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GENETIC VARIATION WITHIN TWO SUBSPECIES OF *ACACIA NILOTICA*

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ABSTRACT

Fifteen enzyme systems were analysed in seeds of two subspecies of *Acacia nilotica*. Allozyme diversity at 37 putative loci was determined from five populations of subspecies *indica* and four populations of subspecies *tomentosa*. Subspecies *tomentosa* had more polymorphic loci (P = 51.4% vs 45.9%), higher mean number of alleles per locus (A = 1.5 vs 1.4) and higher genetic diversity (H_e = 0.112 vs 0.086) than subspecies *indica*. Genetic differentiation between populations was greater in subspecies *indica* (G_{ST} = 0.047 vs 0.015) which is also shown by the lower genetic identity values for subspecies *indica* (I = 0.977) compared to those of the subspecies *tomentosa* (I = 0.997). The results are discussed and compared with those reported for other acacias and other plant taxa.

Key words: *Acacia nilotica,* allozyme, genetic diversity, subspecies, provenance, polyploid

INTRODUCTION

Acacia nilotica (L.) Del. is a commercially important, naturally occurring tetraploid tree species widely distributed in subtropical and tropical Africa and in Asia eastwards to India. Essentially a tree of semiarid and arid regions, *A. nilotica* is mostly restricted below 450 m elevation and grows on a variety of soils. It requires direct sunlight and has a deep and extensive root system. *Acacia nilotica* produces showy bright yellow flowers that are pollinated by bees (TYBIRK 1989). Its seeds exhibit dormancy as the testa is very hard. Mature pods are broken by dry winds or remain indehiscent on the ground and are not designed for long distance dispersal (LUNA 1996). Regeneration may occur close to the parent tree resulting in high degree of inbreeding. This species is exceedingly variable with nine subspecies having been recognised with more or less distinctive morphological, ecological and geographical features (DWIVEDI 1993).

There is considerable variation in the habit ranging from subspecies *tomentosa, nilotica and indica* being ovoid crowned, evergreen or semi evergreen to the deciduous and spreading subspp. *adstringens, subalata* and *kraussiana* while the subsp. *cupressiformis* and subsp. *hemispherica* differ in having narrow, erect and bushy crown respectively (WICKENS 1995). Most of the subspecies are tetraploids though higher ploidy levels are known to occur in subspp. *nilotica* and *tomentosa*. The pollen grains of acacias are grouped into polyads which generally have 16 pollen grains. All seeds within a pod are likely to be full sibs as they are fertilized by pollen from a single polyad. The species is andromonoecious with only 30% of the tree being hermaphrodite and the rest have no female function.

Provenance trials including the different subspecies of *A. nilotica* were carried out in eight countries and coordinated by DANIDA Forest Seed Centre, Denmark (GRAUDAL & THOMSEN 1994). Genetic variation in *Acacia nilotica* has mainly been investigated using morphological and physiological traits and compared to the Australian diploid acacias, very little is known about the polyploid acacias of Africa and Asia (MORAN *et al.* 1989). Knowledge of the genetic structure of each subspecies is basic for an appropriate genetic improvement programme. This investigation was undertaken to determine the extent of genetic diversity in two subspecies of *A. nilotica* namely *A. nilotica* subspecies *indica* (Benth.) Brenan (naturally occurring in Pakistan, India, middle east and Burma) and *A. nilotica* subspecies *tomentosa* (Benth.) Brenan (naturally occurring in African countries like Nigeria, Sudan and Ethiopia (DWIVEDI 1993).

