Electronic Journal of Plant Breeding,
ISSN 0975-928X Vol 7 No 2 DOI: 10.5958/0975-928X.2016.00030.2

# Assessment of genetic variability among Muskmelon (*Cucumismelo* L.) genotypes through biometrical traitsand molecular markers

***Rukam S. Tomar[1], M. V. Parakhia[1], V. M. Rathod[1], J. R. Thakkar[1], V. V. Kothari[1], R. R. Acharya[2]. andB. A. Golakiya[1]**

[1]Department of Biotechnology, Junagadh Agricultural University, Junagadh 362001, Gujarat, INDIA
[2]Main Vegetable Research Station, Anand Agricultural University, Anand, Gujarat, INDIA

*Author for correspondence :-Rukam S. Tomar, rukam@jau.in

(Received: February 2015, Accepted: January 2016)

**Abstract**
Fifty genotypes of muskmelon (*Cucumismelo* L.) were evaluated for variability through yield attributing characters and molecular markers. Analysis of variance for yield attributing character showed significant variation for all the traits, indicating presence of sufficient variability. $D^2$ values distributed all the genotypes in seven clusters. Maximum genetic distance was obtained between clusters II and V, while clusters III and VII displayed the lowest degree of divergence. Total soluble sugars followed by total soluble solids and fruit yield per plant contributed the most towards divergence.Random Amplified Polymorphic DNA (RAPD)and Inter Simple Sequence Repeat (ISSR)analysis using 130and 62primers generated 1108and 462 discrete markers, respectivelyranging from 200-1800 bp in size. The UPGMA analysis showed that genotypes were distributed in different groups based on similarities matrix.The dendrogram obtained by combining the data of both the molecular marker revealed genetic similarities ranging between 57% to 81% with highest genetic similarity between MM-68 and MMM-61. These results suggest that RAPD and ISSR markers are useful for muskmelon genetic diversity analysis from different region of India, which will be helpful for further genetic improvement program of plant. Knowledge on the genetic diversity of muskmelon can be used to future breeding programmes to improve fruit quality.

**Key word:** Muskmelon, Genetic Diversity, RAPD, ISSR

**Introduction:**
Muskmelon (C*ucumismelo L.*) with diploid chromo some number, 2n = 24 belonging to Cucurbitaceae family originated in Asia from India to Persia, with their center of development near what is Iran today. The early travellers introduced it to Europe from where it moved to other part of world. At present, muskmelon is being cultivated throughout the world under both tropical and sub tropical climate conditions (Tomar and Bhalala, 2006).

Melons are relatives of cucumbers, squash, pumpkin and watermelon, all of which are known as cucurbits. Muskmelon is a juicy, tasty and delicious fruit popular for its nutritive value.It contains phytoconstituents like β-carotene, apocarotenoids, ascorbic acid, flavonoids, terpenoids, carbohydrates, amino acids, vitamins, phospholipids and glycolipids.Traditionally it was used for different medicinal aspects like reducing blood pressure, prevent cardiac dysfunction etc. The chronic consumption of muskmelon juice helps to prevent atherosclerosis and liver steatosis. Moreover it has an immense pharmacological properties like antiinflammatory, antioxidant, anti-ulcerogenic, anti cancer, antidiabetic and anti microbial activity (Parle and Singh, 2011). It is highly relished because of its flavour, sweet taste and refreshing effect. It is a good source of dietary fibre, vitamins and minerals (Tomar and Bhalala, 2006).

For any crop improvement programme aimed at achieving maximum productivity, a detailed knowledge of genetic variability and diversity of various quantitative characters, and their contribution to diversity, is essential. The dramatic advances in molecular genetics over the last few years have provided workers involved in the conservation of plant genetic resources with a range of new techniques for easy and reliable identification of plant species. Many of these techniques have been successfully used to study the extent and distribution of variation in species gene-pools and to answer typical evolutionary and taxonomic questions (Rawashdeh, 2011). In order to characterize and evaluate the genetic diversity in muskmelon genotypes molecular markers have been proved to be valuable tool (Ferdaous*et al.*, 2012).Among the different types of molecular markers available, Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeat (ISSR)are useful for the assessment of genetic diversity because of their

simplicity, speed and relatively low cost compared to other molecular markers.RAPD and ISSR have few advantages because they do not require prior knowledge of sequence. Moreover, ISSR markersallow more stringent annealing temperatures and reveal more polymorphic fragments that can be highly variable within a species. RAPD and ISSR techniques have been used for studying genetic variability, gene tagging in various plant species and also for genetic diversity assessment, cultivar fingerprinting and phylogenetic studies (Tomar*et al.*,2014).

