Genetic diversity study of melon native landraces using ISSR markers in Kurdistan region of Iraq

Abstract

Considering the ancient cultivation of various numbers of melons, it seems that there is a valuable and rich gene pool of melon in Kifri city of Kurdistan region of Iraq. In this research, the genetic diversity of 15 indigenous melons of Kifri and 7 non-native melons was analyzed using inter simple sequence repeat (ISSR) markers. A total of 6 primers were used to amplify parts of the genomic DNA of the samples. Genomic DNA was extracted using Salting out method, and polyacrylamide gel was used to separate DNA fragments. In this study, 65 bands were scored and 34 bands had polymorphisms. To evaluate the genetic similarity between the samples, cluster analysis was used using the Jaccard similarity coefficient with the UPGMA method. The average genetic distance between the samples using the Jaccard similarity coefficient was 0.74, and the average polymorphic information content (PIC) was 0.78. The primer (AC)8 G had the highest PIC (0.93). Cluster analysis indicated wide range of diversity across the genotypes used. These genotypes have high diversity which could be used in breeding programs.

Key words: Melon, Genetic diversity, ISSR marker, Kurdistan region, Iraq

Introduction

"A melon is any of various plants of the family Cucurbitaceous with sweet, edible, and fleshy fruit. This fruit has many different genotypes all over the world (Raghami *et al.*, 2014). Awareness of the degree of genetic diversity of the genetic resources of plant species is of particular importance to reduce the amount of genetic resources samples in the gene bank and to check the purity of seeds in plant breeding. This issue is important for plants of the summer family, which are exposed to severe genetic erosion. Considering that in recent years, the area under the cultivation of non-indigenous melons is expanding, the genetic erosion of natives has increased. There are different methods to estimate genetic diversity in plant species, and the use

of molecular markers is one of the most important and powerful tools in this field. DNA (Inter Simple sequence Repeats (ISSR)) predominantly provides dominant markers. This method of investigation the relationship has been highest levels of polymorphism and considered simple, relatively cheap, and easy to conduct" (Xu, 2010). "The Inter Simple-sequence repeats (ISSR) technique markers is available among different types of molecular marker techniques (Williams *et al.*, 1990) and has been the most applicator be the researchers due to its simplicity, cost effective, and fast and easy to perform" (Dos Santos *et al.*, 1994; Williams et al., 1990). So far, some studies have been done through molecular markers to investigate polymorphisms in the genetic reserves of Iraqi native melon populations in order to investigate the genetic relationship. Al- juboori *et al.* (2019) investigated "the genetic diversity of 6 melon genotypes in Iraq. The results of this study showed that there is a high genetic diversity between these 6 genotypes". In another study, Rafat Aziz et al. (2020) studied "Genetic diversity and structure analysis of melon (Cucumis melo L.) genotypes using namely universal rice primer (URP), inter retrotransposon amplified polymorphism (IRAP), sequence-related amplified polymorphism (SRAP), and conserved DNA derived polymorphism (CDDP) markers". Moayedi Nejad *et al.* (2010) investigated "the kinship relationships of 43 Iranian cantaloupe cultivars using the ISSR marker and showed that the ISSR marker is a suitable technique for studying the genetic diversity of cantaloupe. The results of this study indicated a high level of variability in Iraqi melon germplasm, which must be preserved and included in improvement programs for this ancient crop. There are many native varieties of melons in Kifri city, Kurdistan region of Iraq, and economically, this product is very important in people's livelihood. So far, the genetic diversity of native melons of this city has not been investigated". Therefore, the purpose of the current study was to investigate the genetic diversity of several indigenous melon cultivars using ISSR markers.

Materials and Methods

Plant materials

The seeds of 15 genotypes from different native melons of Kifri city and seven genotypes from non-native melons were collected from the fields of this city and then identified.

DNA extraction

In this study, 3 to 5 seeds from each native mass were planted in each pot at a temperature of 25 degrees Celsius and with a day length of 16 hours. 0.2 grams of leaves from the plants at the 2-leaf stage were weighed as a mixture of 22 plants and used for extraction of DNA. Extraction of genomic DNA of each genotypic mass was done using the method of Delaporta et al. (1983).

PCR amplification and electrophoresis

"Six ISSR primers were used to evaluate the genetic diversity of samples" (Stepansky *et al.* 1999). "Primer sequences and their annealing temperatures are presented in Table 1. PCR reactions were performed in 25µL volumes containing 40 ng template DNA, 50 µM of each dNTPs , 1X PCR buffer (100 mM Tris-HCL, 50 mM KCL, 0.01% gelatin, and 0.25% tween 20), 2.5 µM of each primer pair, 1 unit/µl *Taq* DNA polymerase and MgCI2 (0.9–1.5 mM)" as already reported by Rafat Aziz *et al.* (2020). Amplification reactions were carried out in an Eppendorf Thermo Cycler (Eppendorf, Germany) under the following conditions: initial denaturation step at 94 ºC for 7 min, followed by 37 cycles of denaturation at 94 °C for 60 s, annealing (in accordance with Table 1) for 60 s, extension at 72 °C for 120 s and a final extension step at 72 °C for 7 min. The amplification products were separated by gel electrophoresis on a 1.5 % agarose gel in 1× TAE buffer for about 1.5 hours and stained with 0.025% ethidium bromide. After electrophoresis, the PCR products were envisioned under UV light by a gel documentation system.

