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RESEARCH **P**APER

Genetic diversity analysis of pumpkin genotypes (*Cucurbita moschat* Duch ex. Poir) using morphological and RAPD markers

M.S. MURALIDHARA AND N.C. NARASEGOWDA

Department of Horticulture, University of Agricultural Sciences, BENGALURU (KARNATAKA) INDIA
Email : murali7831@gmail.com

The study was conducted to ascertain genetic diversity of 20 pumpkin (*Cucurbita moschat* Duch ex. Poir) genotypes collected from different parts of India, using morphological and random amplified polymorphic DNA (RAPD) markers. The morphological data recorded for growth and yield characters at different levels. CTAB method used for isolation of DNAs from 20 genotypes. A total of 21 markers produced form 3 primers, out of that one band was polymorphic and remaining once were monomorphic. A dendogram grouped the genotypes into 2 clusters A and B at 9 linkage distance. Cluster B was the major group consisted of 12 accessions, CM-14 genotype which is diverse form other genotypes grouped under cluster B. Cluster B again sub divided into 6 sub groups. The second cluster A consisted of 8 accessions separated at 3 linkage distance and divided into 2 groups. The genetic dissimilarity matrix based on Squared Euclidean Distance, showed a maximum dissimilarity (10%) between the genotypes Bangalore local-2 and Magadi local and the minimum dissimilarity (1%) between CO-2 and Solan Badam, CO-2 and Bangalore local-1, KIC-1 and IIHR-5. This analysis showed the potentiality of CM-14 variety due to its less vine length, less number of days to female flower appearance and early maturity as probable parent in hybridization programme.

Key words : Cluster analysis, CTAB, Dissimilarity matrix, Genetic diversity, RAPD

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INTRODUCTION

Pumpkin (*Cucurbita moschata* Duch ex. Poir) being an important Cucurbitaceous vegetable crop cultivated under tropical, sub tropical and temperate regions all over the world. Northern and Southern America are the possible primary centers of origin for this crop. Pumpkin is a monoecious and cross pollinated crop, honey bees play a important role in pollination and this crop offers considerable variation for different traits.

The fruits are sweetish when fully mature and are used for preparing sweets, candy and fermented into beverages. Yellow or orange fleshed pumpkins are rich in carotene. The fruit contains 1.4 g of protein, 0.7 mg of iron, 10 mg of calcium, 2 mg vitamin C, 30 mg of phosphorus and 50µg of carotene in 100 g of edible portion (Bose and Sam, 1990). Apart from nutrition, preliminary research indicates that phytochemicals found in pumpkin may favorably affect insulin and glucose levels in laboratory diabetic models, two compounds trigonelline and nicotinic acid isolated from pumpkin paste are found to reduce blood cholesterol and glucose levels.

Among the Cucurbitaceous vegetables, pumpkin has a place of high value due to its long shelf life, long period of availability, excellent response to vegetable forcing, high nutritive estimates and better transport qualities. It is used at both mature and immature stages as a vegetable. It is also consumed after processing.

In India we can find a large amount of genetic diversity based on morphological characters. Genetic analysis based on quantitative traits has been made in this crop by Gopalakrishnan *et al.* (1980), Kumar (1982), Doijode and Sulladmath (1986) and Lakshmi *et al.* (2002) in India. However, phenotypic characters have limitations as they are influenced by environmental factors and the developmental stages of the plant. In contrast, molecular markers, based on

DNA sequence polymorphism are independent of the environmental conditions and show a higher level of polymorphism.

Random amplified polymorphism DNA markers are more useful for the assessment of genetic diversity due to their simplicity, speed and relatively low cost compared to other molecular markers (Williams *et al*., 1990; Rafalski and Tingey, 1993). RAPD markers have been used extensively in cucurbits to classify accessions (Horejsi and Staub, 1999), to assess the genetic relationship among the different genotypes of *Cucurbita moschata* (Gwanama *et al*., 2000). In the present study, RAPD and morphological markers were used to estimate genetic diversity among twenty genotypes of pumpkin maintained at the Department of Horticulture, University of Agricultural Sciences, Bengaluru.

