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EFFECTS OF SOIL PROPRIETIES ON WATER AND MINERAL NUTRITION OF *BETA MACROCARPA* GUSS.

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Abstract

During their development cycle, plants are subjected to variations of climatic conditions and soil proprieties which affect their growth. Scattered information is available on the mineral status of the halophytes habitat. *B. macrocarpa,* an annual herbaceous plant, was selected among a large number of other species, in coastal and marginal lands in Tunisia. We aimed in this study to focus on soil proprieties and plants mineral status during principal periods of the *B. macrocarpa* growth cycle, in order to understand the relationship between changes in soil fertility on plant growth and mineral acquisition. Results showed that soil fertility influences plant distribution between bared and vegetated soil. Furthermore, salinity increased notably in bared soil. This provides information concerning the role of the microhabitats, formed by halophytes tufts, in keeping adequate soil moisture. Nevertheless, mineral acquisition by plants was similar in both plant types, then this species is able to maintain adequate growth even in relatively poor and desiccated soil. Sodium and chloride were the most abundant ions in plants tissues; this reflects their utilisation in osmotic adjustment. Results herein suggested that the litter formed by halophyte fallen organs and by organic debris at the feet of tufts contributed to localized soil enrichment in N and P.

Introduction

Millions hectares of arable land are too saline for agriculture, and hundreds of thousands hectares of agriculturally productive land are lost annually for food production due to salinization (Anon., 2008). The fact that such soils cannot sustain food crops does not exclude vegetation with halophytic plants, which are well-adapted to the salt and water stress which prevent the growth of most crops (Doddema *et al.,* 1986). Although they represent only 2% of terrestrial plant species, halophytes are present in about the half of the higher plant families and represent a wide diversity of plant forms. Despite their polyphyletic origins, halophytes appear to have evolved the same basic method of osmotic adjustment: accumulation of inorganic salts, mainly NaCl, in the vacuole and accumulation of organic solutes in the cytoplasm. Because of their diversity, halophytes have been regarded as a rich source of potential new crops.

Nutritional status of halophytes is influenced both by substrate characteristics and by plant ion physiology. Halophytes have evolved ion physiology characteristics that allow them to cope with the multiple challenges of saline substrates: toxic ion effects, nutrient limitations, and osmotically reduced water availability (Munns & Tester, 2008).

During their cycle of development, plants are subjected to variations of climatic and edaphic conditions which affect their growth. As the mainstream research was performed mostly in experimental model systems, the possible advantages provided through some traits of the plant's natural habitat were perforce left out and by the same token, the concept that a plant and its habitat form a distinct interactive unit did not attract much investigational interest.

In Tunisia, although scattered information is available on the mineral composition of the habitat of halophytes, few investigators have studied the correlation between mineral changes in the habitat and corresponding effects on ion composition of plant tissues (Sanaâ, 1997; Abdelly, 1997; Serpa *et al.,* 2007).

Beta macrocarpa Guss was selected from large number of other species grown in coastal and marginal lands of Tunisia because of its short growth cycle and its adequate feed biomass production "appreciated by animals and hight nutritional quality" (Abdelly, 1997). Besides, laboratory study showed that this species seems to be promising with adequate salt tolerance (Hessini 2002). However, the study of this species in its own habitat is missing. The purpose of this study is two fold first is to know the nature of this halophyte, sacredly know the differences in soil and plants inside and outside tufts of halophytes where this plant is dominant and to look after physiological and biochemical changes in soil and mineral ion composition of underground and above-ground parts of *B. macrocarpa* through its growing cycle, in order to provide insight into the characteristics that allow this species to cope with coastal and marginal ecosystems.

Materials and Methods

Description of study area and collection procedure: *Beta macrocarpa Guss* was collected from an area in the edge of a saline depression, the sebkha of Soliman in North Tunisia, with the geographical coordinates: 36° 41' 47" N, 10° 29' 30" E (Fig. 1). The mean annual rainfall of this upper semi-arid region is between 350 and 450 mm (Table 1).

The samples selections of soils and plants are carried out from the Sebkha of Soliman (Fig. 1), at three periods of the year during March, September and October, 2002. These dates correspond to the principal periods of development of *B. macrocarpa*. At the beginning of September, the soil being dry, the seeds of *B. macrocarpa* were collected. In October, after the first autumnal rains (Table 1), the annual plants start to develop and in March it is the optimal growth and the beginning of the flowering period. The taking away are carried out inside (in tufts) and outside (naked soil) halophytes tufts (out tufts).

