The Combined Effects of Salts and Calcium on Growth and Mineral Accumulation of *Tetragonia tetragonioides* – A Salt Removing Species

M.A. NEVES, M.G. MIGUEL, C. MARQUES, T. PANAGOPOULOS, J. BELTRAO
Faculty of Engineering of Natural Resources, University of Algarve
Campus de Gambelas, 8005-139 Faro
PORTUGAL
e-mail: maneves@ualg.pt, mgmiguel@ualg.pt, tpanago@ualg.pt, jbeltrao@ualg.pt

Abstract: - High soil salinity levels have drastic effects on the growth and yield of horticultural crops. Salination may be controlled by environmentally safe and clean techniques as the use of salt removing species. *Tetragonia tetragonioides*, a wild halophytic species has been studied for this purpose. It was studied the effect of high salt concentrations on growth and mineral composition and the influence of high calcium concentration on plant response. Plants were grown in 5 litters in randomized pots and were daily irrigated. The NaCl concentration of the irrigation solution ranged from 0 up to 200 mM. Additionally, the irrigation solution provided plants with two calcium concentrations: 2 and 10 mM of $CaCl_2$. *T. tetragonioides* present high ability for sodium and chloride leaf accumulation. Besides that, the species provide more advantages: 1) high biomass production potential; 2) several harvests during the year (summer and winter); 3) high content of minerals; 4) horticultural importance, as a leaf vegetable crop; 6) easy multiplication (seed propagation) and easy crop management; 7) tolerance to drought and warm conditions; 8) soil erosion control due to its excellent soil covering. The obtained results suggest that plants supplemented with the high calcium level had longer stems than plants in the low calcium level, accumulated greater amounts of calcium at high salinity levels and present similar leaves dry matter.

Key words: - Salination process; Salt tolerance; Salt removal; Calcium; Soil reclamation; Drought.

1 Introduction

Conventional techniques to combat the wastewater salination process can be characterized by four generations: 1) Problem of root zone salination by soil leaching, where contamination can be observed [1]; 2) Use of subsurface trickle irrigation - economy of water, and therefore less additional salts; however the problem of groundwater contamination due to natural rain or artificial leaching can occur [2]; 3) Enhanced fertilization increases tolerance to salinity, although sensitivity increases also [3], and contamination increases by other hazardous chemicals such as nitrate [4]; 4) Use of salt tolerant species - this technique will be very useful to the plants, but it does not solve the problem of soil or groundwater contamination [5, 6].

In some ocasions saline water is used in the Mediterranean area for horticultural production- The most effective way to control the salination process and to maintain the agricultural fields sustainability is to combat the salination problems using environmentally safe and clean techniques. Those ate as follows: 1) Use of salt (ions) removing species [6]; 2) Use of drought tolerant crops species, because less water is applied and, therefore, less salts are infiltrated [7]; 3) reduction of salt application by deficit irrigation [8]; 4) application of minimal levels of water enough to obtain a good visual appearance GVA of the landscape [9].

In order to evaluate the wild plants ability to remove salts from soil, several species from the Mediterranean coastal flora, living in saline environments were studied [10, 11]. An exotic leaf vegetable crop, *T. tetragonioides* living at a wild status in the Mediterranean Basin sand dunes, was studied as a salt removal specie; *T. tetragonioides* was chosen during the project first phase among a set of 6 species (*Beta maritima*, *Portulaca oleraceaa*, *Thymus mastichina*, *Lotus creticus* and *Limoniastrum monopetalum*), as the best salt removing crop.

The main reasons to chose this species were: fast rate growth, high biomass production (if properly managed), easy cropping (as winter or summer crop), and absence of diseases and pests of major importance, as well as good acceptance by local consumers as a leafy vegetable [12]. Moreover, it was demonstrated its capability as, beside other interests, as a high biomass horticultural leaf crop, producing plant dry weight 40,000-50,000 kg ha^{-1}, if the plant population density is around 75,000 plants ha^{-1} [13]. In field conditions, some authors [14] found in Poland average productivity of New Zealand spinach over 70t ha^{-1}. In that experiment, with potted plants, the fresh biomass per plant changed from 700 to 900g.

