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# EVALUATION OF GROWTH RESPONSE AND WATER RELATIONS OF TWO BEAN GENOTYPES (*PHASEOLUS VULGARIS* L.) FROM ALGERIAN SEMI-ARID REGIONS GROWN UNDER SALT STRESS

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**Summary.** The performance of two common bean genotypes *Djadida* and *Tema* tested under NaCl stress showed sensitivity to moderate salt concentrations. It appears that salinity produced an imbalance in water relations and affected growth parameters; NaCl application reduced roots, leaves and stems mass production and plant water content. A similar physiological behavior was observed in the two bean genotypes under saline conditions demonstrating the existence of quantitative rather than qualitative differences between the tested genotypes. We noted the superiority of the genotype *Tema* in maintaining water equilibrium by sinking water potential while sustaining higher growth rate under salinity.

***Keywords:*** Growth, *Phaseolus vulgaris*, tema, djadida, salinity tolerance, *w*ater relations.

***Abbreviations:*** Ψw – Water potential; RWC – Relative water content; RGR – Relative growth rate; S.S – Sum of square.

## INTRODUCTION

Drought and high salinity are the two major environmental determinants of plant growth and agricultural productivity in the world (Flowers, 2004). However, 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). The adverse effects of salt stress are usually less severe on salt-tolerant plants such as cotton than on the salt-sensitive species such as beans (Ajmal khan et al., 2006). Generally, exposure to salt stress triggers many common reactions in plants

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that lead to cellular dehydration with concomitant osmotic changes (Sairam and Tyagi, 2004).

Water status is highly sensitive to abiotic stress including salinity and is, therefore, dominant in determining the physiological plant 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). As salt stress occurs frequently and can affect most habitats, plants have developed several strategies to cope with this challenge. Among stress defense mechanisms are the inclusion and exclusion of toxic $Na^+$ ions (Foyer and Noctor, 2005).

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 soil nitrogen in symbiotic association with *Rhizobium* (Neel et al., 2002). Therefore, they 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).

The objective of this study was to evaluate the impact of salt stress on plant water relations and relative growth rate as well as its components in two bean (*P. vulgaris*) genotypes commonly cultivated in semi-arid regions in Algeria. Evaluation of the physiological responses of common bean genotypes to salt stress induced by NaCl could serve for further studies in the field to verify the differential behavior within this species in order to exploit it in breeding programs.

## MATERIALS AND METHODS

### Plant material and culture

Seeds of two common bean (*Phaseolus vulgaris*) genotypes, Tema and Djadida, released by the Technical Institute of Crop Production were surface sterilized with 5% (w/v) commercial bleach sodium hypochlorite solution (NaOCl) three times for 30 min, washed in deionized water, and then germinated in sand. After 7 days, healthy and uniform seedlings with fully developed trifoliate leaves were transferred to aerated 25% 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, relative humidity 70% and day/night temperature 27/20°C. Salt treatments were set up 21 days after sowing on the plants treated with 25% Hoagland solution supplemented with 30 mM, 60 mM, 90 mM, 120 mM and 150 mM NaCl. Plants grown in nutrient solution only served as control. The solutions were renewed twice to three times a week to adjust pH to 5.5 and minimize nutrient depletion. Plants were harvested and analyzed 10 days after salt treatment.

### Water status and growth determination

Predawn water potential ($\Psi w$) was measured the early morning with a pressure chamber of Scholander (1965). The growth parameters were calculated according to Hunt (1990). The mean 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. The leaf relative water content (RWC) of the uppermost fully expanded leaflets was measured before harvesting. The leaflets were detached and weighed (fresh weight, FW), floated on distilled water for 24 h at 5ºC in the dark to allow turgidity to be regained and then re-weighed (turgid weight, TW), then dried for 48 h at 80 °C until constant weight to determine the dry weight (DW). The relative water content was calculated as:

$$RWC\ (\%) = [(FW-DW)/(TW-DW)]x100$$

Absolute growth and relative growth rate were determined following a sequence of one plant from each treatment group until there were at least five replications, leaves, petioles, and stems were excised and their 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 afterwards for 48 h at 80°C to determine their DW.

