# PHOSPHORUS AVAILABILITY, DEFOLIATION TOLERANCE, AND GENETIC DIFFERENTIATION IN WHITE CLOVER

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## ABSTRACT

The genetic composition of mixed populations of white clover growing in low fertility, grass-dominant hill pastures was estimated four and eight years after populations were established. Populations were based on an adapted ('Grasslands Tahora') or poorly adapted ('Grasslands Huia') cultivar, and received either no fertiliser, or 35kg P /ha /year. The P response curve of surviving plants was also determined, and compared with plants grown from 'standard' seedlines of the respective cultivars. Between years 4 and 8, the proportion of the populations constituted by plants true-to-type for the sown cultivar remained steady at 56 - 58% for Tahora, but fell from 43% to 14% for Huia, indicating that selection forces were operating on the introduced populations. Tahora and Huia survivors from fertilised and unfertilised pastures did not differ from each other, or from plants grown from 'standard' seedlines, in their response to added P in the glasshouse. Selection forces do not appear to favour clover plants with superior ability to acquire or utilise available P in this environment; rather, it is suggested that defoliation stress is the dominant selection force.

## KEYWORDS

White clover, genetic differentiation, phosphorus, defoliation, natural selection, stress

## INTRODUCTION

Populations of plant species subject to environmental variation develop genetic, or ecotypic, differences over time. Genetic differentiation occurs in response to stressful levels of environmental factors such as soil type (Snaydon, 1970), salinity (Antlfinger, 1981) and water availability (Farris, 1987). Life history, and morphological and physiological traits, are subject to differentiation. These processes form the basis of conventional plant breeding approaches to overcoming environmental limitations to growth, where ecotypes are compared and screened to identify adapted germplasm. Genetic differentiation usually occurs over long time frames. However, when selection pressure and intraspecific phenotypic variation are high, and generation intervals are short, differentiation may occur within a few years (Endler, 1986).

This paper describes a study of genetic differentiation in populations based on two cultivars of white clover (*Trifolium repens* L.) introduced to a low fertility grassland site where the resulting mixed grass-clover pastures subsequently received contrasting levels of phosphorus (P) fertiliser. One cultivar was notionally adapted to the environment, the other was not. The purpose of the work was to examine the ecophysiological basis of plant selection and breeding programs aimed at improving the P-use efficiency of white clover (Caradus *et al*., 1991). The hypotheses under examination were that, after eight years growth and development: 1) the proportion of the white clover populations comprised by survivors of the original seed introduction would be higher for the adapted cultivar than the poorly-adapted cultivar (providing evidence that selective forces were operating); 2) the survival of the poorly-adapted cultivar would be greater where P fertiliser was applied; and 3) the surviving plants in pastures receiving no fertiliser would have differentiated unique P nutrition characteristics compared to plants grown from 'standard' seedlines populations of the same cultivar. Hypotheses 2 and 3, if supported, would provide evidence that the ability of plants to acquire and/or utilise P was a critical factor in plant survival.

## MATERIALS AND METHODS

'Grasslands Tahora' and 'Grasslands Huia' white clover cultivars were oversown into a low fertility, hill land pasture near Palmerston North, New Zealand, in May/June, 1986. Tahora is a small-leaved variety selected from clover plants collected from hill sites throughout New Zealand and is, notionally at least, well adapted to the site. Huia is a medium leaf size variety based on collections of clover plants from high fertility lowland sites, and is not considered persistent in low fertility hill environments (Charlton, 1984).

In an unimproved state, the experimental site is characterised low soil P levels (5 mg /kg, Olsen test) and pH (5.1), and pastures dominated by *Agrostis capillaris* with only low levels of white clover (1% of total annual dry matter production) and *Lolium perenne* (<5% total DM). Each seedline was sown into four replicate plots (average 0.135 ha) of each of four fertiliser treatments: control (no fertiliser applied); reactive phosphate rock ('RPR') applied at 35kg P /h /year; partially acidulated phosphate rock ('PAPR'), at 35kg P /ha /year; and a superphosphate/RPR blend ('Blend'), also at 35kg P /ha /year. All plots were continuously grazed by sheep at stocking densities varying from around 10 /ha in winter to around 35 /ha in spring. High stocking densities were maintained to ensure maximum utilisation of pasture grown. Pasture mass ranged between 1000 and 2000kg DM /ha throughout the experiment. Full details of the experimental site, treatments and management are in Chapman *et al*. (1993).

