# Genetical and environmental effects on white clover root growth and morphology

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## Abstract

The root systems of white clover (*Trifolium repens* L.) populations collected from sites differing in drought stress, edaphic stresses or defoliation severity were compared. White clover populations adapted to dryland sites were more taprooted than populations from moist environments. Populations adapted to low-P soils tended to be finer-rooted than populations from high-P soils. Aluminium-tolerant populations selected on the basis of shoot growth had greater root growth than Al-susceptible populations when grown in Al-toxic soil. Root structure of white clover has important adaptive significance aiding growth and survival in stressful environments.

***Additional key words:*** *root systems, drought, aluminium-tolerance, phosphorus, root/shoot ratio, taproot, root length, root number.*

## Introduction

Root structure and function has a potentially important influence on growth and survival of plants particularly in environments characterised by drought and low soil fertility. White clover (*Trifolium repens* L.), the most important legume of pastures in New Zealand, is found in a wide range of environments and is often subjected to such environmental stresses. Genetic variation within white clover is considerable for root structure and function (Caradus, 1990).

The aim here is to determine firstly the effect of natural selection on root growth and morphology among lines of white clover of varying origin, and secondly the effect of environmental variables on root characters and whether the effect varies among white clover lines of different origin. The results will contribute to an understanding of the adaptive value of the root characters examined.

## Method and Materials

### Comparison of populations from dry and moist sites

White clover populations were collected from north-facing and south-facing aspects (slopes between 20° and 30°) on three dry hill country (average rainfall less than 760 mm per annum) and three wet hill country (average rainfall greater than 1400 mm) farms in the Wairarapa. The root systems of these populations were compared with those of Huia, Tahora and Whatawhata Early Flowering by growing seedlings in field tiles (9 cm diameter x 37 cm deep) sunk 30 cm into the ground, as described by Caradus *et al.* (1990). There were 10 replicate plants of each line. After 81 days' growth plants were removed, soil was washed from roots and taproot diameter and root and shoot dry weights were measured.

### Comparison of populations from soils with adequate and deficient levels of phosphorus

Four white clover populations were collected from the Rothamsted Park Grass experiment; two from P-rich soils and two from P-deficient soils. They were vegetatively propagated and planted into a low-P soil (1 ppm Olsen P) in 1m x 1m plots. Superphosphate was applied at two rates, either 12 or 125 kg P/ha. There were two replicates. Plots were defoliated infrequently by cutting.

After 1 year's growth, 6- cm diameter cores were taken to a depth of 10 cm during spring and early summer. There were three samples at 6-weekly intervals with two cores taken per plot. Soil was washed from roots and roots were separated into three categories: fine (diameter <1 mm) coarse (diameter >1 mm ≤2 mm) and very coarse (diameter >2 mm). Root length and number in each category were recorded. Leaf, stolon and root components were dried and weighed. A full description of the populations and methods is given by Caradus and Snaydon (1986).

**Root growth of white clover genotypes selected for tolerance and susceptibility to aluminium**

A Wainui silt loam with a pH of 5.0 and bicarbonate-extractable-P level of 7 mg/kg, to which aluminium (Al) was added, was used to select genotypes of Huia white clover for tolerance and susceptibility for Al based solely on shoot growth (Caradus *et al.*, 1987). Four genotypes were selected for tolerance to Al and three genotypes for susceptibility to Al. Pair crosses were made between two of the Al-tolerant genotypes and two of the Al-susceptible genotypes. The seven selected genotypes and two progeny lines were compared with Huia and *Lotus pedunculatus* cv. Maku in soil containing 400 g Al/kg soil. There were three plants per 450 ml pot and four replicates. After 12 week's growth in spring plants were harvested, shoots were separated from roots and both components were dried and weighed.

Data were log-transformed before analysis of variance.

**Effect of soil pH on the rooting patterns of white clover populations**

Eight-week-old stolon tips of five white clover populations (two from high pH and two from low pH treatments of the Rothamsted Park Grass Experiment, plus a Huia seed line) that had been previously rooted in phosphorus (P) -deficient sand were transplanted into glass-fronted containers (11 x 7 x 45 cm high) containing either acid soil (pH 4.5, 5 ppm $NaHCO_3$ extracted P) or calcareous soil (pH 7.1, O ppm P) in spring and grown outside. The containers were positioned at an angle of 15° so that roots would grow toward the glass surface, which was covered by a black plastic shield.

