

# Effect of Sodium Chloride on Water Relations and Some Organic Osmotica in arid Zone Plant Species *Melilotus indica* (L.) All.

Auswirkungen von Natriumchlorid auf Wasserhaushalt und auf einige organische Osmotica in Indischem Steinklee (*Melilotus indica* (L.) All. in ariden Gebieten

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## 1 Introduction

In a previous study (ASHRAF et al, unpublished data) it was found that a salt tolerant natural population of *Melilotus indica* contained high amounts of Na<sup>+</sup>, K<sup>+</sup> and Ca<sup>2+</sup> in its shoots compared with those in a non-tolerant population. The high tolerance of the salt tolerant population was suggested to be due to its ability to maintain relatively low shoot Na/K and Na/Ca ratios.

The present study was undertaken to investigate the role of different organic osmotica such as soluble carbohydrates, soluble proteins, free amino acids, and proline in salt tolerance of the species, since these organic osmotica have been found to have played a crucial role in the salt tolerance of many other plant species (MAAS and NIEMAN, 1978; GREENWAY and MUNNS, 1980; WYN JONES, 1981; ASHRAF and NAQVI, 1992).

## 2 Materials and Methods

Seed of a land race of *Melilotus indica* (L.) All. was collected from a salt affected field (ECe = 7.82 dS m<sup>-1</sup>; pH 0 9.84; SAR = 24.6) and that of a normal line (IM-83, mainly bred for qualitative characters) was obtained from a local seed supplier. Surface sterilized seeds of each population were sown in plastic Petri dishes. After ten days ten seedlings of comparable size of each population were transplanted equidistant from each other into 18 cm size plastic pots containing 4.0 kg well washed and dried sand.

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The experiment was placed in a wire netting house with open sunlight and was arranged in a randomized complete block design with four blocks. Each block contained two populations and four NaCl treatments. The NaCl treatments used were 0 (control), 80, 160, and 240 mol m<sup>-3</sup> (EC = 1.2 (non-saline), 8.9, 17.1, and 24.8 dS/m respectively) in full strength Rorison nutrient solution (HEWITT, 1966). Seedlings were grown for a further 11 days irrigated with full strength Rorison nutrient solution after which time salt treatments were begun by adding aliquots of 40 mol m<sup>-3</sup> on alternate days until the respective treatments were reached. Treatments continued with addition of one litre of the appropriate solution after every three days to each pot.

All the plants were harvested 42 days after the start of the salt treatments. Plant roots were removed carefully from the sand and washed with LiNO<sub>3</sub> solution isotonic with the salt treatment in which plants were growing. Shoots and roots were separated. After recording the fresh weights of all plant parts, plant material was dried at 70°C for one week and dry weight recorded. Fresh plant material was used for the determination of soluble proteins, total free amino acids, proline and water relation parameters, whereas dry plant material was used for the determination of total soluble sugars.

#### *Leaf water potential*

After the completion of 42 days in salt treatment, a fully expanded youngest leaf was excised from each plant at 0900 hours and leaf water potential was measured using a pressure bomb (Chas W. Cook and Sons, Birmingham, U.K.).

#### *Leaf osmotic potential*

A fully expanded youngest leaf was excised from each plant at 0900 hours as for water potential measurement. The leaf material was frozen in 2.0 cm<sup>3</sup> polypropylene tubes for two weeks, thawed and sap was extracted. After centrifugation at 8000 x g for 5 min. the sap was used directly for osmotic potential measurement in an osmometer (Wescor 5500).

Leaf turgor potential was calculated as the difference between osmotic potential and water potential.

#### *Total soluble sugars*

Total soluble sugars were estimated following TREVELYAN and HARRISON (1952). 0.5 g of dried leaves were extracted in 75% ethyl alcohol. 0.2 ml of each sample extract was treated with a 4 ml of anthrone reagent. The absorbance was read at 620 nm using a spectrophotometer (Hitachi, U 2000).

#### *Total soluble proteins*

Total soluble proteins were determined following LOWRY et al (1951). 0.2 g of fresh leaf tissue was homogenized in 4 ml of sodium phosphate buffer (pH = 7.0), centrifuged and the supernatant was used for the estimation of both soluble proteins and total free amino acids.

0.2 ml of sample extract was treated with Folin phenol reagent and the optical densities were read at 620 nm on a spectrophotometer (Hitachi U 2000).

