Chapter 2

Morphological, Biochemical, and Molecular Characterization of Orange-Fleshed Sweet Potato (*Ipomoea batatas* [L.] Lam) Germplasms

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Abstract

The sweet potato is considered as an excellent source of β-carotene and anthocyanins and has a considerable value in the functional food market. In this report, 21 sweet potato (*Ipomoea batatas* [L.] Lam) germplasms were evaluated for genetic diversity using morphological and biochemical and molecular markers. Ten morphological traits were studied, and the mean squares due to germplasm were highly significant for storage root number per plant, individual root weight, storage root (fresh) per plant, storage root (dry) per plant, storage root yield, and storage root length. UPGMA cluster analysis based on morphological traits separated the germplasm into three groups. The similarity coefficient ranged from 0.00 to 0.50 with an average of 0.176. Biochemical analysis, viz. total phenol and antioxidant, was performed to find out superior genotype at biochemical level under given conditions. Maximum total phenol was observed in the genotype "V-12" (1.39 mg), whereas maximum total antioxidant was observed in "Samrat" (0.30 mg). RAPD analysis was carried out, and out of 15 RAPD primers, 10 primers produced 96 reproducible and polymorphic bands. UPGMA cluster analysis based on RAPD data also separated the genotypes into three clusters. The results of the present study can be used for sweet potato crop improvement through molecular breeding and marker-assisted selection for desired traits in future.

Keywords: sweet potato, morphological marker, biochemical, RAPD, UPGMA

1. Introduction

The sweet potato (*Ipomoea batatas* [L.] Lam) is a dicotyledonous plant that belongs to the family Convolvulaceae and plays a critical role in food security after potato and cassava. Its large, starchy, sweet-tasting, tuberous roots are most important root crop worldwide [1]. The young leaves and shoots are sometimes eaten as greens. The sweet potato is native to the tropical regions in America, and its cultivation area covers around 135,000 hectares with an estimated annual production of 1,639,000 MT in India [2]. The roots are used as a source of carbohydrate and dietary fiber. Dietary fiber has the potential to reduce the incidence of a variety of diseases in man, including colon cancer, diabetes, heart diseases, and digestive disturbances [3]. Orange, white, and creamy flesh sweet potato is most commonly grown and eaten. In orange- and yellow-fleshed sweet potato, color is due to the presence of carotenoids of which β-carotene is most abundant. To increase the yield and quality of the sweet potato, it is important to study on molecular and biochemical variation in sweet potato genotypes, but unfortunately, negligible work has been done on sweet potato in spite of fact that lot of variability exists in sweet potato for physiological and biochemical characters, which can be utilized for improving tuber yield coupled with high nutritive value [4].

Food fortification, dietary diversification, and vitamin A supplementation are the recommended strategies to control vitamin A deficiency. Physiological and biochemical factors determine the storage quality of any crop. As the tuber forms major proportion of total dry weight of plant, productivity is largely governed by the process of tuberization and photosynthetic efficiency of the leaf canopy in support of the storage root sink. Both the processes are being controlled by environmental factors [5]. The flesh color of the root varies from various shades of white, cream, yellow to dark-orange depending upon the carotenoid content. In the orange-fleshed sweet potato, the major carotenoid present is β-carotene. Carotenoids have been linked with the enhancement of immune system and decreased risk of degenerative diseases such as cardiovascular problems, age-related macular degeneration, and cataract formation [6]. Interest in carotenoids has increased due to their possible health benefits as carotenoids are often associated with health preventive effort and reduced risk of aged related macular degeneration, anticancerogenic activity, antioxidant capacity, antiulcer activity, and also reduced risk of cardiovascular disorders [7]. Both sweet potato roots and tops are nutritious foods, which could be used to advantage in combating nutritional deficiencies in parts of the developing world if means could be found to overcome resistance to their increased consumption. The roots are used primarily as human food and are eaten boiled, steamed, fried, or baked. Raw leaves and tuber tips are also excellent sources of ascorbic acid and some of the vitamin B, especially riboflavin that is deficient in many Asian diets. However, high percentages of water soluble vitamin are lost on cooking [8]. Recent studies associated with the consumption of carotenoid-rich food showed the decrease of the incidence of certain cancers in human beings [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 have been successfully employed to assess the genomic stability of regenerated plants regardless of the presence or absence of obvious phenotypic alterations earlier. Molecular markers have been proved to be valuable tools in the characterization and evaluation of genetic diversity within and between species and

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population. It has been deserved that different markers might reveal different classes of variation [10, 11]. RAPD markers offer many advantages such as higher frequency of polymorphism, rapidity [12], technical simplicity, requirement of a few nanograms of DNA, no requirement of prior information of any DNA sequence, and feasibility of automation [13]. Hence, the present study is the attempt to determine genetic diversity among sweet potato (*Ipomoea batatas* [L.] Lam) genotypes using morphological, biochemical, and molecular markers.

2. Materials and methods

2.1. Plant material

Morphological and Molecular Characterization of twenty one diverse germplasms of sweet potato (*Ipomoea batatas*) were reported in this research. The experimental materials comprised of 21 diverse germplasms of sweet potato were sown in randomized block design replicated thrice. Each entry was planted in 3 × 2.4 m^2, keeping row to row and plant to plant distance of 60 and 30 cm, respectively. The recommended packages of practices were followed to raise a healthy crop.

2.2. Morphological analysis

The genotypes were evaluated in randomized block design with three replications. Observations on various morphological characters like storage root number per plant, individual root weight (g), storage root (fresh) per plant, storage root dry yield per plant (g), storage root yield per plot (kg), and storage root length (cm) were recorded from five selected plants from each replication, which were averaged and subjected to statistical analysis of all the characters. To test the variation among various genotypes of sweet potato, analysis of variance was carried [14]. Mean value of all the seven morphological characters for each plot of all the three replications was used for simple matching using UPGMA algorithm [15]. Principal component analysis (PCA) was used to depict nonhierarchical relationships among the genotypes. Eigen values and eigenvectors were calculated by the Eigen program using a correlation matrix as input (calculated using standardized morphological data), and a 2D plot was used to generate the two-dimensional PCA plot from NTSYS-pc 2.2 [16].

2.3. Biochemical analysis

2.3.1. Total carotenoids (mg 100 g^{-1} fresh weight)

Flesh was cut into small pieces longitudinally and mixed with 80% aqueous acetone for 2 hours at 50°C using an orbital shaker. Then it was filtered through Whatman paper. Filtrate was kept at −20°C prior to analysis. Five ml of sample extract was mixed with 5 ml distill water and 1 ml of mix (hexane/acetone/methanol) (50/25/25 v/v). Sample was kept at centrifuge at 3000 rpm for 10 min. The absorbance of upper layer was measured at 450 nm. Total carotenoids of the sample were calculated as μg 100 g^{-1} [17]:

$$\text{Total carotenoids } (\mu g\ 100\ g^{-1}) = \frac{A \times \text{Volume of the extract}}{A^{x} \times \text{Sample weight}}$$

where A = absorbance and A^x = absorbance coefficient (2505).

2.3.2. Total antioxidant (mg 100 g^{-1} fresh weight)

Five gram tuber was extracted with 20 ml of 60% methanol (0.1% HCl) and kept overnight. Then it was centrifuged at 10,000 rpm for 15 min at 10°C. The supernatant was taken for analysis. Hundred µl of methanolic extract was mixed with 3 ml of solution (1.2 M sulfuric acid, 46 mM sodium phosphate, and 8 mM ammonium molybdate) and was incubated for 90 min at 95°C in water bath. It was then allowed to cool down at room temperature. Reading of plant sample was read 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 100 g^{-1} [17].

