, respectively. Then the sample was homogenized and the upper phase was filtered and pH determination was taken (Thermo Orion, model 3 Star) and we measured the electrical conductivity by means of a conductivimeter Thermo Orion (model 135A). We also determined soil texture by the hydrometer method, organic material content (Walkley-Black method) and the content (saturated extract method) of phosphorus, calcium, magnesium, carbonates, bicarbonates, sulfates, chlorides and soluble salts (electric conductivity).

### *2.3. Determination of proline*

We worked with plant material such as leaves, stems and roots of *Suaeda nigra* and *Atriplex canescens* by following modification of the technique of Bates et al. (1973), for proline quantification.

#### *2.3.1. Phase 1- plant extract recollection*

The purpose of carrying out the extraction of proline under different methods will reveal the most appropriate method by which this important osmoprotectant can be obtained. We took 9 g of foliar material (with no ribbing and previously grinded for *Atriplex canescens* and without grinding for *Suaeda nigra,* the ones we applied three extraction methods too. The first method (M) consisted in place 3 g from leaf material in a mortar with 30 ml of sulphosalicylic acid and later the sample was macerated. The second method (S) consisted in placing 3 g of foliar material in a glass tube containing 30 ml of sulfosalicylic acid and then we gave it ultrasonic treatment with 3 pulses of 30 seconds with a sonicator (BRANSON, model 150-D). Finally the third method (M+S) consisted in placing method M and S combined. The samples obtained were placed in glass tubes and then were centrifuged 5000 rpm for 5 minutes, finally the supernatant was transferred on a falcon tube and stored at 5°C for 5 minutes.

#### *2.3.2. Phase 2- process of proline extraction*

From the plant extracts we proceed to get the free proline through 2 treatments for each extraction method. The first consisted in a previous dilution (1:2) of plant extract and the second consisted in an after dilution (+1), so we could compare the proline content and have a better evidence of which one was the best methods to extract it.

We took an aliquot of supernatant of 0.6 ml from the sample contained in the falcon tube, for the samples M (1:2), S (1:2) y M+S (1:2) with a previous dilution and then we proceed as described on the method of Bates et al*.,* 1973. Also, we took the organic phase was read by spectrophotometer with a Thermo Fisher Scientific, model Biomate 3 at 518 nm the absorbance was documented. We constructed a calibration curve in which the results were interpolated. The experimental protocol was designed completely randomized with three replicates per treatment. Data were analyzed by ANOVA and Tukey to determine the effects of treatment, with a significance level of 5% using the experimental design software. (Olivares, 1994).

## 3. Results and Discussion

### *3.1. Soil physiochemical analysis*

The results obtained show that the value of pH ranges from 7.71 to 9.08. On the other hand, the conductivity in sample one varies between 1.716 mS $cm^{-1}$ to 39.0 mS $cm^{-1}$ in sample three at 25°C. The results obtained showed that the soil in the region of our study belongs to a clayey soil with a proportion of 3.84% sand, 33.28% silt and clay, which content of organic material is low (0.93%), with an alkaline pH (9.0) and electric conductivity of 27.95 mS $cm^{-1}$, which is classified as extremely salty. Soluble Phosphorous (0.30 mg $L^{-1}$) is considered low, Calcium (374.59 mg $L^{-1}$) is optimum and in soluble Magnesium (42.68 mg $L^{-1}$) is acceptable.

### *3.2. Proline content*

The results obtained showed that there is a wide variability in the content of proline. For *S. nigra* the values vary between 4.621 to 54.662 µmol $mg^{-1}$, independently of the plant tissue used. For this species, most concentration it is accumulated in the leaves. On the other hand, for *A. canescens* the values of proline vary between 0.544 to 7.676 µmol $mg^{-1}$, the highest average value was 3.951 µmol $mg^{-1}$ and was found in the leaves, and the lowest concentration (1.476 µmol $mg^{-1}$) was observed in the stem. The average values of the content of this amino acid for both species, no matter the plant tissue used ranged from 25.614 and 2.457 µmol $mg^{-1}$ for *S. nigra* and *A. canescens*, respectively (Table 1).

About the different methods of proline extraction, the results obtained showed that there is a wide variability; we found for *S. nigra* the values vary between 4.621 to 54.662 µmol $mg^{-1}$ according to the different extraction methods. For this species, the higher concentration of proline was 31.82 µmol $mg^{-1}$ and was got with the macerating method and sonicated with previous dilution [M+S (1:2)], and in average, the least content 20.011 µmol $mg^{-1}$. Now for *A. canescens* the proline content no matter what extraction method was used, it varies between 0.544 to 7.656 µmol $mg^{-1}$.

