Available online at www.sciencedirect.com
ScienceDirect
Flora 204 (2009) 737–746
FLORA
www.elsevier.de/flora

Melilotus indicus (L.) All., a salt-tolerant wild leguminous herb with high potential for use as a forage crop in salt-affected soils

Emad A. Al Sherif

Beni Suef University, Faculty of Science, Botany Department, Beni Suef 62511, Egypt

Received 10 July 2008; accepted 27 October 2008

Abstract

Previously unexploited legume species may offer utilization potential where environmental stresses constrain the use of more conventional forage crops. *Melilotus indicus* (L.) All., Yellow sweet clover, occurs as a weed in different habitats in Egypt. It grows in moderately saline areas, where traditional forage legumes cannot be cultivated. Our extensive field studies have recorded the species in many different habitats ranging from healthy agricultural lands to abandoned saline areas. The studied plants maintained high nodulation capacity (68 – 95%) and nitrogenase activities (about 1.81 μmol C_2H_4 $plant^{-1}$ h^{-1}) in different habitats. Greenhouse experiments demonstrated that seed germination was maintained at 80% when growing on substrats containing 200 mM NaCl and that 25% of the germination capability was preserved when 300 mM NaCl was added to the growth medium. The growth rate of seedlings was not significantly affected by 200 mM NaCl but was reduced by 30% under 300 mM NaCl. It is supposed that *M. indicus* uses a salt inclusion mechanism for maintaining growth under saline conditions, as it accumulated high amounts of Na^+ and Cl^- ions. Leaf succulence and indices of leaf water status did not differ among the salt treatments, whereas relative water content was reduced by only 3% and water content at saturation increased by about 14% at high salt concentrations in the growing medium. Our results suggest recommending the cultivation of *M. indicus* in salt-affected soils, which are widespread and pose a problem for the farmers of Egypt and other countries in the world's arid belt.

Keywords: Salt-affected land; Forage; *Melilotus*; Wild Legumes; Pasture crop; NaCl physiology

Introduction

Exploration of new wild plant species tolerant to severe conditions has recently become an urgent global issue. In developing countries this is now considered with high priority, to meet food and fodder demands of increasing population pressure in the light of limited available resources and food shortages (Al Sherif, 2007; Rumbaugh, 1990). Salinity is one of the most prominent and intractable problems facing farm managers in the world. Approximately 20% of agricultural land and 50% of cropland in the world is salt stressed (FAO, 2008; Flowers and Yeo, 1995). Egypt, a developing country with a population of more than 80 million people, becomes seriously affected by secondary salinity; 33% of its cultivated land, which comprises only 3% of the total land area, is already salinized (Ghassemi et al., 1995). Salt-affected lands are less productive and profitable, particularly if valuable salt-sensitive crops cannot be grown, and soil reclamation is costly. One of

E-mail address: emad_702001@yahoo.com.

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doi:10.1016/j.flora.2008.10.004

the alternative approaches is the use of native species with economic and ecological relevance.

Legumes are a key component of sustainable agriculture and can offer many economic and environmental benefits if grown more widely in crop rotations, because of their ability to fix nitrogen in root nodules in a symbiotic interaction with soil rhizobia. Several environmental conditions limit the growth and activity of nitrogen-fixing legumes. Salinity is the major factor, that threatens legume agriculture in arid and semi-arid climates. However there are plants that grow under saline conditions, and historically these have been opportunistically used as fodder for grazing livestock or as components of mixed rations to replace roughage (Al Sherif, 2007; Al Sherif et al., 2004; Hameed and Ashraf, 2008). The selection of such economic plants, with appropriate management, could result in the rehabilitation and re-vegetation of salt-affected lands, which cannot be cultivated with traditional crops.

Melilotus (sweet clover) is a leguminous genus which includes 25 species, annual and biennial types (Allen and Allen, 1981), characterized by high seed yields, tolerance of temperature extremes, nitrogen fixation rates superior to other legumes, and value in crop rotations (Hirsch, 2002; Hirsch et al., 2000; Stickler and Johnson, 1959). In some countries (e.g. Argentina, Canada, Russia, and Spain) *Melilotus* species are grown in moderately saline areas where traditional forage legumes cannot be grown (Bowman et al., 1998; Maddaloni, 1986; Smith and Gorz, 1965). Variations in salt tolerance have been found between a limited numbers of *Melilotus* species. Coumarine, a secondary plant compound of *Melilotus*, can cause a haemorrhagic condition known as swe et clover disease (Evans and Kearney, 2003; Nair et al., 2006). However preliminary research also suggests that it is possible to undertake management practices to limit high concentration of coumarine (Nair et al., 2006).