RESEARCH METHODOLOGY

The study comprised of 20 genotypes of pumpkin collected for different parts of India: Solan Badam, CM-14, BLGL-1, CO-2, Arka Chandan, Arka Suryamukhi, KIC-1, KIC-2, KIC-3, IIHR-2, IIHR-3, IIHR-5, IIHR-6, IIHR-7, Doddaballapur local, Tarikere local, Magadi local, Bengaluru local-1, Bengaluru local-2 and Bengaluru local-3. Around 10g of just matured leaves, free from pest and disease damage collected from 20 genotypes individually in ice box from field, samples washed with water and wiped with 70 per cent ethenol, then air dried, sealed in polythene bags and stored at 80^0C for DNA extraction.

Morphological characteristics :

The data recorded on morphological characters such as vine length(m), number of leaves, number of branches, days to first male and female flowering, sex ratio, days to 50 per cent female flowering, per cent fruit set, days to first harvest, weight of fruits(kg), weight of seeds per fruit(g), number of fruits per plant, yield per plant(kg), TSS, carotene content and rind thickness. The mean average of 15 plants of each genotype was selected for the determination of morphological characteristics and the critical difference was calculated by using GENRES software (Table A). The phenotypic correlation among characters was carried out using GENRES programme following the randomized complete block design (Table B). A one way ANOVA and backward regression analysis were performed to analyzed correlation between the yield characters using GENRES programme.

Isolation and purification of genomic DNA :

DNA was extracted from the fresh leaf power of pumpkin by cetyl trimethyl ammonium bromide (CTAB) method as described by Simon *et al.* (2007). Leaf powder (0.5g) was mixed with 10ml of extraction buffer, preheated to 65°C, containing 100mM Tris-Base, pH 8.0, 20mM EDTA, pH 8.0, 1.4 M NaCl, 3 per cent (w/v) CTAB, 2 per cent polyvinyl pyrrolidone, and 1per cent β-mercaptoethanol, then incubated at 65°C for 30 minutes with gentle shaking in a thermostat. The mixture cooled to room temperature, to which 10 ml of cold 24 : 1 (v/v) chloroform : isoamylalcohol was added and mixed well. After centrifugation at 7000 rpm for 20 minutes at 4°C, the supernatant was transferred to a fresh tube and the chloroform: isoamylalcohol step was repeated until a clear supernatant was obtained. To the supernatant 5 M NaCl was added (0.5 v/v) and mixed gently followed by addition of 0.8 vol of cold isopropanol to precipitate the DNA. The mixture was incubated overnight at 4°C and centrifuged at 8000 rpm for 20 minutes. The resulting pellet was washed with 70 per cent ethanol, air tried and dissolved in TE buffer (10 mM Tris-HCl and 1 mM EDTA at pH 8). Two µg of RNase was added to each sample which was incubated for 3h at 37°C, mixed with an equal volume of phenol and centrifuged at 6000 rpm for 20 minutes at room temperature and followed by washing with an equal volume of 1:1 (v/v) of phenol: chloroform then with chloroform alone. DNA was precipitated overnight at 4°C with 0.5 vol of 5 M NaCl and 1 vol of cold isopropanol and the resulting pellet obtained after centrifuged was dissolved in TE buffer, analyzed on agarose gel and quantified using spectrophotometer.

PCR amplification :

PCR amplification protocol was according to Williams *et al.* (1990). Out of the 54 primers screened using bulk DNA, 3 showing clear and distinguishable bands were selected for RAPD-PCR analysis (Table C). Reproducibility of the primers was tested twice under similar conditions. PCR reactions were carried out in a volume of 25 µl containing 25 □g DNA, 150 µM each of dNTP, 1.5 mM MgCl2, 1 unit *Taq* DNA polymerase, 5 pmol primer in PCR buffer [50 mM KCl, 10mM Tris-HCl pH 9, 05% (v/v) NP40 and 0.05% (v/v) Triton X-100]. Amplifications were performed in a Corbett Research Tremocycler, programmed for an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for a minute, annealing at 35°C for a minute, primer extension at 72°C for 2 minutes and final extension at 72°C for 5 minutes. PCR products were resolved on a 1.2 per cent (w/v) agarose gel, visualized and documented using an Alpha Digidoc system.

