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. *rehmi* 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; Indraja 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 watermelon is very rare and this is highly needed for the watermelon 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 watermelon 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 (l 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

| S. No. | Genotypes                  | Fruit shape | Rind colour | Flesh colour | Fruit stripes | Seed colour | Organoleptic taste |
|-------|----------------------------|-------------|-------------|--------------|---------------|-------------|-------------------|
| 1     | *Citrus lanatus var. citroides* | Oval        | Light green | Yellow       | Weak          | Red         | Low sweet         |
| 2     | *Citrus lanatus var. lanatus* | Flate globe | Light green | Yellow       | Diffused      | Red         | Low sweet         |
| 3     | *Citrus lanatus var. lanatus* | Round       | Light green | White        | Diffused      | White       | Medium            |
| 4     | *Citrus lanatus var. lanatus* | Round       | Medium green| Yellow       | Clearly defined| Brown       | Low sweet         |
| 5     | *Citrus lanatus var. lanatus* | Round       | Yellow      | Light red    | Weak          | Black       | Low sweet         |
| 6     | *Citrus lanatus var. lanatus* | Round       | dark green  | Reddish Pink | Weak          | Black       | Low sweet         |
| 7     | *Citrus lanatus var. lanatus* | Flate globe | Light green | Light red    | Clearly defined| Brown       | Medium sweet      |
| 8     | *Citrus lanatus var. citroides*| Round       | Medium green| Light red    | Clearly defined| Black       | Medium sweet      |
| 9     | *Citrus lanatus var. citroides*| Round       | Medium green| Yellow       | Diffused      | Brown       | Bitter            |
| 10    | *Citrus lanatus var. citroides*| Round       | dark green  | Orange       | Weak          | Brown       | Bitter            |
| 11    | *Citrus lanatus var. citroides*| Round       | Light green | White        | Diffused      | Brown       | Low sweet         |
| 12    | *Citrus lanatus var. citroides*| Round       | Light green | Light red    | Diffused      | Black       | Bitter            |
| No. | Variety | Shape | Color | Flesh Color | Texture | Taste | Sweetness |
|-----|---------|-------|-------|-------------|---------|-------|-----------|
| 13  | DWM 35  | Flate globe | Yellow | Yellow | Weak | Red | Low sweet |
| 14  | DWM 36  | Oval | Light green | Light green | Weak | Grey | Low sweet |
| 15  | DWM 40  | Flate globe | Yellow | White | Diffused | Black | Bitter |
| 16  | DWM 41  | Round | Yellow | Yellow | Diffused | Grey | Sour |
| 17  | DWM 43  | Round | dark green | Yellow | Weak | Brown | Sour |
| 18  | DWM 51  | Round | Light green | Yellow | Clearly defined | White | Sweet |
| 19  | DWM 66  | Round | Medium green | Reddish Pink | Weak | Black | Low sweet |
| 20  | DWM 67  | Round | Medium green | Reddish Pink | Weak | Black | Sweet |
| 21  | DWM 68  | Round | Medium green | White | Diffused | White | Bitter |
| 22  | DWM 90  | Round | Yellow | Reddish Pink | Absent | Grey | Medium sweet |
| 23  | DWM 95  | Round | Light green | Reddish Pink | Clearly defined | Brown | Low sweet |
| 24  | DWM 100 | Flate globe | Light green | White | Diffused | White | Low sweet |
