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GENETIC DIVERSITY ANALYSIS OF SWEET POTATO [*IPOMOEA BATATAS* (L.) LAM.] GERMPLASMS THROUGH RAPD AND ISSR MARKERS

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Abstract

Sweet potato [*Ipomoea batatas* (L.) Lam.] germplasms are unique as they are heterozygous, vegitatively as well as sexually propagated and polyploid in nature. Forty-four germplasms from local farmer's field and from various centres of India were determined using 15 RAPD and 15 ISSR markers to evaluate their genetic relatedness. Average polymorphisms for RAPD and ISSR markers were found to be 86.72 and 81.64%, respectively. The OPM series primer produced 145 total bands with 138 polymorphic loci and ISSR markers gave 116 total bands with 101 polymorphic loci. The clusters were made using the UPGMA method using both the RAPD and ISSR data and group diverse yet similar germplasm in two different clusters. This gives ample information to use them in further improvement programme.

Introduction

The sweet potato [*Ipomoea batatas* (L.) Lam.] a natural hexaploid (2n = 6x = 90), having basic chromosome number x = 15 belongs to morning glory family, Convolvulaceae. Molecular marker analysis of wild and cultivated sweet potato of Central America revealed highest diversity due to the centre of origin (Huang and Sun 2000). However, the wild ancestor of this plant has not yet been identified. Thus one can consider the centre of origin followed by its domestication to Central or South America. However, one of the studies based on paleobotanical evidence reported that sweet potato genus *Ipomoea* belongs to Meghalaya (India) rather than America (Srivastava *et al*. 2018*)*. Sweet potatoes are cultivated in subtropical, tropical and warm temperate regions of the globe. There are various skin colours present on the crop like purple, brown, yellow, orange and red. Likewise, the red, orange or pink flesh is more favourable due to their sweet taste. These indicate the sufficient variation present in this crop which needs to be addressed using molecular methods.

To avoid confusion among genotypes and improper selection of parents for breeding programme, one must use molecular markers to appropriately characterize and classify them. The biggest advantages of molecular markers are their environmental independent nature; irrespective of time of development; not influenced by epistatic interactions (Koutita *et al*. 2005) and can target any part of the genome (de Vicente and Fulton 2003). It is evident that different molecular markers can target different regions of the genome thus providing more detail and diverse information of actual diversity present within genotypes (Dávila *et al.* 1999). RAPD were the first of the PCR-based markers and were developed independently by Williams *et al.* (1990). The Inter-Simple Sequence Repeats (ISSR) (Zietkiewicz *et al.* 1994) is arbitrary, neutral dominant markers bind to various microsatellite fragments and amplify fragments between them. Both types of marker require no sequence information, cheap and simplicity of use as well as small amount of

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sample requirement. However, very little information is available on use of molecular markers in India for diversity analysis in sweet potato. There was large variation (unpublished data) present with respect to flower shape, leaf shape, yield, height of the plant as well as skin color. Therefore, germplasms were analyzed to study RAPD and ISSR based genetic diversity in a sweet potato germplasms collected from farmer's field of Gujarat and various other collection from India, to compare diversity and phylogenic relatedness observed using RAPD and ISSR in the studied materials, and to assess reliability of both markers for diversity analysis.

Materials and Methods

The present investigation was carried out at AICRP on Tuber Crops, Regional Horticultural Research Station, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari, (20^0 57' North latitude and 72^0 54' East longitude at an altitude of about 11.98 meter above the mean sea level) Gujarat, India.

A total of 44 genotypes of sweet potato, representing local cultivars as well as collection from different sub centres of India (Table 1), were maintained and used for the present study. Young emerged leaf samples of 44 genotypes were collected early in the morning and used for DNA isolation described by Doyle and Doyle (1990) with some modifications**.** Investigation was carried out with 15 decamer RAPD (Table 2) and ISSR markers (Table 3). The amplified PCR products were analyzed through 1.5% agarose gel electrophoresis prepared in 1x TBE buffer.

