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Phylogenetic analysis of *Acacia* (*Mimosaceae*) as revealed from chloroplast RFLP data

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Abstract Chloroplast DNA of 22 species of *Acacia* (Tourn.) Miller was digested with ten restriction endonucleases, Southern-blotted and probed with cloned fragments covering the chloroplast genome of tobacco (*Nicotiana tabacum* L.). Phyletic and phenetic analyses of the resulting 176 polymorphic bands recorded among the 22 species were performed. The phylogram was reconstructed using heuristic search and Wagner parsimony. The resulting most parsimonious consensus phylogram displayed three major phyletic lineages, consistent with the previously established three subgenera of *Acacia*. The 10 species of subgenus *Acacia* and the 6 species of subgenus *Heterophyllum* formed two monophyletic sister clades. The 5 species of subgenus *Aculeiferum* studied and *Acacia albida* (Syn. *Faidherbia albida*) grouped together and were basal to the clades of subgenera *Acacia* and *Heterophyllum*. The phylogram indicated that subgenus *Heterophyllum* diverged earlier from subgenus *Aculeiferum* than did subgenus *Acacia*; however, the phenogram indicated the reverse. The study indicated that *A. nilotica* and *A. farnesiana* are sister species, though *A. nilotica* is Afro-Asiatic and *A. farnesiana* is American. The phenogram separated the three subgenera in agreement with the phylogram, but the two dendrograms differed regarding the topologies of the species and the distance of evolution between subgenera *Acacia* and *Heterophyllum*.

Key words DNA · Molecular evolution · Parsimony · PAUP · Phylogeny · RFLP

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Introduction

The genus *Acacia* (Tourn.) Miller includes over 1200 species distributed in the tropical and subtropical zones of the world (Atchison 1948; Ross 1979). Vassal (1972) divided *Acacia* into three subgenera (*Heterophyllum*, *Aculeiferum* and *Acacia*). Chevalier (1934) transferred *Acacia albida* Del. from the genus *Acacia* to *Faidherbia*. Subsequent reports appeared to concur with the results of Chevalier (Vassal 1972; El-Tinay et al. 1979; Playford et al. 1992), while others did not (Ross 1979; Bukhari 1997a, b; Harrier et al. 1997). *Acacia* species are adapted to dry conditions (Ross 1981) and have agroforestry potentials. They are noted for their multiple uses such as fuelwood, timber, fiber, medicine, food, handicrafts, domestic utensils, environmental protection, soil fertility, shade, game refuge, ornamental planning, gum, fodder and tan (Wickens et al. 1995).

A wide range of techniques have been used to investigate the systematics of the genus *Acacia*: shoot and pollen morphology (Bentham 1875; Vassal 1969, 1972; Guinet and Vassal 1978), histology (Robbertse 1975 a, b, c; Vassal 1975), cytology (Ghimpu 1929; Atchison 1948; Khan 1951; Vassal 1972, 1975; Hamant et al. 1975; Vassal and Lescanne 1976; Guinet and Vassal 1978; Bukhari 1997a, b), isozymes (Moran 1992; Playford et al. 1993; Oballa 1996), immunology (El-Tinay et al. 1979; Brain 1990; Brain and Maslin 1996) and DNA analysis (Playford et al. 1992; Harrier et al. 1997). Most of the characters used in the above studies were quantitative with continuous variations (Guinet and Vassal 1978). Such data tend to group taxa on the basis of present phenetic similarity. Consequently, the estimated phenograms may not reflect similarities inherited through common descent (Palmer et al. 1988). One way to optimize estimates of similarities by decent is to

construct all possible alternative associations among the target taxa and choose those showing a minimum number of character state changes (Camin and Sokal 1965; Farris 1970).

The rate of nucleotide change of the chloroplast genome is much slower than that of the other genomes in the eucaryotic plant cell (Palmer 1987). One mechanism for such a conservative evolution as that found in the angiosperms is the uni-parental clonal inheritance of plastids (Ohyama et al. 1986; Shinozaki et al. 1986; Palmer 1987; Palmer et al. 1988; Hsiao et al. 1995), though a few cases of recombination and bi-parental inheritance have been recorded (Govindaraju et al. 1988). Such a conservative mode of evolution has made chloroplast DNA phylogeny suitable for revealing evolutionary pathways among distantly related taxa, thereby avoiding the consequences of rapid adaptation and repeated interspecific hybridization (Palmer 1987).

