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Research Article

Assessment of molecular diversity through ISSR markers in muskmelon (*Cucumis melo* L.)

Mani Lohani, D. K. Singh, Mangaldeep Sarkar

Abstract

The present investigation was carried out for the assessment of molecular diversity in parental lines of muskmelon to be used for the crossing programs to get hybrids with desirable traits. A total of eight inbred lines of andromonoecious muskmelon were assessed with twenty ISSR (Inter Simple Sequence Repeat) primers. Based on polymorphism four primers were selected for whole molecular analysis. The polymorphism varied from 50.00 to 90.90% with an average of 82.97 percent. Pair-wise similarity matrix in accordance with Jaccard's coefficient revealed the similarity estimation ranging from 55.00% to 75.00%. The maximum similarity value was recorded for the PMM-37 (0.75) with Hara Madhu while the lowest was observed for PMM-4A (0.55) with PMM-16 and between PMM-32 (0.55) and PMM-18. Eight genotypes were categorized into two main clusters. Cluster I included 6 genotypes and Cluster II comprised 2 genotypes with approximately 70.80% similarity among themselves. Cluster I is further subdivided into two sub-clusters IA and IB with 63.00% similarity. Sub-cluster IA further forked into two small clusters IAi and IAii. IAi is comprised of PMM 4A. IAii is further subdivided into 2 groups; Group 1 and Group 2 with approximately 73.60% similarity. Group 1 consists of PMM 16 and group 2 consists of PMM 37 and check variety Hara Madhu with approximately 74.00% similarity. Sub-cluster IB comprised two genotypes PMM 32 and PMM 18 with approximately 70.90% similarity among themselves.

Keywords ISSR, genotypes, genetic polymorphism, muskmelon, molecular diversity, molecular marker

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Authors:
M. Lohani, D. K. Singh
Department of Vegetable Science,
College of Agriculture, G.B.P.U.A.&T.,
Pantnagar, Uttarakhand, India

M. Sarkar ✉
College of Agriculture, Navsari Agricultural University, Waghai, Gujarat, India

✉ mangaldeepsarkar@gmail.com

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Introduction

Muskmelon (*Cucumis melo* L.), one of the vital nutrient-rich cucurbitaceous vegetables is considered to have originated from regions of Persia, North-Western India, or Africa. A current study showed that muskmelon is precisely originated from Asian origin [1]. The crop is so named because of the delightful aroma present in its ripe or horticulturally matured fruits. A wide range of variability in general botany of plant and fruit characters like fruit morphology, its shape and size, colorful rind, texture and taste of the edible part and maturity period makes this vegetable most polymorphic among all the species under the family of cucurbitaceae. As a result, this species forms different groups like Persian types, Casaba and Honey Dew. The most popular one is cantaloupe group, fruits of which are small in size, oval in shape and rind is with heavy netting. Being a warm-season vegetable, it is reported to be grown all over the world in tropical and sub-tropical regions and often treated as fruit. Muskmelon has excellent dietary fiber content and necessary vitamins specifically vitamins A and C with a good amount of minerals.

Indian cultivated muskmelon genotypes fall under several groups belonging to two main clusters, sweet genotypes including Cantalupensis, Momordica, Reticulatus, Flexuosus, Ameri, and Acidulus, and non-sweet genotypes including only Dudaim. Diverse plants of compatible taxonomic groups are expected to give high hybrid strength; hence, it imposes the study of genetic diversity among the collected germplasm that helps to select diverse ideal parents, which upon hybridization might lead to effective gene recombination [2]. An important way to increase productivity in any crop plant is by introgression of useful traits from diverse germplasm into the available genetic pool. The extent of initial divergence determines the success of crop improvement programs to a large extent [3]. Cluster analysis helps in the identification of suitable parental combinations. The knowledge of the genetic diversity of various features, particularly those that provide yield and quality, is most effective in planning the breeding program.

