Effect of salt stress on accumulation of proline and soluble sugars in cladodes and roots of two *Opuntia* species existing in Algerian steppe

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**Abstract**

The accumulation of soluble salts in the soil diminishes significantly the value of the productivity of cultivated or not cultivated species. The introduction of plants tolerant to salinity is one of the techniques used for the valuation of marginal soils. Faced with this problem, the *Opuntia* has a great capacity to adapt to various weather conditions. The objective of the present study was the influence of sodium chloride (NaCl) at different levels (control, 200, 400, 600 meq.l⁻¹) on synthesis of proline and soluble sugars in two species of *Opuntia*. The results show the variability of the proline accumulation and soluble sugars according to the organ, species and dose of salts. The accumulations of these compounds are much more in cladodes compared to the roots. In *O. engelmanii* var. *longuiformis* the content of proline will increase from 0.46 µg/100g FW in the control plants to 0.89 and 0.93 µg/100g FW in the stressed plants at 400 and 600 meq.l⁻¹ of NaCl respectively in young cladode. At *O. streptacantha* Lem. the highest soluble sugars content in the aged cladode (98 µg/100g FW) was recorded with the stress at 600 meq.l⁻¹. This suggests that, both *Opuntia* species are able to accumulate these biochemical compounds, order to ensure the osmotic adjustment under stress conditions at varying proportions.

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Introduction

In arid and semi-arid areas, soil salinization is a major abiotic factor that reduced agricultural yields of many cultures (Zid and Grignon, 1991; Baatour et al., 2004). The accumulation of soluble salts in the soil diminishes significantly the value of the productivity of cultivated or not cultivated lands (Pessarakli, 1999). The FAO (1996) estimates that from 237 millions of hectares of irrigated lands, around of 30 millions are gravely affected for the salt; other 80 millions of hectares have been affected in some grade and around 1.5 millions of hectares lost annually because of watering due to the flooding or the salinity. During the past 45 years, much attention has been given to the effect of NaCl on plant growth and production; however, the fundamental mechanisms determining the responses of plants to salinity are still not understood (Amzallag et al., 1990). In Algeria, saline soils cover an area of 3.2 million ha (Hamdy, 1999). According to Le Houérou (2000), Algeria is among the most affected countries by salinization.

The introduction of plants tolerant to salinity is one of the techniques used for the valuation of marginal soils. Faced with this problem, the Opuntia has great capacity to adapt to a variety of climatic conditions, and has been cultivated in the arid and semi-arid climates of many countries (Pimienta, 1994). Tolerance of cacti to salinity has been studied by (Berry and Nobel, 1985), (McCree and Richardson, 1987), (Silverman et al., 1988), (Gersani et al., 1993), (Le Houérou, 1996), (Nobel, 1998), (Le Houérou, 2002), (Murillo-Amador et al., 2001), (Conyet al., 2006), (Ochoa-Alfaroet al., 2008), (Nieto–Garibayet al., 2011). Among other Opuntia quinimilo from Argentina is reported to be more tolerant to NaCl than other Opuntia species. Another cactus with high tolerance to salinity is Cereus validus (Nobel, 1998).

In this study, our objective is to study the biochemical responses of two Opuntia species in relation to salt stress. Order to valorization these species in the revegetation of degraded areas in arid and semi-arid. In this context these species could be a source of germplasm of great agricultural and ecological interests.

Materials and methods

Plant material and culture conditions

The plant material used in this study consists of cladodes and roots of two Opuntia species: Opuntia engelmanii var. longuiformis and Opuntia streptacantha Lem. grown in the region of M’sila (Algeria).

The cladodes are planted in 2012, in the form of completely randomized design with twenty replications, the cladodes are planted in pots 2 liter volume, filled with sand and exposed to natural growing conditions. The cladodes are watered weekly by distilled water. Salt stress is applied after the second year of culture. Sodium chloride (NaCl) was chosen as a stress agent. Four concentrations of (NaCl) were used: 0 (control), 200, 400 and 600 meq.l⁻¹.

Studied traits

The studied traits included; proline and soluble sugars. After 60 days of application to the salt stress, we performed levies from plant material (young cladodes, aged cladodes and roots) (Fig. 1). 20 samples per species per concentration are intended for the analysis.

