DO *MELILOTUS* SPECIES HAVE A ROLE FOR SALINE AREAS IN AUSTRALIA?

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Summary. The salt tolerance of nine populations of *Melilotus* species was evaluated in adult plants grown in hydroponic solutions at five NaCl concentrations (0, 45, 90, 135 and 180 mM) in the greenhouse. Salinity response curves differed significantly between the nine populations, with material (*M. albus*), that had been collected from degraded areas in Argentina proving to be the most salt-tolerant in relative terms. This result was supported by analyses of shoot and root Cl and Na concentrations which suggested that salt tolerance was associated with ion exclusion from the shoots. We conclude that it is possible to select *Melilotus* species that may be grown successfully in saline areas in Australia.

INTRODUCTION

The genus *Melilotus* (sweet clover), contains many species which produce high quality pasture feed but is not grown widely in Australia compared with other parts of the world (*e.g.* the former USSR, USA, Canada, Spain, China and Argentina). In these countries, *Melilotus* species also have a role in regenerating moderately saline areas where traditional forage legumes cannot be grown (2). In Australia, the area of land affected by salinity is estimated to be three million ha and it is also important to identify species that can tolerate saline conditions yet still provide good quality feed for grazing animals. The research overseas has concentrated on the species *M. segetalis, M. indica* and *M. messanensis*, however there has been little assessment of the salt tolerance of other species such as *M. albus* or *M. officinalis*. The study reported here evaluated the salt tolerance of nine populations of *Melilotus* to quantify the level of salt tolerance that exists within this genus and to identify salt tolerant germplasm that may be suitable for saline areas in Australia.

MATERIALS AND METHODS

The salt tolerance of nine populations and species of *Melilotus* was evaluated in the greenhouse at Tatura, Victoria from December 1994 until March 1995. These populations included three commercially available cultivars (*M. albus* cvv. Polara and El domador, and *M. officinalis* cv. Norgold), three populations of *M. albus* that had been collected from degraded areas in the Entre Rios province of Argentina (32?S, 58?W, neutral soils, 800 mm annual rainfall) by P.M. Evans (*viz.* Pam 15, 19 and 20), two populations that showed promise from research in Spain (*viz. M. albus* Psm 21 and *M. indica* Psm 22), and one line that came from the National Annual Legume Germplasm Centre based in Perth, WA, whose exact origin was unknown (*viz. M. indica* Paum 23). A tenth line, also originating from Spain (*viz. M. italica* Psm 24) was originally included in the experiment but did not survive.

Seeds of each of the nine populations were first germinated under non-saline conditions in trays of vermiculite. At the second trifoliate leaf stage, seedlings were transplanted into individual cells (each 9 cm^3) within polystyrene trays that were floating in half-strength Hoagland solution in stainless steel tanks in the greenhouse (25?C ? 3?C day, 12?C ? 3?C night). Salinity treatments (0, 45, 90, 135 and 180 mM NaCl) were imposed after four weeks, the treatments being reached in increments of 45 mM NaCl over four days. Plants were grown hydroponically in continuously aerated and recirculating solutions. The pH and electrical conductivity of the solutions were monitored and adjusted when necessary every two days and solutions were completely replenished every two weeks. The experiment was a randomised block-split plot design with four replicates. The experimental unit consisted of a row of five or ten plants (depending on the amount of seed available) of each population.

There were three harvests at three-weekly intervals commencing three weeks after the salinity treatments had been imposed. At the first two harvests, the shoots of all plants were cut to 3 cm above the base of the plant. The third harvest was a destructive harvest and root production was also measured. Following

harvest, the plant material was weighed, then dried at 70?C for 48 hours and weighed before it was chemically analysed. Cl, Na, K, Ca and Mg were measured on acid-digested plant material using an Inductively Coupled Plasma Optical Emission Spectrophotometer (Labtam Plasma Scan).

Dry matter and tissue ion data were analysed by Anova with salinity levels fitted as orthogonal polynomials (Genstat 5, Lawes Agricultural Trust, Rothamsted Exp. Station UK). Residuals were checked for normality and homogeneity. There was no difference in the relative performance of the populations between the three different harvests and results are presented for the third harvest only when both the roots and shoots were harvested.

