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Organic solute status and water relations of some salt-tolerant and salt-sensitive accessions of lentil (*Lens culinaris*)

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SUMMARY

Three salt-tolerant (ILL 6451, ILL 6788, ILL 6793) and two salt-sensitive (ILL 6439 and ILL 6778) accessions of lentil (*Lens culinaris* Medic.) were grown for 42 days in sand culture salinized with 0 or 30 mol m^{-3} NaCl in full strength Rorison nutrient solution in order to determine the physiological/biochemical mechanisms responsible for their salt tolerance. All three salt-tolerant accessions produced significantly greater shoot and root biomass than the two salt-sensitive accessions after the salt treatment. Of the salt-tolerant accessions, ILL 6451 and ILL 6788 had significantly higher leaf water potentials but lower osmotic potentials than the other accessions under saline conditions. Leaf diffusive resistance and epicuticular wax content were significantly higher in the two sensitive accessions compared with the tolerant accessions after the salt treatment. Leaf soluble proteins decreased due to NaCl treatment but to the same extent in both tolerant and sensitive accessions. Total free amino acids and non-reducing sugars were relatively low in the two salt-sensitive accessions after the salt treatment. Reducing sugars of the tolerant accessions remained unaffected due to NaCl, whereas those of ILL 6439 increased and of ILL 6778 decreased. Leaf free amino acids and non-reducing sugars appeared to have contributed, to some extent, to the salt tolerance of tolerant accessions.

Key-words: Lens culinaris, lentil, NaCl, organic osmotica, water relations.

INTRODUCTION

Escalating levels of soil salinity are a continuing threat to agricultural productivity in arid and semi-arid regions of the world. The consequent detrimental effects on the growth and yield of salt-sensitive crops are well documented (Carter 1975; Maas & Hoffman 1977; Epstein *et al.* 1980; Greenway & Munns 1980). Many curative and management practices have been adopted by soil scientists to overcome the salinity problem, but these methods are highly expensive and are also not the ultimate solution of the problem. However, one of the possible alternatives is the development of crop cultivars/lines tolerant to high salt concentrations. The biological approach to overcome the salinity problem has received considerable attention in the last few decades (Shannon 1978; Yeo & Flowers 1984; Epstein 1985; Ashraf *et al.* 1986). With this aim in mind 133 local/exotic accessions of

lentil (*Lens culinaris*) were screened for salt tolerance at different growth stages in a previous study (Ashraf & Waheed 1990). A considerable amount of variation in salt tolerance was observed among these accessions. Of the salt-tolerant accessions only ILL 6451, ILL 6788 and ILL 6793 were found to be consistently tolerant at the early and later growth stages. By contrast, of the salt-sensitive accessions, ILL 6439 and ILL 6778 were consistently sensitive at all growth stages.

The present study was undertaken to determine underlying physiological factors responsible for controlling salt tolerance or salt sensitivity in these five lines with their differing degrees of salt tolerance. The knowledge of physiological mechanisms of salt tolerance is extremely important in seeking rapid and objective parameters for mass screening programmes (Maas & Nieman 1978; Greenway & Munns 1980; Yeo & Flowers 1984).

It has been established that in addition to different inorganic ions, organic osmotica such as free amino acids, soluble proteins, and reducing and non-reducing sugars play an important role in osmoregulation in relation to salinity stress (Wyn Jones *et al.* 1979; Wyn Jones 1981; Moftah & Michel 1987; Ashraf 1989). Therefore, one of the major objectives was to draw parallels between growth and different organic osmotica in some selected lines of lentil.

MATERIALS AND METHODS

Seeds of three salt-tolerant (ILL 6451, ILL 6788 and ILL 6793) and two salt-sensitive (ILL 6493 and ILL 6778) accessions/lines of lentil (*Lens culinaris* Medic.) were obtained from ICARDA (International Centre for Agricultural Research in Dry Areas) Aleppo, Syria. About 200 seeds of each accession were sown in Petri dishes. After 7 days, six seedlings of comparable size of each accession/line were transplanted equidistant from each other into each plastic pot of 24 cm diameter and 25 cm deep. Each pot contained 4 kg washed and dried sand. The experiment was conducted in a greenhouse at $22 \pm 4°C$ day temperature and $11 \pm 2°C$ night temperature and 12 h day length of natural sunlight, supplemented by cold white fluorescent tubes (each of 40 W).