2 Material and methods

2.1 Effect of salts (1st experiment)

The experiment was conducted from September to November with potted plants. The species used were *T. tetragonioides* submitted to 5 salinity levels: 0, 25, 50, 75, and 100 mM NaCl, respectively S0; S1; S2, S3 and S4. The conductivity of the irrigation solution were of 0.9; 3.6; 6.0; 8.5 and 10,8 dS m^{-1}, respectively. The number of plants per treatment was 7. Six-leaves plants were transplanted to 5 litters randomized pots, in a 2:1 turf and vermiculite medium, in September 2000. They were irrigated, each two days, with saline water from September to November.

Plant growth was estimated by periodic determination of leaf number, stem length and by the plant dry weight at the end of the experiment. Minerals in leaves were analysed by the dry-ash method [15]. The extraction was made by treating the ash with 6 M HCl, except in the case of Cl^-, in which the extraction was carried out with hot water. The amounts of sodium, potassium, calcium, magnesium and ferrous cations were determined by atomic absorption spectrophotometry. Chloride anions were determined in the aqueous extract by titration with silver nitrate according to Piper [16]. Nitrogen was determined by the Kjeldahl method. Phosphorous was determined by colorimetry method according to the vanadate-molybdate method [15]. All mineral analysis wereonly performed in the leaves.

Stem growth and leaf number were measured periodically and the mineral composition determined at the end of the experiment in November The ability of these species to grow on saline conditions was compared by the relative reduction of growth due to the salinity. Nitrogen content was determined by the Kjeldahl method. Chloride ions were determined by titration with silver nitrate.

Statistical analyses were made with an SPSS computer program. Two-way analyses of variance (ANOVA), least significant difference and Duncan's multiple-range tests (P<0.05) for comparisons between treatments over time were conducted.

2.2 Combined effects of Salts and Calcium (2nd experiment)

The salt concentrations used were 0, 50, 100 and 200 mM. For each salt concentration, two calcium concentrations were used: 2 (Ca1) and 10 mM (Ca2) of $CaCl_2$. Statistical analyses were made with an SPSS computer program. The experimental conditions were improved relatively to the mineral nutrient level (increase of 50%) and correction of pH values (near 6). The sowing date was 15 Apr., the planting was on the 12 May, and the treatments were applied from the 20 May to 30 June. Two-way analyses of variance (ANOVA), least significant difference and Duncan's multiple-range tests (P<0.05) for comparisons between treatments over time were conducted.

3 Results

3.1 Effect of salts (1st experiment)

T. tetragonioides produced significant amounts of dry matter, which ranged from 84 to 62 g/plant. The partition of the plant dry matter to plant organs was changed by the effect of the medium salt concentration (fig. 1). There was an increase of the percentage of dry matter of the leaves in saline conditions and a decrease of dry matter allocated to the seeds with the increasing of salinity.

The percentage of dry matter of stems and roots were constant in all treatments. At the end of the experiment, it was observed a low reduction of the plant stems length and of the dry matter production, more pronounced with the increase of the salinity levels (less 27 % of the dry matter at 100 mM NaCl comparatively to the control treatment).

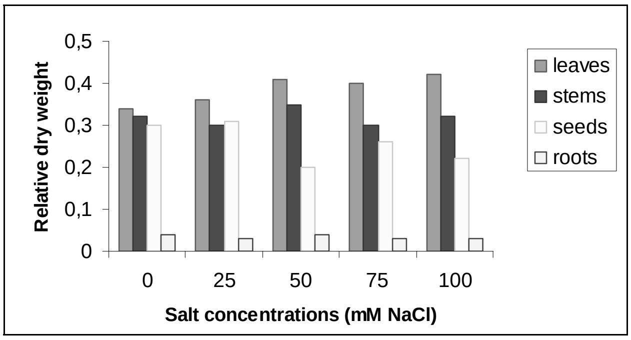

Figure 1 – Partition of dry matter among plant organs at different salinity levels.

T. tetragonioides showed to be relatively tolerant to saline conditions. The stem growth were relatively unaffected by the saline stress, as shown by the little differences among the mean stem length at 3 dates during the experimental period (fig. 2).

The first date was at the beginning of the saline treatment, the second was at the onset of flowering stage, and the third was at onset of fruiting period. However, during the experimental period there was a significant reduction of new leaves production due to salt treatment (fig. 3). This could explain the decrease of plant dry matter at harvest in the most saline treatments relatively to control.