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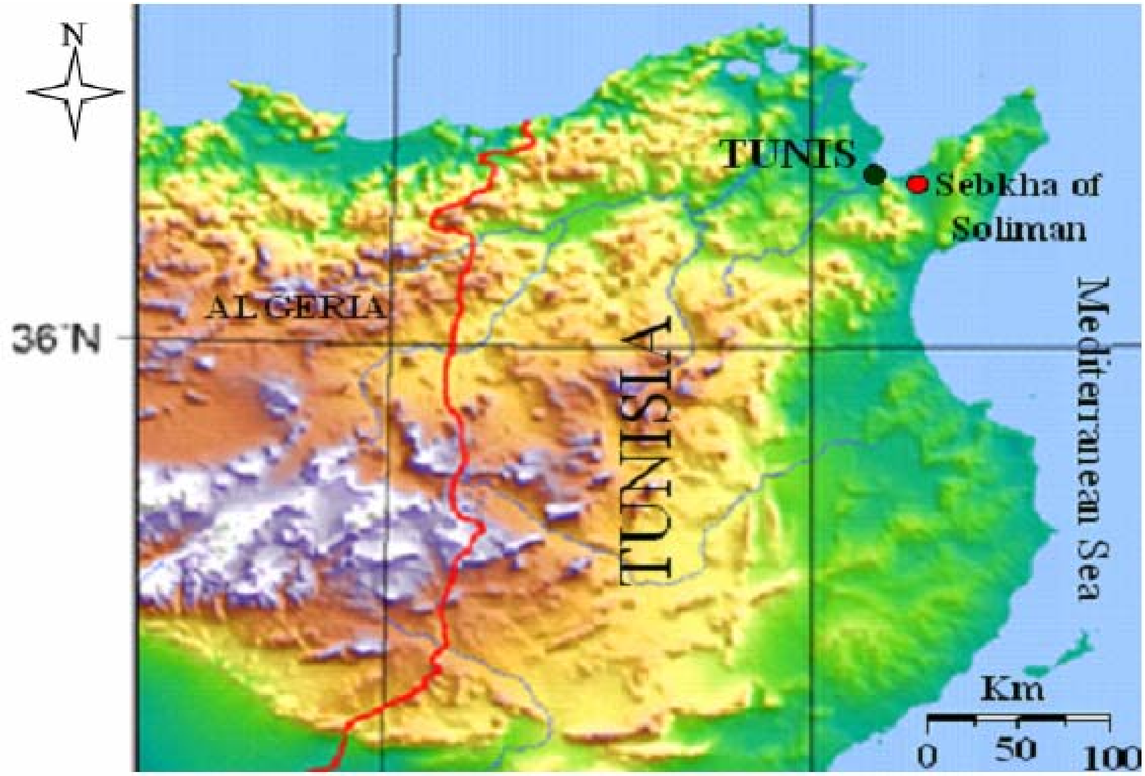

Fig. 1. Location map showing the site (Sebkha of Soliman) from which the concerned plant specie and the soil samples were collected.

Table 1. **Temperatures (°C) and precipitation (mm) in Soliman during 2002.**

Month	Temperature	Precipitation
January	15.8	59
February	16.4	34
March	17.1	38
April	19.4	27
May	22.4	16
June	26.8	08
July	30.4	05
August	30.6	10
September	28.7	29
October	24.9	48
November	20.4	45
December	17	50

B. macrocarpa: an annual herbaceous plant, Chenopodiaceae, Cyclolobeae. It is a halophyte according to the definition of Flowers *et al.,* (1977) i.e., a plant that can survive and complete its life cycle on high salinity substrates, spread in marginal and saline coastal lands, it is a succulent-leafed plant, salt-tolerant (Hessini, 2002).

Soil samples and plants were collected from six sites with 10 samples of soil and plants from each site. Sites were distant from each other by approximately 100m going from the periphery towards the centre of the saline depression. Surface soil (0-20 cm) samples and plants were collected from inside and outside the tufts of halophytes in March, September and October.

This study describes the principal physico-chemical characters of the soil and the ionic composition of *B. macrocarpa* tissues in its natural habitat. The variation of the environmental conditions (temperature, pluviometry, etc.) during the cycle of the plant has an effect not only on the plant ionic composition but also on physico-chemical properties of the soil.

