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NUMERICAL TAXONOMY OF *ABELMOSCHUS* MEDIK. (MALVACEAE) IN INDIA

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Abstract

In the present study, numerical taxonomy approach has been used for the first time to access the taxonomy and species relationships of *Abelmoschus*. Sixteen *Abelmoschus* taxa were subjected to cluster analysis using 52 diagnostic characters related to root, stem, leaf, flower, fruit and seed. In this analysis, the first six principal components (PCs) accounted for the total variance of 79.22%. Similarity values for all 17 operational taxonomic units (OTUs) ranged from 0.18 to 0.82 with an average of 0.34. *Abelmoschus sagittifolius* showed maximum similarity value of 0.82 with *A. moschatus* subsp. *tuberosus*. On the other hand, minimum similarity values (0.18) were observed between *A. tuberculatus* and *A. moschatus* subsp. *tuberosus*, *A. tuberculatus* and *A. sagittifolius*, *A. palianus* and *A. moschatus* subsp. *tuberosus*, and *A. palianus* and *A. sagittifolius*. Neighbour joining (NJ) cluster analysis clearly discriminated 17 OTUs into four major clusters. The present study also validates the utility of morphometric analysis of *Abelmoschus* with respect to the taxonomy and species relationships.

Introduction

Over the years, taxonomy has found important practical applications in various fields of science such as theoretical and applied biology including agriculture, evolutionary study, forestry, public health, biodiversity management and environmental issues (Godfray, 2002). Adanson (1763) proposed that classification should be based on characters covering all aspects of plant such as leaf, flower, fruit, seed, and each character should be given equal importance. As a consequence, a mathematical approach has been established by taxonomists called Numerical Taxonomy (Sokal and Sneath, 1963). Morphological data are considered significant in systematics because variation exhibited by morphological traits is supposed to be categorized by gaps between taxa which reflect their evolutionary arrangement emerged through morphological changes (Otte and Endler, 1989).

Taxonomy of *Abelmoschus* Medik. has a complex history with uncertainty in the generic status and composition of the genus as well as the species concept applied within the genus. The taxonomic treatment for some species of *Abelmoschus* is not consistent. *Abelmoschus manihot* (L.) Medik. and *A. moschatus* Medik. are the most polymorphic species (Hamon and Charrier, 1983). Hochreutiner (1924) described 14 species of *Abelmoschus,* in which *A. moschatus* and *A. manihot* constitute several varieties. However, Sivarajan and Pradeep (1996) did not consider infra-specific classification of *A. manihot* produced by Van Borssum-Waalkes (1966). Paul and Nayar (1988) and Paul (1993) therefore treated *A. manihot* as a single species without any infra-specific classification. Bates (1968) also suggested that all subspecies and varieties of *A. manihot* should

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be compressed in one group. Later, Vredebregt (1991) pointed out that *A. manihot* subsp. *manihot*, *A. manihot* subsp. *tetraphyllus* var. *tetraphyllus* and *A. manihot* subsp. *tetraphyllus* var. *pungens* complex lack discrete species boundaries among them, which further contradicts Hochreutiner (1900), Van Borssum-Waalkes (1966), Paul and Nayar (1988) and Paul (1993). Infraspecific taxonomy of *A. moschatus* is also a matter of debate as many subspecies and varieties have been recognized by Masters (1874), Hochreutiner (1900) and Van Borssum-Waalkes (1966).

Wild species of *Abelmoschus* comprise still larger unexplored variability, not even 2–3% of them have been studied beyond recognizing them as valuable reservoirs of untagged genes of agronomically useful traits (Sandhu *et al.*, 1974). Therefore, a thorough and robust hypothesis is urgently needed on morphological variation and species relationships among all taxonomically valid species of *Abelmoschus* which may provide the species-wise perspective that will be used in okra [*A. esculentus* (L.) Moench.] breeding strategies and effective germplasm management. The main objective of this study were to examine the morphological variation related to root, stem, leaf, flower, fruit and seed characters of *Abelmoschus* taxa by means of numerical taxonomy in order to resolve their relationships.