In this species, the higher concentration of proline was 20.011 µmol $mg^{-1}$ and was observed with the macerate and sonicator method with s subsequent dilution [M+S (+1)]. On the other hand for *A. canescens* the content of proline no matter what extraction method was used, it varied between 0.544 and 7.656 µmol $mg^{-1}$; in this species the higher concentration of proline (3.787 µmol $mg^{-1}$) was seen when the samples were treated with the macerated and sonicator method with previous dilution [M+S (1:2) and with the sonicator method with after dilution was seen the least content on average (1.573 µmol $mg^{-1}$) (Table 2).

The changes in the accumulation of proline in relation to different plant structures (leaves, stems and roots) and the nature and intensity of the stress under natural condition, reflects the capacity of resistance or susceptibility of *Atriplex canescens* and *Suaeda nigra*. The results obtained confirm that the diffusion and accumulation of this amino acid in the leaf, stem and root of both genus, is a function with the concentration of salts in the environment. Di Martino (2006), shows that some species of plants are capable to synthesize compatible organic solutes, like glycinbetaine and proline and the kinetic of accumulation depends on the intensity of the stress and duration of exposure to it.

Table 1: Multiple mean comparison for proline content (µmol $mg^{-1}$) in leaf, stem and root of *Suaeda nigra* and *Atriplex canescens*

| Plant species | Organ | Mean | Standard error | Min. value | Max. value |
|---|---|---|---|---|---|
| *S. nigra* | Leaf | 8.798[a] | ±0.775 | 4.621 | 15.083 |
| | Stem | 31.321[b] | ±2.321 | 14.397 | 47.425 |
| | Root | 36.724[b] | ±1.776 | 25.381 | 54.662 |
| | Total | 25.614 | ±1.933 | 4.621 | 54.662 |
| *A. canescens* | Leaf | 3.951[b] | ±0.434 | 1.494 | 7.676 |
| | Stem | 1.476[a] | ±0.155 | 0.544 | 2.530 |
| | Root | 1.944[a] | ±0.130 | 1.237 | 3.124 |
| | Total | 2.457 | ±0.215 | 0.544 | 7.676 |

Same letters indicate no significant differences

Table 2: Mean multiple comparison for proline content (µmol $mg^{-1}$) in leaf, stem and root of *Suaeda nigra* and *Atriplex canescens* obtained by different extraction methods

| Plant species | Treatment | Mean | Standard error | Min. value | Max. value |
|---|---|---|---|---|---|
| *S. nigra* | M (1:2) | 29.935[a] | ±5.683 | 6.149 | 45.277 |
| | M (+1) | 21.403[a] | ±4.278 | 8.737 | 38.937 |
| | S (1:2) | 31.099[a] | ±5.234 | 8.467 | 54.662 |
| | S (+1) | 20.059[a] | ±3.801 | 5.037 | 31.990 |
| | M+S (1:2) | 31.182[a] | ±4.803 | 9.118 | 47.425 |
| | M+S (+1) | 20.011[a] | ±3.615 | 4.621 | 30.526 |
| | Total | 25.614 | ±1.933 | 4.621 | 54.662 |
| *A. canescens* | M (1:2) | 2.725[ab] | ±0.155 | 2.021 | 3.322 |
| | M (+1) | 1.848[ab] | ±0.130 | 1.494 | 2.484 |
| | S (1:2) | 2.084ab | ±0.288 | 1.032 | 3.463 |
| | S (+1) | 1.573[a] | ±0.323 | 0.544 | 3.236 |
| | M+S (1:2) | 3.787[b] | ±0.780 | 1.880 | 7.676 |
| | M+S (+1) | 2.723[ab] | ±0.776 | 0.881 | 6.086 |
| | Total | 2.457[ab] | ±0.215 | 0.544 | 7.676 |

Now talking about the content of proline, this research shows that under natural conditions the salt stress, *Suaeda nigra* synthesizes and accumulates proline in a higher proportion in leaves stems and roots (25.614 µmol $mg^{-1}$) than *Atriplex canescens* (2.457 µmol $mg^{-1}$). According to Glenn and Brown (1998), the tolerance to the drought deficit and salt stress in *Atriplex canescens* and *Suaeda nigra* its related to a common mechanism of absorption of $Na^+$, that uses directly for osmotic adjustment, however, some authors believe that increasing of the concentration of this component in the stress it's a consequence and not an adaptive response to stress, being for this reason a sign of metabolic alteration (Dix and Pearce, 1981).

## 4. Conclusions

The synthesis and proline accumulation varies significantly between species and analyzed plant structures. In *A. canescens*, the highest proline accumulation was presented in leaves (3.951 µmol $mg^{-1}$) because the accumulation of free proline begins with the roots, but its transportation system transfers it to the stems and compartmentalizes finally in the leaves. Mean while *S. nigra* has in the root (36.724 µmol $mg^{-1}$) where the direction goes from the leaves to the roots. The changes in accumulation of proline in

relation to the different plant structures (leaves, stems and roots), and the nature and intensity of the stress in wild conditions, show the capacity of resistance or susceptibility.

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