Natural Resources for Human Health

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Original Research

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Received 16 March 2026
Revised 17 April 2026
Accepted 16 May 2026
Available Online 02 September 2026

Edited by Manoj G Kulkarni

KEYWORDS:
Acacia nilotica
Sudan
Genetic relationships Dominant DNA markers

Natr Resour Human Health 2026; 6 (4): 445–454
https://doi.org/10.53365/nrfhh/224633
eISSN: 2583-1194

Genetic variability analysis of medicinally important *Acacia nilotica* (L.) Willd. ex Delile tree based on RAPD "Random Amplified Polymorphic DNA" markers

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ABSTRACT: Arbitrarily amplified dominant DNA markers (random amplified polymorphic DNA (RAPD), SCoT, and branch point signal (BPS)) were used to evaluate the genetic relationship between three subspecies of *Acacia nilotica* in the Sudan (nilotica, tomentosa, and adstringens) and a new group that was observed within Sudan's *A.nilotica* populations with intermediate morphological traits between the subspecies tomentosa and nilotica. DNA was extracted from seeds that were collected from 28 *A. nilotica* populations representing the subspecies nilotica (10 populations), subspecies tomentosa (11 populations), the subspecies adstringens (3 populations), and the new group (4 populations) through the agro-ecological zones of the Sudan. A total of 48 primers (8 SCoT, 12 BPS, and 28 RAPD) were tested, of which 16 primers (33.33%) showed polymorphism (2 SCoT, 3 BPS, 11 RAPD) within the studied populations and used for further genetic evaluation. The selected primers amplified 142 bands in the range of 1.39–0.38 kbp, of which 102 were polymorphic (71.27%) among the studied populations with an average of 8.88 bands per primer as well as an average of 59.90% and 0.2175 of the allele frequency and gene diversity (expected heterozygosity), respectively. Spearman's similarity coefficient indicated high genetic similarity (92.07%) between the new group and the subspecies nilotica, followed by 90.09% and 46.29% with subspecies tomentosa and adstringens, respectively. The genetic dendrogram based on UPGMA clustering analysis grouped the new group with the subspecies nilotica and tomentosa in one cluster, indicating hybridization between them, while the subspecies adstringens was alone in the second cluster.

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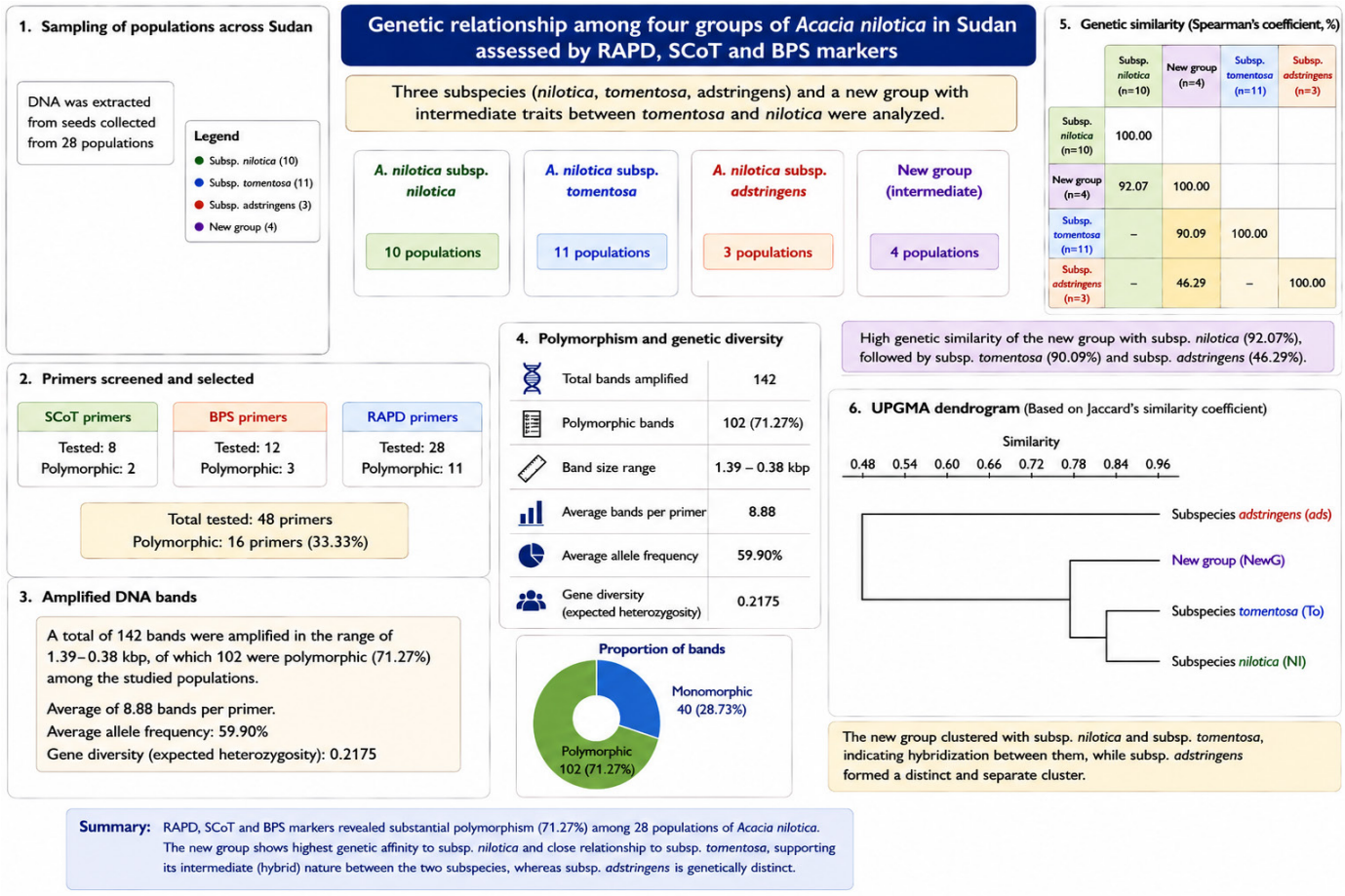
GRAPHICAL ABSTRACT