#### *Total free amino acids*

Total free amino acids of the leaf tissue were determined following the method described by HAMILTON and VAN SLYKE (1943). 1 ml of each sample extract which was extracted during the soluble proteins estimation, was reacted with 1 ml of 10% pyridine and 1 ml of 2 % ninhydrin solutions. The optical densities were read at 570 nm on a spectrophotometer (Hitachi U 2000).

#### *Proline estimation*

0.5 g of fresh leaf tissue from each pot was homogenized in 10 ml of 3% sulfosalicylic acid solution. Proline from the extract was estimated spectrophotometrically following the ninhydrin method of BATES, WALDREN and TEAR (1973).

#### *Statistical analysis of data*

The data for all the variables were subjected to two-way analysis of variance, and the least significant differences (LSD) were calculated following SNEDECOR and COCHRAN (1980) for comparing mean values.

### **3 Results**

Mean data for plant dry weight (Tab. 1) and analysis of variance of the data (Tab. 2) show that NaCl in the rooting medium had a significant adverse effect ( $p < 0.001$ ) on the plant dry matter of both populations of *Melilotus indica*. However, populations differed significantly ( $p < 0.01$ ) and populations x treatments interaction was also significant ( $p < 0.01$ ) showing that populations responded differently to increasing salt concentration. The salt tolerant population had significantly ( $p < 0.05$ ) greater plant dry matter than the normal population at all salt concentrations except 240 mol m<sup>-3</sup>.

Tab. 1: Plant dry weight (g/plant) of two populations of *Melilotus indica* when grown for 42 days in sand culture salinized with varying concentrations of NaCl in full strength Rorison nutrient solution.

Populations	NaCl concentrations mol m <sup>-3</sup>			
	0 (cont.)	80	160	240
Salt tolerant	3.24 a	3.55 a	1.94 a	1.74 a
	x	x	y	y
Normal	2.41 b	3.09 b	1.56 b	1.68 a
	x	y	z	z

Means with the same letters in each column and each row do not differ significantly at 0.05 level.

The results for leaf water potential, osmotic potential, turgor potential, and succulence are presented in Fig. 1 and analyses of variance of the data in Tab. 2.