The two subspecies grow allopatrically having different geographic distribution ranges (Fig. 1). They also differ in their occurrence with subsp. *tomentosa* developing as tall riverine trees mostly around water logged depressions (SUDAN FORESTRY DIVISION 1950) whereas subsp. *indica* naturally occurs in the Indian subcontinent on plains and xerophytic grasslands with

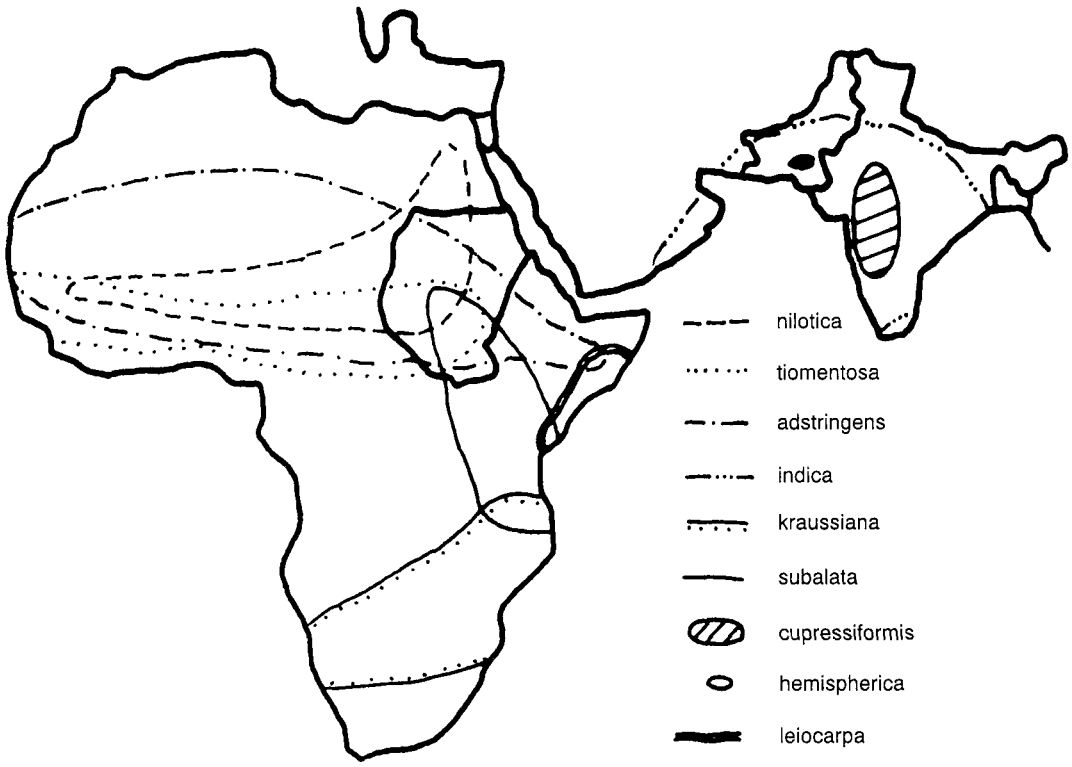

Figure 1. Approximate natural distribution of various subspecies of *A. nilotica* (modified from BRENAN 1983).

poor drainage (SETH 1954). In their natural habitat both subspecies occur as long lived (up to 70 years) medium sized trees. The morphological differences between *Acacia* species common to Africa and India are slight suggesting that their separation is geologically relatively recent.

MATERIALS AND METHODS

Plant Material

Five seedlots of *Acacia nilotica* subspecies *indica* (bulked from 25 mother trees each) that included one from India (T1) and four from Pakistan (T2, T3, T4 and T5) and four seedlots of *Acacia nilotica* subspecies *tomentosa* (bulked from 20–40 trees each) that included four provenances from Sudan (T6, T7, T8, T9) were analyzed (Table 1) to determine the levels and distribution of allozyme genetic diversity within each subspecies. Seed collection was organised by DANIDA Forest Seed Centre, Denmark.

Seed germination

Approximately 100 seeds from open-pollinated flowers from each bulked population were selected at random and germinated. Prior to sowing, the seeds were treated with concentrated sulphuric acid for one hour and then soaked in water overnight to break dormancy. Seeds germinated within ten days in a water saturated vermiculite media under green house conditions (temperature maintained at 30 °C/25 °C day/night). Forty eight seedlings were randomly selected from each of these populations for the allozyme analysis.

