Molecular Taxonomy of some Selected Taxa of Subfamily Mimosoideae

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Abstract: Taxonomic relationships of 12 species representing three tribes of Momosoideae were studied using both the morphological characters and pollen grain characters as well as molecular data obtained by RAPD-PCR, AFLP and ISSR analysis. Nei coeffidient was used to compute the similarity matrices and the UPGMA method was to plot five dendograms demonstrating the relationships among the examined species. The 1st based on morphological and pollen grain character, the 2nd based on molecular criteria obtained by RAPD-PCR analysis, the 3rd based on ISSR analysis, the 4th based on ALFP analysis, and the 5th was based on all the information based on all the molecular parameters. The overall work culminated in discussion of the taxonomic position which was suggested by other workers. The collective dendogram based on the molecular data, even though apparently different from that based on morphological characters show similar relationships with the morphologically based dendogram. For instance, it shows a close relation among *Albizia julibrissin, Al. lebbek* (originally both belong to Ingeae) and *Faidherbia albida* (originally belongs to Acacieae). *Calliandra haematocephala* was separated from other species compared with morphological based dendogram which also separated it but within the same group with *Albizia julibrissin*, *Al. lebbek* and *Faidherbia albida*. This supports the view that *Calliandra haematocephala* represnts a distinctive character in the Mimosoideae. [Journal of American Science 2010;6(10):479-491]. (ISSN: 1545-1003).

Keywords: Mimosoideae, Genetic relationships.

1. Introduction:

The subfamily Mimosoideae includes three tribes Acacieae Benth. Ingeae Benth. and Mimoseae Born (Bentham, 1842). Tribe Acacieae includes only a single genus Acacia Mill as stated by Bentham (1875). However, Vassal (1972&1981) stated that tribe Acacieae contains two genera; the large cosmopolitan genus Acacia and the monotypic African genus Faidherbia A. Chev. Guinet (1981) stated that *Faidherbia* is troublesome as it has stamens that are shortly united at base and has pollen similar to taxa of the Ingeae, but was placed in Acacieae. The tribe Mimoseae Bron shares the character state of free stamens with the Acacieae, but the Mimoseae has as many or twice as many stamens as petals while the Acacieae has numerous stamens (Vassal, 1981). Guinet (1990) noted that the pollen structural symmetry was shared by some Mimoseae and *Acacia* subgenus *Acacia*. She concluded that such conflicting character states would lead to difficulty in making a classification based solely on morphological characters. El Azab (2005) concluded that the genus *Faidherbia* of the tribe Acacieae is better transferred into the tribe Ingeae based on the character of stamen and pollen grains. She suggested that some *Acacia* specie may be differentiated into different groups according to pollen characters. She also noted similar pollen characters in some *Acacia* and *Albizia*.

Many investigators have described in details the use of PCR technique to detect polymorphism among different plants (Weining & Langridge, 1991; Waugh & Powell, 1992). Rashmi *et al*. (2004) studied identification and genetic relationships in six tree species of *Acacia* using RAPD markers. A total 253 distinct DNA fragments were amplified by using 17 random primers which revealed a wide range of variability within the species. They concluded that these RAPD markers have the potential for conservation and characterization of genetic relatedness among the species. Bessaga *et al.*, (2004) analyzed natural populations of *Prosopis* species (Leguminosae: Mimosoideae) by the RAPD technique with the purpose of obtaining markers for species and hybrid identification. Five bands provided a tool for identifying any of the *Prosopis* species studied. Mattagajasingh *et al.*, (2006) employed RAPD technique using 22 primers to assess genetic diversity and inter-specific relationships among nine taxa of *calliandra* (Leguminosae: Mimosoideae). They stated that the intra-generic classification and phylogeny inferred from molecular markers support the traditional classification of the genus based on morphological characters but one species showed different position. Josiah *et al.*, (2008) used ISSR and RAPD markers to detect genetic variation within and among four Kenyan populations of *Acacia senegal*, which were considered as a multipurpose tree species, highly valued for Arabic gum production. The populations were delimited in two groups reflecting geographical sub-structuring and concluded that

conservation should target individual trees within populations and cover the entire ecological amplitude of the populations. Hemeida *et al.*, (2004) used the AFLP marker to fingerprint five *Acacia* species. AFLP data were also analyzed to evaluate the species relationships using different clustering algorithms. They found that AFLP revealed great generic variation in *Acacia*. Finally, they concluded that AFLP is a reliable technique and provides one of the most informative approaches to ascertain genetic relationships in *Acacia*, which may also be true for other related genera.

2. Materials and Methods:

I. Materials

Table 1 shows the source of the studied species and assigned to their tribes as proposed by Elias (1981).

II. Methods

A. Vegetative and Pollen Grain Characters

Morphology of the examined species were carefully described from trees growing in their sites. Characters not investigated by the authors were compiled from Bailey (1976) and Täechkolm (1974). The pollen characters are cited from El Azab (2005).

B. Molecular

This work was carried out at the Environmental Stress Laboratory (ESL), at the Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center (ARC), Giza, Egypt, in cooperation with the Department of Botany, Faculty of Science, Ain Shams University.

DNA Extraction

Several protocols for plant DNA isolation failed to produce good quality DNA from plants. We therfore developed a protocol based on Del Rio et al., 1996 and the Anna Maria et al., 2001 methods by adding absolute methanol to the exraction buffer leaf in order to reduce complex polysaccharide and secondary metabolites.

RAPD-PCR reactions were conducted using 23 random 10-mer primers with the sequences shown in table 2. Amplification was carried out in a Hybaid thermocycler programmed as follows: 94^{o}C/4 min (1 cycle); 94 ^{o}C/1 min, 37 ^{o}C/1 min, 72 ^{o}C/2 min (40 cycles); 72 ^{o}C/7 min (1 cycle) and 4 ^{o}C (infinitive).

Table 1: The studied species are assigned to their taxonomic position according to Elias (1981).

Subfamily	Tribe	Species and Synonomy	Sources
Mimosoideae	Acacieae	1. *Acacia laeta* R.Br.ex.Benth. 2. *A. nilotica* (L.) Dellile 3. *A. saligna* (Labill.) H.L.Wendl. 4. *A. seyal* Delile 5. *Faidherbia albida* (Delile) A. Chev.= (*A.albida* Del.)	Botanic Garden of Aswan Cairo-Alex. Agri. road (K55) Cairo-Suez road (K 66) Orman Botanic Garden Botanic Garden of Aswan
	Ingeae	6. *Albizia julibrissin* Durazz. 7. *Al. lebbek* (L.) Benth. 8. *Calliandra haematocephala* Haussk.	Orman Botanic Garden Orman Botanic Garden Education Botanic Garden & Orman Botanic Garden
	Mimoseae	9. *Adenanthera pavonina* L. 10. *Dichrostachys cineria* (L.) Wight & Arn. = (*Mimosa cineria* (L.)) 11. *Prosopis cineraria* (L.) Druce = (*Prosopis spicigera* (L.)) 12. *P. juliflora* (Swartz.) DC. = (*Mimosa juliflora* (Swartz.) Sw.	Orman Botanic Garden Orman Botanic Garden Zoo Garden – Giza – Egypt Zoo Garden – Giza – Egypt

Table (2): The 23 random 10-mer primer codes and their basic sequences.