1. INTRODUCTION

The subject of infra-specific classification of wild organisms receives more and more sporadic attention from taxonomists, evolutionary biologists, and ecologists to solve the species problem (Styles, 1986; Yatskievych and Moran, 1989). Byrne et al. (2002) stated that a major goal of conservation biology is the preservation of genetic diversity in order to maintain the evolutionary potential of the species. The use of genetic data to determine evolutionary relationships between populations and species significantly contributes to conservation (Frankham et al., 2002; Ndoye-Ndir et al., 2008). Species of a wide geographic distribution and diverse habitats can show extremely high phenotypic variability (Elberse et al., 2003).

Acacia nilotica (family Fabaceae, subfamily Mimosoideae) is a multipurpose tree species (Ndoye-Ndir et al., 2008; Wickens, 1995) with a diverse range of growing environments and a broad ecological amplitude (Brenan, 1983; Elamin, 1973; Mahmood et al., 2005). Its taxonomy is well known worldwide by nine subspecies (Brenan, 1983) of which the subspecies *nilotica*, *tomentosa* and *adstringens*, are widely distributed in the Sudan and play a significant role in rural economy and environmental conservation (El Amin, 1990; Elamin, 1973). Nonetheless, morphological characteristics were some of the first indicators utilized in plant classification, genetic resource management, and breeding initiatives, but they come with various drawbacks, such as limited polymorphism, reduced heritability, delayed expression, and susceptibility to environmental factors (Beyene et al., 2005), especially concerning quantitative traits (Akinyele and Osekita, 2011). The genetic variation within and among the subspecies of *A. nilotica* was assessed based on morphological expressions of vegetative and reproductive characteristics (Mahmood et al., 2005). Furthermore, in the Sudan, Gibreel (2015) indicated based on a detailed morphological and anatomical study of *A. nilotica* the presence of a group of intermediate morphology and anatomy between the subspecies *nilotica* and *tomentosa* suggested further advanced molecular studies to confirm hybridization. On the other hand, DNA markers do not have such limitations since they can be used to detect variation at the DNA level and have proven to be effective tools for distinguishing closely related

genotypes (Beyene et al., 2005). Also, it has been widely used in genetic conservation and taxonomic identification of a species (Ferreyra et al., 2004; Josiah et al., 2010; Mattagajasingh et al., 2006). In spite of the fact that very few is known about the genetics of polyploid populations of forest trees (Bever and Felber, 1991; Fagg and Stewart, 1994) molecular markers were used to verify the genetic relatedness of some *Acacia* tree species (Habeballa et al., 2010; Habeballa, 2008; Harrier et al., 1997; Joly et al., 1992; Miller and Bayer, 2001; Nanda et al., 2004), taxonomic verification of some forestry species and population genetic of some woody species (Abdelkheir et al., 2011; Habeballa et al., 2010; Hamza, 2010; O'mer Basheir and M., 2010). The level of genetic diversity within and among populations of *A. nilotica* at DNA level is not well documented worldwide in relation to previous morphological classification to subspecies (Mahmood et al., 2005; Ndoye-Ndir et al., 2008; Varghese et al., 1999). Also, with no doubt, nothing was known until recently about the genetic variation pattern within and among the currently recognized *A. nilotica* sub-taxa in the Sudan as well as how ecological and/or genetic differences are controlling these patterns of variation and their taxonomic implications at the subspecies level. The present study aims to analyze the genetic variability in *A. nilotica* native to Sudan by studying the genetic relationship among the three subspecies *nilotica*, *tomentosa,* and *adstringens* based on three molecular marker techniques: random amplified polymorphic DNA (RAPD), start codon targeted (SCoT) polymorphism, and branch point signal (BPS) sequences. The study also extends to answer the question about the genetic relationship between the new group (intermediate in morphology between *nilotica* and *tomentosa*), which was observed by the author for the first time and the studied subspecies of *A. nilotica*. This will improve and update the knowledge about *A. nilotica* taxonomy and help to understand the level of morphological and genetic diversity as well as hybridization among its subspecies in the Sudan.

