Introduction

In order to meet the ever increasing demand for medicinal plants, for the indigenous systems of medicine as well as for the pharmaceutical industry, many medicinal plants need to be cultivated commercially, but soil salinity and other forms of pollutions represent serious threats to plant production (Qureshi et al. 2005). In this context, fenugreek (*Trigonella foenum graecum* L.), an annual legume, is extensively cultivated in most regions of the world for its medicinal value (Petropoulos, 2002). In many countries, this species is grown in arid and semi-arid regions where high concentration of salts is an important characteristic of the soils. Soil salinity is one of the major abiotic stresses which affect seeds germination (Misra and Dwivedi, 2004), and cause several problems for plant growth (Shannon et al. 1994; Demiral and Turkan, 2006) especially for glycophytes, by inducing physiological dysfunction (Shannon et al. 1994). NaCl salinity affects water and ion transport processes in plants, which may change the nutritional status and ion balance (Läuchli and Epstein, 1990). Under salt stress, plants have evolved complex mechanisms allowing for adaptation to osmotic and ionic stress caused by high salinity. These mechanisms include the lowering of the toxic ions concentration in the cytoplasm by restriction of Na^+ influx or its sequestration into the vacuole and/or its extrusion (Hajibagheri et al. 1987).

The aim of this work was to study the effect of salt stress on germination, growth, water status and mineral nutrient in local fenugreek accession, cultivated in north of Tunisia.

Materials and methods

Germination study

Seeds of fenugreek (*Trigonella foenum graecum* L.) were sterilized with sodium hypochlorite and rinsed thoroughly with tap water and then with distilled water. To evaluate the effect of NaCl on germination four replicates of 25 seeds for salt treatment, or for the control, were placed on two layers of filter papers in 90 mm Petri dishes. The filter papers were moistened with distilled water for the control, or with 200 mM NaCl for the salt treatment. Water or fresh salt solution was added periodically, maintaining the filter papers moistness during the experiment. The seeds were germinated for 7 days in the dark at 25 ºC in the germination chamber. The number of germinated seeds was counted each 8 hours for 7 days and the percentage of germination was calculated.

Growth study

The experiment was conducted in a conditioned room under artificial light (150 $\mu mol.m^{-2}. s^{-1}$; 16 h photoperiod), at 25°C day/20°C night and air humidity 60-80%. Seedlings of this annual legume, issue from seed germination, were grown into 2.5 L polyethylene pots with nutrient solution, pH 5.5-6.5. After two weeks, two treatments were applied (1) control (nutritive solution) and (2) NaCl (200 mM). To avoid osmotic shock, the NaCl concentrations were increased gradually by 50 mM every day until the desired concentration was reached. Fifty days after the beginning of salt treatment, plants were harvested and divided into leaves, stems and roots. Fresh and dry weights, percentage of mortality and water content were measured. Dried matters of leaves, stems and roots were digested with nitric acid 0.1 N. Cation concentrations in the extracts such as Na^+, K^+ and Ca^{2+} were determined by flame spectrophotometry.

Relative growth rate (RGR) was determined as the rate of increase in total dry weight per unit of plant weight according to Hunt (1982) thus: $RGR = (In\ W_2 - In\ W_1)/(t_2 - t_1)$,

RGR in $g.g^{-1}\ day^{-1}$, where, W total plant weight (g), t the time (days), and the subscripts 1 and 2 are initial and final harvest of biomass yield.

Statistical analysis

Data were analyzed by using the SAS 8.1 software and means were compared using Duncan's tests ($p < 0.05$).