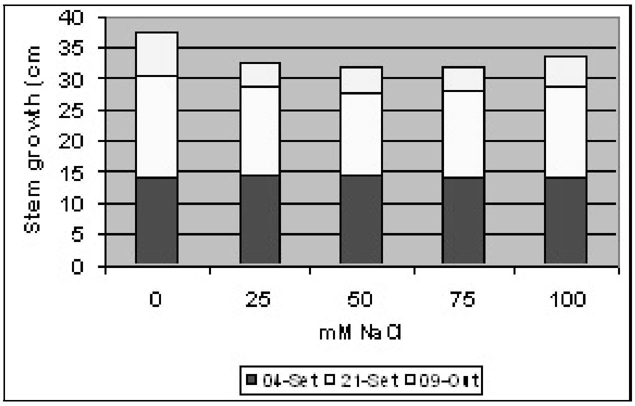

Figure 2 - Mean stem length of *T. tetragonioides* during the experimental period. The plants were submitted to 5 levels of salinity.

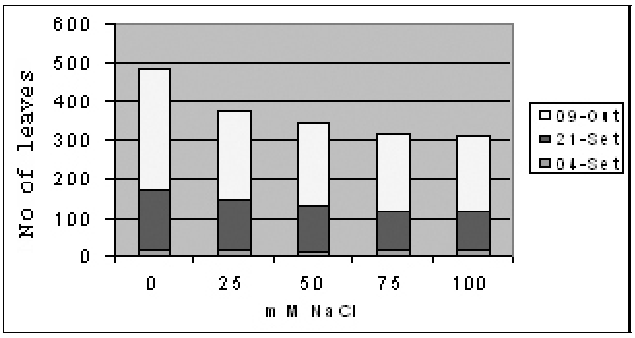

Figure 3 - Mean plant leaf number during the experimental period.

Despite the reduction of biomass production by the salinity, from a horticultural point of view, this could be counterbalanced by the relative increase of the marketable part of the crop (leaves). The reduction of the seeds production by plants in saline conditions showed that the reproductive system was more susceptible than the vegetative system to salinity.

The leaf mineral composition of *T. tetragonioides* (Table 1) shows a high ability to extract salts from soil. The effect of salinity on nitrogen concentration was not affected by the salt concentration. In relation to sodium, potassium and calcium content, there was an increase with the salinity.

3.2 Combined effects of Salts and Calcium (2nd experiment)

There was a clear effect of salinity on growth, as observed by the reduction of the leaf production and the main stem length (Figures 4 and 5, respectively). High calcium level improves the stem growth at high saline conditions (100 e 200 mM NaCl). However, the calcium effect was not apparent in relation with the leaf number that showed, as in the previous experiment, a pronounced reduction with the increase of the salinity level.

The mineral composition was consistent with the previous results. Mineral composition showed that the influence of combined effects of salts and calcium was only relatively strong on the calcium concentration of the plant leaves, increasing its value enhancing the calcium concentration of the nutrient solution (Fig. 6).

On the other hand, the effects of calcium had a very low influence on the mineral concentration of the other studied ions (nitrogen, sodium, chloride, iron, phosphate, chloride and magnesium). The decrease of calcium content from the 50 mM NaCl to the 200 mM NaCl treatment level is in accordance with the expected effect of the Na^+/Ca^+ ratio increase of nutrient solution.

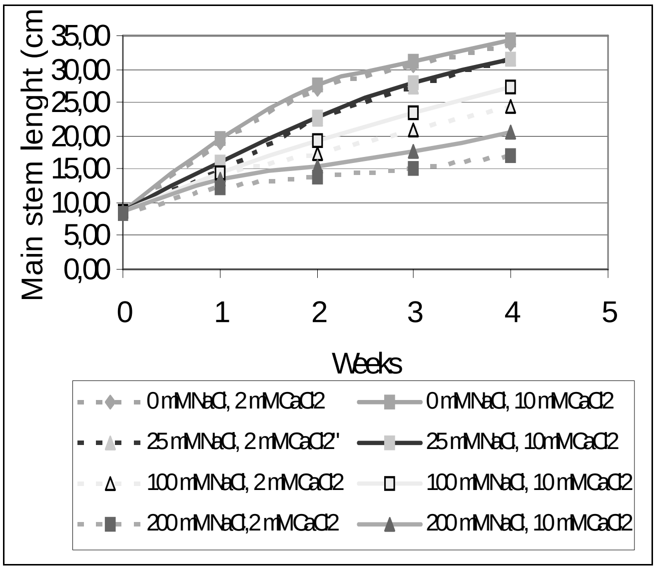

Figure 4 - Plant growth for each salt concentration during a 4-weeks period, according to the $CaCl_2$ concentration (grams of fresh weight per plant).

