

Effect of NaCl on the growth and the ionic balance K^+/Na^+ of two populations of *Lotus creticus* (L.) (Papilionaceae)

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Abstract

Lotus creticus (L.) is a major pastoral and forage legume in the arid climate of Tunisia where salinity is a serious production problem. A laboratory experiment was carried out to assess the physiological behaviour of two populations of *Lotus creticus* (Msarref (Msf) and Oued Dkouk (Odk)) in a solid substratum in the presence of salt. The tested concentrations vary from 0 to 400 mM NaCl. It has been shown that the two populations of *Lotus creticus* are fairly tolerant to salt at growth phase. The growth productions are recorded in absence of salt, mainly at the population Odk. The presence of salt in the medium affects growth of the whole plant for both populations. Compared to root biomass, the aerial one was more affected by salt. For all treatments, plants of the two populations remain able to produce and to allocate dry matter to the different organs. However, the salinity generated a disruption at the level of water feeding of plants of the two populations. Compared to root organ, water contents in aerial organ proved to be the least affected by salt. The survey of the relation of water content of leaves according to its production in biomass showed that the expression of growth potentialities is associated with a better leaves hydration for the population Odk. It seems then that the decrease of growth under saline stress is not associated to a water (osmotic) effect. The survey of the Na^+ / K^+ ratio showed for both studied populations an increase of Na^+ contents in aerial and root organs, with an excess of accumulation of these ions in the aerial organ particularly more marked at the population of Msf. In spite of the predominance of the Na^+ ions, the two populations, mainly Odk, remain capable to assure a K^+ selectivity. This selectivity is ensured mainly at the level of roots where high potassium content is recorded compared to the aerial organ. The tolerance of the two populations is probably acquired by their better faculty to assure K^+ selectivity and to compartmentalize Na^+ ions in leaves. Such a mechanism reflects probably an inclusive behaviour towards salt. This behaviour justifies the faculty of plants to maintain their growth even in very hard salinity conditions.

Keywords: *Lotus creticus*, Growth, Salinity, Osmotic adjustment, Ratio K^+/Na^+ , Tolerance.

Introduction

Plant cover is needed to protect soil from natural and anthropogenic erosion, mainly where climatic conditions constrain plant growth (Vignolio *et al.*, 2005). Soils with poor vegetation cover are susceptible to erosion by rainfall, the erosion increasing with slope (Rundel, 1995; Muzzi *et al.*, 1997; Grace *et al.*, 1998; Wali, 1999; Carroll *et al.*, 2000; K). Desertification in the Mediterranean region is mainly due to vegetation cover reduction and soil erosion (Kosmas *et al.*, 2000). Mediterranean climate is characterized by hot, dry summers and cool, cold winters, which limits the use of different species for soil revegetation (Savé *et al.*, 1999). Therefore, the use of native species for revegetation may be an interesting practice especially in those countries with dry climatic conditions, where salinity is often a serious problem because of the poor quality of irrigation water during the dry season (Sánchez-Blanco *et al.*, 1998). Native species called Mediterranean plants are usually considered more tolerant and adapted to dry conditions and to soil salinity (Caballero and Cid, 1993). Plants exposed to high salt concentrations must withstand both water deficit and ion imbalance imposed by salt excess. Although water deficit always has a negative effect, many crop plants are primarily sensitive to Na⁺ excess (Greenway and Munns, 1980) due to its adverse effects on K⁺ nutrition, cytosolic enzyme activities, photosynthesis and metabolism (Niu *et al.*, 1995; Ben Khaled *et al.*, 2003). The depressive effect of salt on the growth is, according to Hajji *et al.* (1999), the results of (i) a reduction in the osmotic potential of the soil solution around the roots, (ii) an increase in the accumulation of some ions in harmful concentrations in tissues and (iii) a modification of the nutritional statute of the essential ions to the growth and the development. It is known that the capacity of plants to counteract salinity stress strongly depends on the status of their K⁺ nutrition. Increasing K⁺ supply in the root environment may mitigate the reduction of plant biomass due to an increase in salinity (Chow and Tsang, 1990; Delgado and Sánchez-Raya, 1999). It is possible that a high K⁺/Na⁺ ratio is more important for many species than simply maintaining a low concentration of Na⁺ (Gorham *et al.*, 1990; Rubio *et al.*, 1999; Cuin *et al.*, 2003). Potassium starvation regularly accompanies sodium toxicity (Flowers and Läuchli, 1983). Peng *et al.* (2004) have shown that the decline of salt tolerance under low K⁺ conditions might have resulted from increased Na⁺ entrance through the high affinity K⁺ system. However, salt sensitive plants (glycophytes) try to restrict ion movement from roots to shoots whereas salt resistant plants (halophytes) tend to take up Na⁺ ions (Slama, 1987; Hasegawa *et al.*, 2000). In 1999, Amzallag showed that the toxicity of salt is frequently deduced from the negative correlations between the biomass of the aerial organs and its Na⁺ content. Compared to the shoots, several studies have shown that roots are less affected (Greenway and Munns, 1980; Munns and Termaat, 1986; Mohamed *et al.*, 1989). In other studies, it was shown that the growth of aerial organ was inhibited under salt stress by the decrease of root growth (Cramer *et al.*, 1989; Yeo *et al.*, 1991; Rengel, 1992). According to Levigneron *et al.* (1995), the increase of soil salinity is translated by an immediate reduction of shoot growth. The latter is associated to the reduction in the water potential gradient between the plant tissues and the medium. The halophytes and some tolerant glycophytes carry out the osmotic adjustment by concentrating salt in their tissues (Munns, 2002). But the quantities necessary to accumulate become quickly toxic for the sensitive glycophytes which are unable to adjust

