Journal of Agricultural Technology 2011 Vol. 7(4): 1097-1106
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

RAPD markers for genetic variability studies in ashgourd *Benincasa hispida* (Thunb.) Cogn.

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Resmi, J. and I. Sreelathakumary (2011) RAPD markers for genetic variability studies in ashgourd *Benincasa hispida* (Thunb.) Cogn. Journal of Agricultural Technology 7(4):1097-1106.

Genetic relationships were estimated among twenty five ashgourd [*Benincasa hispida* (Thunb.) Cogn.] landraces of different types using PCR-based random amplified polymorphic DNA (RAPD) markers. Twenty nine primers, out of the forty decamer primers yielded amplification products indicating presence of sequence complementary to these primers in the DNA of ashgourd landraces. Pair-wise genetic similarities among the landraces determined using Jaccard's coefficient ranged from 0.14 to 1.00. The dendrogram based on clustering matrix separated the landraces with morphologically distinct smooth textured fruits from waxy textured fruits and the former group showed less divergence than the latter. With in the group of waxy textured fruits, limited variation was detected among landraces with small and medium sized fruits. Further, morphologically similar landraces with large fruits form distinct clusters with in the major clusters. The study demonstrated the usefulness of the RAPD marker analysis in effectively assessing the genetic relationship present among the ashgourd landraces examined.

Key words: RAPD**,** ashgourd, marker, polymorphism, primer

Introduction

Ashgourd is a monotypic genus with only one cultivated species *Benincasa hispida* (Thunb.) Cogn. It is an important warm-season cucurbit vegetable, grown for its succulent hairy fruits, used in confectionary and in ayurvedic medicinal preparations. Although ashgourd is becoming a crop of industrial importance, relatively less attention has been paid towards the morpho-molecular characterization of existing strains available in different parts of the country. The genetic variation as detected by RAPD analysis opens up the avenue for the proper identification and selection of the genotypes that could be used for varietal identification and planning for future crop improvement programme. RAPD marker profiles were highly consistent and useful in establishing the cultivar identity. RAPD analysis has been

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successfully employed to analyse genetic diversity in cucumber (Ping *et al*., 2002), melon (Woo and Hyeon, 2003; Kandasamy, 2004), bottlegourd (Ram *et al.,* 2006), ashgourd (Verma *et al.*, 2007), teasle gourd (Rasul *et al*., 2007), sweetgourd (Rahaman *et al*., 2007) and bittergourd (Rahman et al., 2007; Behera *et al.,* 2008, Resmi, 2009). In the present study, an attempt has been made to evaluate the genetic variability and relationships using PCR-based Random amplified polymorphic DNA (RAPD) marker technique in ashgourd.

Materials and methods

Plant materials

The experimental material consisted of 25 morphologically distinct landraces of ashgourd collected from different agroclimatic regions of Kerala, Tamil Nadu and Karnataka. The details of the landraces and their sources are presented in Table 1

DNA isolation and quantification

High-molecular weight genomic DNA was extracted from tender leaves of 15-20 days old seedlings as per Murray and Thompson (1980) protocol using CTAB. The quality of isolated DNA was tested by agarose gel electrophoresis and further quantitated by spectrophotometry (Spectronic Genesys 5).

RAPD assay

DNA amplification reactions were performed following the protocol of Staub *et al.* (2000) with minor modifications. Polymerase chain reactions of genomic DNA were carried out in 25 µl reaction volume containing 2.5 µl 10x PCR buffer, 1 µl $MgCl_2$, 2 µl each of dNTPs, 10 pM decamer primer (Operon Inc., CA, USA), 1 unit of Taq DNA polymerase (Invitrogen, USA) and 40 ng genomic DNA. Amplification was performed in a thermal cycler (PTC-100, MJ Research Inc.) for an initial denaturation at 94^{o}C for 5 minutes, followed by 44 cycles of denaturation at 94^{o}C for 15 seconds and annealing at 35^{o}C for 15 seconds. An extension at 72^{o}C for 75 seconds was included after the last cycle. The PCR product was analyzed by electrophoresis on 1.2 per cent agarose gel prepared in 1x TAE buffer, visualized under UV-Vis transilluminator after ethidium bromide staining and photographed using gel documentation system (BIO RAD, USA).