Data analysis

"The PCR-generated products were scored as absence (0) or presence (1) of bands. POPGENE software version 1.31 (Yeh et al. 1997) was used to calculate the population's genetic structure (including observed number of alleles per locus (Na), effective number of alleles (Ne), gene diversity (H), and Shannon information index (I))". Cluster analysis based on the Unweighted Pair Group Method with Arithmetic Mean(UPGMA) was analyzed NTSYS pc version 2.02 software (Roelfs et al. 1988). To determine markers performance indexes, the following parameters were calculated: Polymorphism Information Content (PIC) based on the formula PIC

= 1-$\sum_{i=1}^{n}$ Pi (Lynch and Walsh 1998), Marker Index (MI) using the formula MI = PIC × N × β (Powel et al. 1996), Effective Multiplex Ratio (EMR) based on the formula EMR = N × β (Powel et al. 1996) and Resolving Power (RP) using the formula RP = ∑ Ib, where Ib = 1 – (2 x |0.5 – p_i|)(Anderson et al. 1993). In this formula, N is the total number of bands for each pair of primers, β is the polymorphic percentage and p_i is the ratio of accessions comprising the *i*th band.

Table 1: Characteristics of the ISSR loci used in the study

No	Sequence (5'-3')	Annealing temperature (˚C)
P1	TCTCTCTCTCTCTCTCC	52.8
P2	AGAGAGAGAGAGAGAGT	50.8
P3	GAGAGAGAGAGAGAGAYG	54.8
P4	ACACACACACACACACG	52.8
P5	ATGATGATGATGATGATG	52.7
P6	AGTCGTAGTACACACACACACAC	59.4

Results and discussion

The primers used for melon germplasm analysis were able to produce a total of 1342 bands, which showed the number of 1012 polymorphism bands. The results obtained for the primers used in this study are shown in Table 2. The highest amount of PIC and Heterozygosity were related to P3 and P1 Primer, respectively. The highest and lowest percentage of polymorphism was related to P2 and P3 primers, respectively.

Table2: Number of observed alleles, polymorphic bands. Polymorphic%, effective alleles, polymorphic information content (PIC), observed heterozygosity and Shannon index in twenty-two melon landraces

Primer name	Number of observed alleles	polymorphic bands	Polymorphic %	Effective alleles	PIC	Heterozygosity (*Ha*)	Shannon index (*I*)
P1	6	4	66.67%	1.57	0.81	0.31	0.43
P2	11	11	100%	1.49	0.88	0.30	0.46
P3	15	9	60%	1.45	0.93	0.25	0.36
P4	14	12	85.71%	1.46	0.88	0.26	0.37
P5	7	5	71.43%	1.24	0.86	0.18	0.30
P6	8	5	62.5%	1.42	0.85	0.24	0.35
Mean	10.12	7.67	74.39%	1.44	0.87	0.25	0.38

The genetic distance of the studied genotypes varied from 0.56 to 0.96 using the Jaccard similarity coefficient. The high range of genetic distance of the samples in the present study indicates the high diversity of melon genotypes native to the region. Dendrogram obtained from cluster analysis by UPGMA method based on Jaccard similarity coefficient is shown in Fig 1. The greatest genetic distance was between the genotypes Lined Elongated and Ghandak milk Genotypes (both of the indigenous masses) and the least genetic distance was between the two genotypes Safidak Deh Kohaneh and Safidak Edimi. In this study, Khatouni and Kifir masses had a very small genetic distance, so it is possible that these are the same masses or that the genetic locations used in this study was not able to identify the small differences between them.

The results of study showed that primers P2, P3 and P4 showed the highest polymorphism index and could identify the genetic distance of individuals better than other primers, so these primers can be used for the analysis of melon germplasms in future researches. By comparing the Shannon index among the six locus, the highest Shannon value was related to the P2 loci, which seems logical considering a large number of alleles in this loci. On the one hand, the high polymorphism value of native masses shows the efficiency of using the ISSR marker in the study of melon germplasm, and on the other hand, it shows the genetic diversity of this plant in this region. Although an accurate assessment of genetic diversity using a large number of markers is verified with morphological characteristics, however, the results of the present study showed that the primers used in this research were useful for purposes such as differentiation, identification, and evaluation of the diversity of melon populations. Crossing of masses and groups that are genetically more distant can create masses with more diversity. The results of this research can be considered in the direction of reform planning in order to create more diverse figures for different attributes of fashion. Also, these studies can be useful in order to remove duplicate samples in the gene bank. Therefore, it is suggested to identify the groups more markers will be evaluated along with their morphological characteristics and kinship relationship.