Little is known about the phylogeny of *Acacia*, although previous studies have pointed out the necessity of using all available tools to expand our knowledge on the systematics of such a diversified genus as *Acacia* (Vassal 1972; El-Tinay et al. 1979; Guinet and Vassal 1978). In the study reported here 22 species of *Acacia* were surveyed for chloroplast restriction fragment length polymorphisms (RFLPs) to test the suitability of such data in revealing evolutionary trends and phylogenetic relationships within the genus.

Materials and methods

Plant material, DNA isolation and digestion

Twenty-two species of *Acacia*, 10 of which belong to subgenus *Acacia*, 6 to *Heterophyllum* and 6 to *Aculeiferum*, were included in this study. Geographic origin and sources of the accessions are shown in Table 1. A modified method of Doyle and Doyle (1987) was used to prepare whole-cell DNA. One-month-old seedlings were kept in the dark for 36 h. Three grams of expanding leaves from ten seedlings of each accession were harvested. The harvested materials were frozen in liquid nitrogen, then finely powdered with a mortar and pestle. The powder was suspended in extraction buffer [100 m*M* TRIS, pH 8; 1.6 *M* NaCl; 20 m*M* EDTA; 2% cetyltrimethylammonium bromide; 2% (w/v) polyvinylpyrrolidone, MW 40 000]. The concentration of NaCl and polyvinyl pyrrolidone used above were optimal to remove a highly viscous transparent substance that always associated with the isolated DNA. Each of the DNA samples obtained above was diluted to 1.5 ml and further purified using the QIAGEN DNeasy Plant kit according to the manufacturer's instructions, with steps 1–7 excluded. About 4 μg of the purified DNA from each sample was double-digested with 6 units each of the following restriction endonuclease combinations (*Bam*HI + *Bcl*I, *Eco*RI + *Eco*RV; *Hin*dIII + *Nco*I; *Sac*II + *Sal*I;

Table 1 Plant materials used, their geographic origin, source and somatic chromosome numbers

Taxon	Origin	Accession number	Latitude (°)	Longitude (°)	Donor[a]	2n[b]
Subgenus *Heterophyllum* Vassal						
A. sophorae Willd.	Australia	01879/92	42S	148W	DANIDAFSC	26
A. melanoxylon Roxb.	Australia	01878/92	41S	144E	DANIDAFSC	26
A. dealbata Link.	Australia	01876/92	41S	147E	DANIDAFSC	26
A. implexa Benth.	Australia	01877/92	36S	134E	DANIDAFSC	26
A. mearensii Willd.	Kenya	01892/92	1S	38E	DANIDAFSC	26
A. holosericae Cunn. ex Don.	Senegal	01875/92	5N	15W	DANIDAFSC	52
Subgenus *Aculeiferum* Vassal						
A. asak (Forssk.) Willd.	Tanzania	752/91	5S	35E	TNTSP	26
A. caffra (Thunb.) Willd.	Tanzania	754/91	5S	35E	TNTSP	26
A. polycantha Willd.	Tanzania	751A/91	5S	35E	TNTSP	52
A. senegal (L.) Willd.	Sudan	−/90	12N	38E	STSC	26
A. mellifera (Vahal.) Benth.	Sudan	−/90	13N	35E	STSC	26
A. albida Del. [Syn. *Faidherbia albida* (Del.) A. Chev]						
A. albida Del.	Sudan	−/90	17N	33E	STSC	26
Subgenus *Acacia* Vassal						
A. nilotica ssp. *nilotica* Hill.	Sudan	01644/86	14N	32E	DANIDAFSC	52
A. seyal. (Del.) var. *seyal* Mill.	Sudan	−/91	11N	34E	STSC	104
A. tortilis (Forssk.) Hayne	Tanzania	21 A/93	6S	36E	TNTSP	
A. radiana (Savi.) Brenan	Israel	01284/84	31N	35W	DANIDAFSC	78
A. sieberana DC.	Sudan	− /93	14N	36E	STSC	26
A. farnesiana (L.) Willd.	Chile	01470/84	28S	70W	DANIDAFSC	52
A. elatior Brenan	Kenya	01810/88	3S	37E	DANIDAFSC	52
A. drepanolobium Harms ex Siös.	Tanzania	751/91	5S	35E	TNTSP	52
A. ehrenbergiana Hayne	Niger	01561/86	14N	10E	DANIDAFSC	52
A. nubica Benth.	Kenya	01890/92	0	37E	DANIDAFSC	56

