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Short communication

Diversity Analysis of Cashewnut (*Anacardium occidentale* L.) Varieties Grown in Coastal Region of Maharashtra using ISSR Marker

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Cashew (*Anacardium occidentale* L.), a member of family *Anacardiaceae,* is a tropical evergreen tree with chromosome number 2n = 42. India has the distinction of being the world's largest producer of cashewnut. The area under cashew in India was 1.9 mha and recorded 0.725 mt productions in 2014-15 with the productivity of 0.71 t ha^{-1} (DCCD, 2015). Cashew production in Maharashtra was 0.235 mt and the area under cultivation is 0.186 m ha and productivity was 1.26 t ha^{-1} during 2014-2015. The Konkan region of Maharashtra comprising of Thane, Raigad, Ratnagiri and Sindhudurg districts are the major track of cashew cultivation, where farmers have adopted different varieties of cashew nut developed by Dr. B. S. Konkan Krishi Vidyapeeth, Dapoli.

Genetic improvement of cashewnut is limited with respect to yield and quality. It is due to the lack of knowledge of genetic diversity of the indigenous germplasm both in India and other countries. The molecular approach for identification of plant genotypes seems to be more effective than traditional morphological markers because it allows direct access to the hereditary material and makes it possible to understand the relationships between plants (Das *et al.,* 2009). DNA-based genetic markers are useful for varietal identification in cashew. They also could facilitate estimation of genetic diversity and relationship between landraces, selections, and hybrids. Among the different molecular markers, the ISSR (Inter-Simple Sequence Repeat) technique is a powerful, rapid, simple, reproducible and inexpensive way to assess genetic diversity or to identify closely related cultivars in many species (Moreno *et al*. 1998).

Keeping this in view, the present investigation was undertaken to analyse the genetic diversity of 9 varieties of cashew obtained from Regional Fruit Research Station (RFRS), Vengurle, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli., Maharashtra (Table 1). DNA was isolated from tender leaf tissue following the protocol of Edwards (1991) *i.e.,* rapid method. A total of 27 ISSR markers were used to measure genetic diversity between and within samples of the nine different varieties of cashew. A PCR protocol was standardized for all ISSR markers. Each 20 μl PCR contained 50 ng template DNA, 2.5 μl of 10 x PCR buffer, 0.5 μl of 15 mM $MgCl_2$, 1 μl of 10 mM dNTPs (Bangalore Genei Pvt. Ltd., Bangalore, India), 10 pmol of each ISSR primer (Bioresource Biotech Pvt. Ltd., Pune, India) and 3.0 units of Taq polymerase (Bangalore Genei Pvt. Ltd.). Thermal profiles were standardised for each ISSR primer pair (*i.e.* marker) based on its melting temperature using a Master Cycler 2231 gradient-PCR machine (Eppendorf, Hamburg, Germany). The PCR-amplified products were separated by electrophoresis in 2% (w/v) agarose gel at 80 V. ISSR markers across the 9 varieties were scored for their presence (1) or absence (0) of bands for each primer. The binary data so generated was used to estimate the levels of polymorphism by dividing the number of polymorphic bands by the total number of scored bands. Jaccard's similarity co-efficient for each pair wise comparison

between varieties were calculated and similarity co-efficient matrix was generated. This matrix was subjected to unweighted pair group method for arithmetic average analysis (UPGMA) to construct a dendrogram. The similarity co-efficient analysis and dendrogram construction were carried out by using MVSP-A multivariate statistical package-5785 (version 3.1).

