Salinity Effects on the Physiological Response of Two Bean Genotypes (*Phaseolus vulgaris* L.)

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ABSTRACT

The performances of two common bean genotypes; Djadida and Tema were tested under NaCl stress. The plants of three weeks were treated for 10 days with NaCl from 30 mM to 150 mM. It appears that salinity produced an imbalance in water relations and affected growth parameters. NaCl application altered roots, leaves and stems mass production and plant water relations. NaCl application affected adversely roots, leaves and stems mass production and altered plant water relations and stomata density. It should be noted that the same physiological behavior was observed for both bean genotypes subjected to salinity which revealed the existence of a quantitative instead of a qualitative difference between the tested genotypes. Here we observed the superiority of the genotype *Tema*, for maintaining its growth and water relations under salt stress while further researches are necessary to validate our finding under field conditions.

KEYWORDS

Salinity tolerance, Phaseolus vulgaris, water relations, growth, stomata.

تأثير الإجهاد الملحي على الاستجابة الفسيولوجية لنمطين وراثيين لنبات الفاصوليا (*Phaseolus vulgaris* L.)

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المُستلخص

أظهر اختبار قدرات نمطين وراثيين لنبات الفاصوليا، (الجديدة) و (تيما)، بعمر ثلاثة أسابيع تحت تأثير الإجهاد الملحي بكلوريد الصوديوم (من 30 ملمول إلى 150 ملمول) لمدة 10 أيام حساسية لتحمل تركيز معتدل للملح فيما يبدو أن الملوحة تحدث خللا في العلاقات المائية وكثافة الثغور وتؤثر على إنتاج الكتلة الحيوية لكافة اجزاء النبات بما في ذلك الجذور، الأوراق والسيقان. وقد لوحظ نفس السلوك الفسيولوجي للنمطين الوراثيين تحت الإجهاد الملحي على حد سواء مما يدل على وجود فارق كمي بدلا من وجود فارق نوعي بين النمطين الوراثيين الذين تم اختبارهما. و لقد لاحظنا تفوق النمط الوراثي " تيما" فيما يتعلق في المحافظة على توازن علاقاته المائية والنمو تحت تأثير الملوحة. وتوصي الدراسة بإجراء المزيد من البحوث الميدانية لتأكيد النتائج التي حققناها.

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الكلمات الدالة

مقاومة الملوحة، الفاصوليا، العلاقات المائية، النمو، الثغور.

Introduction

Common bean (*Phaseolus vulgaris* L.) is a major vegetable crop for human nutrition in the world (Bayuelo-Jiménes *et al*., 2002a). Beans are grown in a wide range of environments from sea level to high elevations (Pessarkli, 1993). However, common bean and other leguminous are regarded as appropriate crops for the enhancement of bioproductivity and the reclamation of marginal lands, because they not only yield nutritious fodder, protein rich seeds and fruits, but also are known to enrich the soil nitrogen in symbiotic association with rhizobium (Neel *et al*., 2002). They therefore, contribute a lot to the improvement of soil fertility in the semi-dry lands where most of the soils are already salinized (Bayuelo-Jiménes *et al*., 2002b).

Drought and high salinity are the two major environmental determinants of plant growth and agricultural productivity around the world (Flowers, 2004). Saline lands are not only distributed in arid regions but also frequently occur in fertile alluvial plains and many coastal regions (Ajmal khan *et al*., 2006). Generally, exposure to salt stress triggers many common reactions in plants that lead to cellular dehydration with concomitant osmotic changes (Sairam and Tyagi, 2004).

Physiological processes such as water status are highly sensitive to salinity and are, therefore, dominant in determining the plant's response to stress. Salinity reduces the ability of plants to utilize water and causes a reduction in growth rate, as well as changes in plant metabolic processes (Munns, 2002). Water stress induced by salinity may influence plant growth by adverse effects on dry matter partitioning, cell extension, cell division, leaf photosynthesis and/or transpiration (Munns, 2003). The effect of salinity stress on photosynthetic rate and water use efficiency was closely related to leaf anatomical features. The reduction of mesophyl conductance was associated with leaf thickness and smaller intercellular spaces in the mesophyl of salt-stressed leaves, which may have made the part towards the sites of CO_2 fixation more difficult (Omami, 2005).

The objective of this study was to understand the salt stress-induced mechanisms, at the whole plant level, that cause growth reduction by analyzing how salinity affects plant water relations and relative growth rate as well as its components in *P. vulgaris* genotypes. Evaluation of the physiological responses of common bean genotypes to salt stress induced by NaCl could serve for further introduction in the field furthermore to verify whether the existence of differential behavior within this species in order to exploit it for breeding programs.

