



## The response of lucerne (*Medicago sativa* L.) to sodium sulphate and chloride salinity

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### Abstract

Sodium and sulphate-dominated salinity is a serious environmental problem occurring in soils and groundwater in many parts of the world. The effect of Na<sub>2</sub>SO<sub>4</sub> and NaCl, at electrical conductivity levels ranging from 2 to 17 dS m<sup>-1</sup>, on the growth and tissue ion concentrations of 16 lines of lucerne (*Medicago sativa* L.) was examined in the greenhouse over a 2 month period. Averaged across all lines, plants grown at 17 dS m<sup>-1</sup> produced 66% of the dry matter of plants grown at 2 dS m<sup>-1</sup>. However there were significant differences among lines in relative salt tolerance (as defined by the slope of the reduction in dry matter) versus electrical conductivity. Dry matter production was negatively correlated with shoot concentrations of Na<sup>+</sup>, Cl<sup>-</sup> and S<sup>2-</sup> and generally lines that were more tolerant to salinity had lower concentrations of those ions in the shoots. We conclude that lucerne is moderately tolerant to Na<sub>2</sub>SO<sub>4</sub>-predominated salinity, and that the degree of intraspecific variation that exists within this species will allow more tolerant lines to be selected for establishment in conditions where sulphate salinity is a problem.

### Introduction

Much of the research quantifying the salt tolerance of plant species has been based on experiments in which NaCl is the predominant salt. There has been comparatively little research examining plant responses to situations where Na<sub>2</sub>SO<sub>4</sub> dominates. However, Na<sub>2</sub>SO<sub>4</sub> is present at higher concentrations than NaCl in the soils and groundwater in many areas of the world including parts of India, Egypt and California (Banuelos et al., 1993; Manchanda and Sharma, 1989). In the few studies where plant responses to both NaCl and Na<sub>2</sub>SO<sub>4</sub> have been examined and compared, it has been found that the degree of growth suppression differs according to which salt dominates and the species that is being studied (Khan et al., 1995; Manchanda et al., 1982;

Meiri et al., 1971). Ion uptake, salt accumulation and parameters such as transpiration rates, may also be affected to different degrees by the two salts (Meiri et al., 1971).

In the San Joaquin Valley of Central California, Na<sub>2</sub>SO<sub>4</sub> is the predominant salt in farm drainage water that is being reused to irrigate crops in an irrigation system that starts with very salt-sensitive species and progresses to salt-tolerant species as the drainage water becomes more degraded (Rhoades, 1989). This reuse system is being promoted as an environmentally-sound method for the disposal of saline drainage water. For such a system to be successful, it is important to ascertain the tolerance of particular species to Na<sub>2</sub>SO<sub>4</sub>-salinity. Lucerne, a high value fodder crop grown widely in many irrigation areas of the world including the San Joaquin Valley, is moderately sensitive to NaCl (Maas and Hoffman, 1977). However, its tolerance to Na<sub>2</sub>SO<sub>4</sub> has not been published nor is

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Table 1. Sources of the 16 seed lines of lucerne (*Medicago sativa*) that were used in the study

Line	Origin
1. Moapa	Commercial cultivar
2. Cuf 101	Commercial cultivar
3. AZ88NDC	Cultivar developed for tolerance to NaCl (released 1988, University of Arizona)
4. AZ90NDC-ST	Cultivar developed for tolerance to NaCl derived from line 3 – AZ88NDC (released 1990, University of Arizona)
5. B-88 (86292) parent	Line developed by Waterman–Loomis for tolerance to NaCl
6. B-88 (93206) progeny	Line developed by Waterman–Loomis for tolerance to NaCl Progeny of line 5 (B-88 (86292))
7. B204 (91-216) parent	Line developed by Waterman–Loomis for tolerance to NaCl
8. B204 (93 BGX-14) progeny	Line developed by Waterman–Loomis for tolerance to NaCl Progeny of line 7 (B204 (91-216))
9. WL 525 HQ	Commercial cultivar
10. UC Salton	Commercial cultivar
11. Nevada NMP 25	Non-dormant line with root knot resistance (Prosser)
12. Nevada NMP 26	Non-dormant selections from 16 (Prosser)
13. Nevada Syn yy	Progeny from cross between non-dormant material and Northern root knot nematode resistant germplasm
14. MSBCWAn3	Selection from material at Prosser
15. MSACW3An3CLS5	Selection for resistance to bacterial wilt and anthracnose resistance
16. SW32An4P3	Selection from Moapa plus another parent for resistance to anthracnose and phytophthora

it known whether there is any intra-specific variation for tolerance to this salt. The objectives of this study were, firstly, to quantify the tolerance of lucerne to Na<sub>2</sub>SO<sub>4</sub> salinity, and secondly, to assess whether any intra-specific variation in tolerance exists which will enable material to be identified that has greater adaptation to areas, such as the San Joaquin Valley, where Na<sub>2</sub>SO<sub>4</sub>-salinity predominates.