RAPD profile analysis :

Amplified fragments from each primers were manually scored for the presence (1) and absence (0) and a matrix of the different RAPD phenotypes of all 3 primers were assembled for statistical analysis. The sizes of the fragments were estimated using 500 bp standard DNA markers, co-electrophoresed with the PCR products. A genetic dissimilarity matrix was developed using Squared Euclidean Distance,

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Table A : Mean performance for growth and yield parameters of Pumpkin genotypes

Genotypes	Vine length	No. of leaves	No. of branches	Days to first male flowering	Days to first female flowering	Sex ratio	Days to 50% female flowering	Days to first harvest	Per cent fruit set	No. of fruits per plant	Weight of fruit (kg)	Wt. of seeds per fruit (g)	Rind thickness (mm)	TSS (0B)	Carotene (µg/100g)	Yield per plant (kg)
Solan Badam	5.05	49.60	8.00	40.33	41.33	13.17	51.67	73.00	28.53	2.00	2.69	32.40	25.13	3.80	61.30	5.27
CM-14	3.09	72.50	5.33	30.67	34.33	12.20	45.33	75.00	51.33	1.93	4.15	20.47	22.13	3.17	65.43	8.01
BLGL-1	5.50	69.00	6.33	35.33	46.33	10.70	51.67	89.33	95.47	1.97	2.62	21.50	32.63	6.77	58.20	5.10
CO-2	6.52	98.33	7.00	30.33	35.00	8.97	41.67	64.67	41.00	1.85	4.52	55.57	37.17	4.77	74.63	8.51
Arka Chandan	7.37	130.47	10.33	31.67	35.33	20.14	41.00	74.33	83.83	2.95	1.85	43.03	29.20	5.23	62.27	5.23
Arka Suryamukhi	6.57	117.00	6.00	30.33	30.67	8.46	38.67	70.00	96.33	4.10	0.70	24.57	21.70	5.00	65.77	2.89
KIC-1	5.31	93.42	6.33	29.67	32.33	14.27	42.67	69.67	51.67	1.87	4.51	73.47	41.67	6.30	79.00	8.37
KIC-2	6.96	70.67	6.00	38.67	35.33	20.20	45.67	71.00	41.43	2.19	5.00	43.87	42.23	8.03	58.20	11.05
KIC-3	5.56	65.00	5.67	33.00	36.33	9.98	41.67	89.33	81.47	3.02	2.90	18.20	35.17	5.07	58.20	8.68
IIHR-2	5.75	113.47	11.00	37.33	44.33	11.83	63.67	82.67	32.17	2.01	5.82	48.33	41.13	4.93	90.63	11.70
IIHR-3	5.54	94.17	10.00	53.00	59.33	16.11	67.67	93.00	54.23	3.75	2.37	43.37	39.93	4.00	45.10	8.90
IIHR-5	5.76	180.27	9.67	41.00	47.00	17.37	56.33	88.67	63.00	3.43	1.21	25.97	22.37	7.77	54.47	4.07
IIHR-6	5.10	128.07	10.33	41.00	49.00	13.57	51.00	86.00	35.97	1.97	2.31	45.20	23.90	4.27	50.90	4.54
IIHR-7	5.64	119.40	11.67	51.67	62.33	14.38	75.67	96.33	46.03	2.59	1.97	27.60	28.63	6.07	66.00	5.08
Doddaballapur Local	6.38	155.58	8.00	38.33	45.33	10.65	56.67	88.67	30.83	2.68	2.28	23.87	42.97	5.17	55.60	6.20
Tarikere Local	6.69	139.67	10.33	56.33	65.00	15.98	78.33	98.00	65.43	2.79	3.04	18.07	35.00	8.50	74.80	8.49
Magadi Local	6.47	172.00	11.33	50.33	66.00	19.47	77.67	95.33	74.83	4.43	1.55	23.03	24.87	8.00	81.27	6.74
Bengaluru Local-1	4.58	80.97	10.67	51.67	59.33	14.27	61.33	97.00	41.50	2.13	3.27	73.77	40.50	4.60	49.90	6.98
Bengaluru Local-2	5.62	79.33	7.33	33.00	35.67	17.33	45.67	64.67	53.90	2.29	3.03	38.87	26.47	8.70	50.67	6.94
Bengaluru Local-3	6.61	92.67	6.67	35.33	39.67	16.77	51.00	71.00	51.40	1.90	5.04	45.30	49.03	7.00	51.43	9.57
Mean	5.80	106.08	8.40	39.45	45.00	14.29	54.25	81.88	56.02	2.59	3.04	37.32	33.09	5.86	62.69	7.12
S.E. ±	0.502	15.799	1.122	1.718	1.270	1.654	0.696	1.715	1.000	0.409	0.244	2.298	1.161	0.232	0.581	1.045
C.D. (P = 0.05)	1.016	31.984	2.272	3.479	2.571	3.349	1.410	3.471	2.025	0.828	0.494	4.653	2.351	0.469	1.175	2.115

