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Isozyme variation in progenies of plus trees of *Acacia nilotica* (L.) ex Del. ssp. *indica* (Benth.) Brenan from north-western India

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Abstract

***Acacia nilotica* ssp. *indica* (*babul*) is an extremely important agroforestry species in semi-arid regions of Indian sub-continent. Genetic variation among 30 progenies of 'plus' trees of this species collected from 3 states of India, were assessed by esterase and peroxidase isozyme profiles. Rf value in peroxidase isozyme ranged from 0.02 to 0.12 with 4 bands; while it ranged from 0.05 to 0.32 with 10 bands in esterase. The range of similarity index varied from 0.214 to 1.000 for the two enzymes. In general, lesser genetic diversity was observed in the progenies of plus trees from Gujarat, medium in Uttar Pradesh and higher in that of Haryana, and there were also some similarities between certain progenies of the 3 states. The dendrogram revealed the co-efficient level of 0.53 and three clusters, i.e. A, B and C with 6, 8 and 16 progenies, respectively. The study is important for analysis of genetic diversity between progenies and its further use in establishment of seed orchards of *Acacia nilotica* ssp. *Indica*.**

Key words: *Acacia nilotica* ssp. *indica*, plus tree, progeny, esterase, peroxidase

Introduction

Among the biochemical markers, isozymes are simple and reliable and are considered as valuable tool for assessing the genetic structure and variability within and between populations. They have been found to be of particular use at lower taxonomic levels to act as a marker for studies including inheritance and mating systems.

With the development of isozyme analysis and its application to the field of forest genetics, there has been an increasing interest to study the variation present in different tree species and to exploit it for development of strategies in tree improvement programme. Early selection would be possible, if a variant is known to be linked with an interesting morphological trait. This has been successfully performed for agricultural plants while for forest trees partial success has been reported.

Acacia nilotica ssp. indica (Benth.) is an extremely important agroforestry species in semi-arid regions of Indian sub-continent. It is a small sized tree with strong vertical and horizontal root system [1], and a long growing period of more than 300 days with 4 peaks of leaf flush and monolayer canopy [2]. This species has high potential for nitrogen fixation [3], and has been considered as one of the fast growing species of the wastelands, and agroforestry systems throughout India providing strong timber, fodder for goats and sheep, and high quality fuelwood in rural areas [4].

Application of isozyme technique for establishment of variation among provenance/progeny in *Acacia nilotica* ssp. *indica* has not been attempted so far. In the present study, peroxidase and esterase were studied to find out the variation between progenies of 30 plus trees collected from 3 different states of India.

Materials and methods

The seeds of 30 plus trees of *Acacia nilotica* ssp. *indica* were collected from 3 states (10 trees from each state) of north-western India (23°40N to 29°59N lat., 72°37E to 81°00E long., 55 m to 259 m alt., 400 mm to 1050 mm rainfall). The criteria for selection of trees were: (i) straight tree bole, (ii) superiority in height, (iii) conical shape of the canopy, and (iv) free from diseases. Ripe pods were collected from the trees, sun-dried and seeds were separated through manual threshing to ensure the collection of undamaged seeds. They were further cleaned through winnowing [5] and fumigated with aluminum phosphide. The seeds were stored at room temperature in air-tight aluminium cans.

Twenty five seeds of each progeny were germinated in BOD incubator at 25°C, and 7 days old etiolated plumule were crushed with 0.25-0.3 ml extraction buffer at 4°C in a chilled pestle and mortar. The extracts were centrifuged at 10,000 rpm for 20 minutes at 4°C. The clear supernatant was collected and used for electrophoresis.

Esterases were analyzed by the standard procedure described by Dadlani and Varier [6] and peroxidase isozymes were detected by the method of

Mitra *et al.* [7]. Similarity index was calculated following mathematical model given by Nei and Li [8].

Results and discussion

Thirty progenies (ten from each state) of *Acacia nilotica* ssp. *indica* showed wide variation of banding patterns of peroxidase and esterase isozyme systems. These were distinguished not only by their molecular weights, but also by the relative staining intensity i.e. very light, light, medium and dark. Based on variable intensity of bands, zymogram of peroxidase and esterase were prepared. In peroxidase isozyme analysis, only four bands were observed with Rf value ranging from 0.02 to 0.12 whereas in esterase, there were 10 bands with Rf value between 0.05 to 0.32 (Table 1).