Table 1. List of 44 germplasm used in this study.

1.	NSp-1	12.	NSp-12	23.	S-61	34.	Kamala Sundari
2.	NSp-2	13.	NSp-13	24.	765-4	35.	Kishan
3.	NSp-3	14.	NSp-14	25.	C-71	36.	POL-19-8-10
4.	NSp-4	15.	NSp-15	26.	CARISp-1	37.	POL-19-8-2
5.	NSp-5	16.	NSp-16	27.	CIP-440038	38.	S-1156
6.	NSp-6	17.	NSp-17	28.	CIP-440127 (Bhukanti)	39.	S-1281
7.	NSp-7	18.	NSp-18	29.	CIPSWA-2	40.	S-1-60
8.	NSp-8	19.	NSp-19	30.	Co3-4	41.	Sree Bhadra
9.	NSp-9	20.	NSp-20	31.	Gouri	42.	ST-10
10.	NSp-10	21.	187017-1	32.	IGSP.C-15	43.	ST-14
11.	NSp-11	22.	362-7	33.	IGSP-14	44.	SV-998(SV-98

Amplified RAPD and ISSR profile for all genotypes with each primer was recorded as presence (1) or absence (0) of band throughout the amplified profile with a primer. Each amplified band was considered as unique locus. The binary data were used to calculate genetic similarities based on Jaccard's coefficient (Jaccard 1908) and UPGMA (Unweighted Pair Group Method using Arithmetical Averages) and dendrogram was generated employing SAHN tool of NTSYS (Rohlf 2000) to determine the genetic relationship of sweet potato genotypes. Cluster analysis was done based on the un-weighted pair group method with arithmetic means (UPGMA) using NTSYS-pc version 2.11T (Sokal and Michener 1958). Various components were calculated as No. of monomorphic and polymorphic loci, total bands, polymorphism information content (PIC) (Garcia *et al.* 2004**)**, resolving power (Rp) (Prakash and Staden 2007), effective multiplex ratio (EMR), marker index (MI) and polymorphism percentage.

Results and Discussion

Fifteen arbitrary primers gave reproducible, polymorphic and finely resolved bands selected out of 15 primers. A total of 3229 amplicons with 145 loci, of which 138 loci were polymorphic with an average of 86.72 % polymorphism, with an average of 9.67 loci were generated per primer (Table 2, Fig. 1). Jaccard's similarity coefficients based on RAPD markers among the all pair-wise combinations of genotypes ranged from lowest values (0.220) between NSp-13 and NSp-12 indicating maximum genetic divergence to highest value (0.693) between NSp-15 and NSp-14 with an average value of 0.475. The UPGMA clustering algorithm based on RAPD data grouped

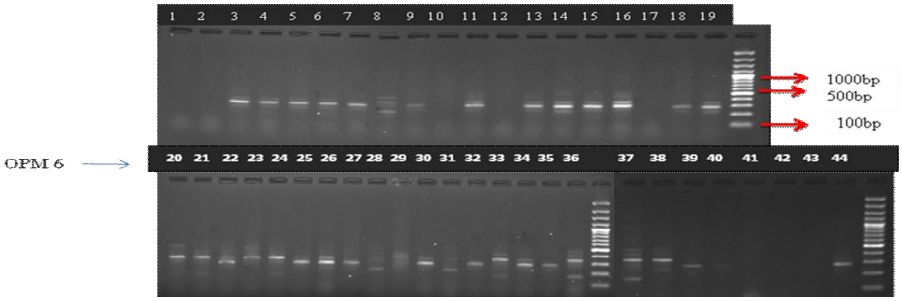

Fig. 1. RAPD profile sweet potato genotypes generated by OPM-17.and OPM 6.

