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Species relationships among wild and cultivated *Abelmoschus* Medik., (Malvaceae) species as reveled by molecular markers

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ABSTRACT

The genus *Abelmoschus* is represented by more than 10 species which shows wide distribution over the diverse agro–climatic conditions of Indian subcontinent. In the present study, total 60 accessions of eleven *Abelmoschus* species were subjected to estimate genetic diversity and species relationships. Out of 200 primers 21 primers was found to be considerable for cluster analysis. All 21 primers produced total 321 amplicons with ranges from nine (OPA-5) to nineteen (105, 966) and average number of amplicons detected were 15.3 per primer. Out of the 321 amplicons amplified, 301 were polymorphic showing 93.77% polymorphism with average of 14.3 per primer. Among the studied species, high genetic diversity was observed in *A. manihot* subsp. *tetraphyllus* var. *tetraphyllus* (0.1711), whereas lowest genetic diversity was observed in *A. manihot* subsp. *tetraphyllus* var. pungens. Among the studied species highest (0.9095) similarity index was observed between *A. esculentus* and *A. caillei* whereas *A. palianus* and *A. crinitus* exhibited lowest (0.5149) similarity index. The UPGMA based cluster analysis clearly distinguishes the *Abelmoschus* species into three major clusters – I, II and III. This study validate the utility of RAPD markers as a reliable tool for phylogenetic relationships as well as effective characterization of *Abelmoschus* genetic resources.

Keywords: *Abelmoschus*, genetic diversity, okra, RAPD, species relationships

INTRODUCTION

Family Malvaceae commonly known as 'mallow family' is housing various economic genera such as *Gossypium* (Cotton), *Corchorus* (Jute), *Hibiscus* (Kenaf) and *Alcea*, *Malva* (ornamentals importance) in tropical and subtropical regions (Sivarajan and Pradeep, 1996). Among that, genus *Abelmoschus* Medik., is very popular one due to its tasty, gelatinous and nutritionally valuable fruit bearing species *A. esculentus* (L.) Moench. It is popularly known as '*bhendi*' in Indian vegetable market. The diverse agro–climatic conditions encompassing variable rainfall and soil regimes have

promoted diversification of the *Abelmoschus* in the Southeast Asia. The genus displays a variable habit, from annual to perennial, herbs to shrubs with leaves are long petiolate, hastate or palmately lobed, pubescent or glabrous, and flowers are white, dark yellow, pink to red colored (Sutar *et al*., 2013). India is the only country with the highest number (92%) of *Abelmoschus* species occurrences which are taxono-mically valid (Hinsley, 2013).

Recently, plant species has been considered as the central units of ecological and evolutionary studies, and therefore, the assessment of genetic diversity and relationships among closely related species is an essential target of current systematic studies as well as crop improvement programmes (Edlley *et al.*, 2012). Species of the genus *Abelmoschus* exhibit several highly variable root, flower, fruit and seed characters that have been used extensively in classification system by several authors (Van Borssum-Waalkes, 1966; Sivarajan and Pradeep, 1996; John *et al.*, 2012; Sutar *et al.*, 2013). A very little is known about the *Abelmoschus* genetic diversity, population structure and phylogeny. Unfortunately, studies using molecular markers in *Abelmoschus* species are lagging behind as compare to other crop species. Sporadic attempts have been made for the assessment of genetic diversity in genus *Abelmoschus*, but all of these restricted to *A. esculentus* or with limited taxon sampling. Recently, several workers have estimated genetic diversity using molecular markers such as isozymes (Torkpo *et al.*, 2006), SDA-PAGE (Osawaru *et al.*, 2014), RAPD (Martinello *et al.*, 2001; Sunday *et al.*, 2008; Nwangburuka *et al.*, 2011; Prakash *et al.*, 2011), ISSR (Yuan *et al.*, 2014), AFLP (Salameh, 2014), SRAP (Gulsen *et al.*, 2007), SSR (Sawadogo *et al.*, 2009), cpDNA and nrDNA sequence data (Ramya and Bhat, 2012) and transcriptome data (Schafleitner *et al.*, 2013). All of these studies have been conducted in fragmented manner resulting in gaps in taxonomic identity analysis and level of genetic diversity, and therefore, limits the utilization of available genetic resources of okra in India.

Therefore, a thorough and robust hypothesis is urgently needed on genetic variation and genetic relationships among all taxonomically valid species of *Abelmoschus* which may provide the species–wise perspective that will be used in okra breeding strategies and elite germplasm management. Due to the simplicity, no prior requirement of sequence information and cost effective-ness, among the molecular markers RAPD has proved as an alternative strategy and an efficient tool in detecting genetic variations and genetic relationships between cultivated species and its wild relatives including *Abelmoschus*. Therefore, present investigation has been conducted with cultivated, wild and newly described taxa from Indian eco-geographical regions to access the level of genetic diversity and to resolve the species relationships of *Abelmoschus* taxa. Results obtained from present study will clarify our understanding of taxono-mically complex but agriculturally importance of Indian *Abelmoschus* genetic resources.

MATERIALS AND METHODS

Plant material

Plants were collected either in the young leaf form (if available) or in the seed form from different eco-geographic regions of India. Taxonomic identification of collected plants was done by the Second author. Plants collected in the seed form were grown in the greenhouse of National Bureau of Plant Genetic Resources, New Delhi and 10-12 days old seedling were harvested for total genomic DNA extraction. Total 60 accessions belonging to 11 *Abelmoschus* species were included in this study (Table 1).