Materials and Methods

(1) Plant Material and Culture

Seeds of two common bean genotypes *Phaseolus vulgaris*, Tema and Djadida, released by the Technical Institute of Crop Production (Algeria) were evaluated under salt stress. The seeds were surface sterilized with 5% (w/v) commercial bleach sodium hypochlorite solution (NaOCl) three times for 30 min with gentle stirring and subsequently washed in deionized water then germinated in sand. After 7 days, healthy and uniform seedlings with fully developed trifoliate leaves were transferred to aerated Hogland nutrient solution and grown in a culture chamber for up to 28 days under controlled conditions with light intensity of about 600 µmol $m^{-2}s^{-1}$ and 14h duration, 70 % relative humidity and 27/20 ºC day/night temperature. Three weeks after sowing, the following salt treatments were set up; 30 mM, 60 mM, 90 mM, 120 mM and 150 mM NaCl against plants grown in the nutrient solution to serve as a control. The solutions were renewed twice to three times a week to adjust pH toward 5.5 and minimize nutrient depletion. Plants were harvested and analyzed after 10 days from salt treatments.

(2) Morpho-Physiological Measures

Predawn water potential (Ψw) was measured with a pressure chamber of Scholander (1965). The leaf relative water content (RWC) of the uppermost fully expanded leaflets was measured before the harvest. The leaflets were detached and weighed (fresh weight, FW), floated on distilled water for 24 h at 5 ºC in the dark to allow turgidity to be regained and then re-weighed (turgid weight, TW), and dried during 48 h at 80 °C until constant weight to determine the dry weight (DW). The relative water content was calculated as RWC (%)=[(FW-DW)/(TW-DW)]x100.

Growth parameters were calculated according to Hunt (1990). The relative growth rate RGR ($g.g^{-1}.d^{-1}$) was calculated as the rate of increase of total dry weight per unit of plant dry weight. Leaves, petioles, and stems were excised and their fresh weight (FW) was immediately recorded. Roots were rinsed three times with distilled water and carefully dampened using tissue paper before their fresh weight was recorded. The samples were dried afterward during 48 h at 80 °C to determine dry weight (DW).

The density of stomata or the number of stomata per unit area was calculated on the upper and the lower epidermis of the uppermost fully expanded leaf. Imprints were made by coating a leaf area with clear nail varnish, covering with 'sellotape', applying pressure and replacing onto a glass microscope slide. Images of each slide were captured using a digital camera attached to a Zeiss microscope.

(3) Statistical Analysis

A randomized complete block design was used. Data were analyzed using the GLM procedure (SAS, 1985). Five replicates per treatment per genotype were used for growth and water relations analyses. Two-way analysis of variance was used to determine significant differences among genotypes for various traits. Treatment means were compared using protected Student-Newmen-Keuls test at $P \leq 0.05$. Many regressions and correlations based on the coefficient of Pearson were also established. Sum of square analysis was introduced in order to determine the genotypic contribution in plants responses regarding the expression of traits.

Results

In general, adverse effects of salinity treatment were observed when NaCl concentrations increased in the medium for all variables, independent of bean genotypes. The variance analyses show significant differences as among saline levels such as between common bean genotypes, for most variables studied. The interaction between the two factors analyzed (bean genotypes and saline levels) was not significant only for stem and root biomass of plants and the relative water content.

Salinity significantly affected leaf water content and water potential (Figure 1). Differences among genotypes were significant at any salt concentration. The results show that leaf water potentials of both common bean genotypes decreased significantly with increasing salt levels in the nutrient solution. Overall, Djadida had less negative values of water potential under NaCl treatments than Tema genotype.