Results and discussion

Germination percentage

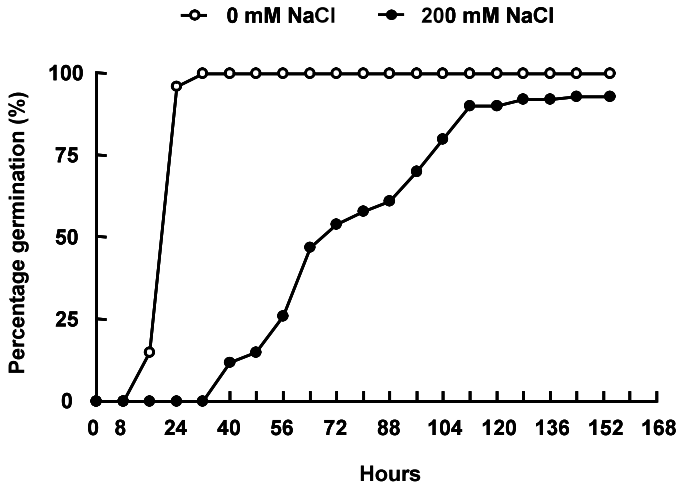

Figure 1. Effect of NaCl treatment on seed germination of fenugreek. Values are means of 4 replicates.

Germination of fenugreek seeds in distilled water reached the maximum in 24 h (Figure 1). Concentration of 200 mM NaCl delayed germination; it did not have much effect on final germination which was 93 percent (Figure 1). Similar results have been reported for beach pea (Todd, 2001). Many authors have reported that increases in salinity lead to a reduction and/or delay in germination of both halophyte and glycophyte seeds (Katembe et al. 1998). Delays in germination by increased salt concentration may be explained by the lower osmotic potential of the solution (Todd, 2001).

Seedling Mortality

The results of short duration studies during the vegetative growth stage showed that NaCl 200 mM caused a slightly increased mortality of plants (6%) (data not shown). Therefore, this species appears to have some potential for growth in saline conditions.

Growth parameters

Dry matter

Adding NaCl to the culture medium significantly reduced shoot biomass of fenugreek (33 %). Maximum reduction was observed in stems (45 %). The root dry matter was not affected (Table 1). The same results were observed in *Lotus creticus* (Rejili et al. 2007). High concentrations of salt have detrimental effects on plant growth (Ashraf and Orooj, 2006). Salinity inhibits plant growth for two reasons: first, water deficit and second salt-specific or ion-excess effects (Munns et al. 2006).

Table 1. Effect of NaCl treatment on the roots, stems and leaves dry matter in fenugreek plants. Significant differences between treatments are indicated with different letters at P< 0.05 (n=10).

NaCl (mM)	Dry weight (g)		
	Roots	Stems	Leaves
0	0.028 a	0.064 a	0.123 a
200	0.029 a	0.035 b	0.090 b

Relative growth rate (RGR)

For a better appreciation of the salt effect on growth, we calculated the relative growth rate (RGR). This parameter makes it possible to evaluate the plants biosynthetic activity under salt stress. Figure 2 showed the RGR values of the roots, stems and leaves of fenugreek plants after 15 days in the absence and presence of NaCl 200 mM. At high salt level (200 mM), the RGR of the leaves and stems was reduced by 17 and 30% respectively, whereas root RGR was not affected. Theses results are in agreement with these shown by Ghars et al. 2008.

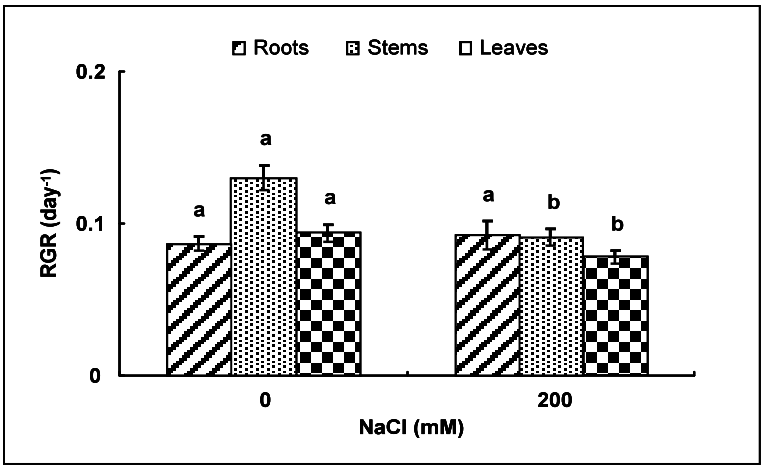

Figure 2. Relative growth rate (RGR) of roots, stems and leaves of fenugreek plants grown for 15 days on two NaCl concentrations (0 and 200 mM). For each plant organ, significant differences between treatments are indicated with different letters at P< 0.05 (n=10).

