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Nematode-resistant, clonal almond rootstock breeding by crossing in Turkey

Halit Seyfettin ATLI[1,*], Canan CAN[2], Mehmet BAŞ[3], Kamil SARPKAYA[4], Ayşe FİDANCI[3], Ertuğrul İLİKÇİOĞLU[4], Nergiz ÇOBAN[4], Sultan BAY TÜRKOĞLU[2]

[1]Department of Horticulture, Faculty of Agriculture, Siirt University, Siirt, Turkey
[2]Department of Biology, Gaziantep University, Gaziantep, Turkey
[3]Atatürk Horticultural Central Research Institute, Yalova, Turkey
[4]Pistachio Research Institute, Gaziantep, Turkey

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Abstract: Nematode-resistant clonal rootstock use is limited in Turkey. Throughout a 3-year-study (2012–2014), targeted rootstock candidates were obtained through hybridization between almond and plum cultivars. Some characteristics, such as strong growth ability, compatibility with other almond cultivars, nematode resistance, and rooting of the cuttings were common among the hybrid types. In the hybridization studies, one almond cultivar (Ferragnes) and 2 plum (Myrobalan and *Pissardi nigra*, both belonging to *Prunus cerasifera*) were used as female parents, and 2 plum (Myrobalan and *Pissardi nigra*) and 2 almond cultivars [Ferragnes and AB3 (*Amygdalus orientalis* Mill. Type)] were used as male parents. In total, 6 combined hybridizations were made: Myrobalan × Ferragnes, AB3 × Myrobalan, *Pissardi nigra* × Ferragnes, AB3 × *Pissardi nigra*, Ferragnes × Myrobalan, and Ferragnes × *Pissardi nigra*. Some hybrid characteristics, such as budding affinity, nematode-resistance, and rooting of the cuttings were considered and the results were evaluated according to the weight-rank method. After consideration of the selected characteristics, the highest scored hybrids, FS2, FS19, FS22, FS23, and FC4, were evaluated as nematode-resistant almond rootstock clone candidates.

Key words: Almond, breeding, nematode, plum, rootstock

1. Introduction

Turkey is one of the most important areas in Eurasia due to its high number of plant species. Such plant diversity is the result of extreme altitudinal differences, providing a wealth of habitats, such as rocky slopes in deep, disjunct valleys. Most agricultural regions in Turkey are famous for their fruit and vegetable production and hope exists regarding strengthening the reputation of horticultural products at home and abroad, helping to raise producer incomes, and building sustainable, inclusive food systems (Seferoğlu, 1991; Hepaksoy, 1994; Ercisli et al., 2003; Doğan et al., 2014; Alp et al., 2016; Gündüz and Özbay, 2018).

Turkey produces 85,000 tons of almonds annually and ranks 6th in global almond production (FAO, 2018). Almond seedling production is generally conducted by rootstocks through direct germination of the seeds, and cv. Texas, cv. Garrigues, or wild bitter almonds are usually the choice for growers. In Turkey, imported GF677, GN, and Rootpack series have been used as clonal rootstocks in almond cultivation for the last 20, 10, and 5 years, respectively. Hence, there is a need to develop clonal rootstock for almond cultivation. In the literature, many studies exist on this topic and more are being conducted (Janick, 1979; Marull et al., 1991; Esmenjaud et al., 1993; Esmenjaud et al., 1995; Felipe et al., 1997; Lecouls et al., 2004; Beckman, 2008). Through the aforementioned research about almond rootstock breeding, valuable rootstock such as Hansen 536, Cornerstone, Nickels, Julior, Nemaguard, Cadaman, Penta, Adesoto, Barrier 1, Guardian, Lovell, Kuban 86, Paramount, Atlas, Viking, Nemared, Marianna 2624, GF677, GF557, Felinem, Garnem, Adafuel, Pollizo, Monegro, and Rootpack were developed.

This study aimed to determine nematode-resistant clonal almond rootstock for use in Turkey, by combining the superior characters of almond and plum cultivars. Nematode-resistant plum and almond varieties were reciprocally hybridized; the F1s that exhibit nematode resistance were tested for production through cuttings and grafting compatibility.

