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RESEARCH ARTICLE

Diversity analysis of Indian sweet potato (*Ipomoea batatas* L. Lam) genotypes using simple sequence repeat markers

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Abstract

Eighty-two genotypes of sweet potato of the Indian collection were subjected to molecular characterization using 29 simple sequence repeat markers (SSR) to study molecular-level genetic diversity. A total of 70 alleles with an average of 0.47 PIC value was recorded. The SAHN dendrogram based on UPGMA clustering method depicted total 6 sub-clusters where the highest K values for different genetically distinct populations interpreted was 5. POPGENE structure revealed the smallest group of total seven genotypes with highest genetic purity from genotypes from the states of West Bengal and Telangana. Factorial analysis majorly differentiated the genotypes between two groups of eastern and middle with collections of southern India. These genotypes collected from diverse geographical regions showed great genetic diversity, possibly having some influence on their adaptation due to geography.

Keywords: Sweet potato, genotypes, characterization, POPGENE structure, SSR, genetic diversity

Introduction

Sweet potato (*Ipomoea batatas* L.), a heterozygous sexually and vegetatively propagated polyploidy species, is an important crop of the Convolvulaceae family, cultivated and consumed worldwide. Cultivated sweet potato has originated from its closest relative *I. trifida* (2n=6x=90) which is evolved from tetraploid (4x) and diploid (2x) progenitors (Nishiyama et al. 1975; Feng et al. 2018). About 13 wild species of the genus belonging to *Ipomoea* section *batatas* and Sweet potato is the only species widely cultivated as a major staple crop in about 100 countries (Wolfe 1992). This nutrition-rich root vegetable crop is widely adopted for cultivation in marginal land, which provides enormous potential for enhancing food security and preventing malnutrition in the developing world. Sweet potato having starchy roots is propagated clonally through stem cuttings. Hybrid or polycross breeding techniques are adopted for the successful breeding of sweet potato. Morphological descriptors are potentially useful for clonal identification and genetic distance estimation. In order to improve character, the breeding strategies adopted include estimating each character's genotypic and environmental variance component.

On the other hand, environmental influence on phenotypically plastic genotypes makes the process of phenotypic evaluation complex (Lin et al. 2007). The advantage of simple sequence repeat markers (SSR) lies in single locus markers with multiple alleles, which imparts more variability than markers, providing an effective way to discriminate between genotypes. SSR microsatellite markers show high level of polymorphism with co-dominant inheritance. Molecular markers have also shown important and critical applications in assessing and conserving genetic variation.

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For genetic diversity analysis, the morphological, agronomic, and molecular marker approaches are often used to generate information in combination to investigate the heterogeneity in different crop species, including sweet potato (Palumbo et al. 2019). Molecular markers such as microsatellites or SSRs play an important role in assessing and conserving genetic diversity due to their efficiency, reliability and reproducibility. Primers designed to flank SSR loci were developed and used to characterize different elite polycross sweet potato cultivars (Hu et al. 2004). Differences in sequences of SSR products were also studied to determine levels of polymorphism among sweet potato cultivars (Buteler et al. 1999). In some cases, though SSR is a co-dominant marker but still considering the critical scenario in case of polyploidy level sometimes considered as dominant markers like AFLP or RAPD. In spite of these possibilities, SSR markers can reveal a higher level of polymorphic bands and had already been developed for sweet potato (Hu et al. 2004). These markers had successfully been employed to determine relationships between different cultivars derived from hybrid or polycross breeding by the breeders in East African and Latin American landraces (Gichuru et al. 2006). The perception of genetic diversity is of prime need for conservation and adequate use of germplasm. The information on genetic diversity at morphological and molecular level in Indian germplasm/ cultivars is limited and scanty. Recently, a few reports on diversity analysis determining the genetic relatedness among a few Indian collections of sweet potato using limited number of molecular markers are published (Paliwal et al. 2020; Murthy et al. 2021). Therefore, the present study was carried out to evaluate the genetic relatedness among different sweet potato cultivars based on SSR markers and examination of validity of the information in relation to the pedigree history of the cultivars.

