Plant and Soil **146**: 131–136, 1992.

PLSO SV44

Variation in growth and ion accumulation between two selected populations of *Trifolium repens* L. differing in salt tolerance

M.E. ROGERS and C.L. NOBLE
Institute for Sustainable Agriculture, Tatura, Vic., 3616, Australia

Key words: salt tolerance, *Trifolium repens*, white clover

Abstract

Two divergent populations of *T. repens* cv. Haifa developed from two generations of recurrent selection for shoot chloride concentration, were grown in the greenhouse at 0 and 40 mol m^{-3} NaCl. Over two harvest cycles at 40 mol m^{-3} NaCl, the population selected for a low concentration of chloride in the shoot maintained a significantly lower chloride and sodium concentration compared with those plants selected for a high shoot chloride concentration. The distribution of chloride in the shoots was further examined in a subsample of plants from both populations. In all plants, concentrations of chloride were lower in the expanding and fully expanded leaves than in the older leaf tissue or petioles.

While there were no significant differences in the photosynthetic rates between lines, shoot yields and relative leaf expansion rates were higher in the low chloride population. Plant death was greater in plants selected for high shoot chloride. These results suggest that selections based on measurements of low shoot chloride concentrations may be successful in developing a cultivar of *T. repens* with improved salt tolerance.

Introduction

In northern Victoria, Australia, white clover (*Trifolium repens* L.) is a major component of perennial pastures that are affected by rising water tables and increasing levels of soil salinity. As a species, *T. repens* is sensitive to NaCl (Gauch and Magistad, 1943), but it is polyploid and cross-fertilizing with considerable plant-to-plant variation for salt tolerance (Noble and Shannon, 1987), indicating that there is potential to improve its salt tolerance by selection and breeding.

Exclusion of sodium and chloride from actively growing tissue is a salt resistance mechanism common in salt-sensitive species such as white clover (Läuchli, 1984). Individual plants may differ in their capacity to regulate and control ion transport and accumulation and, hence, in their salt tolerance (Noble et al., 1983; Winter and Läuchli, 1982). With a view to improving the salt tolerance of *T. repens*, efforts have concentrated on selecting individual plants for low rates of transport of chloride. Two divergent populations of *T. repens* cv. Haifa have been developed based on the concentration of chloride in the shoots. These populations have undergone two generations of recurrent selection following evaluation and selection at 40 mol m^{-3} NaCl and are providing the material for investigations of the physiological and genetic basis for salt tolerance in *T. repens*.

Materials and methods

The effect of NaCl on the growth and tissue ion concentration in two divergent populations of *T. repens* (viz. 'high Cl' plants and 'low Cl' plants) and the parent cultivar Haifa was assessed in a naturally lit greenhouse at Tatura (at night and day temperatures of 10°C ± 3°C and 25°C ± 3°C, respectively). Seeds with a uniform seed weight were first germinated under non-saline conditions in trays of vermiculite and seedlings were transplanted at the second trifoliate leaf stage into cells of polystyrene 'speedling' trays filled with vermiculite. These trays were floated on modified half-strength Hoagland solution (Karmoker and Van Steveninck, 1978) in stainless steel tanks with a volume of 160 L. Salinity treatments of 0 and 40 mol m^{-3} NaCl were imposed after two weeks, the latter being reached in increments of 20 mol m^{-3} NaCl over two days. Plants were grown hydroponically in continuously aerated solutions and the pH and electrical conductivity of the solutions were monitored and adjusted as necessary every three days. The solutions were replenished every two weeks. The experiment was a randomised block-split plot design. The NaCl treatment was applied to the main plots or tanks and there were four replicates. The split plots in each main plot were rows of 10 plants of each of the 'high Cl', 'low Cl' and cv. Haifa populations.

