Journal of Root Crops, 2011, Vol. 37 No. 2, pp. 125-130
Indian Society for Root Crops
ISSN 0378-2409

Assessing Relationship between Indian and Exotic Cultivars of Sweet Potato (*Ipomoea batatas* (L.) Lam.) by Random Amplified Polymorphic DNA

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Received:10 August 2011; Accepted: 24 December 2011

Abstract

Sweet potato (*Ipomoea batatas* (L.) Lam.) is a crop of economic and social importance with nutritive values, high energy yield, wide adaptability and high level of genetic diversity. It is an important source of dietary fibre, minerals, vitamins and bioactive compounds such as β-carotene, phenolic acid and anthocyanin. Sweet potato clones possess diversity in skin and flesh colors (white, cream, yellow, orange and purple) of tubers. Random amplified polymorphic DNA (RAPD) analysis using 21 Indian and exotic cultivars of sweet potato comprising of diversified skin and flesh colour including orange fleshed sweet potatoes with arbitrary 15 decamer primers showed 164 polymorphic bands which fell into three major clusters *viz*., clusters A, B and C. Clusters A and B included both Indian and exotic orange-fleshed sweet potato cultivars, whereas cluster C included mostly white-fleshed cultivars of Indian origin. The predictive linkages will lead to a valuable breeding programme for the genetic improvement of sweet potato and verification of pedigree records.

Key words: Sweet potato, cultivars, *Ipomoea batatas*, RAPD, genetic diversity, β carotene

Introduction

Sweet potato (*Ipomoea batatas* L. (Lam.)) is an important tuber crop grown in many tropical and subtropical regions of the world. It has nutritive values, high energy yield, wide adaptability and high level of genetic diversity. In sub-Saharan Africa, sweet potato plays a major role in providing food for the population and is the second most important crop after cassava (Hijmans et al., 2001). This crop has received much attention for global food security. The importance of sweet potato and the high level of genetic diversity in this crop are reflected in the fact that the field gene banks in national program across Asia and Pacific regions are maintaining more than 16000 accessions (Thomas and Raman, 2000). There are thousands of sweet potato genotypes cultivated around the world. The number of cultivars maintained in India is estimated to be around 2200 (Naskar, 1996). Genetic variation and conservation of diversity within a crop species are the basis for all varietal improvement programmes. Because of biological complexities of sweet potato, information on genetic identity and relationship of sweet potato genotypes is crucial to the improvement of core collection and for tailoring information as germplasm explorations to focus on those areas with maximum genetic diversity. The advancements of plant molecular techniques related to DNA based markers provide powerful and reliable tools for exploring genes of agronomically important traits and in genome mapping.

RAPDs (Random Amplified Polymorphic DNA)

(Williams et al., 1990) are markers which are simple to generate and have been used in many studies (Chalmers et al., 1992; Lashermes et al., 1993), including studies on *Ipomoea* in its centre of origin, Mexico and South America (Jarret and Austin, 1994). Genetic polymorphism of sweet potato accessions has been studied using DNA amplification fingerprinting (DAF) (Prakash et al., 1996) and by RAPD (Dhillon and Ishiki, 1999). The use of molecular markers in sweet potato and other *Ipomoea* species for genetic information such as genetic identity and relationship between genotypes has been well documented. However, there is dearth of information on molecular markers in Indian sweet potato genotypes. Hence, the present study was undertaken to assess the relationship between Indian and exotic cultivars through RAPD.

Materials and Methods

Plant material

Twenty one cultivars of sweet potato were used in the present investigation, which were obtained from the field gene bank of the All India Co-ordinated Research Project on Tuber Crops, Bidhan Chandra Krishi Viswavidyalaya (BCKV) centre, West Bengal, India and the details are given in Table 1.

DNA extraction

Genomic DNA was extracted from the tender leaves of 3-4 weeks old plant using protocol described by Varadarajan and Prakash (1991). One gram leaf sample was required to yield good quality DNA. Tender leaf samples were collected and washed with sterile double distilled water. One gram was taken and crushed in pre-chilled pestle mortar using liquid nitrogen. The DNA pellet was re-suspended in 100 μl of TE buffer (Tris-HCL 10mM and EDTA 1mM pH8.0). Quantification of DNA was done by electrophoresis using 0.8% agarose gel and DNA was diluted to a final concentration of 30-50ng μl^{-1} with sterile distilled water.

