

## DIFFERENCES IN RESPONSE OF SOME TUNISIAN CHICKPEA GENOTYPES (*CICER ARIETINUM* L.) TO SALINITY

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### Abstract

A greenhouse experiment was conducted to assess the effects of salt stress on plant growth and mineral accumulation in three Tunisian chickpea genotypes (*Cicer arietinum* L.). NaCl was added and salinity was maintained at 0, 25 and 50 mM NaCl (EC: 0, 2.65 and 5.3 mS.cm<sup>-1</sup> respectively). In comparison to Chetoui and Kesseb, Amdoun maintained a high level of whole plant growth. Genotypic differences were observed at the level of Na<sup>+</sup>, Cl<sup>-</sup> and K<sup>+</sup> distribution between different plant organs. Amdoun showed the lowest leaf Na<sup>+</sup> and Cl<sup>-</sup> concentrations and the highest K<sup>+</sup> concentrations in these organs, particularly in the presence of high salinity (50 mM). These findings suggest that the relative salt tolerance of Amdoun may be due to its K<sup>+</sup>/Na<sup>+</sup> discrimination. This genotype seem to be able to protect its photosynthetic apparatus against the toxic Na<sup>+</sup> and Cl<sup>-</sup> ions, and to ensure an appropriate K<sup>+</sup> supply to the plants under salt stress.

### Introduction

Chickpea (*Cicer arietinum* L.) is one of the world's most important but lesser-studied leguminous food crops with nearly 10 million hectare grown across the Americas, the Mediterranean basin, East Africa, the Middle East, Asia and Australia (Jayashree *et al.*, 2005). While in the developed world it represents a valuable crop for export, in the developing world it provides a protein-rich supplement to cereal-based diets. Chickpea seed contains 13% to 33% protein, 40% to 55% carbohydrate and 4% to 10% oil (Stallknecht *et al.*, 1995). Fatty acid composition varies with chickpea type but is approximately 50% oleic and 40% linoleic (Duke, 1981). Chickpea seeds are consumed fresh as green vegetable, parched, fried, roasted and boiled; as snack bar, sweet and condiments.

Chickpea is generally grown without irrigation, planted in the post-rainy season, surviving through to harvest on progressively increasing drought and salinity. Thus, chickpea grows during the time of the year when many other legumes are rarely cropped. Efforts to identify genotypes underlying salinity tolerance are mostly focused on model species and major cereal crops such as rice and maize (Bruce *et al.*, 2002; Nguyen *et al.*, 2004) with lesser attention being given to legumes. Although it is considered to be a salt-sensitive species, the selection and breeding of cultivars that can grow under saline conditions constitute a more effective method to minimize the repercussion of exposure to salinity (Ashraf & McNeilly, 2004).

Salinity is a worldwide problem in irrigated areas. In the Mediterranean basin, the percentage of irrigated soils affected by salinity amounts to about 20%, varying from country to country between 7 and 40% (Hamdy *et al.*, 1995). The legumes are generally sensitive to salt stress (Laüchli, 1984). Nevertheless a big inter-specific variability has been observed. It is established that common bean, chickpea and pea are the most sensitive

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legumes to salinity (Cordovilla *et al.*, 1986; Soussi *et al.*, 1999), whereas soybean is the most tolerant (Delgado *et al.*, 1994). In *Melilotus segetalis*, the salt stress (170 mM NaCl) inhibits the vegetative growth, reduced the shoot biomass, as well as the efficiency of these organs to use the nutrients (K, Ca) for the production of biomass (Romero & Maranon, 1996). Salinity also modifies the distribution of the dry matter and nutrients between the organs. These effects depend on the stress severity and the age of the plants. Under moderate salinity, the growth of the roots is more severely affected than shoot driving to a reduction of the ratio, roots / shoots. This effect becomes more pronounced with the age of the plants (Romero & Maranon, 1996). Under these conditions of stress, plants seem to acquire syndromes of resistance to stress with particularly a slow growth, a weak acquirement of the mineral resources and a preferential allowance of the carbon for the roots. Of such features confer to the plants a relative tolerance to the nutritional disruptions (Chapin, 1991) and a better protection against the Na<sup>+</sup> ions and Cl<sup>-</sup> (Romero & Maranon, 1996; Ashraf, 2004; Ashraf *et al.*, 2008).

In saline soils, NaCl toxicity is known to disturb nutrient absorption by plants. In particular, the absorption of K<sup>+</sup> is inhibited; an effect which enhances the content of Na<sup>+</sup> in tissues (Greenway & Munns, 1980). Ashraf & McNeilly (2004) suggested that the maintenance of a high tissue K<sup>+</sup>/Na<sup>+</sup> ratio by a plant under salinity may serve as reliable criteria of salt-tolerance. However, some genotypes may have a certain degree of salt tolerance (Soussi *et al.*, 1999; Rao *et al.*, 2002). Serraj (2002) suggest that differences in salt tolerance occur not only in different species, but also in different genotypes of the same species.

