Indian J. Hort. 68(3), September 2011: 345-350

Multivariate based marker analysis in ashgourd

J. Resmi and I. Sreelathakumary*
Department of Olericulture, College of Agriculture, Vellayani, Thiruvananthapuram 695 522

ABSTRACT

Genetic diversity of 25 ashgourd genotypes of different geographical origin was assessed at the molecular level and compared to morphological traits for degree of divergence. The clustering pattern based on Mahalanobis D^2 statistic indicated that there was no association between geographical distribution of genotypes and genetic divergence. The cluster profile, based on quantitative data and RAPD markers revealed that morphologically distinct and superior lines were genetically differentiable. Further more, the RAPD analysis gave a perfect differentiation of waxy textured group from smooth textured group, which is in line with morphological characterization.

Key words: Genetic divergence**,** ashgourd, marker, polymorphism, RAPD.

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 multivariate analysis using Mahalanobis D^2 statistic, which measures the forces of differentiation at intra- and inter-cluster levels is a valuable tool in obtaining quantitative estimates of divergence. In the process of formulating the ashgourd crop improvement programmes, understanding about the nature and degree of genetic divergence available in the germplasm plays a pivotal role. This technique provides a basis for selection of genetically divergent parents in hybridization programme.

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 analysis has been successfully employed to analyse genetic diversity in cucumber (Ping *et al*., 10), in melon (Woo and Hyeon, 14; Kandasamy, 3) and in bittergourd (Rahman *et al*., 11). In the present study, an attempt has been made to evaluate the genetic variability and relationships using multivariate statistical tools and PCR-based random amplified polymorphic DNA (RAPD) marker technique in ashgourd.

*Corresponding author's E-mail: sreelathakumary@rediffmail.com

MATERIALS AND METHODS

Twenty five morphologically distinct ashgourd genotypes collected from different agro-climatic regions of Kerala, Tamil Nadu and Karnataka were evaluated at the Vegetable Plot in the Department of Olericulture, College of Agriculture, Thiruvananthapuram. The details of the genotypes are furnished in Table 1. The crop was grown in a randomized block design with two replications at a spacing of 4.5 m × 2.0 m. The crop received timely management practices as per package of practices recommendations of Kerala Agricultural University (KAU, 4). Observations were recorded on four randomly selected plants of each genotype in each replication for 11 characters, *viz*., vine length (cm), days to first female flower, node to first female flower, sex ratio, fruit length (cm), fruit girth (cm), fruits per plant, average fruit weight (kg), yield per plant (kg), seeds per fruit and mosaic incidence. The genetic divergence was estimated using D^2 statistics of Mahalanobis (7) and the populations were grouped into clusters by following the method suggested by Tocher (Rao, 12).

High-molecular weight genomic DNA was extracted from tender leaves of 15-20 days old seedlings as per Murray and Thompson (9) protocol using CTAB. The quality of isolated DNA was tested by agarose gel electrophoresis and further quantitated by spectrophotometry (Spectronic Genesys 5). DNA amplification reactions were performed following the protocol of Staub *et al.* (13) 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

Table 1. Details of *Benincasa hispida* genotypes used in the study and their sources.

Sl. No.	Genotype No.	Source	Morphological type	
			Fruit size	Fruit texture
1	BH1	Thakkala (Tamil Nadu-1)	Large	Waxy
2	BH 2	Cherthala (Alappuzha-1)	Large	Waxy
3	BH 3	Vadakkancheri (Thrissur-1)	Large	Waxy
4	BH 4	Balaramapuram (Trivandrum-1)	Large	Waxy
5	BH 5	Co-1, TNAU (Tamil Nadu-2)	Large	Waxy
6	BH 6	Thiruvalla (Pathanamthitta-1)	Large	Waxy
7	BH 7	Kattakada (Trivandrum-2)	Extra large	Smooth
8	BH 8	Cheruplasseri (Palakkad-1)	Very small	Waxy
9	BH 9	Indu, KAU (Trivandrum-3)	Large	Waxy
10	BH 10	Ambalathara (Trivandrum-4)	Small	Waxy
11	BH 11	Vadakara (Kozhikode-1)	Very small	Waxy
12	BH 12	Periya (Wyanad-1)	Medium	Waxy
13	BH 13	Bangalore-1	Medium	Waxy
14	BH 14	Aryanad (Trivandrum-5)	Medium	Waxy
15	BH 15	Neyattinkara (Trivandrum-6)	Extra large	Waxy
16	BH 16	Madhurai (Tamil Nadu-3)	Large	Waxy
17	BH 17	Kalpetta (Wayanad-2)	Large	Smooth
18	BH 18	Ettumannoor (Kottayam-1)	Large	Waxy
19	BH 19	Edathua (Alappuzha-2)	Large	Waxy
20	BH 20	Nagarcoil (Tamil Nadu-4)	Medium	Smooth
21	BH 21	Thodupuzha (Idukki-1)	Small	Waxy
22	BH 22	Pala (Kottayam-2)	Very small	Waxy
23	BH 23	KAU local, KAU (Trivandrum-7)	Large	Waxy
24	BH 24	Kottarakara (Kollam-1)	Medium	Waxy
25	BH 25	Nemom (Trivandrum-8)	Large	Smooth