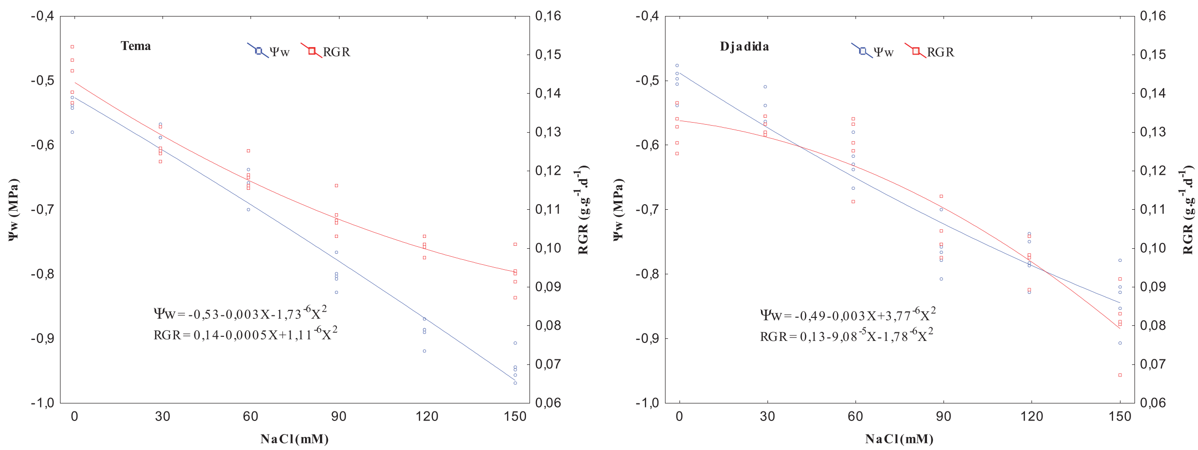

Figure 1: Water Potential and Relative Growth Rate Behavior of Two Genotypes of *Phaseolus Vulgaris* Subjected to NaCl Treatments.

Reduction of water potential was higher in Tema than in Djadida genotype. In fact, it decreases by 75% when control is compared to the treatment with 150 mM NaCl in Tema against a decline less than 50% in Djadida under the same conditions (see figure 1). It should be noted that up to 90 mM NaCl, decrease became more significant in both genotypes. According to the sum of square analysis, 90% of the total variation of water potential in this experiment was attributed to the saline constraint. Similar pattern was observed concerning leaf relative water content. RWC values ranged between 75% and 85% in both genotypes with higher values recorded in Tema genotype. The decrease of RWC for both genotypes tissues under salt stress was around of 85% and was significantly correlated with the decline of water potential (Ψw). Salt treatments contribute by 95% of the total expressed variability in water behavior.

Salinity caused also an increase in the stomata frequency in the upper face of leaves at about 55% for the genotype Tema and about 38% for the genotype Djadida comparing to their respective control. Observed values regarding this character were higher in Djadida genotype leaves under salinity below to 90 mM NaCl to be then comparable under high concentrations (Figure 2a). By the same, in the lower face of leaves, increase was around 43% for Tema genotype and about 21% for Djadida. Under salinity up to 90 mM NaCl, Tema genotype manifested higher values comparing to the other genotype (Figure 2b).

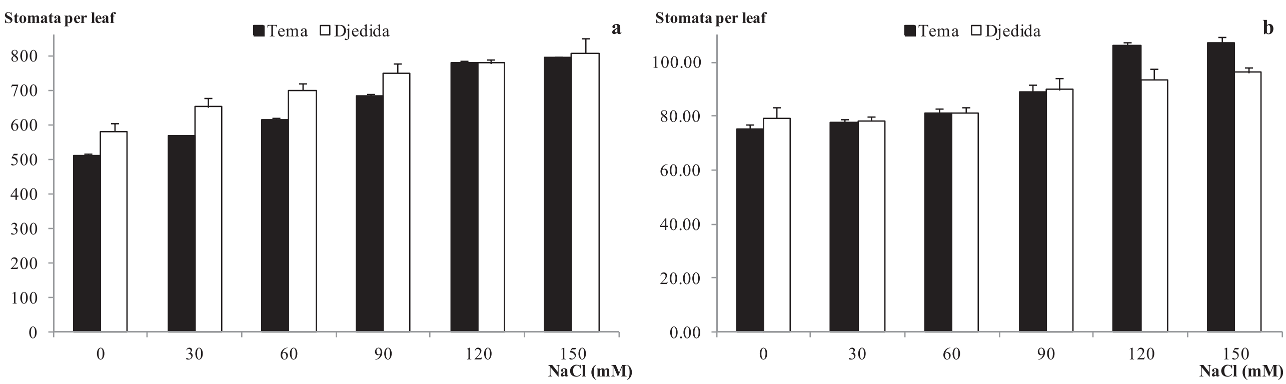

Figure 2 (a) &(b): Stomata Density Per Leaf of the upper (a) and lower (b) Epidermis of Two Genotypes of *Phaseolus vulgaris* Subjected to NaCl Treatments.