Materials and methods

Plant materials

The selected 82 lines of sweet potato were used from the field gene bank of ICAR-All India Coordinated Research Project on Tuber Crops, Bidhan Chandra Krishi Viswavidyalaya (BCKV), India (Table 1). Among total 82 genotypes, lines with prefix of H, S and IB were collected from Hyderabad; prefix of POL and DOP were poly cross lines collected from Dholi (Bihar) and BCSP lines were collected from Kalyani, BCKV. The lines with prefix of X are poly cross collected from Dholi (Bihar), whereas OP lines are open-pollinated genotypes. All the lines are polycross natural selection genotypes collected from different parts of India. Before starting the study, the vine cuttings of the genotypes were maintained in plant growth chamber (GC-1000) in controlled conditions of alternate 16 hours light and 8 hours dark. The plants grown for 30 days were used for the experiment to investigate genetic relationships among sweet potato genotypes including heirloom cultivars and elite collections of this crop.

Plant genomic DNA extraction

The total plant genomic DNA was isolated from 250 mg of young leaves of sweet potato by silica-based spin column using DNeasy Plant Mini Kit (QIAGEN). Concentration of DNA samples was determined using Qubit 3, Nano-drop fluorometer (Thermo) and a total of 5 to 8 ng of total genomic DNA from each of the samples for polymerase chain reactions (PCRs).

PCR amplification of simple sequence repeats

A total of 29 SSR primers characterized by Buteler *et al*. (1999) for sweet potato DNA amplification were used for the reactions (Table 2). A final volume of reaction mixture was 25 µL containing 25 mM $MgCl_2$, 10x PCR buffer, 10 mM dNTPs, 1-µM forward primer, 1-µM reverse primer, 5U/ μL^{-1}U Taq polymerase (TaKaRa Bio, Japan), 5 to 8 ng template DNA, and molecular biology grade water (SIGMA-ALDRICH, Germany) was used for the PCR. The amplification conditions were set up thus: 94°C for 5 minutes; denaturation at 94°C for 30 seconds.; annealing at between 56.0 and 62.0°C for 30 seconds. (depending on the annealing temperature of the primer); polymerization at 72°C for 1 min for 33 cycles, with a final extension at 72°C for 7 min was done in Veritti 96 well Thermal Cycler (Applied Biosystem). Amplification products were separated on a 2% high-resolution agarose gel (SIGMA-ALDRICH) using a 50 bp ladder (New England Bio Labs.). The electrophoresis was conducted on a Hoffar gel electrophoresis system, and the DNA fragments were recorded by a computer automated gel documentation system (Vilber-Lourmet, France). The recorded amplified fragments were analysed by Bio-ID software (Vilber-Lourmet).

Statistical analysis

Each amplified fragment was treated as a unit character and scored as binary codes (1/0=+/-). Only those with medium or high intensity were taken into account. Fragments with the same mobility on the gel but with different intensities were not distinguished from each other when genotypes were being compared. A hierarchical clustering dendrogram of sweet potato genotypes was done based on UPGMA similarity matrix following SAHN method using NTSYS-PC (ver. 2.11X; Exeter Software, N.Y., Rohlf 2000).-Principal coordinate analysis (PCoA) analysis was also performed to visualize individuals in scatter plot. Another method of clustering based on Bayesian model-based clustering analysis was used to find out the optimal number of clusters among sweet potato genotypes using the software STRUCTURE2.3.4 (Pritchard et al. 2000), which allocates individuals into a number of clusters (K) based on the multi-locus genotypic data. The number of clusters (K) which are genetically significantly distinct was defined using the Evanno et al. (2005) method. The admixture model and correlated allele frequencies were applied for each run