In the present work, we have studied the effect of salinity on chickpea growth and mineral distribution of plants when they depend on mineral nitrogen for their N nutrition in three Tunisian chickpea genotypes viz., Amdoun, Kesseb and Chetoui. The mechanisms by which the tolerant genotype of chickpea overcomes the toxicity of sodium and chloride were investigated.

## Materials and Methods

**Plant growth and culture condition:** This work was conducted on three local genotypes of chickpea (*Cicer arietinum*): Amdoun, Kesseb and Chetoui. Healthy seeds of uniform size were disinfected with 2% Calcium hypochlorite solution, and were germinated in 1-liter plastic pots filled with sterile humidified sand. Cultures were conducted in a greenhouse under natural light. Plants were irrigated with the following nutrient solutions: Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O (3.5 mM), KNO<sub>3</sub> (3 mM), NH<sub>4</sub>NO<sub>3</sub> (2mM), MgSO<sub>4</sub>·7H<sub>2</sub>O (1.5 mM), KH<sub>2</sub>PO<sub>4</sub> (1.6 mM), K<sub>2</sub>HPO<sub>4</sub> (0.3 mM), H<sub>3</sub>BO<sub>3</sub> (4 μM), MnSO<sub>4</sub> (4 μM), ZnSO<sub>4</sub> (1 μM), CuSO<sub>4</sub> (1 μM), CoCl<sub>2</sub> (0.12 μM), (Na)<sub>6</sub>(Mo)<sub>7</sub>O<sub>24</sub> (0.12μM) and Fe-K-EDTA (45 μM).

After 2 weeks, plants were separated into three plots of 10 plants each. The first plot received nutrient solution without NaCl (control), while the two other batches received the same nutrient solution containing 25 mM and 50 mM NaCl (EC:2.65 dS m<sup>-1</sup> and 5.3 dS m<sup>-1</sup>, respectively). After 4 weeks of treatment; the plants were separated into shoots and roots, dried at 60 °C for 72 hours, and then pulverized into a fine powder.

**Chemical analysis:** For nitrogen, samples of dry matter were digested in hot concentrated H<sub>2</sub>SO<sub>4</sub>, as described by Kjeldahl. After extraction in 0.5% HNO<sub>3</sub>, K<sup>+</sup>, Na<sup>+</sup> and Cl<sup>-</sup> ions were measured according to Pauwels *et al.*, (1992). Chloride was assayed using a chloridometer (Buchler), while Na<sup>+</sup> and K<sup>+</sup> were quantified by flame emission photometry (Corning, UK).

**Statistical analysis:** Analysis of variance (ANOVA), using the AV1W MSUSTAT program with orthogonal contrasts and mean comparison procedures, was performed to detect differences between the treatments. Separation mean was carried out using the multiple range test with Fisher's least significant difference (LSD) ( $P < 0.05$ ) (Slama *et al.*, 2007).

## Results

**Plant growth:** Independently of salt treatment, Amdoun produced more biomass than the other genotypes, Kesseb and Chetoui. The submission of plants to 25 mM NaCl decreased plant biomass by 8%, 19% and 24% respectively in Amdoun, Kesseb and Chetoui. When plants are subjected to high salt stress (50 mM NaCl), Amdoun remain the most productive genotype with only 10% of biomass decrease when this biomass decreased by 45% and 50%, respectively in Kesseb and Chetoui genotypes (Fig. 1).

In order to check the effect of salt stress on plant organs growth, we calculated root / shoot (R/S) ratio (Table 1). Obtained results show that salinity decreased R/S ratio in all genotypes indicating that roots are more sensitive to salt stress than shoots. In Amdoun, R/S decreased by 8% at 25 mM NaCl and 39% at 50 mM NaCl. In the other genotypes, this ratio decreased by 30% and 49% in Kesseb and 18% and 56% in Chetoui, respectively at 25 and 50 mM NaCl. In the two salt treatments, roots of Amdoun were less affected by salt stress than the other genotypes.

**Nitrogen and mineral distribution:** According to genotypes and salt stress, N concentration was the highest in shoots (Fig. 2A) than the roots (Fig. 2B). The submission of plants to salt stress decreased nitrogen concentration in shoots, particularly in Kesseb and Chetoui genotypes. This decrease was estimated to 14 and 17% in Amdoun, 16 and 33% in Kesseb and 26 and 38% in Chetoui, respectively at 25 and 50 mM NaCl. In the roots, we noted also a significant decrease of N concentration in Kesseb and Chetoui at 50 mM NaCl.

Taken together, the plant growth and nitrogen nutrition results discriminate clearly Amdoun from the other genotypes Kesseb and Chetoui. These results let us think that this genotype is more tolerant to salt stress than the other ones and arrange some specific mechanisms in order to protect its tissues against their overload with toxic ions,  $\text{Na}^+$  and  $\text{Cl}^-$ . Independently of salt severity and genotypes, leaves accumulated less sodium than roots (Fig. 3A,B). At 25 and 50 mM NaCl,  $\text{Na}^+$  accumulation increased three times in leaves of Amdoun, as compared to control plants. However, in Kesseb and Chetoui,  $\text{Na}^+$  accumulation increased three times at 25 mM NaCl and about 4 times at 50 mM NaCl (Fig. 3A). This result indicated that Amdoun genotype had a better leaves protection against toxic sodium. In roots, we noted an increasing gradient of sodium accumulation with salt severity (Fig. 3B).

