Improving the nodulation of regenerating stands of Messina (*Melilotus siculus*) in saline soils

Amanda Bonython[1,3], Ross Ballard[1,3], Nigel Charman[1,3], Andrew Craig[1,3] and Phillip Nichols[2,3]

[1]South Australian Research and Development Institute, Livestock and Farming Systems, GPO Box 397, Adelaide, South Australia, 5001. www.sardi.sa.gov.au Email Amanda.Bonython@sa.gov.au
[2]Department of Agriculture and Food Western Australia, Locked Bag 4, Bentley Delivery Centre, Western Australia, 6983. www.agric.wa.gov.au Email phil.nichols@agric.wa.gov.au
[3]Future Farm Industries Cooperative Research Centre, The University of Western Australia Crawley, WA, 6009. www.futurefarmcrc.com.au

Abstract

Messina (*Melilotus siculus* (Turra) Vitman ex B.D. Jacks) has been identified as the most promising salt and waterlogging tolerant annual pasture legume for areas affected by dryland salinity in southern Australia. Messina is nodulated and fixes nitrogen with *Sinorhizobium medicae*. Currently there is no commercial inoculant available for Messina. The strain used for evaluation purposes ('AM medic' strain WSM 1115) was developed for use on non-saline soils and fails to persist in saline environments (soil surface salinity >10 dS/m), resulting in poor nodulation of regenerating seedlings. Field sites were established in saline environments, at two alkaline sites in South Australia (SA) and one acidic site in Western Australia (WA), to evaluate the effect of 78 strains of rhizobia on the nodulation and growth of regenerating Messina plants. Evaluated rhizobia had been isolated from plants and soils collected from saline environments and were compared to WSM 1115. Visual assessments of plant health and biomass in regenerating plots were used to select a cohort of strains for detailed measurement. Plants were sampled from these treatments and mean nodule number (SA), nodulation score (WA) and shoot and root dry weight (mg/plant) (SA and WA) determined. Four strains (SRDI 554, SRDI 875, SRDI 905 and WSM 4191) produced significantly more nodules per plant ($P<0.05$) than WSM 1115 at both SA sites. Significant differences ($P<0.05$) in shoot and root weight (dry matter mg/plant) were measured at one SA site and at the WA site. Strain WSM 4190 performed best at the WA site. The best performing strains have been established at multiple field sites in 2010 to allow detailed evaluation of their persistence. Nitrogen fixation capacity and acid tolerance will also be assessed in parallel greenhouse studies, with a view to recommending a strain of rhizobia for use in commercial inoculants for Messina.

Key Words

Rhizobium medicae, Sicilian sweet clover, Melilotus messanensis, N_2-fixation

Introduction

In 2000 it was estimated that approximately 5.7 million hectares of Australia's agricultural and pastoral zone was affected by dryland salinity, with the area expected to increase to approximately 17.0 million hectares by 2050 (Dolling et al. 2001). Areas affected by dryland salinity are also prone to waterlogging. Currently there are no commercial annual pasture legumes which can tolerate the combined stresses of salinity and waterlogging experienced in this landscape. Extensive glasshouse (Nichols et al. 2009; Rogers et al*.* 2008) and field (Nichols *et al.* 2008) research has identified Messina (*Melilotus siculus* (Turra) Vitman ex B.D. Jacks (syn. *M. messanensis* (L.) Mill.)) as the most promising salt and waterlogging tolerant annual pasture legume for areas affected by salinity in southern Australia.

The development of Messina as a new pasture species has been severely constrained by nodulation failure in regenerating stands. Currently there is no commercial inoculant available for Messina. Messina is nodulated and fixes nitrogen with *Sinorhizobium medicae* but the current commercial *S. medicae* strain, 'AM medic' WSM 1115, was developed for use on non-saline soils. Research has shown that WSM 1115 fails to persist in the soil over the summer months when soil surface salinity levels are greatest (Bonython et al*.* 2009; Charman et al. 2006). As a result regenerating stands of Messina in saline environments (soil surface salinity >10 dS/m) are poorly nodulated with sub-optimal herbage production. In order to address this, field trials were established in three saline environments (two alkaline sites in South Australia (SA) and one acidic site in Western Australia (WA)) to examine if any of 78 strains of rhizobia sourced from soils and plants collected from saline environments could improve the nodulation and growth of regenerating Messina plants.

