It is the paper published as:

Author: B. J. Scott, M. Ewing, R. Williams, A. Humphries and N. Coombes
Title: Tolerance of aluminium toxicity in annual Medicago species and lucerne.
Journal: Australian Journal of Experimental Agriculture **ISSN**: 0816-1089
Year: 2008
Volume: 48
Issue: 4
Pages: 499-511

Abstract: A rapid (7 day) solution-based screening test was developed using 15 annual *Medicago* cultivars and one *M. sativa*. Based on a relative root regrowth after exposures to aluminium (Al), Zodiac (*M. murex*), Orion (*M. sphaerocarpos*) and the *M. polymorha* cultivars Santiago, Cavalier and Serena had the greatest Al tolerance. Herald (*M. littoralis*) and Rivoli (*M. tornata*) were most sensitive. Ranking for Al tolerance from the solution culture correlated well ($r = 0.80$) with ranking for tolerance of the 16 genotypes grown in an acidic soil (unlimed pH_{Ca} 4.1). We screened 17 Australian populations of lucerne (*M. sativa*) using a 24 h 'pulse' of 75 µmol/L Al, and a three day 'recovery' of 10 µmol/L Al. We identified and recovered plants with a root regrowth of ≥5 mm in all 17 populations with selection intensities of 2 to 4%.

Four of these selected populations (Aurora, UQL-1, A513 and TO2-011) were polycrossed within each population to produce four populations of seed from the cycle 1 selections. The length of root regrowth under Al stress was improved for all four populations of cycle 1 selection ($P \leq 0.001$; from 2.6 mm for the original populations to 6.3 mm for the cycle 1 selections). In a subsequent experiment the cycle 2 selections from Aurora, UQL-1 and TO2-011 had significantly greater root regrowth than both the cycle 1 selections ($P \leq 0.001$; 8.3 cf. 6.6 mm) and the unselected populations (3.0 mm). The selections from TO2-011 appeared to have greater improvement in the average length of root regrowth after 2 cycles of selection. Selected germplasm was more tolerant than GAAT in our evaluation. Based on estimation of realised heritability, it seemed likely that higher selection intensities would give more rapid improvements in tolerance. Our studies have not investigated the physiological basis of any tolerance of Al which we observed.

Author Address: bscott@csu.edu.au

URL: http://dx.doi.org/10.1071/EA07137

Tolerance of aluminium toxicity in annual Medicago species and lucerne.

B.J. Scott[A,B,G], *M.A. Ewing*[A,C], *R. Williams*[A,D], *A.W. Humphries*[A,E] *and N.E. Coombes*[F]

[A]CRC for Plant-based Management of Dryland Salinity, University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia

[B]Previous address: NSW Department of Primary Industries, Agricultural Institute, PMB, Wagga Wagga, NSW 2650, Australia; Currently: EH Graham Centre for Agricultural Innovation (NSW Department of Primary Industries and Charles Sturt University), Faculty of Science and Agriculture, School of Agricultural and Veterinary Sciences, Charles Sturt University, Locked Bag 588, Wagga Wagga, NSW 2678, Australia

[C] Western Australian Department of Agriculture and Food, South Perth, WA, Australia.

[D]Previous address: NSW Department of Primary Industries Tamworth Centre for Crop Improvement, 4 Marsden Park Road, Calala, NSW 2340, Australia; Currently: Queensland Department of Primary Industries & Fisheries, Leslie Research Centre, PO Box 2282 (13 Holberton St.), Toowoomba Queensland 4350, Australia.

[E] South Australian Research and Development Institute (SARDI), GPO Box 397, Adelaide, South Australia 5001, Australia.

[F]NSW Department of Primary Industries, Agricultural Institute, PMB, Wagga Wagga, NSW 2650, Australia.

[G]Corresponding author, email: bscott@csu.edu.au

Short title: Aluminium tolerance in *Medicago* spp.

.

Abstract: A rapid (7 day) solution-based screening test was evaluated using 15 annual *Medicago* cultivars and one *M. sativa*. Three-day-old seedlings were grown for 1 day in an 'Al pulse' solution containing , 50, 75 or 100 μM/L of Al, followed by root staining, and then grown for 3 days in a 'recovery' solution with Al concentrations of 0, 10, 20,or 30 μM/L. New root growth (unstained) was measured. Based on a relative root regrowth index Zodiac (*M. murex*), Orion (*M. sphaerocarpos*) and the *M. polymorha* cultivars Santiago, Cavalier and Serena had greater Al tolerance than the other cultivars; Herald *(M. littoralis*) and Rivoli (*M. tornata*) were most sensitive. Ranking for Al tolerance from the solution culture correlated well (r = 0.80) with ranking for tolerance of the 16 genotypes grown in an acidic soil (unlimed pH_{Ca} 4.1). Plant Al tolerance gave similar ranking to the known ranking in acidic soils.

Using the above screening test, we screened 17 Australian populations of lucerne (*M. sativa*) using a "pulse" of 75μM Al and a "recovery" of 10 μM Al. We identified and recovered plants with a root regrowth of ≥ 5 mm in all 17 populations with selection intensities of 2 to 4%. Four of these selected populations (Aurora, UQL-1, A513 and TO2-011) were polycrossed within each population to produce four populations of seed from the cycle 1 selections. The length of root regrowth under Al stress was improved for all 4 populations of cycle 1 selection ($P \leq 0.001$; from 2.6 mm for the original populations to 6.3 mm for the cycle 1 selections).

In a subsequent experiment the cycle 2 selections from Aurora, UQL-1 and TO2-011 had significantly greater root regrowth than both the cycle 1 selections ($P \leq 0.001$; 8.3 cf 6.6 mm) and the unselected populations (3.0 mm). There was also a significant interaction between the three populations, and the selections from TO2-011 appearing to have greater improvement in the average length of root regrowth after 2 cycles of selection. GAAT

appeared to be similar in tolerance of aluminium to the original populations of UQL-1 and TO2-011. Aurora appeared to be less tolerant.

The approach of selection within locally adapted material, at least in the Australian context, would appear to be helpful. Recurrent selection (selection intensities of 2 - 6%) has improved Al tolerance, as determined by the screening test, and this improvement has continued for two selections cycles. Based on estimation of realised heritability, it seems likely that higher selection intensities would give more rapid improvements in tolerance. Our studies have not investigated the physiological basis of any tolerance of Al which we observed.

Additional keywords: alfalfa, aluminum, soil acidity, soil pH

Introduction

Progress in breeding legumes for improved growth on acidic soils requires both tolerant plants and tolerant rhizobia. In addition, their association, when under acidity stress, needs to produce an effective symbiosis (Munns 1985). In the present study we characterised *Medicago* genotypes for host plant tolerance of aluminium (Al) toxicity, and have included lucerne (*M. sativa).*

Medicago species are known to be very sensitive to Al, and Andrew *et al.* (1973) found that *M. scutellata*, *M. truncatula* and *M. sativa* were the most Al sensitive species of pasture legume they tested. Wheeler and Dodd (1995) also grouped the 6 *Medicago* species they tested in the very sensitive category. However Sledge *et al*. (2005) found a range of tolerances of Al within *M. truncatula*.

On mildly acidic soils in Western Australia growth of annual medic has been linked to the capacity of selected rhizobia to colonise acidic soil and persist (Howieson *et al*. 1988), and to host plant capacity to nodulate under an acidity stress (Howieson and Ewing 1989). Current recommendations are for the use of selected *Rhizobia* in combination with *M. murex* and *M. polymorpha* cultivars (Gillespie 1989; Ewing *et al*. 1989). In eastern Australia on mildly acidic soils, *M. murex* was superior to *M. truncatula* in its field performance (Dear and Jenkins 1992), and improved nodulation in *M. murex* and *M. polymorpha* compared to *M. truncatula* has been recorded (Young and Brockwell 1992). These mildly acidic soils (pH_{Ca} 4.7 or greater) were likely to have little or no exchangeable aluminium. As a result the effect of pH and Ca concentration on nodulation was studied (Ewing and Robson 1990; Howieson *et al*. 1993). However, in severely acidic soils (pH_{Ca} of 4.2, and exchangeable Al of 0.4 cmol(+)/kg) in a pot experiment, Evans *et al*. (1990) reported that *M. murex* (*cv* Zodiac) was more tolerant of than *M. truncatula* (*cv* Jemalong).

Lucerne is sensitive to acidic soils and to Al toxicity (Campbell *et al*., 1988; Parrot and Bouton, 1990; Cocks, 2001), and responsive to lime application on acidic soils (Munns 1965 *a, b* and *c*; Horsnell 1985, Mugwira and Haque 1993; Mullen *et al*. 2006). However acidic subsurface soils cannot be effectively amended with lime, and soil acidity below the surface limed layer of soil is known to impact on the production of lucerne (Pohlman 1946; Bouton *et al*. 1986; Pinkerton and Simpson 1986). This has lead to interest in improving the acidic soil tolerance of lucerne. Improved growth of lucerne roots at depth in acidic soils, either due to deep liming (Simpson *et al*. 1977) or improved plant Al tolerance, should improve the utilisation of deeper soil moisture (Bouton and Radcliffe 1989), and so reduce deep drainage and accretions of water to the water table. Excessive deep drainage and rising water tables can

result in adverse effects in terms of waterlogging and salinisation in some landscapes (Wood 1924; Cocks 2001; Tennant and Hall 2001; McFarlane and Williamson 2002).

Al tolerance of the lucerne plant has involved studies comparing cultivars and populations. Although tolerance differences have been reported (Campbell *et al*. 1989; Bouton; 1996), this approach has not been productive. An alternative approach was to select individual plants from within populations, and to improve tolerance of Al or acidic soils by recurrent selection. Recurrent selection in lucerne for improved Al or acidic soil tolerance has been conducted over two selection cycles in soil (Devine *et al*. 1976; Brooks *et al*. 1982) or over four cycles in both soil and solution culture (Campbell *et al*. 1988). Selection intensity in the first cycle of selection was ≥ 10 % (Devine *et al*. 1976), 10.5% (Campbell *et al*. 1988) or 12 – 18% (Brooks *et al*. 1982). Selection intensities in subsequent cycles were 15.6% in the second selection cycle (Devine *et al*. 1976) or 5.5 to 7.1% in cycles 2 to 4 (Campbell *et al*. 1988).