Methods of measuring traits

Proline is extracted and assayed according to the method of Troll and Lindsley (1954), streamlined and developed by Drier and Goring (1974). Soluble sugars were extracted and assayed according to the method of Schields and Burnett (1960). The contents are expressed by µg/100g Fresh Weight (FW) for proline and soluble sugars.

Statistical analysis

The results obtained were analyzed using the statistical software StatBox Pro. V. 6.4 and the mean of data were compared by Newman-Keuls test at 5 %.

Results

Effect of NaCl on accumulation of Proline
Fig. 2 indicates that proline content increases in the different plant organs according to the increasing salinity. Note also that, young cladodes of *Opuntia engelmanii var. longuiformis* are rich to proline compared to other organs, both in the control plants than those treated with different concentrations of salts. The content of proline will increase from 0.46 µg/100g FW in the control plants to 0.89 and 0.93 µg/100g FW in the stressed plants at 400 and 600 meq.l⁻¹ of NaCl, respectively.

**Table 1.** Effect of salt stress (NaCl) on proline content (µg/100g FW) in different organs of *O. engelmanii var. longuiformis* and *O. streptacantha* Lem. Each value represents an average of 20 samples ± SD. Values bearing the same letter in each column are not significantly different at p <0.05.

<table>
<thead>
<tr>
<th></th>
<th><em>O. engelmanii var. longuiformis</em></th>
<th><em>O. streptacantha</em> Lem.</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Aged cladode</td>
<td>Young cladode</td>
</tr>
<tr>
<td>Control</td>
<td>0.40±0.007ᵇ</td>
<td>0.46±0.006ᵇ</td>
</tr>
<tr>
<td>200 meq</td>
<td>0.75±0.004ᵇ</td>
<td>0.84±0.003ᵇ</td>
</tr>
<tr>
<td>400 meq</td>
<td>0.78±0.003ᵃ</td>
<td>0.89±0.004ᵇ</td>
</tr>
<tr>
<td>600 meq</td>
<td>0.79±0.004ᵃ</td>
<td>0.93±0.003ᵇ</td>
</tr>
<tr>
<td>Probability</td>
<td>0.0001</td>
<td>0.00008</td>
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</tbody>
</table>

We also observe that the accumulation of proline in the roots is about double and three times higher in the treated plants to 400 and 600 meq.l⁻¹ of salt (0.46 and 0.67 µg/100g FW, respectively), compared to the control plants, against to 0.23 µg/100g FW. In aged cladodes, the highest proline content is recorded in the plants treated with 600 meq.l⁻¹ (0.79 µg/100g FW). Furthermore, the quantity of proline accumulated is increased in the direction of roots, aged cladodes and young cladodes. The content of proline accumulated is increased in the control plants toward stressed plants with salts (NaCl) (table 1).

**Table 2.** Effect of salt stress (NaCl) on soluble sugars content (µg/100g FW) in different organs of *O. engelmanii var. longuiformis* and *O. streptacantha* Lem. Each value represents an average of 20 samples ± SD. Values bearing the same letter in each column are not significantly different at p <0.05.

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td></td>
<td>Aged cladode</td>
<td>Young cladode</td>
</tr>
<tr>
<td>Control</td>
<td>54±0.28ᵃ</td>
<td>89±0.35ᵇ</td>
</tr>
<tr>
<td>200 meq</td>
<td>73±0.49ᵇ</td>
<td>92±0.35ᵇ</td>
</tr>
<tr>
<td>400 meq</td>
<td>77±0.42ᵇ</td>
<td>95±0.49ᵇ</td>
</tr>
<tr>
<td>600 meq</td>
<td>80±0.56ᵇ</td>
<td>99±0.49ᵇ</td>
</tr>
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In *Opuntia streptacantha* Lem. the proline content is changing in all organs with increasing salt concentration, compared to control plants (Fig. 2). Proline content fluctuates within the organ and the salt treatment. In roots the accumulation is low with values: 0.13 µg/100g FW, 0.34 µg/100g FW, 0.45 µg/100g FW and 0.44 µg/100g FW within control plant, 200, 400 and 600 meq.l⁻¹ respectively. The quantity of proline accumulated is increased in the direction of roots, young cladodes and aged cladodes (table 1). Statistical analysis (Table 1) indicates that the root system is significantly less rich in proline,
Effect of NaCl on the accumulation of soluble sugars

In *O. engelmaniivar. longuiformis*, the results analysis indicates that the amounts of soluble sugars are variable, in root, aged cladode and young cladode (fig. 3).

