GENETIC VARIATION IN ISOZYME ANALYSIS OF *ACACIA CRASSICARPA*

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NOR AINI, A. S. & CHUBO, J. K. 2003. Genetic variation in isozyme analysis of *Acacia crassicarpa*. The pattern of genetic variation of *Acacia crassicarpa* among eight provenances (four from Papua New Guinea, one from Irian Jaya and three from Queensland) was assayed using the technique of starch gel electrophoresis. Twelve enzyme systems used in the analysis were coded by 23 loci, producing heterozygosity values ranging from 0.064 to 0.100. The proportion of polymorphic loci was found to vary from 56.5 to 60.9% with an average of two alleles per locus. Factors such as the historical and ecological background, selection and reproductive biology were suggested to explain the polymorphism and heterozygosity paradox. Genetic identity of the species varied from 0.9770 between Jardine River and Olive River of Queensland to 0.9955 between Limal-Malam and Samlleberr, both from Papua New Guinea. Clustering by altitude was also observed. High values of genetic similarity showed that the provenances were closely related to each other in spite of coming from different regions.

Key words: *Acacia crassicarpa* - provenance - isozyme - genetic variation - genetic identity

NOR AINI, A. S. & CHUBO, J. K. 2003. Variasi genetik dalam analisis isozim *Acacia crassicarpa*. Pola variasi genetik *Acacia crassicarpa* bagi lapan provenans (empat daripada Papua New Guinea, satu daripada Irian Jaya dan tiga daripada Queensland) dicerakin menggunakan teknik elektroforesis gel kanji. Dua belas sistem enzim yang digunakan dalam analisis dikodkan oleh 23 lokus. Ini memberi nilai keheterozigotan berjulat daripada 0.064 hingga 0.100. Kadar polimorfisme lokus didapati berbeza daripada 56.5 hingga 60.9% dengan purata dua alel bagi setiap lokus. Faktor berasaskan sejarah dan ekologi, pemilihan dan biologi pembiakan juga disyorkan untuk menerangkan tentang paradoks polimorfisme dan keheterozigotan. Identiti genetik spesies ini berubah daripada 0.9770 di antara Jardine River dan Olive River daripada Queensland hingga 0.9955 di antara Limal-Malam dan Samlleberr, kedua-duanya daripada Papua New Guinea. Pengelompokan melalui altitud juga dicerap. Nilai identiti genetik yang tinggi menunjukkan bahawa provenans amat berkaitan antara satu sama lain walaupun berasal dari kawasan yang berbeza.

Introduction

Acacia crassicarpa is a fast-growing tree that thrives well on a variety of soil types including those that are deep siliceous and clays with impeded drainage. It occurs at latitudes ranging from 8 to 20°S with a main occurrence at an altitude of 200 m above sea-level (Turnbull 1986). Botanically, *A. crassicarpa* can grow up to 2 to 3 m

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on coastal beaches and up to 5 to 20 m in less exposed areas. It has also recorded a height of 30 m in some favourable sites. The diameter of *A. crassicarpa*'s stem rarely exceeds 50 cm. The bark is grey-brown in colour while the inner bark is red and fibrous (Pedley 1978). The wood of *A. crassicarpa* is known to be strong and durable with an air density of 710 kg m^{-3} and a basic density of 620 kg m^{-3}. In Papua New Guinea, its wood has been used for heavy construction, boat building, flooring, veneer and posts (Turnbull 1986).

Effective forest management requires a thorough understanding of many aspects, not only the biology and ecology of the species but also the genetics. Genetic studies can identify superior populations or provenances. In fact, genetic variation is essential as an important buffer to the temporal and spatial variation of potential stress factors (Bergmann *et al.* 1989) and can be manipulated by breeders for human use to achieve adaptability to varied environments and to improve desirable traits. This justifies identification, determination, managing and preserving the maximum amount of genetic variation within the species (Soule 1980, Whitmore 1980). Despite its importance, information on the amount and distribution of genetic variability, especially that concerning tropical tree species, is relatively scarce (Hamrick & Loveless 1986).