Devine *et al*. (1976) assessed their acidic soil tolerant and sensitive populations for their growth in a glasshouse experiment in an acidic Tatum soil. They concluded that Al tolerance in lucerne was heritable, and recurrent selection was useful. They noted that only 2% of plants in the tolerant population were in the most tolerant class, and suggest that further progress could be made. Campbell *et al*. (1988) described their recurrent selection as giving "significant but minimal progress". Brooks *et al*. (1982) demonstrated that selection on an acidic soil produced increased yield on that soil. Brooks *et al*. (1982) also noted that their selection in the acidic soil may have been to manganese toxicity. In the field the acid selections performed better than the limed selections under all soils conditions, implying that acid selection improved the vigour of lucerne under a wide range of growth conditions (Brooks *et al*. 1982). A third cycle of selection produced AT (cycle 3 acid selections) and AS

(cycle 3 limed selections). The AT population became known as GAAT (GeorgiA Acid soil Tolerant). These were evaluated in the field on soils either limed or unlimed in the soil surface but with acidic subsoils. Under dry seasonal conditions the AT population exploited more moisture from the 60-75cm depth of the profile than either cv. Apollo or the AS population (Bouton and Radcliffe 1989).

While some advances have been made it has been widely concluded that there is only a small range of tolerance within the tetraploid *Medicago sativa* species. Bouton *et al*. (1999) concluded in their review that "…there has not been enough genetic variation identified within tetraploid lucerne germplasm to result in a commercially useful Al tolerant cultivar". Bouton *et al*. (1999) went on to advance a range of alternative approaches for improving tolerance in lucerne including transfer from the diploid *Medicago sativa* spp. *coerulea* (Sledge *et al*. 2002), asymmetric breeding (Stoutjesdijk *et al*. 1995) and transgenic approaches (Tesfaye *et al*. 2001), all of which have shown some potential.

We have revisited recurrent selection within populations as an approach to improving Al tolerance in lucerne. The method seems to be a reasonable approach, and Campbell *et al*. (1988) and Dall'Agnol *et al*. (1996) suggested that recurrent selection would be useful. We suggest that earlier attempts at recurrent selection of whole plants have not applied sufficient selection intensity in seeking what is likely to be a 'rare' character in lucerne. We aimed to apply greater selection intensity (a few % of plants selected). This called for the development of a rapid screening system where thousands of plants could be tested. In developing the screening system we have used annual *Medicago* as a wide range of stresses can be imposed on a genotype as cultivars are genetically homozygous. In addition their acidic soil tolerance in the field is known. Our research has not investigated the mechanistic basis for any observed tolerance of Al in *Medicago*.

Material and methods

The test developed was a rapid (7 day) test that examined the sensitivity of root tips to high Al and short duration growth of roots in nutrient solutions with added Al using a stain to mark roots. The solution culture Al tolerance was compared to that obtained from a pot experiment using acidic soil (limed and unlimed) supplied with mineral N. This study was a forerunner to the screening of lucerne (*M. sativa*) for tolerance of the plant to Al using the rapid test in solution culture.

Experiment 1 - Solution culture screening of Medicago species

Fifteen annual medics covering a range of performance on acidic soils were tested for their capacity to grow roots under Al stress (see Table 1). A single lucerne cultivar (*Medicago sativa* L *cv*. Aurora) was included for comparison. All annual medics were sourced from the Australian Temperate Pasture Genetic Resource Centre, Adelaide. Aurora seed (used a control throughout all experiments) was commercial certified seed from a single source. Seed size of each *Medicago* was measured by counting out and weighing 50 seeds.

Insert Table 1 near here

The method of screening was to grow plants for 3 days on floating rafts in a tank on a laboratory bench. Temperature was controlled at 21 °C ± 1 °C, with an external water bath, and fluorescent light was provided for 12 hrs each day. The rafts were similar to those described by Polle *et al*. (1978). For the first 24 hrs deionised water as used and for the subsequent 48 hrs a nutrient solution was introduced. Solution pH at all stages of the screening test was maintained at 4.3 by daily correction using 0.1 M HCl. This was followed

by a 24 hr "pulse" of Al by changing the nutrient solution and adding 0, 50, 75 or 100 μM of Al. This exposure had the potential to kill the growing point of the root. Roots were then stained, rinsed in deionised water and placed in new "recovery" nutrient solutions at 0, 10, 20, or 30 μM Al. These solutions had the potential to slow the root growth of roots tips not killed by the pulse of Al. The method is derivative of existing screening methods (Raman *et al*. 2002; JS Moroni unpublished). After 3 days in the "recovery" solution the roots were inspected and new root growth (white) was measured. Cultivars shared a raft and four rafts shared a nutrient solution container. Three seeds of each cultivar were placed randomly in each raft. The process was repeated over 5 weeks to give 5 replications. The design was a split-plot where main plots were Al exposures and split-plots were *Medicago* genotypes.

The nutrient solution used throughout was (μM): Ca, 1000; Mg, 400; K, 1000; NO_3, 3400; NH_4, 600; PO_4, 100; SO_4, 401.1; Cl 78; Na, 40.2; Fe, 20; B, 23; Mn, 9; Zn, 0.8; Cu, 0.30; and Mo, 0.1. Iron was supplied as Fe-EDTA prepared from equimolar amounts of $FeCl_3$ and Na_2EDTA. Al was added as a solution prepared using $AlK(SO_4)_2.12H_2O$.

The stain was a peroxidase stain used previously in wheat (Scott and Fisher, 1989). One litre of stain solution was prepared by adding 9.526g of sodium acetate to approx. 450 mls of water and this solution was adjusted to pH 5 with concentrated acetic acid. Extra water was added to a volume of 500 mls, and 430 mls of this solution was added to 450 mls of water, and 40 mls of 3% hydrogen peroxide. A second solution was prepared by adding 0.5g of o-dianisidine (water soluble) dissolved in 80 mls of water. This was added to the 920 mls of the first solution. The stain was a red to pink colour on the roots. Roots were exposed to the stain for 1 minute then thoroughly rinsed in deionised water before the rafts were floated on the "recovery" solution.

Experiment 2 - Pot experiment in soil with Medicago species.

The soil used was an acidic sandy loam collected from the surface 15 cm depth near Binnaway, central western New South Wales. The soil was dried at 40°and sieved through a 5 mm sieve. This soil type had been used in earlier studies (Evans *et al*. 1990; Ring *et al*. 1993). After collection and drying the soil had a pH_{Ca} of 4.1 (1:5; soil:0.01mol/L $CaCl_2$) and exchangeable cations (cmol(+)/kg) by the method of Gillman and Sumpter (1986) of Ca 0.37, Mg 0.16, K 0.19, Na <0.01, Al 1.30 and Mn <0.01.

The experimental design was 16 genotypes (as for experiment 1) and four lime rates as a split-plot design, in four replicates. The genotypes formed main plots and the lime rates were subplots. The genotypes were the same as those used in the solution culture experiment, and the lime applications were nil, 0.2, 0.6 and 1.2 g of fine analytical reagent grade of lime/pot. This was approximately equivalent to nil, 0.3, 1 and 2 t/ha of lime on pot weight basis. The pots were circular (diameter 8 cm) and 15 cm high and held 760g of air-dry soil. Pots were watered daily to field capacity by weight using deionised water.

Basal nutrients were added by applying half to the pots in solution, drying and mixing prior to the sowing of the experiment, and the second half was applied in solution after germination. Total basal application (kg/ha) was: N, 35 as NH_4NO_3; P, 50 as $Ca(H_2PO_4)_2.H_2O$; K, 50 as K_2SO_4; Mg, 10 as $MgSO_4.7H_2O$; B, 1 as H_3BO_3; Cu, 1.8 as $CuSO_4.5H_2O$; Zn, 1.6 as $ZnSO_4.7H_2O$ and Mo, 0.1 as $Na_2MoO_4.2H_2O$. Plants were harvested after 28 days of growth by cutting the 6 plants to the soil level in each pot. Soil was sampled using a thin corer (5 mm diameter) from 2 replicates only of the pots sown to Aurora lucerne, and roots were washed free of soil. Shoots and roots were dried at 70 °C and weighted. Soil was dried at 40 °C for 48 hrs and pH_{Ca} was determined.

Experiment 3 – Screening of lucerne populations.

Lucerne populations were selected from recently released commercial cultivars and from advanced lines within the breeding programs based at Tamworth (New South Wales; NSW) and Adelaide (South Australia; SA). This approach was adopted so that if we were successful in selecting plants for tolerance of Al, then the populations produced would be agronomically well adapted to current locations and production systems. We screened 5 commercial cultivars which were SARDI 7 (Kobelt 2002) and SARDI 10 (Kobelt 2006) from South Australia (SA), Aurora (Oram 1990) and Venus (Williams 2003) from New South Wales (NSW), and UQL-1 from Queensland (Irwin 2000, UQL; University of Queensland Lucerne). The breeder claims 'substantial introgression' of *M. falcata* genetic material in UQL-1 since it has around 17% variegated flowers. In addition 9 populations from SA and 3 from NSW breeding programs were screened.

Screening was conducted in solution culture as described earlier with a 24 hr pulse of 75 μM Al and a recovery solution over 3 days of 10 μM Al. Seedlings showing maximum regrowth length of roots were recovered and grown in the glasshouse. In practice no plant with less than of 5 mm regrowth was retained. At this root regrowth length we were confident that the root growing point was alive and recovering. At least one raft carrying cv Aurora was in each tank in all screening tests. Recovered plants were established in pots in the glasshouse. The root regrowth length of selected plants was a second measurement of root regrowth made as plants were placed in to pots and 2 to 3 hrs later than the initial measurements. Plants were later sent to Tamworth or Adelaide, either as potted plants or as plants washed free of potting soil, to produce seed. Four populations were chosen to evaluate the impact of a single cycle of selection. The selected plants within the four populations were polycrossed to produce 4 seed lots (cycle 1 seed).