PCR procedure

Amplification of DNA was obtained by the protocol outlined by Williams et al. (1990). The DNA was

Table 1. Description of sweet potato cultivars used for RAPD analysis

Serial No.	Name of cultivars	Place of collection	Skin and flesh colour of tuber
1	CIPSWA-2	*CIP Centre, Bhubaneswar, Orissa	Red skin, orange-fleshed
2	SP-362-7	**CTCRI, RC, Bhubaneswar, Orissa	Red skin, orange-fleshed
3	S-594	CTCRI, RC, Bhubaneswar, Orissa	Light red skin, white-fleshed
4	SP-440038	CIP Centre, Bhubaneswar, Orissa	Deep red skin, orange-fleshed
5	SP-420127	CIP Centre, Bhubaneswar, Orissa	Red skin, orange-fleshed
6	IGSP-15	***IGKV, Chhattisgarh	Light red skin, orange-fleshed
7	S-1156	CTCRI, Trivandrum, Kerala	Red skin, Orange-fleshed
8	SV-98	CTCRI, Trivandrum, Kerala	Red skin, orange-fleshed
9	BCSP-19	BCKV, Kalyani, West Bengal	Light red skin, white-fleshed
10	S-1281	CTCRI, Trivandrum, Kerala	Light red skin, white-fleshed
11	Kamala Sundari	BCKV, Kalyani, West Bengal	Orange skin, orange-fleshed
12	ST-14	CTCRI, RC, Bhubaneswar, Orissa	Red skin, orange-fleshed
13	S-61	CTCRI, RC, Bhubaneswar, Orissa	Light red skin, orange-fleshed
14	90/704	CTCRI, RC, Bhubaneswar, Orissa	Light red skin, white-fleshed
15	S-30/21	CTCRI, RC, Bhubaneswar, Orissa	Light red skin, white-fleshed
16	90/101	BCKV, Kalyani, West Bengal	Red skin, pale orange-fleshed
17	BCSP-14	BCKV, Kalyani, West Bengal	Deep red skin, white-fleshed
18	BCSP-10	BCKV, Kalyani, West Bengal	Deep red skin, white-fleshed
19	BCSP-7	BCKV, Kalyani, West Bengal	Light red skin, white-fleshed
20	WBSP-4	BCKV, Kalyani, West Bengal	White skin, white-fleshed
21	BCSP-18	BCKV, Kalyani, West Bengal	Scarlet red skin, white-fleshed

*CIP: International Potato Centre, **CTCRI: Central Tuber Crops Research Institute, ***IGKV: Indira Gandhi Krishi Viswavidyalaya

amplified with 15 decamer primers from Operon selected by preliminary screening (60 primers) for giving higher levels of polymorphism and reproducible fragment patterns. Ingredients of each reaction included template DNA 25-30 ng, dNTPs 200 μM each, Taq DNA polymerase 1.5 units, $MgCl_2$ 2 mM, 1x PCR reaction buffer (Bangalore Genei Ltd. India) and 15ng of each decamer primer in a total volume of 25μl. Amplification was performed in a thermal cycler (Eppendorf Mastercycler, Germany). Complete reaction consisted of 36 cycles, each cycle consisting of three steps; denaturation at 92°C for 30 sec, annealing at 38°C for 30 sec, extension at 72°C for one min, with an initial denaturation at 94°C for two min and final extension at 72°C for seven min, followed by cooling at 4°C. Amplified fragments were separated on 1.0 % agarose (Sisco Research Laboratory India) gel containing ethidium bromide (0.5μg ml^{-1} of agarose) at 60 Volt for five hours in Tris Acetate EDTA buffer.

Data analysis

Amplified fragments from all the primers, which were reproducible over two amplifications, were scored by the Total Lab gel documentation software (Biotech R&D Laboratories, Yercaud, India). The size of the fragments was estimated by using 1.0 kb ladder marker (Biolab, England). Bands were manually scored, '1' for presence and '0' for absence and the binary data were used for statistical analysis. A genetic dissimilarity matrix was calculated according to Squared Euclidean Distance that estimated all pair-wise differences in the amplification product (Sokal and Sneath, 1973) and the cluster analysis was performed based on Ward's method using a minimum variance algorithm (Ward, 1963).