ng genomic DNA. Amplification was performed in a thermal cycler (PTC-100, MJ Research Inc.) for an initial denaturation at 94°C for 5 min., followed by 44 cycles of denaturation at 94°C for 15 s. and annealing at 35°C for 15 s. An extension at 72°C for 75 s. 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 (Biorad, USA).

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, 2) and was subjected to cluster analysis using UPGMA and dendrogram was generated.

RESULTS AND DISCUSSION

The analysis of variance revealed that the genotypes varied significantly for all the characters under study. After computing D^2 values for all the possible pairs, 25 genotypes were grouped into seven clusters, which indicated a large genetic diversity (Table 2). The cluster I was the largest with eight genotypes, followed by cluster II with four genotypes. Clusters III, IV, V and VI had three genotypes each. The cluster VII included only one genotype. Distribution of genotypes into various clusters showed no uniformity and those belonging to the same place were distributed among different clusters, thus ruling out the association between geographical distribution of genotypes and genetic divergence. Present results support the

Table 2. Grouping of 25 ashgourd genotypes using D^2 statistics.

Cluster No.	Number of genotype(s)	Genotype(s)
I	8	BH 4, BH 10, BH 11, BH 14 , BH 20, BH 21, BH 22, BH 24
II	4	BH 2, BH 5, BH 9, BH 19
III	3	BH 6, BH 15, BH 16
IV	3	BH 12, BH 13, BH 17
V	3	BH 1, BH 3, BH 23
VI	3	BH 7, BH 18, BH 25
VII	1	BH 8

findings of Lovely (6) in ashgourd, Lakshmi *et al*. (5) in pumpkin, Kandasamy (3) in melon, Maharana *et al*. (8) in ivygourd, and Dey *et al.* (1) in bittergourd.

The intra- and inter- cluster distance represent the index of genetic diversity among clusters as given in Table 3. The inter-cluster distances were greater than intra-cluster distances, revealing considerable amount of genetic diversity among the genotypes studied. The cluster VI recorded a maximum intra-cluster distance, whereas cluster VII had the minimum. With respect to inter-cluster distance, cluster V showed the highest divergence from cluster VII suggesting thereby that the genotypes belonging to clusters V and VII may be undertaken in a hybridization programme for evolving good hybrids or segregants. For crop improvement programmes, intercrossing among genotypes with outstanding mean performance was suggested by Lovely (6).

The comparison of cluster means for the different characters indicated considerable differences between the clusters for all the characters (Table 4). Cluster VII (BH 8) comprised of genotypes with smallest fruits, highest fruits per plant, shorter vine length, earliness in flowering and lowest sex ratio, yield and seeds per fruit. Cluster V consisted of genotypes with medium sized fruits with highest mosaic resistance and seeds per fruit. Cluster III had the highest average fruit weight and yield per plant. Cluster VI comprised of large sized fruits with high mosaic incidence. Thus, clusters III and V were superior for most of the biometric characters, whereas clusters I and VI were generally poor. Cluster II and VII was found to be intermediate.

Rational choice of parents on the basis of their genetic diversity can provide scope for rapid improvement. The cluster comprising one genotype with specific valuable traits and other genotypes falling in highly divergent group will help in broadening the existing genetic base and may produce new genotypes with hitherto unknown combination.