2. MATERIALS AND METHODS

2.1. Plants material

Ripe, fresh, and disease-free seeds were extracted from mature, ripe, and healthy pods (50 per tree), which were collected from 28 populations, each with 100 trees (25–35 cm dbh). The trees represent four sub-taxa of *A. nilotica* growing in 13 variable sites, namely the subspecies *nilotica* (10 populations), subspecies *tomentosa* (11 populations), subspecies *adstringens* (3 populations), and the new group (4 populations), which has morphology of pods, seeds, leaves, and bark intermediate between *nilotica* and *tomentosa* (Table 1). The tree sampling procedure and pod harvesting technique were according to Abdelkheir et al. (2011).

2.2. DNA extraction and purification

Total genomic DNA was extracted using DNeasy® Plant Mini Kit (Qiagen GmbH, Hilden, Germany) procedure with slight modification to reduce the effect of polysaccharide and secondary metabolites on the quality of the obtained DNA. The seeds (100 seeds/population) were ground using a Mixer Mill (Retsch MM200, Retsch GmbH, Haan, Germany). An optimal weight of 40 mg of the ground seeds was placed in a 2 ml tube. The DNA was extracted according to the manufacturer's protocol (Quiagen GmbH, Hilden, Germany).

2.3. Polymerase chain reaction (PCR) and electrophoresis

Forty-eight arbitrary primers supplied by different manufacturers [Eurofins MWG Operon, Ebersberg, Germany (primers 1–20) and Carl Roth GmbH, Co.KG, Karlsruhe, Germany (primers 21–41) were tested for PCR reproducibility using a small number of DNA samples. Sixteen primers (2 SCoT, 3 BPS, and 11 RAPD) of clear polymorphism among the studied subtaxa were selected and used for further genetic evaluation (Table 2). PCR was carried out in a final volume of 25 µL containing 8.5 µL RNase-free water (Qiagen GmbH), 12.5 µL TopTaq Master Mix (DNA polymerase, Qiagen GmbH), 1.0 µl of magnesium chloride ($MgCl_2$, Qiagen GmbH), 2 µL primer (10 pmol), and 1.0 µL genomic DNA. Amplifications were performed in steps initiated by denaturation (at 94°C, 3 min) followed by 35 cycles consisting of denaturation (at 94°C, 30 seconds), annealing (TA°C, 30 seconds), extension (at 72°C, 1 min), final extension cycle (72°C, 10 minutes), and cooling of the samples down to 10°C for further use. The annealing temperature (TA°C) was based on the primer used. The extracted DNA samples and PCR products were quantified using gel electrophoresis. The 1.5% gel was prepared in 1xTBE buffer plus ethidium bromide (Serva Electrophoresis GmbH, Heidelberg, Germany). The gels were visualized under UV light (Transilluminator, Vilber Lourmat Deutschland GmbH, Eberhardzell, Germany) and photographed using a VisionCapt program (Vilber Lourmat Deutschland GmbH, Eberhardzell, Germany). The fragment lengths were measured using Bio-1D PC-software (Vilber Lourmat, Eberhardzell, Germany). Consistence of PCR-amplified products was checked by performing all reactions at least twice. Bands that were unambiguous and reproducible in successive amplifications were selected for scoring further analysis.

2.4. Data collection and genetic analysis

The data for marker analysis were scored from the banding pattern. Bands were considered to be the same when they appeared in the same position. A binary data matrix was performed by scoring the presence (1) and absence (0) of bands obtained by each primer across all samples. A locus was considered polymorphic if the frequency of the most common bands did not exceed 0.95. Genetic variation in this study was estimated by calculating the polymorphism (%), average allele frequency, and gene diversity (Mandal and Gupta., 1996; Mariette et al., 2002), using the formula $He = 1 - p_i^2 - q_i^2$ where p_i represents the frequency of the present band and q_i represents the frequency of the absent band for each locus per primer. Spearman's similarity coefficient was calculated from a binary data matrix, and then cluster phenograms were constructed based on Unweighted Pair-Group Method with the Arithmetic Mean (UPGMA) Method (Sneath and Sokal, 1973; Rollf, 1993; Salih, 2014), by using the PAST (PAleontological Statistics) statistics software package version 3.01 (Hammer et al., 2001).

