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Genetic diversity studies in muskmelon using microsatellite markers

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ABSTRACT

Genetic variation knowledge is critical in muskmelon crop improvement. For genetic diversity evaluation in twenty-five muskmelon genotypes twenty microsatellite markers were used in the present study. Nineteen SSRs were polymorphic and reproducible among twenty. The expected heterozygozygosity (He) values varied from 0.39 to 0.46 at an average of 0.42, the observed heterozygozygosity (Ho) was 0.31 to 0.59 with a mean of 0.49 and these values were somewhat more than the expected heterozygosity, indicating that the population is naturally highly heterozygous. PIC values varied from 0.54 to 0.84, with a mean of 0.69. This data confirmed the polymorphism at the molecular level, indicating these genotypes make up a remarkable basis of muskmelon diversity. Cluster analysis was used to assess the relationship between genotypes and genotypes were divided into three clusters.

***Keywords*:** Muskmelon, genetic diversity and microsatellite markers (SSR).

Muskmelon (2n=24) is one of India's most important dessert cucurbits. It is a major riverbed crop, accounting for 80 per cent of total muskmelon cultivation (Nanadpuri, 1989) and thought to be emanated in Tropical Africa, with India is considered as secondary diversity hotspot. Muskmelon is grown on 54 thousand hectares in India, with an estimated annual production of 1,145,000 MT in 2018-2019 (Anon, 2019). Muskmelon gets its name from musky aroma it gives off when the fruits are ripened. Muskmelon is considered as a nutrient dense food due to its high levels of vitamins such as β-carotene, minerals and carbohydrates. Predominantly muskmelon is andromonoecious and is a cross pollinated crop that allows for extensive use of heterosis. In muskmelon, assessing genetic diversity is important for sensible applications like broadening of genetic base and exploiting heterosis. Morphological and molecular markers aid to determine genetic diversity among individuals. For characterizing the relationships between intra-specific and landrace melons across horticultural groups, morphological and molecular variations can be used. Across the world many authors have implemented melon diversity on the basis of phenotypic traits (Staub *et al*., 2004; Laghetti *et al*., 2008; Escribano and Lazaro, 2009; Nasrabadi *et al*., 2012; Henane *et al*., 2015; Trimech *et al*., 2015).The evaluation of diversity on the basis of phenotypic data was hindered due to the lack of adequate data on distinctive phenotypic characters and uncertainty caused by a large variation in a specific trait in a given population because of climatic conditions (Singh *et al*., 2004). On the other hand, using of DNA marker technologies largely overcomes these limitations, and these are widely used in a range of crop plants. Molecular markers extend and supplement morphological and biochemical characterization, providing more accurate and detailed information than traditional phenotypic data and as long as it is a reliable and effective method as it is less influenced by environmental variation.

Simple sequence repeat (SSR) markers are repetitions of the 1 to 6 nucleotide-long DNA motifs arranged in conjunction. They were regarded as powerful polymerase chain reaction (PCR)-based Mendelian markers (Jarne and Lagoda, 1996) on the basis of their polymorphic nature, and readily transferable within the species. A lot of attention has been paid to microsatellites because they are part of or related to some important agronomic genes. All these positive characteristics apart from their multi-allelic and co-dominant nature, significant abundance and requirement of small amount of DNA have bestowed to the tremendous increase of attentiveness in SSRs for diversity assessment. Molecular markers can quickly distinguish muskmelon species and provide reliable information on their phylogenetic relationships using a small amount of DNA. The primary goal of our experiment is assessment of genetic diversity in 25 muskmelon genotypes by SSR markers.

MATERIALS AND METHODS

Plant material

Experiment was carried out using 25 different muskmelon genotypes in Dr.Y.S.R Horticultural University, Andhra Pradesh. Table 1 contains a list of genotypes that were used and their important horticultural traits.

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Genomic DNA isolation

CTAB (CetylTrimethyl Ammonium Bromide) method (Murray and Thompson, 1980) was followed to extract DNA. DNA quality and quantity estimated by 0.8% agarose gel electrophoresis and U.V. Spectrophotometer Nano drop (ND-1000). DNA concentration was estimated and measured at optical density at 260 nm.

Microsatellite/SSR primer selection

In the current study, twenty SSR primer pairs were used for diversity analysis (Table 2). Nineteen primers out of twenty demonstrated improved reactivity with the clear and estimated amplified product sizes (Henane *et al*., 2015 ; Aierken *et al*., 2011).