### Materials and methods

Seed of sixteen lines of lucerne were sown into vermiculite in seedling trays (100 cm × 30 cm) in the greenhouse at Riverside, California (33°58.24' latitude, 117°19.12' longitude, 297 m elevation). Day time air temperatures ranged from 18 to 40 °C (mean = 31 °C), night time air temperatures ranged from 16 to 30 °C (mean = 22 °C) and the relative humidity ranged from 44 to 52%. The seed lines chosen for this study were registered cultivars as well as several breeders lines (Table 1). Several of the lines (viz. lines 3, 4, 5, 6, 7, 8 and 10) had been developed for tolerance to NaCl. Other lines (viz. lines 11, 12, 13, 14, 15 and 16)

had been developed for disease resistance. The cultivars Moapa and CUF101 were chosen because they had been included in previous studies for tolerance to NaCl (e.g. Noble et al., 1984). Following germination and the emergence of the first trifoliate leaf (six days after sowing in vermiculite in trays), seedlings were transplanted into tanks (1.2×0.6×0.5 m deep) containing washed river sand (average bulk density of 1.2 Mg m<sup>-3</sup>). Ten plants of each line were sown in each tank in rows that were spaced 10 cm apart. Average in-row spacing between plants was 0.5 cm.

For the first 2 weeks the seedlings were irrigated four times daily with a modified nutrient solution consisting of 5.0 mM Ca<sup>2+</sup>, 1.25 mM Mg<sup>2+</sup>, 15 mM Na<sup>+</sup>, 2 mM K<sup>+</sup>, 6.9 mM SO<sub>4</sub><sup>2-</sup>, 7 mM Cl<sup>-</sup>, 5.0 mM NO<sub>3</sub><sup>-</sup>, 0.17 mM KH<sub>2</sub>PO<sub>4</sub>, 23 μM Fe as sodium ferric diethylenetriamine pentaacetate (NaFeDTPA) 23 μM H<sub>3</sub>BO<sub>3</sub>, 5 μM MnSO<sub>4</sub>, 0.4 μM ZnSO<sub>4</sub>, 0.2 μM CuSO<sub>4</sub>, and 0.1 μM H<sub>3</sub>MoO<sub>4</sub> added to local tap water. The electrical conductivity of this solution was about 2 dS m<sup>-1</sup>. Each irrigation cycle lasted until the sand was saturated (10 min), after which the nutrient solution drained into 800 L reservoirs for recycling for the next irrigation. Water lost by evapotranspiration

Table 2. Chemical composition of the six salinity treatments used in the study

Treatment	1	2	3	4	5	6
Target	2.0	4.1	7.9	11.9	15.9	20.1
EC (dS m <sup>-1</sup> )						
Actual	2.1	3.8	7.0	9.7	14.1	17.2
EC (dS m <sup>-1</sup> )						
Ion concentration (meq l <sup>-1</sup> )						
Ca	5.1	8.9	16.5	25.2	26.9	26.9
Mg	3.1	6.6	13.1	20.0	30.9	40.2
Na	13.8	29.1	58.2	88.5	137.0	178.0
K	2.1	2.1	2.1	2.1	2.1	2.1
SO <sub>4</sub>	14.0	29.6	59.1	89.8	127.5	158.0
Cl	3.5	14.1	28.2	42.9	66.4	86.3

was replenished each day to maintain constant osmotic potentials ( $\Psi_s$ ) in the solutions.

Two weeks after transplanting, five salinity treatments were imposed by adding specific amounts of the salts MgSO<sub>4</sub>, Na<sub>2</sub>SO<sub>4</sub>, NaCl and CaCl<sub>2</sub> in increments equivalent to 2–4 dS m<sup>-1</sup> per day until the solutions reached final electrical conductivities of 2.1, 3.8, 7.0, 9.7, 14.1 and 17.2 dS m<sup>-1</sup> (Table 2). Salinity treatments were modified according to a concentration based on the model of Simunek and Suarez (1994) which simulates typical soil water interactions to include absorption, desorption, dissolution and precipitation (Table 2).

The experiment consisted of three replicates of the six salt treatments giving a total of 18 sand tanks. This was a split plot design with salt applied at the main plot level and the plant lines at the subplot level. The electrical conductivities of the solutions were measured every two days and samples of the solutions were taken twice during the duration of the experiment for chemical analysis (Ca, Mg, Na, K, P and S) using Inductively Coupled Plasma Optical Emission Spectrophotometry (ICP, Labtam Plasma Scan). Chloride was determined by colorimetric amperometric titration.

All plants were harvested one week after the full salinity treatments had been imposed. At harvest, the row of 10 plants was cut to 2 cm above the base of the plant. Shoot fresh weight and dry weight (dried at 70 °C for 48 h) were measured. Chloride was determined on nitric-acetic acid extracts by colorimetric-amperometric titration. Na, K, Mg, Ca and S were determined on nitric-perchloric acid digests of the

dried, ground plant material by the ICP. Two additional harvests were undertaken at two weeks and four weeks after the first harvest and the shoots were chemically analysed as described previously.

### Statistical Analyses

Plant dry matter production (expressed as production per row of 10 plants) and tissue ion concentrations were analysed by ANOVA with a randomised block structure (Genstat 5.0, Lawes Agricultural Trust, Rothamsted Experimental Station). There was insufficient plant material from Line 4 to undertake any chemical analyses. Residuals were checked for normality and homogeneity. Orthogonal polynomials were fitted to the data with Na<sub>2</sub>SO<sub>4</sub> as a quantitative explanatory variable. The linear and quadratic components, or contrasts, were tested for significance and quantified with P values. The data are plotted as fitted curves with the observed means represented by points. Fischer's unrestricted Least Significant Differences are included to complement the means. For dry weight, the quadratic component was insignificant and the response curves are, therefore, represented as straight lines. For tissue concentrations of Na, Cl and S, the quadratic components were significant and the data are plotted as curves. There was no variation between harvests in the performance of particular lines and the results are presented for harvest 2. Line 1 is included in all graphs to assist in comparing the responses of the 16 lines.