Allozyme analysis

Enzymes were extracted from young leaflets (without the stem) harvested from 25 day old seedlings. The leaf samples were immediately ground in a mortar with a pinch of sand and 4–5 drops of a phosphate-polyvinyl pyrrolidone buffer (MITTON *et al.* 1979) to stabilise the proteins. The crude extract was absorbed onto filter paper wicks which were placed in microtest plates for storage at –70°C.

Samples were run on 10% starch gels using three gel electrode buffer systems (Table 2). The 15 enzyme systems stained (loci in parantheses) were diaphorase (*Dia–1, Dia–2, Dia–3*), triosephosphate isomerase (*Tpi–1, Tpi–2, Tpi–3*), phosphoglucomutase (*Pgm–1, Pgm–2, Pgm–3*), leucine aminopeptidase (*Lap–1, Lap–2, Lap–3*), fluorescent esterase (*Fe–1, Fe–2, Fe–3, Fe–4*), alcohol dehydrogenase (*Adh–1, Adh–2*),

Table 1. Geographic origin of the different sources of *A. nilotica*.

No	Species/subspecies	Source	Country	Latitude	Longitude	Mean annual rainfall (mm)	Number of mother trees
T1	*A. nilotica indica*	Kutch	India	23°50' N	69°48' E	348	25
T2	*A. nilotica indica*	Dargai Jahan-gira	Pakistan	33°50' N	72°20' E	750	25
T3	*A. nilotica indica*	Sukhur	Pakistan	28° N	68° E	100	25
T4	*A. nilotica indica*	Gujrat	Pakistan	32°49' N	73°53' E	500	25
T5	*A. nilotica indica*	Thatta	Pakistan	24°41' N	67° 5' E	204	25
T6	*A. nilotica tomentosa*	Lambewa forest	Sudan	13°07' N	33°56' E	588	25
T7	*A. nilotica tomentosa*	Eddllem	Sudan	14°30 N	32°10' E	270	20
T8	*A. nilotica tomentosa*	Wad.Nimir	Sudan	14°30' N	32°10' E	300	40
T9	*A. nilotica tomentosa*	Lumbowa	Sudan	13° N	34° E	450	26

Table 2. Electrode and gel buffer systems and electrophoretic conditions used to resolve 37 putative loci in *Acacia nilotica*. Buffer systems are modifications of SOLTIS *et al.* (1983). (1 and 2 are modifications of buffer system 6 and 3 is a modification of buffer system 8).

System	Electrode buffer	Gel buffer	Enzyme systems	Initial setting
1	0.4 M NaOH 0.3 M boric acid pH 8.6	0.015 M Tris 0.004 M citric acid pH 7.6	DIA, TPI, PGM LAP, FE, ADH PGI	200V 3 hrs
2	0.05 M NaOH 0.27 M boric acid pH 8.0	0.10 M Tris 0.016 M citric acid pH 8.45	IDH, 6-PGD, SKDH FDP, ACO, MDH	200V 5 hrs
3	0.388M LiOH 0.263 M boric acid pH 8.0	0.004 M LiOH 0.029 M boric acid 0.033 M Tris 0.006 M citric acid pH 7.6	MNR, AAT	40 mA 4.5 hrs

phosphoglucoisomerase (*Pgi–1, Pgi–2, Pgi–3*), iso citrate dehydrogenase (*Idh–1, Idh–2*), 6-phosphogluconate dehydrogenase (*6-Pgd–1, 6-Pgd–2, 6-Pgd–3*), shikimic dehydrogenase (*Skdh–1, Skdh–2, Skdh–3*), fructose 1, 6-di-phosphatase (*Fdp–1, Fdp–2, Fdp–3*), aconitase (*Aco–1, Aco–2*), malate dehydrogenase (*Mdh–1, Mdh–2, Mdh–3*), menadione reductase (*Mnr–1*) and aspartate aminotransferase (*Aat–1, Aat–2*).