2.3.3. Total phenol (mg 100 g^{-1} fresh weight)

Phenolic compounds are well-known phytochemicals found in all plants. They consist of simple phenols, benzoic and cinnamic acid, coumarins, tannins, lignins, lignans, and flavonoids. The total phenolics in sweet potato extracts were estimated by Folin-Ciocalteu colorimetric method. One gm of powdered sample was added in 80% ethanol followed by centrifugation at 10,000 rpm for 20 min, this step was repeated twice. Supernatant was taken and evaporated for dryness. The residue was dissolved in 5 ml of distilled water. Diluted sample extract (1 ml) was added 3.0 ml of 20-fold diluted Folin-Ciocalteu reagent and 2 ml of 20% (w/v) Na_2CO_3. The mixture was incubated in a water bath at 50°C for 1 min and allowed to cool. The absorbance was measured at 650 nm and used to calculate total phenolics content using a standard curve [17].

2.4. Molecular analysis

The genomic DNA was extracted from young leaves (3-week-old plantlets) using CTAB method [18] with slight modifications. Totally, 20 RAPD primers were used for PCR amplification out of which 15 primers produced polymorphic, consistent, and reproducible banding pattern. In brief, reproducible and clear banding patterns were obtained in a reaction mixture of 20 µl containing 1X reaction buffer, 1 unit of *Taq* DNA polymerase, 200 µM each of dNTPs mix, 20 pmol of primer, and 50 ng of template DNA. PCR amplification in the thermocycler (programmable thermal cycler from BIORAD™ International) was programmed for an initial denaturation step of 5 min at 94°C, followed by 40 cycles of denaturation (94°C, 1 min), annealing (37°C, 1 min), and extension (72°C, 1 min) followed by a final extension of 72°C for 5 min and a hold temperature of 4°C. The amplified products were electrophoresed on 1.5% agarose (Sigma chemicals Co. Ltd. India) gels in TAE buffer at 50 volts for 3 hours. The electrophoresed gels were visualized under UV transilluminator and photographed using gel documentation system [19]; 100 bp ladder and 1 Kb DNA ladder were used as standard (Bangalore, Genei, India).

2.5. Data analysis

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

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obtained, and statistical analysis was performed using the NTSYS-pc version 2.02e [16]. The pairwise association coefficient was calculated from qualitative data using Jaccard's similarity coefficient (by means of SIMQUAL procedure of NTSYS-pc), and the cluster analysis was performed (by means of SAHN procedure of NTSYSpc) via unweighted pair group method with arithmetic averages (UPGMA) to develop a dendrogram [15]. A two-dimensional and three-dimensional principal component analysis (PCA) was constructed to provide another means of testing the relationship among the cultivars using the Eigen program (NYSTS-pc). The effective number of alles, Nei's genetic diversity/expected number of heterozygosity, and Shannon's Information index were computed using Popgene software. A two-dimensional and three-dimensional principal component analysis (PCA) was constructed to provide another means of testing the relationship among the cultivars using the Eigen program NTSYS-pc version 2.02 [15].

2.6. Association between genetic, morphological, and biochemical diversity

The cophenetic correlation was calculated to find the degree of association between the original similarity matrix and the tree matrix in morphological, molecular, and biochemical analyses. Using the Mantel test [20], a comparison between all the methods was performed for the accessions for which both data sets were available by calculating the correlation between the three data sets in NTSYS-pc. Using the same software, PCA was also carried out to identify any genetic association among the genotypes.

3. Results and discussions

3.1. Morphological analysis

Morphological characterization is regarded as the first step in description and classification of any germplasm. A sound knowledge of various morphological traits in the breeding material helps classification, identification, naming, and documentation of the entries in a crop. These hasten the process of utilization of genetic material for crop improvement programs. The data of morphological characters were subjected to analysis of variance (ANOVA) for randomized block design (RBD). The mean square values due to genotypes were found significant for all the traits, thereby indicating substantial amount of variability among the genotypes (**Table 1**). The mean squares due to replication were found significant for all the characters, namely, storage root number per plant, individual root weight (g), storage root fresh root per plant, storage root dry yield per plant (g), storage root yield per plot (kg), storage root length (cm), which had high value of variance indicating that the diversity existed, which can contribute to improvement of the crop. The mean performances of different *Ipomoea batatas* L. genotypes for different characters are presented in **Table 1**.

The genotypes Gauri and 187017 showed maximum root number per plant, that is, 7 and C-71, SI-60, SV-71, and SREE VARDHINI, that is, 2 with general mean of 3.86. The perusal of mean table revealed that the test germplasm V-11 was found to be the lowest in root weight (35 g), whereas C-71(1100 g) was the highest among the test germplasm, the germplasm C-71 exhibited highest values of storage root fresh yield per plant (2200 g), while minimum in V-10 (160 g). There was significant difference in yield for all the germplasm with an average value of 433.71 g. The germplasm

SN	Genotype	Storage root number/plant	Individual root wt (g)	Storage root fresh yield/ plant (g)	Storage root dry yield/ plant (g)	Storage root yield/plot (kg)	Storage root length (cm)
1	Gauri	7.00	60.00	420.00	134.40	3.36	20.00
2	ST-10	5.00	65.00	325.00	113.75	2.84	22.00
3	V-10	4.00	40.00	160.00	56.00	1.40	16.00
4	SP-2	4.00	55.00	220.00	77.00	1.92	25.00
5	V-11	5.00	35.00	175.00	51.25	1.28	17.00
6	C-71	2.00	1100.00	2200.00	540.00	13.50	33.33
7	Kamla Sundari	4.00	120.00	480.00	129.60	3.24	10.00
8	Sree Nandini	3.00	150.00	450.00	157.50	3.93	25.00
9	SP-1	3.00	75.00	225.00	78.75	1.95	26.00
10	CIPSWA-2	3.00	80.00	240.00	84.00	2.10	27.00
11	SI-60	2.00	100.00	200.00	70.00	1.75	25.00
12	SV-71	2.00	110.00	220.00	77.00	1.92	23.00
13	440127	3.00	91.67	285.00	99.75	2.50	28.00
14	V-7	3.00	60.00	180.00	63.00	1.57	19.00
15	Khangudu	5.00	144.00	720.00	129.60	3.25	20.00
16	Pol-19-8-2	4.00	124.00	496.00	173.60	4.34	26.00
17	MPUAT-6	4.00	103.00	412.00	144.20	3.60	28.00
18	Varsha	4.00	50.00	200.00	54.00	1.35	8.00
19	ST-14	5.00	110.00	550.00	184.33	4.60	12.00
20	Sree Vardhini	2.00	125.00	250.00	85.00	2.12	17.00
21	187017	7.00	100.00	700.00	199.50	4.99	13.00
	GM	3.86	137.98	433.71	128.68	3.21	20.97
	SE	0.25	7.92	18.04	2.69	0.16	1.12
	CD5	0.71	22.64	51.55	7.67	0.46	3.20
	CD1	0.96	30.31	69.02	10.27	0.61	4.29
	CV	11.21	9.94	7.20	3.61	8.66	9.25

Table 1. Mean values of morphological characteristics observed for sweet potato.

V-11 had minimum (51.25 g) among the test germplasm, while C-71 had maximum (540 g) for dry yield per plant. Storage root yield per plot was maximum in the germplasm C-71 (13.50 kg), whereas V-11 had the minimum value of root yield/plot (1.28 kg). The highest storage root length (cm) was observed in germplasm C-71 (33.33 cm), while the minimum was VARSHA (8 cm).

SN	Characters	Replication	Genotype	Error
	d.f.	[2]	[20]	[40]
1	Storage root number/plant	0.5119	6.3857**	0.1869
2	Individual root weight (g)	318.7778	149072.5125**	188.2
3	Storage root fresh yield/plant (g)	3105.1904	577303.5500**	976
4	Storage root dry yield/plant (g)	53.9544	32674.7156**	21.63
5	Storage root yield/plot (kg)	0.0237	20.4307**	0.07747
6	Storage root length (cm)	4.0635	133.0635**	3.763

*, **, significant at 5% and 1% level of significance, respectively.