Table 1. Isozyme banding pattern of 30 progenies of *Acacia nilotica* spp. *indica*

Band No.	Rf value	HR-1	HR-2	HR-3	HR-4	HR-5	HR-6	HR-7	HR-8	HR-9	HR-10	UP-1	UP-2	UP-3	UP-4	UP-5	UP-6	UP-7	UP-8	UP-9	UP-10	GJ-1	GJ-2	GJ-3	GJ-4	GJ-5	GJ-6	GJ-7	GJ-8	GJ-9	GJ-10
Peroxidase																															
1	0.02	D	M	M	D	M	M	D	M	M	D	M	D	M	L	M	M	VL	VL	M	L	-	-	M	M	M	-	VL	VL	-	-
2	0.06	D	VL	M	D	-	M	M	-	M	M	M	-	-	VL	-	L	L	M	-	L	D	M	M	-	-	D	M	-	M	D
3	0.09	-	M	D	D	D	-	-	D	-	-	-	-	L	-	-	M	L	-	-	M	D	L	L	M	M	M	-	-	M	D
4	0.12	D	D	D	D	D	M	D	D	M	D	-	D	-	D	L	D	M	M	D	D	L	L	D	-	M	M	M	L	M	M
Esterase																															
1	0.05	M	M	M	-	-	L	L	-	L	-	L	-	-	VL	-	-	-	-	-	-	M	-	VL	VL	-	M	L	-	VL	M
2	0.09	M	L	-	-	-	M	M	-	-	-	M	-	-	M	L	-	L	L	M	M	L	-	L	-	-	L	VL	-	-	-
3	0.14	-	-	D	D	M	M	M	D	D	D	D	VL	VL	M	VL	-	L	L	D	D	M	-	D	L	-	VL	VL	L	-	-
4	0.16	D	D	M	M	D	D	D	D	D	D	D	D	D	D	-	L	D	D	D	D	D	L	M	M	L	M	-	M	D	D
5	0.19	D	D	M	M	M	D	D	D	D	D	D	M	M	D	M	M	D	D	D	M	D	M	-	VL	M	L	D	-	L	L
6	0.22	L	-	L	L	-	-	-	L	L	-	M	VL	L	M	-	-	M	M	-	-	-	-	-	-	VL	-	L	L	VL	-
7	0.23	L	L	VL	-	-	-	-	-	L	L	L	VL	D	VL	VL	-	L	D	M	L	M	L	D	M	D	M	M	M	M	M
8	0.25	M	L	L	M	M	L	L	L	L	L	M	L	M	L	L	L	-	-	L	L	L	VL	L	-	L	-	M	VL	-	L
9	0.27	M	M	M	D	D	M	M	M	D	M	M	M	D	L	M	-	-	M	M	L	-	-	L	-	-	-	L	-	-	-
10	0.32	L	VL	L	L	L	VL	VL	L	-	-	L	L	M	VL	L	VL	VL	VL	L	L	-	-	-	L	-	-	-	VL	-	-

D = Dark; M = Medium; L = Light; VL = Very light

Peroxidase

In progenies of Haryana (HR) state, band 1 and 4 were monomorphic while band 2 and 3 were polymorphic. Band 1 showed light intensity in the progenies of HR-2, 3,5,6,8 and 9, and dark intensity in HR-1, 4, 7 and 10, while band 2 showed very light intensity in HR-2 and it was absent in HR-5 and HR-8 progenies. Band 3 showed medium intensity in HR-2 and dark in HR-3, 4, 5 and 8, whereas, band 4 was dark in all except in HR-6 and HR-9 which were having medium intensity.

In progenies of Uttar Pradesh (UP), band 1 was monomorphic and expressed all four types of intensities. Band 2 was absent in UP-2, 3, 5 and 9 and present with very light intensity in UP-4, light in UP-6,7 and 10 and medium intensity in UP-1 and 8. Band 3 was present in UP-3, 6, 7 and 10 having light to medium intensity and was absent in other progenies. Band 4 was absent in UP-1 and 3, light in UP-5, medium in UP-7 and 8 and dark in UP-2, 4, 6, 9 and 10.

In progenies of Gujarat (GJ) state, all the bands were polymorphic. Band 1 was absent in GJ-1, 2, 6, 9 and 10, medium in GJ-3, 4 and 5, very light in GJ-7 and 8 and there was no dark intensity in this band. Band 2 was absent in GJ-4, 5 and 8, dark in GJ-1, 6 and 10 whereas medium in GJ-2, 3, 7 and 9. Band 3 and 4 also showed variations for pattern and intensity.

State-wise comparison of the progenies showed that band 1 was present in all progenies of Haryana and Uttar Pradesh, and it was absent in 5 progenies of Gujarat. Band 2 was found present in 21 progenies and was having very light intensity in 2 progenies, medium in 11 progenies, and dark intensity in 5 progenies. Band 3 was present in 17 out of 30 progenies, and was having dark intensity in 6 progenies, light intensity in 4 progenies and medium intensity in 7 progenies. Band 4 was present in all the progenies of 3 states except UP-1, 3 and GJ-4 (Table 1).