### Statistical analysis

A randomized complete block design was used. Data were analyzed using the GLM procedure (SAS Institute, Cary, NC, 1985). Five to six replicates per treatment were used for growth and water relations analyses. Two-way analysis of variance was used to determine significant differences among genotypes for various traits. 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 (S.S) was introduced in order to determine the genotypic contribution to the plant response.

## RESULTS AND DISCUSSION

Generally, a negative effect of salinity was observed with increasing NaCl concentrations in the medium for all variables regardless of the genotype tested. For all studied parameters, the variance analysis showed significant differences among saline levels as well as between the two genotypes. Additionally, the interaction between the two factors analyzed (bean genotypes and saline levels) was not significant for the expression of stem and root biomass of plants and the relative water content.

Relative growth rate (RGR) of the two genotypes was significantly affected by salinity levels ($P<0.05^{*}$). RGR of Tema plants decreased linearly with the increase of NaCl concentrations in the medium while this parameter in Djadida plants remained almost unchanged until salinity reached a concentration of 60 mM NaCl and declined significantly thereafter (Fig. 1a). Plant height differed more significantly among genotypes ($P<0.01^{**}$) than among salinity levels ($P<0.05^{*}$). A decrease by 80% under saline conditions for Tema plants and around 60% for Djadida plants was observed. The genetic contribution was responsible for 40% of the total variability while salinity constraint contributed by 50%. Plant roots

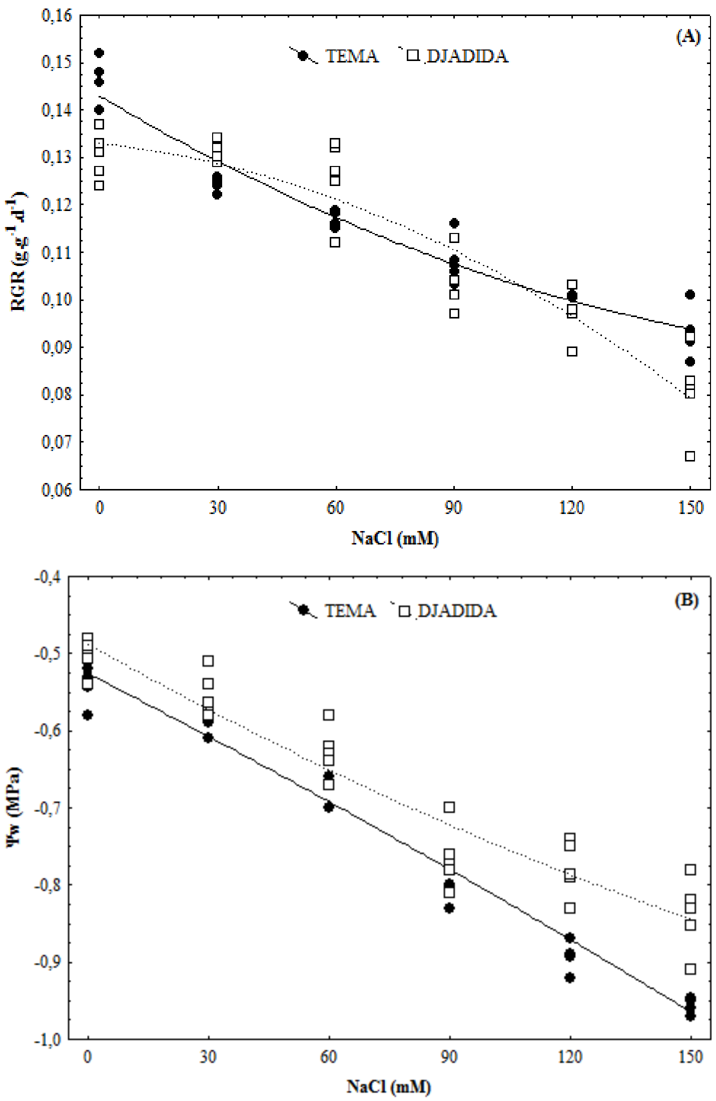

Fig. 1. Relative growth rate (A) and water potential (B) of bean genotypes subjected to salinity.

