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Heritable differences in white clover for response to phosphorus: new prospects for low input pastoral systems

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Abstract

Three large glasshouse experimcnts were conducted to identify white clover (*Trifolium repens* L.) genotypes, and develop experimental seed lines, with increased tolerance of low-phosphorus (P) soils. The first compared the P response of 119 white clover cultivars; the second, the P response of 110 white clover genotypes from 11 selected cultivars; and the third the P response of progeny from crosses between genotypes selected for differences in response to added P. There were significant differences among cultivars and genotypes for shoot dry weight response to added P. Evaluation of progeny showed that high P response was dominant over low P response, the general combining abilities of all high P response genotypes were greater than that of the low P response genotypes, and that narrow sense heritabilities for P response were moderate (≈0.4). The chances of manipulating differences in P response by breeding, application of the results to date and future directions of this work are discussed.

Keywords white clover, *Trifolium repens*, phosphorus response, low-phosphorus tolerance, heritability, breeding, combining ability

Introduction

Low input systems may result in pastures with a reduced sward legume content. White clover (*Trifolium repens* L.), the predominant pasture legume, requires relatively high levels of phosphorus (P). Since many New Zealand soils are naturally P deficient, significant contributions by white clover can be maintained only by application of phosphatic fertiliser. Largely as a result of economic constraints, the application of phosphatic fertilisers to New Zealand pastures declined during the mid 1980s and has not returned to the levels previously considered necessary to maintain pasture productivity. To improve the growth of white clover with reduced inputs of P, a multidisciplinary programme (Dunlop *et al.* 1990) to identify genotypes and develop experimental seed lines of white clover for increased tolerance to low-P soils was begun.

Our initial endeavour was to identify differences within white clover for some aspect of its P nutrition. Plants were categorised on the basis of their response to various levels of applied P because there was no relevant single P level at which plants should be grown. P response was defined as the change in dry matter yield with increasing P supply. High response to P is associated with a rapid increase in dry weight with small increases in P supply, with maximum yields being reached at lower P levels than for plants categorised as having a low response to P.

Intraspecific variation for response to P and tolerance of low P (i.e. an ability to grow well at low levels of P supply) has been previously demonstrated among semi-natural populations of white clover in the field (Caradus & Snaydon 1986a) and glasshouse (Snaydon & Bradshaw 1962; Caradus & Snaydon 1986b; Godwin & Blair 1991). but often the relationship between field and glasshouse responses is poor (Caradus & Snaydon 1986c). Despite this, the present trial was undertaken in the more controlled environment of pots situated in a glasshouse since the prime objective was to obtain material with genetic differences in their response to P (Dunlop *et al.* 1990). In addition, previous field screening trials of this sort have been beleaguered by (a) micro-environment variability requiring high replication and so reducing the size of the germplasm pool for investigation, (b) the often overriding effect of defoliation intensity and (c) at some sites the resurgence of volunteer white clover particularly at higher levels of P supply.

Method and Materials

The P response of white clover cultivars and genotypes was determined in pots in a glasshouse at Palmerston North. The soil used was a Wainui silt loam (Typic Dystrochrept), with a pH of 5.0 and Olsen P of 6mg/kg soil. Phosphorus added as $Ca(H_2PO_4)_2 4H_2O$ was mixed into soil along with sulphur added as $CaSO_4$ (500 mg S/ kg soil) and potassium as KC1 (500 mg K/kg soil). Soil was then incubated at a gravimetric moisture content of 50% for 30 days before being packed into 120 mm diameter pots at a bulk density of 0.7 to 0.8 Mg/m^3. *Rhizobium trifolii* inoculum was added 1 week after planting. Three times a week pots were watered to weight so that soil was at a gravimetric moisture content of 55%. Each trial was grown for 5-6 weeks in either summer or autumn.

Four phases of theprogramme have been completed; a detailed description of these is given in Mackay *et al.* (1990) and Caradus *et* al. (1992). In Phase I the P response of white clover cultivars was determined, in Phase II the P response of white clover genotypes; in Phase III selected genotypes were crossed; and in Phase IV the P response of progeny from these crosses was evaluated.