| 25  | DWM 109 | Cylindrical | Medium green | Light red | Clearly defined | Black | Sweet |
| 26  | DWM 113 | Flate globe | Medium green | Dark Red | Clearly defined | Grey | Very sweet |
| 27  | DWM 114 | Round | Light green | White | Weak | White | Bitter |
| 28  | DWM 115 | Round | Medium green | Reddish Pink | Diffused | Red | Sweet |
| 29  | DWM 117 | Oval | Medium green | White | Weak | White | Sour |
| 30  | DWM 121 | Round | dark green | Reddish Pink | Weak | Brown | Sweet |
| 31  | DWM 122 | Round | Medium green | Light red | Weak | Brown | Medium sweet |
| 32  | DWM 124 | Round | dark green | Reddish Pink | Weak | White | Low sweet |
| 33  | DWM 128 | Round | Light green | Reddish Pink | Weak | Black | Sour |
| 34  | DWM 129 | Round | Medium green | White | Clearly defined | Brown | Sweet |
| 35  | DWM 131 | Round | Light green | White | Diffused | Brown | Low sweet |
| 36  | DWM 133 | Round | Medium green | White | Weak | White | Low sweet |
| 37  | DWM 134 | Flate globe | Light green | Reddish Pink | Clearly defined | Grey | Medium sweet |
| 38  | DWM 136 | Flate globe | Medium green | Reddish Pink | Clearly defined | Black | Low sweet |
| 39  | DWM 140 | Round | Medium green | White | Diffused | Black | Low sweet |
| 40  | DWM 142 | Round | dark green | Light red | Weak | Grey | Sweet |
| 41  | DWM 143 | Round | dark green | White | Weak | Black | Low sweet |
| 42  | DWM 149 | Oval | Light green | Orange | Diffused | Black | Low sweet |
| 43  | DWM 150 | Round | Light green | White | Diffused | White | Low sweet |
| 44  | DWM 159 | Round | Light green | Reddish Pink | Clearly defined | Black | Medium sweet |
| 45  | DWM 162 | Round | Light green | White | Diffused | Brown | Low sweet |
| 46  | DWM 164 | Round | Light green | White | Diffused | Brown | Bitter |
| 47  | DWM 173 | Round | Medium green | Reddish Pink | Clearly defined | Brown | Sweet |
| 48  | DWM 178 | Flate globe | dark green | Reddish Pink | Weak | Brown | Medium sweet |
| 49  | DWM 184 | Round | White | Light red | Weak | Black | Sweet |
| 50  | DWM 196 | Round | Medium green | White | Diffused | White | Bitter |
| 51  | DWM 210 | Round | Yellow | White | Weak | Black | Very Bitter |
| 52  | Sugar Baby Citrullus lanatus var. lanatus | Round | dark green | Dark red | Weak | Black | High sweet |
| 53  | Arka Manik Citrullus lanatus var. lanatus | 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.

### 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   | TCTTGTCGTAATCTCTGC                    | TTCAAGAAGAAATTTGGTCACCT             | 2                 | 0.154          | 0.370          | 0.65      |
| 2      | BVWS01116   | TGGCTTGACTTTTGGGAAACC                 | GAGCTCCCACACCTGAAATTTT              | 2                 | 0.063          | 0.256          | 0.53      |
| 3      | BVWS02421   | GAGGGGACTGATCGAGAA                    | ATCCCTGGTCATTGAGTT                  | 2                 | 0.087          | 0.201          | 0.56      |
| 4      | BVWS02306   | AGGTTGCTGTCCAGAAAGTC                  | TGCAGAAATACAAAAAGTAC                | 2                 | 0.173          | 0.037          | 0.55      |
| 5      | BVWS02380   | TTTGCGTGTTGTTTTGATG                   | CCAGAAGATATCCATCCC                  | 3                 | 0.025          | 0.073          | 0.70      |
| 6      | BVWS02225   | ATGGTATAATCAAGAATTG                   | GATTGTTGAGTAGAAGAAGTA               | 2                 | 0.108          | 0.201          | 0.65      |
