Genetic improvement of strand medic (*Medicago littoralis* Rohde ex Lois.) for Australian farming systems

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Abstract

Strand medic (*Medicago littoralis*) provides the principle source of fixed nitrogen (N) in many neutral to alkaline sandy-loam soils, such as those that dominate the Eyre Peninsula region of South Australia. So well adapted is the species in these environments that it is often grown in near monoculture conditions in the ley phase of these farming systems. This paper reports the efforts made in the genetic improvement of strand medic for traits including herbicide tolerance, improved N_2-fixation, tolerance to root-lesion nematode and resistance to powdery mildew disease. A sulfonylurea herbicide (SU) tolerant line (coded FEH-1) developed by EMS mutagenesis of the strand medic variety Herald, offers significant promise, especially for use in low rainfall regions with alkaline soils, where the breakdown of SU residues is reduced. The herbicide tolerance trait in FEH-1 appears to be controlled by a single dominant gene. Plant selections have also been made for 'symbiotic promiscuity' that will enable strand medic to form a greater proportion of optimal N_2-fixing symbioses with the naturalised rhizobia that proliferate in most soils where the plant is grown. Selection and breeding for tolerance to the root-lesion nematode (*Pratylenchus neglectus*) and resistance to powdery mildew (*Erysiphe trifolii*) are also being undertaken in order to improve productivity.

Media summary

Selection and breeding efforts are in progress at SARDI to develop strand medic varieties with tolerance to sulfonylurea herbicide residues, improved N_2-fixation, tolerance to root-lesion nematode and powdery mildew resistance.

Key Words

Medicago littoralis, sulfonylurea herbicide tolerance, N_2-fixation, Pratylenchus neglectus, Erysiphe trifolii.

Introduction

Annual medics (*Medicago spp.)* deliver multiple benefits to semi-arid farming systems in southern Australia. They provide feed for livestock, benefit the following cereal crop by improving soil fertility through N_2-fixation and also break the disease cycle of several soil-borne pathogens of cereals. These self-regenerating pasture species have relatively high levels of hardseededness, which enables them to persist through cropping phases and regenerate in subsequent years. Strand medic (*M. littoralis* Rohde ex Lois.) is well adapted to neutral to alkaline sandy-loam soils of which there are large areas in the lower rainfall (< 375 mm per annum) cereal livestock zones of temperate Australia (> 5.5 M ha).

Over the past decade there have been reports of declining medic production (Bellotti and Kerby 1993) and, in response, many investigations have sought to identify the causes. Several agro-biological factors have been implicated. There is strong evidence that the sulfonylurea (SU) herbicides play a significant role with reports of severe stunting of medic even at very low residue levels (eg < 1ppb; Heap 2000). A study on an alkaline soil in the Victorian Wimmera found chlorsulfuron reduced medic growth by 89% and

75%, and triasulfuron by 60% and 33%, one and two years after herbicide application respectively (Noy 1996). Symbiotic limitations have also been investigated as a possible cause of declining medic production. What is clear is that most of the alkaline soils where strand medic is grown contain large populations of naturalised rhizobia (Slattery et al.1999; Ballard and Charman 2000; Brockwell 2001). Whilst it is rare that these populations are completely ineffective (fix no N) with strand medic, they are often sub-optimal when their N_2-fixation capacity is compared with an effective soil isolate or a commercial inoculant strain. For example, Ballard and Charman (2000), using inoculant strain WSM826 as a benchmark, found that the strand medic variety Herald achieved only 49% of its symbiotic potential with the soil rhizobia from several regions in South Australia. A similar study on Eyre Peninsula region by Brockwell (2001), while dismissing the symbiosis as a factor in medic decline, showed that nearly 40% of the populations of soil rhizobia were sub-optimal when compared to the most effective soil population. Since the naturalised rhizobia are difficult to displace, plant selection for improved N_2-fixation with the resident rhizobia is probably the only practical way of optimising the symbiosis across an array of environments. Another factor believed to be limiting the production of annual medics is disease. In field experiments where rudimentary disease control was achieved using fungicides and nematicides, root diseases have been shown to account for average production losses of 22% in annual medics (WD Bellotti unpublished data). Nematodes and a suite of fungal pathogens were implicated. More specifically, the root lesion nematode (*Pratylenchus neglectus*) has been shown in growth room studies to cause production losses in Herald strand medic of up to 65% (Hutton et al. 1999). Recently powdery mildew (*Erysiphe trifolii*) has also emerged as a significant factor. The disease was first noted on strand medics by pasture evaluators eight years ago, but outbreaks now occur across Australia (J Howie pers. com.).

This paper describes efforts to develop new strand medic varieties which can be used by farmers to tackle the problems of sulfonylurea herbicide residues in soil, sub-optimal N_2-fixation with the naturalised soil rhizobia, root damage by root-lesion nematode and foliar damage by powdery mildew.