were better developed in Djadida plants which showed higher root length under controlled conditions. For both genotypes, a slight roots expansion was marked under low salinity, but a severe decrease by 50% was observed at higher NaCl concentrations ($P<0.01^{**}$). Variability in roots drop was mainly attributed to the salt constraint than to the genotype (S.S=80%). Results regarding the plant biomass showed inverse relationship with salinity; for both genotypes, the biomass of roots, leaves and stems were adversely affected by NaCl stress. It is striking that independently of saline level, Tema plants had higher leaves and stems biomass weights as well as DW and FW than Djadida plants while high roots DW and FW were more characteristic of Djadida genotype. Biomass reduction tendency was more pronounced in Tema than in Djadida plants with increasing salinity. Leaves fresh weight (LFW) decreased linearly by 43% in Tema plants, however, a significant increase of FW was observed under treatments with 30 mM and 60 mM NaCl in Djadida leaves to decrease thereafter significantly by 47% compared to the control. This variation was attributed mainly to the salt constraint (S.S=86%). A similar linear decrease was observed for leaves DW in both genotypes. The genotypic variability contributed by 20% while 70% of the total variability was due to salinity. A similar significant decrease was noted for fresh and dry stems biomass in both genotypes (by 40% and 70%, respectively) ($P<0.01^{**}$). The genetic part of variability for this reduction was less than 10%. In spite of roots biomass, higher values were recorded in Djadida plants which suffered by 45% decrease against 40% in Tema genotype plants with increasing salinity levels. DW followed the same pattern with less than 30% of reduction under salt stress. This variation was mainly due to the environmental constraint (S.S=90%).

Salinity significantly affected leaf water content and water potential (Fig. 1b). The results showed that leaf water potentials of both common bean genotypes decreased significantly with increasing salt levels in the nutrient solution ($P<0.01^{**}$). Djadida had less negative values of water potential under NaCl treatments than Tema genotype. The reduction was higher in Tema than in Djadida genotype. In fact, it decreased by 175% when control was compared to the treatment with 150 mM NaCl in Tema against a decline less than 150% in Djadida under the same conditions (Fig. 1b). It should be noted that up to 90 mM NaCl decrease became more significant in both genotypes. Only 5% of the total variation of water potential in this experiment was attributed to genotypic diversity while 90% was due to the saline constraint. A similar pattern was observed concerning leaf relative water content (RWC) under stress. RWC values ranged between 75% and 85% in both genotypes with higher values recorded in Tema genotype. The decrease of RWC in the tissues of both genotypes under salt stress was around 85% and it significantly correlated with the decline in water potential ($\Psi$w) ($r=0.78^{**}$). Salt treatments contributed by 95% to the total variability in water behavior of both genotypes.

Although NaCl is the major salt in most salt-affected soils, other salts also present in the soil play a combined role in the salt tolerance of a species (Marschner, 1995). In our study, salinity had adverse effects on biomass and water content of both tested genotypes. Reductions in bean biomass under saline conditions were indicative of severe growth limitations (Gama et al., 2007).