Table 1. Different cultivars of sweet potato used in molecular characterization

S.No.	Cultivar Name	S.No.	Cultivar Name	S.No.	Cultivar Name	S.No.	Cultivar Name
1	DOP-MIX-93/13	23	CROSS-4	45	X-25	67	POL-4-9
2	DOP-H-85-16	24	S-594	46	OP-9	68	POL-20-62
3	POL-19-8/2	25	X-108/1	47	X-108/2	69	POL-21-1
4	DOP-X-9	26	X-9	48	OP-57	70	POL-4-4/5
5	H-200	27	Bidhan Jagannath	49	H-268	71	IB-480
6	DOP-82-6	28	BCSP-2	50	IB-90-11/24	72	IB-81
7	X-134	29	Kamala Sunduri	51	X-142	73	IB-700
8	DOP-MIX-94/38	30	KISAN	52	H-85/16	74	H-620
9	DOP-MIX-93-1	31	BCSP-11	53	BCSP-8	75	H-635
10	86-X-15	32	S-107	54	BCSP-15	76	H-82-6
11	DOP-MIX-94/19	33	BCSP-4	55	S-72	77	H-82-2
12	Sree Kanaka	34	BCSP-5	56	H-42	78	H-85/179
13	DOP-MIX-94-42	35	S-30/25	57	POL-13-4	79	H-633
14	87-X-46	36	S-783	58	BCSP-10	80	H-85/70
15	DOP-92/120	37	S-30	59	BCSP-12	81	H-85/168
16	DOP-MIX-94/36	38	SV-98	60	BCSP-13	82	BCSP-3
17	ST-14	39	OP-34	61	BCSP-14		
18	86-X-16	40	IGSP-6	62	S-1221		
19	S-22	41	IGSP-7	63	S-25		
20	CIPSWA-2	42	IGSP-C/16	64	S-30/15		
21	440038	43	IGSP-C/17	65	IB-90-11/1		
22	X-109/2	44	OP-23	66	BCSP-1		

Table 2. The details of SSR primers with their PIC content used in the study

S. No.	Name	No. of allele	PIC	Sl. No.	Name	No. of allele	PIC
1	IB-S01	2	0.49	16	IB-J10A	2	0.47
2	IB-R03	2	0.5	17	IB-J116A	2	0.21
3	IB-S10	3	0.53	18	IBC12	2	0.5
4	IB-S11	2	0.5	19	JB1809	2	0.49
5	IB-R13	3	0.51	20	IBJ522a	2	0.37
6	IB-R14	3	0.57	21	IBC5	2	0.44
7	IB-R16	3	0.57	22	IBJ544b	2	0.5
8	IB-S18	3	0.43	23	IB-318	3	0.65
9	IB-R19	2	0.46	24	IB-255F1	3	0.41
10	IB-R21	2	0.37	25	IB-255	2	0.49
11	IB-CIP-1	3	0.34	26	IB-286	2	0.46
12	IB-CIP-2	2	0.48	27	ITSSR04	3	0.5
13	IB242	3	0.46	28	IB2/42	2	0.44
14	IB297	3	0.58	29	IB3/31	2	0.36
15	IB324	3	0.5			Mean	0.47

with 10,000 iterations and 100,000 Markov Chain Monte Carlo(MCMC) replications in the structure analysis. The population genetic diversity parameters were estimated on designated populations (K) using Pop Genever 1.32 (Yeh et al. 2000).

Results

In present study, SAHN dendrogram based on UPGMA clustering method depicted a total 6 sub-clusters for 82 sweet potato genotypes (Fig. 1). The Dice coefficient between the different groups of sweet potato genotypes ranged from 0.61 to 1.00. Among the different genotypes only DOP-MIX-94/36 had been separated from all other 81 genotypes sowing maximum dissimilarity, whereas all other 80 genotypes showed up to more than 0.08 Dice coefficients. The two genotypes namely, IGSP-7 and IGSP-C/17 had shown 0.92 dice coefficient. First cluster contained DOP-MIX-93/13 to BCSP-14, 11 genotypes, whereas H-200 to BCSP-3, total 17 genotypes had been grouped under second cluster. Third cluster consisted of 16 genotypes from IB-90-11-1 to H-85-179, and S2-2 up to BCSP-5 had been considered as fourth while last but not the least, the fifth cluster OP-34 to DOP-MIX-94/36 a total of 18 genotypes.