Table 2. Analysis of variance (ANOVA) for morphological traits of experimental data.

The analysis of variance was done for six characters studied, and their mean square values are presented in **Table 2**. The mean squares and mean squares due to replication were also found to be highly significant for all the characters. The overall analysis of ANOVA indicated the presence of high genetic variability in the experimental material, which can be further exploited for sweet potato improvement. Elameen et al. (2011) studied the phenotypic diversity of morphological plant and root descriptor traits in 105 sweet potato germplasms using 27 phenotypic characters using ANOVA. The analysis of variance (ANOVA) revealed highly significant variation among the accessions for 21 out of the 27 characters studied [21].

Comparative analysis of seven morphological characters revealed low level of variation. Pairwise similarity among the genotypes of *Ipomoea batatas* L. ranged from 0.01 to 0.50 with an average of 0.176 based on morphometric data. A dendrogram generated from morphometric data grouped all 21 genotypes into three major clusters (**Figure 1**). The first cluster involved seven germplasms, namely, Gauri, V-7, Sree Nandini, SP-1, CIPSWA-2, 440127, and 187017-1 at similarity coefficient of 0.01. This cluster was further divided into two subclusters A and B. Subcluster A included six germplasms: Gauri, V-7, Sree Nandini, SP-1, CIPSWA-2, and 440127 at similarity coefficient of 0.08. Subcluster B comprises only one germplasm, viz., 187017-1 at similarity level of 0.03, which was most distinct from all other germplasms. The second cluster comprised four germplasms, namely, ST-10, V-11, Khangadu, and ST-14 at similarity value of 0.161. The third cluster was the biggest that included 10 germplasms, namely V-10, Kamla Sundari, POL-19-8-2, MPUAT-6, Varsha, SP-2, SV-71, SI-60, C-71, and Sree Vardhini at similarity value of 0.04. This cluster was further divided into two subclusters C and D. Subcluster C included five germplasms: V-10, Kamla Sundari, POL-19-8-2, MPUAT-6, and Varsha at similarity coefficient of 0.161. Subcluster D was further divided into D1 and D2. Subcluster D1 included SP-2, SV-71, and SI-60 at similarity coefficient of 0.11. SP-2 and SV-71 found to be morphologically quite similar at similarity value of 0.50. Subcluster D2 comprised germplasms C-71 and Sree Vardhini.

Yada et al. (2010) assessed morphological characterization of 1256 Ugandan sweet potato germplasms and grouped the 1256 accessions into 20 major clusters, with the number of

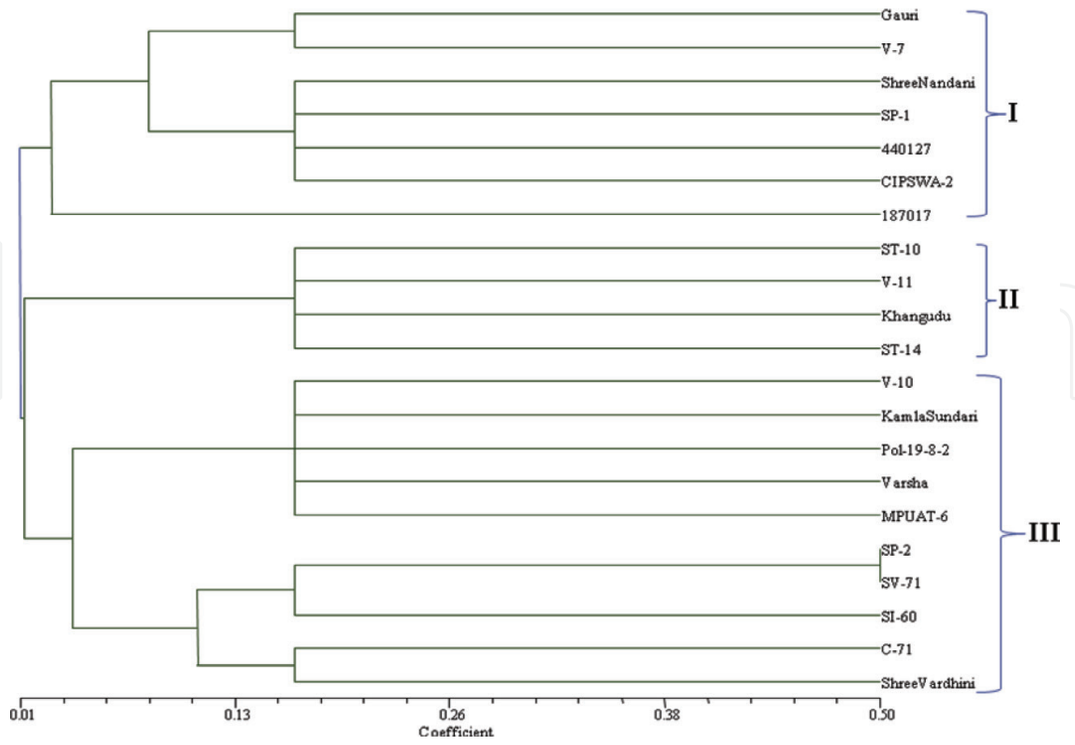

Figure 1. Dendrogram generated for 21 *Ipomoea batatas* L. germplasm using UPGMA cluster analysis based on morphological characters.

accessions per cluster ranging from 15 to 166 [22]. Similarly, Norman et al. (2014) also evaluated the diversity within sweet potato germplasm using factor and cluster analyses and revealed eight clusters at distance coefficient of 0.80 [23].

The two-dimensional plot generated from PCA showed three groups that were found to be similar to the clustering pattern of the UPGMA dendrogram. In the 2D plot, genotype 187017 was found distinct as like UPGMA dendrogram. Genotype C-71 was found along with Sree Vardhini in UPGMA dendrogram, whereas it was most distinct in 2D plot and made separate group (**Figure 2**). The analysis gave five principal components (PCs), out of which the first four principal components contributed 99.81% of the total variability.

The first three principal components accounted for 99.12% of the total variability, in which the highest variation was contributed by the first component (68.48%), followed by second (22.07%) and third components (8.56%). The first PC was influenced by the characteristics of the storage root yield/plot (kg), storage root dry yield/plant (g), storage root number/plant, storage root fresh yield/plant (g), and storage root length (cm). In the second PC, the traits contributing to the total variability were storage root length (cm), storage root number/plant, individual root weight (g), storage root yield/plot (kg), and storage root dry yield/plant (g). Third PC was influenced by the characteristics of the storage root number/plant, storage root fresh yield/plant (g), and storage root length (cm). In the fourth PC, the traits contributing to the total variability were the storage root number/plant, individual root wt (g), storage root yield/plot (kg), and storage root dry yield/plant (g) (**Table 3**).