However, very little work has been carried out on improvement of the muskmelon crop. Assessment of genetic variation using yield attributing characters and molecular markers appears to be an attractive alternative to the conventional diversity analysis and can also aid in management and conservation of biodiversity (Tomar*et al.*,2014).In present study, we evaluated the genetic diversity among fifty different genotypes of muskmelon from different regions of India by using yield attributing traits, RAPD and ISSR markers, in order to establishthe base line to assist future crop improvement and plant breeding program.

## Material and Methods

### *Plant material*

Fifty different genotypes of muskmelon were used to study genetic diversity among them. They were collected from various regions of India like Anand Agricultural University, Anand, Gujarat; IARI, New Delhi; GBPUAT, Pantnagar, Uttarakhand; Punjab Agricultural University, Ludhiana, Punjab; NDUAT, Faizabad, Uttar Pradesh; RAU andDurgapura, Rajasthan (Fig. 1).

### *Yield attributing traits*

To determine the yield and yield attributing traits in muskmelon, 50 genotypes were grown with a spacing of 150 x 90 cm (6 x 4.5 m plot size) in a Randomized Block Design with three replications. Observations were taken on 10 randomly selected plants from each plot. Observations were recorded on the number of the node on which first female flower appeared, days to first picking, fruit weight (kg), fruit length (cm), fruit girth (cm), flesh thickness (cm), number of fruits per plant, fruit yield per plant (kg), moisture content (%),total soluble solids (TSS in %), total soluble sugars (mg $g^{-1}$) and acidity (%). Total soluble solids were determined by Zeiss Hand Refractometer while total soluble sugars and acidity percentage were determined by method described byDubios*et al.*,1956 and Ranganna, 1976 respectively.

### *Isolation of DNA*

Total Genomic DNA was extracted from fresh leaves of muskmelon by CTAB (CetylTrimethylAmmonium Bromide) method. 125 mg of leaf sample were ground to powder in liquid nitrogen using a mortar and pestle and was homogenized with 1 ml of DNA extraction buffer containing 2% CTAB, 1.4 M NaCl, 100 mMTrisHCl (pH - 8.0), 20 mM EDTA (Ethylene DiamineTetra Acetic acid), 0.5 % PVP (Polyvinylpyrrolidone), 0.5% (V/V) β-mercaptoethanol. Mixture was transferred to 1.5 ml centrifuge tube and incubated for 1 hour at 65°C in water bath and was centrifuged at 8400 x g for 8 minutes to settle down cell debris. An equal volume of Chloroform:Isoamyl alcohol (24:1) were added to mixture and centrifuged at 12,096 x g for 10 minutes for protein precipitation. Upperaqueouslayer of solution was mixed with Isopropyl alcohol to precipitate the DNA. DNA pellet was dissolved in TE buffer (pH - 8.0)for further use. DNA was quantified by spectrophotometer (PicodropPET01) using software v2.08 as well as by using 0.8 % agarose gel through electrophoresis. Dilution of DNA was carried out as per the requirement of PCR analysis.

### *RAPD analysis*

Total 130 RAPD primers of Operon Series (OPA to OPZ- 5 primer each) were used to amplify DNA of all fifty genotypes of muskmelon. PCR reactions were conducted in a volume of 20 µL consisting of 1x PCR buffer, 1.5 mM $MgCl_2$, 1 U of *Taq* DNA polymerase, 0.2 mM of dNTPs, 0.2µM of primer and template DNA of approximately 50ng. Amplification reaction was performed in Veriti thermal cycler ofLife Technology. The cycle program included one cycle of 5 min at 94°C, followed by 40 cycles, each consisting of a denaturation step for 1 min at 94°C, an annealing step for 1.5 min at 37°C and an extension step for 2 min at 72°C, followed by final extension step for 10 min at 72°C. After the final cycle, the samples were cooled at 4°C.

### *ISSR Analysis*

The ISSR analysis was carried out using a set of 100 UBC primers (UBC 801 to UBC 900)for DNA amplifications through PCR, out of which only 48 primers responded to all the accessions. PCR reactions were conducted in a volume of 20 µL consisting of 1x PCR buffer, 1.5 mM $MgCl_2$, 1 U of *Taq* DNA polymerase (invitrogen), 0.2 mM of dNTPs, 0.2 µM of primer and template DNA of approximately 50 ng. Amplification reaction was performed in Veriti thermal cycler of Life Technology. The cycle program included an initial denaturation 4 min at 94 °C, followed by 40 cycles of 1 min at 94 °C, 45 sec at 46 °C and 2 min at 72 °C, and 7 min final extension at 72 °C. The amplified fragments were separated on a 1.5% agarose gels in 1x TBE buffer running at 85 V constant for 1h and photographed under UV light in

a gel documentation system (Syngene Doc System).