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Data analysis

Polymorphism was detected by scoring the presence (+) or absence (-) of the reproducible bands and further analyzed the data with NTSYSpc (Version 2.02i) software. The data from the three primers were used to estimate the similarity on the basis of the number of shared bands. A genetic similarity matrix was constructed using Jaccard's coefficient method (Jaccard, 1908) and was subjected to cluster analysis using UPGMA and dendrogram was generated.

Table 1. Particulars of landraces of *Benincasa hispida* used in the study and their sources

Landrace number	Source	Morphological type	
		Fruit size	Fruit texture
BH1	Thakkala (Tamil Nadu-1)	Large	Waxy
BH 2	Cherthala (Alapuzha-1)	Large	Waxy
BH 3	Vadakkancheri (Thrissur-1)	Large	Waxy
BH 4	Balaramapuram (Trivandrum-1)	Large	Waxy
BH 5	Co-1, TNAU (Tamil Nadu-2)	Large	Waxy
BH 6	Thiruvalla (Pathanamthitta-1)	Large	Waxy
BH 7	Kattakada (Trivandrum-2)	Extra large	Smooth
BH 8	Cheruplasseri (Palakkad-1)	Very small	Waxy
BH 9	Indu, KAU (Trivandrum-3)	Large	Waxy
BH 10	Ambalathara (Trivandrum-4)	Small	Waxy
BH 11	Vadakara (Kozhikode-1)	Very small	Waxy
BH 12	Periya (Wyanad-1)	Medium	Waxy
BH 13	Bangalore-1	Medium	Waxy
BH 14	Aryanad (Trivandrum-5)	Medium	Waxy
BH 15	Neyattinkara (Trivandrum-6)	Extra large	Waxy
BH 16	Madhurai (Tamil Nadu-3)	Large	Waxy
BH 17	Kalpetta (Wayanad-2)	Large	Smooth
BH 18	Ettumannoor (Kottayam-1)	Large	Waxy
BH 19	Edathua (Alappuzha-2)	Large	Waxy
BH 20	Nagarcoil (Tamil Nadu-4)	Medium	Smooth
BH 21	Thodupuzha (Idukki-1)	Small	Waxy
BH 22	Pala (Kottayam-2)	Very small	Waxy
BH 23	KAU local, KAU (Trivandrum-7)	Large	Waxy
BH 24	Kottarakara (Kollam-1)	Medium	Waxy
BH 25	Nemom (Trivandrum-8)	Large	Smooth

Results and discussion

RAPD polymorphism

DNA amplification of twenty five landraces of *Benincasa hispida* was studied using forty decamer primers. Twenty nine primers, out of the forty decamer primers yielded amplification products indicating presence of sequence complementary to these primers in the DNA of ashgourd landraces. Eleven primers did not give any amplification. Based on polymorphic and reproducible banding patterns, three primers (Plates 1, 2 and 3) were selected. They were OPA-01, OPA-07 and OPA-13. This could be explained by the capability of individual primers to amplify the less conserved and highly repeated regions of the genomic DNA. There is high possibility for the amplified fragments to contain repeated sequences. Bhat and Jarret (1995) suggested that the number of polymorphisms might be more important than the number of primers for the generation of stable phenogram and it would vary with plant material under investigation and the sequences that are amplified. In bittergourd, Pala (2001) identified six RAPD primers to show genetic relationship among the genotypes while Behera *et al*. (2007) used twenty nine RAPD primers for genetic diversity studies.