their internal osmotic potential (Mott and Steward, 1972; Munns, 1993). Consequently, the most studied criteria used to evaluate the tolerance of plants to saline stress appear (i) ionic transport and regulation such as the Na^+ foliar exclusion, high K^+/Na^+ selectivity of shoots (Schactman *et al.*, 1991; Wolf *et al.*, 1991); (ii) the phloem Na^+ and Cl^- ions retranslocation (Zid and Grignon, 1991); (iii) synthesis of the nitrogenized molecules such as glycine betain (Gerard *et al.*, 1991; Levigneron *et al.*, 1995), polyamines (Le Dily *et al.*, 1991) and proline accumulation (Ullah *et al.*, 1994; EL Haddad and O'Leary, 1994) and (iv) Chlorophyllian fluorescence (Belkhodja *et al.*, 1994).

Lotus creticus, a member of Papilionaceae family, grown on the beaches and cliffs of the Mediterranean coast, could be used for revegetation in saline conditions. Although, its introduction requires knowledge about its eco-physiological characteristics in its natural habitat (Sánchez-Blanco *et al.*, 1998). In earlier report, we have shown that this species is able to support a level of salinity around 300 mM in germinative phase (Rejili *et al.*, 2006). *Lotus creticus* is cultivated in many countries; it is widely grown in arid and semi-arid region where soils contain high levels of salts. However, salt affected soils can be utilized by growing salt tolerant crops because such crops would allow expansion of crop production to areas where conventional reclamation procedures are economically or technically limited. Since *Lotus creticus* is a major pastoral legume crop (Neffati, 1994), it could be grown on salt-affected lands if it possesses high degree of salt tolerance. Keeping in mind the present study was conducted to assess the response of this crop to salt stress since the mechanism by which plant tolerates salt is complex and it differs from species to species (Greenway and Munns, 1980; Ashraf, 1994; Ashraf and Harris, 2004).

Materials and methods

Plant material and growth conditions

Lotus creticus seeds were collected from two sites in Southern Tunisia. Located in Jeffara, the first site (area of Msarref (Msf): $10^{\circ} 59' . 677$ E and $33^{\circ} 10' . 269$ N) formed part of the lower arid bioclimatic stage. The second one which forms part of the Oued dkouk national park (Odk), is localised in the higher Saharan bioclimatic stage ($10^{\circ} 32' . 280$ E and $32^{\circ} 08' . 760$ N). Seeds were sowed in plastic containers filled with a mixture of marketed peat and sterile sand (equal parts by vol.) during eight weeks. The pots were placed under the natural conditions at a temperature ranging between $25/17^{\circ}$ C (day/night). Plants were daily irrigated. Seven weeks after sowing, the pots were left in 5 treatments corresponding to the different NaCl concentrations: 0, 50 mM, 100 mM, 200 mM and 400 mM. Two harvest periods, before (t_1) and after (t_2) application of salt, were realized. At the end of the salinization period, leaves and roots of plants were harvested and washed with distilled water, dried at 80°C and stored at room temperature for mineral analyses.

Mineral content Analyses

Sodium and Potassium contents were determined in a digestion extract with HNO_3 (0.5 N) by atomic absorption spectrometry (Shimadzu, Japan).

Growth and nutrition analysis methods

1.1. Water content (WC)

The organs water contents were defined as follows.

$$\mathbf{WC = (FW - DW) / DW}$$

Where:

DW: dry weight

FW: fresh weight

1.2. Relative average growth (RAG)

The RAG was calculated according to the formula of Hunt (1990) cited by Lachaâl (1998).

$$\mathbf{RAG = (DW_{t_2} - DW_{t_1}) / [DW * (t_2 - t_1)]}$$

Where:

$$DW = (DW_{t_2} - DW_{t_1}) / \ln(DW_{t_2}) - \ln(DW_{t_1})$$

DW_{t_1} = dry matter mass (mg) before the application of salt.

DW_{t_2} = dry matter mass (mg) at the end of salinization period.

1.3. Sensitivity rate index (IS)

The effect of salt on growth can be appreciated by a sensitivity rate index (I_s) calculated according to the following formula:

$$\mathbf{I_s = [(DW_{NaCl} - DW_{control}) / DW_{control}] \times 100}$$

1.4. Ionic Contents

The ionic contents (IC) accumulated in the various organs during the culture were expressed in meq.g^{-1} DW. It was calculated according to the following formula.

$$\mathbf{IC = Ionic\ quantities / DW\ (g)}$$

1.5. K⁺/Na⁺ selectivity

The equivalent ionic fraction K ($K + Na$) is given on the ionic contents of the shoots, stems and roots. Reported to the equivalent ionic fraction in the medium, this ratio defines the K^+/Na^+ selectivity in the plant. This estimate validity was given by Glenn *et al.* (1994).

1.6. Vacuolar compartmentation

To appreciate the Na^+ compartmentalization degree in foliar tissues, we carried out an indirect evaluation by correlating shoot water content with its Na^+ content. When Na^+ is compartmentalized in the vacuole, it was used for the osmotic adjustment and its vacuolar accumulation induced a supplement tissue hydration. However, if Na^+ accumulation was extracellular, it involved tissue dehydration.