The results presented in the present investigation are in support with the earlier studies. Moulin et al. (2012) characterized of 46 sweet potato landraces using morphological descriptors

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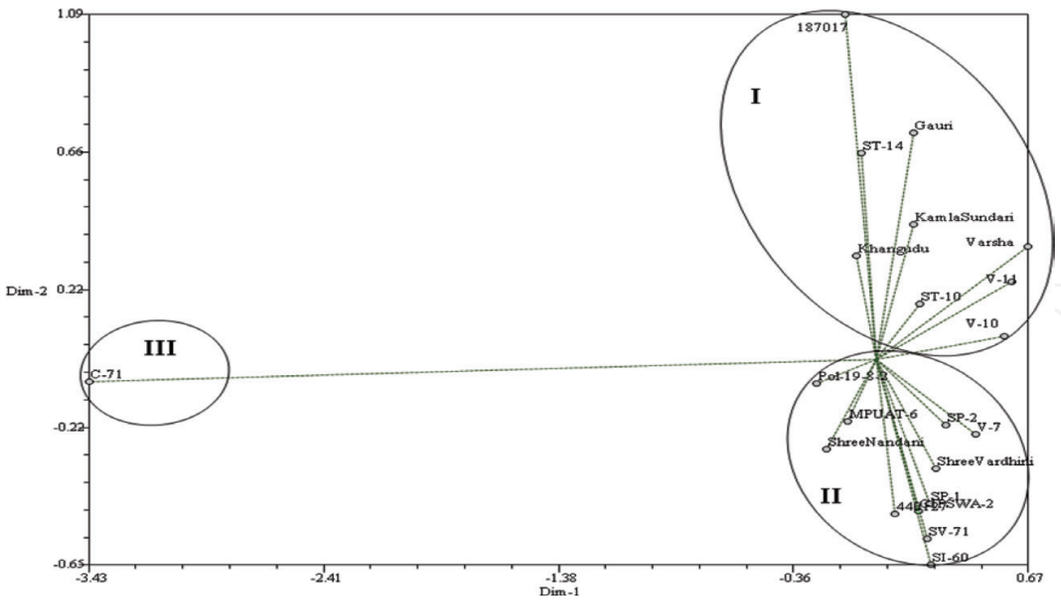

Figure 2. 2D plot generated for 21 *Ipomoea batatas* L. genotypes using UPGMA cluster analysis based on morphological characters.

	PC-1	PC-2	PC-3	PC-4
Storage root number/plant	0.611	0.1762	0.1481	0.3114
Individual root wt (g)	−0.3443	0.0796	−0.0025	0.009
Storage root fresh yield/plant (g)	0.0484	−0.0704	0.0235	−0.0048
Storage root dry yield/plant (g)	0.1317	0.0496	−0.0955	0.0034
Storage root yield/plot (kg)	0.1316	0.0487	−0.0949	0.0032
Storage root length (cm)	0.323	0.5878	0.103	−0.0388

Table 3. Detail of principal components based on morphological traits.

and reported that the morphological characterization was efficient to detect genetic variability among accessions [24]. Amoatey et al. (2016) also reported the significant genetic variability among the 20 accessions of sweet potato studied based on the agromorphological characters, and the hierarchical cluster analysis grouped these accessions into two clusters at a genetic similarity index of 61.60% [25].

3.2. Biochemical analysis of tubers

3.2.1. Total carotenoid (mg 100 g^{-1} fresh weight)

The data presented in **Table 4** indicated that germplasm studied has significant differences for total carotenoids. The range for total carotenoids was 0.76–9.24 mg 100 g^{-1} fresh weight. The mean for total carotenoids was 4.84 mg 100 g^{-1} fresh weight. The maximum total carotenoids were recorded in tubers of "ST-14" (9.24 mg 100 g^{-1} fresh weight), while minimum was recorded in "SREE NANDNINI" (0.76 mg 100 g^{-1} fresh weight).

S.No.	Germplasm	Total carotenoids mg 100 g^{-1} fresh weight	Total Antioxidant mg 100 g^{-1} fresh weight	Total Phenols mg 100 g^{-1} fresh weight
1	Gauri	6.6	0.21	1.28
2	ST-10	1.01	0.28	1.07
3	V-10	1.02	0.18	1.32
4	SP-2	5.4	0.21	1.03
5	V-11	1.12	0.23	1.18
6	C-71	7.91	0.23	1.19
7	Kamla Sundari	6.46	0.2	1.25
8	Sree Nandini	0.76	0.26	1.38
9	SP-1	6.15	0.16	1.18
10	CIPSWA-2	6.91	0.23	0.94
11	SI-60	7.16	0.19	1.36
12	SV-71	1.17	0.19	1.3
13	440127	5.45	0.23	1.34
14	V-7	2.6	0.2	1.35
15	Khangudu	4.55	0.21	0.98
16	Pol-19-8-2	7.95	0.23	1.37
17	MPUAT-6	1.1	0.23	1.21
18	Varsha	4.45	0.17	1.08
19	ST-14	9.24	0.25	1.05
20	Sree Vardhini	0.87	0.19	1.16
21	187017	6.3	0.19	1.03

Table 4. Total carotenoids, total antioxidants, and total phenols in sweet potato tubers.

Liu et al. (2008) reported that the orange-fleshed sweet potato had higher total carotenoids content than yellow-fleshed [26]. Eluagu et al. (2010) reported that utilizing orange-fleshed sweet potato in their raw (unbalanced) form retains nutrient more than in their processed form and total carotenoids in the 10 orange-fleshed clones ranged between 10.32 and 13.99 mg 100 g^{-1} of fresh weight [27].

3.2.2. Total antioxidant (mg 100 g^{-1} fresh weight)

The data presented in **Table 4** indicated that germplasm showed significant differences for total antioxidants. The mean value for total antioxidants was 0.08 mg 100 g^{-1} fresh weight, and it ranged from 0.16 to 0.28 mg 100 g^{-1} fresh weight. The maximum total antioxidants were observed in "ST-10" (0.28 mg 100 g^{-1} fresh weight) followed by "Sree Nandini"

(0.26 mg 100 g^{-1} fresh weight) and "ST-14" (0.25 mg 100 g^{-1} fresh weight). The minimum total antioxidants were observed in "SP-1" (0.16 mg 100 g^{-1} fresh weight). Padda and Picha (2007) quantified the antioxidant activity and phenolic content of sweet potato roots and leaves of different sizes and ages [28]. Khurnpoon and Rungnoi (2012) also estimated the total phenol content and antioxidant activities of 36 sweet potato (*Ipomoea batatas*) cultivars with distinctive flesh color (white, yellow, orange, and purple) grown in Thailand [29].

3.2.3. Total phenol (mg 100 g^{-1} fresh weight)

The data presented in **Table 4** indicated that germplasm showed significant differences for total phenols. The mean value for total phenols was 1.19 mg 100 g^{-1} fresh weight, and it ranged from 0.94 to 1.38 mg 100 g^{-1} fresh weight. The maximum total phenols were observed in "SREE NANDNI" (1.38 mg 100 g^{-1} fresh weight) followed by "POL-19-8-2" (1.37 mg 100 g^{-1} fresh weight) and "SI-60" (1.36 mg 100 g^{-1} fresh weight). The minimum phenols were observed in "CIPSWA-2" (0.94 mg 100 g^{-1} fresh weight). Vyas et al. (2014) evaluated total phenolic content (TPC) of four successive extracts of various parts of *Nyctanthes arbortristis* Linn. TPC revealed that all extracts act as radical scavengers possibly due to presence of polyphenolic compounds and concluded that *Nyctanthes arbortristis* Linn. exhibited strong antioxidant activity and could serve as potential therapeutic plant for various diseases [30].

3.3. Molecular analysis

Out of 15, 10 RAPD primers showed variable degree of amplification and generated total 96 bands, which were polymorphic (**Figure 3**; **Table 5**). Only those fragments that were consistently amplified were considered for analysis. **Table 5** combines the comparative information about total number of fragments with base pair obtained by all the primers in all sweet potato genotypes. The advent of the RAPD provided a competent method to detect DNA polymorphism and generate a large number of molecular markers for genomic applications [31].

RAPD markers are easy and rapid and have the advantage of no prior knowledge of genome sequence. RAPD technique can be used in laboratories with limited resources but requires optimization for reproducible results for each species under research. Once the reaction conditions have been optimized, the technique is consistent and instructive. Silva et al. (2014) studied genetic diversity using RAPD markers. Marker showed that the collection had a high level of polymorphism. By UPGMA, they separated three groups of genotypes and identified two reconstructed populations by structure software [32].

