INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 4, No. 12, p. 54-61, 2014

RESEARCH PAPER OPEN ACCESS

AFLP based genetic diversity assessment among *Cucumis melo* var. *agrestis* genotypes in Southern Caspian coastline

Fatemeh Vafadar Shamasbi[1], Ali Dehestani[2*], Saber Golkari[3]

[1]*Agricultural Biotechnology, Maragheh Islamic Azad University, Maragheh, Iran*

[2]*Genetics and Agricultural Biotechnology Institute of Tabarestan(GABIT), Sari Agricultural Sciences and Natural Resources University, Sari, Iran*

[3]*Dryland Agricultural Research Institute, Maragheh, Iran*

Key words: Wild melon, molecular markers, genetic diversity, amplified fragment length polymorphism (AFLP), cluster analysis.

http://dx.doi.org/10.12692/ijb/4.12.54-61 Article published on June 22, 2014

Abstract

Genetic diversity of indigenous plant population is the prerequisite for any research on plant breeding and improvement, making genetic diversity conservation of critical importance. Genetic diversity of 17 wild melon (*Cucumis melo var. agrestis*) genotypes in southern Caspian Sea region was evaluated using AFLP markers. 386 out of 564 studied primers amplified a total of bands, out of which (68.4%) were polymorphic. The average number of bands per primer was 56.4, while an average number of 38.6 were estimated for polymorphic bands. The mean polymorphic information content (PIC) was estimated to be 0.221, while the highest and lowest PICs of 0.288 and 0161 were recorded for M-GAG, E-CCA and M-GAG, E-CCA primers, respectively. Marker index (MI) values were calculated as 15.51. The principle coordinate (PCoA) of the markers revealed that the first three components accounted for 63.5% of the total variance indicating the markers distribution throughout the genome is mostly non-random. Cluster analysis based on Jaccard similarity index and UPGMA algorithm showed a high variation within the studied genotypes and a mean similarity index of 0.65 was calculated for AFLP markers. Ghaemshahr and Juybar wild melon genotypes showed the highest similarity (0.76) while the least similarity was observed between Tonekabon and Livan wild melon (0.55). The Relatively high genetic diversity observed may be attributed to several factors e.g. nature of melon reproduction (cross-pollination), high differentiation ability of AFLP markers and existence of several undistinguished subspecies.

* **Corresponding Author:** Ali Dehestani ✉ A.Dehestani@Gmail.com,

Introduction

Wild Melon (*Cucumis melo* var. *agrestis*) is an annual and herbaceous plant which belongs to the family Cucurbitaceae. The *cucumis melo.var agrestis* subspecies is highly tolerant to unfavorable growth conditions and genetically distant from European and US commercial melon (Mliki *et al.,* 2001; Dje *et al*., 2006).

Evaluation of the genetic resources for specific traits such as yield, fruit quality, pest and disease resistance are conducted regularly. Furthermore collection and conservation of genetic resources with high level of diversity is necessary for fulfilling future demand of breeding programs (Given, 1987). The amount and distribution of genetic variation in the gene pool have been measured by different biochemical and molecular techniques in several studies (Wachira *et al.,* 1994). Several marker types such as isozymes, restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNA (RAPDs) and simple sequence repeats (SSRs) have been used to assess genetic diversity in melon (Perl-Treves *et al*., 1985; Staub *et al.,* 1987; Neuhausen. 1992; Garcia *et al.* 1998; Katzir *et al.,* 1996). The effectiveness of isozymes is limited by the low number of enzyme detection systems and low isozymic variation in *C. melo* germplasm. On the contrary, successful application of molecular markers in assessing genetic diversity of melon has been reported. Neuhausen (1992) successfully classified 44 *C. melo* lines with RFLP markers. Intraspecific classification of melon using RAPDs and ISSRs in 54 melon accessions in two subspecies (sweet types and exotic types) has been reported (Perl-Treves *et al.,* 1998). Garcia *et al.* (1998) have also successfully used RAPDs for assessing diversity in melon. Among different molecular markers, amplified fragment length polymorphisms (AFLPs) generally reveal a higher number of polymorphisms and do not require DNA sequence information (Vos *et al.,* 1995). Therefore AFLP is considered as a reliable marker choice for genetic diversity studies in a large number of organisms and species (Park *et al.,* 2010).

