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Evaluation of Some New Almond X Peach Hybrids Rootstocks Resistant to Nematode

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Abstract: This study was conducted to assess the morphological, chemical diversity and nematode resistance (*Meloidogyne incognita*) among new six hybrids coming from hybridization between Om Elfahm almond rootstock as mother plant and Okinawa peach rootstock as father plant through two successive seasons (2017 and 2018) in Giza governorate. The chosen hybrids trees were 8 years old grown in clay loamy soil and regular horticulture practices were applied as recommended. The results revealed that all these new hybrids were almost similar in morphological shape. They started flowering in early February and flowering continues for a month for all of the hybrids. Flowering period was approximately 9-15 day. These hybrids start vegetative growth in last week of February and that was nearly the same time for starting fruit set. Fruit set percentage ranged between 25% and 60%. As for vegetative growth, it was clear that hybrid number 4 had the longest branch length while hybrid number 2 had the highest number of shoots per branch. Hybrids number 1&2 have the most leaves number while hybrids number 1, 3 &6 had the largest leaf area. For chemical study, hybrid number 4 had the highest carbohydrate %, nitrogen % and C/N ratio while hybrid number 5 was the highest in chlorophyll percentage. Nematode evaluation cleared that hybrids number 3, 4, 5 and 6 show high resistant to nematode while hybrids number 1&2 show less resistant to it. Simple sequence repeats (SSR) and sequence tagged site (STS) techniques were used to find some molecular genetic markers related to root knot resistance. The primer Pchgms1showed a fragment related to root knot nematode with all new hybrids except hybrid number 6. On the other hand, primer OPA11 gave a fragment in all hybrids. Primer OP834B showed a fragment at molecular size (127bp.) in all hybrids and primer OPAP4 detected a fragment of the expected size (183bp.) appeared only in hybrid number 5.

Key words: Evaluation · Almond X peach hybrids · Nematode resistance (*Meloidogyne incognita*) · Rootstocks · SSR · STS

INTRODUCTION

Prunus belongs to the *Prunoideae*, a subfamily of *Rosaceae* which includes several species. Genus *Prunus* comprises around 98 species which all the stone fruits are included in this group. Three subgenera namely: *Amygdalus* (peaches and almonds), *Prunophora* (plums and apricots) and *Cerasus* (cherries) under *Prunus* are universally accepted. The genus *Prunus* often called the stone fruits and it is one of the most important genera of woody plants.

Almond (*Prunus dulcis miller*) is one of the most important nut crops worldwide and one of the oldest nuts tree [1]. Today it represents the largest production of many nut tree products. Almond grows in regions of the world that are characterized by having a subtropical Mediterranean climate with wet winters and warm dry summers [2].

Production of peach is about 415932 ton in whole cultivated area about 62614fed. Production ranged between 7 ton/fed in new reclaimed land sand which are concentrated in north and northeastern parts of the outvalley, Noubaria and NorthSaini and about 8 ton/fed in the old lands in Menoufia and Behera governorates [3]. Hybridization and crosses have been carried out between almond and peach. It can be readily achieved and have proven to be particularly valuable as rootstocks as well as sources of commercial useful trials [4].

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Breeding of rootstocks play a major role in modern culture. Recently, growers recognized the importance of the rootstock; breeding has an essential value for fruit yield. The most important agricultural traits and the tree as a biotic unit; such as vigor, blossom initiation, fruit set, fruit size and fruit flavor, etc.; may be, substantially, influenced by the rootstock [5, 6]. Moreover, the rootstock determines the ecological fitness of the tree. Rootstocks can be affecting the health status of critical tree phonological stages, tree kilter and tree sensitivity to pests and diseases [7]. Furthermore, in the efficiency of pest and disease management programs and fruit yield [8, 9]. Rootstocks with good ecological fitness are increasingly important in environmentally-friendly fruit production [10].

Cao Ke *et al.* [11] indicated that, the root knot nematode (RKN), *Meloidogyne incognita,* can cause severe crop loss in economically important *Prunus* species like peach, almond, plum and apricot. Some peach rootstocks, including Nemaguard, Nemared and Myrobalan display significant resistance to RKN.

