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Diversity analysis of Sweet Potato (*Ipomoea batatas* [L.] Lam) genotypes using morphological, biochemical and molecular markers

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Sweet potato [*Ipomea batatas* (L.) Lam.] is a nutritious food crop primarily grown by small and marginal farmers. Successful breeding and germplasm conservation programs demands characterization of its germplasm. Here, we tried to determine genetic diversity among 21 sweet potato genotypes using morphological, biochemical and molecular markers. Ten morphological traits were studied and subjected to analysis of variance (ANOVA). Mean square due to germplasm were highly significant as well as wide mean range performance was observed for tuber number per plant, individual tuber weight, tuber fresh yield per plant, tuber dry yield per plant, tuber yield per plot and tuber length. UPGMA (Unweighted Pair Group Method Arithmetic Average) cluster analysis based on morphological traits separated the germplasm into three groups. The genotypes Gautam, Shree Arun, RS-92 and CO-3-4 appeared promising with regard to yield characters. Total phenol was maximum in in V-12 genotype (1.39 mg), while minimum was recorded in Samrat genotype (0.95 mg). The highest total antioxidant was observed in the genotype Samrat (0.30 mg), while minimum was recorded in the genotype Navsari Local (0.16 mg). Molecular diversity analysis was carried out using 25 RAPD (Random Amplified Polymorphic DNA) primers, out of which 13 primers produced 117 reproducible amplicons (106 polymorphic, 7 monomorphic and 4 unique amplicons). UPGMA dendogram based on RAPD data separated the genotypes into two major clusters having the similarity coefficient ranged from 0.56 to 0.76. The results can be used for sweet potato crop improvement through molecular breeding and marker assisted selection of for desired traits in future.

Keywords: Biochemical characterization, Diversity Divergence, UPGMA

Sweet potato, [*Ipomoea batatas* (L.) Lam. Fam. *Convolvulaceae*], is an autohexaploid species (2n=6X=90) and known for its large, starchy, sweet-tasting, tuberous roots suitable for human consumption, animal feed and recently for producing ethanol and its derivatives[1]. Worldwide, sweet potato is the sixth most important food crop after rice, wheat, potatoes, maize, and cassava. In India, sweet potato cultivation in about 122336 hectares during 2018 yielded annual production of 1400281tonnes[2]. Sweet potato is a short duration crop that requires little input and tolerates high temperatures, drought, and also adapts to poor fertile soil, and thereby attractive to small farmers[3]. There is increasing demand of sweet potato as functional food for is nutritive and medicinal values *i.e.*, vitamin A, calcium, ascorbic acid, phenolics, etc. Sweet potato antioxidants *viz.* phenolic compounds, anthocyanin, carotenes, etc. are considered important nutraceuticals on account of many health benefits[4] including inhibition of human colon growth, leukemia and stomach cancer cells[5], apart from pathogenic viruses and fungi[6],and amelioration of diabetes[7].

Genetic diversity is most important factor in breeding and crop improvement programmes. In sweet potato, morphological/phenotypic characterization is done by assessing variations in vine, leaf, flower, tuber characteristics for identification of duplicates, correlation with characteristics of agronomic importance and varietal identification and for genetic distance estimation[8,9]. Assessment of genetic diversity at the molecular level is more meaningful than at the phenotypic level as the later involves data on morphological traits which are environmental dependent. Different molecular marker systems *viz.*, AFLP, ISSR and SSR have been successfully employed to assess the genetic diversity[10,11]. Among such markers, Random Amplified Polymorphic DNA

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(RAPD) is generally favoured because of its sensitivity, simplicity, and cost-effectiveness, coupled with the fact that DNA sequence information is not required for primer design, no radioisotope labeling is needed for sample detection, and only a small amount of template DNA is required[12]. The RAPD technique has been applied to several aspects of sweet potato research, such as cultivar identification[13], diversity assessment[14], estimation of intra clonal variations and genetic diversity[15].

Advancement in genomics and genetics has accelerated molecular breeding strategies for several crop species including sweet potato, essentially to increase the yield and quality with high nutritive value[16]. However, it requires information on molecular and biochemical variation in sweet potato genotypes which is scanty despite the fact that lot of variability exist in sweet potato for physiological and biochemical characters. The present study is possibly the first attempt to determine genetic diversity among 21 sweet potato genotypes using morphological, biochemical and molecular markers.

Material and Methods

The present field investigations were carried out at the Instructional Farm, Rajasthan College of Agriculture, MPUAT, Udaipur (24°35′N, 70°42′E), Rajasthan (India).

Plant material

The experimental material for the present study comprised 21 promising genotypes of sweet potato *viz*. Shankar, Samrat, Shree Rathna (SR), Kalinga, IGPS-14, RS-92, MPUAT-10, Kishan, CO-3-4, Navsari Local, RS-47, RS-43, RS-35, V-17, V-16, V-15, V-13, V-12, V-08, Shree Arun (SA) and Gautam donated by All India Coordinated Research Project on tuber crops, Department of Horticulture, Rajasthan College of Agriculture, MPUAT, Udaipur (Rajasthan), India.

