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USAGE OF BIOLIOGICAL AND MOLECULAR MARKERS FOR DETECTING RESISTANCE CHARACTER AGAINST FUSARIUM WILT IN MELON (*Cucumis melo* L.)

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ABSTRACT

Sixteen melon genotypes were collected from different places by the help of principle project for vegetables improving and hybrids production. These melon genotypes were planted in complete randomized block design at El-Kassasien Horticultural Research Station, Ismaelai Governorate , to obtain some information about genetic relationships among these materials under the artificial infection with Fusarium wilt pathogens and detect some molecular markers related to this biotic stress.

The obtained results could be summarized in the following.

1. A wide variation was found among melon genotypes by the analysis of variance.
2. Data revealed that, melon genotypes with the green flesh color were related to each other and melon genotypes with orange flesh color were also, related to each other, under healthy conditions.
3. Under infection condition, the gene expression had changed for all melon genotypes, and genetic distances were decreased.
4. The genetic distance between Galia, Ideal, Rafegal, Regal, Polidor, and Marsmatrouh was small, meanwhile the plant introduction PI 140471 and PI 266935 had a large genetic distance with the other melon genotypes .
5. The genetic distance between Hale's Best, PI 124111, Cinco, Honeydew orange flesh and Charantaize was small, while the genetic distance between Ismaelawi and all melon genotypes was very large distance, under infection condition.
6. The melon genotypes with Charantaize types, Honeydew orange flesh, Cinco , Charantaize , PI 124111, Hale's Best, were more divergent than plant introductions PI 140471 and PI 266935, which recorded (6.093), (6.570), (6.175), (4.931) and (5.328) genetic distance, respectively
7. The genetic distance between PI 140471 and PI 266935 was very small, which was (0.894). This means that the two plant introduction were similar under infection condition, in the same time the genetic distance between PI 183227 and PI 140471 was most large (10.235).
8. Under infection condition, the maximum inter-cluster distance (2.966) was observed between cluster VII and cluster III, meanwhile, the minimum inter-cluster distance (0.259) was observed between cluster V and cluster I .
9. Data of cluster analysis indicated that, the sixteen melon genotypes were grouped into seven clusters. These cluster grouping were mainly due to differences in their biological performance under healthy and infection conditions.
10. For molecular markers related to Fusarium wilt disease, data revealed that, total number of DNA fragments were forty eight bands, the plant introduction PI 140471 gave alone forty one bands, followed by Galia (inbred line) thirty six bands, while the least genotype amplifying DNA fragments was PI 266935, under infection condition.
11. The dendogram produced from genetic distances between melon genotypes, demonstrated that, the twelve melon genotype were grouped into five sub

clusters. Every sub cluster had two melon genotypes, while the rest melon genotypes Galia and Ideal were alone.

12. Data revealed that, under infection condition the best cluster for the highest yield was the cluster II which included Honeydew orange flesh and Cinco, while cluster II exhibited a great men for earliness, total soluble solids and leaf area . The member of cluster V, Honeydew orange flesh and Cinco should form the basis of selection against Fusarium wilt, also the PI 140471 and PI 266935.

INTRODUCTION

Melon is a valuable crop in East Delta Region, especially under low plastic tunnels, in waiter season. Soil borne diseases are important yield-limiting factors, especially Fusarium root rot and vine decline which have recently observed in many melon fields , causing greet losses in yield.

Symptoms on the aerial part start with yellowing of the crown leaves followed by the collapse of the vines just prior to harvest. In the recent years, the control of this disease have been become more problematic, due to, the continuous use of farmyard manure , without fumigation or without rotation. Use of resistant cultivars is the most effective control method of melon wilt.

Many investigators, reported that, during the past few years, crosses have been made to introduce resistance from cultivar black skin , melon from Taiwan into a commercial Galia melon and into western shipper- type melon lines.

Selection in the segregating populations were conducted , they found that, in addition to the genetic background, other features such as fruit maturity and environmental stresses may contribute to the rate of disease progress (Wolff, 1995,Pivonia *et al.* 1998 and Pivonia *et al.* 1999).

There are differences in the response of melon accessions to Fusarium wilt, Ananas and Honeydew melons tested in the United States were more tolerant to the disease than U.S cantaloupe . (Mertely *et al.*, 1993, Stanghellini *et al.*, 1995, and Wolff and Miller, 1998) .

So, in this investigation we try to classify the genetic melon germplasm into distinct groups or clusters by measure the genetic divergence based on multivariate analysis of quantative traits to fined out a marker related to resistance or tolerance against Fusarium wilt.

Such genetic divergece may be truly represented by empirical D^2 statistic alone (Behl and Singh, 1985) . Genetic diversity among genotypes is due to number and nature of genes and their Functional relationship against environment stress, Nei (1972) and Prasad and Singh (1986).

Also, DNA profiling technique which, recently used to support the morphological identification, some amplified fragments or patterns of fragments may be unique to genotypes and hence could help as a tool of identification of genotypes under infection condition (El-Hawary *et al.*, 2003).

MATERIALS AND METHODS

The present investigation was conducted in the Experimental Farm of El-Kassasien Horticultural Research Station during the growing season of 2006

until the full season of 2007 to get some information about the resistant melon genotypes against Fusarium wilt disease.

Germplasm: Sixteen melon genotypes were used in this investigation, these material were developed by the project of Vegetables Improving and hybrid production. Self pollination and selection was made via 8 generations to make inbred lines, through working in this project.

Isolation of fungi from roots of wilted plants.

Wilted melon plants were collected from the soil of the experimental farm of El-Kassasien and two other farms located around it. Ten plants per field during the autumn growing seasons of 2006 were collected with the its rhizoshere and experimented in the lab of the station. All fields which plants were collected did not treated with any fungicide or fumigation prior to the growing season or through the development of plants.