There were two harvests of the shoots at three-week intervals commencing three weeks after the salinity treatment had been imposed. At harvest, the shoots of all individual plants were cut and plant material was dried at 70°C for 48 hours and weighed before ashing at 460°C overnight. Shoot chloride concentration was measured on individual plants using a Buchler chloridometer based on titration with silver ions, and sodium, potassium, calcium and magnesium were measured using an Inductively Coupled Plasma Optical Emission Spectrophotometer (Labtam Plasma Scan).

The effect of tissue Na or Cl concentration on specific growth mechanisms such as leaf photosynthesis and leaf expansion were measured on plants from the three populations. Leaf expansion rates were measured on three plants of each line in each replicate between weeks 4 and 6 after the salinity treatments had been imposed. Leaves were identified at development stage 0.4 (Carlson, 1966) and their areas were measured non-destructively using a series of templates based on the rating procedure developed by Williams et al., (1964) for *T. subterraneum* (i.e. rating = 10 $\log_e$ 10A where A is the leaf area in square centimetres). Relative leaf expansion rates were then calculated as ($\mathrm{Log_e}A_2 - \mathrm{Loge}A_1/t_2 - t_1$) where A_1 and A_2 are leaf areas at time t_1 and t_2. Leaf areas were measured over five days until leaves had fully expanded.

Leaf photosynthetic rates, at full light and CO_2 levels equal to approximately 340 ppm, were measured using a portable photosynthesis system (Licor 6200) with a chamber attached (volume = 0.6 L) at week 6 after salt had been imposed. One leaf was selected from four plants in each treatment and replicate. The leaf areas were measured on individual leaves before being enclosed into the chamber.

At the completion of the experiment, a subsample of ten plants that were known, from a previous harvest, to cover a range of mean shoot chloride concentrations, were destructively harvested and divided into leaves (old, fully expanded, expanding), and petioles (senesced, old, and young). The yield of these plant parts and their tissue chloride concentrations were measured using the techniques described earlier.

Yield, tissue ion data, relative leaf expansion rates and leaf photosynthetic rates were analysed by anova using Genstat 2.1 (Lawes Agricultural Trust). Plant death was analysed using a generalised linear model with binomial error distribution.

Results

After six weeks exposure to 40 mol m^{-3} NaCl, concentrations of Cl in the shoots of the three populations increased significantly ($p < 0.05$, Table 1), however the 'low Cl' plants maintained a significantly lower concentration of Cl in their shoots ($p < 0.05$, Table 1) than either the 'high Cl' plants or the Haifa population. There was no difference between populations at 0 mol m^{-3} NaCl. Results for shoot concentrations of Na were similar to those of Cl (Table 2), with concentrations being significantly lower in the 'low Cl' population ($p < 0.05$) than in the 'high Cl' and Haifa populations at 40 mol m^{-3} NaCl. Concentrations of potassium in the shoots of 'high Cl' plants were significantly lower than those in the 'low Cl' or Haifa populations ($p <$ 0.1, Table 2). K:Na ratios tended to be more favourable in the 'low Cl' plants than the 'high

Table 1. Shoot yield and tissue chloride concentration following a second cut-regrowth cycle in individual, second generation plants of *T. repens* selected for high and low shoot chloride concentration and plants of the parent cultivar Haifa when grown at 0 and 40 mol m^{-3} NaCl in the greenhouse

Dry weight (g/plant)				Shoot Cl concentration (mol m^{-3} kg^{-1} dry weight)		
NaCl mol m^{-3}	Haifa	High Cl	Low Cl	Haifa	High Cl	Low Cl
0	0.58	0.59	0.59	160	178	150
40	0.41	0.41	0.52	782	893	537

Salinity * line, n = 214;
LSD$_{(p=0.05)}$ = 0.13;
LSD$_{(p=0.1)}$ = 0.11.

Salinity * line, n = 214;
LSD$_{(p=0.05)}$ = 188;
LSD$_{(p=0.1)}$ = 157.