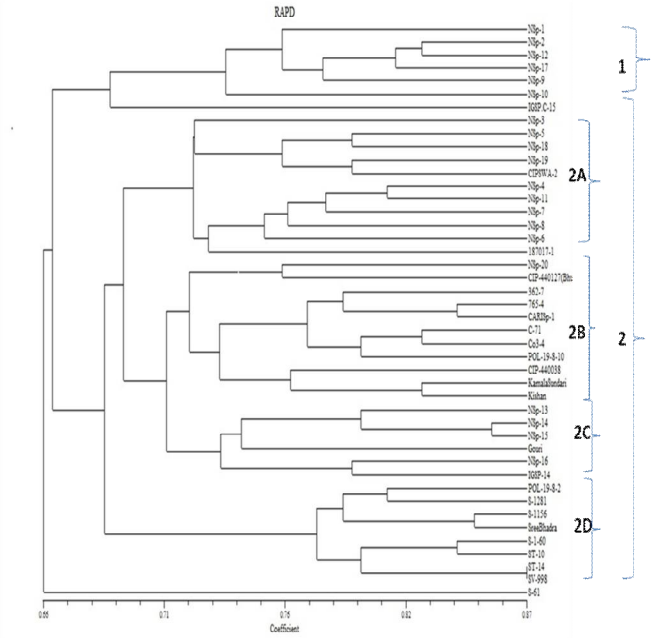

Fig. 2. Dendrogram depicted the classification of the 44 genotypes of sweet potato constructed using UPGMA method based on RAPD. The scale in the bottom is Jaccard's coefficient of similarity.

44 accessions into six groups at a cutoff value of 0.71**.** RAPD clearly distinguished all the species. Among the clusters, most of the samples from sweet potato were clustered into group two (37) than the other, while group one contained 7 genotypes. The second group was sub divided into 5 subgroups, namely 2A to 2E (Fig. 2).

Fifteen arbitrary primers produced a total of 3224 amplicons with 116 loci, of which 101 loci were polymorphic with an average of 87.06 per cent polymorphism (Table 3, Fig. 3) with an average of 7.73 loci were generated per primer (Fig. 4). The values of similarity coefficient obtained in ISSR analysis ranged from 0.21 between IGSP.C-15 and IGSP-14 to 0.79 between S-1-60 and S-1156 of germplasms with an average value of 0.25. The average similarity coefficient among genotypes was 0.47**.** Based on the genetic similarities, 44 genotypes were grouped into two major clusters and five sub clusters at a cutoff value of 0.70 (Fig. 2). The clustering of genotypes proved the suitability of ISSRs in detecting alleles characteristic of genotypes from different species. ISSR clearly distinguished all the species. Among the clusters**,** most of the samples from sweet potato were clustered into group two (2A, 2B, 2C, 2D, 2E) than the other, while group one contained 5 genotypes.

