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Isozyme Variation and Relationships of Selected *Acacia* Species

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Abstract: The investigation of the extent and pattern of genetic variation for isozyme analysis of five selected *Acacia* species *(Acacia crassicarpa, Acacia* hybrid, *Acacia mangium, Acacia auriculiformis* and *Acacia aulacocarpa)* were carried out using the horizontal starch gel electrophoresis. The analyses on 21 enzymes were coded by 43 loci in *A. crassicarpa,* 46 loci in *A. aulacocarpa*, 47 loci in *Acacia* hybrid, *A. mangium* and *A. auriculiformis.* The levels of polymorphic loci for these species ranged from 0.4898 in *A. crassicarpa* and *A. auriculiformis* to 0.6596 in *Acacia* hybrid. Meanwhile, the mean observed heterozygosity varied from 0.1057 in *A. auriculiformis* to 0.1645 in *A. aulacocarpa.* Low levels of genetic variability were found in *A. crassicarpa, A. mangium* and *A. auriculiformis* but were high in the *Acacia* hybrid and *A. aulacocarpa.* The extent of genetic identities ranged from 0.7225 to 0.8778. The least related species were *A. mangium* and *A. auriculiformis,* while the most related species were the *Acacia* hybrid and *A. mangium.* These species were clustered into three main phylogenetic groups. Factors involving geographical distribution, population size, selection and ecological background were suggested to explain for the polymorphism, heterozygosity paradox and the genetic identity of these species. The *Acacia* hybrid was identified to be the most promising species in terms of its genetic variability.

Key words: *Acacia*, isozyme variation, polymorphic, heterozygosity, genetic identity

INTRODUCTION

In Malaysia, the establishment of plantation trial plots and commercial forest plantation in Peninsular Malaysia has started since the 1920's. Due the to problems with recalcitrant seeds and long rotation cycles of indigenous species, forest plantations have resorted to exotic species such as *Tectona grandis, Pinus caribaea, Acacia mangium* and *Gmelina arborea* (Zuhaidi and Ahmad, 1995). The genus *Acacia* has been considered as the most promising species for forest plantation as it covers a wide range of habitat. Similarities in certain morphological characteristics have created identification problems. Thus correct identification of species is crucial especially in initializing a tree improvement program.

Genetic markers derived from electrophoretic analysis can be used to survey the level of genetic diversity within and among populations and also for taxonomic purpose (Ikediobi and Igboanusi, 1983). Isozyme studies (Moran *et al*., 1989a; Hamidi, 1990; Wickneswar and Norwati, 1993; Wickneswari, 1989; John Keen, 1996) conducted on *Acacia* species have been confined to a particular species such as *A. mangium, A. crassicarpa* and *A. auriculiformis.* In fact, very few studies have emphasized on the interspecific variation within the genus.

Trees are genetically variable in their natural population and the amount of variation is dependent on the species level. Sufficient genetic variability is needed to improve forest trees since genetic variation within a population is the raw material upon which evolutionary changes occur. Thus, the amount, the cause and the nature of variation in a species must be determined before embarking on any tree improvement program especially when dealing with exotic species.

Knowledge of genetic diversity among and within species is needed for all conservation purposes. Information on the baseline diversity, either measured or predicted, is essential in deciding what and how to conserve, accession of genetic changes and genetic parameters that are relevant in a conservation plan. It can sometime goes beyond the inventory or description of taxa and genetic variation (Millar and Westfall, 1992). In isozyme analysis, the estimates of genetic variation are usually quantified in terms of number of polymorphic loci per species, the effective number of alleles per locus and the number of heterozygoies loci per individual (Hamrick, 1983).