A dendrogram was constructed using similarity matrix values determined from RAPD data for 21 sweet potato genotypes using UPGMA of NTSYS-pc software (Rholf, 2000). The similarity coefficient for different genotypes was in the range of 0.65–0.83. The average similarity across all the genotypes was found to be 0.74. A dendrogram generated from molecular data grouped all 21 genotypes into five major clusters (**Figure 4**).

The first cluster includes four genotypes, namely, Gauri, CIPSWA-2, Sree Vardhini, and Sree Nandini in which Sree Nandini was most distinct with all the remaining genotypes with similarity value of 0.66. Second cluster comprises only two genotypes like Kamla Sundari and

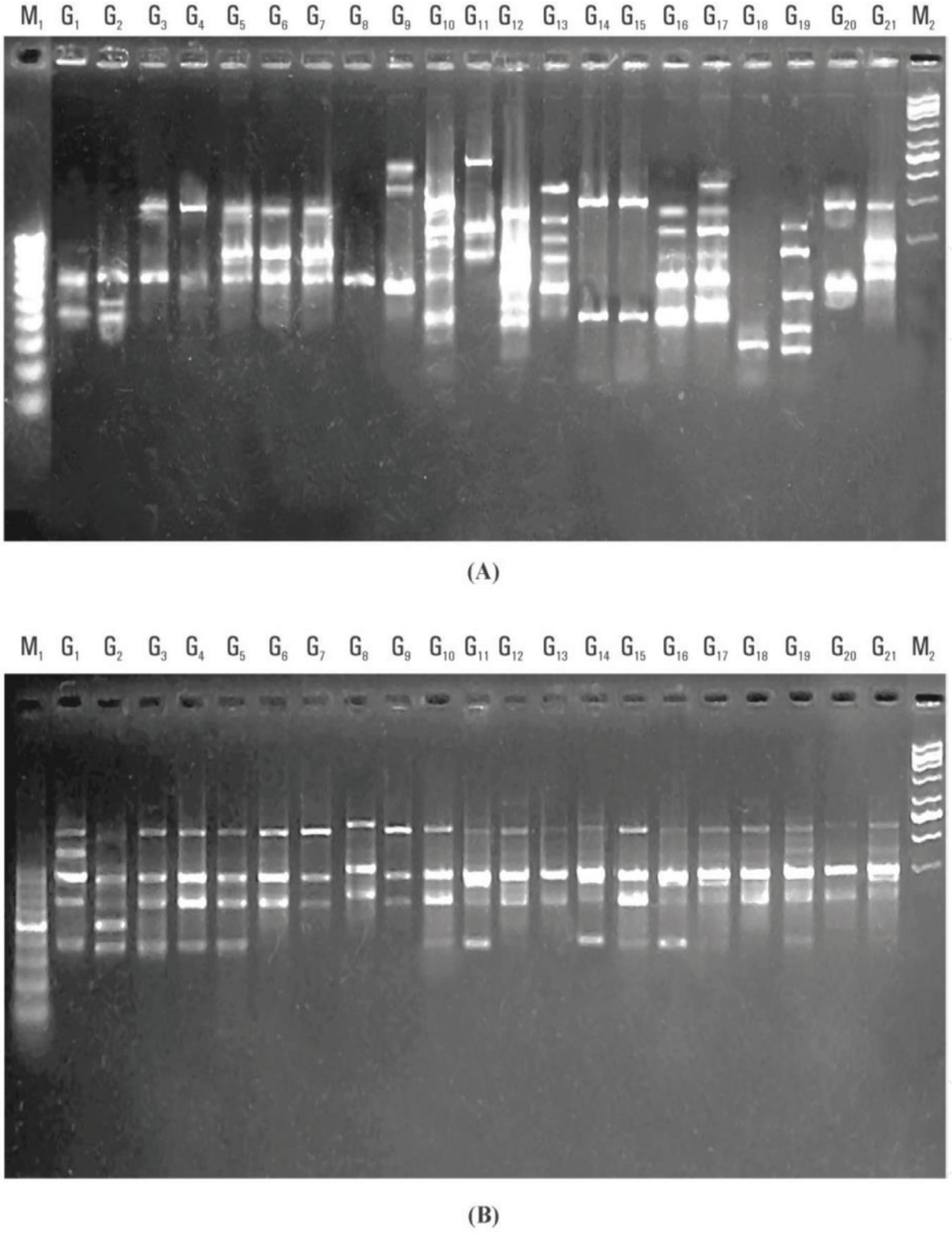

Figure 3. RAPD profiles of *Ipomoea batatas* L. DNA samples obtained from different germplasms using primer (A) OPE-04 and (B) OPE-03.

187017 with similarity value of 0.74. Third cluster comprises two genotypes, namely, SV-71 and Pol-19-8-2 with a similarity value of 0.73. The fourth cluster includes six genotypes, viz., V-11, SP-2, MPUAT-6, SP-1, ST-10, and V-7. The V-11 genotype was the most diverse from other genotypes in this group with similarity value of 0.73. The fifth cluster was the biggest one and comprised eight genotypes, namely, C-71, 440127, Varsha, Mahangudu, V-10, SI-60, and ST-14. Within this cluster, C-71 and 440127 genotypes were the most similar to each other

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S.No	Primers code	Total number of bands (a)	Total number of polymorphic bands (b)	Polymorphism % (b/a*100)
1	OPE-03	10	10	100
2	OPE-04	12	12	100
3	OPA-07	10	10	100
4	OPA-11	8	8	100
5	OPM-03	10	10	100
6	OPM-06	11	11	100
7	OPA-05	7	7	100
8	OPA-09	11	11	100
9	OPP-10	10	10	100
10	OPD-05	7	7	100
Total		**96**	**96**	**100**

Table 5. Polymorphism information of RAPD primers analyzed.

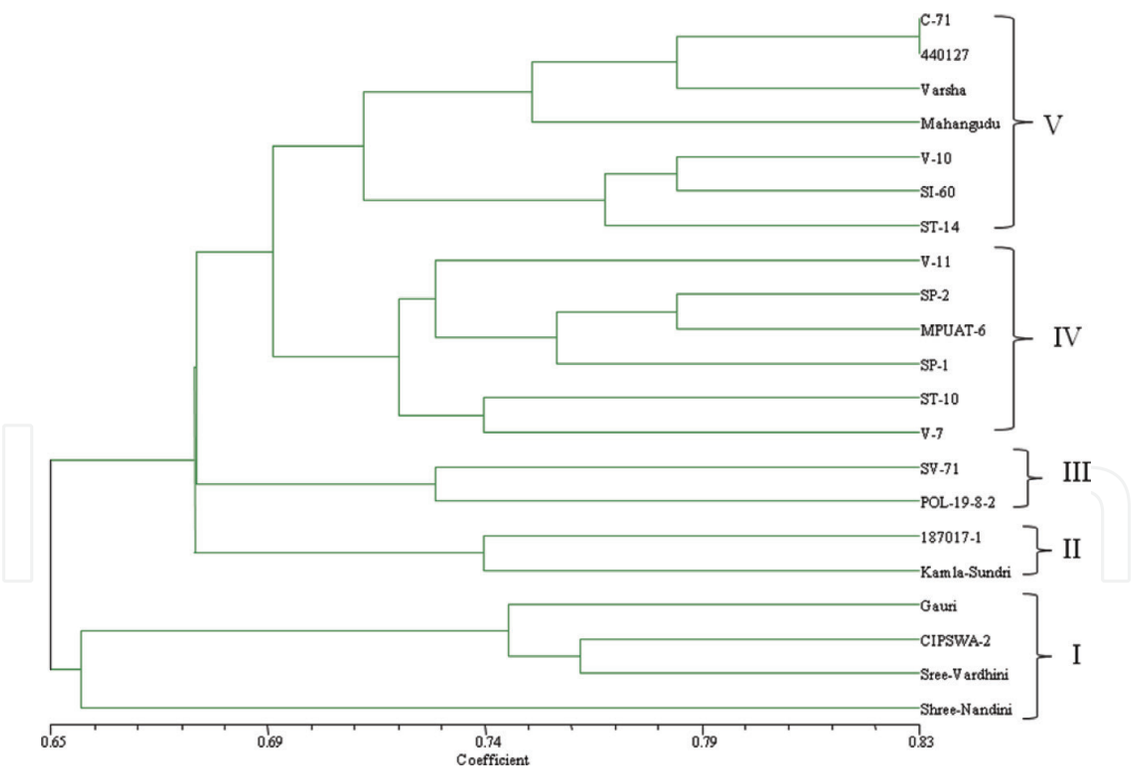

Figure 4. Dendrogram generated for 21 *Ipomoea batatas* L. germplasm using UPGMA cluster analysis based on RAPD marker.