	Primer code	Base sequence		Primer code	Base sequence
1	**A14**	5′ TCTGTGCTGG 3′	13	**O08**	5′ CCTCCAGTGT 3′
2	**B04**	5′ GGACTGGAGT 3′	14	**O09**	5′ TCCCACGCAA 3′
3	**B17**	5′ AGGGAACGAG 3′	15	**O10**	5′ TCAGAGCGCC 3′
4	**B20**	5′ GGACCCTTAC 3′	16	**O11**	5′ GACAGGAGGT 3′
5	**C05**	5′ GATGACCGCC 3′	17	**O12**	5′ CAGTGCTGTG 3′
6	**C11**	5′ CTCACCGTCC 3′	18	**O14**	5′ AGCATGGCTC 3′
7	**F01**	5′ ACGGATCCTG 3′	19	**O16**	5′ TCGGCGGTTC 3′
8	**F09**	5′ GAGGATCCCT 3′	20	**O18**	5′ CTCGCTATCC 3′
9	**O03**	5′ CTGTTGCTAC 3′	21	**O19**	5′ GGTGCACGTT 3′
10	**O04**	5′ AAGTCCGCTC 3′	22	**O20**	5′ ACACACGCTG 3′
11	**O05**	5′ CCCAGTCACT 3′	23	**Z13**	5′ GACTAAGCCC 3′
12	**O06**	5′ CCACGGGAAG 3′			

ISSR reactions were conducting using 7 specific primers (Wolfe & Liston, 1998), as presented in table 3. Amplification was performed according to Nagoka & Ogihara (1997). The reaction mixture consisted of Hot Start Master Mix 12.5 µl, Primer (10 mM) 2.0 µl, Template DNA (50 ng/µl) 1.0 µl, H_2O (dd) Up to 25 µl. Amplification was carried out in a Hybaid thermocycler programmed as follows: 94°C/4 min (1 cycle); 94 °C/1 min, 45 °C/1 min, 72 °C/2 min (40 cycles); 72 °C/7 min (1 cycle) and 4 °C (infinitive). A marker of 1 Kb of a total 14 bands ranging from 10000 to 250 bp (Ameresco) was used as DNA molecular size standard. For both RAPD and ISSR finger printing bands were visualized on UV – transilluminator.

Table 3: ISSR primer names and their nucleotide sequences.

Primer name	Sequence	Primer name	Sequence
HB9	(GT)6 GG	814	(CT) 8TG
HB11	(GT) 6 CC	844A	(CT)8 AC
HB15	(GTG) 3 GC	844B	(CT) 8 GC
17899A	(CA) 6 AG		

AFLP analysis was applied according to Vos *et al.*, (1995) using the AFLP® Analysis System I - invitrogen (cat. no. 10544-013) according to the manufacturer's protocol. Samples were prepared by cutting the genomic DNA with two restriction enzymes (EcoRI and MseI) and ligating with double stranded EcoRI and MseI adaptors. The adaptors were ligated with the overhanging sticky ends produced by the restriction enzymes. Four combinations of EcoRI and MseI were used (E-AAC/M-CAC, E-AAC/M-CTC, E-ACC/M-CTA and E-ACA/M-CAT) used in. AFLP products were detected by electrophoresis in polyacrylamide denaturing sequencing gel. DNA silver staining system (promega, CA, USA) was used for band detection. Only sharp PCR fragments were scored. Fragments at low intensities were only scored as present when they were reproducible in repeated experiments using Gelworks 1D advanced software (UVP Co., UK).

Data analysis

Morphological characters, pollen grain characters as well as PCR amplification products were scored independently as 1 and 0 for each for the presence or absence, respectively for both characters and bands, and the obtained binary data were used for the analyses.

The genetic similarity among studied species was determined by Nei's genetic distance (Nei, 1987) modified to accommodate dominant markers (Labate, 2000) (e.g., RAPD, ISSR and AFLP). A dendrogram was constructed based on a distance matrix using the Unweighted Pair Group Method with Arithmetic averages (UPGMA). All analysis were performed with the NTSYS-pc version 2.02 software package (Numerical Taxonomy System, Exeter Software) (Rohlf, 2000). In addition, correspondence of the morphological character, RAPD, ISSR and AFLP similarity matrices were performed by means of MXCOMP procedure of NTSYS-pc with the null hypothesis that there is no association between these three similarity matrices. The statistical stability of the clusters was estimated by a bootstrap analysis with 1000 replications using Winboot software (Yap & Nelson, 1996).

3. Results and Discussion:

I- Cluster Analysis as Revealed by Morphological and Pollen Attributes.

Description of 79 morphological and pollen characters used for computation and their binary codes (1 & 0) are given in table 4 for numerical analysis. The cluster analysis of both morphological and pollen characters is shown in figure 1. Similarity indices among the studied species (table 5) shows the strongest relationships was between *A. nilotica* and *A. seyal* with similarity index of 89%, followed by that between *Prosopis cineraria* and *P. juliflora* with similarity index of 72%; meanwhile the weakest relationships was scored between *Albizia julibrissin* and *P. juliflora* with a similarity index of 29%.

The dendogram revealed also that the studied species were split into two main clusters. The first cluster contains *Prosopis cineraria* and *P. juliflora* while the second cluster contains the rest of the species. The second cluster was subdivided into two sub-clusters, the first contains *Acacia nilotica*, *A. seyal* and *A. saligna*, the second contains two groups. The first group was subdivided into two subgroups; one of them comprises *Calliandra haematocephala* while the second contained *Albizia lebbek*, *Al. julibrissin* and *Faidherbia albida*. The second group contained *Dichrostachys cineria*, *Adenanthera pavonina* and *Acacia laeta*.

Compared to Elias (1981) the above results could be discussed as follows:

1- At the cluster level

The splitting into two main clusters depends on the number of associated monads whereas, the first cluster including *Prosopis cineraria* and *P. juliflora* characterized with single pollen grain and the second cluster contains the rest of the species which have compound pollen grains.