Drying, crushing, and extraction: Plant and soil samples were dried in an oven at 60°C for 72 hour. Fresh and dry weights were measured. Plants were separated into roots and leaves. Dry organs were reduced to fine powder, and used for cations and anions determination.

To extract minerals from soil, 2.5 g of fine ground soil was put in a centrifuge tube and 2.5 ml of distilled water was added. After 30 min., of agitation, and centrifuged for15 min., (3000-3500 rpm). This operation was repeated twice and each time the supernatant recuperated and leveled with distilled water to 25 ml, the extract was kept at 4°C for different analyses.

Nitrogen and cations assays was performed. Reduced nitrogen content for plants powder and soil fraction, was determined using Kjeldahl Method (Bouat & Crouzet, 1965), nitrate according to Jackson (1958) method and ammonium according to Pauwells *et al.,* (1992) methods.

Cations (K, Na, Mg, and Ca) were extracted by addition of 50 ml nitric acid (0.1 N) to 25 mg of powder and assayed by flame emission photometry (flame spectrophotometer IL 151) on (0.1N) nitric acid extract. Chloride concentration was determined on the same extract by coulombic titration against Ag^+ (Digital chloridometer; Haake-Buchler, KS, USA).

Statistical analysis: Statistical analysis was carried out with the MSUSTAT software, Statistical differences between sites and plants were analyzed by one way analysis of variance (ANOVA), and comparisons of means were affected by the LSD test to determine significant differences between sites and plants.

Results

Soil study: Relative humidity (RH) of in tufts soil was generally higher in October after the first autumn rain (Table 1) and in March but dropped sharply in September, period of seeds sampling. In out tufts soil, RH was generally lower as compared with in tufts soil (Table 2). Soil pH was generally constant in September and March, while it decreased slightly in October. Table 2 shows decreased pH values in out-tufts soil in all sampling dates.

Electric conductivity reached its maximal value in September (seeds maturation period). Furthermore, it appeared to be higher in out-tufts soil (Table 2). Sodium concentration, increased in the top layer of soil in September mainly in out-tufts soil (Table 3) after the high evaporation rate within summer period: June-August (Table 1).

Chloride concentrations were positively correlated with those of Na^+ (Table 3). Inside tufts Na^+ had almost the same amount: about 3 meq per 100g DS. In bare soil, except for March, sodium was accumulated about twice of chloride (Table 3).

Concerning inorganic ions, generally, potassium was accumulated in tufts soil, reaching its maximum content in September and did not change significantly in October (Table 3). Outside tufts K^+ increased gradually from March to October, but it remained always lower than in bare soil.

Phosphorus was generally more accumulated in in-tufts soil and reached its maximum value in September. In out-tufts soil values did not change markedly, however, they remained lower than those of vegetated soil (Table 3).

The highest values of sulphate ions were detected during March in-tufts, in September and especially October sulphate became higher in out-tufts. However, these contents increased from October to March in both soils (Table 3).

Calcium was accumulated more in the naked soil and rose a little during September. Inside tufts (Fig. 2), Ca^{2+} amount was lower. Concerning magnesium, contents decreased from October to March on naked and covered soils (Fig. 3).

Table 2. Variation of relative humidity (RH %), electric conductivity (EC: $mS.m^{-1}$) and pH of in-tufts (IT) and out-tufts (OT) soil in different sampling sites of the Sebkha of Soliman. Values are means of 10 repetitions from six different sites.

	Soil of the Sebkha of sokiman		
	Month	**IT**	**OT**
RH	September	3.9	2.6
	October	17.1	12.5
	March	6.7	6.4
EC	September	0.61	0.62
	October	0.41	0.45
	March	0.5	0.61
pH	September	8.3	7.7
	October	7.3	6.3
	March	8.3	8.2

Table 3. Variation of chemical composition of soils, in-tufts and out-tufts, during the sampling periods.