[a] DANIDAFSC, Danish International Development Agency Forest Seed Center; TNTSP, Tanzanian National Tree Seed Program; STSC, Sudan Tree Seed Center
[b] Bukhari (1997b)

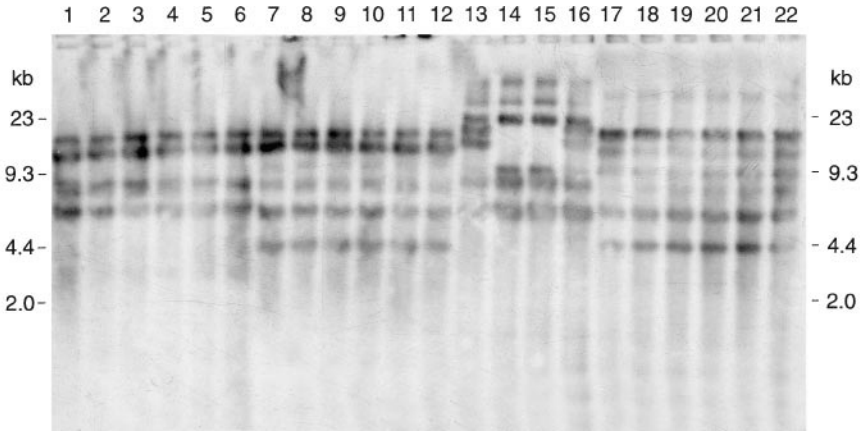

Fig. 1 Southern blot hybridization autophotograph of chloroplast restriction fragments (*Bam*HI + *Bcl*I) of 22 *Acacia* species using a combination of clones five and six of tobacco chloroplast DNA of Sugiura et al. (1986). Molecular weights are given in kilobase pairs. *Lane 1 A. melanoxylon*, *2 A. holosericea*, *3 A. mearensii*, *4 A. dealbata*, *5 A. sophorae*, *6 A. implexa*, *7 A. caffra*, *8 A. mellifera*, *9 A. asak*, *10 A. polyacantha*, *11 A. senegal*, *12 A. albida*, *13 A. tortilis*, *14 A. radiana*, *15 A. drepanolobium*, *16 A. ehrenbergiana*, *17 A. elatior*, *18 A. sieberana*, *19 A. nubica*, *20 A. farnesiana*, *21 A. nilotica*, *22 A. seyal*. Data on the materials and ploidy levels are given in Table 1

*Xba*I + *Xmn*I). The DNA digests were fractionated through a 0.9% agarose gel in TAE buffer (0.04 *M* Tris; 0.02 *M* Na-acetate; 0.002 *M* EDTA; 0.0018 *M* NaCl).

Source of probe and labeling

About 200 ng each of 40 cloned DNA fragments of Sugiura et al. (1986) covering the chloroplast genome of tobacco (*Nicotiana tabacum* L.; *Solanaceae*) was kindly provided by Dr. Bob Jansen (Dept. of Botany, University of Texas, Austin). The vectors (pTZ19R; pBSsk$^+$; pBR322), including the insert, were transferred to a competent *E. coli*. The transformed bacteria were multiplied in LB medium, and the plasmids were isolated using the QIAGEN Plasmid Purification kit. Following vector multiplication, the cloned fragments were excised using the appropriate endonucleases. The inserts were separated from their vectors in a low-melting agarose gel electrophoresis and labeled by random priming with digoxygenin-dUTP. Each labeled probe was used to hybridize up to five separate membranes.

DNA transfer, detection and analysis

DNA transfer and detection were performed according to Boehringer Mannheim (1995). The transferred DNA was fixed at 120°C for 20 min on a positively charged nylon membrane. The immobilized DNA was hybridized with the Dig-labeled probes at 58°C for 16 h. The 40 probes were paired in the order 1 + 2, 3 + 4 up to 39 + 40, and each probe combination was used to hybridize a double-enzyme digest-immobilized DNA. The membranes were washed twice with 2 × SSC; 0.1% SDS at 25°C for 5 min and washed again twice with 0.1 × SSC, 0.1% SDS at 55°C for 15 min. The hybridized probes were detected immunologically, using the chemiluminescence detection kit (Boehringer Mannheim 1995). Following each detection, the probes were stripped off, and the probe-free membranes were rehybridized. Autophotographs were obtained by exposing X-ray films to the filters for 15–20 min.