The experiment had four blocks in a randomized complete block design, each block containing five accessions/lines and two salt treatments. All the pots were irrigated for 4 weeks with 2 litres per pot of full strength Rorison nutrient solution once a week (Hewitt 1966). The pH of the nutrient solution was adjusted to 6·3.

Salt treatment in full strength nutrient solution was begun 4 weeks after the start of the experiment. The concentrations of NaCl used were 0 (control), and 30 mol m^{-3}. The salt concentration was increased in aliquots of 10 mol m^{-3} on alternate days until the appropriate salt treatments were reached. Treatments continued with the addition of 2 litres of the appropriate solution to each pot once a week. Six weeks after the salt treatment, just before the initiation of flowering, the following physiological/biochemical parameters were measured. The time from sowing to opening of first flower in all accessions ranged from 72 to 76 days.

Leaf water potential

The youngest fully expanded leaf was excised from each plant and the leaf water potential measurements were made with a water potential apparatus (Chas W. Cook and Sons, Birmingham, U.K.).

Osmotic potential

About 0·5–1·0 g of the youngest fully expanded leaves were excised from each plant at 09.00 h. The leaf material was frozen in 2 cm^3 polypropylene tubes for 2 weeks, thawed, and frozen sap was extracted by crushing the material with a metal rod. After centrifugation at 8000 × **g** for 4 min, the sap was used directly for osmotic potential determination in an osmometer TP 10 B (Camlab Limited).

Leaf diffusive resistance

Leaf diffusive resistance was measured with an automatic porometer (MK_3 Delta-T Device). The pump rate of the instrument was adjusted at a pump-down time of 2 s. The RH range was adjusted to 40–50%. Then a fully expanded leaf from each plant was inserted into the cup with the sensor head to measure leaf diffusive resistance. Leaf diffusive resistance data were measured three times a day, i.e. at 09.00, 12.00 and 17.00 h and pooled to calculate mean leaf diffusive resistance per day.

Epicuticular wax

After a number of preliminary experiments to compare chloroform and carbon tetrachloride solvents, varying extraction times, volume of the solvent per wash, and the number of washes required, the procedure described here maintained a high level of accuracy and repeatability for lentil. Care was taken during processing to minimize cuticular and subepidermal tissue damage.

Leaves were randomly taken from each plant and their area was measured by the graphic method. The leaf samples (1·0 g) were washed with 40, 30 and 30 ml of carbon tetrachloride for 30 s per wash. The extract thus obtained was filtered, evaporated to dryness and the remaining wax was weighed. Wax content was expressed as μg wax cm^{-2}.

Determination of soluble proteins

Total soluble proteins were determined as described by Lowry *et al.* (1951). 0·2 g of fresh 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.

Sample extract (0·2 ml) was taken in different culture tubes and was treated with Folin phenol reagent. The optical densities were read at 620 nm on a spectrophotometer (Hitachi, U2000). Soluble proteins were calculated as follows:

$$\text{Total soluble proteins mg g}^{-1}\text{ fresh weight} = \frac{\text{Reading of sample} \times \text{Volume of sample} \times \text{Dilution factor}}{\text{Weight of fresh tissue} \times 1000}$$

Determination of free amino acids

Total free amino acids were determined following the ninhydrin method (Hamilton & Van Slyke 1943).

For the estimation of total free amino acids 1 ml of each sample, which was extracted during the soluble protein estimation, was reacted with 1 ml of 10% pyridine and 1 ml of 2% ninhydrin solutions in different culture tubes. The optical densities of these coloured solutions were then read at 570 nm. Amino acids were calculated as follows:

$$\text{Total free amino acids mg g}^{-1}\text{ fresh weight} = \frac{\text{Reading of sample} \times \text{Volume of sample} \times \text{Dilution factor}}{\text{Weight of fresh tissue} \times 1000}$$

Plants from each pot were harvested 6 weeks after the start of salt treatment just before the onset of flowering. Plant roots were removed carefully from the sand and were washed in distilled deionized water. Fresh and dry weights of all the samples were recorded and dry material was used for the estimation of reducing and non-reducing sugars.

