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TOWARDS IMPROVING THE ALUMINIUM TOLERANCE OF WHITE CLOVER

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Abstract

The objective of this study was to use two growth media to determine the extent of intraspecific variation for aluminium (Al)-tolerance within white clover (*Trifolium repens* cv. Grasslands Huia). A further objective was to evaluate the sensitivity of the germinating and establishing seedling to Al. Addition of Al (500 mg kg-1 of soil) as $Al_2(SO_4)_3$ to the Wainui silt loam (Typic Dystrochrept) which caused severe reductions in shoot growth of 30 d seedlings, only slightly reduced the germination or establishment of the seedling. This is an important finding as little would be gained from improving, by selection, Al tolerance in white clover if the plant was unable to germinate and establish in such unfavourable conditions. Sufficient intraspecific variation in Al tolerance exists within white clover to select for a superior Al-tolerant cultivar.

Keywords: White clover, *Trifolium repens,* aluminium tolerance, soil culture, solution culture.

INTRODUCTION

White clover *(Trifolium repens* L.), by far the most important legume species in New Zealand agriculture, shows less aluminium (Al) tolerance than some other legume species, such as *Lotus pedunculatus* Cav. (Scott & Lowther 1980). In fact At-toxicity restricts the distribution and growth of white clover on acid soils. This could result from reduced root elongation as has been shown in other species (Suthipradit & Alva 1986). In a comprehensive review of data obtained from a national series of field trials on lime, which covered all the major soil types in New Zealand, Edmeades and Wheeler (1985) concluded that Al-toxicity could be a factor limiting pasture production on a number of North Island hill country soils and on upland soils in the South Island. Over three million hectares or over 20% of agricultural soils in New Zealand could therefore be involved.

There is evidence of intraspecific variation in Al tolerance within white clover (Caradus 1984), suggesting there is scope for selecting and breeding a more Al tolerant cultivar of this species. Soil (Foy *et a/.* 1982) and solution culture (Furlani *et a/.* 1982) are the two commonly used media for screen genotypes for Al tolerance. The objective of this study was to use these two media to determine the extent of intraspecific variation for Al tolerance within white clover.

In selecting for Al tolerance in the adult plant the sensitivity to Al of the germination and establishment of the seedling becomes a very important feature, one often overlooked. 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. A further objective of this study was to evaluate the sensitivity of the germinating and establishing white clover seedling to Al.

MATERIALS AND METHODS

Effect of Alumlnium on Germination

Scarified seed of white clover, cv. 'Grasslands Huia', were germinated at 25°C on moist filter paper impregnated with $Al_2(SO_4)_3$. There were six levels of Al (0, 0.19, 0.74, 3.70, 7.41 and 18.5 mM), and four replicates of 100 seeds at each level. Number of germinated seeds were counted at 3 d, and radicle lengths of 10 randomly selected germinated seedlings were measured at 4 d, in each replicate.

Screening for Aluminium Tolerance in Solution and Soil Culture

All experiments took place in a heated glasshouse.

Solution Culture: Static, aerated nutrient solution (Pritchard et *al.* 1984) was used with an Al level, as $Al_2(SO_4)_3$, of 0.22 mM in the initial screening of 10 d old white clover seedlings, which had been germinated without Al and grown in sand. Those genotypes which showed the most rapid increase in root growth at this Al level were selected. A further screening of this material was made at 0.30 mM Al so that only 2 to 3% of the original number remained. A total of 120 genotypes were screened in each batch at these two Al levels (during autumn and winter 1985). Three of the selected genotypes flowered and were interpollinated.

Soil *Culture:* In this and the other soil culture study reported, a Wainui silt loam (Typic Dystrochrept) a Yellow Grey Earth/Yellow Brown Earth with a pH of 5.0 and low P level (bicarbonate extractable P 7 mg kg-') was used. To obtain a range of Al levels, Al_2 (SO+ was added and the soil incubated at a gravimetric moisture content of 48% for 30 d before packing into trays (430 x 300 mm) or pots (450 ml) at a bulk density of 0.8 Mg m^{-3}. Trays or pots were watered to weight on a regular basis to maintain a gravimetric moisture content of 55%. Once a week 100 ml of full strength nutrient solution (Pritchard et a/. 1984) was added to each tray.