Tree breeding process had benefits from using DNA molecular markers associated with genes for horticultural traits through marker-assisted selection (MAS). MAS would also allow screening for economically important traits in seedlings, which is especially useful for traits expressed only in fully mature trees. Additionally, MAS could expedite difficult screening procedures such as testing for disease or insect resistance also MAS can greatly improve the efficiency of peach breeding for resistance to root-knot nematodes [12].

Hence, the aim of this work was to study the morphological characters, vegetative growth, flowering parameters and nematode resistance of six new stone fruit hybrids which coming from hybridization between Om Elfahm almond cv. as a mother plant and Okinawa peach rootstock as father plant [13].

MATERIALS AND METHODS

The present study was conducted throughout two seasons 2017 and 2018 on new six mature almond x peach rootstocks (coming from hybridization between Om Elfahm almond rootstock as a mother plant and Okinawa peach rootstock as a father plant). These trees are eight years old planted in Horticulture Research Institute experimental orchard farm, Giza. These trees were planted under clay loamy soil conditions and regular horticulture practices applied as recommended.

Morphological Measurements: Four branches in four directions of each hybrid were labeled for studying morphological and flowering parameters which included:

Flowering Parameters: Beginning date of flowering, date of vegetative bud growth, beginning of fruit set and fruit set percentage were measured. Also, period of flowering in days for each hybrid was determined.

Vegetative Growth Parameters: Stem characters; Cylindrical shape, glabrous surface. Leaf characters; Irregular, alternate arrangement. Stipulate ordinary base. Leaf shape; ovate or serrate. Crenate leaf margin. Apex; obtuse or tapering. Base of blade, symmetrical. smooth upper surface and smooth lower surface coarse. Average of branch length. Average number of shoots per branch. Average of shoots length. Leaves number per shoot. Leaf area (cm^2) was measured at the end of August. Samples were collected from the fourth to the sixth leaves from the selected shoots by using Portable area meter model (LI -3000).

Chemical Analysis: At August samples were taken randomly from the fourth to the sixth leaves from the top of the previously tagged shoots of each tree (three leaves × four shoots). Leaf samples were washed with tap water and dried at 70°C till constant weight and then ground and stored for analysis. The ground samples were digested with sulphoric acid and hydrogen peroxide according to Evenhui [14].

Carbohydrates % were determined according to Dubois *et al.* [15].

Nitrogen % was determined according to the method of Pregl [16].

Leaf chlorophyll % was recorded using Minolta chlorophyll meter SPAD-502 (Minolta camera. Co, LtD, Japan) at the field. Average of ten recordings was taken from the middle of the leaves from the middle of canopy tree [17].

Evaluation of Hybrids to Root Knot Nematode Resistance: Decline soil and root samples were collected and prepare for nematode analysis from trees randomly selected in each sampling site. The roots of the selected plants were carefully surveyed to a soil depth of 5 to 40 cm. Roots along with surrounding soil were put into polythene bags. Roots gently washed then placed in the mist chamber for egg hatching. The hatching J2s were collected 5-day period after which the total number of hatched J2s plus those extracted from pot soil using the modified Baermann

funnel technique was counted. Therefore, roots were removed from the chamber and stained with acid fuchsin in cold lacto-phenol. Stained roots were rinsed in water and cut into pieces to facilitate counting of galls, females, egg masses and immature stages. Rate of nematode reproduction (Rr) was calculated by dividing final population (Pf) by the initial population (Pi). Gall indices were established using a 1-6 scale of Barker [18], 1=0galls; 2=1-10galls; 3=11-30galls; 4=31-70galls; 5=71-90galls and 6=91-100galls/ plant. The resistance rating and of each hybrid was estimated according to the scale of Taylor and Sasser [19], based on nematode reproduction and root galling. In this scale, I= immune (plant does not allow penetration of the nematode); HR= highly resistance (nematode invade root but there is little or no reproduction); R= resistant (limited reproduction with final nematode population lower than initial, incipient galling); MR= moderately resistance (final population equal or slightly higher than the initial, galling scare although noticeable) and S= susceptible nematode densities increase rapidly, causing abundant galling.

DNA Extraction: Young fresh leaf samples were collected separately from each genotype. Plant tissues were ground under liquid nitrogen to a fine powder, bulked DNA extraction was performed using DNeasy plant Mini Kit (QIAGEN). DNA isolation was achieved according to Mohamed [20].

Simple Sequence Repeats (SSRs): PCR reaction was conducted using 1 primer. Its name and sequence are shown as follow.

