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Genetic variation of high yielding drought resistant sweet potato as evident by RAPD markers

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Abstract

Detailed agronomical analysis and random amplified polymorphic (RAPD) markers were used for determining the genetic diversity among ten varieties of sweet potato (*Ipomoea batatas* (L.) Lam), one of the leading tuber crop belonging to the family Convolvulaceae. Varieties developed by Central Tuber Crops Institute, Trivandrum and its Regional Centre, Bhubaneswar with diverse parentage from diverse eco-geographical areas was analyzed for their genetic relationship. A total 1035 amplicons were generated among the ten varieties out of which ~79% bands were found polymorphic. Inter-varietal polymorphism among ten varieties of *I. batatas* varied between 60.0 to 89.7%. Clustering based on similarity index was done following Unweighted Pair Group with Arithmetic mean (UPGA) method and intra-genetic relationships were analysed. The resultant dendrogram of the RAPD data exhibited prominent patterns of inter-varietal relationships that are discussed in the light of the their physio-morphological characters. It was evident from RAPD data that high degree of genetic divergence exists only in var. Kalinga and var. Sourin; two pink skinned tuber producing varieties. However, no much genetic variation were found among var. ST-10, ST-13 and ST-14 except presence of 3000bp marker in 'ST-14' and 800bp marker in 'ST-14' and 'ST-13' besides their common marker of 900bp in all the studied varieties in OPD-12. Omission of 600bp and 700bp DNA bands in 'ST-10' differentiate with other 'ST-13' and 'ST-14' in OPN-4. 'Kalinga' and 'Sourin' showed more closer genetic affinity forming an out group from the rest of the varieties as per the phylogenetic tree is concerned. It suggests their adaptation in various conditions suitable for the specific habitat of particular varieties.

Key words: Agronomic traits, *Ipomea*, RAPD markers, tuber crop, tuber yield

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1. Introduction

The widespread loss of the world's biological diversity is one of the most serious global crises today. The sweet potato, *Ipomoea batatas,* is a member of the family Convolvulaceae. The sweet potato is the only member of the genus *Ipomoea* whose roots are edible. South America and the Mayas of Central America grew several cultivars of sweet potato and they called the plant "cassiri". However, this plant is now cultivated throughout tropical and warm temperate regions. The sweet potato apparently was introduced into Japan from China, sometime around 1700 by way of the Ryuku Islands (Boswell and Bostelman, 1949). In India sweet potato was introduced by Portuguese travelers. In Kyushu today, it is called kara-imo (ka-ra-e-mo), meaning Chinese potato. The sweet potato is a crop plant whose large, starchy, sweet testing tuberous roots are an important root vegetable. The young leaves and shoots are sometimes eaten as greens. The edible tuberous root is long and tapered, with a smooth skin whose colour ranges between red, purple, brown and white. The growing of sweet potatoes is a billion-dollar industry. Currently, North Carolina provides 40% of the annual U.S. production of sweet potatoes. In many African countries sweet potatoes form a large part of the food of the people, ranking seventh among food crops in annual production in the world (Bovell-Benjamin 2007). However, the use of sweet potatoes in the United States is relatively low compared to other staple crops and generally regionalized for both growth and nutrition. It's economic importance and general consumption appear to be increasing with the advent of improved varieties as well as improved storage and processing facilities. In India, sweet potato is grown in all states except humid and temperate environment in an area of 0.14 million hectares producing 1.17 million tones of tuber. The crop is cultivated by small and marginal farmers for consumption by roosting, baking, boiling, frying and as subsidiary vegetable. Foliage and unmarketable tubers are used to fed other livestock and particularly to pig feed (Naskar *et al*., 2007.)