Our results showed that both bean genotypes had the same behavior under saline conditions, and, in addition, that NaCl application affected the whole plants, including roots, leaves and stems mass production as well as plant water relations. Physiologically, it seems to be 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, which was 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 ($r=0.82^{**}$) as reported by Pessarkli (1993). Hillel (1999) reported that plant water content was drastically influenced by high salinity levels due to reduction of tissue osmotic potential, and, consequently, less root water absorption. This result was confirmed in our experiment when the decrease of RWC in both genotypes significantly correlated with the decline in water potential ($\Psi$w) ($r=0.78^{**}$). In contrast, Hu and Schmidhalter (2005) concluded that the reduction of water uptake due to salinity can be compensated by other parts with lower salinities and increasing root activity. This tendency was analyzed in our study by root length ($r=-0.84^{**}$) plus fresh ($r=-0.80^{**}$) and dry ($r=-0.83^{**}$) biomass yields.

Also, both roots dry weight and roots length of the tested genotypes were reduced as salinity increased in the medium ($r=-0.9^{**}$). Our results (Fig. 2) are in agreement with those of Wignarajah (1992) that salinity affected shoot growth more than root growth, but contradict the

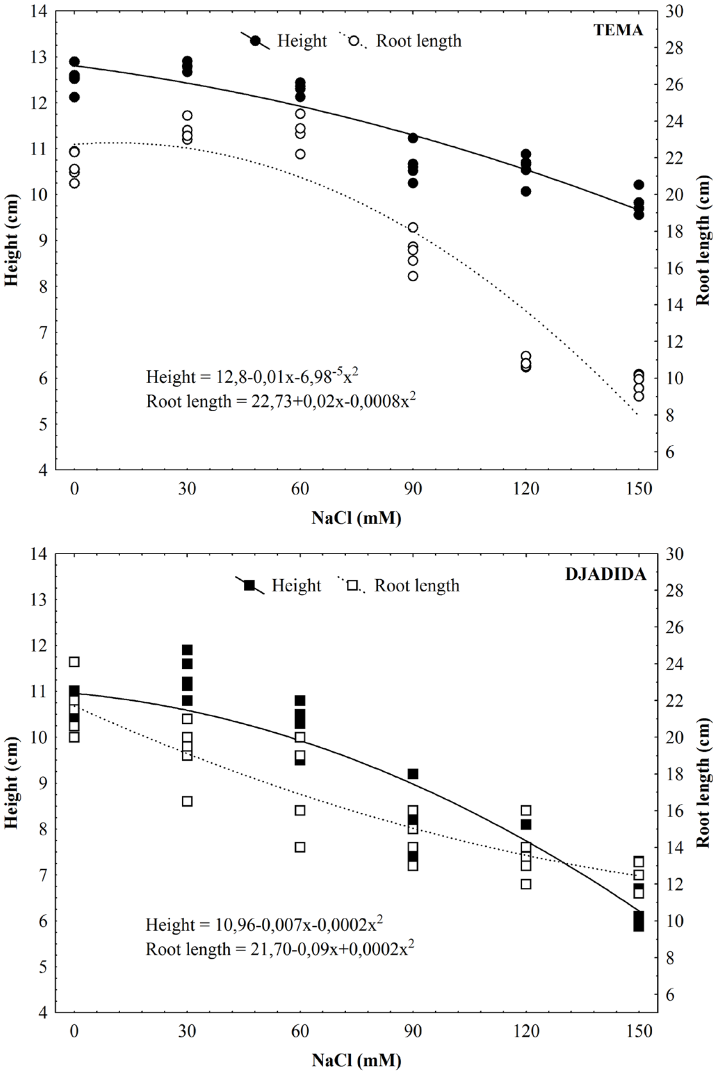

Fig. 2. Plants height and roots length of bean genotypes subjected to salinity.

findings of Cordovilla et al. (1999) that roots were more sensitive than shoots. As also observed for other dependent variables, root biomass decreased linearly with increasing salinity levels, as concluded by Bayuelo-Jiménez et al. (2002b). These authors also observed significant interaction between saline levels and bean species investigated, which demonstrated genetic variability between species and interdependence between factors. As 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 the soil. Nevertheless, according to Munns (2002) little is known about the salinity effects 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 the nutrient solution.