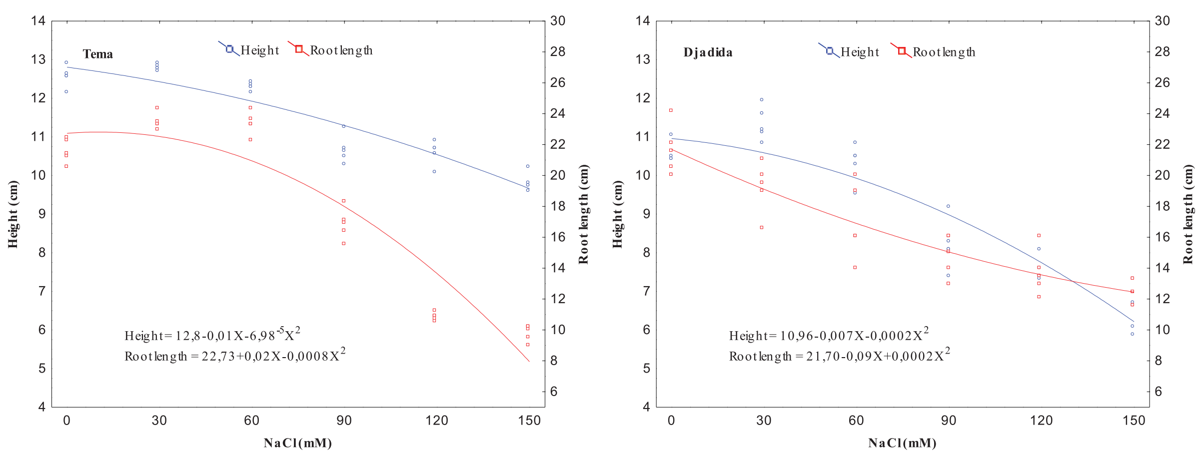

Figure 3: Plants Height and Roots Length of Two Genotypes of *Phaseolus vulgaris* Subjected to NaCl Treatments.

Relative growth rate (RGR) was also significantly affected by salinity levels as reflected by the genotypes. RGR decreased drastically in Tema under salt stress while it increased under the saline conditions until 60 mM NaCl and then declined considerably thereafter in Djadida (see figure 1). Differences in plant heights were highly significant among genotypes than among salinity levels. Decrease was of 80% under saline conditions for Tema however it was around 60% for Djadida. Genetic difference between genotypes was responsible for 40% of the total variability expressed for this trait. Plant root length was more developed in Djadida which showed higher values for unstressed conditions. For both genotypes, a slight amelioration was noted under low salinity level followed by severe decrease of 50% under higher NaCl concentrations (P<0.01**). About 80% of this decline was attributed to the salt stress (Figure 3).

Result regarding the plant biomass showed inverse relationship with salinity. In both genotypes, the growth parameters regarding roots, leaves and stems were adversely affected by the salt stress. It is striking to find out that independent of saline level; Tema genotype has presented higher leaves and stems biomass weights, dry and fresh weights, than Djadida genotype while root dry and fresh weights were much higher in the Djadida genotype. With salinity increased to 150 mM, biomass reduction tendency was more pronounced in Tema than in Djadida genotype (Figure 4).