Polymerase Chain Reaction amplification for SSR markers

The SSR markers were created using twenty primers. The PCR reaction was performed in 96 well microtier plate using an Eppendorf Master Cycler gradient. The details of the PCR programe are given in Table 3. After that each DNA sample was run on 1.5% agarose gels containing ethidium bromide (EtBr) (0.5 mg l^{-1}) later viewed under an ultraviolet transilluminator Gel doc (Alpha Innotech Multi image light cabinet filter positions).

Genetic data analysis

The genetic relationships between muskmelon genotypes were discovered using molecular data analysis. Amplification profiles of various primer combinations for all genotypes were compared on an agarose gel with a 100 bp size DNA ladder. For each primer, the amplified fragments were manually scored for presence (denoted as 1) and absence (denoted as 0). The similarity matrices were subjected to the Weighted Neighbor Joining method for cluster analysis using Darwin Software 6 version (Posada and Crandall, 2001) and Genalex software for expected heterozygozity (He) and observed heterozygozity (Ho), and Cervus software 3.07 version for Polymorphic Information Content (PIC).

RESULTS AND DISCUSSION

19 of the 20 SSR primers demonstrated amplification and polymorphism (Table 4). Amplification profile of markers were given in Fig. 1 and 1a.The genetic variations observed in muskmelon accessions may be the cause of the polymorphism. Heterozygosity can be used to measure the amount of variation in a given population and its distribution across alleles of an examined locus. Expected heterozygozity (He) may be defined as the chance that an individual will be heterozygous in a locus and values in the range of 0.39 to 0.46. Maximum genetic variability accompanied with highest He was observed in the primer CMN 22-16 (0.46). The primer CMBR 105 recorded lowest He value (0.39), followed by CMN 04-03 and AC2ms-1 (0.40). In a population the percentage of heterozygous individuals defined as observed heterozygosity (Ho) and it is in the range 0.31 to 0.5. Primers CMN 22-16 and CMN 21-41 recorded maximum Ho value (0.59), demonstrating that among genotypes they may provide clear genetic profiles and primer CMN 4-40 recorded the lowest Ho value (0.31).

The observed heterozygosity (Ho) per locus tested in this study was more than in Tzitzikas *et al*.(2009), which could be attributed pronounced diversity in the muskmelon germplasm. A higher level of heterozygosity may be observed in the first and foremost studied genotypes which were developed by local farmers because there may be a chance of cross pollination with different accessions. However, the slightly lower values of heterozygozity observed in this study might be due to the absence of intercrossing between genotypes (Raghami *et al*., 2014).

PIC values estimated markers perceptiveness which constitute the variation in alleles which is represented as no.of alleles lopercus as well as their relative frequencies. Expected PIC values of 19 SSR markers in this study were greater than the zero. In the present study SSR markers revealed genetic diversity greately in 25 muskmelon genotypes and high PIC varied from 0 .54 to 0.84. The locus CMN 04-03 had the highest polymorphic information content of 0.84, followed by CMN 04-07 (0.81), CMBR 12 (0.80), TJ 10 (0.80), MU 118 (0.79), and CMBR 105. (0.79).

Genetic diversity assessment

SSR markers are considered to be effective tool in muskmelon for genotype identification and discrimination, assessing genetic diversity and determining genetic relationships. The dissimilarity matrix values were used to cluster 25 muskmelon genotypes into three major clusters (I, II, and III) using Weighted Neighbor Joining method (Fig 2). Clusters I and II had the most genotypes, while Cluster III had only three genotypes. Similar clustering was observed in muskmelon by Kacar *et al*. (2012); Imen *et al*. (2015) and in cucumber by Yang *et al*. (2015). Based on rind colour and the shape of the fruit, two sub-clusters were observed in Cluster I, Ia (8 genotypes) and Ib (4 genotypes). Genotypes in Ia had a characteristic cream rind colour, whereas genotypes in Ib had a green to greenish yellow rind colour. Genotypes of IC 321375, IC 321375-1 and Suvarna were grouped together in sub-cluster Ia. Suvarna, on the other hand, differed from IC 321375 and IC 321375-1 genotypes due to the fruit shape, leaf size and size of seeds. Because of the