Results and Discussion

In RAPD analysis, a total of 185 unambiguous readable and reproducible genomic DNA fragments were amplified from the 15 decamer primers used. The number of amplified fragments varied from 7-18 with an average of 12.3 fragments per primer, and the maximum number of products were observed with the primer OPM-09 and the size of fragments ranged between 250bp - 2000bp (Table 2). Out of these 185 fragments, 164 were polymorphic with an average of 10.93 per primer and 88.64% of the total fragments were polymorphic. The amplification products obtained with primers OPH-14, OPM-09, OPA-01and RP-3 were highly informative (Fig. 1 a-d). In every PCR reaction, DNA fragments were not found in the negative control while similar banding pattern were amplified in positive control indicating contamination free PCR ingredients and consistency of the protocol. Monomorphic bands were not observed with any polymorphic primer. These results were confirmed in repeated three runs of DNA of 21 sweet potato cultivars with respective primers. The level of variation among these cultivars was large enough to permit the development of species specific profiles from the RAPD data (Fig.1 a-d). The combinations of primers produced some unique finger prints for 21 cultivars exhibiting close relationship.

Based on estimated proximity matrix scale generated from the data of RAPD, highest genetic dissimilarity coefficient value was noticed between the genotype Kamala Sundari and S-61 (100) followed by 90/101 and S-61 (97) and the closest proximity

Table 2. Nucleotide sequence of Operon and other primers used to distinguish, estimate genetic distance and create dendrograms of the 21 sweet potato cultivars

Primer	Nucleotide sequence	Total number of bands	Number of polymorphic bands
OPH-14	5'-ACCAGGTTGG-3'	18	17
OPM-09	5'-GTCTTGCGGA-3'	19	18
OPO-09	5'-TCCCACGCAA-3'	12	9
OPQ-01	5'-GGGACGATGG-3'	10	7
OPQ-09	5'-GGCTAACCGA-3'	12	11
OPZ-11	5'-CTCAGTCGCA-3'	11	10
RP-3	5'-GTCTGACGGT-3'	13	12
RA-02	5'-CAGCTCAAGT-3'	12	12
RP-1	5'-CGATCGAGGA-3'	11	9
RA-04	5'-GCAGAGCATC-3'	13	13
OPA-01	5'-CAGGCCCTTC-3'	11	9
OPA-02	5'-TGCCGAGCTG-3'	12	11
OPA-03	5'-AGTCAGCCAC-3'	10	8
OPA-04	5'-AATCGGCGTG-3'	9	7
OPA-05	5'-AGGGGTCTTG-3'	12	11
Total		185	164