List of names and nucleotide sequences of the used SSR primers.

Name	Primer sequence
Pchgms1	F:GGGTAAATATGCCCATTGTGCAATC
	R:GGATCATTGAACTACGTCAATCCTC

Amplification was performed according to Yamamoto and Hayashi [21] with 35 cycles at 94°C for 1 min, 55°C for 1 min and 72°C for 2 min, for denaturation, annealing and primer extension, respectively.

Sequence Tagged Site (STS):

List of names and nucleotide sequences of the used STS primers.

Name	Primer sequence
OPA11	F: 5' TGCAACGTCACATTTTAACC 3'
	R: 5' GATCCAGCAGAGAAAACGAG 3'
OPB	F:GCAGTCAAAAATTTCAAACC
	R:TCCGATTCGAGCCCACTACA
OPP4	F:TTAAGACACCCAAACGATTTCA
	R:TGGGCATTTTGAGGTATCTG

The amplification was carried out according to Sosinski *et al.* [22] as follows: PCR reactions were performed by an initial denaturation for 4 min at 94°C followed by 32 cycles of: 45 s at 94°C, 30 s at 52-55°C (depends on primer as shown in table, 13), 30 s at 72°C; and a final extension of 5 min at 72°C.

The PCR Products Were Separated: The bands were visualized by ethidium bromide under UV florescence. Gels were photographed and scanned with Bio-Rad video densitometer Model 620, at a wave length of 577.

Data Analysis: The investigation was planned out as a factorial experiment in a complete randomized block design. The statistical analysis of the present data was carried out according to Sendecor and Cochran [23]. Significant differences among the means of various treatments were established by LSD at 5%level of probability. Data were analyzed by MSTAT-C.

RESULTS AND DISCUSSION

Beginning of Flowering, Flowering Period, Beginning of Vegetative Growth, Beginning of Fruit Set and Fruit Set%: Concerning beginning of flowering and flowering period in days Fig. (1 & 2) showed that, hybrids ranked trends may be due to different chilling requirements among hybrids. Herein, Hybrid No.3 was the first one to start flowering in both 2017 and 2018 (20/2 and 8/2 respectively) but in regard to its flowering period in days, it lasted for 11days in both seasons, while hybrid No. 4 was the last one to start flowering in both years 1/3 in the first season and 15/2 in the second season respectively and had the shortest flowering period in both seasons (9 days). Moreover, the longest flowering period was obtained from hybrid No. 1& 6 they lasted for 15 days approximately in both seasons. Other hybrids were in between the obtained values.

As for beginning of vegetative growth, Table 1 cleared that, hybrid No. 3 was the first one to start vegetative growth in first of March in the first season 2017 and 18 of February in the second season 2018. On the other hand, hybrid No. 4 was the last one to give vegetative growth (15th of March 2017 and 22nd of February 2018). Other hybrids were in between. While in 2018 hybrids began vegetative growth in different dates ranged from 18 to 20 February. It was clear from the obvious data that 2018 was cooler than 2017 that hybrids had their chilling requirements and start flowering early.

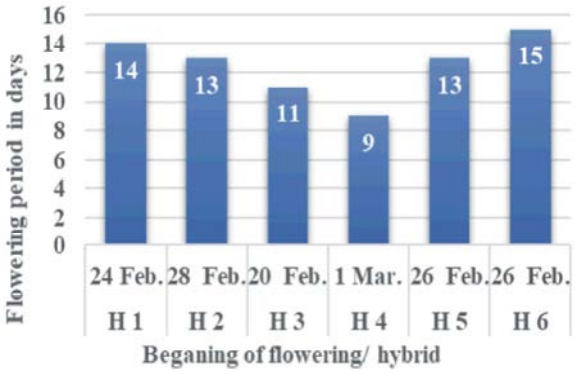

Fig. 1: Flowering period (days) for new 6 hybrids in 2017

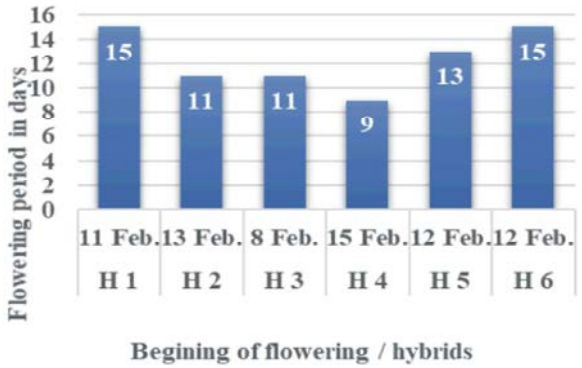

Fig. 2: Flowering period (days) for new 6 hybrids in 2018

Referring to the effect of hybrids on the beginning of fruit set Table (1) showed that, hybrid No. 3 was the earliest to start fruit set in both seasons (25/2/2017 and 18/2/2018). On the other hand, hybrid No.4 was the last in this respect. It started fruit set on 7/3/2017 and 25/3/2018 and the other hybrids were in between.

