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Allozyme Differentiation in Two Subspecies of *Acacia nilotica**

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Isozyme variation at seven polymorphic loci was studied in two subspecies of *Acacia nilotica*. High estimates of genetic diversity (53%) and higher degrees of genetic differentiation (43%) were observed. The implications of these findings for the development of *in situ* conservation strategies are discussed.

Key Words: Allozyme, *Acacia nilotica*, Electrophoresis, Polymorphic, Genetic variation

Introduction

Acacia is a large woody genus comprising more than 1000 species occurring naturally in all the continents except Europe (Moran et al. 1989). In recent years, some of these species including *Acacia nilotica* have emerged as a major plantation species of significant economic importance. The species is suitable for plantation both as monoculture as well as in agroforestry systems. It is one of the most important, versatile and multipurpose tree species within the genus *Acacia*. The species is very popular among the farmers of Asia and Africa for its valuable use as timber, firewood, fodder, bark tanin, gum etc. and also for its natural ability to fix atmospheric nitrogen which in turn replenish fertility of the soil (Fagg 1995). *A. nilotica* has an extensive and diverse natural distribution from Egypt, Mauritiana southwards to southern Africa and Asia eastward to India (Dwivedi 1993). The diversity of site and climatic conditions under which *Acacia nilotica* grows has led to the evolution of an extremely variable species. As a result, the species is presently recognized into nine subspecies with more or less distinctive morphological, ecological and geographical features. The great versatility of the species, in terms of both wide adaptation and multipurpose use, has stimulated interest in the genetic improvement of the species (Mandal et al. 1994). However, information on which to base genetic improvement and conservation effort is neither available at morphological nor at molecular level. In effect, conservation cannot be separated from breeding (Namkoong 1984). To the best of information this is the first report describing variation at molecular level in the natural population of *A. nilotica*.

In this study we examined the extent and pattern of allozyme variation in two populations, one each of two subspecies namely *A. nilotica* spp. *leiocarpa* and *A. nilotica* sp. *kraussiana* using isozyme techniques.

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Materials and Methods

Seed material: Two subspecies of *Acacia nilotica* viz., *leiocarpa* and *kraussiana* were selected for the present study. Bulked open pollinated seeds, one sample each of the two subspecies, were obtained from the Oxford Forestry Institute. Equal quantity of seeds from 25 randomly selected trees of each subspecies were mixed to form the bulk seed sample. Seeds of the subspecies *kraussiana* were collected from the Chantalo areas of district Mangochi of Malawi (14°19′ S, 34°48′ E, 409 m). Seeds of the other subspecies were collected from Sabaki areas of Malindi district of Kenya (3°11′ S, 40°07′ E, 20 m).

Table 1 *Composition of gel and electrode buffer systems*

Gel buffer	Electrode buffer	Enzyme system
0.0014 M EDTA 0.05 M Histidine - HCl (*p*H 7.0)	0.125 M Tris (*p*H 7.0)	6 PGD EST
0.0045 M Histidine - HCl 0.0037 M NaOH (*p*H 6.5)	0.41 M Sodium citrate 0.003 Citric acid (*p*H 6.5)	MDH
0.0052 Histidine - HCl 0.042 M Tris 0.0029 M $LiOH.H_2O$ 0.022 M H_3BO_3 (*p*H 8.2)	0.029 M $LiOH.H_2O$ 0.192 H_3BO_3 (*p*H 8.1)	ADH

Electrophoresis: Electrophoresis was done following the method (with suitable modification) given by Tanksley and Orton (1983). Three hundred seeds of each subspecies were used for the study. The seeds were germinated on moist filter paper in petridishes for 6 days for electrophoresis. Germination was facilitated by nicking the seed coat to allow water absorption. Seeds were extracted on ice using extraction buffer having *p*H 7.5 (KH_2PO_4 0.13 g, Cystine 0.36 g, EDTA 0.33 g, Sucrose 13.60 g. *Tris* 2.40 g, Sodium citrate 0.36, H_2O 100 ml) and absorbed onto filter paper wicks for loading and running on 11% starch gel matrix. The detail composition of gel buffer and electrode buffer are given in table 1.