Morphological traits analysis

The experiment was laid out in randomized block design in three replications and plot size was 3 × 2.4m with spacing of 60 × 30 cm. The observations were recorded on 5 randomly selected plants for each entry (genotype) from all three replications. Various qualitative and quantitative morphological characters, such astuber shape, predominant skin colour, flesh tuber colour, predominant flesh colour, tuber number per plant, individual tuber weight (g), tuber fresh yield per plant (g), tuber length (cm), tuber dry yield per plant (g) and tuber yield per plot (kg) were recorded and were averaged and subjected to statistical analysis of all the characters.

Total antioxidant (mg 100 g^{-1})

Five gram tuber was extracted with 20 mL of 60% methanol (0.1% HCl), kept overnight, centrifuged at 10000 rpm for 15 min at 10ºC, and the supernatant was taken for analysis. About 100 µL of methanolic extract was mixed with 3 mL of solution (1.2 M sulphuric acid, 46 mM sodium phosphate and 8 mM ammonium molybetate) and was incubated for 90 min at 95ºC in water bath. It was allowed to cool down to 25ºC. Reading of plant sample was taken using spectrophotometer at a wavelength of 695 nm and ascorbic acid was taken as standard. Standard curve was plotted with the absorbance readings of standard and plant sample which gave value of total antioxidant in mg per 100 g[17].

Total phenol (mg 100 g^{-1})

Ten grams of potato flour were mixed with 80 mL methanol and kept overnight. The suspension was filtered through Whatman No. 1 filter paper and the filtrate was diluted to 100 mL with methanol. Total phenolic content determination was based on a method as described earlier[18].

RAPD analysis

The genomic DNA was extracted from 3 wk old leaves of 21 genotypes of sweet potato by CTAB extraction method of Doyle & Doyle[19] with slight modifications. Total 25decanucleotide RAPD primers were screened for PCR amplification using BioRad thermocycler by employing the procedure reported by Kaur *et al.*[20]. Upon completion of the reaction, the amplified products were separated on 1.2% agarose gel in 1X TAE buffer using ethidium bromide (EtBr) staining dye and photographed using gel documentation system (Alpha DigiDoc, Germany). The amplicons obtained from different RAPD markers were scored based on the presence (taken as 1) or absence (taken as 0) of bands for each primer. Accordingly, a rectangular binary matrix is obtained and statistical analysis was performed using the NTSYS-pc version 2.02e[21].

Results and Discussion

Genetic diversity studies can identify novel alleles which might improves plant and yield performance under adverse conditions. DNA fingerprinting is a routine method employed to study the extent of genetic diversity across a set of genotypes or cultivars and group them into specific categories. The well characterized germplasm collections are critical for providing genetic materials needed for plant breeding and the associated studies to produce need based plant genotypes with resistance/tolerance to pests, disease, and environmental stress[22]. Various types of markers, such as morphological, biochemical and molecular markers are used for this purpose[20]. Here, we estimated morphological, biochemical and molecular diversity of sweet potato genotypes for framing effective breeding programmes.

Morphological analysis of Sweet potato genotypes

Morphological characterization is regarded as the first step in description and classification of any germplasm[23]. Sound knowledge on various morphological traits in the breeding material helps in classification, identification, naming and documentation of the entries in a crop, and thereby hastens the process of utilization of genetic material for crop improvement programmes[24].

In the present study, the range and mean values of all the qualitative and quantitative data of 10 morphological characters were data presented in Table 1. The quantitative data of 6 morphological characters were subjected to analysis of variance (ANOVA) for Randomized Block Design (RBD). The mean square values due to treatments were found significant for all the traits thereby indicating substantial amount of variability among the genotypes (Table 2). Tuber shape, predominant skin colour, storage flesh tuber colour and predominant flesh colour varies among the sweet potato genotypes. Perusal of mean performance revealed that narrow mean range was found for the characters, such as tuber number per plant (2-6.5), individual tuber weight (85-190 g), tuber fresh yield per plant (212.5-910 g), tuber length (12-45 cm), tuber

Table 2 — ANOVA for various characters in sweet potato

Characters	Source of variation		
	Replications	Treatments	Error
Degree of freedom	2	20	40
Storage tuber no./plant	0.0095	4.446**	0.0517
Individual tuber weight(g)	21.0080	2090.971**	42.1565
Storage tuber flesh yield/plant (g)	103.6856	76702.221**	685.1521
Storage tuber dry yield/plant(g)	10.2046	7839.057**	51.3376
Storage tuber yield /plot (kg)	0.0434	48.028**	0.4341
Storage Tuber length (cm)	0.8663	185.843**	2.2938

[*= Significant at 1 % level; **= Significant at 5 % level]