Table 1: Muskmelon genotypes used in present study

S.No.	Genotype	Source	Fruit shape	Fruit surface	Rind colour	Flesh colour	Cluster
1	IC 321327	NBPGR, Jodhpur, Rajasthan	Ovate	Sutures	Blackish green	Yellow	II (IIa)
2	IC 321370	NBPGR, Jodhpur, Rajasthan	Oblate	Sutures	Orange	Cream	II (IIb)
3	IC 321344	NBPGR, Jodhpur, Rajasthan	Oblate	Sutures	Orange	White	II (IIb)
4	IC 321333	NBPGR, Jodhpur, Rajasthan	Elongate	Smooth	Light yellow	Cream	II (IIa)
5	IC 315330-2	NBPGR, Jodhpur, Rajasthan	Elongate	Grainy	Blackish green	White	II (IIa)
6	IC 315323	NBPGR, Jodhpur, Rajasthan	Globular	Sutures	Pale green	Green	II (IIa)
7	IC 321374	NBPGR, Jodhpur, Rajasthan	Globular	Sutures	Orange	Cream	III
8	IC 321328	NBPGR, Jodhpur, Rajasthan	Pyriform	Grainy	Grey	Cream	I (Ia)
9	IC 315330	NBPGR, Jodhpur, Rajasthan	Elongate	Sutures	Blackish green	Pale orange	I (Ia)
10	IC 321380	NBPGR, Jodhpur, Rajasthan	Flattened	Smooth	Cream	Pale orange	I (Ia)
11	IC 321375	NBPGR, Jodhpur, Rajasthan	Oblate	Lightly corked, sutures	Light yellow	White	I (Ia)
12	IC 321343	NBPGR, Jodhpur, Rajasthan	Flattened	Smooth	Orange	Orange	I (Ia)
13	IC 321375-1	NBPGR, Jodhpur, Rajasthan	Oblate	Lightly corked, sutures	Light yellow	White	I (Ia)
14	IC 321329-1	NBPGR, Jodhpur, Rajasthan	Ovate	Sutures, smooth	Green	Cream	I (Ib)
15	IC 321366	NBPGR, Jodhpur, Rajasthan	Ovate	Smooth	Yellow	Orange	II (IIa)
16	Papasa	Vontimitta, Kadapa, Andhra Pradesh	Elongate	Smooth	Yellow	Orange	II (IIa)
17	Sirangi	Vontimitta, Kadapa, Andhra Pradesh	Pyriform	Grainy	Cream	Cream	II (IIa)
18	Sharbathi	Vontimitta, Kadapa, Andhra Pradesh	Globular	Lightly netted	Light yellow	Cream	III
19	Alpur-1	Vontimitta, Kadapa, Andhra Pradesh	Globular	Grainy	Yellow	Orange	III
20	Improved Sharbathi	Vontimitta, Kadapa, Andhra Pradesh	Globular	Heavily netted	Light yellow	Cream	I (Ia)
21	Suvarna	Vontimitta, Kadapa, Andhra Pradesh	Pyriform	Smooth	Pale green	White	I (Ia)
22	Alpur green	Vontimitta, Kadapa, Andhra Pradesh	Elliptical	Smooth	Cream	Green	I (Ib)
23	KSP 1060	Vontimitta, Kadapa, Andhra Pradesh	Globular	Sutures, smooth	Light yellow	Orange	I (Ib)
24	Alpur orange	Vontimitta, Kadapa, Andhra Pradesh	Elliptical	Smooth	Orange	Orange	I (Ib)
25	ArkaJeet	IIHR, Hessaraghatta, Bengaluru	Oblate	Smooth	Yellow	Cream	II (IIa)