An evaluation of  $\text{Cl}^-$  accumulation in Amdoun shows that this genotype systematically accumulates less  $\text{Cl}^-$  in its leaves than Kesseb and Chetoui (Fig. 4A). In these organ,  $\text{Cl}^-$  accumulation increase with increasing NaCl concentration in the medium. In roots, Amdoun accumulated less chloride than the other genotypes at 25 mM NaCl. At 50 mM NaCl, an inverse behavior was observed (Fig. 4B). As for sodium, it seems that at high salinity Amdoun genotype accumulated high chloride in its roots in order to protect leaves against their overload with this ion. In addition, the obtained results showed that in leaf tissue, the levels of chloride accumulation surpassed those of sodium accumulation. It is known that this repartitioning of ions is an exclusive character in some glycophyte plants and particularly chickpea.

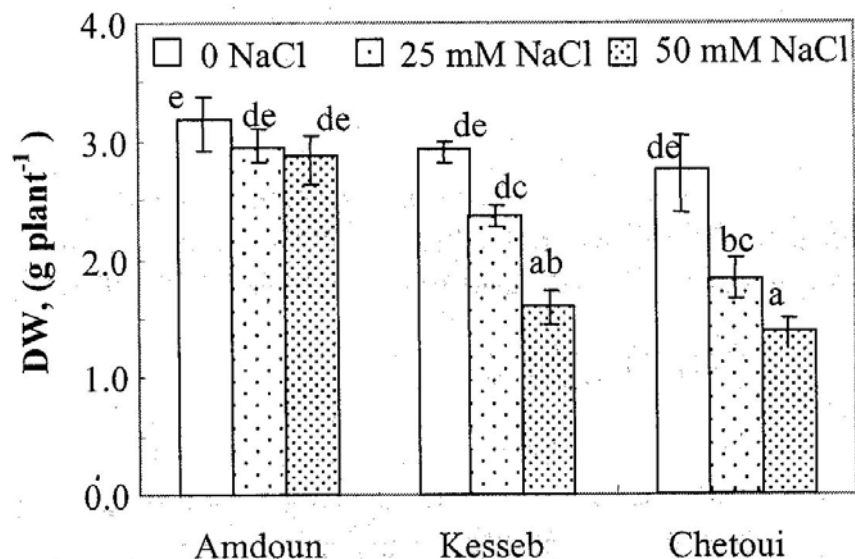


Fig. 1. Effect of salt stress on growth of chickpea (*Cicer arietinum* L.) genotypes. Data are means of 10 replicates ( $\pm$  standard error;  $p = 0.05$ ), harvested 42 days after sowing.