Table 1 — Morphological characteristics studied for 21 genotypes of Sweet potato

Genotypes	Tuber shape	Predominant skin colour	Fresh tuber colour	Predominant flesh colour	Tuber no. /plant	Individual tuber wt. (g)	Tuber flesh yield/plant (g)	Tuber dry yield/plant (g)	Tuber yield /plot (kg)	Tuber length (cm)
Shankar	Elliptic	Red	White	Cream	2.5	120	300	90	7.5	24
Samrat	Irregular, curved	Cream	Whitish cream	Cream	2	125	250	65	6.25	16
Shree-Rathna (SR)	Spherical	Purple	Cream	Orange	3	130	390	97.5	9.75	25
Kalinga	Round to elliptic	Purple red	White	White	3.5	150	525	131.25	13.125	25
IGSP-14	Cylindrical	Pink	Cream	White	4.5	115	517.5	155	12.92	30
RS-92	Cylindrical	Red	Cream	Yellowish	6.5	95	617.5	154	15.45	30
MPUAT-10	Round to elliptic	White	White	White	3.5	95	332.5	83	8.3	27
Kishan	Round to elliptic	Purple	Dull white	Creamy white	3.5	135	472.5	141	11.8	35
CO-3-4	Long oblong	Dark purple	Cream	Cream	6.5	140	910	295	22.75	45
Navsari-L	Oblong	White	Dull white	White	3.5	100	350	105	8.75	28
RS-47	Cylindrical, deep	Purple	Cream	White	2.5	85	212.5	63.75	5.3	18
RS-43	Cylindrical	Dull white	Whitish cream	White	2.5	95	237.5	59	5.9	20
RS-35	Cylindrical	White	Whitish cream	White	2.5	100	250	75	6.25	22
V-17	Round to oblong	Purple	Cream to purple	Cream/Purple	3.5	90	315	78	7.875	17
V-16	Round to elliptic	Purple	Cream to purple	Cream/Purple	3.5	92	322	96	8.0	18
V-15	Spherical	Purple	Cream to purple	Cream/Purple	3.5	96	336	84	8.4	22
V-13	Round to elliptic	Purple	Cream to purple	Cream/Purple	4.5	105	472.5	118	11.8	16
V-12	Round to elliptic	Purple	Cream to purple	Cream/Purple	4.5	105	472.5	141	11.8	18
V-8	Round to elliptic	Purple	Cream to purple	Cream/Purple	3.5	100	350	105	8.75	22
Shree-Arun (SA)	Cylindrical	Pink	White cream	White cream	3.5	150	525	131	13.125	35
Gautam	Elliptic	Cream	White	Dark cream	2	190	380	98.8	9.5	12
		Mean			3.57	114.90	406.57	112.68	101.57	24.05
		Range			2-6.5	85-190	212.5-910	59-295	5.3-22.75	12-45
		S.E.m±			0.131	3.749	15.112	4.137	0.380	0.874
		CV (%)			6.67	5.92	6.74	6.66	6.80	6.60

dry yield per plant (59-295 g) and tuber yield per plot (5.3-22.75 kg).

Pairwise similarity among the genotype of sweet potato ranged from 0.03 to 0.80 with an average of 0.42 based on morphometric data. A dendrogram was constructed using SM similarity coefficient values determined from morphometric data for 21 sweet potato genotypes using UPGMA (Unweighted Pair Group Method Arithmetic Average) of NTSYS software (Fig. 1A). The relationship among the genotypes clearly divided them into two major clusters. The first cluster comprised 17 genotypes and subdivided into subcluster IA and IB. Subcluster IA comprised of 6 genotypes namely, Shankar, RS-47, RS-43, Samrat, RS-35, Gautam. In this subcluster, Samrat and RS-35 were most similar to each other morphologically with similarity value of 0.35. Subcluster IB also comprised 11 genotypes *viz.*, Kalinga, Shree-Arun, MPUAT-10, Navsari Lacal, V-8, V-15, V-17, V-16, Kishan, V-13 and V-12. In this subcluster, Navsari Local and V-8 were most similar to each other morphologically with similarity value of 0.83. The second cluster comprising 3 genotypes of sweet potato, namely IGSP14, RS-92 and CO-3-4. In this subcluster, IGSP14 and RS-92 were most similar to each other morphologically with similarity value of 0.70. In the dendrogram, Shree-Rathna genotype was diverse from remaining genotypes with similarity value of 0.03 and stood apart.

