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Determination of Molecular Markers by RAPD Technique Associated with Four Melon Genotypes under 50% Irrigation Conditions

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Abstract

Seven random primers were selected as molecular marker for drought stress tolerance among four melon genotypes. According to results, 69 polymorphic bands were obtained out of 72 bands. Jaccard coefficient was calculated to study genetic diversity and relationship among genotypes using unweighted pairwise group method with arithmetic mean (UPGMA). The range of genetic similarity ranged from 0.167 to 0.324. In addition, two separated groups with two genotypes in each were identified by phylogenetic tree in form of topology. In the context of the analysis of single marker, 14 RAPD markers were associated with the studied morphological traits, which make them valuable source for genetic expression in plant breeding programs.

Key words : Melon (*Cucumis melo*), Random Amplified Polymorphic DNA (RAPD), Jaccard coefficient, genetic similarity, genetic distance.

Melons (*Cucumis melo* L., 2n=2x= 24) are a significant sweet horticulture crop (Adeyeye *et al*., 2017; Ansari *et al*., 2018 and Sergio *et al*., 2021). According to Aragão *et al*., (2013) it belongs to Cucurbitaceae family, which also include number of other significant crops like cucumber, watermelon, pumpkin, and squash.

Genetic diversity is a practical and attractive method for finding drought tolerant genotypes (Nwokwu *et al*., 2018; Vidya *et al*., 2019; Hussein and Selim, 2020; Shi *et al*., 2021; Silva *et al*., 2021 and Haitham and Sayed, 2024). As reported by many researchers, the most widely methodology for genetic diversity is RAPD technique (Premkrishnan and Vadivel, 2012) due to its speed, low cost, lack of tissue and environmental influences, and high degree of polymorphism without require prior DNA sequence knowledge (Singh *et al*., 2011 and Kaçar *et al*., 2012). So, RAPDs called dominant markers (Sharma *et al*., 2018 and Clisneide *et al*., 2022), and consider effective method for finding genes linked to desired traits without necessity mapping complete genome (Behra *et al*., 2008; Nadeem *et al*., 2018 and Lohani *et al*., 2023). Hence, the purpose of this investigation is to find single RAPD markers linked to studied traits as indicator of drought stress tolerant.

Materials and Methods

Plant Materials and Experimental Location : Four Melon genotypes (G-1201, G-C-61, G-I-115 and G-I-153) were provided by Genetic Dept., Faculty of Agric., Sohag Univ., Sohag, Egypt. To extract DNA, plants were cultivated and recorded (Haitham and Sayed, 2024). The genotypes leaves were sliced and frozen in freezer -20°C.

DNA Extraction : The current study was carried out in the molecular biology laboratory of the department of genetic, faculty of agriculture, Sohag university, Sohag, Egypt. Using cooled mortar and pestle, leaves were crushed in extraction buffer according to Doyle and Doyle (1990) Cetyl Trimethyl Ammonium Bromide (CTAB) approach with some modifications. Electrophoresis on 0.8% agarose gel were used to measure the amount and quality of DNA. For amplification in polymerase chain reaction (PCR), extracted DNA samples were diluted to 50 ng/µl.

RAPD Amplification : RAPD-PCR amplification was employed with seven random decamer primers (OPA-2, OPA-3, OPA 15, OPY-5, OPP-05, OPAW-10 and OPG-09) supplied from department of genetic, faculty of agriculture, Sohag university, Sohag, Egypt. Next, PCR amplifications were performed in 25 µl volume containing 3 µl template DNA (50 ng/µl), 3.5 µL of primer 10 pmol, 12.5 µl GoTaq® Green Master Mix (Promerga, United States) and 7 µL of free nuclease water. PCR reactions were performed with DNA thermal cycler (Thermal Cycler 96-lambet, USA).

Amplification conditions were as follows: initial denaturation at 94°C for 5 minutes, followed by 1 minute's denaturation at 94°C for 45 cycles, and 30 seconds at annealing temperature (depends upon the primer), 1.30 minutes polymerization at 72°C and 7 minutes final extension at 72°C. After completion, 12.5 µl volume was loaded on 1.5 % (w/v) agarose gel stained with ethidium bromide. The size of amplified DNA fragments was estimated with standard molecular size markers (100 – 3000 bp). The gel documentation system (USA, Sambrook *et al.*, 1989) was used to view gel under ultraviolet lamp.