A study of genetic variability of a number of muskmelon germplasm collected from different regions of India demonstrated unique genetic divergence [4]. Therefore, the Indian muskmelon gene bank may be appraised as an important gene reservoir for crop improvement. World gene banks include Indian melon accessions commencing from Northern Rajasthan, Central areas of Madhya Pradesh, and Uttar Pradesh [5]. Expression of morphological characters is governed by many genes; therefore, remarkably controlled by ecological factors and crop stages of development. Thus, analysis of genetic diversity based on those morphological traits is treated as inefficient while DNA or molecular markers are at the top of polymorphism and free from environmental connections [6, 7]. These genetic markers have been utilized efficiently and are reported as the best technique to understand genetic range and relationship [8, 9]. Their judicial utilization in the management of germplasm and marker-aided selection has been done to improve the competence and efficacy for both germplasm curation and advancement [10]. Several factors like the ease in availability of marker, its lower cost, experimental size and preference between an elevated standard predictable heterozygosity, and a high potent multiplex ratio make a marker truly useful to take in genetic diversity. Inter-simple sequence repeats (ISSR) is a dominant and semi-arbitrary DNA marker augmented by PCR in the presence of a single primer compatible with a target microsatellite region of a genome. It is easy and quick to handle as RAPD and it does not demand prior data of the genome, cloning, or individual primer design. It has greater reproducibility than RAPD due to elevated annealing temperature and the cost of testing is lesser than the same of AFLP [11]. ISSR has been utilized in the assessment of the genetic diversity of different cucurbitaceous vegetables [12] and phylogenetic findings in *Cucumis spp.*, *Citrullus spp.*, and *Praecitrullus fistulosus* [13] along with several subspecies of *Cucurbita pepo* [14]. Diversity at the molecular level with morphological variability has been experimented several times in melon species [15-18]. The present study describes molecular diversity among eight inbreed parental lines based on twenty ISSR markers.

Methodology

Materials and methods

A total of eight inbred lines of muskmelon were used in this experiment and opted from the available source of muskmelon germplasm in PCPGR (Pantnagar Centre of Plant Genetic Resources), Pantnagar, Uttarakhand, India (Table 1). The whole experiment of DNA extraction, PCR amplification, gel electrophoresis, and documentation was performed at the Bio Control Laboratory facility, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India. The genomic DNA was extracted from a mass of leaf tissues of seedlings which are three weeks old and the same was taken from 5 plants as of each inbred line. Three-gram leaf tissue was grounded in liquid nitrogen before formation of a very fine powder. Further DNA was taken out by following the CTAB method [19] with minor modifications. The isolated DNA solution was purified by RNase and quantified using a NanoDrop Spectrophotometer (Systronics India Ltd.). An anticipated value of the purity of the DNA was resolute using the ratio amid the readings at 260 nm and 280 nm (OD260 / OD280). The documented value at 260 nm was employed to estimate the concentration of the DNA present in the sample and utilized for supplementary analyses. The selection of ISSR markers was based on their previous performance successfully in other cucurbits.

Table 1. Detail of inbreed lines used in study

S.N.	Inbreed line	Sex form	Source	Morphological traits
1.	PMM-4A	Andromonoecious	PCPGR, Pantnagar	Compact plant with short internode. Flattened fruit with creamy colour
2.	PMM-1	Andromonoecious	PCPGR, Pantnagar	Multilateral growth with long internode. Globular fruit with creamy colour
3.	PMM-32	Andromonoecious	PCPGR, Pantnagar	Bushy plant growth with short internode. Globular fruit with light green colour
4.	PMM-13	Andromonoecious	PCPGR, Pantnagar	Indeterminate plant gowth with long internode. Oblate fruit with pale green in colour
5.	PMM-16	Andromonoecious	PCPGR, Pantnagar	Compact plant with short internode. Elliptical fruit with light yellow in colour
6.	PMM-18	Andromonoecious	PCPGR, Pantnagar	Multilateral growth with long internode. Globular fruit with light green in colour
7.	PMM-37	Andromonoecious	PCPGR, Pantnagar	Determinate plant growth with short internode. Oblate fruit with creamy colour
8.	Hara Madhu	Andromonoecious	PCPGR, Pantnagar	Determinate growth with long internode. Ovate fruit with light orange in colour

