



## Research Article

# Genetic diversity analysis among different horticultural groups of indigenous and exotic *Citrullus* landraces using microsatellite markers

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### Abstract

The study utilised a total of twenty simple sequence repeat (SSR) markers to investigate the genetic diversity of 53 watermelon accessions from different groups of *Citrullus* i.e., *citroides*, *lanatus*, and *colocythis*. Twelve of the twenty SSR markers were shown to be highly polymorphic and were statistically analyzed using Power Marker and NTSYSPc softwares. A total of 33 alleles were generated by polymorphic SSR markers, on an average of 2.6 alleles per loci. Heterozygosity among accessions for individual loci varied from zero to 0.17 (BVWS02306 marker). The gene diversity and PIC values varied between 0.08 to 0.72 and 0.46 to 0.90, respectively and the primer which had the highest gene diversity and PIC values was BVWS02422. The UPGMA-based dendrogram classified all the 53 accessions into two major clusters at 48 per cent similarity. All 41 cultivated watermelon accessions were grouped in cluster I and the remaining 12 accessions from wild types were grouped in cluster II. Thus, this study highlights the importance of molecular markers in the identification of wild and cultivated lines and their exploitation in future breeding programs.

**Keywords:** Watermelon, genetic diversity, microsatellite, Jaccard similarity coefficient

### INTRODUCTION

Watermelon, a member of the Cucurbitaceae family, is a morphologically versatile, out crossing horticultural crop of significant economic importance. Watermelon fruits are rich in many health benefiting compounds including citrulline, lycopene, arginine, and glutathione. It is being grown world over in about 100 countries which accounts for 7 % of the total area under vegetable crops. There are four species in the *Citrullus* family (*C. lanatus*, *C. ecirrhosus*, *C. rehmii* and *C. colocythis*). *Citrullus colocythis*, which is prevalent in central Africa, is most likely to be the progenitor of domesticated *Citrullus lanatus* var. *lanatus*. The fruit features such as size, shape, colour, flavor, structure and nutritional composition differ enormously in Watermelon (Robinson and Decker-Walters, 1997). The evaluation of genetic diversity among watermelon accessions from various groups

is very crucial for their exploitation in plant breeding programmes to pick genotypes with a wide range of characters including fruit traits. Thus, systematic assessment of germplasm is critical for current and future crop genetic refinement (Reddy *et al.*, 2013; Senthilvadivu, 2018; Indraj *et al.*, 2021). In, India, huge variability is noted in different regions and there is an urgent need to characterize these lines using the descriptors and molecular tools for their exploitation in breeding programmes and conservation. The application of molecular markers for the genetic diversity studies in water melon is very rare and this is highly needed for the water melon improvement. Keeping this view, the present study was carried out to determine the genetic variation of 53 watermelon accessions using 20 SSR markers.

## MATERIALS AND METHODS

The water melon germplasm of 53 accessions from different groups of *citroides*, *lanatus*, and *colocythis*, were obtained from various agro-climatic regions of India and were maintained at the Division of Vegetable Science, Indian Agricultural Research Institute, New Delhi, India, which is geographically located at 228.61 m (750 feet) above mean sea level, with 28°08N latitude and 77°12E longitude. The experiment was conducted in randomized block design with three replications during *kharif* season of 2018. The plants were transplanted on raised beds of 2.5 m apart with 0.75 m spacing between the plants. All the recommended agronomic practices along with plant protection measures were followed to raise a successful crop. The morphological features of the different accessions are mentioned in **Table 1**. The genomic DNA of 53 watermelon accessions from various groups was extracted using Murray and Thomson's traditional CTAB method with minor modifications (1980). The DNA quantity was assessed on 0.8 % agarose gel with lambda uncut DNA and the quality was determined using a spectrophotometer. The standard working concentration of 20 ng/l DNA sample was used in PCR and stored at 4 °C. Twenty polymorphic SSR primer pairs uniformly distributed across the watermelon genome were chosen from the previous study (Zhang *et al.*, 2012). For PCR, stock primers were diluted with nuclease-free water and stored at 4°C. PCR amplification was carried out in a 10µL reaction mixture that included 1µL of PCR buffer (1X), 0.2µL of dNTP (0.25 mM), 0.5µL of individual primers (1 M), 2µL of genomic DNA (20 ng), 0.2 µL of Taq DNA polymerase and 6. of nuclease free water. The PCR protocol included a 5 min. initial denaturation stage at 94°C, followed by 35 cycles of 94°C (20 seconds), at 55°C annealing for 20 seconds, elongation for 90 seconds at 72°C and an final extension for 8 min. at 72°C.

