International Journal of Agriculture Sciences
ISSN: 0975-3710 & E-ISSN: 0975-9107, Volume 10, Issue 16, 2018, pp.-7025-7028
Available online at https://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000217

Research Article

MOLECULAR CHARACTERISATION OF CASHEW (*Anacardium occidentale* L.) VARIETIES GROWN IN MAHARASHTRA USING ISSR MARKER

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Received: August 18, 2018; Revised: August 25, 2018; Accepted: August 26, 2018; Published: September 15, 2018

Abstract: The present study was carried out to standardize a DNA isolation protocol for cashew and to estimate genetic diversity among 9 cashew varieties developed by Dr. B. S. K. K. V. Dapoli, Maharashtra through the characterization by using 27 inter-simple sequence repeat (ISSR) markers. The DNA was extracted from the green leaf samples collected from 9 Cashew varieties. A protocol using 0.095 g/ml glucose, 0.025 g/ml polyvinylpyrrolidone, 0.0045 g/ml sodium bisulphite, 0.0055 g/ml sodium dodecyl sulphate, and 50 µl/ml sarcosine produced good quality DNA. A total of 1152 bands were scored out of which 882 were polymorphic, which showed 73.52% polymorphism. The size of amplified fragment ranged from 300 bp to 2000 bp. The primer UBC-843 recorded minimum PIC (Polymorphic information content) value 0.25, whereas primer UBC-876 gave maximum PIC value 0.91. The average PIC value was 0.70 among the all 9 varieties. The Jaccards's Similarity Co-efficient ranged from 0.381 (between varieties Vengurla-2 and Vengurla-8) to 0.649 (between varieties Vengurla-4 and Vengurla-5). The maximum similarity coefficient indicates that all type varieties have narrow genetic base.

Keywords: Cashew, Molecular Marker, ISSR, Genetic variation

Citation: Kambale S.R. and Bukya Anil (2018) Molecular Characterisation of Cashew (*Anacardium occidentale* L.) varieties Grown in Maharashtra using ISSR marker. International Journal of Agriculture Sciences, ISSN: 0975-3710 & E-ISSN: 0975-9107, Volume 10, Issue 16, pp.- 7025-7028.

Introduction

Cashew (*Anacardium occidentale* L.) belongs to family *Anacardiaceae* and native to tropical America, from Mexico and West Indies to Brazil and Peru. The cashew tree is also pantropical, especially in coastal areas. Major producers of cashew nuts are India, Tanzambique and Kenya [1]. India has the distinction of being the world's largest producer of cashew nut. The area under cashew in India was 1027.20 thousand hectare and recorded 725.42 thousand metric tons production in 2014-15 with the productivity of 706 Kg/ha [2]. Genetic improvement of cashew nut yield and quality is still not much researched. Genetic improvement is limited by the lack of knowledge of genetic diversity of the indigenous germplasm in both 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 [3]. DNA-based genetic markers are useful for varietal identification in cashew but also they could facilitate estimation of genetic diversity and relatedness between landraces, selections, and hybrids. Among the different molecular markers, the ISSR technique is a powerful, rapid, simple, reproducible and inexpensive way to assess genetic diversity or to identify closely related cultivars in many species [4]. Hence the present investigation was carried out to standardize a DNA isolation protocol for cashew and to estimate genetic diversity among different varieties of cashew.

Materials and Methods

Plant material

In the present investigation 9 varieties of Cashew obtained from Regional Fruit Research Station (RFRS), Vengurle, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli., Maharashtra, were used for diversity analysis [Table-1].

DNA extraction

DNA was isolated by following the protocol of Edwards, (1991) [5] *i.e.,* Rapid method with slight modifications. Three different solutions (T1, T2, and T3) were tested to extract cashew leaf DNA [Table-2].

Table-1 Details of varieties used in the study

SN	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

The young newly emerged leaves of grafts (0.1 g each) were collected from the 9 cashew varieties and used to extract genomic DNA. Purification of DNA was done to remove RNA and proteins. RNA was removed by RNase treatment and proteins were removed by proteinase-K treatment. The size range and concentration of genomic DNA in each sample was determined after electrophoresis using a λ uncut DNA ladder in 1% (w/v) agarose gels.

DNA amplification

A set of 27 primers composed short tandem repeat sequence with anchor and representing different microsatellites (di and tri repeats) have been used for present study as genetic primers in PCR amplification of inter simple sequence repeats (ISSR) regions as according to method of [6]. The ISSR markers were used for to measure genetic diversity between and within samples of the nine different varieties of cashew [Table-3].

