Improving forage legume options for saline environments – *Melilotus* species

Mary-Jane Rogers[1,2], Timothy Colmer[1,3], Kirsten Frost[1,3], Dave Henry[1,4], David Cornwall[1,2], Elizabeth Hulm[1,4], Steve Hughes[1,5] and Andrew Craig[1,6]

[1] Cooperative Research Centre for Plant-Based Management of Dryland Salinity, Perth, WA www.crcsalinity.com.au . Email MaryJane.Rogers@dpi.vic.gov.au
[2] Department of Primary Industries Victoria, Tatura, Victoria 3616
[3] School of Plant Biology, University of Western Australia, Crawley, Western Australia, 6009
[4] CSIRO Livestock Industries, Wembley, Western Australia 6913
[5] South Australian Research and Development Institute, Waite Institute, Adelaide, South Australia 5001
[6] South Australian Research and Development Institute, Struan Agricultural Centre, Naracoorte South Australia 5271

Abstract

Research within the CRC for Plant-Based Management of Dryland Salinity has systematically evaluated the salt and waterlogging tolerances of over 100 species of forage legumes, including 19 species of *Melilotus.* The research has involved glasshouse assessments of agronomic traits such as dry matter (DM) production and root growth and development; physiological measurements of tissue ion concentrations and root porosity, and *in vitro* estimates of nutritive value. The weed risk potential of each introduced species has also been considered. The species that have performed best in all these characters have been sown in field plots in Western Australia and South Australia in autumn 2006. These plant lines have shown high relative salt tolerance as measured by DM production, low concentrations of Na^+ and Cl^- in the shoots when grown under saline conditions, good waterlogging tolerance as indicated by high levels of shoot and root growth and root porosity, and adequate nutritive values. The field evaluations will further characterise the lines, enabling selections of new cultivars for possible future release to Australian growers.

Key Words

Salt tolerance, discharge environments, plant stress, waterlogging tolerance

Introduction

The *Melilotus* genus is closely related to the *Medicago* and *Trigonella* genera. There are approximately 25 species with annuals and biennials/perennials (Allen and Allen 1981). Species are moderately winter-hardy, drought resistant and are valued as pasture forage. In many countries of the world (e.g. Argentina, Spain, Canada and Russia), *Melilotus* species have a role in regenerating moderately saline areas where traditional forage legumes cannot be grown (Maddaloni, 1986). However, this genus is not widely grown in Australia partly because of concerns relating to high coumarin levels and weediness of some species (Evans and Kearney, 2003). A national project, funded by the CRC Plant-Based Management of Dryland Salinity, commenced in late 2003, with a principal aim of evaluating and developing plants that can be grown in saline, discharge areas of southern Australia. The initial focus of this research has been towards legume species, since these species tend to be more salt sensitive than grass species, yet they are critical to the present forage system providing both nitrogen (via nitrogen fixation) and forage of high nutritive value. *Melilotus* species were identified as priority species, so research in this project commenced by assessing the salt and waterlogging tolerance of 19 available species of *Melilotus.*

Methods

Glasshouse salinity tolerance assessment- Tatura, Victoria

Seedlings of 19 *Melilotus* species (Table 1) plus 3 control species (*Trifolium fragiferum, Trifolium michelanium* and *Medicago sativa*) were transplanted into stainless steel tanks (120 L) filled with water

and modified Hoagland nutrient solution that was continuously aerated. The mean glasshouse temperatures for the duration of this experiment were 20.0 ? 4?C day/5.7 ? 2?C night. Each *Melilotus* species was represented by 5 accessions selected from the Genetic Resources Centre at SARDI in Adelaide. The experiment was a split plot design with 4 salinity treatments and 4 replicates using 16 tanks in total. The experimental unit was a row of 18 plants. The nutrient solutions in the tanks were replenished weekly.

After a first destructive harvest (95 days after sowing - DAS) of half the plants (9 plants), the NaCl treatments (0, 80, 160 and 240 mM NaCl) were imposed in increments of 80 mM/day until full treatments had been reached. The second harvest took place 126 DAS and 28 days after the full salinity treatments had been imposed. At harvest fresh weights of plant shoots and roots were measured and plant material was dried at 70?C for 48 hours to determine dry matter (DM) production. Samples of dry shoots and roots were analysed for tissue ion concentrations (Cl^-, Na^+, and K^+) by the University of Western Australia, Perth and for nutritive value by CSIRO Livestock Industries, Perth.

