Plant parameters identified for evaluating varietal performance of acid tolerance in lucerne

Xianguang Zhang, Alan Humphries and Geoff Auricht

South Australian Research and Development Institute (SARDI), GPO Box 397, Adelaide 5001.
www.sardi.sa.gov.au Email zhang.xianguang@saugov.sa.gov.au

Abstract

Validating plant response to soil acidity using glasshouse pot cultures is an essential step in the process of selective breeding for acid-tolerant lucerne. The objective of this study was to identify plant parameters that can be effectively used to assess varietal performance in acid soils. A glasshouse pot experiment using acid soils was conducted to quantify variations and differences in plant establishment, plant growth and development of a range of lucerne genotypes with known aluminium (Al) tolerance response. Plant parameters examined include morphological and/or agronomic traits at their early plant growth stages (up to 90 days after sowing).

Significant differences in dry matter production were detected among the lucerne genotypes when grown in a typical acid soil (pH_{CaCl2} 4.5; Al 3.3 ppm). Al-tolerant genotypes had better plant establishment in the acid soil than an Al-sensitive one, and they produced significantly higher dry root weight and total dry weight on a pot basis. Al-tolerant genotypes were also able to form root nodules in the acid soil without artificial inoculation. Plant establishment, number of nodules formed, and dry root weight and/or total dry weight were positively correlated. These results clearly showed the advantage of Al-tolerant lucerne in early dry matter production in the acid soil conditions, through a more functioning root system. It is suggested that dry root weight and total dry weight during early plant growth are useful plant parameters for indicating relative acid tolerance in lucerne. Issues of how soil acidity affects plant establishment and plant production in relation to water regime management and/or nitrogen fertiliser application were also discussed.

Key Words

aluminium tolerance, dry root weight, total dry weight, agronomic traits, morphological traits, correlations.

Introduction

Soil acidification is a major land degradation problem in Australia and many other countries around the world (Carver and Ownby 1995). Aluminium (Al) toxicity associated with acid soils is a particular growth-limiting factor as it restricts rooting depth and branching in plants, which in turn decreases plant production. In Australia, lucerne (*Medicago sativa* L.) is the most widely grown perennial pasture legume and occupies some 3 million hectares, much of which is found in the cereal belt. However, the value and the use of lucerne as a forage plant are limited by its lack of tolerance to soil acidity (Cocks 2001), it is therefore imperative to breed for acid-tolerant lucerne, in the light of achieving agricultural sustainability with economic benefit and environmental friendliness.

Attempts to breed for acid tolerance in lucerne have been made worldwide over the past 2 decades, with varying initial successes (Bouton 1996). In recent years, we have been systematically using a breeding strategy to breed for acid-tolerant lucerne, focusing on breeding for Al tolerance using a hydroponic system and field selection of adapted plants on acid soils as the first approach. So far some progresses have been made in breeding improved Al-tolerant lucerne, as indicated by enhanced root regrowth of Al-tolerant selections in solution cultures with toxic Al concentrations imposed. But little is known of the agronomic advantage of those Al-tolerant lucerne genotypes in acid soils, in terms of plant establishment, growth and development. This leads to the adoption and use of the second step and approach – validating the performance of Al-tolerant selections in acid soil pot cultures before any sound conclusions are drawn about their suitability and adaptation to field conditions of targeted acid soils. This process entails the identification of plant parameters that can be reliably used to assess relative acid tolerance response of lucerne germplasm, irrespective of their origin, breeding history or backgrounds.

Previous glasshouse experiments indicated the varying effects on plant performance of cultue media (e.g., normal potting mixture, acid soil or limed acid soil), cultivars, fertilisers, or water regimes, and/or their interactions in lucerne (Zhang et al. 2004). It was, nevertheless, evidenced that soil acidity affects plant establishment, early seedling growth and plant production. Integrated analyses showed that plant dry matter production, such as dry root weight, dry shoot weight, or total dry weight, was more profoundly reduced than other agronomic characters examined in acid soil pot cultures (our unpublished data). But the insignificant effects of cultivars with a narrow range of variation in acid tolerance made the evaluation of little use. This problem was corrected and improved in later experiments with the inclusion of a wider range of lucerne genotypes or selections differing in Al tolerance response, based on magnitudes of their root regrowth in solution culture with imposed toxic Al concentrations. The use of lucerne genotypes with differential Al tolerance response is thus essential for the purpose of identification and validation.