3. RESULTS AND DISCUSSION

The differences due to genetic make-up or to environmental influence are the main factors behind the variation, which occurs among organisms (Elrod and Stansfield, 2003). However, members of the same species may show throughout their life cycle continuous phenotype variation while their genotype is relatively stable (Elmund et al., 2004). Thus, variation due to the genetic make-up of organisms is more

Table 1
The collection sites, location, general habitat condition, and the identified sub-taxa of *A. nilotica* in each site.

Collection sites	Location	Location within agro-ecological zone (Harrison and Jackson 1958)	General habitat condition	Identified sub-taxa of *A. nilotica*
Shambat (Sah)	15° 39′ N, 32° 30′ E	Semi-desert: *A. tortilis* – Maerua crassifolia Desert Shrubland, average yearly precipitation 75–300 mm/year	Private farms, seasonal streams, and along the River Nile banks	NI, To, NewG
Alsunt (Alsu)	15°35′ N, 32° 30′ E		Riverine forest reserve, on White Nile River, loamy soil	NI, To
Alsnait (Alst)	14 ° 44′ N, 33° 22′ E	Semi-desert: Grassland on clay soils, with dominance of *A. mellifera*, mean annual rainfalls 400 mm	Riverine forest reserve, on Blue Nile River, loamy soil	NI, To, NewG
Aldenaigila (Alden)	13° 53′ N, 32° 37′ E		Riverine forest reserve, on Blue Nile River, clay soil	NI, To, NewG
Abu Geili (AbG)	13° 34′ N, 32° 35′ E	Woodland savanna with low precipitation on clay soils, average annual rainfall ranges from isohyets 400–600 mm	Riverine forest reserve, on Blue Nile River, clay-loam soil	NI, To
Allmbwa (Alm)	13° 00′ N, 33° 59′ E		Riverine forest reserve, on Blue Nile River, clay-loam soil	NI, To, NewG
Um Zibil (UmZ)	13° 23′ N, 34° 38′ E	Woodland savanna with low rainfall on clay soils has a mean annual precipitation ranging between isohyets of 400–600 mm	River forest reserve, Al Rahd seasonal River, clay soil	NI, To
Aldeisa (Alde)	12° 03′ N, 34° 18′ E	Woodland savanna with low rainfall on clay soils, average yearly precipitation ranges between isohyets of 600–800 mm	Riverine forest reserve, west of the Blue Nile River, clay-loam soil	NI, To, NewG
Sawlail (Saw)	12° 19′ N, 34° 21′ E		Riverine forest reserve, east of the Blue Nile River, clay-loam soil	NI, To, NewG
Klikis (Kli)	12° 58′ N, 32° 53′ E	Woodland savanna with low rainfall on sandy soils experiences mean annual rainfalls ranging from isohyets 400–600 mm	Riverine forest reserve, White Nile River, sandy-loam soil	To
El Ain (Ela)	12° 59′ N, 30° 20′ E	Semi-desert: Grassland found on sandy soil with average annual precipitation of 150–450 mm	Pristine woodland sanctuary, hilly incline, loamy soil	NI, TO, NewG, ads
Nabak (Nab)	12° 31′ N, 29° 55′ E	Savanna with low rainfall on sandy soils, average yearly precipitation varies from 300 to 500 mm in the southern region	Nature reserve, dune slope, sandy-clay terrain	Ads
Kadogli (Kadg)	11° 01′ N, 29° 42′ E and 11° 02′ N, 30° 04′ E	Savanna with low rainfall on sandy soils experiences mean annual rainfalls from 600 to 800 mm towards the south	Area of natural forest, sloped mountains, and soil of sandy clay	Ads

Where NI: subsp. *nilotica*; To: subsp. *tomentosa*; NewG: new group; ads: subsp. *adstringens.*

Table 2
The name, sequence and annealing temperature of each arbitrary SCoT, BPS, and RAPD primers which tested for PCR.

No.	Primer code	Primers sequences (5' to 3')	Annealing temperature (T_A°C)
1	SCoT15	ACGACATGGCGACCGCGA	61
2	SCoT21	ACGACATGGCGACCCACA	58
3	BPS3	TGAGTCCAAACTAAC	42
4	BPS6	TGAGTCCAAACTGAT	42
5	LA2a	CGTGCAGGTGTTAGTA	49
6	SC10/17	GTTAGCGGCG	27
7	SC10/19	CGTCCGTCAG	27
8	SC10/22	CTAGGCGTCG	27
9	SC10/24	ACCCATGCGG	27
10	KN8205M	TCGCCGCAAA	32
11	KN9825D	AAGCGACCTG	32
12	KN8202B	ACCTCAGCTC	32
13	KN8207M	AGCGTCACTC	32
14	KO8111D	CCCAGTCACT	32
15	KO8118D	CAGTGCTGTG	32
16	KO8124B	CTCGCTATCC	32