In the present study, the genetic relationships within the wild melon populations were assessed by AFLP technique.

Materials and methods

Seventeen geographically distinct genotypes of wild melon collected from Mazandaran and Golestan provinces in southern Caspian Sea region were used in this study. The exact locations of sampling have been presented in Table 1. Seeds of collected samples were germinated in vitro and fully expanded leaves were collected for DNA extraction.

DNA Extraction

Total genomic DNA was extracted according to the method of Doyle and Doyle (1990) with some modifications. An additional precipitation step with 5M NaCl was included to reduce polysaccharide pollution. DNA quality and concentration were checked by spectrophotometer at 260 nm.

AFLP analysis

AFLP analysis was performed following the protocol of Keygene N.V. (Zabeau and Vos. 1993) with minor modifications. About 250 ng of genomic DNA was double-digested using *Eco*RI and *Mse*I following the protocol of Vos *et al.* (1995). The DNA fragments were ligated to *Eco*RI and *Mse*I specific adaptors. The adaptor-ligated DNA was diluted with TE buffer and subjected to pre-selective amplification.

Pre-selective reactions were performed in 20µl volume containing 2µl of diluted DNA, 200 µM of dNTPs, 2.5 mM of $MgCl_2$, 0.2 mM of each *Mse*I adaptor + C and *Eco*RI adaptor + A primers, 2 µl *Taq* buffer and 1 unit of *Taq* polymerase (Fermentas, Germany). The PCR amplifications were subjected to 30 cycles of 94°C for 30 s, 56°C for 60 s and 72°C for 60 s, with a final extension of 7 min (Bio- RAD Thermo cycler). The PCR product was diluted 20-folds in water and used for the selective amplification. After pre-amplification 5 µl of the PCR product was checked on 1% agarose gel.

The selective amplification mixture (25µl) was

prepared with 2µl of diluted DNA, 200 µM of dNTPs, 2.5 mM of $MgCl_2$, 10 µM of each *Eco*RI + 3 and *Mse*I + 3 primers, 2.5 µl *Taq* buffer and 1 unit of *Taq* polymerase (Fermentas, Germany).

The Touchdown-PCR was carried out as following cycling program: 94°C for 30s, 65°C (-0.7°C/cycle) for 30s and 72°C for 60s over 12 cycles. At 56°C (optimal annealing temperature) 23 more cycles were performed to complete the selective amplification reaction (Bio- RAD Thermo cycler).

The PCR product was mixed with 20 µl formamide loading buffer (98% formamide, 10 mM EDTA, 0.005% each of xylene cyanol FF and bromophenol blue) and denatured by incubation for 5 min at 94°C. The denatured PCR products were separated on a 6% denaturing polyacrylamide gel.

Data analysis

The AFLP fragments between 50 and 850 bp were scored as present (1) or absent (0) for each primer combination and used for analysis.

Principal coordinates analysis (PCoA) was performed using molecular data to obtain a graphical representation of the relationship structure of the different wild melon genotypes. Computations were done using the procedures in the NTSYS pc 2.2 software.

The genetic similarity (GS) between pairs was estimated according to the Jaccard coefficient [Jaccard 1908; GS (ij) = 2a/(2a + b + c)], where GS (ij) is the measure of genetic similarity between individuals i and j, a is the number of polymorphic bands that are shared by i and j, b is the number of bands present in i and absent in j and c is the number of bands present in j and absent in i. The statistical analysis was carried out using the NTSYS- pc 2.2 software. Cluster analysis was performed on the genetic distance matrix by the unweighted pair-group method (UPGMA, Sneath and Sokal 1973) and a tree was constructed using NTSYS pc 2.2. The goodness of fit of the clustering to the data matrix was calculated and the reliability and robustness of the dendrograms were tested by bootstrap analysis with 1,000 replications to assess branch support using PHYLIP 3.6 software.

Polymorphic Information Content (PIC) is a parameter that refers to the value of a marker for detecting the degree of polymorphism within a population and depends on the number of detectable alleles and the distribution of their frequency (Ramamoorthi *et al.,* 2009).