A PCR protocol was standardized for all ISSR markers. Each 20 µl PCR contained 50 mg template DNA, 2.5 µl of 10× PCR buffer, 0.5 µl of 15 mM MgCl2, 1 µl of 10 mM dNTPs (Bangalore Genei Pvt. Ltd., Bangalore, India), 10 mol-1 of each ISSR primer (Bio resource 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) [Table-3]. The PCR-amplified products were separated by electrophoresis in 2% (w/v) agarose gels at 80 V.

Data analysis

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 un-weighted 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 multi variety statistical package-5785 (version 3.1).

Results and Discussion

DNA isolation

DNA was isolated from young tender leaves of each cashew using the rapid protocol of Edwards (1991) [5], *i.e.*, Rapid method. Various concentrations of glucose, Polyvinylpyrrolidone (PVP), and Sodium Dodecyl Sulphate (SDS), were tested [Table-2]. A combination of 0.095 g/ml glucose, 0.025 g/ml PVP, 0.0045 g/ml sodium bisulphite, 0.0055 g /ml SDS, and 50 µl/ml Sarcosine produced the highest yields and best quantity DNA without any contamination by phenolic compounds. A similar concentration of PVP was used by [7]. 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 [8].

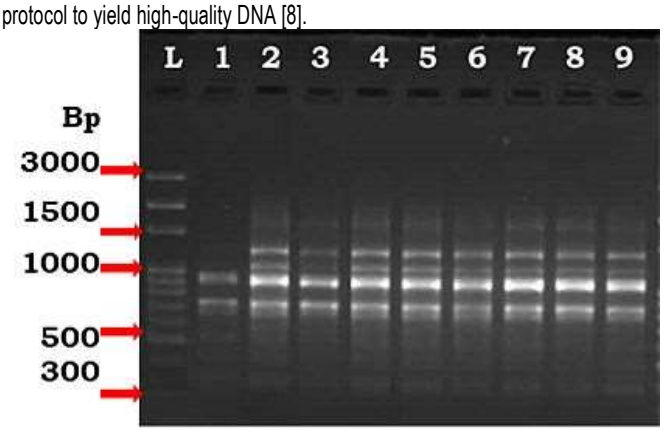

Fig-1 Gel photograph of ISSR profile of cashew varieties produced by primer UBC-811

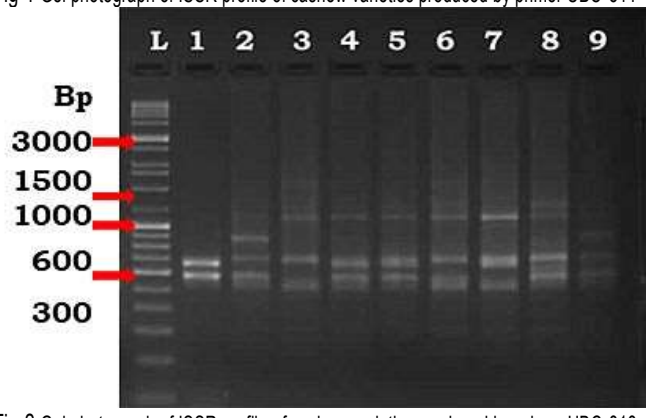

Fig-2 Gel photograph of ISSR profile of cashew varieties produced by primer UBC-818

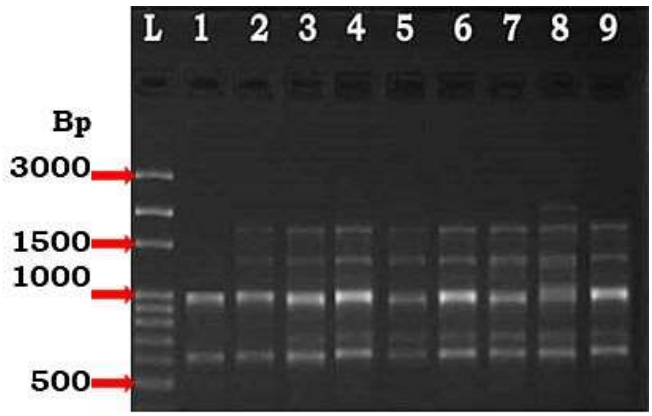

Fig-3 Gel photograph of ISSR profile of cashew varieties produced by primer UBC-886

Table-2 Chemical composition of the three extraction solutions T1–T3 used to isolate genomic DNA from cashew leaves.

