'Breeding for Success: Diversity in Action' C.F. Mercer (ed).
Proceedings of the 13th Australasian Plant Breeding Conference,
Christchurch, New Zealand 18-21 April 2006. pp. 1053-1058.

Phenotyping and pattern analysis of key root morphological traits in a white clover mapping population.

MZZ Jahufer[1,2], *DA Care*[1,2], *SN Nichols*[1,2], *JR Crush*[1,2], *Li Ouyang*[1,2], *A Dunn*[1,2], *JL Ford*[1,2], *AG Griffiths*[1,2], *CG Jones*[1], *CS Jones*[1,2]

[1]Pastoral Genomics, P.O. Box 109185, Newmarket, Auckland, New Zealand (address for correspondence)
[2]AgResearch Grasslands, Private Bag 11008, Palmerston North, New Zealand.

Abstract. Application of molecular marker technology to identify Quantitative Trait Loci (QTL) associated with key root traits is expected to enhance white clover breeding programmes, to improve characteristics such as increased efficiency in phosphorous (P) uptake and summer moisture stress tolerance. The phenotypic diversity for a range of root morphological traits among 500 mapping family (F1) progeny was characterised using plants grown hydroponically. The root traits measured were: dry weight, length, surface area, diameter, volume, numbers of root tips and root forks. Variance component analysis indicated significant ($P<0.005$) genotypic variance among the 500 progeny for all the root traits measured. For most root traits the differences between the minimum and maximum values also indicated a large range of phenotypic variation among the 500 progeny. Pattern analysis helped identify progeny groups as well as providing a graphical representation of the association among the root traits. One of the progeny groups contained members with root trait morphology indicating the potential to enhance soil penetration per unit volume of soil, which could result in improved soil P uptake. The genotype-by-root trait matrix of individual progeny means generated from this study will be used for preliminary QTL analysis.

Introduction

The agronomic importance of root morphology and root architecture in white clover (*Trifolium repens* L.) relates particularly to their effects on the phosphate (P) nutrition of the plant. In the sward, white clover has to compete with companion grasses for nutrients such as P as well as water. Consequently, in New Zealand pastoral agriculture fertiliser P inputs are usually the single largest discretionary item in farm accounts.

Phosphate diffuses very slowly in soil (Nye 1977), and movement of P to the root surface is the rate limiting step in P acquisition by plants. Plants with finely divided roots that permeate a large volume of soil are more efficient in obtaining P than plants with coarse, unbranched roots. This has been shown experimentally for white clover grown with browntop (*Agrostis tenuis*) (Jackman and Mouat 1970).

It is known that genetic variation in root morphology is extensive in white clover, and the narrow sense heritabilities for the limited range of root traits that have been described are of sufficient magnitude to suggest a good response to selection (Caradus and Woodfield 1998). Key traits likely to contribute to an increase in soil volume explored per unit investment in root carbon include, increased root length, reduced root diameter, increased branching and longer root hairs.

For traits that are difficult to measure, the application of indirect selection, using trait-associated molecular markers is likely to improve the efficiency of applied plant breeding programs (Collard *et al.* 2005). Measurement of root morphology is complicated, time consuming and expensive. The identification of Quantitative Trait Loci (QTL), and associated molecular markers, for key root traits is likely to

significantly enhance white clover breeding to develop new cultivars with improved characteristics such as: increased efficiency in P uptake and tolerance to summer moisture stress.

This paper describes the characterisation of phenotypic diversity for a range of root morphological traits among 500 F1 mapping population progeny. The objective of the full project is to identify QTL for the breeding of new white clover cultivars for the New Zealand pastoral industries.

Materials and methods

A mapping population for root trait characterisation was developed by pair crossing two white clover genotypes with contrasting root morphology. Seed was harvested from both parents. A random sample of 500 F1 progeny, consisting of 250 seeds from each reciprocal cross, was used to generate the 500 plants which were later cloned for phenotyping.

Interaction of roots with the physical and chemical soil composition generates confounding effects which often make root experimental data difficult to interpret and extrapolate across environments. In order to generate a set of data that had a known and controlled set of environmental conditions, the progeny clones were grown using hydroponics. The hydroponic solution used in the experiment was based on the chemical composition of New Zealand pasture topsoils (Edmeades *et al.* 1985, Blamey *et al.* 1991).