2- At the sub-cluster level

The first sub-cluster contains *Acacia nilotica*, *A. seyal* and *A. saligna* which are separated at taxonomic

distance 0.59 have common morphological characters evergreen leaves, symmetrical leaf base, head inflorescence , many distinct stamens heteropolar pollen). The clustering of these *Acacia* agrees with Elias (1981). El Azab (2005) proposed that the transfer of *A. saligna* from Acacieae to Mimoseae due to the occurrence of some common features of pollen characters which was also stated by Guinet (1969) and Sorsa (1969). However, results in the present work do not support their proposal.

In the second sub-cluster *Calliandra haematocephala* which was separated at taxonomic distance 0.58 represents a distinctive taxon confirming the view of Guinet & Hernandez (1989) who stated that *Calliandra haematocephala* is very isolated genus within Mimosoideae. In sub-group two *Albizia lebbek*, *Al. julibrissin* and *Faidherbia albida* were separated at taxonomic distance of 0.62; all have in common many stamens connate at the base and common pollen grain characters (numerous monads, heteropolar and porate). Guinet (1981) stated that the genus *Faidherbia* (which originally belongs to Acacieae) raise the problem of limits between Ingeae and Acacieae. In this work, the genus *Faidherbia* is better included within the Ingeae. This claim is supported by El Azab (2005) who found that this genus differs mainly from Acacieae in having 28 to 32 monads; thus resembling the Ingeae species. In this context, Elias (1981) stated that this genus is distinct from Acacieae in pollen characters; and is better transferred to tribe Ingeae or at least it represents a link based on the many distinct stamens which become connate at base. Earlier to this Bentham (1842) suggested that Acacieae and Ingeae are very close. Guinet (1990) also believed that Acacieae and Ingeae have always been considered as very close entities. In conclusion, the Acacieae can be distinguished from Ingeae by having free staminal filaments while Ingeae has united filaments. Vassal (1981) reached the same conclusion. In the present work, unless the filament character is concerned, no morphological characters can separate Ingeae from Acacieae.

The second group contains *Dichrostachys cineria*, *Adenanthera pavonina* and *Acacia laeta* which are separated at taxonomic distance of 0.64, all having common morphological characters (free stamen, asymmetrical leaf base, spike inflorescence and straight pod) and also have common pollen characters (16 monads, acalymmate and heteropolar). The separation of *Acacia laeta* with the Mimoseae species is in agreement with Guinet (1969), who stated that there is close pollen similarity between Acacieae and Mimoseae. However, El Azazb (2005) on pollen bases stated that this species is better included within Ingeae. The classification of *Acacia laeta* as well as *Faidherbia albida* and *Acacia saligna* should better be based on both parameters viz. morphological and pollen character.

2. Polymorphism detected by RAPD analysis

RAPD analysis for the studied 12 species utilizing 23 primers produced 277 total bands incuding 60 specific markers table 6. All species gave a specific marker ranging from one band for *Dichrostachys cineria* to ten bands for *Faidherbia albida*. Primer B17 scored the largest number of markers (6 markers) while B04, O12 & O20 gave no specific markers.

Genetic relationships and cluster analysis as revealed by RAPD data

The dendogram based on RAPD-PCR divided the studied taxa into two main clusters; the first cluster includes *Faidherbia albida* and *Albizia lebbek*, while the second cluster includes the remaining taxa. The highest similarity index 60.5% was recorded between *Prosopis juliflora* and *P. cineraria*, while the lowest similarity index (25%) was recorded between *Calliandra haematocephala* and *Prosopis juliflora* (Table 7; Fig. 2)

Polymorphism detected by ISSR analysis

A high level of polymorphism was generated utilizing the seven ISSR primers. A total number of 50 ISSR bands were obtained. Of these, 49 bands were polymorphic (98%) and only one was monomorphic (2%) banding (Table 8). The specific markers generated by ISSR primers were including 8 positive markers and one negative marker (Table 9).

Seven species (*Acacia laeta*, *A. seyal*, *Albizia julibrissin*, *Calliandra haematocephala*, *Dichrostachys cineria*, and *Prosopis cineraria*) did not reveal specific marker while the largest number of markers was produced by *A. nilotica*, 2 positive markers, with fragment size 2120 bp and 1905 bp with HB11 and 814 respectively.

Similarly, *Adenanthera pavonina* produced 2 positive markers with fragment size 2120 bp and 395 bp against HB15 and 844A respectively. The lowest number of markers, 1 positive marker, was produced by *Faidherbia albida* with fragment size 350 bp against HB11, *Albisia lebbek* with fragment size 390 bp against 814 and *Acacia saligna* with fragment size 775 bp against 17899A. *P. juliflora* produced 1 negative marker with fragment size 1345 against HB9. 775 bp against 17899A. *P. juliflora* produced 1 negative marker with fragment size 1345 against HB9. In conclusion, all ISSR primers used in the present study successfully distinguished between the studied species in term of all banding pattern. This is in agreement with Wolfe *et al.*, (1998) who stated that the main advantages of ISSR are higher variability and rigid banding pattern.

3. Cluster Analysis and Genetic Relationships as Revealed by ISSR Data

The similarity indices of the studied species utilizing ISSR analysis are given in table 10. The strongest genetic relationships based on ISSR data was scored between *A. saligna* and *A. seyal* with similarity index of 81% followed by those between *Al. julibrissin* and *A. seyal* (69%) and between *Al. lebbek* and *Calliandra haematocephala* (65%). Similarly, the similarity index was 65% between *Dichrostachys cineria* and *P. cineraria.* Low genetic similarity was scored between *A. saligna* and *P. juliflora* (21%) followed by that between *Al. julibrissin* and *P. juliflora* (32%).

The dendogram developed for the studied species divided them into two clusters as shown in figure 3. The first cluster is subdivided into two sub-clusters; the first sub-cluster separated *Adenanthera pavonina* as a single taxon. The second group is subdivided into two subgroups; the first contains *Dichrostachys cineria* and *P. cineraria* and the second includes *P. juliflora* only. The second sub-cluster contains two groups; first separated *Faidherbia albida* and the second contains *Albizia lebbek* and *Calliandra haematocephala.* The second cluster is subdivided into two sub-clusters; the first contains *A. laeta* and *A. nilotica*. The second is subdivided into two groups; the first group includes *A. saligna* and *A. seyal*, while the second group separated *Al. julibrissin* alone.