Concerning fruit set percentage in both seasons under study (Table 1); hybrids ranged between 60 % (hybrids 1, 3 and 6) then 50% hybrids 2 and 5. While hybrid No, 4 was the lowest one. There were no differences between the two seasons under study.

These results in agreement with those reported by Razavi *et al.* [24] and Liang Guo *et al.* [25] who reported that over the past 50 years, heat accumulation during tree dormancy increased significantly, while chill accumulation remained relatively stable. Heat accumulation was the main driver of bloom timing, with the effects of variation in chill accumulation negligible in Bejings cold winter climate.

Vegetative Growth Parameters

Stem and Leaf Characters: As shown in Table (2) there were no differences between all studied morphological characters of the considered hybrids. All the new rootstocks under study have cylindrical stem shape with smooth surface. At the same time all of them had irregular alternate leaf arrangement with stipulate ordinary leaf base. Also, all hybrids had hastate leaf shape with crenate leaf margin.

All the studied hybrids leaves had cordate apex with symmetrical blade base. Moreover, it was noticed that all new hybrids had smooth upper surface with coarse lower surface. These results are in agreement with Neveen and Azza [26] and Atalla [27] who reported that the leaf base of almond is acute and hastate in peach and the margin in stone fruit (except apricot) is always serrate.

Average of Branch Length, Shoots No. Per Branch, Shoots Length per Branch, Leaves No. Per Shoot and Leaf Area: Regarding various measurements i.e. average of branch length, shoots number per branch, shoots length per branch, leaves no. per shoot and leaf area of the considered hybrids data presented in Table (3) showed that:

About the average of branch length, Table 3 displays that, branch length was significantly longer in hybrid No. 4 as compared to the other hybrids in both years (113.2cm. and 120.2cm. in 2017 and 2018 respectively). On the other hand, hybrid No. 3 recorded the shortest significant average of branch length length in both seasons (56.2cm. in 2017 and 50, 4cm. in 2018). Other hybrids were in between the two significant values.

As for number of shoots per branch (Table 3) declared that, hybrids No. 2 and 4 recorded the highest significant number of shoots per branch. Hybrid No. 2 recorded (13.1-12.6) 2017 and 2018 respectively and hybrid No. 4 recorded 10.7 in the first season and 12.4 in the second season respectively. On the other hand, hybrid No. 3 and 5 recorded the lowest value for No. of shoots per branch with insignificant differences. Hybrid No. 3 recorded (5.1&4.2) and hybrid No. 5 was (5.7&6.3) in both seasons respectively.

Referring to average of shoot length per branch, Table (3) cleared the differences between hybrids in shoot length. Data cleared that hybrid No. 2 attained the highest significant value in both years (56.9cm & 51.1cm). While, hybrid No. 6 recorded the lowest significant value (13.2cm in 2017 and 15.9cm in 2018).

Concerning the average of leaves number per shoot, Table (3) shows that hybrids No. 1 and 2 obtained the highest significant value. Hybrid No.1 recorded 23.8 in 2017 and 21.5 in 2018 and hybrid No. 2 got 25.1 and 22.9 in both seasons. On the other hand, hybrid No. 4 shows the lowest significant number of leaves in both years under study (12.3 in 2017 and 14.3 in 2018).