***Data Analysis***

Genetic divergence based on yield and yield attributing traitswere estimated by usingMahalanobis $D^2$ statistics (Mahalanobis, 1936)between different pairs of genotypes. However, different clusters were generated through Tocher's method as given by Rao, 1952. DNA banding patterns generated by RAPD and ISSR markers were scored for the presence (1) or for absence (0) of eachamplified band. RAPD and ISSR data generated were used to analyse pairwise similarity co-efficient(Jaccard, 1908) using the similarity for qualitative data (SIMQUAL) format of NTSYS-pc version 2.1 (Numerical Taxonomy and Multivariate Analysis System) software package (Rohlf, 1993). The module SAHN performed sequential, agglomerative, hierarchical and non-overlapping clustering by unweighted pair-group method, arithmetic average (UPGMA). The module Tree Plot was used to convert the data generated by SAHN into a dendrogram. Pairwise distance matrix was calculated using the Jaccard similarity coefficient (Sneath and Sokal, 1973).

**Results and Discussion**

***Yield attributing traits***

Seven clusters were formed on the basis of Mahalanobis's $D^2$ values, of which maximum number of similar genotypes (24)appeared in cluster I. Clusters II and V were composed of12 and 8 genotypes, respectively, whereas, clusters III, IVand VI, VII were composed of two genotypes and a singlegenotype, respectively (Fig.2). Intra- and inter-cluster average values ranged from0.00 to 41.24. Since clusters VI and VII consistedof a single genotype, the intra-cluster distance was zero(0). The maximum intra-cluster distance was observed for cluster V (41.24). The inter-cluster distance was maximumbetween clusters II and V (138.78), followed by clusters IVand V (124.24), clusters V and VI (104.56) and clusters Iand II (83.55). The minimum inter-cluster distance wasobserved between clusters III and VII (21.35), followed byclusters IV and VI (21.46). Data further reveals that therewas good scope for selection within a cluster as indicatedby the high magnitude of intra-cluster distance amongclusters. Cluster VI and VII, with single genotype, indicatedan independent identity and importance, due to variousunique traits possessed by the genotypes. Mean values of clusters for various traits arepresented in graph (Fig.3). Almost all the clusters were highlydistinct from each other in all the characters studied. ClusterIII exhibited the highest mean value for the number of thenode at which the first female flower appeared (6.7), fruitweight (0.91), fruit length (18.85), flesh thickness (2.50)and moisture content (91.90). Cluster II exhibited highestvalues for total soluble solids (11.50) and total soluble sugars(6.77), and cluster VI for number of fruits per plant (5.41)and fruit yield per plant (3.28). Clusters IV, V and VIIexhibited highest value for fruit girth (43.4), acidity percentage (0.21) and days to first picking (81.67),respectively.Cluster II showed the lowest mean values for fruitweight (0.60), fruit length (17.15), flesh thickness (2.02)and moisture percentage (88.74); cluster I exhibited thelowest mean value for fruit weight (0.60) and fruit girth(36.03); cluster V had the lowest mean values for fruit yieldper plant (2.03) and total soluble sugars (3.01), and, clusterIII for number of fruits per plant (2.51) and total solublesolids (8.61). Clusters IV, VI and VII showed the lowestmean values for acidity percentage (0.01), days to firstpicking (75.67) and the number of the node at which thefirst female flower appeared (2.33), respectively.Contribution of different traits to diversity indicatesthat total soluble sugars (74.45) provide the highestcontribution to diversity, followed by total soluble solids(22.25) and fruit yield per plant (2.11). Thus, these threecharacters need more attention for improvement ofmuskmelon. Genetic diversity observed among the genotypes may be due to factors like history of selection, heterogeneity, selection under diverse environments and genetic drift.

***RAPD analysis***

A total of 130decamer oligonucleotides primer were used to investigate genetic relationship between fifty different genotypes of muskmelon. Each of the random primers produced distinct polymorphic banding patterns in all genotypes examined. In the present study, the total number of fragments produced by one hundred thirty primers was 1108.The number of bands produced per primer ranged from 4 to 10. Average number of bands per primer was 8.52, while average number of polymorphic bands was 6.20. A maximum number of 10 amplicons were amplified with primer OPD-07, while the minimum number of fragments (4) was amplified with primer OPC-06. The highest number of polymorphic bands (6) was obtained with primers OPA-03, OPH-08, OPG-02, OPN-07 and OPY-05. Primer OPH-08 exhibited the highest percentage (80.0%) of polymorphism.

The genetic similarity coefficient based on RAPD markers of the 50 different genotypes were used to generate a dendrogram using the UPGMA method. The obtained dendrogram was divided into two main clusters; cluster A included 17 genotypes while cluster B included 33 genotypes (Fig.4). The main cluster (Cluster A) included two sub clusters A1 & A2. Subcluster A1 contained 15 genotypes, while sub cluster A2 contained 2 genotypes, AMM-

99-113 and AMM-00-7. Second main cluster B contained thirty three genotypes, which were also divided into two sub cluster B1 and B2. Sub cluster B1 included 23 genotypes, while sub cluster B2 included 10 genotypes. The estimated genetic similarities ranged from 47% to 89% revealing very high levels of genetic variability among the studied genotypes. The highest genetic similarity (89%) was between AMM-99-122 and AMM-8. This was followed by 86% genetic similarities between the accession MM-28 and NDM-21, AMM-99-112 and AMM-02-22.