2. Materials and methods

2.1. Plant materials

One almond (cv. Ferragnes) and 2 plum (Myrobalan and *Pissardi nigra* cultivars, both belonging to *Prunus cerasifera*

* Correspondence: hsatli@yahoo.com

Ehrh) were used as female parents, and 2 almond [cv. Ferragnes and AB3 (*Amygdalus orientalis* Mill. Type)] and 2 plum (Myrobalan ve *Pissardi nigra*) cultivars were used as male parents in the cross combinations. The plums were resistant and the almonds were susceptible to nematodes (Esmenjaud et al., 1993; Esmenjaud et al., 1995). A total of 2593 F1 seeds were obtained through 6 reciprocal cross combinations and were used as the plant materials in this study. Hybridizations were carried out in the province of Gaziantep at the Pistachio Research Institute.

2.2. Pollen collection, crosses, and germination ratios

Pollen collections from the male parents were conducted at the pink bud stage and the anthers were left at the laboratory for 1 day after removal from the trees. Emasculation was done using scissors at the pink bud stage and the crossings were conducted by smearing the pollens onto the stigma. The pollen viability was determined via the triphenyl tetrazolium chloride method and germination was examined on media containing 10% sucrose (Atlı and Kaşka, 2001). The crosses were as follows: Myrobalan × Ferragnes (CF), Myrobalan × AB3 (CA), *P. nigra* × Ferragnes (SF), *P. nigra* × AB3 (SA), Ferragnes × Myrobalan (FC), and Ferragnes × *P. nigra* (FS). The nematode-sensitive parents (AB3 and Ferragnes) were reciprocally crossed.

2.3. Dormancy break, planting, and seedling development of the F1 seeds

The F1 seeds were dried on cage wire, the pericarp was broken, 0.3% fungicide was applied to the seeds, and they were left at 4 °C in wet perlite to break the dormancy. The seeds were germinated 2.5 months later and planted in 4-L plastic tubes containing 1:1:1:1 (soil:peat:sand:manure) (Atlı et al., 2011). They were grown in fully controlled greenhouses. The hybrid seedlings were transferred to field conditions in May at 1 × 0.5 m intervals.

2.4. Grafting, budding success rates, and grafting compatibility

In order to define grafting compatibility, 26 hybrid plants that had reached at least 1.5-m in height and contained 10 lateral branches or shoots were grafted with cv. Ferragnes using the T-shaped bud grafting method. Budding success was defined through scion development. Grafting compatibility was determined on 10 individual F1 plants that were chosen according to rooting success, appropriate shoot development, health, and strong growth. Macroscopic and microscopic analyses were conducted on longitudinal cross-sections taken 12 months after grafting (Sass, 1964; Tekintaş, 1991; Özban and Özmutlu, 1994).

2.5. Rooting through cuttings of hybrid plants

From the 26 F1 plants, 24 cuttings, each 15–25 cm in length, were taken to use in the rooting experiments. Of those, 12 were put into 500 ppm Indole-3-butyric acid (IBA) solution, while the other 12 were put into 1000 ppm IBA for 5 s (Atlı et al., 2011). Next, the cuttings were planted on rooting media containing wet perlite. Root development was defined 2.5 months later and they were planted in plastic tubes.

2.6. Plant growth values of hybrid individuals

The growth parameters of the 26 F1 individuals were determined by inspecting their diameters at 25 cm above ground level with a digital compass (Yıldız, 2001). The hybrids were divided into 4 groups:

24 mm and below:	weak
25 mm–30 mm:	medium
31 mm–35 mm:	medium-strong
36 mm and higher:	strong

The seedling vigor (general appearance) of the hybrids was divided into 5 groups (1–5).

2.7. Data analyses

Modified balanced scaling methods were used in the data evaluation, and the nematode resistance, propagation with cuttings, and budding affinity characters were considered (Table 1) (Düzgüneş, 1963; Atlı, 2008).