Although sweet potato breeding was stared in 1904 (Tioutine, 1935) but cytological analysis were started for the breeding work in interspecific hybridization (Tring *et al.*, 1957). RAPD, ISSR and AFLP markers were used to identify the duplicates or distinguishing closely morphologically similar varieties or accessions by few workers (Sagredo *et al*., 1998, Zhang *et al.*, 1998, 2000). Interspecific genetic relationships were although established among *Ipomoea lacunose, I. ramoni, I. trichocarpa* and *I. triloba* (Jones and Deonier, 1965) using RAPD marker long back but not much of work have been done in various Indian varieties of sweet potato except genome variation and RAPD marker variation in interspecific level by Dhillon and Ishiki (1999). Moreover, Gichuki *et al.* (2003) could established genetic polymorphism among 74 sweet potato varieties collected from different agroclimatic zones of 23 sweet potato producing countries. Microsatellite, DAF, RAPD and AFLP markers were used to analyze the genetic variability among the species and to establish the taxonomic relationships of *I. batatas* (Buteler *et al*., 1999, Connolly *et al.*, 1994, Jarret and Austin, 1994, He *et al*., 1995, Sagredo *et al*., 1998). Therefore, RAPD is a very reliable and easier, low cost technology in DNA fingerprint to establish genetic polymorphism even in varietal level. This communication is a part of the ongoing research on genetic relationship analysis among various sweet potato varieties available in Central Tuber Crop Research Institute (CTCRI), Bhubaneswar, we intended to establish the genetic relationships and phylogenetic affinities among ten popular Indian high yielding varieties of sweet potato considering morphological characters together using RAPD markers.

2. Materials and methods

2.1 *Plant materials*

Ipomoea batabas (L) Lam. varieties collected from the experimental germplasm garden of Central Tuber Crop Research Institute, Bhubaneswar have been depicted in Table 1. An equal quantities of young leaf sample from single plant of each variety were collected separately and stored in a -85^{0}C freezer for DNA extraction.

2.2 *Morphological data collection*

The genotypes were grown in field during June 2007 and harvested after 120 DAP (Days After Planting). Recommended cultural practices were followed. Morphological and storage root data were collected based on Biodiversity International Descriptors. The morphological characters were recorded from the middle sections of the main stem. Observations on morphological characters were recorded 12 weeks after planting of primary vine, storage root characters were recorded using medium to large sized storage roots from the entire harvest of the genotype. Yield data were recorded based on root yield. The genotypes comprise of different accessions indigenous and exotic from different eco-geographical areas. Out of the 10 genotypes, 7 were released varieties and 3 were known clones. Thus, the material represented a wide range of geographic diversity.

2.3 *Isolation of DNA*

For DNA isolation, 5g of leaf tissue were ground to fine powder with liquid nitrogen and the powder was suspended in 30 ml of suspension buffer (pH 8.0) containing 50 mM EDTA, 100 mM Tris-HCl, 0.8 M NaCl, 0.5 M sucrose, 2% Triton X 100 and 0.1% β-mercapto-ethanol that incubated at 60^{0}C for 30 min. The suspension was centrifuged at 10,000g for 15 min at room temperature and the pellet was suspended in 20ml of extraction buffer (20mM EDTA, 100 mM Tris-HCl, 1.5M NaCl, 2% CTAB and 1% β-mercaptoethanol, pH 8.0). Again, the suspension was incubated at 60^{0}C for 45 min followed by chloroform: isoamyle alcohol (24:1) extraction and ethanol precipitation at –20^{0}C for 2h.

DNA was hooked out and dried with vacuum drier and TE (10 mM Tris-HCl, 1mM EDTA) was added to dissolve the DNA. The DNA was further purified with RNAse at 37^{o}C for 1h followed by chloroform: isoamylalcohol extraction and ethanol precipitation in the presence of 0.3M sodium acetate (pH 5.2). The DNA was spooled out, washed in 70% ethanol, air dried and dissolved in TE buffer and the DNA concentration was estimated in Versafluor TM Fluorometer (Bio-Rad, USA) using Hoechst 33258 as the dye. The DNA was diluted to final concentration of 25ng μl^{-1} using TE buffer for using as template for RAPD analysis.

2.4 *RAPD analysis*

RAPD profiles were generated by using single decamer random oligonucleotide primers (Operon Technologies, Alameda, USA) in polymerase chain reaction (PCR) following the standard protocol of Williams *et al.* (1990). The sequence of primer is given in Table 2. Each polymerase chain reaction (PCR), 25µl of amplification mixture was taken that contains 25ng of genomic template DNA, 200µM of each dNTP, 25ng of primer, 0.5unit of Taq DNA Polymerase (Bangalore Genei Pvt. Ltd., Bangalore, India) and 10× PCR assay buffer (50mM KCl, 10mM Tris-HCl, 1.5mM $MgCl_2$, pH 9.0). The PCR reaction of the cocktail was carried out in a GeneAmpPCR 2400 thermal cycler (Perkin Elmer, USA) programmed for 45 cycles. The first cycle was programmed for 5 min at 94^{o}C for denaturation, 1min at 40^{o}C for primer annealing and 2min at 72^{o}C for DNA polymerization. In the next 44 cycles the period of denaturation was maintained at 1min while the primer annealing and DNA polymerization was same as in the first cycle. An additional cycle of 15min at 72^{o}C was used for primer extension. The amplified samples were stored at 4^{o}C and separated by electrophoresis in 1.5% agarose gel in 1× TAE buffer for 3h at 50V. To determine the size of the amplified DNA fragments. Gene Ruler 100bp DNA ladder plus (MBI Fermantas, Lithuania) was used as size standard. DNA fragments were visualized by staining the gel with ethidium bromide and photographed in Bio-Rad (USA) Gel documentation system using Quantity One software for documentation.