DNA extraction and RAPD profiling

Total genomic DNA of *Abelmoschus* species was extracted following Doyle and Doyle (1987) with some modifications [i.e. grinding of leaves with 100-300mg of PVPP (Polyvinylpolypyrrolidone)] to reduce polysaccharides compounds. The quantity of genomic DNA was estimated using NanoDrop® ND-1000 spectrophotometer (NanoDrop Technologies Inc., USA) and samples were diluted to a concentration of 20 ng/µl. Initially, 100 RAPD primers (Kit 2 and Kit 10 of GENEMEDS Synthesis, USA) and 100 RAPD primers (OPERON series, USA) were screened for clear and strong amplifications. Based on the survey, total 21 RAPD primers producing clear and polymorphic amplifications were selected for the final analysis (Table 2). PCR amplification was optimized and conducted in a reaction buffer of 25µl containing 1X PCR buffer; 1 Unit Taq DNA polymerase (Life Technologies, India); 0.2 mM each of dATP, dGTP, dCTP and dTTP; 3 mM of $MgCl_2$, 0.1 µM of respective primer and 40 ng of genomic DNA. PCR amplification was carried out in BIOER XP Thermal Cycler (Hangzhou Bioer Technologies, China). The thermal cycling program for RAPD was as follows: initial denaturation at 94°C for 6 min, followed by 40 cycles of

Table 1: Source of populations of the *Abelmoschus* species used in the present study.

Sr. No.	Species	Code	Collection no.	District	State
1	*A. esculentus* (L.) Moench.	escu_1	SUA-1	Kolhapur	Maharashtra
2		escu_2	SUA-14	Kolhapur	Maharashtra
3		escu_3	SUKA-3	Bilaspur	Himachal Pradesh
4		escu_4	A-4	Cultivar	Maharashtra
5		escu_5	VRO-6	Cultivar	Uttar Pradesh
6		escu_6	Var AA	Cultivar	Kerala
7	*A. caillei* (A.Chev.) Stevels	cail_7	IC587017	Darjeeling	West Bengal
8		cail_8	NMB2924	Almora	Uttarakhand
9		cail_9	NMB2913	Almora	Uttarakhand
10		cail_10	EC305619	Not Available	Bangladesh
11	*A. moschatus* subsp. Medik. m*oschatus*	mosm_11	SUA-19	Barddhman	West Bengal
12		mosm_12	SUA-20	Kolhapur	Maharashtra
13		mosm_13	SUA-22	Kolhapur	Maharashtra
14		mosm_14	SUA-32	Thiruvanatpuram	Kerala
15		mosm_15	IC 140985	Akola	Maharashtra
16		mosm_16	IC 140986	Akola	Maharashtra
17		mosm_17	IC 141056	West Siang	Arunachal Pradesh
18	*A. palianus* Sutar et al.	pali_18	SRYA-54	Durg	Chhattisgarh
19	*A. tuberculatus* Pal & Singh	tube_19	SUA-46	Dharwad	Karnataka
20		tube_20	SUA-49	Kolhapur	Maharashtra
21		tube_21	SRYA -6	Mandla	Orissa
22		tube_22	SRYA -7	Mandla	Orissa
23	*A. manihot* (L.) Medik subsp. *tetraphyllus* (Roxb. ex Hornem.) Borss. Waalk. var. *tetraphyllus*	mant_23	SUA-24	Pune	Maharashtra
24		mant_24	SUA-33	Kolhapur	Maharashtra
25		mant_25	SUA-35	Kolhapur	Maharashtra
26		mant_26	SUA-36	Kolhapur	Maharashtra
27		mant_27	SUA-38	Nanded	Maharashtra
28		mant_28	SUA-42	Kolhapur	Maharashtra
29		mant_29	SUA-48	Kolhapur	Maharashtra
30		mant_30	SRYA -8	Kabridham	Orissa
31		mant_31	SRYA -9	Mandla	Orissa
32		mant_32	SRYA -10	Damoh	Madhya Pradesh
33		mant_33	IC140982	Pratapgarh	Uttar Pradesh
34		mant_43	SUA-43	Mangalore	Karnataka
35		mant_44	SRYA -14	Sagar	Madhya Pradesh
36		mant_45	SRYA -16	Hoshangabad	Madhya Pradesh
37		manm_46	SUKA-1	Panchkula	Haryana
38		mant_47	IC141019	Raigargh	Maharashtra
39		mant_48	IC141029	Thane	Maharashtra
40		mant_49	IC141013	Sindhudurg	Maharashtra