There were four plants per container, the two nearest the glass front being planted 1.5 cm from the glass.

Two levels of superphosphate were applied to the soil surface, one equivalent to 10 kg P/ha and the other 200 kg P/ha. All containers received 100 kg K/ha, added as muriate of potash, 50 kg Mg/ha, added as magnesium sulphate, and *Rhizobium trifolii* inoculant. There were two replicates. The glass front of each container was divided into eight zones 5 cm deep. The number of root tips visible in each zone was counted after 3, 5, 7 and 9 weeks. After the final root count, the glass fronts were removed and the soil profile cut into 5 cm depths; roots were washed out in a 2 mm sieve and shoots removed from roots in the top 5 cm zone, for dry weighing.

## Results and Discussion

**Drought tolerance and root structure**

White clover populations collected from, and presumably adapted, to dryland sites were larger leaved and had greater taproot diameters than populations collected from wet sites (Table 1). Similarly, taproot diameter of populations was significantly and negatively correlated with annual average rainfall at each site and the percentage water in soil samples taken at the time of population collection (Table 2). A previous study comparing dryland populations collected from throughout New Zealand with bred lines showed that some populations had larger and more numerous taproots than was expected on the basis of their shoot type (Caradus and Woodfield, 1986).

Root/shoot ratio did not differ between populations from dry and wet sites (Table 1) but there was a suggestion that root systems of populations from drier sites were larger than those from wet sites (Table 2). A study at a dryland site in South Africa has shown that high yields of white clover cultivars were associated with both number of nodal taproots per unit of stolon length and total root mass (Smith and Morrison, 1983). The cultivar that gave the highest yields under their dryland conditions had the greatest weight of nodal taproot and also the greatest weight of fibrous root. Thomas (1984) comparing cultivars S184 and Olwen found that reduction in root density due to drought did not reduce their ability to recover from drought, but the greater root density of S184 compared with Olwen may have made S184 more efficient at absorbing fresh application of water and nutrients, and hence have had some effect in improving its rate of growth.

While in the present study there were no significant differences between populations collected from drier north-facing aspects compared with moister south-facing aspects, a previous study (Caradus, 1981) has shown that moist hill country populations from north-facing aspects have significantly more fibrous root than those from south-facing aspects.

High root length, volume and density and more extensive rooting at depth have been associated with drought tolerance in a number of other species including rice (Parao *et al.*, 1976), wheat (Hurd, 1971), bean (White and Castillo, 1989), soybean (Raper and Barber, 1970) and maize (Lorens *et al.*, 1987).

**Edaphic tolerance and root structure**

Populations collected from, and presumably adapted to, low-P soils (Caradus and Snaydon, 1986) had larger and finer root systems (Table 3). Low-P populations had

**Table 1. Comparison of white clover populations collected from 'wet' and 'dry' farms. Values are means of 6 populations. Data from Caradus *et al.* (1990).**

| | Farm Type | | | |
|---|---|---|---|---|
| | Dry | Wet | Significance | $LSD_{0.05}$ |
| Leaflet width (mm) | 10.6 | 9.2 | * | 1.0 |
| Taproot diameter (mm) | 3.3 | 2.9 | * | 0.3 |
| Shoot dry weight (g) | 3.1 | 2.1 | ns | - |
| Root dry weight (g) | 0.9 | 0.6 | ns | - |
| Root/shoot ratio | 0.33 | 0.32 | ns | - |

**Table 2. Correlation of plant characters with collection site variables of 12 white clover populations, df=10. Significant (P<0.05) coefficients are underlined.**