Phase I- Phosphorus response of white clover cultivars

Seedlings of 119 white clover cultivars and breeding lines from 25 different countries (Mackay *et al.* 1990) were grown at 11 P levels with replication varying from 3 for 0, 40, 120, and 200 mg P/kg soil, 2 for 400, 500, and *600* mg P/kg soil, to 1 for 20.80 and 300 mg P/kg soil. There were 3 seedlingsperpot. The study wasconducted during April and May 1987.

Phase II - Phosphorus response of white clover genotypes

Ten genotypes randomly selected from 11 cultivars (Table 1) shown in Phase I to vary in their response to added P were grown in potting mix for 6 months until a minimum of 30 stolon tips were produced. Stolon tips consisting of 1 or 2 nodes were removed and pre-rooted in sand for 10 days before transplanting singly into pots. There were7 P levels, 0, 60, 150, 250, 350, 450, and 550 mg P/kg soil with 3 replicates. The study was conducted during December and January 1988/89.

Phase III - Selection and crossing programme

On the basis of data from Phase 2, 6 high-P-responding genotypes from high-responding cultivars and 5 low-P-responding genotypes from low-responding cultivars were selected. These 11 genotypes were interpollinated by hand crossing in all combinations (a diallel cross) during winter 1989, with reciprocals kept separate.

Phase IV - Progeny testing

The 110 progeny lines from the diallel cross *were* compared with the 11 parent genotypes to determine mode of inheritance of P response. Seedlings were grown to provide stolon tips for direct comparison with the cloned parent genotypes. There were 3 plants per pot, and 7 P levels with 3 replicates as in Phase II. The study was conducted during December and January 1989/90.

Measurements and data analyses

Leaf number, stolon length, total shoot, leaf and stolon dry weight, proportion of leaf to total shoot weight and individual leaf weight in Phases I, II and IV were measured. Data were analysed by analysis of variance. Where appropriate, data were log-transformed to ensure homogeneity of variance. Principal component analysis was used to summarise the data and quadratic response curves ($y = a + bx + cx^2$) were fitted to the first principal component (Mackay *et al.* 1990). All P response functions presented are derived from this analysis. Quadratic curves were fitted to quantify response to P such that genotypes or lines designated as high responders had a larger positive linear coefficient 'b' and a larger, but negative quadratic coefficient 'c' than those genotypes or lines designated as low responders.

The fist principal component accounted for 70-85% of thevariation in each Phase and was a measure of plant size predominantly incorporating dry weight measurements, leaf number and stolon length (Mackay *et al.* 1990; Caradus *et al.* 1992). The second principal component accounting for about 10% in each Phase was dominated by the morphological measurements - proportion of leaf and individual leaf weight (i.e. leaf size). While genotype selections were made on the basis of principal component 1, only total shoot weight data will be presented here. Individual leaf weight will be used to compare morphologies.

Results

Intraspecific variation for P response

There was considerable variation among cultivars and genotypes for Presponse as indicated by highly significant ($P<0.001$) cultivar x P level (Phase I) and genotype x P level (Phase II) interactions for shoot dry weight and individual leaf weight. Example curves showing the degree of variation for Presponse between cultivars and genotypes are given in Figures 1 and 2 respectively. At very low levels of P supply (20 and 40 mg P/kg soil) there was a 2.5-2.8 fold variation among cultivars for shoot dry weight; at medium-low levels (80 and 120 mg P/kg soil) a 3.5-6.5 fold variation; at medium-high levels (200 and 300 mg P/kg soil) a 3.8-4.8 foldvariation; and at high levels (400 and 500 mg P/kg soil) a 3.1-3.9 fold variation (Figure 1).

Table 1 Description of cultivars used to provide genotypes for identification of genotype P response differences.