***ISSR Analysis***

For ISSR analysis, 100 primers (UBC series) were screened from which 62 primers produced scorable patterns. Amplification profiles were screened for the presence of polymorphism among 50 muskmelon genotypes. A total of 462 fragments were generated by the 62 primers with an average of 7.5 fragments per primer. Primer UBC-842 yielded the highest number of amplicons (10), while primer UBC-828 detected the lowest number (4 amplicons). The number of polymorphic markers also varied between primers, ranging from 1 to 6, with none of the primer yielding only monomorphic bands. The average number of polymorphic fragments per primer among the 50 muskmelon genotypes was 4 with UBC-842 generating maximum 6 polymorphic bands. Moreover, the size of the amplified fragments varied with different primers, ranging from 200 to 1800 bp. The scored data obtained from the 48 primers were used to determine the genetic similarity among the fifty muskmelon genotypes using the Dice coefficient.

Based on the 248 polymorphic ISSR fragments generated by 62 primers, a dendrogram (Fig.5) was constructed using UPGMA cluster analysis. The obtained dendrogram was divided into two main clusters; cluster A included 48 genotypes while cluster B included 2 genotypes (Fig.5). The main cluster (cluster A) included two sub clusters A1 & A2. Sub cluster A1 contained 40 genotypes, while sub cluster A2 contained 8 genotypes. Cluster B contained only 2 genotypes, GMM-2 and AMM-99-125. The estimated genetic similarities ranged from 54% to 90% revealing high levels of genetic variability among the studied genotypes. The highest genetic similarity (90%) was between AMM-10 and AMM-00-6. This was followed by 89% genetic similarities between the accession AMM-00-25 and AMM-99-113.

***RAPD and ISSR combined Analysis***

The genetic similarity coefficient based on RAPD and ISSR markers of the 50 different genotypes were carried out. Cluster analysis was performed on the basis of similarity coefficient generated from combined RAPD and ISSR profiles. The obtained dendrogram was divided into two main clusters; cluster A included 20 genotypes while cluster B included 30 genotypes (Fig. 6). The main cluster (cluster A) included two sub clusters A1 & A2. Subcluster A1 contained 18 genotypes, while sub cluster A2 contained 2 genotypes, AMM-02-27 and PMM-97-10. cluster B contained 30 genotypes, which divided into two sub cluster B1 and B2. Sub cluster B1 included 26 genotypes. Sub cluster B2 included 4 genotypes namely AMM-15, PUSA-MADHURAS, GMM-2 and AMM-99-125. The estimated genetic similarities ranged from 57% to 81% revealing high levels of genetic variability among the studied genotypes. The highest genetic similarity (81%) was between MM-68 and MMM-61. This was followed by 80% genetic similarities between the accession, AMM-99-112 and AMM-99-113.

The presence of unique RAPD and ISSR markers among the various muskmelon genotypes indicates the advantage of this approach for fingerprinting purposes. Therefore, the results of the present study revealed that DNA markers represent an efficient tool for estimating the genetic variability and the genetic relationships among closely related genotypes of muskmelon. This could represent a useful tool in muskmelon improvement programs.

**References**

Dubios, M., Gilles, K.A., Hamilton, J.K.,Rebers, P.A. and Smith, F. 1956. Colorimetric method for determination of sugar and related substances. *Analytical Chemistry*, **28:** 350-352.

Ferdaous, G., Walid, E., Hedia, H., Khadija, F., Leila, T., Nidhal, M., Tebra, T., and Ali, F. 2012. The use of ISSR and RAPD markers for genetic diversity among South Tunisian Barley.*International Scholarly Research Network,* doi:10.5402/2012/952196.

Jaccard, P. 1908.Nouvellesrecherchessur la distribution florale. *Bulletin de la Société vaudoise des sciences naturelles,***44:** 223-270.

Mahalanobis, P.C. 1936. On the generalized distance in statistic. Proc. Nat'l. Instt. Sci.,(India), **2:** 49-55.

Parle, M. and Singh, K. 2011. Muskmelon is eat must melon.*International research journal of pharmacy*, **2**: 52-57.

Ranganna, S. 1976. Analysis of fruit and vegetable products. McGraw-Hill Publishing Company Ltd., New Delhi, India.

Rao, C.R. 1952. Advanced Statistical methods in biometrical research.John Wiley and Sons, Inc., New York, 236-278.

Rawashdeh, I.M. 2011. Genetic diversity analysis of *chilleafragrantissima*(Forskal)SchultzB ip populations collected from different regions of jordanusing RAPD Markers.*Jordan Journal of Biological Sciences*,**4**: 21-28.