All parts of the root tissue were sampled for the determination of their infection with Fungi by the members of plant pathology lab in El-Kassasien and in pathology lab of Suez canal Univ. Fac. Agric., and pathology lab. in Fac. of Sci., Benha University. Roots of each plant were plated on five petri plates , nine segments of taproot were plated on three plates, and two additional plates were plated with six segments of secondary and tertiary roots. Brown to black root lesions were observed on roots later found to be infected with Rhizoctonia and small and light brown lesions were observed on roots infected with Pythium. Root tissues were plated on potato dextrose agar medium amended with chloramphenicol at 250 mg/Liter. Results declared that symptoms of Fusarium colonization was detected (Schmitthennspell, 1979 and Pivonia *et al.*, 1997).

Preparation of inoculum

The three isolates of fungi, Fusarium, Rhizoctonia and Pythium were separately grown on PDA at 27 °C for 10 days, then were added to sterilized barely then inoculated until used, respectively. Plastic cylinders (pot) with open ends, 30 cm diameter and 35 cm high were filled with sterilized soil by formalin 5 % (1.5 kg soil/pot), then inoculum of the three pathogens were added, (250 gm/ 1.5 kg sterilized soil) , then the combination of the three species of fungi were mixed with the soil. Melon seeds of sixteen genotypes were planted in the 15th January, ten seeds per pot under the greenhouse condition. The pots were arranged in a complete randomized block design with three replicates. Each treatment was consisted of 3 pots. Normal recommended agricultural practices were done.

After 33 days of sowing date at wilt incidence, the lower edges of pots were cutted to allow for roots to develop, also, after the fruit setting only, one fruit was left per plant and the others were thinned to permit for plant to complete its leaf cycle, Pivonia *et al.* (1997). The following data were recorded on individual plants which randomly chosen: plant height, number of male and perfect flowers per plant, time of opening 50 % of male and prefect flowers per plant, leaf area (cm^2) which described by Radford (1967)., chlorophyll content (a, b and carotinoids), according to the method described by Wettestein (1957), yield components were also recorded on individual plants as follow: average fruit weight, fruit length and diameter, flesh thickness and total soluble solids.

Pathogenicity test : Pathogenicity test was carried out according to (Ali, 2000).

Reaction of certain genotypes to Fusarium oxysporum F. sp. Melonis, Rhizoctonia and Pythium under greenhouse condition: was made according to (Ciccarese, *et al.*, 1987).

Statistical analysis

Morphological, yield and quality characters were statistical analyzed on plot mean basis. Analysis of variance (ANOVA) appropriate to complete block design was performed to test the significance of observed differences among melon genotypes. The least significant differences (LSD) test criterion at ($P > 0.05$) was used to evaluate the differences between all the melon genotypes.

To study clustering patterns, the studied melon genotypes based on mean performance of morphological and yield characters were analyzed using hierarchical Euclidean cluster analysis developed by Hair *et al.* (1987) to assess genetic divergence in sixteen melon genotypes. The multivariate analysis was done by SPSS program (SPSS 1995) running on a IBM-compatible personal computer.

Molecular Markers:

Six random primers used for RAPD analysis were provided by Op technology (USA) with the following sequences:

Primer code	Sequence (5 to 3)
OPC-20	ACT TCG CCA C
OPB-17	AGG GAA CGA G
OPC-08	TGG ACC GGT G
OPB-11	GTA GAC CCG T
OPD-03	GTC GCC GTC A
OPB-13	TTC CCC CGC T

Total genomic DNA was extracted from the true leaves of melon genotypes. RCR was performed in 30 ul volumes tubes according to Williams *et al.* (1990). Amplification was carried out in a DNA thermocycle (MWG-BIO TECH Primuse) programs as follows : 94 °C/4 min (1 cycle), 94 °C/30 sec., 35 °C/1min and 72 °C/2 min (40 cycles); (1 cycle) 72 °C for 5 min. , then 4 °C infinitive.

RESULTS AND DISCUSSION

Analysis of genetic variations

The analysis of variance for mean performance of the studied characters, summarized in Table (1), revealed that differences in genotypes were highly significant for most of studied quantative characters, except number of male flowers, meanwhile highly significance were observed for infection treatments but the interaction between genotypes and infection treatments were only significant for number of perfect flowers, crop growth rate, leaf area, carotinoids contents and average fruit weight, while the average fruit length, diameter, flesh thickness and total soluble solids were highly significant .

Table (1): Analysis of variance for fifteen quantative characters of sixteen melon genotypes .

Source of variation	Mean square (MS)															
	d.f.	Plant height	No. of male flowers	No. of prefect flowers	Crop growth rate	Time of opening 50 % of flowers		Leaf area	Chlorophyll contents (mg / gm / frech weight)			Average fruit weight	Average fruit length	Average fruit diameter	Flesh thickness	Total soluble solids
						male	female		a	b	Carotinoids					
Replications	2	1.697	120.09	0.465	0.002	1.885	63.78	1.010	0.004	0.003	0.007	10675.8	0.75	0.020	0.0001	0.009
Genotypes	15	194.03**	265.53*	0.450*	0.026*	21.36**	50.52**	866.40**	0.024**	0.058**	0.016**	157997.5**	35.33**	28.20**	2.027**	7.954**
Infection	1	715.04**	6534.0*	19.82**	0.414**	150**	776.34**	1941.12**	0.119**	0.335**	0.693**	424782.1**	203.29**	118.37**	13.79**	131.83**
Geno. X infection interaction	15	38	94.33	0.341*	0.031*	1.422	0.388	65.97*	0.001	0.003	0.007*	11366.9*	4.389**	6.214**	0.331**	1.825**
Error	62	32.34	161.02	0.159	0.012	1.630	2.064	24.62	0.001	0.0045	0.002	4036.0	1.076	1.329	0.066	0.435

These results indicate that, the melon genotypes were genetically differed in their genetic background and there are great genetic diversity among them. Prasad *et al.* (2001) and El-Shimi *et al.* (2003).