No.	Cultivar	P response category	Classification[a] group	Morphological[b] rating	Agronomic[c] rating	Origin
1	Gwenda	very high	Intermediate	0.67	0.77	U K
2	Trifo	very high	large	1.15	0.85	Denmark
3	Ladino Gigante Lodigiano	high	ladino	1.39	0.98	Italy
4	Viglasska	high	Intermediate	1.00	0.60	Czechoslovakia
5	Huia	medium high	intermediate	1.00	1.00	New Zealand
6	Isolation V	medium low	small	0.68	0.89	New Zealand
7	Dusi	low	ladino	1.46	0.96	South Africa
8	Crau	very low	large	1.47	1.15	France
9	El Lucero	very low	large	1.06	0.99	Argentina
10	G.23	very low	large	1.27	1.17	New Zealand
11	Luclair	medium low (but very low yielding)	intermediate	1.03	0.94	France

[a] from Caradus et al. 1969
[b] relative to Hula based on leaf size, from Caradus et a/. 1991
[c] relative to Huia based on clover content in a grazed grass sward from Caradus et al. 1991

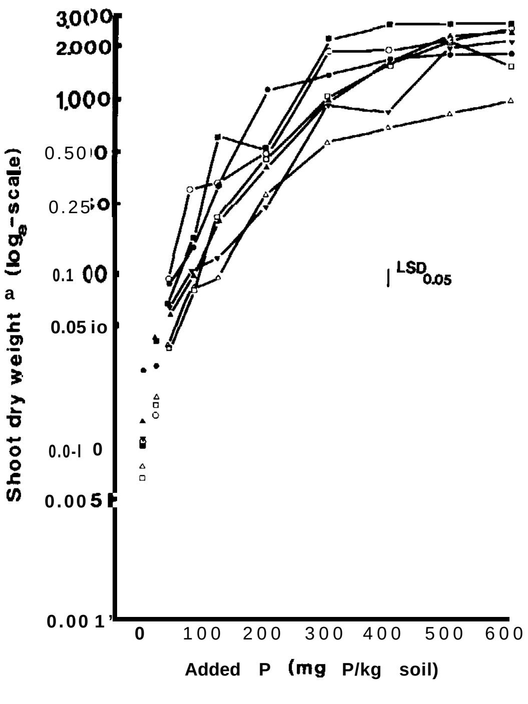

Figure 1 Variation among white clover cultivars for shoot dry matter response to added phosphorus. in Phase I. Cultivars included Trifo (■), Ladino Gigante Lodigiano (0). Merwi (A), Duron (▼), Gwenda (●), Dusi (0) and Luclair (A).

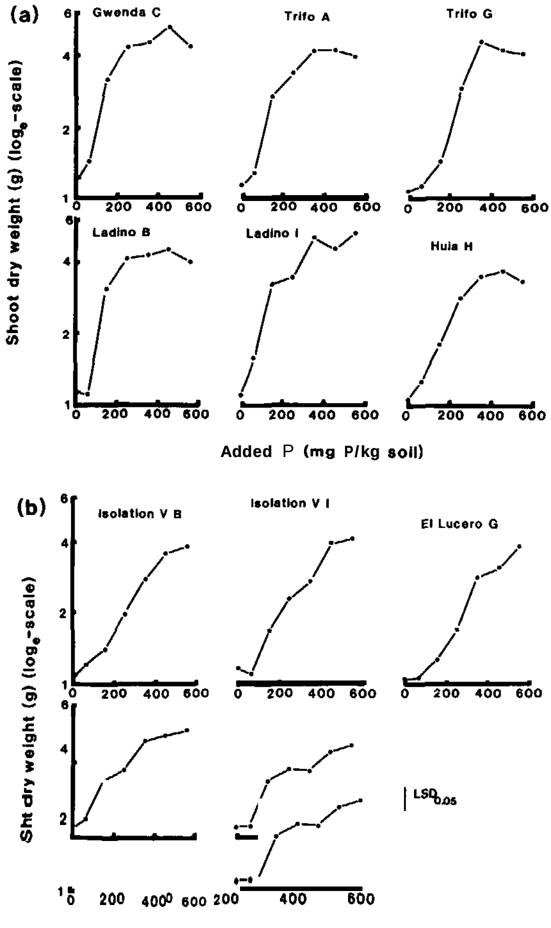

Figure 2 Variation among white clover genotypes for shoot dry matter response to added phosphorus in Phase II, comparing those designated as (a) high P responders and (b) low P responders. Genotype numbers correspond with cultivar numbers in Table 1.