reliable in taxonomy than environmentally induced variation (Akinyele and Temitokan, 2005). The pressure of natural selection is one of several ways by which genotype and environment may interact and affect the genetic constitution of a population, and which may lead in the longer term to evolutionary changes (Akinyele and Osekita, 2011; Mather and Jinks, 1971). RAPDs techniques as molecular tool showed potential in the analysis of both among and within subspecies genetic diversity (Ndoye-Ndir et al., 2008). This study is the first on *A. nilotica* DNA-based genetic diversity characterization in the Sudan. However, the level of morphological diversity of the species in the Sudan is well known and studied among three main subspecies of a wide distribution and variety of habitats namely *nilotica*, *tomentosa,* and *adstringens* (El Amin, 1990; Elamin, 1973; Gibreel, 2015). Recently, a new group was observed in many areas with morphological characteristics intermediate between the subspecies *tomentosa* and *nilotica*. The present study investigates for the first time the genetic relatedness among these groups using different molecular genetic markers. The employed markers are prevalent in nature, and a wide variety of random primers can produce a vast amount of genetic information throughout the whole genome. This has made them useful for species where the DNA sequence is not available (Kremer et al., 2005). The subspecies *tomentosa*, *nilotica*, *adstringens,* and the new group were represented by 10, 11, 3, and 4 populations, respectively, and were analyzed by 16 primers that were employed in this study and assumed a random sample of the genome (Ndoye-Ndir et al., 2008). The primers generated 142 bands of an average size of 1.39 kbp–0.38 kbp and ranging in number from 5 by LA2a to 11 by SC10/17, SC10/22, and KN8202B with an average of 8.88 bands per primer (Table 3). Among the 142 amplified fragments, 103 were polymorphic (72.54%). The banding pattern obtained by BPS6 indicated a high degree of polymorphism. The selected primers showed polymorphism ranging from 100% (SCoT2, BPS6, SC10/17, SC10/22, and SC10/24) to 30 % (KN8207M) (Table 3). The obtained polymorphism in this study (71.27%) indicates a high genetic variability among the new group and the three subspecies. Similarly, Ndoye-Ndir et al. (2008) reported 94.2% polymorphism among the nine subspecies of *A. nilotica* (*nilotica*, *tomentosa*, *adstringens*, *leiocarpa*, *indica*, *subulata*, *cupressiformis*, *jacquemontii*, *kraussiana*) based on RAPDs. Furthermore, the average gene diversity (expected heterozygosity) among the subspecies *tomentosa*, *nilotica*, *adstringens,* and the new group was 0.2175, ranging from 0.3592 obtained by the primers KN9825D to 0.0039 by KO8111D (Table 3). From the genetic point of view, plant species are highly polymorphic with a mean genetic diversity (Expected heterozygosities "He") of 15% (0.15), whereas plant populations are relatively less polymorphic with a mean genetic diversity of 11% (0.11) (Hamrick and Godt, 1990). This result agreed with Varghese et al. (1999), who reported that *A. nilotica* shows a quite high level of genetic diversity, much of which occurs among its subspecies. The level of polymorphism and gene diversity among the subspecies is relatively higher than within each subspecies. However, the polymorphism and gene diversity were 62.41% and 0.1892 within the subspecies *tomentosa*, 48.82% and 0.1615 within the subspecies *nilotica*, 42.74% and 0.1536 within the new group, while within the subspecies *adstringens*, a very low level of 25.78% polymorphisms and gene diversity of 0.1060 was scored. However, Varghese et al. (1999) reported 45.9% polymorphism and 0.087 an expected heterozygosity for the subspecies *indica* less than 51.470% polymorphism and 0.112 an expected gene diversity for *tomentosa*. Hamrick and Godt (1990) also found comparable average values for 473 additional plant species exhibiting diverse life history traits. Hamrick et al. (1992) indicated 65% of polymorphism and 0.177 as an expected heterozyosity for long-lived woody perennials. Anticipated heterozygosities (He) noted for different animal-pollinated acacias were 0.133 for Australian acacias (Moran et al., 1989), 0.081 for *A. auriculiformis*, and 0.107 for *A. melanoceras* (Hamrick and Murawski, 1991). Joly et al. (1992) and Playford et al. (1993) reported higher genetic diversity values of 0.454 and 0.33 for *A. albida* and *A. Melanoxylon*, respectively (Table 4).

Table 3

Genetic characteristics obtained by 16 arbitrary primers among *A. nilotica* subspecies *nilotica* (10 populations), *tomentosa* (11 populations), *adstringens* (3 populations), and the new group (4 populations) of *A. nilotica* growing at different sites in the Sudan.