Experiment 4 – Evaluation of original populations (cycle 0) and cycle 1

Seed of the original populations of Aurora, UQL-1, A513 and TO2-011 were retested on rafts as described in experiment 3. Also included was seed from the plants previously selected for tolerance (cycle 1). The experimental design was a split-plot with 8 populations of lucerne (Aurora, UQL-1, A513 and TO2-011, each with cycle 0 and cycle 1 seed) sharing a tank, which was placed into a water bath. Each population was loaded onto two rafts (100 seeds per raft and 16 rafts). Two tanks were run each week for 6 weeks giving 8 populations in two replicates for 6 weeks. At the end of each 7 day run roots of all plants which were stained were individually measured and recorded to the nearest mm.

Plants from cycle 1 seed with the longest root regrowth were recovered and planted into pot as described earlier to give a second selection cycle and subsequently were sent to the breeding programs at Tamworth and Adelaide to produce seed (cycle 2 seed).

Experiment 5 – Evaluation of original populations (cycle 0), cycle 1, and cycle 2.

The original population, seed from cycle 1 and cycle 2 selected plants of Aurora, UQL-1 and TO2-011 were compared using screening similar to experiment 4. A small quantity (2 g) of GAAT (Georgia Acid soil Tolerant) was available and was tested. This seed was sourced from the USA and obtained via the Australian Temperate Pasture Genetic Resource Centre in Adelaide (South Australian No. 34943). The experimental design was for cycle 0, cycle 1 and cycle 2 seed and GAAT to be loaded on to rafts with a single raft per population (100 seeds per raft and 10 rafts) this was placed in a tank in the water bath. The original populations and GAAT were loaded on to additional rafts (4 rafts) and placed in a separate container immersed in the larger container containing the 10 rafts. This permitted Al stress to be imposed on the

plants grown on the 10 rafts while no Al stress was imposed on the plants grown in the smaller container. Two replicates were run each week for 7 weeks giving 10 populations in two replicates for 7 weeks under Al stress and 4 populations in two replicates for 7 weeks without Al stress but germinated and exposed to the same operations. The Al stress in solution culture was as described earlier with a 24 hr pulse of 75 μM Al, and a recovery solution over 3 days of 10 μM Al.

Plants from cycle 2 seed with the longest root regrowth were recovered and planted into pots as described earlier to give a third selection cycle and subsequently were sent to the breeding program at Tamworth to produce seed (cycle 3 seed).

Statistical analysis and estimates of heritability

All regressions, correlations and analyses of variance were conducted using Genstat (Payne et al. 1993). Data were transformed using square root transformations where needed to stabilise variance across the range of means. The Genstat REML (residual maximum likelihood) linear mixed model directive was used in the analyses of experiments 1, 4 and 5 to allow the modelling of variance components in the experimental designs.

Realised heritability of the aluminium tolerance character in lucerne was calculated using the method of correlation, introduced by Wright (1921). This calculation uses the ratio of the single-generation progress of selection to the selection differential of the parents.

Soil analysis

Soil samples were analysed for pH in calcium chloride (pH_{Ca}). Exchangeable cation measurements were conducted using the barium chloride/ammonium chloride method (Gillman and Sumpter 1986).

Results

Experiment 1 – Medicago species in nutrient solution

Measurements of root elongation in the recovery solution over 3 days are given in Table 2. Poor germination due to hardseededness in the *Medicago polymorpha* genotypes and in *M. orbicularis* resulted in numerous missing values. The data on *M. orbicularis* has not been presented for experiment 1, and the data on *M. polymorpha* is less reliable than other data presented for this experiment.

Insert Table 2 near here

A relative root regrowth index was derived by expressing the regrowth averaged over all Al stresses as a % of the unstressed nil Al treatment for all cultivars. This index integrated across the range of Al treatment stresses. Zodiac (*M. murex*), Orion (*M. sphaerocarpos*) and the *M. polymorpha* cultivars Santiago, Cavalier and Serena were not significantly different, and had greater Al tolerance than most other cultivars (Table 3). Rivoli (*M. tornata*) and Herald (*M. littoralis*) cultivars appeared to be sensitive.

Insert Table 3 near here

There was a considerable range in root length when no Al stress was imposed, ranging from 49.1 mm for Sava to 9.4 mm for Rivoli (Table 2). Using the data presented in Table 2, there was a relationship between seed size and root growth with no Al stress ($P \leq 0.05$; variance accounted for [VAF] = 22%) or with Al stress (average over all Al stresses

treatments; $P \leq 0.05$; VAF = 26%; data not presented), where large seed size gave greater root regrowth.

The root regrowth (averaged over all Al stresses) was not significantly related to the root growth under the nil Al treatment (VAF = 8%). In other words an index based on a direct measure of root regrowth under Al stress, as would occur when screening lucerne, may include only a small component of seedling vigour (root regrowth under no Al stress) if any, in this Al tolerance index.

The root regrowth rank under each treatment (Table 2) was correlated with the rank for relative root elongation index (Table 3) derived from all Al exposures, and is presented in Table 4. The use of the recovery phase only of the screening test (nil Al pulse) gave regrowth of roots significantly related to the relative regrowth index where 20 and 30 μm Al was used. When Al was not added in the 3 day recovery solution, and the pulse of Al alone was used, the correlation was only significant at the 75μm Al rate. Root regrowth under any combination of Al pulse and exposure to Al in recovery gave more consistent and improved correlations with the results of the entire experiment (r = 0.60 to 0.94).

Insert Table 4 near here

Experiment 2 – Medicago species in soil

Soil pH_{Ca} at the end of the pot experiment after lime additions of nil, 0.3, 1 and 2 t/ha of lime (approx) were 4.15, 4.30, 4.75 and 5.45 ($P \leq 0.01$; sed 0.134; df 3). At harvest visual inspection showed no nodules forming on the roots, and plants did not appear to be N deficient, indicating that the mineral N in the pots was adequate for the supply of N for the lucerne plants.

Lime increased the yield of dry matter of shoots, roots and total (shoots + roots), cultivar total yield varied and there was an interaction between lime rate and cultivar (all terms significant at $P \leq 0.001$). As shoot yield and root yield were correlated (r = 0.80), only total plant yield data is presented (Table 5). Maximum yields were obtained at either 1 or 2 t/ha of lime.

Insert Table 5 near here

In order to further examine the interaction term an index was used to indicate responsiveness to lime application by the cultivars. For each cultivar the yield of the two low lime rates (nil and 0.3 t/ha; yield under acidic soil stress), was expressed as a % of the yield from the two higher lime rates (1 and 2 t/ha; yield with no acidic soil stress; Table 5). Zodiac (*M. murex*), Orion (*M. sphaerocarpus*) and the *M. polymorpha* cultivars Serena, Cavalier and Scimitar rank as the most tolerant of acidic soil, that is they were less responsive to liming. Harbinger *(M. littoralis*) was most sensitive, but was not different from Rivoli (*M. tornata*), *M. orbicularis*, Herald (*M. littoralis*) Jester (*M. truncatula*) or Aurora (*M. sativa*). There was a correlation (r = 0.84) between ranking for tolerance of aluminium in solution culture in the screening test with ranking of tolerance derived from response in plant growth to lime applied to an acidic soil (unlimed pH_{Ca} 4.1; Fig. 1).

Insert Fig. 1 near here

Experiment 3 – Screening of lucerne populations.

Within the 17 cultivars and populations of *M. sativa* screened it was possible to identify and recover plants with a root regrowth of ≥ 5 mm and to maintain selection intensities of between 2 and 4% (Table 6). However, the length of root regrowth and frequency of plants selected

differed between populations. In some populations plants with shorter root regrowth were selected (eg TO1–007; selection intensity of 1.98% and average root regrowth of 6 mm), while in other populations plants were selected more frequently with a longer root regrowth (eg L97a; selection intensity 3.33% and average root regrowth of 14 mm).

Insert Table 6 near here

Experiment 4 – Evaluation of original populations (cycle 0) and cycle 1

Across all four populations the length of root regrowth under Al stress was improved following one cycle of selection ($P \leq 0.001$) from 2.6 mm for the original populations to 6.3 mm for the cycle 1 selections. The improvement from one cycle of selection in TO2-011 appeared to be greater than in the other populations (Table 7). This appeared, in part, to be due to the almost complete elimination in cycle 1 of plants with regrowth of 2 mm of less (Fig 2). The original populations differed in their average length of root regrowth under the conditions of the screening. The average length of regrowth of roots of Aurora (1.8 mm; Table 7) was significantly less than all other original populations ($P \leq 0.05$). A513 was not different from TO2-011, but both were was significantly less than UQL-1 ($P \leq 0.01$; 3.7 mm). The cycle 1 populations also varied with Aurora and A513 (4.7 and 5.2 mm respectively) not different, but less than UQL-1 ($P \leq 0.05$) and TO2-011 ($P \leq 0.01$). TO2-011 was significantly greater ($P \leq 0.01$) than all other cultivars. The significant change in ranking suggests that the TO2-011 improved more than the other three populations with one cycle of selection.

Insert Table 7 and Fig. 2 near here

Experiment 5 – Evaluation of original populations (cycle 0), cycle 1, cycle 2 and GAAT.