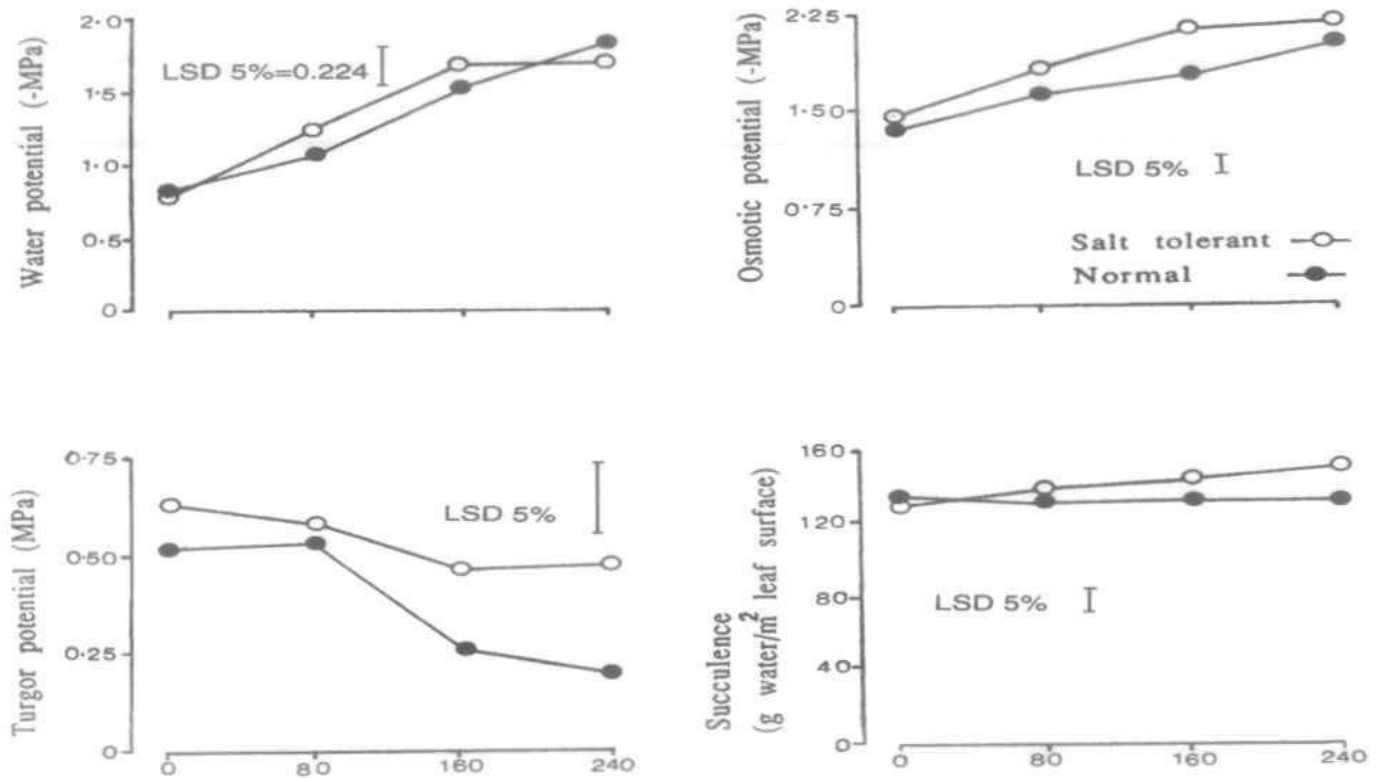


Fig. 1: NaCl concentrations mol m<sup>-3</sup>. Leaf water potential, osmotic potential, turgor potential and succulence of two populations of *Melilotus indica* grown for 42 days in sand culture salinized with varying NaCl concentrations in full strength Rorison nutrient solution.

Tab. 2: Mean squares from analysis of variance of data for plant dry weight, leaf water potential, osmotic potential, turgor potential, and succulence of two populations of *Melilotus indica* grown for 42 days in sand culture salinized with varying concentrations of NaCl in full strength Rorison nutrient solution.

Source of variation	df	Plant dry weight	Leaf water potential	Leaf osmotic potential	Turgor potential	Succulence
Blocks	3	0.091 NS	0.018 NS	0.004 NS	0.008 NS	58.6 NS
Populations (P)	1	0.586 **	0.067 NS	0.089 **	0.281 ***	548.6 ***
Treatments (T)	3	1.291 ***	0.734 ***	0.159 ***	0.302 ***	369.2 ***
P x T	3	0.322 **	0.051 NS	0.049 **	0.079 **	318.6 ***
Error	21	0.065	0.025	0.0098	0.016	41.4

\*\*, \*\*\* significant at 0.01 and 0.001 levels, respectively; NS, non-significant.

The populations did not differ for leaf water potential, and their leaf water potential declined linearly with the increase in salt concentration of the growth medium. The leaf osmotic potential of both populations also decreased consistently with increase in

NaCl concentration. However, the salt tolerant population had significantly ( $p < 0.05$ ) lower osmotic potential than the normal population at all NaCl treatments. Although NaCl in the rooting medium had an adverse effect on the turgor potential of both populations, a significant adverse effect of NaCl was observed in the normal population. The salt tolerant population had significantly ( $p < 0.05$ ) higher leaf turgor than the normal population at 160 and 240 mol m<sup>-3</sup> NaCl. Leaf succulence was also significantly ( $p < 0.05$ ) higher in the salt tolerant population compared with the normal population at 160 and 240 mol m<sup>-3</sup> NaCl.

Mean data for leaf total soluble sugars, soluble proteins, free amino acids and proline are presented in Fig. 2 and analysis of variance summaries of the data in Tab. 3.