with similarity value of 0.83 (**Figure 3**). Wang et al. (2009) assessed genetic distance and cluster analysis in 30 sweet potatoes based on the 26 RAPD primers and showed that the genetic distance among the 30 sweet potato varieties ranged from 0.0390 to 0.4306 with an average of

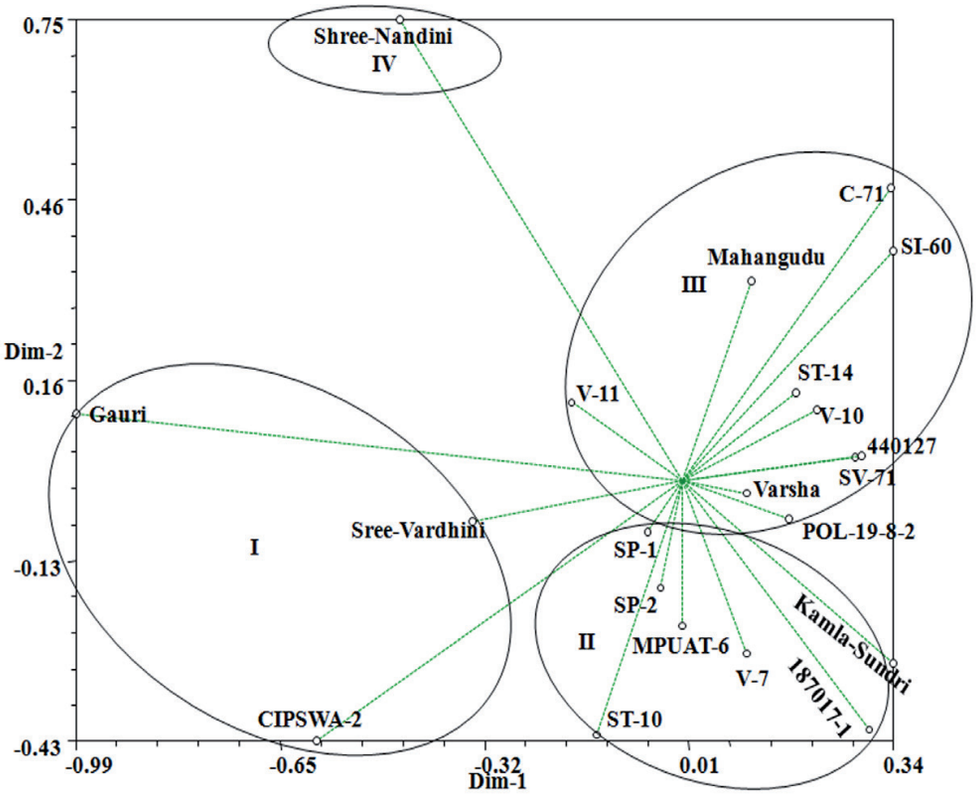

Figure 5. 2D plot generated for 21 *Ipomoea batatas* L. germplasms using UPGMA cluster analysis based on RAPD marker.

0.3086 [33]. The dendrogram based on RAPD markers indicated that the sweet potato varieties coming from the same regions or having the same parents were clustered into the same groups.

The two-dimensional plot generated from PCA showed four groups that were found to be similar to the clustering pattern of the UPGMA dendrogram (**Figure 5**). In the 2D plot, genotype Sree Nandini was found most distinct, whereas it was present along with Sree Vardhini in UPGMA dendrogram.

The analysis gave 19 principal components (PCs), out of which the first 10 principal components contributed 72.42% of the total variability. The first three principal components accounted for 30.14% of the total variability, in which the highest variation was contributed by the first component (12.30%), followed by the second (9.52%) and third components (8.31%). Similar clustering pattern was detected by Moulin et al. (2012) using eight RAPD primers. That results revealed that these eight primers with 44 sweet potato accessions generated a total of 93 scorable fragments, 88 of which (94.6%) are polymorphic [34]. Genetic relationship among sweet potato genotypes was also visualized by performing PCA based on RAPD data. The results of PCA were comparable to the cluster analysis with minor differences. Genotypes grouped within the same cluster in the dendrogram were also occupying the same position in two-dimensional and three-dimensional scaling based on molecular data.

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3.4. Association between molecular and morphological diversity

Cluster analysis was performed for both morphological and molecular data using the unweighted pair group method using arithmetic mean (UPGMA) algorithm, from which dendrograms depicting the similarity among germplasm were drawn and plotted using NTSYS-pc. The correlation was calculated to find the degree of association between the original similarity matrix in both morphological and molecular analyses. Using the Mantel test, a comparison between both methods was performed for the genotypes and showed moderate level of correlation between molecular and morphological data, i.e., r = 0.21279. This finding is agreed with that of Elameen et al. (2011) who studied the phenotypic diversity of morphological plant and root descriptor traits in 105 sweet potato germplasms using 27 phenotypic characters. Cluster analysis was conducted using the unweighted pair group method with arithmetic mean (UPGMA) [21].

3.5. Cumulative data analysis of morphology, biochemical, and molecular markers

Pairwise similarity among the genotypes ranged from 0.58 to 0.77 with an average of 0.67 based on combined morphometric, biochemical, and molecular markers data. The highest similarity (77%) was observed between C-71 and 440127 genotypes, whereas the lowest was observed between Sree Nandini and 187017-1 with a similarity value of 0.50. A dendrogram

