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Analysis of genetic variability in sweet potato accessions using Start Codon Targeted (SCoT) polymorphism

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Abstract

Sweet potato is a native species of South America, belonging to the family Convolvulaceae and usually considered as the only species of *Ipomoea* of economic importance. It is a hexaploid species derived from the ancestral diploid species, *I. trifida* and is one of the important vegetable crops in tropical and sub-tropical areas of the world. To study the variability of the crop, different molecular markers were used, among them SCoT markers can be used for genetic diversity and mapping studies. In this study SCoT polymorphism was used for studying genetic diversity of 40 Sweet potato accessions from ICAR-CTCRI and CIP using 10 SCoT primers. A total of 128 bands were generated, of which 75 were polymorphic among accessions. The primer SCoT 11 produced the highest number of bands (25), while the primer SCoT 21 produced lowest number of bands (7) with a product size range from 200 bp to 2 kb with a polymorphism of 56.5%. The Hierarchical cluster analysis among the sweet potato lines using SCoT marker resulted in grouping of genotypes into three main clusters. Almost all the released varieties from ICAR-CTCRI included in the present study were clustered together in cluster I and the CIP highly carotene clones were grouped under II and III clusters. These results indicated that SCoT marker is useful to detect polymorphism and distinguish variability between the sweet potato lines.

Keywords: Genetic diversity, SCoT primers, sweet potato, cluster analysis

Introduction

Sweet potato (*Ipomoea batatas* (L.) Lam), an significant food crop is grown in the tropics, sub-tropics and warm temperate regions of the world for its edible storage

roots. It is a cross-pollinated and hexaploid (2n=6x) crop with 90 chromosomes (Jones, 1965) and one of the world's seventh important food crops, especially in developing countries (CIP, 1996). Asia and Africa account for 95% of the world's sweet potato production. According to the Food and Agriculture Organization (FAO) statistics, world production of sweet potato in 2013 was more than 110 MT, and Asia accounts almost 86% of the world's production. China is the biggest producer of around 79 MT from about 3.5 M ha. Sweet potatoes are rich in dietary fibre, minerals and antioxidants. Now sweet potato is used as staple food, animal feed, industrial and potential raw material for alcohol production. The purple and orange fleshed genotypes can produce sizable quantities of anthocyanin and carotenoid plant pigments. In addition, the high β- carotene content of orange-fleshed sweet potato (OFSP) plays a crucial role to prevent vitamin A deficiency especially night blindness and maternal mortality.

The heterozygous nature of sweet potato clones is mainly because of the obligatory out crossing nature allowing a wide range of genetic recombination with natural seed production (Nayar *et al*., 1984). For breeding of the sweet potato, a large number of varieties and lines have been classified into several cross incompatibility groups depending on their self-incompatibility phenotypes (Shinjo and Omura, 1962; Martin and Cabanillas, 1968). Despite its importance, there have been only very few genetic studies on sweet potato, maybe due to its self-incompatibility, hexaploid nature, ploidy level with large chromosome number (2n=90) (Magoon *et al*., 1970; Ozias-Akins and Jarret, 1994).

The study of phenotypic and genetic diversity to identify groups with diverse genotypes is important for conserving, evaluating and utilizing genetic resources and further for developing new crop varieties (Maric *et al.*, 2004). Different molecular markers have been used to assess its genetic diversity *viz.*, RAPD (Welsh and McClelland, 1990; Williams *et al.*, 1990), AFLP (Zang *et al*., 2004), ISSR (Huang and Sun, 2000), SSR (Jarret *et al*., 1994). These works confirmed that sweet potato is a crop with high genetic polymorphism, also represented in the great diversity observed in morphological and tuber traits (Woolfe, 1992). Among all the molecular marker systems, Start Codon Targeted (SCoT) polymorphism is a new marker type, and not yet used in the sweet potato diversity studies. It is a novel, simple, and reliable gene-targeted marker technique based on the translation start codon (Collard and Mackill, 2009; Xiong *et al*., 2009).

