Agrotechniques in Industrial Crops

Journal Homepage: https://atic.razi.ac.ir

Evaluation of Genetic Distance and Similarity among Native Genotypes of Seeded Watermelon (*Citrullus lanatus* var. *citroides*) in the Khorasan Region Using Microsatellite Markers

Saeedreza Vessal[*1], Leila Samiei[1], Hassan Porsa[1], Bahman Fazeli-Nasab[2,3]

[1]*Research Center for Plant Sciences, Ferdowsi University of Mashhad, Mashhad, Iran*
[2]*Department of Biotechnology and Plant Breeding, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad 9177948978, Iran*
[3]*Department of Agronomy and Plant Breeding, Agriculture Institute, Research Institute of Zabol, Zabol, Iran*

ARTICLE INFO

Original paper

Article history:
Received: 14 Mar 2023
Revised: 18 May 2023
Accepted: 22 Jun 2023

Keywords:
Edible seed as nut
Genetic diversity
PIC
SSR
UPGMA

ABSTRACT

Citrullus lanatus var. *citroides* is one of the Khorasan region's major rainfed and cash crops. It is typically grown on marginal lands and mostly dependent on rainfall. Microsatellite markers were therefore employed in this study to determine the genetic diversity among the genotypes from these important production zones in the provinces of Razavi and North Khorasan in order to be used in the future breeding programs of this crop. With the aid of 15 pairs of microsatellite primers (SSR), the genetic diversity among 17 genotypes of *C. Lanatus* var. *citroides* collected from various parts of Khorasan province along with one edible variety (Ed) was examined in this study. These markers allowed for the DNA fingerprinting of genotypes, in which 11 markers were polymorphic. In two cases, the polymerase chain reaction (PCR) products were monomorphic and shared by all genotypes, but in the other two, no detectable PCR product was observed. The range of the polymorphic information content (PIC) was 0.13 to 0.86. The genetic similarity among genotypes was determined to be between 15% and 55% using the Jaccard similarity matrix in the NTSYS program (Ver. 2.02). Three major groups were formed by clustering 17 genotypes using the UPGMA method and the Jaccard similarity coefficient. These findings demonstrated that the Ed variety was classified into a different group due to its significant genetic differences from other seeded watermelon genotypes. While verifying the significant amount of genotype diversity, the results of principal component analysis (PCA) of the data were highly in agreement with those of cluster analysis, making the similarities of genetic relationships very clear. This study could be significant in terms of the first steps toward identifying genetic diversity and maintaining genetic reserves in native genotypes of seeded watermelon for future breeding purposes.

DOI: 10.22126/ATIC.2023.9177.1100

1. Introduction

The cultivars of watermelon are classified into edible (dessert) and seeded (edible seed as nut) varieties. The annual plant-seeded watermelon also known as *C. Lanatus* and of the *citroides* variety belongs to the Cucurbitaceae family and is regarded as a semi-arid crop (Grumet *et al.*, 2021). In general, Khorasan, Gorgan, Khuzestan, Azerbaijan, Jiroft, Bandar Abbas, Sistan and Baluchistan, Kashan, and Fars are Iran's major watermelon production regions (Honari *et al.*, 2017).

In some parts of the country, seeded watermelon is one of the plants that is occasionally grown as a second crop following winter crops due to factors including a rather short time from planting to ripening, low production costs, increased profitability, and a suitable resistance to drought. In addition, farmers have shown increasing interest in this plant due to its capacity to be grown as a rain-fed crop when compared to other agricultural plants (Zargaran Khouzani, 2021). The size, shape, color, and pattern of the fruit skin, along with the color of the seed coat and the ripening period vary across *C. Lanatus* cultivars grown in various parts

* Corresponding author.
E-mail addresses: vessal@um.ac.ir

of the nation (Guo *et al.*, 2019), These traits demonstrate the great genetic diversity in this plant. In the three provinces of Khorasan, seeded watermelon is one of the cash crops for rain-fed conditions, however, the precise cultivated area and yield for this kind of watermelon are not available.

When investigating diversity, certain techniques and statistical models based on physical attributes, morphological data, or molecular characteristics of individuals are used to express differences or similarities of species, populations, or even individuals (Chu *et al.*, 2022; Pal *et al.*, 2020). Finding out the genetic diversity of plant materials is the first step in identifying and preserving genetic resources in gene banks and applying them in breeding programs (Janipour *et al.*, 2018). Identifying the best parental combinations for plant breeding, assessing the degree of differentiation for classifying samples, developing the main collection, and assessing the amount of genetic erosion in the gene bank collection can all be achieved with the help of the information obtained from genetic diversity and differentiation studies (Jomeh Ghasem Abadi *et al.*, 2019b; Pahlavan *et al.*, 2021; Vaez-Sarvari *et al.*, 2022). Compared to their wild relatives, modern agriculture has decreased the genetic diversity of cultivated plants. Therefore, it is essential to study genetic diversity in order to develop cultivars with a higher yield, better quality, and greater tolerance against abiotic and biotic stresses such as resistance to pests and diseases (Anter, 2023).

Numerous morphological, biochemical, and molecular data can be used to estimate genetic diversity. Molecular markers, on the other hand, are one of the most effective and reliable methods for assessing genetic variation (Asha *et al.*, 2023). Among these, DNA-based molecular markers providing information directly from each plant's genome, are not affected by environmental factors and can be valuable for assessing genetic variation (Vaez-Sarvari *et al.*, 2022; Vivodik *et al.*, 2022).

The co-dominance, reliability, and reproducibility of the results of simple sequence repeat (SSR), or microsatellites, make them one of the most practical and successful marker systems based on DNA. Variations in the number of repeats in the microsatellite sections of these markers can result in a high level of polymorphism, which is very helpful in various sorts of genetic studies. Additionally, microsatellites have frequently been utilized to distinguish between pure lines, cultivars, and wild species (Maragal *et al.*, 2023; Zhou *et al.*, 2023).