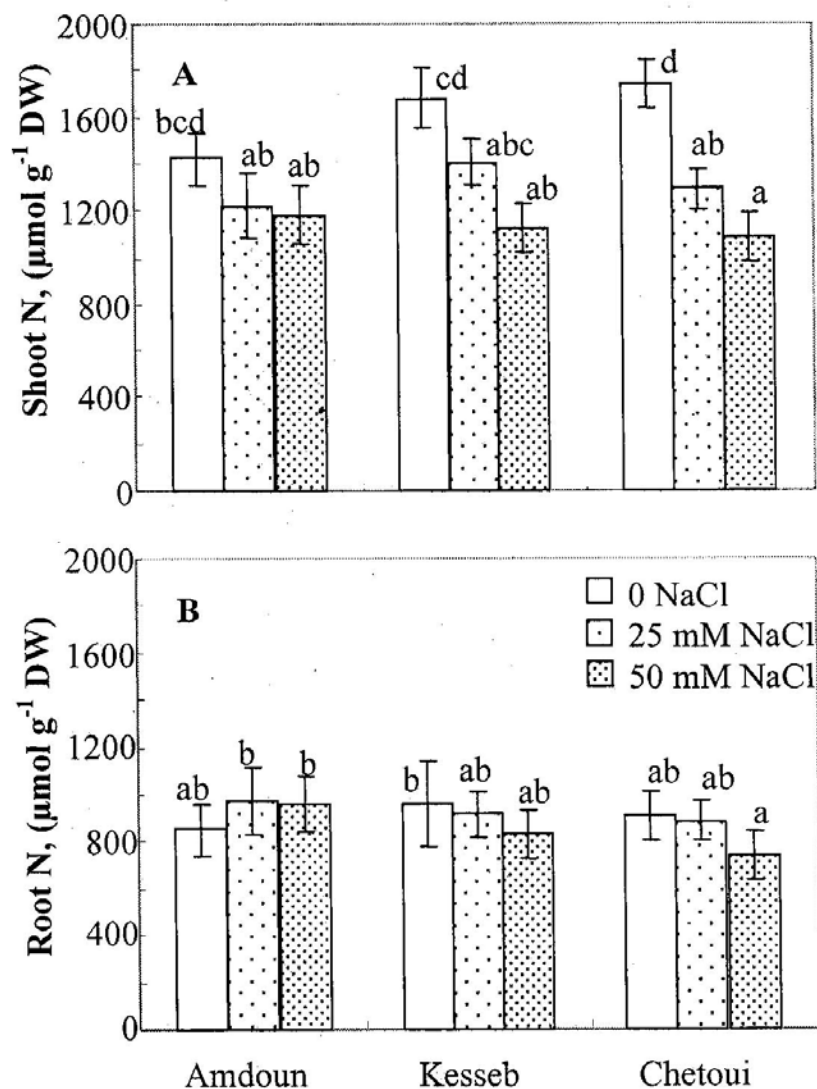


Fig. 2. Effect of salt stress on nitrogen assimilating activity in chickpea (*Cicer arietinum* L.) genotypes. Data are means of 10 replicates ( $\pm$  standard error;  $p = 0.05$ ), harvested 42 days after sowing.

**Table 1. Variation of the Root/Shoot ratio (R/S) in three chickpea (*Cicer arietinum* L.) genotypes, according to salt concentration.**

NaCl (mM)	0	25	50
Amdoun	1.08 ± 0.085	0.88 ± 0.071	0.66 ± 0.042
Kesseb	1.16 ± 0.10	0.81 ± 0.065	0.60 ± 0.039
Chetoui	1.19 ± 0.091	0.98 ± 0.081	0.52 ± 0.031

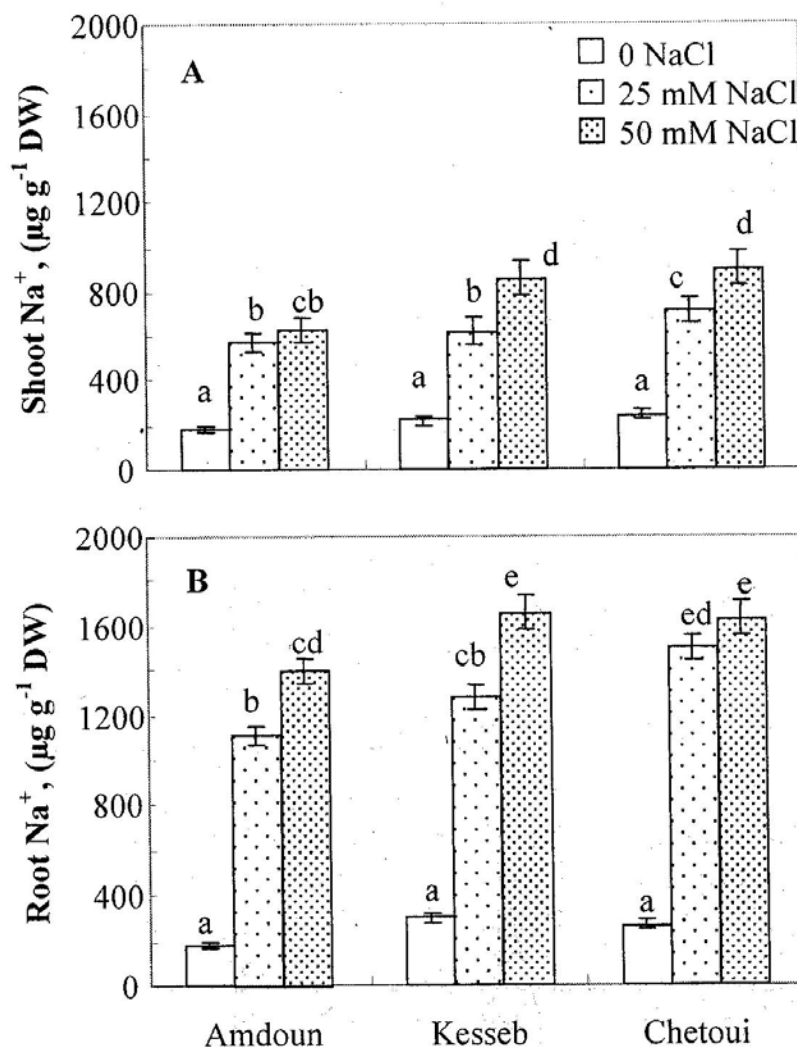


Fig. 3. Sodium accumulation in leaves (a) and roots (b) of chickpea (*Cicer arietinum* L.) genotypes, in response to. Data are means of 10 replicates ( $\pm$  standard error;  $p = 0.05$ ), harvested 42 days after sowing.

In leaves, K<sup>+</sup> accumulation increased slightly when plants are subjected to 25 mM NaCl. With 50 mM NaCl, plants of Amdoun continue to accumulate potassium at high level, whereas no significant modification was observed in Kesseb and Chetoui (Fig. 5A).