This experiment confirmed that the original population of Aurora had less length of root regrowth under Al toxicity than either UQL-1 or TO2-011 ($P \leq 0.01$; Table 8). Over the three cultivars the cycle 1 selections, as in experiment 4, were a significant improvement in length of root regrowth under Al stress over the original cultivars ($P \leq 0.001$; 3.0 mm in cycle 0 to 6.6 mm in cycle 1). In addition this improvement in root regrowth length continued with the cycle 2 selections significantly greater than the cycle 1 selections ($P \leq 0.001$; 6.6 to 8.3 mm). There was also a significant interaction between the three populations and the cycle of selection. In the cycle 1 selections TO2-011 was ranked as having the greatest regrowth of roots, although TO2-011 was not significantly different from UQL-1, unlike experiment 4. However, in the second selection cycle TO2-011 had greater length of root regrowth than UQL-1 (9.9 *cf* 8.1 mm; $P \leq 0.01$). This suggests that TO2-011 had greater improvement under selection than either Aurora or UQL-1. In the cycle 2 selections of TO2-011 there appeared to be very few plants with root regrowth of 2 mm or less (11%) compared to Aurora (23%) and UQL-1 (16%; Fig 3).

Insert Table 8 and Fig. 3 near here

Comparisons with the original populations of Aurora, UQL-1, TO2-011 and GAAT under both Al stress and no Al stress showed a significant interaction term between aluminium treatment and the four populations of lucerne (Table 9). This interact was due to the greater decline in the root length of Aurora on the transformed scale (2.78), compared to the other populations of lucerne (UQL-1, 2.40; TO2 – 011, 2.49 and GAAT, 2.50). This suggested greater sensitivity of Aurora to Al stress and that GAAT had no greater Al tolerance than in the Australian germplasm UQL-1 and TO2 - 011.

Insert Table 9 near here

Recurrent selection.

The second cycle of selection, conducted within experiment 4, resulted in a reduced selection intensity of about 5 to 6% (Table 10). However the average length of regrowth of roots of selected plants appeared to be improved with regrowth varying from 13.3 to 17.9 mm despite the lower selection intensity, when compared to the first cycle of selection (Table 6; 8 to 11 mm).

A third cycle of recurrent selection in three populations was conducted by selecting within the cycle 2 populations during experiment 5. The selection intensity was in the range of about 5.5 - 6%, similar to the second selection cycle of 5 - 6% (Table 10). The mean length of root regrowth of selected plants in the third cycle (15.1 to 18.5 mm; Table 10) improved only slightly on the length of regrowth of selected plants in the second selection cycle (13.3 – 17.9 mm), and this may foreshadow a reducing benefit for continued recurrent selection using our system of screening.

Insert Table 10 near here

Heritability

Table 11 shows the realised heritability of aluminium tolerance in four populations, calculated using the information presented in Tables 6, 7, 8 and 10. Realised heritabilities from all germplasm sources ranged from 10 to 90%. After 2 cycles of selection, UQL-1 had the lowest estimate of heritability, and the breeders line T02-011 had the greatest response to selection. Realised heritabilities decreased in each population with recurrent selection.

Insert Table 11 near here

Discussion

Annual Medicago species

This study identified differences in the aluminium tolerance of roots among annual medics. The tolerance ranking was broadly consistent between the solution culture screening test and the soil evaluation. The rank order of cultivars was in agreement with general field observation with *M. murex*, *M. sphaerocarpos* and *M. polymorpha* cultivars being the most tolerant (Dear and Jenkins 1992; Ewing *et al*. 1989; Gillespie 1989; Young and Brockwell 1992).

The general ranking of performance of cultivars for performance of acidic soils has been ascribed previously to their propensity to nodulate, combined with the choice of suitable rhizobia. However the plant Al tolerance in the present study indicated a similar ranking to the nodulation ranking in acidic soil. This suggests that both nodulation behaviour and plant tolerance of Al have probably been co-selected when these plants are grown on acidic soil in their native range. This is likely as these cultivars are based on direct selections from plants collected around the Mediterranean. The third character in the acidic soil tolerance set (manganese toxicity tolerance) has been researched by others and *M. murex* (cv. Zodiac) was more Mn tolerant than a *M. polymorpha*, which in turn, was more tolerant than *M. tornata* (Carneiro *et al*. 2001). We suggest that the existence of all three characteristics (Al tolerance, Mn tolerance and superior nodulation) within the annual medic *M. murex* may contribute to performance on acidic soil in the field. The importance of each may vary with soil characteristics and severity of stress but impairment of N_2 fixation may be the primary failure of *Medicago* under acidic soil stress (Munns 1965 *a*, *b* and *c*; Robson and Loneragan 1970).

Rapid screening test

We used this screening test to identify individual plants that were Al tolerant from within cultivars of lucerne. The test was developed for use by plant breeders and is highly visual. This speeds the assessment and identification of the few putatively tolerant plants within the population. Root regrowth can be measured or estimated on a very small subset of individuals that can be rescued and transplanted. This test has been established at Tamworth New South Wales in the lucerne breeding program.

The screening test itself may have some potential to confuse "vigour" (growth with no stress) with true tolerance of aluminium. However, there was no significant relationship of regrowth with no Al (vigour) and the regrowth with Al stress. Further the good correlation between rankings based on absolute root length and rankings based the tolerance index derived from root regrowth in annual medics (Table 4), indicated that progress with selection in lucerne may be possible despite possible confusion between vigour and Al tolerance.

Aluminium tolerance in lucerne and response to selection

The character (putative Al tolerance) was located in all *Medicago sativa* cultivars and populations tested. This suggests that an approach of selection within locally adapted material, at least in the Australian context, would prove helpful. However the frequency of the character varied.

Recurrent selection has improved Al tolerance, as determined by the screening test, and this improvement has continued for two selections cycles. We succeeded in applying a selection intensity of 2 to 4% in the first cycle of selection (Table 6), but reduced this to 5 to 6% in subsequent selection cycles (Tables 10). This was a decision on our part to preserve

diversity in the selected populations by selecting a reasonably large number of plants (35 to 45 plants).

The first cycle of selection made progress in all four populations tested, however the breeding line TO2 – 011 from Tamworth, appeared to make greater progress than other cultivars. The trait is likely to be under genetic control of a polygenic system, due to the continuous variation observed for the root regrowth trait (Fig 2 and 3) and continued improvement in root regrowth with a second selection cycle may indicate continued aggregation of multiple genes.

Realised heritabilities for our germplasm sources ranged from 10 to 90%, depending on germplasm and cycle of selection (Table 11). Response to selection varied among the four source populations and indicated that the choice of source germplasm will be a factor for success in producing populations of lucerne that are tolerant to aluminium stress.

The response to selection decreased with each generation in each of the source populations. For example, in TO2-011 heritability decreased from the range 74% (experiment 5) to 90% (experiment 4) in the first cycle of selection, to 23% in a second cycle of selection. A decrease in response to selection is common in lucerne, as reported for germination at low temperature by Klos and Brummer (2000). The results suggest that further improvements in putative aluminium tolerance beyond 2 cycles of selection will need to be achieved through pyramiding aluminium tolerance genes from a greater number of source populations. Based on our estimates of realised heritability, the use of a higher selection intensity (0.2 to 0.8% compared with 2 to 4%), would make it possible to achieve the same response to selection in 1 cycle of selection that we observed in 2 cycles of selection.

Comparison with GAAT

Our evaluation of GAAT indicated that Al tolerance, in our screening system, differed only marginally from the original populations of Australian cultivars. GAAT has been shown to be Al tolerant in callus culture (Parrot and Bouton 1990), and in Al toxic nutrient solutions when compared to cultivar Regen-SY (Tesfaye *et al*. 2001). The possibilities are that the Al stress imposed in our screening test was too high and identified no advantage in GAAT. Alternatively GAAT may have been selected in a soil for characteristics other than or in addition to Al tolerance. In particular we note the authors Brooks *et al*. (1982) quoted Mn toxicity tolerance to be a probable character for which they had selected. However selections from within our populations showed a marked improvement over GAAT under our test conditions.

Aluminium tolerance in lucerne in soil and field

It has not been established that Al tolerance selected in lucerne with our screening test relates to performance in acidic soils. We were aware of the warning by Campbell *et al*. (1988) that plants of intermediate response may be variable between their response to Al in solution culture and their response in acidic soils. However the correlation within the annual medics between tolerance by the screening test and performance in an acidic soil low in Mn, and with mineral N supplied, indicated that our screening method is likely to be testing for Al tolerance. Similar research has indicated that in barley the screening test (the basis of our current test) rankings correlate broadly with growth in an acidic soil (JS Moroni unpublished).

When compared to the original populations, the cycle 2 populations we have selected may not show improved performance in acidic soils in the field. We believe that the primary cause of failure of *Medicago* species in moderately acidic soils is related to nodulation failure (Munns 1965a, b, c; Robson and Loneragan 1970). Plants with improved root growth in Al

toxic acidic soils may express some advantage, but only where nodulation is not restricted, and where Mn toxicity is not an issue. Such a situation may exist on some soil types where the surface soil has been limed to provide a site for nodulation, and where the capacity of the plant to exploit water and nutrients at depth may be expressed in plant growth or persistence. Alternatively, nodulation may occur at depth in a less acidic soil layer while the surface soil is more acidic (Evans *et al*. 2005). Improved Al tolerance could enhance nutrient and water uptake in the acidic surface soil layers. Screening lucerne for improved nodulation with new, more acid tolerant strains of rhizobia is occurring in tandem with our research for improved root elongation (Charman, *et al.* this edition).

We believe that future progress in selection for better performance of lucerne in the field will involve screening for Al tolerance, Mn tolerance and capacity to nodulate. It might also involve improved rhizobia (Charman, *et al.* 2008 this edition). We therefore believe that selection for these additional characters (nodulation and Mn tolerance) should continue in parallel with current efforts on Al tolerance. Success in the field may then be possible. We do not see that aspirations for considerable tolerance in *M. sativa* to acidic soils are realistic, but it may be possible to reproduce the performance of *M. murex* or *M. polymorpha* within *M. sativa*.

Acknowledgements

The authors thank Rod Fisher and Shirani Katupitya (NSW Department of Primary Industries, Wagga) who assisted in laboratory and glasshouse, and Tim O'Brien (NSW DPI, Tamworth) and Eric Kobelt (South Australian Research and Development Institute, Adelaide) for the production of seed from selected populations. The study was funded by the CRC for Plant-

based Management of Dryland Salinity through a project supervised by Geoff Auricht (SARDI) and was supported by NSW DPI and SARDI.