Conclusion

In the current study, the genetic diversity of 15 native melons of Kifri, Kurdistan region of Iraq and 7 non-native melons was analyzed by ISSR markers. A total of 6 primers were used to amplify parts of the genomic DNA of the samples. Based on the results, 65 bands were scored and 34 bands had polymorphisms. Cluster analysis showed wide range of diversity across the

genotypes used. The obtained results showed that these genotypes have high diversity which could be used in breeding programs.

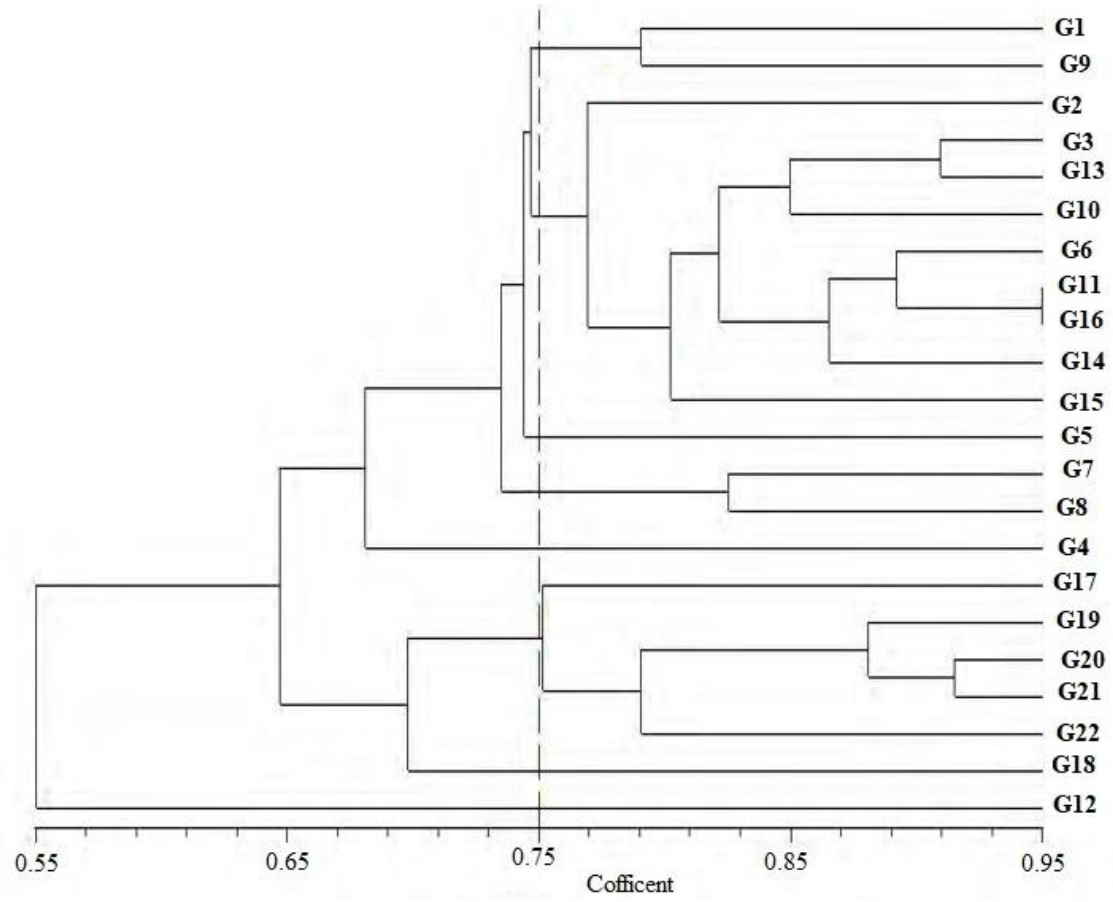

Figure 1: Dendrogram of genetic diversity among cultivars and populations based on Jacard Similarity Coefficient and UPGMA method. (G1: Stretched white, G2: Sefidak dost, G3: Sefidak Deh Koneh, G4: Garmek, G5: Ghandak, G6, Semsar, G7: Ajghoni, G8: Ghandak gazinak, G9: Pakistani, G10: Shadgan, G11: Khatoni, G12: Ghandak milk, G13: Sefidak Edimi, G14: Tashkandi, G15: Ashti, G16: Mashhadi, G17: Chat ashtori, G18: Kshideh khatdar, G19: Abiari, G20: Sefidak Janabad, G21: Sefidak Jeznik, G22: Sefidak Gard)

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