Melilotus indicus is a wild herb, very common in different habitats in Egypt. Its suitability to saline conditions in many regions of the world, as compared with other species (for example, *M. albus*), has been evaluated (Evans and Cameron, 1998; Evans and Kearney, 2003; Evans et al., 2001; Maddaloni, 1986; Rogers and Evans, 1996). Although there are many previous laboratory studies on salt tolerance in *M. indicus*, there have been no field studies. It is clear, however, that salt tolerance may differ between laboratory or glasshouse and natural field conditions, owing to the complex interaction of a number of edaphic and climatic factors. The present work investigates the performance of *M. indicus* under both field and laboratory conditions to evaluate its potential for use as a fodder crop in salt-affected soil in Egypt.

Material and methods

Field studies

Because of the ubiquitous nature of the studied species in Egypt, three different habitats were chosen. (1) Healthy arable soil (including barley fields in coastal land), characterized by high crop production, (2) Salt-affected, less productive soil, and (3) newly reclaimed land, with recent agricultural management. For the vegetation surveys a simplified method describing species presence was performed for the three different habitats during the period December 2006–May 2007. Fifty homogeneous stands ($10 \times 10\,m^2$) were selected, 20 in healthy soils, 20 in salt-affected soil, and 10 in newly reclaimed lands. The homogeneity was judged by the general physiognomy of the vegetation and the physiography of the sites. Specimens were identified with the help of local standard floras (Boulos, 1995; Täckholm, 1974).

Nodulation status and nitrogenase activity

Twenty plants were collected from each of the three different habitats for nodulation percentage determination, and 10 plants for enumeration of nodule number and chemical analysis. The nitrogen-fixing activity (nitrogenase activity) of the legume–*Rhizobium* symbiosis was determined according to the methods described by Witty and Minchin (1988).

Greenhouse experiments

Seed germination

Healthy uniform seeds of *M. indicus* were collected from salt-affected land (EC = 6.7 dS/m, determined in saturated soil paste extract by conductivity measurement), at Beni Suef Governorate, Egypt. For the germination tests, the seeds were sown in sterilized Petri dishes on a double layer of filter paper moistened with 5 ml of the treatment solution. The treatment solutions for salinity tests were distilled water (control), 100, 200, and 300 mM NaCl. Three replicates of 25 seeds were used in each treatment; germination was under conditions of natural light and room temperature (during December 2006, with average temperature 18.1 °C). Seeds were considered to be germinated after the radicle reached 1 cm. Germination speed (vigor value) was calculated using the following formula (Bradbeer, 1988): $V = (a/1 + b/2 + c/3 + d/4 + \cdots + x/n) \times 100/S$, where a, b, c,…x, respectively, represent the number of seeds that germinated after 1, 2, 3,…n days of incubation, and S is the total number of germinated seeds. The experiment was replicated twice and extended for 15 days.

Pot experiment

Seedlings were transplanted into pots (15 cm diameter) filled with sterilized clay loamy soil, and watered by the mineral nutrient solution twice a week. The mineral solution contained the following, in mmoles/liter: $CaCl_2 \cdot 2H_2O$, 1.0; $MgSO_4 \cdot 7H_2O$, KNO_3 1.65; K_2SO_4, 0.50, and $NaH_2PO_4 \cdot 2H_2O$, 0.65; and micronutrients (in micromoles/liter): $FeSO_4 \cdot 7H_2O$, 27.0; $MnCl_2 \cdot 4H_2O$, 1.13; $CuSO_4 \cdot 5H_2O$, 0.08; $ZnSO_4 \cdot 7H_2O$, 0.19; $NaMoO_4 \cdot 2H_2O$, 0.05, and H_3BO_3, 5.77. The pH of the nutrient solution was adjusted to 7. Pots were arranged in randomized blocks in the greenhouse. The average mean temperature was 18.1 °C. Photoperiod was about 12 h; no artificial illumination (light) was supplied. The treatment solutions of NaCl (0, 100, 200, and 300 mM) were applied 2 weeks after transplanting. The salt treatments continued for 2 weeks and pots were flushed thoroughly with distilled water once a week to avoid salt accumulation in the root zone. As the plants grew in size, the volume of liquid was increased and the differences between salt concentrations were kept constant.