Water status

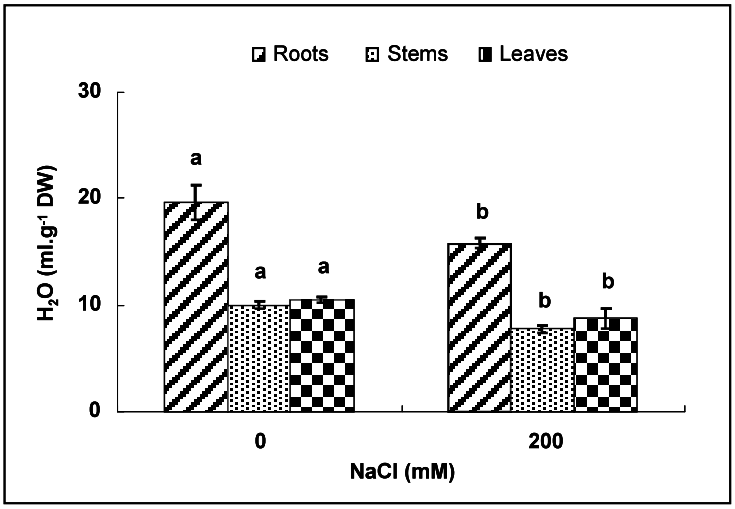

Figure 3. Water content in roots, stems, and leaves in fenugreek plants cultivated under absence or presence (200 mM) of NaCl levels. For each plant organ, bars with the different letters are significant at 5 % level. Mean for n=10.

Similarly, our results show that salt stress decreases significantly water content of different organs (Figure 3). These are consistent with reports in *Lotus creticus* (Rejili et al. 2007). The most pronounced effect was observed in roots. In the absence or presence of salt, the aerial organs and roots of fenugreek presented different water contents. The water content of the roots is higher than of the shoots.

Nutrition

Salt stress causes disturbances in the mineral nutrition of fenugreek plants. In the presence of NaCl, the amounts of K^+ and Ca^{2+} absorbed by roots and transported to the shoots were restricted. Potassium and calcium content of plants decreased significantly in saline conditions whereas Na^+ contents significantly increased (Figure 4). Consequently, K^+/Na^+ and Ca^{2+}/Na^+ ratios decreased significantly in all the organs of fenugreek with the addition of 200 mM NaCl in the culture medium (Table 2). Both ratios were markedly higher in shoots as compared to those in roots. Similar results have been reported for *Ammi majus* L. (Ashraf et al. 2004).