Polymorphism detected by AFLP analysis

A total of 433 major AFLP bands were observed with 100% polymorphism. The number of amplicons / combinations were 137, 125, 86 and 85 amplicon and the fragment size scored ranged from 1171 to 193 bp, 936 to 111 bp, 1173 to 49 bp and 952 to 214 bp with the primer pair combination E-AAC / M-CAC, E-AAC/M-CTC, E-ACC/M-CTA and E-ACA/M-CAT respectively (Table 11).

Species-Specific Markers Based on AFLP

In the current study, a total of 104 AFLP species-specific markers from 433 bands were identified. All species produced specific positive markers identified by the four AFLP combinations (Table 11). The highest number of specific markers (35) was detected by the primer combination E-AAC/M-CTC followed by 26 markers scored by the primer combinations E-ACC/M-CTA and E-AAC/M-CAC. While the lowest number of specific marker, 17, was detected by the primer combination E-ACA/M-CAT. AFLP analysis generated the highest number of bands due to the high number of loci identified and showed a higher discriminatory power to detect genetic variability among species.

4. Cluster Analysis and Genetic Relationships as Revealed by AFLP Data

In the present work, the genetic similarity indices (Table 12) show the strongest relationship between *Dichrostachys cineria* and *Prosopis juliflora* (43%) and the lowest (19%) between *Prosopis cineraria* and *Acacia laeta*.

The dendogram based on AFLP data separated *A. nilotica* as one distinct taxon, the rest of the species as shown in figure 4. The latter is subdivided into six groups. The first group includes *Acacia laeta* and *Acacia saligna*; the second group contains *A. seyal* and *Faidherbia albida*; the third group contains *Albizia julibrissin*; the fourth group includes *Dichrostachys cineria*, *Prosopis juliflora* and *Adenanthera* pavonina; the fifth group contains Prosopis *cineraria*; and the sixth group includes *Al. lebbek* and *Calliandra haematocephala*.

5. Cluster Analysis and Genetic Relationships Based on Combined Data (RAPD, ISSR and AFLP)

The similarity index based on the combined data (Table 13) showed that the strongest genetic relationship scored was 44% between *Dichrostachys cineria* and *Prosopis cineraria*, while the lowest genetic relationship scored was 22% between *Acacia laeta* and *Calliandra haematocephala*. The dendogram subdivided the species into two clusters as shown in figure 5. The first cluster contains *Acacia laeta* and *A. nilotica* at genetic distance 0.39. The second cluster contains the rest of the species. The latter is subdivided into two subclusters, the first separated *Calliandra haematocephala* at a genetic distance of 0.33; while the second subcluster contains two groups, the first group contains *Albizia julibrissin*, *Al. lebbek* and *Faidherbia albida* at a genetic distance of 0.34 and the second group contains *Acacia saligna* and *A. seyal* at a genetic distance of 0.40 in the first subgroup; while the second subgroup separated *Adenanthera pavonina* at a genetic distance of 0.38; the third subgroup contains *Dichrostachys cineria*, *Prosopis cineraria* and *P. juliflora* at a genetic distance of 0.44.

Table 4: Data matrix of vegetative and pollen morphology of the studied species

Attributes	Characters		1	2	3	4	5	6	7	8	9	10	11	12
Whole Plant	Duration	Ever green	1	1	1	1	1	0	0	0	0	1	1	0
		Deciduous	0	0	0	0	0	1	1	1	1	0	0	1
Stem	Texture	Glabrous	0	0	0	0	0	1	1	1	0	0	0	0
		Pubscent	0	0	1	0	0	0	0	0	1	1	0	0

		Spiny (prickly)	1	1	0	1	1	0	0	0	0	0	1	1
Leaf	Composition	Compound	1	1	0	1	1	1	1	1	1	1	1	1
		Phyllode	0	0	1	0	0	0	0	0	0	0	0	0
	Pinnae Arrangement	Opposite	1	1	1	1	1	1	1	1	0	1	1	1
		Alternate	0	0	0	0	0	0	0	0	1	0	0	0
	Pinnae Shape	Obovate	1	0	0	0	0	0	0	0	0	0	0	0
		Ovate	0	0	0	0	1	0	1	0	1	0	1	1
		Oblong	1	0	0	1	1	1	1	1	1	0	0	0
		Lanceolate	0	0	1	0	0	0	0	1	0	0	0	0
		Linear	0	0	1	0	0	0	0	0	0	1	0	1
	Pinnae Apex	Acute	0	0	1	0	0	1	0	0	0	1	0	0
		Obtuse	1	1	0	1	0	0	1	1	1	1	0	1
		Mucronate	0	0	0	0	1	0	1	1	0	0	1	0
	Pinnae Base	Symmetrical	0	1	1	1	0	1	0	1	0	0	1	0
		Asymmetrical	1	0	0	0	1	0	1	0	1	1	0	1
	Stipules	Present	1	1	0	1	1	0	0	1	0	1	1	1
		Absent	0	0	1	0	0	1	1	0	1	0	0	0
		Foliceous / scaly	1	0	0	0	0	0	0	1	0	0	1	0
		Spiny	0	1	0	1	1	0	0	0	0	1	0	1
	Glands	Main Rachis	0	0	0	0	1	0	1	0	0	0	0	0
		Petiole	0	1	0	1	0	0	0	0	0	0	0	0
		Pinnae	0	1	0	1	0	0	0	0	0	0	0	0
Flower	Inflorescent type	Head	0	1	1	1	0	1	1	1	0	0	0	0
		Spike	1	0	0	0	1	0	0	0	1	1	1	1
	Calyx color	Green	0	1	1	1	1	1	1	1	1	1	1	1
		Colored	1	0	0	0	0	0	0	0	0	0	0	0
	Crolla (color)	Green	0	0	0	0	0	0	0	0	0	0	0	1
		Yellow	0	1	1	1	1	0	1	0	1	1	1	0
		Pink	1	0	0	0	0	1	0	1	0	1	0	0
	Stamen no.	Ten	0	0	0	0	0	0	0	0	1	1	1	1
		Many	1	1	1	1	1	1	1	1	0	0	0	0
		Distinct	1	1	1	1	0	0	0	0	1	1	1	1
		Connate	0	0	0	0	1	1	1	1	0	0	0	0
Fruit (Pod)	Shape	Linear	1	1	1	1	0	0	0	1	1	1	1	1
		Strap	0	0	0	0	1	1	1	0	0	0	0	0
	Hardness	Hard	0	0	0	0	1	0	1	1	1	1	1	1
		Soft	1	1	1	1	0	1	0	0	0	0	0	0
Fruit (Pod)	Dehiscence	Dehiscent	1	0	1	0	0	0	0	1	1	0	0	0
		Indihescent	0	1	0	1	1	1	1	0	0	1	1	1
	Constriction	Present	0	1	1	1	0	0	0	0	0	0	0	1
		Absent	1	0	0	0	1	1	1	1	1	1	1	0
	Apex	Beaked	0	0	0	0	0	0	0	0	0	0	0	1
		Beakless	1	1	1	1	1	1	1	1	1	1	1	0
	Texture	Glabrous	1	0	1	1	1	1	0	0	1	1	1	1
		Hairy	0	1	0	0	0	0	1	1	0	0	0	0
	Color	Straw-yellow	0	0	1	0	0	0	1	0	0	0	1	1
		Red-brown	1	1	0	1	1	1	0	1	1	1	0	0
	Pod appearance	Twisted	0	0	0	1	1	0	0	0	0	0	0	0
		Straight	1	1	1	0	0	1	1	1	1	1	1	1
Pollen Morphology	Number/anther	Numerous	0	0	0	0	0	0	0	0	1	1	1	1
		Eight (polyads)	1	1	1	1	1	1	1	0	0	0	0	0
		Eight (octads)	0	0	0	0	0	0	0	1	0	0	0	0
	No. of monads	Single	0	0	0	0	0	0	0	0	0	0	1	1
		Numerous	1	1	1	1	1	1	1	1	1	1	0	0
	Polarity	Isopolar	0	0	0	0	0	0	0	0	0	0	1	1
		Heteropolar	1	1	1	1	1	1	1	1	1	1	0	0
	Type	Calymmate	0	0	0	0	0	0	0	1	0	0	0	0
		Aclaymmate	1	1	1	1	1	1	1	0	1	1	0	0
	Aperture occurrence	Distal	1	1	1	1	1	1	1	1	1	1	1	1
		Proximal	1	1	1	1	1	1	1	0	1	1	1	1
	Aperture type	Porate	1	0	0	0	1	1	1	1	0	1	0	0
		Colporate	0	1	0	1	0	0	0	0	0	0	0	0
		Pseudocolpi	0	0	1	0	0	0	0	0	1	0	0	0
		Composite	0	0	0	0	0	0	0	0	0	0	1	1
	Pollen collumela	Distinct	0	1	0	1	1	0	0	1	0	1	1	1
		Indistinct	1	0	1	0	0	1	1	0	1	0	0	0