SCoT marker polymorphism was described by Collard and Mackill (2009a), based on the short conserved regions of plant genes that are surrounded by the ATG translation start codon (Joshi *et al*., 1997; Sawant *et al*., 1999). The principle of SCoT marker is the single primer amplified region since it uses a single primer as a forward and reverse primer, like the RAPD or ISSR technique. Most primers differed from each other by at least one nucleotide with an emphasis on variations at the 3*'* end, which has been shown to be critical for primer-template specificity (Kwok *et al*., 1990; Sommer and Tautz, 1989). Thirty six primers were designed and successfully utilized in rice (Collard and Mackill, 2009), peanut (Xiong *et al*., 2009, 2010, 2011), longan (Chen *et al*., 2010), mango (Luo *et al*., 2010, 2011), potato (Gorji *et al*., 2011), sugarcane (Liping *et al*., 2012) and Giloe (Singh *et al*., 2013) for accession

identification and genetic diversity analysis. The current study aims to evaluate the efficiency of SCoT markers in sweet potato and to analyze the genetic diversity between CIP accessions and those from ICAR-CTCRI released varieties that would be useful for developing improved sweet potato lines and for enriching the germplasm collection.

Materials and methods

Plant materials

A total of 40 genotypes (Table 1) including 25 OFSP seedling progenies from CIP and 15 released varieties of ICAR-Central Tuber Crops Research Institute (ICAR-CTCRI), Thiruvananthapuram, Kerala, India taken from the sweet potato improvement project were used to analyze the level of genetic variability among them.

Genomic DNA extraction

DNA was extracted from 100 mg of young leaves collected from each accession using the Cetyl Trimethyl Ammonium Bromide (CTAB) method of Doyle and Doyle (1990) with minor modifications. The isolated DNA stock samples were stored at -20°C and the quality was checked by electrophoresis on 0.8 % agarose stained with ethidium bromide (EtBr). The quantity of DNA was accessed using Spectrophotometer.

SCoT primers

Primers were designed from consensus sequence derived from the previous studies by Joshi *et al*. (1997) and Sawant *et al*. (1999). For primer design, the ATG codon (+1, +2, and +3), 'G' at position +4, 'C' at position +5, and A, C, and C at positions +7, +8, and +9, respectively, were fixed. All primers were 18-mer and GC content ranged between 50% and 72% (Collard and Mackill, 2009).

PCR amplification and electrophoresis

The PCR conditions were optimized for the SCoT primers. A total of 20 SCoT primers were initially screened for polymorphism in sweet potato accessions. Among them, 10 primers which gave reproducible clear and distinct bands were selected for analyzing all the 40 accessions. The PCR reaction was optimized and performed with each reaction containing 10µM of each dNTP, 2 µl of each primer, 1U of Taq polymerase and 10-50ng of template DNA in a 20µl reaction. The PCR cycle of 94°C for 3 min, followed by 35 cycles of 94°C for 1 min, 50°C for 1 min, 72°C for 2 min, and a final extension at 72°C for 5 min was used for all the primers.

PCR amplified products were resolved on 2 % agarose gel and 100 bp DNA ladder (Fermentas, USA) was used as standard size marker. Agarose gel were visualized and documented under Ultra violet light using Alpha Imager 1200^{TM} (Alpha Innotech Corporation, USA).

Genetic Data analysis

For SCoT analysis, DNA bands obtained with all the primers were scored visually for the presence (1) or absence (0) of bands for all 40 accessions and the binary data matrix was constructed. Only clear bands were scored. Data analysis was performed using the NTSYS-pc software (Rholf, 2002, version 2.1).