Table 2: SSR primers used for diversity assessment in 25 muskmelon genotypes

Sl. No.	Primer code	Microsatellite primer sequence (5′→3′)	Reference allele length (bp)	Observed allele length range (bp)	Anneling temperature (°C)	Polymorphic bands (No.)
1.	CMGAN 80	F: TATATTGATTGCTGGGAAAGG R:CTTTTTTGGCTTTATTGGGTC	100	83-121	53^0 C	5
2.	CMAGN 68	F:TGGAAGGAAATTAGCATGCAC R: GCCACTCTGTCTTTCTTCC	115	100-133	53^0 C	4
3.	CMBR 2	F:TGCAAATATTGTGAAGGCGA R: ATCCCCACTTGTTGGTTTG	114	71-123	53^0 C	7
4.	CMBR 12	F:ACAAACATGGAAATAGCTTTCA R: GCCTTTTGTGATGCTCCAAT	134	44-117	53^0 C	7
5.	CMBR 22	F:TCCAAAACGACCAAATGTTCC R: ATACAGACACGCCTTCCACC	177	81-119	53^0 C	7
6	CMBR 83	F:CGGACAAATCCCTCTCTGAA R: GAACAAGCAGCCAAAGACG	142	76-100	53^0 C	4
7	CMBR 105	F:TGGTAAGCATTTTGAAATCACTTTT R: TTCCAGACATCTAAAGGCATTG	139	215-264	53^0 C	6
8	CMN 04-03	F:ATCACAGAGACCGCCAAAAC R: GGTTGAAGATTGCGCTTGAT	218	241-283	53^0 C	8
9	CMN 22-16	F: CAGAGGAGGTGGAACTAACCA R: CCATTTTCAACCTCCCAAGA	233	204-222	53^0 C	5
10	CMN 61-44	F: TGTTGGAGTTTAATGAGGAAGGA R: AGAGAAGATGAATGGGGCAC	233	195-288	53^0 C	5
11	CMN 04-07	F: GAAAGCATTAAATATGGCATTGG R: AAGCTTAACAGCTTCCAGGG	286	223-287	53^0 C	7
12.	CMN 21-41	F: GAGGAAATTTTGGAGTTTTTCAA R: TTCCAGACATCTAAAGGCATTG	281	219-231	53^0 C	3
13.	CMN 4-40	F:CACCTGACGATAGGGGTGTT R: AGTATTCGGGTTGGCAAAAA	212	248-264	53^0 C	4
14.	CMCTN 5	F:TCACCTTAAAGTTTAGCCCC R: AAAAATGCAATGAACTGAGCGC	115	133-173	53^0 C	5
15.	CMCTN 86	F:TGTGACAGTTATCAAGGATGC R: AAGGGAATGCATGTGGAC	175	141-183	53^0 C	7
16	TJ 10	F:TACGAGGAAAACGCAAAATCA R: TGAACGTGGACGACATTTTT	155	58-100	53^0 C	6
17	TJ 27	F:TAAGCGGAACAAGCTCATCTC R: CAAAAGCATCAATTGCTTGAA	170	88-132	53^0 C	5
18	MU 118	F:TGTGTGCTGTACTCCTGAAA R: CGGTTCTTTCTTCTCTTCCT		279-351	53^0 C	7
19.	AC2 ms-1	F:TCTTTTGTTCTTGGTTGTGAGT R: GATTGCTTTATTTTGAATCTTTTG	200	100-144	53^0 C	5

Table 3a: PCR amplification for SSR marker

	Stock concentration	Volume taken
Taq buffer + Kcl,+$MgCl_2$	10X	2.5µl
Forward Primer	10p mole	2.5µl
Reverse Primer	10p mole	2.5µl
DNTPs	10mM	0.5µl
Taq DNA polymerase	500U	0.1µl
DNA	100ng	2µl
Sterile water		14.9µl
		25µl

Table 3b: PCR amplification profile for SSR markers

Profile–I	Initial denaturation	94^{o} C	2 minutes
Profile – II	Denaturation	94^{0} C	1 minute
Profile – III	Primer annealing	53^{0} C	1 minute
Profile – IV	Primer extension	72^{0} C	1 minute
Later profile- II to profile – IV were repeated		35 cycles	
Profile – V	Complete primer extension	72^{0} C	2 minutes
Profile – VI	Soak temperature	4^{0} C	Till removal