Methods

Site characterisation

Field trials were sown in June 2008. Sites 1 and 2 were sown on alkaline saline sands near Keith in the south-east of SA (pH_{H2O} 7.8-8.7, summer soil surface ECe >13 dS/m). Site 3 was sown on an acidic saline sand near Darkan, WA (pH_{H2O} 5.7, summer soil surface ECe 38.8 dS/m). An assessment of soil collected from each site before sowing showed an absence of rhizobia able to nodulate Messina.

Experimental treatments and trial establishment

Each site contained 81 treatments, comprising 78 experimental strains of rhizobia, two sources of WSM 1115 (Table 1) and an un-inoculated control. All rhizobia were applied to seed at approximately double the commercially recommended rate and the seed pelleted with fine lime (Seedcote[TM]). The seed used was a composite of five Messina accessions, SA# 36981, SA# 36983, SA# 40000, SA# 40001 and SA# 40005. Inoculated seed was hand-sown into 2 m x 1 m plots at a rate of 10 kg/ha. Treatments were arranged in a randomised block layout with four replications.

Table 1. Strains of *Sinorhizobium* species evaluated in the field and collection site details.

Strain annotation	Origin of strain	Description of collection sites	ECe (dS/m) at collection
WSM 1115[A]			
SRDI 554	West Beach, SA	Estuary bank	Unknown
SRDI 809 – SRDI 814	Globe Park, SA	Mangrove margin	42
SRDI 821 – SRDI 825	Port Wakefield, SA	Salt scald	32
SRDI 830 – SRDI 834	St Kilda, SA	Mangrove margin	25
SRDI 838 – SRDI 843	St Kilda, SA	Salt scald	43
SRDI 874 – SRDI 875	Ouyen, Victoria	Sodic soil near salt scald	Unknown
SRDI 876 – SRDI 877	Granite Island, SA	Cliff face on ocean side of island	Unknown
SRDI 881 – SRDI 885	Goolwa, SA	Beach near Murray River mouth	Unknown

SRDI 887 – SRDI 892	Cooke Plains, SA	Samphire flat	High
SRDI 897 – SRDI 899	Keith, SA	Old Messina trial site sown 2003	Unknown
SRDI 900 – SRDI 905	Robe, SA	Drain & roadway near saline lake	27
SRDI 912 – SRDI 914	Robe, SA	Beach	4
SRDI 918 – SRDI 937	Robe, SA	Adjacent to saline lake	2 – 15
WSM 4118	Hadera, Israel	Adjacent to brackish lake	Unknown
WSM 4121	Afula, Israel	Adjacent to brackish lake	Unknown
WSM 4123	Karkur, Israel	Native pasture	Unknown
WSM 4189 – WSM 4191	Bunbury, WA	Adjacent to brackish lake	5
WSM 4192 – WSM 4203	Waterloo, WA	Irrigated pasture	3 to 37

[A]Two WSM 1115 treatments were included in the field trials. One (annotated WSM 1115c) used a commercial packet of Group AM inoculant. The second (annotated WSM 1115e) used the strain sourced from the Australian Inoculants Research Group and was grown in peat culture using the same method used for all other experimental strains.

Sites were visually assessed for nodulation in October 2008. Twelve isolates (SRDI 810, SRDI 812, SRDI 821, SRDI 823, SRDI 877, SRDI 888, SRDI 903, SRDI 904, SRDI 937, WSM 4195, WSM 4199 and WSM 4200) and the un-inoculated control were noted as producing poor levels of nodulation. The nodulation of all other strains of rhizobia was satisfactory.