Month	Soil of the sebkha of soliman													
	Na^+ (meq. $100g^{-1}$ DS)		Cl^- (meq.$100g^{-1}$ DS)		K^+ (meq. $100g^{-1}$ DS)		PO_4^{3-} (meq. $100g^{-1}$ DS)		SO_4^{2-} (µg. g^{-1} DS)		Mg^{2+} (meq. 100g $^{-1}$ DS)		Ca^{2+} (meq. $100g^{-1}$ DS)	
	IT	OT	IT	OT	IT	OT	IT	OT	IT	OT	IT	OT	IT	OT
September	3.1	8.2	2.1	4.1	1.6	0.7	5.5	3.0	26	27	0.25	0.22	3.2	3.8
October	2.9	6.3	1.9	3.9	1.5	0.8	4.6	2.9	18	25	0.3	0.3	3.1	3.5
March	3.1	3.2	3	3.2	0.2	0.3	4.2	3.9	31	28	0.2	0.2	2.8	3.1

Regarding nitrogen fractions, independently of sampling date, organic nitrogen amount in the soil inside and outside tufts did not exceed 1.6 mg. g^{-1} of dry soil. Inside the tufts, nitrogen decreased from September to March. However, in October, a slight decrease was observed due to its absorption by plants which had already begun their growth (Table 4). In bare soil, N was rather regular during all the cycle. The amount on nitrate did not change significantly inside tufts, but, a considerable increase was noted in this nutrient during September outside tufts. Results showed its accumulation was in outside tufts especially in periods of seeds maturity (September) and in the beginning of vegetative growth of plants (October). Ammonium content inside tufts decreased from 40 to 35 µg N. g^{-1} dry soil, in September and March respectively. In bare soil, NH_4^+ content remained appreciably constant during the growth period and it exceeded that of vegetated soil only in October.

Plants study: Shoots and roots dry matter showed a significant increase during plant growth, between October and March. This growth was more important in shoots (Fig. 2A). Generally, Shoot/Root ratio in October is lower than in March (data not shown). In October, we observed a reduction in this ratio for out-tuft plants. Plants beginning their vegetative growth during this month seemed to allocate more biomass to their roots in bare soils. We observed an increase in the number of leaves and their respective areas from October to March. Nevertheless, leaf area decreased significantly in out-tufts plants mainly in March, while leaf number remains constant in both soil types (Fig. 2 B). also It was also noted that in-tufts plants had thicker leaves than those of out-tuft.