MATERIALS AND METHODS

Five *Acacia* genus i.e., *A. mangium, A. auriculiformis, A. aulacocarpa, A. crasslcarpa* and

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Acacia hybrid (*A. mangium* x *A. auriculiformis*) were sampled. *A. mangium* and *A. auriculiformis* were collected from a species trial plot at Universiti Putra Malaysia, Serdang, Selangor (latitude 03°02'N, longitude 101°42'E, 20 m a.s.1). *A. crassicarpa* was sampled from a provenance trial in UPM, Serdang, Selangor (latitude 03°02'N, longitude 101°42'E, 32 m a.s.1) whereas *A. aulacocarpa* from Compartment 10, Block C of Sg. Buluh Reserve Forest, Selangor (latitude 03°13'N, longitude 101°34'E, 40 m a.s.1). The *Acacia* hybrid was obtained from the Forest Research Institute of Malaysia (FRIM), Kepong, Selangor (latitude 03°13'N, longitude 101 °38'E, 38 m a.s.1).

Electrophoresis: An amount of 0.6 g of leaf sample was homogenized in liquid nitrogen and was ground using pestle and mortar until powder form and the extraction buffer was added. Samples were kept in a freezer until ready for use. Starch gel was prepared using 10.5% hydrolyzed potato starch. Electrophoretic run was carried out in a 4°C refrigerator with the appropriate electric current or voltage depending on the buffer used different combinations of enzyme and buffer systems were used.

Four electrophoretic buffer systems were used i.e., Histidine (H), Lithium (L), Morpholine Citrate (MC), Tris Citrate (TC). The recipes for these buffers were adopted from the previous experiments on *Acacia* species (Moran *et al*., 1989a; Wickneswari and Norwati, 1993; John Keen, 1996). The recipes were as below:

- Histidine (H) - Gel buffer: 0.005 M histidine HCI at pH 8.0. Tray buffer: 0.41 M tri sodium citrate pH 8.0.
- Lithium (L) - Gel buffer: 9 parts of 0.065 M tris plus 0.001 M citric acid (monohydrate) and 1 part of tray buffer, pH 8.2. Tray buffer 0.05 M lithium hydroxide, 0.19 M boric acid pH 8.5
- Morpholine Citrate (MC) - Gel buffer 1 in 20 dilution of tray buffer. Tray buffer 0.04 M citric acid (monohydrate) pH 6.1
- Tris Citrate (TC) - Gel buffer: 0.1 M tris 0.0069 M citric acid (monohydrate) at pH 8.6. Tray buffer: 0.3M boric acid, 0.1 M NaOH pH 8.6

Alleles of each loci were labeled as Very Fast (VF), Fast (F), Medium (M), Slow (S), Very Slow (VS) whereas each locus was numbered according to its decreasing anodal mobility. The parameters calculated include genetic frequencies, the proportion of polymorphic loci and the observed expected heterozygosities according to Ferguson (1980). A dendrogram was constructed using the Unweighted Pair Group Method with Arithmetic Means (UPGMA) based on the genetic identity values.

RESULTS AND DISCUSSION

Based on the interspecific variation of the 21 enzymes used, (Table 1), the average number of alleles per locus ranged from 1.87 to 2.04. The observed heterozygosities for the species were 0.1496±0.0303 for *A. crassicarpa,* 0.1496±0.0271 for *A.* hybrid, 0.1206±0.0249 for *A. auriculiformis,* 0.1057±0.0217 for *A. mangium* and 0.1645±0.0282 for *A. aulacocarpa.* Meanwhile, the expected heterozygosities for the respective species were 0.2713±0.0404, 0.3437±0.0370, 0.2612±0.0381, 0.2671±0.0407 and 0.2991±0.0383. These values were accordant with the range of heterozygosity reported on tropical trees i.e., 0.1000 to 0.2160 by Hamrick and Loveless (1986) and Loveless and Hamrick (1987). When compared to the interspecific variation of the same genus, these values were found to be within the range of *A. crassicarpa* (Moran *et al*., 1989a; John Keen, 1996), *A. auriculiformis* (Moran *et al*., 1989a; Wickneswari and Norwati, 1993) and *Acacia mangium* (Moran *et al*., 1989a; Hamidi, 1990). The values however were found to be lower than those reported on *Acacia albida* (0.3120) (Joly *et al*., 1992) and *A. melanoxylon* (0.1770) (Playford *et al*., 1993).