In recent years, numerous studies on watermelon (*C. Lanatus*) and other members of the Cucurbitaceae have been carried out in important global centers to assess the level of genotypic diversity in various aspects. In the majority of these studies, the presence of high diversity among the studied samples has been confirmed. It seems that cross-pollination and the activities of pollinating insects are the reasons for this high diversity (Hanif *et al.*, 2022).

In research (Joobeur *et al.*, 2006), 37 microsatellite primers have been identified and used to differentiate edible (*C. lanatus*) and seeded watermelon (*C. lanatus* var. *citroides*) accessions. Additionally, these primers' polymorphic information content (PIC) ranged from 0.39 to 0.97. In another study (Joobeur *et al.*, 2006; Mujaju *et al.*, 2010), both RAPD and SSR markers have been employed to study seeded watermelon diversity. In addition, the genetic diversity of seeded watermelon has been examined using 23 microsatellite primers that were evenly dispersed throughout the genome. With a polymorphism information content range of 0.45 to 0.82, these primers were able to amplify 2-7 alleles (Zhang *et al.*, 2012). In a study, 121 polymorphic fragments from 49 different types of seeded watermelon were discovered after genetic analysis using 30 microsatellite primers from melon and watermelon. Results from these primers have been addressed as being quite helpful for assessing diversity (Kwon *et al.*, 2010). Several cucurbits, such as watermelon, cucumber, gourds, and pumpkin, have been studied using microsatellite markers. (Adeyemo *et al.*, 2020; Gbotto *et al.*, 2022) The polymorphic information content (PIC) was between 0.18 to 0.64 at the moment (Mhlaba *et al.*, 2018).

There are not many studies specifically on seeded watermelon (*C. Lanatus* var *citroides*), even though there have been many studies on the genetic variety of many species of sweet or edible watermelon (*C. Lanatus*). Seeded watermelons (*C. Lanatus*) are mainly grown in the provinces of Khorasan, particularly Razavi and North Khorasan, due to appropriate climatic conditions. In some regions, such as the Jovin and Sabzevar areas, this product is regarded as one of the farmers' primary crops; nevertheless, little research has been done on the seeded watermelon genotypes of

this region so far. Microsatellite markers were therefore employed in this study to determine the genetic diversity among the genotypes from these important production zones in the provinces of Razavi and North Khorasan to be used in the future breeding programs of this crop.

2. Materials and methods

2.1. Plant materials

In the current study, the edible and commercial cultivar Crimson Sweet (control) was compared with 18 genotypes collected from various regions of the Razavi and North Khorasan provinces (Table 1), which were quite distinctive in terms of morphological characteristics. There was a minimum distance of 30 km between the selected genotypes. To get rid of the sticky residue on the seeds, they were first thoroughly rinsed. They were then planted in one-liter pots and grown in the greenhouse condition after being germinated in Petri dishes and a germinator at a temperature of 25±1°C for 48 hr.

2.2. DNA extraction

DNA extraction was performed according to Kwon et al.'s method (Kwon *et al.*, 2010) as a mixture (equal share of all repeats in the tissue used for extraction). In other words, rather than removing individual plants, each genotype's plant samples from all related replicates are ground together to determine the average level of variation within that genotype.

The extracted samples were electrophoresed in 1% agarose gel and TAE buffer to assess the DNA's quality, in terms of lack of protein contamination or DNA breakages. The concentration of all extracted samples used in the PCR reaction was set at 10 ng/l to repeat the results. Those DNA samples were chosen for the next steps, which had a sharp band and no streak. Additionally, the spectrophotometric method was used to determine the amount of DNA. The amount of DNA solution's absorption was checked at 260 and 280 wavelengths.

Table 1. Genotype name and their source used in this research.

Genotype name	Genotype source	Location
Ed (Crimson Sweet)	Commercial (OP)	Niagara Company, USA
C1	Native	Esfarayen (North Khorasan Province in Iran)
C2	Native	Esfarayen (North Khorasan Province in Iran)
C3	Native	Esfarayen (North Khorasan Province in Iran)
C4	Native	Esfarayen (North Khorasan Province in Iran)
C5	Native	Esfarayen (North Khorasan Province in Iran)
C6	Native	Esfarayen (North Khorasan Province in Iran)
C7	Native	Esfarayen (North Khorasan Province in Iran)
C8	Native	Esfarayen (North Khorasan Province in Iran)
C9	Native	Esfarayen (North Khorasan Province in Iran)
C10	Native	Esfarayen (North Khorasan Province in Iran)
C11	Native	Esfarayen (North Khorasan Province in Iran)
C12	Native	Esfarayen (North Khorasan Province in Iran)
C13	Native	Esfarayen (North Khorasan Province in Iran)
C14	Native	Esfarayen (North Khorasan Province in Iran)
M3-1	Native	Sabzevar (Razavi Khorasan province, Iran)
M7-1	Native	Sabzevar (Razavi Khorasan province, Iran)
M7-2	Native	Sabzevar (Razavi Khorasan province, Iran)

2.3. Primers and polymerase chain reactions

In this research, 11 microsatellite markers reported in different studies were used (Table 2) (Levi *et al.*, 2006; Mujaju *et al.*, 2010; Zhang *et al.*, 2012). Red master mix-Amplicon 2X, from the Amplicon Company, 7.5 microliters, 1 microliter of DNA sample with a concentration of 10 ng/microliter, 0.5 microliters of each of the primers (forward and reverse), and 5.6 microliters of nuclease-free water were used in the PCR reaction (Mujaju *et al.*, 2010). Initial annealing was done at 94 ºC for 4 min followed by 35 cycles of 94 ºC for 30 s, annealing at the appropriate temperature for each primer pair (Table 2) for 30 s, and extension at 72 ºC for 30 s. At the end of the cycles, the final expansion was done at 72 ºC for 7 minutes.

2.4. Electrophoresis of PCR products

A current of 80 mA was used to electrophorese PCR results in 2% agarose gel for two hours. Five microliters of polymerase chain reaction product and three microliters of 0.5% Green-weaver were added to each well.