	Pollen Sculpturing	Faint reticulate	1	0	0	0	0	0	0	0	0	0	0	0
		Faveolate	0	1	0	0	0	0	0	0	0	0	0	0
		Faint reticulate-psilate	0	0	1	0	0	0	0	0	0	0	0	0
		Psilate-faveolate	0	0	0	1	0	1	1	0	0	0	0	0
		Faveolate-rugulate	0	0	0	0	1	0	0	0	0	0	0	0
		Rugulate-fossulate	0	0	0	0	0	0	0	1	0	0	0	1
		Scabrate-psilate	0	0	0	0	0	0	0	0	1	0	0	0
		Verrucate	0	0	0	0	0	0	0	0	0	1	0	0
		Faveolate-psilate	0	0	0	0	0	0	0	0	0	0	1	0

Table 5: Similarity index of the studied species based on morphological data.

Species	1	2	3	4	5	6	7	8	9	10	11	12
1	1											
2	0.585	1										
3	0.563	0.603	1									
4	0.606	0.892	0.594	1								
5	0.606	0.585	0.406	0.667	1							
6	0.603	0.548	0.59	0.603	0.603	1						
7	0.515	0.523	0.5	0.515	0.697	0.762	1					
8	0.554	0.531	0.413	0.492	0.523	0.613	0.615	1				
9	0.625	0.444	0.548	0.469	0.531	0.525	0.594	0.476	1			
10	0.667	0.615	0.531	0.606	0.667	0.54	0.515	0.523	0.688	1		
11	0.476	0.516	0.426	0.508	0.571	0.367	0.444	0.452	0.525	0.635	1	
12	0.406	0.476	0.355	0.469	0.469	0.295	0.406	0.381	0.516	0.625	0.721	1

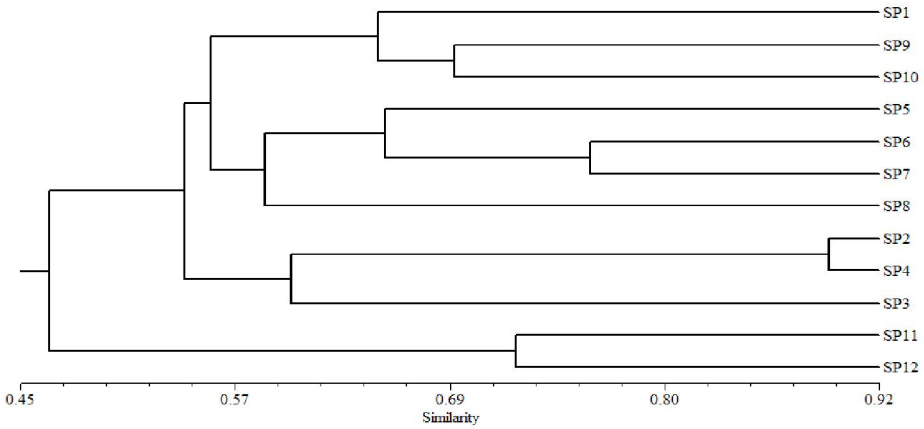

Figure 1: Dendogram indicating the relationships between the studied species based on morphological analysis. SP1 up to SP12 refer to the species indicated in table 1.

Table 6: Number of amplified fragments and specific markers of the studied species based on RAPD-PCR analysis.

Primers	TAF	PB	1		2		3		4		5		6		7		8		9		10		11		12		TSM
			AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	
A14	10	10	2	0	2	2	4	1	1	0	2	0	1	0	2	0	2	0	3	1	1	0	1	0	3	0	4
B04	12	12	7	0	5	0	6	0	6	0	2	0	3	0	1	0	4	0	7	0	6	0	3	0	0	0	0
B17	17	17	6	0	5	0	3	1	4	0	8	2	7	0	4	0	4	1	6	1	4	0	7	0	9	1	6
B20	12	12	3	1	3	0	2	1	3	0	4	0	3	0	3	1	1	1	2	0	1	0	2	0	3	0	4
C05	11	11	5	0	4	0	2	0	5	0	4	0	3	0	4	1	2	0	4	0	3	0	5	1	4	0	2
C11	7	7	3	0	2	0	1	0	1	0	1	1	2	0	3	1	1	1	1	0	2	0	3	0	1	0	3
F01	13	13	5	0	5	0	3	0	4	0	6	1	5	0	5	0	3	0	4	0	4	0	4	0	5	0	1
F09	14	14	4	0	5	0	4	0	3	0	4	0	4	1	3	0	2	0	6	1	5	0	6	1	4	0	3
O03	12	12	1	0	1	0	3	1	4	1	3	1	3	0	4	0	1	0	3	0	3	0	3	0	4	2	5
O04	17	17	5	0	3	0	5	1	6	0	5	1	6	1	4	0	5	0	5	0	5	0	5	0	6	1	4
O05	12	12	4	0	5	0	2	0	2	0	4	0	3	0	2	0	6	1	4	0	2	0	2	0	2	0	1