On 4% ultra high resolution agarose gels, the amplified products were resolved and visualized using the Syngene Gel Documentation System. Out of twenty, 12 highly polymorphic SSR markers were used to rank the genetic profile of 53 watermelon accessions based on differences in their allele size. Power Marker version 3.25 program (<http://www.powermarker.net>) was used to calculate major allele frequency, polymorphism information content (PIC), gene diversity, and heterozygosity based clustering (Liu and Muse, 2005). NTSYS-pc v2.1 software was used to measure the relationship between genetic similarities found by SSR markers and genetic similarity coefficients (Jaccard's distance or genetic distance) (Rohlf, 2000). The dissimilarity coefficients of Jaccard's were used as input for the construction of a dendrogram/phylogenetic tree using the unweighted pair-group method with arithmetic mean (UPGMA).

## RESULTS AND DISCUSSION

In the present study, a total of 20 SSR markers were selected for diversity assessment of 53 watermelon accessions collected from different geographical regions of India. Twelve markers were observed to be highly polymorphic while, eight markers were found to be monomorphic in the studied material resulting in 60 per cent average polymorphism. The sequence information of 12 polymorphic SSR markers is presented in **Table 2**. Gel images of the representative polymorphic marker BVWS02390 is presented in **Fig. 1**. In 53 watermelon germplasm, 33 alleles were found, with an average of 2.6 alleles per locus. The number of alleles per locus varied between 2 and 6. Since the germplasm was mostly wild, all of the markers studied showed a high degree of molecular polymorphism. The marker, BVWS02422, recorded the maximum number of alleles *i.e.*, six among the 53 genotypes which might be due to

**Table 1. Morphological characterization of 53 watermelon accessions**

S. No.	Genotypes Horticultural group	Fruit shape	Rind colour	Flesh colour	Fruit stripes	Seed colour	Organoleptic taste
1	DWM 4 <i>Citrullus lanatus</i> var. <i>citroides</i>	Oval	Light green	Yellow	Weak	Red	Low sweet
2	DWM 7 <i>Citrullus lanatus</i> var. <i>lanatus</i>	Flate globe	Light green	Yellow	Diffused	Red	Low sweet
3	DWM 8 <i>Citrullus lanatus</i> var. <i>lanatus</i>	Round	Light green	White	Diffused	White	Medium
4	DWM 13 <i>Citrullus lanatus</i> var. <i>lanatus</i>	Round	Medium green	Yellow	Clearly defined	Brown	Low sweet
5	DWM 15 <i>Citrullus lanatus</i> var. <i>lanatus</i>	Round	Yellow	Light red	Weak	Black	Low sweet
6	DWM 16 <i>Citrullus lanatus</i> var. <i>lanatus</i>	Round	dark green	Reddish Pink	Weak	Black	Low sweet
7	DWM 19 <i>Citrullus lanatus</i> var. <i>lanatus</i>	Flate globe	Light green	Light red	Clearly defined	Brown	Medium sweet
8	DWM 22 <i>Citrullus lanatus</i> var. <i>citroides</i>	Round	Medium green	Light red	Clearly defined	Black	Medium sweet
9	DWM 27 <i>Citrullus lanatus</i> var. <i>citroides</i>	Round	Medium green	Yellow	Diffused	Brown	Bitter
10	DWM 30 <i>Citrullus lanatus</i> var. <i>citroides</i>	Flate globe	dark green	Orange	Weak	Brown	Bitter
11	DWM 32 <i>Citrullus lanatus</i> var. <i>citroides</i>	Round	Light green	White	Diffused	Brown	Low sweet
12	DWM 34 <i>Citrullus lanatus</i> var. <i>citroides</i>	Round	Light green	Light red	Diffused	Black	Bitter