Statistic analysis

The follow-up of the various parameters under salt stress was given on the basis of nine replications for each treatment (three). The measured parameters were subjected to an

analysis of the variance (ANOVA test). The confidence interval was calculated at the threshold of 95 %.

Results

1. Plant growth

1.1. Whole plant growth

The dry matter of whole plants cultivated under salt stress is given in Figure 1. In the absence of salt, plant growth was maximum and the Msf population plants were less productive than those of the Odk population. Such a result was confirmed by the Anova analysis which revealed a highly significant population effect ($P < 0.01$; Table 1). The presence of salt to 100 mM reduced significantly the dry matter production of both populations ($P < 0.01$). Compared to the control, the degree of reduction reached 50 % for the Odk population whereas it was only 33 % for the Msf population. At high levels of salinity (200 to 400 mM), plant biomass of both populations was statistically identical.

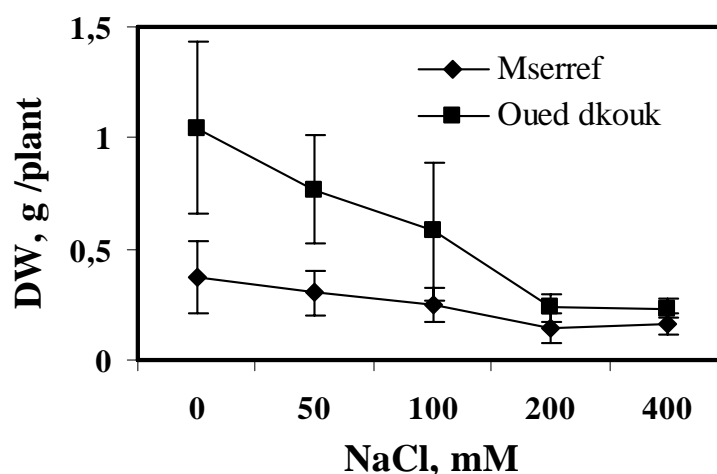


Figure 1. Effect of different NaCl concentrations on the whole plant dry weight production of the two populations of *Lotus creticus*. Each point represents the average of 9 individual measurements. Confidence interval was calculated at the threshold of 95%. (Initial dry matter of Msf = 0,0735 g/plant and that of Odk = 0,0919 g/plant)

Table 1. Results of Anova Test showing the effect of salinity on dry matter production of the whole plant.

Source	df	F	Significance
Population	1	25,323	0,000
Treatment	4	9,324	0,000
Interaction	4	3,090	0,020

1.2. Different organ growths

The effect of salt stress on the dry matter of the different organs is illustrated in Figure 2. In the absence of salt, the shoots dry matter of Msf population was significantly lower than those of Odk population ($P < 0.01$; Table 2). The presence of salt affected significantly the shoots biomass ($P < 0.01$). Compared to the control, dry matter decreased by 50 % for both populations at 100 mM of NaCl. At high salinity (200 and 400 mM), the shoots remained able to produce dry matter. The allocated dry matter represented, compared to control, approximately 40 % and 20 % for the two populations Msf and Odk respectively. The analysis of salt effect on stem biomass of both populations showed that, up to 100 mM, NaCl contribution did not modify the biomass of these organs. It was only with very high levels of salt (200 and 400 mM) that stem dry matter presented a highly significant reduction ($P < 0.01$; Table 2). The reduction reached 33 % for Msf population and 50 % for the Odk population. Compared to the aerial organs, the roots dry matter was not affected by salt stress.