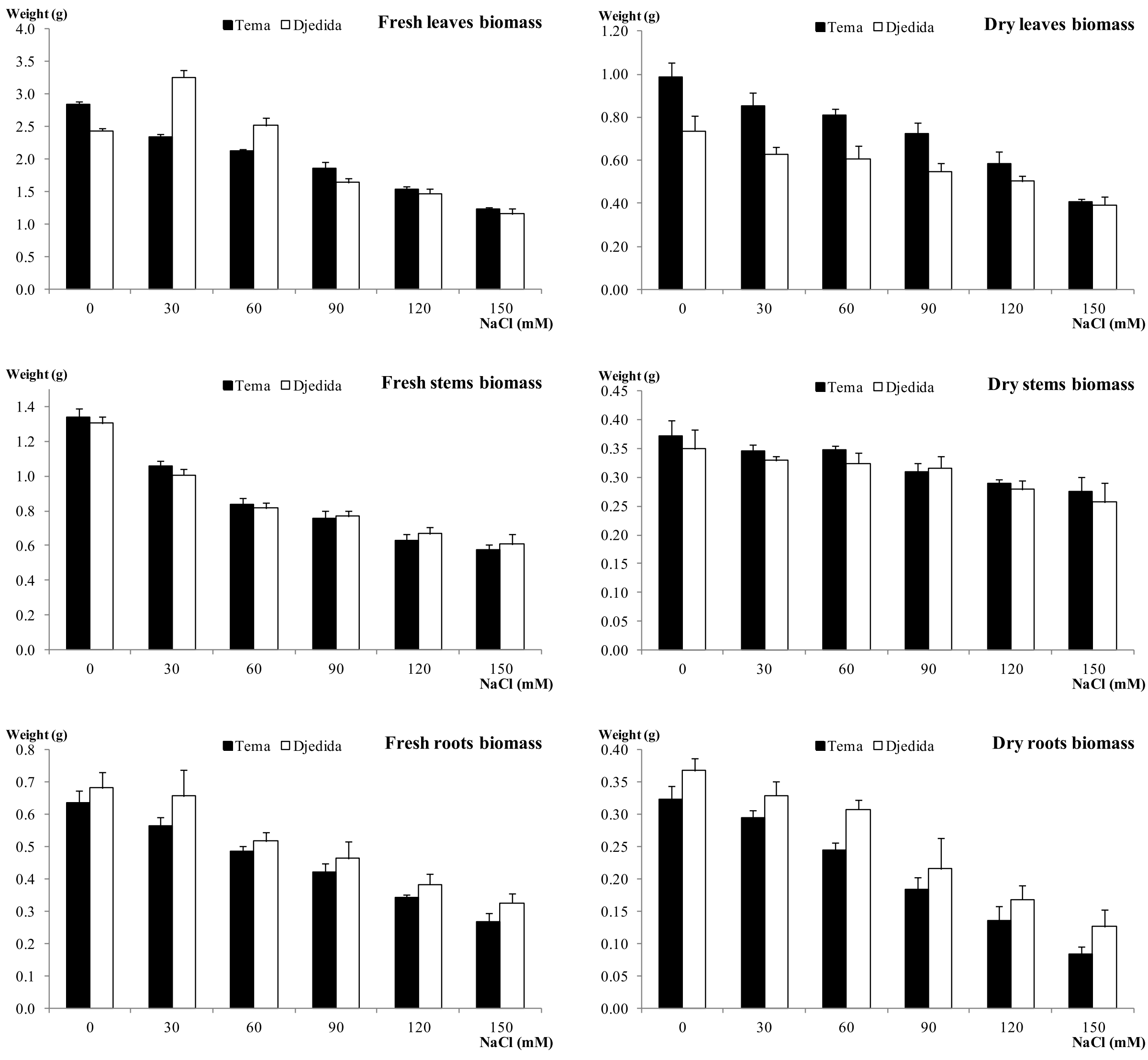

Figure 4: Fresh and Dry Organs Biomass of Two Genotypes of *Phaseolus Vulgaris* Subjected to NaCl Treatments.

Leaves fresh weight (LFW) decreased linearly by 43% in Tema however, significant increase of fresh weight was observed under treatments of 30 mM and 60 mM NaCl in Djadida leaves to decrease after that significantly by 47% compared to control. This variation was attributed mainly to salt constraint (86%). Similar linear decrease was observed for leaves dry weight in both genotypes, genotypic variability contributes by 20% while 70% of the total variability was due to salinity.

Similar significant decrease was noted for fresh and dry stems biomass of 40% and 70% respectively in both genotypes. The genetic part of this reduction was less than 10%. In spite of roots biomass, higher values were recorded in Djadida which suffered by 45% decrease under salinity against 40% in Tema genotype as function of salinity. Dry weight followed the same pattern with less than 30% reduction under stress. This variation expressed for this trait was mainly due to salinity (90%).

Discussion

Although NaCl is the major salt in most salt-affected soils, other salts play a combined role in the salt tolerance of a species (Marschner, 1995). Salinity had adverse effects on water content and biomass. Reductions in bean biomass under saline condition were indicative of severe growth limitations (Gama *et al*., 2007).

These results show that both bean genotypes had the same behavior under saline conditions. In addition, NaCl application affected all the plants mass production (roots, leaves and stems) and plant water relations. Physiologically, it is a quantitative rather than a qualitative difference between these genotypes. These results support similar findings of Foolad (1996) in tomato and Bayuelo-Jiménez *et al*. (2002b) in bean.