## Results

### Dry Matter Production

The 16 lines of lucerne differed significantly ( $P < 0.001$ ) in both absolute dry matter production and in relative dry matter response to Na<sub>2</sub>SO<sub>4</sub> (as defined by the slope of the response curve) (Figure 1). Moapa (line 1) proved to be one of the most salt tolerant, as well as one of the highest producing lines, especially at moderate to high salinity levels (10–17 dS m<sup>-1</sup>), and was significantly ( $P < 0.05$ ) more salt tolerant than the lines Nevada NMP25 (line 11), WL525 (line 9) and Cuf 101 (line 2). The salt tolerance of all the other lines tested (viz. lines 3, 4, 5, 6, 7, 8, 10, 12, 13, 14, 15, 16) did not differ significantly from Moapa although there was a large amount of variation within some of these, especially the material that was still in the early stages of cultivar development (e.g.

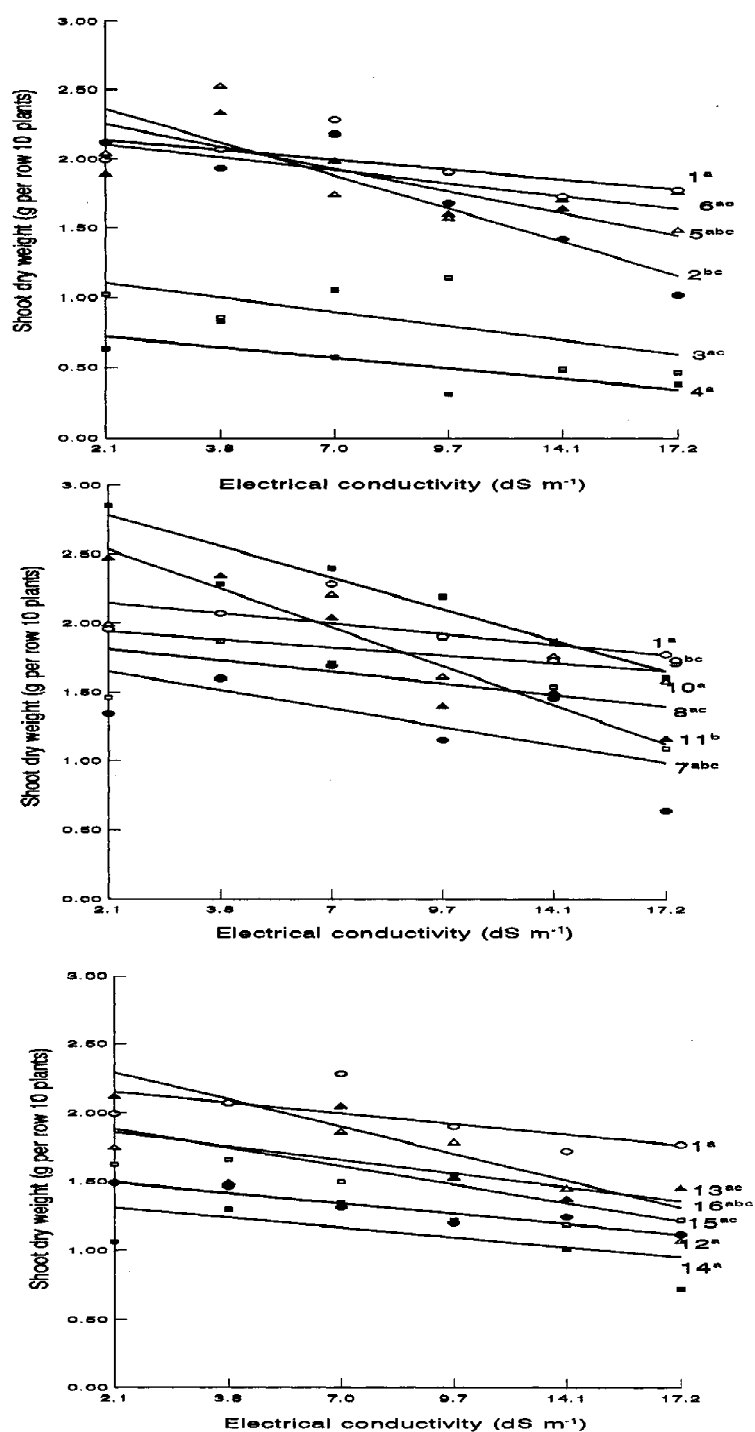


Figure 1. The effect of salinity on dry matter production in 16 lines of *M. sativa*. Line 1 is included in all figures for comparison. (a) Lines 1, 2, 3, 4, 5, 6; (b) Lines 1, 7, 8, 9, 10, 11; (c) Lines 1, 12, 13, 14, 15, 16.  $\circ$  = Line 1,  $\bullet$  = Lines 2, 7, 12,  $\square$  = Lines 3, 8, 13,  $\blacksquare$  = Lines 4, 9, 14,  $\triangle$  = Lines 5, 10, 15,  $\blacktriangle$  = Lines 6, 11, 16. Significance of effects: line  $P < 0.001$ ,  $\text{Na}_2\text{SO}_4$  (linear)  $P < 0.014$ , (quadratic)  $P = 0.465$ .  $\text{Na}_2\text{SO}_4 \times$  line (linear)  $P = 0.075$ , (quadratic)  $P = 0.207$ . Slopes with any similar superscript are not significantly different ( $P > 0.05$ ).