The objective of this study was to identify plant parameters that can be consistently used to assess varietal performance in acid soils, using genotypes of differential Al tolerant responses. A group of morphological, agronomic traits were examined on individual plants grown in a glasshouse pot culture of acid soils to determine the most useful plant parameters and the likely associations between these and root regrowth. The degree to which these were indicative of relative Al tolerance, was also tested.

Methods

Four lucerne genotypes (cultivars or selections) with similar winter vigour but different Al tolerance were chosen from a current lucerne breeding program. They included SARDI Ten, GAAT‘S’, A140 and A135. SARDI Ten was an Australian commercial cultivar with no Al tolerance (as indicated by short regrowth root length in a toxic Al solution). GAAT‘S’ and A140 were Al-tolerant genotypes: with GAAT‘S’ being a selection for Al tolerance from GAAT, a USA introduction (Bouton 1996) and A140 being a clonal selection for good seed set from GAAT‘S’. A135 was chosen as an intermediate Al-tolerant line with good acid soil adaptation. These results of Al tolerance response were from a previous solution test.

The pot culture experiment was conducted during early March–early June 2006 in controlled glasshouse conditions (with temperature ranged from 21–24?C) on the Waite Campus, using a RCBD with 3 replicates. The soil used was a typical acid soil, collected from a farmer’s paddock located in Mt Pleasant of South Australia, with pH 4.5 (in CaCl2) and 3.3 ppm Al. Pots, 20 cm in diameter, were filled with 4.8 kg soil each; watered to 100% field capacity (FC) then sown and applied with a complete fertiliser ‘Thrive’ (N 27.0%, P 5.5%, K 7.0%) at 50 kg N/ha. Each pot was sown with 20 imbibed seeds. Two weeks later, emerged seedlings were thinned to 10 plants per pot. Plants were watered every 2 days to maintain 100% FC for the first week, and 80% FC every 3–4 days for the remaining period. Measurements were taken for plant height and number of trifoliate leaves per plant on 40-d-old plants. Plants were harvested when they were 90 days old and washed under running tap water. Individual plants were checked and recorded for number of nodules formed. Plants were then separated into shoots and roots, and bulked on a pot basis; they were dried to constant weight and weighed. ANOVA was performed on data collected, using Microsoft Excel, with results reported on a pot basis.

Results and discussions

Morphological characters

At early growth stages up to 45 days, A135 was apparently bigger, taller and more vigorous than SARDI Ten and A140; A140 had smaller leaves and was more compact than GAAT‘S’.

Results (Table 1) indicated considerable differences in the morphological traits measured, including plant height, among the 4 genotypes tested. In terms of trifoliate leaf development, either on the main or 2nd stem, SARDI Ten was slower than the other 3 genotypes. A140 had slightly more trifoliate leaves than

GAAT‘S’, on the main stem (NTL-MS) or on the 2nd stem from the axil of the unifoliate leaf (NTL-2S/UL). A135 (10%) and GAAT‘S’ (7%) even bore a few trifoliate leaves on the 2nd stem from the axil of their 1st trifoliate leaf (NTL-2S/1TL).

Agronomic performance

Throughout the pot culture, leaves of both GAAT‘S’ and A140 appeared dark green whilst A135 and SARDI Ten had a few old leaves turn yellow at 75 and 80 days after sowing, respectively. At day 90, both GAAT‘S’ and A135 had 100% plants established, followed by A140. Visually, nearly all individual plants, except those of SARDI Ten, had secondary roots formed. Roots of GAAT‘S’ were thicker than those of A140; and A135 was intermediate.

Table 1. Mean performance (?*s.e.m.*) for some morphological traits of 4 lucerne genotypes tested in a glasshouse pot culture using an acid soil.

Measurements were taken on 40-d-old plants. NTL-MS = Number of Trifoliate Leaves on the Main Stem; NTL-2S/UL = No. of Trifoliate Leaves on the 2nd Stem from the axil of the Unifoliate Leaf; NTL-2S/C = No. of Trifoliate Leaves on the 2nd Stem from the axil of the Cotyledons; NTL-2S/1TL = No. of Trifoliate Leaves on the 2nd Stem from the axil of the 1st Trifoliate Leaf.