O06	11	11	4	0	2	0	4	0	2	0	2	0	1	0	5	1	2	0	2	0	4	0	5	0	4	0	1
O08	8	8	3	1	3	0	4	1	4	0	2	0	2	0	2	0	2	1	2	0	3	0	3	0	2	0	2
O09	12	12	3	1	1	0	4	0	4	0	3	1	3	0	4	0	2	0	2	0	4	0	5	0	4	0	3
O10	8	8	3	1	3	0	4	1	2	0	2	0	2	0	3	0	1	0	1	0	4	0	3	0	3	0	2
O11	13	13	3	0	3	0	3	0	3	0	4	0	5	0	5	0	4	0	4	0	4	1	3	0	3	1	2
O12	16	16	7	0	5	0	6	0	7	0	3	0	6	0	5	0	7	0	7	0	6	0	6	0	3	0	0
O14	11	11	6	0	6	0	7	0	6	1	5	0	4	0	4	0	5	0	2	0	2	0	3	0	5	0	1
O16	13	13	2	1	4	0	2	0	3	1	2	0	3	1	3	0	2	0	3	0	3	0	3	0	4	0	3
O18	16	16	4	0	5	0	2	0	4	0	8	1	5	0	4	2	2	0	4	0	4	0	3	1	2	0	4
O19	9	9	3	0	3	0	2	1	1	0	3	2	4	1	2	0	2	0	2	0	2	0	2	1	2	0	5
O20	11	11	2	0	2	0	2	0	4	0	4	0	4	0	3	0	3	0	3	0	4	0	3	0	2	0	0
Z13	12	12	3	0	3	0	4	1	3	0	5	0	4	1	5	0	3	1	3	0	3	0	5	1	2	0	4
Total	277	277	88	5	80	2	79	9	82	3	86	10	83	5	80	6	66	6	80	3	79	1	85	5	77	5	60

TAF= Total Amplified Fragment; PB= Polymorphic Bands; AF= Amplified fragment per taxa; SM= Specific marker per taxa including either the presence or absence of a band in specific taxa; TSM= Total number of Specific Marker across taxa; 1-12 species as listed in table 1.

Table 7: Similarity index of the studied species based on RAPD data.

Sp.	1	2	3	4	5	6	7	8	9	10	11	12
1	1											
2	0.536	1										
3	0.369	0.3	1									
4	0.318	0.321	0.457	1								
5	0.307	0.357	0.31	0.318	1							
6	0.351	0.331	0.429	0.376	0.398	1						
7	0.343	0.41	0.261	0.294	0.379	0.402	1					
8	0.271	0.272	0.286	0.268	0.31	0.28	0.311	1				
9	0.371	0.44	0.352	0.385	0.323	0.333	0.288	0.452	1			
10	0.443	0.34	0.377	0.435	0.323	0.346	0.3	0.384	0.544	1		
11	0.393	0.412	0.339	0.371	0.312	0.345	0.313	0.289	0.39	0.476	1	
12	0.364	0.382	0.357	0.377	0.339	0.35	0.316	0.25	0.333	0.41	0.605	1

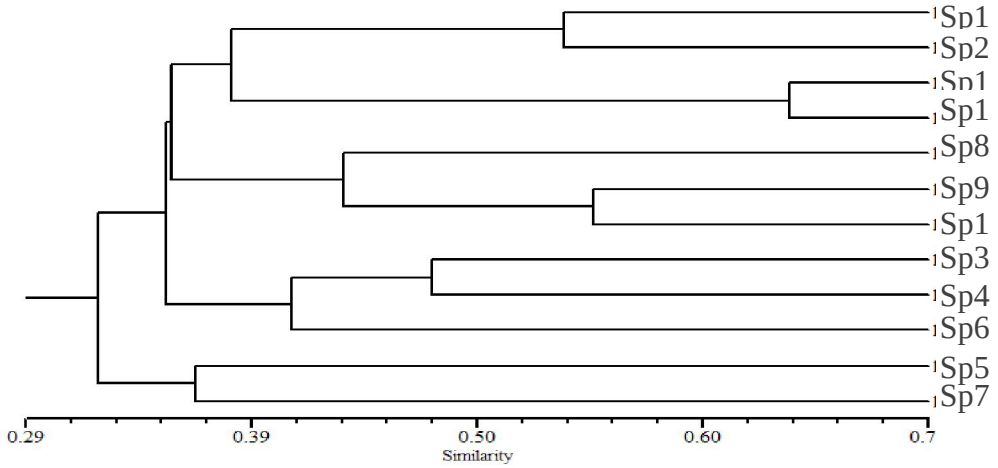

Figure 2: Dendogram illustrating the relationships between the studied species based on RAPD analysis. SP1 up to SP12 refer to the species names as indicated in table 1.

Table 8: Number of amplified fragments and specific markers of studied species based on ISSR analysis.

Primers	TAF	PB	1		2		3		4		5		6		7		8		9		10		11		12		TSM
			AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	
HB9	6	6	1	0	2	0	3	0	3	0	3	0	2	0	2	0	4	0	2	0	2	0	3	0	2	1	1
HB11	8	8	3	0	3	1	2	0	2	0	3	1	3	0	1	0	3	0	1	0	5	0	3	0	4	0	2
HB15	9	9	2	0	2	0	1	0	2	0	4	0	2	0	3	0	4	0	2	1	2	0	2	0	4	0	1
17899A	6	6	2	0	2	0	1	1	1	0	4	0	2	0	2	0	2	0	4	0	3	0	3	0	4	0	1
814	9	9	4	0	3	1	3	0	1	0	4	0	3	0	4	1	2	0	2	0	4	0	3	0	2	0	2
844A	8	8	3	0	5	0	4	0	4	0	3	0	3	0	2	0	2	0	3	1	2	0	2	0	2	0	1
844B	4	3	3	0	2	0	3	0	2	0	2	0	2	0	3	0	3	0	3	0	3	0	3	0	3	0	0
Total	50	49	18	0	19	2	17	1	15	0	23	1	17	0	17	1	20	0	17	2	21	0	19	0	21	1	8