Materials and Methods

Taxon sampling and taxonomic treatment

On the basis of distribution data obtained from literature survey, several field trips were undertaken during 2010–2012 to collect and study different taxa of *Abelmoschus* occurring in India (Table 1). Confirmation of collected specimens was ensured with the help of information gathered from floras, published reports (Van Borssum-Waalkes, 1966; Paul and Nayar, 1988; Sivarajan and Pradeep, 1996) and the herbarium specimens. A standard procedure of using herbarium material was applied (Edlley *et al.*, 2012). Morphological characters of plants related to root, stem, leaf, flower, fruit and seed were described from their natural habitats, to avoid any ambiguity in the characters due to environmental effect. Seed related characters were taken from Patil *et al.* (2015).

Character selection and data analysis

Species and in some cases their populations were used as operational taxonomic units (OTUs) for numerical taxonomy based on morphological data. Characters selected for morphological description of *Abelmoschus* species were those reported by Bisht *et al.* (1993, 1995), Sivarajan and Pradeep (1996) and based on field observations. A total 52 diagnostic characters related to habit, stem, leaf, flower, fruit and seed were chosen and scored for each OTU (Table 2).

The characters were converted into binary states and multi-states (interval) code. Standardization to $\mu = 0$ and $\sigma = 1$ of morphological data were done based on YBAR option with the software NTSYSpc ver. 2.10e (Rohlf, 1992). Neighbour joining tree was constructed using euclidean distance with the same software. Principal components (PCs) analysis was performed to analyze non-hierarchical relationship among the OTUs. This analysis was executed by calculating the eigenvectors and eigen values from Eigen programme in the NTSYS software. Morphometric analyses of quantitative data related to leaf, flower and fruit were done using SPSS version 11.5 for Windows.

Results

Morphological observations

Morphological evaluation of *Abelmoschus* species demonstrated that characters related to root, pubescent stem, leaf, flower, fruit and seed were significantly different between species. Root of *A. enbeepeegearense* John *et al.*, *A. crinitus* Wall. and *A. sagittifolius* (Kurz.) Merr. *ss.*, was tuberous, while rest of the species was non-tuberous. *Abelmoschus enbeepeegearense*, *A. crinitus*, *A. manihot* (L.) Medik. subsp. *tetraphyllus* (Roxb. *ex* Hornem.) Borss. var. *pungens* (Roxb.) Hochr. and *A. moschatus* Medik. subsp. *moschatus*, had conspicuous stem, while rest of the species had glabrous stem. Flowers of *A. ficulneus* and *A. angulosus* var. *angulosus* had white corolla, while *A. angulosus* var. *purpureus* had pink corolla. On the other hand, rest of the species

Table 1. Studied taxa of *Abelmoschus* along with their codes, accession numbers, places of collection (latitude/longitude) and altitude.

Sl. No.	Taxon	Code	Accession/ collector no.	Place of collection (Latitude/Longitude)	Altitude (m)
1.	*Abelmoschus esculentus* (L.) Moench.	AES	Var. AA	NA	NA
2.	*A. caillei* (A. Chev.) Stevels	ACA	NMB2924	N5^o 26.860'/E20^o 88.221'	1012
3.	*A. moschatus* Medik. subsp. *moschatus* (odourless seed)	AMO	EC316073	NA	NA
4.	*A. moschatus* Medik. subsp. *moschatus* (musk scented seed)	AMM	IC141056	N8^o 38.999'/E77^o 03.698'	124
5.	*A. moschatus* Medik. subsp. *tuberosus*	ATR	IC324070	NA	NA
6.	*A. sagittifolius* (Kurz.) Merr. ss.	ASG	W357	N19^o 17.265'/E77^o 30.977'	487
7.	*A. tuberculatus* Pal & Singh	ATB	IC550656	N19^o 24.909'/E78^o 03.337'	432
8.	*A. ficulneus* (L.) Wight &Arn.	AFI	IC141001	N15^o 30.040'/E74^o 59.587'	644
9.	*A. crinitus* Wall.	ACR	N/SS2759	N19^o 43.478'/E78^o 17.201'	470
10.	*A. manihot* (L.) Medik. subsp. *manihot*	AMN	TCR2305	N16^o 40.857'/E74^o 12.759'	569
11.	*A. manihot* (L.) Medik subsp. *tetraphyllus* (Roxb. *ex* Hornem.) Borss. Waalk.	AMT	IC141019	N23^{o}34.630'/E78^o 33.261'	1828
12.	*A. manihot* (L.) Medik. subsp. *tetraphyllus* (Roxb. *ex* Hornem.) Borss. var. *pungens* (Roxb.) Hochr.	AMP	NMB2933	N19^o 11.795'/E73^o 42.307'	904
13.	*A. angulosus* var. *grandiflorus* Thwaites	AAG	IC470751	N12^o 26.429'/E75^o 39.666'	694
14.	*A. angulosus* var. *purpureus* Thwaites	AAP	AP1	N13^o 25.799'/E75^o 44.921'	1606
15.	*A. angulosus* var. *angulosus* Sivrajan & Pradeep	AAA	AA1	NA	NA
16.	*A. enbeepeegearense* John *et al.*	AEN	JRN/09/25	NA	NA
17.	*A. palianus* Sutar *et al.*	APA	SUA54	NA	NA

