Genetic variation captured in Dieback Resistant Jarrah clones

Report to ALCOA Ltd

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Conserving the nature of WA

29 November 2004

Executive summary

Dieback Resistant Jarrah clones have been developed, and established in seed production orchards as sources of resistant seed for rehabilitation of forest sites. A genetic analysis of the clones in the Dieback Resistant Jarrah program was undertaken to assess the identity of the different samples of clonal germplasm, and to investigate the level of diversity captured in the clonal program compared to that in the natural populations of the species.

Genetic analysis of 119 samples representing accessions of 70 clones from four germplasm sources, tissue culture, Pinjarra Seed Orchard, Marrinup Orchard, and mother plants from the Huntley Orchard, was undertaken using RFLP loci. The identity of the clonal samples was determined by comparison of genotypes, and the level of diversity in the clones was compared to the level of diversity identified in a previous study of natural populations.

The study showed most clones had identical genotypes between different accessions but there was a lack of similarity between different accessions in 25% of clones. Many of these (14% of total clones) showed differences at one or two loci and most likely represent some effect of the tissue culture process on the pattern of variation at these loci. The remainder of the clones (12%) showed differences at a large number of loci and most likely represent clerical error in mis-labelling of samples.

There was a high level of genetic diversity captured in the Dieback Resistant Jarrah clones compared to a genetic survey of natural populations, and the clones were not differentiated from a sample of individuals from the natural populations.

Introduction

Jarrah (*Eucalyptus marginata*) is a long-lived hardwood species, endemic to Western Australia and is the dominant species through a large part of the forested area of the south-west. Jarrah is susceptible to the root pathogen *Phytophthora cinnamomi*, which causes dieback disease in many species in the south-west forests of Western Australia. Dieback has a significant impact on the jarrah forest and management of the impact and spread of dieback is major aspect of forest management strategies. Forest management and regeneration after tree harvesting is usually via natural revegetation, but bauxite mining also occurs within jarrah forests and these mining sites are generally rehabilitated using seed stocks (Wheeler *et al.* 2003). Some resistance to *P. cinnamomi* has been observed in jarrah and a genetic basis for this resistance has also been identified (McComb *et al.* 1994, Stukely and Crane 1994). Seed resistant to infection by *P. cinnamomi* would be the first choice for the rehabilitation of mining sites as well as for the regeneration of forest in areas badly affected by the pathogen. Clonal lines with *P. cinnamomi* resistance have already been selected and used to establish seed orchards to provide such resistant seed.

The design of seed orchards is important if the quality of seed produced is to effectively meet the objectives of the planting. In seed orchards for production plantations the main objective is to provide seed that is representative of the selection applied in the breeding program. In the case of jarrah dieback seed orchards, the seed will be used to rehabilitate forest areas, and therefore the objective is not only to provide a source of dieback resistance seed, but to ensure that the seed is representative of the genetic diversity in the jarrah forest. Selection of breeding material from restricted areas or establishment of breeding material from limited collections can lead to loss of genetic diversity (El-Kassaby 2000). For example, assessment of the Subjanjeriji seed orchard of *Acacia mangium* showed that only 56% of the total gene diversity of the species had been captured in the orchard as it had been established with collections from a limited area of the species distribution (Butcher *et al.* 1996). Estimates of the level of diversity captured in breeding programs based on the regions from which germplasm collections have been made, were 36% for *A. aulacocarpa*, 67% for *E. pellita*, but 81% for *A. auriculiformis* (Moran *et al.* 2000). The level and structuring of diversity in natural populations is an important determinant of the source area for germplasm collection if such collections are to represent the species diversity (Moran *et al.* 2000). Good preliminary genetic studies and careful selection of germplasm can mean that a highly varied and representative seed orchard can be established with a limited number of founding individuals (Marcucci Poltri *et al.* 2003). An earlier study into the population genetics of jarrah in the south-west of Western Australia (Wheeler *et al.* 2003) analysed 30 nuclear RFLP loci in 15 populations from across the species range. Moderate levels of genetic diversity were detected, but there was little genetic differentiation among the populations.

The overall aim for jarrah forest rehabilitation would be to provide seedlings that were resistant to dieback and were as genetically diverse as natural jarrah populations. This study conducted a genetic analysis of the clones in the Dieback Resistant Jarrah program to assess the identity of different accessions of clonal germplasm, and to investigate the level of diversity captured in the clonal program.