The experimental layout was a row-column spatial design with repeated checks (Gleeson 1997, Kempton and Gleeson 1997). The two parental genotypes that were crossed to generate the F1 mapping population were used as the repeated checks (50 clones of each parental genotype). The 100 checks (in total) were distributed among the 500 progeny. This experimental design facilitated phenotyping of 500 progeny, which would have been difficult to undertake had a design with replication been used.
The time from establishment of the progeny clones in the hydroponic system to the collection of material for root measurements was four to five weeks. The traits measured were: ***SD***, shoot dry weight (g), ***RD***, root dry weight (g), ***RL***, root length (cm), ***RS***, root surface area (cm^2), ***RDI***, root diameter (mm), ***RV***, root volume (cm^3), ***RT***, No. root tips, ***RF***, No. root forks, ***TL***, ratio RT/RL (no./cm), ***TRD***, ratio RT/RD (no./mg), ***FL***, ratio RF/RL (no./cm), ***FRD***, ratio RF/RD (no./mg), ***RLRD***, ratio RL/RD (cm/mg), ***STD***, stolon dry weight (g). At the time of root trait measurement, clonal shoot and stolon dry weight were also recorded.

The data were examined using the variance component analysis procedure, REML option, in GenStat 6.2 (2002). A mixed linear spatial model was used in the analysis using the REML algorithm. The combination of variation across columns, rows and also the variation across the clonal checks, enabled adjustment of the genotype means (Best Linear Unbiased Predictors BLUP's), for spatial variation. The resulting genotype-by-trait BLUP matrix was used for pattern analysis (Watson *et al.* 1995, Kroonenberg 1994, Gabriel 1971).

Results and discussion

There was significant ($P<0.005$) genotypic variance among the 500 progeny for all the root morphological traits, as well as stolon dry weight (Table 1). Although measurement of root trait morphology was the main objective of the study, shoot development, during the time allocated for the establishment of roots, was also

measured and is presented as dry weight (STD). Shoot dry weight (STD) did not vary significantly (*P>0.05*) among the 500 progeny (Table 1). This could have been due to the relatively short period (4 to 5 weeks) of growth of the progeny clones before this trait was measured. For most root traits the differences between the minimum and maximum values also indicated a large range of phenotypic variation among the progeny. The two parents of the mapping population showed significant differences (*P<0.05*) between each other for all the root traits measured. Compared with parent 2, parent 1 had higher expression for all root traits, except for the traits RDI, TL and FL.

Table 1. Means, maximum and minimum and genotypic variance components (σ^2_g) with associated standard errors (±SE), for the traits measured on the 500 white clover F1 mapping population 1 progeny and their two parents.
Traits: *SD*, shoot dry weight (g), *RD*, root dry weight (g), *RL*, root length (cm), *RS*, root surface area (cm^2), *RDI*, root diameter (mm), *RV*, root volume (cm^3), *RT*, No. root tips, *RF*, No. root forks, *TL*, ratio RT/RL (no./cm), *TRD*, ratio RT/RD (no./mg), *FL*, ratio RF/RL (no./cm), *FRD*, ratio RF/RD (no./mg), *RLRD*, ratio RL/RD (cm/mg), *STD*, stolon dry weight (g).

	SD	RD	RL	RS	RDI	RV	RT
Progeny mean	0.0831	0.0130	253	33	0.4432	0.3526	176
Minimum	0.0728	0.0076	144	18	0.4014	0.2245	106
Maximum	0.1082	0.0298	636	91	0.5353	0.8395	407
Parent 1	0.0883	0.0149	328	40	0.4014	0.4004	221
Parent 2	0.0779	0.0111	179	26	0.4850	0.3047	130
Parental difference	n.s.	0.0038	149	14	0.0836	0.0957	91
σ^2_g	1595	$*2.26\times10^{-5}$	$*103.45\times10^{2}$	*175	$*1.2\times10^{-3}$	*0.01	*4171
±SE	1275	0.53×10^{-5}	28.25×10^{2}	37	0.78×10^{-3}	0.003	1065
l.s.d (0.05)	n.s.	0.002	50	6	0.02	0.06	32
	RF	TL	TRD	FL	FRD	RLRD	STD
Progeny mean	627	0.8035	15	1.9766	36	19	0.0078
Minimum	97	0.6149	12	0.7880	5	14	0.0058
Maximum	2914	1.4117	33	6.2250	155	48	0.0156
Parent 1	263	0.7175	17	1.9090	40	23	0.0087
Parent 2	130	0.8896	12	2.0440	33	15	0.0069
Parental difference	133	0.1721	4	0.1350	7	8	0.0018
σ^2_g	$*154.25\times10^{3}$	*0.05	*11.7	*0.51	*214	*16.5	$*5.7\times10^{-6}$
±SE	10.7×10^{3}	0.02	5.2	0.05	19	4.4	2.6×10^{-6}
l.s.d(0.05)	30	0.10	1.8	0.34	6.8	2.0	0.001