Table 1: Continued…

Sr. No.	Species	Code	Collection no.	District	State
41	A. manihot (L.) Medik. subsp. *tetraphyllus* (Roxb. ex Hornem.) Borss. var. pungens (Roxb.) Hochr.	manp_50	IC253297	Almora	Uttarakhand
42		manp_51	IC429939	Garhwal	Uttarakhand
43		manp_52	NMB 2933	Uttarkashi	Uttarakhand
44	A. ficulneus (L.) Wight & Arn.	ficu_34	SRYA -4	Ganjam	Orissa
45		ficu_35	SRYA -5	Kabridham	Orissa
46		ficu_36	SRYA -12	Sagar	Madhya Pradesh
47		ficu_37	SRYA -13	Damoh	Madhya Pradesh
48		ficu_38	SRYA -15	Hoshangabad	Madhya Pradesh
49		ficu_39	SRYA -17	Hoshangabad	Madhya Pradesh
50		ficu_40	SUKA-2	Panchkula	Haryana
51		ficu_41	SUKA-5	Madurai	Tamil Nadu
52		ficu_42	SUA-47	Satara	Maharashtra
53	A. angulosus Thwaites	angg_53	SUA-39	Udupi	Karnataka
54		angg_54	SUA-40	Coorg	Karnataka
55		angg_55	SUA-44	Chikmagalur	Karnataka
56		anga_56	SUKA-10	Idduki	Kerala
57		anga_57	SUKA-11	Idduki	Kerala
58		angp_58	SUKA-7	Nilgiri	Tamil Nadu
59	A. crinitus Wall.	crin_59	SRYA -1	Sambalpur	Orissa
60	A. moschatus Medik. subsp. tuberosus (Span.) Borss.	most_60	SUA-31	Nanded	Maharashtra

denaturation (94°C) for 1 min, annealing (35°C) for 1 min, primer extension (72°C) for 1 min, followed by an extension at 72°C for 10 min. The amplification products of RAPD were analyzed by electrophoresis on ethidium bromide stained 1.8% agarose gel and profile was photographed under UV transilluminator using gel documentation system GBOX iChemi XT (SYNGENE, UK). A 100bp DNA ladder (MBI Fermentas, Germany) was used as standard.

Data analysis

The amplification profile of 60 accessions was carefully compared to each other across the lanes with respect to their molecular weight. Each band was scored manually for presence (1), absence (0) or missing (9) in RAPD profile of all samples. In order to generate UPGMA dendrogram of 60 accessions, 21 polymorphic RAPD primers were used for cluster analysis using the program NTSYSPC 2.1 (Rohlf, 2000). Further, Nei's unbiased genetic distance between the different species was calculated by POPGENE version 1.32 software (Yeh *et al.*, 1999). Various genetic differentiation parameters such as observed number of alleles per locus (Na), effective number of alleles per locus (Ne), Nei's gene diversity (H), Shannon's information index (I), number of polymorphic loci (P), percent polymorphic loci and estimate of gene flow (Nm) were also computed by POPGENE 32 software.

RESULTS AND DISCUSSION

In this study, out of 200 RAPD surveyed 21 RAPD primers were selected based on the extent of polymorphism observed in the clear amplification products (Figure 1). All 21 primers produced total 321 amplicons with ranges from nine (OPA–5) to nineteen (105, 966) and average number of amplicons detected were 15.3 per primer (Table 2). The molecular weight of the amplicons varied from 100bp to 3500bp (923). Out

of the 321 amplicons amplified, 301 were polymorphic showing 93.77% polymorphism with average of 14.3 per primer. This facts suggest that all studied primers has significant potential for DNA fingerprinting and diversity analysis and therefore can be economically used for the study of genetic parameters in *Abelmoschus* populations.

The inter-population genetic diversity analysis is summarized Table 3. Among the studied species, high genetic diversity in terms of mean gene diversity (H) was observed in *A. manihot* subsp. *tetraphyllus* var. *tetraphyllus* (0.1711), whereas lowest genetic diversity was observed in *A. manihot* subsp. *tetraphyllus* var. *pungens*. Level of genetic diversity was found to be low (PPL = 13.53% to 13.86%) in cultivated species populations than wild species populations (PPL = 4.29% to 62.05%). Also, similar trend was observed for mean number of effective alleles (Ne), Shannon's informative index and percentage of polymorphic loci (Table 3). Nei's genetic identity and genetic distances between the eleven *Abelmoschus* species are shown in Table 4. Among the studied species highest (0.9095) similarity index was observed between *A. esculentus* and *A. caillei* whereas *A. palianus* and *A. crinitus* exhibited lowest (0.5149) similarity index.

The genetic relationships among the studied accessions obtained by employing UPGMA cluster analysis clearly distinguishes the *Abelmoschus* species into three major clusters – I, II and III (Figure 2). Cluster I is represented by two cultivated species (*A. esculentus* and *A. caillei*) as well as two wild species (*A. tuberculatus* and *A. moschatus* subsp. *moschatus*). Cluster II is the only cluster comprising maximum number of species which again categorized into two sub-groups viz., sub–group II-A: *A. manihot* subsp. *tetraphyllus* var. *tetraphyllus*; *A. ficulneus*; *A. crinitus*; *A. moschatus* subsp. *tuberosus*; sub–group II-B: *A. manihot* subsp. *tetraphyllus* var. *pungens* and all three varieties of *A. angulosus*. As expected, the newly described taxa (*A. palianus*) clustered separately (Cluster III) with an accession (angg_54) of *A. angulosus*.

Table 2. Sequence of the RAPD primers used for *Abelmoschus* species DNA amplification, their fragment size intervals and % polymorphism.