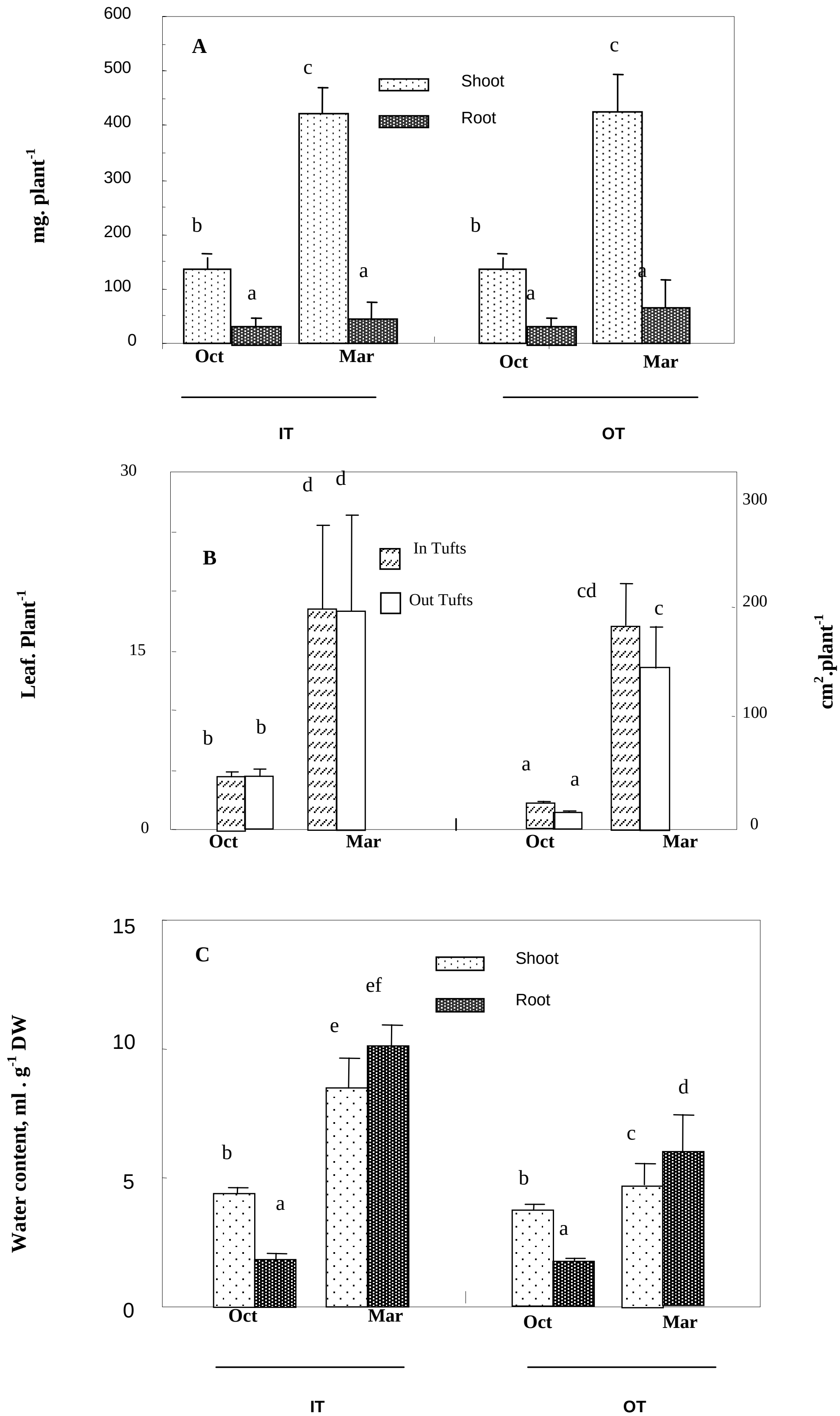

Fig. 2. Variation of biomass accumulation (A), Leaf number and leaf area (B) and water content (C) in shoots and roots of in-tufts and out-tufts growing *B macrocarpa* plants. Values (means of 10 replicates ± SD) followed by the same letters are not significantly different at 5% according to Fisher's LSD test. IT denotes in tufts and OT denotes out tufts.