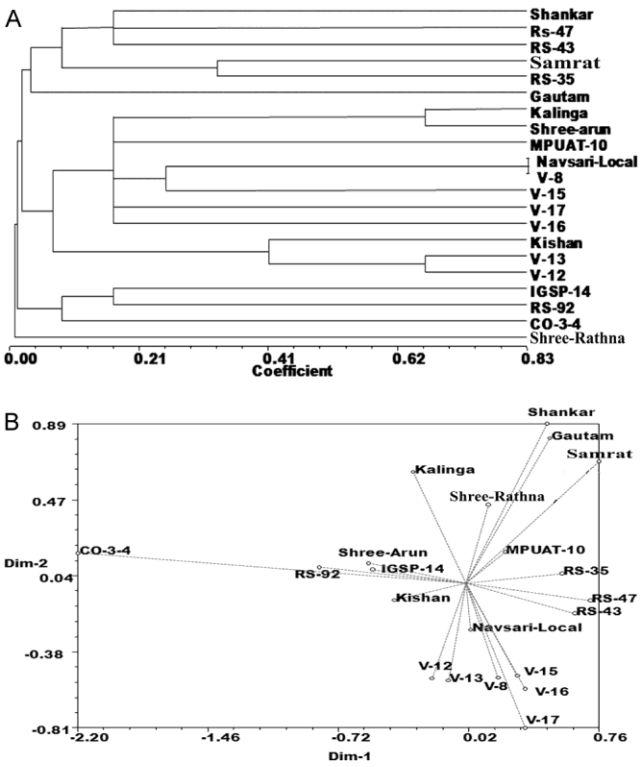

Fig. 1 — Dendrogram generated from 21 sweet potato genotypes based on morphological characters (A) UPGMA cluster analysis; and (B) 2-D plot of Principal component analysis

Based on Mantel Z statistics[25], the estimated correlation coefficient (r) was 0.15 which was considered a good fit of the UPGMA cluster pattern to the data. The two-dimensional plot generated from PCA showed 3 groups that were found to be similar to the clustering pattern of the UPGMA dendrograms for most of the genotypes except for Shree-Rathna and Co-3-4 (Fig. 1B). In the 2-D plot, genotype CO-3-4 was found distinct as depicted in UPGMA dendogram. All the genotypes *viz.*, V-8, V-12, V-13, V-15, V-16, V-17 and Navsari Local were also found together in one group like UPGMA dendogram.

The analysis gave 8 principal components (PCs), out of which the first 7 principal components contributed 99.96% of the total variability (Table 3). The first 5 principal components accounted for 95.53% of the total variability, and the first 3 accounted for 81.69% of the variance, in which the highest variation was contributed by the first component (45.04%), followed by second (24.24%) and third components (12.41%). The first PC was influenced by the characteristics as tuber shape, predominant skin colour, fresh tuber colour, predominant flash colour, and individual tuber weight and tuber length. In the second PC, the traits contributing to the total variability were storage tuber shape, predominant skin colour, fresh tuber colour, predominant flash colour, average storage tuber number/plant and storage tuber length.

The results presented in the present investigation are in support with the earlier studies. Moulin *et al.*[26] characterized 46 sweet potato landraces using morphological descriptors and reported that the

Table 3 — Eigenvectors of morphological variables explained by first 2 principal components (PC)

Morphological traits	PC-1	PC-2
Storage root shape	0.10	0.46
Predominant skin colour	0.10	0.47
Storage fresh root colour	0.04	0.44
Predominant fresh colour	0.20	0.32
Average storage root no./plant	–0.07	0.13
Individual root weight(g)	0.27	–0.04
Storage root fresh yield/plant(g)	–0.06	–0.01
Storage root dry yield/plant(g)	–0.09	–0.01
Storage root yield/plot(kg)	–0.06	–0.01
Storage root length(cm)	0.04	0.09

morphological characterization was efficient in detecting genetic variability among accessions. Rosero *et al.*[27] reported the significant genetic variability for 20 characters among the 70 accessions of sweet potato studied based on the 49 agro morphological characters studied and clustering analysis revealed low variability in traits related with flowering and higher diversity in root traits. Zhang *et al.*[28] performed phenotypic characterization for association analysis in sweet potato using the quality traits *viz.*dry matter (%), starch content (%), amylose content (%), amylopectin content (%) and ß-carotene content (mg/100g flesh wt.) among 239 sweet potato genotypes.

Biochemical analysis in sweet potato genotypes

The data presented in Table 4 indicate that genotypes showed significant differences for total antioxidants and total phenols. The mean value for total antioxidants was ranged from 0.16 to 0.30 mg 100 g^{-1} fresh wt. The maximum total antioxidants were observed in Samrat (0.30 mg 100 g^{-1} fresh wt.) followed by 'V-12, Kishan' (0.29 mg 100 g^{-1} fresh wt.) and 'CO-3-4' (0.28 mg 100 g^{-1} fresh wt.). The minimum total antioxidants were observed in Navsari Local (0.16 mg 100 g^{-1} fresh wt.).

The mean value for total phenols was 1.23 mg 100 g^{-1} fresh weight and it ranged from 0.95 mg 100 g^{-1} fresh weight to 1.39 mg 100 g^{-1} fresh weight. The maximum total phenols were observed in V-12 (1.39 mg 100 g^{-1} fresh wt.) followed by V-13 (1.38 mg 100 g^{-1} fresh wt.) and Shree Arun (1.37 mg 100 g^{-1} fresh wt.). The minimum total phenol was observed in Samrat (0.95 mg 100 g^{-1} fresh wt.).