Morphological traits	SARDI Ten	GAAT‘S’	A140	A135
Plant height (cm)	13.1 ? 0.38	13.5 ? 0.49	10.3 ? 0.10	17.4 ? 0.64
NTL-MS	5.5 ? 0.13	6.9 ? 0.07	7.3 ? 0.39	6.7 ? 0.10
NTL-2S/UL	1.8 ? 0.51	2.8 ? 0.60	3.4 ? 0.26	3.2 ? 0.30
NTL-2S/C	0.1 ? 0.14	1.2 ? 0.40	0.9 ? 0.42	0.6 ? 0.15
NTL-2S/1TL	0	0.1 ? 0.03	0	0.2 ? 0.12

There were significant differences for root nodulation among the 4 genotypes. Both GAAT‘S’ and A135 formed clusters of nodules; A140 had fewer nodules, either singularly or in cluster; but no nodules were found on roots of SARDI Ten. GAAT‘S’ might have formed nodules earlier than A140. A135 had nodules formed but visual observations showed the leaf-yellowing symptoms. It is not known whether A135 produced sufficiently effective nodules for its symbiotic nitrogen fixation. Other issues concerning the ability of plants to form root nodulation include the duration of plants cultured in this acid soil since no nodules were ever found in younger plants harvested (<90 days) from previous pot experiments with other genotypes using the same acid soil. Root nodulation appeared to be stage-dependent; other confounding factors may include cultural management practices such as water regime (i.e. regular watering or simulating FC) and fertiliser application (i.e. the amount and the stage of plant growth).

ANOVA indicated significant differences for dry matter production on a pot mean basis among these 4 genotypes (Table 2). SARDI Ten showed a lower dry shoot weight (4.89 g) than A135 (6.04 g); GAAT‘S’ and A140 were intermediate. Differences for dry root weight were much larger: SARDI Ten (2.86 g) was only half of GAAT‘S’ (6.00 g) or A135 (5.25 g); A140 was also significantly lower than GAAT‘S’ or A135 (Figure 1). The same result was obtained for the total dry weight. These results clearly showed the advantage of Al-tolerant lucerne in dry matter production (of roots and the total) in the acid soil conditions, through a supposedly more functioning rooting system. There were, however, no significant differences for the dry matter production on a plant basis. This is a confounding issue affected by the disparate number of plants established per pot. Use of larger sample size might be one solution.

Table 2. Mean performance for some agronomic traits of 4 lucerne genotypes tested in a glasshouse pot culture using an acid soil.

Plants were harvested at 90 days after sowing. Values within each row followed by the same letter(s) indicate non-significance (or *ns*) at *P* = 5%; * significant.

Agronomic traits	SARDI Ten	GAAT'S'	A140	A135	Significance
Plants established (%)/pot	77 a	100 b	90 ab	100 b	*
No. of nodules/pot	0 a	11.3 b	6.7 ab	9.0 b	*
No. of nodules/plant	0 a	1.1 b	0.7 ab	0.9 b	*
Dry shoot weight (g/pot)	4.89a	5.49 ab	5.56 ab	6.04 b	*
Dry root weight (g/pot)	2.86 a	6.00 c	3.91 b	5.25 c	*
Total dry weight (g/pot)	7.75 a	11.48 c	9.48 b	11.29 c	*
Dry shoot weight (g/plant)	0.653	0.549	0.618	0.604	*ns*
Dry root weight (g/plant)	0.388	0.600	0.435	0.525	*ns*
Total dry weight (g/plant)	1.041	1.148	1.053	1.129	*ns*
Ratio of dry shoot wt to total dry wt	0.633 c	0.478 a	0.587 bc	0.535 ab	*
Ratio of dry root wt to total dry wt	0.367 a	0.522 c	0.413 ab	0.465 bc	*

Associations among plant attributes

Linear correlation analyses further revealed the associations between Al tolerance, as indicated by long regrowth root length (RRL) in solution culture with imposed Al stress (3.5 μm Al; pH 4.5), and dry matter production in pot culture of the acid soil (Table 3). Among those traits showing significantly positive correlations were number of nodules and dry root weight (DRW), total dry weight (TDW) or RRL; DRW and TDW or RRL.