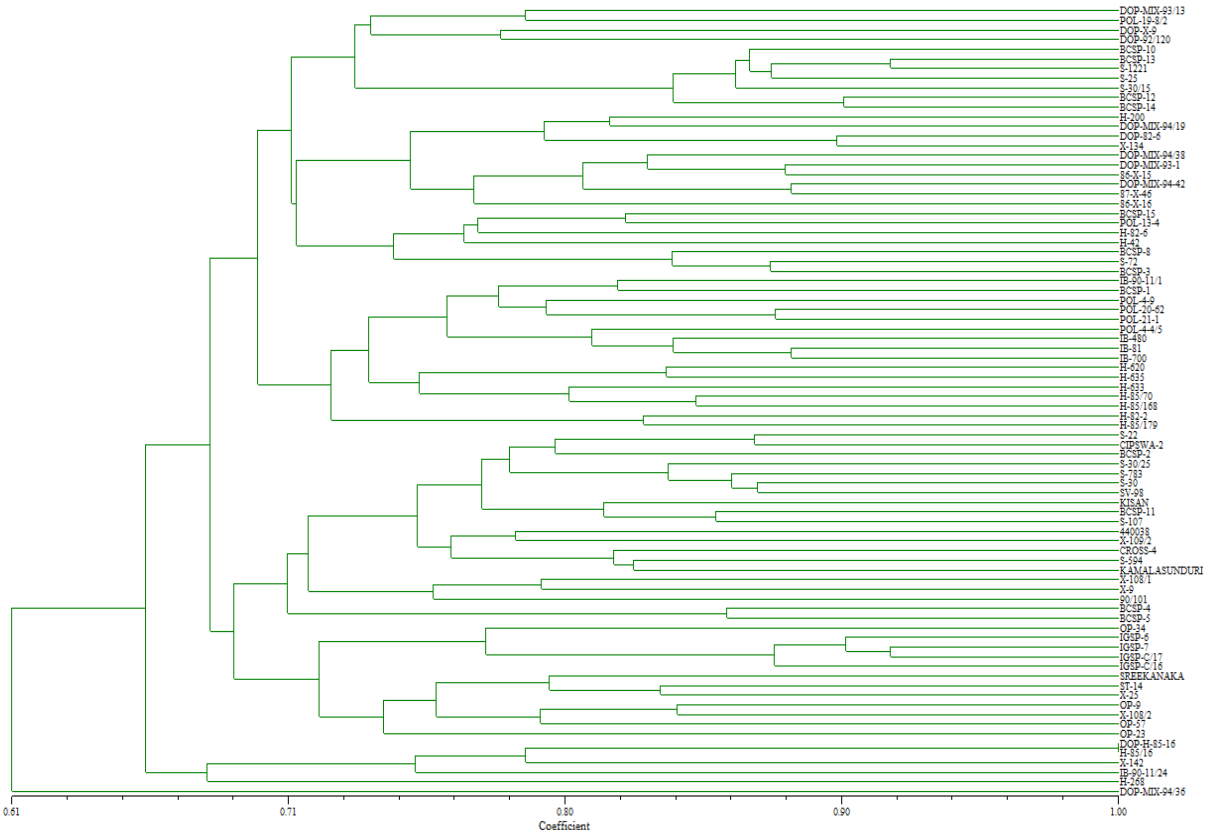

Fig. 1. UPGMA cluster differentiating the potato genotypes based on the similarity matrix generated from SSR molecular markers

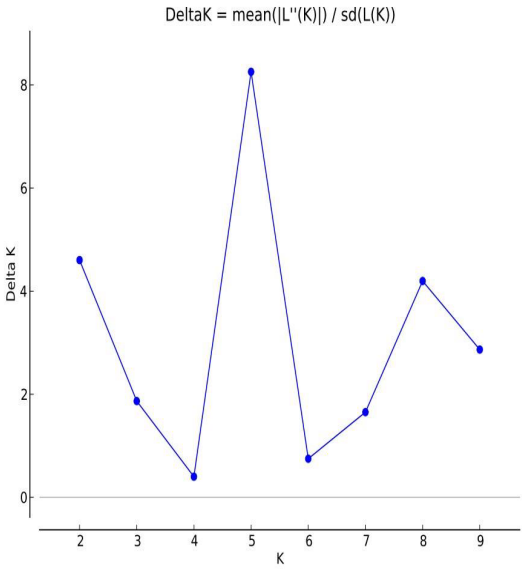

Fig. 2a. K value is plotted against delta K for a population of 82 sweet potato genotypes

Based on the 29 polymorphic SSR markers data across the 82 genotypes, 5 distinct populations were estimated as described by Evanno et al. (2005) (Fig. 2a). The individuals were arranged depending on the estimated membership coefficient value («Q») in every cluster. In this case the smallest group shown in red 58 to 64 a total of seven genotypes has highest genetic purity most of which belongs to collections from West Bengal and Telangana regions. The third, fourth and fifth populations have more admixture.

