nt and Soil 123, 101–105 (1990).
Kluwer Academic Publishers. Printed in the Netherlands.

1/1/1990 PLSO 8316 62185

Variation for aluminium tolerance in white clover

A.D. MACKAY, J.R. CARADUS and M.W. PRITCHARD
Grasslands Division, Department of Scientific and Industrial Research, Private Bag, Palmerston North, New Zealand

Received 23 June 1989. Revised January 1990

Key words: aluminium tolerance, cultivar, *Trifolium repens* L., white clover

Abstract

Aluminium (Al) tolerance of fourteen white clover (*Trifolium repens* L.) cultivars from eleven countries was compared in the greenhouse in the Wainui silt loam (Typic Dystrochrept) to which Al had been added at nine levels (0, 2.5, 5, 20, 50, 150, 250, 500 and 750 mg kg^{-1} of soil) as $Al_2(SO_4)_3$ and incubated for 30 days. None of the white clover cultivars, including those either referred to as Al-tolerant, Dusi and Pathfinder, or from countries that have large areas of acid soils, El Lucero M.A.G, Bayucua, Bage and Zapican, showed greater Al-tolerance than 'Grasslands Huia' white clover. Subsequent screening for Al-tolerance can therefore be restricted to germplasm with wide agronomic adaptation.

Introduction

Intraspecific variation for aluminium (Al) tolerance has been observed in many crop and herbage species (Devine *et al.*, 1976; Fageria, 1985; Foy *et al.*, 1965; Mesdag and Slootmaker, 1969; Walker *et al.*, 1975), including white clover (*Trifolium repens*), a perennial legume (Caradus, 1987). This variation in Al-tolerance has been used successfully to breed Al-tolerant cultivars of a number of important crop plants (Devine *et al.*, 1976; Reid *et al.*, 1969). To date, selection for Al-tolerance within white clover has not been attempted.

Aluminium toxicity is a problem in many regions of the world, due not only to natural weathering cycles, but also to the continuous cropping and farming of fragile soils (Clark, 1982). In Australia, intensification of agricultural practices has increased soil acidity and this can lead to an increase in active Al levels (Davidson, 1987). In Europe the use of large quantities of nitrogenous fertilizers is causing the slow acidification of many low buffered soils.

White clover is found in many regions of the world (Caradus, 1986). It grows in a range of environments and is productive at a range of soil pH and fertility levels. Two white clover cultivars, Dusi and Pathfinder, have been referred to as Al-tolerant (Hall, 1948; Smith and Morrison, 1983), while other cultivars, El Lucero M.A.G. Bayucua, Bage and Zapican, have been selected and bred in countries that have large areas of acid soils. These are often referred to as acid tolerant and consequently may be tolerant to excessive amounts of Al. A comparison of the Al-tolerance of these cultivars could, therefore, be the first step in a breeding programme. White clover has become an increasingly important legume in the economy of a growing number of pastoral industries around the world and in some countries an alternative to nitrogen fertilizer. Improved Al-tolerance could be a desirable trait to incorporate into this important pasture plant.

The objective of the present study was to determine in soil, the Al-tolernace of white clover cultivars from different regions of the

9516

world, as the first step in a programme to breed an Al-tolerant white clover cultivar.