The Polymorphism Information Content (PIC) value that measure the informativeness of the genetic marker was calculated as $\sum [2 \text{ pi } (1\text{-pi})]/n$ (where pi is the frequency of the allele in the set of 17 genotypes) (Powell *et al.* 1996).

The marker index (MI) is determined according to Powell *et al* (1996) to obtain a measure of the utility of the marker system. MI calculation was PIC$\times \alpha \times \beta$, Where the proportion of polymorphic bands is α and β is the number of loci per assay unit.

The analysis of molecular variance was done using Gen Alex 6.5 software to describe the population structure.

Results and discussion

AFLP profile analysis

The present study revealed that there is a large genetic diversity in wild melon. For AFLP analysis, ten selective primer combinations were used to assay genetic diversity among 17 wild melon genotypes. Initially 15 AFLP markers were screened based on their ability to amplify polymorphic and reproducible DNA bands. Among them only 10 AFLP primers produced clear and reproducible fragments with multiband patterns in each genotype that were selected for further analysis and considered as informative and polymorphic primers.

A total of ten AFLP primer combinations (Table 2) were selected based on the number of fragments amplified in each genotype and a significant

polymorphism was observed among closely related genotypes. Results showed that a total of 564 bands were generated, of which more than 68% were polymorphic. AFLP fingerprinting of 17 genotypes of wild melon with ten primer combinations (Table 2) revealed a total number of 386 unambiguous polymorphic amplified DNA fragments.

Table 1. Origin of the 17 *C. melo*.var *agrestis* genotypes studied in this research.

Code	Sampling region	Geographical Location
1	Tonekabon	50.2E, 24.3N
2	Mahmoodabad	1.8E, 1.3N
3	Amol	32.1E, 36.5N
4	Babol	26.2E, 24.5N
5	Ghaemshahr	41.6E, 44.2N
6	Joybar	39.8E, 12.4N
7	Sari	45.8E, 48.3N
8	Sorak	25.1E, 51.8N
9	Neka	26.1E, 38.7N
10	Rostamkola	49.1E, 30.4N
11	Alitape	9.45E, 55.8N
12	Behshahr	30.3E, 27.2N
13	Khalilsahr	47.6E, 8.9N
14	Livan	23.7E, 56.8N
15	Bandargaz	52.8E, 26.5N
16	Kordkuy	35.4E, 31N
17	Gorgan	6.9E, 15.6N

Table 2. The 10 AFLP primers used for assessment of genetic diversity in wild melon genotypes.

Primer	Total No. of bonds	No. of Polymorphic bonds	Polymorphism (PI) (%)	PIC*	MI	Nei index	Shannon index
M-GAG E-CCA	38	32	84	0.288	24.19	0.21	0.33
M-AAG E-CTA	55	45	81	0.23	18.63	0.17	0.28
M-CCC E-TCC	39	27	69	0.176	12.14	0.12	0.14
M-AAG E-GTG	64	50	78	0.267	20.82	0.20	0.32
M-AAG E-CTC	69	52	75	0.257	19.27	0.24	0.33
M-AAG E-TCC	63	37	58	0.209	12.12	0.16	0.25
M-GAG E-CTC	56	32	57	0.161	9.17	0.13	0.21
M-GAG E-TCC	64	45	70	0.265	18.55	0.21	0.33
M-CCC E-GTG	69	37	53	0.171	9.06	9.06	0.24
M-CCC E-CTC	47	29	61	0.188	11.46	11.46	0.25

*PIC=2pi (1-pi).

However, only 68.4% of polymorphism was observed within the wild melon genotypes. Some bands, marked were found to be monomorphic across all the genotypes. The most polymorphic bands were generated by primer E-CCA, M-GAG. Accordingly, of the bands in this experiment 84% were polymorphism across wild melon genotypes by this primer. Each primer combination was able to distinguish the 17genotypes. The average number of polymorphic bands was 38.6. The level of polymorphism ranged from 84% (E-CCA/M-GAG) to 53% (E-GTG/M-CCC).

Data analysis showed that the PIC value varied from 0.161 to 0.288, with an average of 0.243. The highest PIC value was obtained for primer (E-CCA, M-GAG) and the lowest obtained for primer (E-CTC, M-GAG). As a result, (E-CCA, M-GAG) with the highest polymorphic bands and PIC value was recognized as the most appropriate and discriminating primer to estimate genetic similarity among genotypes of *cucumis melo. var agrestis*.