Visual assessment of plant health and biomass

In 2009, regenerating plots at Site 1 and Site 3 were visually assessed, using a 0-5 scale at Site 1 and a 0-10 scale at Site 3, to rank plant health and biomass from poor (score 0, no healthy plants) to good (maximum score, large number of healthy plants). Plants that were green and growing vigorously were deemed healthy and were readily distinguished from unhealthy plants that were typically small, yellow and symptomatic of poor nitrogen fixation. A visual assessment at Site 2 was less insightful due to the high occurrence of weeds. Data from Sites 1 and 3 was used to select experimental strains for further evaluation.

Nodulation assessment and shoot and root dry weight

Thirteen experimental strains from Site 1 and five experimental strains from Site 2 (Table 2) were selected for nodulation assessment on 28 July 2009 and 18 August 2009, respectively. The WSM 1115 and un-inoculated treatments were also assessed. Ten plants were randomly selected in each plot and carefully removed. The number of nodules was ascertained on each root system. Eight experimental strains, WSM 1115c and the un-inoculated control were harvested on 8 September 2009 from Site 3. Sampling strategy and laboratory assessment were modified due to the advanced stage of plant growth. The nodulation of ten healthy plants was scored using a 0-10 scale based on the number, size and distribution of nodules on the root system, where 0 = no nodules, 10 = > 2 crown nodules that are > 1 cm? in size (Dr. R. Yates, 15 September 2010, Centre for *Rhizobium* Studies, WA, pers. comm.). At all three sites shoot and root dry weight (mg/plant) was determined.

Statistical analyses

All data was analysed using GenStat (12th Edition) (Payne *et al.* 2009). The visual assessment scores were analysed spatially as linear mixed models ($P<0.05$). All other results were analysed using a general analysis of variance ($P<0.05$).

Results and Discussion

Visual assessment of plant health and biomass

At all sites the plant health and biomass score of the WSM 1115 treatments was poor and in all but one instance the WSM 1115 scores were not significantly greater than the un-inoculated control (data not shown). Several experimental strains improved plant health and biomass scores compared to the WSM 1115 treatments. Three strains at Site 1, SRDI 876 (score 4.0), SRDI 905 (score 3.5) and SRDI 4191 (score 3.2), and two strains at Site 3, SRDI 905 (score 4.4) and WSM 4191 (score 4.7), significantly improved plant health and biomass scores compared to the WSM 1115 treatments.

Nodulation assessment and shoot and root dry weight

Several strains produced more nodules than WSM 1115 (Table 2). Strains SRDI 554, SRDI 875, SRDI 905 and WSM 4191 significantly increased the number of nodules per plant compared to the WSM 1115 treatments at both Sites 1 and 2. These strains (except SRDI 875) were also amongst the best strains at Site 3, although they did not significantly improve nodulation score compared to WSM1115c. At Site 3, only strain WSM 4190 had a significantly better nodulation score compared to WSM 1115c. Strains SRDI 810 and SRDI 877 were identified as having poor levels of nodulation in the year of establishment, and at Site 1 this was again evident in regenerating plants.

Table 2: Number of nodules per plant (Sites 1 and 2), nodulation score (Site 3) and shoot and root dry weight (mg/plant) of regenerating Messina plants.

Treatment	Number of nodules per plant		Nodulation score	Shoot dry weight (mg/plant)			Root dry weight (mg/plant)		
	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
Uninoculated	0.7	1.5	0.1	40	83	31	12	32	19
WSM 1115c	1.0	6.4	2.4	43	602	245	15	157	48
WSM 1115e	2.2	4.5		74	511		22	134	

SRDI 554	21.0	28.0	4.7	78	331	173	29	89	55
SRDI 810	5.5			80			28		
SRDI 813			3.7			319			81
SRDI 832	5.4			79			26		
SRDI 840	4.9			143			42		
SRDI 875	11.7	19.8	1.9	183	505	175	53	112	39
SRDI 876	20.5	16.4	2.6	190	514	246	53	105	70
SRDI 877	6.0			142			33		
SRDI 881			4.9			235			74
SRDI 900	13.1			117			36		
SRDI 905	28.5	19.0	5.2	132	315	230	47	83	68
SRDI 914	9.4			133			35		
SRDI 934	5.4			106			35		
WSM 4123	7.7			183			50		
WSM 4190			6.0			765			152
WSM 4191	15.8	25.1	5.5	119	303	456	36	73	107
Mean	9.9	15.1	3.7	115.1	395.5	287.5	34.5	98.1	71.3
LSD ($P<0.05$)	7.8	11.7	3.1	80.8	440.0	491.3	19.0	105.2	82.9