Growth measurement

Fresh weights of roots and shoots were measured. Samples were dried at 70 °C for 24 h and the dry weight determined.

Plant water relations measurement

Five mature leaves per treatment were sampled at the end of experiment. In these leaves, the following parameters were examined: composite leaf area (LA), measured with an LI-3100 LA meter (Li-Cor, Lincoln, NE), fresh mass (FM), fresh mass at full turgor (TM), measured after immersion of leaf petioles in distilled water for 48 h in the dark, and dry mass (DM), measured after oven drying at 70 °C to constant weight. Additionally, the specific leaf area (SLA), (the ratio of LA to DM of individual leaves), and leaf tissue density ($D = (DM/FM) \times 1000$ (Dijkstra, 1989)) were calculated, as were relative water content, ($RWC = (FM-DM)/(TM-DM) \times 100$), succulence ($S = (FM-DM)/LA$), water content at saturation ($WCS = (TM-DM)/DM$), and water saturation deficit ($WSD = (TM-FM)/(TM-DM) \times 100$).

Plant and soil analysis

Samples of oven-dried plant shoots were ground, 0.1 g was weighed into a 10 ml vial, 10 ml of 0.5 M HNO_3 added, and samples placed on a shaker at 20 °C for 2 days. The extract was used to estimate K^+ and Na^+ using a flame photometer (Jenway, Dunmow, UK). Chloride was determined using a Buchler–Cotlove Model 4-2008 chloridometer (Buchler Instruments, Fort Lee, USA).

For each habitat, five soil samples were collected from profiles of 0–25 cm depth. These five were then pooled together to form one composite sample, air dried, and thoroughly mixed. Textures were determined by the hydrometer method, providing quantitative data on the percentage of sand, silt, and clay. The concentrations of soil minerals Na^+, K^+, Fe^{3+}, Ca^{2+}, and Mg^{2+} in soil were determined using a Perkin 403 atomic absorption spectrophotometer (Perkin-Elmer Corp., Norwalk, CT, USA) according to the Analytical Methods for Atomic Absorption Spectrophotometry (1983). Chlorides were quantified by titrating 5 ml of the 1:5 soil/distilled water extract against 0.01 N silver nitrate solution using potassium chromate (1%) as indicator. Sulphates were determined spectrophotometrically using a JENWAY 6300 spectrophotometer (Jenway, Dunmow, UK) using barium chloride and hydrochloric acid (5 ml water soil extract + 5 ml 1 N HCl + 0.5 g $BaCl_2$) and the absorbances read at 606 nm; pH and conductivity of the soil samples were determined in saturated soil paste extract by pH and conductivity meters, respectively, and carbonates and bicarbonates by titrating 5 ml of the 1.5 soil/distilled water extract against 0.01 N HCl using phenolphthalein and methyl orange as indicators (Jackson, 1962). Protein content of plants collected from different habitats was determined following the method of Lowry et al. (1951), using bovine serum albumin as a protein standard.

Statistical analysis

The least significant differences between the mean values were calculated as recommended by Bailey (1994). Terms were considered significant at $P = 0.05$.

Results

Field studies

Habitat characteristics and floristic composition

Table 1 shows large variations in soil physicochemical properties of the investigated *M. indicus* habitats, which ranged from sandy to loamy clay soils, and from healthy (EC = 0.75 dS/m) to salt-affected soils (EC = 6.7 dS/m). Salt-affected soil showed higher concentrations of Na^+, Cl^-, Mg^{2+}, and SO_4^{2-} than healthy soils by 7, 2, 1.4, and 1.5 fold, respectively. Floristic analysis (Table 2) showed the highest incidence of the studied species in healthy soil, followed by salt-affected soil, while the least was recorded at the newly reclaimed land. The species most associated with salt-affected soils were *Cynodon dactylon*, *Phragmites australis*, and *Spergularia marina*.