Indeed, we note that the carbohydrate compounds are more concentrated in the cladodes than in the roots, as well in the control plants than those treated with different concentrations of salts. This accumulation is higher in young cladodes especially when plants are stressed at 600 and 400 meq.l⁻¹ with values 99 µg/100g FW and 95 µg/100g FW respectively. The increase in salinity level, there was an increase; in soluble sugars content was noticed. A 35.18%, 42.59% and 48.14% increase in the stressed outplants with 200, 400 and 600 meq.l⁻¹ respectively, compared to the control plants. Whereas in roots, soluble sugars amount is about 63 µg/100g FW with stressed outplants by 600 meq.l⁻¹ (table 2).

At *O. streptacantha* Lem. the quantity of soluble sugars accumulated is increased in the direction of roots, young cladodes and aged cladodes. The highest soluble sugars content in the aged cladode (98 µg/100g FW) was recorded with the stress at 600 meq.l⁻¹. Besides, the amount of soluble sugars in roots is lowest (69 µg/100g FW) at plants treated with 600 meq.l⁻¹, in comparison with the controls (17 µg/100g FW) (fig. 3, table 2). When *O. streptacantha* Lem. was treated with NaCl with different concentration there was significant difference was noticed for soluble sugars content in roots, young cladodes and aged cladodes.

**Discussion**

The biochemical response, analyzed through the expression of the accumulation of proline and soluble sugars of both species of *Opuntia*: *O. engelmaniivar. longuiformis* and *O. streptacantha* Lem. in salt stress conditions with (NaCl) 200, 400 and 600 meq.l⁻¹, show that the plants accumulate these compounds in different organs. This accumulation varied from one organ to another, from one species to another, depending on the intensity of concentration salt stress.

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**Fig. 1.** Different organs of Opuntia plant (YC: Young cladode; AC: Aged cladode; R: Root): (A) *O. engelmaniivar. longuiformis*; (B) *O. streptacantha* Lem. intended for the analysis.

**Fig. 2.** Proline content in different organs of Opuntia plants (*O. engelmaniivar. longuiformis* and *O. streptacantha* Lem.) stressed by NaCl for two months.
In *O. engelmannii var. longuiformis*, the accumulation of proline is in the direction root, cladodes, as well in control plants toward stressed plants with different concentrations. In the cladodes, the proline is preferentially concentrated in young cladodes at significantly higher levels when the salinity of NaCl increases. These results are in conformity with the study of Hadjadj et al. (2011) on Atriplex. Indeed, in the *O. engelmannii var. longuiformis* stressed by NaCl (200, 400 and 600 meq.l^{-1}) proline content increasing much more in young cladodes comparatively to aged cladodes. Ould El Hadj-Khelil (2001) noticed that in tomato plants under stress of 200 mM NaCl, the accumulation of proline is greater in young leaves than in the basal ones, and application of a second salt treatment generates a further increase in the amino acid. This increase is more important when leaf tissues are young and salinity is high. As against Martinez et al. (2005) showed that exposure of plants *Atriplex halimus* L. at 50 mM of NaCl induced accumulation of proline in aged leaves, relatively of the young leaves.

![Fig. 3. Soluble sugars content in different organs of Opuntia plants (O. engelmannii var. Longuiformis and O. streptacantha Lem.) stressed by NaCl for two months.](image)

For *O. streptacantha* Lem. in aged cladode the quantity of proline accumulated is increased with 0.70 µg/100g FW at 400 meq.l^{-1}. In roots the accumulation is high with 0.45 µg/100g FW at 400 meq.l^{-1} comparatively to control plant with 0.13 µg/100g FW. These results are in accordance with the work of (De-la-cerda et al., 2001) have found that exposure of two sorghum genotypes (*Sorghum bicolor* L. Moench) one sensitive and the other tolerant to 100 mM of NaCl, causes an increase in proline in all parts of the plants two genotypes and more particularly in the aged leaves: third and fourth leaf from the apex.