Materials and methods

Soil and aluminium additions

The Wainui silt loam (Typic Dystrochrept) with a pH of 5.0 and available P (estimated by the bicarbonate method of Olsen *et al.*, 1954) of 8 mg kg^{-1} was used. The phosphorus level would be regarded as low, restricting white clover growth. The soil was collected from a hill pasture to a depth of 100 mm and sieved (<6 mm) moist. To obtain a range of Al levels, $Al_2(SO_4)_3$ dissolved in water, was added to the soil which was then incubated at a gravimetric moisture content of 48% for a minimum of 30 days. After sieving measurements were made of exchangeable cations (three sequential extractions with netural 1 *M* ammonium acetate at a solution:soil ratio of 5:1 and a 30-min shake at each extraction) by atomic absorption spectrophotometry and extractable Al (three sequential extractions with 1 *M* KCl at a solution:soil ratio of 10:1 and a 30-min shake at each extraction) by inductively coupled optical plasma emission spectrometry. Results are presented in Table 2. In the presence of the SO_4^- ion in solution and at a pH of 4.5 to 5.0 the inorganic monomeric Al species present would include Al^{3+}, $Al(OH)^{2+}$, $Al(OH)_2^+$, $Al(OH)_3^+$, $Al(SO_4)^+$, with $Al(OH)^{2+}$ and $Al(OH)_2^+$ predominant. These two species are thought to be the most important in terms of reducing root growth (Alva *et al.*, 1986). Soil was packed into trays (430 × 300 mm) at a bulk density of 0.8 gm cm^{-3}. The study reported was conducted in a heated greenhouse.

Comparison of white clover cultivars

Based on the findings of a preliminary study, nine Al levels (0, 2.5, 5, 20, 50, 150, 250, 500 and 750 mg kg^{-1} of soil) were used to compare the Al-tolerance of 14 white clover cultivars. Scarified seed of each cultivar (Table 1) and *Lotus pedunculatus* cv., 'Grasslands Maku' was germinated at 25°C on moist filter paper. *Lotus pedunculatus* was included in the study as an indicator of a low fertility, Al-tolerant pasture legume (Davis, 1981). The germinated seed was sown in trays to that there were 12 seedlings of each line at each Al level in each replicate. There were two trays at each Al level at each replicate. Each Al level was replicated 3 times. After sowing, a cocktail, containing a wider range of strains of *Rhizobium trifolii* held by DSIR Biotechnology, Private Bag, Palmerston North, New Zealand, and known to effectively nodulate and fix nitrogen with a wider range of white clover cultivars was applied in a suspension sprayed over the soil surface. Trays were watered to weight twice weekly maintain a gravimetric moisture content of 55%. Once weekly 250 mL of nutrient solution (Pritchard *et al.*, 1984) was added to each tray. The number of seedlings established was counted and cotyledon

Table 1. Name, country of origin, and grasslands number of the fourteen white clover cultivars

Number	Name	Country of origin
1	Grasslands Huia	New Zealand
2	Grasslands Pitau	New Zealand
3	Grasslands Tahora	New Zealand
4	Grasslands Kopu	New Zealand
5	Dusi	South Africa
6	Pathfinder	Canada
7	Bagé	Brazil
8	Bayucua	Uruguay
9	Estanzuela Zapican	Uruguay
10	Haifa	Australia
11	El Lucero M.A.G.	Argentina
12	Quin Zhen	China
13		Devon, UK
14		Costa Rica

size measured at 7 d, and shoot dry weight at 45 d.

Statistical analysis

All data was log transformed and subjected to an analysis of variance with LSD used to compare white clover cultivars. Since no large differences were found for data analysed on a per row basis or on a per surviving plant basis all analyses were carried out on a per row basis.

Results and discussion

Establishment of seedlings

The establishment of pre-germinated seedlings of 9 of the 14 white clover cultivars and Maku lotus was unaffected by the addition of Al (Fig. 1). Even with the addition of 500 and 750 mg Al kg^{-1} of soil, which raised extractable Al levels to 115 and 243 mg kg^{-1} of soil, respectively, or in terms of exchangeable Al, 1.27 and 2.43 meq, respectively, levels in the critical range for white clover (Edmeades *et al.*, 1983), the establishment of these 9 cultivars was not affected. This is an important finding, as little would be gained from selecting and releasing an Al-tolerant white clover cultivar when the plant is unable to germinate and establish in unfavourable environments.

The establishment of Pathfinder and Dusi, which have been referred to as Al-tolerant cultivars (Hall, 1948; Smith and Morrison, 1983, respectively), was reduced by the addition of 250 and 500 mg Al kg^{-1} of soil, respectively. El Lucero M.A.G. from Argentina appeared to be the most sensitive cultivar to changes in Al, with the addition of as little as 50 mg Al kg^{-1} of soil severely reducing the number of seedlings established at 7 days. Bagé from Brazil which has large areas of acid soils, was as sensitive to Al as the line from Costa Rica.