lines 7, 14, 15). AZ88NDC (line 3) and in particular its progeny AZ90NDC-ST (line 4) – lines which had been especially developed for tolerance to NaCl – were less productive than other lines with respect to dry matter but showed a good degree of tolerance to Na<sub>2</sub>SO<sub>4</sub>-dominated salts.

The responses (and slope of the response curve) of several of the progeny lines, e.g. lines 6 and 8 were similar to those of their parental lines 5 and 7 (Figure 1). None of the progeny lines, namely 4, 6, 8 and 12, showed a significant improvement in relative salt tolerance compared with the parent material. However, absolute dry matter production had been improved (in most cases) under the respective salt tolerance breeding program. SW32An4P3 (line 16) performed similarly to its parent line Moapa (line 1) but did not produce more dry matter under saline conditions perhaps because a degree of salt tolerance was lost during selection for anthracose and phytophthora resistance.

#### Tissue Concentrations

##### Na, Cl and SO<sub>4</sub>

Tissue concentrations of Na, Cl and S increased significantly ( $P < 0.001$ ) with increasing external concentrations of these ions (Figures 2, 3 and 4 and Table 2). There were also significant differences ( $P < 0.001$ ) between plant lines and significant ( $P < 0.001$ ) salinity-line interactions.

The cultivar Moapa, amongst the most salt tolerant lines in terms of dry matter production, also had the lowest concentrations of Na, Cl and S in the shoot. Lines B-88 (5) and WL525 HQ (9) responded in a similar way to Moapa for all three ions. Shoot ion concentrations of Na and S for line SW32An4P3 (16 – derived from Moapa) were similar to Moapa but concentrations of Cl were greater. Concentrations of Na, Cl and S in Nevada NMP 26 (12) were amongst the highest of all lines and were always significantly ( $P < 0.001$ ) greater than Moapa.

The shoot ion responses showed variation among the parent-progeny lines that had been developed specifically for tolerance to NaCl. The response curves for lines B204 91-216 (7 parent) and B204 93 BGX-14 (8 progeny) did not differ. However the response for lines B-88 86292 (5) and B-88 93206 (6) were significantly ( $P < 0.001$ ) different, with concentrations of all three ions (Na, Cl and S) being lower ( $P < 0.001$ ) in the parent line (5) compared with the progeny (line 6). Tissue concentrations of Cl and Na

were greater in line Nevada NMP26 (12 progeny) than in line SW32An4P3 (16 parent), but there was no difference in the slopes of the response curves for these ions.

Averaged across all lines, concentrations of Na, Cl and S were all significantly ( $n = 266$ ,  $P = 0.001$ ) negatively correlated with dry matter production (viz. Na:  $r = -0.364$ , Cl:  $r = -0.304$ , S:  $r = -0.365$ ) suggesting that low concentrations of these ions are associated with higher levels of shoot dry matter.

##### Ca, Mg, K and P

Tissue ion concentrations of Ca, Mg and K decreased significantly ( $P < 0.001$ ) in all plant material with increasing external salinity. (Table 3). The response of plant tissue concentrations of P was varied ( $P = 0.26$ ) but showed a decreasing trend as concentrations of Na<sub>2</sub>SO<sub>4</sub> increased.

Individual lines differed significantly ( $P < 0.001$ ) in shoot concentrations of all four elements (Ca, Mg, K and P). For Ca, line Nevada NMP25 (11) had the highest shoot concentration of Ca (368 mmol kg<sup>-1</sup> dwt across all salinity levels) compared with 298 mmol kg<sup>-1</sup> dwt for line MSACW3An3CLS5 (15). For Mg, the range was from 163 mmol kg<sup>-1</sup> dwt for line 15 to 136 mmol kg<sup>-1</sup> dwt for line Nevada NMP26 (12). For K, mean concentrations ranged from 1145 mmol kg<sup>-1</sup> dwt for line B204 93 BGX-14 (8) to 971 mmol kg<sup>-1</sup> dwt for line AZ88NDC (3), and for P the range was from 79 mmol kg<sup>-1</sup> dwt (line UC Salton (10)) to 70 mmol kg<sup>-1</sup> dwt (line B204 91-216 (7)). There was a significant salinity-line interaction only for Ca ( $P = 0.03$ ).

#### Discussion

This study has shown lucerne to be moderately tolerant to EC levels up to 17 dS m<sup>-1</sup> where Na<sub>2</sub>SO<sub>4</sub> is the major salt. For example, at 17 dS m<sup>-1</sup>, dry matter production averaged across all 16 lucerne lines was reduced to 66% of the production at 2 dS m<sup>-1</sup>. By extrapolation with other studies (e.g. Brown and Hayward, 1956; Mohammad et al., 1989; Noble et al., 1987), we can speculate that lucerne is more tolerant to salinity where Na<sub>2</sub>SO<sub>4</sub> predominates than to situations where NaCl is the dominant salt. For example, at 14 dS m<sup>-1</sup> from NaCl, the growth of six cultivars of lucerne was reduced to 42% of that at the control treatment, (Brown and Hayward, 1956), but further studies are required to confirm this finding.