2.5. Molecular data analysis

In the beginning, numbering was done from the highest and heaviest band to the lowest and lightest band to tally the bands associated with each indicator. The bands were given numbers, graded depending on the existence (one) or absence of a band (zero) and the resulting numerical data were then examined by NTSYS ver. 2.02 software. Based on zero and one data, there are various criteria for determining a genotype's genetic distance or similarity. Among the various criteria, Jaccard similarity coefficients (GSJ) (Jaccard, 1912), Nei and Li or Dice (GSNL) (Nei and Li, 1979) and simple matching (GSSM) are the most commonly used coefficients. The software created the similarity matrix for the experiment's data based on all three of the aforementioned coefficients. The most effective dendrogram was chosen and created using the cophenetic correlation coefficient. The best compatible similarity coefficient and clustering algorithm was found to be the Jaccard similarity coefficient and UPGMA algorithm based on calculations.

For each set of primers, the polymorphism information content or PIC diversity index was determined using the formula PIC= 1-$\sum$ Pi^2. The frequency of the i^{th} allele in a microsatellite locus, which is enlarged for n alleles, is represented by Pi in this formula.

3. Results

Eleven of the 15 pairs of examined microsatellite primers (SSR) showed appropriate polymorphism (Fig. 1). Out of 11, nine primers displayed polymorphism. In total, 65 bands were amplified in all genotypes, of which 63 were polymorphic. Both primes 5 (P5) and 8 (P8) failed to generate any bands. Only one monomorphic band was seen for primers P4 and P11 (Table 1). The primer number P3 was associated with the lowest amount of polymorphism (only 2 alleles), and the highest amount of polymorphism (13 alleles), with the highest PIC (0.86) associated with primer P6 (Table 2). In the current study, there was also a strong association between each primer's polymorphism index and the number of observed polymorphic bands (Fig. 2).

The Jaccard similarity matrix using the NTSYS ver. 2.02 software for all studied genotypes revealed that the C8 and C12 genotypes have the most similarities (55%), while the C9 and Ed (edible watermelon) genotypes share the least similarities (15%). The NTSYS ver. 2.02 software was used to classify genotypes into four separate groups based on the UPGMA method and Jaccard similarity coefficient (Fig. 3). The edible watermelon (Ed) was classified separately from the other genotypes in a single cluster. This genotype's average similarity to other genotypes was only 24%, in which the lowest correspondence was observed with the C9 genotype based on the Jaccard similarity matrix.

The second group, consisting of the two genotypes C6 and C7, is divided from the third branch by 33.5% similarity. The two genotypes C8 and C12, which shared the most genetic resemblance with the C13 genotype among all genotypes, were assigned to the third branch. In general, the third group showed two sub-branches, with two genotypes C10 and M7-1 in the first sub-branch and other genotypes broken up into various branches in the second. The C4 genotype with less similarity to the other genotypes in the fourth group was assigned to a different sub-branch (Fig. 3).

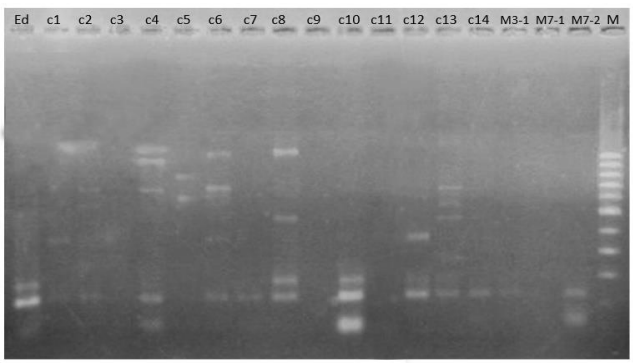

Figure 1. Genotype fingerprints using the P6 marker. C1 to C17, M3-1, M7-1 and M7-2 are seeded genotypes compared with Ed as an edible cultivar; M is the size marker (DNA-Leiter; 15628050).

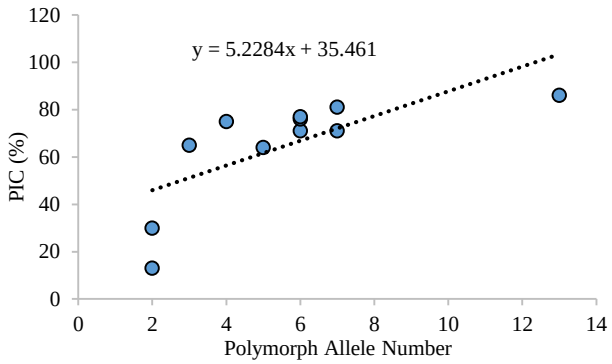

Figure 2. Relationship between polymorphic formation content (PIC) and the number of polymorphic bands produced by each SSR primer.

Table 2. The specifications of the primers used and other related information.