Table B : Phenotypic correlation co-efficient for quantitative characters in pumpkin

Characters	No. of leaves	Vine length	No. of branches	Days to first male flowering	Days to first female flowering	Sex ratio	Days to 50% female flowering	Days to first harvest	Fruits set per cent	Number of fruits per plant	Fruit weight	Weight of seeds per fruit	Rind thickness	TSS	Carotene	Correlation with yield
No. of leaves	1	0.319	0.482*	0.265	0.378	0.256	0.417	0.38	0.097	0.499*	-0.43	-0.217	-0.192	0.282	0.208	-0.254
Vine length		1	0.131	0.01	-0.021	0.233	0.03	-0.088	0.235	0.334	-0.109	-0.053	0.236	0.457*	0.127	0.097
No. of branches			1	0.652**	0.693**	0.363	0.683**	0.56*	-0.173	0.25	-0.272	0.092	-0.087	0.059	0.132	-0.12
Days to first male flowering				1	0.935**	0.32	0.89**	0.789**	-0.165	0.274	-0.249	-0.085	0.042	0.175	-0.084	0.005
Days to first female flowering					1	0.246	0.939**	0.858**	-0.077	0.292	-0.294	-0.141	-0.002	0.179	0.012	-0.057
Sex ratio						1	0.255	0.063	-0.004	0.111	-0.032	0.113	0.015	0.497*	-0.128	0.075
Days to 50% female flowering							1	0.779**	-0.157	0.287	-0.163	-0.2	0.053	0.251	0.2	0.081
Days to first harvest								1	0.091	0.326	-0.376	-0.285	-0.012	0.042	-0.06	-0.113
Fruits set per cent									1	0.477*	-0.49*	-0.425	-0.323	0.259	0.017	-0.341
Number of fruits per plant										1	-0.653**	-0.394	-0.309	0.149	0.011	-0.154
Fruit weight											1	0.47*	0.643**	-0.026	0.294	0.798**
Weight of seeds per fruit												1	0.473*	-0.158	0.011	0.278
Rind thickness													1	0.069	-0.01	0.641**
TSS														1	0.084	0.076
Carotene															1	0.262
Correlation with yield																1

* and ** indicate significance of values at P=0.05 and 0.01, respectively

Table C : Details of three polymorphic randomly selected primers for RAPD analysis

Sr. No.	Primers	Sequence	No. of bands	Monomorphic bands	Polymorphic bands	Polymorphic unique band
1.	OPC2	5' GTGAGGCGTC 3'	9	9	0	0
2.	OPA3	5' AGTCAGCCAG 3'	8	8	0	0
3.	OPA20	5' GTTGCGATCC 3'	4	3	1	1
Total			21	20	1	1

which estimates all pair wise differences in the amplification products and a cluster analysis was based on Ward's method using a minimum variance algorithm (Ward, 1963).

RESEARCH FINDINGS AND ANALYSIS

In any crop improvement programme evaluation for assessing the extent of variability in the genotypes of different geographical origin is most important. A wide range of genetic variability exists in pumpkin for both quantitative and qualitative characters. The present investigations revealed low to medium genetic diversity in pumpkin. Hence, there is an immediate need to maintain and conserve their genetic resources. The DNA fingerprints developed for the pumpkin genotypes would be of immense use in precise identification of these genotypes individually which would be useful in patenting and acquiring plant varietal rights to safeguard the genetic resources and selection of superior parental combinations useful in hybridization programmes.