NaCl levels increase in the nutrient solution affected plant growth and development of both genotypes, thus it is in agreement with the investigation of Santana *et al*. (2003) who studied the influence of salinity on some bean species. The total water uptake decreased with increasing salinity, and the decrease patterns were similar to those of dry matter production as reported by Pessarkli (1993). Hillel (1999) reported that plant water content is drastically influenced by high salinity levels due to reduction on tissue osmotic potential, and, consequently, less root water absorption. In our experiment, the decrease of RWC in both genotypes was significantly correlated with the decline in water potential (Ψw) (r=0.78**). In contrast, Hu and Schmidhalter (2005) concluded that, the reduction on water uptake as function of salinity can be compensated by other parts with lower salinities and increasing root activity; this tendency was analyzed in our study by root length and fresh and dry biomass yields.

Both root dry weight (RDW) and root length of the genotypes were adversely reduced as salinity increased (r=-0.94**). Our results (Fig. 2&3) are in agreement with those of Wignarajah (1992) that salinity affected shoot growth more than root growth but contradict the findings of Cordovilla *et al*. (1999) that roots were more sensitive than shoots. As also observed for other dependent variables, root biomass had a linear decrease with salt increase (Bayuelo-Jiménez *et al*., 2002b). These authors also observed significant interaction between saline levels and bean species investigated, that demonstrates genetic variability between species and interdependence between factors. As also reported by Storey *et al*. (2003), the root system is one of the most important characters for salt stress because roots are in contact with soil and absorb water from soil, nevertheless Munns (2002) suggests that little is known about salinity effect on root system. However, Bayuelo-Jiménez *et al*. (2002a,b) reported that salt-tolerant species of Phaseolus maintained relatively high root growth even at 180 mM NaCl in nutrient solution.

The consequent increase in root to shoot growth seems to be associated with increased salinity tolerance in these species. It is possible that under salt stress the plant spends more photosynthetic energy on root production in search of water and/or reducing water loss and thus maintains a relatively high water relations (Kafkafi, 1991). Probably, avoidance of salinity by intensive root development was dependent on species or genotypes.

In *Phaseolus vulgaris* genotypes, concentrations higher than 60 mM NaCl caused stunted growth due to salt-induced reduction in photosynthates (Brugnoli and Lauteri, 1991). In this experiment, relative growth rates (RGR) increased under saline condition of 60 mM NaCl and then declined considerably for both genotypes (see figure).

Plant stem growth was significantly reduced by salinity (r=-0.9**). The direct contact of roots with the adversely saline environment contributes with a faster and higher salt absorption that deleteriously affects plant organs interfering the stem growth (Taiz and Zeiger, 2002).

Reductions in the biomass of *Phaseolus vulgaris* genotypes under saline condition were indicative of severe growth limitations. Salinity had adverse effects not only on the biomass, but also on other morphological parameters such as plant height, root length and shoot/root ratio especially for plants of indeterminate growth of Tema.

In several legumes, such as soybean (Grattan and Maas, 1988), faba bean (Belkhodja, 1996) and bean (*Phaseolus vulgaris* L.) (Wignarajah, 1992), salinity was reportedly found to reduce shoot and root weights. The degree of reduction in dry matter yield increased with the increasing salt-stress level and over time (Haouala *et al*., 2007). At high salinities, growth reduction might either be caused by a reduced ability to adjust osmotically as a result of saturation of the solute uptake system, or because of excessive demand on the energy requirements of such systems (Zhu, 2003). Other factors, such as nutrient deficiencies, may also play an important role (Marschner, 1995). It is hypothesized that increased medium salinity could restrict the synthesis of plant growth promoters such as cytokinins and increase the production of inhibitors such as abscisic acid (Xiong and Zhu, 2003).

The increased stomata density at higher salinity agrees with the observations in beans (Kaymakanova, 2008). This could be explained with the dwindling of the leaf cells as a result of the xeromorphic structure of the salt-treated plants. According to Culter *et al*., (1977) the reduction in sell size which generates stomata density increase appears to be a major response of cells to water deficiency that may be caused either by drought or salinity stress. Carimi *et al*., (2005) confirmed, as well, the decrease of the cell size including stomata in salt-treated plants. Water status is highly sensitive to salinity and therefore is dominant in determining the plant responses to stress (Stepien et *al*., 2006). Some authors have considered the reduction in cell size under drought to be drought adaptation mechanism (Omami, 2005).

Conclusion

In conclusion, NaCl application affected adversely roots, leaves and stems mass production and altered plant water relations and stomata density. It should be noted that the same physiological behavior was observed for both bean genotypes subjected to salinity which revealed the existence of a quantitative instead of a qualitative difference between the tested genotypes. Here we observed the superiority of the genotype Tema, for maintaining its growth and water relations under salt stress while further researches are necessary to validate our finding under field conditions.

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