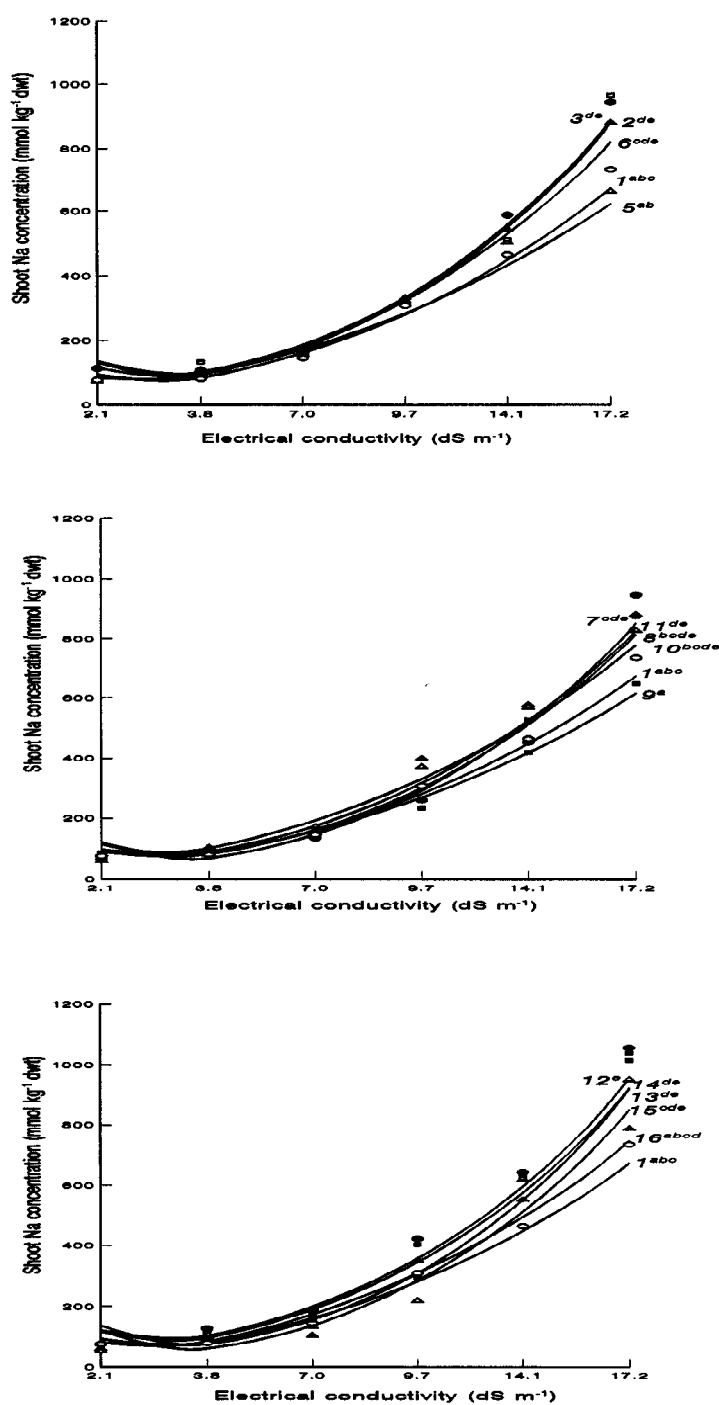


Figure 2. The effect of salinity on tissue Na concentration in 16 lines of *M. sativa*. Line 1 is included in all figures for comparison. (a) Lines 1, 2, 3, 4, 5, 6; (b) Lines 1, 7, 8, 9, 10, 11; (c) Lines 1, 12, 13, 14, 15, 16.  $\circ$  = Line 1,  $\bullet$  = Lines 2, 7, 12,  $\square$  = Lines 3, 8, 13,  $\blacksquare$  = Lines 4, 9, 14,  $\triangle$  = Lines 5, 10, 15,  $\blacktriangle$  = Lines 6, 11, 16. Significance of effects: line  $P < 0.001$ ,  $\text{Na}_2\text{SO}_4$  (linear)  $P < 0.001$ , (quadratic)  $P < 0.001$ .  $\text{Na}_2\text{SO}_4 \times$  line (linear)  $P = 0.002$ , (quadratic)  $P = 0.004$ . Slopes with any similar superscript are not significantly different ( $P > 0.05$ ).