At Site 1 strains SRDI 875, SRDI 876 and WSM 4123 significantly increased both shoot and root dry weight compared to the WSM 1115 treatments (Table 2). On average these strains increased shoot and root dry weight by 150 % and 136 % respectively compared to WSM 1115e. Shoot and root dry weight did not vary significantly between experimental strains and the WSM 1115 treatments at Site 2. This may reflect the later harvest (August) at this site and the associated mortality of poorly nodulated seedlings in some plots. At Site 3, the strain with the highest nodulation score (WSM 4190) also had increased shoot (+212 %) and root dry weight (+217 %) compared to WSM 1115c. Although SRDI 554 had a high number of nodules per plant at Sites 1 and 2, shoot and root dry weight was similar to WSM 1115 and inferior to the best experimental treatments.

Conclusion

Several strains of rhizobia have been identified that improved the nodulation and growth of regenerating Messina plants in saline soils compared to the current commercial 'AM medic' strain, WSM 1115. Of particular interest are the strains SRDI 554, SRDI 875, SRDI 905, WSM 4190 and WSM 4191.

Interestingly, these strains, sourced from various Australian soils, outperformed strains collected from naturalised Messina pastures in Israel. Strains sourced from WA (WSM 4190 and WSM 4191) tended to perform better in that state, possibly as a consequence of their better adaptation to acid soils. Despite some obvious differences in strain performance at the various locations (e.g. strain SRDI 875) there appears to be a reasonable prospect that a single strain can be identified to cover the broad range of environments where Messina is likely to be grown.

The best performing strains have been established at multiple field sites in 2010 to allow detailed evaluation of their persistence. Nitrogen fixation capacity and acid tolerance will also be assessed in parallel greenhouse studies, with a view to recommending a strain of rhizobia for use in commercial inoculants for Messina.

Acknowledgements

This research was funded by the Future Farm Industries CRC and undertaken by the South Australian Research and Development Institute and the Department of Agriculture and Food Western Australia.

References

Bonython AL, Charman N, Ballard R, Craig AD and Nichols PGH (2009). Selection of rhizobia to improve the nodulation of *Melilotus siculus* growing in saline environments. Proceedings of the 15th Australian Nitrogen Fixation Conference, 8 to 13 November, Margaret River, Western Australia.

Charman N, Ballard R and Craig A (2006). *Melilotus siculus* (syn *messanensis*) is constrained by a lack of suitable rhizobia. Proceedings of the 13th Agronomy Conference, 10 to 14 September, Perth, Western Australia.

Dolling PJ, Moody P, Noble A, Helyar KR, Hughes B, Reuter D and Sparrow L (2001). Australian Dryland and Salinity Assessment 2000: Extent, impacts, processes, monitoring and management options. National Land and Water Resources Audit, Commonwealth of Australia, Canberra, Australia.

Nichols PGH, Craig AD, Rogers ME, Albertsen TO, Miller S, McClements DR, Hughes SJ, D'Antuono MFD and Dear BS (2008). Production and persistence of annual legumes at five saline sites in southern Australia. Australian Journal of Experimental Agriculture 48, 518-535.

Nichols PGH, Malik AI, Stockdale M and Colmer TD (2009). Salt tolerance and avoidance mechanisms at germination of annual pasture legumes and their importance for adaptation to saline environments. Plant and Soil 315, 241-255.

Payne RW, Murray DA, Harding SA, Baird DB and Soutar DM (2009). GenStat for Windows (12^{th} Edition), Lawes Agricultural Trust, Rothamstead Experimental Station, VSN International, Hemel, Hampstead.

Rogers ME, Colmer TD, Frost K, Henry D, Cornwall D, Hulm E, Deretic J, Hughes SJ and Craig AD (2008). Diversity in the genus *Melilotus* for tolerance to salinity and waterlogging. Plant and Soil 304, 89-101.