Genetic diversity within population and within species determines the rate of adaptive evolution and the extent of response in traditional crop improvement (Perez de la Vega 1993). In the past, studies on genetic variation through morphological characteristics are complex and are affected by environmental variation. These studies have been surpassed only for DNA analysis by means of the analysis of biochemical markers, such as isozymes, which are less affected, if at all, by the environmental factors. Isozymes have been extremely useful in providing knowledge of the genetic composition of populations (Perez de la Vega 1993). Isozymes as genetic markers have also facilitated the solving of practical problems encountered in forest tree breeding and genetic research (Rudin 1976).

Genetic markers derived from enzyme electrophoretic analysis have been reported on temperate and tropical trees such as *Pseudotsuga menziesii* (Yeh & O'Malley 1980, Mejnartowicz & Lewandowski 1994, Prat & Arnal 1994), *Pinus strobus* (Beaulieu & Simon 1994), *P. nigra* (Aguinagalde & Bueno 1994), *Eucalyptus* species (Moran & Bell 1983) as well as *Acacia auriculiformis, A. crassicarpa* (Moran *et al.* 1989a), *A. mangium* (Hamidi 1990), *Shorea curtisii, S. leprosula* (Daim 1993), *S. parvifolia* and *S. acuminata* (Kong 1994, Daisy 1995).

Materials and methods

Sample collection

Leaf samples from eight provenances were collected from a three-year-old *A. crassicarpa* provenance trial of six blocks at Universiti Putra Malaysia, Serdang, Selangor. Details of the provenances are given in Table 1. Samples were transported to the Physiology Laboratory in a container containing crushed ice.

Table 1 Details of eight provenances of *Acacia crassicarpa* used in this study

Plot No.	Provenance	Geographic region	Parent trees	Latitude		Longitude		Altitude (m)
				Degree	Minute	Degree	Minute	
1	Jardine River Bamaga	Queensland	15	11	23	142	22	20
2	Bimadebun	Papua New Guinea	40	8	37	141	55	25
3	Old Zim WP	Papua New Guinea	5	8	40	143	06	20
4	Bensbach	Papua New Guinea	35	8	53	141	17	25
5	Limal-Malam	Papua New Guinea	30	8	40	142	43	40
6	Samlleberr, Irian Jaya	Indonesia	5	8	40	140	00	40
7	Olive River	Queensland	5	12	19	142	18	60
8	Claudie River	Queensland	4	12	48	143	18	20

Isozyme assay

Electrophoretic run

Leaf samples were homogenised together with the buffer in liquid nitrogen and were ground into powder using mortar and pestle. The homogenate and a tracking marker were then absorbed onto filter wicks (0.6 cm × 0.4 cm, Whatman No.3) before being inserted into the prepared 10.5% starch. The gel was placed between two electrode tanks containing 500 ml of buffer. Three types of system were used, i.e. Histidine, Morpholine Citrate and Lithium, adopted from previous studies by Daim (1993), Kong (1994) and Daisy (1995) on other tropical species.

Electrophoresis was performed in a 4 °C refrigerator with an electric supply of 65mA for the Histidine and Morpholine Citrate systems and 80mA for the Lithium system for four hours. The 12 enzymes used were alcohol dehydrogenase (ADH), glucose dehydrogenase (GDH), glutamate oxaloacetate transaminase (GOT), esterase (EST), isocitrate dehydrogenase (IDH), leucyl aminopeptidase (LAP), malic enzyme (ME), 6-phosphogluconic dehydrogenase (6PGD), phosphoglucose isomerase (PGI), phaephoglucomurase (PGM), shikimate dehydrogenase (SDH) and tetrazolium oxidase (TO).

In each enzyme, the locus was labelled as fast (F), medium (M) and slow (S) according to its decreasing anodal mobility. The frequency of the allele, the number of heterozygotes per locus as well as the genetic identity between two populations were estimated using formulae adapted from Nei (1972). Phylogenetic analysis was conducted using Nei's unbiased genetic distances (Nei 1972) among provenances and a dendrogram was constructed using UPGMA cluster analysis.

Results

From the isozyme analysis, a total of 23 loci were scored with 60% polymorphism. The numbers of alleles per locus were found to range between 2.087 and 2.174 (Table 2). ADH, 6PGD and PGI were found to be controlled by two loci, with both polymorphic and monomorphic bands. EST was controlled by three loci with two

loci detected as monomorphic and one locus as polymorphic. However, GDH, LAP and ME were controlled by only one locus where both homozygote and heterozygote were observed. On the other hand, GOT was found to be controlled by one polymorphic locus and two monomorphic loci.