2.5 *RAPD data scoring and analysis*

In RAPD analysis, the presence or absence of the bands were taken into consideration and the difference in the intensity of the band was ignored. From RAPD data a binary matrix was obtained. The matrix elaborated utilizing the multivariate analysis program NTSYS-pc (Rohlf, 1993). The binary matrix was transformed in a similarity matrix using the Jacquard's similarity coefficient. The cluster analysis was carried out using the UPGMA (Unweighted pair group mean average) method.

3. Results

3.1 *Morphological characteristics*

Detailed morphological characters were tabulated in Table 1 which revealed some interesting information. There were variations in the genotypes in respects of morphological and storage root characters (Table 1). The plant type of the genotypes were spreading and semi-erect. Leaves were green with variation in immature leaf colour. Flowering of the genotypes ranged from profuse to moderate except 'Kishan' which was shy flowering type. The storage root yield ranged from 19.0 – 29.04 t/ha. 'ST-10' showed maximum yield with high dry matter (DM) and starch content. 'ST-14' and 'Gouri' which have dark orange flesh reach in β-carotene. Rest of the varieties had white flesh

colour with variation in skin colour (Table 1). All the genotypes had good cooking quality. Thus, most of them were suitable for table purpose while 'ST-10', 'ST-13' and 'ST-14' were suitable for table and processing purpose.

3.2 *RAPD analysis*

Ten varieties of *I. batabas* collected from experimental garden of Central Tuber Crop Research Institute (CTCRI), Regional Centre, Dumduma, Bhubaneswar showed some amount of DNA marker variations in variety level. The number of amplification products ranged from 33 to 70 for ten different varieties (Table 2, Figs. 1A and 1B) and polymorphism was between 1.06% in between 'ST-10' and 'ST-13' to 73.69% in between 'Kishan' and 'Kalinga'. RAPD profiles of ten varieties of sweet potato shared a number of common bands for all primers. The total unique bands obtained from 20 primers were 72 (3.6%) while the monomorphic bands percentage found 5.55%. Similarities index showed a maximum closeness of 98.94% between 'ST-13' and 'ST-10' while least similarities was found between 'Gouri' and 'Kalinga' (Table 3). The profiles were very distinct in the studied ten genotypes.