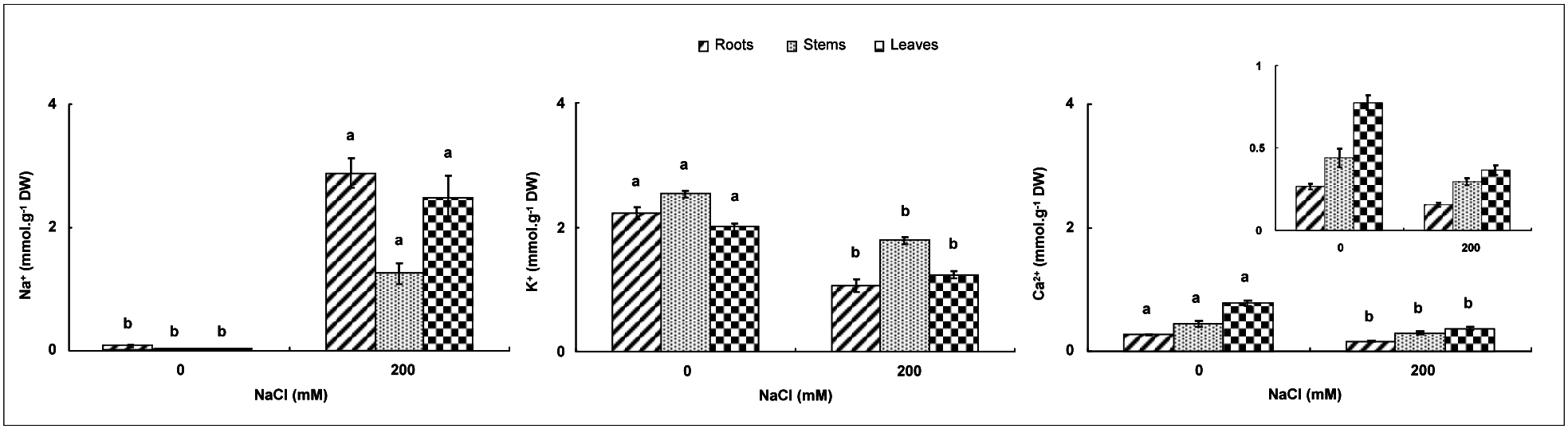

Figure 4. Na, K, and Ca concentrations in roots, stems and leaves of fenugreek submitted to salt levels (0 and 200 mM). For each plant organ, means (n=10) with the different letters differ significantly at the 5% level.

Table 2. K^+/Na^+ and Ca^{2+}/Na^+ ratios in roots, stems and leaves of fenugreek plants were subjected to salt levels (0 and 200 mM). Means with the same letters do not differ significantly at the 5% level.

Plant part	NaCl level (mM)	K/(K+Na)	Ca/(Ca+Na)
Roots	0	0.97 a	0.80 a
	200	0.27 b	0.05 b
Stems	0	0.99 a	0.96 a
	200	0.59 b	0.19 b
Leaves	0	0.99 a	0.98 a
	200	0.33 b	0.13 b

To appreciate the capacity of the plant to compartmentalize the Na^+ in the leaf vacuoles, we have correlated leaf water content with its Na^+ content according to Oertli's hypothesis (Flowers et al. 1991). Our results showed that in plants treated by NaCl (200 mM), the water content of leaves remained stable with the increase of Na^+ contents (Figure 5). Thus, sodium appears well sequestered inside the vacuole, which might play a role in osmotically adjustments in the plant under saline conditions. These observations suggest that fenugreek developed an inclusion mechanism for the Na^+ vacuolar compartmentation (Munns et al. 2000).

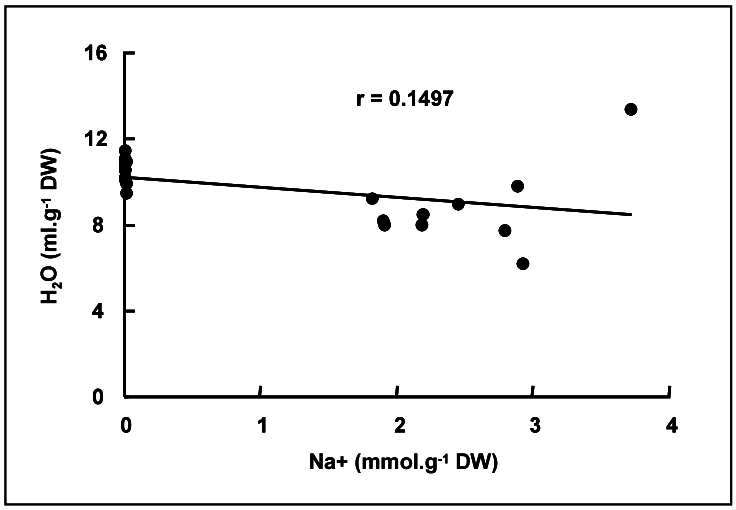

Figure 5. The relationship between sodium and water contents of leaves of fenugreek plants grown on NaCl concentrations (0 and 200 mM). Data for salt treatments were pooled.

Conclusion

Under these culture conditions our study demonstrated some potentiality of salt tolerance during germination and vegetative growth stage in fenugreek. Results show that tolerance to high salinity is associated with maintenance of high germination percentage and the growth parameters, and consequently its survival, during its vegetative growth stage. For adaptation to ionic stress at this stage, fenugreek plants seem to develop a sodium inclusion mechanism by lowering the toxic concentration of ions in the cytoplasm by sequestration of Na^+ into the vacuole, which allows osmotic adjustment.

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