Where an enzyme stain revealed more than one zone of activity, the fastest migrating zone was designated locus 1 and the next locus 2 etc. This procedure was also followed in numbering alleles within each locus. Interpretation of putative loci from the banding pattern observed were based on segregation patterns with reference to typical subunit structure (HARRIS & HOPKINSON 1976; GOTTLIEB 1981). The different phenotypes were distinguished not only by the position of the bands but also by the relative staining intensity of the bands. Differences in staining intensity were interpreted as differences in allelic dosage in this tetraploid species. Allozyme analysis was carried out on the assumption that the auto tetraploid *A. nilotica* displays tetrasomic inheritance (see MANDAL *et al.* 1994) . This was indicated by the maximum of four allozyme alleles per locus observed in an individual plant and the lack of fixed heterozygotes and the variation in gene dosage levels detected in the isozyme loci.

Data analysis

Statistics of genetic diversity were calculated at the population and subspecies levels. Five genetic parameters namely percent polymorphic loci (*P*), mean number of alleles per locus (*A*), mean number of alleles per polymorphic locus (*AP*), observed heterozygosity (H_o) and expected heterozygosity ($H_e = 1 - \Sigma p_i^2$; also referred to as genetic diversity) were calculated assuming that the individuals were tetraploids (HAMRICK & GODT

Table 3. Summary of genetic diversity within five populations of *A. nilotica* ssp. *indica* and 4 populations of *A. nilotica* ssp. *tomentosa* based on 37 putative loci

Population	P	A_p	A	H_o (s.e.)	H_e (s.e.)
A. nilotica subspecies *indica*					
T1	35.1	2.08	1.38	0.123 (0.04)	0.075 (0.026)
T2	35.1	2.0	1.32	1.135 (0.04)	0.088 (0.026)
T3	40.5	2.0	1.41	0.176 (0.06)	0.098 (0.03)
T4	35.1	2.0	1.35	0.134 (0.04)	0.082 (0.025)
T5	35.1	2.0	1.35	0.148 (0.05)	0.088 (0.028)
	36.2	2.02	1.36	0.143	0.086
Mean	45.9	2.02	1.41	0.143	0.087
A. nilotica subspecies *tomentosa*					
T6	43.2	2.0	1.43	0.177 (0.05)	0.111 (0.03)
T7	43.2	2.12	1.54	0.191 (0.05)	0.106 (0.029)
T8	40.5	2.0	1.41	0.183 (0.05)	0.108 (0.03)
T9	43.2	2.06	1.46	0.193 (0.05)	0.122 (0.031)
	42.5	2.05	1.46	0.186	0.112
Mean	51.4	2.05	1.50	0.192	0.112
t-value	17.29**	3.08*	8.28**	14.3**	16.25**

1989; NEI 1973). Total genetic diversity (H_T) and mean diversity within populations (H_S) were calculated for each polymorphic locus. Genetic differentiation between populations was estimated by $G_{ST} = D_{ST}/H_T$ where $D_{ST} = H_T - H_S$. Mean values were calculated for each subspecies and genetic parameter. Levels of statistical significance among the subspecies for each parameter were determined by t-test. Variance estimates were made using the jackknife procedure of (WEIR & COCKERHAM 1984). Nei's genetic identities were calculated for pairwise comparisons of divergence between populations (NEI 1972; 1977).

RESULTS

Genetic variation in *A. nilotica* subspecies *indica*

Seventeen of the 37 loci resolved (45.9%) were polymorphic in at least one population (Table 3). The common allele was the same in every population with the exception of *6-Pgd–3, Pgm–3 and Aco–1*. Rare alleles were present at *Adh–2, Mnr–1, Tpi–1, Fe–3* and *Fe–4* in 4 of the populations (except T4).

Genetic diversity measures within populations are presented in Table 3. The mean number of alleles per locus within each population ranged from 1.32 to 1.41 (mean = 1.36). The percentage of polymorphic loci was the same in four populations (35.1%) with only population T3 from Sukhur in Pakistan having more polymorphic loci (40.5%). The mean number of alleles per polymorphic locus was the same for four of the populations (except T1 = 2.08). The mean observed heterozygosity ($H_o = 0.143$) was higher than the mean expected heterozygosity ($H_e = 0.086$).