Tissue ion analyses

0.1 g of dried plant material was weighed into a 10 ml vial and 10 ml of 0.5 M HNO_3 added, with samples placed on a shaker at 20?C for 2 days. The extract was diluted appropriately, with K^+ and Na^+ measured using a flame photometer (Jenway Ltd, model PFP7, Essex, UK). Chloride was determined using a Buchler-Cotlove chloridometer (Buchler Instruments, Model 4-2008, Fort Lee, USA).

Nutritive value analyses

The dried samples were ground to pass through a 1 mm sieve using a cyclone grinder (CYCLOTECH 1093 Sample Mill). Near infrared reflectance spectra were collected for the region from 400-2500 nm with a scanning monochromator (model 6500 NIRSystems Inc. Silver Spring, MD USA). NIR was used to analyse for Dry Matter Digestibility (DMD) with calibrations based on the methods of Clark *et. al* (1982).

Waterlogging tolerance assessments- University of Western Australia, Perth

The waterlogging tolerances of the same 19 species of *Melilotus* and 3 control species were evaluated at the University of Western Australia, Perth in two experiments of 9 and 10 species respectively. The research took place in a controlled environment room (20?C/15 ^oC day/night temperature, with 12 hour photoperiod, irradiance of 375 – 490 ?mol quanta m^{-2} s^{-1}, PAR). There were 2 treatments, aerated or stagnant/waterlogged and 3 replicates. Pots were arranged randomly within each replicate and were rotated every second day.

Scarified, sterilised seeds were imbibed and then transplanted into 50% aerated nutrient solution (identical to that used in the salinity assessments) in sealed 4.5 L pots covered in aluminium foil to ensure roots were grown in darkness. There were 8 plants per pot inserted into holes in the lid. When the plants were 2 weeks old, the solution was changed to full strength concentration. Nutrient solutions were renewed weekly.

An initial harvest of 4 plants per pot was carried out 4 weeks after imbibition. The stagnant treatment was then imposed on half the pots by bubbling with N_2 gas until the O_2 concentration in the solution was approximately 10% of that in air-saturated solution. The pots were then left stagnant for 24 hours after which the solution was replaced with stagnant agar nutrient solution (0.1% w/v dissolved agar added to the standard nutrient solution to prevent convective movements). Prior to adding to the pots, the solution was bubbled with N_2 overnight to displace the O_2 out of solution. Nutrient solutions were renewed weekly.

The final harvest of 4 plants per pot occurred after 4 weeks of treatments. The shoot was cut from the root and the lateral roots were separated. Roots and shoots were oven dried (70?C) and weighed. Root porosity (proportion of gas volume per root unit volume) was measured in both main and lateral roots (Raskin 1983).

Results and Discussion

Salinity tolerance

The growth of most species of *Melilotus* was reduced by the salinity treatments (Table 1) – however there were differences amongst species in the degree of growth reduction and therefore in relative salt tolerance. For example, at 240 mM NaCl, the growth of *M. messanensis, M. suaveolens*, *M. tauricus* and *M. wolgicus* was at least 85% of that under non-saline conditions (0 mM NaCl). By contrast, DM production in plants of *M. speciosus* and the control species *T. michelianum* was only approximately 30% of that at 0 mM NaCl. There was a large amount of variation in DM production amongst the *Melilotus* species with some species e.g. *M. speciosus*, producing significantly more DM under control (0 mM NaCl) conditions compared with slower growing perennial species such as *M. suaveolens* and *M. polonicus*.

Nutritive value assessments were limited by the amount of DM available for each species. There were significant differences in DMD levels between *Melilotus* species (Table 2). When the DMD results were adjusted for the soluble salt content, there was a clear downward trend in DMD as an effect of increasing NaCl concentrations however, even at the lower DMD range the forage should be acceptable to maintain a 40 kg ewe (ARC, 1984). Concentrations of Cl (Table 2) and Na (not presented) in shoot tissues, increased significantly with increasing NaCl concentrations in all species, and there were significant differences between species at 160 mM NaCl. Species such as *M. messanensis,* that showed high relative salt tolerance in terms of dry weight, also had lower concentrations of Cl in the shoots compared with *M. speciosus* – a species that displayed low relative salt tolerance. In many agricultural species, the ability to regulate the uptake and translocation of Na and Cl to prevent excessive accumulation of these ions in the leaves is recognised as a mechanism of salt tolerance (Munns 2005).