Factorial analysis (Fig. 3) differentiated all the genotypes into two groups indicating, in the left quadrant with green blue and in right quadrant black orange color spots. Effective number of alleles in different population ranged from 1.4576 (Population I) to 1.9253 (Population III). The highest Shannon's information index was found in Population III (0.6880). Though maximum expected heterozygosity was found in population III (0.4644) but maximum observed heterozygosity found in population IV (0.4244). Nei's Expected heterozygosity was maximum in population III (0.4528) and average expected heterozygosity found in population IV (0.4289). All the remaining populations showed average expected heterozygosity (Table 3).

Discussion

Landraces can be considered as the repository of genetic resource which had been evolved through a continuous selection on account of various stresses. These are the main source of useful traits and variability that helps in widening the genetic base as well as helping us for crop improvement. In the case of sweet potato, clonal propagation had been proved to be fruitful in conserving genetic diversity. It is well-known that the polymorphism in sweet potato is mostly contributed by large genome size, self-incompatibility, and

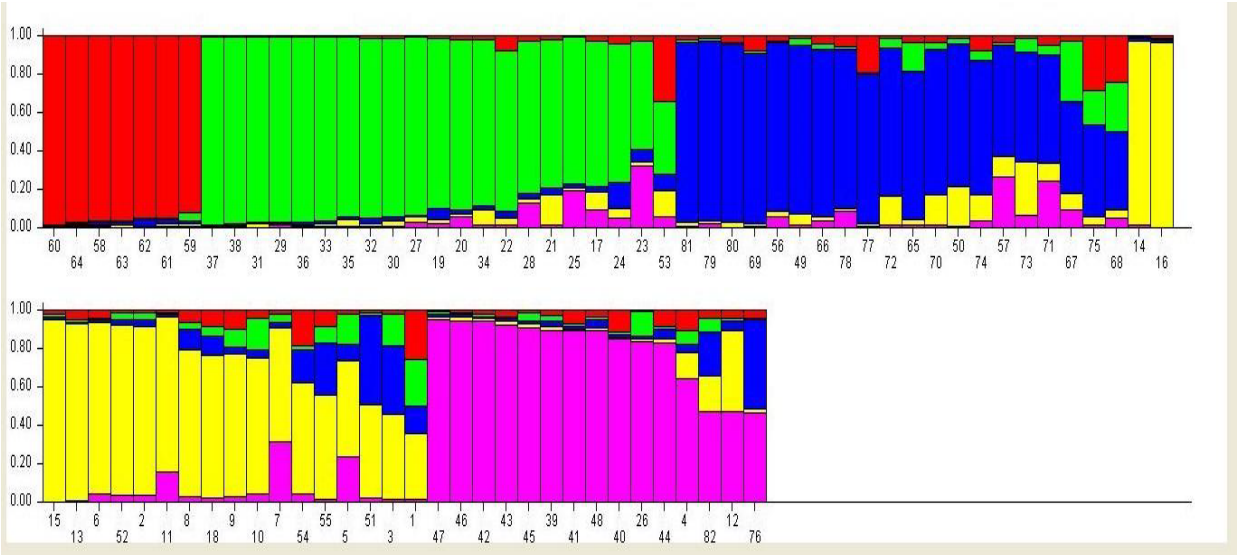

Fig. 2b. Population structure of 82 sweet potato genotypes for k=5

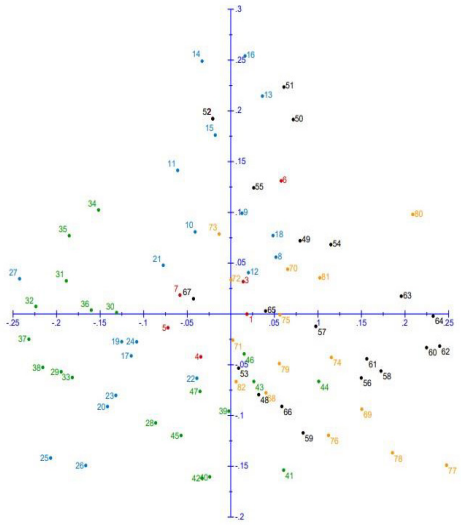

Fig. 3. Principal coordinate analysis of 82 sweet potato genotypes based on SSR markers