Materials and Methods

Plant material

A total of 119 clonal samples were analysed for variation at 27 nuclear RFLP loci. These 119 samples were made up of accessions from 70 clones (Table 3), with replicate samples being obtained from four different sources, tissue culture (T, n = 52), adult trees planted in the Marrinup Orchard (A, n = 20), Pinjarra Seed Orchard trees (P, n = 33) and mother plants in the Huntley Orchard (M, n = 14). The tissue culture samples were obtained as subcultures from the ALCOA tissue culture laboratory at Marrinup, and leaf samples of trees from the Marrinup, Pinjarra and Huntly orchards were provided by ALCOA. The majority of the clones originated from the northern jarrah forest and 14 originated from the southern jarrah forest.

DNA was extracted from leaves using the protocol described in Byrne *et al.* (1998), with addition of sodium sulfite in the extraction buffer (Byrne *et al.* 2001, Wheeler *et al.* 2003). Sample DNA was then digested with two restriction enzymes, *Bgl*II and *Eco*RV, Southern blotted and hybridised with 25 RFLP probes selected from the eucalypt genetic linkage map (c113, c116, c135, c136, c170, c299, c333, c395, c411, c451, g059, g067, g086, g095, g099, g142, g154, g174, g183, g195, g233, g243, g250, g256, g261; Byrne *et al.* 1995). Restriction digestion and hybridisation followed the protocol of Byrne and Moran (1994) and the probe plasmids were amplified and labelled with ^{32}P using the random priming method.

Data analyses

The resulting banding patterns from the 25 probes revealed 27 loci that were interpreted according to a Mendelian multi-allelic model. The fidelity of the genotypes of the clones represented by different accessions was identified by directly comparing the genotypes of the samples.

Standard genetic diversity indices (number of alleles per locus, percentage polymorphism, and observed heterozygosity) were calculated using BIOSYSL (Swofford and Selander 1981) for the clone samples as a whole, and by the origin of the germplasm sampled.

The genetic diversity captured in the clones was compared to that represented in a genetic analysis of 15 natural populations by comparison with data obtained in a previous study (Wheeler *et al.* 2003). The genetic indices (number of alleles per locus, percentage polymorphism, and observed heterozygosity) for the clone samples were compared to that captured in the sampled individuals from the natural populations treated as a whole, and compared to the mean of the population values. Total gene diversity (H_T), F_{ST} estimation and differences in allele frequencies (with correction for multiple tests) were calculated by FSTAT (version 2.9.3.2, Goudet 2001). Genetic distance (Nei's unbiased estimation) between the clones and the natural sample were calculated using Popgene (version 1.3.1, Yeh *et al.* 1999). Analysis of molecular variance (AMOVA) was carried out using GenAlEx (version 5.1, Peakall and Smouse 2001).

The relationships between clones and individuals from the natural populations was represented using multidimensional scaling in two-dimensions with the statistical package SPSS for Windows (release 12.0.2), using Euclidian distances between the samples. The program can only compute up to 100 cases for the multidimensional scaling so 100 samples were selected from the total of 231 (tissue culture, Pinjarra Seed Orchard and natural populations combined) by choosing 50 clonal samples (44 tissue culture samples and six Pinjarra Seed Orchard samples, the only Pinjarra Seed

Orchard samples that were not duplicated by a tissue culture sample) and 50 natural population samples (every population was still represented, but only by three or four samples). Euclidian distances between the samples were calculated based on their allelic genotype.

Results

Fidelity of clone germplasm

Out of the 70 different clones, 47 were represented by replicated samples obtained from different origins (45 individuals represented by two sample origins, and two by three different sample origins) (Table 1). Of these 47 individuals, 29 (61.7%) had identical genotypes between germplasm obtained from different sources, including the two clones where germplasm was sampled from three different origins. The remaining 18 clones (25.7%) were represented by samples with different genotypes (Table 1). For ten of these clones (14.4% of total) the samples showed different genotypes at only one locus (eight clones) or two loci (two clones). In eight of the clones (11.8% of total 70) the samples had different genotypes at numerous (seven or more loci) loci.