The isolation of high-quality DNA is important for all molecular biological analyses, because contaminants such as proteins, polyphenols, and polysaccharides can interfere with key enzymes. Thus, it is important (i) to choose the most appropriate part of the plant to use as the source of DNA; and (ii) to establish an optimum extraction protocol to yield high-quality DNA (Angeles *et al.,* 2005). The quality DNA was isolated from all the nine varieties. The marker analysis helps to understand the genetic makeup of the germplasm and also make it possible to analyse genetic diversity within species as well as between species. A total of 1152 scorable DNA fragments were produced and among them 882 DNA fragments were found to be polymorphic in the 9 cashew varieties. The minimum number of polymorphic fragments produced by the primer UBC-843 (04) while the maximum numbers of polymorphic fragments were produced by the primer UBC- 876 (85) (Figure 1). Average number of polymorphic bands observed per primer was 32.66 (Table 2). The average percentage polymorphism across the 27 primers among the varieties found to be 73.52%. Thimmappaiah *et al.* (2016) also recorded such type of polymorphism level in cashewnut by using RAPD and ISSR markers. It indicates that ISSR markers are applicable to all genotypes and cashew nuts. The primers produced high degree of polymorphism with an average of 73.52%.

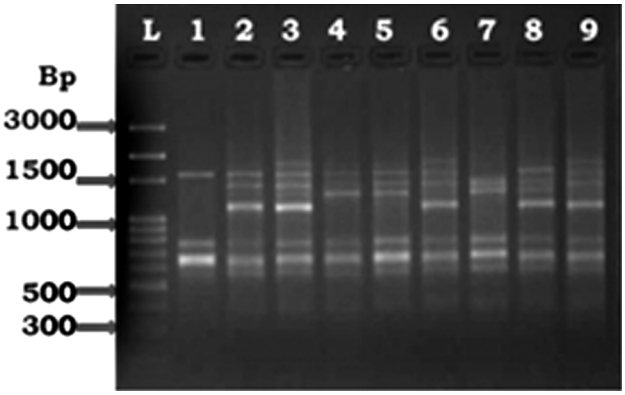

Fig. 1. *Gel photograph of ISSR profile of cashew varieties produced by primer UBC-825*

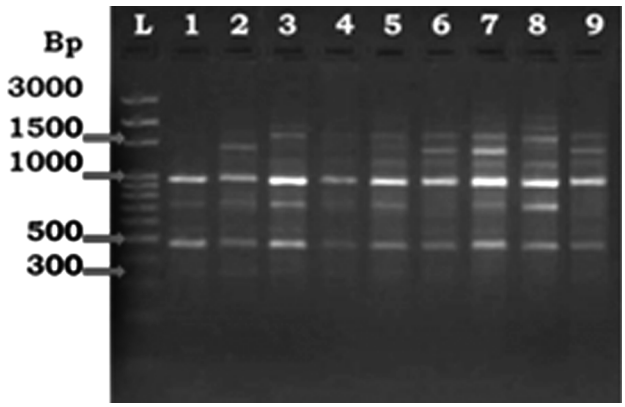

Fig. 2. *Gel photograph of ISSR profile of cashew varieties produced by primer UBC-834*

Table 1. *Details of varieties used in the study*

Sr. No.	Varieties	Abbreviation	Parents
1	Vengurla-1	V-1	Selection of Ansur – 1
2	Vengurla-2	V-2	Selection of WBDC
3	Vengurla-3	V-3	Vengurla-1 x Vetore-56
4	Vengurla-4	V-4	Midnopur Red x Vetore-56
5	Vengurla-5	V-5	Ausur Early x Mysore Kotekar
6	Vengurla-6	V-6	Vetore 56 x Vengurla-1
7	Vengurla-7	V-7	Vengurla-3 x VRI-1
8	Vengurla-8	V-8	Vengurla-4 x VRI-1
9	Vengurla-9	V-9	Vengurla-4 x M-10/4