| 7      | BVWS02398   | ATGGAAGTTTTTGGGACCTTGT               | TACCAGTTCCATTGAAGACAC               | 2                 | 0.000          | 0.171          | 0.53      |
| 8      | BVWS00547   | TTGGTGGAAATTTTGAGATCC                | TCATTAGGAGGCACTGCC                 | 2                 | 0.153          | 0.282          | 0.74      |
| 9      | BVWS02390   | TGGGCTTGACTTTTGGG                    | TCATTCCAGAGACTGTTCC                | 1                 | 0.000          | 0.146          | 0.46      |
| 10     | BVWS02417   | CCAGCAGTGGACAAACAGAAA                | CCTCAGTCACCCTTGAACCA               | 3                 | 0.043          | 0.651          | 0.81      |
| 11     | BVWS02422   | ACCTGATAATCTCCAGTCGTCCTT             | AGTCATATTTGAGAGGCAAGC              | 6                 | 0.083          | 0.802          | 0.90      |
| 12     | BVWS02335   | ATCCAAATGCTTTGTCGTC                  | TAACAGCCGCGATCTGACC                | 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 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 BVWS236 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
| EJPB | DWM-15 | DWM-184 | DWM-131 | DWM-27 | DWM-22 | DWM-129 | DWM-40 | DWM-68 | DWM-162 | Arka_Manik | DWM-7 | DWM-51 | 1 | 0.94 | 1 |
|------|--------|--------|--------|-------|-------|--------|-------|-------|-------|---------|-------|-------|---|------|---|
| EJPB | DWM-109 | 0.83 | 0.88 | 0.83 | 0.83 | 0.71 | 0.79 | 0.76 | 0.88 | 1 | |
| EJPB | DWM-34 | 0.35 | 0.31 | 0.35 | 0.35 | 0.41 | 0.33 | 0.23 | 0.31 | 0.36 | 0.71 | 0.31 | 0.42 | 0.31 | 1 |
| EJPB | DWM-43 | 0.52 | 0.48 | 0.52 | 0.52 | 0.58 | 0.5 | 0.39 | 0.48 | 0.55 | 0.5 | 0.48 | 0.61 | 0.48 | 0.71 | 1 |
| EJPB | DWM-128 | 0.78 | 0.82 | 0.78 | 0.78 | 0.67 | 0.74 | 0.81 | 0.82 | 0.94 | 0.43 | 0.82 | 0.7 | 0.82 | 0.32 | 0.5 | 0.82 | 0.82 | 0.94 | 0.78 | 0.65 | 0.38 | 1 |
| EJPB | DWM-7 | 0.71 | 0.75 | 0.71 | 0.67 | 0.67 | 0.75 | 0.75 | 0.15 | 0.75 | 0.63 | 0.75 | 1 | |
| EJPB | DWM-134 | 0.67 | 0.71 | 0.67 | 0.67 | 0.57 | 0.63 | 0.8 | 0.71 | 0.61 | 0.35 | 0.71 | 0.6 | 0.71 | 0.19 | 0.35 | 0.71 | 0.71 | 0.61 | 0.67 | 0.86 | 0.8 | 1 |
| EJPB | DWM-36 | 0.45 | 0.41 | 0.45 | 0.45 | 0.4 | 0.43 | 0.38 | 0.41 | 0.48 | 0.5 | 0.41 | 0.42 | 0.41 | 0.65 | 0.74 | 0.41 | 0.41 | 0.48 | 0.39 | 0.33 | 0.36 | 0.29 | 0.33 | 0.35 | 0.33 | 0.72 | 0.52 | 0.32 | 1 |
| EJPB | DWM-95 | 0.89 | 0.84 | 0.89 | 0.89 | 0.86 | 0.95 | 0.74 | 0.84 | 0.75 | 0.42 | 0.84 | 0.81 | 0.84 | 0.37 | 0.48 | 0.84 | 0.84 | 0.75 | 0.89 | 0.68 | 0.37 | 0.7 | 0.65 | 0.7 | 0.63 | 0.6 | 0.84 | 0.68 | 0.46 | 0.89 | 0.68 | 0.76 | 1 |
| EJPB | DWM-113 | 0.68 | 0.64 | 0.68 | 0.68 | 0.82 | 0.73 | 0.55 | 0.64 | 0.57 | 0.46 | 0.64 | 0.77 | 0.64 | 0.41 | 0.46 | 0.64 | 0.64 | 0.5 | 0.68 | 0.43 | 0.45 | 0.43 | 0.45 | 0.68 | 0.65 | 0.76 | 0.44 | 0.67 | 0.64 | 0.59 | 0.61 | 0.5 | 1 |
| EJPB | DWM-131 | 0.89 | 0.94 | 0.89 | 0.89 | 0.85 | 0.94 | 0.82 | 0.94 | 0.83 | 0.4 | 0.94 | 0.8 | 0.94 | 0.3 | 0.46 | 0.94 | 0.94 | 0.74 | 1 | 0.67 | 0.35 | 0.78 | 0.72 | 0.76 | 0.75 | 0.94 | 0.94 | 0.94 | 0.57 | 0.85 | 0.59 | 0.39 | 0.47 | 0.64 | 0.86 | 0.89 | 1 |
| EJPB | Sugar_Baby | 0.68 | 0.72 | 0.68 | 0.68 | 0.67 | 0.74 | 0.61 | 0.72 | 0.63 | 0.32 | 0.72 | 0.62 | 0.72 | 0.32 | 0.5 | 0.72 | 0.72 | 0.55 | 0.65 | 0.84 | 0.67 | 0.42 | 0.78 | 0.74 | 0.68 | 0.55 | 0.75 | 0.67 | 0.43 | 0.53 | 0.55 | 0.7 | 0.52 | 0.59 | 0.78 | 0.78 | 0.78 | 0.74 | 0.72 | 1 |
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.

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