Values for G_{ST} ranged from 0.001 (*Fdp–2*) to 0.172 (*Fe–4*) with a mean G_{ST} of 0.047 indicating that about 95.3% of the allozyme variation occurred within populations (Table 4). The mean genetic identity among the five populations was 0.977 with a range of 0.960 to 0.996 (Table 5). The most divergent population was T4 which had genetic identities with other populations ranging from 0.960–0.971.

Genetic variation in *A. nilotica* subspecies *tomentosa*

Nineteen of the 37 loci resolved (51.4%) were polymorphic in atleast one population (Table 3). The common allele was the same in every population with the exception of *Dia–3*. Rare alleles were present at *Adh–2, Mdh–3, Fdp–3, Fe–4* and *Aco–1* in the populations T7 and T8.

The mean number of alleles per locus within each population ranged from 1.41 to 1.54 (mean = 1.46) (Table 3). The percentage polymorphic loci was the same for three of the populations (except T8). The mean number of alleles per polymorphic locus ranged

Table 4. Statistics of population genetic structure and genetic diversity for polymorphic loci in two subspecies of *A. nilotica*

A. nilotica subspecies *indica*					*A. nilotica* subspecies *tomentosa*				
Locus	H_T	H_S	D_{ST}	G_{ST}	Locus	H_T	H_S	D_{ST}	G_{ST}
Aat–1	0.423	0.404	0.019	0.046	*Adh–1*	0.060	0.059	0.001	0.015
Lap–2	0.098	0.095	0.003	0.031	*Adh–2*	0.006	0.006	0.0001	0.012
Mnr–1	0.006	0.006	0.0001	0.013	*Mnr–1*	0.443	0.439	0.005	0.011
Tpi–1	0.006	0.006	0.0001	0.013	*Mdh–3*	0.088	0.084	0.004	0.049
Fdp–2	0.499	0.499	0.001	0.001	*Tpi–1*	0.114	0.111	0.003	0.023
Fdp–3	0.408	0.404	0.004	0.009	*Tpi–2*	0.011	0.011	0.000	0.004
Fe–2	0.035	0.034	0.0002	0.005	*Tpi–3*	0.500	0.500	0.0003	0.001
Fe–3	0.004	0.004	0.000	0.008	*Fdp-2*	0.505	0.505	0.0001	0.0003
Fe–4	0.309	0.256	0.353	0.172	*Fdp–3*	0.007	0.007	0.0001	0.012
6Pgd–1	0.177	0.173	0.004	0.021	*Fe–4*	0.383	0.371	0.012	0.031
6Pgd–3	0.472	0.421	0.051	0.108	*6Pgd–1*	0.230	0.227	0.003	0.014
Pgm–1	0.008	0.008	0.0001	0.009	*6Pgd–3*	0.500	0.500	0.002	0.004
Pgm–2	0.125	0.116	0.009	0.070	*Pgm–1*	0.155	0.152	0.003	0.020
Pgm–3	0.091	0.082	0.009	0.103	*Pgm–2*	0.016	0.016	0.0002	0.011
Dia–1	0.016	0.016	0.0001	0.008	*Dia–1*	0.155	0.148	0.007	0.045
Dia–2	0.438	0.408	0.030	0.068	*Dia–2*	0.498	0.497	0.001	0.003
Aco–1	0.437	0.389	0.048	0.110	*Dia–3*	0.148	0.146	0.002	0.014
					Aco–1	0.016	0.016	0.0002	0.011
					Pgi–2	0.489	0.488	0.0004	0.001
Mean	0.209	0.195	0.014	0.047	Mean	0.228	0.225	0.002	0.015

Table 5. Nei's statistic of genetic identity between five populations of *A. nilotica* subspecies *indica* and four populations of *A. nilotica* subspecies *tomentosa*.