Table 2. *Primer wise amplification and % polymorphism of cashew varieties*

Sr.No.	Primer Name	No. of Polymorphic Bands	Total No. of Bands	Polymorphism %	Range of Amplification (bp)	PIC
1	UBC-807	38	38	100	600-1800	0.82
2	UBC-811	28	37	75.68	300-2000	0.71
3	UBC-812	24	42	57.14	300-1300	0.71
4	UBC-813	25	25	100	900-1700	0.68
5	UBC-814	21	39	53.84	300-1600	0.76
6	UBC-815	13	22	59.09	300-2000	0.46
7	UBC-816	33	42	78.57	300-1300	0.79
8	UBC-817	25	34	73.53	500-1500	0.68
9	UBC-818	33	51	64.7	400-1300	0.79
10	UBC-824	15	24	62.5	500-1200	0.53
11	UBC-825	45	54	83.33	400-2000	0.84
12	UBC-834	53	62	85.48	300-2000	0.85
13	UBC-841	47	74	63.51	300-1700	0.85
14	UBC-843	4	13	30.76	700-1200	0.25
15	UBC-844	28	37	75.67	400-2000	0.71
16	UBC-845	0	9	0	1300-1400	0
17	UBC-854	14	14	100	300-1000	0.64
18	UBC-857	33	42	78.57	500-1700	0.79
19	UBC-876	85	85	100	300-2000	0.91
20	UBC-878	20	20	100	400-1800	0.6
21	UBC-879	76	85	89.41	400-2000	0.89
22	UBC-881	26	35	74.26	800-1900	0.77
23	UBC-884	45	54	83.33	400-1300	0.82
24	UBC-885	45	54	83.33	300-1300	0.82
25	UBC-886	49	49	100	600-2000	0.84
26	UBC-889	22	67	32.84	300-1300	0.64
27	UBC-891	35	44	79.55	300-1800	0.77
	Total	882	1152	1985.1	–	–
	Average	32.667	42.667	73.522	–	0.70

Average 43 bands per primer were amplified. The percentage of polymorphism across the cashew genotypes ranged from 30.76-100.00 per cent.

The maximum PIC information produced by the primer UBC-876 (0.91) followed by UBC-879 (0.89) and UBC-834 (0.85) (Figure 2) while the minimum PIC value was given by the primer UBC-843 (0.25). The average PIC value obtained for each primer was 0.70. Dasmohapatra *et al.* (2014) also obtained similar results that the PIC value was highest for the primer AM-15 (0.82) followed by primer AM-15 (0.81) while, the lowest PIC value was recorded by the UBC-825 (0.35). They also recorded the mean PIC value of 0.60 for fourteen ISSR primers in twenty five cashew genotypes.

The overall range of the similarity among 9 varieties of cashew ranged from 0.381 to 0.649 which indicates there was variability among the cashew varieties under study. The high similarity between varieties indicates that these varieties are having narrow genetic base which has been

Table 3. *Clustering pattern of 9 varieties of cashew*

	Cluster	No. of Genotypes	Name of the Genotype
I	IA	1	Vengurla-9.
	IB	2	Vengurla- 8, Vengurla-7.
II	IIA	3	Vengurla-6, Vengurla-5, Vengurla-4.
	IIB	3	Vengurla-2, Vengurla-3, Vengurla-1.

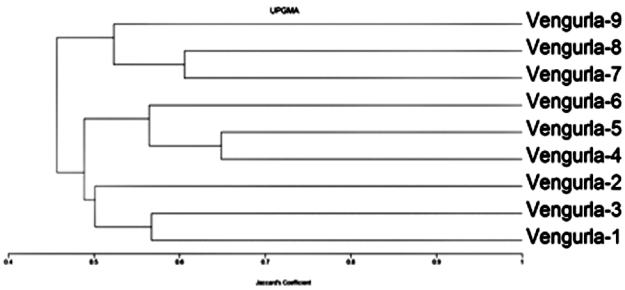

Fig. 3. *Dendrogram constructed using Jaccards Similarity Coefficient*

detected by molecular characterization. The ISSR primers have a high potential to reveal polymorphism and to determine intra and inter genomic diversity (Paul *et al*., 2013). The cluster analysis was carried out based on the ISSR profile (Table 3). Two major clusters are formed with two subclusters in each group. The dendrogram based on Jaccard's similarity coefficient was constructed using UPGMA after analysis of banding patterns generated by all the accessions with 27 primers across the 9 varieties of cashew genotypes (Fig. 3).

The study indicated that ISSR markers are suitable for the assessment of genetic diversity among different varieties of cashew. Such information may be useful for selecting the diverse parents and monitoring the genetic diversity for improvement of cashew. Similarly, these varieties will be protected from commercial exploitation, since the genetic profile is available.

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