Esterase

In progenies of Haryana state, band number 4, 5, 8 and 9 were monomorphic while the remaining 6 bands were polymorphic. In case of progenies of Uttar Pradesh band 5 and 10 were monomorphic, while in case of Gujarat all the bands were polymorphic except band number 6. Almost all the progenies showed dark and medium intensity in band 3, 4 and 5 whereas band 1, 2, 6, 8 and 10 showed medium, light and very light intensity and even absent in some of the progenies. Band 5 was having dark and medium intensity in

progenies of Haryana and UP. This band was either absent or was having wide variation from very light to dark intensities in progenies of Gujarat. For band 4, 19 out of the 30 progenies showed dark intensity, while for band 3 only 9 progenies showed dark band. Band 9 showed dark and medium intensity in all progenies of Haryana, in 6 progenies of the Uttar Pradesh and none from Gujarat state.

Total number of bands in progenies of Gujarat, Haryana and Uttar Pradesh states were 59, 74 and 78, respectively. Among 30 progenies, only UP-1 and 4 showed all the ten bands with varying intensity, whereas lesser i.e. 4 bands were observed in UP-6 and GJ-2 (Table 1).

There are only a few studies made on reproductive biology of *Acacia nilotica* on the basis of which the wide variation found in the present study, could be explained. Nevertheless, Tybirk [9] described the floral structure of *Acacia nilotica* in Kenya. According to this study, there are two types of flowers i.e. unisexual and hermaphrodite in *Acacia nilotica*, and out of which 1/3rd are hermaphrodite of which about 30% set seeds indicating cross pollination behavior of the species.

Mandal and Gupta [10] also revealed a high estimate of genetic diversity (53%) and high degree of genetic differentiation (43%) within population of *Acacia nilotica*. Similar ranges of polymorphism produced by different enzyme systems were also reported in *Eucalyptus* ssp. [11]. Handa *et al.* [12] studied isozymes on *Populus ciliata* × maximowiczii hybrids and observed that peroxidase and esterase were polymorphic. Zhu *et al.* [13] observed differences between the provenances of *Magnolia officinalis*.

The similarity matrix computed revealed wide genetic similarities from 0.214 to 1 among the progenies. The similarity matrix was further divided into ten equal parts with class interval of 0-10; from which only 46 pairs were having lower similarity of 10 per cent, whereas 138 pairs were observed with similarity ranging from 80 to 90 per cent. The highest similarity value (more than 80%) was observed among 30 pairs (Fig. 1).

On the dendrogram (Fig. 2), the similarity and dissimilarity have been shown among 30 progenies. Genetic variability within cluster was minimum in Gujarat progenies. Haryana and Uttar Pradesh progenies had more similarities and Gujarat formed a distinct cluster except GJ-3, 5, 7 and 8. HR-6 and HR-7 were similar as far as these two isozymes are considered. In general, there were higher similarity index values indicating high degree of resemblances among the progenies irrespective of their geographical origin.

Similarly, progenies of Haryana i.e. HR-4, 5 and 8 formed close relationship with UP-3 in group 'B'; three sets of cluster were formed between UP-6 and GJ-5; UP-2 and GJ-8 and HR-4 and 8. The cluster HR-4 and 8 made sub-group by joining with HR-5 and UP-3 and other two clusters joined directly with subgroup formed in 'B' group. Similarly, 'C' group was having 16 progenies of 3 subgroups and made 5 clusters and it included the highest (100 per cent) similarity in progenies of HR-6 and 7 (Fig. 2).

The dendrogram obtained by the UPGMA clustering method revealed the genetic relationship of 30 progenies of *A. nilotica* spp. indica. The phenetic tree or dendrogram constructed through hierarchical analysis revealed the coefficient level of 0.53 and three clusters, i.e. A, B and C with 6, 8 and 16 progenies, respectively. The branching (cluster) was observed up to the co-efficient level of 1.0, which indicated high level of similarity among the progenies. Progenies of Uttar Pradesh and Haryana showed similarities and got intermixed while 6 of Gujarat progenies formed a separate cluster. Hence, only Gujarat entries have diverse genetic background and not that of Uttar Pradesh and Haryana. There was no such distinct grouping of progenies of same geographic origin and were spread throughout the major cluster and occurred intermingled