*NA = not available

Table 2. Description of 52 morphological characters used in the cluster analysis of 16 taxa of *Abelmoschus*.

Sl. No.	Character	Code	Description/Value
	Habit:		
1	Growth habit	GRH	erect (0) medium (1) procumbent (2)
	Root:		
2	Root type	ROT	non-tuberous (0) tuberous (1)
	Stem:		
3	Branching habit	BRH	non-branched (0) branched only at base (1) branched evenly (2) branched only at top (3)
4	Stem pubescence	STP	glabrous (0) slight (1) conspicuous (2)
5	Stipule shape	STS	long linear (0) linear lanceolate (1) triangular (2) short linear (3)
	Leaf:		
6	Leaf colour	LEC	green (0) green with red veins (1) dark green (2) light green (3)
7	Leaf length	LEL	in cm
8	Leaf width	LEW	in cm
9	Leaf length : width ratio	LLW	-
10	No. of lobes	NLN	5 (0) more than 5 (1) less than 5 (2)
11	Leaf texture	LTX	glabrous (0) slight (1) conspicuous (2) wooly (3)
12	Leaf margin	LMR	crenate (0) dentate (1) undulate (2) entire (3) serrate (4) serrulate (5)
	Flower:		
13	Flower stalk	FST	straight (0) drooping (1)
14	Pedicel length	PDL	in cm
15	No. of epicalyx segment	NES	in no.
16	Shape of epicalyx segment	SHE	linear (0) lanceolate (1) triangular (2) ovate (3) broadly lanceolate (4) deltoid (5)
17	Persistence of epicalyx	PEE	caducous (0) partially persistent (up to seven days) (1) persistent (2)
18	Flower length	FLL	in cm
19	Flower diameter	FDM	in cm
20	Flower length : diameter ratio	FLD	-
21	Flower length : pedicel length ratio	FLP	-
22	No. of petals	NPT	5 (0) more than 5 (1)
23	Petal colour	PTC	yellow (0) light yellow (1) dark yellow (2) red (3) pink (4) white (5)
24	Length of style	LST	in cm
25	No. of stigma lobes	NSL	5 (0) 6 to 8 (1)
26	Stigma colour	SCO	red (0) dark red (1) light red (2) white (3) pink (4)
	Fruit:		
27	Fruit colour	FCO	green (0) dark green (1) yellow green (2)