Jaccard's similarity coefficients were used to generate dendrogram using Unweighted Pair Group Method with Arithemetic Average (UPGMA) (Sneath and Sokal, 1973) and relationships between the accessions were represented in the dendrogram. Principal component analysis (PCA) was performed by using FAMD Software version 1.25 (Schluter and Harris, 2006). AMOVA analysis was performed using the Arlequin software package, version 2000 (Schneider *et al*., 2000).

Table 1: List of the sweet potato genotypes used in this study

Sl No.	Accession name	Tuber Flesh colour	Origin	Sl No.	Accession name	Tuber Flesh colour	Origin
1	162-5	OFSP	CIP	21	75-3	OFSP	CIP
2	58-3	OFSP	CIP	22	309-9	OFSP	CIP
3	97-13	OFSP	CIP	23	196-2	OFSP	CIP
4	35-9	OFSP	CIP	24	148-26	OFSP	CIP
5	98-1	OFSP	CIP	25	148-22	OFSP	CIP
6	160-2	OFSP	CIP	26	Sree Varsha	WFSP	ICAR-CTCRI
7	130-8	OFSP	CIP	27	Sree Nandhini	Cream	ICAR-CTCRI
8	390-3	OFSP	CIP	28	Sree Vardhini	White	ICAR-CTCRI
9	130-17	OFSP	CIP	29	Sree Ratna	Yellow	ICAR-CTCRI
10	130-3	OFSP	CIP	30	Gowri	Orange	ICAR-CTCRI
11	281-9	OFSP	CIP	31	Sankar	Pale yellow	ICAR-CTCRI
12	526-12	OFSP	CIP	32	Sree Arun	white	ICAR-CTCRI
13	581-6	OFSP	CIP	33	Sree Varun	Cream	ICAR-CTCRI
14	130-2	OFSP	CIP	34	Kalinga	Cream	ICAR-CTCRI
15	64-1	OFSP	CIP	35	Gautham	Orange	ICAR-CTCRI
16	261-4	OFSP	CIP	36	Sourin	Cream	ICAR-CTCRI
17	327-16	OFSP	CIP	37	Kishan	Cream	ICAR-CTCRI
18	427-10	OFSP	CIP	38	ST-14	OFSP	ICAR-CTCRI
19	582-46	OFSP	CIP	39	S1	WFSP	ICAR-CTCRI
20	148-7	OFSP	CIP	40	ST -13	PFSP	ICAR-CTCRI

OFSP: Orange Fleshed Sweet potato; WFSP: White fleshed sweet potato; PFSP: Purple Fleshed Sweet potato

Table 2: Polymorphism detected with 10 SCoT primers in sweet potato accessions

Sl No.	Primer ID	Primer Sequence (5^1-3^1)	No. of amplified bands	No. of polymorphic bands	Polymorphic ratio (%)
1	SCoT 1	CAACAATGGCTACCACCA	20	15	75.0
2	SCoT 2	CAACAATGGCTACCACCC	15	08	53.3
3	SCoT 5	CAACAATGGCTACCACGA	08	05	62.5
4	SCoT 11	AAGCAATGGCTACCACCA	25	14	56.0
5	SCoT 14	ACGACATGGCGACCACGC	16	09	56.3
6	SCoT 15	ACGACATGGCGACCGCGA	10	06	60.0
7	SCoT 20	ACCATGGCTACCACCGCG	08	05	62.5
8	SCoT 21	ACGACATGGCGACCCACA	07	03	33.3
9	SCoT 35	CATGGCTACCACCGGCCC	09	05	55.6
10	SCoT 40	CAATGGCTACCACTACAG	10	05	50.0