Table 2. List of ISSR markers used in the present experiment

S.N.	Genei Code no.	Sequence 5'- 3'	GC content (%)	Tm (°C)
1	Primer 809	AGAGAGAGAGAGAGAGYG	52.7	50.5
2	Primer 843	CTCTCTCTCTCTCTCTRA	47.2	47.1
3	UBC 854	TCTCTCTCTCTCTCTCAGG	52.6	51.5
4	UBC 855	ACACACACACACACACCTT	47.3	54.6
5	UBC 856	ACACACACACACACACCTA	47.3	53.8
6	UBC 861	ACCACCACCACCACCACC	66.6	60.6
7	UBC 890	ACGACTACGGTGTGTGTTTGTGT	47.8	58.8
8	UBC 840	GAGAGAGAGAGAGAGACTT	47.3	49.2
9	UBC 808	AGAGAGAGAGAGAGAGC	52.9	48.8
10	UBC 825	ACACACACACACACACT	47	51.4
11	ISSR CR-2	CACACACACACACACAAG	50	51.6
12	UBC 866	CTCCTCCTCCTCCTCCTC	66.6	55.7
13	UBC 846	CACACACACACACACART	47.2	51.8
14	Sola 1	BDBACAACAACAACAACA	37	47.8
15	Sola 5	DBDACACACACACACAC	49	50.1
16	Sola 11	GAGCAACAACAACAACAA	38.8	48.7
17	B 5	GAGAGAGAGAGAGAGAT	47	45.4
18	B 10	AGAGAGAGAGAGAGAGG	52.9	48.2
19	Sola 2	DDCGACGACGACGACGA	62.7	57.4
20	Sola 4	VHVGTGTGTGTGTGTGTG	53.7	53.7

Among 20 markers used, only 4 were selected due to their polymorphism shown in muskmelon (Table 2). PCR amplifications were done in 25 µL (final reaction) volume having 20 ng template DNA, 10 mM dNTPs, 0.50 µL Taq DNA polymerase (Bangalore Genei Pvt. Ltd., Bangalore, India) with 0.8 mM of each primer (1.5 µL). Amplification reactions were done in 0.2 ml tubes by using an Eppendorf Thermal cycler (Bio-Rad). The amplification comprised of a 5-minute preliminary denaturation at 94°C subsequently the same process at 94°C for 1 minute. Annealing of the primer was carried out at 51°C for 1.30 minutes followed by an extension phase at 72°C for 2 minutes. The reactions were later subjected to 35 further cycles after the attainment of the final annealing temperature. This was followed by a final extension for 7 min at 72°C. PCR products were separated in 1.5% agarose gels in 1X TBE buffer by electrophoresis for 3-4 hours at 80 V using a horizontal gel electrophoresis system (Sigma-Aldrich). Gels were stained with ethidium bromide. The amplified fragments were seen and snapped under UV light. Reproducible DNA bands were manually scored. Fragile bands with insignificant intensity were barred from the ultimate data analysis. Each parental DNA band profiles were scored in a binary fashion (1 indicating its presence; 0 indicating its absence). The binary data were utilized for the computation of pair-wise similarity coefficient. An unweighted pair group method with the arithmetic averages (UPGMA) was employed to construct a dendrogram. The computer program NTSYS-pc Version 2.0 was used for the computation of multivariate analysis [20].

Results and Discussion

In this present experiment, only four ISSR primers (Sola-2, 4, 5, and B-5) were selected for diversity analysis, therefore, it is crucial to draw solid conclusions associated with diversity among the muskmelon inbreeds. The similarity value among eight inbred lines ranged from 55% to 75% (Table 3). The supreme similarity value was noted for PMM-37 (0.75) with Hara Madhu. The lowermost resemblance was recorded for PMM-4A (0.55) with PMM-16 and PMM-32 (0.55) with PMM-18. The UPGMA (un-weighted pair group method with arithmetic mean) analysis was carried out and a dendrogram (Fig. 1) was created utilizing Jaccard's similarity matrix of ISSR markers relating data produced out of four polymorphic primers on eight inbreed lines of muskmelon. Eight genotypes