Table 2. Shoot sodium and potassium concentrations following a second cut-regrowth cycle in individual, second generation plants of *T. repens* selected for high and low shoot chloride concentration and plants of the parent cultivar Haifa when grown at 0 and 40 mol m^{-3} NaCl in the greenhouse

Shoot Na concentration (mol m^{-3} kg^{-1} dry weight)				Shoot K concentration (mol m^{-3} kg^{-1} dry weight)		
	Haifa	High Cl	Low Cl	Haifa	High Cl	Low Cl
0	99	80	87	1074	1168	1157
40	796	934	635	952	907	1043

Salinity * line, n = 214;
LSD$_{(p=0.05)}$ = 127;
LSD$_{(p=0.1)}$ = 106.

Salinity * line, n = 214;
LSD$_{(p=0.05)}$ = 156;
LSD$_{(p=0.1)}$ = 131.

Cl' plants at 40 mol m^{-3} NaCl although these values were not significantly different (viz. 1.95 compared with 1.20) and both ratios were above the value (1.0) required for optimal efficiency (Greenway and Munns, 1980). Concentrations of calcium in the shoots decreased at 40 mol m^{-3} NaCl ($p < 0.05$) but there was no difference between populations ($p = 0.208$) (viz. mean concentrations were 399 mol m^{-3} Ca at 0 mol m^{-3} NaCl compared with mean concentrations of 267 mol m^{-3} Cl at 40 mol m^{-3} NaCl for the three populations combined). There were no significant differences in the shoot concentrations of magnesium between plant populations ($p = 0.324$) or at either NaCl concentration ($p = 0.693$) (viz. mean concentrations for the three populations combined were 105 mol m^{-3} Mg at 0 mol m^{-3} NaCl compared with 102 mol m^{-3} Mg at 40 mol m^{-3} NaCl).

Shoot yields at harvest 2, both in the 'high Cl' and Haifa populations, decreased significantly at 40 mol m^{-3} NaCl ($p < 0.05$, Table 1) and were lower than the yield of 'low Cl' plants at this concentration ($p < 0.1$, Table 1). The yield results from harvest one also revealed that the 'low Cl' plants were higher yielding than the Haifa plants ($p < 0.1$, data not presented).

Information on Cl distribution in three plants with different mean shoot Cl concentrations is shown in Table 3. In plants with a lower overall mean shoot Cl concentration, concentrations were lower in every plant part compared with those plants with a higher mean shoot Cl concentration. Concentrations also tended to be higher in the older leaves and petioles than in the expanding leaves.

Leaf expansion rates were also sensitive to increased levels of NaCl and were significantly lower at 40 compared with 0 mol m^{-3} NaCl ($p < 0.05$, Table 4). At 40 mol m^{-3} NaCl, relative leaf expansion rates for the 'low Cl' and Haifa plants were significantly higher ($p < 0.05$, Table 4) than those for the 'high Cl' plants which would account for some of the observed differ-

Table 3. The distribution of chloride in the shoots of three plants of *T. repens* differing in mean shoot chloride concentration when grown at 40 mol m^{-3} NaCl for six weeks

	Mean plant	Chloride concentration (mol m^{-3} kg^{-1} dry weight) Leaves			Petioles		
		Old	Fully expanded	Expanding	Senesced	Old	Young
Low Cl	708	1354	564	211	846	1721	592
Haifa	1027	1336	846	367	1185	2623	715
High Cl	1577	2228	1100	370	1628	2376	821

Table 4. Relative leaf expansion rates and leaf photosynthesis rates in second-generation plants of *T. repens* selected for high and low shoot chloride concentration and plants of the parent cultivar Haifa when grown at 0 and 40 mol m^{-3} NaCl in the greenhouse

NaCl mol m^{-3}	Relative leaf expansion rates			Leaf photosynthesis rate μ mol^{-2} s^{-1}		
	Haifa	High Cl	Low Cl	Haifa	High Cl	Low Cl
0	0.271	0.302	0.290	7.60	6.68	7.91
40	0.142	0.065	0.166	7.59	7.35	8.43

Salinity * line, n = 25
$LSD_{(p=0.05)} = 0.040$;
$LSD_{(p=0.1)} = 0.033$.