Table 4 — Total phenolsand total antioxidants(mg 100g^{-1} fresh weight)in sweet potato tubers

Genotypes	Total Phenols mg 100 g^{-1} fresh wt.	Total Antioxidant mg 100 g^{-1} fresh wt.
Shankar	1.31	0.17
Samrat	0.95	0.30
Shree Rathna	1.04	0.19
Kalinga	1.10	0.26
IGSP-14	0.98	0.18
RS-92	1.13	0.20
MPUAT-10	1.28	0.25
Kishan	1.23	0.29
CO-3-4	1.30	0.28
Navsari Local	1.08	0.16
RS-47	1.02	0.21
RS-43	1.19	0.24
RS-35	1.37	0.26
V-17	1.34	0.22
V-16	1.32	0.24
V-15	1.37	0.26
V-13	1.38	0.23
V-12	1.39	0.29
V-08	1.35	0.25
Shree Arun	1.37	0.27
Gautam	1.33	0.23

These results were well supported by the similar reports from Khurnpoon & Rungnoi[29] who studied the total phenol content and antioxidant activities of 36 sweet potato cultivars with distinctive flesh colour (white, yellow, orange and purple) grown in Thailand. Mohanraj & Subha[30] have also reported that the sweet potato tubers are rich in secondary metabolites and 4-ipomeanol from sweet potato is a potential chemotherapeutic agent for cancer.

Molecular diversity analysis

The advent of the RAPD provided an efficient method to detect DNA polymorphism and generate a large number of molecular markers for genomic applications. RAPD markers are simple, rapid and have the advantage of no prior knowledge of genome sequences. All the 21 sweet potato genotypes were examined for DNA polymorphism using 25 random RAPD oligonucleotide primers.

Out of 25 primers, 13 primers produced amplification whereas, 12 primers *viz.*, 0PA-07, OPA-09, OPE-03, OPE-04, OPM-03, OPM-04, OPM-05, OPZ-01, OPZ-02, OPZ-04, OPZ-05, OPZ-09 did not show any amplification. Out of 13, all the primers showed variable degree of polymorphism ranging from 75-100%. These primers on 21 sweet potato genotypes generated 117 total bands, out of which 106 were polymorphic and 4 were unique amplicons. Primers, namely OPP-02, OPJ-04 OPZ-03 and OPD-05 produced unique amplicon in genotypes Navsari Local, Kalinga IGPS-14 and CO-3-4, respectively. This information can be further utilized for genotype identification.

In all the genotypes, evaluated primers produced minimum 4 and maximum 13 bands and their sizes ranged between 100 and 3000bp. The average number of amplicons per primer was found to be approximately 11.11%. Electrophoresis pattern of RAPD profile were studied and only the fragments which were consistently amplified were considered for analysis (Fig. 2).