Pre-germinated seed of Huia were sown at a **rate** of 214 seedlings per tray, with 2 trays per Al level. Ten Al levels (0, 1, 2, 5, 7, 15, 20, 50, 150 and 500 mg kg-' of soil) were used. Measurements were made of the number of seedlings established and cotyledon diameter at 5 d, and seedling dry weight at 30 d. Before the final harvest at 30 d a selection was made, on the basis of shoot size, of the 10 most Al-tolerant plants and the 10 plants which showed the least tolerance to Al from the 214 plants in each of the two replicates to which 500 mg Al kg-' of soil was added. The Al intolerant selections were split into two groups. The recovery growth rate of one group was slow, 'the other rapid when planted out.

Soil pH_w, at a water:soil ratio of 2.5:1, exchangeable cations, and exchangeable Al, extracted with 1M KCl at a solution:soil ratio of 10:1 and a 30 min shake *were* measured at 30 d. The pH of the Wainui soil was affected only by additions of Al above 150 mg kg^{-1} of soil (Table 1). Additions of Al above 20 mg kg^{-1} of soil resulted in marked increases in exchangeable Al.

Table 1: Effect of Al addition on the pH_w, exchangeable cations, and increases in extractable Al of the Wainui silt loam.

Al addition (mg kg^{-1})	pH_w	Exchangeable cations Ca (mmol (+) kg")	Mg	Na	K	Increase in' extractable Al (mg kg^{-1})
0	4.69	37	12	1.4	3.5	0
1	4.62	35	11	1.5	3.4	0
2	4.62	37	12	1.4	3.4	0
5	4.63	37	12	1.5	3.7	15
7	4.62	37	12	1.5	3.4	11
15	4.63	36	12	1.5	3.4	20
20	4.62	37	12	1.4	3.3	20
150	4.47	36	12	1.5	2.5	71
500	4.30	36	12	1.4	2.5	115
750	n.d.	36	13	1.6	n.d.	243

n.d. not determined

* Al is not expressed in terms of exchangeable Al because at this pH several Al species would be present.

Evaluation of Aluminium Tolerant Selections

Seed-line comparison: Pre-germinated seed of 15 white clover lines, including the Al-tolerant selection from solution culture (Table 2), and *Lotus pedunculatus* cv.

Table 2: Description of white clover lines included in the experiment to identify intraspecific variation for Al-tolerance.

Grasslands number	Cultivar	Country of origin
C962	Pathfinder	Canada
C2600	El Lucero MAG	Argentina
c3351		Costa Rica
C5848		Devon, United Kingdom
C5890	Pitau	New Zealand
C5892	Haifa	Australia
C6205	Tahora	New Zealand
C6350	Dusi	South Africa
C6479	Bayucua	Brazil
C6480	Zapican	Uruguay
C6484	Huia	New Zealand
C6598	Bage	Brazil
C6726	Al tolerant selection from solution culture	New Zealand
C6765	Kopu	New Zealand
C6837	Quin Zhen	China

'Grasslands Maku' were sown so that there were 12 seedlings of each line per tray with three replicate trays. Nine Al levels (0, 2.5, 5, 20, 50, 150, 500 and 750 mg kg^{-1} of soil) were used. After sowing *Rhizobium trifolii* was applied. Number of seedlings established was counted and cotyledon size measured at 7 d, number of seedlings with a spade leaf at 11 d, and shoot dry weight at 45 d.

Stolon tip propagation comparison: Pre-rooted stolon tips of 30 genotypes (i) five genotypes of Huia; five selections for Al tolerance in (ii) solution culture and (iii) soil culture; five selections for Al-intolerance in soil culture with (iv) slow and (v) rapid recovery growth rates after transplanting and (vi) five genotypes of Maku lotus were examined. Two pre-rooted stolon tips were planted per pot.

Pots were arranged in a randomised block design with three replicates. Four Al levels (0, 50, 250 and 750 mg kg-' of soil) were used. After planting, **Rhizobium** *trifolii* was applied. Shoot size was estimated by counting the number of leaves per pot at 50 days.

RESULTS AND DISCUSSION

Effect of Aluminium on Germination, Establishment and Initial Growth

Germination at 3 d was insensitive to Al up to 3.7 mM Al (Fig. 1). The reduction at 18.5 mM Al was 30%. This is reasonably consistent with results for some grass species (Hackett 1964) and for soybeans (Suthipradit & Alva 1986).