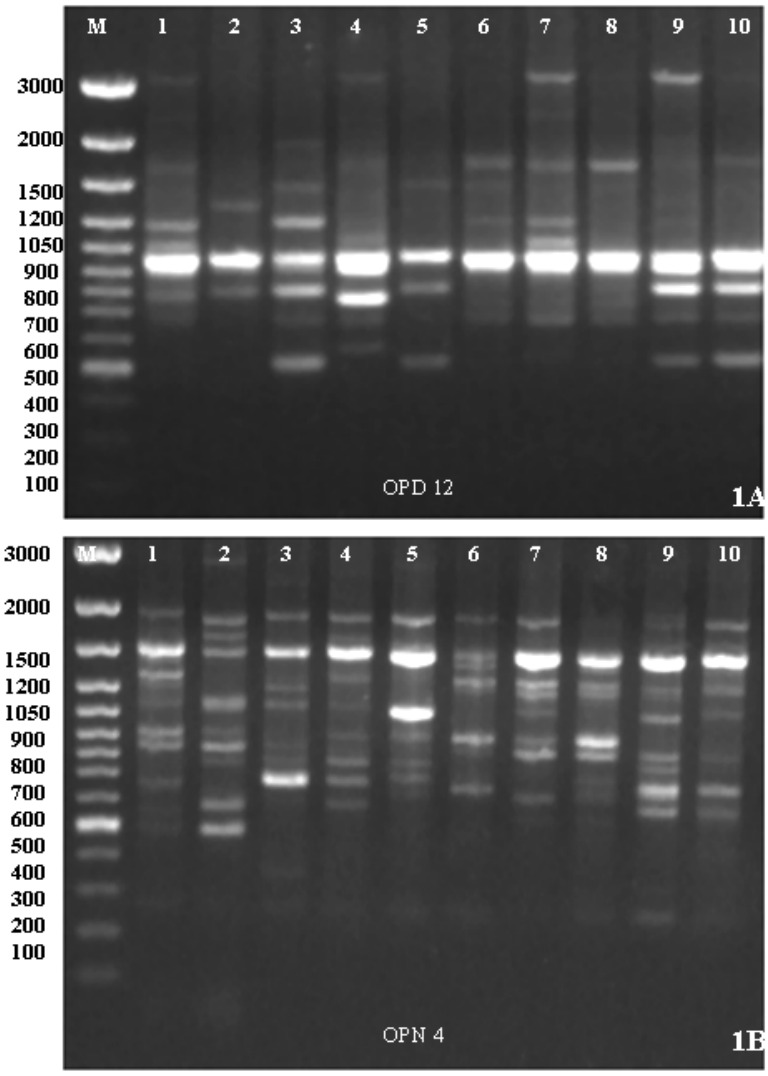

Figure. 1A & 1B. RAPD profile of ten cultivars of sweet potato amplified by OPD-12 and OPN-4 primer respectively. M= Marker DNA (DNA Ladder plus, MBH, Fermentas), 1=Kalinga, 2=Gouri, 3=Kishan, 4=Sree Nandini, 5=Sankar, 6=Saurin, 7=Goutam, 8=ST-10, 9= ST-14, 10=ST-13.

In OPN-4, one high DNA markers of 2000bp were found in all varieties except 'Sourin', 'ST-10' and 'ST-14'. The unique DNA bands of 800bp and 1800bp were only obtained in 'ST-14' and 'ST-13'. A prominent DNA marker of 1000bp was the characteristics of 'Sankar' while 'Sourin', 'Sree Nandini' and 'Kishan' were distinguished from other varieties having 650pb, 600bp and 500bp respectively. DNA band with 3000bp, was only found in 'Goutam' and 'ST-14' in OPD-12 primer. In that same primer DNA bands of 850bp found common in between 'ST-13' and 'ST-14'. A marker band of 700bp and 500bp found unique in 'Sree Nandini' and 'Kishan'. The DNA band with 900bp was found to be varietal marker in OPD-12 primer (Figs. 1A and 1B).

3.3 *Cluster analysis*

Pair wise comparisons were made for the RAPD profiles obtained from RAPD markers of eight populations of *I. batatas* which clustered in a single tree with two branches, one having 'Kalinga' and 'Sourin' producing a out group; while rest of the varieties were found in the other branch of the tree (Fig. 2). All the ST varieties exhibited closer affinity having closer genetic similarity with 'Goutam'. 'Gouri' was found to be distantly related with other members in the cluster followed by 'Kishan' having pink skin colour of the tuber (Table 1, Figure. 2). Furthermore, 'Sankar' had the relatively more genetic similarity with 'Goutam' and all the three 'ST' varieties.

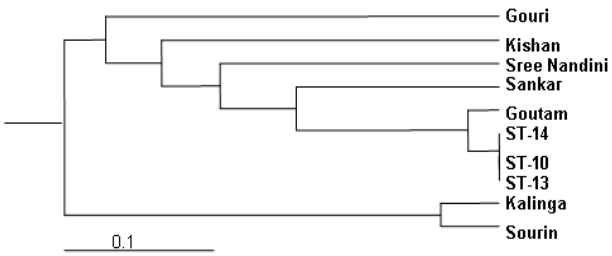

Figure 2. Dendrogram representing clustering of different Indian varieties of sweet potato based on the Jaccard's similarity indices from RAPD analysis using random primers.

4. Discussion

The average number of amplification product obtained with one primer for ten varieties was 51.75 of which 7.6% products were polymorphic. 'Kalinga' and 'Kishan' showed a maximum number of polymorphic bands with 73.6% of polymorphism (Figs. 1A and 1B). The profiles were very distinct in the ten varieties studied. RAPD profiles of ten varieties were amplified by different primers out of which OPN-1, OPN-4 and OPD-12 showed distinct DNA profile for each variety without any significant variation among the plants collected (Figs. 1A and 1B). This type of genetic variation in variety level was found in other cultivars of sweet potato (Gichuki *et al.*, 2003).