Data analysis

Bands on the autophotograph were recorded as present (1) or absent (0) and used to create a data matrix. Each restriction site was considered as an unordered, undirected and independent character. The data were analyzed using the computer program Phylogenetic Analysis Using Parsimony (PAUP for Apple MacIntosh, version 3.1.1; Swofford 1993). Wagner parsimony phylograms were reconstructed using heuristic searching. Random tree branch swapping functions were employed to examine alternative trees of identical length. Some 150 bootstrap resampling replicates of the data were performed according to Felsenstein (1985) to estimate the statistical significance of the branches.

Cluster analysis on the above scoring was also performed using the NTSYS (Numerical Taxonomic and multivariate System) software package, version 1.70 (Rohlf 1992). The coefficients of association were obtained using the 'Simqual' (similarity for qualitative data) option, and the resulting data were clustered using the 'SAHN' (Sequential Agglomerative Hierarchical Nested clustering and 'UPGMA' options (Sneath and Sokal 1973).

Results

The tobacco (*Solanaceae*) chloroplast probes used in this study produced resolvable signals with the present material (*Mimosaceae*, Fig. 1). A total of 176 scoreable polymorphic bands were obtained from the 100 Southern blot profiles. Heuristic search under Wagner parsimony revealed three equally and most parsimonious phylograms of 411 steps (consistency index = 79.5%, retention index = 76%). The majority-rule consensus of the three equally parsimonious trees assigned the *Aculeiferum* as the basal clade in the genus and the other two subgenera as monophyletic sister clades with *A. senegal* and *A. caffra* as the overall common ancestors. However, this ancestry is supported by only 49% bootstrap confidence limits. Most branches in all of the 150 replicates were supported by strong bootstrap confidence limits, but supports for the *Aculeiferum* clade and *A. nubica* were relatively low (Fig. 2). The three major clades of the 22 species studied were consistent with the three subgenera of *Acacia*. The phylogram indicates that subgenus *Heterophyllum* evolved before subgenus *Acacia*. Interestingly, *A. albida*