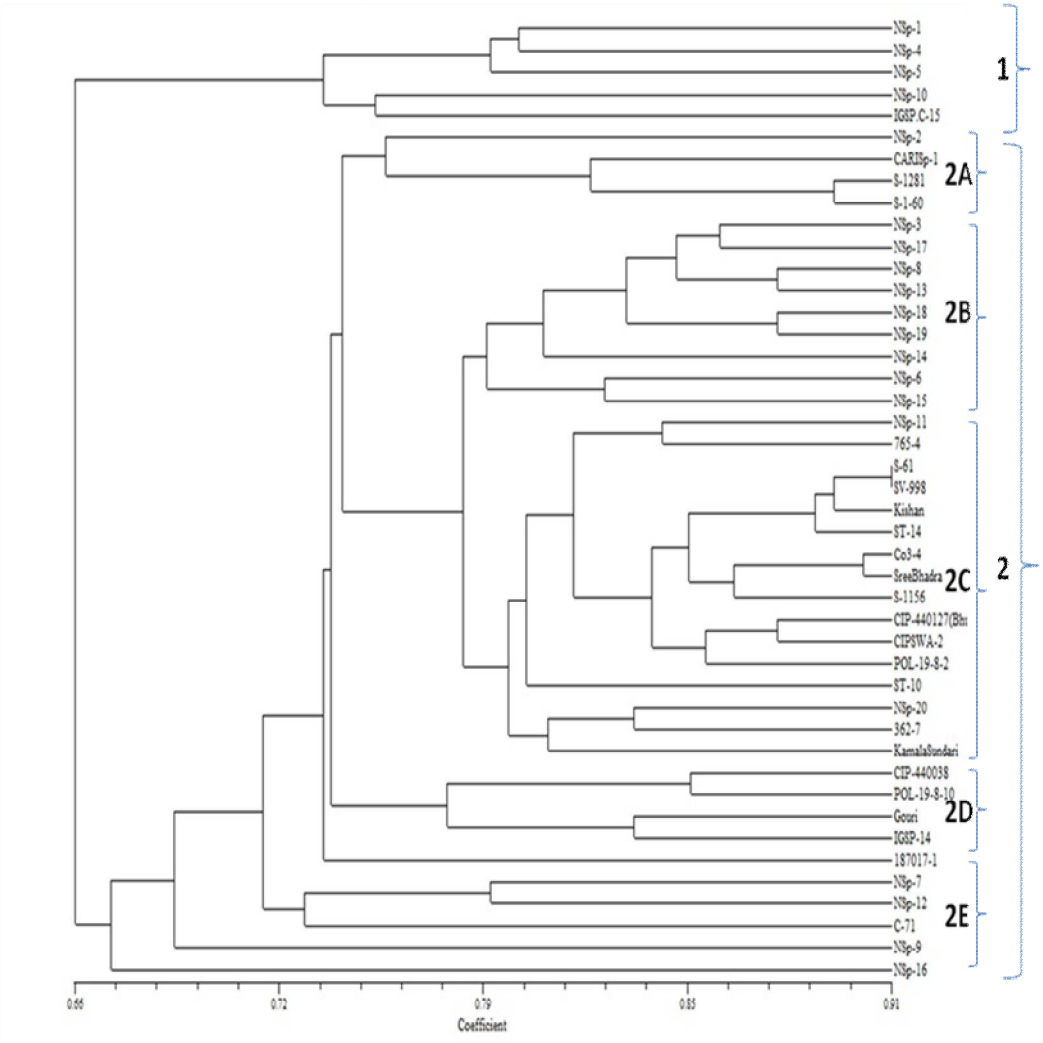

Fig. 3. Dendrogram depicted the classification of the 44 genotypes of sweet potato constructed using UPGMA method based on ISSR. The scale in the bottom is Jaccard's coefficient of similarity.