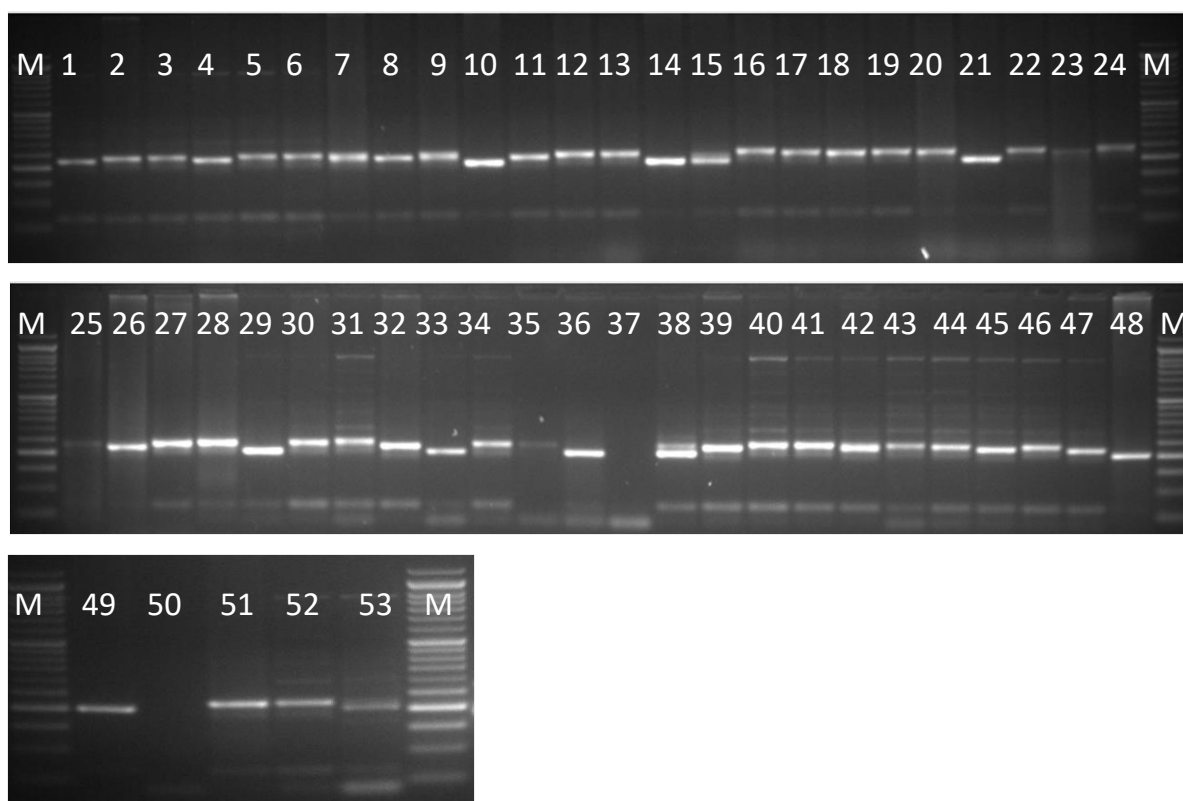
13	DWM 35	<i>Citrullus lanatus</i> var. <i>citroides</i>	Flate globe	Yellow	Yellow	Weak	Red	Low sweet
14	DWM 36	<i>Citrullus lanatus</i> var. <i>citroides</i>	Oval	Light green	Yellow	Weak	Grey	Low sweet
15	DWM 40	<i>Citrullus lanatus</i> var. <i>citroides</i>	Flate globe	Yellow	White	Diffused	Black	Bitter
16	DWM 41	<i>Citrullus lanatus</i> var. <i>citroides</i>	Round	Yellow	Yellow	Diffused	Grey	Sour
17	DWM 43	<i>Citrullus lanatus</i> var. <i>citroides</i>	Round	dark green	Yellow	Weak	Brown	Sour
18	DWM 51	<i>Citrullus lanatus</i> var. <i>lanatus</i>	Round	Light green	Yellow	Clearly defined	White	Sweet
19	DWM 66	<i>Citrullus lanatus</i> var. <i>lanatus</i>	Round	dark green	Reddish Pink	Weak	Black	Low sweet
20	DWM 67	<i>Citrullus lanatus</i> var. <i>lanatus</i>	Round	Medium green	Reddish Pink	Weak	Black	Sweet
21	DWM 68	<i>Citrullus lanatus</i> var. <i>lanatus</i>	Round	Medium green	White	Diffused	White	Bitter
22	DWM 90	<i>Citrullus lanatus</i> var. <i>lanatus</i>	Round	Yellow	Reddish Pink	Absent	Grey	Medium sweet
23	DWM 95	<i>Citrullus lanatus</i> var. <i>lanatus</i>	Round	Light green	Reddish Pink	Clearly defined	Brown	Low sweet
24	DWM 100	<i>Citrullus lanatus</i> var. <i>lanatus</i>	Flate globe	Light green	White	Diffused	White	Low sweet
25	DWM 109	<i>Citrullus lanatus</i> var. <i>lanatus</i>	Cylindrical	Medium green	Light red	Clearly defined	Black	Sweet
26	DWM 113	<i>Citrullus lanatus</i> var. <i>lanatus</i>	Flate globe	Medium green	Dark Red	Clearly defined	Grey	Very sweet
27	DWM 114	<i>Citrullus lanatus</i> var. <i>lanatus</i>	Round	Light green	White	Weak	White	Bitter
28	DWM 115	<i>Citrullus lanatus</i> var. <i>lanatus</i>	Round	Medium green	Reddish Pink	Diffused	Red	Sweet
29	DWM 117	<i>Citrullus lanatus</i> var. <i>lanatus</i>	Oval	Medium green	White	Weak	White	Sour
30	DWM 121	<i>Citrullus lanatus</i> var. <i>lanatus</i>	Round	dark green	Reddish Pink	Weak	Brown	Sweet
31	DWM 122	<i>Citrullus lanatus</i> var. <i>citroides</i>	Round	Medium green	Light red	Weak	Brown	Medium sweet
32	DWM 124	<i>Citrullus lanatus</i> var. <i>lanatus</i>	Round	dark green	Reddish Pink	Weak	White	Low sweet