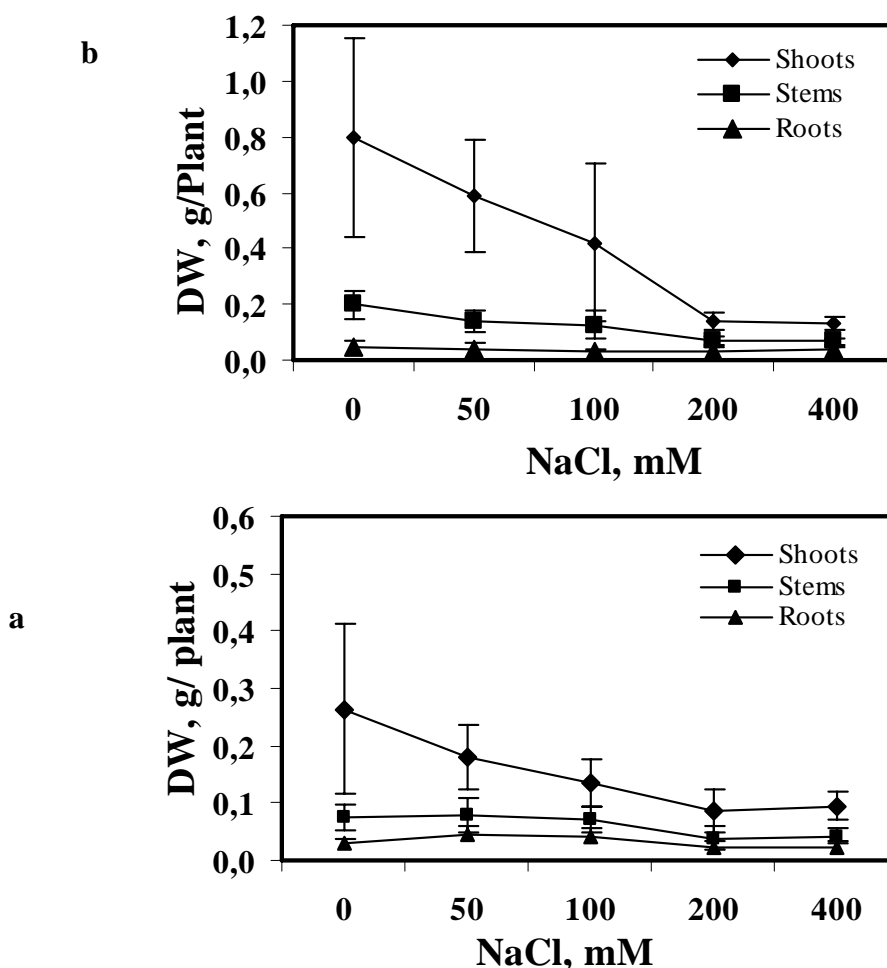


Figure 2. Effect of different NaCl concentrations on shoots, stems and roots dry weight production of the two populations of *Lotus creticus* (a - Msarref and b - Oued dkouk). Each point represents the average of 9 individual measurements. The confidence interval was calculated at the threshold of 95%.

Table 2. Results of Anova Test showing the effect of salinity on the three organs dry matter production.

Sources	Variables	df	F	Significance
Population	DW Shoots	1	20,938	0,000
	DW Stems	1	31,500	0,000
	DW Roots	1	1,182	0,280
Treatment	DW Shoots	4	31,316	0,000
	DW Stems	4	9,314	0,000
	DW Roots	4	2,025	0,099
Interaction	DW Shoots	4	25,922	0,000
	DW Stems	4	2,851	0,029
	DW Roots	4	1,036	0,394

1.3. Relative average growth (RAG)

Figure 3 shows that the growth activity of aerial organs was significantly higher for Odk population than for Msf ($P < 0.05$; Table 3). The roots RAG was, however, identical for both populations ($P > 0.05$). The presence of NaCl at levels exceeding the 100 mM resulted in a significant reduction of the aerial organs RAG for both populations ($P < 0.01$). The roots RAG was, however, significantly insensitive ($P > 0.05$).

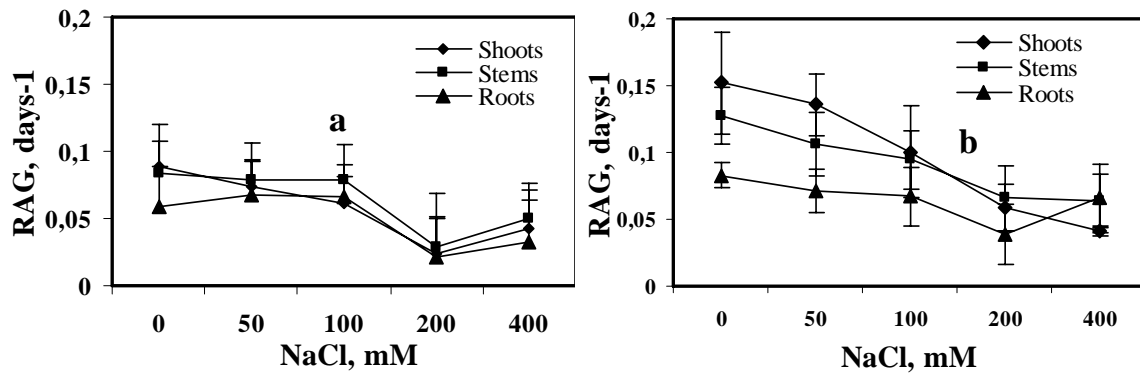


Figure 3. Relative average growth (RAG) expressed in quantity of biomass produced per unit of dry weight and unit of time ($g \cdot g^{-1} DW$) of roots, stems and shoots at both populations of *Lotus creticus* (a - Msarref and b - Oued dkouk) cultivated under salt stress (50 to 400 mM). An average of 9 repetitions and confidence interval was calculated at the threshold of 95%.

1.4. Sensitivity rate index (IS)

As shown in Figure (4), roots proved to be the least sensitive organs to salt for both populations (treatment effect: $P < 0.05$; Table 4). On the contrast, aerial organs did not

express its maximum growth potentialities. It tolerated at 100 mM levels concentrations.

Table 3. Results of Anova Test showing the effect of salinity on Shoots, Stems and Roots RAG.

Sources	Variables	df	F	Significance
Population	RAG Shoots	1	20,414	0,000
	RAG Stems	1	9,594	0,003
	RAG Roots	1	2,307	0,133
Treatment	RAG Shoots	4	13,727	0,000
	RAG Stems	4	6,190	0,000
	RAG Roots	4	2,888	0,027
Interaction	RAG Shoots	4	1,830	0,131
	RAG Stems	4	0,437	0,781
	RAG Roots	4	0,728	0,575

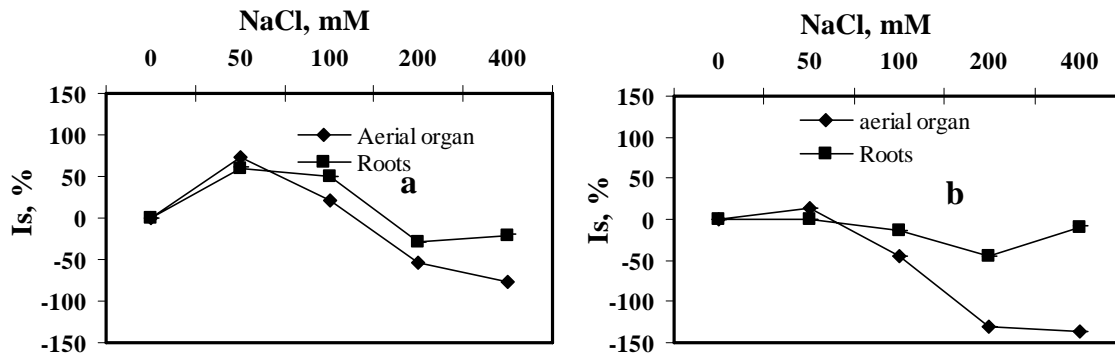


Figure 4. Sensibility rate index evolution of aerial and root organs of the two populations of *Lotus creticus* (a - Msarref and b - Oued dkouk) cultivated under salt stress (0 to 400 mM). An average of 9 repetitions and confidence interval was calculated at the threshold of 95%.