Of the ten clones that had differences between samples at only one or two loci, five (50%) of these involved the locus c299. In addition, 90% (all except clone number 44) involved a sample originating from tissue culture. In most cases the differences between the genotypes of the samples for these ten clones involved similar alleles, rather than completely different alleles. In all nine cases involving tissue culture samples, seven were homozygous for the tissue culture sample and heterozygous for the paired sample, and in all but one of these cases alleles were shared between the paired samples. Of the two cases involving heterozygous tissue culture samples, one allele was shared in both cases with the paired sample. The one case that did not involve a tissue culture sample was between a heterozygous mother plant sample and a homozygous adult plant from the Huntley Orchard sample where at two loci there was one shared allele between the samples.

Table 1 Genotypic comparisons between clonal accession samples from different sources.

Sample ID#	Source of samples *	Identical genotypes between sample	Loci with differences between

		sources?	sample sources
1	T,A,P	Yes	
2	T,P	Yes	
4	T,A,P	Yes	
5	T,P	Yes	
6	A,P	Yes	
14	A,P	Yes	
15	T,P	Yes	
19	T,P	Yes	
20	T,P	Yes	
22	T,P	Yes	
23	T,P	Yes	
24	T,P	Yes	
25	T,P	Yes	
26	T,P	Yes	
28	T,P	Yes	
29	T,P	Yes	
30	T,P	Yes	
31	T,M	Yes	
32	T,P	Yes	
35	T,A	Yes	
36	A,M	Yes	
40	A,M	Yes	
48	T,M	Yes	
54	T,M	Yes	
56	T,M	Yes	
58	T,M	Yes	
59	T,P	Yes	
63	T,P	Yes	
67	T,P	Yes	
7	T,P	No	250, 256, 67, 59, 451, 154, 261(L), 299
12	T,A	No	174, 261(L), 136, 233, 250, 256, 67
18	A,P	No	142, 250, 256, 67, 116, 183, 59, 195, 154, 174, 86, 261(L), 113, 299
21	T,P	No	174, 333, 261(L), 135, 136, 395, 250, 256, 67, 183, 59, 451
51	T,M	No	154, 136, 299, 395, 250, 256, 67, 116, 183, 59, 451
60	T,M	No	86, 261(L), 113, 299, 395, 250, 256, 67, 183, 59, 451
61	T,M	No	195, 86, 261(L), 261(S), 113, 136, 183, 59
66	T,M	No	154, 261(L), 299, 256, 67, 116, 183, 59, 451
10	T,P	No	299
11	T,P	No	299
13	T,P	No	299
16	T,P	No	299. 411
42	T,A	No	299
44	A,M	No	233, 183
57	T,P	No	411
65	T,P	No	170
68	T,M	No	116
70	T,M	No	154

* T= tissue culture, A= adult plants in Marrinup Orchard, P= Pinjarra Seed Orchard, M= mother plants in Huntley Orchard.

The following samples were represented by tissue from only one source: 3, 8, 9, 17, 27, 33, 34, 37, 38, 39, 41, 43, 45, 46, 47, 49, 50, 52, 53, 55, 62, 64, 69

None of the clones that showed discrepancies between samples obtained from different sources had genotypes that matched any other clonal samples that were genotyped.

Genetic diversity analyses

The majority of the loci were polymorphic with only three loci monomorphic across all clonal sample groups. The number of alleles per locus across all groups ranged from one to 12 while the maximum number of alleles in the tissue culture and Pinjarra Seed Orchard accessions was ten and 11 respectively.

When all the clonal samples were considered together, irrespective of their origin, and compared with the combined data from the natural population study (Wheeler *et al.* 2003) it was apparent that most of the alleles found in the populations had been captured within the clones (Table 2). In total there were 170 alleles found in the natural population study, 122 (72%) of these were captured in the combined clonal sample dataset, with 107 (63%) captured in both the tissue culture and Pinjarra Seed Orchard clonal sample datasets. In addition to the alleles identical to those in the natural populations, 33 new alleles were found in the clonal samples (with 28 in the tissue culture samples and 22 in the Pinjarra Seed Orchard samples). Of the 27 loci, eight (29.6%) had 100% of the original alleles captured in the clones and there were only two loci (7.4%) where less than 50% of the natural population alleles were captured in the clones. There were 16 loci where alleles were identified in the clones that had not previously been found in the population study. When samples from tissue culture and the Pinjarra Seed Orchard were analysed alone there was a similar, but slightly lower, level of retained genetic diversity compared to that in all the clones sampled. With the tissue culture samples, seven loci (25.9%) retained 100% of the alleles in the natural populations and only four loci (14.8%) had less than 50% of the natural population alleles. In the Pinjarra Seed Orchard samples five loci (18.5%) retained 100% of the alleles and only four (14.8%) had less than 50% of the natural population alleles.