Genetic diversity for sixteen melon genotypes based on means of morphological and physiological performance

Data presented in Table (2) declared that , genetic distance were mostly large between Ismaelawi genotype and all other melon accessions, especially with PI 140471 (17.677) and PI 266935 (17.598), respectively. Also,, the plant introduction PI 266935 was genetically large divergent with the other melons which have green flesh color like Galia, Ideal, Rafegal, Regal, Polidor, Marsmotrouh and PI 183227, meanwhile it was related to PI 140471 rather than any genotype (2.03) genetic distance .

The same trend was observed between plant introduction of PI 140471 and other melon genotypes with green flesh color, which the genetic distance was (9.770), (8.883), (12.081), (10.096), (10.208), (8.883) and (9.840), respectively.

On the other hand, the melon genotypes with orange flesh color like PI 124111, Hale's Best, Cinco, Honeydew orange flesh and Charantaize were moderately related to each other which recorded the least genetic distances between each other, (2.164), (1.890), (1.477), and (1.120), genetic distance.

Also, data in Table (2) showed that, honey dew orange flesh and Cinco and Charantaize and Hales Best were recorded large genetic distances with plant introductions of PI 140471 and PI 266935.

These results indicated that, the melon genotypes with green flesh color were related to each other, while the melon genotypes with orange flesh color were also related to each other, while they diversed between all of them. These results were found in agreement with those obtained by Silberstein *et al.* (1999).

Under infection conditions, Data in Table (3) declared that, the gene expression of all melon genotypes had changed, and the calculated genetic distance were decreased, for all melon genotypes. Data showed that, Marsamatrouh was genetically similar to Galia , Ideal, Rafegal, Regal and Polidor, while plant introductions of PI 140471, and PI 266935 were genetically diversed than Galia, Ideal, Rafegal, Regal, Polidor, Marsamatrouh and PI 183227.

Meanwhile , honey dew orange flesh, Hale's Best PI 124111, Cinco and Charantaize were genetically diversed than PI 266935 and PI 140471. The most large distance were observed between PI 265935 and PI 183227 (10.291), PI 183227 and PI 140471 (10.235) and Ismaelawi, PI 140471, PI 266945 (12.338) and (12.764), respectively.

Also, under infection condition, the genetic distance between PI 140471 and PI 266935 was very small (0.894) indicating that these melon genotypes could be used in a breeding program for resistance- tolerane genotypes against Fusarium wilt . However, this improve that, the gene expression of melon genotypes with orange flesh were similar, also, melon genotypes with green flesh were similar under infection condition, at the same time, the plant introductions of PI 140471 and PI 266935 were similar in their gene expression under infection condition.

Table (2):Genetic distance among sixteen melon genotypes under healthy condition, based on means of morphological and physiological performance

Genotypes	Galia	Ideal	Rafegal	Regal	Polidor	Marsamatrouh	PI 140471	PI 183227	PI 266935	Honeydew green flesh.	Hale's Best	PI 124111	Cinco	Honeydew orange flesh	Charantaize	Ismaelawi
Galia	0.00															
Ideal	1.864	0.00														
Rafegal	2.409	3.943	0.00													
Regal	1.678	3.215	2.443	0.00												
Polidor	1.843	3.125	2.676	1.557	0.00											
Marsamatrouh	0.589	2.186	2.349	1.552	1.690	0.00										
PI 140471	9.770	8.883	12.081	10.096	10.208	9.840	0.00									
PI 183227	3.475	5.238	2.283	2.474	3.160	3.195	12.212	0.00								
PI 266935	9.593	8.499	11.927	10.060	10.160	9.751	2.030	12.305	0.00							
Honeydew green flesh	2.052	1.710	4.264	3.000	2.473	2.135	8.290	5.098	8.086	0.00						
Hale's Best	2.479	2.563	4.646	2.668	2.787	2.554	7.606	4.955	7.503	1.634	0.00					
PI 124111	4.243	3.793	6.466	4.475	4.768	4.330	5.703	6.691	5.6737	3.211	2.164	0.00				
Cinco	1.955	1.148	4.178	3.144	3.095	2.215	8.325	5.244	7.963	1.305	1.890	3.179	0.00			
Honeydew orange flesh	1.735	1.445	4.017	2.734	2.767	1.927	8.297	4.871	8.004	1.137	1.477	2.977	0.662	0.00		
Charantaize	1.938	2.684	3.880	1.920	2.020	1.798	8.447	4.012	8.474	1.693	1.120	3.004	2.175	1.680	0.00	
Ismaelawi	8.119	9.544	5.836	7.863	7.887	7.948	17.677	5.877	17.598	9.754	10.232	12.133	9.782	9.644	4.931	0.00

Table (3) :Genetic distance among sixteen melon genotypes under infection conditions, based on means of morphological and physiological performance

Genotypes	Galia	Ideal	Rafegal	Regal	Polidor	Marsamatrouh	PI 140471	PI 183227	PI 266935	Honeydew green flesh.	Hale's Best	PI 124111	Cinco	Honeydew orange flesh	Charantaize	Ismaelawi
Galia	0.00															
Ideal	1.160	0.00														
Rafegal	0.529	1.238	0.00													
Regal	1.084	2.183	1.244	0.00												
Polidor	1.282	2.229	1.473	0.765	0.00											
Marsamatrouh	0.504	1.447	0.824	0.987	1.090	0.00										
PI 140471	6.225	6.714	6.109	6.045	6.767	6.332	0.00									
PI 183227	4.414	4.643	4.625	4.309	3.630	4.169	10.235	0.00								
PI 266935	6.224	6.643	6.108	6.098	6.770	6.350	0.894	10.291	0.00							
Honeydew green flesh	2.069	2.484	2.011	2.367	2.976	2.233	4.625	6.345	4.589	0.00						
Hale's Best	1.354	2.328	1.416	0.869	1.514	1.395	5.328	5.080	5.335	1.799	0.00					
PI 124111	1.861	2.812	1.787	1.327	1.958	1.897	4.931	5.498	4.967	1.965	0.911	0.00				
Cinco	1.039	0.886	1.142	1.825	1.892	1.316	6.570	4.596	6.515	2.338	1.939	2.555	0.00			
Honeydew orange flesh	0.930	1.046	1.008	1.632	1.850	1.202	6.093	4.859	6.012	1.975	1.560	2.133	0.637	0.00		
Charantaize	1.386	2.451	1.573	0.574	0.911	1.137	6.175	4.131	6.264	2.619	1.195	1.576	2.166	2.012	0.00	
Ismaelawi	6.717	6.649	6.895	6.790	6.099	6.545	12.738	2.684	12.764	8.675	7.533	7.995	6.645	7.016	6.683	0.00

These results indicate that, the plant introductions of PI 140471 and PI266395 were the most diverse than all melon genotypes followed by PI 124111, thereby they could be useful in breeding program for raising a new hybrid tolerant or resistant to Fusarium root rot.