Sr. No.	Primer name	Sequence (5'–3')	Loci size range (S_R)	No of total bands (N_T)	No. of polymorphic bands (N_P)	% polymorphism (P_P)
1	103 (Kit 2)	CCGAGGCAAG	200-1500	18	16	88.8
2	105 (Kit 2)	CACATGCAAG	250-1500	19	18	94.7
3	118 (Kit 2)	ACAGACGAAG	250-1700	15	15	100.0
4	923 (Kit 10)	GCCCTGCGTA	100-3500	18	18	100.0
5	926 (Kit 10)	TCAGCAGCAT	200-2000	15	14	93.3
6	951(Kit 10)	GCTCGCTCAT	200-1200	13	13	100.0
7	955 (Kit 10)	GCCGAGAGAT	200-1600	16	14	87.5
8	966 (Kit 10)	GATGGGTGAT	250-1800	19	18	94.7
9	995 (Kit 10)	GTGTCGGAAA	200-1400	16	15	93.7
10	996 (Kit 10)	GCACCTGAAA	150-1400	17	15	88.2
11	OPA-5	AGGGGTCTTG	400-1200	9	9	100.0
12	OPA-12	TCGGCGATAG	300-1300	10	10	100.0
13	OPA-18	AGGTGACCGT	450-1100	12	11	91.7
14	OPC-8	GTCCACACGG	300-1500	16	14	87.5
15	OPD-3	GTCGCCGTCA	350-1500	15	12	80.0
16	OPD-19	CTGGGGACTT	300-2500	18	17	94.4
17	OPM-1	GTTGGTGGCT	400-2000	17	17	100.0
18	OPM-3	GGGGGATGAG	300-1500	15	14	93.3
19	OPN-2	ACCAGGGGCA	400-1400	12	11	91.6
20	OPN-6	GAGACGCACA	250-1250	13	13	100.0
21	OPN-20	GGTGCTCCGT	300-1800	18	17	94.4

Table 3: Comparison of genetic variation statistics (allele frequency, gene diversity, Shannon's information index and polymorphism) among the populations of *Abelmoschus* species based on RAPD data.

Species population	Na	Ne	H	I	PL	PPL
A. esculentus						
Mean	1.1386	1.0937	0.0523	0.0768	42	13.86 %
St. Dev.	0.3461	0.2631	0.1403	0.2010		
A. caillei						
Mean	1.1353	1.0967	0.0553	0.0807	41	13.53 %
St. Dev.	0.3426	0.2549	0.1427	0.2066		
A. moschatus subsp. m*oschatus*						
Mean	1.3465	1.1807	0.1084	0.1661	105	34.65 %
St. Dev.	0.4767	0.3132	0.1712	0.2493		
A. tuberculatus						
Mean	1.2244	1.1433	0.0839	0.1247	68	22.44 %
St. Dev.	0.4179	0.2935	0.1636	0.2386		
A. manihot subsp. *tetraphyllus* var. *tetraphyllus*						
Mean	1.6205	1.2749	0.1711	0.2677	188	62.05 %
St. Dev.	0.4861	0.3262	0.1776	0.2558		
A. ficulneus						
Mean	1.4026	1.1880	0.1161	0.1808	122	40.26 %
St. Dev.	0.4912	0.3016	0.1686	0.2472		
A. manihot subsp. *tetraphyllus* var. *pungens*						
Mean	1.0429	1.0265	0.0161	0.0241	13	4.29 %
St. Dev.	0.2030	0.1301	0.0774	0.1149		
A. angulosus						
Mean	1.3267	1.1884	0.1129	0.1706	99	32.67 %
St. Dev.						
A. crinitus						
Mean	1.0000	1.0000	0	0	0	0
St. Dev.	0	0	0	0		
A. moschatus sub. *tuberosus*						
Mean	1.0000	1.0000	0	0	0	0
St. Dev.	0	0	0	0		
A. palianus						
Mean	1.0000	1.0000	0	0	0	0
St. Dev.	0	0	0	0		

Abbreviations: NA = mean number of observed alleles; NE = mean number of effective alleles; H = mean gene diversity; I = Shannon's Information index–mean; PL = number of polymorphic loci; PPL = percentage of polymorphic loci; and St. Dev. = standard deviation.

Table 4: Nei's genetic identity (above diagonal) and genetic distance (below diagonal) between the populations of *Abelmoschus* species. Species code corresponds to the Table no 1.

Species	escu	cail	mosm	pali	tube	mant	ficu	manp	angg	crin	most
escu	****	0.9095	0.7242	0.6149	0.7676	0.7365	0.6977	0.5767	0.6579	0.6345	0.6724
cail	0.0948	****	0.7656	0.6223	0.7984	0.7691	0.7242	0.6116	0.7125	0.6945	0.7296
mosm	0.3226	0.2671	****	0.7557	0.8154	0.8526	0.8279	0.7262	0.8237	0.7129	0.7900
pali	0.4862	0.4744	0.2802	****	0.6250	0.6305	0.6070	0.5462	0.6258	0.5149	0.6106
tube	0.2645	0.2252	0.2041	0.4700	****	0.8427	0.7915	0.6710	0.7681	0.6798	0.7373
mant	0.3059	0.2625	0.1595	0.4612	0.1712	****	0.9494	0.7661	0.8930	0.7780	0.8226
ficu	0.3600	0.3227	0.1889	0.4992	0.2338	0.0519	****	0.7273	0.8557	0.7769	0.8154
manp	0.5505	0.4917	0.3199	0.6048	0.3990	0.2665	0.3185	****	0.8366	0.6607	0.7153
angg	0.4187	0.3390	0.1939	0.4687	0.2638	0.1132	0.1558	0.1785	****	0.7576	0.8172
crin	0.4549	0.3645	0.3384	0.6639	0.3859	0.2510	0.2524	0.4145	0.2775	****	0.7261
most	0.3969	0.3153	0.2357	0.4934	0.3048	0.1953	0.2040	0.3351	0.2019	0.3201	****