Electrophoresis was conducted using horizontal gel tank systems linked to a 500mA power source. Gels were run at a constant current of 40mA with a power rating of 100y/110y. Most enzymes required 5-6 hrs, but esterase needed 3 hrs for complete run of the gel. Enzymes were detected *in situ* with specific histochemical stains. Two enzyme systems viz. Malate dehydrogenase (MDH) and Esterase (EST) were studied and four loci were scored (Mdh-1, Mdh-2, Mdh-3 and Est-2) for *kraussiana*. In the case of *leiocarpa*, four enzyme systems viz. Alcohol dehydrogenase (ADH), Esterase (EST), 6-Phosphogluconate dehydrogenase (6 PGD) and Malate dehydrogenase (MDH) were studied and four loci (Adh, Est-1, 6 Pgd and Mdh-1) were scored.

Estimates of genetic variation: Genotypes of the seedlings were determined directly from the gels. Genotypes of the mother trees were estimated following Ritland and Jain (1981) as given in Ritland (1990) multilocus estimation programme (MLT). A locus was considered polymorphic if the frequency of the most common allele did not exceed 0.95. Estimates of the genetic variation parameters were based on Nei (1978). Genetic variation within each population was estimated by calculating percent polymorphic loci (P), mean number of alleles per locus (A) and gene diversity (H) or expected gene diversity. The total gene diversity (H_T) was calculated as the expected heterozygosity when both the population were treated as a single panmictic unit.

Results and Discussion

Table 2 presents estimates of allelic frequencies at 7 variable loci in the two populations, one each of the two subspecies of *A. nilotica*. None of the loci were mono-

Table 2 *Observed allelic frequencies in two populations of* ***A. nilotica***

A. nilotica spp. *leiocarpa*			*A. nilotica* spp. *kraussiana*		
Locus	Allele	Frequency	Locus	Allele	Frequency
Adh-1	1	0.314	Est-2	1	0.578
	2	0.021		2	0.422
	3	0.633			
Est-1	1	0.371	Mdh-1	1	0.214
	2	0.348		2	0.386
	3	0.225		3	0.476
	4	0.071			
6 Pgd	1	0.305	Mdh-2	1	0.592
	2	0.677		2	0.368
	3	0.018		3	0.045
Mdh-1	1	0.380	Mdh-3	1	0.570
	2	0.477		2	0.310
	3	0.133		3	0.142

Table 3 *Estimates of mean sample size per locus (N), mean number of alleles per locus (A), percentage of polymorphic loci (P), index of genetic diversity (H_e) for seven loci in two populations of* ***A. nilotica***

Population	N	A	P[1]	He[2]
leiocarpa	300	3.25	99.32	0.566
kraussiana	300	2.75	99.00	0.509
Average	300	3.00	99.16	0.534

[1]Loci were designated polymorphic if the frequency of most common allele was less than 0.95

[2]Unbiased estimate (Nei 1978)

morphic in both the populations. At most of the loci appreciable variation in allelic frequencies was observed.

Estimates of measures of genetic variation were generally very similar in both the populations (table 3). Each population was polymorphic, on the average, at 99% of their loci and most such loci segregated for 2 to 4 alleles. Maximum (4) and minimum (2) alleles were observed in Est-1 in *leiocarpa* and Est-2 in *kraussiana*. All other loci had 3 alleles. Average number of alleles per locus over population was 3.00.

Results of hierarchical partitioning of the gene diversity statistics are presented in table 4. The total gene diversity varied from 0.583 (Mdh-1) to 0.922 (Est-1) and averaged 0.845. The mean diversity within population was 0.485. Thus, while 57.4% of the total gene diversity resides within the population, inter population differentiation accounts for 42.6%. Gst, the principal measure of genetic differentiation ranged from 28% (Est-1) to 60% (Mdh-1) and averaged 43% for the species.

Plant species are highly polymorphic with a mean genetic diversity of 15%, whereas plant populations are relatively less polymorphic with a mean genetic diversity of 11% (Hamrick & Godt 1989). In comparison, the two subspecies of *A. nilotica* is highly diverse with an average of 3.00 alleles per locus and a mean genetic diversity of 53%. Levels of genetic diversity observed in the present materials are generally consistent with the estimates of genetic diversity noted for other Acacia species including *A. tortilis* (Olng'otie 1991). Angiosperm trees have an average genetic diversity of 17% ranging from 1.7% in *Acacia mangium* (Moran et al.