Salinity * line, n = 25;
$LSD_{(p=0.05)} = 3.033$;
$LSD_{(p=0.1)} = 2.475$.

Table 5. Plant death in second-generation plants selected for high and low shoot chloride concentration and in plants of the parent cultivar Haifa when grown at 0 and 40 mol m^{-3} NaCl in the greenhouse for six weeks

NaCl (mol m^{-3})	Predicted plant mortalities (proportion of plants that died					
	Haifa	s.e.	High Cl	s.e.	Low Cl	s.e.
0	0.0242	(0.0155)	0.0271	(0.0154)	0.0288	(0.0177)
40	0.1258	(0.0516)	0.3029	(0.0715)	0.1462	(0.0549)

ences in yield between populations. However, despite differences in leaf expansion rates, there were no significant differences in individual leaf photosynthesis rates between populations or between NaCl concentrations (Table 4). Individual leaf photosynthesis values tended to be higher at 40 mol m^{-3} NaCl, but rates varied greatly between leaves even of the same treatment despite similar leaf areas.

Throughout the experiment, the number of plants that died was significantly larger in the 'high Cl' plants at 40 mol m^{-3} NaCl than in the 'low Cl' or Haifa plant populations ($p < 0.001$, Table 5).

Discussion

For all plant characteristics measured in this experiment the 'low Cl' plants were superior to those of the 'high Cl' or Haifa plants in the presence of 40 mol m^{-3} NaCl. The concentrations of Cl and Na in the shoots were lower, the concentrations of K were higher rendering a more favourable K:Na ratio, plant death was lower and shoot yield was higher. This suggests that firstly salt tolerance in *T. repens*, in common with other salt-sensitive species such as *Festuca* (Hannon and Barber, 1972), grapevine (Downton, 1977), soybean (Abel, 1969; Läuchli and Wieneke, 1979) and rice (Yeo and Flowers, 1982), is correlated with restricted and regulated Cl and Na translocation in the shoot. Secondly, that there is significant variation in Cl uptake and distribution within *T. repens* and that this variation can be selected for and incorporated into a breeding program.

To date, there has been little research on the salt tolerance of *T. repens*. This species is generally classified as salt-sensitive (Gauch and Magistad, 1943; Smith and McComb, 1983) although it is recognised as being genetically and phenotypically variable (Burdon, 1980). Ab-Shukor et al., (1987) demonstrated that several natural populations of *T. repens* exhibited high to very high salt tolerance (comparable to that of *T. alexandrinum*) in terms of root growth at 150 to 200 mol m^{-3} NaCl, but made no measurements of tissue ion concentrations. Our limited research examining Cl distribution throughout the plant revealed that the Cl concentration in the leaves tended to be about one third to one half that of the petioles. These results are similar to those of research on ion distribution in *T. alexandrinum* by Winter and Läuchli (1982), where chloride concentrations per gram dry weight in the petioles was about three times that of the leaves (allowing for differences in water content of the two organs). These authors concluded that *T. alexandrinum* uses several mechanisms to cope with moderate salinity levels including retranslocation of Na and Cl out of young leaves. In *T. repens*, further research is now possible using these divergent plant selections to identify how Cl and Na exclusion is regulated and to study ion compartmentation. Early indications using a scanning electron microscope with X-ray microprobe suggest that chloride is concentrated in the vacuoles of the palisade and spongy mesophyll cells within the leaf tissue (Rogers and Noble, unpublished).

The effects of NaCl on other physiological mechanisms in *T. repens* were varied. Measurements of leaf expansion rates were sensitive to shoot Na and Cl concentrations, whereas rates of photosynthesis in expanding leaves were insensitive. This suggests that concentrations were not sufficiently high to affect photosynthesis or chlorophyll activity. Other authors have drawn similar conclusions in cereals (Rawson et al., 1988) and spinach (Robinson et al., 1983). Although there were significant differences in leaf expansion rates between populations, the difference in overall yield was less significant, implying that the allocation of assimilates between roots, stems and leaves may differ between selected populations.