For the PCR amplification we followed the standard protocol (Williams *et al*., 1990). The amplification using 1 unit of *Taq* DNA polymerase and 1.5 mM $MgCl_2$ produced clear banding pattern. Out of 54 primer screened 3 resulted in moderately good polymorphism (Table C) and others not gave any amplification. The DNA amplification profile of 20 pumpkin genotypes with 3 random primers produced a 21 fragments with a average of 7 segments per primer of the 21 bands 1 was very polymorphic with one genotype, remaining segments were monomorphic and common to all genotypes. This was comparable with the results obtained by Gwanama *et al.* (2000) in pumpkin (144 bands from 16 primers), Jack *et al*. (2004) in muskmelon (135 bands from 24 primers), Dey *et al*. (2006) in bitter gourd (208 bands from 29 primers) and Rathod (2007) in bitter gourd (143 bands from 14 primers). The present investigation revealed that there was a low to medium genetic diversity in pumpkin. Based on RAPD polymorphism, the primer namely OPA20 (1) showed the polymorphic band. Pattern of RAPD fragment produced by the random primer OPC02 is shown in the Fig.1. The genetic dissimilarity (GD) values showed in Table 1, ranged from 1 (CO-2, Solan Badam, BL-1, KIC-1 and IIHR-5) to 10 (BL-2 and ML) with an average of 5.5 across all 20 genotypes. This range of dissimilarity value suggested low to moderate diversity presence in the accessions, this was not much higher than that reported in 31 (landraces) of *Cucurbita moschata* by using 31 RAPD primers by Gwanama *et al*. (2000).

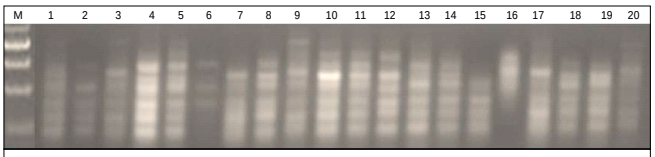

Fig. 1 : RAPD gel profile of pumpkin accessions amplified using OPC02 primer.

A tree diagram was developed for 20 accessions of *Cucurbita moschata* is given in the Fig. 2. The linkage distance varied from 1 to 16 units. The whole dendrogram was classified in to two major groups (A and B). The most important major group B consisted of 12 accessions and CM-14 was grouped under B. These clusters were separated at 9 units of linkage distance and sub divided into 6 sub groups. The second major group A consisted of 8 accessions and this cluster was separated at 3 units of linkage distance and sub divided into 2 groups.

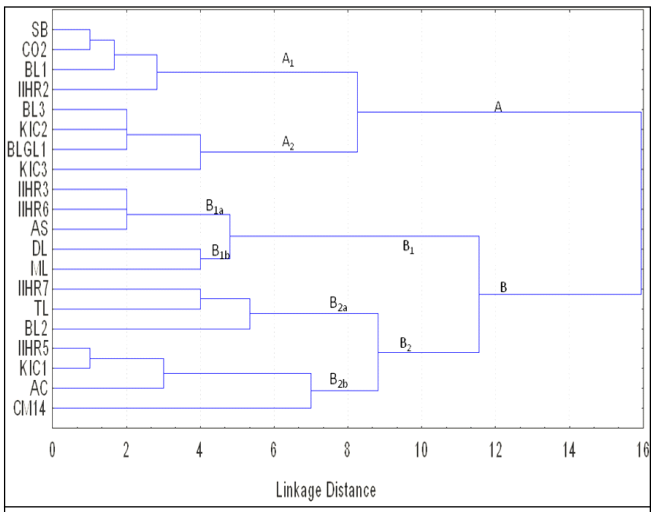

Fig. 2 : Dendogram showing the genetic divergence among twenty pumpkin accessions using cluster analysis of RAPD data

The major group B was sub divided in to six sub groups (B1, B2, B1a, B1b, B2a, B2b) comprising different accessions in each group. The group B1 again divided into B1a and B1b. The sub group B1a had IIHR-3, IIHR-6 and Arka Suryamukhi divided with B1b at 2 units of linkage distance. The B1b group contained Doddabalapur Local and Magadi Local were separated at a linkage distance of 4. The B2a group contained IIHR-7, Tarikere Local and Bengaluru Local-2

which were separated at a linkage distance of 5.5 with B2b. The sub group B2b consisted of CM-14, which was separated at 7 linkage distance. The sub group B2b had Arka Chandan, KIC-1 and IIHR-5 were separated at 3 linkage distance.