Table 1. Soil physicochemical characters of different habitats where *M. indicus* was recorded.

	Healthy soil	Salt affected soil	Newly reclaimed land
K (mg/kg)	1099 ± 45.70[a]	1016.5 ± 14.00	1.26 ± 00.11
Na (mg/kg)	345 ± 41.50	2547.0 ± 85.00	8.24 ± 00.12
Mg (mg/kg)	711 ± 25.30	1050.7 ± 50.00	6.10 ± 00.18
Ca (mg/kg)	174.1 ± 31.20	195.6 ± 24.00	17.39 ± 00.30
Fe (mg/kg)	22.3 ± 01.30	78.8 ± 11.40	38.20 ± 03.50
SO4 (mg/kg)	2145 ± 41.30	3936 ± 32.00	153.4 ± 15
Cl (mg/kg)	2314 ± 53.21	4775 ± 53.00	15.82 ± 01.20
HCO_3 (mg/kg)	231 ± 02.30	395 ± 11.10	18.46 ± 02.50
EC (dS/m)	0.75 ± 0 0.11	6.7 ± 1.50	0.55 ± 00.09
pH	7.12 ± 00.20	7.38 ± 0.11	7.14 ± 00.50
Soil texture	Clay loamy	Clay loamy	Sandy

[a] ± SE ($N = 4$).

Table 2. Percent presence of plant species (having > 10% in at least one stand) associated with *Melilotus indicus* at the four different habitats.

	Healthy soil	Salt affected soil	New reclaimed land
Melilotus indicus (L.) All.	36	31	9
Alhagi graecorum Boiss.	0	12	0
Avena fatua L.	32	0	1
Bassia indica (Wight) AJ Scott.	0	12	0
Beta vulgaris L.	24	19	1
Brassica nigra (L.) Koch.	36	3	2
Chenopodium murale L.	41	6	2
Cichorium endivia L.	53	1	1
Conyza bonariensis (L.) Cronquist	0	3	0
Coronopus squamatus (Forssk.) Asch.	31	0	0
Cynodon dactylon (L.) Pers.	3	45	2
Cyperus rotundus L.	20	0	0
Daucus syrticus Murb.	32	0	0
Echinochloa colona (L.) Link.	32	25	2
Emex spinosa (L.) Campd.	16	0	0
Fumaria bracteosa Pomel	3	0	0
Hordeum murinum L.	0	12	0
Launea nudicamlis (L.) Hook	0	0	12
Lolium perenne L.	26	15	7
Malva parviflora L.	51	0	1
Medicago polymorpha L.	3	0	4
Medicago intertexta (L) Mill.	7	10	1
Oxalis corniculata L.	6	0	0
Phragmites australis (Cav.) Trin.ex Steud.	3	54	0
Phalaris minor Retz.	16	0	2
Plantago lagopus L.	3	0	0
Plantago major L.	7	0	0
Poa annua L.	33	5	0
Phyla nudiflora (L.) Greene	9	0	0
Sisymbrium irio L.	2	0	8
Sonchus oleraceus L.	54	1	18
Spergularia marina (L.) Griseb.	0	62	0
Trifolium resupinatum L.	19	0	1
Vicia sativa L.	8	0	7

Nodulation, nitrogenase activity, and shoot content of protein, Na^+, K^+, and Cl^-

The studied plants showed high nodulation percentages (ranging between 68% and 95%) and nitrogenase activities (average 1.81 μmol C_2H_4 $plant^{-1}$ h^{-1}) at all different habitats (Table 3). Protein content, as expected for a legume, was high (ranging between 21% and 30%), with the highest values recorded in plants collected from salt-affected soils and the lowest in plants collected from newly reclaimed land. Shoot systems of plants collected from salt-affected soils exhibited higher concentrations of Na^+ and Cl^- than those collected from healthy soils by more than two- and threefold, respectively, and showed a reduction in K^+ content of about 38% (Table 3).

Greenhouse experiments

Germination and growth

Germination percentage and vigor value of the studied species was not significantly ($p = 0.05$) affected at 100 or 200 mM NaCl, and 25% germination ability was maintained at 300 mM NaCl (Fig. 1a). Root dry weight showed a slight increase at 100 and 200 mM NaCl and decreased by only about 8% at 300 mM NaCl. Shoot dry weight was not significantly affected at 100 or 200 mM NaCl, but a 30% reduction at 300 mM NaCl was recorded (Fig. 1b).