The clover populations that developed from sowing were an admixture of the introduced cultivar and volunteer plants of the resident clover ecotype (a small-leaved, acyanogenic strain). In January, 1990, and again in January, 1994, 50 white clover plants were collected at random from all 16 plots of each pasture type (Tahora-sown or Huia-sown) and grown on in individual pots to determine the genetic composition of populations. The proportion of the sample population that was 'true-to-type' for the sown cultivar was estimated using leaf cyanogenic potential as a genetic marker, as described by Chapman *et al*. (1993). A subsample population of 80 true-to-type white clover plants was established for each cultivar x fertiliser treatment combination (20 plants from each replicate plot).

A glasshouse experiment, conducted from December, 1995 to March, 1996, compared the P response characteristics of the subsample populations collected from the control and Blend treatments in 1994 with each other, with plants grown from 'standard' Tahora and Huia seedlines, and with plants of the resident ecotype. Stolon cuttings from all 80 subsample plants were transplanted into 1 litre pots filled with an infertile (6mg /kg P, Olsen test) Typic Dystrochrept soil to which had been added 0, 30, 60, 150, 300 or 500 mgP /kg dry soil. Four stolon cuttings were planted in each pot. There were 10 replicate pots of each population (n=7) x P level (n=6) combination. Plants were allowed to grow for 47 days before all herbage except for residual stolon material was harvested, dried and weighed. Plant regrowth was also harvested 21 days later.

## RESULTS AND DISCUSSION

**Genetic composition of field populations**. In 1990, plants true-to-type for the sown cultivar constituted a mean 57.% of the clover population in Tahora-sown pastures and 43.4% of the clover population in Huia-sown pastures (Table 1). By 1994, eight years after sowing, the proportion of true-to-type plants in the Huia-sown pastures had fallen to just 13.7%, whereas it remained steady in the Tahora-sown pastures. The size of the populations (number of plants per m$^2$) did not differ notably between years. Thus, hypothesis 1 is supported. Selection pressures clearly were operating in the experimental environment. Further, the data in Table 1 confirm that the objective in breeding Tahora, *viz* to improve upon the persistence of Huia in intensively-grazed hill pasture systems (Williams 1983), was achieved. However, there were no significant effects of fertiliser treatment on the genetic composition of pastures (Table 1). Thus, hypothesis 2 is not supported.

**P nutrition characteristics of surviving plants.** There was a significant (P<0.05) effect of population on plant yield in both harvests of the glasshouse experiment, due to the lower response of the resident ecotype to added P compared to most of the other populations (Figure 1). There were no significant interactions between populations and P levels for yield from either harvest. Thus there is no evidence that genetic differentiation for P nutrition characteristics occurred during eight years of population growth and development in the field, and hypothesis 3 is also not supported.

## CONCLUSIONS

These results suggest that, within populations, selection forces do not favour plants with greater ability to acquire or utilise P in this hill environment. The finding that the medium-leaved cultivar Huia failed to persist suggests that defoliation stress is the dominant selection force in operation. Compared to small-leaved plant types such as Tahora, Huia is less able to position leaves below grazing height in the face of continual, frequent defoliation (Sheath and Hodgson, 1989), and thereby maintain a positive carbon economy (Korte and Parsons, 1984). There appear to be limited prospects for altering the P response characteristics of white clover populations in intensively-grazed hill pastures through intraspecific selection for variation in P nutrition characteristics.

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**Table 1**
Percentage of plants in white clover populations based on Grasslands Tahora ('Tahora-sown') or Grasslands Huia ('Huia-sown') cultivars and receiving different fertiliser treatments that were true-to-type for the sown cultivar four (1990 sampling) or eight (1994 sampling) years after sowing.

| | Year of sampling | | | |
|---|---|---|---|---|
| | 1990 | | 1994 | |
| Fertiliser treatment [1] | Tahora-sown | Huia-sown | Tahora-sown | Huia-sown |
| Control | 42.5 | 41.1 | 33.4 | 13.6 |
| RPR | 62.3 | 36.5 | 50.4 | 8.9 |
| PAPR | 60.7 | 49.8 | 58.9 | 13.2 |
| Blend | 65.3 | 46.0 | 81.9 | 19.1 |
| Significance of main effects | | | | |
| - fertiliser | NS | | NS | |
| - pasture type | P<0.05 | | P<0.001 | |

[1] see text for description

**Figure 1**
Yield response (gDM per pot) of Grasslands Tahora and Grasslands Huia white clover plants originating from different field populations, or from standard seedlines, to added P: a) first harvest (47 days growth); b) second harvest (21 days regrowth).
Tahora ex control treatment
Huia ex control treatment
Tahora ex Blend treatment
Huia ex Blend treatment
Tahora ex standard seedline
Huia ex standard seedline
Resident ecotype