Primer name	Original name of primer	Motif	Tm	Produced alleles	Diversity alleles	PIC (%)
P1-F	MCPI-07-M13F	GGTTATGGCCATCTCTCTGC	56	5	5	64
P1-R	MCPI-07-R	GAGAGTGGGCGTAAGGTGAG				
P2-F	MCPI-32-M13F	AAGGCTGCAGAGACCATGAC	50	8	7	81
P2-R	MCPI-32-R	GAACGGGCAAGAAGTAGTAA				
P3-F	MCPI-28-M13F	AATGTTAAGCAGTAAGCACATG	56	2	2	13
P3-R	MCPI-28-R	GTTAAGTGGAAGAGGCCACA				
P6-F	MCPI-37-M13F	AATCTTCCCCATGCCAAAAC	51	13	13	86
P6-R	MCPI-37-R	CTTCCCTCCCAAACCTTCAG				
P7-F	MCPI-21-M13F	AAAGTTTTCATGCCAACGTATC	49	7	7	71
P7-R	MCPI-21-R	TCAGCCAATATGGTCAAATAGC				
P9-F	MCPI-14-M13F	TCAAATCCAACCAAATATTGC	47	6	6	76
P9-R	MCPI-14-R	GCAACCACTACAAAGGAAGAG				
P10-F	ASUW13-F	CTAGAGAAACCCCATC	47	6	6	71
P10-R	ASUW13-R	CTCCACTCACATACACAG				
P12-F	BVWS00209-F	TGCTTCAAAATCTATTCACAATTTGC	50	3	3	65
P12-R	BVWS00209-R	TTCTTGGTTTCGGGTTTCTTTACA				
P13-F	BVWS00228-F	GGAAGAGTGAGGTGATAAATCAATATGT	51	6	6	77
P13-R	BVWS00228-R	AATTGGCCCAAATATCCATATGAC				
P14-F	BVWS01734-F	AAAATTACATCTTAAATGCGCC	56	2	2	30
P14-R	BVWS01734-R	GGAACATTGACTTCAATCAGCA				
P15-F	BVWS00233-F	AAACCATGATTTTACAGGGGATCA	56	5	4	75
P15-R	BVWS00233-R	TTTCTGTCTTCTTTTGACCAATGC				

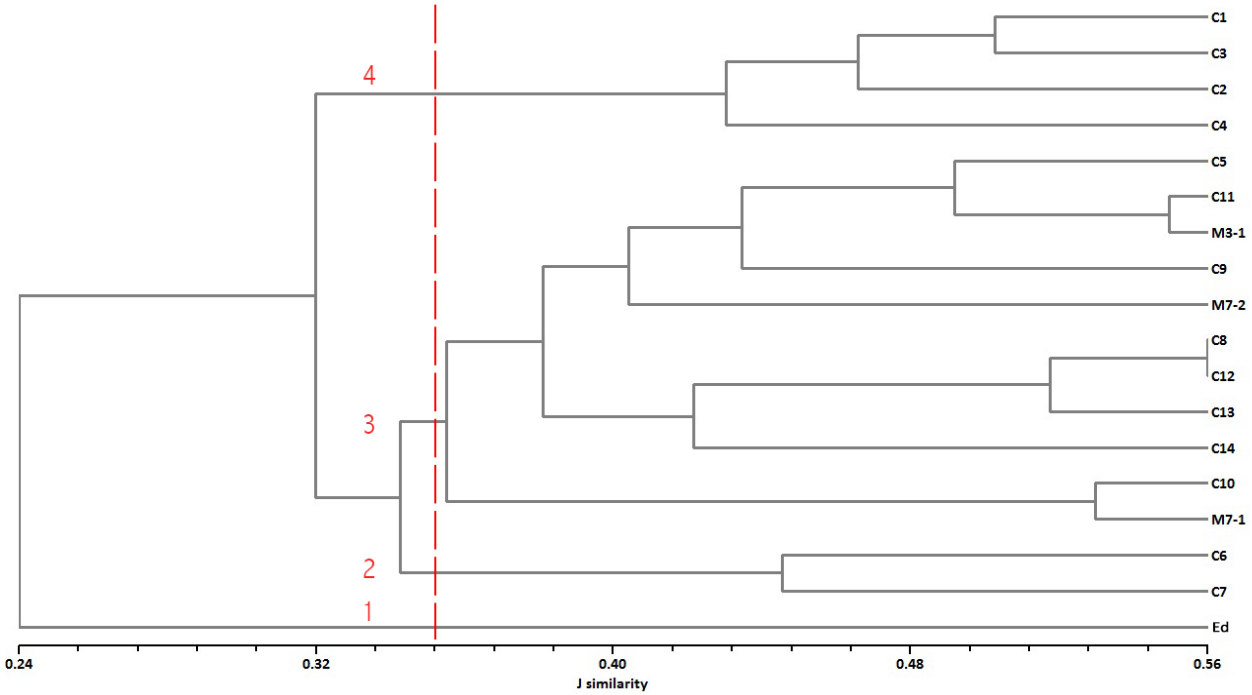

Figure 3. Cluster analysis of seeded watermelon genotypes and edible cultivar based on Jaccard similarity coefficient and UPGMA method.

Useful information on genetic diversity among genotypes can be found in the two-dimensional diagram of principle components analysis (PCA), which is typically applied in addition to cluster analysis (Fig. 4). It can be inferred that the primers used had a uniform and appropriate distribution at the genome level based on the cumulative percentage of the variance of the first two components, which justifies a small percentage of the total variance (Table 3). Fig. 4 shows dashed lines connecting every genotype at a single point; these lines are angled concerning one another; the greater the angle, the farther apart two genotypes are from one another; and the longer the line specific to a genotype, the greater the deviation from the average diversity among genotypes, which can be analyzed depending on the direction of this line

(Shaygan *et al*., 2021). Based on this, genotypes can be divided into three broad categories. Fig. 3 shows that the bigger group (left side), which also includes the numerous sub-branches in this category, has more variability in the direction of the line of each genotype than the other two groups.

There can be some subtle discrepancies between the results in Fig. 3 and 4. This is because, in PCA, primers with low values for determining the relationship or distance are ignored from the analysis and in turn, the outcomes of these two analyses can be different to some extent. In other words, before performing PCA, all primers evaluated between genotypes had the same value in the study of diversity. However, by using this method, the true value of each trait in determining the relationship or distance between genotypes will be determined, and the components that have Eigenvalues higher than one will be referred to as principal components.