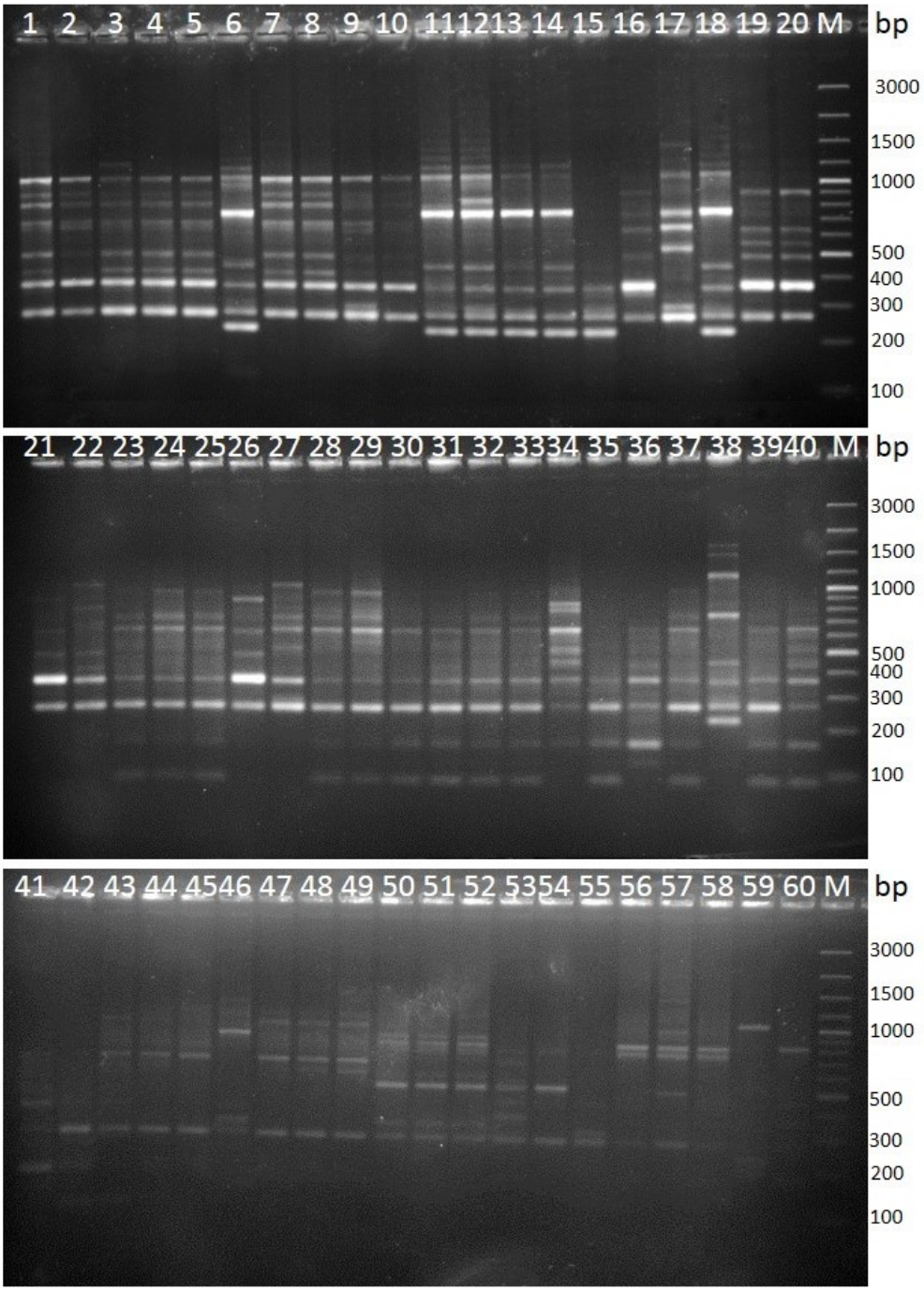

Figure 1. RAPD profile obtained with primer, 926 (Kit 10) indicating the extent of polymorphism observed in the *Abelmoschus* species. The lanes marked 1 to 60 correspond to the species code and accessions as listed in Table 1. The lane marked 'M' is DNA molecular weight standard, 100bp ladder from MBI Fermentas, Germany.

DISCUSSION

As mentioned above, one of the major problems hindering effective utilization of *Abelmoschus* germplasm is the unexplored genetic diversity and unresolved species relationships. In addition, our knowledge on correct species identification of wild species of *Abelmoschus* is still limited, hindering the efficient use in breeding practices and priority basis conservation of its valuable germplasm. Indian sub–continent has been considered as a center of diversity for *Abelmoschus*. So far, genetic diversity and interspecific relationships of Indian taxa have remained unexplored and unresolved. In the present study, we assessed RAPD data for eleven *Abelmoschus* species (60 accessions) in order to analyze the genetic diversity and