Water relations and shoot contents of Na^+, K^+, and Cl^-

The most sensitive parameter was LA, which declined by 34%, 44%, and 52% at 100, 200, and 300 mM NaCl, respectively. SLA showed an increase (around 14%) under salinity stress (Table 4). Leaf density (*D*) showed a slight (4%) reduction at 300 mM NaCl. No significant differences were observed among salt treatments in respect of leaf water status, and RWC exhibited only 3% reduction at the higher salt concentration. WCS showed significant increase (14%) at high salt concentrations than control.

Shoot Na^+ and Cl^- content (Fig. 2) increased significantly ($P = 0.05$) with increasing NaCl concentration, reaching 25- and 13-fold, respectively. In contrast concentrations of K^+ decreased with increasing levels of NaCl by about 16%, 24%, and 34% at 100, 200, and 300 mM NaCl, respectively.

Table 3. Nodulation, nitrogenase activity and protein content of *Melilotus indicus* collected from different habitats.

	Healthy soil	Salt-affected soil	New reclaimed land
Nodule no $plant^{-1}$	63 ± 3.2[a]	58 ± 20.4	42 ± 4.7
Nodulation (%)	95 ± 3.3	81 ± 40.1	68 ± 6.2
Nitrogenase activity (μmol C_2H_4 $plant^{-1}$ h^{-1})	2.4 ± 0.06	1.05 ± 0.03	1.98 ± 0.02
Protein (g/kg dry wt.)	283 ± 18.4	306 ± 17.6	215 ± 21.5
Na^+ (mmol/g DM)	1.03 ± 0.05	2.32 ± 0.10	0.12 ± 0.01
Cl^- (mmol/g DM)	0.53 ± 0.01	1.8 ± 0.02	0.24 ± 0.03
K^+ (mmol/g DM)	1.95 ± 0.03	1.21 ± 0.04	1.01 ± 0.07

[a] ±SE ($N = 4$).

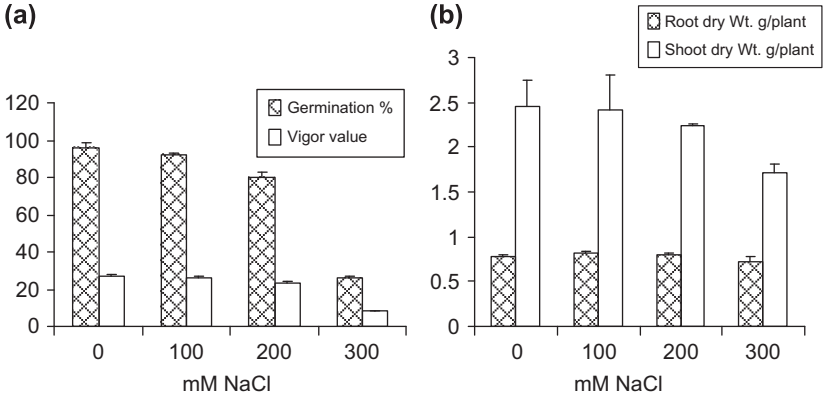

Fig. 1. Effect of salinity on germination and vigor value (germination speed)–a , and on root and shoot dry weights–b.

Table 4. Effect of salinity (NaCl) concentration in the nutrient solution, on leaf area (LA), specific leaf area (SLA), density of the leaf tissue (D), relative water content (RWC), succulence (S), water content at saturation (WCS) and water saturation deficit (WSD) of *Melilotus indicus* ($n = 5$).