Sl. No.	Character	Code	Description/Value
28	Fruit shape	FSH	lanceolate (0) ovoid (1) lanceolate-ovoid (2) broadly ovoid (3) widely elliptic (4)
29	Fruit beak	FBE	non-beaked (0) beaked (1)
30	Fruit length	FRL	in cm
31	Fruit width	FRW	in cm
32	Fruit length : width ratio	FLW	-
33	Fruit pubescence	FPB	tomentose (0) glandular hairy (1) soft strigulose (2) densely hispid (3) hirsute (4) tuberculate hairy (5)
34	Fruit tuberculation	FTB	non-tuberculate (0) tuberculate (1)
35	Fruit dehiscence	FDH	laterally (0) apically (1)
	Seed: a. macro-morphology		
36	Seed odour	SOD	odourless (0) musk scented (1)
37	Seed size	SDS	large (0) medium (1) small (2)
38	Seed shape	SSH	obovate (0) globose (1) reniform (2) sub-reniform (3)
39	Seed colour	SCO	dark brown (0) brown (1) greenish (2) blackish (3)
40	Seed texture	STX	glabrous (0) pubescent (1)
41	Hilum position	HLP	terminal (0) sub-terminal (1)
42	Hilum shape	HLS	ovate (0) broad ovate (1) triangular (2) round (3)
	b. micro-morphology		
43	Trichome	TRC	absent (0) present (1)
44	Trichome density	TRD	sparse (0) dense (1)
45	Trichome type	TRT	spiral (0) non-spiral (1)
46	Seed sculpture	SSC	reticulate (0) reticulate-foveate (1)
47	Epidermal cell shape	ECS	polygonal (0) tetra-hexagonal (1) pentagonal-hexagonal (2)
48	Anticlinal wall shape	AWS	undulate (0) striate (1)
49	Anticlinal wall thickness	AWT	thin (0) thick (1)
50	Anticlinal wall level	AWL	raised (0) grooved (1)
51	Periclinal wall level	PWL	convex (0) concave (1) flat (2)
52	Periclinal wall texture	PWT	tuberculate (0) smooth (1) wavy (2) not noticeable (3)

had yellow corolla. *Abelmoschus angulosus* var. *grandiflorus* Thwaites, *A. angulosus* var. *angulosus* Thwaites, *A. angulosus* var. *purpureus* Thwaites, *A. ficulneus* (L.) Wight & Arn., and *A. sagittifolius* had ovoid fruits, while *A. palianus* fruits were broadly ovoid. Fruits dehiscence was apically in *A. ficulneus*, *A. tuberculatus* Pal & Singh, *A. manihot*, *A. palianus* Sutar *et al.* and *A. crinitus*, while rest of the species laterally dehiscence. Seeds of *A. moschatus* subsp. *moschatus* had musk scent, and the remaining species were odourless.

Using the seed morphological characters, the studied taxa of the *Abelmoschus* revealed two basic types of seeds i.e., Type I: Seeds with deciduous trichomes and Type II: Seeds with persistent trichomes. *Abelmoschus esculentus*, *A. caillei*, *A. crinitus*, *A. moschatus* subsp. *moschatus*, *A. moschatus* subsp. *tuberosus* and *A. enbeepeegearense* belong to the Type I. In contrast, Type II comprises *A. ficulneus*, *A. tuberculatus* and *A. manihot* subsp. *tetraphyllus* var. *pungens*, *A. manihot* subsp. *manihot*, *A. manihot* subsp. *tetraphyllus* var. *tetraphyllus*, *A. angulosus* var. *grandiflorus*, *A. angulosus* var. *purpureus*, *A. angulosus* var. *angulosus* and *A. palianus*.

Numerical taxonomic analysis

The ratio of leaf length to leaf width and flower length to flower diameter did not show variation among the studied OTUs. Pearson's correlation analysis was done to determine the correlation among leaf, flower and fruit characters (Table 3). The highest positive correlation value (*r*p) was observed between FDM to FLL (0.878) followed by LEW to LEL (0.862) and FRL to LEL (0.816) at 0.01 level of significance. On the other hand, the lowest positive correlation value was observed between PDL to LEL (0.041) followed by FLP to LEL (0.052) and FRW to FLD (0.070). However, negative correlation was also observed between LLW to LEW (-0.437), FRW to FLP (-0.538) and FLP to PDL (-0.741).