Table 4. Results of Anova Test showing the effect of salinity on the sensibility rate index of aerial (ISA) and root (ISR) organs.

Sources	Variables	df	F	Significance
Population	ISA	1	3,579	0,062
	ISR	1	3,183	0,078
Treatment	ISA	4	4,237	0,004
	ISR	4	2,668	0,038
Interaction	ISA	4	0,236	0,917
	ISR	4	1,171	0,330

3. Organ hydration

The water content of the aerial and root organs under salt stress is given in Figure 5. In the absence of salt, the aerial organs of the two populations presented different water contents. The variance analysis confirmed that population factor acted significantly on water content of these organs ($P < 0.01$; Table 5). For Msf population, the root water content represented half of the aerial organs. Salinity has significantly improved water content of the aerial organs for both populations ($P < 0.01$; Table 5). This improvement was reached at 100 mM for both populations. Beyond these concentrations, the water contents of the aerial organs decreased considerably with the increase of salt. This was confirmed by the variance analysis which showed that the "population" and "treatment" factors acted significantly on this parameter ($P < 0.01$). The interaction between these factors was also significant at the threshold of 1% ($P < 0.01$). The roots water state was not affected by salinity. At 400 mM, the organ hydrations (aerial and root) of both populations were identical.

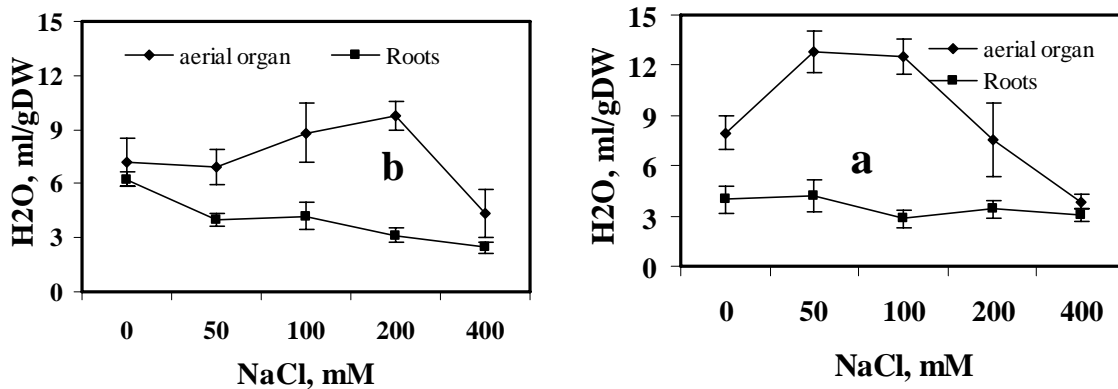


Figure 5. Effect of salinity on the aerial and root water content of the two populations of *Lotus creticus* (a - Msarref and b - Oued dkouk). An average of 9 repetitions and confidence interval was calculated at the threshold of 95%.

Table 5. Results of Anova Test showing the effect of salinity on aerial (AWC) and root (RWC) organs water content.

Sources	Variables	df	F	Significance
Population	AWC	1	12,074	0,001
	RWC	1	0,030	0,862
Treatment	AWC	4	27,337	0,000
	RWC	4	20,910	0,000
Interaction	AWC	4	10,981	0,000
	RWC	4	14,034	0,000

It was showed previously that the growth of both populations was affected by salt stress. However, a significant improvement of the water status of the aerial organs was showed. It seems that the growth decreasing under salt stress wasn't associated to a water (osmotic)

effect but it was probably the consequence of a massive accumulation of toxic ions and/or of limited nutrient absorptions (ionic effect).

4. Ionic contents

4.1. Sodium

Figure 6 shows that in the absence of salt, the both organs populations presented very low Na⁺ contents. These contents didn't exceed the 0,5 meq.g⁻¹ DW. The presence of salt involved a Na⁺ aerial tissues improvement with an excess of accumulation for Msf population. At 400 mM, the values reached 5 meq/gDWAP for Msf population, were only 2.5 meq.g⁻¹ DWAP for Odk population. The both population's roots were definitely less rich in Na⁺; the sodic contents exceeded hardly the 2.5 meq.g⁻¹DWR. Significant differences in Na⁺ accumulation between the two populations were revealed by the Anova analysis at two factors (*P*< 0.05) (Table 6).

