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Short Communication

Isoenzyme variation in four *Acacia* species

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Many species of *Acacia* are grown as multipurpose trees in monoculture plantations as well as in multispecies agroforestry systems in the tropics including India. The various classifications of acacias are not comprehensive and still debatable. Study of the isoenzyme pattern is considered as an important tool to understand the genetic relationship between individuals, provenances, species and also for the identification of hybrids. Depending on the molecular weight of these zwitterions, their mobility in an electrophoretic medium varies. This differential movement is a direct manifestation of the genetic make up responsible for such multiple forms of isoenzymes. Genetically, the production of isoenzymes of multiple forms or molecular weight is accounted to the allelic variation of the organisms. Thus isoenzyme of a particular molecular weight can be considered as a direct manifestation of the blue print of the specific gene loci. Isoenzymes are more advantageous to study the variation in species or provenance due to their co-dominant expression, freedom from environmental influences and ease with which large number of individuals can be simultaneously evaluated over many loci [1].

In the present study, analysis of two isoenzymes namely, esterase (Est) and glutamate oxaloacetate transminase (GOT) was carried out using vertical slab poly acrylamide gel electrophoresis (PAGE) in four species of acacias to understand their genetic relationships. The species selected for the study were *A. auriculiformis* A. Cunn. Ex Benth., *A. mangium* Willd, *A. ferruginea* DC and *A. nilotica* (Linn.) Willd ex. Del. The former two species having phyllodes are native to Australia, Papua New Guinea and Indonesia while the latter two with bipinnate leaves are endemic to the Indian subcontinent. The isozymes were extracted using the extraction buffer from mature leaves/phyllodes of 8 month old seedlings, run on a poly acrylamide gel and stained following standard procedures.

With reference to their relative mobility (Rm) a total of seven isoenzyme bands (three of Est and four of GOT) were obtained for the two enzyme systems in the four species of acacias (Fig. 1). The bands are designated based on their mobility. The most mobile one in each was assigned number 1 and next in order of mobility as 2 and so on.

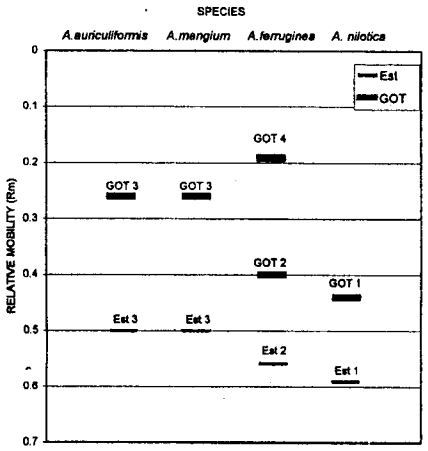

Fig. 1. Zymogram of enzymes estrase (Est) and glutamate oxaloacetate transminase (GOT) in four species of *acacias*

Out of the three isoenzyme bands obtained for esterase, Est-3 (Rm = 0.50) was present in *A. auriculiformis* and *A. mangium*. Est-2 (Rm = 0.56) of *A. ferruginea* and Est-1 (Rm = 0.59) of *A. nilotica* were different from each other and also from that of the former two species in their mobility. The enzyme GOT was separated into four isoenzyme bands. The band GOT-3 (Rm = 0.26) was present both in *A. auriculiformis* and *A. mangium*. There were two bands of GOT for *A. ferruginea*, GOT-4 (Rm = 0.19) and GOT-2 (Rm = 0.40). The most mobile was GOT-1 of *A. nilotica* (Rm = 0.44).

It is seen that both *A. auriculiformis* and *A. mangium* possess identical monomorphic gene loci for the two enzyme systems as evidenced by the uniform banding of Est and GOT with Rm = 0.50 and 0.26, respectively. A similarity index value of one between the two species (Table 1) is also indicative of their

genetic similarity. In an earlier study it has been shown that out of the eight gene loci obtained for *A. mangium* and *A. auriculiformis* using four enzyme systems, three were identically present in both species indicating their genetic relatedness [2]. The genetic relationship of these two species is further evidenced by their ability to provide natural hybrids [3]. It has also been suggested that *A. mangium* is evolved from *A. auriculiformis* relatively recently [4].

For the other two Acacias namely, *A. ferruginea* and *A. nilotica* the banding pattern of the two enzyme systems was distinctly different among them and also from the former two acacias. Esterase exhibited only one band each at Rm = 0.56 and Rm = 0.59, respectively, in these species indicating the monomorphic nature of the gene in them. The gene for GOT, on the other hand, appeared to be polymorphic in *A. ferruginea* producing two isoenzyme bands at Rm = 0.19 and 0.40. *A. nilotica* had only one band for GOT (Rm = 0.44). Thus these two acacias which are believed to be evolved in the Indian subcontinent appear to be distinctly different from each other as far as the gene loci for these two enzymes are concerned. This observation points to the possibility that *A. nilotica* and *A. ferruginea* got differentiated at an early stage of evolution. They can be considered as genetically different from the two Australian species studied. Out of the six pair-wise comparisons involving the four species of acacias studied, only one namely, *A. auriculiformis* with *A. mangium* alone showed a similarity index (SI) of one (Table 1). All the rest had S.I. = 0 indicating lack of genetic similarity for the enzymes studied. Though all the acacias studied except *A. nilotica* possessed 2n = 26 chromosomes, the enzyme systems revealed clear genetic distinction between the two groups. *A. nilotica*, known to be an autotetraploid with 2n = 52 [5] was also found to be genetically different from all the other species studied. Polyploid nature of this species might have also contributed to its species identity as indicated by the banding intensity of enzymes assayed. With comparable amounts of leaf extracts, the banding intensities of these isoenzymes were more in *A. nilotica* compared to the other three species studied.

Table 1. Similarity indices of the four *Acacia* species for esterase (Est) and glutamate oxaloacetate transminase (GOT) enzymes*

	A. auriculiformis	A. mangium	A. ferruginea	A. nilotica
Est	1			
A. auriculiformis				
GOT	1			
Est	1	1		
A. mangium				
GOT	1	1		
Est	0	0	1	
A. ferruginea				
GOT	0	0	1	
Est	0	0	0	1
A. nilotica				
GOT	0	0	0	1

*Estimated by the method of Sokel and Sneath (1963)

Certain isoenzymes are seen to be exclusive to a particular species. Such isoenzyme can be selected as marker for identification of inter-specific hybrids in the natural environment. Based on an earlier study it has been proposed that glutamate dehydrogenase can be used as a marker for identification of natural hybrids of *A. auriculiformis x A. mangium* [2]. According to the present study, it appears that esterase and glutamate oxaloacetate transminase can not be used as markers in these two species since they carry identical monomorphic gene loci for these enzyme systems and therefore their isoenzyme bands are similar in their mobility. However, both these enzyme systems can be employed as marker in hybridization studies involving the two Australian species or between species of these two groups.

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