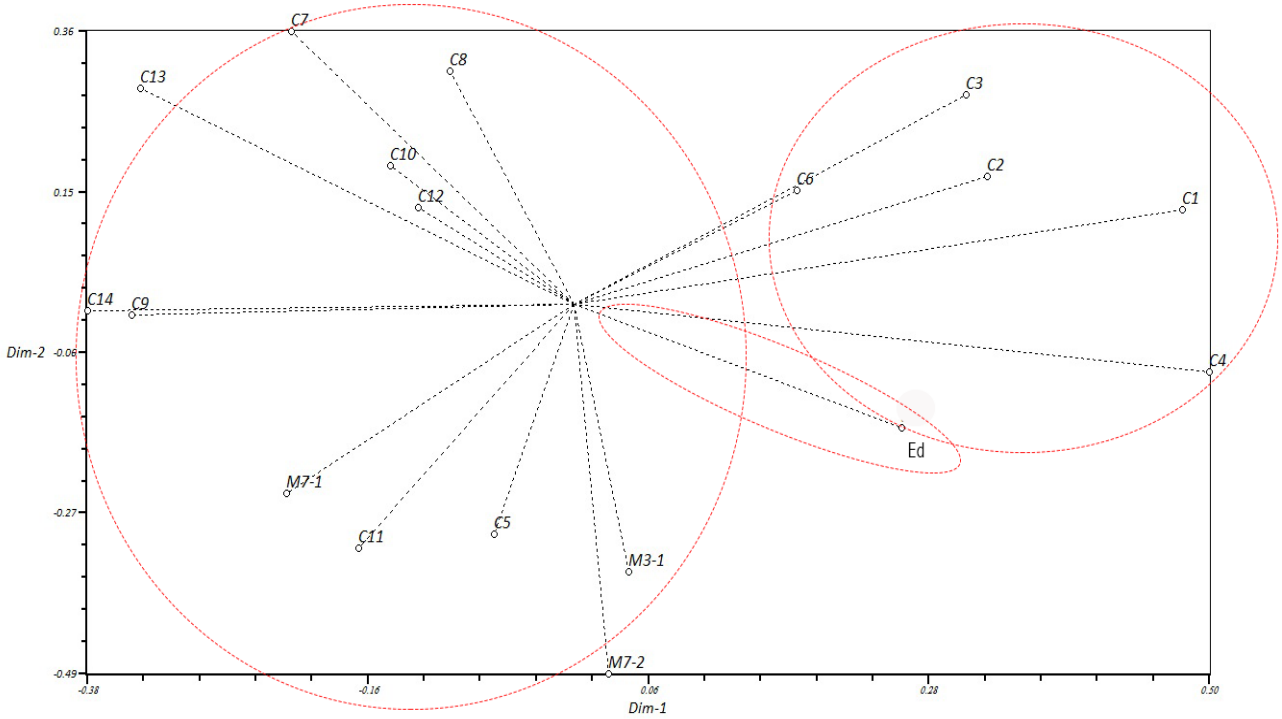

Figure 4. Genotypes are grouped using principal component analysis (PCA) using the first two components and the Jaccard similarity coefficient.

Table 3. The breakdown into main components (according to the Jaccard similarity matrix).

Components	Eigenvalues	Variance (%)		
		Expected	Observed	Cumulative
1	1.31	19.41	11.91	11.91
2	1.14	13.86	10.35	22.27
3	0.97	11.08	8.85	31.12

4. Discussion

For polymorphism and polymorphism index, many values have been recorded so far. The polymorphism index ranged from 0.075 to 0.37 in a study (Bahraminejad *et al*., 2012). The average polymorphism index in other studies is 0.51 (Bryan *et al*., 1997), from 0.07 to 0.8, with an average of 0.56, is the polymorphism index (Maccaferri *et al*., 2003), specifically for the motif dinucleotide values of 0.9 have been reported for the average polymorphism index (Prasad *et al*., 2000). Also, in other research, the average polymorphic index equals 0.86 (Mujaju *et al*., 2010), the polymorphic index ranges from 0.35 to 0.82 (Deng *et al*., 2015), Polymorphic index from 0.4 to 1 and with an average of 0.833 (Zhou *et al*., 2003) and the average polymorphic index equals 3. 0 (Bohn *et al*., 1999) is also reported. Also, in research (Mujaju *et al*., 2010), despite having a high polymorphism of 79%, one of the primers had the lowest polymorphism (13%) in the most recent analysis. The polymorphic index cannot have a fixed number and depends on variables such as the number of bands produced by each location, and even both the number of genotypes and primers have a positive correlation with the polymorphic index,

according to the findings of this study and all the results mentioned above (Janipour *et al.*, 2018; Jomeh Ghasem Abadi *et al.*, 2019a; Jomeh Ghasem Abadi *et al.*, 2019b; Pahlavan *et al.*, 2021). Whereas in a research (Roder *et al.*, 1995) with 18 genotypes and 15 primers, the average polymorphism index in wheat was 0.63; when the number of genotypes was reduced to 6, the average polymorphism index resulted in 0.54.

The average number of bands generated at each site is positively correlated with the degree of polymorphism (Jomeh Ghasem Abadi *et al.*, 2019a; Jomeh Ghasem Abadi *et al.*, 2019b), in the current study, there is also a strong association between each primer's polymorphism index and the number of polymorphic bands produced.

The average amount of polymorphic information in the current study was greater than 50% (PIC>0.5). Therefore, the applied markers can be regarded as effective markers to divide our seeded watermelon genotypes into distinctive categories (Kwon *et al.*, 2010). The outcomes confirm that these primers could be successfully applied to other similar studies in watermelon in the future.

It is clear that seeded watermelons have a great deal of variation and that this variability can be leveraged to enhance both quantitative and qualitative traits given the low level of similarity across the genotypes employed in this study. Although, it has been noted that the quantity of genotypes and primers has some influence on the similarity coefficient. For example, in research (Parashar *et al.*, 2014), the similarity coefficient in cumin using the ISSR marker was from 0.63 to 0.94 (Parashar *et al.*, 2014), but in other studies from 0.33 to 0.78 (Seidler-Łożykowska *et al.*, 2014), an average of 0.57 (Bohn *et al.*, 1999), an average of 0.44 (Maccaferri *et al.*, 2003), an average of 0.43 (Agrama and Tuinstra, 2003).