33	DWM 128	<i>Citrullus lanatus</i> var. <i>lanatus</i>	Round	Light green	Reddish Pink	Weak	Black	Sour
34	DWM 129	<i>Citrullus lanatus</i> var. <i>lanatus</i>	Round	Medium green	White	Clearly defined	Brown	Sweet
35	DWM 131	<i>Citrullus lanatus</i> var. <i>lanatus</i>	Round	Light green	White	Diffused	Brown	Low sweet
36	DWM 133	<i>Citrullus lanatus</i> var. <i>lanatus</i>	Round	Medium green	White	Weak	White	Low sweet
37	DWM 134	<i>Citrullus lanatus</i> var. <i>lanatus</i>	Flate globe	Light green	Light red	Diffused	Grey	Medium sweet
38	DWM 136	<i>Citrullus lanatus</i> var. <i>lanatus</i>	Flate globe	Medium green	Reddish Pink	Clearly defined	Black	Low sweet
39	DWM 140	<i>Citrullus lanatus</i> var. <i>lanatus</i>	Round	Medium green	White	Diffused	Black	Low sweet
40	DWM 142	<i>Citrullus lanatus</i> var. <i>lanatus</i>	Round	dark green	Light red	Weak	Grey	Sweet
41	DWM 143	<i>Citrullus lanatus</i> var. <i>lanatus</i>	Round	dark green	White	Weak	Black	Low sweet
42	DWM 149	<i>Citrullus lanatus</i> var. <i>lanatus</i>	Oval	Light green	Orange	Diffused	Black	Low sweet
43	DWM 150	<i>Citrullus lanatus</i> var. <i>lanatus</i>	Round	Light green	White	Diffused	White	Low sweet
44	DWM 159	<i>Citrullus lanatus</i> var. <i>lanatus</i>	Round	Light green	Reddish Pink	Clearly defined	Black	Medium sweet
45	DWM 162	<i>Citrullus lanatus</i> var. <i>lanatus</i>	Round	Light green	White	Diffused	Brown	Low sweet
46	DWM 164	<i>Citrullus lanatus</i> var. <i>lanatus</i>	Round	Light green	White	Diffused	Brown	Bitter
47	DWM 173	<i>Citrullus lanatus</i> var. <i>lanatus</i>	Round	Medium green	Reddish Pink	Clearly defined	Brown	Sweet
48	DWM 178	<i>Citrullus lanatus</i> var. <i>lanatus</i>	Flate globe	dark green	Reddish Pink	Weak	Brown	Medium sweet
49	DWM 184	<i>Citrullus lanatus</i> var. <i>lanatus</i>	Round	White	Light red	Weak	Black	Sweet
50	DWM 196	<i>Citrullus lanatus</i> var. <i>lanatus</i>	Round	Medium green	White	Diffused	White	Bitter
51	DWM 210	<i>Citrullus colocynthis</i>	Round	Yellow	White	Weak	Black	Very Bitter
52	Sugar Baby	<i>Citrullus lanatus</i> var. <i>lanatus</i>	Round	dark green	Dark red	Weak	Black	High sweet
53	Arka Manik	<i>Citrullus lanatus</i> var. <i>lanatus</i>	oval	Light green	Dark red	Clearly sweet	Black	High sweet