The proportion of polymorphic loci for the 5 selected *Acacia* species ranged from 48.94 to 65.96% (Table 1). This was found to be moderately high when compared to other species of the same genus (Wickneswari and Norwati, 1993; John Keen, 1996; Playford *et al*., 1993; Khasa *et al*., 1994; Moran *et al*., 1989b). However, the values were relatively lower than other tropical species (Joly *et al*., 1992; Danzamann and Burchert, 1983; Chamberlain *et al*., 1994; Kim *et al*., 1997). Among the species studied, *Acacia* hybrid and *A. aulacocarpa* were found to produce high proportion of polymorphic loci of 0.6596 and 0.5745, respectively (Table 1).

Heterozygosity in isozyme data has been reported to be influenced by factors such as the number and types of enzyme gene loci studied. Most of the enzymes used in this study i.e., ADK, GD, MDH, MR and SDH were polymorphic thus made the estimation of the variation to be relatively high. Furthermore, discrepancies in diversity estimation among different studies, even on the same species, could also occur if the number of loci controlling an enzyme were interpreted differently by different investigators. In addition, the number of populations screened could also influence the estimation, especially when the population size differed among studies.

Normally, good growth is expected when trees were grown in research plots or artificial stands due to the

Table 1: Variation and relationship of selected *Acaia* species

	Species				
Alleles	*A. crasssicarpa*	*A.* hybrid	*A. auriculiformis*	*A. mangium*	*A. aulacoccarpa*
Mean H_o	0.1496	0.1496	0.1206	0.1057	0.1645
SE	0.0303	0.0271	0.0249	0.0271	0.0283
Mean H_e	0.2713	0.3437	0.2612	0.2671	0.2991
SE	0.0404	0.0370	0.0381	0.0407	0.0383
Loci scored	43	47	47	47	46
Proportion of polymorphic loci	0.4894	0.6596	0.5106	0.4894	0.5745

Table 2: Nei's (1978) coefficient of genetic identities among five *Acacia* species of selected *Acacia* species

Species	*Acacia* hybrid	*A. auriculiformis*	*A. mangium*	*A. aulacocarpa*
A. crassicarpa	0.7406	0.7432	0.7451	0.7960
Acacia hybrid		0.8690	0.8778	0.8548
A. auriculiformis			0.7225	0.8660
A. mangium				0.7458

uniform treatment and considerable stable environment. This climatic stability was hypothesized to have provided a refuge for the species planted and permit the species to be less variable and carry a relatively low proportion of polymorphic loci. Harper (1977), Mitton and Aadalora (1981) has reported that trees in a monoculture plantation would allocate more energy for their growth and less energy to increase heterozygosities and proportion of polymorphic loci. This could probably explain for the low heterozygosities (i.e., 0.150, 0.121 and 0.106) and proportion of polymorphic loci (0.4894, 0.5106 and 0.4894) for *A. crassicarpa, A. auriculiformis* and *A. mangium* (Table 1). This findings could further be supported with a finding by Dusan (1992) on *Picea abies,* where low level of heterozygosities (H = 0.275) were found in trees raised in artificial stand when compared to those found in a virgin jungle (H = 0.322).

Higher genetic diversity and proportion of polymorphic for a species can be useful for adaptation, especially when it is planted under an undesirable and harsher conditions. Normally, polymorphism is required as part of the adaptive strategies for a population from a heterogeneous environment of the forest (Feret and Bergmann, 1976). Such adaptation would normally be expressed in a form of higher heterozygosity and this will enable the species to survive (Rosman and Nor Aini, 1993). In this study, the high value of the parameters in *A. aulacocarpa* were essential to fit the harsher growing conditions since the population was planted in a rocky area and exposed to high wind speed from a highway nearby. Furthermore, high density planting of the species on this site might have forced the species to undergo competition for space and energy. Yap (1987) has earlier reported that the site has been abandoned and has caught fire before planting. Thus this species was believed to possess high heterozygosities which enable them to survive under these limiting factors.