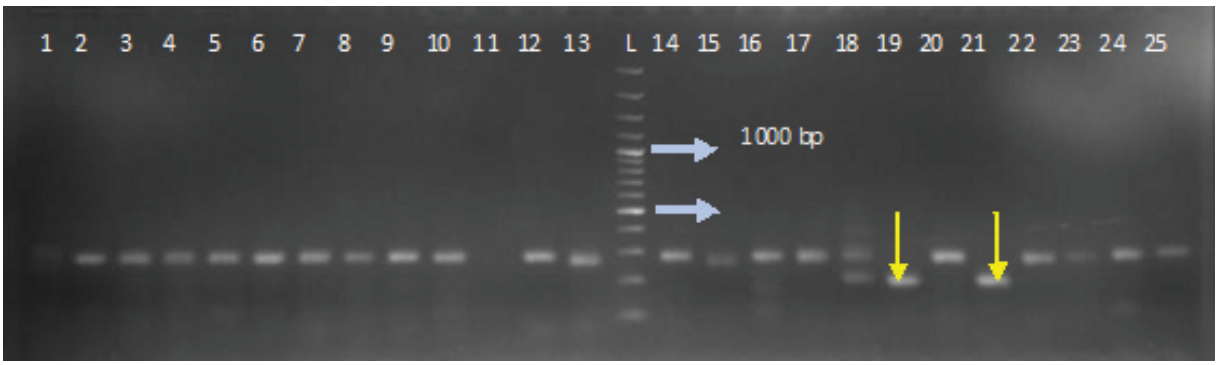
Table 4: Genetic diversity assessment parameters in 25 genotypes of muskmelon using SSR data

Sl. No	Primer	Na	Ne	I	Ho	He	PIC
1	CMGAN 80	2	1.732	0.601	0.507	0.413	0.70
2	CMAGN 68	2.000	1.823	0.637	0.547	0.447	0.64
3	CMBR 2	2.000	1.716	0.594	0.493	0.407	0.76
4	CMBR 12	1.960	1.774	0.605	0.480	0.422	0.80
5	CMBR 22	2.000	1.740	1.740	0.560	0.413	0.80
6	CMBR 83	2.00	1.798	0.625	0.453	0.436	0.69
7	CMBR 105	1.880	1.726	0.558	0.373	0.391	0.79
8	CMN 04-03	2.000	1.706	0.585	0.507	0.400	0.84
9	CMN 22-16	2.000	1.855	0.649	0.587	0.458	0.63
10	CMN 61-44	2.000	1.789	0.620	0.507	0.431	0.68
11	CMN 04-07	2.000	1.773	0.615	0.507	0.427	0.81
12	CMN 21-41	2.000	1.806	0.630	0.587	0.440	0.57
13	CMN 4-40	1.960	1.765	0.597	0.307	0.416	0.67
14	CMCTN 5	2.000	1.756	0.608	0.413	0.420	0.45
15	CMCTN 86	1.920	1.766	0.583	0.560	0.409	0.69
16	TJ 10	2.000	1.780	0.615	0.480	0.427	0.72
17	TJ 27	2.000	1.846	0.641	0.520	0.451	0.54
18	MU 118	1.960	1.960	0.606	0.440	0.424	0.79
19	AC2 ms-1	1.960	1.725	0.585	0.427	0.404	0.70
	Average	**1.981**	**1.780**	**0.668**	**0.487**	**0.423**	**0.698**

Na: Number of different alleles, Ne: Number of effective alleles, I: Shannon's information index, Ho: Observed Heterozygosity, He: Expected Heterozygosity, PIC: Polymorphic Information Content

cylindrical fruit shape, IC 321380, IC 315330, and IC 321343 formed one sub-cluster, but IC 321343 formed a separate sub-cluster due to flesh colour (light orange). IC 321328 and Improved Sharbathi formed one sub cluster because of the round fruit shape. In sub cluster Ib out of four genotypes, Alpur green and IC 321329-1 formed one group because of the flesh colour which was light green to cream in colour and Alpur orange and KSP 1060 formed one group because deep orange flesh colour.

Cluster II was further subdivided into two subgroups based on fruit shape and fruit surface type. Fruits in sub cluster IIa have a round to cylindrical shape and a smooth surface. Genotypes in sub cluster IIb had oblate fruit



Lane 1	IC 321370	Lane 9	Arka Jeet	Lane 17	IC 321380
Lane 2	IC 321344	Lane 10	IC 321366	Lane 18	Suvarna
Lane 3	IC 321333	Lane 11	IC 321374	Lane 19	IC 321375-1
Lane 4	IC 321327	Lane 12	Sharbarhi	Lane 20	IC 321343
Lane 5	IC 315330-2	Lane 13	Alpur- 1	Lane 21	IC 321375
Lane 6	Papasa	Lane 14	I.Sharbathi	Lane 22	IC 321329-1
Lane 7	Sirangi	Lane 15	IC 321328	Lane 23	Alpur green
Lane 8	IC 315323	Lane 16	IC 315330	Lane 24	KSP 1060
				Lane 25	Alpur orange