Analysis of the 52×17 correlation matrix data set resulted in 14 eigenvectors (PCs). Out of 14 PCs, first six PCs were retained because they had eigenvalues of equal or higher than 1. For each PC, a component loading of more than 0.05 was considered as being significant. In this analysis, the first six PCs (PC1 = 23.48%, PC2 = 19.34%, PC3 = 12.13%, PC4 = 10.28%, PC5 = 7.47% and PC6 = 6.52%) accounted for the total variance of 79.22% differentiating the 17 OTUs. The first axis (PC-1) was highly influenced by STS, SHE, NSL, FTB, SOD, SCO and HLS, and defined 23.48% of the overall variance. These characters show considerable significant values of taxonomic importance with respect to the species differentiation. For the second axis (PC-2), the characters contributing to the total variability were BRH, FCO, FLW, SDS, SOD and HLP with 19.34% of variance. In the third axis (PC-3), characters such as GRH, LEC, NLN, LMR, NPT, AWL and PWL showed significant value of taxonomic importance to discriminate the 17 OTUs.

Similarity values of all 17 OTUs ranged from 0.18 to 0.82 (Table 4). *Abelmoschus sagittifolius* showed maximum similarity value of 0.82 with *A. moschatus* subsp. *tuberosus*, whereas minimum similarity value (0.18) was observed between *A. tuberculatus* and *A. moschatus* subsp. *tuberosus*, *A. tuberculatus* and *A. sagittifolius*, *A. palianus* and *A. moschatus* subsp. *tuberosus*, and *A. palianus* and *A. sagittifolius*. Neighbour joining (NJ) cluster analysis clearly discriminated 17 OTUs producing four major clusters (Fig. 1).

Cluster I: *A. esculentus*, *A. caillei*, *A. tuberculatus* and *A. ficulneus*

Cluster II: *A. moschatus* subsp. *moschatus* (musk scented seed), *A. moschatus* subsp. *moschatus* (odourless seed), *A. moschatus* subsp. *tuberosus*, *A. sagittifolius*, *A. crinitus*, *A. enbeepeegearense* and *A. manihot* subsp. *tetraphyllus* var. *pungens*

Cluster III: *A. angulosus* var. *grandiflorus*, *A. angulosus* var. *angulosus*, *A. angulosus* var. *purpureus* and *A. palianus*

Cluster IV: *A. manihot* subsp. *manihot* and *A. manihot* var. *tetraphyllus*

Discussion

Plant species have been considered as the central units of ecological and evolutionary studies, and therefore, the identification of boundaries among closely related species is an essential target of current systematic studies (Edlley *et al.*, 2012). In this study, morphological variation based on 52 characters (qualitative and quantitative) related to habit, root, stem, leaf, flower, fruit and seed were analyzed, which gave new insights into their potential taxonomic values for the species differentiation in the genus *Abelmoschus.*

Focusing on the root type in *Abelmoschus* species the present study revealed that there are only three species, which have tuberous root and others are non-tuberous. The characters such as shape of stipule, number of lobes in leaf, leaf margin, shape and nature of epicalyx segment, petal colour, number of stigma lobe, fruit colour, fruit tuberculation, seed odour, seed colour and seed size significantly contributed to separating the studied taxa and have always been central diagnostic characters in the genus *Abelmoschus* (Medikus, 1787; Van Borssum-Waalkes, 1966;

Table 3. Correlation coefficients among leaf, flower and fruit variables (quantitative) in *Abelmoschus* taxa. Abbreviations of characters correspond to Table 2. ** indicates significant correlation at the 0.01 level; * indicates significant correlation at the 0.05 level.

		Leaf			Flower						Fruit		
		LEL	LEW	LLW	PDL	FLL	FDM	FLD	FLP	LST	FRL	FRW	FLW
Leaf	LEL	-											
	LEW	0.862**	-										
	LLW	0.070	-0.437	-									
Flower	PDL	0.041	0.147	-0.061	-								
	FLL	0.158	0.324	-0.264	0.490*	-							
	FDM	0.112	0.338	-0.397	0.479	0.878**	-						
	FLD	0.155	-0.010	0.322	-0.081	0.070	-0.405	-					
	FLP	0.052	-0.110	0.206	-0.741**	0.085	-0.038	0.235	-				
	LST	-0.248	-0.151	-0.035	0.599*	0.762**	0.637**	0.097	-0.039	-			
Fruit	FRL	0.816**	0.583*	0.273	-0.114	-0.012	-0.175	0.404	0.183	-0.287	-		
	FRW	0.224	0.420	-0.293	0.773**	0.652**	0.557*	0.070	-0.538*	0.455	0.082	-	
	FLW	0.614**	0.264	0.481	-0.428	-0.294	-0.350	0.216	0.448	-0.406	0.842**	-0.390	-