Several genotypes had a high Presponse (measured as a large negative quadratic coefficient in the fitted response curves) and were low-P tolerant (i.e. high yielding at moderately low levels of P supply (150 mg P / kg soil)) (Figure 3a). These were generally large-leaved genotypes predominantly from the cultivars Trifo, Ladino Gigante Lodigiano, Dusi and G.23 (Table 2). The cultivars Viglasska, Huia and Isolation V had no genotypes that were low-P tolerant. All cultivars had genotypes that had both low P response and were low-P intolerant.

A number of genotypes could also be identified as high responders and high yielders at high levels of P supply (450 mg P/kg soil) (Figure 3b). These once again were large-leaved genotypes predominantly from the cultivars Trifo, Ladino Giganteum Lodigiano, Dusi and G.23 (Table 2). Isolation V, El Lucero and Luclair had no genotypes categorised as high yielding.

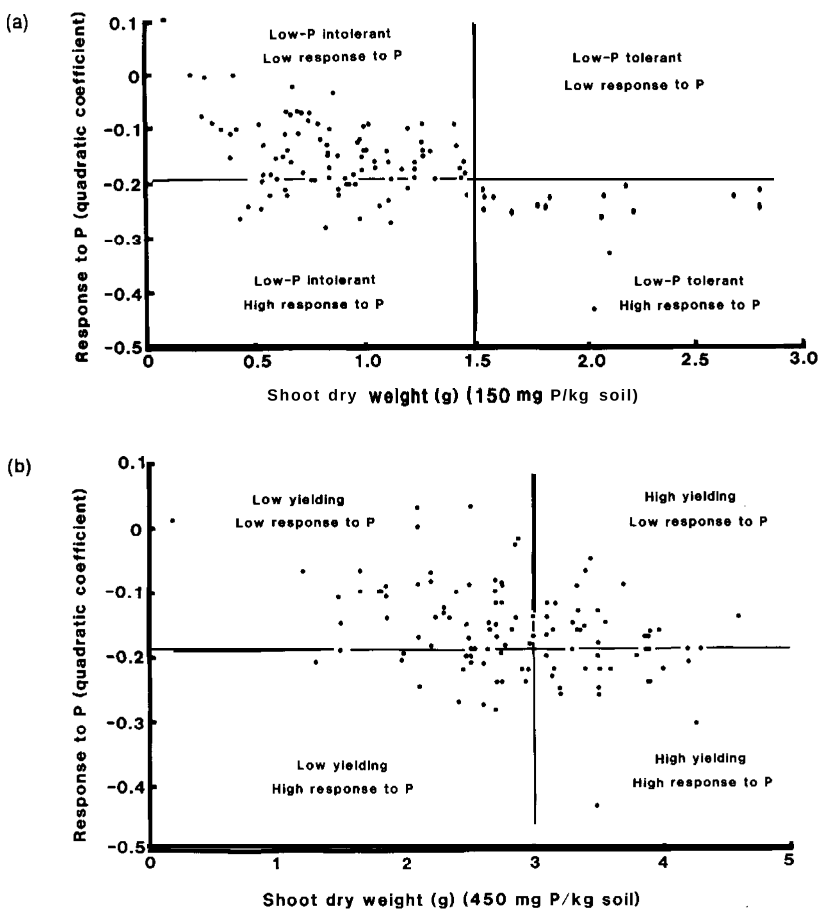

Figure 3 The relationship between P response. estimated as the quadratic coefficient of the fitted response curve, with yield at (a) a moderately low level of P supply (150 mg P/kg soil) and (b) a sufficient level of P supply (450 mg P/kg soil).

Selection of cultivars and genotypes with different P responses

Cultivars were grouped by P response curve shape using cluster analysis (Mackay *et al. 1990*). Response at very low levels of P supply ranged from very high (e.g. Gwenda) to very low (e.g. Dusi), and change in response with increasing level of added P ranged from a rapid reduction (e.g. Gwenda), through only a slight reduction (e.g. Merwi) to a slight increase (e.g. Duron) (Figure 1). The 11 cultivars selected to provide genotypes for characterisation of genotype P response covered a wide range of origins and morphological types (Table 1).

Table 2 Number of genotypes within cultivars exhibiting high or low P-response and either(i) tolerance or intolerance to low levels of P supply (Figure 3a) or (ii) high or low yield at high levels of P supply (Figure 3b). Mean individual leaf sizes (dry weights) are given with standard errors for cultivars and categories.