In Amdoun roots, we noted a gradual decreasing of potassium accumulation with NaCl concentration. In Kesseb and Chetoui, potassium accumulation decreased at 25 mM NaCl but not at 50 mM NaCl. These results support our previous hypothesis concerning leaves protection. In fact, it seems that at high salinity, Amdoun root accumulates the toxic ions, sodium and chloride, in order to protect the other organs. This mechanism is poorly pronounced in Kesseb and chetoui plants.

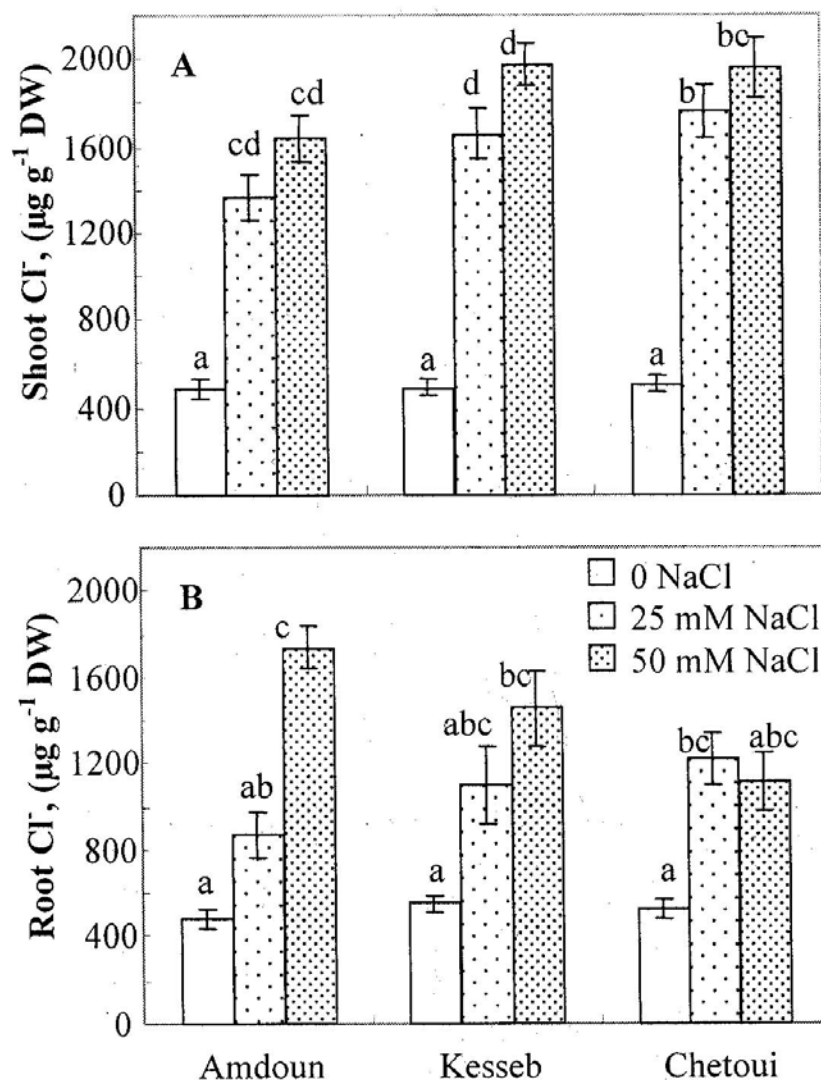


Fig. 4. Effect of salt stress on chloride accumulation in leaves (a) and roots (b) of chickpea (*Cicer arietinum* L.) genotypes. Data are means of 10 replicates ( $\pm$  standard error;  $p = 0.05$ ), harvested 42 days after sowing.

## Discussion

Our results demonstrated that salinity significantly affects whole plant growth in chickpea. Nevertheless, genotypic differences were observed when three genotypes were exposed to salt stress. Specifically, Amdoun expressed a high potential of growth, whereas, the growth of Kesseb and Chetoui was severely hindered under salt stress. In agreement to previous studies, this deleterious effect was more pronounced in Chetoui and Kesseb than Amdoun. In fact, it has been largely documented that salt stress limits plant productivity in legumes through diminished photosynthetic efficiency and carbon metabolism (Ferri *et al.*, 2000; Garg & Singla, 2004). Velagaleti & Marsh (1989) demonstrated that salt stress inhibit the shoot growth and decreased the intrinsic photosynthetic capacity. Lodhli *et al.*, (2009) showed a decreased water content of both root and shoot portions in the presence of NaCl in the rooting medium of wheat. In Maize, the reduction of the growth under salt stress is related to sensitivity of the photosynthetic parameters (Cha-um & Kirdmanee, 2009). In accordance with plant growth, nitrogen assimilation decreased under salt stress, but Amdoun genotype

maintains high capacity of nitrogen assimilation than the other genotypes. In general, only 20-50% of the soil nitrogen is recovered by the annual crops (Khan *et al.*, 2009), salinity reduces more N accumulation in plants (Feigin, 1985). This result let us think that this genotype maintains a better photosynthetic apparatus under salt stress. In fact, the calculation of the salt intensity index (Table 2) based on the plant dry weight of all stressed (DWs) and control (DWc) plants ( $SII = 1 - DWs / DWc$ ; Fischer & Maurer (1978) demonstrated that Amdoun maintained the lowest values at low (25 mM NaCl) and high salt stress (50 mM NaCl). At 25 mM NaCl, SII was 2 times more important in Kesseb than Amdoun and 3 times more important in Chetoui than Amdoun. At 50 mM NaCl, SII was 4.5 times more important in Kesseb than Amdoun and 5 times more important in Chetoui than Amdoun. These results confirm the relative tolerance of Amdoun genotype and the particular sensitivity of Chetoui.