Radicle elongation was more sensitive to Al than seed germination (Fig. 1). Addition of Al above 0.93 mM significantly reduced radicle elongation at 4 d. The reduction in radicle length at 18.5 mM Al was nearly 80% (Fig. 1).

Establishment of pre-germinated seedlings in soil was reduced by Al at levels above 150 mg kg^{-1} of soil as was cotyledon size at 7 d (Fig. 2). However, even when 750 mg Al was added, the reduction in establishment and cotyledon size was only 17% and 32%, respectively (Fig. 2). It appears that germination and establishment, including the appearance of the cotyledons of the seedling, are tolerant to very high concentrations of Al in solution. This is an important finding, because there would be little to be gained from breeding and releasing a plant with improved Al tolerance if the plant were unable to germinate and establish under such unfavourable conditions.

With the emergence of the spade leaf and the first trifoliate leaf, however, the seedling becomes more sensitive to Al (Fig. 3). Whereas the reduction in

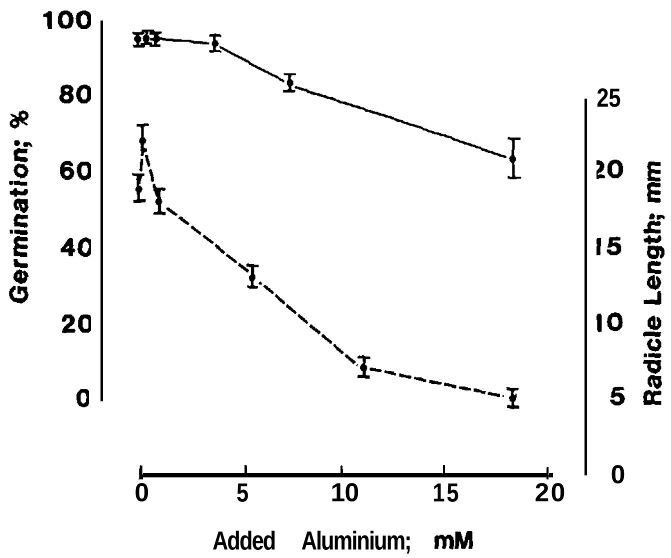

Figure 1: Effect of increasing additions of Al on (a) germination (———) at 3 d and (b) radicle length (-- —) at 4 d of white clover.

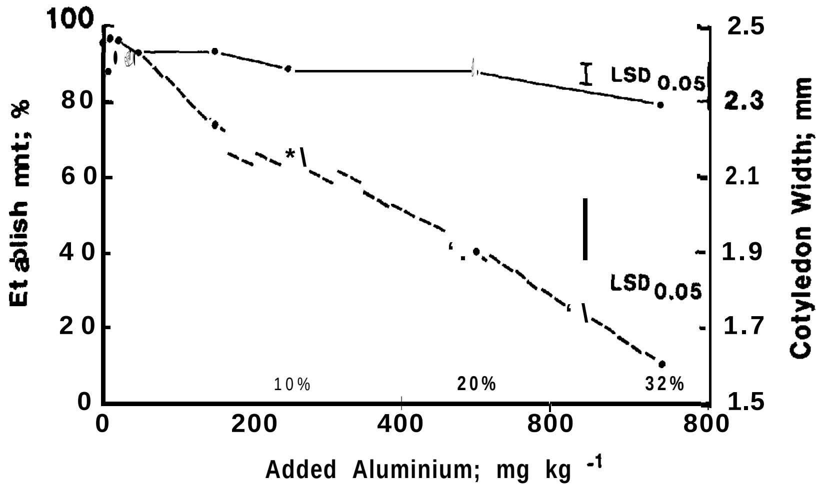

Figure 2: Effect of increasing additions of Al to the Wainui soil on (a) establishment 7 d (———) and (b) cotyledon size 9 d (- — —) after planting of pre-germinated white clover seed.

establishment and cotyledon growth was small at Al levels over 150 mg kg^{-1} of soil; shoot growth of the white clover 30 d after planting was severely reduced (Fig. 3).