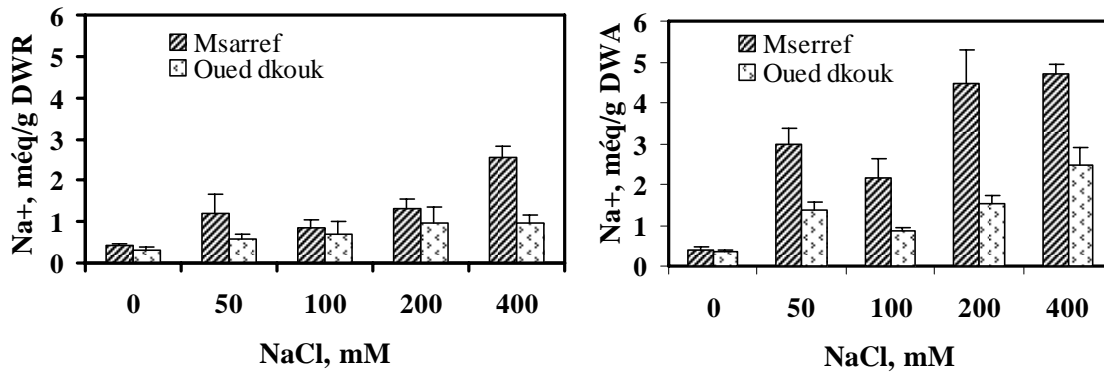


Figure 6. Variation of the sodium content in the aerial (DWAP) and root (DWR) organs of the two populations of *Lotus creticus* cultivated under salt stress (0 to 400 mM). An average of 9 repetitions and confidante interval was calculated at the threshold of 95%.

Table 6. Results of Anova Test showing the effect of salinity on aerial (ASC) and root (RSC) organs sodium content.

Sources	Variables	df	F	Significance
Population	ASC	1	55,593	0,000
	RSC	1	1,262	0,265
Treatment	ASC	4	24,255	0,000
	RSC	4	5,701	0,000
Interaction	ASC	4	4,207	0,004
	RSC	4	4,183	0,004

4.2. Potassium

The potassium content of the aerial and root organs is presented in Figure 7. In the absence of salt, the organs K^+ contents were significantly more important for Msr population than for Odk. This was confirmed by the variance analysis where a highly significant population effect was revealed ($P < 0.01$; Table 7). The presence of salt induced a potassium content reduction, particularly in the aerial organs. This was confirmed by the Anova test which detected a highly significant treatment effect at the threshold of 1% ($P < 0.01$). The rooted potassium nutrition was statistically identical at the both populations ($P < 0.05$). The roots K^+ content proved to be insensitive under salt stress.

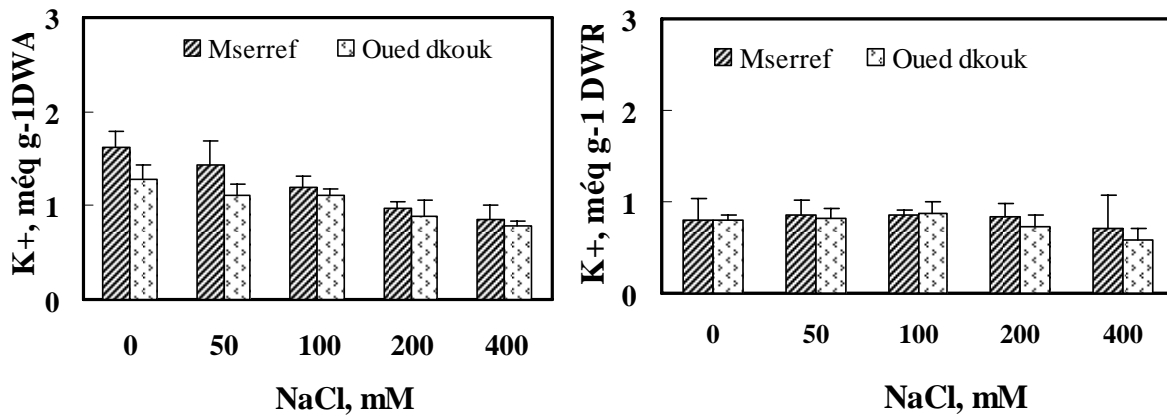


Figure 7. Variation of the potassium content in the aerial (DWA) and root (DWR) organs of the two populations of *Lotus creticus* cultivated under salt stress (0 to 400 mM). An average of 9 repetitions and confidence interval was calculated at the threshold of 95%.

Table 7. Results of Anova Test showing the effect of salinity on sodium content of aerial (APC) and root (RPC) organs.

Sources	Variables	df	F	Significance
Population	APC	1	13,509	0,000
	RPC	1	0,749	0,390
Treatment	APC	4	20,411	0,000
	RPC	4	1,592	0,184
Interaction	APC	4	1,680	0,163
	RPC	4	0,265	0,900

5. Vacuolar compartmentation

The data of Figure 8, correlating shoots water content with its Na^+ content, showed that the presence of salt in the medium provoked a sodium accumulation in the photosynthetic organs for both populations. This foliar accumulation was associated with water content

stability. These observations suggest that the Na^+ ion underwent a certain compartmentation mechanism. However, this sodic accumulation made probably the plants to osmotically adjust themselves and to maintain its water potential gradient, essential for the water circulation from the medium to the plant organs.