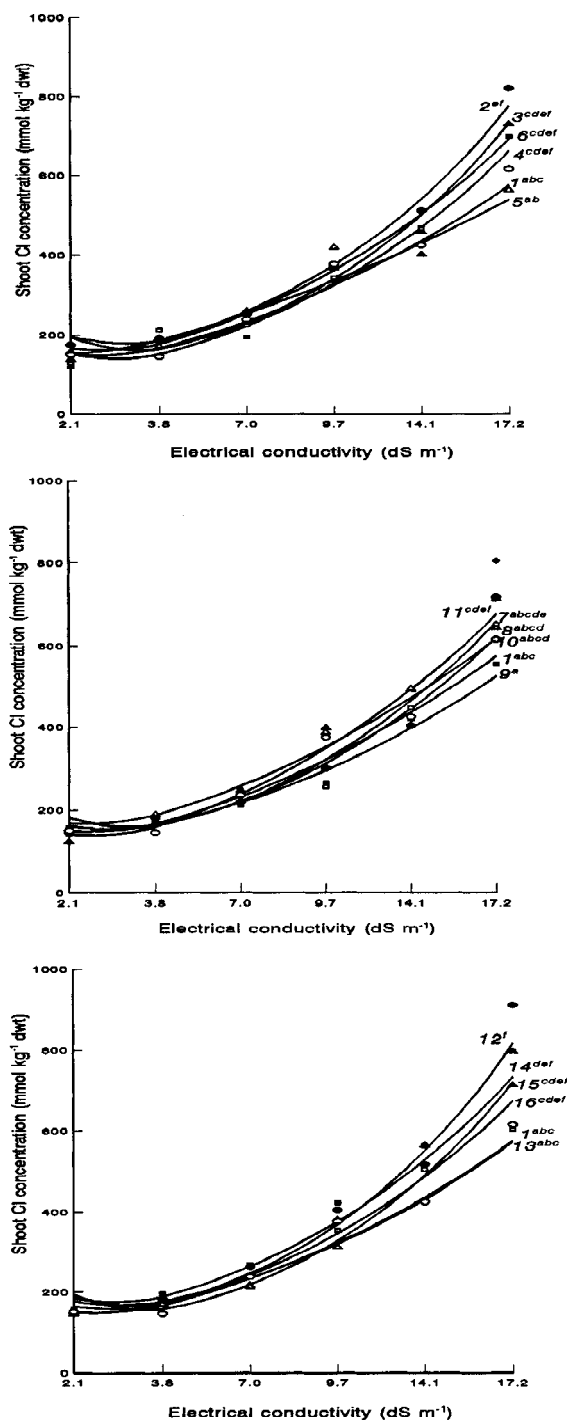


Figure 3. The effect of salinity on tissue Cl concentration in 16 lines of *M. sativa*. Line 1 is included in all figures for comparison. (a) Lines 1, 2, 3, 4, 5, 6; (b) Lines 1, 7, 8, 9, 10, 11; (c) Lines 1, 12, 13, 14, 15, 16.  $\circ$  = Line 1,  $\bullet$  = Lines 2, 7, 12,  $\square$  = Lines 3, 8, 13,  $\blacksquare$  = Lines 4, 9, 14,  $\triangle$  = Lines 5, 10, 15,  $\blacktriangle$  = Lines 6, 11, 16. Significance of effects, line  $P < 0.001$ ,  $\text{Na}_2\text{SO}_4$  (linear)  $P < 0.001$ , (quadratic)  $P = 0.002$ ,  $\text{Na}_2\text{SO}_4 \times$  line (linear)  $P = 0.002$ , (quadratic)  $P = 0.002$ . Slopes with any similar superscript are not significantly different ( $P > 0.05$ ).

A greater sensitivity to NaCl compared with  $\text{Na}_2\text{SO}_4$  (of the same electrical conductivity levels) has been shown in other species including french beans (Meiri et al., 1971), sorghum (Khan et al., 1995), wheat (Manchanda et al., 1982) and chickpea (Sharma et al., 1993) and appears to be related to greater uptake rates of chloride compared with sulphate on an equivalent basis (Meiri et al., 1971). In our study, Cl concentrations in the external solution were approximately half that of  $\text{SO}_4$ , yet lucerne plants growing at EC levels of  $3.8 \text{ dS m}^{-1}$  had shoot concentrations of Cl of around  $200 \text{ mmol kg}^{-1} \text{ dwt}$  compared with S concentrations of around  $120 \text{ mmol kg}^{-1} \text{ dwt}$ . Where Cl is the major external salt, shoot Cl concentrations may rise even higher (e.g. levels increased to around  $500 \text{ mmol kg}^{-1} \text{ dwt}$  in lucerne plants irrigated with water at  $4.5 \text{ dS m}^{-1}$  the field, Noble et al., 1987). Chloride has also been found to have a greater effect on plant development and water balance than sulphate. For example, in beans, transpiration rates were found to be suppressed to a greater degree by chloride salinity than by sulphate salinity (Meiri et al., 1971).

There was considerable variation in tolerance to  $\text{Na}_2\text{SO}_4$  between lucerne lines. The lines Moapa (line 1), Salton (line 10), WL525 (line 9) and SW32An4P3 (line 16) were found to have superior salt tolerance and/or produced more dry matter than other lines under moderate to high concentrations of  $\text{Na}_2\text{SO}_4$ . This intra-specific variation for tolerance is similar to that found for tolerance to NaCl in lucerne (e.g. Al-Khatib et al., 1994; Brown and Hayward, 1956; Mohammad et al., 1989; Rumbaugh and Pendery, 1990; Yapulnik and Heuer, 1991) and can be attributed to the heterogeneous nature of lucerne (Al-Khatib et al., 1994), to the degree of natural selection that may have occurred at the collection site (Rumbaugh and Pendery, 1990) and to the deliberate selection for certain traits that has occurred under the particular breeding programs.