Variation for Aluminium Tolerance and the Effects of Selection

A significant ($P<0.001$) line x Al interaction was obtained on log-transformed data, for pm-germinated seed of 15 white clover lines, including the Al tolerant selection from solution culture (Table 2), and Maku lotus. The interaction can be

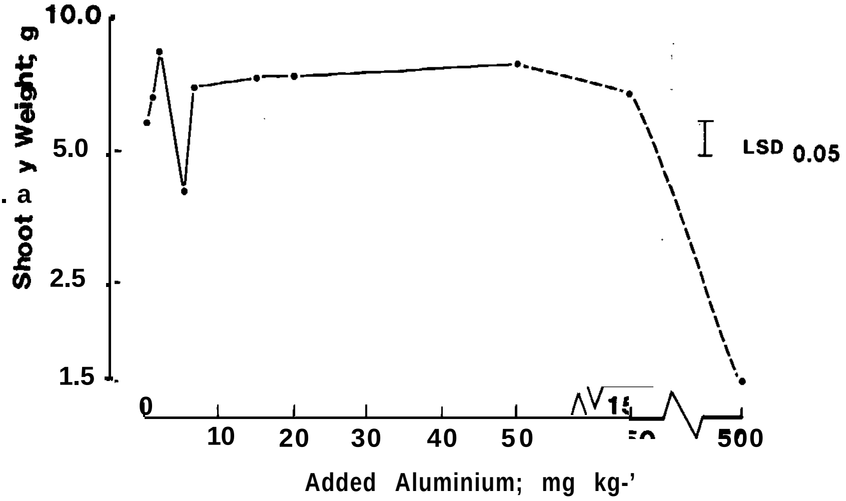

Figure 3: Effect of increasing additions of Al to the Wainui soil on shoot dry weight 30 d after the planting of pre-germinated white clover seed.

explained by differences in the extent of the reduction in shoot growth at 2.5 mg Al kg-' of soil between lines; e.g. compare Maku and the Al-tolerant selected seed line (Fig. 4). When comparing Huia and the Al-tolerant selection from solution culture, the interaction was not due to an improvement in Al tolerance of the selection (Fig. 4). Selection had simply enhanced growth rate at both zero added Al, and at each of the treatments to which Al had been added. This finding highlights one of the real difficulties of selecting for improved nutritional characters or increased tolerance because of the confounding effect of growth rate (Antonovics et *al.* 1967). The difference between Huia and Maku lotus was also 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 (Table 2), including those either referred to in the literature as Al-tolerant (e.g. Dusi, Smith & Morrison 1983; Pathfinder, Hall 1948) or from countries that have large areas of acid soils (e.g. El Lucero MAG, Bayucua, Bage and Zapican), showed greater Al tolerance than Huia. Tahora, a hill country white clover selection also failed to show any Al tolerance compared with Huia.

A significant ($P<0.001$) line x Al interaction was obtained when pre-rooted stolons of 30 genotypes, including Al-tolerant and Al-intolerant selections from soil and solution culture were compared for Al tolerance. The mean of the three selected genotypes from solution which formed the parents for the seed line (Fig. 4) did not show any greater Al tolerance than the mean of the five Huia genotypes (Fig. 5). Only one (No. 14) of the five selections from solution culture which were evaluated showed greater tolerance than Huia (Fig. 5). However, two (Nos. 16 and 17) of the five of the selections for Al tolerance from soil, showed greater Al tolerance than Huia. The most Al-tolerant selection from soil had a similar shoot size as the mean of the five lotus genotypes in soil to which 750 mg Al had been added (Fig. 5). With one exception, the Al-intolerant selections were inferior to the five Al-tolerant selections from soil; one of the poorest Al-intolerant genotypes was genotype 29 (Fig.' 5). At present the criteria used for screening plants showing Al tolerance is not rigid enough on the first selection to discriminate between plants with superior Al