The accumulation of proline in young cladodes may result from the strong accumulation of salts in the latter. Ould el-Hajj khelil (2001) reports that older basal leaves are involved in the sequestration of excess Na^+, this is done to benefit young leaves growing, which appear to be protected while Na^+ in excess. Huber (1974) showed that the salt inhibits the catabolism of the amino acid at the proline dehydrogenase, an enzyme involved in the degradation of proline. It could also correspond to the stimulation of the synthesis. Indeed, (Hu et al., 1992) assert that in *Vigna aconitifolia*, agenecoding for synthetase pyrroline-5-carboxylate, is highly expressed in leaves and roots of plants treated with 200 mM of NaCl.

The accumulation of proline would be caused by the increased synthesis from glutamate and ornithine (Hare and Cress, 1997). Under stress conditions, the amino acid metabolism is greatly altered and protein synthesis increases as a result of these metabolic conditions, the synthesis of proline can be promoted by an increase in concentrations of metabolites accompanied by a production of precursors proline.
and may be the main cause of the accumulation of proline in the tissues of plants exposed to stress conditions (Silveira et al., 2001).

Also, our results show that the amount of accumulated proline in both species of Opuntia is fairly high, which suggests that this osmolyte is among the most effective biochemical parameters for this plant's resistance to extreme environmental conditions and expressing markedly in the cladodes. It would migrate from the various organs to these cladodes for purposes of protection and increased photosynthetic efficiency because some studies have reported that proline is synthesized in the leaves and transported to the resistance sites of aggressions (Vezina and Paquin, 1984; LaLiberte and Paquin, 1984) others report that the proline migrates to the leaves for stressed plants; such is the case for sorghum (Weimberger et al., 1984), eggplant (Joshi, 1984), cotton (Boutelier, 1986), vines (Imamul Huq and Larher, 1984), beans (Belkhodja, 1996; Belkhodja and Benkablia, 2000). Bellinger et al. (1989) reported that this accumulation is not a sensibility indicator of plant to stress, but an indicator of acquisition to tolerance for stresses.

Concerning soluble sugars, according to the results on accumulation of soluble sugars in the root and cladodes of O. engelmanii var. longuiformis and O. streptacantha Lem. it appears that these carbohydrate compounds have also increased depending to the increase in salt concentration for both species. The accumulation of soluble sugars occurs significantly in cladode than in roots under normal conditions or under salt stress in Opuntia. Belfakih et al. (2013), showed an increase in the content of soluble sugars in a banana variety (dwarf) in a salt stress situation. Ben Khaledet et al. (2003) reported that exposure of plantlets to clover (Alexandria) at 2, 4, 6 and 8 gl⁻¹of NaCl, induced an accumulation of soluble sugars in the leaves. This increase was 37% to 4 gl⁻¹ and 57% to 8 gl⁻¹of NaCl. These results reflect firstly, the variability of biochemical metabolism of both Opuntia species under salt stress, and secondly, express their ability to synthesize and accumulate the soluble sugars in different organs. Sugars play an important role in osmotic adjustment, well as at of stabilization of some proteins. The accumulation of sugars seems to induce gelation of the cell contents by saturating the intracellular environment, this phenomenon allows avoiding the crystallization of contained molecules in cell, therefore limit the damage of cellular structures (Dubos, 2001). There seems to be some proportionality between the amounts of accumulated proline and those of soluble sugars. Therefore, the increase content of proline, under the effect of saline treatment, is proportional to the increase in quantity of soluble carbohydrates. These results explain the existence of a connection between the biosynthesis pathway of proline and the accumulation of soluble sugars; in general, they could provide the carbon precursor and the chemical energy required for the synthesis of proline. The α-ketoglutarate, intermediary respiratory tract during glucose oxidation, play a particularly important role such as acceptor amino group of glutamine, for glutamate conversion. This one; the main precursor of proline during a stress situation (Hopkins, 2003).

Conclusion
Accumulation of proline and soluble sugars in both species of Opuntia would be involved in osmotic adjustment mechanisms and would also serve such as osmoprotecteurs. These biochemical elements can be considered as « Biochemical markers » of degree of tolerance to salt stress and consequently can be used to early selection of salt tolerant species. However, quantitative variation depends upon the species, the organ and level of stress provoked. This variation is probably related to the role of this amino acid at the cellular level and its involvement in osmotic adjustment. According to our findings, the two Opuntia species are able to grow in the presence of different levels salinity ranging from 200 to 600 meq.l⁻¹ NaCl.

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