Tolerance to NaCl in many forage legume species including lucerne, white clover and subterranean clover is related to the capacity of the plant to limit the transport of  $\text{Na}^+$  and  $\text{Cl}^-$  to the shoots (Noble et al., 1984; Rogers et al., 1993; West and Taylor, 1981). Plants that are more tolerant of NaCl are usually more efficient at restricting the accumulation of these ions to prevent toxic concentrations affecting plant processes and subsequent growth (Winter and Lauchli, 1982). This study with  $\text{Na}_2\text{SO}_4$  showed a general relationship (with some exceptions) between low shoot concentrations of  $\text{Na}^+$  and  $\text{S}^{2-}$  and salt tolerance in terms of dry matter production, with the more tolerant lines

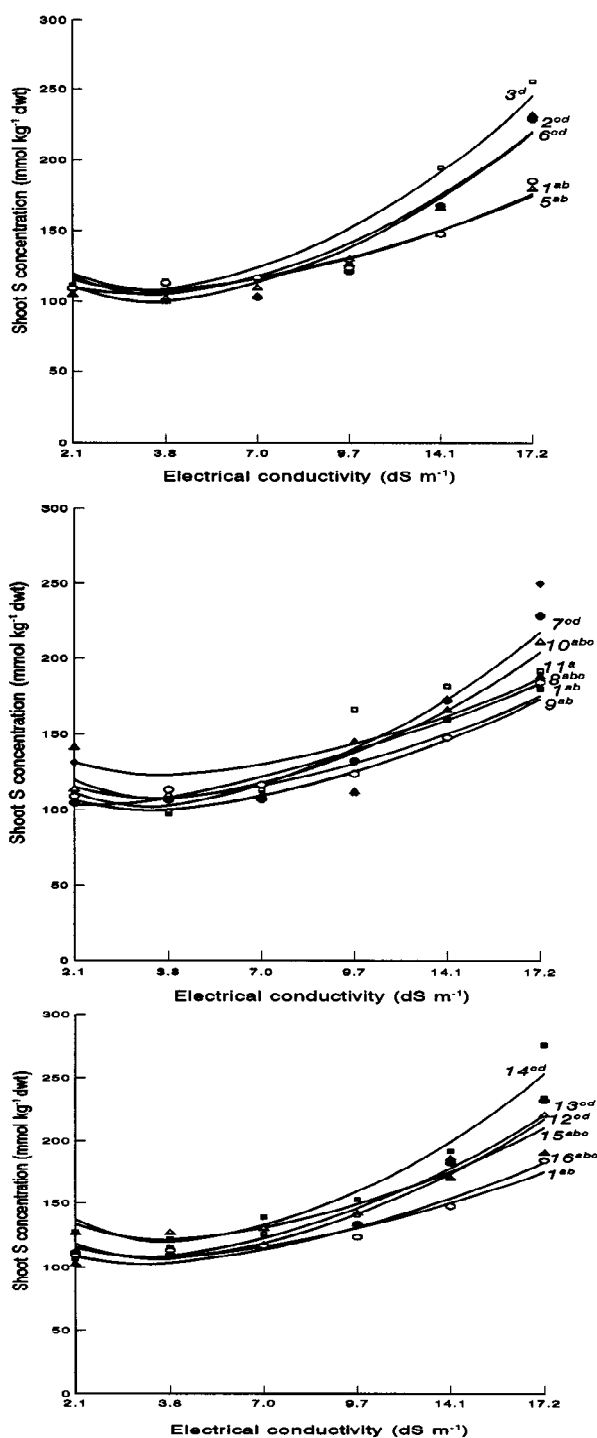


Figure 4. The effect of salinity on tissue  $\text{SO}_4$  concentration in 16 lines of *M. sativa*. Line 1 is included in all figures for comparison. (a) Lines 1, 2, 3, 4, 5, 6; (b) Lines 1, 7, 8, 9, 10, 11; (c) Lines 1, 12, 13, 14, 15, 16.  $\circ$  = Line 1,  $\bullet$  = Lines 2, 7, 12,  $\square$  = Lines 3, 8, 13,  $\blacksquare$  = Lines 4, 9, 14,  $\triangle$  = Lines 5, 10, 15,  $\blacktriangle$  = Lines 6, 11, 16. Significance of effects line  $P < 0.001$ ,  $\text{Na}_2\text{SO}_4$  (linear)  $P < 0.001$ , (quadratic)  $P < 0.001$ .  $\text{Na}_2\text{SO}_4 \times$  line (linear)  $P = 0.003$ , (quadratic)  $P = 0.021$ . Slopes with any similar superscript are not significantly different ( $P > 0.05$ ).