Table 4. Pairwise similarity matrix of 16 *Abelmoschus* taxa based on simple matching coefficient from the matrix of 52 characters. OTUs codes used correspond to the Table 1. Bold represents minimum and underline represents maximum value of similarity.

OTUs	AES	ACA	AMO	AMM	ATR	ASG	ATB	AFI	AMN	AMT	AMP	ACR	AAG	AAA	AAP	AEN	APA
AES	-																
ACA	0.62	-															
AMO	0.42	0.34	-														
AMM	0.42	0.32	0.70	-													
ATR	0.26	0.24	0.52	0.46	-												
ASG	0.26	0.24	0.54	0.46	<u>0.82</u>	-											
ATB	0.36	0.32	0.30	0.30	**0.18**	**0.18**	-										
AFI	0.28	0.28	0.20	0.20	0.20	0.20	0.36	-									
AMN	0.30	0.28	0.34	0.30	0.28	0.28	0.32	0.40	-								
AMT	0.28	0.28	0.30	0.26	0.30	0.30	0.36	0.42	0.70	-							
AMP	0.22	0.20	0.34	0.30	0.30	0.30	0.34	0.36	0.34	0.38	-						
ACR	0.28	0.30	0.38	0.32	0.38	0.38	0.24	0.20	0.28	0.38	0.28	-					
AAG	0.34	0.34	0.32	0.30	0.26	0.26	0.36	0.36	0.54	0.48	0.36	0.26	-				
AAA	0.30	0.28	0.32	0.30	0.22	0.22	0.32	0.30	0.46	0.42	0.36	0.28	0.62	-			
AAP	0.28	0.28	0.30	0.26	0.26	0.26	0.36	0.34	0.40	0.40	0.36	0.24	0.66	0.52	-		
AEN	0.38	0.30	0.48	0.48	0.44	0.44	0.28	0.22	0.24	0.24	0.24	0.48	0.26	0.28	0.28	-	
APA	0.38	0.36	0.34	0.32	**0.18**	**0.18**	0.38	0.32	0.44	0.44	0.40	0.42	0.46	0.52	0.38	0.38	-