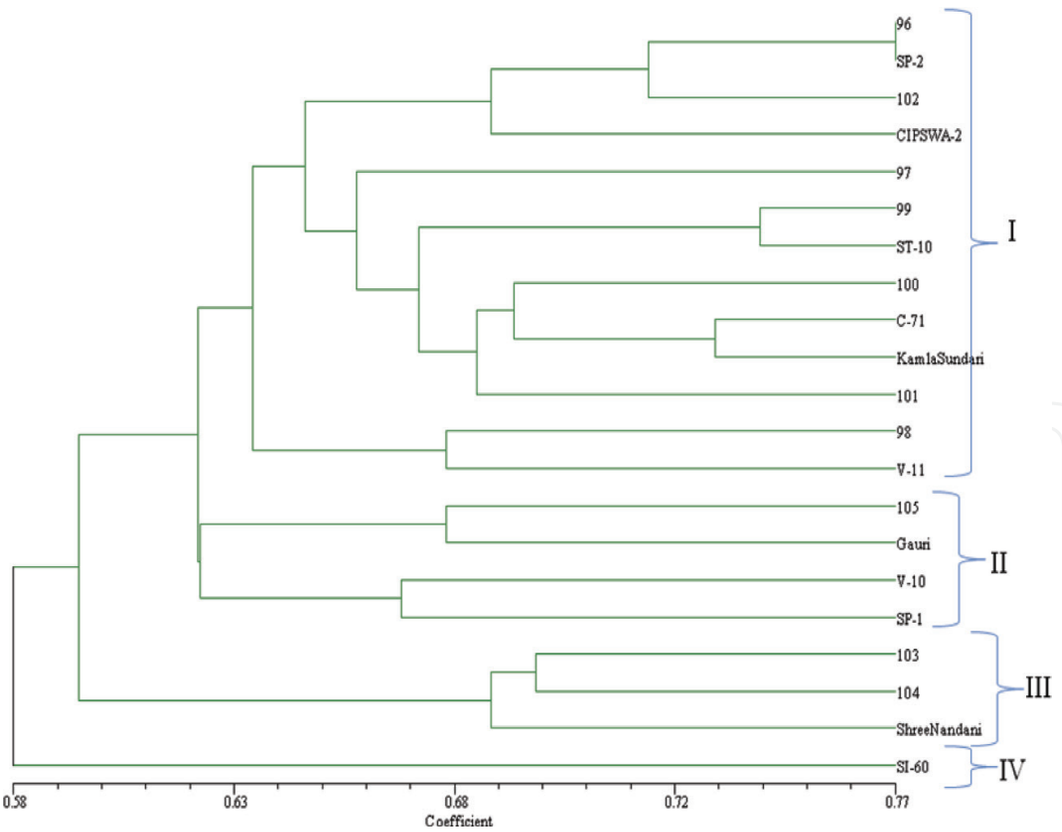

Figure 6. Dendrogram generated for 21 *Ipomoea batatas* L. germplasms using UPGMA cluster analysis based on cumulative morphological, biochemical, and molecular data.

based on combined morphometric, biochemical, and molecular marker data clustered all 21 genotypes into four major clusters (**Figure 6**).

The first cluster was the biggest one and comprised 13 genotypes, namely, C-71, 440127, Varsha, Mahangudu, V-11, V10, SI-60, SP-2, MPUAT-6, ST-14, SP-1, ST-10, and V-7. Within this cluster, C-71 and 440127 were the most similar morphologically, biochemically, and genetically, showing a similarity value of 0.77. In this group, V-11 was distinct from the other genotypes, with a similarity value of 0.65. The second cluster comprised four genotypes: 187017-1, Kamla Sundari, SV-71, and Pol-19-8-2. Third cluster comprised three genotypes, namely, Gauri, CIPSWA-2, and Sree Vardhini. Within this cluster, Gauri and CIPSWA-2 were most similar to each other with a similarity value of 0.70. Fourth cluster was smallest one and comprised only one genotype, namely, Sree Nandini, which was highly distinct from other genotypes with a similarity coefficient of 0.58. Based on Mantel Z-statistics (Mantel, 1967), the correlation coefficient (r) was estimated as 0.14. This value was considered a good fit of the UPGMA cluster pattern to the cumulative morphological, biochemical, and molecular data.

The 2D plot generated from the PCA of the combined morphological, biochemical, and molecular data (**Figure 7**) also supported the clustering pattern of the UPGMA dendrogram. In the 2D plot, genotype SP-1 was grouped in cluster III, whereas in UPGMA clustering, it was grouped in cluster I. However, in 2D plot, Sree Nandini genotype grouped with Gauri, CIPSWA-2, and Sree Vardhini genotypes, whereas it was in separate clusters in UPGMA pattern.

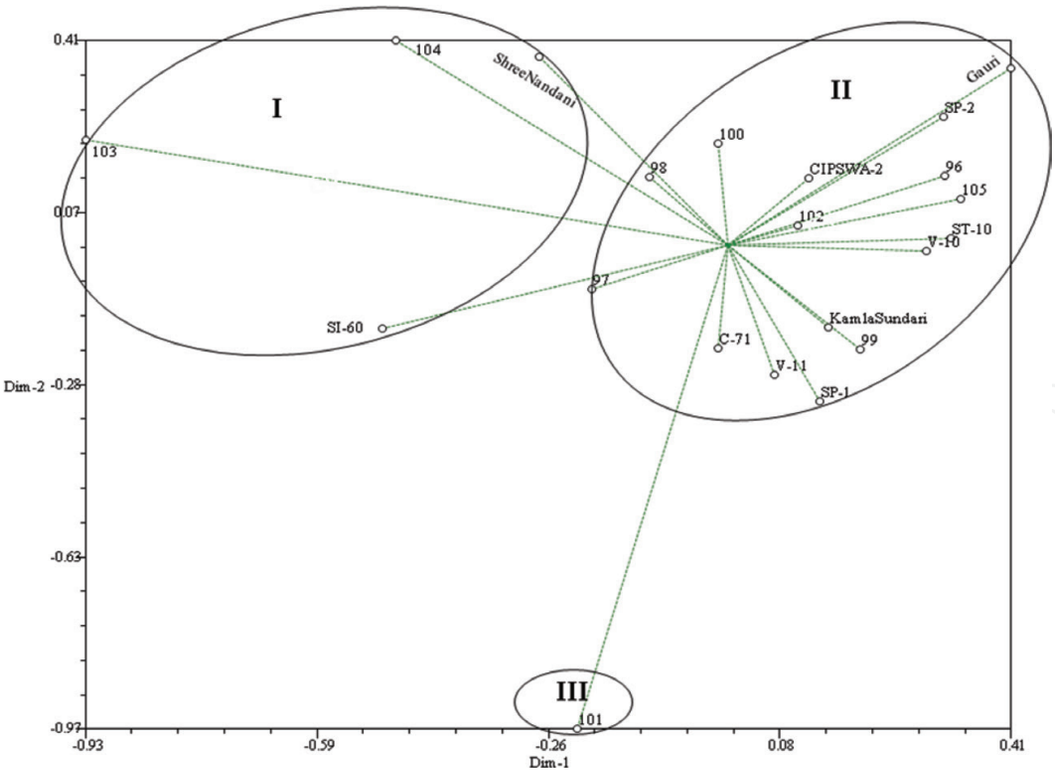

Figure 7. 2D plot generated for 21 *Ipomoea batatas* L. germplasms using UPGMA cluster analysis based on cumulative morphological, biochemical, and molecular data.

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The analysis gave 19 PCs, out of which the first 10 PCs contributed 72.34% of the total variability of the analyzed genotypes. The first five PCs accounted for 44.88% of the total variability; the first three accounted for 30.01% of the variance, in which maximum variability was contributed by the first component (11.40%), followed by the second (9.63%) and third (8.96%) components. Kaur et al. (2016) reported the UPGMA dendrogram based on the combined morphological and molecular markers in which the 23 mungbean genotypes were divided into three main clusters, showing a close genetic relationship, which might be due to their close genetic bases [35].

4. Conclusion

Findings of the present study revealed that 21 sweet potato germplasms were moderate to high diversity based on molecular, biochemical, and morphological assessment approaches. The results obtained will serve as a guide for the basis of genotype management and crop improvement programs. Designing effective breeding programs is largely dependent on understanding the genetic diversity of the relevant germplasms. Here, we reported our detailed analysis of representative sweet potato accessions cultivated using morphological, biochemical, and molecular markers. Our results demonstrated significant genetic diversity in the orange flashed sweet potato germplasm collection. 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. To create new hybrid varieties with new alleles and increased genetic diversity, sweet potato accessions with a wide genetic background that includes introduced varieties should be used in breeding programs.

From the discovery of first molecular marker, there was a continuous development in the molecular markers technology from RFLP to SNPs and a diversity of array-technology-based markers. Advancements in the nanopore-based sequencing technologies have led to development of low-cost sequencing with high throughput. In spite of the presence of these highly advanced molecular marker tools and techniques, the outcomes of such technologies are yet to come due to inaccurate phenotyping. Application of molecular marker technologies also lies in the areas of plant biology like systematics, population genetics, evolutionary biology and conservation genetics, genomics, identification of the wild progenitors of domestic species, and the establishment of geographic patterns of genetic diversity. The success of molecular marker technology for bringing crop improvement depends on the positive interaction between plant breeders and biotechnologists.