duplication of non-coding regions or presence of pseudo alleles in many wild genotypes which have not gone under much selection pressure for desirable traits of interest. The polymorphic information content ranged from 0.46 for BWS02390 to 0.90 for BVWS02422 primer pairs (Table 2). Mujaju *et al.* (2010) also reported similar results

in a sample of 97 watermelon lines, with polymorphic information content (PIC) ranged from 0.47 to 0.77 for the RAPD primers and from 0.39 to 0.97 for the SSR loci. The gene diversity ranged from 0.146 to 0.802 with BVWS02422 primer having the largest gene diversity. The similarity coefficient values ranged from 0.11

**Table 2. List of polymorphic markers along with their sequences, allele number, heterozygosity, gene diversity and PIC values**

S. No.	Primers	Forward primer	Reverse primer	Number of alleles	Heterozygosity	Gene diversity	PIC value
1	BVWS00681	TCTTGTCGTGCAATCTCTGC	TTCAAGAAGAAAATTGGTCACCT	2	0.154	0.370	0.65
2	BVWS01116	TGGCTTGAATTTTGAAACC	GAGCTTCCACACCTGAATTTT	2	0.063	0.256	0.53
3	BVWS02421	GGAGGGCAATACGATGAGAA	ATCCTGGGTCATTGCAGATT	2	0.087	0.201	0.56
4	BVWS02306	AGGTTGCTGTCCAGAAGGTC	TTCGCCAAATACAAAAGTAC	2	0.173	0.037	0.55
5	BVWS02380	TTTCGCTGTCTTGTTTTGA	CCGAAGAATATCCATCCCCT	3	0.025	0.073	0.70
6	BVWS02225	ATGTCATAATCCTAAGTTGA	GATTTGTGGATGAAGAGTA	2	0.108	0.201	0.65
7	BVWS02398	ATGGAATGCTTTGGGACTTG	TCCACAGTTCATTGAAGACACA	2	0.000	0.171	0.53
8	BVWS00547	TGGTGTGAAAATGAAGTCCC	TCATTAGGAGGCAGTGCAAA	2	0.153	0.282	0.74
9	BVWS02390	TGGTTCAAGTACTTTGGGG	TCTTCTCCCATTGCCGTAC	1	0.000	0.146	0.46
10	BVWS02417	CCAGCAGTGACCAACAAGAA	CCTTCAGTCACCTTCAAGCA	3	0.043	0.651	0.81
11	BVWS02422	ACCTGATAACTCGTGCGCTT	AGCTCAATTTACAGGCGAC	6	0.083	0.802	0.90
12	BVWS02335	ATCCAAATGCTTGTCCGTC	TAACTAGCCGGCATCTGACC	4	0.087	0.597	0.75



**Fig. 1. SSR allelic profiling for genetic diversity analysis in watermelon with SSR BVWS02390. M:100 bp ladder; Lane 1–53 are different watermelon accessions**

(DWM 210 & DWM 41) to 1.00 (DWM 115 & DWM 173, DWM 178, DWM 13, DWM 66, DWM 122) indicating the existence of good amount of variability among the lines studied for their exploitation in the breeding programmes (Table 3). Heterozygosity for specific individual loci among accessions varied from 0.0 to 0.17, with the BVWS02306 marker having the highest value (0.17). Sheng *et al.* (2012) reported genetic similarity coefficients within the 95 Chinese cultigens of watermelon in the range of 0.37 to 0.99.

