Research Article

Molecular Diversity Among Sesame Varieties Of Tamil Nadu

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Abstract:
Fourteen Inter Simple Sequence Repeat (ISSR) markers were used to estimate the molecular genetic diversity among 10 sesame varieties cultivated in Tamil Nadu. The 14 ISSR primers in 10 sesame genotypes yielded 66 reproducible amplified bands. The number of amplified bands varied from 1 to 7. Out of 66 bands, 65 were polymorphic (98.5%). Average number of bands and polymorphic bands per primer were 4.7 and 4.6 respectively. The polymorphic information content (PIC) value ranged between 0.50 and 0.85. NTSYS-pc (ver 2.02) software was used to calculate the Jaccard's similarity coefficients. The maximum similarity was observed between VRI 1 and VRI 2 followed by Paiyur 1 and TMV 6 and less similarity between VRI 1 and SVPR 1. Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster analysis grouped the 10 genotypes in to five groups at 0.60 similarity coefficient. Varieties CO 1, SVPR 1 and TMV 7 were clustered in to three separate clusters indicating most divergent among the released varieties of Tamil Nadu.

Key words: Genetic diversity, ISSR markers, Marker Aided Selection, PIC value

Introduction

Sesame (*Sesamum indicum* L.) is an important crop in tropical and subtropical areas (Ashri, 1998). Sesame seed, which is highly nutritive (50% oil and 25% protein), is traditionally used for direct consumption and as a source of oil of excellent quality due to the presence of antioxidants (Namiki, 1995) like sesamin, sesaminol, sesamol, sesamolinal, and squalene (Mohamed and Awatif, 1998) and also contains large level of polyunsaturated fatty acids (Davidson, 1999). Despite the nutritional value and historic and cultural importance of sesame, the research on sesame has been scanty. At present, no international CGIAR (Consultative Group on International Agricultural Research) agency is mandated to study sesame.

Information on genetic diversity and relationships among populations is important for ongoing plant breeding programs since a wide range of genetic diversity among parents is essential for crossing programs (Ganesh and Thangavelu 1995). Genetic diversity in crop species can be determined using morphological and agronomic characteristics as well as isozyme and DNA marker analysis (Liu, 1997; Reiter *et al*., 1993). However, the use of morphological and agronomic characteristics is associated with a strong influence from environmental factors and is therefore dependent on the conditions during cultivation. DNA markers overcome this limitation as they are not influenced by the environment and they occur in more or less unlimited numbers.

In order to enhance efficiency of the sesame breeding programme, the use and inclusion of molecular techniques are needed in the frame of marker-assisted selection approach. But information on the genetic diversity in sesame is limited as well. Only a few reports are available on the use of molecular markers such as isozyme (Isshiki and Umezaki, 1997), RAPD (Bhat *et al.*, 1999), ISSR (Kim *et al.*, 2002), AFLP (Uzun *et al.*, 2003) and SSR (Dixit *et al.*, 2005). In order to enhance the efficiency of the sesame breeding

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programme, the use and inclusion of molecular techniques are needed in the frame of marker-assisted selection approach.