Table 2 Comparison of the presence of alleles among natural populations (Wheeler *et al.* 2003), combined clonal samples, clonal samples from tissue culture alone and clonal samples from the Pinjarra Seed Orchard alone.

Locus	No. of alleles in natural pops	Combined clonal data		Tissue culture clones alone		Pinjarra Seed Orchard alone	
		Identical to natural pop alleles (%)	New to clones	Identical to natural pop alleles (%)	New to clones	Identical to natural pop alleles (%)	New to clones
g183	6	4 (67%)		4 (67%)		4 (67%)	
g67	9	8 (89%)	3	7 (78%)	3	8 (89%)	1
g59	9	5 (56%)	1	5 (56%)	1	5 (56%)	1
c116	6	4 (67%)		4 (67%)		4 (67%)	
g256	6	6 (100%)	2	6 (100%)	1	5 (83%)	1
g250	6	5 (83%)	2	4 (67%)	1	4 (67%)	1
g233	9	7 (78%)	1	6 (67%)	1	7 (78%)	1
g142	1	1 (100%)	1	1 (100%)		1 (100%)	1
g99(L)	1	1 (100%)		1 (100%)		1 (100%)	
g99(S)	1	1 (100%)		1 (100%)		1 (100%)	
c395	4	4 (100%)		4 (100%)		4 (100%)	
c451	9	6 (67%)		4 (44%)		4 (44%)	
c299	16	11 (69%)	1	8 (50%)	1	7 (44%)	1
c136	9	7 (78%)	4	4 (44%)	4	6 (67%)	2
g261(L)	8	7 (88%)	5	6 (75%)	4	6 (75%)	5
g174	7	5 (71%)	5	4 (57%)	5	5 (71%)	2
g86	7	4 (57%)	2	4 (57%)	2	4 (57%)	2
c333	3	3 (100%)	2	3 (100%)	2	3 (100%)	2
g154	9	3 (33%)		3 (33%)		3 (33%)	
g195	4	3 (75%)		3 (75%)		3 (75%)	
c170	2	1 (50%)	1	1 (50%)		1 (50%)	1
c113	9	9 (100%)		8 (89%)		7 (78%)	
g243	2	1 (50%)		1 (50%)		1 (50%)	
g95	3	2 (67%)		2 (67%)		2 (67%)	
c135	10	5 (50%)	1	5 (50%)	1	5 (50%)	
g261(S)	7	2 (29%)	1	1 (14%)	1	1 (14%)	1
c411	7	7 (100%)	1	7 (100%)	1	5 (71%)	
Total	170	122 (72%)	33	107 (63%)	28	107 (63%)	22

Allelic diversity measures within clonal sample groups were moderate and comparable to those found in the natural population study (Wheeler *et al.* 2003) (Table 3). In fact, there was a higher proportion of polymorphic loci and significantly ($p < 0.05$) more alleles per locus with all the clonal groups when compared with the average for the natural populations. However, when the natural populations were combined the proportion of polymorphic loci was equal to the highest value obtained from the clonal samples and the average number of alleles per locus was 6.3, larger than what was found with any of the clonal groups (Table 3, Figure 1). However the number of alleles at frequencies higher than 5% and the effective number of alleles were similar between the clones and the natural samples (Figure 1). The level of rare alleles (frequency less than 5%) was lower in the tissue culture and Pinjarra Seed Orchard datasets, 51.8% and 49.8% respectively, compared with 63.0% in the combined natural population dataset. Private alleles (alleles present in only one population) accounted for the extra alleles found in the combined natural population data (Figure 1). A significantly ($p < 0.05$) higher level of observed heterozygosity (H_O) was found with the clonal data than with the average of the natural populations but not with the combined population data (Table 3).