The consequent increase in root to shoot growth seemed to be associated with increased salinity tolerance in this species.

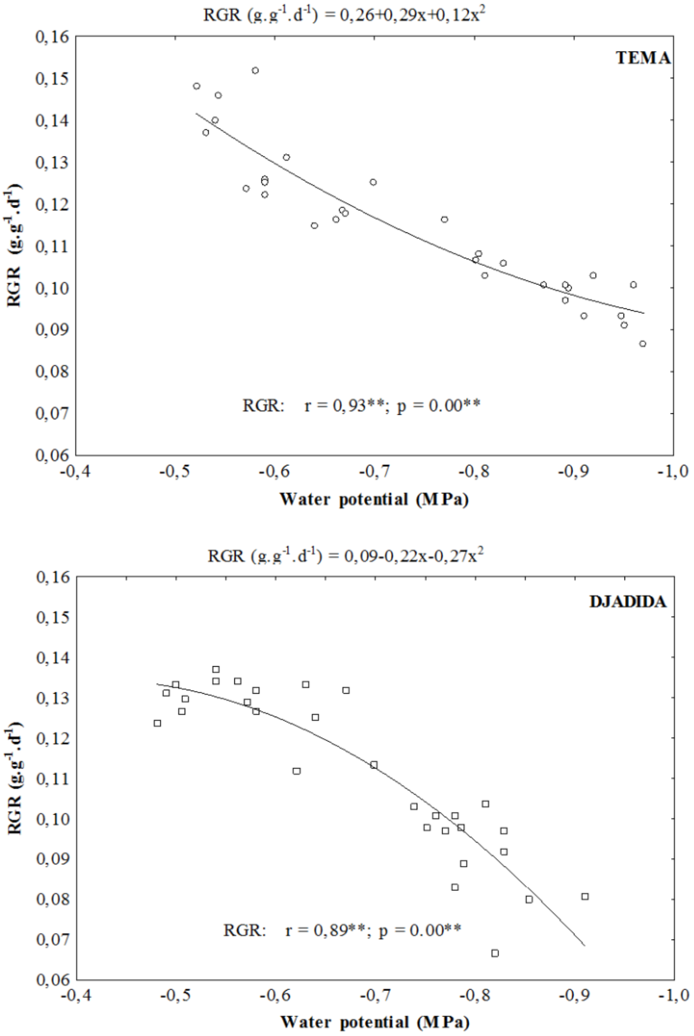

Fig. 3. Relative growth rate evolution against water potential of bean genotypes subjected to salinity.

It is possible that under salt stress the plant spends more photosynthetic energy for root production in search of water and/or reducing water loss and thus maintains relatively high water relations (Kafkafi, 1991). Probably, avoidance of salinity by intensive root development is 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 in both genotypes (Fig. 3).

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

Reductions in the biomass of *Phaseolus vulgaris* genotypes under saline conditions were indicative of severe growth limitations (r=-0.93**). Salinity had adverse effects not only on the biomass, but also on other morphological parameters such as plant height (r=-0.7**), root length (r=-0.86**) and shoot/root ratio especially in 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 found to reduce shoot and root weights. The degree of reduction in dry matter yield increased with increasing salt-stress level and over time (Haouala et al., 2007). At high salinities, growth reduction might be caused either by a reduced ability to adjust osmotically as a result of saturation of the solute uptake system, or because of an 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).

In conclusion, NaCl application affected the whole plants, including roots, leaves, stems mass production and plant water relations. A similar physiological behavior under saline conditions was observed in the two bean genotypes demonstrating existence of a quantitative rather than a qualitative difference between the tested genotypes. We noted the superiority of the genotype Tema to maintain water equilibrium by sinking water potential while sustaining higher growth rate under salinity. Further research is required to confirm these results under field conditions.

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