Materials and methods

Plant material: The material for the study comprised 10 sesame varieties of Tamil Nadu viz., Paiyur 1, SVPR 1, CO 1, TMV 3, TMV 4, TMV 5, TMV 6, TMV 7, VRI 1 and VRI 2 collected from Department of Oilseeds, Tamil Nadu Agricultural University, Coimbatore and used for molecular diversity analysis. DNA was extracted from young leaves on each individual plant. Leaf tissue samples were collected and immediately stored at -20° C. Extraction of the DNA from the samples was carried out using High-throughput mini DNA extraction protocol of Doyle and Doyle (1990). Young fresh leaves of 10-15 days old were collected and grinded in pestle and mortar by using preheated (around 65ºC) CTAB buffer. Around 450 µl of CTAB buffer was added. Extracted samples were incubated in the water bath for 30 min at 65º C. After incubation, around 450 µl of chloroform: isoamyl alcohol (24:1) was added into the tubes and inverted twice to mix. Then the tubes were kept in centrifuge for 10 minutes at 12000 rpm. Then the aqueous layer was transferred into the new eppendorf tubes. An amount of equal volume of iso propanol (stored at -20 ºC) was added to each sample and inverted once in mix and kept overnight at 4 ºC. The samples were centrifuged at 5500 rpm for 15 min on the next day. The supernatant was discarded from each sample and the pellets settled in the bottom were air dried for 30 min. A quantity of 100 µl of TE buffer was added into each sample and stored it overnight at 4 ºC. RNAse (3 µl) was added into each sample to exclude the RNA contamination on the following day. An amount of 200 µl chloroform: isoamyl alcohol (24:1) was added into the tubes and centrifuged at 5000 rpm for 5 minutes and the supernatant was taken into the fresh tubes. To which twice the volume of absolute ethanol and $1/10^{th}$ volume of 3M sodium acetate was added and kept the samples at -4ºC for overnight. Centrifuge the tubes at 5500 rpm for 15 minutes. The supernatant was discarded and 200 µl of 70 per cent ethanol was added and centrifuged at 5000 rpm for 5 minutes. The supernatant was discarded and the pellet was air dried for 30 minutes. The pellet was resuspended by using 100 µl TE buffer and kept at -20 ºC for long term use.

ISSR methodology: The quality of the DNA was checked using 0.8 per cent agarose gel. Then the DNA was diluted using TE buffer according to the desired concentration. DNA amplifications were performed in a 10 µl reaction volume containing approximately 1.0 µl 10 X Taq buffer + $MgCl_2$ (15mM), 1.0 µl dNTP, 1.0 µl Primers (10 µ M), 0.1 µl Taq polymerase (3 IU / µl), 4.9 µl Sterile double distilled water and 2.0 µl Template DNA (10ng/ µl). PCR reactions were performed in a thermal cycler (Applied Biosystems) and programmed to a touchdown temperature cycle at 94ºC initial denature for 3 min, 94ºC denature for 3 sec, annealing at 63ºC for 30 sec, extension at 72ºC for 1 min for 19 cycles. Then 19 cycles at 94ºC initial denature for 15 sec, annealing at 55ºC for 30 sec, extension at 72ºC for 1 min. The PCR products were run with gel electrophoresis system on a 1.5% agarose gel at 120 V in Tris-borate-EDTA (TBE) buffer. A 100 bp standard molecular weight ladder was used in the electrophoretic runs. The UV transilluminated gels were photographed with a gel documentation and image analysis system. A total of 14 primers were selected to carry out the analysis in the ten varieties. Total bands were scored visually and polymorphic bands were analyzed as presence (1) or absence (0). Phylogenetic relations were determined by the UPGMA method using the Jaccard's similarity coefficient (NTSYS pc ver 2 software).

Results and Discussion

The PCR amplification using 14 primers in 10 sesame genotypes yielded 65 reproducible amplified bands. The number of amplified bands varied from 1 to 7. Out of 66 bands, 65 were polymorphic (98.5%). Average number of bands and polymorphic bands per primer were 4.5 and 4.3 respectively. The Polymorphic Information Content (PIC) value as a relative measure of polymorphism level ranged between 0.496 (ISSR 809) to 0.854 (ISSR 861) (Table 1).

The similarity matrix was computed using ISSR markers based on Jaccard's coefficient using NTSYS-Pc programme (Rohlf, 1990). The similarity coefficients based on 14 ISSR markers ranged from 0.24 to 0.80. The maximum similarity (0.80) was observed between the VRI 1 and VRI 2 followed by Paiyur 1 and TMV 6 (0.79). Low similarity was observed between SVPR 1 and VRI 1 (0.24) (Table 2).