Scoring and data analysis : Clear and intense bands were scored, while week bands against smear background weren't considered for additional analysis. DNA were scored for presence (1) or absence (0) bands at each locus with different molecular weight sizes in form of binary matrix. To reflect variation in priming sequence, MVPS 3.1 software was used to calculate binary data in form of genetic distance and phylogenetic tree based on unweighted pair group approach with the arithmetic average (UPGMA) according to Jaccard (1908). For effective amplification, single-marker analyses were used to determine how the markers relate to phenotypic variation.

Results and Discussion

For improving melon plants, breeders need to know more information about genetic diversity to study in reality the relationship between populations by determining alleles that may influence melon plant's ability to survive in its existing habitat (Dhillon *et al.*, 2012 and Mani *et al.*, 2023). Therefore, description values of the examined morphological traits were displayed in Table-1, to identify genes that are linked to them.

Regarding polymorphism analysis, seven RAPD primers with 50% or higher GC content were selected to calculate DNA amplicon size and polymorphism among four melon genotypes (Table 2) the total number of bands for every primer were noted independently to calculate polymorphic bands. The total numbers of amplified bands were 72 bands with an average 10.29. while, the polymorphic bands were 69, and the PCR amplified product's total sizes varied from 240 bp to 2900 bp. These results were in agreement with that found by Kong *et al.*, (2007), who used EST-SSR markers in melo and showed that the alleles numbers ranged from 2 to 5 with an average per locus 2.9. in addition, Dantas *et al.* (2012) examined 18 RAPD primers in 43 melon accessions gather from northeast Brazil and found 96.53% polymorphic bands. Likewise, Yildiz *et al.* (2011) found polymorphisms of 72.11% and 86.79% in RAPD markers in Turkish melon accessions.

Based on similarity matrix data (Table 3) the maximum similarity value (0.324) was scored between genotypes G-1201 and G-C-61, indicating moderate diverse at genetic level. Meanwhile, the minimum similarity value of

(0.167) was showed between G-1201 and G-I-115, demonstrating more divers with highly polymorphic. On the other hand, the phylogenetic tree analysis was divided into two main groups (Fig. 1). Genotypes G-1201 and G-C-61 were grouped at similarity coefficient 0.324, while at a similarity coefficient 0.269 was grouped genotypes G-I-153 and G-I-115, and all of groups was cluster in similarity coefficient 0.228.

The relationship between RAPD markers and variation in estimated morphological traits was determine by single marker analysis. The analysis of variance (Tables 4 and 5 and Fig. 2) revealed highly significant association ($P<0.05$, $P<0.01$) between RAPD markers with flowers numbers, green leaves numbers and brown leaves numbers (OPA-21350bp, OPA-2700bp, OPA-2600bp, OPA-2480bp), (OPP-051250bp, OPP-051000bp, OPP-05700bp, OPP-05600bp), (OPG-092900bp, OPG-092700bp, OPG-091450bp, OPG-09900bp) and (OPA-15600bp, OPA-15500bp). Suggesting that, these markers may be useful as a genetic marker for further genetical studies. Depending on te type of primer, a high degree of polymorphism bands indicates wide range of genetic variation

Table 1. Descriptive analysis of the examined morphological traits under 50% irrigation in the studied four melon genotype

	Minimum	**Maximum**	**Mean ± S. Dev.**	**Variance**
1° Leaf Score Tolerance	0.30	0.56	0.41 ± 0.11	0.01
2° Leaf Score Tolerance	0.70	1.40	1.05 ± 0.29	0.08
Flowers Numbers	0.00	0.91	0.28 ± 0.43	0.19
Vine Length	44.1	73.6	61.78 ± 13.96	194.96
Green Leaves Numbers	2.80	4.10	3.60 ± 0.56	0.31
Brown Leaves Numbers	1.78	4.27	2.84 ± 1.21	1.46

Table 2. Seven RAPD primers with polymorphic and monomorphic corresponding bands and their size range in four melon genotypes

Primers Code	**Sequence (5'---3')**	**Size Range (bp)**	**Total bands**	**Polymorphic bands**	**Monomorphic bands**	**Polymorphic loci (%)**	**Polymorphic information content (PIC)**
OPA-2	TGCCGAGCTG	420 - 1800	10.00	9.00	1.00	90.00	0.98
OPA-3	AGTCAGCCAC	240 - 2000	11.00	10.00	1.00	90.91	0.97
OPA 15	TTCCGAACCC	400 - 2850	11.00	11.00	0.00	100.00	0.96
OPY-5	GGCTGCGACA	400 - 1600	6.00	6.00	0.00	100.00	0.97
OPP-05	CCCCGGTAAC	240 - 2000	11.00	10.00	1.00	90.91	0.97
OPAW-10	GGTGTTTGCC	400 - 2850	11.00	11.00	0.00	100.00	0.97
OPG-09	CTGACGTCAC	400 - 2900	12.00	12.00	0.00	100.00	0.97
Total			72.00	69.00	3.00		