The considerable genetic variation among the genotypes demonstrates the genetic richness of these genotypes, demonstrating their importance for choosing superior genotypes to be used in crossbreeding blocks to build synthetic cultivars with the highest level of heterosis (Fallahi *et al.*, 2022; Gao *et al.*, 2022; Mahapatra *et al.*, 2022; Ouyang *et al.*, 2022; Sakran *et al.*, 2022; Suri *et al.*, 2022; van Hulten *et al.*, 2018). This study unequivocally demonstrates that SSRs can be a perfect tool for assessing the genetic diversity and relationships within the population. Although precise assessment of variation needs many markers and morphological features, valid genetic diversity assessments can still be made. The results, however, demonstrated that the set of chosen markers was successful in the identification and evaluation of the variation, as well as genetic relations among seeded watermelon genotypes. This is also critical for the management of genetic resources in terms of removing redundant accessions and also determining seed purity.

5. Conclusion

In summary, it can be inferred from the findings of molecular data that the genotypes of seeded watermelon (*C. lanatus* var. *citroides*) found in the provinces of Razavi and North Khorasan are highly diverse. Such genetic variability has been demonstrated in other research investigations on watermelon (*C. Lanatus*) genotypes carried out in various parts of the world (Zhou *et al.*, 2023; Amzeri *et al.*, 2021; Singh *et al.*, 2022). From the perspective of race, the high degree of genetic diversity in this crop, some of which can be attributed to its cross-pollination, can be viewed as a significant breeding advantage, because it allows appropriate selection for hybridization between genotypes with significant differences to produce heterosis in key agricultural traits.

Conflict of Interests

All authors declare no conflict of interest.

Ethics approval and consent to participate

No human or animals were used in the present research.

Consent for publications

All authors read and approved the final manuscript for publication.

Availability of data and material

All the data are embedded in the manuscript.

Authors' contributions

All authors had an equal role in study design, work, statistical analysis and manuscript writing.

Informed Consent

The authors declare not to use any patients in this research.

Funding/Support

This research was supported by the Ferdowsi University of Mashhad.

Acknowledgement

We appreciate Ferdowsi University of Mashhad for funding (ID: 2.27348) and facility supports to accomplish this research.

References

Adeyemo O., Adegoke S., Oladapo D., Amaghereonu C.C., Thomas A., Ebirikwem E.E., Adeyinka B., Amoda W. 2020. Transferability of SSR Markers used for Assessment of Genetic Relationship in Five Species/Genera in Cucurbitaceae. Egyptian Journal of Botany 60(1): 275-286. https://dx.doi.org/10.21608/ejbo.2019.13562.1324

Agrama H., Tuinstra M. 2003. Phylogenetic diversity and relationships among sorghum accessions using SSRs and RAPDs. African Journal of Biotechnology 2(10): 334-340. https://doi.org/10.5897/AJB2003.000-1069

Amzeri A., Badami K., Gita P., Syah M.A., Daryono B.S. 2021. Phenotypic and genetic diversity of watermelon (*Citrullus lanatus*) in East Java, Indonesia. Biodiversitas Journal of Biological Diversity 22(11). https://doi.org/10.13057/biodiv/d221161

Anter A.S. 2023. Induced Mutation to Enhance Plant Biodiversity and Genetic Resources for Intensification of Crop Production to Mitigate Climatic Changes. IntechOpen. https://doi.org/10.5772/intechopen.108117

Asha K.I., Aswani S.A., Radhika N.K., Krishnan B.P. 2023. Genetic variability and diversity analysis of Chinese potato (*Solenostemon rotundifolius* (poir.) JK Morton) germplasm using morphological and molecular markers. South African Journal of Botany 155: 171-177. https://doi.org/10.1016/j.sajb.2023.02.014

Bahraminejad A., Mohammadi-Nejad G., Abdul Kadir K., Bin Yusop M.R., Samia M.A. 2012. Molecular diversity of Cumin (*Cuminum cyminum* L.) using RAPD markers. Australian Journal of Crop Science 6(2): 194-199. http://www.cropj.com/bahramnejad_6_2_2012_194_199.pdf

Bohn M., Utz H.F., Melchinger A.E. 1999. Genetic similarities among winter wheat cultivars determined on the basis of RFLPs, AFLPs, and SSRs and their use for predicting progeny variance. Crop Science 39(1): 228-237. https://doi.org/10.2135/cropsci1999.0011183X003900010035x

Bryan G., Collins A., Stephenson P., Orry A., Smith J., Gale M. 1997. Isolation and characterisation of microsatellites from hexaploid bread wheat. Theoretical and Applied Genetics 94(5): 557-563. https://doi.org/10.1007/s001220050451

Chu S., Wang S., Zhang R., Yin M., Yang X., Shi Q. 2022. Integrative analysis of transcriptomic and metabolomic profiles reveals new insights into the molecular foundation of fruit quality formation in *Citrullus lanatus* (Thunb.) Matsum. & Nakai. Food Quality and Safety 6: fyac015. https://doi.org/10.1093/fqsafe/fyac015

Deng S.Y., Wang X.R., Zhu P.L., Wen Q., Yang C.X. 2015. Development of polymorphic microsatellite markers in the medicinal plant *Gardenia jasminoides* (Rubiaceae). Biochemical Systematics and Ecology 58: 149-155. https://doi.org/10.1016/j.bse.2014.11.009

Fallahi F., Abdossi V., Bagheri M., Ghanbari Jahromi M., Mozafari H. 2022. Genetic diversity analysis of Eggplant Germplasm from Iran: assessments by morphological and SSR markers. Mol Biol Rep 49(12): 11705-11714. https://doi.org/10.1007/s11033-022-07768-5

Gao Z., Yun L., Li Z., Liu Q., Zhang C., Ma Y., Shi F. 2022. Hybrid purity identification using EST-SSR markers and heterosis analysis of quantitative traits of Russian wildrye. PeerJ 10: e14442. https://doi.org/10.7717/peerj.14442