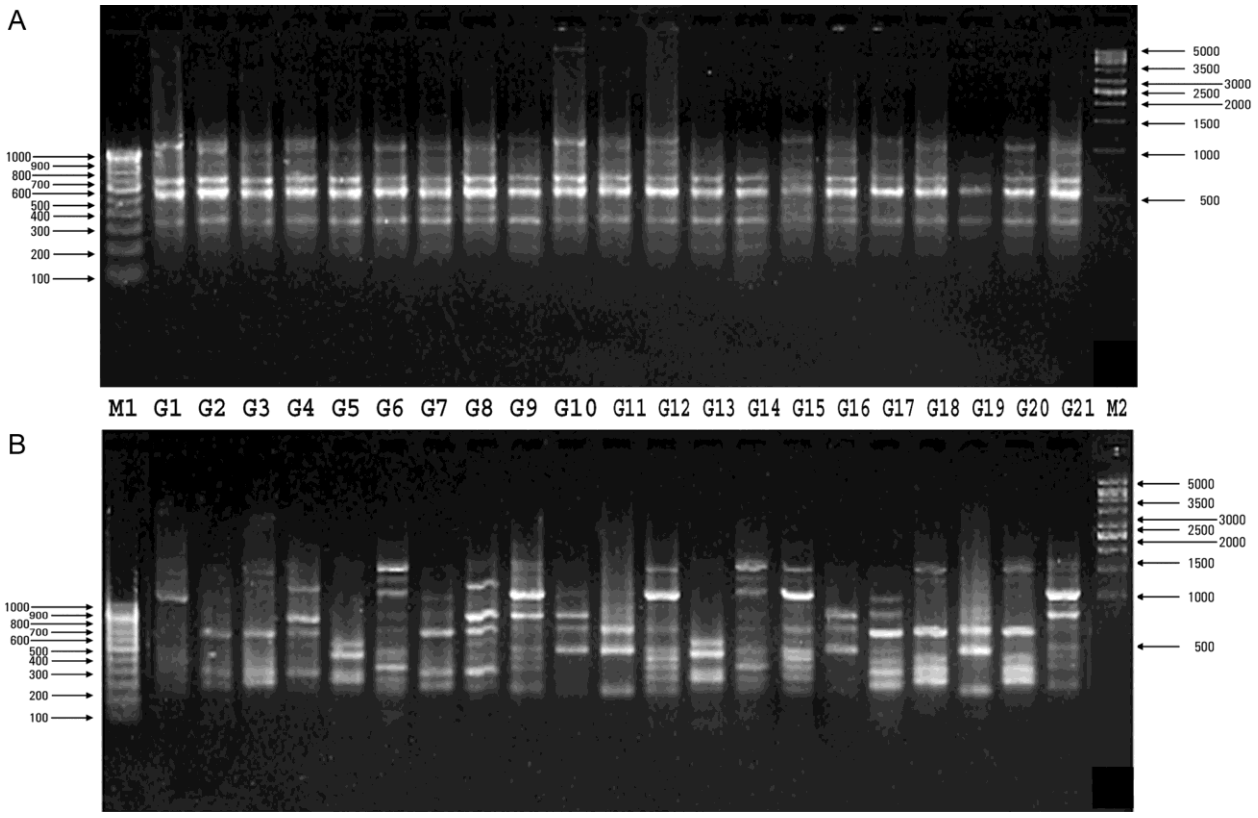

Fig. 2 — RAPD profiles generated by primer (A) OPA-06; and (B) OPA-08. [The lane (G1-G21) corresponding to the sweet potato genotypes (M1: 100bp and M2: 1000bp DNA ladders)]

Average polymorphism was found to be 90.60%. The DNA amplification and polymorphism generated among various sweet potato genotypes using random primers are presented in Table 5.

Primer OPZ-03 produced 13 scorable bands, out of which 12 bands showed polymorphism and also a unique band of 950 bp was amplified in IGPS-14 genotype. Primer OPD-05 generated 8 scorable bands, out of which 6 are polymorphic. Primer OPP-01 amplified 6 polymorphic amplicons. Interestingly, amplicon size of 500 bp was amplified by primer OPP-01, in all sweet potato genotypes except MPUAT-10 and Kishan. Primer OPP-02 amplified a total of 8 bands, 7 of which were polymorphic. A unique amplicon was amplified by primer OPP-10 in Navsari Local and also a unique amplicon was amplified by primer OPE-03 only in CO-3-4 (Table 6). The most informative primers were OPA-06, OPA-08, OPE-17, 0PP-01 and OPZ-08 and showed 100% polymorphism.

Moulin *et al.*[26] also reported 8 RAPD primers selected for the analysis of the 44 sweet potato

Table 5 — DNA amplification profile and polymorphism generate in sweet potatousing 13 RAPD primers

Primer	MW (bp)	Total no. of bands	Total no. of polymorphic band	Poly morphism (%)
OPA-03	400-1450	6	5	83.33
OPA-06	200-1200	9	9	100
OPA-08	200-2000	9	9	100
OPA-10	250-2000	8	7	87.50
OPD-05	300-1600	8	6	75.00
OPE-17	250-2750	11	11	100
OPJ-04	300-3000	11	9	81.81
OPP-01	500-2000	6	6	100
OPP-02	250-3000	8	7	87.50
OPP-10	250-2750	8	7	87.50
OPZ-03	100-1000	13	11	84.61
OPZ-06	300-2000	7	6	85.71
OPZ-08	250-1800	13	13	100
Total		117	106	90.6

Table 6 — Unique alleles obtained using RAPD primers

Primer code	Total no. of bands	No. of unique allele	Allele size (bp)	Mr. range	Geno types
OPZ-03	13	1	950	100-1000	IGPS-14
OPP-02	8	1	3000	250-3000	Navsari Local
OPD-05	8	1	900	300-1600	CO-3-4
OPJ-04	11	1	2500	300-3000	Kalinga

accessions generated a total of 93 scorable fragments, 88 of which (94.6%) were polymorphic. Palumbo *et al.*[31] detected 117 marker alleles using 11 EST-SSR primers in accession pool of sweet potato and reported the minimum of 6 (J206A) to a maximum of 16 (GDAAS0757) allele with an average of 10.5 per locus detected using the nine SSR primers generated a total of 50 fragments (100% polymorphic).

The pairwise Jaccard's similarity coefficient among all of the 21 genotypes ranged from 0.58 to 0.76. The maximum similarity of 0.76 was observed between genotypes Samrat and Shree-Arun, indicating that they are genetically quite similar, whereas IGSP-14 showed the minimum similarity coefficient of 0.58. Average similarity across all the genotypes was 0.67. The dendrogram (Fig. 3A) clearly indicates that IGPS-14 to be most distinct from the remaining genotypes. Based on the relationship among the genotypes, we divided them into five main clusters. The first cluster includes two genotypes namely, Shankar and Kalinga which were similar to each other with similarity value of 0.67. The second cluster was biggest one and comprising 9 genotypes *viz.*, Samrat, Shree-Arun, RS-47, RS-35, Kishan, CO-3-4, RS-43,V-16 and V-08. Within this cluster, Samrat and Shree-Arun was genetically most similar to each other with similarity value of 0.76. Third cluster comprised with two genotypes like Shree Rathna and V-12. These genotypes were genetically similar to each other with similarity value of 0.69. The fourth cluster comprising 7 genotypes namely, Gautam, RS-92, V-13, V-17, MPUAT-10, Navsari Local and V-15. Within this cluster, Gautam and RS-92 were closely related to each other at a similarity coefficient 0.74. Fifth cluster have only one genotype IGPS-14 which was most distinct from remaining all the genotypes with similarity value of 0.76.