Table 1. The effect of NaCl on the shoot dry matter production of selected species of *Melilotus* and three control species (*M. sativa, T. fragiferum* and *T. michelianum*)

Species	Shoot dry weight at 0 mM (g/plant)	Relative dry matter at 240 mM NaCl (% growth compared with growth at 0 mM NaCl)	Salt tolerance ranking*
M.albus annual	0.20	62	Intermediate
M.albus perennial	0.33	55	Intermediate
M.altissimus	0.09	51	Intermediate
M.dentatus	0.02	67	Intermediate
M.elegans	0.26	60	Intermediate
M.hirsutus	0.05	82	Tolerant
M.indicus	0.27	69	Intermediate

M.infestus	0.27	41	Sensitive
M.italicus	0.31	65	Intermediate
M.messanensis	0.40	89	Tolerant
M.neapolitanus	0.11	62	Intermediate
M.officinalis	0.08	77	Intermediate
M.polonicus	0.03	80	Tolerant
M.speciosus	0.86	31	Sensitive
M.suaveolens	0.01	89	Tolerant
M.sulcatus ssp. brachystachys	0.21	47	Sensitive
M.sulcatus ssp. segatalis	0.21	73	Intermediate
M.tauricus	0.04	108	Tolerant
M.wolgicus	0.03	101	Tolerant
T.fragiferum (C1)	0.11	68	Intermediate
T.michelanium (C2)	0.31	31	Sensitive
Medicago sativa (C3)	0.19	75	Intermediate

l.s.d.(P=0.05) relative growth, species*salinity =36

* Tolerant –shoot DM > 80% of control, Intermediate - shoot DM 50-80% of control, Sensitive – shoot DM <50% of control

Table 2. The effect of NaCl on dry matter digestibility (soluble salts subtracted) and shoot tissue Cl concentrations in four species of Melilotus and the control species T. michelianum

	% Dry matter digestibility (on a DM basis with soluble salts subtracted) at:	Shoot tissue Cl concentration (?mol/g dwt) at NaCl concentrations of:

	0 mM NaCl	160 mM NaCl	0 mM NaCl	160 mM NaCl
M. albus *perennial*	73.5	66.0	67	1451
M. italicus	73.3	66.3	102	2201
M. messanensis	70.6	66.3	160	1330
M. speciosus	69.6	62.1	62	2272
T. *michelianum*	75.4	71.9	208	1936
SED (P=0.05) Salinity*Species		0.835		
l.s.d. (P=0.05) Salinity*Species				519

Waterlogging tolerance

There were significant differences amongst *Melilotus* species in the effect of waterlogging on shoot and root growth (Table 3), with several species (e.g .*M. messanensis* and *M. sulcatus* ssp*. segetalis*) showing good

tolerance to the hypoxic conditions. All species appeared to adapt to the waterlogging by increasing root porosity in the main and lateral roots (data not presented). There was no consistent relationship between salt tolerance and waterlogging tolerance.

Table 3. The effect of growth in stagnant solution culture on shoot and root dry weight in 10 species of Melilotus and 3 control species (M. sativa, T.fragiferum and T. michelianum)

	Shoot dry weight (g/plant)		Root dry weight (g/plant		Waterlogging tolerance ranking*
Species	Aerated	Stagnant as % control	Aerated	Stagnant as % control	
M. *messanensis*	2.4	102	0.3	84	Tolerant
M. neapolitanus	1.0	18	0.3	11	Sensitive
M. officinalis	1.5	33	0.9	37	Sensitive

M. polonicus	0.6	52	0.4	49	Intermediate
M. speciosus	3.9	35	1.1	33	Sensitive
M. suaveolens	0.5	70	0.6	61	Intermediate
M. sulcatus (ssp. brachystachys)	1.5	27	0.5	16	Sensitive
M. sulcatus (ssp segetalis).	1.6	87	0.5	96	Tolerant
M. tauricus	0.6	45	0.4	34	Sensitive
M. wolgicus	0.8	22	0.6	25	Sensitive
M. sativa (C1*)*	5.8	31	1.2	2	Sensitive
T. fragiferum (C2*)*	1.5	101	0.3	84	Tolerant
T. michelianum (C3)	3.9	103	0.5	150	Tolerant
LSD (P=0.05) species*stagnant	0.7		0.2		

* Tolerant – shoot DM >80% of control, Intermediate – shoot DM 50-80% of control, Sensitive – shoot DM <50% of control

Weed risk

A major consideration with this research is the weed potential of target species. Several *Melilotus* species (viz. *M. dentatus, M. elegans, M. neopolitanus, M. polonicus, M. suaveolens* and *M. wolgicus*) have now been recognised as significant weed risks, and research on these species will not be continuing.

Conclusion

This research has identified several species of *Melilotus* that offer potential for saline areas. *M. messanensis*, in particular – a species with low coumarin levels- performed well under both saline and waterlogged glasshouse conditions. Research on this species is continuing with further glasshouse studies on 30 individual accessions, as well as efforts to identify suitable matching rhizobia for field establishment. Field experiments have now commenced in South Australia and Western Australia.

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