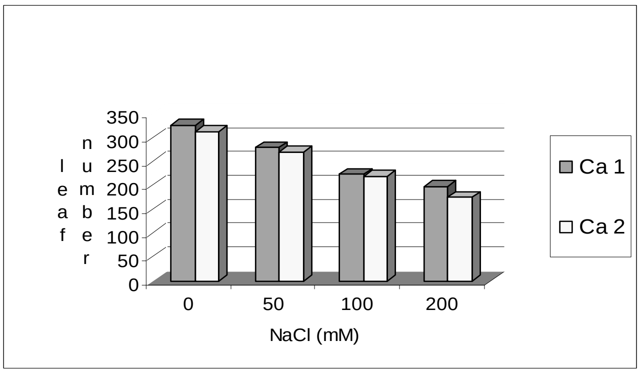

Figure 5 - Mean number of plant leaves at the end of the experiment for the different salt treatments and the two calcium levels (2 and 10 mM $CaCl_2$).

Table 1 – Mineral and nitrogen composition of the *T. tetragonioides* leaves, according to the salt treatment: Values are expressed in mol. kg^{-1} plant dry weight.

Salt	Nitrogen	Sodium	Potassium	Calcium	Iron	Magnesium	Chloride	Phosporous
S0	1.78	1.33	0.76	0.26	$2.28\ 10^{-3}$	0.24	0.08	0.18
S1	1.78	1.49	0.83	0.30	$2.39\ 10^{-3}$	0.24	0.08	0.17
S2	1.77	1.66	0.91	0.35	$2.49\ 10^{-3}$	0.24	0.09	0.15
S3	1.77	1.83	0.98	0.40	$2.59\ 10^{-3}$	0.24	0.10	0.14
S4	1.76	2.00	1.06	0.45	$2.69\ 10^{-3}$	0.23	0.11	0.13

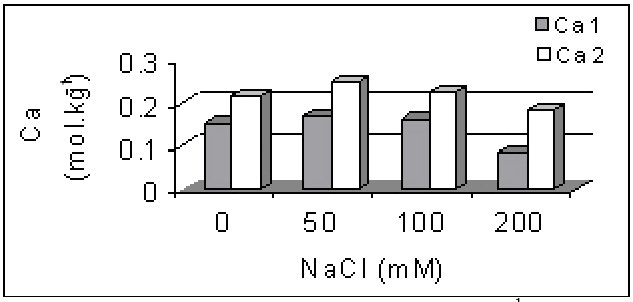

Figure 6 - Calcium leaf concentration (mol. kg^{-1} plant dry weight) at different NaCl levels in the irrigation water and the two calcium levels (2 and 10 mM $CaCl_2$).

4 Conclusions

T. tetragonioides is a valuable salt (ions) removal species. In both experiments, The obtained results suggested that plants supplemented with the high calcium level had longer internodes than plants in the low calcium level, at high salinity levels. It was shown that this new technique to control salinity is a potent and environmental clean tool to maintain the sustainability of the agricultural areas and reclaim soil; however, additional research is needed. Taken into account the values of sodium and chloride content observed in *T. tetragonioides*, it could be extrapolated potential soil removal of these mineral elements by the species on different saline conditions. From the data presented, it could be expected that in very saline soils, that species could extract just to 700 kg ha^{-1} of sodium and 490 kg ha^{-1} of chloride. *T. tetragonioides* partition among plant organs was affected by the medium salt concentration. There was an increase of the percentage of dry matter of the leaves in saline conditions and a decrease in seeds. The percentage of dry matter of stems and roots were constant in all treatments.

The reduction of biomass production by the salinity, from a horticultural point of view, could be counterbalanced by the relative increase of the marketable part of the crop (leaves). The reduction of seed production in saline conditions showed that the reproductive system was more susceptible to salinity than the vegetative system. The effect of salinity on nitrogen composition was affected by the salt concentration. In relation to sodium content, there was a significant increase with salinity. The potassium concentration was higher in the control than in the saline treatments. The excellent growth and development of *T. tetragonioides* in saline conditions in comparison with other species suggest to study that species more deeply and to provide a complete characterisation of their agronomic behaviour. New experiments carried out with increasing the level of salinity and studying the effect calcium medium concentration on production, mineral composition and nutritional factors.