	Salinity (NaCl) concentration				LSD ($P = 0.05$)
	0 mM	100 mM	200 mM	300 mM	
LA (cm^2/leaf)	6.8	4.5	3.8	3.2	0.86
SLA ($m^2\,kg^{-1}$)	1021.80	1186	1150	1169	13.3
D ($g\,kg^{-1}$)	84.5	81.9	80.5	80.3	11.3
RWC (%)	84.4	83.1	82.3	81.6	2.5
S ($mg\,H_2O\,cm^{-2}$)	0.011	0.01	0.01	0.011	0.001
WCS ($g\,H_2O\,g^{-1}$ DM)	12.35	13.06	13.42	14.20	1.70
WSD (%)	20.4	14.8	17.4	18.3	1.1

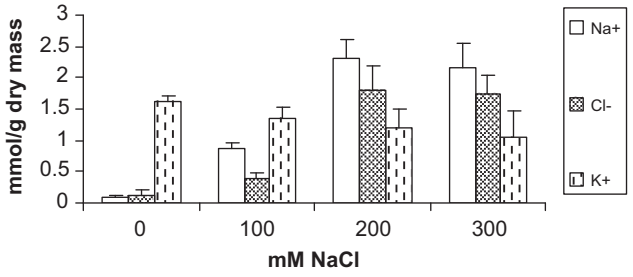

Fig. 2. Effect of salinity on Na^+, Cl^-, and K^+ content on shoot system of *Melilotus indicus*.

Discussion

Habitat characters and floristic composition

The ability of the studied species to grow in a wide range of conditions indicates its adaptability and wide ecological amplitude. Previously it was recorded in habitats differing in soil conditions in Egypt (El Hadidi and Kosonova, 1971; Shaltout and El Fahar, 1991) and in Pakistan (Nasir and Ali, 1977). The competitive ability of *M. indicus* in comparison with plants characterized by salt tolerance such as *S. marina* confirms its salt tolerance.

Nodulation, nitrogenase activity, and shoot content of protein, Na^+, K^+, and Cl^-

The variation in nodulation percentage among individuals collected from different habitats can be explained by the different prevailing environmental conditions. Nodulation status depends on many factors, including the legume stage and edaphic factors, and, especially, moisture content (Graham and Vance, 2000; Vance, 1998). The high nodulation percentage and nitrogenase activity recorded in the *M. indicus* plants gives the species economic importance as it can be used to enhance soil fertility. The lowest nodulation percent (68%), recorded at the newly reclaimed land, is likely to be due to decreases in population levels of rhizobia in this habitat. Salinity is known to principally affect the infection process, by inhibiting root hair growth and by decreasing the number of nodules per plant, and the amount of nitrogen fixed per unit weight of nodules (Hafeez et al., 1988). *M. indicus* has an indeterminate nodule structure (Al Sherif et al., 2004; Zahran, 1998). This type of structure helps it to fix nitrogen in salt-affected soil. Bordeleau and Prevost (1994) and Sinclair and Serraj (1995) reported that nitrogen fixation in indeterminate nodules is more tolerant to salt stress than in determinate ones. Nitrogen (N) is one of the major limiting nutrients for most crops and other plant species (Newbould, 1989), but, on the other hand, saline environments are generally deficient in nitrogen (Amonkar and Karmarkar, 1995; van Hoorn et al., 2001). The nitrogen-fixing ability of the studied species, especially in salt-affected areas, predestines it to re-vegetate salt-affected soils (without the need to apply any chemical nitrogen), an important process for the stabilization and reclamation of the plant growth substrate (Läuchli and Epstein, 1990).

The increased protein content of plants collected from salt-affected soils suggests a mechanism for salt tolerance. Proteins in plants grown under saline conditions may provide a storage form of nitrogen that is re-utilized when stress is over, and may play a role in osmotic adjustment (Singh et al., 1987). A higher content of soluble proteins has been observed in salt-tolerant than in salt-sensitive cultivars of barley (Hurkman et al., 1989), sunflower (Ashraf and Tufail, 1995), finger millet (Uma et al., 1995), and rice (Lutts et al., 1996; Pareek et al., 1997; Rains, 1989).

Greenhouse experiments

Germination and growth

Because the early stages of plant growth are sensitive to habitat salinity (Baldwin et al., 1996; Mariko et al.,

1992), on germination represents a bottleneck in the life cycle of many species. Therefore the high germination percentage and vigor value (germination speed) recorded under salinity stress is a very important character from the ecological point of view. Maranon et al. (1989) stated that seed populations of *M. indicus* are sensitive to 50 mM or even to 10 mM NaCI. In contrast, in this study, seeds were collected from plants living in saline soils, which would be expected to exhibit salt tolerance during the germination stage, as a result of natural selection. Many studies have shown that populations collected from saline sites are more salt tolerant than populations collected from non-saline sites (Ashraf et al., 1994, Bazzaz, 1973; Hameed and Ashraf, 2008; Marañón et al., 1989; Rogers and Evans, 1996; Ungar, 1978).