**(P<0.005), n.s., non-significant*

Pattern analysis

Pattern analysis enabled a graphical summary of the large 500 mapping family (F1) progeny-by-root trait (12 traits) matrix to be generated. The resulting biplot, figure 1, enabled examination of the patterns of variation among the 500 progeny based on the 12 traits, the association among the traits and also identification of novel genotypes.

The clustering procedure in pattern analysis helped identify 6 progeny groups (Fig. 1). The groups 1, 2, 3, 4, 5 and 6 consisted of 13, 237, 93, 5, 43 and 111

individuals, respectively. Group 1 consisting of 13 individuals had the highest mean root dry weight, length, surface area, volume, and numbers of tips and forks (results not presented). This group had a low mean root diameter. Parent 1 occupied group 6 and Parent 2 group 3.

Principal component analysis of the 500 progeny and their two parents, based on the expression of 12 root traits, generated a biplot which provided a graphical summary of the complete data set (Fig. 1). The biplot was further enhanced by the allocation of symbols to indicate the 6 progeny groups generated from cluster analysis. While progeny group 2 occupied an intermediate position relative to the directional vectors in the biplot, groups 1, 3, 4 and 5 contained progeny that had above population mean expression for the traits represented by the adjacent vectors. As the association among directional vectors is represented by the angle between them, the smaller the angle the stronger the positive correlation and vice versa, the traits root dry weight, volume, length, surface area, and numbers of tips and forks, appeared to have a strong positive correlation among each other (Fig.1). The position of group 1 relative to the directional vectors for these traits indicated that this group consisted of progeny with high expression for the traits. These traits were negatively correlated with root diameter (RDI) (Fig. 1).

Group 1 contained plants with root systems that were characterised by low root diameter, large numbers of forks and tips, high root volume and length. The root dry weight of members of group 1 was also high in comparison to other groups. In terms of pedigree of the members of group 1, nine of the thirteen progeny were produced by parent 1. Whether this is a result of maternal effects will be examined further. The levels of expression of root traits by the plants in group 1 indicate that their root system morphology has the potential to enhance soil penetration per unit volume, resulting potentially in a positive impact on P uptake.

The phenotypic mean genotype-by-root trait data matrix generated from this experiment will be combined together with the microsatellite genotypic data from the mapping population, for QTL identification. Once the QTL-associated markers for the root traits have been identified, the members of group 1 could be used to confirm marker genotypes associated with high levels of trait (RD, RV, RT, RF, RS and RL) expression. Progeny in group 1 could also be pair crossed to other white clover plants with different root morphology and superior above ground plant type, to generate 'bridging' populations that could be used to test the stability of the QTL.