References

Andrew CS, Johnson AD, Sandland RL (1973) Effect of aluminium on the growth chemical composition of some tropical and temperate pasture legumes. *Australian Journal of Agricultural Research* **24**, 325-339.

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

Bouton JH, Radcliffe DE (1989) Effects of acid soil selection on agronomically important traits in alfalfa. In *Proceeding of the XVI International Grasslands Congress* Nice, France p 377-378.

Bouton J, Sledge M, Parrott W (1999) Breeding for aluminium tolerance. In "The Alfalfa Genome Conference", Madison Wisconsin. Conference/Symposium held August 1 –3, 1999. (http://www.naaic.org/TAG/TAGpapers/Bouton/BoutonAlPaper.htm)

Bouton JH, Sumner ME, Hammel JE, Shahandeh H (1986) Yield of an alfalfa germplasm selected for acid soil tolerance when grown in soils with modified subsoils. *Crop Science* **26**, 334-336.

Brooks CO, Bouton JH, Sumner ME (1982) Alfalfa, Medicago sativa L., in highly weathered, acid soils III The effects of seedling selection in an acid soil on alfalfa growth at varying levels of phosphorus and lime. *Plant and Soil* **65**, 27-33.

Campbell TA, Nuernberg NJ, Foy CD (1989) Differential responses of alfalfa cultivars to aluminium stress. *Journal of Plant Nutrition* **12**, 291-305.

Campbell TA, Elgin JH, Foy CD, McMurtrey JE (1988) Selection in alfalfa for tolerance to toxic levels of aluminium. *Canadian Journal of Plant Science* **68**, 743-753.

Carneiro JP, Varennes Ade, Amante H, de Varennes A (2001) Manganese toxicity in three species of annual medics. *Journal of Plant Nutrition* **24**, 1957-1964.

Charman N, Ballard RA, Humphries AW, Auricht GC (2008) Improving lucerne nodulation at low pH: Contribution of rhizobial and plant genotype to the nodulation of lucerne seedlings growing in solution culture at pH 5. *Australian Journal of Experimental Agriculture* (in press; this issue)

Cocks PS (2001) Ecology of herbaceous.s 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.

Dall'Agnol M, Bouton JH, Parrott WA (1996) Screening methods to develop alfalfa germplasms tolerant of acid, aluminium toxic soils. *Crop Science*. **36**, 64-70.

Dear BS, Jenkins L (1992) Persistence, productivity and seed yield of *Medicago murex, M. truncatula, M. aculeata* and *Trifolium subterraneum* on an acid red earth soil in the wheat belt of eastern Australia. *Australian Journal of Experimental Agriculture* **32**, 319– 329.

Devine TE, Foy CD, Fleming AL, Hanson CH, Campbell TA, McMurtrey JE III, Schwartz JW (1976) Development of alfalfa strains with differential tolerance to aluminum toxicity. *Plant and Soil* **44**, 73-79.

Evans CM, Fettell NA, Brockwell J (2005) Populations of *Sinorhizobium meliloti* congregate in the 30-60 cm section of the soil profile in a stand of dryland lucerne (*Medicago sativa*): is this where lucerne fixes its nitrogen? *Australian Journal of Experimental Agriculture* **45**, 225-230.

Ewing MA, Revell CK, Thorn CW (1989) Annual Medics*: Medicago polymorpha* L. var. *brevispina* (Benth.) Heyn (burr medic) cv. Santiago. *Australian Journal of Experimental Agriculture* 29, 297-298.

Ewing MA, Robson AD (1990). The effect of solution pH and external calcium concentration on the early growth and nodulation of several annual *Medicago* species. *Australian Journal of Agricultural Research* **41**, 933-939.

Evans J, Dear B, O'Connor GE (1990) Influence of an acid soil on the herbage yield and nodulation of five annual pasture legumes. *Australian Journal of Experimental Agriculture* **30**, 55-60;

McFarlane DJ, Williamson DR (2002) An overview of water logging and salinity in southwestern Australia as related to the 'Ucarro' experimental catchment. *Agriculture and Water Management* **53**, 271-275.

Gillespie DJ (1989) The development of Murex medic as a new pasture species for acid soils in Australia. *Proceedings of the XVI International Grassland Congress*, 4-11 October, Nice, France, p 215-216.

Gillman GP, Sumpter EA (1986) Modification to the compulsive exchange method for measuring exchange characteristics of soils. *Australian Journal of Soil Research* **24**, 61-6.

Howieson JG, Ewing MA (1989) Annual species of *Medicago* differ greatly in their ability to nodulate on acid soils. *Australian Journal of Agricultural Research* **40,** 843-850.

Howieson JG, Ewing MA, D'Antuono MF (1988) Selection for acid tolerance in *Rhizobium meliloti*. *Plant and Soil* **105**, 179-188.

Howieson JG, Robson AD, Ewing MA (1993) External phosphate and calcium concentrations, and pH, but not the products of rhizobial nodulation genes, affect the attachment of *Rhizobium meliloti* to roots of annual medics. *Soil Biology and Biochemistry* **25**, 567-573.

Horsnell LJ (1985) The growth of improved pastures on acid soils, 2. The effect of soil incorporation of lime and phosphorus on the growth of subterranean clover and lucerne pastures and on their response to topdressing. *Australian Journal of Experimental Agriculture* **25**, 157-163.

Irwin JAG (2000) 'UQL-1'. *Plant Varieties Journal* **13**, 41-42.

Klos KLE, Brummer CE (2000) Response of six alfalfa populations to selection under laboratory conditions for germination and seedling vigor at low temperatures. *Crop Science* **40**, 959-964.

Kobelt ET (2002) 'Super 7'. *Plant Varieties Journal* **15**, 43-44.

Kobelt ET (2006) 'SARDI Ten'. *Plant Varieties Journal* **19**, 189-194.

Mullen CL, Scott BJ, Evans CM, Conyers MK (2006) Effect of soil acidity and liming on lucerne and following crops in central-western New South Wales. *Australian Journal of Experimental Agriculture* **46**, 1291–1300

Mugwira LM, Haque I (1993) Screening Forage and Browse Legumes Germplasm to Nutrient Stress .1. Tolerance of Medicago-Sativa L to Aluminum and Low Phosphorus in Soils and Nutrient Solutions. *Journal of Plant Nutrition* **16**, 17-35.

Munns DN (1965*a*) Soil acidity and the growth of a legume. I. Interactions of lime with nitrogen and phosphate on growth of *Medicago sativa* L. and *Trifolium subterraneum* L. *Australian Journal of Agricultural Research* **16,** 733-41.

Munns DN (1965*b*) Soil acidity and the growth of a legume. II. Reactions of aluminium and phosphate in solution and effects of aluminium, phosphate and calcium, and pH on *Medicago sativa* L. and *Trifolium subterraneum* L. in solution culture. *Australian Journal of Agricultural Research* **16,** 743-55.

Munns DN (1965*c*) Soil acidity and growth of a legume. III. Interaction of lime and phosphate on growth of *Medicago sativa* L. in relation to aluminium toxicity and phosphate fixation. *Australian Journal of Agricultural Research* **16,** 757-66.

Munns DN (1985) Acid soil tolerance in legumes and rhizobia. *Advanced Plant Nutrition* **2,** 63-91.

Pinkerton A, Simpson JR (1986) Responses of some crop plants to correction of subsoil acidity. *Australian Journal of Experimental Agriculture* **26**, 107 - 13.

Oram RN (1990) 8. Lucerne, a. *Medicago sativa* L. (lucerne) cv. Aurora. In 'Register of Australian Herbage Plant Cultiivars' pp. 205-206. (CSIRO Australia, Melbourne).

Parrot WA, Bouton JH (1990) Aluminum tolerance in alfalfa as expressed in tissue culture. *Crop Science* **30**, 387-389.

Payne RW, Lane PW, Digby PGN, Harding SA, Leech PK, Morgan GW, Todd AD, Thompson R, Tunicliffe Wilson G, Welham SJ, White RP (1993) Genstat 5 Release 3 Reference Manual. (Clarendon Press: Oxford, U.K.).

Pohlman GG (1946) Effect of liming different soil layers on yield of alfalfa and on root development and nodulation. *Soil Science* **62,** 255-66.

Polle E, Konzak AF, Kittrick JA. (1978) Visual detection of aluminum tolerance levels in wheat by hematoxylin staining of seedling roots. *Crop Science* **18**, 823-827.

Raman H, Moroni JS, Sato K, Read BJ, Scott BJ (2002) Identification of AFLP and microsatellite markers linked with an aluminium tolerance gene in barley (*Hordeum vulgare* L). *Theoretical and Applied Genetics* **105**, 458-464

Ring SM, Fisher RP, Poile GJ, Helyar KR, Conyers MK, Morris SG (1993) Screening species and cultivars for their tolerance to acidic soil conditions. *Plant and Soil* 155/156, 521-524.

Robson AD, Loneragan JF (1970) Growth of annual *Medicago* species on an acidic soil. *Proceedings of the XIth International Grasslands Conference*, p 434.

Scott BJ, Fisher JA (1989) Selection of genotypes tolerant of soil acidity. *In* "Soil Acidity and Plant Growth". (Ed. AD Robson). Academic Press, Australia. p 167 - 204.

Sledge MK, Bouton JH, Dall'Agnoll M, Parrot WA, Kochert G (2002) Identification and confirmation of aluminium tolerance GTL in diploid *Medicago sativa* subsp. *coerulea*. *Crop Science* **42**, 1121-1128.

Sledge MK, Pechter P, Payton ME (2005) Aluminum tolerance in *Medicago truncatula* germplasm. *Crop Science* **45**, 2001-2004.