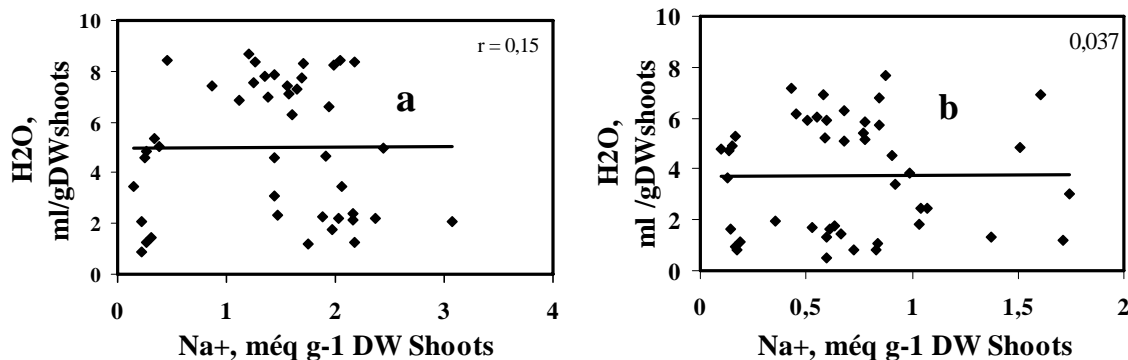


Figure 8. Correlation between foliar water content and its sodium content of the two populations of *Lotus creticus* (**a** - Msarref and **b** - Oued dkouk) cultivated under salt stress (0 to 400 mM).

Discussion

Results relating to plant cultivated stages in presence of different salt concentrations showed that Odk population was more productive than Msf population in absence of salt (population effect: $P < 0.05$). The presence of salt in the medium affected both biomass production and plant development of both populations (treatment effect: $P < 0.01$). Concerning biomass production, our results confirmed that dry matter of the aerial organs (Figure 2) was significantly affected by NaCl levels exceeding the 100 mM. This reduction appeared relevant for Odk population. The same result was shown by Sánchez-Blanco *et al.* (1998) on plants of *Lotus creticus* spp *creticus* cultivated on hydroponic medium. Le Houérou (1986) showed that *Lotus creticus* was able to support 100 mM of NaCl concentrations. Abdelly (1992) showed that salt delayed new shoots development and induced a reduction of its expansions in medic plant. Erdei and Kuiper (1979) showed that growth of *Plantago media* was reduced at as low concentration as 25 mM NaCl and the plants were killed at 75 mM, while the salt tolerant *Plantago maritima* still maintained growth at 300 mM NaCl.

The effect of salinity on biomass depends on plant size and its relative average growth (RAG). The depressive action of salt on growth appeared by a significant reduction of the aerial organ growth activity (Figure 3). For instance, shoots were more affected than stems for both populations. Compared to the aerial organs, the roots dry matter was not affected by salt stress. The same results were shown by Vinit- Dunand *et al.* (2002) in cucumber plants. Several authors suggested that, under saline stress, the osmotic effect is responsible for the aerial organ growth reduction (Muuns and Termaat, 1986; Yeo *et al.*, 1991; Rengel, 1992). Indeed, the data of Figure 9, correlating the foliar biomass with its water content for both populations, showed that expression of growth potentialities was independent, at Msf

population, of its hydration. However, an improvement of the water status was observed for Odk population. This result pleaded in favour of the previous hypothesis.

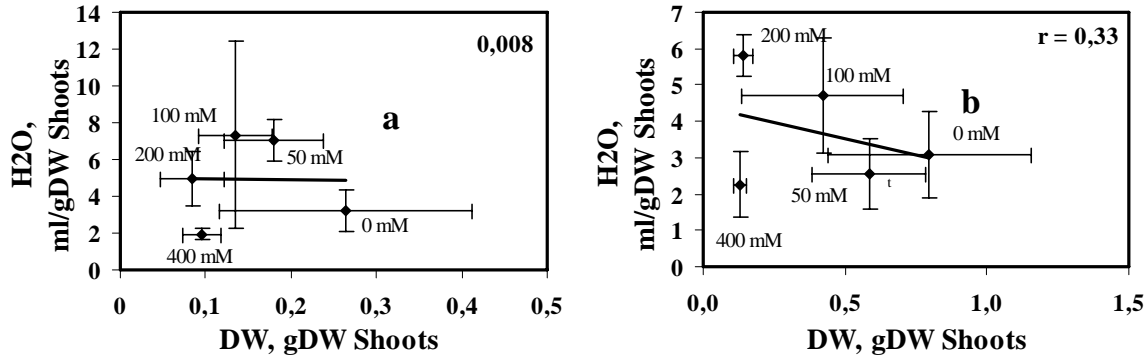


Figure 9. Correlation between the aerial organs growth and its water content of the two populations of *Lotus creticus* (a - Msarref and b - Oued dkouk) cultivated under salt stress (0 to 400 mM). An average of 9 repetitions and confidence interval was calculated at the threshold of 95%.

Plants exposed to saline stress were prone to an osmotic stress and to specific toxicity effects of Na⁺ and Cl⁻ ions (Bernstein and Hoyward, 1958; Shannon, 1984; Ayer and Westcot, 1985; Hajji *et al.*, 1999). Flowers *et al.* (1977) summarized the depressive effect of salinity on the growth by a nutritional and/or hydrous imbalance. The significant correlation between the aerial biomass production and its Na⁺ content suggest that, for both populations, the growth decrease was due to the ionic toxicity (Figure 10).