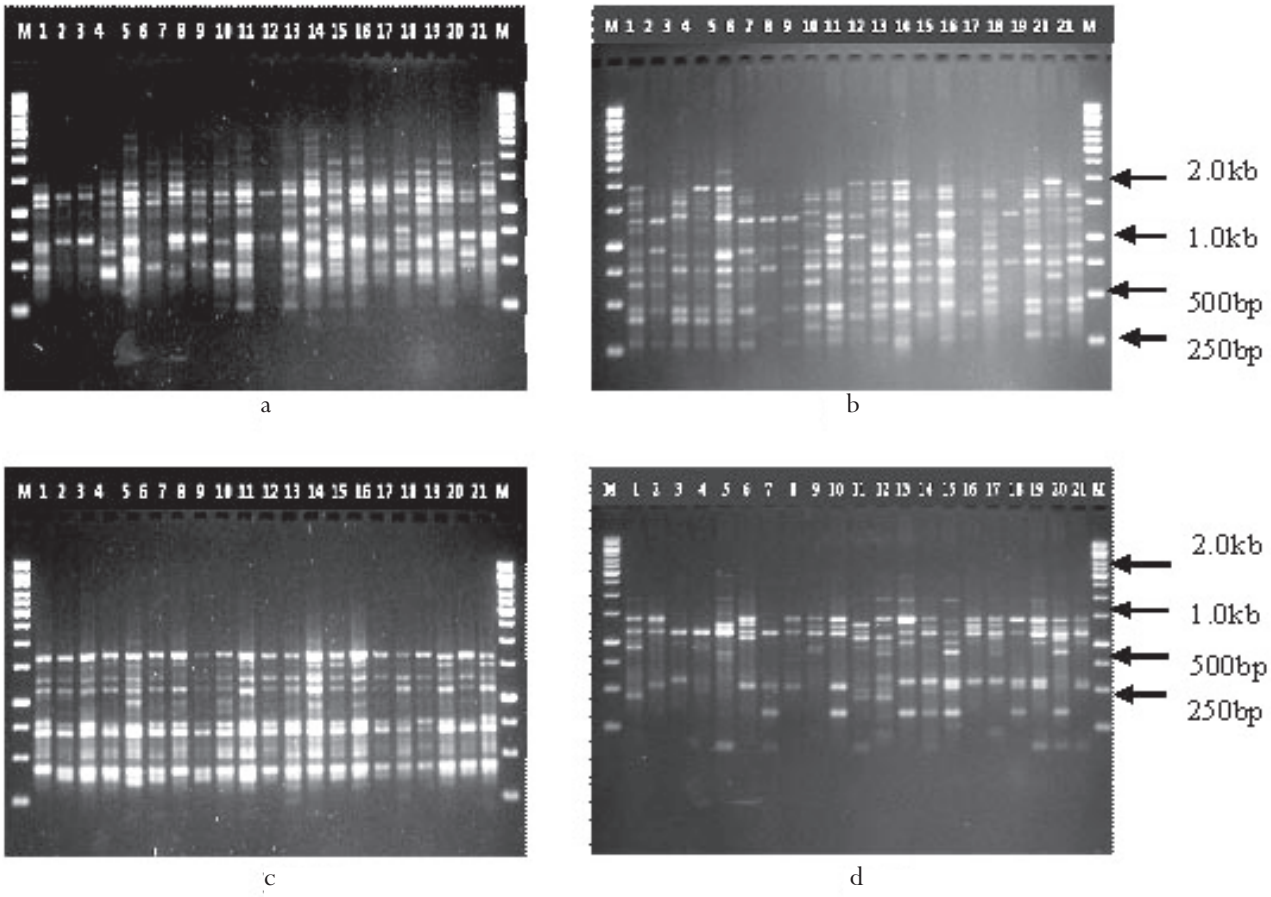

Fig. 1 a-d. RAPD pattern obtained from 21 cultivars of sweet potato with the primer (a) OPH-14 (b) OPM-09 (c) OPA-01and (d) RP-3

value was noticed between BCSP-7 and BCSP-18 (29) followed by SV-98 and SP-362-7 (33) (Table 3). The dendrogram made with four RAPD primers showed that the 21 sweet potato cultivars formed three distinct major clusters A, B and C (Fig.2). The maximum distance between all these clusters was nearly 175 units. The distance between cluster A and B was nearly 110 units, while cluster C showed the maximum distance from A and B. The cluster A contained nine orange-fleshed sweet potato cultivars/genotypes *viz.*, CIPSWA2, SP 420127(CIP), S1156, S594, IGSP15, BCSP19, WBSP4, S1281 and ST14. The cluster B consisted of seven orange- fleshed and white-fleshed sweet potato cultivars i.e., SP362-7, SV98, Kamala Sundari, 90/704, SP 440038, 90/101 and BCSP10. The three cultivars, SP440038, 90/101 and BCSP10 in

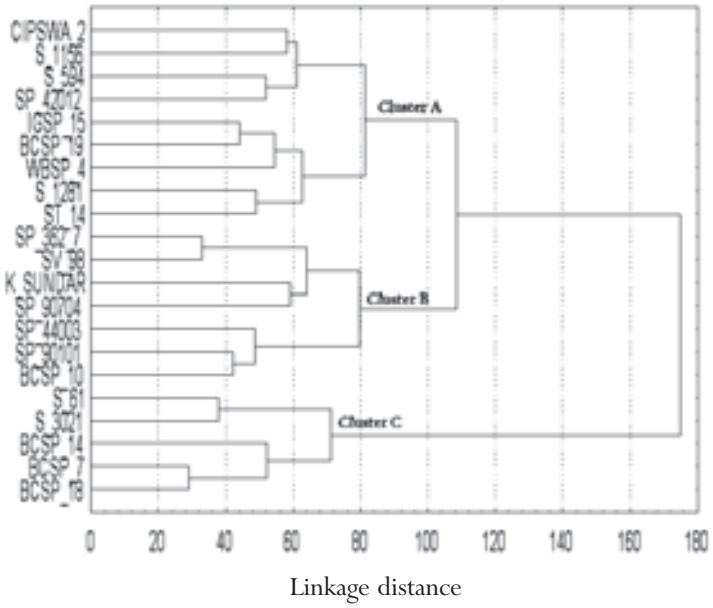

Fig. 2. Dendrogram constructed for 21 cultivars of sweet potato with 4 RAPD primers (pooled)

Table 3. Squared Euclidean distance matrix of 21 sweet potato cultivars based on RAPD markers