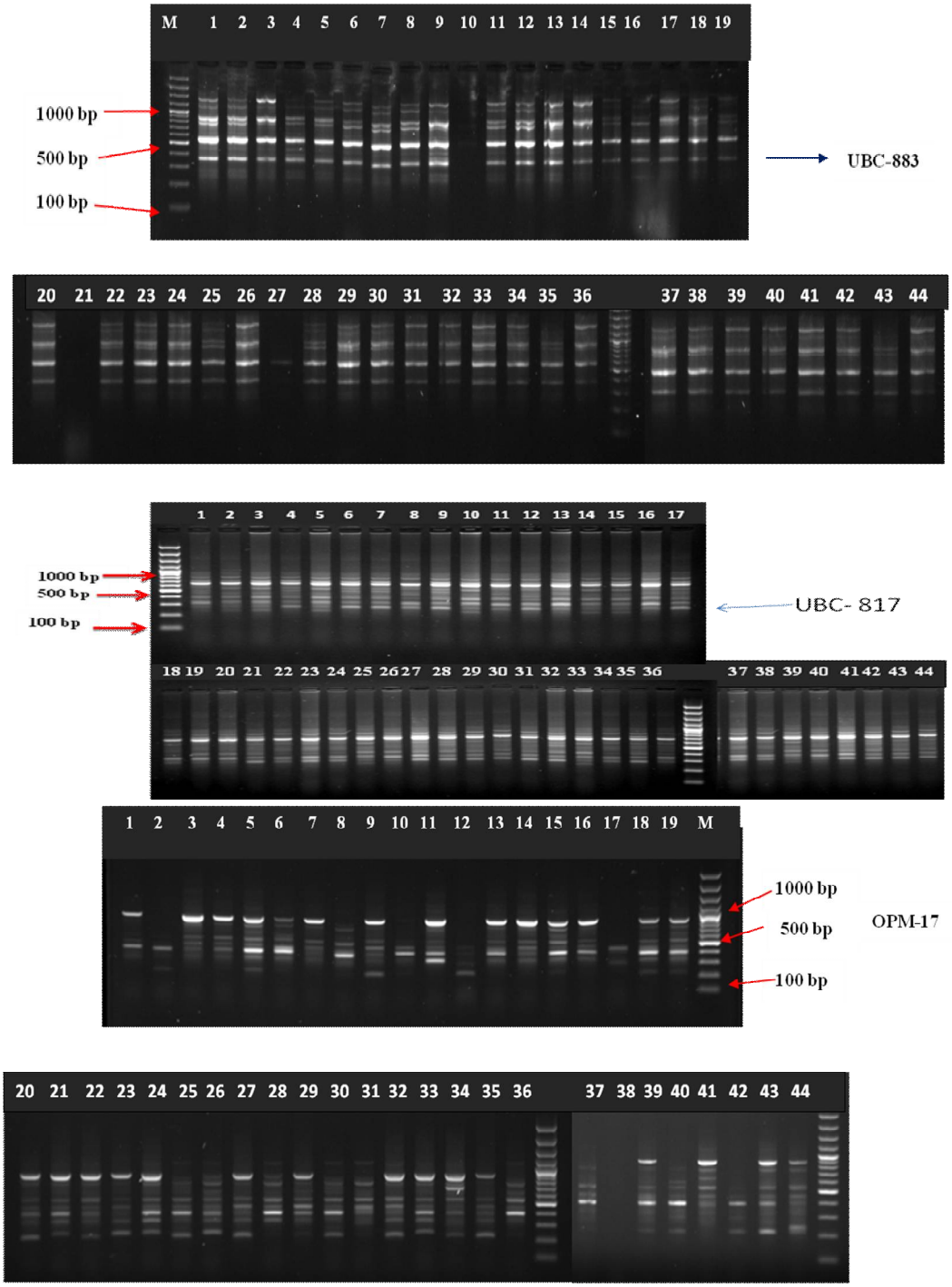

Fig. 4. ISSR profile of sweet potato genotypes generated by UBC-883,UBC 817.

Average polymorphism for RAPD analysis was found to be 86.72 %, whereas for ISSR it was 81.64% (Fig. 5). The cophenetic correlation value, r = 0.96 and r = 0.98 were studied for RAPD and ISSR, respectively and found that both markers are good fit for cluster analysis.