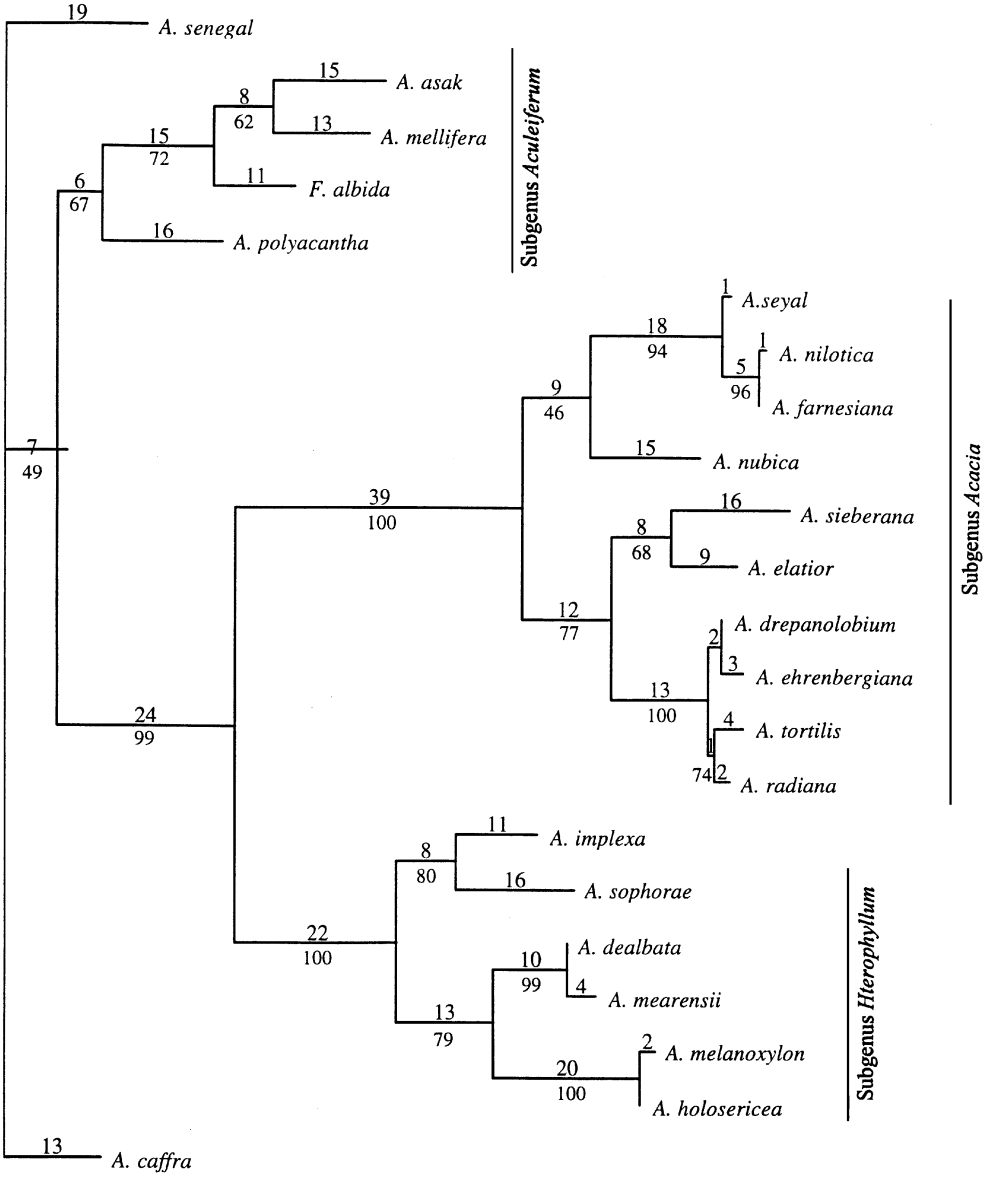

Fig. 2 Majority rule consensus phylogram of three minimal-length trees based on length polymorphism of restriction fragments of chloroplast DNA of 22 *Acacia* species. The phylogram was constructed using the Wagner parsimony method (Swofford 1993). The Felsenstein's bootstrap confidence levels from 150 replicates of heuristic search analysis are given *below* each branch. The estimated number of changes in a restriction site following divergence from the nearest ancestral node are given *above* each branch. Branch lengths are proportional to the evolutionary distance between taxa. The phylogram has 411 steps (consistency index = 79.5%, retention index = 76%). Data on the materials and ploidy levels are given in Table 1

was placed within the 6 studied species of subgenus *Aculeiferum*.

In the present study, *A. tortilis* showed the highest absolute number (99) of unshared bands and mean distance (56) with *A. polyacantha*, while *A. nilotica* and *A. farnesiana* showed the lowest number of unshared bands (1) and mean distance (0.01). Moreover, mean distance and absolute number of unshared bands among all the 22 studied species increased with increasing distance of evolution. Pairwise mean distance and absolute number of unshared bands are presented in Table 2.

Differences in ploidy levels within each of the three subgenera were not reflected in this chloroplast-based phylogeny. For instance, *A. seyal* (2n = 8x) appeared as a sister clade of *A. nilotica* (2n = 4x) and *A. farnesiana* (2n = 4x), whereas *A. tortilis* (2n = 4x) was the nearest sister to *A. radiana* (2n = 6x). Likewise, *A. melanoxylon* (2n = 2x) appeared as a close sister to the tetraploid *A. holosericea* (2n = 4x). Besides, the interspecific relationships based on chloroplast phylogeny were not always concordant with the species geographical distribution. For instance, *A. nilotica* is closer to *A. farnesiana* than it is to *A. seyal*, and these branches are supported by high bootstrap confidences (Fig. 2).

The phenogram produced by NTSYS separated the three subgenera of *Acacia* in concordance with the phylogram produced by PAUP. However, substantial reshuffling of the position of species within each subgenus occurred. Furthermore, subgenus *Acacia* appeared more evolved than subgenus *Heterophyllum* in the phenogram, which is in contrast with the phylogram (Figs. 2, 3). Again, the phenogram placed *A. albida* within subgenus *Aculeiferum* at a 77%