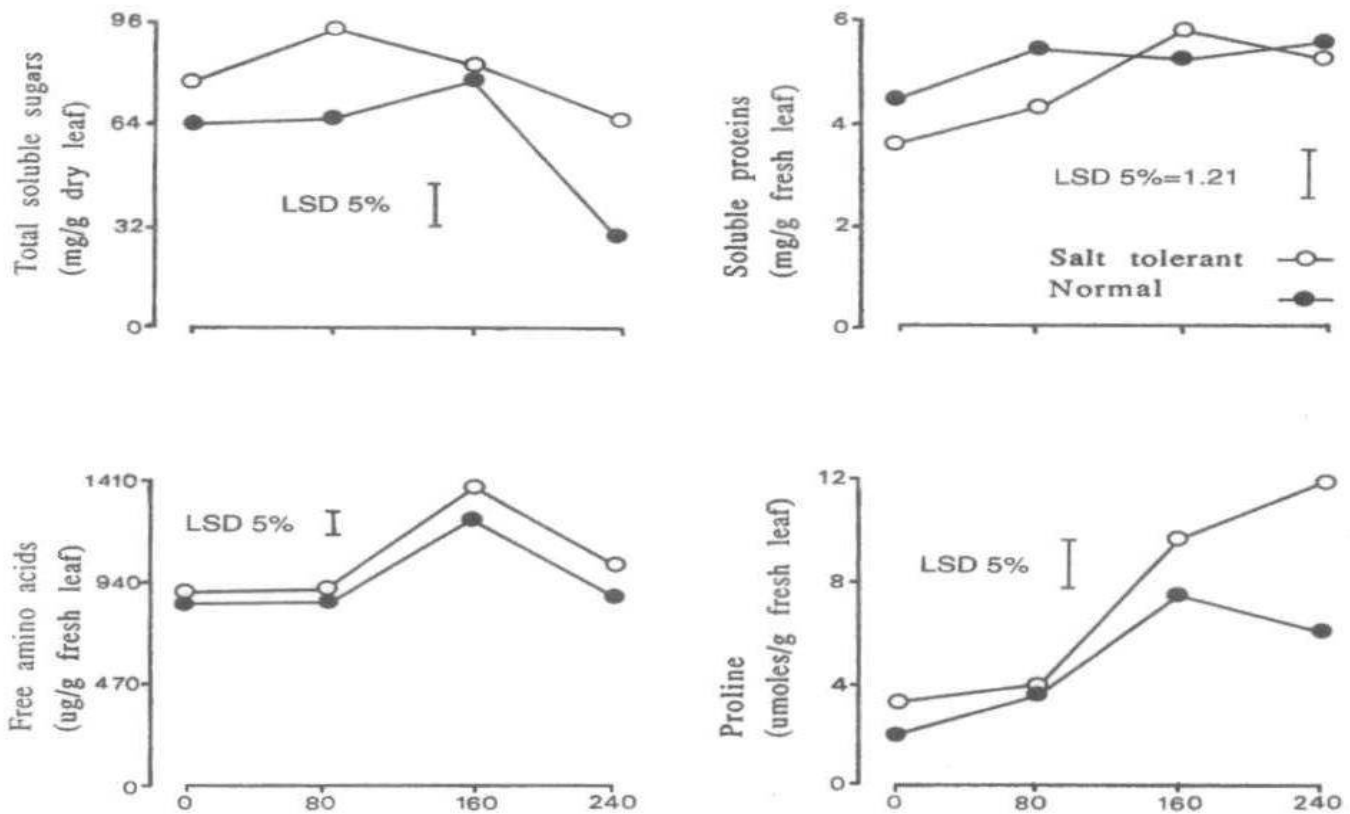


Fig. 2: NaCl concentrations mol m<sup>-3</sup>. Leaf total soluble sugars, soluble proteins, free amino acids and proline of two populations of *Melilotus indica* grown for 42 days in sand culture salinized with varying concentrations of NaCl in full strength Rorison nutrient solution.

The pattern of accumulation of soluble sugars in the two populations was different. Soluble sugars in the salt tolerant population increased at 80 and 160 mol m<sup>-3</sup> compared with those in control, whereas at the highest NaCl treatment the sugar quantity was almost the same as in control. By contrast in the normal population amount of sugars remained almost uniform at the first three treatments, whereas at the highest treatment a decline in the sugar content was observed. However, the salt tolerant population had significantly greater ( $p < 0.05$ ) soluble sugars than the normal population at 80 and

240 mol m<sup>-3</sup> NaCl. No significant effect of NaCl was observed on the leaf soluble proteins of both populations and populations also did not differ for this variable.

Tab. 3: Mean squares from analysis of variance of data for leaf soluble sugars, soluble proteins, free amino acids and proline of two populations of *Melilotus indica* when grown for 42 days in sand culture salinized with varying concentrations of NaCl in full strength Rorison nutrient solution.