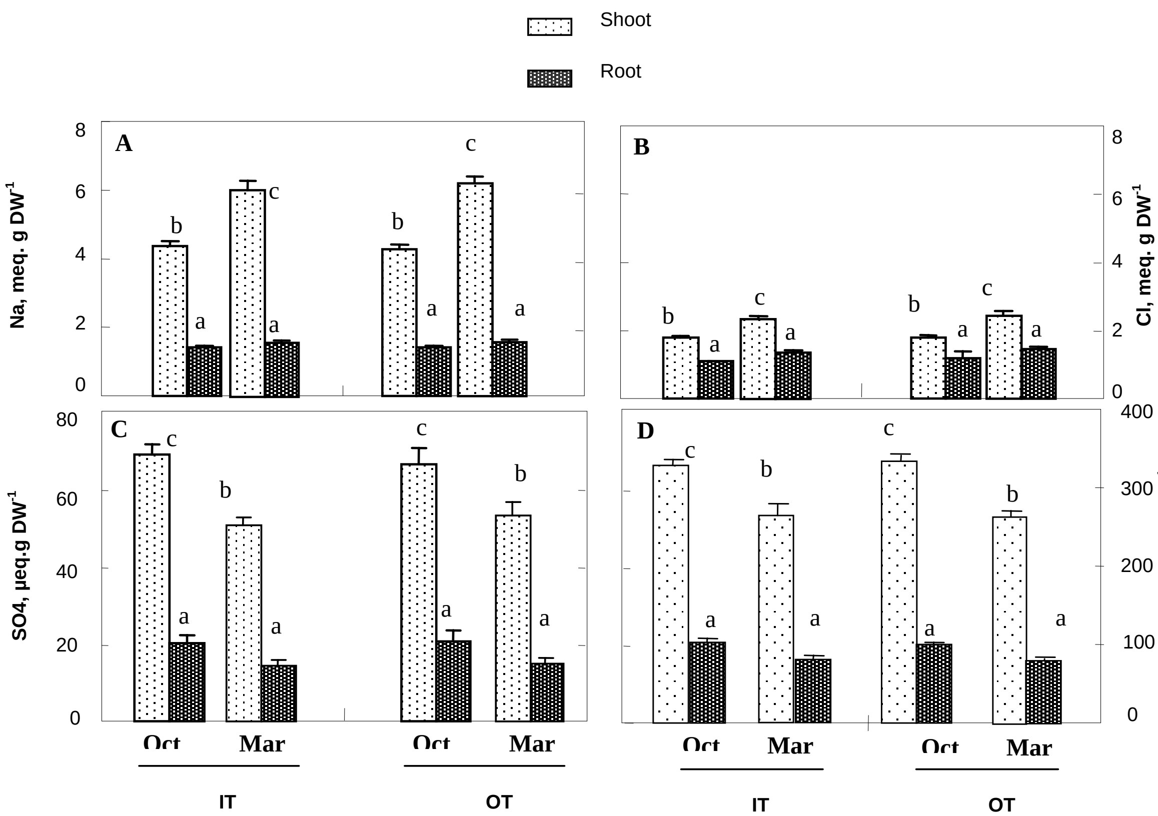

Fig. 3. Variation of Sodium (A) Chloride (B), Sulphate (C) and Phosphorus (D) in shoots and roots of in-tufts and out-tufts growing *B macrocarpa* plants. Values are means of 10 replicates, the same letters are not significantly different at 5% according to Fisher's LSD test. IT denotes in tufts and OT denotes out tufts.

Table 4. Variation of nitrogen content (mg. g $^{-1}$DS) in the soil of the Sebkha of Soliman during the three sampling months in (IT) and out (OT) the halophyte tufts.

Month	Soil of the sebkha of soliman					
	NH_4^+		NO_3^-		N org	
	IT	OT	IT	OT	IT	OT
September	0.04	0.034	0.04	0.09	1.58	1.35
October	0.031	0.035	0.037	0.05	1.35	1.11
March	0.035	0.034	0.05	0.044	1.38	1.36

Tissue water content varied greatly with growth of plants. In October (beginning of the vegetative growth), water content of shoots did not exceed 5 ml. g DW^{-1} which represented twice higher than roots. In March, water content increased either in shoots and roots. In the other hand, results showed decreased water content mainly in shoots of out-tufts plants, essentially in March (Fig. 2C).

Sodium and chloride concentrations expressed in meq. g DW^{-1} were lower in both shoots and roots, in October. In March, there was a marked increase in the contents of these two ions in whole plant. However, the sodium content always exceeded those of chloride in both soil kinds and sampling periods (Fig. 3A,B). Generally, these two ions are preferably accumulated in shoots. However, no differences, between both kinds of soil and both sampling months, were detected.

Concerning phosphorus, Figure 3D shows that from October to March a significant reduction was detected in *B. macrocarpa* plants. However, independently of the type of soil these contents were more significant in shoots than in roots. A similar pattern of accumulation was also observed in Sulphate content in *B. macrocarpa* plants (Fig. 3C). However, these contents remains always very low compared to those of phosphorus.

Potassium contents were higher in roots than in shoots; nevertheless, they did not exceed 1.5 meq. gDW^{-1}. We noted a more significant accumulation of potassium in October than in March, in both plants parts, independently of the soil type (Fig. 4A). Calcium and magnesium were accumulated more in shoots than in roots. From October to March, there was a reduction in these cations contents independently of plant part and soil type (Fig. 4B; 4C).

Figure 4D shows that plants collected in October accumulated more reduced nitrogen compared to those collected in March; however, shoots contents were always higher than those of roots. Ammonium contents were accumulated more by plants collected in October than March, mainly in shoots; in roots, however, contents were very low. No difference was found between plants collected out tufts and in tufts of halophytes (Fig. 4E). Nitrates seemed to be accumulated more in plants collected in October than in March and outside that inside halophytes tufts. Generally NO_3^- is preferably accumulated in shoots (Fig. 4F).