RESULTS

Shoot growth decreased with increasing NaCl concentration in all nine populations of *Melilotus* ($p<0.05$, Fig. 1). There were differences in salt tolerance ($p<0.05$) between the populations (which were not related to membership of a particular species of *Melilotus*) and the cultivars Norgold and El domador were the most salt-sensitive in relative terms - as shown by the slope of the decline in dry matter. These two cultivars produced significantly more shoot dry matter than other populations at lower NaCl concentrations, but, at the higher salinity levels, production was only around 10% of that at 0 mM NaCl. In contrast, the shoot dry matter production of the *M. albus* populations Pam 15 and 19 did not decrease until approximately 80 mM NaCl, and, at 180 mM NaCl, plant yield was reduced to 39% and 27% respectively of the yield at 0 mM NaCl. The results for root growth (not presented) were similar although there were larger differences between populations and, with the exception of Psm 22, all populations were significantly more salt-tolerant than cv. Norgold.

Concentrations of Cl in the shoots increased with increasing NaCl concentrations in all nine populations of *Melilotus* ($p<0.001$, Fig. 2). Shoot Cl concentrations in the cv. El domador increased rapidly at external NaCl concentrations greater than 45 mM and this response was different from those observed in lines Psm 22 and Pam 15 ($p<0.05$) where shoot concentrations of Cl tended to plateau at external NaCl concentrations of around 60 mM. The response curves for shoot Na concentrations were very similar to those shown for Cl concentration (not presented). In contrast, concentrations of Na in the roots were highest in line Pam 15 ($p<0.05$) at 180 mM NaCl, and lowest in line Paum 23 - a line that had one of the highest shoot concentrations of Na (data not presented). Shoot K:Na ratios decreased significantly with increasing external NaCl concentration but were variable between plants so that there were no differences between populations.

Figure 1. The predicted responses to NaCl in shoot growth of nine populations of *Melilotus* species. The slopes of populations with any similar superscript are not significantly different ($P>0.05$). Significance of effects: population $p<0.001$, NaCl (linear) $p<0.001$, NaCl*population (linear) $p=0.043$.

Figure 2. The predicted responses to NaCl in the shoot Cl concentrations of nine populations of *Melilotus*. Populations with any similar superscript are not significantly different ($P>0.05$). Significance of effects: population $p<0.001$, NaCl (linear) $p<0.001$, NaCl*population (linear) $p<0.001$.

DISCUSSION

In Australia, *Melilotus* has not been widely sown because of concerns relating to a high coumarin content in the shoots which can cause tainting in milk and butter. This concern, however, has been alleviated by the release of low coumarin varieties such as Norgold and El domador and there should be now fewer reservations to growing *Melilotus* over a wide range of environments - including saline areas. The results from our study show that there are significant interspecific and intraspecific variations in salt tolerance within *Melilotus* species which is consistent with findings in other forage legume species such as subterranean clover (5) and white clover (4). The ecotypes of *M. albus* that were collected from degraded areas in Argentina by Evans (*viz* Pam 15 and 19) were significantly more salt-tolerant than other material especially the commercial cultivars El domador (*M. albus*) and Norgold (*M. officinalis*). This suggests that a degree of natural selection for salt tolerance may have occurred at the collection site - a phenomenon that has also been observed in evaluations of salt tolerance at germination in *Melilotus* species (3).

Salt tolerance in *M. indica* has been associated with the ability of plants to store high concentrations of Na, K and Ca in the shoots (1), and to maintain high ratios of shoot K/Na and Ca/Na. Whilst we found significant differences between populations with respect to shoot ion concentrations, it was difficult to relate tolerance to any specific ion exclusion or inclusion mechanism. If anything, the results suggest that salt tolerance in *M. albus* may be associated with Na and Cl exclusion from the shoots and accumulation in the roots, as was observed in Pam 15 - the most salt tolerant line. Ion exclusion from the shoots is a common salt tolerance mechanism in forage legumes (*e.g. 4*, 5, 6).

The *Melilotus* genus is closely related to the *Medicago* genus and therefore *Melilotus* plants may provide useful alternatives to *Medicago sativa* (lucerne) plants in areas that are affected by salinity. This conclusion is also supported by research from pot studies of both species (1). This initial study has been important in identifying salt-tolerant germplasm within the genus *Melilotus,* especially within *M. albus*. Four field experiments have now been sown in western Victoria to fully assess the role of this species in saline areas in Australia.

ACKNOWLEDGEMENTS

We thank D. Graham and F. Robertson for technical assistance and D. Smith and R. Baigent for chemical analyses. This research was funded by the Victorian State Salinity Program.

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