The proportions of polymorphic loci were similar for all provenances (0.609) except for Jardine River with 0.565. The average number of alleles per locus ranged from 2.087 to 2.174 while the observed heterozygosities ranged from 0.064 to 0.100. The genetic identities were found to range from 0.9770 to 0.9955 (Table 3).

Table 2 Summary of selected genetic diversity parameters of eight *Acacia crassicarpa* provenances

Range	Jardinne River (QLD)	Bimadebun (PNG)	Old Zim (PNG)	Bensbach (PNG)	Limai-Malam (PNG)	Samlleberr (IND)	Oliver River (QLD)	Claudie River (QLD)
Range observed heterozygosity (Ho)								
Minimum	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Maximum	0.267	0.250	0.283	0.217	0.200	0.183	0.233	0.250
Mean	0.083	0.079	0.100	0.093	0.074	0.064	0.074	0.096
S.E.	0.026	0.024	0.030	0.027	0.022	0.019	0.024	0.028
Range expected heterozygosity (He)								
Minimum	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Maximum	0.625	0.646	0.630	0.602	0.606	0.590	0.580	0.596
Mean	0.225	0.219	0.237	0.230	0.190	0.196	0.194	0.228
S.E.	0.069	0.066	0.069	0.067	0.057	0.061	0.061	0.066
Loci scored	23	23	23	23	23	23	23	23
Proportion of polymorphic loci	0.565	0.609	0.609	0.609	0.609	0.609	0.609	0.609
Average number of alleles per loci	2.087	2.174	2.130	2.174	2.130	2.130	2.174	2.174

Table 3 Nei's genetic identity among eight provenances of *Acacia crassicarpa*

Provenance	Bimadebun (PNG)	Old Zim (PNG)	Bensbach (PNG)	Limal-Malam (PNG)	Samlleberr (IND)	Olive River (QLD)	Claudie River (QLD)
Jardine River (QLD)	0.9909	0.9827	0.9917	0.9830	0.9851	0.9770	0.9883
Bimadebun (PNG)		0.9949	0.9895	0.9894	0.9900	0.9910	0.9919
Old Zim (PNG)			0.9832	0.9900	0.9886	0.9896	0.9893
Bensbach (PNG)				0.9910	0.9927	0.9860	0.9929
Limal-Malam (PNG)					0.9955	0.9915	0.9954
Samlleberr (IND)						0.9867	0.9906
Olive River (QLD)							0.9925

Discussion

Genetic polymorphism in natural population is part of the adaptive strategies of populations in heterogeneous environments. However, genetic polymorphism in a population can be affected by the physiological and evolutionary adaptive potential. An extreme environmental condition can cause a decrease in the heterozygosity level since only special genotypes can survive in such environments. Mejnartowicz (1983), Muller-Starck (1985), Geburek *et al.* (1987), Prus-Glowacki and Godzik (1995), Prus-Glowacki and Nowak-Bzowy (1992), and Scholz and Bergmann (1993) and have reported adaptations of forest trees to the environments. Moreover, genetic variation was closely related with the evolutionary potential of a species (Godt & Hamrick 1995).

In this study, the average values of the observed heterozygosity for all provenances were low, ranging from 0.064 to 0.100. Samlleberr of Irian Jaya, Indonesia, produced the lowest heterozygosity value while Old Zim in Papua New Guinea gave the highest value as indicated in Table 2. In terms of mean heterozygosities, both geographical regions were similar to those reported by Moran *et al.* (1989a). The observed heterozygosities, on the other hand, were found to fall within the range reported by Moran *et al.* (1989a) on *A. crassicarpa*, Wickneswari and Norwati (1993) on *A. auriculiformis*, and Hamidi (1990) on *A. mangium*.

The range of mean heterozygosities was higher than that reported by Wickneswari and Norwati (1993) on *A. auriculiformis* but was lower than those reported on similar and other species of the same genus, e.g. *A. auriculiformis* (Moran *et al.* 1989a), *A. mangium* (Moran *et al.* 1989b, Hamidi 1990) and *A. melanoxylon* (Playford *et al.* 1993). The values of polymorphism ranged from 58.5 to 60.8%, which were similar to those reported by Hamrick and Loveless (1989) on tropical tree species in Central America (mean 60%). This result, on the other hand, was higher than those reported by Hamrick *et al.* (1981) on conifer (0.677) as well as other Australian trees.