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Conflict of interest

All the authors declare that there is no conflict of interest.

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References

[1] Wadl PA, Olukolu BA, Branham SE, Jarret RL, Yencho GC, Jackson DM. Genetic diversity and population structure of the USDA sweetpotato (*Ipomoea batatas*) germplasm collections using GBSpoly. Frontiers in Plant Science. 2018;**21**:1166. DOI: 10.3389/fpls.2018.01166. eCollection 2018

[2] Horticultural Statistics at a Glance. Horticulture Statistics Division, Department of Agriculture, Ministry of Agriculture & Farmers Welfare. New Delhi, India: Government of India; 2017. p. 481

[3] Palmer JK. Carbohydrates in sweetpotato. In: Villareal R, Griggs T, editors. Proceedings of the First International Symposium; 23-27 March 1981; Taiwan. China: AVRDC; 1982. pp.137-138

[4] Wang A, Li R, Ren L, Gao X, Zhang Y, Ma Z, et al. A comparative metabolomics study of flavonoids in sweet potato with different flesh colors (*Ipomoea batatas* (L.) lam). Food Chemistry. 2018;**260**:124-134

[5] Ghosh SP, Ramanujan T, Jos JS, Moorthy SN, Nair RG. Tuber Crops. New Delhi: Oxford IBH Publishing; 1988. 146 p

[6] Byers T, Perry G. Dietary carotenes, vitamin C and vitamin E as protective antioxidants in human cancers. Annual Review of Nutrition. 1992;**12**:139-159

[7] Montilla EC, Hillebrands S, Winterhalter P. Anthocyanins in purple sweet potato (*Ipomoea batatas*) varieties. Fruits, Vegetable and Cereal Science and Biotechnology. 2011; **5**(2):19-24

[8] Woolfe JA, editor. Sweet Potato an Untapped Food Resource. UK: Cambridge University Press; 1992. 643 p

[9] Gester H. Anti carcinogenic effect of common carotenoid. International Journal for Vitamin and Nutrition Research. 1993;**63**:93-121

[10] Powell W, Morgante M, Andre C. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Molecular Breeding. 1996;**2**:225-238

[11] Russel JR. Direct comparison of levels of genetic variations among barley accessions detected by RFLPS, SSRS and RAPDS. Theoretical and Appliied Genetics. 1997;**95**:714-722

[12] Fahima T, Sun GL, Beharav A, Krugman T, Beiles A, Nevo E. RAPD polymorphism of wild emmer wheat populations, *Triticum dicoccoides* in Israel. Theoretical Applied Genetics. 1999;**98**:43-47

[13] Subudhi PK, Huang N. RAPD mapping in a doubled haploid population of rice (*Oryza sativa* L.). Hereditas. 1999;**130**:2-9

[14] Panse VG, Sukhatme PV. Statistical Methods for Agricultural Workers. New Delhi: Indian Council of Agricultural Research; 1975. 361 p

[15] Sneath PHA, Sokal RR. Numerical Taxonomy. San Francisco: Freeman; 1973. 575 p

[16] Rohlf FJ. NTSYS-PC Numerical Taxonomy and Multivariate Analysis System, Version 2.02. Setauket, New York: Exeter Software; 2000

[17] Thimmiah SR. Estimation of chlorophyll. In: Standard Method of Chemical Analysis. New Delhi: Kalyani Publisher; 1999. 307 p

[18] Murray M, Thompson WF. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Research. 1980;**8**:4321-4326

[19] Sambrook J, Fritschi EF, Maniatis T. Molecular Cloning: A Laboratory Manual. 2nd ed. New York, USA: Cold Spring Harbor Laboratory Press; 1989. 1546 p

[20] Mantel NA. The detection of disease clustering and a generalized regression approach. Cancer Research. 1967;**27**:209-220

[21] Elameen A, Larson A, Klemsadel SS, Fjellheim S, Sundheim L, Mosalla S, et al. Phenotypic diversity of plant morphological and root descriptor traits within a sweet potato, *Ipomoea batatas* (L.) germplasm collection from Tanzania. Genetic Resources and Crop Evolution. 2011;**58**:397-407

[22] Yada B, Tukamuhabwa P, Wanjala B, Kim DJ, Skilton RA, Alajo A, et al. Characterization of Ugandan sweetpotato germplasm using fluorescent labeled simple sequence repeat markers. Hortscience. 2010;**45**(2):225-230

[23] Norman PE, Beah AA, Samba JA, Tucker MJ, Benya MT, Fomba SN. Agro-phenotypic characterization of sweet potato (*Ipomoea batatas* L.) genotypes using factor and cluster analyses. Agricultural Science Research Journal. 2014;**4**(2):30-38

[24] Moulin MM, Rodrigues R, Gonçalvez LSA, Sundré CP, Pereira MGA. Comparison of RAPD and ISSR markers reveals genetic diversity among sweet potato landraces (*Ipomoea batatas* (L.) Lam.). Acta Scientiarum Agronomy. 2012;**34**:139-147

[25] Amoatey HM, Sossah FL, Ahiakpa JK, Quartey EK, Appiah AS, Segbefia MM. Phenotypic profiles of different accessions of sweet potato (*Ipomoea batatas* L. Lam) in the coastal savanna agro-ecological zone of Ghana. African Journal of Agricultural Research. 2016; **11**(26):2316-2328

[26] Liu SC, Lin JT, Yang DT. Determination of Cis-and Trans- α and β carotenoids in Taiwanese sweet potatoes (*Ipomoea batatas* L.*)* harvested at various times. Journal of Food Chemistry. 2008;**116**(3):605-610

[27] Eluagu E, Onimawo N, Ignitiatus A. Effect of processing on the antinutritional factors of orange fleshed sweet potato (*Ipomoea batatas* (L.) Lam) flours. Electronic Journal of Environmental, Agricultural and Food Chemistry. 2010;**9**(6):1000-1005

[28] Padda MS, Picha DH. Antioxidant activity and phenolic composition in 'beauregard' sweet-potato are affected by root size and leaf age. Journal of American Society Horticulture Science. 2007;**132**:447-451

[29] Khurnpoon L, Rungnoi O. The correlation between total phenol and antioxidant capacity of sweet potato (*Ipomoea batatas* L.*)* with varying flesh color. Acta Horticulturae. 2012;(945):413-419. DOI: 10.17660/ActaHortic.2012.945.56

[30] Vyas S, Kachhwaha S, Kothari SL. Comparituve analysis of the in vitro antioxidant activity and polyphenolic content of successive extracts of *Nyctanthes arbourtristis* Linn. International Journal of Pharmacy and Pharmaceutical Sciences. 2014;**6**:0975-1491

[31] Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acid Research. 1990;**18**:6531-6535

[32] Silva AVCD, Andrade LNTA, Rabbani ARC, Nunes MUC, Pinheiro LR. Genetic diversity of sweet potatoes collection from Northeastern Brazil. African Journal of Biotechnology. 2014;**13**(10):1109-1116

[33] Wang Q, Zhang L, Wang B, Yin Z, Feng C, Wang Q. Sweet potato viruses in China. Crop Protection. 2009;**29**:110-114

[34] Moulin MM, Rodrigues R, Gonçalves LSA, Sudré CP, Santos MH, Silva JRP. Collection and morphological characterization of sweet potato landraces in north of Rio de Janeiro state. Horticultura Brasileira. 2012;**30**:286-292

[35] Kaur G, Joshi A, Jain D, Choudhary R, Vyas D. Diversity analysis of green gram (*Vigna radiata* (L.) Wilczek) through morphological and molecular markers. Turkish Journal of Agriculture and Forestry. 2016;**40**(2):229-240