Table 3 Descriptive genetic diversity parameters for jarrah (*Eucalyptus marginata*) samples from natural populations and dieback resistant clones. Standard errors in parentheses.

Sample	Mean sample size	Mean no. of alleles per locus	Percentage of loci polymorphic[a]	Observed heterozygosity
Mean of 15 natural populations	10 (0.0)	2.7 (0.06)	67.1	0.298 (0.007)
Combined data from 15 natural populations	145.6 (0.1)	6.3 (0.7)	88.9	0.309 (0.050)
Mother plants from Huntley	13.5 (0.3)	3.4 (0.4)	74.1	0.353 (0.054)
Adult plants from Marrinup	19.1 (0.5)	3.8 (0.4)	77.8	0.328 (0.053)
Pinjarra seed orchard	30.6 (0.7)	4.8 (0.5)	88.9	0.323 (0.050)
Tissue culture	47.6 (1.4)	5.0 (0.6)	81.5	0.309 (0.051)

a A locus is considered polymorphic if more than one allele was detected

Total gene diversity for the natural populations and the clonal samples together was $H_T = 0.366$, compared to $H_T = 0.345$ for natural populations alone (Wheeler *et al.* 2003). Nei's unbiased genetic distance was low between the combined natural population data and the tissue culture and Pinjarra Seed Orchard data (0.039 and 0.038 respectively). Genetic differentiation, F_{ST}, was low at 0.046 (SE = 0.014), when all 15 natural populations and the tissue culture and Pinjarra Seed Orchard samples were analysed. Pairwise F_{ST} values between the combined natural population data and the tissue culture or Pinjarra Seed Orchard data were low, $F_{ST} = 0.0310$ and $F_{ST} = 0.0304$ respectively. Allele frequencies were significantly different ($p = 0.05$) between the combined natural population dataset and both the tissue culture and Pinjarra Seed Orchard datasets. The analysis of molecular variation (AMOVA) showed the majority of the variance was found among individuals within populations (90%), and the other hierarchical levels accounted for low, but significantly different from zero, amounts of the genetic variation (among individuals within populations, 90% of the variation, $\Phi st = 0.10$, $p = 0.001$; among populations within groups, 2% of the variation, $\Phi sr = 0.03$, $p = 0.001$; among groups, 8% of the variation, $\Phi rt = 0.08$, $p = 0.001$).

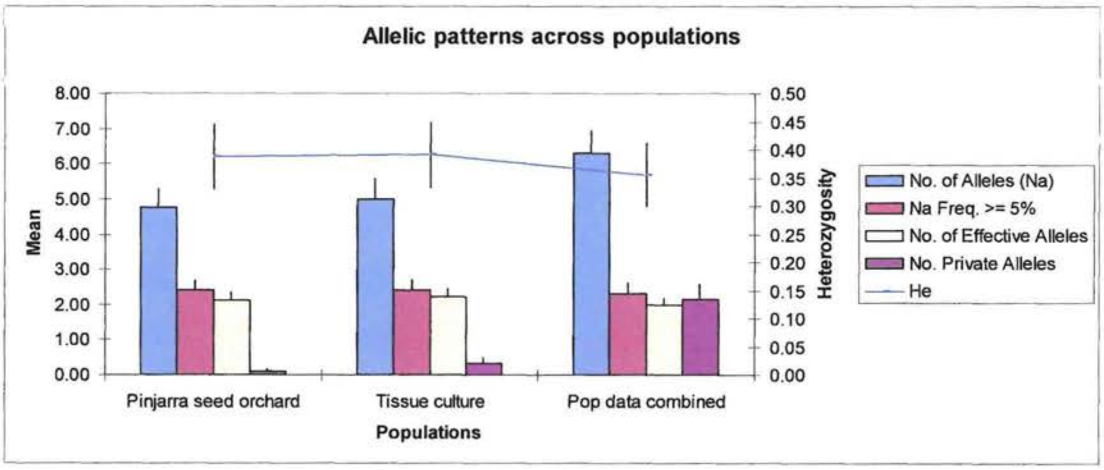

Figure 1 Allelic patterns found in two clonal sample datasets and in the combined natural population dataset.