DNA amplification of 25 genotypes of *Benincasa hispida* was studied using 40 decamer primers. Twenty nine primers, out of the forty decamer primers yielded amplification products indicating presence of sequence complementary to these primer in the DNA of ashgourd genotypes. Eleven primers did not give any amplification. Based on polymorphic and reproducible banding patterns, three primers (Fig. 1, a,b, & c) were selected. The molecular survey of the ashgourd germplasm by RAPD using these three selected primers resulted in 20 scorable bands (average of 6.66 bands per primer) of which two were monomorphic and rest, 18 were polymorphic (90.0 %). The number of bands ranged from 1 to 8 with an average of 3 per primer. 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

Table 3. Average inter- and intra-cluster D^2 values among eight clusters in 25 ashgourd genotypes.

Cluster	I	II	III	IV	V	VI	VII
I	397.30	10662.02	23094.50	2580.15	32854.00	1321.92	1322.18
II		456.32	2890.19	3354.86	6621.94	13099.44	17412.36
III			286.55	11357.85	1243.44	25733.91	32266.60
IV				417.05	18283.41	4198.01	6413.52
V					401.25	36516.94	43823.68
VI						549.72	1997.78
VII							0.00

Diagonal elements = intracluster values

Off-diagonal elements = intercluster values

Table 4. Cluster means of eleven biometric characters in ashgourd.

Cluster	Vine length (cm)	Days to first female flower	Node to first female flower	Sex ratio	Fruit length (cm)	Fruit girth (cm)	Fruits per plant	Av. fruit weight (kg)	Yield per plant (kg)	Seeds per fruit	Mosaic incidence (V.I.)
I	578.44	55.93	22.59	10.12	24.69	37.07	5.42	1.51	5.77	225.50	47.81
II	438.00	57.03	21.94	13.84	38.95	63.58	2.72	5.39	13.16	955.25	51.25
III	483.17	56.90	22.43	13.21	43.13	66.85	2.46	7.93	17.70	1352.67	53.33
IV	540.67	58.83	26.04	12.61	37.85	49.25	3.25	2.89	8.78	559.33	51.67
V	529.17	55.45	21.46	12.40	39.86	68.93	2.38	6.78	14.36	1556.83	45.83
VI	706.00	55.45	20.28	10.51	55.28	47.49	2.56	6.92	15.02	186.00	57.50
VII	388.50	46.25	27.63	8.59	13.65	22.20	8.00	0.28	1.99	73.50	47.50