| | Leaflet width | Taproot diameter | Shoot dry weight | Root dry weight | Root/shoot ratio |
|---|---|---|---|---|---|
| Average annual rainfall | -0.47 | <u>-0.63</u> | -0.44 | -0.55 | -0.34 |
| Percent water in soil sample* | -0.47 | <u>-0.61</u> | -0.54 | <u>-0.59</u> | 0.12 |
| Soil Olsen P | 0.33 | 0.46 | <u>0.64</u> | <u>0.66</u> | -0.08 |
| Soil pH | <u>0.59</u> | 0.53 | 0.24 | 0.30 | 0.23 |
| Soil % N | -0.52 | -0.34 | -0.04 | -0.06 | -0.16 |
| Soil % organic matter | <u>-0.61</u> | -0.57 | -0.35 | -0.40 | -0.15 |
| Soil SO4-S | -0.26 | -0.09 | -0.01 | -0.01 | -0.01 |
| Soil K (meq S)% | <u>0.62</u> | <u>0.72</u> | <u>0.74</u> | <u>0.79</u> | 0.20 |
| Soil Ca (meq %) | <u>0.67</u> | 0.57 | 0.37 | 0.43 | 0.15 |
| Soil Mq (meq %) | <u>0.65</u> | 0.49 | 0.44 | 0.45 | -0.03 |

* at time of collection in autumn

**Table 3. Root morphology of Rothamsted populations collected from high-P and low-P soils when grown in pure clover swards. Values are means of three harvests, taken using 283 $cm^3$ cores in spring and early summer of the second year's growth, and two P levels.**

| | Population Group | | | $LSD_{0.05}$ & |
|---|---|---|---|---|
| Root character | low-P | high-P | Significance | $LSR_{0.05}$[1] |
| **Total root length (cm)** | 615 | 396 | * | x1.44 |
| Proportion of root length | | | | |
| - fine (≤1 mm diam.) | 0.962 | 0.937 | ns | - |
| - coarse (>1≤2 mm diam.) | 0.037 | 0.054 | ns | - |
| - very coarse (>2 mm diam.) | 0.002 | 0.010 | * | 0.006 |
| **Total root number** | 493 | 286 | * | x1.42 |
| Proportion of root number | | | | |
| - fine root (≤1 mm diam.) | 0.986 | 0.980 | * | 0.004 |
| - coarse root (>1 mm diam.) | 0.014 | 0.020 | * | 0.004 |
| **Average root weight (mg)** | 0.39 | 0.46 | ns | - |
| **Root length/weight (cm/mg)** | 3.47 | 3.43 | ns | - |
| **Total root weight (mg)** | 183 | 123 | * | x1.38 |

[1] LSR - least significant ratio from untransformed log-data

longer roots and more roots, with a lower proportion of very coarse roots based on root length, and a higher proportion of fine roots based on root number (Table 3). While there was no difference between low-P and high-P populations for root/shoot ratio when growth at high levels of P supply, populations from low-P soils had a higher root/shoot ratio than populations from high-P soils when grown at low levels of P supply (Table 4).

A frequently branched, fine root system is more effective in absorbing nutrients and while species comparisons tend to support this there are relatively few recorded instances of such demonstrations within species (Caradus, 1990). Using similar populations to those in the present study Caradus and Snaydon (1988b) found in a nutrient solution study that populations from low-P soils had lower root elongation rates and shorter average root lengths than populations from high-P soils.

**Table 4. Effect of P supply on the root/shoot ratio of Rothamsted populations collected from high-P and low-P soils and grown in pure clover swards. Values are means of three harvests taken using 283 $cm^3$ cores in spring and early summer of the second year's growth.**

| Population Group | P level (kg/ha) 12 | 125 |
|---|---|---|
| Low-P | 0.20 (0.457)[1] | 0.17 (0.423) |
| High-P | 0.17 (0.431) | 0.18 (0.439) |
| Significance of interaction | ** | |
| $LSD_{0.05}$ | (0.012) | |

[1] arcsin square root transformed

White clover genotypes selected for tolerance to Al on the basis of shoot growth and progeny from crosses between two such genotypes had larger root systems than genotypes selected for susceptibility to Al and progeny from crosses between two of them (Table 5). There was, however, no difference between genotypes for root/shoot ratio. In another forage legume, lucerne, selection for Al-tolerance based on shoot growth alone has proven more successful than when selection was based on root size and morphology (Bouten *et al.*, 1982). The Al-tolerant selections of lucerne based on shoot growth similarly had better root growth in acid subsoil than Al-susceptible selections.