These findings were found in agreement with those found by Ameral *et al.*, 1996 and El-Shimi, 2003.

The average inter cluster and intra-cluster genetic distance are presented in Table (4) in natural condition.

Table (4): Intra cluster and Intr- cluster genetic distance among sixteen melon genotypes, in healthy condition.

Cluster	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII
Cluster I	1.145						
Cluster II	0.660	0.00					
Cluster III	0.522	1.125	0.240				
Cluster IV	0.459	0.829	0.571	0.194			
Cluster V	1.086	1.673	0.614	1.143	0.278		
Cluster VI	2.049	1.453	2.541	2.117	3.108	0.00	
Cluster VII	2.522	3.133	2.038	2.492	1.493	4.546	0.00

Data reveled that, the maximum inter-cluster distance was observed between cluster VII and cluster VI (4.546), and between cluster VII and cluster III (3.133), followed by a distance of (3.018) between cluster VI and cluster V, suggesting wide range of diversity between all of them.

The minimum inter cluster distance (0.459) was observed between cluster IV and cluster I, also, between cluster IV and cluster III (0.571), indicating close relationship among the included genotypes.

Under infection condition, data presented in Table (5) declared that , the maximum inter cluster distance (2.966) was observed between cluster VII and cluster III followed by cluster VI and cluster III, (1.721), followed by a distance of (1.621) between cluster V and cluster III, meanwhile, the minimum inter cluster distance (0.259) was observed between cluster V and cluster I. These results indicated that, gene expression of melon genotypes had changed according to their ability against disease severity and their tolerance against the pathogen. These results were in agreement with Wahab and Gopalakrishnan (1993).

Table (5): Intra cluster and intr- cluster genetic distance among sixteen melon genotypes, in infection condition.

Cluster	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII
Cluster I	0.091						
Cluster II	0.293	0.187					
Cluster III	1.600	0.506	0.115				
Cluster IV	0.536	0.582	0.184	0.00			
Cluster V	0.259	0.462	1.621	0.533	0.082		
Cluster VI	0.319	0.589	1.1721	0.641	0.236	0.00	
Cluster VII	1.423	1.482	2.966	1.931	1.475	1.439	0.347

Table 5The intra- cluster distance ranged from (0.00) between the genotypes of

cluster II up to (4.546) between the genotypes of cluster VI, under healthy condition, mean while, the maximum intra cluster distance (2.966) was observed between the genotypes of the cluster III, indicting that, these melon genotypes could be useful in breeding program against Fusarium root rot, while the minimum intra cluster distance was observed between the genotypes of cluster I (0.091).

Based on the extent of relative dissimilarity among genotypes based on morphological characters, the sixteen melon genotypes were grouped into seven clusters. The grouping pattern and distribution of melon genotypes into different clusters are given in (Tables 6 and 7). Cluster II and VII, had single genotype.

Table (6) The grouping pattern and distribution of melon genotypes into clusters, under healthy condition.

Cluster	No. of genotypes	
I	2	Hales Best and Charantaize
II	1	PI 124111
III	4	Galia, Regal, Polidor, Marsmatrouh
IV	4	Ideal, Honeydew green flesh, Cinco and Honeydew orange flesh
V	2	Rafegal and PI 183227
VI	2	PI 140471 and PI 266395
VII	1	Ismaelawi

The data suggested that , PI 124111 genotypes, (cluster II) and Ismaelawi, (cluster VII) most diverged from the other genotypes, while the plant introductions of PI 140471 and PI 266395 came together in cluster VI. Also, Marsamatrouh Egyptian melon landrace came with Galia, Regal , and Polidor in the same cluster, while Cinco and Honey dew orange flesh were in the same cluster (V). Under infection conditions, the grouping pattern of the melon genotypes had changed according to the ability genotypes to tolerate the disease severity.

Data in Table (7) indicated that, honeydew green flesh came in single cluster of IV as alone, while Ideal was positioned in cluster VI as alone. Meanwhile Regal, Polidor, hales Best, PI 124111 and Charantaize came in the cluster II together. While Cinco and Honey dew orange flesh positioned in one cluster of cluster VI . On the other hand, PI 183227 and Ismaelawi had the same cluster of VIII.

These results indicated that, these genotypes were genetically most diversed, and the ability to resist Fusarium root rot were differed according to the genetic material. Also, results indicted that, Honey dew green flesh and Ideal were mostly diverged than the others, under infection condition, and the plant introductions of PI 140471 and PI 266935 were similar in their resistance to Fusarium root rot. Meanwhile, Cinco and Honey dew orange flesh are similar in their genetic background against Fusarium root rot.

Table (7) Grouping pattern of the sixteen melon genotypes under infection conditions.