Rohlf, F.J. 1993. NT-SYS-pc: Numerical taxonomy and multivariate analysis system, Version 2.11 W, Exteer Software, Setauket.

Sneath, P.H.A. andSokal, R.R. 1973.Numerical t axonomy. freeman press, San Francisco, CA, USA

Tomar, R.S. and Bhalala, M.K. 2006.Heterosis studies in muskmelon (*Cucumismelo* L.).*Journal of Horticulture Science*, **1**: 144-147.

Tomar, R.S. and Bhalala, M.K. 2006. Combining ability studies in muskmelon (*Cucumismelo* L.).*Journal of Horticulture Science*, **1**: 109-115.

Tomar, R.S., Parakhia, M.V., Kavani, R.H., Dobariya, K.L., Thakkar, J.R., Rathod, V.M., Dhingani, R.M. and Golakiya, B.A. 2014. Characterization of castor (*Ricinuscommunis* L.) genotypes using different markers.*Research Journal of Biotechnology*, **9**: 06-13.

Tomar, R.S., Kulkarni, G.U., Parakhia, M.V., Thakkar, J.R.,Rathod, V.M., Solanki, R.K. and Golakiya, B.A. 2014. Genetic diversity analysis in coriander (*Coriandrumsativum*) genotypes through morphological and molecular c haracterization.*Research Journal of Biotechnology*,**9**: 01-11.