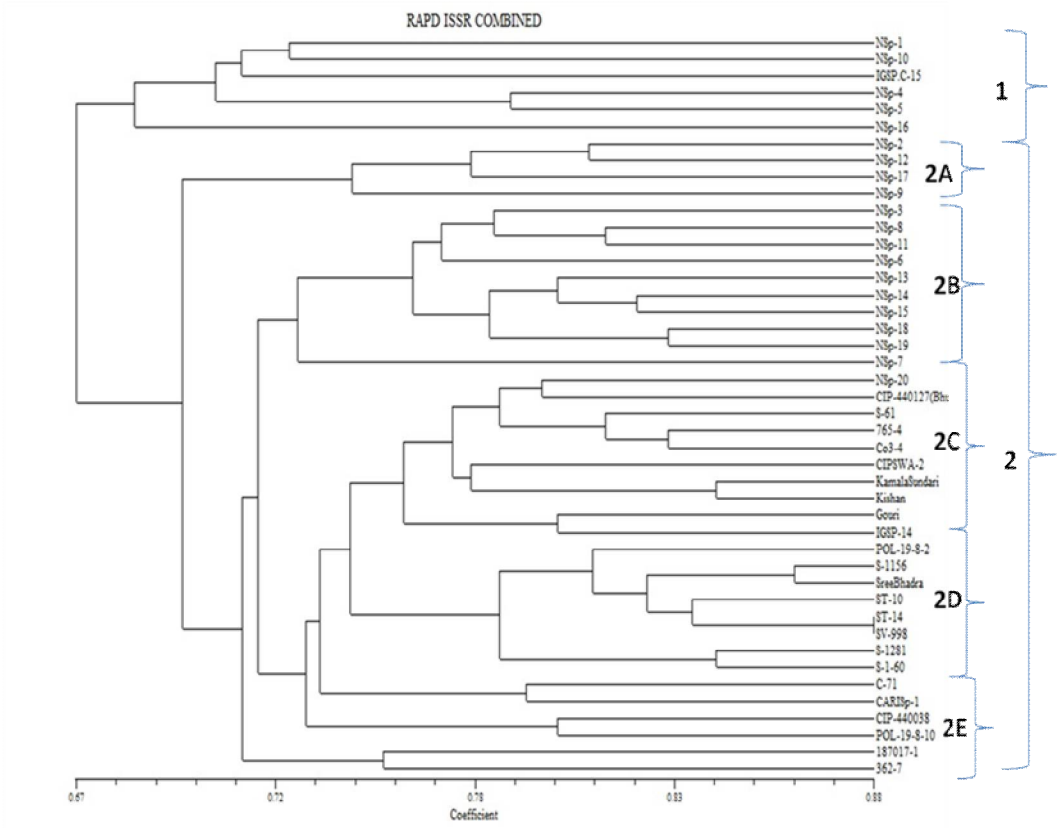

Fig. 5. Dendrogram depicted the classification of the 44 genotypes of sweet potato constructed using UPGMA method based on RAPD and ISSR markers. The scale in the bottom is Jaccard's coefficient of similarity.

In the present study, 44 sweet potato germplasms collected from local farmers' field as well as from the various research stations of India were studied for their genetic relatedness. There is always doubt regarding origin of sweet potato, as one of the groups from India has proved its origin in Meghalaya region of India (Srivastava *et al.* 2018). However, several other reports indicated that it belongs to America. Therefore, there is a need to identify variation present in them to use them in breeding programme. Hybridization of diverse germplasms and its adaptation to the local environments are results of continuous evolution of gene pool and variation in the traits. During this investigation, two sequence independent markers systems RAPD and ISSR were used. PIC analysis provided detail information about the polymorphism generated by each marker as a result most suitable and polymorphic marker can be selected for diversity analysis (Powell *et al.* 1996). In the present study, mean PIC% of 0.46 and 0.58 was obtained with ISSR marker and RAPD marker, respectively. The higher PIC value for the RAPD than that of the ISSR marker observed makes its MI (measure of the efficiency to detect polymorphism) more informative. The

Table 2. Statistics of amplified fragments generated in 44 genotypes of sweet potato based on RAPD primers.