Cotyledon size of the establishing seedling at 7 days was not affected by the addition of up to 150 mg Al kg^{-1} of soil (Fig. 1). Cultivars did not differ in cotyledon size at any Al level at 7 days, despite marked variation in establishment of some white clover cultivars at Al additions of 500 and 750 mg kg^{-1} of soil. It appears that establishment of the seedling which includes the appearance of the cotyledons, is tolerant to very high levels of Al in soil.

Growth of established seedlings

Compared with the small reduction in establishment and cotyledon size of most cultivars caused by the addition of 150 mg Al kg^{-1} of soil at 7

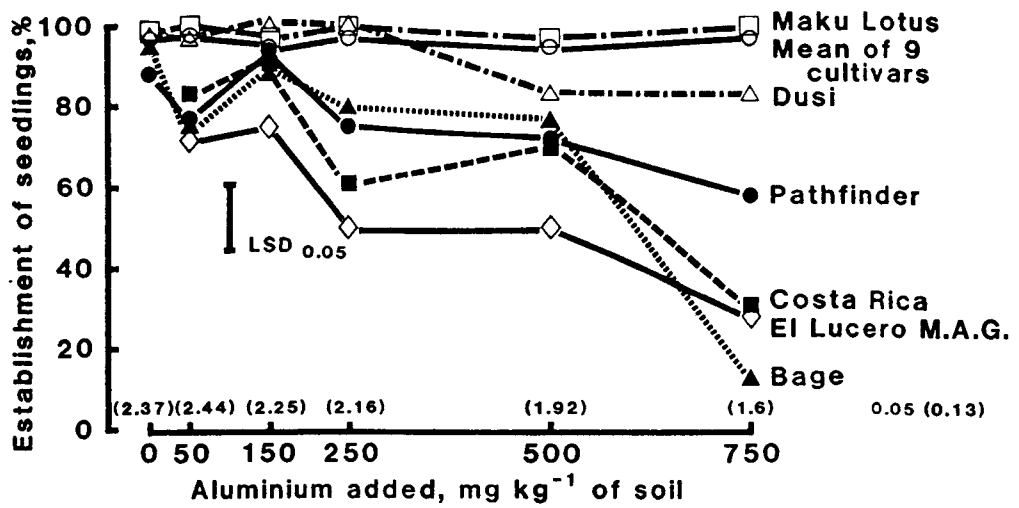

Fig. 1. Establishment of seedlings of fourteen white clover cultivars and Maku lotus at 7 d in the Wainui silt loam to which 0, 50, 150, 250, 500 and 750 mg Al kg^{-1} of soil had been added. *Values in brackets* are average cotyledon size of all 14 white clover cultivars seedlings at each Al addition. There was no cultivar × Al level interaction for cotyledon size.