The unique DNA bands of 500bp, 700bp, 1300bp were only obtained in 'Kishan', 'Sree Nandini' and 'Gouri' respectively in OPD-12 while an unique band of 800bp separate out 'ST-10' from 'ST-13' and 'ST-14' (Figs. 1A and 1B). DNA markers of 900bp and 1500bp found common and very distinct in all varieties were produced by OPD-12 and PON-4 respectively. However, 'Sourin', 'ST-10' and 'ST-14' showed a week marker of 1500bp in OPN-4 which demarked these varieties from rest of the varieties. 'Sankar' showed a prominent unique marker of 1000bp in OPN-4 which was found to be the characteristic of this variety. DNA band with 500bp 600bp, and 700bp were unique to 'Gouri', 'Kissan' and 'Sree Nandini' OPN-4 primer.

Phylogenetic analysis of all the ten varieties of *I. batatas* showed a single tree with 'Kalinga' and 'Sourin' different group. That confirms the significant genetic variability between these varieties that might have originated from different ancestors in the process of natural evolution. While rest of the varieties found in a same major branch of the tree with closer genetic affinity among 'ST-10', 'ST-13' and 'ST-14' – all have different promising qualities. Furthermore, a closer genetic similarity of ST variety with 'Goutam' and distant relation with 'Gouri' as well as 'Kishan' confirms promising breeding possibilities among these varieties in crop improvement programme with regard to tuber quality and yield (Table 1, Fig. 2). Interestingly, two varieties i.e. 'Kalinga' and 'Sourin' could be used as parents in breeding programme taking advantages of their wide genetic variability with the other studied varieties for higher yield and wide adaptability in various ecological zones of all the ST varieties.

Therefore, depending on the genetic architecture of these varieties and their edaphic preferences and adaptations, different varieties would likely to display varying degrees of polymorphism. Present observations on *I. batata* do support this presumption. This is because of its several and varied adaptation and distributional patterns. RAPD was analyzed on ten varieties and the variability patterns were scored on the basis of amplified products. However the overall polymorphism in this species is not that of high magnitude in varietal level. The observed inter-varietal divergence could be ascribed to the fluctuating microclimatic conditions in the regions where these genotypes inhabit. The analysis was carried out not only on a pair wise combination of variability but also accounted for the entire information given by all the genotypes against all the primers used.

The genetic resources of a species exist at two fundamental levels: i) genetic differences between individuals within local population and ii) genetic differences between different local varieties. However, only in the last 15 years, through the eletrophoretically detection of genetic variation at many protein loci, has it been practical to describe the amounts and distribution of genetic variation in natural populations (Nei, 1975). Significant genetic differentiation was reported to find variety specific fragments associated with soil types (Dawson *et al.*, 1993). Efforts to pressure genetic resources must take in to account the components of genetic variation, both within and between local populations.

The distribution of genotypes observed on the dendrogram is represented in Fig. 2 showing the RAPD data as a powerful tool in assessing the genetic diversity. Both molecular and phenotypic measures of divergence should be considered while measuring the diversity (Dizon *et. al.*, 1992, Waples, 1995). The evolutionary potential of individual population can be predicted from their genetic make up. The isolation combined with genetic drift/divergence selection will generate unique and irreplaceable combinations of genotypes, which may or may not be manifest as differences in phenotype. This process of isolation and divergence is well studied here using molecular markers. It is concluded that genetic diversity due to geographically isolation is not potentially replaceable. The genetic diversity within the species limits the response to selection especially in stressful or ecologically marginal environment. However, this is an preliminary report of Indian varieties of sweet potato for molecular markers and phylogenetic relationships. The development of SCAR markers from the distinct variety specific RAPD markers will be of immense help in identifying varietal ambiguity. The probe can also be of used in identifying DNA markers and its localization on chromosome. Analysis of more numbers of varieties in genetic level could have thrown more light on their genetic relationships along with morphological traits which will be of immense help in guiding the breeding programme in sweet potato for their improvement.