Table 1: Beginning of vegetative growth, beginning of fruit set and fruit set % of new 6 hybrids

	Beginning of vegetative growth		Beginning of fruit set		Fruit set%	
Hybrid Number	2017	2018	2017	2018	2017	2018
Hybrid 1	5/3	23/2	2/3	18/2	60	60
Hybrid2	7/3	23/2	6/3	20/2	50	50
Hybrid3	1/3	18/2	25/2	18/2	60	60
Hybrid 4	15/3	22/2	7/3	25/2	25	25
Hybrid5	9/3	20/2	6/3	20/2	50	50
Hybrid6	5/3	18/2	5/3	20/2	60	60

Table 2: List of vegetative growth parameters of new 6 hybrids

Hybrid Number	Hybrid 1	Hybrid 2	Hybrid 3	Hybrid 4	Hybrid 5	Hybrid 6
Stem characters						
Cylindrical shape (+)	+	+	+	+	+	+
Glabrous surface (+)	+	+	+	+	+	+
Leaf characters:						
Arrangement: Irregular alternate (+)	+	+	+	+	+	+
Base: stipulate (-)	-	-	-	-	-	-
Leaf shape: hastate(+), Ovate (-)	+	+	+	+	+	+
Leaf margin: crenate(+)	+	+	+	+	+	+
Apex:						
Cordate(+), obtuse(-)	+	+	+	+	+	+
Base of blade: symmetrical(+)	+	+	+	+	+	+
Upper surface:						
Smooth (+)	+	+	+	+	+	+
Lower surface: coarse(+), Smooth(-)	+	+	+	+	+	+

Table 3: Average of branch length, shoot number per branch, shoot length, leave number per shoot and leaves area in new 6 hybrids in both 2017 and 2018

	Average of branch length (cm)		Average of shoots number per branch		Average of shoot length		Leaves number per shoot		Leaves area (cm^2)	
Hybrid Number	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
Hybrid 1	104.9ab	100.7b	8.2b	7.1bc	47.2b	40.8b	23.8a	21.5ab	3.65a	3.34a
Hybrid 2	81.4b	85.3c	13.1a	12.6a	56.9a	51.1a	25.1a	22.9a	2.85b	2.78b
Hybrid 3	56.2c	50.4d	5.1c	4.2c	41.5b	37.3b	17.5c	18.0c	3.21ab	3.08ab
Hybrid 4	113.2a	120.2a	10.7a	12.4a	28.5c	22.3c	12.3d	14.3d	2.97b	3.00ab
Hybrid 5	51.4c	55.8d	5.7bc	6.3bc	31.7c	35.7b	21.5b	18.8bc	2.84b	2.72b
Hybrid 6	78.7c	80.3c	6.9bc	7.5b	13.2d	15.9d	15.9c	12.7d	3.11ab	2.89ab

*Means within each column followed by the same letter(s) are not significantly different at 5% level

About the effect of hybrids on leaf area, it was clear in Table 3 that hybrid No. 1, 6 and 3 gave the highest significant value in both years' hybrid No.1 recorded 3.65 and 3.34 and hybrid No. 6 recorded 3.11 and 2.89 in 2017 and 2018 respectively. Meanwhile, hybrid No. 3 recorded 3.21 in 2017 and 3.08 in 2018.on the other hand hybrids No. 2, 4 and 5 recorded the lowest significant value.

These results are in line with Neveen and Azza [26], who mentioned that GF677 rootstock (hybrid between peach × almond) induced the highest significant shoot length and average length of internodes followed by Nemaguard.

Hybrids Content of Carbohydrates%, Nitrogen%, C/N Ratio and Chlorophyll%: Data presented in Table (4) showed the effect of different hybrids on chemical measurements (carbohydrate, nitrogen, C/N ratio and chlorophyll concentration)

As for the effect of hybrids on carbohydrate percentage, Table 4 showed that hybrid no 4 recorded the highest significant carbohydrate concentration in both years (55.4 %, in 2017 and 58.1 % in 2018) followed by hybrid No.1 (54.8 % in the first season and 53.7 % in the second season). On the other hand, hybrid No.5 recorded the lowest concentration as it recorded 40.2 % in 2017 and 39.8 % in 2018). Other hybrids were in between.