Simpson JR, Pinkerton A, Lazdovskis J (1977) Effects of subsoil calcium on the growth of some lucerne genotypes (*Medicago sativa* L.) in acidic soil profiles. *Australian Journal of Agricultural Research* **28**, 629-638.

Stoutjesdijk PA, Larkin PJ, Sale PWG (1995) Improving the acid-soil tolerance of *Medicago sativa* by asymmetric hybridisation. . In "Plant-soil interactions at low pH: principles and management", (eds Date RA, Grundon NJ, Rayment GE, Probert ME). Proceedings of the Third International Symposium, Brisbane, Queensland, Australia, 12-16 September 1993. Kluwer Academic Publishers, Dordrecht, Netherlands, p 455-457.

Tennant D, Hall D (2001) Improving water use of annual crops and pastures-limitations and opportunities in Western Australia. *Australian Journal of Agricultural Research* **52**, 171–182.

Tesfaye M, Temple SJ, Allan DL, Vance CP, Samac DA (2001) Overexpression of malate dehydrogenase in transgenic alfalfa enhances organic acid synthesis and confers tolerance to aluminum. *Plant Physiology* **127**, 1836-1844.

Wheeler DM, Dodd MB (1995) The effect of aluminium on the growth of a range of temperate legume species and cultivars: a summary of results. In "Plant-soil interactions at low pH: principles and management", (eds Date RA, Grundon NJ, Rayment GE, Probert ME). Proceedings of the Third International Symposium, Brisbane, Queensland, Australia, 12-16 September 1993. Kluwer Academic Publishers, Dordrecht, Netherlands, 433-437.

Williams R (2003) 'Venus'. *Plant Varieties Journal* **16**, 47-48.

Wright S (1921) Correlation and causation. *Journal of Agricultural Research* **20**, 557-585

Wood WE (1924) Increase of salt in soil and streams following the destruction of native vegetation. *Journal of the Royal Society, Western Australia* **10**, 35-47.

Young RR, Brockwell J (1992) Influence of soil pH on the development of symbiosis in field-grown acid-sensitive and acid-tolerant annual medics. *Australian Journal of Experimental Agriculture* **32**, 167-173.

Table 1. Cultivars tested for Al tolerance in a short duration nutrient solution system (experiment 1).

Cultivar name	Species	Origin	South Australian No.
Paragosa	*M. rugosa*	Portugal	416
Harbinger	*M. littoralis*	Iran	421
Sava	*M. scutellata*	Germany	5615
	M. orbicularis		
Rivoli	*M. tornata*	Morocco	9553
Serena	*M. polymorpha*	Australia	15004
Zodiac	*M. murex*	Sardinia	23101
Santiago	*M. polymorpha*	Chile	25714
Caliph	*M. trunculata*	Australia	27783
Mogul	*M. trunculata*	Australia	27784
Orion	*M. sphaerocarpos*	Sicily	27802
Herald	*M. littoralis*	Australia	30796
Jester	*M. trunculata*	Australia	36437
Cavalier	*M. polymorpha*	Australia	36438
Scimitar	*M. polymorpha*	Australia	36439

Table 2. Root growth of *Medicago* species (mm) over 3 days in nutrient solution with a range of Al concentrations (μM), following a "pulse" exposure to Al in solution for 1 day (experiment 1).

Data in brackets are square root transformed.

Cultivar (*species*)	Prior 1 day	Root growth during 3days of Al exposure			
	PULSE	NIL Al	10μm	20μm	30μm
Serena (*M. polymorpha*)	NIL	18.0 (4.24)	14.0 (3.74)	10.3 (3.21)	2.4 (1.53)
	50μm	10.1 (3.17)	7.8 (2.80)	5.2 (2.27)	0.9 (0.96)
	75μm	12.3 (3.51)	10.6 (3.25)	8.4 (2.89)	2.9 (1.70)
	100μm	3.8 (1.95)	3.2 (1.80)	3.9 (1.98)	0.6 (0.79)
Cavalier (*M. polymorpha*)	NIL	19.6 (4.43)	9.9 (3.15)	16.1 (4.01)	9.1 (3.01)
	50μm	23.9 (4.89)	13.9 (3.73)	21.2 (4.61)	15.7 (3.96)
	75μm	17.8 (4.22)	10.1 (3.18)	17.7 (4.21)	13.7 (3.70)
	100μm	12.3 (3.51)	0.0 (0.00)	17.3 (4.16)	13.3 (3.64)
Orion (*M. sphaerocarpos*)	NIL	23.1 (4.80)	23 (4.80)	15.7 (3.96)	15.2 (3.90)
	50μm	19.0 (4.36)	20.1 (4.48)	13.3 (3.65)	15.6 (3.95)
	75μm	13.4 (3.66)	15.2 (3.90)	10.5 (3.23)	13.4 (3.66)
	100μm	10.6 (3.25)	13 (3.60)	12.1 (3.48)	15.2 (3.90)
Zodiac (*M. murex*)	NIL	17.6 (4.20)	15.2 (3.91)	12.3 (3.50)	8.1 (2.84)
	50μm	15.9 (3.99)	14.6 (3.82)	11.8 (3.43)	9.7 (3.12)
	75μm	13.7 (3.71)	13.3 (3.65)	11.7 (3.42)	10.5 (3.24)
	100μm	7.8 (2.79)	8.1 (2.85)	10.0 (3.17)	8.9 (2.98)
Santiago (*M. polymorpha*)	NIL	10.1 (3.18)	11.0 (3.32)	6.8 (2.60)	5.0 (2.25)
	50μm	9.1 (3.02)	10.8 (3.29)	n.a	6.7 (2.58)
	75μm	6.3 (2.51)	8.3 (2.88)	5.5 (2.34)	0.0 (0.00)
	100μm	4.7 (2.16)	7.0 (2.65)	7.0 (2.65)	7.7 (2.78)
Scimitar (*M. polymorpha*)	NIL	13.1 (3.63)	7.8 (2.78)	4.6 (2.14)	1.7 (1.30)
	50μm	7.6 (2.76)	4.2 (2.05)	2.0 (1.41)	0.0 (0.00)
	75μm	4.7 (2.18)	2.5 (1.58)	1.2 (1.10)	0.6 (0.75)
	100μm	4.1 (2.02)	2.3 (1.53)	2.5 (1.59)	0.0 (0.00)
Caliph (*M. truncatula*)	NIL	19.1 (4.37)	8.6 (2.93)	6.3 (2.50)	1.1 (1.05)
	50μm	15.8 (3.97)	7.0 (2.65)	5 (2.23)	1.3 (1.14)
	75μm	9.1 (3.02)	3.3 (1.82)	2.4 (1.56)	0.4 (0.60)
	100μm	6.2 (2.49)	1.9 (1.40)	2.8 (1.69)	0.5 (0.71)
Paragosa (*M. rugosa*)	NIL Al	23.9 (4.89)	16.0 (4.00)	7.6 (2.75)	2.8 (1.66)
	50μm	10.7 (3.28)	6.3 (2.51)	1.6 (1.28)	0.3 (0.54)
	75μm	10.7 (3.28)	6.9 (2.62)	2.4 (1.55)	0.9 (0.95)
	100μm	5.0 (2.23)	2.8 (1.69)	1.4 (1.16)	0.3 (0.55)
Harbinger (*M. littoralis*)	NIL	14.4 (3.80)	7.8 (2.79)	4.3 (2.06)	1.6 (1.26)
	50μm	7.7 (2.77)	3.5 (1.88)	1.4 (1.17)	0.5 (0.72)
	75μm	3.3 (1.81)	1.1 (1.04)	0.2 (0.49)	0.0 (0.17)
	100μm	1.8 (1.36)	0.5 (0.70)	0.5 (0.69)	0.0 (0.00)
Mogul (*M. truncatula*)	NIL	21.9 (4.69)	9.6 (3.11)	4.4 (2.09)	1.8 (1.32)
	50μm	14.7 (3.83)	5.6 (2.38)	1.9 (1.38)	0.0 (0.00)
	75μm	8.4 (2.89)	2.4 (1.55)	0.5 (0.72)	0.2 (0.43)
	100μm	5.7 (2.38)	1.3 (1.15)	0.7 (0.86)	0.3 (0.57)
Jester (*M. truncatula*)	NIL	11.2 (3.35)	2.9 (1.71)	1.5 (1.22)	0.0 (0.00)
	50μm	7.8 (2.79)	1.6 (1.28)	0.6 (0.80)	0.0 (0.00)
	75μm	4.7 (2.16)	0.6 (0.76)	0.2 (0.44)	0.1 (0.24)

	100µm	4.4 (2.09)	0.7 (0.81)	1.1 (1.03)	0.7 (0.82)
Aurora (*M. sativa*)	NIL	21.1 (4.59)	8.1 (2.85)	2.6 (1.62)	0.8 (0.92)
	50µm	13.7 (3.70)	4.4 (2.09)	0.7 (0.87)	0.3 (0.52)
	75µm	8.4 (2.89)	1.9 (1.39)	0.1 (0.34)	0.0 (0.12)
	100µm	6.5 (2.54)	1.3 (1.16)	0.0 (0.00)	0.2 (0.42)
Sava (*M. scutellata*)	NIL	49.1 (7.01)	25.5 (5.05)	11.8 (3.43)	9.0 (2.99)
	50µm	25.0 (5.00)	10.1 (3.17)	2.5 (1.57)	2.2 (1.49)
	75µm	19.5 (4.41)	7.2 (2.69)	1.6 (1.25)	1.7 (1.30)
	100µm	10.2 (3.19)	2.5 (1.58)	0.5 (0.69)	0.5 (0.73)
Rivoli (*M. tornata*)	NIL	9.4 (3.07)	5.3 (2.31)	2.0 (1.41)	0.1 (0.37)
	50µm	4.0 (2.00)	1.9 (1.36)	0.2 (0.47)	0.0 (0.00)
	75µm	1.2 (1.08)	0.3 (0.56)	0.0 (0.00)	0.0 (0.00)
	100µm	1.0 (1.02)	0.4 (0.60)	0.0 (0.00)	0.0 (0.00)
Herald (*M. littoralis*)	NIL	12.7 (3.56)	6.5 (2.54)	0.2 (0.40)	0.1 (0.37)
	50µm	3.3 (1.81)	0.9 (0.92)	0.0 (0.00)	0.0 (0.00)
	75µm	2.4 (1.56)	0.6 (0.79)	0.0 (0.00)	0.0 (0.00)
	100µm	1.4 (1.17)	0.3 (0.51)	0.0 (0.00)	0.0 (0.00)

REML variance components analysis (transformed data)

Pulse	***	Cultivar	***
Recovery	***	Cultivar*Pulse	**
Pulse*Recovery	***	Cultivar*Recovery	***
		Cultivar*Pulse*Recovery	n.s.
sed ; *df = 735*			(0.571)

***, $P \leq 0.001$**; $P \leq 0.01$; n.s. not significant; n.a., not available.

Table 3 Cultivars ranked by Al tolerance based on an index of percentage of root growth under all 15 Al stress treatments as a % of the root growth with no Al stress (experiment 1).