A. nilotica subspecies *indica*					*A. nilotica* subspecies *tomentosa*			
T1	T2	T3	T4	T5	T6	T7	T8	T9
T1	0.996	0.977	0.971	0.980	T6	0.999	0.998	0.997
T2		0.984	0.971	0.981	T7		0.997	0.998
T3			0.960	0.983	T8			0.996
T4				0.969				

from 2.0 to 2.12 (mean = 2.05). This subspecies also had a higher mean observed heterozygosity (H_o = 0.186) than the mean expected heterozygosity (H_e = 0.112). The G_{ST} values were low ranging from 0.0003 (*Fe–3*) to 0.049 (*Tpi–1*) with a mean G_{ST} of 0.015 (Table 4). Thus, about 98.5% of the genetic diversity in this subspecies occurred within populations. Genetic identity values between the populations, were high and ranged from 0.996 to 0.999 (mean = 0.997) (Table 6).

DISCUSSION

Genetic diversity measures for both the subspecies (*indica* P = 45.9%, A = 1.41, H_e = 0.087; *tomentosa* P = 51.4%, A = 1.50, H_e = 0.112) are similar to the mean values reported for 473 other plant species with a wide variety of life history characteristics (HAMRICK & GODT 1989), but was lower than the values reported for long-lived woody perennials (P = 65.0%, A = 2.22, H_e = 0.177; HAMRICK *et al.* 1992). Expected heterozygosities reported for other animal pollinated acacias were 0.133 for Australian acacias (MORAN *et al.* 1989), 0.081 for *Acacia auriculiformis* (WICKNESWARI & NORWATI 1993) and 0.107 for *Acacia melanoceros* (HAMRICK & MURAWSKI 1991). Higher genetic diversity values were reported for *Acacia albida* (H_e = 0.454, JOLY *et al.* 1992) and *A. melanoxylon* (H_e = 0.33, PLAYFORD *et al.* 1993). Significant differences in

genetic diversity occur among different categories of plants like those with regional distribution and those in different successional stages. Dicots generally have 30% lower values than monocots and gymnosperms (HAMRICK & GODT 1989). Though *A. nilotica* has a wide distribution range, the different subspecies are confined to distinct geographic regions. They occur as medium sized long duration trees in arid conditions but along the river systems of Nile and Indus they occur gregariously and thrive in water logged conditions. Morphological differences between populations of subspp. *indica and tomentosa* are evident in field trials. Populations of subsp. *indica* generally have an erect habit compared to the relatively bushy form of African counterparts. Subsp. *adstringens* has a very conspicuous bushy and spreading tree form (Unpublished results of field trials at Coimbatore).

Within populations, subspecies *indica* had significantly lower genetic diversity values than subspecies *tomentosa*. (Table 3). The within population variation was higher than the between population variation in both subspecies. However, H_T and H_S values were significantly higher and the mean G_{ST} was significantly lower in the *tomentosa* populations (Table 4). The genetic diversity within populations reported for acacias (HAMRICK *et al.* 1992, $H_e = 0.096$) is close to the value obtained for subspecies *indica* but the G_{ST} value reported (0.206) is considerably higher than the values obtained for either sub species of *A. nilotica*. Within population differentiation would be dependent on the nature of the sampled population. In case of isolated trees sampled from farmlands and mixed forests there would be considerable selfing and regeneration of related individuals close to the parent trees. Differences could also occur when seed collections are made from natural and modified environments especially in the case of subsp. *indica* which is widely grown and pods are often collected from a few trees to be raised in agroforestry conditions. A high level of selfing was observed in the mating system analysis of scattered *A. nilotica* ssp. *leiocarpa* trees from Africa (MANDAL *et al.* 1994). The low G_{ST} values obtained may be because only a few populations from a limited region of the natural range have been covered in this study. Allozyme studies of other acacias have demonstrated clustering of populations into major geographic regions (*Acacia auriculiformis* – WICKNESWARI & NORWATI 1993; *Acacia albida* – JOLY *et al.* 1992) and in *Acacia melanoxylon,* geographic separation has led to differences suggesting the presence of two subspecies (PLAYFORD *et al.* 1993). Furthermore, for *Tectona grandis*, KERTADIKARA & PRAT (1995) identified clear separation of the Indian provenances from African and Indonesian provenances.