The molecular survey of the ashgourd germplasm by RAPD using three selected primers resulted in 20 scorable bands (average of 6.66 bands per primer) of which 2 were monomorphic and rest, 18 were polymorphic (90.0 %). The detection of polymorphisms herein is in large part due to the use of highly informative primers identified during primer-screening using a small set of diverse ashgourd landraces. The number of bands ranged from 1 to 8 with an average of 3 per primer. A maximum of 8 polymorphic bands were obtained in PCR amplification from genomic DNA of the landraces used in present study. However, in the case of *C. lanatus*, Levi *et al.* (2001) reported an average of 21.2 polymorphic products per primer. The higher number of polymorphic products indicates that the genotypes used in present study posses a higher degree of polymorphism. The proportion of polymorphic loci obtained in the present study was lower (90 %) than that reported in teasle gourd (95%) (Rasul *et al.*, 2007) but higher than that in *C. melo* (18 %) bittergourd (36.5%) (Dey *et al.*, 2006), ashgourd (73 %) (Pandey *et al.*, 2008) and ridgegourd (81.5%) (Hoque and Rabbani, 2009). In contrast, while analyzing the genetic diversity among some accession of the *Cucurbita maxima*, *Cucurbita pepo*, *Cucurbita ficifolia* and *Lagenaria siceraria* using 26 primers, Ferriol et al. (2003) observed 57% polymorphic bands from a total of 92 consistent bands. Such a high level of polymorphism may be attributed to the use of different species

and genus rather than use of cultivars or inbred lines within a species. The size of the DNA bands ranged from 500 to 2500 bp.

The primer OPA-07 (7 bands) was unique as it could distinguish maximum of the landraces tested. The highest number of scorable bands (8 bands) was given by OPA-13 of which one of the bands produced was monomorphic. The primer OPA-01 produced five scorable bands of which one band was monomorphic for all the landraces.

The estimation of Jaccard's similarity coefficients and construction of dendrogram by using UPGMA revealed the presence and extent of genetic similarities among the twenty five landraces of ashgourd examined. Pair-wise genetic similarities among the landraces determined using Jaccard's coefficient ranged from 0.14 to 1.00 (Table 2). Cluster analysis revealed that at about 0.35 similarity coefficient, the twenty five landraces of ashgourd grouped into two clusters (Fig. 1). Landraces with morphologically distinct smooth and waxy textured fruits grouped into two major clusters with an exception of BH 19 falling in the first cluster. This substantiates the moderately broad distribution of genetic variability, which can be attributed to the broad genetic base in their origin.

Landraces with smooth textured fruits (BH 7, BH 17, BH 20 and BH 25) again grouped into two with two members each at 59 per cent similarity. This grouping was in concordance with their average fruit weight. BH 7 showed 74.8 per cent similarity with BH 25 while BH 17 showed 80.2 per cent similarity with BH 20. The waxy textured group formed a more divergent cluster than smooth textured group. With in the group of waxy textured fruits, limited variation was detected among landraces with small sized fruits. Morphologically similar landraces BH 11, BH 14 and BH 24 are grouped together and showed 100 per cent similarity.

Landraces with medium sized fruits also showed limited variability. They formed five subclusters with in the waxy textured group with 100 percent similarity for BH 2 with BH 23 and BH 3 with BH 9 respectively. Further, landraces classified as belonging to the same morphotypic group did not always cluster together. This was evidenced from the results of RAPD analysis that morphologically similar landraces with large fruits form distinct clusters with in the major clusters. BH1, BH 5, BH 6, BH 7, BH 15, BH 18 and BH 25 with high average fruit weight formed distinct clusters under molecular study.

In the present study, RAPD marker analysis revealed and grouped ashgourd landraces into eight clusters according to their genetic relationships reliably. The results indicated that most of the collected landraces examined were genetically distinct, and these differences can be provided for the

development of strategies for genetic analysis and crop improvement in this species.The clusters based on RAPD analysis using three primers depict wide genetic variation among the landraces examined and provided varietal profiles. It can easily differentiate *B. hispida* landraces, even the closely related ones. Polymorphism obtained in the present study will be further useful in fingerprinting and in determining genetic diversity among the ashgourd