Cultivar	High P response		Low P response		High P response		Low P response		Mean leaf weight (mg)
	low-P tolerant	low-P intolerant	low-P tolerant	low-P intolerant	high yielding	low yielding	high yielding	low yielding	
Gwenda	1	2		7	1	2	1	6	13.0 ± 1.22
Trifo	3	2		5	4	1	5		24.0 ± 1.35
Ladino G.L.	6			4	6		3	1	35.2 ± 1.91
Viglasska		5		5	1	4	1	4	17.3 ± 0.86
Hula		5		5	1	4	1	4	16.1 ± 0.57
Isolation V		3		7		3		7	10.5 ± 1.15
Dusi	3	2		5	5		5		39.2 ± 2.93
Crau	2		1	7	1	1	5	3	26.5 ± 2.13
El Lucero	1	3		6		4		6	16.1 ± 0.93
G.23	3	2		5	3	2	1	4	22.3 ± 1.22
Luclair	1	2		7		3		7	23.4 ± 1.90
Mean leaf weight (mg)	32.9 ± 2.42	17.6 ± 1.53	32.9 ± ▪	21.0 ± 0.99	32.6 ± 2.30	16.6 ± 1.23	27.6 ± 1.56	17.8 ± 0.92	

Genotypes were ranked for P response based on the magnitude of both linear and quadratic response coefficients. Of the top 20 genotypes, 65% came from cultivars previously identified as having a medium to very high P response, while for the bottom 20 genotypes, 70% came from cultivars previously identified as having medium to very low P response. The P responses of the 11 selected genotypes are given in Figure 2.

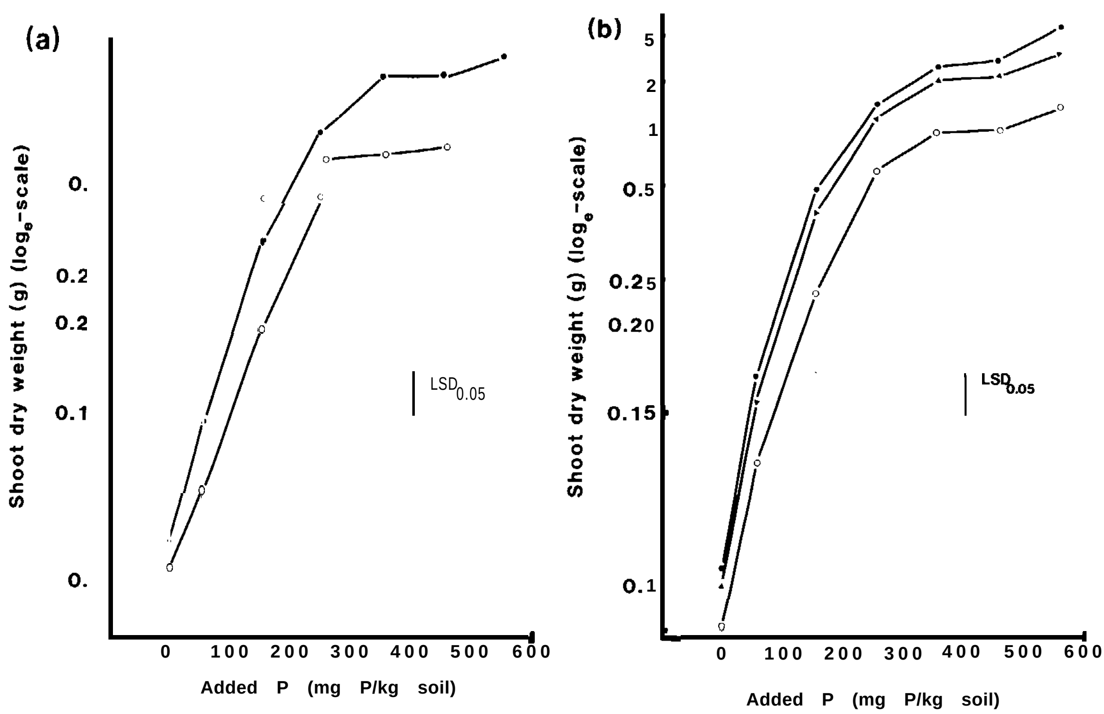

Figure 4 Comparison in Phase IV of (a) parent genotypes selected as high P responders (●) and low P responders (O) and (b) progeny from crosses either between high P responders (●), low P responders (o) or high and low P responders (A).