outcrossing. Though as a hexaploid crop species (2n = 6x =90), dominant markers are desirable for polymorphism analysis, sweet potato, an auto-hexaploid with many chromosomes, makes a better choice for SSR markers. SSRs are well-established markers that have been used in several studies in sweet potato in phylogenetic studies and designation of gene pools. The SSR markers may also aid in testing hybrids and marker-assisted breeding programmes of sweet potato and other clonally propagated crops.

The genotype DOP-MIX-94/36 was proved to be the totally different out group with maximum nodal length which was also reflected by earlier in UPGMA clustering (Fig. 1). The maximum bootstrap value was found in the node of division of genotype 49 (H-268) and 50 (IB-90-11/24) showing maximum similarity were collected from Telangana and Andhra Pradesh. These two may be the duplication of same genotype which may be treated as different ecotypes. Koussao et al. (2014) used morphological characters and molecular markers to differentiate a set of 112 accessions from Burkina Faso, West Africa and found 11 duplicates, while 28 SSR markers were more informative in discriminating the accessions that identified five duplicates.

The genotypes, BCSP-4 (33) and BCSP-5 (34), collected from two different regions of Bengal, showed great similarity as clustered very closely with bootstrap value 74. The genotypes such IGSP-6 (40), IGSP-7 (41), IGSP-C/16 (42) and IGSP-C/17 (43), which are collections from Chhattisgarh, also made robust cluster with higher bootstrap values. The genotypes depicted in green color from 28 to 48 numbers are basically from West Bengal, Telangana and Madhya Pradesh collections, though one Bihar (Dholi) collection

Table 3. Summary diversity of five sweet potato populations

Populations	Ae (Mean ± St dev)	I (Mean ± St dev)	Ho (Mean ± St dev)	Nei's He (Mean ± St dev)	Hae (Mean ± St dev)
Population I	1.46 ± 0.45	0.38 ± 0.32	0.29 ± 0.36	0.25 ± 0.22	0.39 ± 0.1
Population II	1.78 ± 0.5	0.62 ± 0.26	0.39 ± 0.29	0.40 ± 0.12	0.39 ± 0.1
Population III	1.93 ± 0.5	0.69 ± 0.21	0.42 ± 0.28	0.45 ± 0.12	0.39 ± 0.1
Population IV	1.82 ± 0.37	0.66 ± 0.20	0.42 ± 0.31	0.39 ± 0.12	0.43 ± 0.1
Population V	1.79 ± 0.45	0.63 ± 0.24	0.39 ± 0.29	0.41 ± 0.15	0.39 ± 0.1

Ae: Effective number of alleles; I: Shannon's Information index; Ho: Observed heterozygosity; Nei'sHe: Nei's Expected Heterozygosity; Hae: Average Expected Heterozygosity

X-25 was also mixed with. Overall, moderate diversity was observed between the genotypes, indicating a prime need for further collections and/or incorporation of more divergent genotypes for a successful breeding programme. Similar diversity analysis studies on sweet potato using microsatellite markers could also differentiate genotypes. A moderate genetic diversity among 192 genotypes using 10 SSR markers had been reported in Uganda (Yada et al. 2010). In the similar way a low genetic diversity had also been reported in East African sweet potato (Gichuru et al. 2006), whereas a similarity of 15 to 78% had been reported in different Indonesian sweet potato accessions (Soegianto et al. 2011). Though Eastern Africa is the second most diversity rich zone followed by Central America (Villordon et al. 2007), but the narrow region might contribute lower diversity for the collection of genotypes. Some studies also indicate a good amount of molecular diversity present in sweet potato genotypes of Brazil (Veasey et al. 2008) and China (Li et al. 2009). Anglin et al. (2021) genotyped a huge collection of sweet potato with a panel of 20 SSR markers to assess genetic identity, diversity and population structure and reported intraspecific relationship in the population which uncovered high level of redundancy in material from Peru and Latin America.