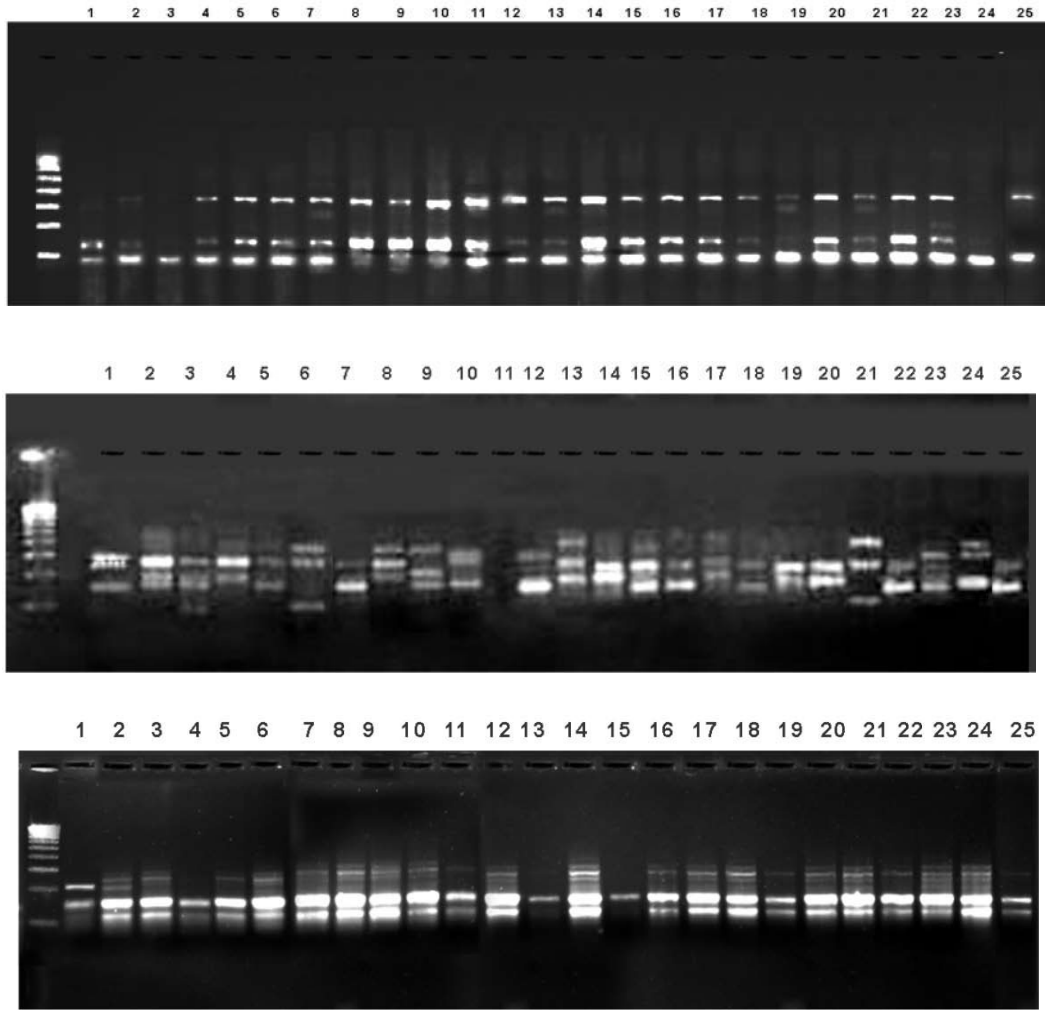

Fig. 1. DNA amplification profiles of twenty five *B. hispida* genotypes using the Primer (a) OPA-01, (b) OPA-07, and (c) OPA-13.

the genotypes 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 genotypes.

Pair-wise genetic similarities among the genotypes determined using Jaccard's coefficient ranged from 0.14 to 1.00. Cluster analysis revealed that at about 0.35 similarity coefficient, the twenty five ashgourd genotypes of grouped into two clusters (Fig. 2). Genotypes 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. Genotypes 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 genotypes with small sized fruits. Morphologically similar genotypes BH 11, BH 14 and BH 24 are grouped together and showed 100 per cent similarity. Genotypes with medium sized fruits also showed limited variability. They formed five sub-clusters with in the waxy textured group with 100 per cent similarity for BH 2 with BH 23 and BH 3 with BH 9, respectively. Further, genotypes 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 genotypes 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 has revealed and grouped ashgourd genotypes into eight clusters according to their genetic relationships reliably. The clusters based on RAPD analysis using three primers depict wide genetic variation among the genotypes examined. It can easily differentiate *B. hispida* genotypes, 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 genotypes.

In comparing clustering pattern of quantitative traits with RAPD markers, genetic dissimilarity was noticed. Credibility of genetic diversity analysis involving 11 quantitative traits and 20 RAPD markers could not be established beyond doubt, since; pedigree details of the genotypes involved were not available. Though