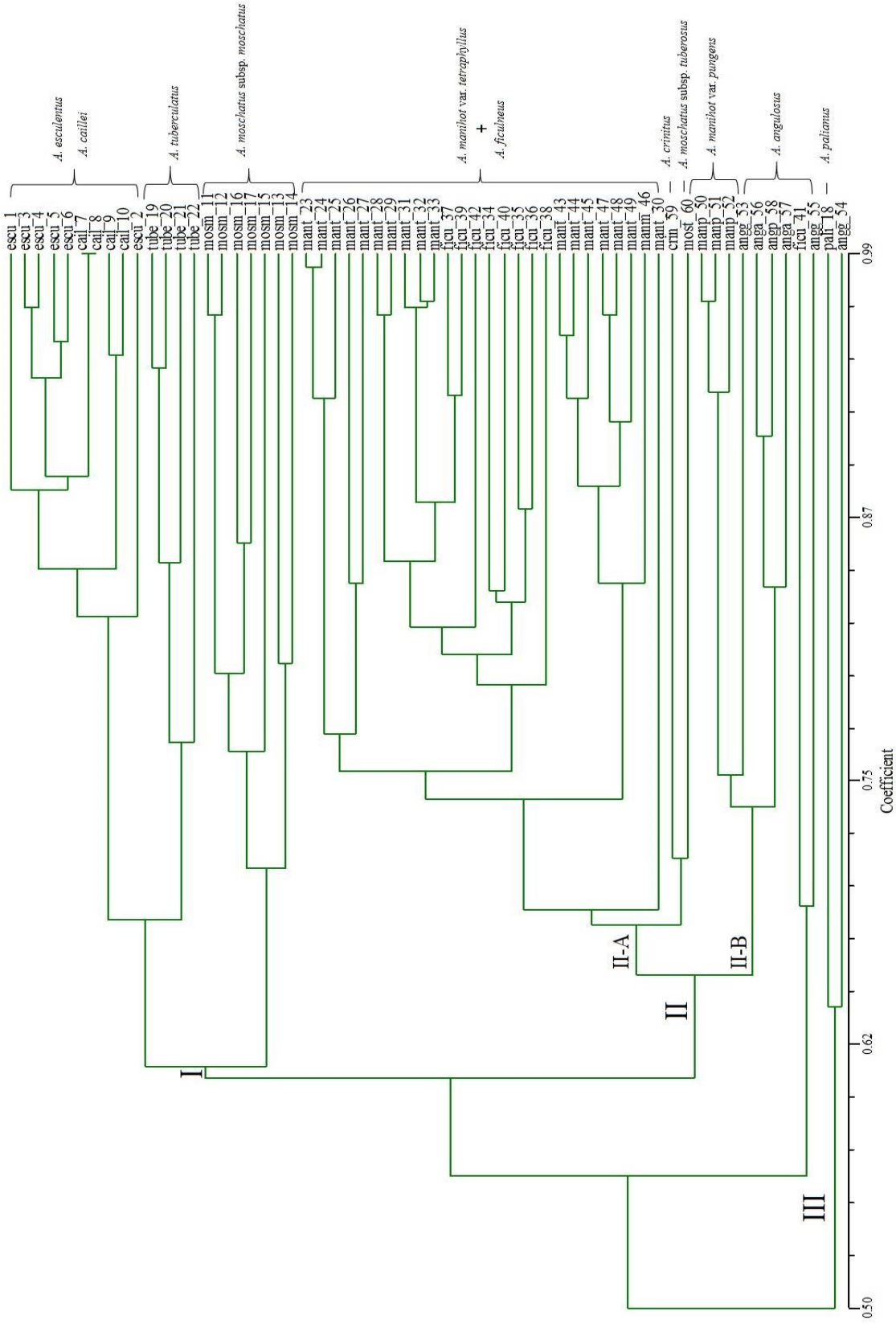

Figure 2. UPGMA tree based on RAPD data analysis using NTSYSPC 2.1 software (Rohlf, 2000). Three major cluster were identified as indicated.

species relationships will not only help in comparative assessment genomics of *Abelmoschus* but also help in developing superior varieties through marker assisted selection (MAS).

Present study indicate that RAPD markers has significant potential in the study of *Abelmoschus* genetic resources, where genetic characterization of the *Abelmoschus* nuclear genome is still lacking. Our study examines a considerably larger number of species and its populations (except *A. palianus*, *A. crinitus* and *A. moschatus* subsp. *tuberosus*) and loci than examined in earlier studies. The observed total polymorphism percentage (93.77%) for the amplified 21 RAPD primers indicate that these RAPD primers can be useful to detect species-specific distinctness and natural interspecific gene introgression. The observed low level of genetic diversity in cultivated population suggesting that cultivation may be seriously influence genetic variation of present-day cultivars. The estimated Nei's genetic identity/distance revealed highest affinity between *A. esculentus* and *A. caillei* among the studied species. This may be attributed to the commonness of morphological similarities especially of vegetative and reproductive characters shared by these two cultivated species. These finding further supports Patil *et al.*, (2015) who shown that these cultivated taxa (*A. esculentus* and *A. caillei*) shared maximum similarity in seed macro- and micro-morphological characters (seed size, hilum shape and trichome type) than other wild species. Additionally, viable seed formation and low extent of crossability barriers has also been reported between these two species (Patil *et al.*, 2013) further support sister species relationships of *A. caillei* to *A. esculentus* in our resulted UPGMA tree. These facts have been suggesting that *A. caillei* has strong gene pool bonding with *A. esculentus* which can be used as a potential bridging parent to transfer alien genes for deployment in okra breeding programs.