Levels of genetic diversity within populations are influenced by several characteristics of the species (HAMRICK & GODT 1989). Seed dispersal, breeding system and geographic range all have predictive value. More widespread species as well as species that are out-crossed tend to have more within population diversity. Unlike other out crossed long lived trees considerable selfing (60% of seeds set through self pollination) and inbreeding occur in *A. nilotica* subsp. *leiocarpa* (MANDAL *et al.* 1994). The pods remain indehiscent and the full sibs regenerate naturally around the mother trees (FAGG 1995) and hence gregarious populations of the species are likely to contain a high proportion of related trees. However these pods could be dispersed by wild herbivores and domestic livestock giving a patchy distribution of seeds over short areas. In subsp. *tomentosa* during floods the pods are often carried over long distances and deposited along the banks of the rivers giving rise to gregarious stands. Thus populations along the same river system may be related resulting in low between population variation. The populations of subsp. *tomentosa* studied are located along the Blue Nile and White Nile river systems in Sudan. Differences in self incompatibility occur between the different subspecies and between trees of a population. Contrary to the trend in subsp. *leiocarpa* very high level of outcrossing was reported in subsp. *kraussiana*. There were however significant differences in outcrossing rate between trees (MANDAL & ENNOS 1995) emphasising the importance of selecting trees for seed collection. Higher total diversity and within population differentiation noticed in subsp. *tomentosa* could be due to the occurrence of certain hybrids in the populations (BRENAN 1983) as four subspp. *tomentosa, nilotica, adstringens* and *subalata* are known to grow sympatrically over extensive areas in their natural range in Sudan (Fig.1). Higher ploidy levels are also known to occur in this subspecies. Hybridization has been reported to occur between subsp. *indica* and subsp. *hemispherica* (ALI & QAISER 1980) resulting in considerable variability in the population. Several natural interspecific hybrids of acacias have been reported from Africa and it is suspected that there may be many more as the full extent of hybridization and introgression is largely unknown (ROSS 1979). Further investigation into these aspects and the degree of self incompatibility and selfing rate in the two subspecies would be necessary to understand the situation better. Controlled hybridization would be possible between outcrossed individuals of these subspecies. Higher between population differentiation in subsp. *indica* is understandable because populations occurring over a wide natural range covering large areas of the Indian subcontinent (often in dry thorn

forests) would be separated more and regeneration through root suckers is also known in this subspecies. In this study, however, the four populations of subspecies *tomentosa* from a limited geographic area in Africa were found to have higher levels of allozyme variation than the five populations of subspecies *indica* from India and Pakistan. Thus, it appears that subspecies *indica* may be less genetically variable than subspecies *tomentosa*. Some of the higher overall variation in subspecies *tomentosa* is due to the higher proportion of polymorphic loci and slightly more alleles per polymorphic locus. However, the higher mean H_T value observed for *tomentosa* also indicates that alleles in this subspecies have more even frequencies. The larger differences observed between the two species for mean population values of *P, AP* and H_e are largely due to differences in how genetic diversity is distributed within and among populations represented in this study. The *indica* populations of *Acacia nilotica* which had a maximum spread of 10° latitude had lower genetic identity values (mean = 0.977) compared to the *tomentosa* populations (mean = 0.997) which had only a maximum spread of 1.3° latitude.

In summary, our analysis of a limited subset of populations within two of the nine subspecies of *A. nilotica* indicate that the two subspecies maintain somewhat less variation than comparable woody species. Furthermore, the two subspecies were quite different genetically with only 12 of the 23 loci polymorphic across the two subspecies being polymorphic in both subspecies. Thus, while it would appear that *A. nilotica* contains quite high levels of genetic diversity much of the diversity within the species occurs among its subspecies. A more comprehensive study including a higher proportion of the subspecies would be required to resolve what proportion of the total genetic diversity in this species occurs among subspecies, among populations within subspecies and within local populations.

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