In this experiment we were concerned with the effect of NaCl on shoot growth and ion relations in *T. repens*. Subsequent research will examine ion distribution and growth in both roots and shoots, and the heritability of salt tolerance. To date, the results are encouraging indicating that selections based on measurements of shoot Cl may be successful in developing a cultivar of *T. repens* with improved salt tolerance.

Acknowledgements

We thank Mrs M Filip and Mr I Treacy for providing excellent technical assistance. This research was funded by the Victorian State Salinity Program.

References

Abel G H 1969 Inheritance of the capacity for chloride inclusion and chloride exclusion by soybeans. Crop Sci. 9, 697–698.

Ab-Shukor N A, Kay Q O N, Stevens D P and Skibinski D O F 1988 Salt tolerance in natural populations of *Trifolium repens* L. New Phytol. 109, 483–490.

Burdon J J 1980 Intra-specific diversity in a natural population of *Trifolium repens*. J. Ecol. 68, 717–735.

Carlson G E 1966 Growth of clover leaves: Developmental morphology and parameters at ten stages. Crop Sci. 6, 293–294.

Downton W J S 1977 Photosynthesis in salt-stressed grape leaves. Aust. J. Plant Physiol. 4, 183–192.

Gauch H G and Magistad O C 1943 Growth of strawberry clover varieties and of alfalfa and ladino clovers as affected by salt. J. Am. Soc. Agron. 35, 871–880.

Greenway H and Munns R 1980 Mechanisms of salt tolerance in nonhalophytes. Annu. Rev. Plant Physiol. 31, 149–190.

Hannon N J and Barber H N 1972 The mechanism of salt tolerance in naturally selected populations of grasses. Search 3, 259–260.

Karmoker J L and Van Steveninck R F M 1978 Stimulation of volume flow and ion flux by abscisic acid in excised root systems of *Phaseolus vulgaris* L. cv. Redland Pioneer. Planta 141, 37–43.

Läuchli A 1984 Salt exclusion; an adaptation of legumes for crops and pastures under saline conditions. *In* Salinity Tolerance in Plants: Strategies for Crop Improvement. Ed. R C Staples. pp 171–187. Wiley, New York.

Läuchli A and Wieneke J 1979 Studies on the growth and distribution of Na^+, K^+ and Cl^- in soybean varieties differing in salt tolerance. Z. Pflanzenernaehr. Bodenkd. 124, 3–13.

Noble C L and Shannon M C 1987 Strategies for irrigation of white clover with saline water on the heavy clay soils of northern Victoria. Proc. Fourth Aust. Agron. Conf. Melbourne, 305 p.

Noble C L, Halloran G M and West D W 1984 Identification and selection for salt tolerance in lucerne (*Medicago sativa* L.). Aust. J. Agric. Res. 35, 239–252.

Rawson H M, Richards R A and Munns R 1988 An examination of selection criteria for salt tolerance in wheat, barley and triticale genotypes. Aust. J. Agric. Res. 39, 759–772.

Robinson S P, Downton W J S and Millhouse J A 1983 Photosynthesis and ion content of leaves and isolated chloroplasts of salt-stressed spinach. Plant Physiol. 73, 238–242.

Smith M K and McComb J A 1981 Use of callus cultures to detect NaCl tolerance in cultivars of three species of pasture legumes. Aust. J. Plant Physiol. 8, 437–442.

Williams R F, Evans L T and Ludwig L J 1964 Estimation of leaf area for clover and lucerne. Aust. J. Agric. Res. 15, 231–233.

Winter E 1982 Salt tolerance of *Trifolium alexandrinum* L. II. Ion balance in relation to its salt tolerance. Aust. J. Plant Physiol. 9, 227–237.

Yeo A R and Flowers T J 1982 Accumulation and localisation of sodium ions within the shoots of rice (*Oryza sativa*) varieties differing in salt resistance. Physiol. Plant. 56, 343–348.