A dendrogram was constructed for 10 genotypes using Jaccard's similarity index values using the NTSYS- pc ver 2.02. In Sequential Agglomerative Hierarchical Non overlapping (SAHN) UPGMA were used to generate dendrogram. Based on the dendrogram, the 10 genotypes formed four clusters at similarity index of 0.55 (Figure 1) as cluster I (Paiyur 1, TMV 6, TMV 5, TMV 3, TMV 4, VRI 1 and VRI 2), cluster II (CO1), cluster III (TMV 7) and cluster IV (SVPR 1).
The ISSR technique was previously performed in sesame to study the genetic relationship of a sesame germplasm by Kim *et al*. (2002). The technique does not need any prior information about DNA sequence and overcomes many of the technical limitations of RAPD and AFLP because of its high reproducibility and simplicity (Danilova and Karlov 2006; Ratnaparkhe et al. 1998). Since there is no sequence information and molecular studies are limited in sesame, ISSR-PCR offers great applicability to identify molecular markers.

The present study detected a high level of polymorphism for sesame between the different sesame varieties of Tamil Nadu. The PCR amplification showed that out of 66 bands, 65 were polymorphic (98.6%). It is also comparable with the 100% of polymorphism reported by Salazar *et al*. (2006) from a study of sesame from Venezuela using RAPD. In their result, all primers used for analysis were highly polymorphic (100%). Average number of bands and polymorphic bands per primer were 4.5 and 4.3 respectively. The Polymorphic Information Content (PIC) value as a relative measure of polymorphism level ranged between 0.496 (ISSR 809) to 0.854 (ISSR 861). The higher PIC value indicated the informativeness of the primer. Among the primers used in the study four primers *viz*., 826, 841, 847 and 861 exhibited the PIC value from 0.856 to 0.771. These primers can provide the basis for sesame DNA profile system.
Based on this similarity index, genotypes SVPR 1 and VRI 1 (0.24) were identified as diverse genotypes; VRI 1 and VRI 2 (0.80) followed by Paiyur 1 and TMV 6 (0.79) were identified as close genotypes.

The cluster I (Paiyur 1, TMV 6, TMV 5, TMV 3, TMV 4, VRI 1 and VRI 2) was found to be largest one with seven genotypes. Cluster II (CO1), cluster III (TMV 7) and cluster IV (SVPR 1) had one genotype each. Genotypes from diverse clusters may be inter crossed to generate higher variability. Hence genotypes namely Paiyur 1, TMV 3, TMV 4, TMV 5 and TMV 6 (cluster I) may be crossed with cluster II (CO1), cluster III (TMV 7) and cluster IV (SVPR 1) to create more variability.

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Table 1. Percentage of polymorphism and Polymorphic Information Content (PIC) value for the ISSR primers

Sl. No	Primers	Total no. of bands	No. of polymorphic bands	PIC value
1	807	5	5	0.748
2	808	2	2	0.526
3	809	4	3	0.496
4	826	7	7	0.803
5	841	7	7	0.791
6	847	6	6	0.771
7	855	4	4	0.708
8	857	5	5	0.716
9	861	7	7	0.854
10	862	5	5	0.669
11	865	3	3	0.512
12	866	4	4	0.660
13	889	4	4	0.688
14	890	3	3	0.647
	Total	**66**	**65**	
		4.5	**4.3**	

Table 2. Jaccard's similarity coefficient- 10 genotypes

	Paiyur 1	SVPR 1	TMV 4	CO 1	TMV 3	TMV 5	TMV 6	TMV 7	VRI 1	VRI 2
Paiyur 1	1.00									
SVPR 1	0.37	1.00								
TMV 4	0.48	0.42	1.00							
CO 1	0.68	0.43	0.52	1.00						
TMV 3	0.70	0.49	0.46	0.63	1.00					
TMV 5	0.67	0.33	0.50	0.70	0.64	1.00				
TMV 6	0.79	0.33	0.47	0.67	0.61	0.77	1.00			
TMV 7	0.40	0.31	0.38	0.37	0.35	0.40	0.46	1.00		
VRI 1	0.61	0.24	0.45	0.49	0.49	0.53	0.60	0.50	1.00	
VRI 2	0.60	0.33	0.53	0.57	0.54	0.58	0.58	0.52	0.80	1.00

Fig. 1. Diversity among Sesame varieties of Tamil Nadu