Estimation of reducing and non-reducing sugars

Dried leaves (0·5 g) were extracted in 75% ethyl alcohol. The extract was passed through an ion exchanger (Cationic resin Amberlite IR-120) to separate sugars from free amino acids.

Total sugars were estimated by the method described by Trevelyan & Harrison (1952): 0·2 ml of each sample extract was treated with 4 ml of anthrone reagent. The absorbance was read at 620 nm against a blank using a spectrophotometer (Hitachi, U2000). The total amount of sugars (mg g^{-1} dry weight) was calculated from a standard curve.

Total reducing sugars were estimated by the method described by Hulme & Narian (1931). Sample extracts of 1 ml each were treated with sodium carbonate and potassium ferricyanide solutions and then with potassium iodide-zinc sulphate-sodium chloride solution and sulphuric acid solution. 1% starch solution was used as an indicator. The violet coloured reaction mixture was then titrated with standard sodium thiosulphate solution to a colourless end-point. The total reducing sugars were calculated using the following formula:

$$S = b\,(T + a)$$

where S is amount of reducing sugars; b is a terminal factor value = 0·340; T is volume of sodium thiosulphate used; and a is a terminal factor value = 0·05. Total non-reducing sugars were calculated by subtracting total reducing sugars from total sugars.

RESULTS

The data for fresh and dry weights of shoots and roots of five accessions of lentil are presented in Fig. 1 and analysis of variance of the data in Table 1. Salt in the rooting medium caused a significant reduction in fresh and dry weights of shoots of all accessions (shoot fresh wt $P \leqslant 0{\cdot}01$; shoot dry wt $P \leqslant 0{\cdot}001$), whereas the overall effect of NaCl on either root fresh weights or dry weights was non-significant. However, accessions × treatment interactions were significant (shoot fresh wt $P \leqslant 0{\cdot}05$; shoot and root dry wts $P \leqslant 0{\cdot}01$; root fresh wt $P \leqslant 0{\cdot}001$). Accessions ILL 6451, ILL 6788 and ILL 6793 produced significantly greater ($P \leqslant 0{\cdot}05$) fresh and dry biomass of shoots compared with ILL 6439 and ILL 6778 in the salt treatment. In root fresh biomass there was a gradual decline from ILL 6451 (the most tolerant) to ILL 6778 (the most sensitive). The root dry matters of ILL 6451 and ILL 6788 were not affected by the NaCl treatment, whereas those of the other accessions declined significantly. ILL 6793 was moderately tolerant in both fresh and dry weights of roots.

Leaf water potential (Table 2) of all accessions decreased after the salt treatment compared with the controls. ILL 6451 and ILL 6788 had significantly higher ($P \leqslant 0{\cdot}05$) leaf