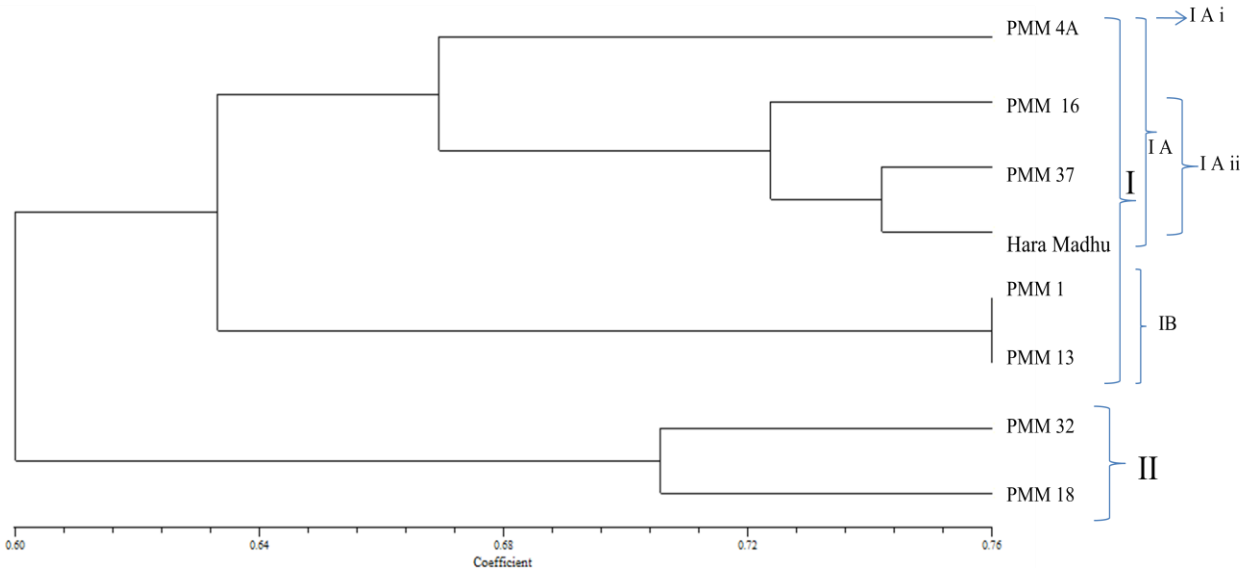

Figure 1. UPGMA dendogram of muskmelon parents based on Jaccard's Coefficient

Table 3. Pair wise similarity matrix in accordance with Jaccard's coefficient for 8 genotypes of muskmelon

Genotypes	PMM-4A	PMM-1	PMM-32	PMM-13	PMM-18	PMM-16	PMM-37	Hara Madhu
PMM-4A	1.00							
PMM-1	0.65	1.00						
PMM-32	0.58	0.60	1.00					
PMM-13	0.64	0.76	0.65	1.00				
PMM-18	0.67	0.62	0.55	0.67	1.00			
PMM-16	0.55	0.56	0.71	0.62	0.62	1.00		
PMM-37	0.65	0.60	0.60	0.62	0.73	0.64	1.00	
Hara Madhu	0.69	0.64	0.67	0.65	0.73	0.60	0.75	1.00

were categorized into two major clusters. Cluster I consisted of 6 genotypes and Cluster II comprised 2 genotypes with approximately 70.8% similarity among themselves. Cluster I is further subdivided into two sub-clusters IA and IB with 63% similarity. Sub-cluster IA further forked into two small clusters IAi and IAii. IAi is comprised of PMM 4A. IAii is further subdivided into 2 groups. Group 1 and Group 2 with approximately 73.6% similarity. Group 1 consists of PMM 16 and group 2 consists of one genotype namely PMM 37 and check variety Hara Madhu with approximately 74% similarity. Sub-cluster IB comprised two genotypes PMM 32 and PMM 18 with approximately 70.9 % similarity among themselves. Yeboah et al. [21] created a genetic map in accordance with ISSR and SRAP markers in cucumber and noticed 26% (ISSR) and 20% (SRAP) polymorphism. The above results are in conformity with Levi et al. [13] in Citrullus and Sikdar et al [22] in the Cucurbitaceae family.

