ALLOZYME ANALYSIS IN SEED AND TREE IDENTIFICATION IN NEW ZEALAND

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ABSTRACT

Allozyme analysis by electrophoresis has the potential for many practical uses in tree breeding programmes, orchard management, and seed or clone identification. Of two studies carried out in New Zealand, one was on identification of the race to which three unidentified *Pinus muricata* D. Don seedlots belonged. The other involved identification of a mislabelled seed orchard clone of *Pinus radiata* D. Don.

Keywords: seedlot identification; clone identification; electrophoresis; *Pinus radiata; Pinus muricata.*

INTRODUCTION

The technique of starch gel electrophoresis can be used to identify different genetic, or allelic, forms of the same enzyme (allozymes) and hence distinguish between different genotypes. These genetic markers can be a useful tool for distinguishing between different clones or different strains of forest trees. Such identification is possible in most forest tree species because considerable effort is usually made to incorporate a broad base of genotypes, and hence a large amount of allozyme variation, in breeding programmes. A wide variety of uses have been found in seed orchard management. Hunter (1977) used allozyme analysis to detect labelling errors in a *Pinus taeda* L. (loblolly pine) seed orchard and found that six of the 22 clones had at least one mislabelled ramet. Other studies include an assessment of pollen contamination from outside a seed orchard (Fast *et al.* 1986), a study to quantify inbreeding within seed orchards (e.g., Sorenson 1982), and a study to distinguish between different strains of plantation tree species (Millar 1983). Butcher & Fountain (1987) suggested that allozyme analysis could be used to identify graft-incompatible clones before grafting is carried out. In this paper two New Zealand examples of allozyme analysis in forestry are reported.

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MATERIALS AND METHODS

Standard starch gel electrophoresis techniques were used. Approximately 10 mg of each of the samples to be analysed were crushed in three drops of a vegetative extraction buffer, pH 8.0 (Feret 1971), to preserve the activity of the enzymes. The extracts were then absorbed on to filter paper wicks. These wicks were then inserted into a 12.5% (w/v) starch gel 70 mm wide, 230 mm long, and 10 mm thick. Approximately 90 samples can be inserted along the length of the gel. The starch gel was buffered at pH 8.5 with a tris-citrate gel buffer and the electric circuit was completed with a lithium borate electrode buffer pH 8.1 (Ridgeway *et al.* 1970). A current was then applied across the gel to separate out different allelic forms of the same enzyme along the width of the gel. Sufficient allozyme separation was usually achieved in 3–4 hours. The starch gel was then sliced horizontally into nine slices, each approximately 0.8 mm thick. Each slice could be stained for a different enzyme system. If different forms of an enzyme were present, coloured bands were seen at different different distances from the point where the wicks are inserted (Fig. 1). When no genetic variation was present at an enzyme locus, a single continuous band was seen across the gel.

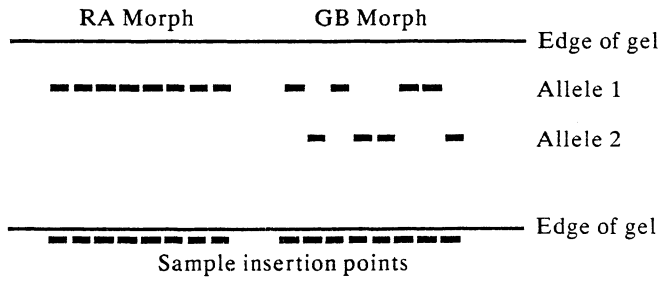

FIG. 1 — Section of starch gel after the gel has been stained for the enzyme alcohol dehydrogenase (ADH).

The samples were of two types—vegetative buds, and seeds. The vegetative tissue is diploid and so both alleles at a particular locus can be identified from one sample. The megagametophyte storage tissue inside the seed, as opposed to the developing embryo, is haploid and so only one of the alleles of the female parent tree is expressed. In order to identify both alleles at a particular locus the haploid storage tissue of eight seeds from each tree was analysed. Analysis of eight seeds means that there is less than a 0.8% chance that a second allele will be missed and that a tree will be wrongly described as a homozygote when it is actually heterozygous.