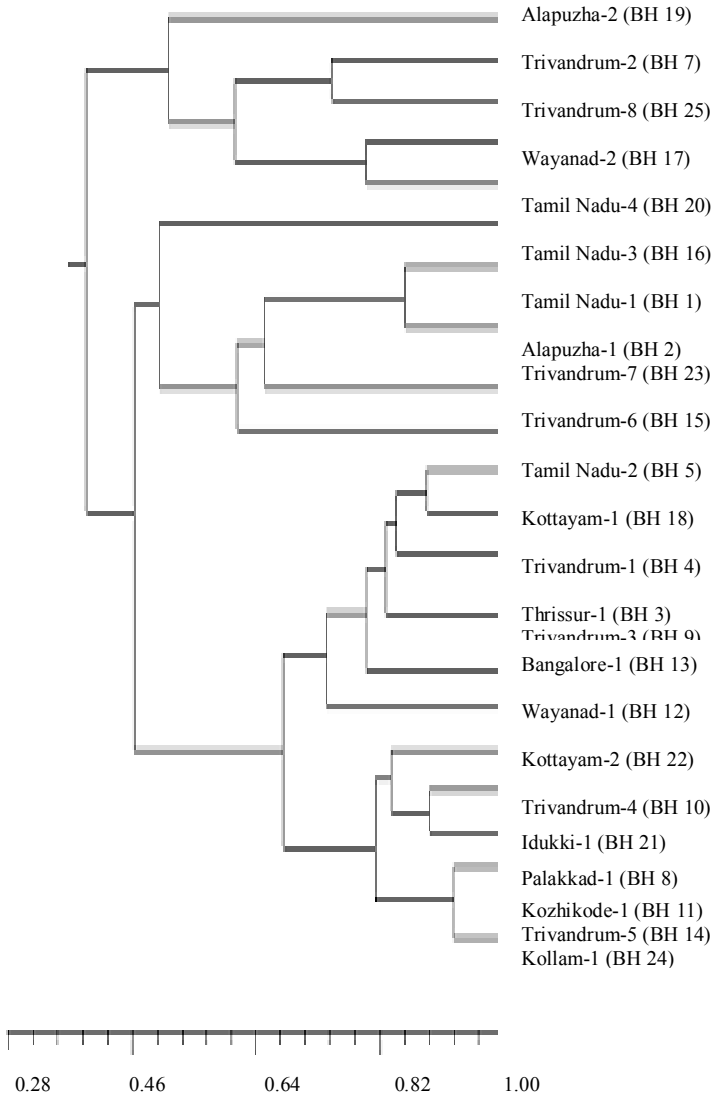


Fig 1. Jaccard's Similarity Coefficient

landraces. Thus the study revealed that RAPD technique can be suggested as an objective and viable alternative or supplement to ampelography for ashgourd identification. For future studies on analysis of ashgourd landraces, wider genetic base and greater number of RAPD primers are to be included for accurate results. Finally, the results support the idea that RAPD technique being relatively simpler, quicker, inexpensive, non-radioactive, versatile and universal can detect sufficient polymorphisms for germplasm characterization and genetic distance studies. This marker system has the ability to amplify DNA from dispersed polymorphic loci and has its power to detect small genetic differences.

Plate 1. Amplification profiles of the DNA of twenty five landraces of *B. hispida* using the primer OPA-01

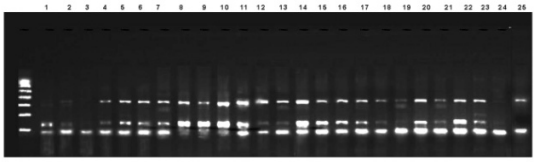


Plate 2. Amplification profiles of the DNA of twenty five landraces of *B. hispida* using the primer OPA-07

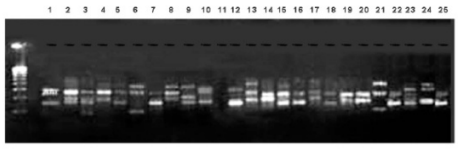
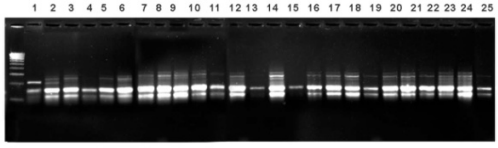


Plate 3. Amplification profiles of the DNA of twenty five landraces of *B. hispida* using the primer OPA-13