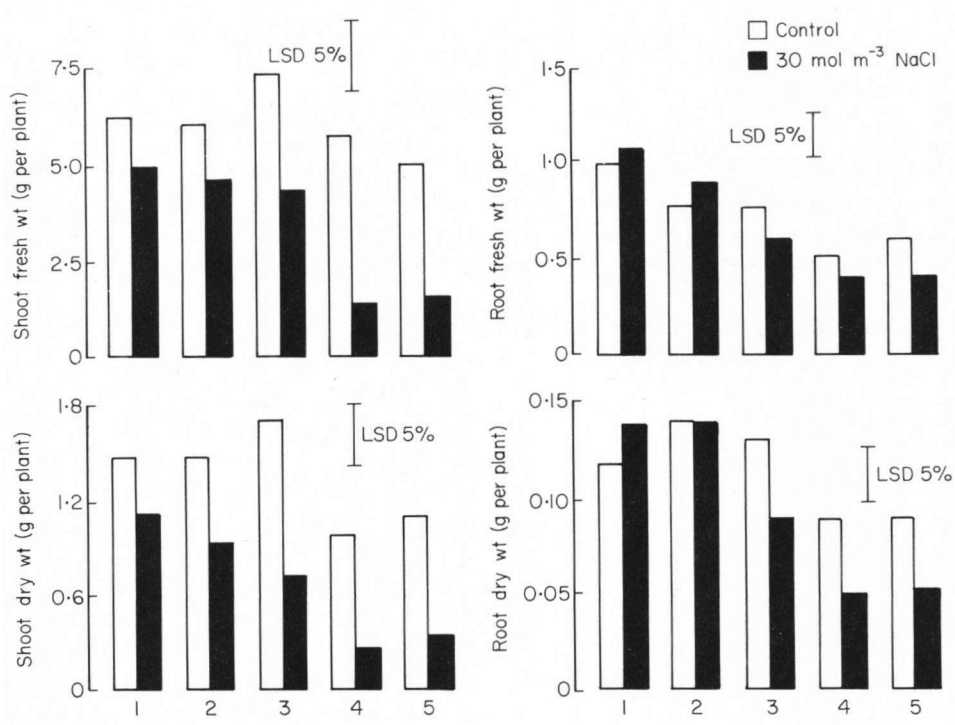

Fig. 1. Mean fresh and dry weights of shoots and roots (g per plant) of five accessions of lentil after 42 days' growth in sand culture salinized with 0 or 30 mol m^{-3} NaCl in full strength Rorison nutrient solution: accessions (1) ILL 6451 (2) ILL 6788 and (3) ILL 6793 are salt-tolerant; accessions (4) ILL 6439 and (5) ILL 6778 are salt-sensitive.

Table 1. Analysis of variance (mean squares) of fresh and dry weights of shoots and roots of five accessions of lentil after 42 days' growth in sand culture salinized with 0 or 30 mol ml^{-3} NaCl in full strength nutrient solution

Source of variation	df	Fresh wt		Dry wt	
		Shoot	Root	Shoot	Root
Blocks	3	1·21 NS	0·029 NS	0·04 NS	0·0005 NS
Accessions (Acc)	4	21·69***	0·119**	0·71***	0·0019**
Treatments (T)	1	13·73**	0·034 NS	0·56***	0·0006 NS
Acc × T	4	6·61*	0·245***	0·25**	0·0018**
Residual	27	1·66	0·026	0·06	0·0004

*$P<0·05$, **$P<0·01$ and ***$P<0·001$ levels.
NS, not significant.

water potential than the other accessions. Leaf osmotic potential (Table 2) of all accessions decreased significantly after the salt treatment (Table 3). ILL 6451 and ILL 6788 had significantly lower ($P\leqslant 0·05$) leaf osmotic potential than the other

Table 2. Mean leaf water potential (− MPa), osmotic potential (− MPa), leaf diffusive resistance (s cm^{-1}), and epicuticular wax content (μg cm^{-2}) of five accessions of lentil after 42 days' growth in sand culture salinized with 0 or 30 mol m^{-3} NaCl in full strength Rorison nutrient solution

Accession number/name	(NaCl concentrations mol m^{-3}) Water potential		Osmotic potential		Leaf diffusive resistance		Wax content	
	0 (Control)	30	0 (Control)	30	0 (Control)	30	0 (Control)	30
1. ILL 6451	1·3a*	1·6a	1·6a	2·4ab	3·9ab	4·5ab	583·9a	286·4a
2. ILL 6788	1·2ab	1·7ab	1·6a	2·5a	4·4a	4·2a	434·0a	346·6a
3. ILL 6796	1·0b	1·9bc	1·4a	2·1bc	2·3b	4·8ab	416·6a	470·7ab
4. ILL 6439	1·2ab	2·1c	1·4a	2·0c	3·5ab	6·4bc	479·3a	548·3bc
5. ILL 6778	1·0b	2·0c	1·4a	1·9c	4·0ab	8·2c	443·9a	678·1c

*Means with the same letters in each column do not differ significantly at the 5% level.