The highly divergent genotype, DWM 210, is an Indian collection from *Citrullus colocynthis* and showed high levels of dissimilarity from other accessions *i.e.*, DWM 41 (0.11) and DWM 27 (0.19) of *C. lanatus* var. *citroides* and Sugar baby (0.32) and DWM 142 (0.31) of *C. lanatus* var. *lanatus* genotypes. Further, this genotype showed moderate similarity to genotype, DWM 4 (0.71) of *C. lanatus* var. *citroides*. Gama *et al.* (2013) noted similar results for similarity coefficient (34 - 100 %) based on 34 alleles of ten microsatellite loci among 17 watermelon genotypes. The watermelon germplasm used in the present study comprised of wild germplasm from *C. lanatus* var. *citroides* and *Citrullus colocynthis* which is the reason for the wider range of similarity coefficient in this study and confirm the higher level of genetic diversity. Since SSR markers are the most consistent and are reliant on the genome of a given crop/species, they are a strong predictor of molecular diversity, and the classification obtained will be consistent even with the inclusion of newer markers, and there is less risk of a variation in this grouping pattern.

Fifty three watermelon accessions were broadly classified into two different clusters at 48% similarity (Fig. 2). Cluster I had 41 genotypes from cultivated types *Citrullus lanatus* var. *lanatus* genotypes. The genotype, sugar baby, was grouped with DWM 129 and it was separated from other members of cluster I B group at 65 per cent similarity which indicated that this variety was quite diverse from all other genotypes from *Citrullus lanatus* var. *lanatus*. These results are in agreement with Gama *et al.* (2013) who grouped 17 genotypes in two groups at 0.42 similarity with *Citrullus colocynthis*, positioned as an out group in the dendrogram. All wild watermelon genotypes from *Citrullus lanatus* var. *citroides* *i.e.*, DWM 35, DWM 36, DWM 22, DWM30, DWM4, DWM 34, DWM 43, DWM 32, DWM 40, DWM41, DWM 27 and DWM 210 of *Citrullus colocynthis* were grouped together in cluster II.

Thus, the genotypes were classified into two groups using SSR markers that correspond to a cultivated/wild scheme of botanical classification. However, the presence of subgroups in each group was recognized using molecular marker based classification. Thus, molecular markers may help select diverse parents from within the cultivated group of germplasms. All the germplasms in cluster II had white flesh colour and are bitter in taste. Thus, this study highlighted the importance of molecular markers in diversity studies in watermelon and their exploitation in genotypes future breeding programmes for the introgression of new genes from the related species to the cultivated ones. Further, this also useful in planning future collection and maintenance of the country's watermelon germplasm. Few of the wild accessions used in this study