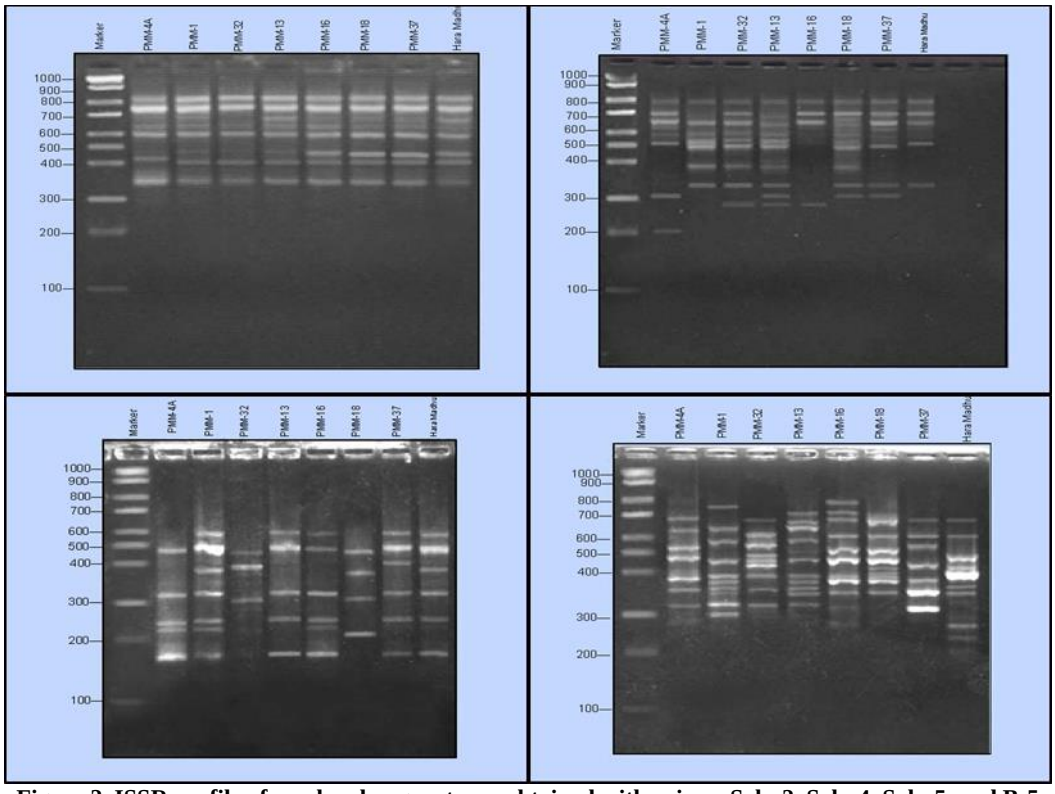

Figure 2. ISSR profile of muskmelon genotypes obtained with primer Sola-2, Sola-4, Sola-5, and B-5

Table 4. Total number of bands and polymorphism showed by suitable ISSR markers

S.N.	Primer	Total number of ISSR loci	Polymorphic loci	
			Number	Percent
1	Sola-2	8	4	50
2	Sola-4	11	10	90.90
3	Sola-5	11	10	90.90
4	B-5	17	15	88.23
5	UBC 856	47	39	82.97

The whole number of ISSR bands produced by four primers for eight genotypes was 47 (Table 4). The amplification product with the most suitable primer Sola-2, Sola-4, Sola-5, and B-5 (Fig. 2) revealed that total number of ISSR loci ranged from 8 (Sola-2) to 17 (B-5). The maximum number of polymorphic bands was witnessed in B-5 (17) followed by Sola-4, Sola-5(11), and Sola-2 (8). The polymorphism (percent) varied from 50.00 (Sola-2-5) to 90.90 (Sola-4 and Sola-5) with an average of 82.97 percent. Genetic diversity study is vital for crop improvement since they amend in reckoning and determining genetic correlation in germplasm, which supports in effective deployment of genetic resources. The major results of these surveys are the identification of distinct parental combinations for the characters of significance and the development of their segregating offspring with maximum genetic variability and exceptional combination [23, 24]. Alleles of magnitude in Indian muskmelon germplasm were detected and registered in earlier findings, which makes it feasible to create high-yielding and abiotic as well as biotic stress-tolerant cultivars via several breeding approaches [25-28].

Conclusion

The present experiment confirms the prevalence of an appreciable quantity of divergence among the inbred lines, which can be preferred for cross-breeding, and the emergence of the elite population in the muskmelon breeding program.

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