Primer	Sequence (5'- 3')	Temp. (^{0}C)	TB	MB	PB	P %	PIC	Rp	EMR	MI	Band size
OPM-01	GTTGGTGGCT	38	6	6	0	0	0	0	0	0	210 - 620
OPM-02	ACAACGCCTC	38	8	0	8	100	0.73	7.40	8	5.84	260 - 800
OPM-03	GGGGGATGAG	38	9	0	9	100	0.57	11.27	9	5.13	300 - 700
OPM-04	GGCGGTTGTC	38	9	0	9	100	0.50	11.36	9	4.50	220 - 1200
OPM-05	GGGAACGTGT	38	8	0	8	100	0.41	11.86	8	3.28	280 - 1160
OPM-06	CTGGGCAACT	38	5	0	5	100	0.79	8.81	5	3.95	300 - 480
OPM-07	CCGTGACTCA	38	9	0	9	100	0.85	6.13	9	7.65	310 - 1140
OPM-09	GTCTTGCGGA	38	12	0	12	100	0.61	12.9	12	7.32	100 - 1250
OPM-10	TCTGGCGCAC	38	14	0	14	100	0.79	10.50	14	11.06	160 - 1180
OPM-11	GTCCACTGTG	38	12	0	12	100	0.72	10.54	12	8.64	180 - 960
OPM-12	GGGACGTTGG	38	12	0	12	100	0.50	14.31	12	6.00	110 - 1030
OPM-14	AGGGTCGTTC	38	12	0	12	100	0.73	9.63	12	8.76	230 - 880
OPM-16	GTAACCAGCC	38	13	0	13	100	0.42	17.36	13	5.46	200 - 1500
OPM-17	TCAGTCCGGG	38	8	1	7	87	0.43	11.81	6.12	2.63	270 - 1000
OPM-20	AGGTCTTGGG	38	8	0	8	100	0.66	8.68	8	5.28	180 - 900
Average			9.67	0.47	9.20	86.72	0.58	10.17	9.14	5.70	

Tm - Annealing temperature; TB - Total no. of amplified bands; MB - Monomorphic bands; PB - Polymorphic bands; P% - Polymorphism; PIC - Polymorphism information content; MI - Marker index; EMR - Effective multiplex ratio; Rp - Resolving power.

Table 3. Statistics of amplified fragments generated in 44 genotypes of sweet potato based on ISSR primers.

Primer	Sequence (5'- 3')	Temp. (^{0}C)	TB	MB	PB	P %	PIC	Rp	EMR	MI	Band size
UBC-847	CACACACACACACACARC	47	7	0	7	100	0.65	14.27	7	4.55	320 - 860
UBC-848	CACACACACACACACARG	47	5	0	5	100	0.53	12.63	5	2.65	480 - 1100
UBC-817	CACACACACACACACAA	47	9	9	0	0	0	0	0	0	260 - 700
UBC-871	TATTATTATTATTATTAT	51	7	0	7	100	0.75	5.63	7	5.25	190 - 1090
UBC-872	GATAGATAGATAGATA	51	3	0	3	100	0.36	4.77	3	1.08	300 - 700
UBC-873	GACAGACAGACAGACA	51	15	0	15	100	0.53	18.00	15	7.95	160 - 1050
UBC-875	CTA GCT AGC TAG CTAG	48	6	2	4	66.66	0.51	7.36	2.6	1.36	350 - 900
UBC-877	TGC ATG CAT GCA TGCA	48	11	0	11	100	0.55	12.36	11	6.05	150 - 860
UBC-878	GGATGGATGGATGGA	48	5	0	5	100	0.41	7.50	5	2.05	180 - 930
UBC-881	GGGTGGGGTGGGGTG	47	9	1	8	88.0	0.54	11.31	7.11	3.84	210 - 730
UBC-882	VBVATATATATATATAT	51	7	3	4	57.14	0.23	11.72	2.2	0.52	120 - 900
UBC-883	BVB TAT ATA TAT ATA TA	51	8	0	8	100	0.47	9.77	8	3.76	250 - 1200
UBC-884	HBHAGAGAGAGAGAGAG	47	9	0	9	100	0.51	11.18	9	4.59	200 - 800
UBC-886	VDV CTC TCT CTC TCT CT	47	9	0	9	100	0.50	11.22	9	4.50	230 - 1300
UBC-888	BDBCACACACACACACA	47	6	0	6	100	0.39	9.27	6	2.34	200 - 1000
Average			7.73	1	6.73	81.64	0.46	9.81	6.46	3.37	