Primer		Fragments (Bands)		% of polymorphic bands	Allele frequency	Gene diversity (He)
Primer code	Primers sequences 5′ to 3′	Total	Number of polymorphic bands			
SCoT15	1.5–0.265	9	3	33.33	0.1156	0.0308
SCoT21	1.522–0.467	10	10	100	0.1	0.3154
BPS3	1.2–0.326	10	5	50	0.1	0.1418
BPS6	1.578–0.363	10	10	100	0.1	0.2981
LA2a	0.859–0.382	5	3	60	0.2	0.2521
SC10/17	1.336–0.260	11	10	90.91	0.0909	0.2945
SC10/19	1.392–0.372	8	7	87.50	0.125	0.2272
SC10/22	1.177–0.388	11	10	90.91	0.0909	0.3295
SC10/24	2.641–0.285	9	9	100	0.1045	0.2893
KN8202B	1.278–0.267	11	5	45.45	0.0909	0.1110
KN8205M	1.350–0.511	6	4	66.67	0.1667	0.1676
KN8207M	1.347–0.309	10	3	30	0.1	0.0644
KN9825D	1.215–0.417	8	7	87.5	0.125	0.3592
KO8111D	1.355–0.364	9	8	88.89	0.1111	0.3340
KO8118D	1.593–0.585	9	3	33.33	0.1111	0.0268
KO8124B	0.885–0.476	6	4	66.67	0.1667	0.2382
Average	**1.3893–0.3773**	**8.88**	**6.45**	**71.27**	**0.1187**	**0.2175**
Total		**142**	**102**	**1131**	**1.8984**	**3.4799**

Table 4

Genetic characteristics obtained by 16 arbitrary primers (average sequences 5′ to 3′ of 1.3893–0.3773 Kbp) among populations within *A. nilotica* subspecies *nilotica* (10 populations), *tomentosa* (11 populations), *adstringens* (3 populations), and the new group (4 populations) growing at different sites in the Sudan.

Fragments		*A. nilotica* subtaxa			
		Nilotica	*Tomentosa*	*Adstringens*	*New group*
Amplified	Total	142	142	142	142
	Average	8.88	8.88	8.88	8.88
Number of absent fragments	Total	23	8	36	25
	Average	1.45	0.5	2.25	1.56
Number of polymorphic fragments	Total	69	90	36	61
	Average	4.31	5.62	2.25	3.81
Number of monomorphic fragments	Total	50	44	70	56
	Average	3.13	2.75	4.38	3.5
% of polymorphic fragments	Total	781.06	998.59	412.53	683.79
	Average	48.82	62.41	25.78	42.74
Allele frequency	Total	1.6996	1.6954	1.6887	1.7084
	Average	0.1062	0.1059	0.1055	0.1068
Gene diversity index (expected heterozygosity)	Total	2.5832	3.0279	1.6964	2.4578
	Average	0.1615	0.1892	0.1060	0.1536

A matrix of Spearman's rho similarity coefficient (Table 5) indicated 0.8187 (81.87%) average genetic similarity among the studied subspecies and the new group, ranging from 0.9391 (93.91 %) as the highest average between the subspecies *nilotica* and *tomentosa,* and the lowest 0.4629 (46.29%) between the new group and the subspecies *adstringens*. The cluster phenogram revealed two main groups of *A. nilotica* (Figure 1), one group comprises the subspecies *adstringens* as a distinct group, while the subspecies *tomentosa*, *nilotica,* and the new group were in the second group with close genetic similarity. Also, the result of principal component analysis based on the covariance matrix described 85.67, 9.26, and 5.07% of the total variance in this study (Table 6). However, the subspecies *nilotica* and *tomentosa* were close to each other or overlapped, whereas the subspecies *adstringens* and the new group were clearly separated. Each formed a distinct group far from the other subspecies (Figure 2). Our results give good hints that the new group is of hybrid origin between the subspecies *nilotica* and *tomentosa*. However, this finding is consistent with Ali and Faruqi (1969a) and (Ali and Faruqi, 1969b) who, for the first time, demonstrated hybridization in the *A. nilotica* complex. Moreover, hybridization between subspecies *indica* and subspecies *hemispherica* (Ali and Faruqi, 1969a, b) was further confirmed by cytological evidence (Khatoon and Ali, 2006). Ndoye-Ndir et al. (2008) indicated that differentiation within a population relies on the characteristics of the sampled group. If isolated trees are sampled from agricultural lands and diverse woodlands, there would be significant self-pollination and renewal of closely related individuals near the parent trees (Varghese et al., 1999). A significant degree of selfing was noted in the mating system examination of isolated *A. nilotica* subspecies leiocarpa trees from Africa (Mandal et al., 1994).