Methods

Herbicide tolerance: A SU tolerant line of strand medic (FEH-1) was developed by EMS mutagenesis of the variety Herald (Heap 2000). This tolerant line (as male parent) was crossed to a sensitive line in order to study the genetics of the tolerance trait. The dominant FEH-1 leaf pigmentation enabled the identification of hybrids. Self-fertilised seed was collected from the hybrids and 170 F_2 plants were screened by applying triasulfuron at 10 g a.i. ha $^{-1}$ after 21 days growth in a glasshouse. The progenies of the surviving F_2 plants were also screened as above. The tolerance of FEH-1 was also compared under field conditions to its sensitive parent, Herald. Chlorsulfuron and triasulfuron were applied at 0, 50, 100 and 200% of recommended label rates (i.e. 0, 7.5, 15 & 30 and 0, 11.25, 22.5 & 45 g a.i. ha $^{-1}$ respectively) in May 2001 at Minnipa Agricultural Centre, SA (red sandy loam, $pH_{(w)}$ 8.4). In the following year (2002), Herald and FEH-1 were sown as single spaced seeds into the SU residue treatments. Measurements taken during 2002 included shoot growth at 11, 15 and 17 weeks post sowing.

Selection for improved N_2 -fixation: The N_2-fixation capacity of 500 F_2 and F_3 medic plants was assessed using a visual score of dry weight, in the glasshouse. The plants had been generated from a cross between Herald and a medic line (coded RP) that had previously been selected for improved N_2-fixation with naturalised soil rhizobia. Fifty plants of each parent line were also included as controls. Surface sterilised seedlings were sown into 70 cm^3 pots, containing vermiculite saturated with a N-limited nutrient solution. The seedlings were inoculated four days later with *Sinorhizobium meliloti* strain SRDI148 (isolated from soil, Gunnedah, NSW) that was previously shown to be effective at N_2-fixation with RP, but not with Herald. Plants were grown for 35 days after inoculation and a visual score of dry weight (1 = small chlorotic plants through to 5 = large dark green plants) recorded as an index of nitrogen fixation for all plants prior to the best F_2/F_3 plants being removed to enable further breeding work. Dry weight (mg) of the remaining plants was determined. The relationship between mean dry weight (y) and score (x) is described by the equation $y = 2.8x^2 + 10x$.

Tolerance to root-lesion nematode: A cross was made between a strand medic line (RH-1 as female) with putative tolerance to root-lesion nematode and the variety Herald. Hybrid plants were identified on the basis of leaf pigmentation associated with Herald. Seed from a hybrid plant was collected, and 154 progeny (F_2) entered into a growth room based screening assay where they were compared to Herald and RH-1. Surface sterilised, pre-germinated seedlings were planted into 300 cm^3 pots (one seedling per pot) of sterile potting media. Four days after planting, a suspension of nematodes in 1 ml of water was applied to each seedling. A second application (1 ml) of nematodes was added at 11 days to give a total of 20 nematodes per gram of soil. Plants were grown for 48 days, roots washed and root damage measured (score 0 = no damage; 1-4 = light browning, no loss of lateral roots; 5-7 = extensive browning, moderate loss of lateral roots; 8-10 = extensive browning, no lateral roots and lesioning on tap root). A score of ≤ 4 was considered indicative of tolerance.

Powdery mildew resistance: Eighty lines of strand medic were screened for resistance to powdery mildew (*Erysiphe trifolii*) in a glasshouse. The pathogen was maintained *in-situ* on actively growing medic plants in the screening glasshouse. Seed of the medic lines was sown into 500 cm^3 pots (6 per pot with 5 replications) and assessed for cotyledon and leaf infection 28 days after sowing (score 0 = no mycelium visible; 1-2 = mycelium on lower leaves; 3-4 = mycelium on some upper leaves; 5 = mycelium visible on all leaves).

Results and discussion

Herbicide tolerance: Chi-square values indicated that the F_2 data fit a 3:1 (tolerant vs. sensitive) Mendelian inheritance (data not presented). Some of the F_3 families were homozygous tolerant while others showed a 3:1 (tolerant vs. sensitive) segregation for the tolerance trait, suggesting its control by a single dominant gene. In the field at Minnipa, FEH-1 showed good tolerance to increasing rates of herbicide at all three growth stages (Table 1.). In comparison, Herald shoot dry weight (per plant) declined markedly at rates greater than 50 %. There were also unexpected differences between FEH-1 and Herald in the control treatments at 15 and 17 weeks post-sowing. This has been previously reported at a different site (Howie et al. 2002) and is possibly due to the presence of pre-existing SU herbicide residues affecting plant growth of sensitive plants as their roots explore the soil profile at greater depth (as moisture becomes limiting).

Table 1. Mean shoot dry weight (g/plant) of Herald and FEH-1 after 11, 15 and 17 weeks growth in soil treated with sulfonylurea herbicides (chlorsulfuron and triasulfuron) at 0, 50, 100 and 200 % recommended label rates. Data in parentheses have been square root transformed and should be used to make statistical comparisons at 17 weeks growth.