Cluster	No. of genotypes	
Cluster I	3	Galia, Regal, and Marsmatrouh
Cluster II	5	Regal, Polidor, Hales Best, PI 124111 and Charantaize
Cluster III	2	PI 124111 and PI 266935
Cluster IV	1	Honey dew green flesh,
Cluster V	2	Cinco and Honey dew orange flesh
Cluster VI	1	Ideal
Cluster VII	2	PI 183227 and Ismaelawi

Data in Table (8) declared that, cluster III recorded the first rank for plant height and number of male flowers/ plant, while cluster V recorder the first rank for number of prefect flowers and crop growth rate. This indicated that, for mass production the cultivars of cluster V Cinco and Honey dew orange flesh consider the best genotypes for yielding under infection conditions, which gave highest yield and total phenols. For earliness the cultivars of cluster II Regal, Polidor, Hales Best, Pi 124111 and Charantaize recorded the first rank and also, for leaf area. For chlorophyll contents (a, b and carotinoids) the cultivars of cluster VII (PI 183227 and Ismaelawi) recorded the first rank. Meanwhile, the same cultivars of cluster VII recorded the first rank for average fruit weight and fruit length, while the cluster II recorded the first rank for total soluble solids.

Molecular markers

The results of DNA polymorphism and densitometric analysis for twelve melon genotypes are presented in Table(9). All the melon genotypes were analyzed via RAPD - PCR technique to find out some molecular genetic markers related to infection condition. For the primers, all the melon genotypes successfully amplified DNA fragments. The primer OPC-20 gave the least number of bands (5 bands), while the primer OPB-13 gave the highest number of bands (12 bands), followed by the primer OPD-03 (11 bands), meanwhile, the primers OPC-08 and OPB-11 were equals in band numbers (6 bands).

The highest molecular weight was recorded by the primer OPB-11 at the band No.-1 in honey dew orange flesh (1933 bp), which was monomorphic band. Also, band number 1 with the primer OPD-03 had high molecular weight (1638 bp) but it was polymorphic band.

For the studied genotypes, data in (Table 10) declared that, PI 140471 gave the highest number of markers under infection case, (41 markers) followed by Galia, (36 markers), Rafegal (35 markers) Regal (34 markers), Polidor and PI 183227 (33 markers) ,while the least markers was recorded by the PI 266935. Also, Honey dew orange flesh gave (30 markers), Charantaize (31 markers).

Table (8): Mean values of characters within clusters for different melon genotypes, under infection condition.

Cluster	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII
Plant height	127	133.79	136.83	125.66	76.83	127	135.83
Number of male flowers	89.11	88.59	96.83	79.33	85.66	93	94.16
Number of prefect flowers	2.44	2.80	2.49	2.00	3.18	2.95	2.55
Crop growth rate	1.24	1.29	1.27	1.24	1.32	126	1.25
Time of opening 50 % of male flowers	53.33	53.66	56.33	54.33	54.66	56.33	56
Time of opening 50 % of prefect flowers	66	65.40	67.5	70	66.50	67	71
Leaf area	41.96	57.60	54.28	34.85	34.77	29.54	56.07
Chlorophyll a	0.1348	0.1898	0.2232	0.2072	0.2043	0.1843	0.2461
Chlorophyll b	0.2445	0.3301	0.3545	0.3433	0.2904	0.2774	0.3873
Carotenodes	0.1213	0.1900	0.0983	0.0622	0.1492	0.1389	0.3256
Total phenols	0.045	0.219	0.495	0.039	0.839	0.345	0.268
Average fruit weight	461.92	448.86	240.55	382.81	457.49	472.11	724.01
Average fruit length	9.62	10.10	5.50	10.50	10.08	9.40	12.01
Average fruit diameter	9.44	8.58	4.92	10.83	8.58	9.86	10.24
Flesh thickness	1.7	1.73	0.68	1.50	1.50	1.87	1.91
Total soluble solids	8.49	9.26	7.25	8.5	8.66	8.63	8.58

Table (9): DNA polymorphism using randomly amplified polymorphic DNA, OPC-20, OPB-17, OPC-8, OPB-11, OPD-03 and OPB –13 primers twelve melon genotypes

	Bands number	Molecular weight bp	1 Galia	Ideal	Rafegal	Regal	Polidor	6 PI 140471	7 PI 183227	PI 266935	Honeydew , Green flesh	PI 124111	Honeydew , Orange flesh	Charantaize
OPC-20	1	1165	1	1	1	0	0	1	1	0	0	0	1	1
	2	1012	1	1	1	1	1	1	1	1	1	1	1	1
	3	914	1	1	1	0	1	1	1	1	1	1	1	1
	4	845	1	1	1	1	1	1	1	0	1	0	1	1
	5	772	1	0	1	0	0	0	0	0	0	0	0	0
Total			5	5	5	2	3	4	4	2	3	2	4	4
OPB-17	1	1209	0	0	1	1	1	1	0	0	0	0	0	0
	2	1148	0	0	1	1	1	1	0	0	0	0	0	0
	3	1043	1	1	1	1	1	1	1	1	1	1	1	1
	4	955	0	0	0	0	0	0	0	0	0	1	0	0
	5	855	0	0	1	1	1	1	0	0	0	0	0	0
	6	770	1	1	1	1	1	1	0	0	0	0	0	0
	7	393	1	1	1	1	1	1	1	1	1	1	1	1
	8	385	0	0	1	1	1	1	0	0	0	0	0	0
Total			3	3	7	7	7	7	2	2	2	3	2	2
OPB-08	1	1263	1	1	1	0	1	1	1	0	1	1	1	1
	2	900	1	1	1	1	1	1	1	1	1	1	1	1
	3	783	1	1	1	1	1	1	1	1	1	1	1	1
	4	695	1	1	1	1	1	1	0	0	0	1	1	1
	5	541	1	1	1	0	1	1	0	0	0	2	1	1
	6	307	1	1	1	1	1	1	1	1	1	1	1	1
Total			6	6	6	4	6	6	4	3	4	5	6	6
OPB-11	1	1933	0	0	0	0	0	0	0	0	0	0	1	0
	2	1467	0	0	0	0	0	0	0	0	0	0	1	0
	3	1250	1	1	1	1	1	1	1	1	1	1	1	1
	4	1000	1	1	1	1	1	1	1	1	1	1	1	1
	5	508	0	0	1	1	1	1	1	0	1	0	1	1
	6	234	0	0	1	1	1	1	1	0	1	0	1	1
Total			2	2	4	4	4	4	4	2	4	2	6	4
OPD-03	1	1638	1	1	0	0	0	1	1	0	0	0	0	0
	2	1475	1	1	0	0	0	1	1	1	1	1	0	0
	3	1325	1	1	1	1	1	1	1	1	1	1	1	1
	4	1163	1	1	1	1	1	1	1	1	1	1	1	1
	5	1038	1	1	0	1	0	1	1	1	1	1	0	0
	6	854	1	1	0	1	0	1	1	0	1	1	0	0
	7	692	1	1	0	1	0	1	1	1	1	1	0	0
	8	615	1	1	0	1	0	1	1	1	1	1	0	0
	9	470	1	1	1	1	1	1	1	1	1	1	1	1
	10	350	0	0	0	0	0	0	0	1	0	0	0	0
	11	250	0	0	0	0	0	0	0	1	0	0	0	0
Total			9	9	3	7	3	9	9	9	8	8	3	3
OPB-13	1	1214	1	0	1	1	1	1	1	0	0	0	0	1
	2	1114	1	1	1	1	1	1	1	0	0	0	0	1
	3	1062	1	0	1	1	1	1	1	1	1	1	1	1
	4	1024	1	0	1	1	1	1	1	1	1	1	1	1
	5	963	1	1	1	1	1	1	1	1	1	1	1	1
	6	950	0	0	0	0	0	1	0	0	0	1	0	1
	7	840	1	0	1	1	1	1	1	0	0	0	1	1
	8	780	1	0	1	1	1	1	1	0	1	0	1	1
	9	565	1	0	1	1	1	1	1	0	1	0	1	1
	10	517	1	0	1	1	1	1	1	0	1	0	1	1
	11	393	1	0	1	1	1	1	1	0	1	0	1	1
	12	219	1	0	0	0	0	0	0	0	1	0	1	1
Total			11	2	10	10	10	11	10	3	8	4	9	12