Table 3. Genetic similarity values among the 17 genotypes of *Cucumis melo. Var agrestis* as determined by 10 AFLP markers using Jaccard similarity coefficient.

	g1	g2	g3	g4	g5	g6	g7	g8	g9	g10	g11	g12	g13	g14	g15	g16	g17
g1	1																
g2	0.7	1															
g3	0.66	0.69	1														
g4	0.65	0.67	0.7	1													
g5	0.63	0.65	0.67	0.72	1												
g6	0.62	0.64	0.64	0.69	0.76	1											
g7	0.61	0.61	0.64	0.67	0.68	0.73	1										
g8	0.61	0.63	0.61	0.63	0.67	0.68	0.72	1									
g9	0.57	0.6	0.59	0.59	0.62	0.63	0.66	0.65	1								
g10	0.6	0.61	0.62	0.61	0.64	0.63	0.68	0.7	0.72	1							
g11	0.59	0.57	0.61	0.63	0.65	0.65	0.63	0.66	0.67	0.72	1						
g12	0.57	0.6	0.6	0.6	0.66	0.64	0.62	0.67	0.65	0.68	0.68	1					
g13	0.58	0.56	0.6	0.6	0.62	0.63	0.61	0.65	0.65	0.68	0.74	0.7	1				
g14	0.55	0.58	0.58	0.59	0.61	0.59	0.61	0.63	0.62	0.69	0.64	0.69	0.66	1			
g15	0.58	0.59	0.58	0.58	0.6	0.61	0.62	0.6	0.62	0.6	0.62	0.61	0.63	0.68	1		
g16	0.55	0.59	0.57	0.59	0.6	0.58	0.59	0.59	0.61	0.64	0.6	0.62	0.63	0.7	0.71	1	
g17	0.59	0.61	0.6	0.62	0.6	0.61	0.59	0.62	0.59	0.64	0.6	0.62	0.61	0.62	0.65	0.66	

Based on the dataset of AFLPs, a dendrogram was generated using Jaccard's similarity coefficient and the UPGMA method. In this dendrogram, individuals from the same population or accessions from the same genotype grouped together, forming four major clusters. A Mantel test showed, the highest value was observed for UPGMA based on Jaccard's coefficient (r = 0.77). Genetic similarity was calculated from the Jaccard's similarity coefficient (r) value for all accessions which varied from 0.55 to 0.76 (Table 3), with an average of 0.65 implying a medium level of genetic variation between investigated genotypes. The G5 and G6 genotypes from two geographically close territories (Juybar and Ghaemshahr, both located Mazandaran province) were the most closely related genotypes, with similarity value of 76%, followed by G1 and G14 genotypes from a more distance origin (Tonekabon and Livan) with similarity value of 55%, all belong to the same genotypes (*Cucumis melo var. agrestis*).

Cluster analysis

The resultant dendrogram (figure 1) grouped the Gorgan, Kordkuy and Bandargaz genotypes in a separate cluster. Second group consisted of six genotypes (livan, Khalilshahr, Behshahr, Alitape, Neka and Rostamkola). Sari, Sorak, Juybar, Ghaemshahr, and Babol situated in a seprate cluster. Next group consisted of two subgroup. A small subgroup only included genotype of Amol, and genotypes of Tonekabon and Mahmoodabad were in second subgroup.

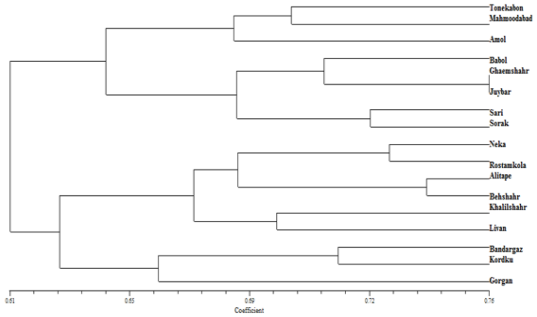

Fig. 1. Dendrogram based on Jaccard's similarity coefficient and UPGMA algorithm showing the genetic relationship among 17 genotypes of *Cucumis melo.var agrestis* analyzed by AFLP markers.