SN	Components	T_1 g/ml	T_2 g/ml	T_3 g/ml
1	Glucose (0.5M)	0.09	0.095	0.1
2	PVP (3%)	0.015	0.025	0.035
3	Sodium Bisulphite (0.4%)	0.004	0.0045	0.005
4	Sodium Lauryl Sulphate (0.5%)	0.005	0.0055	0.006
5	Sarcosyl (5%)	40 µl	50 µl	60 µl

ISSR analysis

The marker analysis helps to understand the genetic makeup of the germplasm and also make it possible to analyze genetic diversity within species as well as between species. In the present study 9 varieties used for ISSR analysis with 27 random primers [Table-1]. 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 was UBC-843 (04); the maximum number of polymorphic fragments was produced by the primer UBC- 876 (85). Average number of polymorphic bands observed per primer was 32.66. The average percentage polymorphism across the 27 primers among the varieties found to be 73.52 %. Similar results were reported by Thimmappaiah *et al*., 2016 [9]. A study was initiated on 172 cashew varieties used for ISSR analysis with 10 primers. A total of 56 polymorphic bands with 91.8% polymorphism were generated. The primers produced high degree of polymorphism with an average of 73.52%. Average 43 bands per primer were amplified. Among the 27 generic primers 6 primers UBC-807, UBC-813, UBC-854, UBC-876, UBC-878 and UBC-886 revealed 100 % polymorphism. The percentage of polymorphism across the cashew genotypes ranged from 30.76-100 percent [Table-4]. Similar results noted in study [10] the primers percentage of polymorphism ranged from 33.33-100 percent among all cashew genotypes.

The PIC value was calculated for the 27 ISSR primers given in [Table-4]. In the present study the maximum PIC information produced by the primer UBC-876 (0.91) 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. The similar result was obtained by Dasmohapatra *et al*., (2014) [10] 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). The mean PIC value for fourteen ISSR primers was 0.60 obtained in twenty five Cashew genotypes.

Genetic relationship among germplasms

The genetic distance was computed considering the 9 genotypes from the pooled data. The overall range of the similarity among 9 varieties of cashew was found to be very wide ranging from 0.381 to 0.649 which indicates there was high variability among the cashew varieties under study. Based on the similarity matrix and clustering pattern, the varieties-4 and varieties-5 were found to have maximum similarity coefficient 0.649; while the lowest similarity coefficient (0.381) were observed in between the varieties-2 and varieties-8 which was suggesting a large differentiation in the germplasm of cashew. The ISSR primers have a high potential to reveal polymorphism and to determine intra and inter genomic diversity [11].

Table-3 Sequences and annealing temperatures of the 27 ISSR primers

SN	Primer	Primer sequence	Standardized Annealing temperature	GC Content
		(5' – 3')	(°C)	(%)
1	UBC – 807	AGA GAG AGA GAG AGA GT	45.5	45
2	UBC – 811	GAG AGA GAG AGA GAG AC	43.4	43.3
3	UBC – 812	GAG AGA GAG AGA GAG AC	52	44.4
4	UBC – 813	CTC TCT CTC TCT CTC TT	47.05	43.5
5	UBC – 814	CTC TCT CTC TCT CTC TA	47.05	41.4
6	UBC – 815	CTC TCT CTC TCT CTC TG	49.5	45
7	UBC – 816	CAC ACA CAC ACA CAC AT	51.4	51.2
8	UBC – 817	CAC ACA CAC ACA CAC AA	47.05	52.8
9	UBC – 818	CAC ACA CAC ACA CAC AG	47.9	52.1
10	UBC – 824	TCT CTC TCT CTC TCT CG	56.7	49.1
11	UBC – 825	ACA CAC ACA CAC ACA CT	50.4	49.3
12	UBC – 834	AGA GAG AGA GAG AGA GT	50.4	49.8
13	UBC – 841	GAG AGA GAG AGA GAG AC	45.4	45.7
14	UBC – 843	CTC TCT CTC TCT CTC TRA	50	37.6
15	UBC – 844	CTC TCT CTC TCT CTC TRC	52	39.4
16	UBC – 845	CTC TCT CTC TCT CTC TRG	50	43.4
17	UBC – 854	TCT CTC TCT CTC TCT CRG	54.8	51.1
18	UBC – 857	ACA CAC ACA CAC ACA CCG	51.7	57.1
19	UBC – 876	GAT AGA TAG ACA GAC A	40	36.4
20	UBC – 878	GGA TGG ATG GAT GGAT	50	29
21	UBC – 879	CTT CAC TTC ACT TCA	40	42.2
22	UBC – 881	GGG TGG GGT GGG GTG	50	66.5
23	UBC – 884	HBH AGA GAG AGA GAG AG	40	35
24	UBC – 885	HBH AGA GAG AGA GAG AG	40.7	41.7
25	UBC – 886	VDV CTC TCT CTC TCT CT	51.4	36.9
26	UBC – 889	DBD ACA CAC ACA CAC AC	47.05	39.4
27	UBC – 891	AGA TGT GTG TGT GTG TG	50	51.8