Recent research using 240 accessions in China revealed that there was little difference in the level of genetic diversity between landraces and improved varieties. This was likely because the accessions from various regions were traded and used. In order to further widen the genetic base of sweet potato cultivars, more efforts should be undertaken to collect and use sweet potato germplasm resources (Zhao et al. 2022). It may be useful to the plant breeders that, close genetic links may offer a route for the introduction of high-yielding and resistant genes into cultivars used by farmers and commercial growers (David et al. 2018).

STRUCTUE analysis also showed existence of admixture among the designated population. The analysis indicated that the third, fourth and fifth population (Fig. 2b) may not be a stable population as they are having more admixtures and are still in evolving phase. The factorial analysis also showed that the samples were clustered randomly with some genotypes clustering together with respect to the geographic locations. Overall, the factorial analysis reinstated the results obtained from STRUCTURE analysis. The genotype DOP-MIX-94/36 which is totally an ungrouped one and did not exhibit any kind of grouping pattern based on their geographical origin (Fig. 1). Previous studies had also demonstrated this type of genetic diversity analysis in case of sweet potato (Yada et al. 2010; Roullier et al. 2013). Probably self-incompatibility and vegetative propagation are two major factors affecting genetic constitution of sweet potato, and geographic origin has less impact. Self-incompatibility also imparts limitation in inbreeding and allele fixation, leading to prevention of new natural combinations (Yada *et al.* 2010). Further, research needs to be carried out to find the factors defining the population structure (Bruckner 2004). Recent research on the genotypes of African and American ancestry revealed substantial genetic diversity and relatively close genetic gaps between the genotypes. Recently, the population structure analysis has demonstrated support for four ancestral populations with many of the accessions having lower levels of gene flow from the other populations (Anglin et al. 2021). This was especially true of germplasm derived from Peru, Ecuador, and Africa. In present study, three separate genetic groups were identified by cluster analysis among the 31 genotypes, and the clustering patterns generally matched the geographical origin and ancestry of the genotypes.

Selection of genetically similar parents leads to the restriction of genetic variability in the offspring. Continuation of this condition contributes to a narrower genetic base with slower genetic gain. Hence, risk of crop vulnerability increases as adaptation through selection is more priority compared to new variability generation (Wilkinson *et al*. 2001). In these circumstances, germplasm collection and preservation is the best way to mitigate the demand of the growing human population. This holds true in every aspect of sweet potato cultivation as it is the clonally propagated crop. Hence, morphological traits, geographical origin, and genetic diversity are of immense importance (Kim et al. 2017). Previously performed studies confirmed high levels of heterozygosity like 0.75 for Kenya, 0.60 for Latin American, and 0.37 for Tropical American accessions result (Roullier et al. 2013). In this regards our Indian genotypes showed a maximum observed moderately higher heterozygosity 0.4244 which is in between of the earlier (Table 3). Higher levels of genetic diversity and heterozygosity may occur by the outcrossing and self-incompatibility in case of sweet potato. Self-incompatibility in the field level can give rise to much genetic diversity from crossing (Yada et al. 2010). Understanding the genetic diversity of the relevant germplasm is crucial for designing efficient breeding strategies. The genotypes of sweet potatoes showed modest genetic diversity according to earlier marker analysis findings. Although sweet potatoes are extremely heterozygous, the genetic variety of advanced varieties was also impacted by the breeding process's limited use of parent selection. According to earlier literature, breeding efforts should make advantage of accessions with a diverse genetic background, including introduced types, to produce new hybrid varieties of sweet potatoes with novel alleles and greater genetic diversity (Paliwal 2020).

These genotypes were collected from different areas of Indian subcontinent sowing great genetic diversity, which produced mainly two major groups under factorial analysis. They were of eastern to middle and southern part of India, indicating that sweet potato have some geographical effect on their adaptation. The present information would

be helpful for future marker-assisted breeding program of sweet potato.

Authors' contribution

Conceptualization of research (JC); Designing of the experiments (JC); Contribution of experimental materials (JC); Execution of field/lab experiments and data collection (PPS, NK, SGR); Analysis of data and interpretation (PPS, NK, G); Preparation of the manuscript (PPS, NK, G).

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