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(Received 22 March 2011; accepted 30 May 2011)

Table 2. Similarity matrix for 25 landraces of *B. hispida* generated using RAPD

x	BH1	BH2	BH3	BH4	BH5	BH6	BH7	BH8	BH9	BH10	BH11	BH12	BH13	BH14	BH15	BH16	BH17	BH18	BH19	BH20	BH21	BH22	BH23	BH24	BH25
BH1	1.00																								
BH2	0.40	1.00																							
BH 3	0.33	0.57	1.00																						
BH 4	0.14	0.37	0.71	1.00																					
BH 5	0.28	0.50	0.85	0.62	1.00																				
BH 6	0.33	0.57	0.71	0.50	0.62	1.00																			
BH 7	0.30	0.50	0.42	0.25	0.37	0.42	1.00																		
BH 8	0.22	0.27	0.50	0.36	0.45	0.50	0.44	1.00																	
BH 9	0.20	0.36	1.00	0.45	0.44	0.40	0.40	0.42	1.00																
BH 10	0.22	0.27	0.50	0.36	0.60	0.50	0.44	0.80	0.42	1.00															
BH 11	0.28	0.50	0.85	0.62	0.70	0.62	0.37	0.45	0.44	0.60	1.00														
BH 12	0.25	0.44	0.55	0.40	0.50	0.55	0.33	0.70	0.43	0.54	0.50	1.00													
BH 13	0.25	0.33	0.50	0.50	0.42	0.50	0.16	0.20	0.40	0.20	0.42	0.22	1.00												
BH 14	0.22	0.27	0.50	0.36	0.45	0.50	0.44	0.80	0.42	0.80	1.00	0.54	0.20	1.00											
BH 15	0.25	0.14	0.50	0.50	0.42	0.28	0.16	0.33	0.40	0.33	0.42	0.22	0.50	0.33	1.00										
BH 16	0.20	0.36	0.45	0.33	0.54	0.45	0.40	0.72	0.46	0.72	0.54	0.80	0.18	0.58	0.18	1.00									
BH 17	0.35	0.44	0.55	0.40	0.50	0.55	0.70	0.70	0.40	0.70	0.50	0.77	0.22	0.70	0.22	0.80	1.00								
BH 18	0.22	0.27	0.50	0.36	0.45	0.50	0.44	0.80	0.42	0.80	0.45	0.54	0.20	0.70	0.33	0.58	0.70	0.35							
BH 19	0.30	0.42	0.47	0.37	0.50	0.37	0.50	0.40	0.56	0.40	0.50	0.44	0.14	0.55	0.33	0.36	0.44	0.55	1.00						
BH 20	0.35	0.30	0.55	0.40	0.50	0.55	0.50	0.89	0.40	0.88	0.50	0.60	0.22	0.88	0.37	0.63	0.77	0.88	0.44	1.00					
BH 21	0.25	0.44	0.45	0.40	0.50	0.55	0.50	0.70	0.80	0.70	1.00	0.77	0.22	1.00	0.22	0.80	0.50	0.70	0.44	0.77	1.00				
BH 22	0.50	0.50	0.46	0.42	0.57	0.42	0.60	0.44	0.40	0.44	0.57	0.50	0.16	0.44	0.40	0.40	0.50	0.44	0.80	0.50	0.50	1.00			
BH 23	0.25	1.00	0.55	0.40	0.50	0.55	0.50	0.70	0.80	0.70	0.50	0.77	0.22	0.70	0.22	0.80	0.33	0.70	0.44	0.77	0.46	0.50	1.00		
BH 24	0.22	0.40	0.50	0.36	0.45	0.50	0.44	0.60	0.72	0.63	1.00	0.88	0.20	1.00	0.20	0.90	0.88	0.63	0.40	0.70	0.88	0.44	0.88	1.00	
BH 25	0.36	0.60	0.40	0.48	0.42	0.40	0.45	0.43	0.60	0.43	0.42	0.47	0.40	0.43	0.40	0.40	0.47	0.43	0.50	0.47	0.64	0.64	0.47	0.43	1.00