Table 3. Analysis of variance (mean squares) of leaf water potential, osmotic potential, leaf diffusive resistance and epicuticular wax content of five accessions of lentil after 42 days' growth in sand culture salinized with 0 or 30 mol m^{-3} NaCl in full strength Rorison nutrient solution

Source of variation	df	Water potential	Osmotic potential	Leaf diffusive resistance	Wax content
Blocks	3	0·023 NS	0·035 NS	12·3 NS	25 764·2 NS
Accessions (Acc)	4	0·492***	0·518***	59·4***	307 234·2***
Treatment (T)	1	0·580***	0·366**	66·7***	39 682·6 NS
Acc × T	4	0·132*	0·178*	29·9**	102 411·4**
Residual	27	0·039	0·048	7·2	19 641·6

*$P<0·05$, **$P<0·01$ and ***$P<0·001$.
NS, not significant.

accessions. Leaf diffusive resistance (Table 2) of ILL 6796, ILL 6439 and ILL 6778 increased significantly after the salt treatment, whereas that of ILL 6451 and ILL 6788 remained unaffected. ILL 6439 and ILL 6778 had a significantly greater leaf diffusive resistance than the other accessions after the salt treatment. Epicuticular wax content (Table 2) of ILL 6778 increased and that of ILL 6451 decreased, whereas that of the remaining three accessions remained unchanged. ILL 6439 and ILL 6778 had a significantly greater ($P \leqslant 0·05$) epicuticular wax content than the other accessions.

The data for leaf soluble proteins, total free amino acids and reducing and non-reducing sugars of five accessions of lentil are presented in Fig. 2 and their analysis of variance in Table 4. Leaf soluble proteins of all accessions generally decreased after the salt treatment compared with those of controls. ILL 6439 had the highest and ILL 6778 the lowest leaf soluble proteins of all accessions. Although there was no significant effect of NaCl on the free amino acids of all accessions, the accessions differed significantly ($P \leqslant 0·001$). ILL 6451 was the highest and ILL 6439 and ILL 6778 the lowest in total free amino acids of all accessions after the salt treatment. Reducing sugars (Table 4) of ILL 6439 increased and those of ILL 6778 decreased after the salt treatment, whereas those of the remaining accessions remained unaffected. ILL 6439 had a significantly ($P \leqslant 0·05$) higher content of reducing sugars than the other accessions after the salt treatment. Non-reducing sugars of all accessions decreased significantly ($P \leqslant 0·001$) due to the effect of NaCl. ILL 6439 and ILL 6778 accumulated less non-reducing sugars than the other accessions after the salt treatment. ILL 6788 was the highest in non-reducing sugar accumulation of all accessions after the salt treatment.

DISCUSSION

The results presented here for shoot and root biomass confirm the high salt tolerance of two accessions (ILL 6451 and ILL 6788), the moderate tolerance of one (ILL 6793), and the salt sensitivity of two accessions (ILL 6439 and ILL 6778), as was observed in a previous study (Ashraf & Waheed 1990).

Osmoregulation is an important process governing salt tolerance in plants because it reduces the cell osmotic potential to a level which provides high turgor potential for