Geographic range relating to its distribution could also give a significant effect on the levels of genetic diversity among species. Conkle (1992) and Hamrick *et al.* (1992) reported that widespread species with significant ranges of latitudes and elevations have higher proportion of polymorphic and heterozygosities than the more geographically restricted species. In this case, *A. aulacocarpa* is more widely spread with distribution ranging from New South Wales to southern Papua New Guinea (6-31°S) than *A. auriculiformis* (5-17 °S), *A. crassicarpa* (8-20 °S) and *A. mangium* (1-18 °S). Thus, *A. aulacocarpa* should have higher values for these genetic parameters. In addition, Moran *et al.* (1989b) reported that *A. mangium* has a remarkably low level of isozyme variation because of its restricted occurrence. The higher values of heterozygosities and polymorphism in A. hybrid were mainly due to the combined genotypes of *A. mangium* and *A. auriculiformis,* which leads to heterosis and hybrid vigor.

Nei's coefficient of genetic identities for all possible combinations among five species (Table 2) and genetic identities ranged from 0.7314 to 0.8879. The combination of *A. auriculiformis-Acacia* hybrid exhibited the highest genetic identity with I = 0.8879, while the *A. crassicarpa-A. auriculiformis* combination gave the lowest genetic identity (I) of 0.7314.

The dendrogram constructed based on genetic identities (Table 2) to observe the relationships among the species formed 3 main clusters or phylogenetic groupings. The first cluster with the highest genetic identity of 0.8778 was between *A.* hybrid and *A. mangium* indicating that *A.* hybrid and *A. mangium* were genetically similar. In addition, this can also suggests that they might share similar ancestry. Similar explanation can also be used to explain the second grouping between *A. auriculiformis, A. aulacocarpa* and *A. crassicarpa,* which formed another phylogenetic arm.

Since the hybrid in this study was a combination between *A. mangium* and *A. auriculiformis,* the clustering of *A. mangium-Acacia* hybrid was mainly due to the similarity of genetic structure within them. *A. auriculiformis* as another parent tree for the hybrid progeny, although it did not form the same cluster, produced high genetic identity of 0.8690 with *Acacia* hybrid.

A. auriculiformis formed a clustered with *A. aulacocarpa* with genetic identity value of 0.8660. Clustering of *A. auriculiformis-A. aulacocarpa* was expected since their morphologies have been reported as similar by Turnbull (1986). However *A. crassicarpa,* which shares similar morphology, formed another phylogenetic arm. Wright (1976) noted that the closer the two species in terms of their taxonomic relationship could indicate the great possibility of hybridizing them. This information can be used as an empirical basis to study the possibility of natural hybridization and controlled pollination among possible related species.

The result of this study indicated that the variations among these species were relatively low with heterozygosities ranging from 0.1056 in *A. mangium* to 0.1645 in *A. aulacocarpa.* Meanwhile, the proportion of Polymorphic loci (P) was found to range from 0.4894 *A. crassicarpa* to 0.6596 in *Acacia* hybrid. Levels of genetic identities obtained from this study were relatively high ranging from 0.7225 to 0.8778. The lowest genetic identity is between *A. mangium* and *A. auriculiformis,* while the highest is between *Acacia* hybrid and *A. mangium.* These species formed three main phylogenetic groups. Group I consisted of *Acacia* hybrid and *A. mangium,* while Group II consisted of *A. auriculiformis* and *A. aulacocarpa.* Group III formed a single phylogenetic arm of *A. crassicarpa.*

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