Source of variation	df	Soluble sugars	Soluble proteins	Free amino acids	Proline
Blocks	3	102.4 NS	0.52 NS	7816.4 NS	3.68 NS
Populations (P)	1	1258.1 ***	1.69 NS	48471.9 **	17.48 **
Treatments (T)	3	328.8 *	1.56 NS	29089.6 **	26.63 ***
P x T	3	639.6 ***	1.43 NS	28343.3 **	6.61 *
Error	21	88.4	0.73	5408.1	2.02

\*, \*\*, \*\*\*, significant at 0.05, 0.01, and 0.001 levels, respectively; NS, non-significant.

Free amino acids in the leaves of the salt tolerant population increased with the increase in salt concentration of the growth medium, whereas those of the normal population had significantly ( $p < 0.05$ ) greater amount of free amino acids than the normal population at the two higher salt concentrations. Proline content of both populations increased consistently with the increase in salt concentration. However, the salt tolerant population had significantly greater amount of proline than the normal population at 160 and 240 mol m<sup>-3</sup> NaCl.

#### 4 Discussion

In biomass production the salt tolerant population of *Melilotus indica* excelled the non-tolerant population when subjected to salt stress.

Salt tolerant plants, when subject to saline conditions, maintain their intra-cellular osmotic potential lower than that of the saline growth medium so as to maintain turgor potential, otherwise they would experience physiological drought and hence poor growth (MAAS and NIEMAN, 1978; GREENWAY and MUNNS, 1980; WYN JONES, 1981). The salt tolerant population maintained considerably low osmotic potential and had high turgor potential compared with the non-tolerant population. These results can be related to salt tolerance.

If parallels are drawn between the low osmotic potential of the salt tolerant population and different organic osmotica determined in this study, high concentrations of soluble carbohydrates in the salt tolerant population may have been substantially responsible for maintaining osmotic potential. These results can be related to the argument of CRAM (1976) that sugars contribute up to 50% of the total osmotic potential in glycophytes growing under saline conditions. But such positive relationship of soluble



sugars with osmotic potential was not observed in *Brassica carinata*, the most salt tolerant of the species belonging to the genus *Brassica* (ASHRAF and NAQVI, 1992). Total free amino acids and, in particular, proline are known to have a considerable role in osmoregulation in plants subject to saline conditions (WYN JONES, et al, 1977; STOREY, AHMAD and WYN JONES, 1977; RAINS, 1981).

Although proline was much higher in the salt tolerant population than in the non-tolerant population, its concentration was so low that it does not seem to have played a significant role in maintaining the osmotic potential low. In contrast, the concentration of free amino acids may have played a significant role in reducing the osmotic potential of the salt tolerant population.

Overall it can be concluded that salt tolerance of *Melilotus Indica* is partly controlled by the synthesis of high amounts of organic osmotica such as soluble sugars, free amino acids and proline in addition to the maintenance of low tissue Na/K and Na/Ca ratios under salt stress.

## 5 Summary

Two populations of *Melilotus indica* (L.) All. (a salt tolerant and a non-tolerant, IM-83) were grown for 42 days in sand culture salinized with 0 (control), 80, 160, and 240 mol m<sup>-3</sup> NaCl. The salt tolerant population produced significantly greater plant dry biomass than the non-tolerant population. The salt tolerant population maintained low leaf osmotic potential and high turgor potential compared with that of the non-tolerant line. The salt tolerant population maintained low leaf osmotic potential and high turgor potential compared with that of the non-tolerant line. The considerably low leaf osmotic potential of the salt tolerant population can be easily related to its high contents of leaf soluble sugars, free amino acids and proline. Of these parameters soluble sugars played a major role in lowering the osmotic potential of the salt tolerant population and hence seemed to have contributed partly to its high salt tolerance.

## Zusammenfassung

Zwei indische Steinkleesorten (*Melilotus indica* (L.) All., eine lokale salztolerante und die nicht salzverträgliche Sorte IM-83) wurden 42 Tage lang in salzhaltigen Sandkulturen mit 0 (Kontrolle), 80, 160 und 240 mol m<sup>-3</sup> NaCl angebaut und geprüft.

Die salztolerante Sorte hatte einen signifikant höheren Trockenmasse-Ertrag als die nicht tolerante. Ferner behielt die salztolerante Sorte ein niedrigeres osmotisches Potential in den Blättern und einen höheren Tugordruck.

Dieses beträchtlich niedrigere osmotische Potential der salztoleranten Sorte kann aus dem höheren Gehalt an löslichen Zuckern, freien Aminosäuren und Prolin abgeleitet werden.

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(Yellow sweet clover)