## Screening native Fabaceae species for tolerance to aluminium

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## Abstract

Thirteen native legume species collected from a wide range of Australian environments were screened for their tolerance to aluminium relative to three commercial pasture legume species. The seedlings were grown in a glasshouse on hydroponic culture maintained at pH 4.5. Aluminium treatment consisted of a 24-hour period when plants were placed on a 2.7 mM solution of aluminium chloride. Three species did not survive and four species grew poorly regardless of treatment. All control species had reduced yields of leaves and roots when treated with aluminium. Several native species were identified as potentially tolerant to aluminium and acidic growing conditions.

## Keywords

Australian, Legume, Forage

#### Introduction

Australian agriculture covers a diverse range of landscapes, and plants are often subjected to hostile weather and soil conditions. Native or introduced species able to tolerate extreme conditions such as low pH or high aluminium concentrations are often used to establish ground cover and provide some fodder from areas considered unproductive when sown to traditional pasture species. Plant improvement by selection has increased the productivity of species such as Lotus and Ornithopus for such environments but other limitations, for instance phytotoxicity, can still limit utilisation by grazing animals.

The use of molecular techniques allows the genetic mechanisms for tolerance to be identified and transferred into species bred for high levels of quality forage or grain production. The aim of this bio prospecting is to elucidate the tolerance mechanisms in native species and to use this knowledge in the development of crops and pastures with enhanced tolerance.

#### Methods

All species were germinated in a growth room on water agar following surface sterilisation with 3% calcium hypochlorite solution. Once cotyledons had emerged the seedlings were transferred to trays containing sand approximately 5 cm deep and placed in a glasshouse for two weeks, at which point they were transferred to hydroponic culture and randomised across tubs. Two separate experiments were conducted each containing a number of native and three control legumes (see Table 1).

Two replicates were randomised across each of 20 aerated hydroponic tubs containing 25 L of nutrient solution (3). Aluminium treatment consisted of half of the plants being transferred to 15 L tubs containing the treatment solution for 24 hours, the remainder continued on the original solution. After 24-hrs treatment the roots were allowed to drain and plants were then returned to the original tank (3). All solutions were maintained at a pH of 4.5 over the course of the experiments by the addition of  $H_2SO_4$ . Harvests, for dry weight (DWt) of leaves, stems and roots, took place 2 weeks after treatment, to allow time for differences in growth rate to become apparent. The native species were harvested 13-14 weeks, and the control species were harvested 9-10 weeks after germination so that plants were harvested at the same physiological age.

#### **Results and Discussion**

Three species did not survive and four species grew poorly regardless of treatment. All control species had reduced yields of shoots and roots when treated with aluminium in Experiment 1, while this was only true of *Medicago sativa* Experiment 2. Several of the native species showed no significant reduction in growth parameters and others responded to aluminium treatment with a significant increase in the measured parameters (see Table 1).

The control pasture species were chosen to represent a range of aluminium and acid tolerance and to thus provide a ranking benchmark for the native species (1, 5). *Medicago* species tend to be sensitive, while *Lotus pedunculatus* is highly tolerant of both low pH and high aluminium concentrations. *Trifolium repens* is tolerant of low pH, but can show a range of responses to aluminium. The cultivar Mink was bred from lines that are in the mid-range of tolerance to aluminium for the species.

Table 1. Responses of a range of legume species to treatment with 2.7mM aluminium (log transformed data) shown as: treatment (control)