Data in brackets are square root transformed.

Cultivar (*species*)	Tolerance index	
	All Al/nil (%)	Square root transformed mean
Serena (*M. polymorpha*)	88.8	(9.43)
Cavalier (*M. polymorpha*)	70.8	(8.42)
Orion (*M. sphaerocarpos*)	67.7	(8.23)
Zodiac (*M. murex*)	64.5	(8.03)
Santiago (*M. polymorpha*)	63.3	(7.96)
Scimitar (*M. polymorpha)*	38.2	(6.18)
Caliph (*M. truncatula*)	35.1	(5.93)
Paragosa (*M. rugosa*)	30.9	(5.56)
Harbinger (*M. littoralis*)	24.9	(4.99)
Mogul (*M. truncatula*)	20.9	(4.58)
Jester (*M. truncatula*)	20.5	(4.53)
Aurora (*M. sativa*)	20.0	(4.48)
Sava (*M. scutellata*)	16.3	(4.04)
Rivoli (*M. tornata*)	14.4	(3.80)
Herald (*M. littoralis*)	10.3	(3.21)
REML variance components analysis (transformed data)		
Cultivars		***
sed		(1.367); *df = 44*

***, $P \leq 0.001$

Table 4 Spearman rank order correlations (r) of root growth (mm) in each treatment with the relative index (%) based on all rates of Al exposure (experiment 1).

Prior 1 day PULSE	3 days of Al exposure			
	NIL Al	10μm	20μm	30μm
NIL Al	0.23	0.47	0.76**	0.64*
50μm	0.41	0.70**	0.86***	0.63*
75μm	0.57*	0.78**	0.88***	0.82***
100μm	0.41	0.48	0.94***	0.60*

***, $P \leq 0.001$; **, $P \leq 0.01$; *, $P \leq 0.05$

Table 5 The total plant yield (shoots plus roots; g/pot) of *Medicago* species grown in acidic soil either unlimed or limed at various rates in a glasshouse experiment (experiment 2). The index used was the % yield from the lime rates 0 and 0.3 t/ha of yield at lime rates 1 and 2 (t/ha).

Data in brackets are square root transformed.

Genotype	Lime applied (t/ha equivalent) [final pH_{Ca}]				Index (%)	
	0 [pH 4.15]	0.3 [pH 4.30]	1 [pH 4.75]	2 [pH 5.45]		
Zodiac (*M. murex*)	0.38	0.62	0.69	0.64	72.5	(8.52)
Serena (*M. polymorpha*)	0.19	0.31	0.40	0.31	71.7	(8.47)
Cavalier (*M. polymorpha*)	0.14	0.37	0.41	0.51	69.2	(8.32)
Scimitar (*M. polymorpha*)	0.22	0.38	0.41	0.50	65.0	(8.06)
Orion (*M. sphaerocarpos*)	0.32	0.41	0.60	0.62	63.8	(7.99)
Santiago (*M. polymorpha*)	0.19	0.28	0.38	0.46	53.3	(7.30)
Paragosa (*M. rugosa)*	0.21	0.27	0.41	0.49	52.8	(7.27)
Mogul (*M. trunculata*)	0.17	0.26	0.46	0.42	51.3	(7.16)
Sava (*M. scutellata*)	0.36	0.54	0.77	1.04	50.2	(7.09)
Caliph (*M. trunculata*)	0.14	0.17	0.30	0.35	50.0	(7.07)
Aurora (*M. sativa*)	0.10	0.12	0.21	0.26	46.2	(6.80)
Jester (*M. trunculata*)	0.10	0.21	0.38	0.39	45.1	(6.72)
Herald (*M. littoralis*)	0.01	0.13	0.17	0.14	42.1	(6.49)
M. orbicularis	0.08	0.13	0.27	0.29	39.8	(6.31)
Rivoli (*M. tornata*)	0.18	0.38	0.65	0.60	39.0	(6.25)
Harbinger (*M. littoralis)*	0.05	0.09	0.16	0.24	36.5	(6.05)
	Analyses of variance					
Cultivars	***				***	
Lime	***				n.a.	
Cultivars * lime	***				n.a.	
sed	0.054; *df = 137*				(0.424); *df = 39*	

***, $P \leq 0.001$; n.a. not applicable

Table 6 The number of aluminium tolerant plants selected, approximate selection intensity and average length of regrowth of roots of selected plants from 17 populations of lucerne (experiment 3).

Cultivar/ population	Estimated number of seedlings tested	Number of tolerant plants selected	Selection intensity (%)	Average root regrowth of selected plants (mm)
Cultivars				
SARDI 7	1890	41	2.17	11
SARDI 10	1890	45	2.38	10
UQL-1	1260	38	3.02	11
Venus	1080	30	2.78	9
Aurora	1260	34	2.70	8
South Australian lines				
A513	1980	48	2.42	10
A442	1980	42	2.12	10
L97a	1080	36	3.33	14
G906a	855	14	1.64	8
A34w	675	18	2.67	11
L94b	675	13	1.93	7
L94a	675	25	3.70	8
L92a	675	20	2.96	9
L76c	675	27	4.00	9
NSW lines				
TO1 -007	1260	25	1.98	6
TO2 -011	1260	37	2.94	9
TO2 -010	1260	36	2.86	9

Table 7 A comparison of average length of root regrowth (mm) of original populations (cycle 0) and after one cycle of selection (cycle 1) of four populations of lucerne in experiment 4.

Data in brackets are square root transformed.

Cultivar/population	Cycle 0 (original population)		Cycle 1	
Aurora	1.81	(1.34)	4.73	(2.17)
UQL-1	3.71	(1.93)	7.23	(2.69)
A513	2.37	(1.54)	5.17	(2.27)
TO2-011	2.60	(1.61)	8.38	(2.89)
REML variance components analysis (transformed data)				
Population	***			
Cycle	***			
Population * cycle	***			
Average sed	(0.0696); *df = 179*			

***, $P \leq 0.001$

Table 8 A comparison of average length of root regrowth (mm) under aluminium stress of original populations (cycle 0), and after one cycle, or two cycles of selection of three populations of lucerne in experiment 5.

Data in brackets are square root transformed.

Cultivar/population	Cycle 0 (original population)		Cycle 1		Cycle 2	
Aurora	1.94	(1.392)	5.14	(2.268)	6.94	(2.635)
UQL-1	3.83	(1.956)	7.20	(2.683)	8.12	(2.849)
TO2-011	3.37	(1.836)	7.52	(2.743)	9.94	(3.152)
REML variance components analysis (transformed data)						
Population	***					
Cycle	***					
Population * cycle	***					
Average sed; *df = 158*	(0.07188)					

***, $P \leq 0.001$

Table 9 A comparison of average length of root regrowth (mm) of four populations of lucerne with and without aluminium stress in a solution culture experiment (experiment 5).

Data in square brackets are square root transformed.

Cultivar/population	Nil Al	+ Al
Aurora	17.40 (4.171)	1.94 (1.392)
UQL-1	18.96 (4.354)	3.83 (1.956)
TO2-011	18.69 (4.323)	3.37 (1.836)
GAAT	16.59 (4.073)	2.47 (1.573)
REML variance components analysis (transformed data)		
Population	***	
Aluminium	***	
Population *Aluminium	***	
Average sed for comparisons within nil Al, excluding GAAT; *df = 158*		(0.0677)
Average sed for comparisons within + Al, excluding GAAT; *df = 158*		(0.0730)
Average sed for comparisons between nil and + Al, excluding GAAT; *df = 158*		(0.0933)
Average sed for comparisons with GAAT; *df = 158*		(0.1270)

***, $P \leq 0.001$

Table 10 The number of aluminium tolerant plants selected, selection intensity and average length of regrowth of roots of selected plants from 4 populations of lucerne in a second selection cycle (concurrent with experiment 4), and three populations of lucerne in third cycle of selection (concurrent with experiment 5).

Cultivar	Number of seedlings tested	Number of tolerant plants selected	Selection intensity (%)	Average root regrowth of selected plants (mm)
		Second cycle of selection (experiment 4)		
Aurora	754	47	6.23	13.3
UQL-1	764	48	6.28	16.3
TO2 -011	851	48	5.64	17.9
A513	972	48	4.94	13.9
		Third cycle of selection (experiment 5)		
Aurora	737	41	5.56	15.1
UQL-1	691	42	6.08	16.4
TO2 -011	758	42	5.54	18.5

Table 11. The realised heritability (%h^2) of putative aluminium tolerance in four lucerne populations under selection for aluminium tolerance.