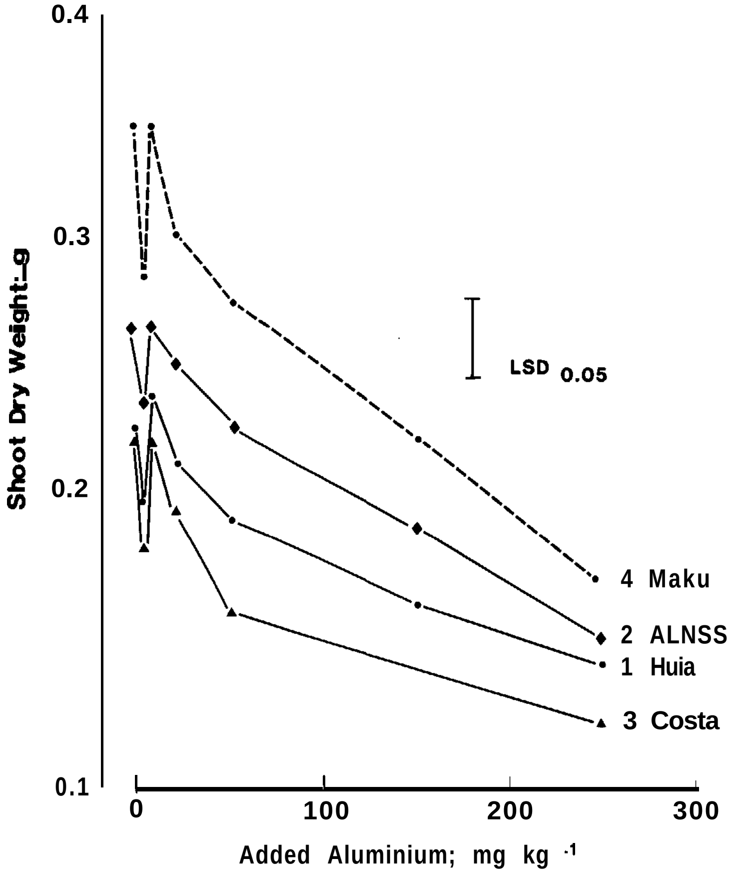

Figure 4: Effect of increasing additions of Al to the Wainui soil on the shoot growth of (1) Huia white clover, (2) Al-tolerant white clover selection (ALNSS), (3) a white clover line from Costa Rica and (4) Maku lotus.

tolerance and those simply with superior growth rates. A second screening of selected material appears necessary to separate these two groups.

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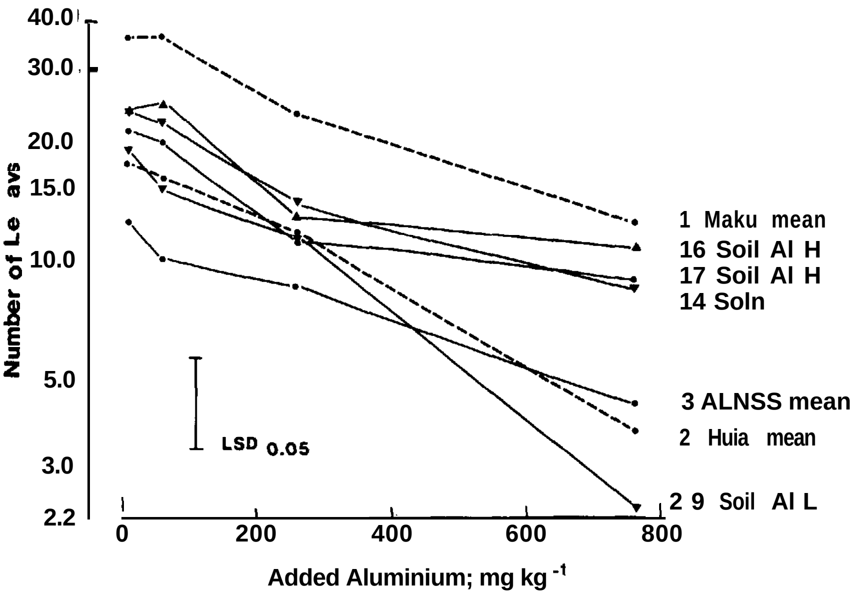

Figure 5: Effect of increasing additions of Al to the Wainui soil on shoot size of (1) the mean of five Maku lotus genotypes, (2) the mean of five Huia genotypes, (3) the mean of the three parents of the Al-tolerant seedline (ALNSS), (16 and 17) genotypes selected for Al tolerance in soil (soil AIH), (14) one genotype selected for Al tolerance in solution culture (soln) and (29) a genotype selected for Al intolerance in soil (soil AIL) 50 d after planting pre-rooted stolon tips.

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