Analysis of molecular variance (AMOVA)

To estimate variance components and to determine the contribution of each of the total variability, molecular variance (AMOVA) was performed using Gen Alex ver. 6.5 software. Analysis results showed that the total contribution of variation within populations, variation between the more than variation within populations showed 79% of total variation. This indicates a relatively good distribution of AFLP is a genome-wide.

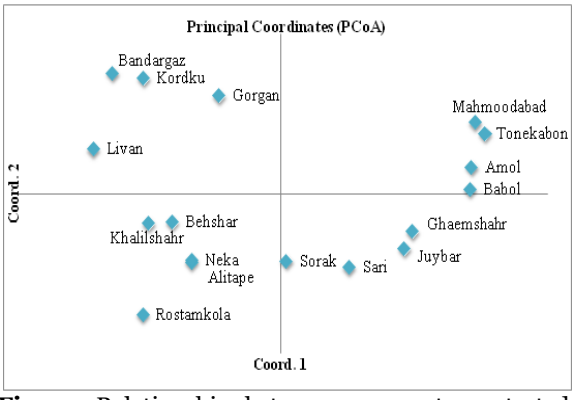

Fig. 2. Relationship between 17 genotypes tested using principal component analysis using software Gen Alex. The first component (PC1) and the second component (PC2) occur on 63.5% Overall of the variation.

Molecular analysis of the samples tested using Nei's genetic distance based on Nei is shown in table 2, also the amount of genetic variation between samples, coefficients of similarity matrix based on Nei (1972) was calculated. Based on Shannon the primer combination E-CCA, M-GAG amount (0.33) and the primer combination E-TCC, M-CCC (0.14) were obtained highest and lowest variability. Average of AFLP markers in this study, 15.51, and the highest and lowest, respectively, the primer combination E-CCA, M-GAG with 24.19 and E-GTG, M-CCC with 9.06.

The principle coordinate analysis (PCoA) of the markers revealed that the first three components described 63% of the total variance indicating the markers distribution throughout the genome is mostly non-random.

This result is related with the dendrogram generated employing UPGMA and is further emphasizing of the genetic similarities delineated by us.

These results confirm the usefulness of AFLP technique for fingerprinting in *cucumis melo. var agrestis* genotypes. Another goal of this research was to optimize the use of AFLP markers to provide a clear picture of the extent of genetic base of wild melon genotypes.

This method based on truncated genomic fragments by polymerase chain reaction for using the DNA of each creature with every amount complexity. dna amplification with this method does not require previous knowledge of the sequence, with a limited number of primer pairs takes place. AFLP technique makes sure and the reliability is high because of the reaction conditions completely appropriate for the connection primers used. There reproducibility of the RFLP and capability chain reaction polymerase can be combined subscales.

Conclusions

Genetic diversity helps organisms to deal better with environmental changes. Genetic diversity indicated differences among the C. melo var agrestis studied. The different estimators of genetic variability employed indicated that the wild melon genotypes had average variability. The AMOVA showed a small genetic differentiation among the C.melo var agrestis populations analyzed, thus these populations were genetically similar. The study showed that the average variation observed, due to the limited number of samples of wild melons, is not accurately assessing the amount of variation within the population estimate.

In the present research 283 bands were amplified using 10 AFLP primers, out of which 564 (68.4%) were polymorphic. The average number of bands per primer was 56.4, while an average number of 38.6was estimated for polymorphic bands.

The highest genetic similarity was observed between two genotypes within *Cucumis melo*. var agerstis of

Ghaemshahr and Juybar, collected from two adjacent territories, with a similarity index of 0.76. The lowest genetic similarity was observed between two genotypes from livan and Tonekabon with a similarity index of 0.55. Cluster analysis based on Jaccard's similarity coefficient and UPGMA algorithm divided genotypes into four major clusters. Also it demonstrated AFLP marker as a powerful tool to genetically discriminate different genotypes of *Cucumis* species.

Acknowledgements

This work was funded by grants from the Genetics and Agricultural Biotechnology Institute of Tabarestan (GABIT), Sari, Iran, We are grateful to the Director, Genetics and Agricultural Biotechnology Institute of Tabarestan.