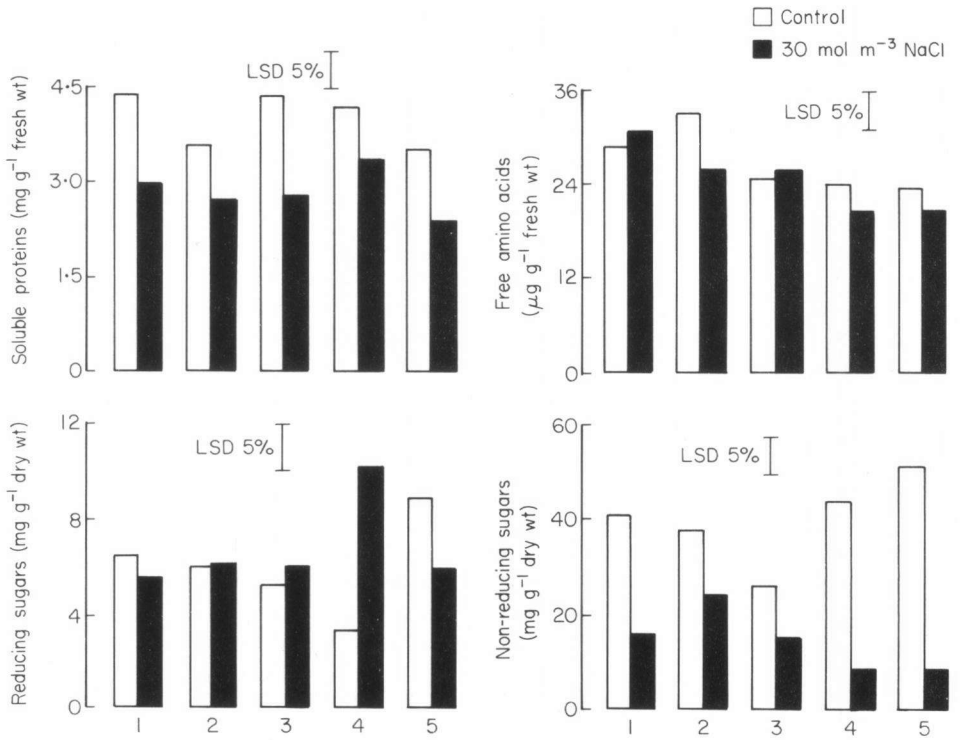

Fig. 2. Mean leaf soluble proteins (mg g^{-1} fresh leaves), free amino acids (μg g^{-1} fresh leaves), and reducing and non-reducing sugars (mg g^{-1} dry leaves) of five accessions of lentil after 42 days' growth in sand culture salinized with 0 or 30 mol m^{-3} NaCl in full strength Rorison nutrient solution. Key to accessions as in Fig. 1.

Table 4. Analysis of variance summaries (mean squares) of soluble proteins, free amino acids, and reducing and non-reducing sugars of five accessions of lentil after 42 days' growth in sand culture salinized with 0 or 30 mol m^{-3} NaCl in full strength Rorison nutrient solution

Source variation	df	Soluble proteins	Free amino acids	Reducing sugars	Non-reducing sugars
Blocks	3	0·93 NS	12·4 NS	1·8 NS	31·4 NS
Accessions (Acc)	4	1·09**	86·7***	18·6***	259·9***
Treatments (T)	1	1·98***	15·6 NS	5·1 NS	278·6**
Acc × T	4	0·84*	37·3**	10·3**	192·8**
Residual	27	0·21	8·8	2·2	36·9

*$P<0·05$, **$P<0·01$ and ***$P<0·001$.
NS, not significant.

maintaining growth (Cram 1976; Maas & Nieman 1978; Greenway & Munns 1980). The decrease in the solute potential of plants grown under salt stress may be due to either water loss or an increase in dissolved solutes, or a combination of both. The significantly lower

leaf osmotic potentials of the two salt-tolerant accessions, ILL 6451 and ILL 6788, after the salt treatment cannot be explained in view of the accumulation of different organic osmotica determined in this study. It is possible that other organic osmotica and inorganic solutes that have not been determined in this study might have played a role in maintaining low osmotic potentials of the tolerant accessions and high osmotic potentials in sensitive accessions. Nevertheless, the accumulation of relatively high amounts of soluble non-reducing sugars and free amino acids in the tolerant accessions, ILL 6451 and ILL 6788, might have played a significant role in maintaining vigorous growth under saline conditions by regulating appropriate metabolic reactions.