Table 2 Pairwise comparison matrices of 22 species of *Acacia* based on restriction site differences obtained using PAUP 3.1.1 (Swofford 1993). The figures above the diagonal represent mean distance, and the ones below the diagonal show the absolute numbers of unshared bands

	seneg.	*asak*	*caffra*	*mellif.*	*polya.*	*albida*	*seyal*	*sieber.*	*nubica*	*elatior*	*drepa.*	*tortilis*	*implex*	*dealb.*	*sopho.*	*melan*	*radia.*	*nilot.*	*meari.*	*farnes*	*ehren.*	*holos.*
A. senegal	–	0.21	0.18	0.24	0.19	0.26	0.45	0.49	0.48	0.52	0.50	0.51	0.43	0.44	0.43	0.47	0.53	0.46	0.42	0.45	0.49	0.47
A. asak	36	–	0.22	0.16	0.23	0.19	0.53	0.51	0.56	0.48	0.50	0.53	0.42	0.40	0.47	0.45	0.52	0.51	0.40	0.52	0.49	0.45
A. caffra	32	38	–	0.21	0.18	0.25	0.48	0.51	0.48	0.50	0.50	0.52	0.39	0.40	0.41	0.47	0.53	0.48	0.39	0.47	0.49	0.46
A. mellifera	42	28	36	–	0.22	0.18	0.53	0.47	0.53	0.44	0.53	0.55	0.44	0.39	0.47	0.49	0.54	0.51	0.39	0.52	0.53	0.48
A. polyacantha	34	40	32	38	–	0.24	0.48	0.46	0.48	0.50	0.55	0.56	0.42	0.45	0.40	0.47	0.55	0.49	0.43	0.48	0.54	0.46
A. albida	46	34	44	32	42	–	0.53	0.48	0.55	0.47	0.50	0.51	0.43	0.43	0.47	0.40	0.52	0.50	0.43	0.49	0.51	0.42
A. seyal	79	93	85	93	85	93	–	0.25	0.19	0.27	0.24	0.27	0.44	0.52	0.46	0.55	0.26	0.04	0.51	0.03	0.26	0.53
A. sieberana	87	89	89	83	81	85	44	–	0.28	0.14	0.21	0.23	0.51	0.48	0.54	0.50	0.22	0.23	0.48	0.24	0.22	0.51
A. nubica	85	99	85	93	85	97	34	50	–	0.23	0.29	0.30	0.43	0.51	0.44	0.56	0.30	0.22	0.51	0.22	0.31	0.55
A. elatior	92	84	88	78	88	82	47	25	41	–	0.18	0.20	0.45	0.45	0.51	0.48	0.19	0.26	0.47	0.27	0.20	0.48
A. drepanolobium	88	88	88	94	96	88	43	37	51	32	–	0.04	0.48	0.45	0.53	0.43	0.03	0.25	0.46	0.26	0.02	0.44
A. tortilis	89	93	91	97	99	89	48	40	52	35	7	–	0.47	0.44	0.51	0.41	0.03	0.28	0.46	0.28	0.06	0.42
A. implexa	75	73	69	77	73	75	78	90	76	79	85	82	–	0.23	0.15	0.28	0.48	0.45	0.24	0.44	0.50	0.28
A. dealbata	77	71	71	69	79	75	92	84	90	79	79	78	40	–	0.26	0.18	0.46	0.51	0.02	0.51	0.47	0.17
A. sophorae	76	82	72	82	70	82	81	95	77	90	94	89	27	45	–	0.31	0.52	0.46	0.27	0.45	0.54	0.30
A. melanoxylon	83	79	83	87	83	71	96	88	98	85	75	72	50	32	55	–	0.42	0.54	0.21	0.55	0.44	0.01
A. radiana	93	91	93	95	97	91	46	38	52	33	9	10	84	80	91	74	–	0.27	0.47	0.27	0.05	0.43
A. nilotica	80	90	84	90	86	88	17	41	39	46	44	49	79	89	80	95	47	–	0.51	0.00	0.27	0.53
A. mearensii	73	71	69	69	75	75	90	84	90	83	81	80	42	9	47	36	82	89	–	0.51	0.48	0.19
A. farnesiana	79	91	83	91	85	87	6	42	38	47	45	50	78	90	79	96	48	7	90	–	0.27	0.53
A. ehrenbergiana	87	87	87	93	95	89	46	38	54	35	11	10	88	82	95	78	8	47	84	48	–	0.46
A. holosericea	83	79	81	85	81	73	94	90	96	85	77	74	50	30	53	8	76	93	34	94	80	–