References

Djè Y, Kouonon LC, Zoro Bi AI, Gnamien GY, Baudoin JP. 2006. Study of botanic, agronomic characters and floral biology of African melon (*Cucumis melo L.* var agrestis Naudin, Cucurbitaceae). Biotechnologie, Agronomie, Sociétéet Environment **10(2),** 109-119.

Garcia E, Jamilena M, Alvarez JI, Arnedo T, Oliver JL, Lozano R. 1998. Genetic relationships among melon breeding lines revealed by RAPD markers and agronomic traits. Theor Appl Genet **96,** 878–885.

Given DR, Bran well D, Haman O, Heywood V, Synge H (Eds). 1987. What the conservationist requires of ex situ collections. Botanic Gardens and the World Conservation Strategy. Academic Press, London , 103–116.

Jaccard P. 1908. Nouvelles researches surla distribution florale. BullSoc Vaud Sci Nat **44,** 223–270.

Katzir N, Danin-Poleg Y, Tzuri G, Karchi Z, Lavi U, Cregan PB. 1996. Length polymorphism and homologies of microsatellites in several *Cucurbitaceae* species. Theor Appl Genet **93,** 1282–1290.

Mantel NA. 1967. The detection of disease clustering and a generalized regression approach. Cancer Res **27,** 209–220.

Mliki A, Staub JE, Zhangyong S, Ghorbel A. 2001. Genetic diversity in melon (Cucumis melo L.). An evaluation of African germplasm. Genet. Resour. Crop Evol **48,** 587–597.

Neuhausen SL. 1992. Evaluation of restriction fragment length polymorphism in *Cucumis melo*. Theor Appl Genet **83,** 379–384.

Perl-Treves R, Zamir D, Navot N, Galun E. 1985. Phylogeny of *Cucumis* based on isozyme variability and its comparison with plastome phylogeny. Theor Appl Genet **71,** 430–436.

Purseglove JW. 1968. Tropical crops: dicotyledons, vol. 2. Longmans Green, London.

Rasul MG, Hiramatsu M, Okubo H. 2007. Genetic relatedness (diversity) and cultivar identification by randomly amplified polymorphic DNA (RAPD) markers in teasle gourd (MomordicadioicaRoxb). Sci. Hortic **111,** 271–279.

Rohlf FJ. 2000. NTSYS-pc numerical taxonomy and multivaria tee analysis system version 22.Exeter Publications, Setauket.

Sneath PHA, Sokal RR. 1973. Numerical taxonomy. WH Freeman, San Francisco, California.

Lübberstedt T, Melchinger AE, Dußle C, Vuylsteke M, Kuiper M. 2000. Relationships among early European maizeinbreds: IV. Genetic diversity revealed with AFLP markers and comparison with RFLP, RAPD, and pedigree data. Crop Sci. **40,** 783–791.

Nei M. 1978. Estimation of average heterozygosity

and genetic distance from a small number ofindividuals. Genetics **87,** 583–590.

Park YH, Kim KH, Choi YM, Choi HS, Chae Y, Park KS, Chung SM. 2010a. Evaluation of TYLCV-resistant Tomato Germplasm Using Molecular Markers. Kor J Hort Sci Technol **28,** 89-97.

Powell W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey Sand Rafalski A. 1996. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Mol. Breed **2,** 225–238.

Staub JE, Frederick L, Marty TC. 1987. Electrophoretic variation in cross-compatible wild diploid species of *Cucumis*. Can JBot **65,** 792–798.

Sneath PHA, Sokal RR. 1973. Numerical taxonomy. WH Freeman, San Francisco, California.

Wachira FN, Waugh R, Hackett CA, Powell W. 1995. Detection ofgenetic diversity in tea (*Camellia sinensis*) using RAPD markers. Genome **38,** 201–210.

Wickremaratne MR. 1981.Variation in some leaf characteristics in tea (*Camellia sinensis* L.) and their use in the identification of clones. Tea Q **50,** 183–189.

Wickremasinghe RL. 1979. Tea In: Mrak EM, Steward GF (eds) Advances in food research, vol. 24. Academic Press, New York, 229–286.