For preselected random primers (OPC-20, OPB-17, OPC-08, OPB-11, OPD-03 and OPB-13) were used in the present study to clear the gene expression of the twelve melon genotypes under infection condition, were illustrated in (Fig 1 and Table 10). Total number of DNA fragments wee fourty eight , which the genotype PI 140471 gave fourty one DNA fragments followed by Galia thirty six DNA fragments, while the least genotype amplifying DNA fragments was PI 266935. The results of RAPD analysis showed that, primer OPB-13 recorded the first rank for polymorphic applications, which gave twelve DNA fragments followed by OPD-03 primer, eleven DNA fragments, meanwhile, the least DNA fragments produced were with the primer OPC-08. Meanwhile, the primer OPB-13 exhibited the highest rank of polymorphism (92 %), followed by OPC-20, while the primer OPC-08 gave the least polymorphism (50 %).

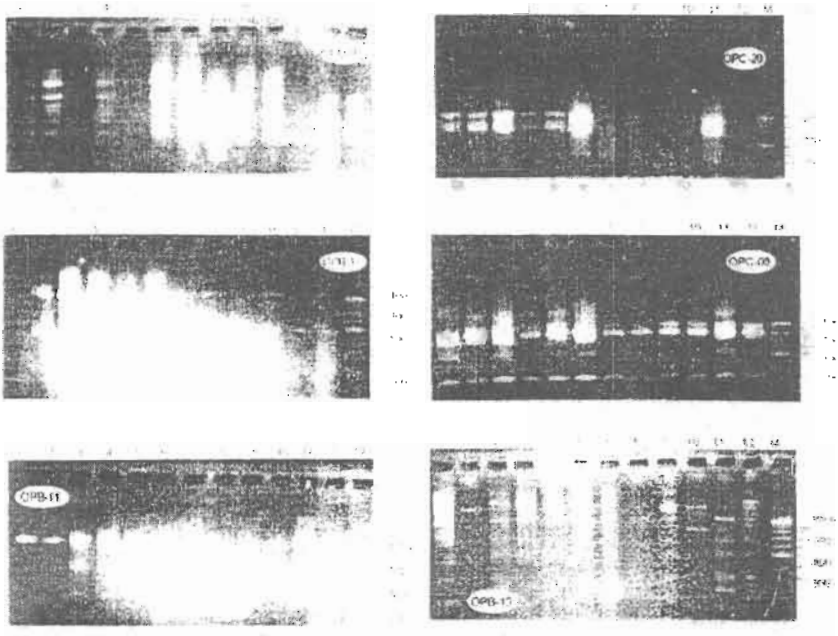

Fig. (1):RAPD profiles detecting markers among melon genotypes generated by primers OPC-20, OPD-03, OPB-17, OPC-08, OPB-13 and OPB-11

These results were in agreement with those obtained by Guirgis (2000), who reported that, total of 97 polymorphic DNA fragments resulting from 10 random primers could be identified by him and were used for the computation of the genetic similarities, and added that DNA amplification of melon and cucumber DNA sequences revealed that a high degree of genetic variability among the used genotypes.

The same results were obtained by Atta and El-Shimi (2003), they reported that , 82 % polymorphism were revealed in the ten tested melon genotypes.

Table (10): Number of amplifying DNA fragments of polymorphic bands produced by each RAPD primer for ever twelve melon genotypes

	1 Galia	2 Ideal	3 Rafegal	4 Regal	5 Polidor	6 PI 140471	7 PI 183227	8 PI 266935	9 Honeydew, Green flesh	10 PI 124111	11 Honeydew, Orange flesh	12 Charantaize	Total number of amplicons	Polymorphic amplicons	Polymorphism %
OPC-20	5	4	5	2	3	4	4	2	3	2	4	4	5	4	80
OPB-17	3	3	7	7	7	7	2	2	2	3	2	2	8	6	75
OPC-08	6	6	6	4	6	6	4	3	4	5	6	6	6	3	50
OPB-11	2	2	4	4	4	4	4	2	4	2	6	4	6	4	67
OPD-03	9	9	3	7	3	9	9	9	8	8	3	3	11	8	73
OPB-13	11	2	10	10	10	11	10	3	8	4	9	12	12	11	92
Total	36	26	35	34	33	41	33	21	29	24	30	31	48	36	75
Average	6	4.3	5.8	5.7	5.5	6.8	5.5	3.5	4.8	4	5	5.2	8	6	

We can concluded that, the expression of melon genotype PI 140471 under infection condition was the best, followed by Galia , Polidor, Rafegal, Honey dew orange flesh and Charantaize.