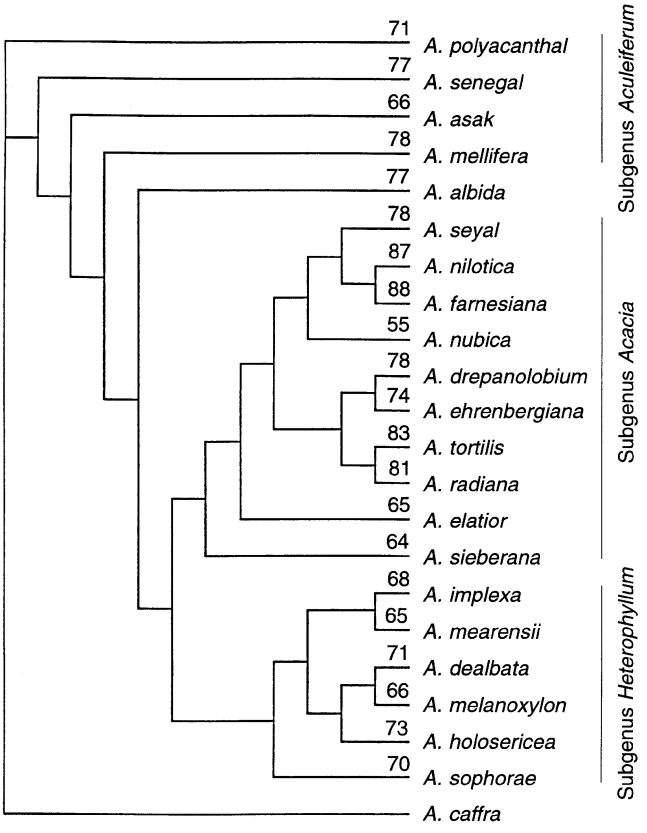

Fig. 3 Cluster analysis performed on chloroplast RFLPs of 22 *Acacia* species using the NTSYS. The coefficients of association were obtained using the 'Simqual' option. The coefficients of similarity between each pair were calculated using the 'Qualitative' and 'UPGMA' (Unweighted Pair-Group Method using Arithmetic averages) options and clustered using the 'SAHN' option (Sneath and Sokal 1973). Confidence limits are given above each branch. Data on the materials and ploidy levels are given in Table 1

confidence level and *A. nubica* within subgenus *Acacia* at a 55% level (Fig. 3).

Discussion

Resolvable bands were obtained in this study even-though chloroplast probes from a distantly related taxon were hybridized to a small amount of total DNA of *Acacia*. This result attests for the conservative mode of chloroplast DNA evolution. The good resolution of bands was probably enhanced by the existence of multiple copies of chloroplast DNA molecules per cell (Palmer et al. 1988).

The phylograms of this study indicated that subgenus *Aculeiferum* is ancestral to subgenera *Acacia* and *Heterophyllum*, with *A. senegal* and *A. caffra* appearing as the overall common ancestors. The ancestry of *A. senegal* and *A. caffra*, however, had a weak statistical support (49% bootstrap confidence level). This result suggests that this ancestry is not very conclusive and that the representatives of the common ancestors were not included in the study. Previous morphological and chromosomal evidence has suggested that section *Filicinae* (not included in the present study) is ancestral to the other sections of subgenus *Aculeiferum* and that subgenera *Acacia* and *Heterophyllum* were derived from a common descent (Atchison 1948; Vassal 1972; Vassal and Lescanne 1976; Guinet and Vassal 1978).

The present phylogram contrasted with the results from studies performed on grasses (Bennett 1972) in which nuclear genome size was seen to increase during evolution. Our results and observations on the mass of nuclear DNA (Bukhari 1997a) suggest that genome size may either increase or decrease during the evolution of *Acacia* subgenera. For instance, subgenus *Acacia* which appears in the present study to have evolved relatively recently, has 1.09 pg nuclear DNA (Bukhari 1997a) and is at least tetraploid (n = 52) with small chromosomes. Subgenus *Heterophyllum* has 1.583 pg and is most often diploid (2n = 26) with larger chromosomes (Bukhari 1997a, b). Both seem, as the present study indicates, to have evolved from a diploid (2n = 26, 1.150 pg) member of subgenus *Aculeiferum* that has medium-sized chromosomes. Changes in chromosome size are usually related to the divergence of subfamilies and genera (Hsiao et al. 1993). This conforms with our present results.