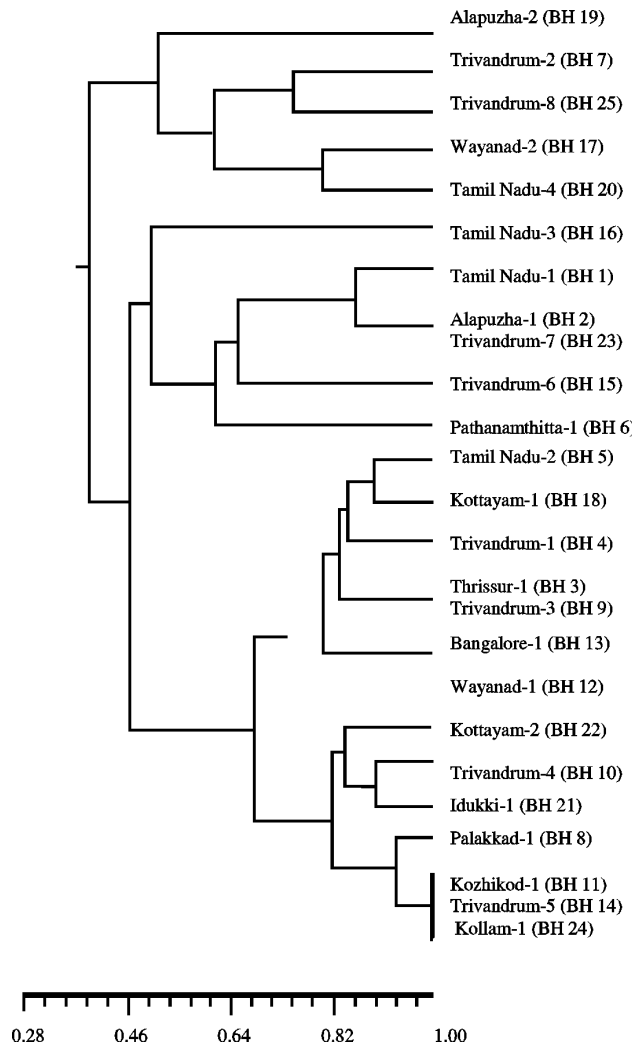

Fig. 2. Dendrogram based on Jaccard's similarity coefficient.

the genotypes are known for their geographical origin, the latter could not be used as a criterion to establish relationship between genotypes, since genotypes from the same geographical origin had clustered separately. By characterizing all the twenty five genotypes of *B. hispida* using morphological (D^2 analysis) and molecular (RAPD marker analysis) methods revealed that morphologically distinct and superior lines were genetically differentiable. Also the RAPD analysis gave a perfect differentiation of waxy textured group from smooth textured group, which is in line with morphological characterization.

REFERENCES

1. Dey, S.S., Behera, T.K., Munshi, A.D. and Sirohi, P.S. 2007. Studies on genetic divergence in bittergourd (*Momordica charantia* L.). *Indian J. Hort.* **64**: 53-57.

2. Jaccard, P. 1908. Nouvelles rescherches suv la distribution florale. *Bull. Soc. Vandoise des Sciences Neturelles*, **44**: 223-70.

3. Kandasamy, R. 2004. Morphological, biochemical and molecular characterization in landraces of melon (*Cucumis melo* L.). Ph.D. thesis, Kerala Agricultural University, Thrissur.

4. KAU. 2007. *Package of Practices Recommendations – Crops*. Directorate of Extension, Kerala Agricultural University, Thrissur, 334 p.

5. Lakshmi, L.M., Haribabu, K. and Reddy, G.L.K. 2003. Genetic divergence in pumpkin. *Indian J. Hort.* **60**: 363-67

6. Lovely, B. 2001. Evaluation of genetic divergence in ashgourd. M.Sc. thesis, Kerala Agricultural University, Thrissur.

7. Mahalanobis, P.C. 1928. A statistical study at Chinese head measurements. *J. Asiatic Soc.* **25**: 31-77.

8. Maharana T., Mandal, P., Sahoo, G.S. and Mahapatra, B. 2006. Multivariate analysis of genetic divergence in kunduru (*Coccinia grandis* (L.) (Voigt). **In**: *Abstracts. First International Conference on Indigeydnous Vegetables and Legumes*, 12 - 15 December 2006, Hyderabad, India, pp. 70.

9. Murray, M. and Thompson, W. 1980. The isolation of high molecular weight plant DNA. *Nucl. Acids Res.* **8**: 4321-25.

10. Ping, Z.G., Ping, X.C. and Xiang, L.X. 2002. Analysis of 50 cucumber accessions by RAPD markers. *Hunan Agric. Sci. Tech. Newslett.* **3**: 10-14.

11. Rahman, M.A., Rabbani, M.G., Ahammed, G.J., Akhter, J. and Garvey, E.J. 2007. Genetic diversity of bittergourd germplasm of Bangladesh. **In**: *Book of Abstract- Conference on Promotion of Biotechnology in Bangladesh-National and International Perspectives.* April 6-8, 2007, pp. 100.

12. Rao, C.R. 1952. *Advanced Statistical Methods in Biometric Research*. John Wiley and Sons Inc., New York, 374 p.

13. Staub, J.E., Danin-Poleg, Y., Fazio, G., Horejsi, T., Reis, N. and Katzir, N. 2000. Comparative analysis of cultivated melon groups (*Cucumis melo* L.) using RAPD and simple sequence repeats markers. *Euphytica*, **115**: 225-41.

14. Woo, L.S. and Hyeon, K.Z. 2003. Genetic relationship analysis of melon (*Cucumis melo*) germplasm by RAPD method. *J. Korean Soc. Hort. Sci.* **44**: 307-13.

Received: December, 2009; Revised: August, 2010; Accepted : May, 2011