Pinus muricata

A steep latitudinal cline occurs in *Pinus muricata* in its native habitat in Northern California. Over approximately 2 km, the green foliage of the southern populations is replaced by the blue foliage of the northern populations. Millar's electrophoretic study (1983) showed that the two races have different allele frequencies at five of the loci studied. One of these, glutamate oxaloacetate transaminase (GOT-1), can be used to

distinguish between the two races. In the green race the most anodal allele (Fast) at the GOT-1 locus had a frequency of 0.97. In the blue race the frequency of this allele was 0.23. In New Zealand it is important to be able to distinguish between the two races because the blue race performs well while the green race performs relatively poorly. In the first example four seedlots of *P. muricata* were tested to determine from which race they originated. The race of the parental trees from which these seedlots were collected was unknown. Consequently it was not possible to distinguish between the seedlots on the basis of parental foliar characteristics.

The haploid storage tissue from 20 seeds of each *P. muricata* seedlot was analysed for the glutamate oxaloacetate transaminase (GOT) enzyme system (enzyme commission code E.C.2.6.1.1.) using the method described above. Enzyme stain recipes follow Pitel & Cheliak (1984). The frequencies of the two alleles at the GOT-1 locus were then compared with the frequencies of these two alleles in the two races reported by Millar (1983).

Pinus radiata

The second example concerns a mislabelled clone of *Pinus radiata.* Careful observations of the general appearance of ramets of clone 65 at Amberley seed orchard showed that two distinct morphs could be distinguished. The colour of the new spring growth, the flowering and branching habits, the time of flushing, and the number of male cones could all be used to divide the clone into two distinct morphs which have been designated RA and GB respectively. There were no ramets of intermediate appearance and no ramets which showed a different combination of the distinguishing traits. Stock material of clone 65 held at Rotorua could also be divided into the same two morphs. However, it was impossible on the basis of the general appearance of the ramets to decide if the two morphs were genetically different clones and to determine which of them was the true parent of clone 65 offspring, highly regarded for their genetic quality. Allozyme studies of the New Zealand land race of *P. radiata* have shown that 42.5% of the analysed enzyme loci were variable (Moran & Bell 1987). This means that it should be possible to identify any genetic differences between the two morphs if a large number of genetic loci are surveyed using this technique.

The haploid storage tissue of *P. radiata* seed collected from 10 F_1 progeny of the true clone 65 and seed collected from the two morphs of clone 65 in Rotorua, and vegetative buds from the two morphs at Amberley seed orchard were analysed for 15 enzyme systems: acid phosphatase (E.C.3.1.3.2.), alcohol dehydrogenase (E.C.1.1.1.1.), glucose-6-phosphate dehydrogenase (E.C.1.1.1.49.), glutamate dehydrogenase (E.C.1.4.1.3.), glutamate oxaloacetate transaminase (E.C.2.6.1.1.), isocitrate dehydrogenase (E.C.1.1.1.42.), leucine-amino peptidase (E.C.3.4.11.1.), malate dehydrogenase (E.C.1.1.1.37.), malic enzyme (E.C.1.1.1.40.), mannose-6-phosphate isomerase (E.C.5.3.1.8.), peptidase (E.C.3.4.13.11.),6-phosphogluconate dehydrogenase (E.C.1.1.1.44.), phosphoglucose isomerase (E.C.2.7.5.1.), phosphoglucomutase (E.C.2.7.5.1.), and shikimic acid dehydrogenase (E.C.1.1.1.25.). Some of these enzymes are controlled by more than one locus and so the total number of loci examined in this study was 24. All of these enzymes are variable in the New Zealand land race of *P. radiata* (Moran & Bell 1987).