The sub group A1 also contained four accessions *viz.*, Solan Badam, CO-2, Bengaluru Local-1 and IIHR-2. This sub group was clustered at 3 linkage distance. The sub group A2 contained four accessions *viz.*, Bengaluru Local-3, KIC-2, BLGL-1 and KIC-3. Where, KIC-3 separated at 4 linkage distance with other members of the group. This sub group was clustered at 4 linkage distance. It is very interesting that the accession CM-14 was the only entity grouped separately at the highest linkage distance (7). The total dissimilarity behavior of CM-14 is probably due to its less vine length, less number of days to female flower appearance and early maturity. The accessions Bengaluru Local-3, IIHR-6, IIHR-3, BLGL-1 and Arka Suryamukhi showed the same linkage distance and probably this would mean that they are all hailing from a single geographical locality or due to some similar vegetative characters. Similar reports are available on the geographical area, in pumpkin (Gwanama *et al*., 2000) and muskmelon (Jack *et al*., 2004).

The present study revealed that morphological variation based on 27 quantitative traits and molecular diversity according to 3 random primers did not show any similarity. The clustering according to molecular analysis did not match with quantitative traits. Similarly Youn and Chung (1998) also did not observe any correlation between the grouping based on RAPD markers and morphological data, in the case of pumpkin. Chowdhury *et al*. (2001) in soyabean, Ferriol *et al*. (2003) in *Cucurbita maxima*. The main reason of mismatch between clustering based on RAPD and quantitative traits may be that most of the quantitative traits are highly influenced by environment. Besides, RAPD markers are randomly distributed throughout the genome and in majority of cases most regions of the genome (nearly 90%) are not expressed at phenotypic level (Dahlberg, 2000). So, it is very difficult to find out similarity between grouping based on the RAPD and quantitative traits. A poor correlation was also reported when a large number of agronomically neutral botanical traits are used (Ortiz, 1997).

Based on quantitative data the potentiality of the pumpkin accessions with respect to yield and its attributing characters and the genetically divergent genotypes identified in the present study will be used in the future breeding programmes for increasing productivity of pumpkin.

Table 1 : Genetic dissimilarity matrix of twenty pumpkin genotypes according to squared euclidean distances

	SB	BL1	IIHR3	IIHR2	IIHR6	IIHR7	IIHR5	DL	TL	ML	AC	AS	CM14	KIC1	CO2	BL2	BL3	KIC2	BLGL1	KIC3
SB	0																			
BL1	2	0																		
IIHR3	4	4	0																	
IIHR2	2	2	4	0																
IIHR6	4	4	2	4	0															
IIHR7	4	4	4	4	4	0														
IIHR5	5	3	5	5	5	3	0													
DL	5	7	5	7	3	5	4	0												
TL	4	4	4	6	6	4	3	5	0											
ML	5	7	3	5	5	7	6	4	5	0										
AC	6	4	4	6	2	6	3	3	6	5	0									
AS	2	4	2	4	2	4	5	3	4	3	4	0								
CM14	7	5	7	5	5	7	6	8	7	8	5	7	0							
KIC1	4	2	4	4	4	4	1	5	4	5	2	4	5	0						
CO2	1	1	5	3	5	5	4	6	3	6	5	3	6	3	0					
BL2	7	7	9	9	7	5	6	6	5	10	7	7	6	7	6	0				
BL3	3	3	7	5	5	7	6	6	5	8	5	5	4	5	2	4	0			
KIC2	3	3	7	5	5	7	4	4	5	6	3	5	6	3	2	6	2	0		
BLGL1	3	3	7	5	7	7	4	6	5	6	5	5	6	3	2	6	2	2	0	
KIC3	5	5	7	7	7	9	6	6	7	6	5	5	8	5	4	6	4	4	2	0

LITERATURE CITED

Bose, T.K. and Som, M.C. (1990). *Vegetable crops in India*, 1st reprint, Good Association Printers, Calcutta. pp.148-150.