Gbotto A.A., Yao N.K., Kitavi M., Osama S.K., Habimana R., Koffi K.K., Bi I.A.Z. 2022. Genetic characterization of oleaginous bottle gourd (*Lagenaria siceraria*) germplasm from Côte d'Ivoire using agromorphological and molecular markers. Plant Genetic Resources 2022: 1-12. https://doi.org/10.1017/S1479262122000247

Grumet R., McCreight J.D., McGregor C., Weng Y., Mazourek M., Reitsma K., Labate J., Davis A., Fei Z. 2021. Genetic Resources and Vulnerabilities of Major Cucurbit Crops. Genes (Basel) 12(8): 1222. https://doi.org/10.3390/genes12081222

Guo S., Zhao S., Sun H., Wang X., Wu S., Lin T., Ren Y., Gao L., Deng Y., Zhang J., Lu X. 2019. Resequencing of 414 cultivated and wild watermelon accessions identifies selection for fruit quality traits. Nature genetics 51(11): 1616-1623. https://doi.org/10.1038/s41588-019-0518-4

Hanif R., Yaqoob M., Ayoub L., Irshad S., Siraj M., Wani F.F., Bhat S., Farook U.B., Sheikh M.A., Rasool J., Mathumitaa P. 2022. Role of insect pollinators in pollination of cucumber. Role of insect pollinators in pollination of cucumber. The Pharma Innovation Journal 11(4S): 1348-1354. https://www.thepharmajournal.com/archives/2022/vol11issue4S/PartQ/S-11-4-34-737.pdf

Honari F., Vessal S., Babaean Jelodar N.A. 2017. Assessment of Genetic Diversity for some Candidate Edible Seed Watermelon Genotypes using SSR Markers and Morphological Traits. Journal of Crop Breeding 9(22): 14-22. http://dx.doi.org/10.29252/jcb.9.22.14

Jaccard P. 1912. The distribution of the flora in the alpine zone. New phytologist 11(2): 37-50. https://doi.org/10.1111/j.1469-8137.1912.tb05611.x

Janipour L., Fahmideh L., Fazeli-Nasab B. 2018. Genetic evaluation of different population of Cumin (*Cuminum cyminum* L.) using DNA molecular markers. Journal of Cellular and Molecular Researches 31(1): 16-32. (In Farsi). https://dorl.net/dor/20.1001.1.23832738.1397.31.1.1.7

Jomeh Ghasem Abadi Z., Fakheri B., Fazeli-Nasab B. 2019a. Bioinformatics Analysis and characterization of RuBisCO's large subunit in some lettuce genotypes. Agricultural Biotechnology Journal 11(3): 1-18. (In Farsi). https://doi.org/10.22103/jab.2019.14215.1140

Jomeh Ghasem Abadi Z., Fakheri B., Fazeli-Nasab B. 2019b. Study of the Molecular Diversity of Internal Transcribed Spacer Region (ITS1.4) in Some Lettuce Genotypes. [Research].

Journal of Crop Breeding 11(29): 29-39. (In Farsi). https://doi.org/10.29252/jcb.11.29.29

Joobeur T., Gusmini G., Zhang X., Levi A., Xu Y., Wehner T., Oliver M., Dean R. 2006. Construction of a watermelon BAC library and identification of SSRs anchored to melon or *Arabidopsis* genomes. Theoretical and Applied Genetics 112: 1553-1562. https://doi.org/10.1007/s00122-006-0258-6

Kwon Y.S., Oh Y.H., Yi S.I., Kim H.Y., An J.M., Yang S.G., Ok S.H., Shin J.S. 2010. Informative SSR markers for commercial variety discrimination in watermelon (*Citrullus lanatus*). Genes & Genomics 32: 115-122. https://doi.org/10.1007/s13258-008-0674-x

Levi A., Thomas C., Trebitsh T., Salman A., King J., Karalius J., Newman M., Reddy O., Xu Y., Zhang X. 2006. An extended linkage map for watermelon based on SRAP, AFLP, SSR, ISSR, and RAPD markers. Journal of the American Society for Horticultural Science 131(3): 393-402. https://doi.org/10.21273/JASHS.131.3.393

Maccaferri M., Sanguineti M., Donini P., Tuberosa R. 2003. Microsatellite analysis reveals a progressive widening of the genetic basis in the elite durum wheat germplasm. Theoretical and Applied Genetics 107: 783-797. https://doi.org/10.1007/s00122-003-1319-8

Mahapatra S., Sureja A.K., Behera T.K., Verma M. 2022. Assessment of genetic diversity of ninety-one bottle gourd [*Lagenaria siceraria* (Mol.) Standl.] genotypes from fourteen different agro-climatic zones of India using agro-morphological traits and SSR markers. Molecular Biology Reports 49(7): 6367-6383. https://doi.org/10.1007/s11033-022-07446-6

Maragal S., Rao E.S., Reddy D.L. 2023. Genetic mapping and meta-analysis identifies several candidate genes for watermelon (*Citrullus lanatus*) fruit quality traits. Scientia Horticulturae 308: 111545. https://doi.org/10.1016/j.scienta.2022.111545

Mhlaba Z.B., Amelework B., Shimelis H.A., Modi A. T., Mashilo J. 2018. Genetic interrelationship among tepary bean (*Phaseolus acutifolius* A. Gray) genotypes revealed through SSR markers. Australian Journal of Crop Science 12(10): 1587-1595. https://doi.org/10.21475/ajcs.18.12.10.p1129

Mujaju C., Sehic J., Werlemark G., Garkava-Gustavsson L., Fatih M., Nybom H. 2010. Genetic diversity in watermelon (*Citrullus lanatus*) landraces from Zimbabwe revealed by RAPD and SSR markers. Hereditas 147(4): 142-153. https://doi.org/10.1111/j.1601-5223.2010.02165.x

Nei M., Li W.H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences 76(10): 5269-5273. https://doi.org/10.1073/pnas.76.10.5269