Table 3. Concentrations of Ca, Mg, K and P in the shoots of 15 lines of lucerne grown at 6 different concentrations of Na<sub>2</sub>SO<sub>4</sub>. (Note: there was insufficient material of Line 4 to undertake chemical analyses)

Ion	Tissue ion concentrations (mmol kg <sup>-1</sup> dwt)															
	EC level	Line														
		1	2	3	5	6	7	8	9	10	11	12	13	14	15	16
Ca	2.1	454	450	431	464	446	450	455	447	443	537	436	462	442	382	443
	3.8	409	437	313	380	416	392	383	395	432	434	407	408	408	383	386
	7.0	315	336	443	344	314	332	349	336	339	360	341	395	320	317	334
	9.7	362	362	342	338	340	353	339	348	363	338	317	353	313	288	309
	14.1	302	263	304	292	262	374	279	278	282	298	268	286	266	231	267
	17.2	223	212	240	244	257	234	224	246	247	239	176	220	227	187	239
<i>Contrasts: Salinity</i> $P < 0.001$ , <i>cultivar</i> $P < 0.001$ , <i>Salinity-cultivar</i> $P = 0.031$																
Lsd ( $P = 0.05$ ) salinity-cultivar = 60.3																
Mg	2.1	132	141	137	137	144	145	146	142	131	139	127	132	151	153	144
	3.8	139	140	139	124	138	144	132	120	133	128	131	137	149	154	144
	7.0	129	127	162	135	123	148	132	122	127	129	124	138	141	158	125
	9.7	139	149	150	146	143	164	143	136	147	144	132	157	149	155	142
	14.1	163	157	185	164	155	173	160	155	158	169	153	171	177	178	157
	17.2	163	173	174	164	187	195	169	173	172	170	146	183	188	182	165
<i>Contrasts: Salinity</i> $P = 0.003$ , <i>cultivar</i> $P < 0.001$ , <i>Salinity-cultivar</i> $P = 0.595$																
Lsd ( $P = 0.05$ ) salinity-cultivar = 19.8																
K	2.1	1248	1290	1261	1293	1277	1300	1283	1349	1306	1155	1234	1226	1327	1342	1227
	3.8	1237	1171	882	1219	1241	1225	1251	1144	1193	1164	1214	1237	1264	1265	1204
	7.0	1254	1219	1117	1156	1332	1290	1222	1251	1224	1243	1142	1185	1190	1270	1159
	9.7	1037	1104	989	1079	983	1124	1035	1058	1018	1116	1010	1101	1122	1136	937
	14.1	956	987	910	1021	1029	1004	954	1031	969	935	947	1016	1051	1086	894
	17.2	852	718	664	855	680	696	788	797	778	691	696	775	777	772	610
<i>Contrasts: Salinity</i> $P < 0.001$ , <i>cultivar</i> $P < 0.001$ , <i>Salinity-cultivar</i> $P = 0.122$																
Lsd ( $P = 0.005$ ) salinity-cultivar = 132.2																
P	2.1	83.4	77.9	70.8	77.9	77.2	72.8	71.6	70.3	82.1	67.4	81.1	71.9	72.1	70.1	72.0
	3.8	66.0	65.4	64.4	66.8	66.9	61.6	65.5	63.0	70.1	65	67.2	74.2	81.1	75.6	82.2
	7.0	78.2	75.8	82.4	79.4	79.9	75.5	72.3	79.0	81.4	72.9	84.1	74.2	81.1	75.6	82.2
	9.7	82.7	77.7	62.8	76.1	72.9	67.7	62.2	66.5	80.2	79.2	78.4	67.8	76.6	67.5	64.5
	14.1	83.8	73.3	69.3	79.2	76.1	72.8	78.7	76.7	81.3	78.2	81.4	73.7	79.3	72.7	76.7
	17.2	69.3	71.3	70.8	73.3	78.9	68.1	70.6	76.2	81.2	74.8	67.7	74.4	79.1	69.4	72.4
<i>Contrasts: Salinity</i> $P = 0.26$ , <i>cultivar</i> $P < 0.001$ , <i>Salinity-cultivar</i> $P = 0.935$																
Lsd ( $P = 0.05$ ) salinity-cultivar = 12.42																

having lower tissue concentrations of these ions. This suggests that, as with tolerance to NaCl, the restriction of S to the shoots is a mechanism of tolerance to high external concentrations of Na<sub>2</sub>SO<sub>4</sub>.

Within the lucerne germplasm used in this study, there was no difference in tolerance to Na<sub>2</sub>SO<sub>4</sub> among

material that had been selected specifically for tolerance to NaCl, based on dry matter production under saline conditions, e.g. lines 3, 4, 5, 6, 7, 8, and lines being developed for superiority in other traits (e.g. tolerance to diseases such as in lines 12, 13, 14, 15, 16), and between the two commercial cultivars of lucerne

(e.g. lines 1 and 10) which had been developed originally for resistance to spotted alfalfa aphid. Generally the response curves of the sibling lines – lines 3 and 4, 5 and 6, 7 and 8, 12 and 13, and 1 and 16, were very similar irrespective of what had been the major focus of each respective breeding program. Our results did show however, that the selection and breeding programs for tolerance to NaCl had been successful in improving absolute dry matter production in lucerne. For example, lines 6 and 8 produced more dry matter than their respective parent lines 5 and 7. Identifying suitable selection criteria to use when breeding for tolerance to stresses such as salinity is difficult (Flowers and Yeo, 1995; Noble and Rogers, 1992), and there have been arguments that it would be more efficient to select for dry matter production under non-saline conditions (Richards, 1983). However, this study confirms that selection and breeding to increase salt tolerance may be more successful if selection is based directly on the physiological mechanisms, such as chloride or sodium exclusion that confer tolerance, rather than on dry matter production under saline conditions.

This study has been successful in identifying some lines of lucerne which are more tolerant and/or produced greater amounts of dry matter when exposed to salinity. As with tolerance to NaCl, there appears to be a general relationship between salt tolerance and the capacity to restrict the accumulation of Na and S in the shoots.

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