Table 4: Carbohydrates %, Nitrogen%, C/N ratio and Chlorophyll% content in 6 new hybrids in 2017 and 2018

	Carbohydrate %		Nitrogen%		C/N ratio		Chlorophyll %	
Hybrid Number	2017	2018	2017	2018	2017	2018	2017	2018
Hybrid1	54.8ab	53.7b	2.21a	2.14a	24.79	25.09	35.2c	43.8a
Hybrid 2	50.2bc	49.5bc	2.13a	2.07a	23.56	23.9	36.7c	39.1c
Hybrid 3	42.7d	44.5cd	2.04a	2.27a	20.93	19.6	42.5a	40.6bc
Hybrid 4	55.4a	58.1a	2.15a	2.16a	25.76	26.89	37.1bc	38.2c
Hybrid 5	40.2d	39.8d	2.23a	2.11a	18.02	18.86	41.5a	43.1ab
Hybrid 6	48.3c	47.8c	2.18a	2.20a	22.15	21.72	39.9ab	40.6bc

*Means within each column followed by the same letter(s) are not significantly different at 5% level

Table 5: Evaluation of new six hybrids resistance to root knot Nematode (*Meloidogyne incognita*)

	2nd stage juveniles in 250 g soil	Galls/1(g) of root		Females / 1 (g) of root	Egg masses /1(g) of root		Development stages/1(g) of root	
Hybrid Number	Average No.	Average No.	RGI	Average No.	Average No.	EI No.	Average No.	Resistance
Hybrid1	54a	5.6a	2	2a	1	1	2.6	R
Hybrid2	43b	4ab	2	1.3ab	1	1	2.3	R
Hybrid3	24c	4ab	2	0.6b	0.6	0	2	HR
Hybrid4	20c	3bc	2	0.6b	0	0	2	HR
Hybrid5	19c	3bc	2	0.6b	0	0	1.3	HR
Hybrid6	10d	2c	1	0.3b	0	0	0.6	HR

*Means on each column followed with the same letter(s) are not significant different at 5 % level.

*Root gall index (RGI) or egg-masses index (EI) was determined according to the scale given by Taylor& Sasser [19] as follows: 0= no galls or egg masses, 1= 1-2 galls or egg masses , 2= 3-10 galls or egg masses, 3= 11- 30 galls or egg masses, 4= 31-100 galls or egg masses and 5= more than 100 galls or egg masses.

R = Resistance, HR = High Resistance

About nitrogen percentage, Table 4 showed that there were no differences between hybrids in nitrogen concentration.

Referring to the effect of hybrids on C/N ratio, it was clear that hybrid No. 4 recorded the highest significant value in both years (25.76 % and 26.89 %). The opposite was recorded with hybrid No. 5 as it recorded the lowest significant values (18.02 % in 2017 and 18.86 % in 2018).

As for the effect of hybrids on chlorophyll percentage, it was clear from data in Table 4 that hybrid No. 5 recorded the highest significant value (41.5 % in 2017 and 43.1 % in 2018). On the opposite hybrid No.2 showed the lowest significant one (36.7% in 2017 and 39.1 % in 2018). Other hybrids were in between the two extremes.

These results are in agreement with those found by Attalla [27] who revealed that the highest number of leaves is developed on Okinawa peach, local apricot and sweet almond during the season. Leaf measurements indicate a significant elongation of peach leaves than the other stocks.

Evaluation of Hybrids to Root Knot Nematode *Meloidogyne incognita* Resistance: Data given in Table (5) indicated that all hybrids slightly varied in their resistance to the root knot nematode *Meloidogyne incognita*. However, hybrids 3, 4, 5 and 6 considered as highly resistant (HR), neither galls nor J2s were detected in soil or root tissues at the end of the evaluation. On the other hand, the rest hybrids (1&2) were considered resistance (R) where there were a low number of galls and final population (PE). Galls recorded with a few numbers only in the roots of the two hybrids.

Hybrid No.6 had the lowest galls of root (2gall/lg) with average 0.3 of females and 0.6 average of development stages. Differences were slightly significant between hybrid No.6 and both hybrids No. 4& 5 in average No. of galls and egg mass.

On the other hand, hybrids No.1 had the highest number of galls/root (5.6 gall/lg) with average2 females and 2.6development stages of roots. Hybrids No. 1&2 have slightly differences in all.

These obtained data strongly supported by the findings of Marull, *et al.* [28] who evaluated *Prunus* rootstock against *Meloidogyne incognita*. He cleared that, Garfi × Nemared (G×N) and Hansen were highly resistant to nematode but other rootstocks were susceptible showing high galling indices and population increases.