The identity of the ancestral species of cultivated okra is a matter of uncertainty still. Very little efforts have been made to find out the probable parents of cultivated okra (Joshi and Hardas, 1956; Joshi *et al.*, 1974). This has led to assumption that one of the parents of *A. esculentus* (n=65) should have been *A. tuberculatus* (n=29) and either of the two Indian species, namely *A. ficulneus* and *A. moschatus* possibly play a role of complementary genome. Cluster I revealed strongly supported sister species relationship of *A. tuberculatus* with *A. esculentus* fully support Joshi and Hardas (1956) and Joshi *et al.*, (1974). Regarding *A. ficulneus*, RAPD data analysis showed distant relationships between *A. ficulneus* and *A. esculentus*. This may be attributed to the extensive morphological differences between *A. ficulneus* and *A. esculentus*. *Abelmoschus ficulneus* has typical white colored flower and stigma whereas *A. esculentus* has typical light yellow colored flower and dark red stigma (Sutar *et al.*, 2013). However, *A. ficulneus* has been reported as a probable progenitor of *A. esculentus* based on *psb*A-*trn*H sequence analysis Ramya and Bhat (2012). Altogether, present analysis indicate that *A. tuberculatus* (n=29) may have major contribution to the origin of cultivated okra. Also, extensive grouping of *A. esculentus* with three species (*A. caillei*, *A. tuberculatus* and *A. moschatus* subsp. *moschatus*) indicated its broad genetic background which may rise through the free gene flow and domestication processes.

Another two varieties of *A. manihot* subsp. *tetraphyllus*, namely var. *tetraphyllus* and var. *pungens*, surprisingly found in two separate sub-groups, further did not support the varietal classification of IBPGR. In our analysis sister species relationships were observed between var. *tetraphyllus–A. ficulneus* (within sub-group II-A) and var. *pungens –A. angulosus* (within sub-group II-B). Additionally, var. *pungens* was highly incompatible than var. *tetraphyllus* with *A. esculentus* indicated that var. *pungens* is highly distant taxa[23]. Moreover, our critical observation on epicalyx and seed morphology of *A. manihot* subsp. *tetraphyllus* var. *pungens* further suggested that this unique taxon should be merged in *A. angulosus* or elevate at species level (Patil *et al.*, 2015). Moreover, observed highest diversity with respect to the mean gene diversity and percentage of polymorphic loci in *A. manihot* subsp. *tetraphyllus* var. *tetraphyllus* may be attributed to the wide occurrence of this taxa throught India. Therefore present analysis indicates its scope as a potential wild relative to improve the present okra cultivars as well as to develop new verities.

The sister species relationship of *A. moschatus* subsp. *tuberosus* to *A. crinitus* and phylogenetically distant from *A. moschatus* subsp. *moschatus* as observed in cluster analyses is fully disagree with Van Borssum-Waalkes (1966) and IBPGR (1991). The observed genetic differentiation was obvious between these species due to the prominent differences in root type and seed property. Both of species (*A. moschatus* subsp. *tuberosus* and *A. crinitus*) are characterized by typical tuberous type of root while rest of the *Abelmoschus* species (including *A. moschatus* subsp. *moschatus*) have non-

tuberosus root. Our exclusive result on species relationships further supports support Bates (1968), who proposed to elevate *A. moschatus* subsp. *tuberosus* to specific rank. Moreover, the current taxonomic status of *A. sagittifolius* as synonym of *A. moschatus* subsp. *tuberosus* remains therefore in question since taxon sampling in this analysis is still too limited to draw any strong conclusion. Additionally, based along the present fact obtained from *psb*A-*trn*H sequence analysis (PP, data not published), it intimates that these two species are separate biological entities and should be handled as a separate taxon rather than synonyms.

As expected newly described taxa, *A. palianus* inferred sister relationships with *A. angulosus* var. *grandiflorus*. This again well supported by morphological similarities as observed by Sutar *et al.*, (2013) which showed that morphological characters can reflect the genetic characteristic. The result obtained confirms the potential of RAPD markers for the identification and species division in *A. angulosus*. Based on the variation in flower color, Sivarajan and Pradeep (1996) have defined three varieties namely, *A. angulosus* var. *grandiflorus* (yellow corolla), *A. angulosus* var. *angulosus* (white corolla) and *A. angulosus* var. *purpureus* (pink corolla). In UPGMA tree all three varieties clearly showed distantness from each other with significant level of genetic diversity (0.1129). This diversity of *A. angulosus* reflects adaptation to the wide conditions of environment which may be beneficial to screen target gene for improvement of present okra cultivars.

Our preliminary phylogenetic analysis of *Abelmoschus* provides a first phylogenetic framework for future research to perceive some conformity with classification based on morphology. We conclude that, the RAPD markers is highly informative to understanding the level of genetic diversity and relationships among *Abelmoschus* species of Indian occurrences would provide an important approach in molecular taxonomy as well as future breeding programs in *Abelmoschus*.

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REFERENCES

Bates DM (1968) Notes on the cultivated Malvaceae. 2, *Abelmoschus*. *Baileya* 16: 99–112

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of leaf tissue. *Phytochem Bulletin* 19: 11–15.

Edlley MP, Marccus A, Anderson AA, Clarisse PS, Fabio P (2012). Integrating different tools to disentangle species complexes: A case study in *Epidendrum* (Orchidaceae). *Taxon* 61: 721–734.

Gulsen O, Karagul S, Abak K (2007) Diversity and relationships among Turkish okra germplasm by SRAP and phenotypic marker polymorphism. *Biol Bratislava* 62: 41–45.