Table 9: Species-Specific markers based on PCR with RAPD and ISSR Primers

Species	RAPD-PCR		ISSR	
	+ve Markers	-ve Markers	+ve Markers	-ve Markers
1	RA-B20-1800 bp, RA-O08-595 bp RA-O09-305 bp, RA-O10-525 bp RA-O16-240 bp	-	-	-
2	RA-A14-460 bp, RA-A14-655 bp	-	HB11-2120 bp 814-1905 bp	-
3	RA-A14-1560 bp, RA-B17-425 bp, RA-B20-450 bp, RA-O03-135 bp, RA-O04-205 bp, RA-O08-2600bp, RA-O10-1455 bp, RA-O19-1155 bp, RA-Z13-2500 bp	-	17899A-775 bp	-
4	RA-O03-165 bp, RA-O14-210 bp, RA-O16-1005 bp	-	-	-
5	RA-B17-1595 bp , RA-B17-230 bp, RA-C11-720 bp, RA-F01-235 bp, RA-O03-335 bp, RA-O04-2585 bp, RA-O09-970 bp, RA-O18-55 bp, RA-O19-1915 bp, RA-O19-925 bp	-	HB11-350 bp	-
6	RA-F09-415 bp, RA-O04-176- bp, RA-O16-350 bp, RA-O19-360 bp, RA-Z13-1145 bp	-	-	-
7	RA-B20-2255 bp, RA-C05-865 bp, RA-C11-125 bp, RA-O06-1240 bp, RA-O18-310 bp, RA-O18-125 bp	-	814-390 bp	-
8	RA-B17-345 bp, RA-B20-1360 bp, RA-C11-285 bp, RA-O05-200 bp, RA-O09-1920 bp, RA-Z13-750 bp	-	-	-
9	RA-A14-285 bp, RA-B17-2620 bp, RA-F09-835 bp	-	HB15-2120 bp 844A-395 bp	-
10	RA-O11-1360 bp	-	-	-
11	RA-C05-425 bp, RA-F09-750 bp, RA-O18-11- bp, RA-O19-615 bp, RA-Z13-1040 bp	-	-	-
12	RA-B17-2980 bp, RA-O03-1065 bp, RA-O03-675 bp, RA-O04-285 bp, RA-O11-730 bp	-	-	HB9-1345 bp

TAF= Total Amplified Fragment; PB= Polymorphic Bands; AF= Amplified Fragment per taxa; SM= Specific Marker including either the presence or absence of a band in specific taxa; TSM= Total number of Specific Marker across taxa.

Table 10: Similarity index of the studied species based on ISSR data.

Species	1	2	3	4	5	6	7	8	9	10	11	12
1	1											
2	0.59	1										
3	0.57	0.50	1									
4	0.48	0.53	0.81	1								
5	0.34	0.38	0.45	0.42	1							
6	0.51	0.50	0.65	0.69	0.55	1						
7	0.57	0.50	0.53	0.56	0.55	0.59	1					
8	0.47	0.51	0.38	0.46	0.56	0.43	0.65	1				
9	0.46	0.50	0.35	0.38	0.45	0.53	0.53	0.49	1			
10	0.51	0.45	0.42	0.44	0.55	0.47	0.53	0.44	0.47	1		
11	0.65	0.53	0.44	0.41	0.43	0.56	0.56	0.46	0.61	0.65	1	
12	0.46	0.45	0.21	0.28	0.55	0.32	0.37	0.44	0.47	0.62	0.60	1

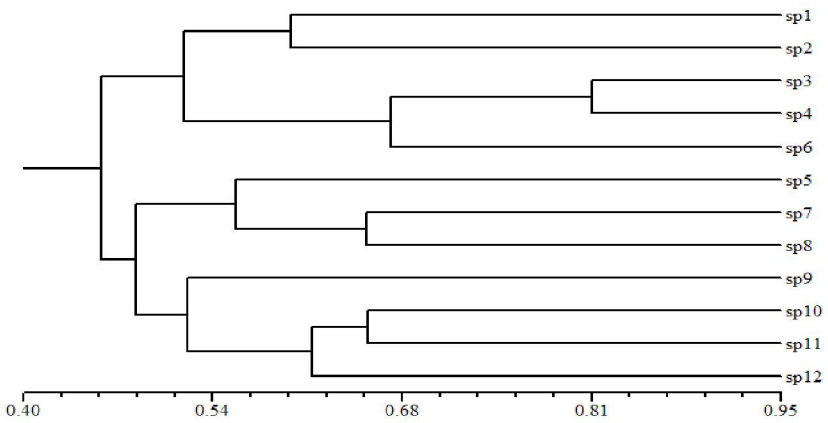

Figure 3: Dendogram illustrating the relationships between the studied species based on ISSR analysis. SP1 up to SP12 refer to the species indicated in table (1).

Table (11): Number of amplified fragments and specific markers of the studied species based on AFLP analysis.

Primer Combination	TAF	PB	Species Specific Markers (SM)												TSM
			1	2	3	4	5	6	7	8	9	10	11	12	
E-AAC/M-CAC	137	137	2	6	3	1	0	3	5	1	1	0	3	1	26
E-AAC/M-CTC	125	125	1	3	4	3	4	6	2	4	4	1	0	3	35
E-ACC/M-CTA	86	86	3	3	6	1	0	3	4	3	0	0	2	1	26
E-ACA/M-CAT	85	85	0	1	3	0	0	0	2	1	5	1	3	1	17
Total	433	433	6	13	16	5	4	12	13	9	10	2	8	6	104

TAF= Total Amplified Fragment; PB= Polymorphic Bands; TSM= Total number of Specific Marker across taxa.

Table 12: Similarity index of the studied species based on AFLP data.

Species	1	2	3	4	5	6	7	8	9	10	11	12
1	1											
2	0.201	1										
3	0.3	0.265	1									
4	0.223	0.281	0.3	1								
5	0.302	0.193	0.277	0.39	1							
6	0.26	0.224	0.269	0.274	0.231	1						
7	0.223	0.237	0.281	0.233	0.336	0.362	1					
8	0.144	0.251	0.301	0.318	0.332	0.258	0.376	1				
9	0.194	0.218	0.282	0.243	0.235	0.331	0.355	0.263	1			
10	0.312	0.286	0.344	0.356	0.352	0.351	0.308	0.338	0.412	1		
11	0.201	0.194	0.27	0.302	0.296	0.294	0.256	0.329	0.275	0.424	1	
12	0.218	0.201	0.308	0.316	0.329	0.363	0.338	0.299	0.339	0.435	0.301	1

Table 13: Similarity index of the studied species based on combined data.