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Table 1. Agronomic characters of ten varieties of sweet potato

Varieties	Plant type	Leaf shape	Types of leaf lobes	Shape of central leaf lobes	Mature leaf colour	Immature leaf colour	Flowering	Storage root skin colour	Flash colour	Shape	Yield t/ha
Sourin	Spreading	Lobed	Slight	Semi elliptic	Green	Purplish green	Moderate flowering	Red	Cream	Round elliptic	22.43
Kalinga	Spreading	Lobed	Deep	Elliptic	Green	Green with purpole edge	Moderate flowering	Purple red	Cream white	Round elliptic	20.2
Kishan	Semi compact	Trangular	Moderate	Semi elliptic	Green	Green with purple margin	Shy flowering	Reddish purple	Cream	Elliptic	21.4
Goutam	Spreading	Trangular	Slight	Semi elliptic	Green	Light purple	Moderate flowering	White	Deep orange	Round to ovate	20.5
Gouri	Semi-erect	Lobed	Deep	Elliptic	Green	Slight purple	Profusely flowering	Purple red	Deep orange	Obovate to round elliptic	21.14
ST-14	Semi compact	Trangular	Very slight	Trangular	Green	Green with purple	Profusely flowering	Pale yellow	Deep orange	Round elliptic	23.43
ST-13	Semi compact	Lobed	Slight	Semi elliptic	Green	Green with purple	Profusely flowering	Dark purple	Pigmented with anthocyanin	Long elliptic	24.15
ST-10	Semi compact	Trangular	Slight	Semi elliptic	Green	Green with purple	Profusely flowering	White Red Cream	White	Round	29.04
Sankar	Spreading	Hastate	Deep	Elliptic	Green	Green with purple	Moderate flowering		Pale yellow	Elliptic	18.0
Sree Nandini	Spreading	Unifoliate with entire margin	Very slight	Teeth	Green	Light green	Moderate flowering		White	Round elliptic	20.0

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Table 2. RAPD primers, their nucleotide sequence &number of RAPD bands generated from ten different varieties of sweet potato

Primer	Primer Sequence (5'------3')	Total No of bands	Total number of bands			Polymorphic (%)	Range of amplicons in base pairs (bp)
			polymorphic	monomorphic	Unique		
OP-A3	AGTCAGCCAC	52	43	8	1	82.69	200-3000
OP-A5	AGGGGTCTTG	42	35	5	2	83.09	500-2000
OP-A8	GTCACGTAGG	58	48	6	4	82.75	150-2500
OP-A10	GTGATCGCAG	38	26	8	4	68.42	400-3000
OP-A11	CAATCGCCGT	35	28	6	1	60.00	200-2800
OP-A14	TCTGTGCTGG	33	22	6	5	66.66	300-2200
OP-A16	AGCCAGCGAA	39	30	5	4	76.92	400-2900
OP-D2	GGACCCAACC	65	53	6	6	81.53	400-1000
OP-D8	GTGTCCCCCA	45	37	4	4	82.22	300-2500
OP-D12	CACCGTATCC	68	59	5	4	86.76	200-3000
OP-D11	AGCGCCATTG	40	26	2	8	65.00	200-1800
OP-D16	AGGGCGTAAG	64	55	6	3	85.93	200-2200
OP-D18	GAGAGCCAAC	48	38	9	1	79.16	300-2500
OP-N1	TCGCCGCAAA	66	57	4	5	86.36	200-2800
OP-N4	CAGCGACTGT	70	59	4	7	84.28	200-3000
OP-N5	GACCGACCCA	64	55	8	1	85.93	500-2500
OP-N10	ACAACTGGGG	68	61	5	2	89.70	200-3000
OP-N11	TCGCCGCAAA	46	38	4	4	82.60	400-2700
OP-N14	TCGTGCGGGT	40	31	6	3	77.50	100-1500
OP-N15	CAGCGACTGT	54	47	4	3	87.03	200-3000
Total	20 primers	1035(51.75)	848 (42.4)	111 (5.55)	72 (3.6)	79.72 (mean)	

Table 3. Similarities index among the ten varieties of *Ipomoea batatas* using RAPD markers

ST-10	ST-13	ST-14	Sourin	Sankar	Kalinga	Gouri	Goutam	Sree Nandini	
ST-13	98.94								
ST-14	98.84	96.76							
Sourin	50.41	50.40	50.40						
Sankar	72.48	72.48	72.48	55.55					
Kalinga	49.18	49.18	49.18	92.06	56.16				
Gouri	53.54	53.54	53.54	23.52	44.68	26.86			
Goutam	95.79	95.78	95.78	48.85	73.88	47.69	53.33		
Sree Nandini	61.87	61.87	61.67	32.50	62.26	27.84	35.71	65.3	
Kishan	60.29	60.29	60.29	25.97	40.77	26.31	37.03	62.5	46.16

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