Hinsley SR (2013) Malvaceae Info, viewed 29 January 2014, from http://www.malvaceae.info/Genera/*Abelmoschus*/*Abelmoschus*.php.

IBPGR (1991) Report of International workshop on okra genetic resources. National Bureau for Plant genetic Resources (NBPGR), New Delhi, India

John KJ, Scariah S, Nissar VA, Bhat KV, Yadav SR (2012) *Abelmoschus enbeepeegearense* sp. nov. (Malvaceae), an endemic species of okra from Western Ghats, India. *Nordic Journal of Botany* 30: 1–6.

Joshi AB & Hardas MW (1956) Alloploid nature of okra, *Abelmoschus esculentus* (L.) Moench. *Nature* 178: 190

Joshi AB, Gadwal VR & Hardas MW (1974) Evolutionary studies in world crops. In Diversity and change in the Indian sub–Continent. Ed., Hutchinson JB, London: Cambridge University Press. Pp. 99–105

Martinello GE, Leal NR, Amaral AT, Pereira MG, Daher RF (2001) Comparison of morphological characteristics and RAPD for estimating genetic diversity in *Abelmoschus* spp. *Acta Horticulture* 546: 101–104.

Nwangburuka CC, Kehinde OB, Ojo DK, Popoola AR, Oduwaye O, Denton OA, Adekoya M (2011) Molecular characterization of twenty–nine okra accessions using the Random Amplified Polymorphic DNA (RAPD) molecular marker. *Acta SATECH* 4: 1–14.

Osawaru ME, Ogwu MC, Omologbe J (2014) Characterization of three Okra [*Ablemoschus* (L.)] accessions using morphology and SDS-PAGE for the basis of conservation. *Egyptian Academic Journal of Biological Science* 5(1): 55–65.

Patil P, Malik SK, Negi KS, John J, Yadav S, Chaudhari G & Bhat KV (2013) Pollen germination characteristics, pollen–pistil interaction and reproductive behaviour in interspecific crosses among *Abelmoschus esculentus* Moench and its wild relatives. *Grana* 52: 1–14

Patil P, Malik SK, Sutar S, Yadav S, John J, Bhat KV **(**2015) Taxonomic importance of seed macro- and micro-morphology in *Abelmoschus* Medik. (Malvaceae). *Nordic Journal of Botany* DOI: 10.1111/njb.00771.

Prakash K, Pitchaimuthu M, Ravishankar KV (2011) Assessment of genetic relatedness among okra genotypes [*Abelmoschus esculentus* (L.) Moench] using RAPD markers. *Electronic Journal of Plant Breeding* 2(1): 80–86.

Ramya P, Bhat KV (2012) Analysis of phylogenetic relationships in *Abelmoschus* species (Malvaceae) using ribosomal and chloroplast intergenic spacers. *Indian Journal of Genetics and Plant Breeding* 72: 445–453.

Rohlf FJ (2000) NTSYS–pc: numerical taxonomy and multivariate analysis system, ver. 2.10e, Exeter Ltd., Setauket, NY.

Salameh NM (2014) Genetic Diversity of Okra (*Abelmoschus esculentus* L.) Landraces from Different Agro-Ecological Regions Revealed by AFLP Analysis. *American-Eurasian Journal of Agriculture & Environment Science* 14 (2): 155–160.

Sawadogo M, Ouedraogo JT, Balma D, Ouedraogo M, Gowda BS, Botanga C, Timko MP (2009) The use of cross species SSR primers to study genetic diversity of okra from Burkina Faso. *African Journal of Biotechnology* 8: 2476–2482.

Schafleitner R, Kumar S, Chen–Yu L, Hegde SG, Ebert A (2013) The okra (*Abelmoschus esculentus*) transcriptome as a source for gene sequence information and molecular markers for diversity analysis. *Gene* 517: 27–36.

Sivarajan VV, Pradeep AK (1996). Malvaceae of Southern Peninsular India. Daya Pub. House, Delhi, India. pp. 44–73.

Sunday EA, Ariyo OJ, Robert L (2008) Genetic relationships among West African okra (*Abelmoschus caillei*) and Asian genotypes *(Abelmoschus esculentus)* using RAPD. *African Journal of Biotechnology* 7: 1426–1431.

Sutar SP, Patil P, Aitawade M, John J, Malik S, Rao S, Yadav S, Bhat KV (2013) A new species of *Abelmoschus* Medik. (Malvaceae) from Chhattisgarh, India. *Genetic Resources and Crop Evolution* 60: 1953–1958.

Torkpo SK, Danquah EY, Offei SK, Blay ET (2006) Esterase, total protein and seed storage protein diversity in okra (*Abelmoschus esculentus* L. Moench). WAJAE 9: 1–7

Van Borssum–Waalkes J (1966) Malesian Malvaceae Revised. *Blumea* 14: 89–105.

Yeh FC, Yang RC, Boyle T (1999) POPGENE ver. 1.32. Microsoft Windows-based Freeware for Publication Genetic Analysis. University of Alberta and the Centre for International Forestry Research, Edmonton. http://www.ualberta.ca/~fyeh/.

Yuan CY, Zhang C, Wang P, Hu S, Chang HP, Xiao WJ, Lu XT, Jiang SB, Ye JZ, Guo XH (2014) Genetic diversity analysis of okra (*Abelmoschus esculentus* L.) by inter-simple sequence repeat (ISSR) markers. *Genetic and Molecular Research* 13 (2): 3165–3175.