Conclusion

Validating varietal performance for acid tolerance using glasshouse pot cultures is important in ultimately developing robust and feasible methodologies to breed for acid-tolerant lucerne cultivars. The inclusion of a range of lucerne genotypes differing in Al tolerance response in this pot experiment, combined with results obtained in a previous pot experiment, facilitated the identification of critical plant parameters to discriminate between acid-tolerant and acid-intolerant lucerne. Al-tolerant lucerne has the advantage of producing higher root dry matter and total dry matter in acid soils under the pot cultural conditions, apparently through improved plant establishment and a supposedly better functioning rooting system. Dry root weight and total dry weight of pot cultured plants at their early growing stages are therefore useful plant parameters of indicating relative performance of acid tolerance in lucerne. We recommend that they be used to identify acid-tolerant lucerne in future screening and evaluation of lucerne germplasm.

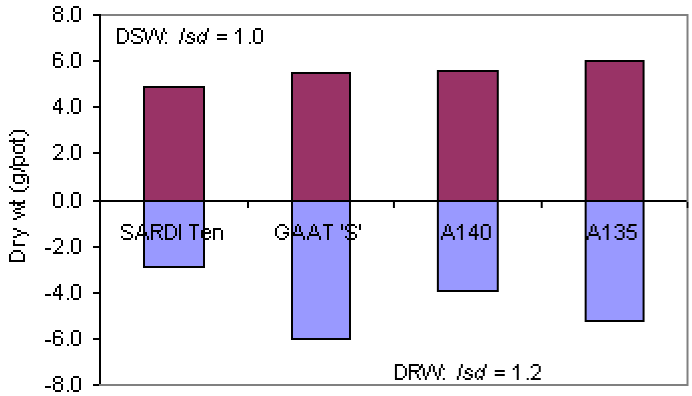

Figure 1. Comparisons of dry shoot weight (DSW, above *x* axis) and dry root weight (DRW, beneath *x* axis) of four lucerne genotypes at harvest (90-d-old plants, on a pot mean basis: g/pot).

Table 3. Linear correlation between traits using the four genotypes.

*, ** significant at 5% (*r* = 0.950) or 1% (*r* = 0.990) level of probability (*df* = 2). DRW, DSW, TDW stand for dry root weight, dry shoot eight and total dry weight, respectively. Data for RRL (regrowth root length), RL (root length) and TRL (total root length) were from a previous screening test of Al tolerance using a hydroponic system.

Traits	Establishment	Nodules	DRW	DSW	TDW	RRL	RL	TRL
Establishment (%)	1							
No. of nodules/pot	0.977*	1						
DRW (g/pot)	0.958*	0.962*	1					
DSW (g/pot)	0.858	0.760	0.682	1				
TDW (g/pot)	0.994**	0.971*	0.980*	0.813	1			
RRL (cm)	0.948	0.994 **	0.955 *	0.688	0.946	1		

RL (cm)	0.928	0.831	0.874	0.886	0.935	0.771	1	
TRL (cm)	0.991**	0.996**	0.975*	0.788	0.989*	0.982 *	0.878	1

Acknowledgement

The authors thank Trevor Rowe for his technical assistance in sampling during the course of the experiment. GRDC is acknowledged for funding this research. Allied lucerne projects funded by ACIAR and CRC Plant-based Management of Dryland Salinity assist in providing a critical mass for research in acidity tolerance.

References

Bouton JH (1996) Screening the alfalfa core collection for acid soil tolerance. Crop Science 36, 198–200.

Carver BF and Ownby JD (1995) Acid soil tolerance in wheat. Advances in Agronomy 54, 117–173.

Cocks PS (2001) Ecology of herbaceous perennial legumes: a review of characteristics that may provide management options for the control of salinity and waterlogging in dryland cropping systems. Australian Journal of Agricultural Research 52, 137–151.

Zhang XG, Garnett T, Davies K, Peck D, Humphries A and Auricht G (2004). Genetic evaluation and improvement of acid stress tolerance in lucerne breeding: Handbook and Abstracts for 4th International Crop Science Congress (eds. T Fischer, N Turner, J Angus, L McIntyre, M Robertson, A Borrell, D Lloyd), Brisbane, Australia. (26 September–1 October 2004).