Species	1	2	3	4	5	6	7	8	9	10	11	12
1	1											
2	0.392	1										
3	0.347	0.306	1									
4	0.29	0.331	0.404	1								
5	0.304	0.286	0.312	0.374	1							
6	0.315	0.299	0.358	0.344	0.329	1						
7	0.301	0.331	0.304	0.283	0.369	0.395	1					
8	0.226	0.287	0.313	0.322	0.33	0.282	0.366	1				
9	0.284	0.319	0.308	0.311	0.29	0.344	0.336	0.356	1			
10	0.387	0.331	0.37	0.399	0.359	0.362	0.322	0.366	0.465	1		
11	0.317	0.305	0.319	0.346	0.311	0.335	0.299	0.327	0.34	0.47	1	
12	0.305	0.298	0.317	0.344	0.348	0.356	0.335	0.296	0.348	0.447	0.45	1

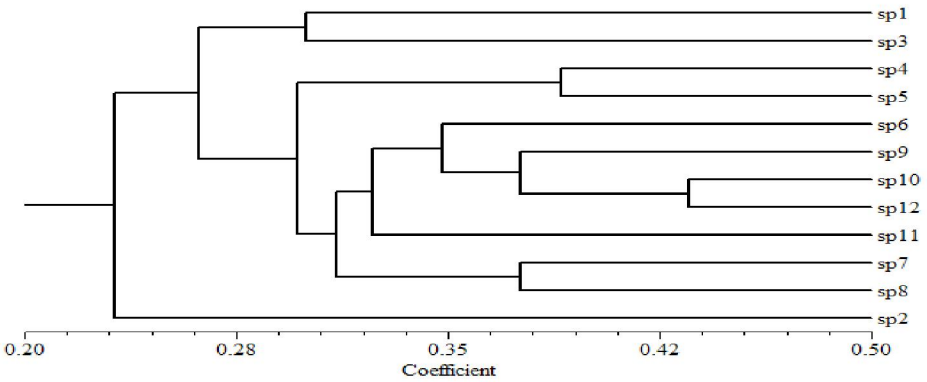

Figure 4: Dendogram illustrating the relationships between the studied species based on AFLP analysis. SP1 up to SP12 refer to the species indicated in table 1.

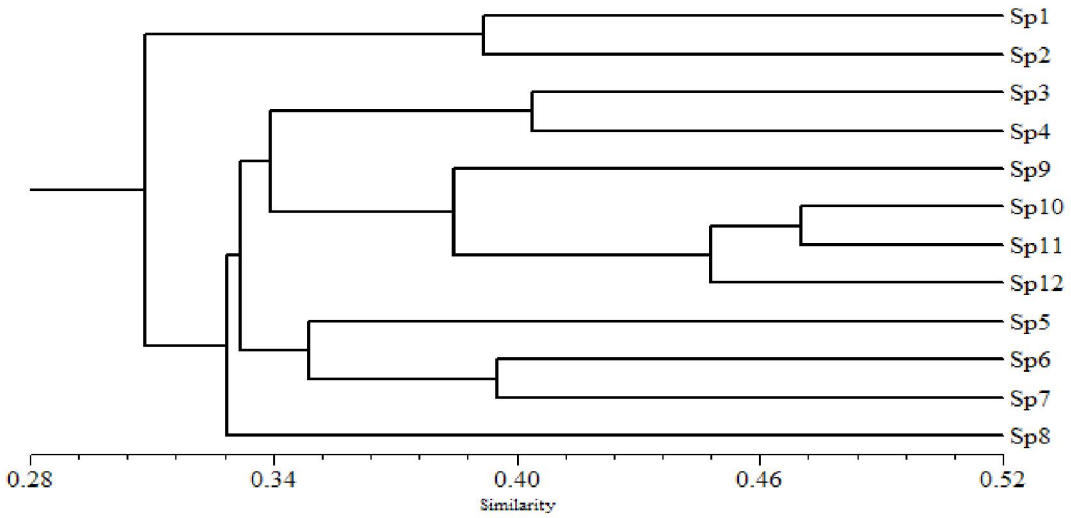

Figure 5: Dendogram illustrating the relationships between the studied species based on combined analysis. SP1 up to SP12 refer to the species as listed in table 1.

4. Conclusions:

The collective dendogram based on the molecular data, even though apparently different from that based on morphological characters show similar relationships with the morphologically based dendogram. For instance, it shows a close relation among *Albizia julibrissin, Al. lebbek* (originally both belong to Ingeae) and *Faidherbia albida* (originally belongs to Acacieae) as mentioned by Joseph *et al.*, (2001) who stated that the tribe Ingeae is nested within Acacieae. This claim is also supported by Elias (1981) and El Azab (2005) who recommended that the genus *Faidherbia* is better included within the Ingeae.

The collective dendogram clearly separated *Calliandra haematocephala* from other species compared with morphological based dendogram which also separated it but within the same group with *Albizia julibrissin, Al. lebbek* and *Faidherbia albida*. This supports the view that *Calliandra haematocephala* represnts a distinctive character in the Mimosoideae. This is in agreement with Guinet & Hernandez (1989) who stated that *Calliandra* is a very isolated genus within Mimosoideae. However, the combined dendogram grouped *Adenanthera pavonina, Dichrostachys cineria, Prosopis cineraria* and *P. juliflora* (originally Mimoseae as stated by Elias, 1981) without changes in their position in Mimosoideae; and also grouped *Acacia saligna* and *A. seyal* together grouped *Acacia laeta* and *A. nilotica* without any change in their position in tribe Acacieae.

The combined dendogram show some differences as compared with the dendograms of RAPD and AFLP due to the difference in number of markers. Nevertheless, ISSR dendogram is almost similar to the

collective dendogram and morphologicaly based dendogram. At the cluster level, first cluster contains *A. laeta*, *A. nilotica*, *A. saligna*, *A. seyal* and *Al. julibrissin* confirms the close relationships between Ingeae and Acacieae (Bentham, 1842). The second cluster was subdivided into two sub-clusters, the 1st contain *Faidherbia albida* (originally tribe Acacieae), *Albizia lebbek* and *Calliandra haematocephala* (originally tribe Ingeae). This supports the proposed transfer *Faidherbia albida* from Ingeae to Acacieae. This view is also supported by Elias (1981), Guinet (1981) and ElAzab (2005) on the basis of pollen grain characters. In second sub-cluster, *Adenanthera pavonina*, *Dichrostachys cineria*, *P. cineraria* and *P. juliflora* (originally belongs to Mimoseae). This is in agreement with Elias (1981).

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