Based on Mantel Z-statistics, the correlation coefficient (r) was estimated as 0.21. The r value of 0.21 was considered a good fit of the UPGMA cluster pattern to the data. Genotypes grouped within the same cluster in the dendrogram were also occupying the same position in two dimensional scaling based on molecular data generated from PCA. In the 2-D plot, genotype IGSP-14 was found along with V-15 whereas it was most distinct in UPGMA dendogram. (Fig. 3B). Similar clustering pattern was detected by Moulin *et al.*[26]. Genetic relationship among sweet potato genotypes were also visualized by performing PCA based on

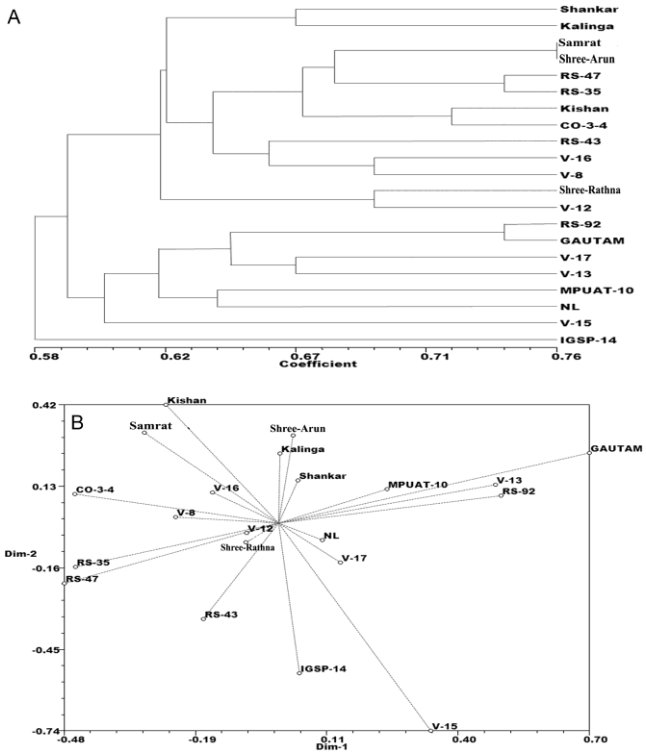

Fig. 3 — (A) UPGMA dendrogram; and (B) 2-D plot of 21 genotypes of sweet potato generated based on RAPD data

RAPD data. Sasai *et al.*[32] performed SSR, Retrotransposon, and SNP markers based linkage analysis to identify genomic regions controlling root-knot nematode, *Meloidogyne incognita* in sweet potato and detected highly effective QTLs for resistance. Thus, such studies could be helpful in selecting sweet potato cultivars that are having resistance to specific abiotic and biotic stress.

Cumulative data analysis of morphology and molecular markers

Pairwise similarity among the genotypes ranged from 0.54 to 0.70 with an average of 0.62 based on combined morphological and molecular data. The highest similarity (70%) was observed between the Samrat and Shree-Arun genotypes, whereas the lowest was observed in IGSP-14 with a similarity value of0.54. A dendrogram based on combined morphological, and RAPD data clustered all 21 genotypes into 3 major clusters (Fig. 4A). The first cluster is biggest one and comprised 13 genotypes *viz.*, Shankar, Kalinga, Samrat, Shree-Arun, CO-3-4, RS-47, RS-35, RS-43, V-8, V-16, Shree-Rathna, Kishan and V-12. Within this cluster, Samrat and Shree-Arun genotypes were the most similar morphologically and genetically, showing a similarity value of 0.70. In this group,