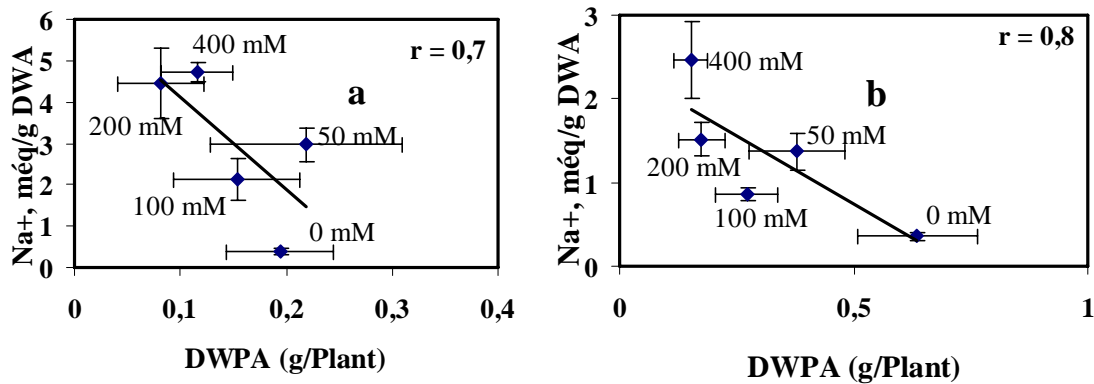


Figure 10. Correlation between the aerial organs biomass and its sodium content of the two populations of *Lotus creticus* (a - Msarref and b - Oued dkouk) cultivated under salt stress (0 to 400 mM). The confidence interval was calculated at the threshold of 95%.

Generally, the most salt tolerant plants accumulate Na^+ in their shoots whereas sensitive plants don't. In the first type, called "Includers", salt was trapped and accumulated in the aerial organs cells, mainly in its vacuoles (Yeo and Flowers, 1986; Levigneron *et al.*, 1995). In the second type, "Excluders", the salt conveyed to the shoots, fault to be trapped, was re-exported towards the roots by the phloemic tissue (Lessani and Marschner, 1978; Wieneke and Laüchli, 1980; Slama, 1982; Fortmeir and Schubert, 1995). Our results (Figure 9) showed that the two studied populations accumulated Na^+ ions in its photosynthetic organs. This accumulation was associated with a water content stability. Such a mechanism reflects probably an inclusive behaviour of the plants and a good aptitude to use the dominant ions (Na^+) for the osmotic adjustment. Consequently, plants have probably adapted to the osmotic stress by either closing their stomata or increasing the osmotic pressure of the leaf cells. This osmotic behaviour on solid substratum was also shown by Sánchez-blanco *et al.* (1998) on hydroponic medium.

The maintenance of suitable potassic nutrition to support growth of different organs requires a good selectivity, in the aerial organs, of K^+ absorption, accumulation and transport compared to Na^+ . Many studies on halophytes and on some tolerant glycophytes plants showed that a high foliar K^+/Na^+ ratio is a salt tolerance criterion (Gorham *et al.*, 1990; Schactman *et al.*, 1991; Wolf *et al.*, 1991; Yeo, 1998). Our results (Figure 11) showed that organs of two populations, in particular Odk, remained strongly selective for K^+ ions. However, the rather weak correlation detected at the Msf population seems to be the consequence of the strong Na^+ and K^+ competition. The later limits the potassium absorption, an essential ion for plant growth and development.

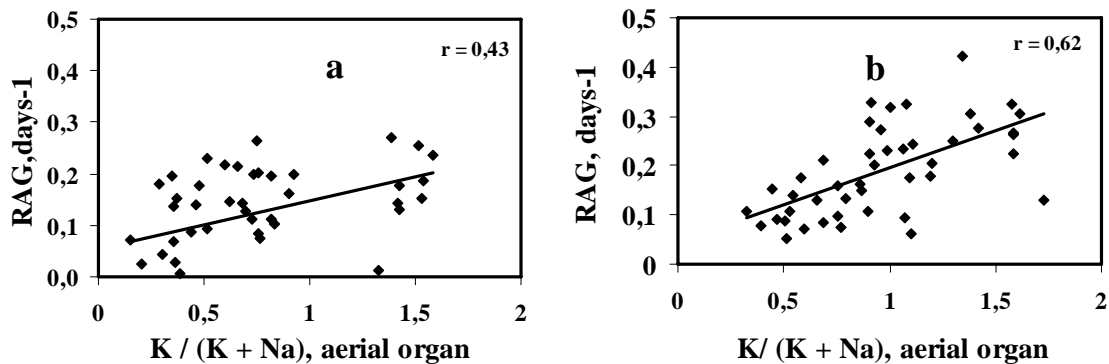


Figure 11. Correlation between RAG and ionic selectivity of the aerial organs of *Lotus creticus* populations (a: Oued dkouk et b: Msarref) cultivated under salt stress (0 to 400 mM). Each point represents an individual measurement.

Conclusion

The comparative study about the effect of salinity on growth of two populations of *Lotus creticus* showed that the Odk population has a significant higher production than Msf in absence of salt. The presence of salt (50 to 400 mM) affected negatively both populations' growth. Such negative effect is more obvious on aerial organs than on roots. Contrarily to

the Msf population, where it is almost stable, the Odk population leaf water content was significantly augmented with the increase of salt concentrations. In both populations, an increase in the contents of Na⁺ in both parts, above and underground, was recorded even though, an excess of accumulation of these ions was more marked in the aerial organ of the Msf population. The presence of high quantities of Na⁺ in the aerial biomass may be attributed to the export, towards the aerial organ of the roots absorbed Na⁺ essence. To escape from the ionic effects of Na⁺, both populations probably express a certain mechanism of compartmentation. In addition, the high levels of Na⁺ generated a kind of competition on the level of the sites of K⁺ absorption, mainly at the Msf population, and thus limited the absorption of this essential element for plants growth and development.

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