Table 4 *Estimates of gene diversity and genetic differentiation at seven loci within* ***A. nilotica***

Locus	Gene diversity			
	H_t	H_s	Dst	Gst
Adh-1	0.875	0.501	0.374	0.427
Est-1	0.922	0.660	0.262	0.284
Est-2	0.880	0.488	0.392	0.445
6 Pgd	0.872	0.449	0.423	0.485
Mdh-1	0.583	0.229	0.354	0.607
Mdh-2	0.893	0.513	0.380	0.425
Mdh-3	0.890	0.559	0.331	0.371
Mean	0.845	0.485	0.359	0.434

H_t, total; H_s, intra and Dst, inter-population genetic diversity; Gst, coefficient of gene differentiation

1989) to 47% in *Metrosideros rugosa* (Aradya et al. 1991). Evidently, *A. nilotica* has a high level of genetic variation (present study) relative to other tropical angiosperm tree species. There may be three major factors, polyploidy, geographic range and breeding systems, responsible for this high level of genetic variation. It has been shown that polyploid species are endowed with higher levels of genetic variation relative to diploids (Wolf et al. 1990). *A. nilotica* is a polyploid, highly widespread in its distribution and has excellent colonizing ability. Further the species is principally melittophilous and follows a mixed mating system (Mandal et al. 1994, Mandal & Ennos 1995).

The results of the present study can be effectively used while formulating sampling or *in situ* conservation strategies for the two subspecies. It is suggested that much smaller population size (Dyson 1974) will be required to capture the total genetic variability for such purpose. However, more extensive data on the degree of genetic differentiation using more population will be required before this can be done effectively.

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References

Aradya K M, Muller-Dombois D and Ranker T A 1991 Genetic evidence for recent incipient speciation in the evolution of Hawaiian *Metrosideros; Heredity* **67** 129-138

Dwivedi A P 1993 *Babul: A Multipurpose Tree of Dry Areas* (Dehra Dun : ICFRE)

Dyson W G 1974 A note on the conservation of tree species *in situ* in *Report of the Third Session of the FAO Panel of Experts on Forest Gene Resources*, 76-79 (*Rome: FAO*)

Fagg C W 1995 *Acacia*-options for tree improvement in India with reference to sampling strategies in Africa; in *Proc. Seed Technology Workshop* (Coimbatore: IFGTB)

Hamrick J L and Godt M J W 1989 *Plant Population Genetics, Breeding and Genetic Resources* (Massachusetts: Sinauer)

Mandal A K, Ennos R A and Fagg C W 1994 Mating system analysis in natural population of *Acacia nilotica* spp. *leiocarpa*; *Theor. Appl. Genet.* **89** 931-935

—— and —— 1995 Mating system analysis in natural population of *Acacia nilotica* spp. *kraussina; For. Eco. Manag.* **79** 235-240

Moran G F, Muona O and Bell J C 1989 Breeding systems and genetic diversity in *Acacia auriculiformis* and *A. crassicarpa; Biotropica* **21** 250-256

——, —— and —— 1989 *Acacia mangium*: A tropical forest tree of the coastal lowlands with low genetic diversity; *Evolution* **43** 231-235

Namkoong G 1984 Strategies for gene conservation in forest tree breeding; in *Plant Genetic Resources* eds. C W Yeatman, D Kaftan and G Wilkes; *AAAs Select. Symp.* **87** 79-89

Nei M 1978 Estimation of average heterozygosity and genetic distance from a small number of individual; *Genetics* **89** 583-590

Olng'otie P A S 1991 *Acacia tortilis. A study of genetic structure and breeding systems*, Ph. D. Thesis, Oxford University, Oxford.

Ritland K 1990 A series of FORTRAN computer programs for estimating plant mating systems; *J. Heredity* **81** 235-237

—— and Jain S K 1981 A model for the estimation of outcrossing rate and gene frequencies using *n* independent loci; *Heredity* **47** 35-52

Tanksley S D and Orton T J (eds.) 1983 *Isozymes in Plant Genetics and Breeding*, (Amsterdam: Elsevier)

Wolf P G, Soltis D F and Soltis P S 1990 Chloroplast DNA and allozymic variation in diploid and autotetraploid *Heuchera grossulariifolia; Amer. J. Bot.* **77** 232-244