The plot of sample relationships produced by multidimensional scaling (Figure 2) was a good fit to the data (R-squared = 0.80) and shows a cloud of samples with no definite structure.

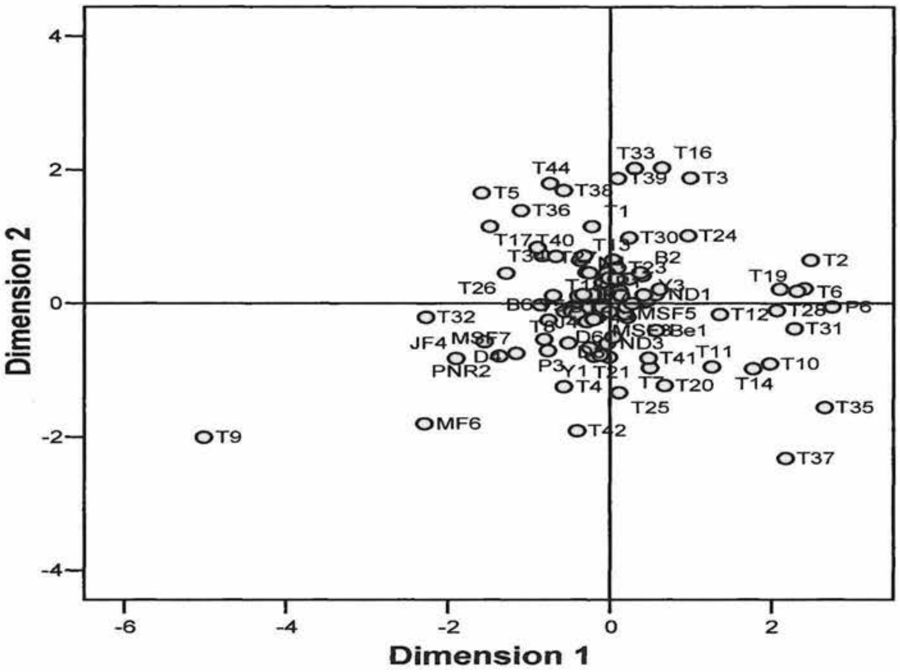

Figure 2 Two-dimensional multidimensional scaling using the Euclidian distance model among 100 jarrah samples (50 clonal and 50 natural population samples).

Discussion

Clone fidelity

The majority of the clones where germplasm from different sources was available showed identical genotypes among the different accessions, indicating that the fidelity of the clones has been maintained through the tissue culture process to deployment of the germplasm in plantations. However, a relatively large proportion of the clones showed non-identical genotypes between the different samples, indicating that the fidelity of the clones has not been maintained in these cases. Within the clones where discrepancies between samples were identified there were two groups with different patterns of discrepancy, suggesting different causes for the inconsistencies. Discrepancy in genotype of different samples for a large number of loci indicates that these samples represent different genetic individuals, and suggests that there have been errors in the identification of clone samples at some stage in the process. Clerical error in sample identification would usually occur through inaccurate labelling, and can occur at many stages of the deployment process, either in the tissue culture laboratory, in field planting, in collection of samples for analysis or in the genetics laboratory. It is unlikely that misidentification of samples has occurred in the genetic laboratory as, if that were the case, the discrepant samples would be expected to match a genotype of another processed sample, and this did not occur. A mix up in identification of samples is perhaps most likely to occur in the field planting process, where samples undergo multiple handling steps and where greater clerical recording of sample position occurs. Processes to ensure accurate identification of samples are usually present in tissue culture laboratories, so it is less likely that misidentification would occur during this stage. Errors in identification of individuals have been found in many cases where the identification of planted individuals has been checked using molecular markers, although this is not always formally reported in the scientific literature. Errors in identity of ramets from four out of ten *Eucalyptus* clones were found in an investigation by Keil and Griffin (1994) and these errors appeared to be due to mis-labelling of samples. Errors in clonal identity have also been found in sitka spruce (Van de Ven and McNicol 1995), loblolly pine (Wheeler and Jech 1992), and *Pinus sylvestris* (Harju and Muona 1989).

While some clerical identification error has been identified in these clone samples, it is likely that this error involves misidentification with other Dieback Resistant Jarrah clones, particularly the clones planted in the Pinjarra Seed Orchard, and therefore does not mean that the dieback resistance of the misidentified clones should be questioned.