Experiment (selection cycle)	Source populations			
	Aurora	UQL –1	A513	TO2-011
Exp 4 (cycle 0 to cycle 1)	47	48	37	90
Exp 5 (cycle 0 to cycle 1)	53	47	n.a.	74
Exp 5 (cycle 1 to cycle 2)	22	10	n.a.	23

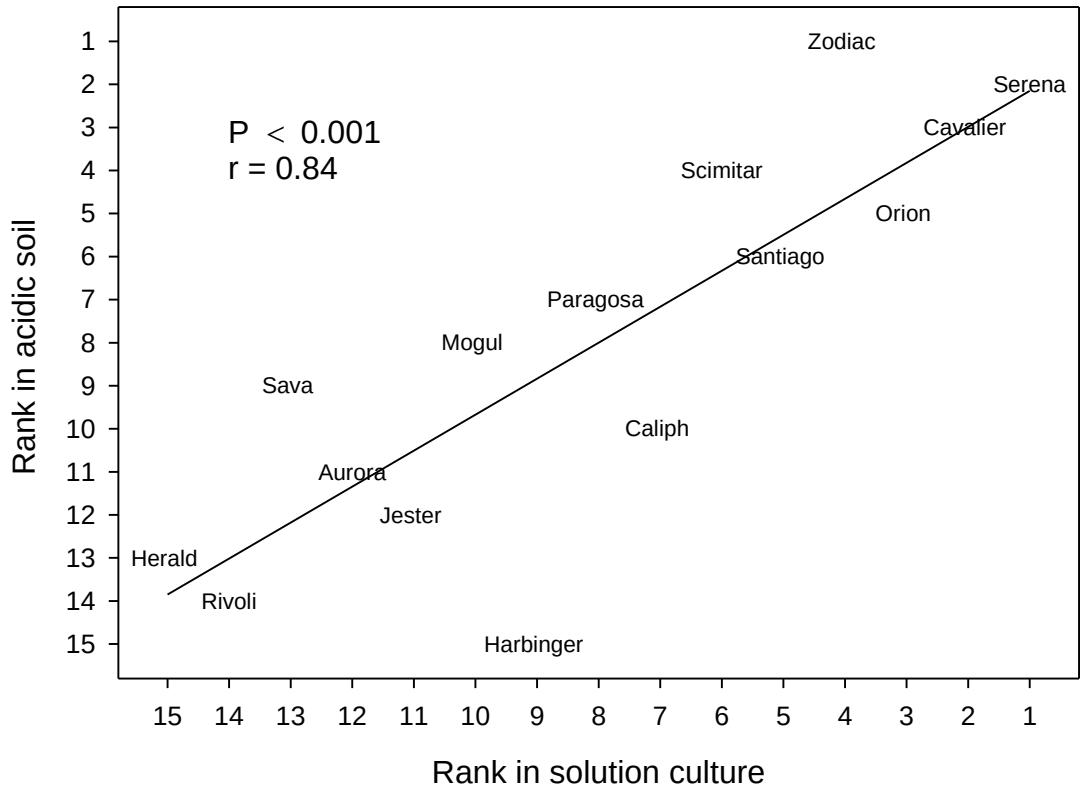

Fig. 1 Spearman rank order correlation between root regrowth under aluminium stress in solution culture (experiment 1) and relative total plant growth over 28 days in an acidic soil in a glasshouse experiment (experiment 2) for a range of *Medicago* species and cultivars.

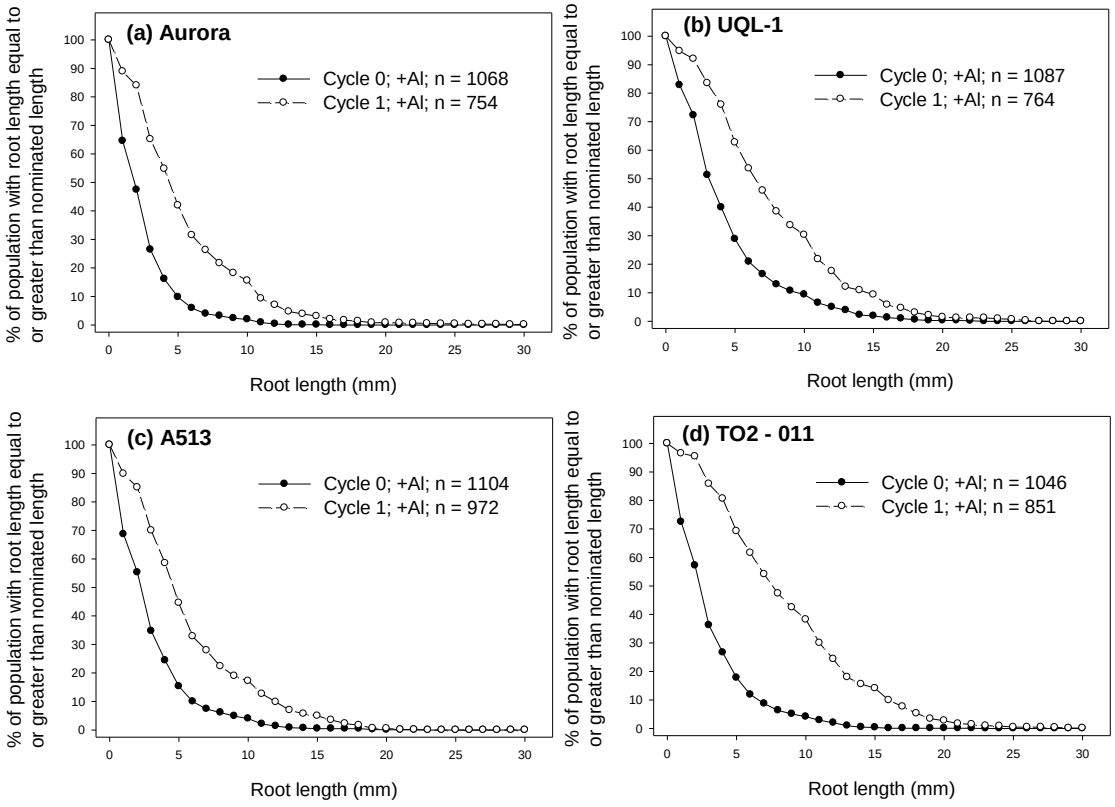

Fig. 2 The distribution of length of regrowth of roots of lucerne (*Medicago sativa*) under aluminium (Al) stress in nutrient solution for two cultivars (Aurora, a and UQL-1, b) and two breeders lines (A513, c and TO2-011, d) compared to populations after one cycle of selection for putative Al tolerance in experiment 4.

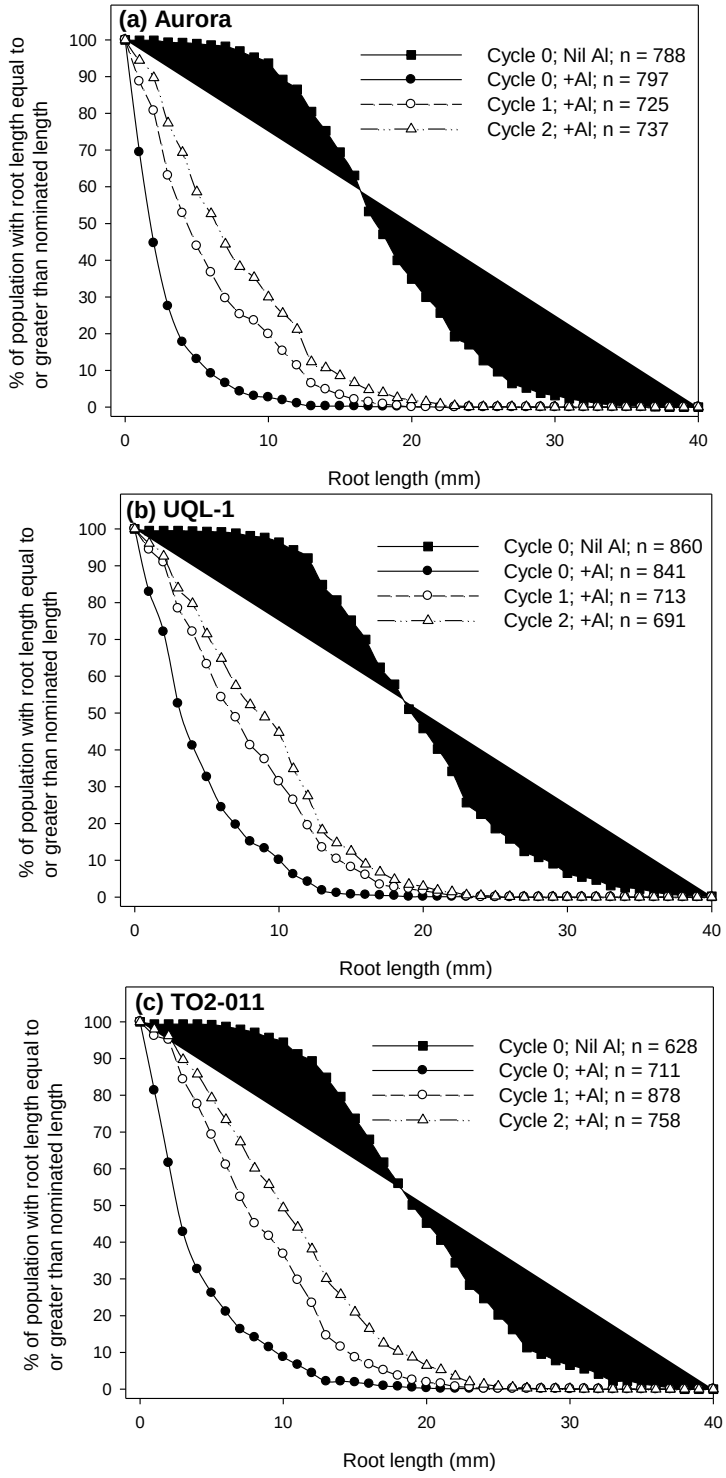

Fig. 3 The distribution of length of regrowth of roots of lucerne (*Medicago sativa*) for two cultivars (Aurora, a and UQL-1, b) and one breeders lines (TO2-011, c) under aluminium (Al) stress and no Al stress in nutrient solution, compared to selected populations after one or two cycles of selection for putative Al tolerance under Al stress in experiment 5.