Cultivars	Matrix file input																				
	CIPSW A-2	SP-362 -7	S-594	SP-440038	SP-420127	IGSP-15	S-1156	SV-98	BCSP-19	S-1281	K-Sundari	ST-14	S-61	SP-90704	S-3021	SP-90101	BCSP-14	BCSP-10	BCSP -7	WBSP -4	BCSP -18
CIPSWA-2	.000																				
SP-362-7	55.000	.000																			
S-594	55.000	58.000	.000																		
SP-440038	52.000	55.000	59.000	.000																	
SP-420127	59.000	72.000	52.000	67.000	.000																
IGSP-15	62.000	45.000	55.000	60.000	57.000	.000															
S-1156	58.000	55.000	57.000	58.000	61.000	48.000	.000														
SV-98	68.000	33.000	69.000	58.000	89.000	56.000	58.000	.000													
BCSP-19	58.000	55.000	67.000	48.000	63.000	44.000	60.000	58.000	.000												
S-1281	52.000	69.000	61.000	52.000	57.000	54.000	68.000	74.000	50.000	.000											
K-Sundari	59.000	58.000	66.000	55.000	76.000	65.000	65.000	55.000	55.000	73.000	.000										
ST-14	63.000	66.000	68.000	67.000	56.000	47.000	65.000	71.000	59.000	49.000	66.000	.000									
S-61	69.000	86.000	76.000	83.000	52.000	65.000	79.000	93.000	71.000	55.000	100.000	56.000	.000								
SP-90704	68.000	59.000	71.000	62.000	77.000	58.000	62.000	48.000	54.000	60.000	59.000	57.000	89.000	.000							
S-3021	65.000	78.000	62.000	77.000	56.000	69.000	79.000	93.000	71.000	51.000	94.000	60.000	38.000	81.000	.000						
SP-90101	64.000	53.000	69.000	46.000	79.000	56.000	60.000	52.000	50.000	70.000	61.000	71.000	97.000	50.000	79.000	.000					
BCSP-14	56.000	77.000	65.000	72.000	55.000	56.000	64.000	88.000	62.000	58.000	77.000	49.000	57.000	66.000	63.000	76.000	.000				
BCSP-10	58.000	55.000	71.000	48.000	73.000	56.000	60.000	56.000	58.000	68.000	73.000	73.000	77.000	64.000	69.000	42.000	62.000	.000			
BCSP-7	66.000	83.000	75.000	78.000	53.000	68.000	80.000	96.000	68.000	56.000	89.000	57.000	43.000	74.000	49.000	82.000	46.000	70.000	.000		
WBSP-4	58.000	45.000	65.000	60.000	67.000	50.000	64.000	60.000	54.000	62.000	63.000	57.000	79.000	60.000	69.000	58.000	62.000	60.000	62.000	.000	
BCSP-18	51.000	70.000	68.000	67.000	58.000	67.000	67.000	85.000	59.000	47.000	74.000	56.000	54.000	65.000	50.000	73.000	47.000	61.000	29.000	53.000	.000

the separate sub-cluster were developed by selection of Indian isolates.The five cultivars i.e. BCSP7, BCSP14, BCSP18, S3021 and S61 found in cluster C are white-fleshed sweet potato from India (Table 3, Fig.2). Closest relationships were observed between BCSP7 and BCSP18 grouped together in cluster C. The primers, OPM-09 and RP-3 generated markers enabling the discrimination of orange-fleshed and white-fleshed cultivars of sweet potato of both exotic and Indian collections (Fig. 1b and 1d). The orange coloured flesh and skin of Kamala Sundari was distinct among the 21 cultivars (Fig. 1a). Orange-fleshed sweet potato formed a more divergent group from the white-fleshed cultivars. Limited variations were observed among the white-fleshed cultivars collected from a particular region. The cultivars BCSP 7, BCSP 14, BCSP 18 which originated from the same geographical conditions showed highest level of similarities.

High genetic variability among the cultivated sweet potato was reported based on RAPD (Connolly et al., 1994, He et al., 1995, Jarret and Austin, 1994). Genetic polymorphism was identified using DNA amplification fingerprinting (DAF) of 70 accessions collected around the world which showed very high variability (Prakash et al., 1996). Primers generated 46 polymorphic markers among 29 *Ipomoea batatas* accessions from different geographical locations around the world. One primer having 10 products enabled discrimination of all 29 accessions studied (Dhillon and Ishiki, 1999). The efficiency of RAPD markers in determining polymorphism in sweet potato lines is high. The present study attributed high unit of diversity between exotic and Indian cultivars which allowed distinction between orange-fleshed and white-fleshed sweet potato. Furthermore, RAPD data from the present study seems to support the pedigree information of sweet potato.

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