Chowdhury, A.K., Srinivar, P., Tongpamnak, P. and Saksoong, P. (2001). Genetic diversity based on morphology and RAPD analysis in vegetable soybean. *Korean J. Crop Sci.*, **46**: 112-120.

Dey, S.S., Singh, A.K., Chandal, D. and Behera, T.K. (2006). Genetic diversity of bitter gourd (*Momordica charantia* L.) genotypes revealed by RAPD markers and agronomic traits. *Sci. Hort.,* **109** (1) : 21-28.

Doijode, S.D. and Sulladmath, U.V. (1986). Genetic variability and correlation studies in pumpkin (*Cucurbita moschata* Poir.). *Mysore J. Agri. Sci.,* **29**: 59-61.

Dahlberg, J.A. (2000). Classification and characterization of sorghum. In: Smith, C.W., Ferderiksen, R.A. (Eds), Sorgham: Origin, history, technology and production. John Wiley and Sons, Inc., New York, pp 99-130.

Ferriol, M., Pico, M.B. and Nuez, F. (2003). Genetic diversity of some accessions of *Cucurbita maxima* from Spain using RAPD and SBAP markers. *Genetic Resour. & Crop-Evolution,* **50**(3): 227-238.

Gopalakrishnan, T.R., Gopalakrishnan, K.G. and Peter, K.V. (1980). Variability, heritability and correlation among some polygenic characters in pumpkin. *Indian J. Agric. Sci.*, **50**(12): 925-930.

Gwanama, C., Labuschagne, M.T. and Botha, A.M. (2000). Analysis of genetic variation in *Cucurbita moschata* by random amplified polymorphic DNA (RAPD) markers. *Euphytica,* **113** (1): 19-24.

Horejsi, T. and Stub, J.E. (1999). Genetic variation in cucumber (*Cucumis sativus* L.) as assed by random amplified polymorphic DNA. *Genetic & Crop Evolution,* **46** (4) : 337-350.

Jack, E., Staub Ana, I.L., Opez-ses, E. and Nikolaos, Fanourakis. (2004). Diversity among melon landraces (*Cucumis melo* L.) from Greece and their genetic relationships with other melon germplasm of diverse origins. *Euphytica,* **136** (2) : 151–166.

Kumar, R.J. (1982). Variability and correlation studies in pumpkin (*Cucurbita moschata* Poir). M.Sc. (Hort.) Thesis, Haryana Agricultural University, Hissar, HARYANA (INDIA).

Lakshmi, L.M., Haribabu, K. and Reddy, G.L.K. (2002). Genetic variability studies in pumpkin *(Cucurbita moschata* Duch ex. poir). *J. Res., ANGRAU,* **30**(1): 82-86.

Ortiz, R. (1997). Morphological variation in *Musa* germplasm. *Genetic Res. & Crop Evolution,* **44** (5) : 393-402.

Rafalski, J.A. and Tingey, S.V. (1993). Genetic diagnosis in plant breeding: RAPDs microsatellite and machines. *Trends Genet.*, **9** (8) : 275-280.

Simon, L., Shyamalamma, S. and Narayanaswamy, P. (2007). Morphological and molecular analysis of genetic diversity in jack fruit. *J. Hort. Sci. & Biotec.,* **82**(5) : 764-768.

Rathod, Vijaykumar (2007). Studies on genetic variability and molecular characterization of bitter gourd (*Momordica charantia* L.) genotypes. M.Sc. (Hort.) Thesis, University of Agricultural Sciences, Bengaluru, KARNATAKA (INDIA).

Williams, J.G.K., Kubelik, A.R., Livik, K.J., Rafalski, J.A. and Tingery, S.V. (1990). DNA polymorphisms amplified arbitrary primers are useful as genetic markers. *Nucleic Acids,* **18** (22) : 6531-6535.

Ward, J.H. (1963). Hierarchic grouping to optimize an objective function. *J. American Stat. Assoc.,* **58** : 236-239.

Youn, S.J. and Chung, H.D. (1998). Genetic relationship among the local varieties of the Korean native squashes (*Cucurbita moschata*) using RAPD technique. *J. Korean Soc. Hort. Sci.,* **39** (5) : 517-521.