**Figure 1.Source of genotype.**

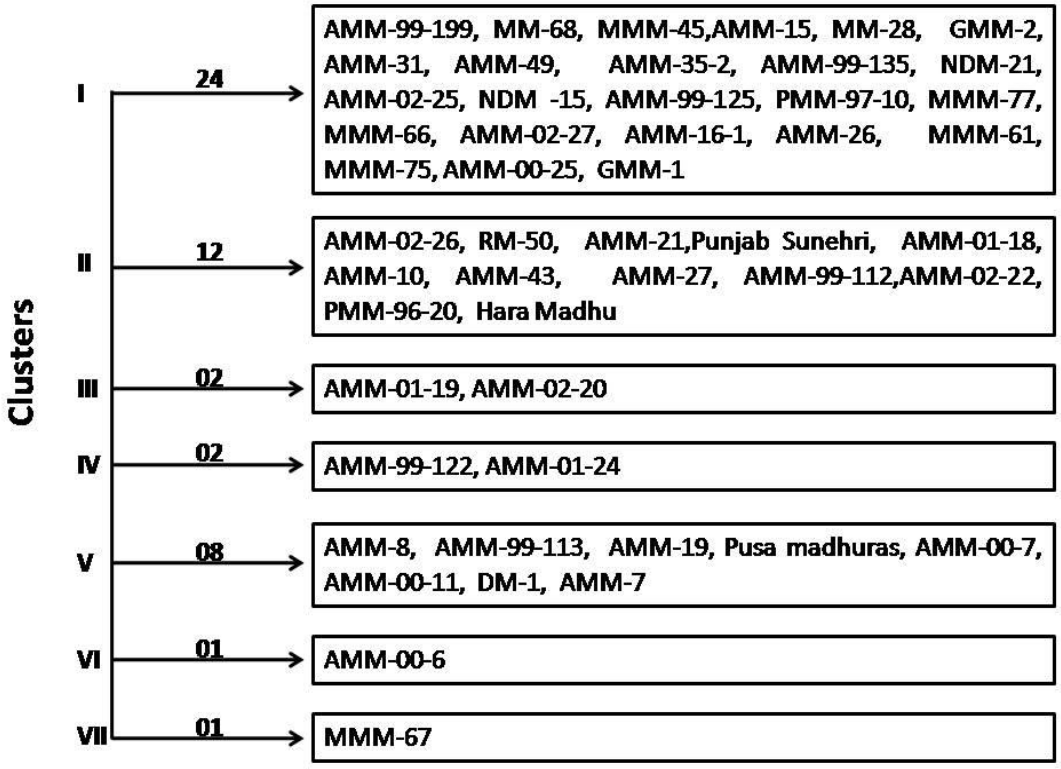


**Figure 2. Cluster groups of 50 genotypes formed on the basis of $D^2$ Statistics in Muskmelon.**

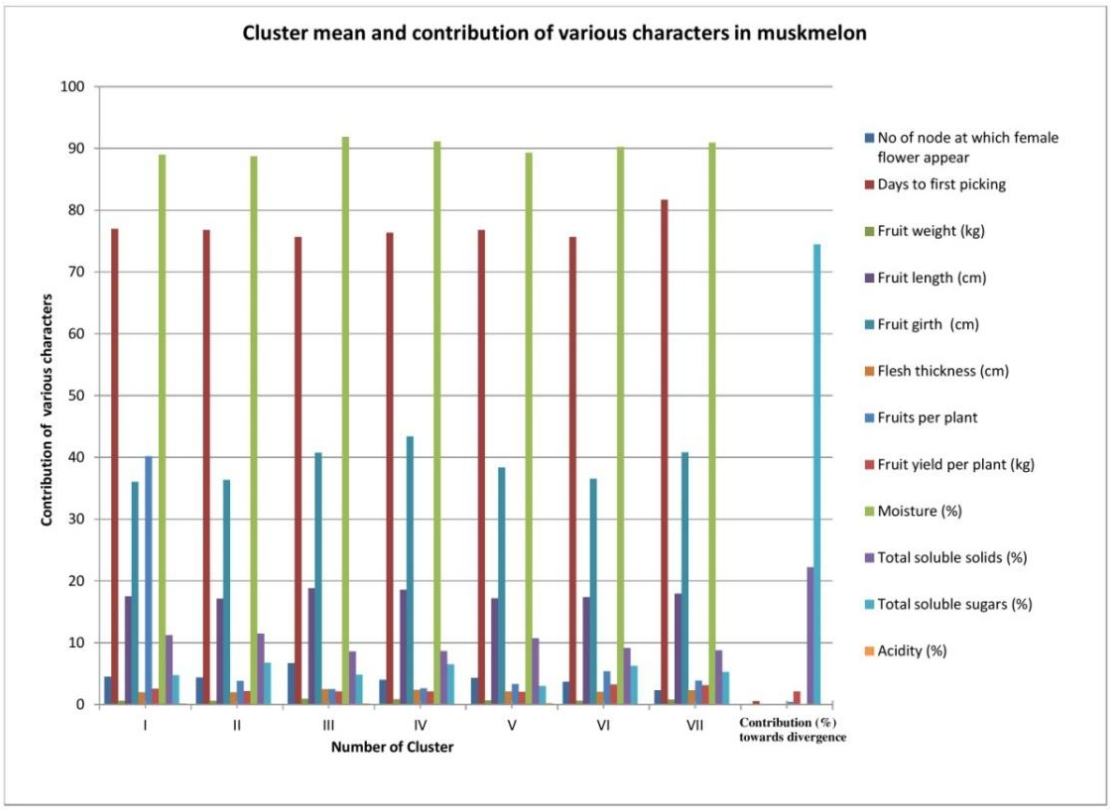