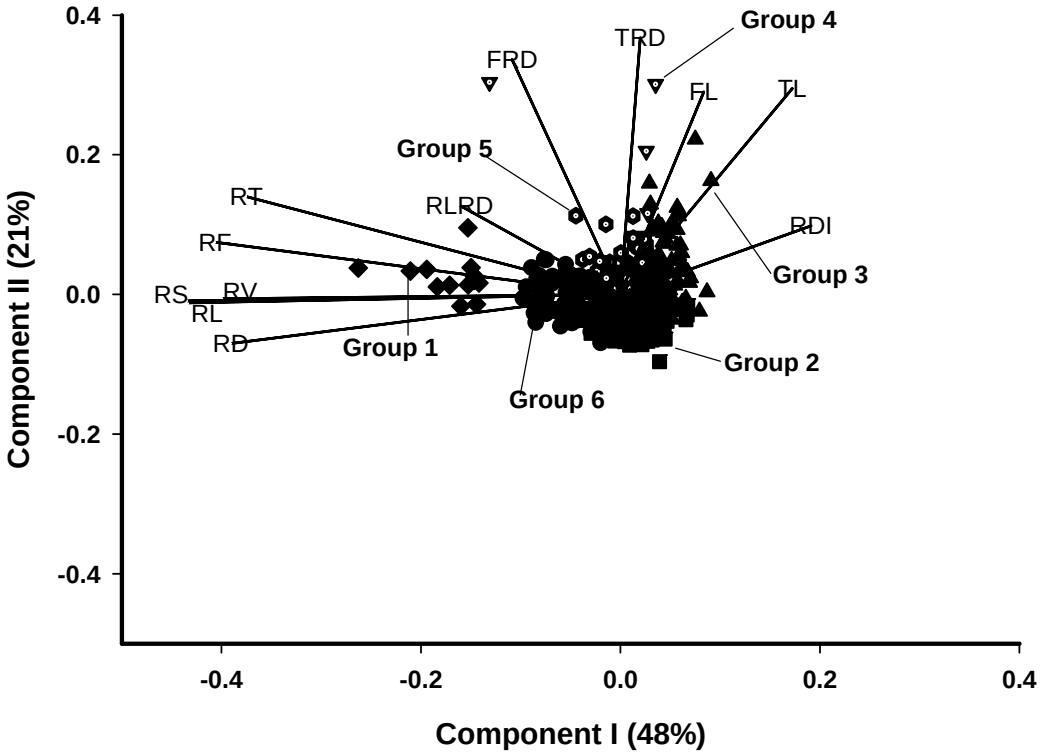

Fig. 1. Biplot generated using genotype root trait means from 500 white clover mapping population 1 progeny. Components 1 and 2 account for 48% and 21% of total variation, respectively. The symbols indicate progeny groups 1 to 6. The directional vectors represent the traits: *RD*, root dry weight (g), *RL*, root length (cm), *RS*, root surface area (cm^2), *RDI*, root diameter (mm), *RV*, root volume (cm^3), *RT*, No. root tips, *RF*, No. root forks, *TL*, ratio RT/RL (no./cm), *TRD*, ratio RT/RD (no./mg), *FL*, ratio RF/RL (no./cm), *FRD*, ratio RF/RD (no./mg), *RLRD*, ratio RL/RD (cm/mg).

Acknowledgment

The authors wish to acknowledge the financial support of Pastoral Genomics, through which this research project was conducted.

References

Blamey FPC, Edmeades DC, Asher CJ, Edwards DG, Wheeler DM 1991. Evaluation of solution culture techniques for studying aluminium toxicity in plants. *In*: 'Plant soil interactions at low pH'. (Eds. R.J. Wright, V.C. Baligar, R.P. Murrmann) pp. 905-912 (Dordrecht, Kluwer Academic Press).

Caradus JR, Woodfield DR 1998. Genetic control of adaptive root characteristics in white clover. *Plant and Soil 200*: 63-69.

Collard BCY, Jahufer MZZ, Brouwer JB, Pang ECK 2005. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. *Euphytica 142*: 169-196.

Edmeades DC, Wheeler DM, Clinton OE 1985. Corrigenda - The chemical composition and ionic strength of soil solutions from New Zealand topsoils. *Australian Journal of Soil Research* 23: 151-165.

Gabriel KR 1971. The biplot graphical display of matrices with application to principal component analysis. *Biometrika* 58: 453-67.

GenStat. 2002. GenStat for Windows. Release 6.1. Sixth Edition. VSN International Ltd., Oxford.

Gleeson AC 1997. Spatial analysis. In 'Statistical Methods for Plant Variety Evaluation' (Eds RA Kempton and PN Fox) pp. 68-85 (Chapman and Hall, London).

Jackman RH, Mouat MCH 1970. The effect of browntop (*Agrostis tenuis* (Sibth.) and increasing phosphorus deficiency on the growth of white clover (*Trifolium repens* L.). In '*Proceedings of the XI International Grassland Congress'*, 354-357.

Kempton RA, Gleeson AC 1997. Unreplicated trials. In 'Statistical Methods for Plant Variety Evalution'. (Eds. R.A. Kempton and P.N. Fox) pp. 86-100 (Chapman and Hall, London).

Kroonenberg PMK 1994. The TUCKALS line: A suite of programs for three-way data analysis. *Computational Statistics and Data Analysis 18*: 73-96.

Nye, PH 1977. The rate-limiting step in plant nutrient absorption from soil. *Soil Science* 123**:** 292-297.

Watson SL, DeLacy IH, Podlich DW, Basford KE 1995. GEBEI An analysis package using agglomerative hierarchical classificatory and SVD ordination procedures for genotype x environment data. *Centre for Statistics Research Report*, Deparment of Agriculture, The University of Queensland, Australia.