Fig. 1: Amplification profile of CMN 61-44 in 25 muskmelon genotypes

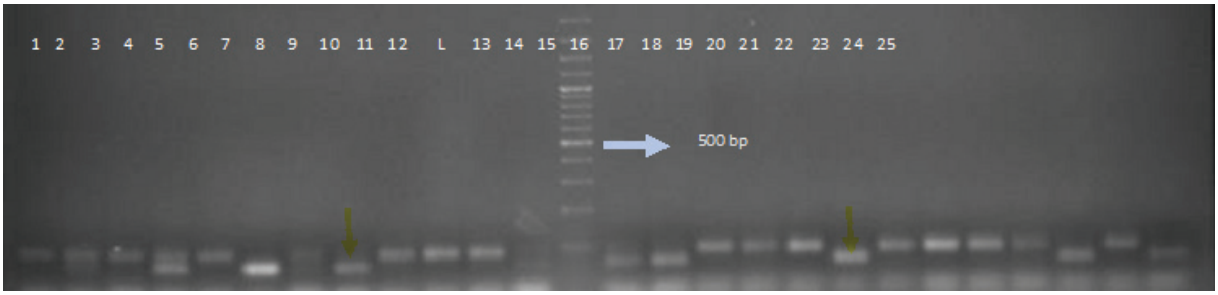


Fig. 1a: Amplification profile of CMBR 12 in 25 muskmelon genotypes

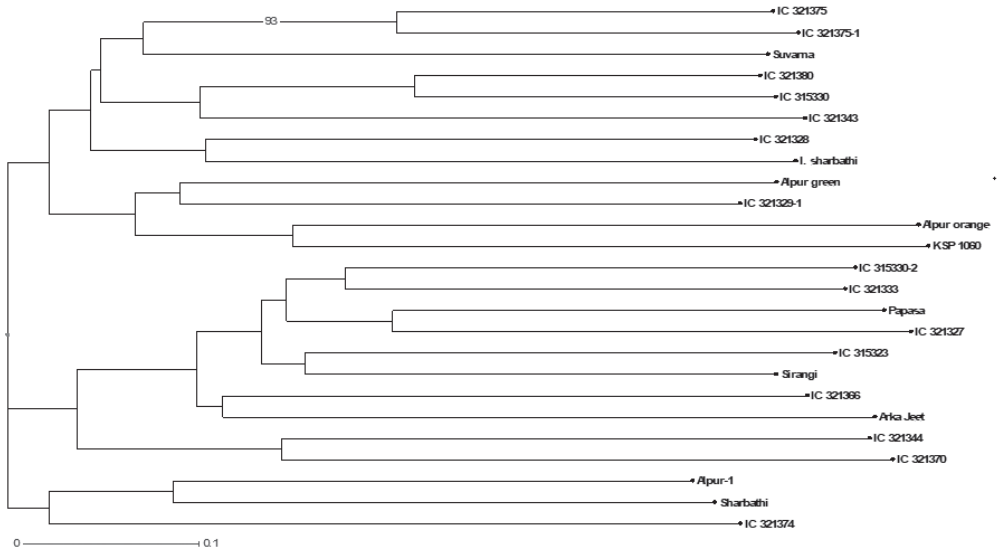


Fig. 2: Weighted Neighbor dendrogram obtained by 19 SSR primers in muskmelon genotypes

shapes with grooved surfaces. Because of their cream rind colour, white flesh colour and similar stem thickness, sub cluster IIa genotypes IC 315330-2 and IC 321333 formed one sub cluster. Because of their cream flesh colour, Papasa and IC 321327 formed one group. The round fruit shape of IC 321366 and ArkaJeet grouped them together. Because of their oblate fruit shape, grooved surface, and cavity orientation, genotypes IC 321344 and IC 321370 clustered together in sub cluster IIb. Cluster III was the smallest, with only three genotypes namely Alpur-1, Sharbathi and IC 321374.

CONCLUSION

The current study clearly shows that genotypes genetic diversity plays a prominent role in muskmelon genetic diversity preservation and enhancement. However, this study suggests that further molecular characterization of the muskmelon gene pool with huge number of genotypes and SSR markers is required to gain an indulgent of the molecular complexity concerned in the gene pool as well as to derive accurate phylogenetic relationships among them.

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