Table-4 Primer wise amplification and % polymorphism of cashew varieties

SN	Primer Name	No. of Polymorphic Bands	No. of Monomorphic Bands	Total No. of Bands	Polymorphism %	Range of Amplification (bp)	PIC
1	UBC-807	38	0	38	100	600-1800	0.82
2	UBC-811	28	9	37	75.68	300-2000	0.71
3	UBC-812	24	18	42	57.14	300-1300	0.71
4	UBC-813	25	0	25	100	900-1700	0.68
5	UBC-814	21	18	39	53.84	300-1600	0.76
6	UBC-815	13	9	22	59.09	300-2000	0.46
7	UBC-816	33	9	42	78.57	300-1300	0.79
8	UBC-817	25	9	34	73.53	500-1500	0.68
9	UBC-818	33	18	51	64.7	400-1300	0.79
10	UBC-824	15	9	24	62.5	500-1200	0.53
11	UBC-825	45	9	54	83.33	400-2000	0.84
12	UBC-834	53	9	62	85.48	300-2000	0.85
13	UBC-841	47	27	74	63.51	300-1700	0.85
14	UBC-843	4	9	13	30.76	700-1200	0.25
15	UBC-844	28	9	37	75.67	400-2000	0.71
16	UBC-845	0	9	9	0	1300-1400	0
17	UBC-854	14	0	14	100	300-1000	0.64
18	UBC-857	33	9	42	78.57	500-1700	0.79
19	UBC-876	85	0	85	100	300-2000	0.91
20	UBC-878	20	0	20	100	400-1800	0.6
21	UBC-879	76	9	85	89.41	400-2000	0.89
22	UBC-881	26	9	35	74.26	800-1900	0.77
23	UBC-884	45	9	54	83.33	400-1300	0.82
24	UBC-885	45	9	54	83.33	300-1300	0.82
25	UBC-886	49	0	49	100	600-2000	0.84
26	UBC-889	22	45	67	32.84	300-1300	0.64
27	UBC-891	35	9	44	79.55	300-1800	0.77
	Total	882	270	1152	1985.1		
	Average	32.667	10	42.667	73.522	Average	0.70

Cluster analysis

The cluster analysis was carried out based on the ISSR profile. The results based on the ISSR profile broadly grouped the 9 cashew varieties into two main clusters (I and II). The first cluster (I) was formed by the two subclasses. The clustering pattern of 9 varieties of cashew was given in [Table-5].

Table-5 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.

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. The dendrogram and similarity coefficient values give an idea about the nature of the individual sample in the whole sample set and all genotypes into two main cluster [Fig-4].

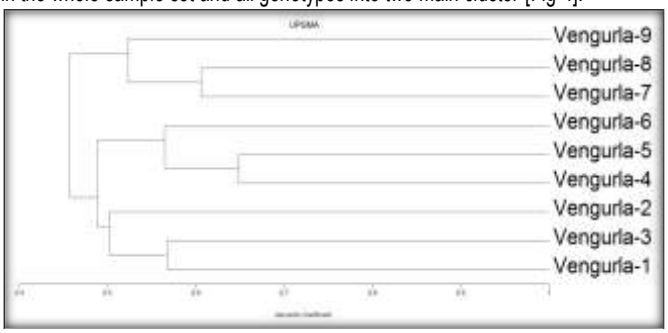

Fig-4 Dendrogram constructed using Jaccards Similarity Coefficient.

Conclusion

The ISSR markers revealed substantial polymorphism in Cashew with suitable for the assessment of genetic diversity. ISSR marker detects distinctness of varieties at molecular level which help in identification of desirable varieties and its utilization for further breeding programme. This information may be useful for selecting the diverse parents and monitoring the genetic diversity periodically for improvement of Cashew.

Application of research: Identification of desirable varieties and its utilization for further breeding programme.

Research Category: Molecular characterisation

Abbreviations: g- gram, ml- millilitre, %- percentage, mM- mill molar

Acknowledgement / Funding: Author thankful to Plant Biotechnology Centre, Dr Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, 415712, Maharashtra, India

***Research Guide or Chairperson of research: Dr Anil Bukya**
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Research project name or number: PhD Thesis

Author Contributions: All author equally contributed

Author statement: All authors read, reviewed, agree and approved the final manuscript

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Ethical Committee Approval Number

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