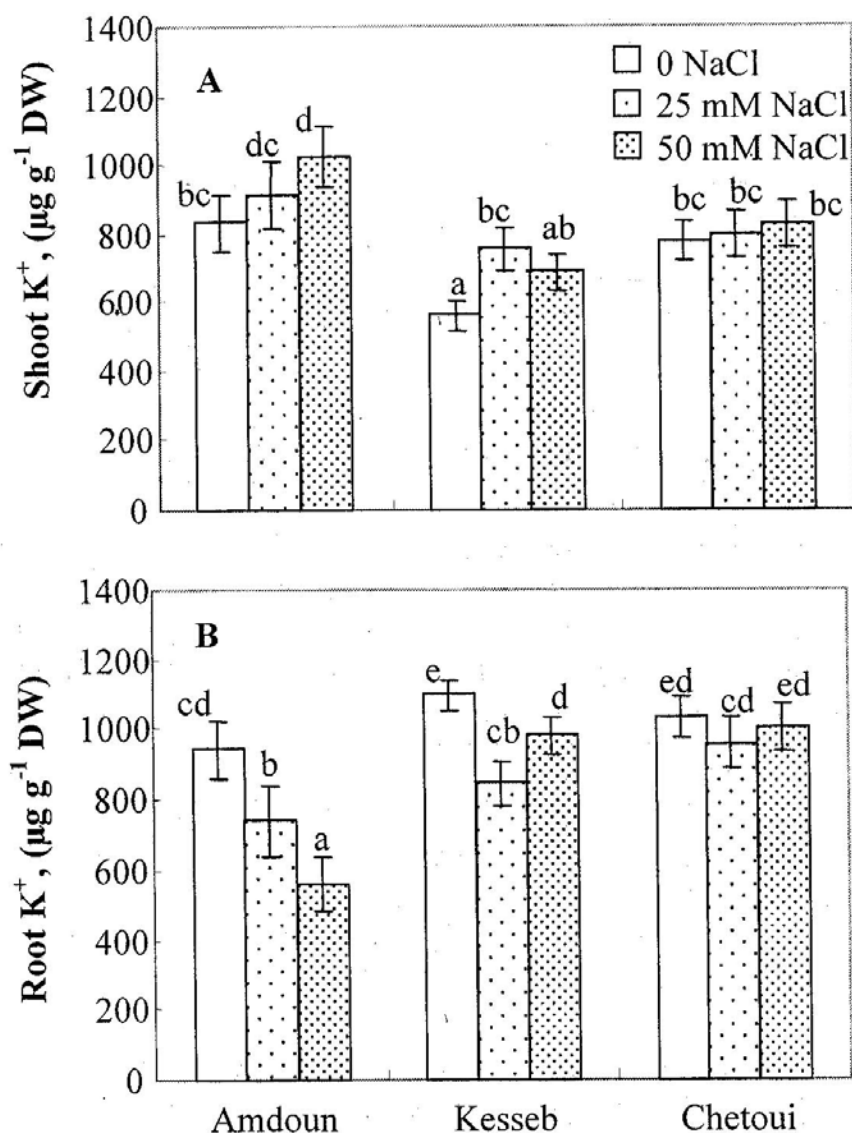
The mineral distribution in plants seems to play a primordial role in this performance. In fact, our  $Na^+$  and  $Cl^-$  analyses demonstrated that Chickpea accumulated sodium and chloride mainly in roots. It is reasonable to consider that this mechanism may in fact ensure a limited Na and Cl toxicity in shoots. Amdoun is the most performant genotype in this mechanism at high salinity (50 mM NaCl). Although the values of K/Na ratio (Table 3) decreased with salinity in all genotypes, K/Na ratios obtained from leaves are usually higher than those in roots. In Amdoun, the K/Na ratio was the highest in leaves (1.61) and the lowest (0.42) in roots at 50 mM NaCl, comparatively to the other genotypes. This result indicates that this particular genotype (Amdoun) had much more potassium than sodium concentrations in leaves and the inverse behavior in roots. These last organs play the role of barrier for sodium translocation to shoots. In the other genotypes, we noted less Na accumulation in roots than in leaves and as a result, photosynthetic apparatus suffer greater damage from exposure to these toxic ions. In fact, the Figure 6 which lies shoot growth to their sodium concentration show that these organs are highly dependent from their sodium content. Amdoun genotype had the lowest Na concentration with the highest shoot growth, particularly at 50 mM NaCl. The same behavior was observed when representing shoot growth in connection with their chloride. Amdoun genotype had the lowest chloride concentration even at high salinity (Fig. 7). Several studies indicated that the adverse effects of salinity on photosynthesis have usually been associated with chloride and sodium toxicity and/or disruption of leaf water relations (Walker *et al.*, 1982; Lloyed *et al.*, 1987, 1989, 1990). Generally the variation in ions concentration on different organs has been associated with tolerance of legumes to salt stress (Fortmeier & Schubert, 1995; Tejera *et al.*, 2006). Salinity induced a clear increase of shoot sodium concentration in chickpea genotypes and the effect was less pronounced in Amdoun. This genotype accumulates sodium and chloride, mainly in roots. Amzallag *et al.*, (1990) and Behl & Jeschke (1981) demonstrated that ABA treatment reduces Na and not alters K in shoot tissue of *Sorghum* and increases the vacuolar Na and strong inhibits the xylem transport in barley roots. Concerning the ionic toxicity, it is established that this toxicity is reduced in common bean by two physiological mechanisms: (a) sodium exclusion in leaves (Jacoby, 1968) and/or (b) restriction of sodium transport to shoots (Montero *et al.*, 1997). In addition, the maintenance of high K/Na ratio has been suggested as criteria of salt-tolerance in brassicas (Ashraf & McNeilly, 2004). Our results in Table 3 showed a significant decrease of the K/Na ratio with salt stress in all genotypes, similar to those reported in shoot of rice (Bohra *et al.*, 1995). Bohra *et al.*, (1995) reported increases in the K/Na ratio with ABA and concluded that hormone might be a mean to alleviate reduction in K/Na with saline stress and thus facilitates salinity tolerance.