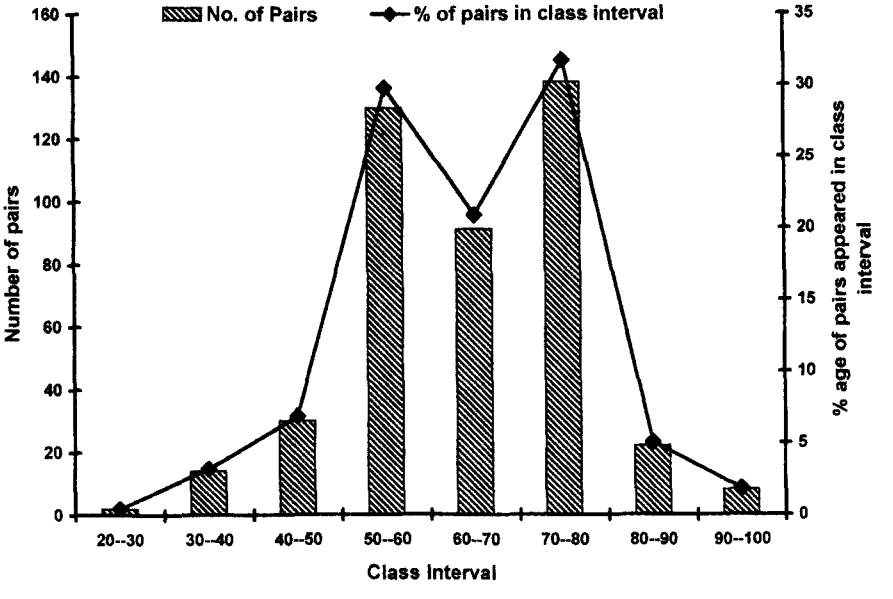

Fig. 1. Class interval and pair percentage of similarity value of 30 progenies of *Acacia nilotica* ssp. *indica*

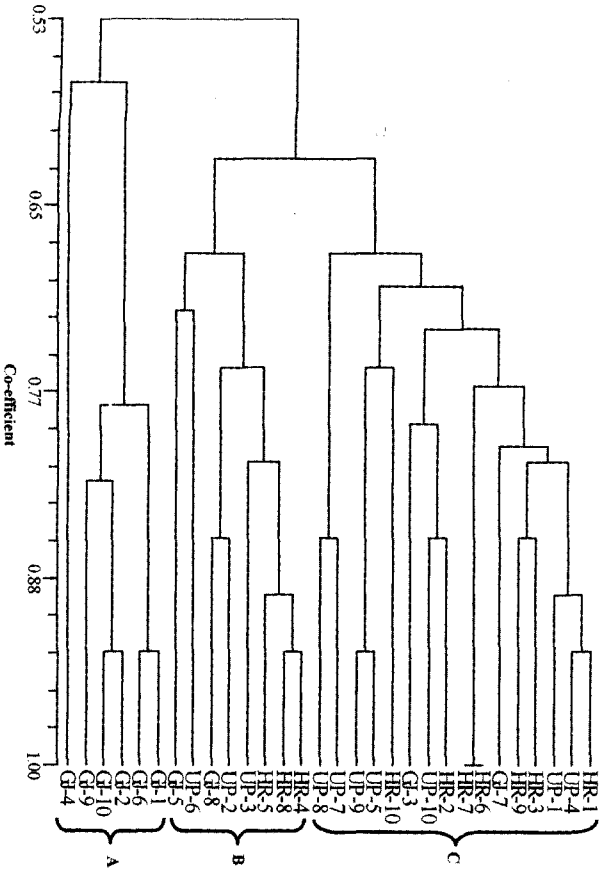

Fig. 2. Phenetic tree constructed from distance matrix of biometric of 30 progenies of *Acacia nilotica* ssp. *indica*

with seed sources from various states. Such matching reports of indistinct grouping of the genotypes of same geographic origin were also observed in *Azadirachta indica* [14].

According to our analysis, in general, less genetic diversity was observed within Gujarat seed sources, medium within Uttar Pradesh seed sources and wider genetic diversity within that of Haryana. Kundu [15] also demonstrated that allozyme data may not correspond with morphometric traits when measuring genetic distance. A number of other studies published on *Acacia* [16], *Eucalyptus* spp [17], Bamboo spp. [18], *Havea brasiliensis* [19], *Tamarindus indica* [20] and *Populus deltoides* [21] also reported provenance variations in isozymes.

Conclusions

Progenies of Uttar Pradesh and Haryana showed similarities, while 6 of Gujarat progenies formed a separate cluster. Hence, only Gujarat entries have diverse genetic background and not that of Uttar Pradesh and Haryana. There was no such distinct grouping of seed sources of same geographic origin and were spread throughout the major cluster and occurred intermingled with seed sources from various states.

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