From a number of studies (Cram 1976; Greenway & Munns 1980; Bates & Hall 1981; Ashraf 1989) it is evident that mechanisms of salt tolerance and drought tolerance share, to some degree, osmotic adjustment phenomenon. But from the results presented here for the salt tolerance of five accessions of lentil, and from the early findings of Ashraf *et al.* (1992) on the drought tolerance of nine accessions including the five examined in this study, no such relationship between the two mechanisms was found. For example, ILL 6439, which is highly sensitive to salt, was highly tolerant to drought. Of the three salt-tolerant accessions only one, ILL 6451, showed positive correlation between salt tolerance and drought tolerance, whereas the remaining two, ILL 6788 and ILL 6793, showed a negative relationship.

There was a negative correlation between data for leaf water potential and leaf diffusive resistance of both salt-tolerant and salt-sensitive accessions. For example, the salt-sensitive accessions, ILL 6439 and ILL 6778, had a significantly lower water potential than the tolerant accessions, but had a greater leaf diffusive resistance. The leaf diffusive resistance regulates water evaporation and CO_2 diffusion (Jordan *et al.* 1975; Bates & Hall 1981). It is also known that severe plant water deficits are correlated with an increase in leaf diffusive resistance. But the correlation between increase in leaf diffusive resistance due to osmotic stress and leaf water potential is controversial. For example, Bates & Hall (1981) and Osonubi (1985) observed in cowpea that the increase in leaf diffusive resistance was independent of leaf water potential. Similarly, Blackman & Davies (1985), working with maize, found that both water potential and turgor potential had no correlation with the increase in stomatal resistance.

Deposition of wax on the leaf surface of both sensitive accessions was higher than that of the tolerant accessions. The lower epicuticular wax content of the tolerant accessions differing in tolerance can be related to their lower leaf diffusive resistance, compared with the sensitive accessions. These findings are in close conformity with the early findings of Johnson *et al.* (1983) and Jordan *et al.* (1984) who concluded that epicuticular wax plays a pivotal role in minimizing evaporative losses.

Leaf soluble proteins generally decreased in all accessions with the addition of NaCl in their rooting medium and there was no clear difference between accessions differing in salt tolerance. Thus, accumulation of soluble proteins cannot be used as an indicator for salt tolerance in lentil. This is in contrast to the findings of Langdale *et al.* (1973) that salinity enhances protein synthesis in cereals and of Helal *et al.* (1975) that salinity promotes the conversion of N into proteins.

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REFERENCES

Ashraf, M. (1989): The effect of NaCl on water relations, chlorophyll, and protein and proline contents of two cultivars of blackgram (*Vigna mungo* L.). *Plant Soil* **119:** 205–210.

Ashraf, M., Bokhari, M.H. & Chishti, S.N. (1992): Variation in osmotic adjustment of accessions of lentil (*Lens culinaris* Medic.) in response to drought stress. *Acta Bot. Neerl.* **41:** 51–62.

Ashraf, M., McNeily, T. & Bradshaw, A.D. (1986): The response of selected salt tolerant and normal lines of four grass species to NaCl in sand culture. *New Phytol.* **104:** 453–461.

Ashraf, M. & Waheed, A. (1990): Screening of local/exotic accessions of lentil (*Lens culinaris* Medic.) for salt tolerance at two growth stages. *Plant Soil* **128:** 167–176.

Bates, L.M. & Hall, A.E. (1981): Stomatal closure with soil water depletion not associated with changes in bulk leaf water status. *Oecologia* **50:** 62–65.

Blackman, P.G. & Davies, W.G. (1985): Root to shoot communication in maize plants—the effects of soil drying. *J. Exp. Bot.* **36:** 39–48.

Carter, D.I. (1975): Problems of salinity in agriculture. In: Poljakoff Mayber, A. and Gale, J. (eds.): *Plants in Saline Environments*, 25–35. Springer-Verlag, Berlin.

Cram, W.J. (1976): Negative feedback regulations of transport in cells. The maintenance of turgor, volume and nutrient supply in plants. II. Part A. Cells. *Encyclopaedia of Plant Physiology*, New series, Vol. 2, pp. 284–316. Springer-Verlag, Berlin.

Epstein, E. (1985): Salt tolerant crops: Origins, development and prospects of concept. *Plant Soil*, **89:** 187–198.