Genetic diversity levels in populations are affected by various traits of the respective species (Hamrick and Godt, 1990). Seed dispersal, breeding method, and geographic distribution all provide predictive insights. Additionally, species that are more common and those that engage in outcrossing generally exhibit greater diversity within populations. In contrast to other long-lived outcrossed trees, significant self-fertilization

Table 5
Values of the genetic similarity index among the subspecies of *A. nilotica* and the new group created by Spearman's rank correlation coefficient, known as Spearman's rho (Hollander 1973).

Genotypes	Genotypes			
	Nilotica	***Tomentosa***	**New group**	***Adstringens***
Nilotica	1	0.9391	0.9207	0.4806
Tomentosa	0.9391	1	0.9009	0.4825
New group	0.9207	0.9009	1	0.4629
Adstringens	0.4806	0.4825	0.4629	1

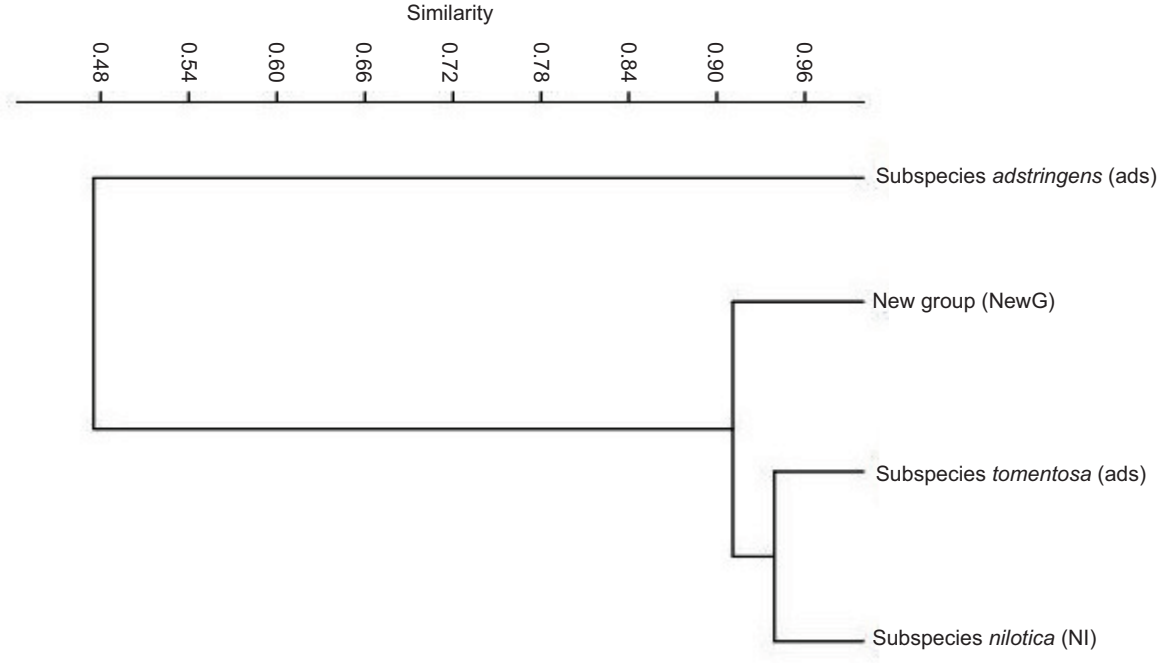


Figure 1. Dendrogram illustrating genetic relatedness among the subspecies *tomentosa*, *nilotica*, *adstringens,* and the new group of *A. nilotica* generated by the UPGMA cluster Analysis based on 102 polymorphic marker bands.

Table 6
Principal Component analysis generated among the three subspecies of *A. nilotica* (*nilotica*, *tomentosa*, *adstringens*) and the new group.

PC	Eigenvalue	% variance
1	6.34	85.67
2	0.69	9.26
3	0.38	5.07

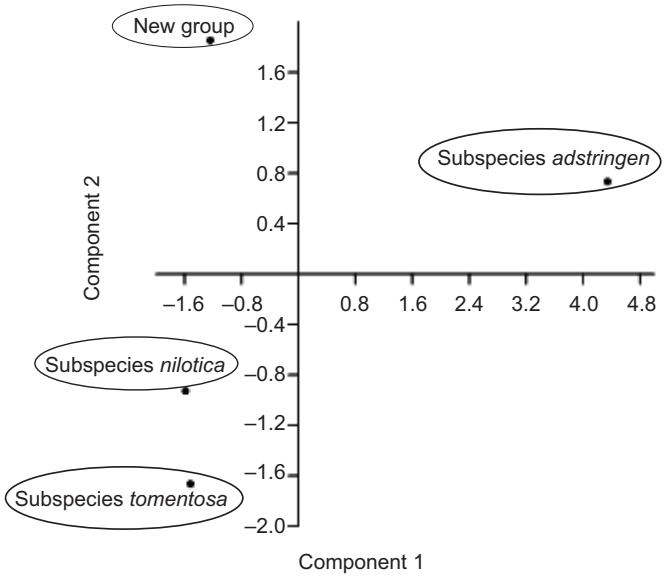


Figure 2. Plotting of the first two principal components, showing the genetic relationship between the subspecies *tomentosa,* subspecies *nilotica*, subspecies *adstringens,* and the new group (expected hybrid between *nilotica* & *tomentosa*) of *A. nilotica*.