Heritability of P response

Comparison of P response groups of parent genotypes, categorised as either high responders or low responders, showed that there was asignificant (P<0.001) difference between groups and a significant (P<0.01) P response group x P level interaction, confirming that P response did differ among groups. Progeny were analysed by response type group of parents i.e. progeny from crosses between either high-responding genotypes, low-responding genotypes, or high- and low-responding genotypes. There was asignificant (P<0.001) difference between groups and a significant (P<0.001) cross-response-type x Plevel interaction. Progeny from crosses between high-responding genotypes had a higher P response than progeny from crosses between low-responding genotypes (Figure 4). Progeny from crosses between high-responding and low-responding genotypes were intermediate, but closer tothe progeny from crosses within the high-responding parent genotype group (Figure 4). This indicates that high P response was dominant over low P response.

Broad sense heritability for P response (i.e. the interaction between P level and genotype), calculated from variance components from analysis of variance among genotypes, was 0.52. Narrow sense heritabilities for P response calculated by regressing progeny on mid-parent were 0.46 for the linear coefficient (i.e. response over low P levels) and 0.33 for the quadratic coefficient (i.e. response at medium to high P levels).

Discussion

Intraspecific variation in dry matter response to P is not uncommon and has been demonstrated in a number of crop or forage species. These include rice (Pillai *et al.* 1984). maize (Sherchand & Whitney 1985), sorghum (Brown & Jones 1977), wheat (Batten *et al.* 1984), barley (Boken 1970). soybean (Dunphy *et al.* 1966), perennialryegrass and cocksfoot (Crossely &Bradshaw 1968). Within maize, rice and wheat large differences have also been shown for tolerance and growth on P-deficient soils (Clark 1990).

Variation among genotypes for response to P and tolerance of low P was partly associated with leaf size (Table 2), with large-leaved types being both more responsive to P and tolerant of low P than small-leaved types. The more responsive genotypes also tended to be more productive and higher yielding. This has been demonstrated in other species such as cocksfoot (Crossley & Bradshaw 1968). soybean (Dunphy *et al.* 1966) and bean (Haag *et al.* 1978). However, variation among cultivars of white clover for response to P was unrelated to plant type and country of origin (Mackay *et al.* 1990).

Variation within white clover for P response is most likely the result of variation in a number of morphological and physiological characters. Plant characters that may have an impact on. or are an integral part, of the P nutrition of white clover for which intraspecific variation has been demonstrated include root morphology (Caradus 1977, 1979; Caradus & Snaydon 1988). P distribution between plant parts (i.e. leaf, stolon and root) (Caradus & Williams 1981; Caradus 1986; Godwin & Blair 1991). P distribution between different metabolic forms (Caradus & Snaydon 1987; Hart & Colville 1988), total P concentration (Caradus 1986) and the interaction of the host genotype with rhizobium isolate (Mytton 1975; Hardarson & Jones 1979) or mycorrhizal strain (Crush & Caradus 1980).

Differences in Presponse havebeenidentified within white clover, and it is assumed that those with high P responses and thus better growth at low levels of P supply are the most desirable types, but a number of 'hurdles' are still to be surmounted and 'steps' to be taken before a so-called P-efficient or low-P-tolerant white clover cultivar can be made available. These include (a) the often poor relationship between glasshouse and field Presponse studies (Caradus & Snaydon 1986c) (b) the impact of defoliation on intraspecific variation for Presponse (Hoglund & Brock 1983). (c) the transfer of the genes controlling high P response to agronomically suitable germplasm, (d) the direct effect of *Rhizobium* isolates, and their interaction with host genotype and hence level of nitrogen supply on P response.

P response in terms of rate of dry weight increase with increasing level of P supply does vary within white clover, it is under genetic control and high P response is dominant over low P response. Groups of genotypes with a response in dry matter production to increases in level of P supply have been successfully identified. The challenge now is to transfer characters conferring a high P response to germplasm suitably adapted to other stresses imposed in infertile grazed swards.

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