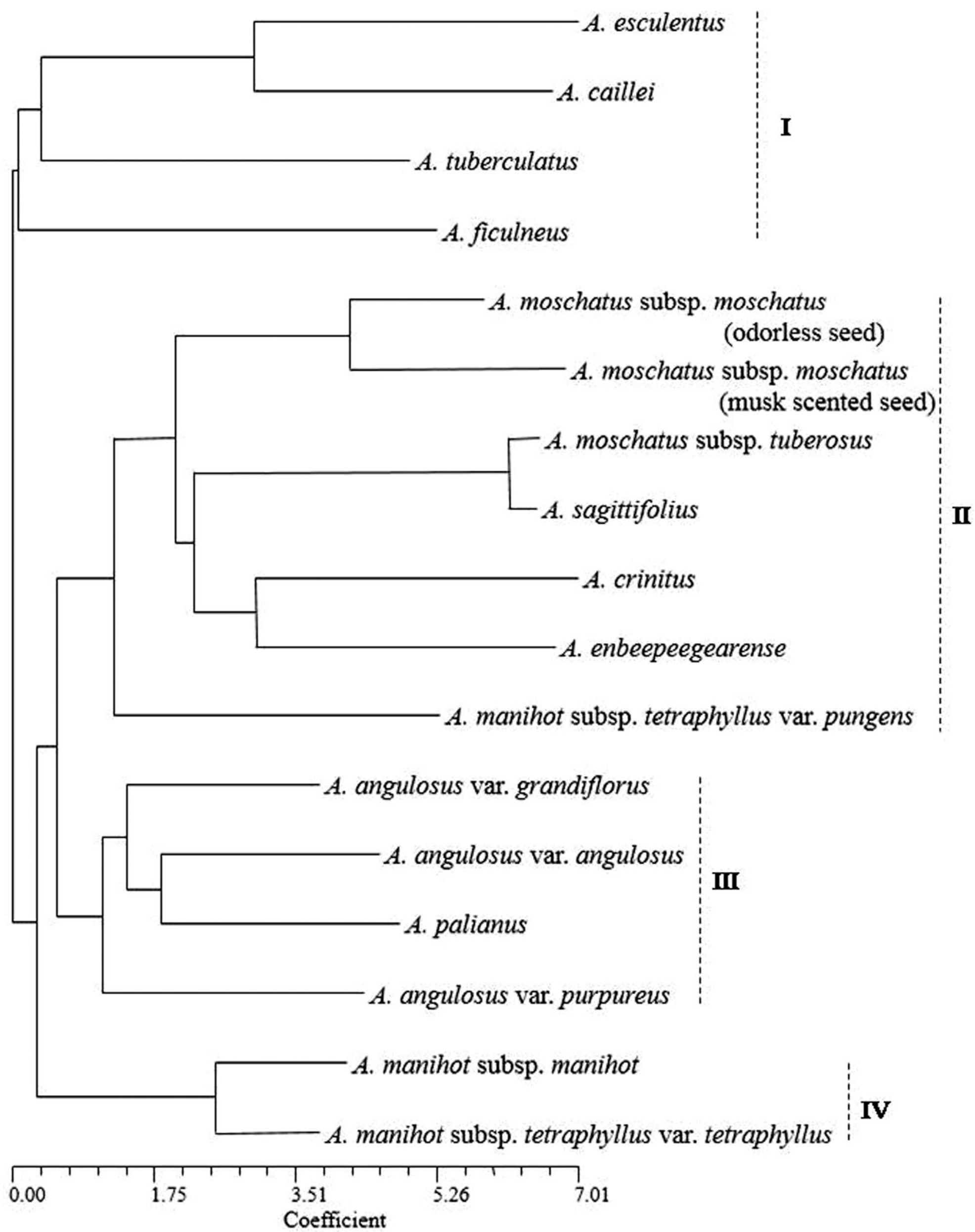

Fig. 1. Dendrogram obtained from neighbour joining (NJ) method showing the relationships of *Abelmoschus* taxa employed in the study.

Paul and Nayar, 1988; Vredebregt, 1991; Sivarajan and Pradeep, 1996; John *et al*., 2012; Sutar *et al*., 2013). The large positive correlation value as observed between leaf length and fruit length, pedicel length and fruit width, flower length and flower diameter were found to be most important and can be useful in a combination for more precise identification of *Abelmoschus* species.

In relation to the origin of cultivated okra, *A. tuberculatus* was found to be closely related to the *A. esculentus* in NJ tree, which further supports the hypothesis of Masters (1875) and Joshi *et al.* (1974). On the basis of species relationships as revealed by NJ tree, it is also assumed that *A. ficulneus* might have contributed to the *A. esculentus* genome as a second parent. The conspicuous presence of trichome on the seeds of *A. tuberculatus* is in partial agreement with Van Borssum-Waalkes (1966) who treated it as a wild form of *A. esculentus* since it generally grows along the roadsides and grassy slopes. Among the cultivated okra *A. esculentus* and *A. caillei* have great similarities in reproductive features. These species generally pose challenge for identification. The results of this study confirmed that cultivated species *A. esculentus* (Asian genotype) and *A. caillei* (introduced genotype) are morphologically distinct and easy to recognize. RAPD based characterization (Sunday *et al.*, 2008) revealed significant differences between *A. esculentus* and *A. caillei* accessions which further confirms our findings about their differentiation.