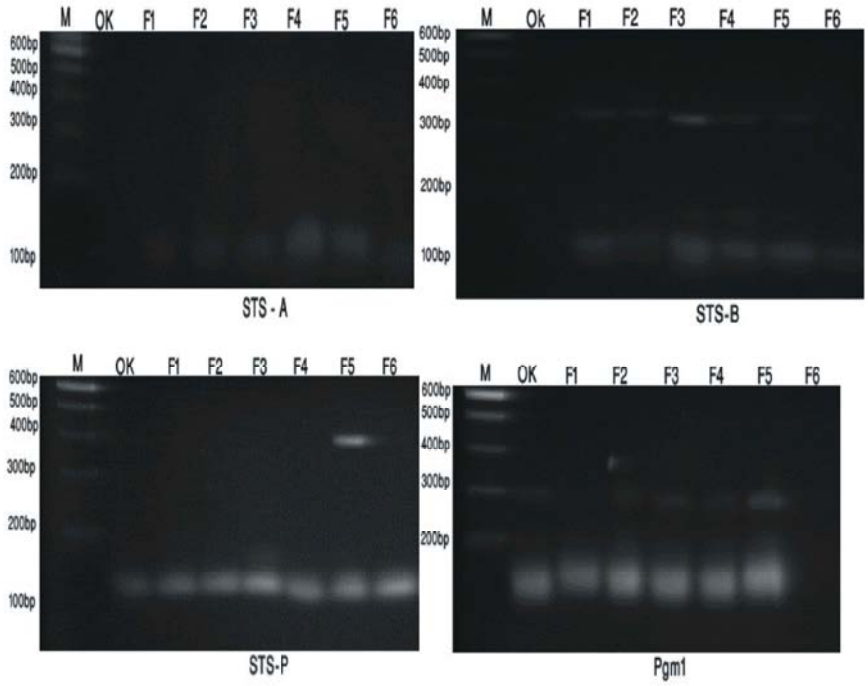

Fig. 3: Polymorphism using SSR-PCR of the new hybrid rootstocks (Om Elfahm X Okinawa) amplified with primer Pchgms1and STS-PCR of the new hybrid rootstocks amplified with primer OPA11, OPB, OPP
(M) DNA ladder marker (bp) (OK) Okinawa (1-6) Hybrids number

Table 6: SSR and STS markers linked to nematode resistance, size of the corresponding bands (bp) and the number hybrids revealing the markers

Marker type	Primer	Band size (bp.)	Hybrid Number
SSR	Pchgms1	194	1, 2, 3, 4 & 5
STS	OPA11	166	1, 2, 3, 4, 5 & 6
	OP834B	127	1, 2, 3, 4, 5 & 6
	OPAP4	183	1, 2, 3, 3, 5 & 6

Detection of Molecular Markers Related to Root-Knot Nematode: In the present investigation one SSR and three STS primers pairs were employed to screen for nematode resistant. The sequences of these primers were obtained from published data by Yamamoto and Hayashi [21] respectively.

The results generated from STS-PCR and SSR-PCR profiles were used to illustrate the genetic relationships among the studied rootstocks as shown in Figure (3).

As shown in (Fig. 3 and Table 6). The SSR primer amplified the specific marker in the expected size (194 bp) as reported by Yamamoto and Hayashi [21] in all the tested hybrids except for hybrid no.6. On the other hand, OPA11 revealed the resistant marked at expected size (166bp) for the hybrid no. 1, 2, 3, 4, 5 and 6. However, OP834B amplified the resistant marker (127bp) for hybrids (1, 2, 3, 4, 5 and 6). Moreover, OPAP4 amplify the marker responsible for nematode resistant for all the investigated hybrids at molecular weight (183bp).

These results are in agreement with Bergougnoux, *et al*. [29] who obtained three RAPD markers, one AFLP and three SSR linked to nematode resistance and from these markers, one RAPD and one AFLP markers, were converted to SCAR (Sequence Characterized Amplified Region) markers which located at less than 1cm from the gene. These markers have been evaluated for marker assisted selection (MAS) in European rootstock (peach, almond or almond-peach) hybrids and proved to be usable reliably in particular to detect the Ma alleles (resistant alleles).

CONCLUSION

It is clear from previous study that hybrids number 3, 4, 5 and 6 had the highest value of main stem length, largest leaf area and chlorophyll % content. Moreover, Nematode evaluation cleared that hybrids number 3, 4, 5 and 6 show highly resistant to Nematode

(*Meloidogyne incognita*) while hybrids 1 &2 show less resistant to it. So, we can consider these rootstocks as promising rootstocks for peach cultivars.

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