Our two dendrograms suggest that subgenera *Acacia* and *Heterophyllum* are sister clades descended from subgenus *Aculeiferum*. In contrast, cytological studies (Bukhari 1997b) have indicated that the chromosomes of subgenera *Heterophyllum* and *Aculeiferum* are represented in subgenus *Acacia*, implying that subgenus *Acacia* is a hybrid between subgenera *Heterophyllum* and *Aculeiferum*. The present phylogeny, however, failed to support this, since it showed that subgenera *Acacia* and *Heterophyllum* were monophyletic. Palmer (1987) pointed out that chloroplasts of most angiosperms, though not yet studied thoroughly in *Acacia*, are slow-evolving and are clonally and uni-parentally inherited. It can be suggested that *Heterophyllum* first descended from *Aculeiferum* and that after a long period of evolution a member of *Heterophyllum* back-crossed with a maternal member of subgenus *Aculeiferum*. As a result, the hybrid product inherited nuclear DNA from both parents, but only the chloroplast of *Aculeiferum*. After spontaneous chromosome doubling (Bukhari 1997b), the hybrid evolved into subgenus *Acacia*.

Except for *A. albida*, the present phylogram is basically in agreement with previous taxonomic studies on *Acacia* based on morphology (Vassal 1972; Guinet and Vassal 1978; Grosso et al. 1994), cytology (Atchison 1948; Vassal 1972; Guinet and Vassal 1978), nuclear DNA analysis (Playford et al. 1992) and biochemistry (Conn et al. 1989; Brain 1990). Chevalier (1934) suggested the removal of *A. albida* from the genus *Acacia*

to a monotypic genus of *Faidherbia*. Several subsequent reports appear to support the studies of Chevalier (Vassal 1972; El-Tinay et al. 1979; Playford et al. 1992). On the other hand, chromosomal studies (Khan 1951; Bukhari 1997b), amount and quality of seed storage proteins (Bukhari, unpublished data) and nuclear DNA amounts (Bukhari 1997a), as well as results from the present study, suggest that *A. albida* fits into subgenus *Aculeiferum*. Random amplified polymorphic DNA) (RAPD) and morphological markers (Harrier et al. 1997) showed that *A. albida* is not independent from the genus *Acacia*. Various morphological characters are known to be shared among some species of subgenera *Acacia* and *Aculeiferum*. For instance, *A. lahti*, *A. horrida* and *A. bussei* (subgenus *Acacia*) have spicate inflorescences, whereas *A. mellifera* ssp. *detinens* (subgenus *Aculeiferum*) has capitate inflorescences (Ross 1979). Likewise, *A. albida* has spinescent stipules (Robbertse 1975a) and capitate inflorescences (Ross 1979).

In the present phylogenetic study, mean distance and absolute number of unshared bands among all the 22 studied species increased with increasing distance of evolution. On the other hand, our two dendrograms differed in the distance of evolution between subgenera *Acacia* and *Heterophyllum*. Other studies have shown that subgenus *Aculeiferum* has the least advanced morphological characters (Atchison 1948; Vassal 1972; Guinet and Vassal 1978) and is closer to subgenus *Heterophyllum* than to subgenus *Acacia* (Conn et al. 1989; Grosso et al. 1994). It appears that the use of molecular data in phylogeny can be impaired by convergence and parallel evolution and that parsimony methods are suitable in reconstructing dendrograms from such data.

The branch connecting *A. nubica* in both dendrogrames was supported by a low confidence limit. Unlike species of subgenus *Acacia*, which have 2n multiples of 13, *A. nubica* has a 2n multiple of 14. Besides, the tetraploid *A. nubica* is only distantly related to the subgenus *Acacia*. The systematics of *A. nubica* deserves further investigation.

The present phylogeny established a close relationship between *A. nilotica* and *A. farnesiana* that is well-supported by bootstrap confidence. *A. nilotica* extends naturally in tropical Africa and Asia while *A. farnesiana* is believed to be native to tropical America although it is now cosmopolitan within the range of *Acacia* (Khan 1951). On the other hand, the present interspecific associations based on chloroplast phylogeny are not always concordant with those of the species reported by Bukhari (1997b). Such misplacement in lower taxa may be the consequence of the clonal inheritance of chloroplasts in contrast with plant nuclear genome which evolves relatively rapidly and assumes recombination and hybridization (Palmer et al. 1988). It is unfortunate that the present study did not include other *Acacia* species native to America. One can only argue that either the American species of subgenus *Acacia* are phylogenetically very closely related to their counterparts in Africa and Asia or that *A. farnesiana* is originally native to the Old World but recently dispersed across the New World. The former explanation appears more plausible since chloroplast DNA is known to evolve slowly and is thus not very useful in resolving narrow phylogenetic relationships.

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