Species (cultivar)	Stem DWt	Leaf DWt	Shoot DWt	Root DWt	Longest root			
Experiment 1								
Medicago sativa (Trifecta)	-1.0 (0.1) *	-0.6 (0.4) *	-0.1 (1.0) *	-0.7 (-0.1) *	5.6 (6.0)*			
Lotus pedunculatus (Maku)	-1.4 (-1.0) *	-0.8 (-0.2) *	-0.3 (0.3) *	-1.8 (-1.2) *	5.2 (5.5)*			
Trifolium repens (Mink)	-1.3 (-0.8)*	-1.1 (-0.7) *	-0.5 (-0.01)*	-1.4 (-1.1) *	5.4 (5.6)*			
Hardenbergia violacea	-2.3 (-2.4)	-1.1(-1.0)	-0.8 (-0.8)	-1.5 (-1.6)	6.1 (6.2)			
Dillwyinia sericea	-4.1 (-4.0)	-2.8 (-2.7)	-2.5 (-2.4)	-3.3 (-3.4)	5.3 (5.2)			
Hovea montana	-5.2 (-5.1)	-3.8 (-3.9)	-3.6 (-3.6)	-4.2 (-4.6) *	4.7 (4.7)			
Pultenaea scabra <sup>1</sup>	-5.1 (-5.0)	-4.1 (-4.3)	-3.8 (-3.9)	-4.7 (-4.8)	4.8 (4.8)			
Senna artemisiodes <sup>1</sup>	-4.7 (-4.8)	-3.4 (-3.3)	-3.1 (-3.1)	-4.6 (-4.2) *	4.8 (5.0)*			
Bossiaea foliosa <sup>1</sup>	-5.1 (-5.4)	-4.1 (-4.3)	-3.7 (-4.0)	-4.8 (-5.4) *	4.1 (3.9)*			
Chi-sq prob	<0.001	<0.001	<0.001	0.002	0.004			
Experiment 2: <sup>2</sup>								
Medicago sativa (Trifecta)	0.6 (1.3) *		1.2 (1.8) *	0.3 (0.6) *	6.4 (6.3)			

Lotus pedunculatus (Maku)	0.8 (0.9)		1.5 (1.5)	-0.3 (-0.4)	5.8 (5.8)
Trifolium repens (Mink)	0.3 (0.3)		0.8 (0.9)	-0.5 (-0.5)	6.1 (6.2)
Bossiaea obcordata	-4.6 (-4.7)		-3.5 (-3.6)	-3.9 (-4.8) *	4.6 (4.5)
Daviesia latifolia	-4.4 (-4.3)		-1.9 (-1.9)	-3.7 (-3.0) *	4.9 (5.3) *
Glycerhiza acanthocarpa	-1.8 (-1.8)		-0.6 (-0.5)	-1.5 (-1.9) *	6.2 (6.2)
Pultenaea scabra <sup>3</sup>	-5.0 (-4.8)		-4.2 (-4.0) *	-4.2 (-4.3)	5.2 (5.0) *
Swainsona lessertifolia	-2.8 (-3.0) *		-1.5 (-1.6)	-2.6 (-2.9) *	5.2 (5.0) *
Chi-sq prob	0.005	N. S.	0.042	0.011	0.023

\* Significant change in yield due to aluminium treatment.

1 These species exhibited poor growth on hydroponics regardless of treatment.

# 2 Viminaria juncea, Gastrolobium racemosum and Pultenaea subspicata: not measured due to poor growth

# 3 Seed was germinated for Experiment 1. Seedlings were 90 days older than other species at harvest

The native legumes screened in these experiments are slow growing relative to the control species. As a result, the post treatment growth period used in this experiment was longer than is usually the case for 'pulse' treatment experiments where plants are exposed to aluminium for only a short period of time (2). Work on short-term responses to aluminium stress in wheat has shown that after an initial reduction in relative elongation rate of the roots, there is a subsequent recovery period (4). This acclimation process could explain the variation seen in the responses of the control species over the two experiments, and account for the increase in yields seen, particularly in the second experiment where the plants had more daylight hours available.

# Conclusions

Further experimentation, to describe in greater detail the mechanism of the tolerance seen here, is planned. Once a highly tolerant species has been identified it will enter the DNRE's Functional Genomics program and the genetic mechanisms for the tolerance will be identified and transferred into species bred for high levels of quality forage or grain production.

# References

(1) Edmeades DC, Blamey FPC, Asher CJ, Edward DG (1991) Aust. J. Agr. Res. 42, 559-569.

(2) Howeller RH (1991) Plant-soil interactions at low pH: principles and management. pp:885-904. (Kulwer Academic Publishers).

(3) McFarlane N, Reed K, Morgan J, John U, Spangenberg G (2002) 12<sup>th</sup> Aust. Plant Breeding Conf. (In Press).

(4) Parker DR (1995) Plant-soil interactions at low pH: principles and management. pp:317-323. (Kulwer Academic Publishers).

(5) Wheeler DM, Dodd MB (1995) Plant and Soil 173, 133-145.