As concluding remarks, it can be suggested that that *T. tetragonioides* is an important salt removing species because:

- The higher biomass production potential: apart the growth rate, this species can produce several yields during the year (summer and winter).
- High content of minerals.
- Horticultural importance as a leafy vegetable for human consumption.
- Easy multiplication (seed propagation) and easy crop management.
- Species tolerant to drought and hot conditions.
- Absence of diseases and pests of major importance.
- Protection from soil erosion due excellent soil covering.

Acknowledgements

This research is part of a project funded by the European Union for the project “Control of salination and combating desertification effects in the Mediterranean region. Phase II”. Program INCO-DC project nº18-CT98-0266.

References

[1] Ben Mechlia, N, Salinity control in the absence of a drainage system under semi-arid conditions, *Acta Horticulturae*, Vol.573, 2002, pp. 175-180.

[2] Oron, G., Beltrão, J., Complete environmental effluent disposal and reuse by drip irrigation. Fragoso, M.A.C.; van Beusichem, M.L. (eds.). Optimization of Plant Nutrition. *Developments in Plant and Soil Sciences,* Vol.53, 1993, pp. 153-156.

[3] Silberbush, M, Ben Asher, J., Simulation of nutrient uptake by plants from hydroponics as affected by salinity build up and transpiration, *Acta Horiculturae,* Vol.573, 2002, pp.97-106.

[4] Beltrão, J**.**, Ben Asher, J., The effect of salinity on corn yield using CERES-Maize model, *Irrigation and Drainage Systems,* Vol.11, 1997, pp.15-28.

[5] Hamdy, A., Sustainable use and management of non-conventional water resources in the arid regions, *Acta Horiculturae,* Vol.573, 2002, pp. 159-174.

[6] Cuartero, J., Pulido, J. M., Gómez-Guillaumón, M. L., Alvarez, M., Salt removal potential of barley, alfalfa, *Atriplex patula* and *A. prostate, Acta Horiculturae,* Vol.573, 2002, pp. 387-396.

[7] Glenn, E.P, Brown, J.J., Blumwald, E., Salt tolerance and crop potential of halophytes, *Critical Reviews in Plant Sciences,* Vol.18, 1999, pp. 227-255.

[8] Nasr, Z., Ben Mechlia, N., Deficit irrigation to reduce salinization in an apple orchard, *Acta Horticulturae,* Vol.573, 2002, pp. 283-287.

[9] Beltrão, J., Gamito, P., Guerrero, C., Arsénio, A., Brito, J., Grass response to municipal wastewater as compared to nitrogen and water application, In: D. Anaç & P. Martin-Prével (Eds.). *Improved Crop quality by Nutrient management,* Kluwer Academic Publishers. 1999, pp. 263-266.

[10] Shaaban, M. M., El-Fouly, M.M., Nutrient contents and salt removal potential of some wiold plants grown in salt affected soils, *Acta Horiculturae,* Vol.573, 2002, pp. 377-385.

[11] Panagopoulos, T., Jesus, J., Antunes, M.D.C. and Beltrao, J. Analysis of spatial interpolation for optimising management of a salinized field cultivated with lettuce. *European Journal of Agronomy* Vol. 24, 2006, pp. 1-10.

[12] Neves, M.A., Miguel M.G., Panagopoulos, T., Beltrao, J. Salt removing species - an environmetally safe and clean technique to control salinity. 6th International Conference of European Water Resources Association. 7-10 September 2005. Palais de l'Europe. Menton, France, 2005.

[13] Beltrão, J., Jesus, S.B., Trindade, D., Miguel, M.G., Neves, M.A., Panagopoulos, T., Ben Asher, J., Combined effects of salts and nitrogen on the yield function of Lettuce, *Acta Horticulturae,* Vol.573, 2002, pp. 363-368.

[14] Jaworska, G., Kmiecik, W., Content of selected mineral compounds, nitrates III and V, and oxalates in spinach (Spinacia oleracea L.) and New Zealand spinach (Tetragonia expansa Murr.) from spring and autumn growing seasons, *Food Science and Technology*, Vol.2, 1999.

[15] Chapman, H.D., Pratt, P.F. *Methods of analysis for soils, plants and waters.* University of California, Division of Agriculture Science, Riverside. 1961.

[16] Piper, C.S. *Soils and Plant analysis*. Inter-Science Publishers Inc., New York, 1945.