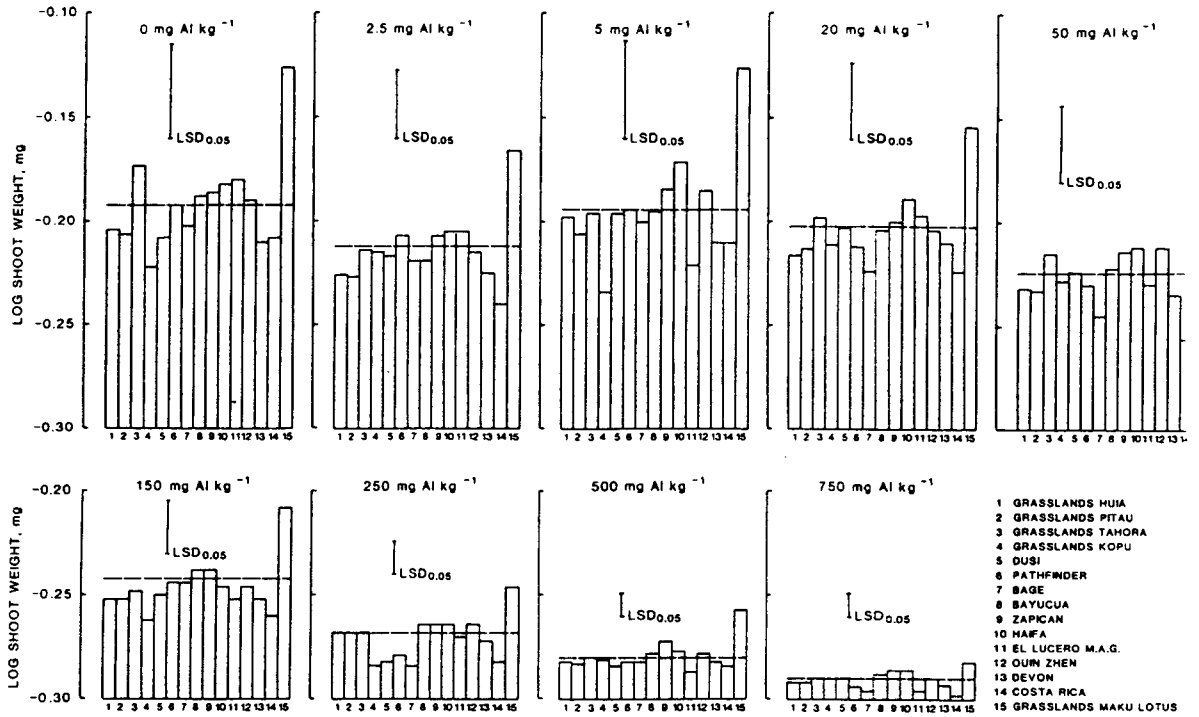

Fig. 2. Shoot growth of fourteen white clover cultivars and Maku lotus at 45 d in the Wainui silt loam to which 0, 2.5, 5, 20, 150, 250, 500 and 750 mg Al kg^{-1} of soil had been added. Mean weight is indicated at each Al level by a *horizontal dashed li*

days (Fig. 1), shoot growth of all white clover cultivars 45 d after planting was severely reduced at this level (Fig. 2). The severe reduction in shoot growth at 45 d with the addition of 150 mg Al kg^{-1} of soil demonstrates that as nutrient (aluminium) and water uptake and translocation through the developing transport system increases, the plant becomes more sensitive to Al in the external soil solution. At an Al ad tion of 250 mg kg^{-1} of soil, KCl extractable approached 1 meq.

A significant ($P < 0.001$) cultivar × Al int action was obtained for log shoot dry weight 14 white clover lines and Maku lotus (Fig. The interaction resulted from differences in t extent of the reduction in shoot growth

Table 2. Effect of added Al of pH_w, exchangeable cations and amount of extractable Al of the Wainui silt loam

Al addition	pH_w	Exchangeagle cations				Extractable
		Ca	Mg	Na	K	
(mg kg^{-1})		(m mol (+) kg^{-1})				(mg kg^{-1})
0	4.7	37	12	1.4	3.5	0
2.5	4.6	37	12	1.4	3.4	0
5	4.6	37	12	1.5	3.7	15
20	4.6	37	12	1.4	3.3	20
50	4.6	36	13	1.5	3.3	32
150	4.5	36	12	1.5	2.5	71
250	4.4	37	13	1.5	2.7	87
500	4.3	38	12	1.4	2.5	115
750	n.d.	36	13	1.8	n.d.	243

n.d. not determined.

[a] Aluminium is not expressed in terms of exchangeable Al because at this pH several Al species would be present. To calculate exchangeable Al (mg al kg^{-1} ÷ 90 = meq).

2.5 mg Al kg^{-1} of soil between cultivars rather than from differences in the tolerance of these cultivars to increasing additions of Al. While the addition of 1, 5 and 20 mg Al kg^{-1} of soil had no effect on shoot growth, the addition of 2.5 mg Al significantly reduced the shoot growth of several cultivars, including Huia, Tahora and the line from Costa Rica, to a similar extent as the addition of 50 mg Al. This did not result from the effect of added Al on either pH or extractable Al (Table 2).