Epstein, E., Rush, D.W., Kingsbury, R.W., Kelley, D.B., Cunningham, G.A. & Wrona, A.F. (1980): Saline culture of crops: a genetic approach. *Science* **210:** 399–404.

Greenway, H. & Munns, R. (1980): Mechanisms of salt tolerance in non-halophytes. *Ann. Rev. Plant Physiol.* **31:** 149–190.

Hamilton, P.B. & Van Slyke, D.D. (1943): Amino acid determination with ninhydrin. *J. Biol. Chem.* **150:** 231–233.

Helal, M., Koch, K. & Mengel, K. (1975): Effect of salinity and potassium on the uptake of nitrogen and on nitrogen metabolism in young barley plants. *Physiol. Plant.* **35:** 310–313.

Hewitt, E.J. (1966): *Sand and Water Culture Methods Used in the Study of Plant Nutrition.* Commonwealth Agricultural Bureau Technical Communication No. 22. 2nd edn. CAB Slough, U.K.

Hulme, A.C. & Narian, R. (1931): The ferricyanide method for the determination of reducing sugars. A modification of the Hagedora-Jonsen-Hanes technique. *Biochem. J.* **25:** 105–161.

Johnson, D.A., Richards, R.A. & Turner, N.C. (1983): Yield, water relations, gas exchange, and surface reflectance of near isogenic wheat lines differing in glaucousness. *Crop Sci.* **23:** 318–325.

Jordan, W.R., Brown, K.W. & Thomas, J.C. (1975): Leaf age as a determinant in stomatal control of water loss from cotton during water stress. *Plant Physiol.* **56:** 595–599.

Jordan, W.R., Shouse, P.T., Blum, A., Miller, F.R. & Monk, R.K. (1984): Environmental physiology of sorghum II. Epicuticular wax load and cuticular transpiration. *Crop Sci.* **24:** 1168–1173.

Langdale, G. Wn., Thomas, J.R. & Littleton, T.G. (1973): Nitrogen metabolism of Star grass as affected by nitrogen and soil salinity. *Agron. J.* **65:** 468–470.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randal, R.J. (1951): Protein measurement with Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.

Maas, E.V. & Hoffman, G.J. (1977): Crop salt tolerance—current assessment. *J. Irrig. Drain. Div. Amer. Soc. Civil Eng.* **103:** 115–134.

Maas, E.V. & Nieman, R.H. (1978): Physiology of plant tolerance to salinity. In: (Eds. ASS, CSA & SSSA) *Crop Tolerance to Suboptimal Land Conditions*, pp. 277–299, ASS, CSA. Madison, Wisconsin.

Moftah, A.E., & Michel, B.E. (1987): The effect of NaCl on solute potential and proline accumulation in soybean leaves. *Plant Physiol.* **83:** 238–240.

Osonubi, O. (1985): Responses of cowpeas (*Vigna unguiculata* (L.) Walp) to progressive soil drought. *Oecologia* **66:** 554–557.

Shannon, M.C. (1978): Testing salt tolerance variability among tall wheatgrass lines. *Agron. J.* **70:** 719–722.

Trevelyan, W.E. & Harrison, J.S. (1952): Studies on yeast metabolism. I. Fractionation and microdetermination of cell carbohydrates. *J. Biol. Chem.* **50:** 298–310.

Wyn Jones, R.G., Brady, C.J. & Speirs, J. (1979): Ionic and osmotic regulation in plants. In: Laidman, D.L. & Wyn Jones, R.G. (eds): *Recent Advances in the Biochemistry of Cereals*, pp. 63–103. Academic Press, London.

Wyn Jones, R.G. (1981): Salt tolerance. In: C.B. Johnson (ed.) *Physiological Processes Limiting Plant Productivity*, 271–292. Butterworths, London.

Yeo, A.R. & Flowers, T.J. (1984): Mechanisms of salinity resistance in rice and their role as physiological criteria in plant breeding. In: Staples, R.C. & Toenniessen, G.H. (eds): *Salinity Tolerance in Plants—Strategies for Crop Improvement*, 151–170. John Wiley & Sons, New York.