**Table 2. Salt intensity index (SII) in three chickpea (*Cicer arietinum* L.) genotypes, according to salt concentration. This parameter was calculated on the basis of plant growth,  $SII = 1 - DW_s / DW_c$ .**

NaCl (mM)	25	50
Amdoun	0.08 ± 0.011	0.10 ± 0.012
Kesseb	0.19 ± 0.015	0.45 ± 0.039
Chetoui	0.24 ± 0.018	0.50 ± 0.031

**Table 3. Variation of the potassium/sodium ratio (K/Na) in leaves and roots of three chickpea (*Cicer arietinum* L.) genotypes, according to salt concentration.**

NaCl (mM)	Leaves			Roots		
	0	25	50	0	25	50
Amdoun	4.47±0.35	1.59±0.12	1.61±0.13	3.7±0.31	0.67±0.07	0.42±0.04
Kesseb	2.55±0.18	1.21±0.11	0.80±0.06	3.62±0.28	0.66±0.05	0.59±0.05
Chetoui	3.23±0.25	1.13±0.09	0.91±0.08	3.88±0.32	0.64±0.06	0.62±0.05



**Fig. 5. Potassium accumulation in leaves (a) and roots (b) of chickpea (*Cicer arietinum* L.) genotypes. Data are means of 10 replicates (± standard error;  $p = 0.05$ ), harvested 42 days after sowing.**