RS-43 was distinct from the other genotypes, with a similarity value of 0.60. The second cluster comprised 7 genotypes *viz*., RS-92, Gautam, V-13, V-17, MPUAT-10, Navsari Local and V-15. Within this cluster, RS-92, and Gautam were observed to be quite similar with a similarity value of 0.68, whereas V-15 found most distinct from remaining other genotypes with similarity coefficient of 0.56. The third cluster comprised only one genotype IGSP-14. This genotype is most distinct morphologically and genetically from all other genotypes with similarity value of 0.54. The 2-D plot generated from the PCA of the combined morphological and RAPD data (Fig. 4B) also supported the clustering pattern of the UPGMA dendrogram. Further, the analysis based on cumulative data of morphological, biochemical and molecular observations generated 19 principal components (PCs), out of which the first 10 PCs contributed 69.03% of the total variability of the analysed germplasm (Table 7). The first 5 PCs accounted for 41.27% of the total variability; the first 3 accounted for 34.39% of the variance, in which maximum variability was contributed by the first component (10.12%), followed by the second (8.83%) and third (8.26%) components. Kaur *et al.*[20] reported a UPGMA dendogram based on the combined morphological and molecular markers *viz*., RAPD, ISSR and SSR, in which the 23 green gram genotypes were divided into three main clusters, showing a close genetic relationship which might be due to their close genetic bases. Galal & El Gendy[33] revealed that there is a wide variation among the 3 sweet potato genotypes in most of the morphological and agronomic characters in addition to their biochemical/nutritional values, whereas molecular characterization through RAPD analysis cannot be useful in separating genotypes according to their morphological, agronomic or chemical characters. David *et al.*[34] showed a successful separation of gene pools in a large set of parental sweet potato genotypes using SSR markers which may help in the search for gene pools in other clonally propagated crops for testing of heterosis exploiting breeding schemes. For plant breeders close genetic relationships associated could provide an avenue for introgression of high yielding and resistant genes into commercial and farmers' varieties.

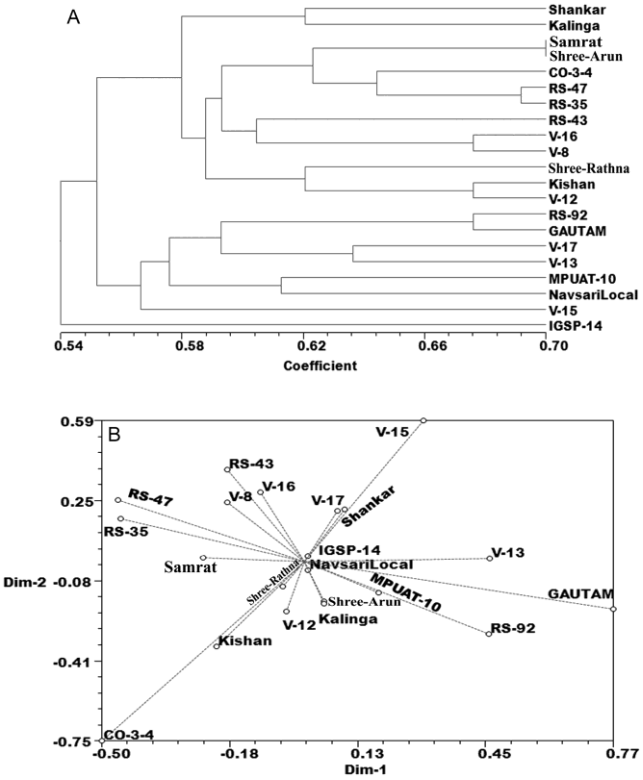

Fig. 4 — (A) UPGMA dendrogram and (B) 2-D plot of 21 genotypes of sweet potato generated based on combined morphometric, biochemical and RAPD data

Table 7 — Eigenvectors of combined morphological variables and molecular data

Principal components (PCs)	Eigenvalue	Percent	Cumulative
1	2.024251	10.1213	10.1213
2	1.765492	8.8275	18.9487
3	1.65257	8.2628	27.2116
4	1.436191	7.181	34.3925
5	1.374245	6.8712	41.2637
6	1.335934	6.6797	47.9434
7	1.168772	5.8439	53.7873
8	1.117968	5.5898	59.3771
9	1.023777	5.1189	64.496
10	0.905826	4.5291	69.0251
11	0.8931	4.4655	73.4906
12	0.843482	4.2174	77.708
13	0.772756	3.8638	81.5718
14	0.713624	3.5681	85.1399
15	0.609324	3.0466	88.1866
16	0.587455	2.9373	91.1238
17	0.545783	2.7289	93.8528
18	0.483894	2.4195	96.2722
19	0.417469	2.0873	98.3596

Conclusion

The results revealed that 21 sweet potato germplasms characterized and analysed in this study were moderate to high diversity based on molecular, biochemical and morphological assessment approaches. The results obtained will serve as a guide for the basis germplasm management and crop

improvement programmes. A result of morphological characteristics like tuber and quality traits contributes to effective conservation and utilization of the sweet potato genetic resources. Results of biochemical/ nutraceuticals, such as phenols and antioxidant study indicated its importance as functional food. Designing effective breeding programs is largely dependent on understanding the genetic diversity of the relevant germplasm. Here, we reported our detailed analysis of representative sweet potato accessions cultivated using morphological, biochemical and molecular markers. Our results demonstrated moderate genetic diversity in the sweet potato genotypes. Although, sweet potato is highly heterozygous, the limited scope of parent selection in breeding also affected the genetic diversity of advanced varieties of sweet potato. Our findings suggest that to create new hybrid varieties with new alleles and increased genetic diversity in sweet potato, accessions with a wide genetic background including introduced varieties should be used in breeding programs. However, genetic characterization based on the conventional characters should be complemented with molecular characterization to reveal genetic diversity for better plant breeding and crop improvement projects.

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Conflict of interest

None to declare.

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