Data presented in Table (11) reveled that, the highest value of similarity coefficient was observed between Polidor and Regal (0.958) followed by Honey dew green flesh and PI 183227 (0.917), then Charantaize and Honey dew orange flesh (0.896). the least values recorded between melon genotypes were, PI 124111 and Rafegal (0.479), PI 266935 and Rafegal (0.458).

There results indicated that, the gene expression of these melon genotypes had changed according to infection severity, and same melon genotypes like PI 124111, PI 266935 and Rafegal may be used as parents for breeding program against Fusarium wilt as moderately resistant parents, while the strong parents were PI 140471 and Honeydew orange and Cinco.

Phylogentic relationship under infection condition.

The dendogram tree (Fig. 2) among melon genotypes for polymorphism detected on DNA levels and across the two levels as well as the pair wise markers differences were detected by DLCE computer package. The analysis was based on the number of markers that were different between any given of pair genotypes. The least average difference (the strongest relationship) was scored between PI 266935 and Polidor , between PI 140471 and PI 266935.

Dendogram analysis separated the twelve melon genotypes into two major clusters. Within the first cluster, three sub clusters can be observed, namely sub cluster I, which involved Rafegal,Polidor, Regal and PI 140471, sub cluster II, which involved Honeydew oreng flesh and Charantaize, sub clucter III, which involved PI 183227, Honeydew green flesh and Galia .This indicate that, Honeydew and Charantaize were related to each other, under the infection condition. On the other hand, the melon genotypes PI 266935, PI 124111 and Ideal belonging to the last cluster while, PI 266935 and PI 124111 were apparently related to each other, under the infection condition , while Ideal grouped alone.

These findings were found in agreement with those obtained by Guirgis (2000) and Stuab *et al.* (1997).

Table (11): The similarity coefficient among twelve melon genotypes based on combined analysis of amplified RAPD fragments after using all primers.

Genotypes	Galia	Ideal	Rafegal	Regal	Polidor	PI 140471	PI 183227	PI 266935	Honeydew , green flesh	PI 124111	Honeydew , orange flesh	Charantaize
Galia	1											
Ideal	0.792	1										
Rafegal	0.729	0.56	1									
Regal	0.708	0.583	0.813	1								
Polidor	0.688	0.563	0.958	0.854	1							
PI 140471	0.813	0.688	0.833	0.854	0.833	1						
PI 183227	0.854	0.729	0.708	0.771	0.708	0.833	1					
PI 266935	0.604	0.729	0.758	0.563	0.500	0.500	0.667	1				
Honeydew , green flesh	0.771	0.688	0.667	0.771	00708	0.750	0.917	0.7 50	1			
PI 124111	0.667	0.792	0.479	0.583	00521	0.604	0.688	0.854	0.771	1		
Honeydew , orange flesh	0.708	0.583	0.771	0.625	0.771	0.646	0.729	0.563	0.771	0.583	1	
Charantaize	0.771	0.604	0.83	0.688	0.833	0.750	0.729	0.542	0.750	0.604	0.896	1

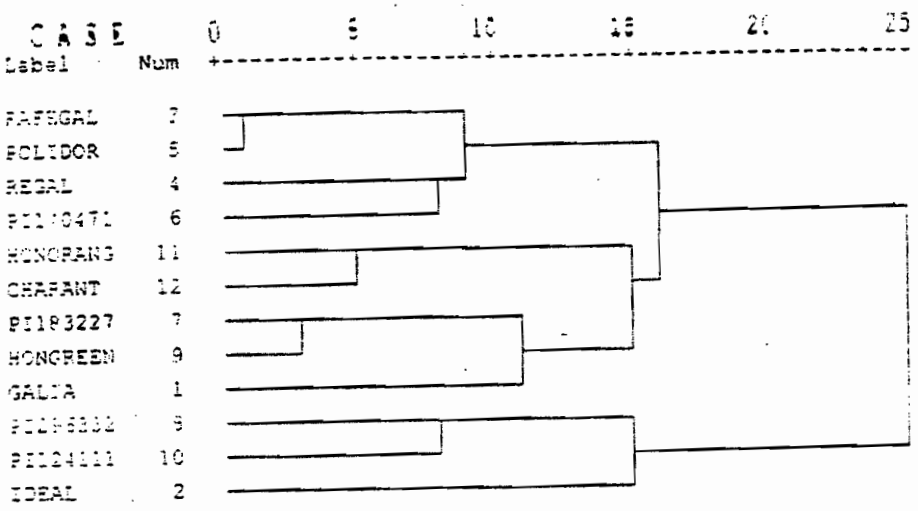

Fig. (2):Dendogram tree demonstrating the relationship among the melon genotypes based on RAPD markers

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استخدام بعض الواسمات البيولوجية والجزئية فى تحديد صفة المقاومة لمرض الذبول الفيوزارمي فى الكانتلوب

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تم تجميع ستة عشر سلالة من الكانتلوب يشمل أصناف بستانية– وسلالات نقية (مرباه داخليا لمدة ٨ أجيال متتالية ومدخلات نباتية– وقد زرعت هذه السلالات التراكيب الوراثية فى تجارب مصممة بطريقة القطاعات الكاملة العشوائية فى ثلاث مكررات بمحطة بحوث البساتين بالقصاصين فى أعوام ٢٠٠٦ ، ٢٠٠٧ وذلك للبحث عن المعلمات البيولوجية التى تتعلق بمعدل اداء التراكيب الوراثية ن وكذلك المعلمات الجزئية– تحت ظروف الإصابة بالمسبب المرضى للذبول.