Results

SCoT marker Analysis

The sweet potato accessions used in present study include *viz.*, OFSP hybrids from CIP and ICAR-CTCRI released varieties. Out of 40 accessions, 25 accessions were collected from CIP-seedling progenies and 15 accessions were collected from ICAR-CTCRI. SCoT primers were screened to study the diversity among the sweet potato accessions, out of which all primers amplified clear and reproducible bands (Table 2). These primers were selected for diversity studies of 40 sweet potato accessions, which produced a total of 128 bands with an average of 12.8 bands per primer ranging from 7 (SCoT 21) to 25 (SCoT 11) per primer. Among them 75 bands were polymorphic between accessions resulting in 56.5 % polymorphism. The primer SCoT 1 showed the maximum number of 15 polymorphic bands from a total of 20 amplified bands with maximum polymorphism of 75 %, followed by the primer SCoT 5 and SCoT 20 produced 62.5 %, SCoT 15 with 60% and the least polymorphism showed in the primer SCoT 21 with 33.3 %. The banding pattern of the SCoT marker is similar to the RAPD profile and the amplified product size of the primer ranges from 200 bp to 2 kb.

Cluster analysis

Based on the UPGMA clustering algorithm from SCoT marker, the 40 genotypes were grouped into three main clusters I, II and III (Fig. 4) at 75% with a short index length of 0.74 - 0.87. The first cluster (I) is composed of 11 accessions, majority of which are the released varieties of ICAR-CTCRI including Kishan, Sree Nandhini, Gautham, Sourin, Gowri, Sree Arun and Kalinga. The CIP and high carotene clones were clustered under II and III clusters including Sree Varsha, Sree Rethna, Sree Vardhini and Sankar. The second cluster (II) included 17 lines and the third cluster (III) consisted of twelve lines of CIP- orange fleshed hybrids. These results indicated the efficiency of SCoT markers in detecting polymorphism among sweet potato lines.

Analysis of genetic diversity

Analysis of molecular variance (AMOVA) based on SCoT data shows that a high percentage of the total genetic diversity of sweet potato populations in this study were distributed with an average of 95.29 % of the genetic diversity distributed within populations and only 4.71 % among populations (Table 3).