The low value of observed heterozygosity when compared to the expected heterozygosity suggests that trees under artificial stands tend to lose their genetic variability. This is evident where studies conducted on natural forest stands were found to give higher heterozygosity. Thus it is estimated that planting species under man-made forest might have caused a depression in the gene pool of many populations where family structure is avoided and a small probability of mating among relatives occurs (Mejnartowicz & Lewandowski 1994). This is further supported by the restricted gene flow within provenances, which enhances mating of neighbouring trees thus causing heterozygosity reduction (Kong 1994). The fact that *Acacia* populations are highly outcrossing (Moran *et al.* 1989b) and pollinated by mainly insects, especially bees and birds (Bernhardt *et al.* 1984, New 1984, Knox *et al.* 1985), further enhances this low heterozygosity factor. Such a fact is evident when only a small group of trees is involved.

Genetic identities as determined using the Nei's indices were estimated on the eight provenances. The values of genetic identities ranged from 0.9770 (Jardine River–Olive River) to 0.9955 (Limal Malam–Samlleberr). The values of genetic identities were, however, reported to be slightly higher than those recorded by Hamidi (1990) on *A. mangium* (0.9865), and Wickneswari and Norwati (1993) on *A. auriculiformis* (0.8869). From the dendrogram (Figure 1), two distinct clusters were formed, i.e. Jardine River, Claudie River, Limal-Malam, Bensbach and Samlleberr forming a cluster, and Bimadebun, Old Zim and Olive River forming the other. Provenances could also be discerned into three groups according to their altitudinal ranges (20–25, 40 and 60 m). Provenances with similar altitudinal ranges were found to form similar clusters, justifying that genetic variation is related to altitudes.

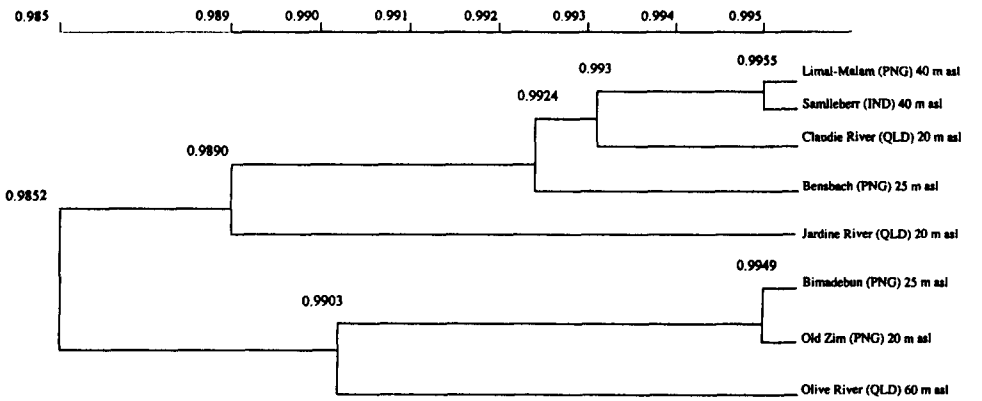

Figure 1 A dendrogram of genetic identities between the eight *Acacia crassicarpa* provenances based on the UPGMA method

No clear pattern of clusters relating to geographic region was observed. The unorganised clustering might be related to the historical formation, especially on effects of the continental drift (glaciation), where it was believed that Papua New Guinea and Australia had a land connection 10 000 years ago (Boland *et al.* 1984). The Torres Straits, for example, which formed and separated the two regions formed and disappeared several times due to the retreat of the sea-levels during glaciation. Thus it is predicted that the existed plants came from plant communities that survived the interglacial period and re-colonised from small scattered refugees (Moran *et al.* 1989b).

In addition, genetic similarities and clustering could be influenced by ecotypic and ecoclinal variation in a species as well as other environments which do not depend solely on geographic distances of the species, making such studies not so reliable (Falkenhagen 1985). This is evident in this study where factors such as land conditions, altitudes and the number of trees vary among provenances.

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