Tm - Annealing temperature; TB - Total no. of amplified bands. MB - Monomorphic bands. PB - Polymorphic bands; P% - Polymorphism; PIC - Polymorphism information content. MI - Marker index. EMR - Effective multiplex ratio. Rp - Resolving power.

highest MI (11.06) was obtained by OPM-10 with mean of 5.70 using RAPD primer. On the other hand, UBC-873 ISSR primer gave highest MI (7.95) with mean of 3.37 using ISSR primer. This reflects the efficiency of marker to simultaneously analyse large number of bands (Powell *et al.* 1996). Therefore, PIC value and MI information together have been used to assess the informativeness of the markers. EMR is the product of the fraction of polymorphic bands and the number of polymorphic bands. Thus, higher MI and PIC will lead to higher EMR. Higher EMR indicated their potential discriminatory power within the genotypes studied. In the present assessment, EMR varied from 5 to 14 (average 9.14) with RAPD marker and 2.6 to 15 (average 6.46) with ISSR marker. Prevost and Wilkinson (1999) analyzed the data using resolving power.

RAPD showed the highest polymorphism level (95.17 %) than ISSR (87.06 %). Moulin *et al.* (2012) used RAPD and ISSR markers to study diversity present in 59 sweet potato landraces (*Ipomoea batatas* (L.) Lam.) obtained from rural area and 19 landraces from local markets. They obtained cophenetic correlation coefficients (CCC) of 0.80 for RAPDs and 0.89 for ISSRs. When, there is large number of germplasms as well as more number of primers used for screening and evaluation, the result would be more reliable and accurate. In the present analysis, mean PIC% of 0.462 with ISSR marker while mean PIC % of 0.58 with RAPD primer was obtained. Gwandu *et al.* (2012) observed 11 - 22 alleles per locus when studying elite sweet potato genotypes from Tanzania. Further, Rodriguez-Bonilla *et al.* (2014) reported a number of alleles ranging from 4 - 25 per locus when characterizing sweet potato germplasm from Puerto Rico. The high number of alleles in sweet potato could be due its hexaploidy nature (Karuri *et al.* 2010, Rodriguez-Bonilla *et al.* 2014). Several scientists have given probable reasons for this vast heterozygosity at the intravarietal level in sweet potato. The high level of genetic diversity found in sweet potato accessions could be a result of genetic makeup of sweet potato, accumulation of random mutations (Hernandez *et al.* 1964) resulted from asexual propagation of sweet potato via stem cuttings and adventitious buds arising from storage roots (Villordon and LaBonte 1996), along with selection, self-incompatible nature of the plant, out crossing nature of the plant as well as hexaploid nature (Yada *et al.* 2010) and geographic and environmental factors, which make local populations of this species an important genetic resource (He *et al.* 2006). Historically farmers have relied only on morphological traits in order to distinguish between different cultivars of a crop or by the degree of perception (selection for perceptual distinctiveness) they have considered morphological variation that constitutes a landrace as an identification unit (Veasey *et al.* 2007). In sweet potato, cultivation constraints such as lack of space between cultivars in the field, vine spreading and morphological variation can cause further confusion regarding the exact identity of the cultivars being grown in a field. In this study, more than 20 germplasm of NSP series which are locally selected clones from the farmer's field were used. The assumption that these vines were progenies produced through vegetative propagation. The genetic distances based on the Jaccard similarity index, using ISSR and RAPD markers, did not correlate with geographic distance between the sampling locations, demonstrating that the accessions did not have distance-related genetic variability. This result could be due to the widespread practice of exchanging accessions between neighboring farmers and relatives, resulting in the same genotype having different names in different localities.

The present study revealed good variability among genotypes using molecular analysis. No duplicates were found among the genotypes and these can be maintained as core collection and used for future diversity studies. The highly polymorphic RAPD and ISSR primers identified can be used for further genetic diversity characterization in future. The result from the present study serves to facilitate the development of better varieties by plant breeding and marker assisted breeding programs.

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