There was no significant difference between populations for total root number when grown in either acid or calcareous soil, nor was there a significant interaction between population type and soil type, when populations were grouped on the basis of pH of the soil from which they were collected. However, there was a significant (P<0.01) population type x depth interaction for root production rate during the final two weeks, because populations from alkaline soils produced fewer roots in the top of the profile and more in the bottom of the profile than populations from acid soils (Figure 1).

This apparent lack of differentiation between groups of semi-natural populations of white clover for response to soils of varying pH is perhaps not surprising since variation for Al-tolerance among similar populations from soils with large differences in pH and Al content could not be demonstrated (Caradus, 1987). Genotype differences in Al-tolerance have been shown (Caradus, 1987; Caradus *et al.*, 1987) but these could not be related to the soil-type from which genotypes were collected. A similar situation has been documented for cotton: cultivars developed on non-acid soils were more Al-tolerant than those from acid soils (Foy *et al.*, 1967). In cereals, however, level of expressed Al-tolerance is closely related to the soil type from which the population originated or was selected (Polle and Konzak 1990).

**Table 5. Comparison of genotypes selected for tolerance (T) and susceptibility (S) to aluminium and progeny lines with Huia and *Lotus pedunculatus* cv. Maku at 400 mg Al per kg soil.**

| Genotype: Line or Cultivar | Shoot dry weight (mg) | Root dry weight (mg) | Root/shoot ratio |
|---|---|---|---|
| Huia | 237 | 104 | 0.44 |
| Maku lotus | 944 | 322 | 0.36 |
| T.77 | 588 | 202 | 0.35 |
| T.97 | 439 | 195 | 0.45 |
| T.28 | 673 | 199 | 0.31 |
| T.81 | 555 | 206 | 0.39 |
| S.110 | 482 | 206 | 0.43 |
| S.129 | 262 | 74 | 0.29 |
| S.8 | 387 | 157 | 0.43 |
| T.77 x T.97 | 909 | 329 | 0.37 |
| S.129 x S.8 | 176 | 85 | 0.50 |
| Significance | *** | *** | ns |
| $LSR^1_{0.05}$ | x1.77 | x1.80 | - |

[1] LSR - least significant ratio from untransformed log-data

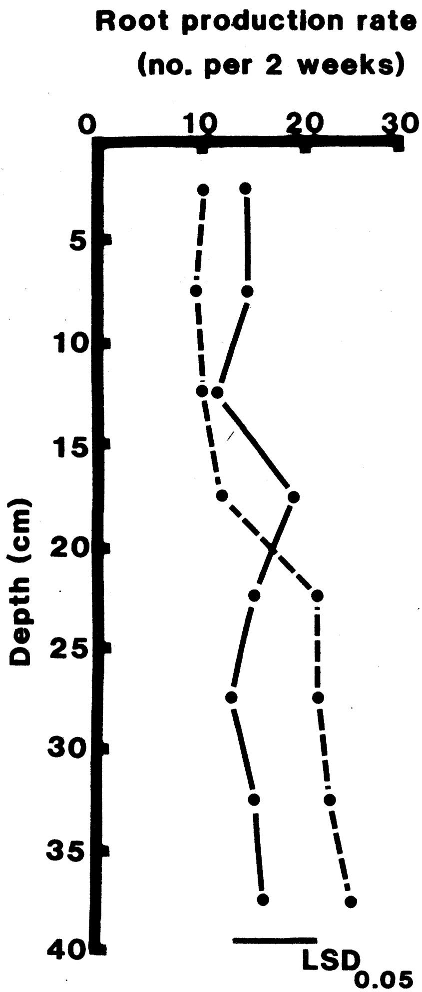


**Figure 1. Root production rate in glass-fronted containers measured on glass surface between weeks 7 and 9 of white clover populations collected from acid (————) and alkaline (- - -) soil. Each value is the mean of two P levels and two soil types.**

## Conclusion

Populations supposedly 'adapted' to drought prone-areas were more taprooted than those from wetter areas. Populations presumably 'adapted' to low-P soils had more finely branched roots than those from high-P soils. Al-tolerant genotypes selected on the basis of shoot growth, and progeny from them, had greater root growth than Al-susceptible genotypes. However, populations from low pH, high-Al soils showed no better adaptation to acid soils than populations from high pH calcareous soils. These studies have shown that root structure of white clover can have important adaptive significance aiding its growth and survival in stress environments.

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