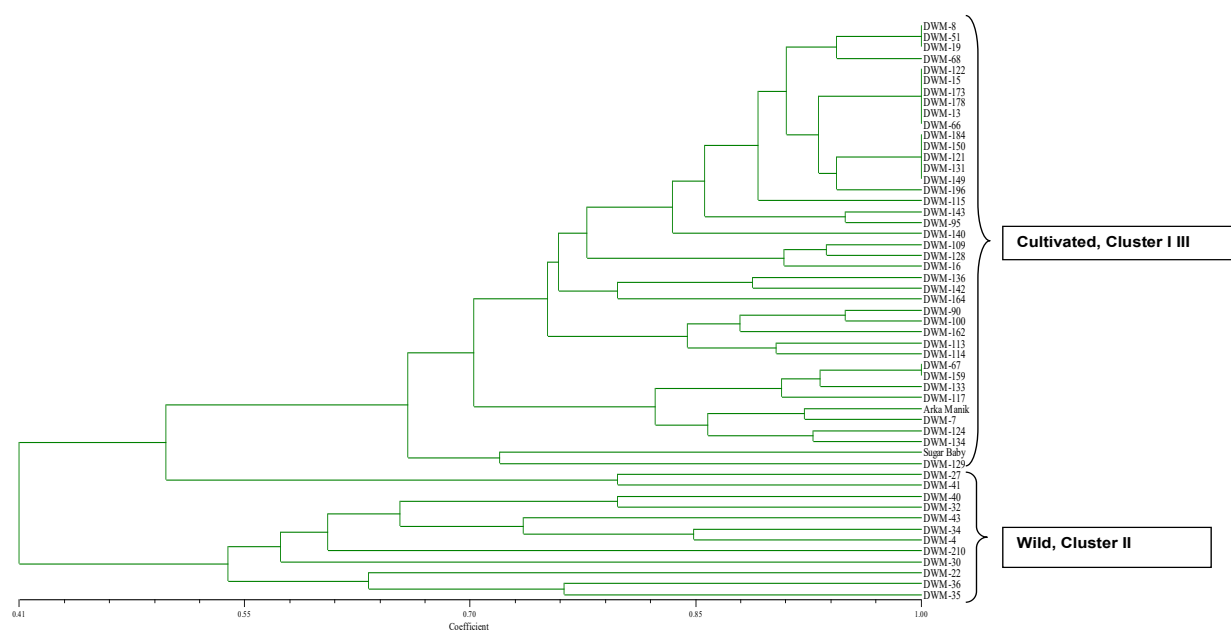


Fig. 2. Dendrogram showing the UPGMA cluster based on Jaccard's similarity coefficient of 53 watermelon accessions



recorded beneficial traits which may be useful to the plant breeders to develop cultivars with a broader genetic base that can better adapt to climate change.

## REFERENCES

- Gama, R.N., Santos, C.A.F., Dias, R.D.C.S. and Souza, F.F. 2013. Molecular characterization of watermelon cultivars using microsatellite markers. *Hortic Bras.*, **31**: 522-527. [\[Cross Ref\]](#)
- Indraja, G., Syed, S., Madhumathi, C., Priya, B.T. and Sekhar, M.R. 2021. Genetic variability studies for horticultural traits in muskmelon (*Cucumis melo* L.). *Electronic Journal of Plant Breeding*, **12**(1): 170-176. [\[Cross Ref\]](#)
- Liu, K. and Muse, S. V. 2005. Power Marker: Integrated analysis environment for genetic marker data. *Bioinformatics.*, **21**: 2128-2129. [\[Cross Ref\]](#)
- Mujaju, C., Sehic, J., Werlemark, G., Garkava-Gustavsson, L., Fatih, M. and Nybom, H. 2010. Genetic diversity in watermelon (*Citrullus lanatus*) landraces from Zimbabwe revealed by RAPD and SSR markers. *Hereditas*, **147**: 142-153. [\[Cross Ref\]](#)
- Murray, M.G. and Thompson, W.F. 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res.*, **8**: 4321-4325. [\[Cross Ref\]](#)
- Reddy, M.P., Sudheer, P.D., Mastan, S.G., Rahman, H., Carels, N. and Bahadur, B. 2013. Karyology and Genomics of *Jatropha*: Current status and future prospects. *Jatropha, challenges for a new energy crop*, Pp. 301-320. [\[Cross Ref\]](#)
- Robinson, R.W, Decker-Walters D.S 1997. Cucurbits: CABI, Wallingford, United Kingdom.
- Rohlf, F. J. 2000. NTSYS-pc: Numerical taxonomy and multivariate analysis system, version 2.1. Exeter Software, Setauket, N.Y.
- Senthilvadivu, G. 2018. Genetic divergence studies in muskmelon (*Cucumis melo* L.). *Electronic Journal of Plant Breeding*, **9**(3): 985-992. [\[Cross Ref\]](#)
- Sheng, Y., Luan, F., Zhang, F. and Davis, A.R. 2012. Genetic diversity within Chinese watermelon ecotypes compared with germplasm from other countries. *J. of the American Society for Hort. Sci.*, **137**: 144-151. [\[Cross Ref\]](#)
- Zhang, H., Wang, H., Guo, S., Ren, Y., Gong, G., Weng, Y., Xu, Y. 2012. Identification and validation of a core set of microsatellite markers for genetic diversity analysis in watermelon *Citrullus lanatus* Hunb. Matsum. & Nakai. *Euphytica*.**186**: 329-342. [\[Cross Ref\]](#)