Herbicide	11 weeks		15 weeks		*17weeks	
Rate (%)	Herald	FEH-1	Herald	FEH-1	Herald	FEH-1
0	0.429	0.385	1.545	4.478	3.89 (1.97)	11.81 (3.44)
50	0.317	0.464	1.588	4.195	2.15 (1.47)	11.96 (3.46)
100	0.193	0.496	0.750	4.330	1.91 (1.38)	15.53 (3.94)
200	0.126	0.473	0.617	4.706	1.07 (1.03)	15.26 (3.91)
LSD 5%	0.146		0.924		(0.52)	

Selection for improved N_2-fixation: When Herald was inoculated with SRDI148, the plants appeared N-deficient and their score was always ≤ 3 (Figure 1). Conversely, when RP was inoculated with SRDI148, an effective symbiosis generally resulted with about 80% of plants achieving score 5. More than 40% of F_2 plants and 20% of F_3 plants scored 5 indicating they had retained the improved N_2-fixation trait from RP (many were also noted to have leaf markers typical of Herald). Twelve plants were subsequently selected and have since been tested at the F_4 generation for their reaction to bluegreen and spotted alfalfa aphid to ensure that the recombinants retain the aphid resistance of the Herald parent (RP is highly susceptible to the bluegreen aphid). The progenies (F_5) of selections combining both improved N_2-fixation and aphid resistance are presently being re-tested using SRDI148. Further selections from this screening will be assessed for agronomic performance under field conditions.

Tolerance to root-lesion nematode: RH-1 had a mean root damage score of 5 compared to Herald, which recorded a mean score of 8 (Figure 2). The root damage scores in the F_2 ranged from 3 to 9 and the phenotypes could not be classified into discrete classes (tolerant/susceptible). This indicated that the tolerance was under multigenic control. Plants with low damage scores will be used for further breeding and selection. Further screening of more F_2 plants and the F_3 generation will help determine the nature of inheritance.

Powdery mildew resistance: Large variation in seedling resistance of strand medic lines to powdery mildew was observed. Scores ranged from 0.9 to 3.8 across the 80 lines. The commercial varieties were moderately susceptible with Herald, Harbinger and Harbinger AR scoring 3, 3.2 and 3.2 respectively. One line was identified with a good level of putative resistance. The resistance of this line will be verified in a second round of screening that incorporates powdery mildew isolates sourced from different locations, prior to the commencement of any breeding effort.

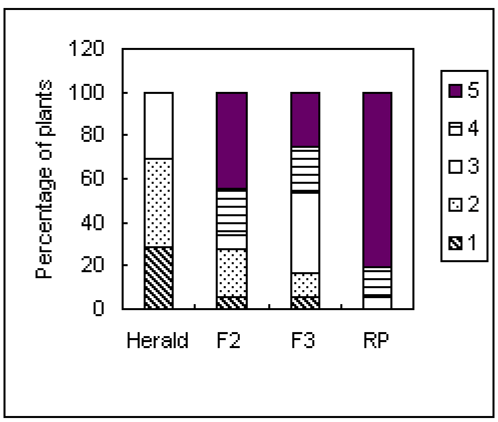

Figure 1. Percentage of Herald, RP, F_2 and F_3 plants in each category of dry weight score (1 =low, 5 = high) after 35d growth in N-deficient media after inoculation with *S. meliloti* strain SRDI148.

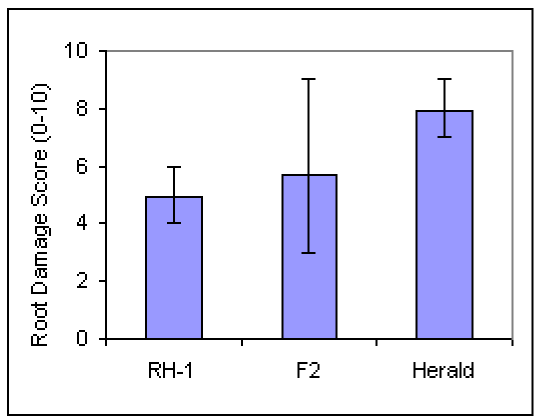

Figure 2. Mean and range of root damage score for Herald, RH-1 and 154 F2 plants (0 = no damage, 10 = severe damage). Vertical bars indicate the highest and lowest values of root damage within each medic population.

Conclusion

Research has highlighted the potential to develop improved strand medic varieties to overcome some of the factors that have been implicated in the sub-optimal production of medic pastures. As the development of the SU herbicide tolerant line is the most advanced, having shown improved production in field experiments, it will be used as the platform for the introgression of traits for improved N_2-fixation and disease resistance. In addition, as the SU tolerance appears to be inherited as a single gene, this trait can also be readily incorporated by crossing into other medic species with different environmental adaptations.

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