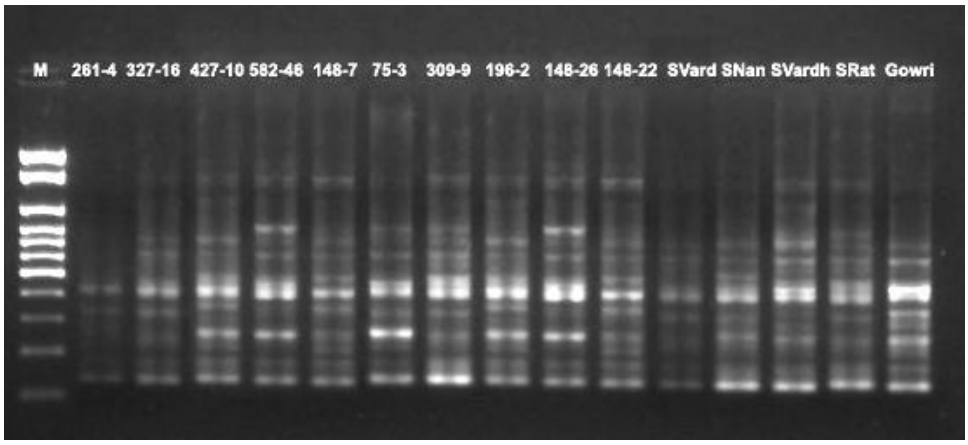

Figure 1: Amplification profile of primer SCoT 11

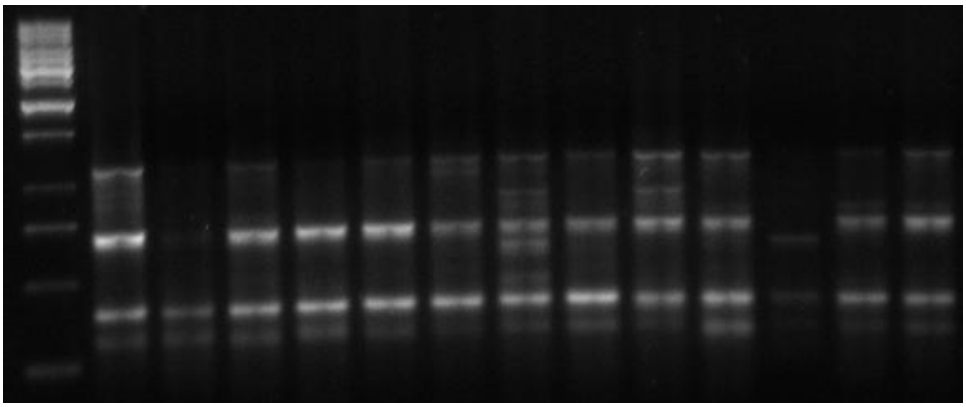

Figure 2: Amplification profile of primer SCoT 40

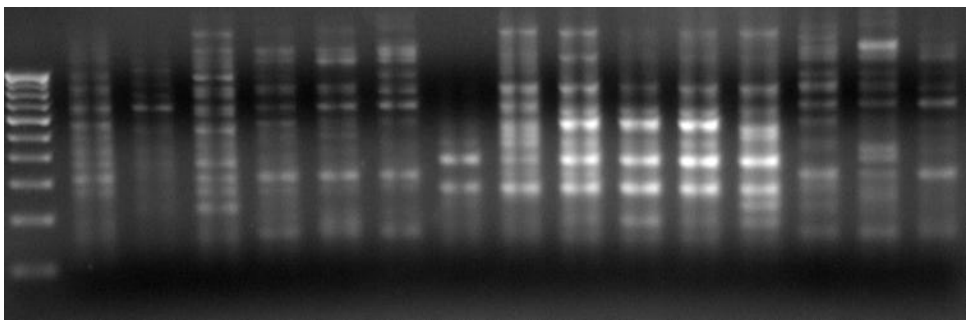

Figure 3: Amplification profile of primer SCoT 14

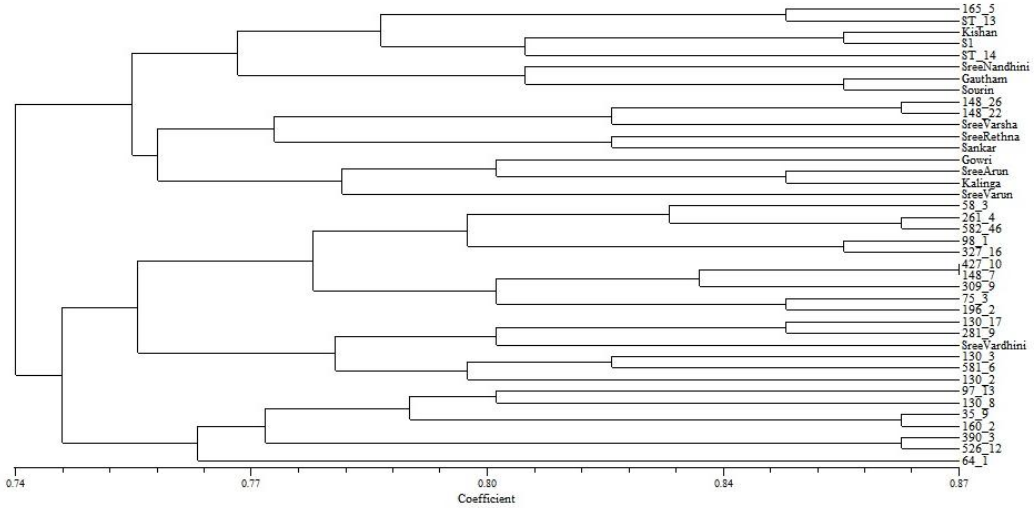

Figure 4: UPGMA dendrogram of sweet potato accessions based on SCoT analysis

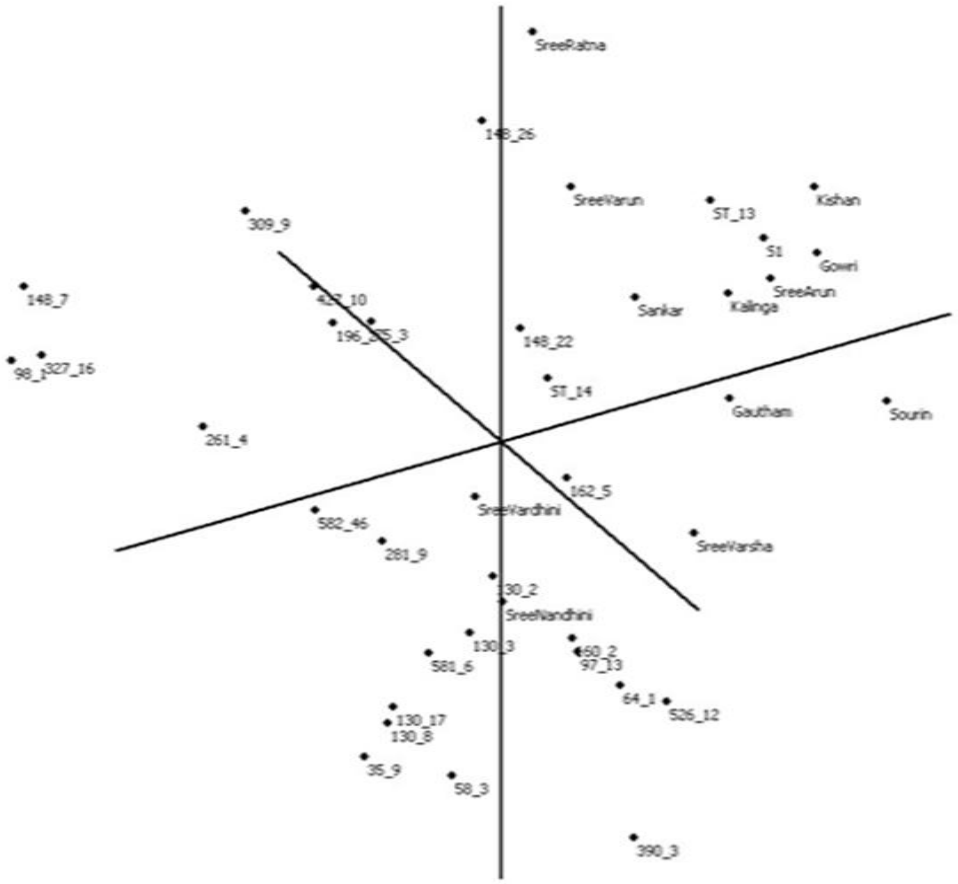

Figure 5: Principal coordinate analysis (PCA) for the SCoT evaluation of the 40 sweet potato accessions.

PCA

Based on the PCA scatter plot of SCoT marker, 40 genotypes were grouped into four main groups I, II, III and IV (Fig. 5). The first group (I) (30%) is composed of 12 accessions, majority of them were ICAR-CTCRI released varieties. The CIP and highly carotene clones were grouped under II (20%) and III (40%) including Sree Nandhini and Sree Vardhini. The fourth cluster group (IV) included 4 lines of CIP-orange fleshed hybrids. These results also indicated that SCoT markers could detect polymorphism among sweet potato lines.

Table 3: Analysis of molecular variance (AMOVA) among sweet potato lines using SCoT primers

Source of variation	d.f. squares	Sum of components	Variance of variation	Percentage
Among populations	4	74.659	0.76531	4.71
Within populations	36	511.262	15.49278	95.29
Total	40	585.921	16.25809	

Discussion

Sweet potato is an important tuber crop having significant value in nutritional as well as health aspects and it is rich in carotenoids and anthocyanins. Genetic diversity in sweet potato has been reported using morphological markers (Karuri *et al.*, 2010). Use of gene targeted markers has advantageous over the use of random type markers (RAPD and ISSR) because they measure genetic diversity from the gene regions and indirectly reveals functional diversity present in the crop.