**Figure 3. Cluster mean and contribution of various characters in muskmelon**

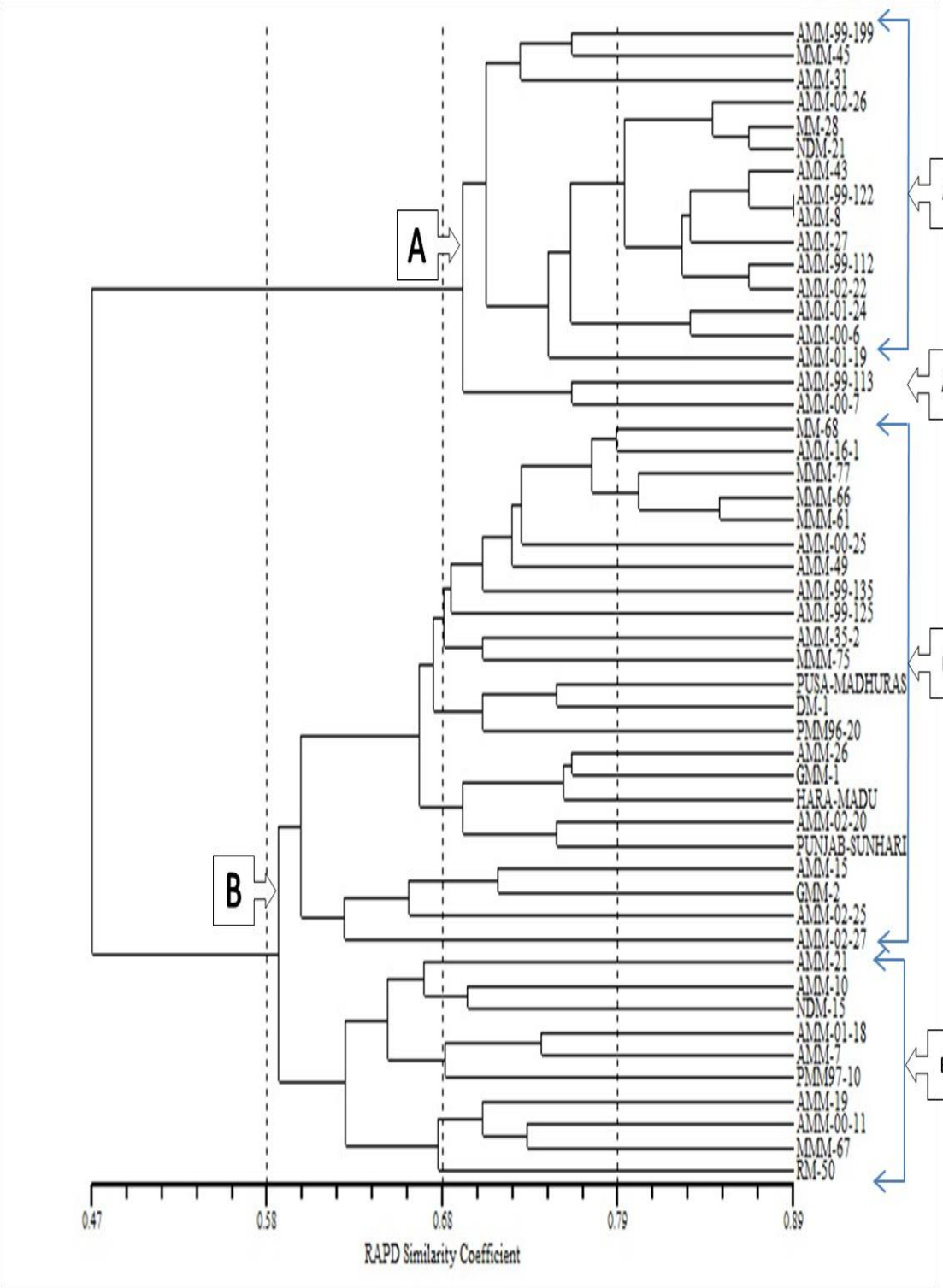


**Figure 4.UPGMA dendrogram displaying the genetic similarity estimates according to RAPD within the 50 genotypes of Muskmelon.**