The pattern of discrepancy between different samples for the second group of clones involved different genotypes at only one or two loci, and these genotypes were generally related. These differences may indicate sample identification error between close relatives (for example, full sibs), however, the clones in the Dieback Resistant Jarrah program are generally unrelated so it is unlikely that this is a valid explanation. The differences may represent mis-scoring of genotypes, however the genotypes have been independently scored by two people and re-checked, and the technique used for the analysis (RFLPs) is a robust technique which is not prone to unreliability. It is possible that the differences in genotype represent somatic mutation occurring in the tissue culture samples. Over time in tissue culture cell replication control mechanisms are relaxed and a greater degree of mutation is revealed. Somatic mutation, or somaclonal variation, defined as the genetic variation induced by in-vitro techniques, has been reported in many species (Gallego *et al.* 1997 and references therein). The level of somatic mutation during tissue culture can be quite high, up to 40% of rye plants showed variation in one study (Linacero *et al.* 2000) particularly if the tissue culture is maintained over long periods of time, although generally the level of variation is less than this, for example 16.5% in turmeric shoots (Salvi *et al.* 2001). The pattern of variation identified in the jarrah clone accessions could commonly be explained by the loss of a restriction enzyme cutting site (generating a homozygous state compared to a heterozygous state) and this could arise from a single base mutation within the cutting site. This would also be consistent with the over representation of discrepancies involving locus c299 if this locus represented a region of DNA more susceptible to mutation in the relaxed control environment of tissue culture. However, somatic mutation is often induced by a callus phase and the dieback resistant jarrah tissue culture program has not involved a callus phase. The use of hormonal treatments to induce shoot and root growth is also known to lead to changes in gene expression, and may involve processes such as DNA methylation. Changes in DNA methylation can also lead to inability of restriction enzymes to cut DNA even though the recognition site is still present. This would also be a feasible explanation for the changes in genotype that were observed between the clonal samples at a few loci. Thus, if the clones where samples showed differences for only one or two loci represent some form of soma-clonal variation, the differences between the samples are likely to involve only a few bases and do not represent major changes in the genome of the clone. Therefore, the genetic basis for the *P. cinnamomi* resistance in these clones should not be affected and would not reduce their utility for reafforestation programs.

Genetic diversity

The level of genetic diversity in the jarrah clonal samples was comparable to the diversity found in the natural populations with similar levels of polymorphic loci, heterozygosity and number of effective alleles. The majority of the alleles found in the populations were captured within the clonal groups and most of the alleles that were not present in the clonal groups were at low frequency (<0.5) in the natural populations. This meets the suggested criteria of Marshall and Brown (1975) that the aim of sampling for genetic resource conservation should be to include, with 95% probability, an allele occurring with frequency of <0.5. In addition, there were 16 loci where new alleles were also identified in the clones, but were not present in the natural population survey.

Not only was there comparable genetic diversity within the clonal datasets compared with the natural populations, when the two groups of data were directly compared they showed a high level of similarity in genetic composition. The low genetic distances and the low level of differentiation (as estimated by F_{ST}) between the combined natural populations and both the tissue culture and Pinjarra Seed Orchard samples indicate a high level of genetic similarity between these clones and the natural individuals. There were significant differences in allele frequencies between the clones and the natural individuals, but this would be a result of a sampling effect, particularly given that the majority of variation is partition between individuals within populations and the high number of rare alleles observed in the natural population data.

Implications

This study has shown that the level of diversity captured in the Dieback Resistance Jarrah clones is representative of the diversity in the natural populations. In particular both the current tissue culture collection and the clones in the Pinjarra Seed Orchard, the main sources of ongoing germplasm, have captured high levels of the species diversity and are representative of this diversity. This suggests that the use of seed produced from seed orchards derived from Dieback Resistant Jarrah clones for regeneration of jarrah forest sites will not have a negative impact on the genetic diversity present within the species, and may enhance the conservation of the genetic resources of the species.

Acknowledgements

ALCOA Ltd provided funding for this study and David Williams of ALCOA provided tissue culture and leaf samples. Bronwyn Macdonald carried out the laboratory work with assistance from Jackie Ujetz. Deryn Alpers carried out the data analysis and assisted with compilation of the report.

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