The SCoT marker technique was employed in the present study for several reasons. Firstly, it is a type of targeted molecular marker technique categorized by simplicity and reproducibility. Its PCR products were resolved by performing agarose gel electrophoresis. Related to arbitrary markers such as RAPD, SCoT markers are highly reproducible due to the use of longer primers. SCoT is a novel marker system and preferentially detects polymorphisms in coding sequences, because the primers were designed to amplify from the short conserved region surrounding the ATG translation start codon (Xiong *et al*., 2009; Collard and Mackill, 2009). Therefore, amplification products generated from the SCoT marker technique may be connected to functional genes and their corresponding traits. SCoT markers can generate presence or absence of dominant markers caused by sequence variations and co-dominant markers caused by insertions and deletions.

In the present study, the use of 10 primers yielded a total of 128 fragments, among which 75 bands were polymorphic with a mean of 2.61 polymorphic bands per primer with 56.5% polymorphism.

Generally SCoT markers showed relatively less number of amplicons in comparision to RAPD markers because SCoT is gene based markers and it amplifies only the functional genes, comprises of smaller portion of total genome therefore less bands per primer is expected whereas, RAPD markers are independent of it therefore more band is expected. Evidently, the SCoT marker technique can detect high to medium

polymorphism compared with other molecular marker techniques used in domesticated sweet potato. However, it is difficult to compare the amount of SCoT polymorphisms in the current study with previous studies that employed other molecular marker techniques because of the difference in the number of primers, accessions, and varieties used. This is the first report of studying the genetic variability in sweet potato using SCoT markers.

In SCoT primer designing, change in single nucleotide within the last three nucleotides at the 3^1 end will affect the banding pattern. In the present study, SCoT primers SCoT 1, SCoT 2 and SCoT 14, SCoT 15 differ only in the last nucleotide at the 3^1 end yet produced different profiles. SCoT primers also varied only in the second last nucleotide and SCoT primers differed only at the third last nucleotide yet also produced very different DNA marker profiles. Two primers differed by a single nucleotide at the 5^1end (SCoT 1 and SCoT 11), also generated different DNA marker profiles.

SCoT markers have been used in previous studies to analyze the level of polymorphism in cultivars of different crops such as mango (73.82%) (Luo *et al*., 2011), peanut (38.22 %) (Xiong *et al*., 2010), *Cicer* species (100 %) (Amirmoradi *et al*., 2012).

SCoT markers studies in mango accessions showed polymorphism of 73.82%. Genetic similarity between accessions was in the range of 66.2 – 94.2% with an average of 78.8%. Moreover, SCoT markers can generate more polymorphism than ISSR markers among the closest mango accessions (Luo *et al*., 2011). In peanut accessions, the percentage of polymorphism is 38.22 (Xiong *et al*., 2010). SCoT polymorphism in *Cicer* species produce a total of 112 bands among 38 accessions belonging to eight annual *Cicer* species using 9 SCoT markers, of which 109 were polymorphic. The number of bands ranged from 7 (SCoT1) to 17 (SCoT13) with an average of 12.4 per primer. The overall size of amplified products ranged from 220 bp to 2.25 kb. *Cicer* species has indicated that SCoT primers generate a DNA fingerprint comparable to those generated by RAPD markers, but the bands were sharp, clear and 100 % polymorphic (Amirmoradi *et al*., 2012).

In summary, SCoT markers successfully evaluated the genetic relationships among the sweet potato accessions used and generated a high level of polymorphism. The 40 genotypes were clustered into three clusters using the UPGMA dendrogram. The results of the present study will be useful for the management of germplasm, improvement of the current breeding strategies and for the release of new cultivar.

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