وقد أظهرت النتائج مايلى:–

١ . كانت هناك فروق معنوية فى متوسط أداء جميع التراكيب الوراثية ، وكذلك ظهر مدى واسع من الاختلافات بينها لجميع الصفات المدروسة.

٢ . أظهرت النتائج أن التراكيب الوراثية للكانتلوب ذات اللحم الداخلى الأخضر (طراز جاليا) كانت متقاربة لبعضها ، كذلك التراكيب الوراثية للكانتلوب ذات اللحم الداخلى البرتقالى (طراز شرانتيز) كانت أيضا متقاربة فيما بينها وذلك تحت الظروف الطبيعية.

٣. حدث تغير فى التعبير الجينى لجميع التراكيب الوراثية وذلك تحت الظروف الصناعية بالمسبب المرضى للذبول فنجد أن المسافة الوراثية قد انخفضت قيمتها مقارنة بالظروف الطبيعية.

٤. كانت المسافة الوراثية بين التراكيب الوراثية الأتية صغيرة وهى جاليا- اديال- رافيجال- ريجال- بوليدور- مرسى مطروح وذلك بعد حدوث الإصابة، وفى الوقت نفسه كانت المسافة الوراثية كبيرة بين المدخل النباتى PI 140471 وجميع التراكيب الوراثية المستخدمة ، وكذلك المدخل النباتى PI 266935.

٥ كما أشارت النتائج الى أن المسافة الوراثية بين المدخل النياتى PI 140471 ن والمدخل النباتى PI 266935 كانت صغيرة (٠,٨٩٤) مما يؤكد القرابة الوراثية بينهما، بينما المسافة الوراثية كبيرة جدا بين المدخل النباتى PI 140471 والمدخل النباتى PI 183227 تحت ظروف الإصابة مما يؤكد اختلافهما فى المنشأ.

٦. أظهرت نتائج التحليل العنقودى للبيانات البيولوجية للتراكيب الوراثية للكانتلوب أن جميع التراكيب الوراثية للكانتلوب المستخدمة فى الدراسة ترتبت فى ٧ مجاميع ، وان أقصى مسافة وراثية قد سجلت بين المجموعة السابعة والمجموعة السادسة حيث كانت (٤,٥٤٦) ، وفى نفس الوقت كانت أقل مسافة وراثية موجودة بين المجموعة الرابعة والمجموعة الأولى (٥,٤٥٩) تحت الظروف الطبيعية.

٧. تحت ظروف الإصابة بفطريات الذبول حدث تغير فى التعبير الجينى لجميع التراكيب الوراثية- فنجد أن المجموعة الأولى تضم جاليا، رافيجال ، مرسى مطروح ، والمجموعة الثانية تضم: ريجال- بوليدور- هالز بست- المدخل النباتى PI 124111 – شرانتيز؛ أما المجموعة الثالثة فتضم : PI 140471 -PI 266935؛ أما المجموعة الرابعة فتضم: هنى ديو (جرين) بمفرده ؛ والمجموعة الخامسة فتضم: سينكو ، هنى ديو (أورانج) ؛ والمجموعة السادسة فتضم:اديال بمفرده ، والمجموعة السابعة فتضم: الأسماعيلاوى والمدخل النباتى PI 183227.

٨. تشير هذه النتائج الى أن أفضل أداء بيولوجى كان للمدخل النباتى PI 140471 وكذلك PI 266935 تحت ظروف الإصابة بمسببات الذبول- وأيضا للصنفين البستانيين سينكو ، هنى ديو (أورانج) ويمكن إدخالهم فى برنامج تربية لانتاج هجن مقاومة للذبول الفيوزارمى.

٩. بالنسبة لتكنيك الDNA فقد أشرت النتائج الى أن أفضل أداء كان من نصيب المدخل النباتى PI 140471 الذى أعطى أعلى رقم من المعلمات الجزيئية تحت ظروف الأصابة يليه جاليا- ثم رافيجال- ثم ريجال ثم شارنتيز يليه هنى ديو (أورانج) – وكان أقلهم المدخل النباتى PI 266935 .

١٠. وقد أشارت تحاليل القرابة الوراثية الى أن التراكيب الوراثية المختلفة للقاوون قد توزعت فى ٣ مجاميع رئيسية ؛٥ مجاميع فرعية بينما جاءت التراكيب الوراثية جاليا- اديال- المدخل النباتى PI 140471 بمفردها وكذلك ريجال.

١١. كما أشارت بيانات التحليل العنقودى الى أن:- أفضل التراكيب الوراثية التى يمكن أن تعطى أعلى محصول تحت ظروف الأصابة هما الصنف البستانى هنى ديو (أورانج) ، والصنف سينكو ، بينما تعتبر التراكيب الوراثية للمجموعة النباتية مبكرة النضج وتضم ريجال ، بوليدور، هالز بست والمدخل النباتى PI 124111 وكذلك الشرانتيز ، بينما تعتبر أفراد المجموعة الثالثة الأفضل فى الحصول على أعلى عدد من الأزهار المذكرة وكذلك صفة طول النبات وتضم المدخل النباتى PI 140471 ، PI 266935 ، بينما أعطت أفراد المجموعة الرابعة هنى ديو (جرين) أفضل قيمة لصفة قطر الثمرة ، وأعطت المجموعة الخامسة التى تضم سينكو ، هنى ديو (أورانج) أعلى القيم لصفات عدد الأزهار الكاملة/ نبات ، معدل نمو النبات ، أعلى تركيز للفينولات ؛ بينما أعطت المجموعة السادسة التى تضم اديال ميزة نسبية فى ميعاد تفتح الأزهار المذكرة على النبات وسمك اللحم ، بينما تميزت أفراد المجموعة السابعة وتضم الأسماعيلاوى والمدخل النباتى PI 183227 بأنها أعطت أعلى القيم من كلوروفيلات أ، ب، والكاروتينويدات وكذلك أعلى متوسط وزن للثمرة وطول وقطر الثمرة .