Owing to the close relationships within the species in Cluster II, we observed some common features, such as seed shape and remnants of trichomes on concentric rows in *A. moschatus* subsp. *moschatus, A. moschatus* subsp. *tuberosus, A. enbeepeegearense* and *A. crinitus.* These characters are confined to these species only indicating their taxonomically diverse nature. Investigations further revealed the remarkable variations in seed coat patterns of two very close taxa, i.e. *A. moschatus* subsp. *tuberosus* and *A. moschatus* subsp. *moschatus* supporting Bates (1968), who proposed to elevate *A. moschatus* subsp. *tuberosus* to the specific rank. Taking only taxonomic treatment into consideration, the present study also assumed that *A. moschatus* subsp. *tuberosus* and *A. sagittifolius* are not two separate entities but same, since both taxa have tuberous root type and yellow flower. Seed odour was found to be distinguishing characters for the correct identification of *A. moschatus* subsp. *moschatus* from other species of *Abelmoschus.* Another interesting new entity *A. enbeepeegearense* recently described by John *et al.* (2012) from the Southern Western Ghats showed intermediate characters (seed shape and seed colour) of *A. moschatus* subsp. *moschatus, A. moschatus* subsp. *tuberosus* and *A. crinitus*. However, seed coat features present in this taxon fully support its elevation as a separate species.

Among the species complex in *Abelmoschus, A. manihot* has been considered a highly variable taxa. Interestingly, for perennial taxa *A. manihot* subsp. *tetraphyllus* var. *pungens*, the present findings contradict with Hochreutiner (1900), Van Borssum-Waalkes (1966), Paul and Nayar (1988) and Paul (1993) who treat it as a variety of *A. manihot* subsp. *tetraphyllus*. In the NJ dendrogram obtained from 52×17 data matrix, this taxon showed distant position from *A. manihot* subsp. *tetraphyllus* var. *tetraphyllus* and *A. manihot* subsp. *manihot.* Vredebregt (1991) also demonstrated that *A. manihot* subsp. *tetraphyllus* var. *pungens* was not much different from var. *tetraphyllus.* In contrast, *A. manihot* subsp. *tetraphyllus* var. *pungens* was the only taxon which showed triangular hilum when it was rounded in *A. manihot* subsp. *tetraphyllus* var. *tetraphyllus*. Thus, hilum shape played a decisive role in differentiating these two taxa. In view of taxonomic significance, epidermal cell features differentiate *A. manihot* subsp. *tetraphyllus* var. *pungens* from widely distributed *A. manihot* subsp. *tetraphyllus* var. *tetraphyllus* and *A. manihot* subsp. *manihot.* Apart from morphological variability in *A. manihot* complex, species reflected great distinctness in seed micro-morphological characters which implies a need to study the specimens of *A. manihot* subsp. *manihot, A. manihot* subsp. *tetraphyllus* var. *tetraphyllus* and *A. manihot* subsp. *tetraphyllus* var. *pungens* using advance molecular markers for precise species differentiation and ranking.

The result obtained confirms the usefulness of seed morphology for identification and categorization of sub-specific taxa of *A. angulosus*. Based on variation in flower color, Sivarajan and Pradeep (1996) defined three varieties of *A. angulosus,* namely *A. angulosus* var. *grandiflorus* (yellow corolla), *A. angulosus* var. *angulosus* (white corolla) and *A. angulosus* var. *purpureus* (pink corolla). The present study significantly provides two more important seed characters which differentiate these varieties: *A. angulosus* var. *grandiflorus* (epidermal cell– tetra or pentagonal, elongate), *A. angulosus* var. *angulosus* (epidermal cell– polygonal) and *A. angulosus* var. *purpureus* (epidermal cell– tetra or pentagonal, not elongate) and therefore, confirm the treatment of Sivarajan and Pradeep (1996). The present study also confirms the uniqueness of recently described *A. palianus* (Sutar *et al.*, 2013). As observed in NJ tree, *A. palianus* was found to be closely related to *A. angulosus*.

In conclusion, classical taxonomy i.e. morphological descriptors, floras, type designations, and identification keys are still important and therefore the present study on *Abelmoschus* provides primary means and promotes further investigations in systematics and genomics.

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