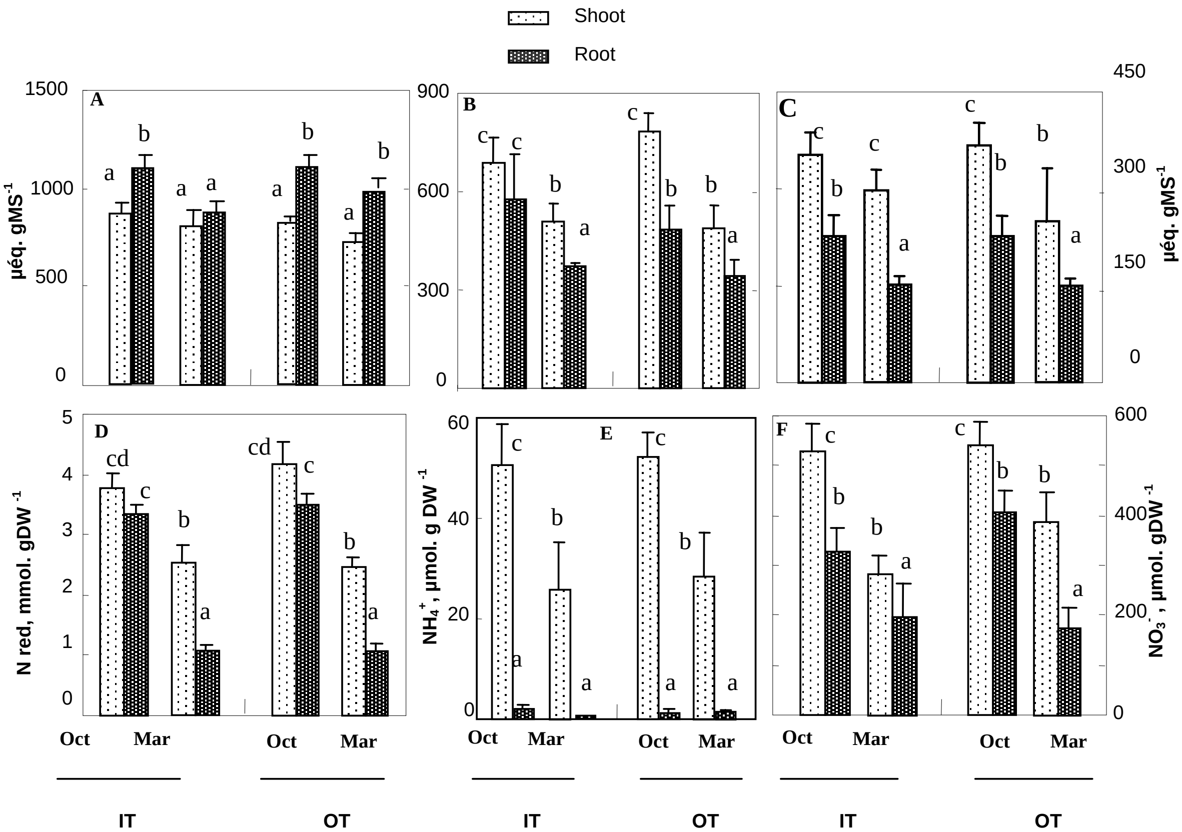

Fig. 4. Variation of Potassium (A), Calcium (B), Magnesium (C), N red (D), ammonium (E) and nitrate (F) contents in shoots and roots of in-tufts and out-tufts growing *B. macrocarpa* plants. Values (means of ten replicates ± SD) followed by the same letters are not significantly different at 5% according to Fisher's LSD test. IT denotes in tufts and OT denotes out tufts.