RESULTS AND DISCUSSION

Pinus muricata

The results for the four seedlots of *P. muricata* are given in Table 1. The 20 alleles at the GOT-1 locus sampled from seedlot 1 contained one Fast and 19 Slow alleles. Seedlot 2 contained six Fast and 14 Slow alleles. Seedlot 3 contained only the Fast allele, and the fourth seedlot contained 18 Fast and two Slow alleles. The probabilities of the four seedlots being drawn from the blue and green races were calculated by expansion of the binomial $(0.23 + 0.67)^{20}$ for the blue race and $(0.97 + 0.03)^{20}$ for the green race (Table 2). The results show that the probability that seedlots 3 and 4 do not originate from the blue race is greater than 99.9%. The probability that seedlots 1 and 2 do not originate from the green race is also greater than 99.9%. Using the 95% confidence level (probability ≤ 0.025 in the one-tailed example given in Table 2), seedlots 1 and 2 are not excluded from the blue race and seedlots 3 and 4 are not excluded from the green race. These results show that only seedlots 1 and 2 can be considered to be from the blue race of *P. muricata.*

TABLE 1–Allele frequencies at the GOT-1 locus for the four seedlots. Sample size = 20.

Seedlot	Fast allele	Slow allele
1	0.05	0.95
2	0.30	0.70
3	1.00	0
4	0.90	0.10

TABLE 2– Probabilities of the four seedlots originating from a given race. (a) Probability of obtaining the result by chance if all seedlots originate from the blue race. (b) Probability of obtaining the result by chance if all seedlots originate from the green race.

Seedlot	Number of Fast alleles	Probability (1-tailed)
(a) All seedlots from the blue race		
1	1	0.04
2	6	0.15
3	20	1.72×10^{-13}
4	18	3.83×10^{-10}
(b) All seedlots from the green race		
1	1	2.25×10^{-28}
2	6	1.55×10^{-17}
3	20	0.54
4	18	0.12

Pinus radiata

The results from the starch gel electrophoresis of the *P. radiata* vegetative samples from the RA and GB morphs at Amberley, and the seed from the two morphs at Rotorua showed that the two morphs were genetically identical for 22 of the 24 loci analysed. Allelic differences between the two morphs were found for two enzyme

systems, alcohol dehydrogenase (ADH) and glutamate dehydrogenase (GDH). The RA morph from Amberley and Rotorua was homozygous at both loci and the GB morph was heterozygous at both loci. This shows that the two morphs are genetically different and that the morph designated RA at Amberley is identical to the morph designated RA at Rotorua. Similarly, the morph designated GB at Amberley is identical to that designated GB at Rotorua.

The F_1 progeny of the true parent were monomorphic at the GDH locus, i.e., all progeny trees were homozygous. At the ADH locus the progeny were polymorphic: three of the 10 F_1 progeny trees analysed were heterozygous and the remaining seven trees were homozygous. The allele frequencies for these two loci in the RA morph, the GB morph, and the F_1 progeny are given in Table 3.

TABLE 3–Allele frequencies for the two loci that can be used to distinguish between the two morphs

Locus	Allele	Allele frequencies		
		RA morph	GB morph	F_1 progeny
ADH	1	1.00	0.50	0.85
	2	0	0.50	0.15
GDH	1	1.00	0.50	1.00
	2	0	0.50	0

The results for the GDH locus show that the GB morph cannot be the maternal parent of the 10 F_1 progeny plants. That is, if the GB morph was the parent the F_1 progeny would have to contain both GDH alleles instead of being homozygous at this locus. At the ADH locus the progeny are polymorphic but this does not mean that the maternal parent must be heterozygous. The maternal parent could be homozygous and the second allele could come from the paternal parent. The F_1 progeny of the true parent, clone 65, were derived from open-pollinated crosses so there is no information about the paternal parents. It is possible to state, on the basis of the information from the GDH locus, that the GB morph is not the true parental clone 65. The RA morph could be the maternal parent on the basis of the electrophoresis data, although these data in themselves do not prove RA to be clone 65.

CONCLUSIONS

This study illustrates two of the many potential uses of starch gel electrophoresis in forestry research. The results show that allozyme analysis by starch gel electrophoresis can be used to distinguish between individual clones and between races of forest trees. The two examples show that the technique has practical uses in orchard and seed management.

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