Little inhibition in seedling growth was recorded in media containing up to 200 mM NaCl, but 300 mM NaCl was inhibitory to plant growth. Harmful effects of salinity are thought to result from low water potentials, nutrient deficiencies, ion toxicities, or a combination of these factors. Nutrient deficiencies can occur in plants when high concentrations of Na^+ in the soil reduce the amounts of available K^+, Mg^{2+}, and Ca^{2+} (Epstein, 1972) or when Na^+ displaces membrane-bound Ca^{2+} (Cramer et al., 1985). In addition, Na^+ may have a direct toxic effect, such as when it interferes with the function of potassium as a cofactor in various reactions. Many of the harmful effects of Na^+, however, seem to be related to the structural and functional integrity of membranes (Kurth et al., 1986). The present study showed that salinity reduced shoot growth more than root growth. These results agree with the generalized assumption that root growth is almost always less affected than shoot growth by salinity (Läuchli and Epstein, 1990; Munns and Termaat, 1986). Salinity-induced dry matter reduction has been reported as more severe in the shoot than in the root for some cultivated legumes, for example soybean (Bernstein and Ogata, 1966; Shalhevet et al., 1995), alfalfa (Esechie et al., 2002; Khan et al., 1994), and chickpea (Lauter et al., 1980). However, these generalizations were based on a few case studies of short-term effects (5–14 d), and were more important in grass species (e.g., barley – Delane et al., 1982; sorghum – Weimberg et al., 1984, wild growing C4 grasses – Naidoo et al., 2008). More long-term studies with a wider taxonomic base would be needed to reach general conclusions on the differential allocation of biomass to root and shoot in response to salinity.

Water relations and shoot contents of protein, Na^+, K^+, and Cl^-

The reduction in LA under saline conditions was due to reduced growth. Maintenance of RWC under salinity (decreased by only 3% at high salt concentrations) indicates the good salt tolerance of the studied species. No change in leaf succulence was observed in the present study in contrast with results obtained with the same species by Ashraf (1993), who recorded an increase in leaf succulence at 160 and 240 mM NaCl. WCS showed an increase at higher salt concentration because the solute content of cells is higher in saline than non-saline conditions, due largely to the accumulation of Na^+ and Cl^-. The increased solute content of cells in salt-treated plants causes more water to be taken up than under control conditions (Munns et al., 2006). Greenhouse experiments confirm the increase of protein content under salinity stress, which was found also in field-grown plants. Proteins may be synthesized *de novo* in response to salt stress or may be present constitutively at low concentrations and increase when plants are exposed to salt stress (Pareek et al., 1997). A higher content of soluble proteins has been observed in salt-tolerant than in salt-sensitive cultivars of non-legumes such as barley (Hurkman et al., 1989), sunflower (Ashraf and Tufail, 1995), finger millet (Uma et al., 1995) rice (Pareek et al., 1997;Lutts et al., 1996; Rains, 1989), and wheat (Ashraf and O'Leary, 1999).

Higher plants possess several mechanisms for salt tolerance. Of these, salt inclusion or salt exclusion has long been recognized in different plant species in relation to salinity (Greenway and Munns, 1980; Maas and Niemann, 1978; Wyn Jones, 1981). The present study shows that *M. indicus* uses a salt inclusion mechanism for maintaining growth under saline conditions as it accumulated high levels of Na^+ and Cl^-. The present study confirms the finding of Ashraf et al. (1994), who stated that the tolerance of *M. indicus* is associated with ion (Na^+ and K^+) inclusion rather than exclusion. The same results were obtained with other leguminous species such as *Trifolium alexandrinum* (Ashraf, 1989) and *Lupinus luteus* (Van Steveninck et al., 1982).

Conclusion

The high ability of the studied species to germinate, grow, and fix nitrogen under salt stress in both field and laboratory studies recommends its cultivation as a fodder crop and as a soil melioration plant on salt-affected soils.

Acknowledgement

The author is very grateful to Dr. Andrew Crombie, Biological Scientific Department, Warwick University, UK, for revising the manuscript.

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