Discussion

Electric conductivity varied between 0.5 and 0.6 mS. cm^{-1} in-tufts to 0.8 $mS.cm^{-1}$ out-tufts. These values characterize moderately salinized soil. Plants had their maximum biomass production in low salinity (March). Furthermore, differences in salinity out-tufts as compared to in-tufts values did not alter biomass accumulation in *B. macrocarpa* plants (Fig 2) which revealed he facultative halophytic behaviour of these plants. Indeed the desalinisation of in-tufts soil by the superficial roots of halophytes could be responsible of the microgradient of salinity (Abdelly *et al.,* 2006). Our data suggested also that in-tufts soil was more humid. This can also explain the higher succulence in leaves of in-tufts plants and their higher water content as compared to isolated ones (Fig. 2C). A similar study near the Dead Sea reported 30% of relative humidity in covered soil while it did not exceed 12% in bare soil (Doddema *et al.,* 1986). These values are higher than those measured in the Sebkha of Soliman. This clearly indicated that our sampling was done in areas relatively far from the sea. However, as the soil moisture decreased, mainly out the halophytes tufts, *B. macrocarpa* seemed to conserve a good hydratation of root tissues, while shoot water content reduction did not exceed 40% as compared with values of in-tufts plants. This provides information concerning the role of these microhabitats, formed by halophytes tufts, in keeping adequate soil moisture, and at the same time, they play a crucial role in preventing rapid water evaporation from soil. The role that a dense plant cover plays in facilitating infiltration and capillary flow towards the surface (Willianson, 1986; Cantero *et al.,* 1998) seems to be in accordance with our findings. Indeed, the slowly drying soil protects the root system from rapid desiccation which in its turn prolongs the period of steady nutrient uptake and slows down the dynamics of plant desiccation (Rakic *et al.,* 2007). Nevertheless, mineral acquisition by plants was similar in both zone (Fig. 3). This indicates that this space is able to maintain adequate growth even in relatively poor and desiccated soil. Toxic ion such as sodium and chloride were the most abundant ions in the plants (Fig. 3) and their concentrations increased with increasing biomass accumulation without any symptoms of toxicity in the aerial parts, independently of the soil type. The use of metabolically undesirable ions by halophytes is widely reported and reflects their utilisation for osmotic adjustment (Flowers, 1985; Flower *et al.,* 1991; Youssef *et al.,* 2003), a process in which Na can be much more suitable than K (Marschner, 1995; Pathan *et al.,* 2007) since it accumulates preferentially in vacuoles (Koyro & Huchzermeyer, 1997). Additionally, *Beta macrocarpa* plants allocated more sodium and chloride to their shoots; these contents were similar in both plants types. Plants which are in the category of includers (*Aster tripolium*; *Batis maritima*; *Sesuvium verrucosum* and *Sesuvium* *portulacastrum*) concentrate more sodium in the shoots compared with the excluders (*Avicennia germinans*; *Limoniastrum multiflorum*; *Rottboellia fasciculata* and *Spartina alterniflora*) (Daoud *et al.*, 2001). *Beta macrocarpa* leaves accumulated sodium, however, they seem to restrain this accumulation when soil EC increases (mainly in in-tufts soil). Moreover, seeds were also able to tolerate elevated EC (measured in September) and grew well in laboratory experiments. Potassium contents were higher in roots than in shoots (Fig. 4). The roots of some salt tolerant species have a greater affinity for K^+ in preference to Na^+ than sensitive species (Breckle, 1990; Grattan & Grieve 1992; Reimann & Breckle 1993; 1995), but the degree of preference for K^+ over Na^+ varies (Breckle, 1990; Grattan & Grieve 1992; Curtin *et al.*, 1993). Nutritional and photosynthetic disorders may occur in plants growing with Ca^{2+}/Mg^{2+} imbalances (Grattan & Grieve 1999). On the other hand, variation in the soil type did not affect Ca uptake and transport through the plant although increased salinity is reported to reduce the amount of Ca in the plant (Marschner, 1995; Koyro & Huchzermeyer, 1997). Yamanouchi *et al*., (1997) reported that Ca increases salt tolerance of plants. They suggested that ameliorating effect of Ca is in accordance with its functions in membrane integrity and control of selectivity in ion uptake and transport. In-tufts soil seems to be more fertile (Table 3, 4), mainly in September (seeds collect). The litter formed by halophyte fallen organs and by organic debris accumulated by the wind at the feet of tufts, could contribute to localized soil enrichment in N and P (Abdelly *et al.*, 2006). This can also be attributed to the co-existence, of legumes in halophyte tufts, (mainly *Medics*) that can fix atmospheric nitrogen (Abdelly, 1997). The highest nitrogen content was measured in September when its uptake became low (Table 4). Differences between both soil types were more pronounced in September and October. In March, increased nitrogen uptake with increasing plant growth maintained nitrogen content similar in both soil types (Table 4). However, the soil of the Sebkha of Soliman seems to be well provided with nitrogen (Heller, 1969). Ammonium contents in soil did not change with plants growth or soil types (Table 4). However, increasing nitrate content out-tufts showed that probably, *B. macrocarpa* plants preferred nitrate. Nevertheless, more investigations are needed to confirm this hypothesis.

Conclusion

Halophytes represent a considerable potential as crop plants and tufts of halophyte which seems to be a very important micro-habitat that need more interest. Moreover, increased research on the selection of halophytic species which have an economic utilisation may enable the rehabilitation and revegetation of salt-affected lands given that the appropriate soil and irrigation management is applied. On the other hand, the concept that a plant and its habitat form a distinct interactive unit should attract much investigational attention in order to better understanding of plants behaviour against many environmental stresses and constraints at the same time.

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