Ouyang Y., Li X., Zhang Q. 2022. Understanding the genetic and molecular constitutions of heterosis for developing hybrid rice. J Genet Genomics 49(5): 385-393. https://doi.org/10.1016/j.jgg.2022.02.022

Pahlavan A., Solouki M., Fakheri B., Fazeli-Nasab B. 2021. Using Morphological and Phytochemical Traits and ITS (1, 4) and rbcl DNA Barcodes in the Assessment of Different *Malva sylvestris* L. Genotypes. Journal of Medicinal plants and By-product 10(1): 19-35. (In Farsi). https://doi.org/10.22092/jmpb.2020.343520.1232

Pal S., Revadi M., Thontadarya R., Reddy D.L., Varalakshmi B., Pandey C.D., Rao E.S. 2020. Understanding genetic diversity, population structure and development of a core collection of Indian accessions of watermelon (*Citrullus lanatus* (Thunb.) Matsum. and Nakai). Plant Genetic Resources 18(5): 359-368. https://doi.org/10.1017/S1479262120000386

Parashar M., Jakhar M., Malik C. 2014. A review on biotechnology, genetic diversity in cumin (*Cuminum cyminum*). International Journal of Life Science and Pharma Research 4(4): L17-L34.

Prasad M., Varshney R.K., Roy J., Balyan H., Gupta P. 2000. The use of microsatellites for detecting DNA polymorphism, genotype identification and genetic diversity in wheat. Theoretical and Applied Genetics 100(3-4): 584. https://doi.org/10.1007/s001220050077

Roder M.S., Plaschke J., König S.U., Börner A., Sorrells M.E., Tanksley S.D., Ganal M.W. 1995. Abundance, variability and chromosomal location of microsatellites in wheat. Molecular and General Genetics MGG 246(3): 327-333. https://doi.org/10.1007/BF00288605

Sakran R.M., Ghazy M.I., Rehan M., Alsohim A.S., Mansour E. 2022. Molecular Genetic Diversity and Combining Ability for Some Physiological and Agronomic Traits in Rice under Well-Watered and Water-Deficit Conditions. Plants (Basel) 11(5). https://doi.org/10.3390/plants11050702

Seidler-Łożykowska K., Kuczyńska A., Mikołajczyk K., Nowakowska J., Bocianowski J. 2014. Estimation of genetic distance among genotypes of caraway (*Carum carvi* L.) using RAPD-PCR. Acta Scientiarum. Agronomy 36(2): 183-188. https://doi.org/10.4025/actasciagron.v36i2.18197

Shaygan N., Etminan A., Majidi Hervan I., Azizinezhad R., Mohammadi R. 2021. The study of genetic diversity in a minicore collection of durum wheat genotypes using agro-morphological traits and molecular markers. Cereal Research Communications 49: 141-147. https://doi.org/10.1007/s42976-020-00073-6

Singh U., Kumar S., Yadav K. 2022. Genetic variability and correlation studies in various genotypes of watermelon [Citrullus lanatus (Thunb.) Mansf.]. Journal of Agriculture and Ecology 13: 65-78. https://doi.org/10.53911/JAE.2022.13107

Suri G.K., Braich S., Noy D.M., Rosewarne G.M., Cogan N.O.I., Kaur S. 2022. Advances in lentil production through heterosis: Evaluating generations and breeding systems. PLoS One 17(2): e0262857. https://doi.org/10.1371/journal.pone.0262857

Vaez-Sarvari H., Emamjomeh A., Fazeli-Nasab B. 2022. Evaluation of genetic diversity of *Cantaloupe* landraces based on the internal transcriptional spacer regions (ITS1, 4). International Journal of Vegetable Science: 1-13. https://doi.org/10.1080/19315260.2022.2051664

van Hulten M.H.A., Paulo M.J., Kruijer W., Blankestijn-De Vries H., Kemperman B., Becker F.F.M., Yang J., Lauss K., Stam M.E., van Eeuwijk F.A., Keurentjes J.J.B. 2018. Assessment of heterosis in two Arabidopsis thaliana common-reference mapping populations. PLoS One 13(10): e0205564. https://doi.org/10.1371/journal.pone.0205564

Vivodik M., Balazova Z., Chnapek M., Hromadova Z., Mikolasova L., Galova Z. 2022. Molecular characterization and genetic diversity studie of soybean (*Glycine max* l.) Cultivars using

rapd markers. Journal of microbiology, biotechnology and food sciences 12: e9219-e9219. https://doi.org/10.55251/jmbfs.9219

Zargaran Khouzani M.R. 2021. Utilization of camelthorn (Alhagi maurorum) to produce organic rain-fed watermelon to promote the sustainable development of the agricultural sector in arid and semi-arid regions. Central Asian Journal of Environmental Science and Technology Innovation 2(4): 156-163. https://www.cas-press.com/article_138802.html

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* Thunb. Matsum. & Nakai. Euphytica 186: 329-342. https://doi.org/10.1007/s10681-011-0574-z

Zhou C., Wang P., Zeng Q., Zeng R., Hu W., Sun L., Liu S., Luan F., Zhu Q. 2023. Comparative chloroplast genome analysis of seven extant Citrullus species insight into genetic variation, phylogenetic relationships, and selective pressure. Scientific Reports 13(1): 6779. https://doi.org/10.1038/s41598-023-34046-6

Zhou H.F., Xie Z.W., Ge S. 2003. Microsatellite analysis of genetic diversity and population genetic structure of a wild rice (*Oryza rufipogon* Griff.) in China. Theoretical and Applied Genetics 107(2): 332-339. https://doi.org/10.1007/s00122-003-1251-y

HOW TO CITE THIS ARTICLE

Vessal S, Samiei L., Porsa H., Fazeli-Nasab B. 2023. Evaluation of Genetic Distance and Similarity among Native Genotypes of Seeded Watermelon (*Citrullus lanatus* var. *citroides*) in the Khorasan Region Using Microsatellite Markers. *Agrotechniques in Industrial Crops* 3(2): 74-83. 10.22126/ATIC.2023.9177.1100