The difference between Huia and Maku lotus was due to growth rate rather than Al tolerance, since the decline in shoot growth was parallel for these two species as added Al increased. None of the white clover cultivars, including those either referred to in the literature as Al-tolerant *e.g.* Dusi (Smith and Morrison, 1983) Pathfinder (Hall, 1948) or from countries that have large areas of acid soils (*e.g.* El Lucero M.A.G, Bayucua, Bagé and Zapican), showed greater Al tolerance than Huia (Fig. 2). Tahora, a hill country white clover selection also failed to show any superior Al tolerance over Huia. Selection for Al-tolerance can therefore be restricted to germplasm with wide agronomic adaptation.

Acknowledgements

The technical assistance of Desley Johnson, Allison MacKay and Jacqui Ackland is acknowledged.

References

va A K, Blamey F P C, Edwards D G and Asher C J 1986 An evaluation of various aluminium indices to predict aluminium toxicity in nutrients solutions. Commun. Soil Sci. Plant Anal. 17, 1271–1280.

Caradus J R 1986 World checklist of white clover varieties. N.Z.J. Expt. Agric. 14, 119–164.

Caradus J R 1987 Intraspecific variation for tolerance to aluminium toxicity in white clover. J. Plant Nutr. 10, 821–830.

Clark R B 1982 Plant response to mineral element toxicity and deficiency. *In* Breeding Plants for Less Favourable Environments. Eds. M N Christian and C F Lewis. pp 71–142. John Wiley, New York.

Devine T E, Foy C D, Fleming A L, Hanson C H, Campbell T A, McMurtrey III J E and Schwartz J W 1976 Development of alfalfa strains with differential tolerance to aluminium toxicity. Plant and Soil 44, 73–79.

Davidson S 1987 Combating soil acidity: Three approaches. Rural Res. 134, 4–10.

Davis M R 1981 Growth and nutrition of legumes on a high country yellow-brown earth subsoil. I. Phosphate response of Lotus, Trifolium, Lupinus, Astragalus, and Coronilla species and cultivars. N.Z.J. Agric. Res. 24, 321–332.

Edmeades D C, Smart C E and Wheeler D M 1983 Aluminium toxicity in New Zealand soils: Preliminary results on the development of diagnostic criteria N.Z.J. Agric. Res. 26, 493–501.

Fageria N K 1985 Influence of aluminium is nutrient solutions on chemical composition in two rice cultivars at different growth stages. Plant and Soil 85, 423–429.

Foy C D, Armiger W H, Briggle L W and Reid D A 1965 Differential aluminium tolerance of wheat and barley varieties in acid soils. Agron. J. 57, 413–417.

Hall M 1948 Five hundred varieties of herbage and fodder plants. Bulletin 39 Aberystwyth Commonwealth Bureau of Pasture and Field Crops.

Mesdag J and Slootmaker L A J 1969 Classifying wheat varieties for tolerance to high soil acidity. Euphytica 18, 36–42.

Olsen S R, Cole C V, Watanabe F S and Dean L A 1954 Estimation of available P in soils by extraction with sodium bicarbonate. USDA Circular 939.

Pritchard M W, Lee J, Dunlop J and Sedcole J R 1984 Effects of aluminium and micro-nutrients on the sorption of phosphorus by *Trifolium repens* L. cv. 'Grasslands Huia' from nutrient solutions during plant induced pH changes. Plant and Soil 81, 389–402.

Reid D A, Jones G D, Armiger W H, Foy C D, Kogh E J and Starling T M 1969 Differential aluminium tolerance of winter barley varieties and selections in associated greenhouse and field experiments. Agron. J. 61, 218–222.

Smith A and Morrison A R J 1983 A deep-rooted white clover for South African conditions. Proc. Grassld Soc. South Africa 18, 50–52.

Walker M E, Marchant W H and Ethredge W J 1975 Effects of soil pH on forage yield and chemical composition of sorghum and millet. Agron. J. 67, 191–193.