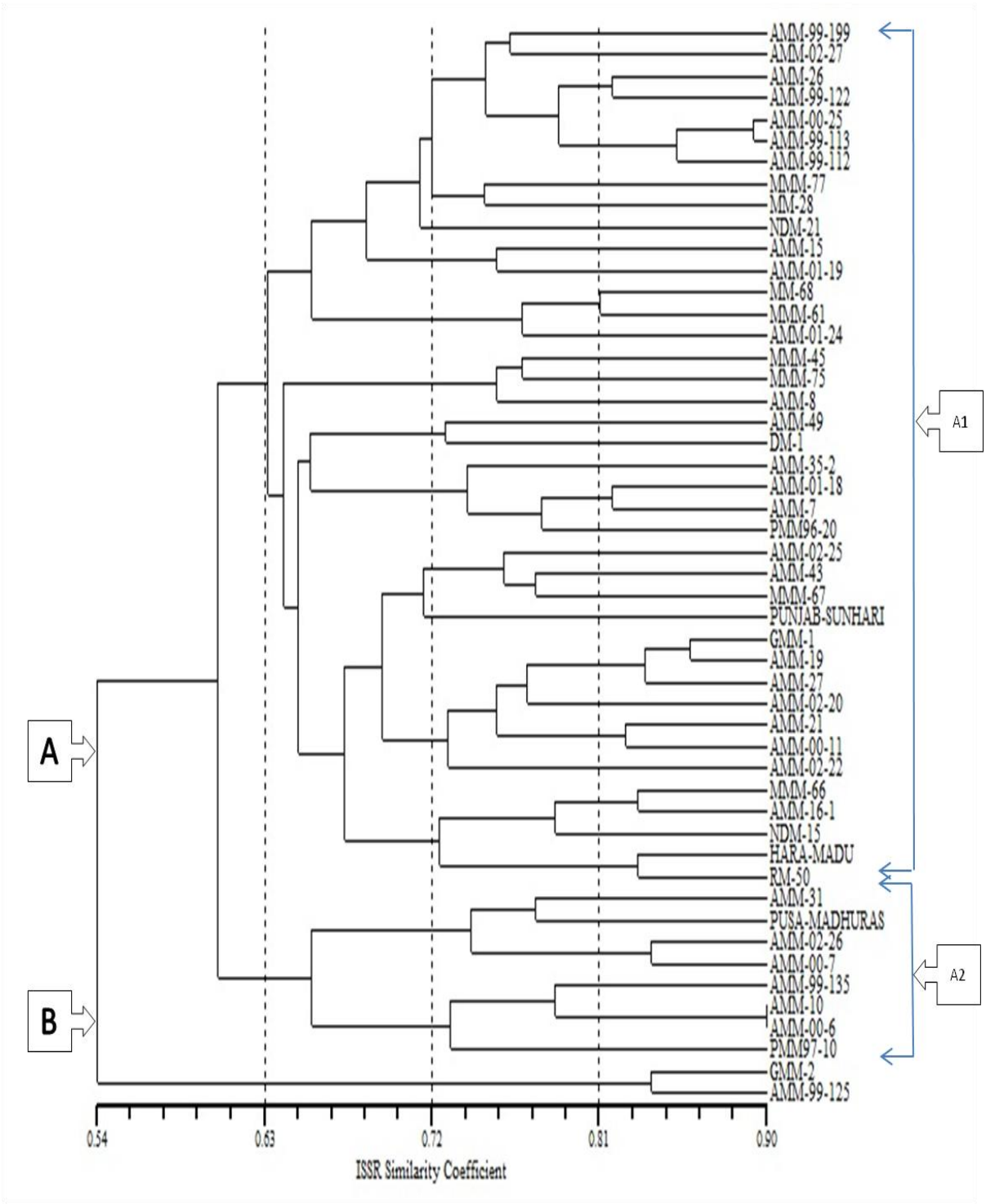


**Figure 5.UPGMA dendrogram displaying the genetic similarity estimates according to ISSR within the 50 genotypes of Muskmelon.**

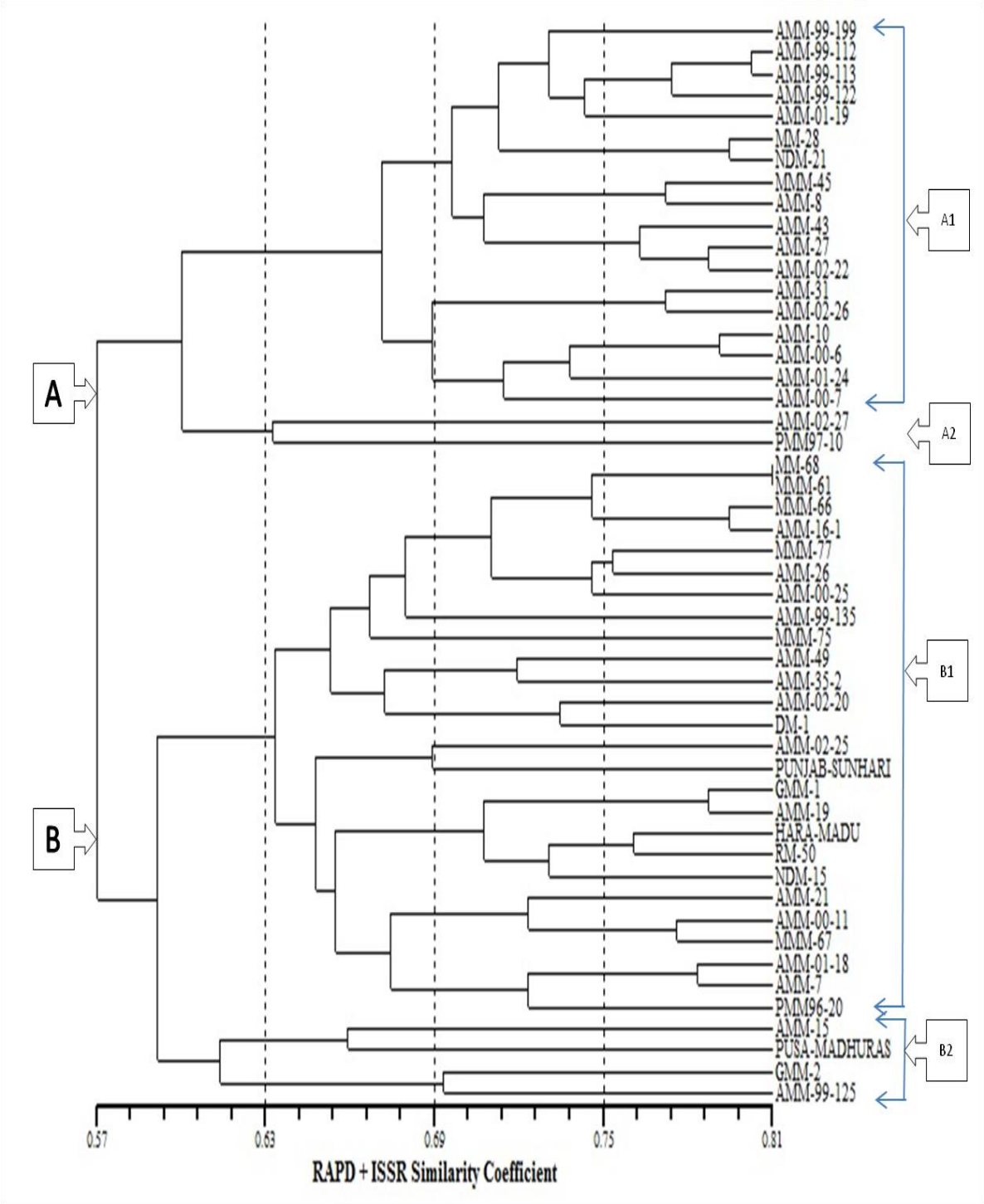


**Figure 6. UPGMA dendrogram displaying the genetic similarity estimates according to RAPD + ISSR within 50 genotypes of Muskmelon.**