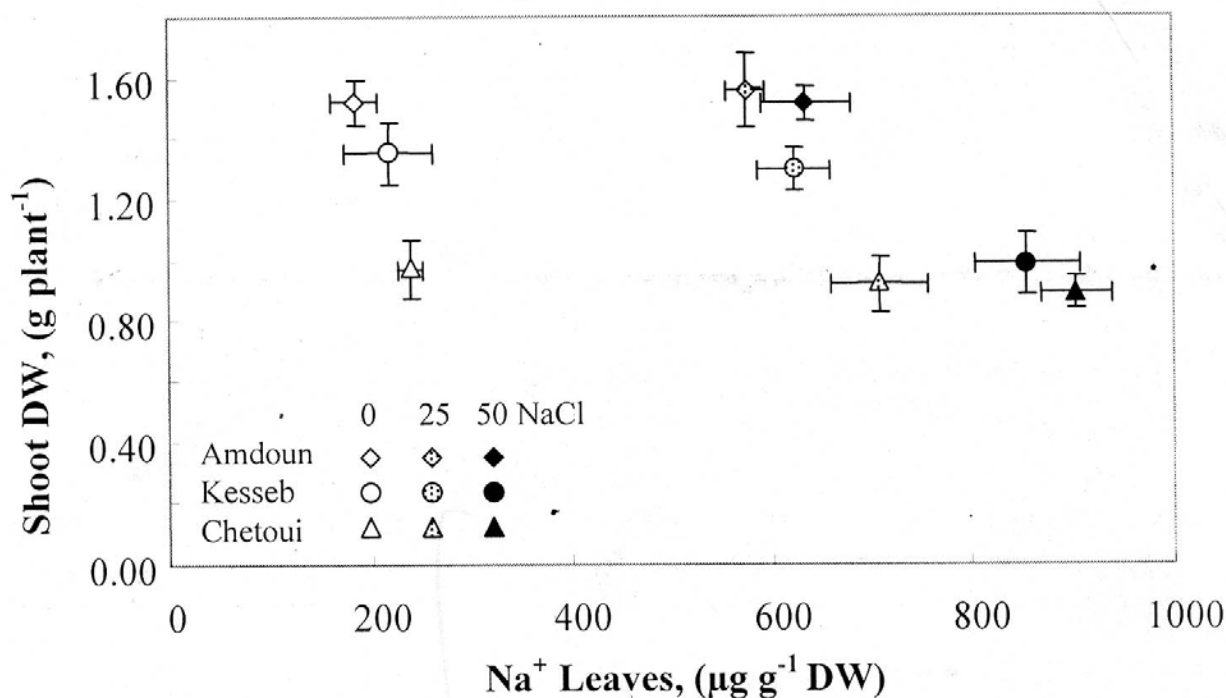


Fig. 6. Relationship between shoot growth (g.plant<sup>-1</sup>) and their sodium concentration (µmol .gDW<sup>-1</sup>). Vertical and horizontal bars represent ± standard errors of means of 10 replicates.

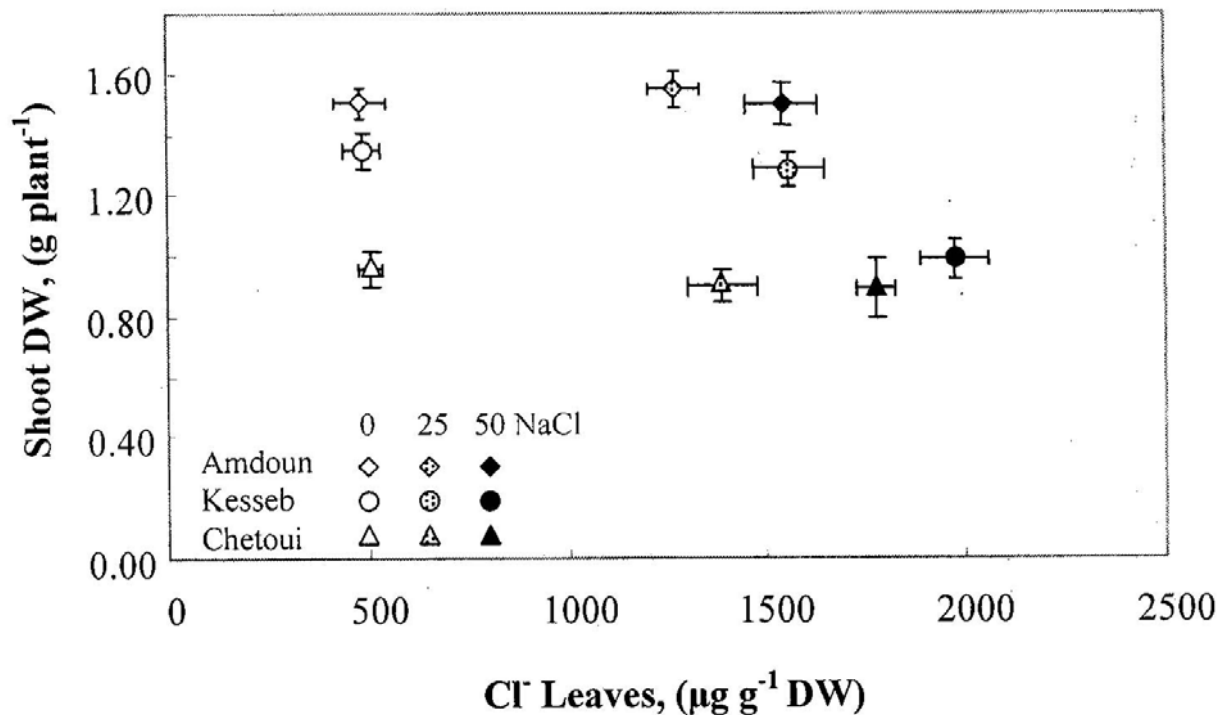


Fig. 7. Relationship between shoot growth (g.plant<sup>-1</sup>) and their chloride concentration (µmol .gDW<sup>-1</sup>). Vertical and horizontal bars represent ± standard errors of means of 10 replicates.

It is thought that chickpea plants can exclude and/or accumulate sodium in roots which results in a reduction of root osmotic potential (Tejera *et al.*, 2006). Taken together, our descriptions on the variability of chickpea growth and mineral nutrition in response to salt stress are in good agreement with the earlier reports of Pessaraki & Zhou (1990) and Tejera *et al.*, (2006). The relative tolerance of Amdoun genotype can be

explained by an efficient compartmentalization of the toxic ions, Na and Cl, in roots in order to protect the photosynthetic apparatus. The maintaining of an important root growth shown in this genotype can also play an important role in this compartmentalization. Our current data support notion that these nutritional and physiological indicators are important for the selection of salt-tolerant chickpea genotypes growing under symbiotic conditions.

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