Table 3. Genetic distance among four melon genotypes based on binary matrix of similarity based on Jaccard coefficient using UPGMA method

	G-1201	**G-C-61**	**G-I-115**	**G-I-153**
G-1201	1.000			
G-C-61	0.324	1.000		
G-I-115	0.167	0.259	1.000	
G-I-153	0.263	0.222	0.269	1.000

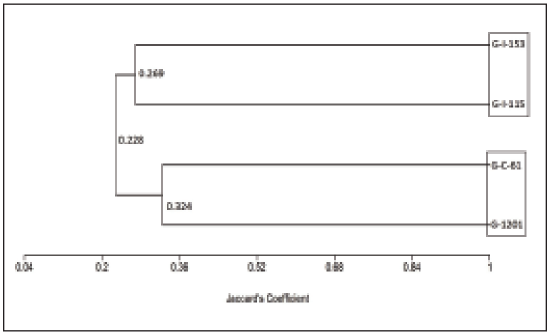

Fig. 1. Dendrogram generated using UPGMA cluster analysis among four melon genotypes based on Jaccard coefficient.

among studied genotypes (Karimi *et al*., 2016 and Clisneide Coelho *et al*., 2022). Sensory *et al*., (2007), who noticed genetic diversity between Turkish melons by through phenotypic traits and RAPD markers. According to these results, the Turkish melon genotypes showed very high level of genetic variation. This finding is in consistent with our study.

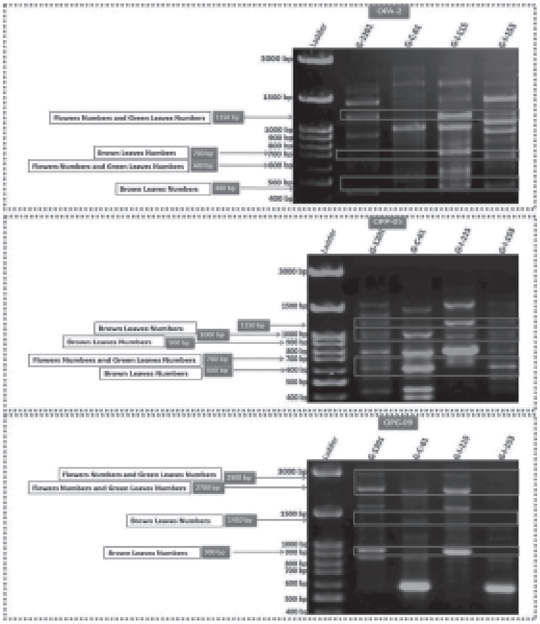

Fig. 2. Significant Bands RAPD-PCR Markers.

Table 4. ANOVA single factor based on RAPD-PCR data

	Flowers Numbers		
S. O. V	**D. F.**	**SS**	**MS**
Between Bands	1	0.532	0.532**
Error	2	0.027	0.013
Total	3	0.559	
	Green Leaves Numbers		
S. O. V	**D. F.**	**SS**	**MS**
Between Bands	1	0.853	0.853**
Error	2	0.087	0.043
Total	3	0.940	
	Brown Leaves Numbers		
S. O. V	**D. F.**	**SS**	**MS**
Between Bands	1	3.990	3.990**
Error	2	0.388	0.194
Total	3	4.378	

Table 5. Single Markers RAPD-PCR

Flowers Numbers and Green Leaves Numbers				
Single	**OPA-2**	**OPP-05**	**OPG-09**	**OPA-15**
Markers	1350 bp	700 bp	2900 bp	600 bp
	600 bp		2700 bp	500 bp
	Brown Leaves Numbers			
Single	**OPA-2**	**OPP-05**	**OPG-09**	
Markers	700 bp	1250 bp	1450 bp	
	480 bp	1000 bp	900 bp	
		600 bp		

Conclusion

According to showed phylogenetic tree, the genotypes that were closer to one another were more similar than those that were farther apart. Furthermore, identified single markers may be useful as a genetic marker for further genetical studies. However, to find more appropriate markers, further researches must be done in the studied genotypes.

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Author Contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Data Availability

All data available within the article.

Conflict of interest

The authors declare no conflict of interest for this research

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