(60% of seeds produced via self-pollination) and inbreeding take place in *A. nilotica* subspecies leiocarpa (Mandal et al., 1994). In this scenario, the pods stay indehiscent, and the full siblings grow naturally near the mother trees (Fagg, 1992); therefore, dense populations of the species probably include a large percentage of related trees. Nevertheless, these pods may be scattered by wild grazers and farm animals, resulting in an uneven spread of seeds across limited regions. According to reports, the subspecies tomentosa's pods are frequently transported over great distances during floods, accumulating along riverbanks and resulting in dense groupings (Ndoye-Ndir et al., 2008). The populations within the same river system might be interconnected, leading to minimal variations between them. The subspecies tomentosa typically thrives along the Blue and White Nile river systems in Sudan, and they occasionally establish mixed populations with the subspecies nilotica. As a result, variations in self-incompatibility exist among various subspecies and among individual trees within a population. In contrast to the pattern observed in subspecies leiocarpa, a significantly elevated degree of outcrossing was noted for the subspecies kraussiana (Ndoye-Ndir et al., 2008). There were, nonetheless, important variations in the outcrossing rate among trees (Mandal and Ennos, 1995), highlighting the significance of choosing trees for seed collections. Brenan (1983) observed that subspecies tomentosa exhibited greater overall diversity and increased differentiation within populations. This result may be attributed to the presence of specific hybrids among the populations, as the four subspecies tomentosa, nilotica, adstringens, and subalata are recognized to coexist in large regions within their natural habitat in Sudan. Hybridization has been documented between subspecies indica and subspecies hemispherica (ALI and QAISER, 1980), leading to significant variability within the population. Numerous natural interspecific hybrids of acacias have been documented in Africa, and it is believed that there are likely many additional hybrids, as the complete scope of hybridization and introgression remains largely unclear (Ross, 1979). Hybridization could be controlled among outcrossed individuals of these subspecies. Greater population differentiation in subspecies indica is understandable since populations found across a vast natural range spanning large regions of the Indian subcontinent (often in arid thorn forests) would be more isolated, and regeneration through root suckers is also recognized for this subspecies. Hence, it seems that subspecies nilotica, tomentosa, and the new group might exhibit lower genetic variability compared to subspecies adstringens. A portion of the greater overall variation in subspecies tomentosa can be attributed to the increased percentage of polymorphic loci and a marginally higher number of alleles per polymorphic locus. In addition, the higher average gene diversity (He) further supports the presence of increased genetic variability within this subspecies.. The significant variations noted in average polymorphism and gene diversity among the three subspecies and the new group in this study are likely attributed to differences in the distribution of genetic diversity both within and between the populations analyzed here. Mahmood et al. (2005) and Schmid and Weiner (1993) reported that floral and fruiting traits are genetically controlled and therefore are less liable to change than certain vegetative expressions that may evolve very quickly in response to the variability of the habitat and which seem likely to be environmentally controlled. Thus, genetically controlled expressions may not show any differentiation despite the variability of the environment (Jasienski et al., 1997; Mahmood et al., 2005). It is necessary for better management, utilization, and conservation of such forestry species to identify the level of morphological and genetic diversity within and among its populations when and

where they interact with their environment. Effective and rigorous measurements are necessary to reverse the current tendencies leading to an impoverishment of genetic resources. Those must be based on a better understanding of the species and the ecosystems, as well as the extent of biological diversity and genetic variation. It is crucial to support the dynamic interaction between subspecies and their environment because it constitutes a genetic source of diversity.

4. CONCLUSION AND RECOMMENDATIONS

This study provides, for the first time, data on the genetic variation in *A. nilotica* across the Sudan. However, additional field surveys are needed in other areas to characterize more plant material. Future research should also focus on several important traits, such as wood and tannin production and the nutritious value of seeds, which were not included in this study. The results from this study show that there is a large genetic variability in *A. nilotica* in the Sudan, which could offer considerable opportunities for the selection of trees with improved productivity.

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ACKNOWLEDGMENT

The authors extend their appreciation to Prince Sattam bin Abdulaziz University for funding this research work through the project number (2025/03/34164).

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