3. Results and discussion

3.1. Pollen viability and pollen germination rates of the parental genotypes

The pollen viability of the parents was generally high and type AB3 had the highest value (95.8%) for pollen viability and germination followed by *P. nigra*, cv. Ferragnes, and Myrobalan (Table 2).

Griggs et al. (1953) determined the 2 almond pollen germination means as 76.5%. The results obtained through this study are in accordance with that of Griggs et al. (1953). However, the rates of the plums were lower than that in the present study and the differences may have been due to the plant materials used.

3.2. Fruit setting rates of the hybrid combinations and number of F1 seeds

The highest and lowest fruit setting rates were obtained with Myrobalan × Ferragnes (66.4%) and Ferragnes × *P. nigra* (4.7%), respectively (Table 3). Generally, when the almond was used as the female parent, fruit formation was low, while when using Myrobalan as the female parent, the fruit setting increased. The highest and lowest hybrid fruit numbers were with Myrobalan × Ferragnes (998 fruit) and *P. nigra* × Ferragnes (128 fruit), respectively (Table 3).

Seed germination rates were higher in the crosses when almond was used as the female parent compared to those of the combinations where plum was used as the female parent. The highest seed germination rate was obtained with Ferragnes × *P. nigra* combinations (81.4%), followed by Ferragnes × Myrobalan (55.7%), *P. nigra* × AB3 (26.5%), *P. nigra* × Ferragnes (16.4%), and Myrobalan

Table 1. Modified balanced scaling points.

Characters	Coefficient	Classes	Class intervals	Balance point
Resistance to nematodes	40	Sensitive Resistance	-- --	1 9
Propagation with cuttings	30	High Medium Low	50% and higher 25%–49% 24% and lower	9 6 1
Budding affinity	30	High Medium Low	Very good Good Not compatible	9 6 1

Table 2. Pollen viability and germination ratios of parental genotypes used in crosses.

Parents	Pollen viability (%)	Pollen germination (%)
Cv Ferragnes	89.3	48.3
AB3 (*A. orientalis* Mill.)	95.8	92.4
Myrobalan (*Prunus cerasifera* Ehrh.)	86.2	24.2
Pissardi nigra (*Prunus cerasifera* Ehrh.)	91.0	23.1

Table 3. Fruit setting rates, fruit and germinating seed numbers, and hybrid germination rate.

Combinations	Fruit setting rate (%)	F_1 seeds (number)	Germinating seeds (number)	Germination rate (%)
Myrobalan × Ferragnes	66.4	998	124	12.4
Myrobalan×AB3 (*A.orientalis*)	42.4	779	103	13.2
P. nigra × Ferragnes	20.3	128	21	16.4
P. nigra × AB3	13.9	181	48	26.5
Ferragnes × Myrobalan	9.9	237	132	55.7
Ferragnes × *P. nigra*	4.7	270	220	81.4
TOTAL	-	2593	648	-

× AB3 (13.2%). The lowest rate was determined with Myrobalan × Ferragnes (12.4%) (Table 3).

Setting of the fruits following crossing in the hybrids where almond was used as the female parent was low when compared to the hybrids where Myrobalan was used as the female parent (Table 3). However, the seed germination rate was higher in hybrids where almond was used as the female parent than in those of the hybrids where plum was used as the female parent. Similarly, Hartmann and Kester (1983) depicted better seed germination rate with almond than with plum.

3.3. Grafting success, compatibility and rooting of hybrid individuals

The graft success rates of the 26 F1 hybrids were 100%, 83.3%, and 50% for 22, 3, and 1 individuals, respectively (Table 4). Previous studies reported high grafting success with almond (Atlı, 2008; Atlı et al., 2014).

The rooting rates were low with both dosages of IBA; however, 1000 ppm IBA induced more root development than that of 500 ppm IBA (Table 4).

The data obtained in the rooting studies were fewer than the results obtained by Atlı et al. (2014). These conflicts may have been due to parental differences in which the hybrids originated from plum in the present study, whereas Atlı et al. (2014) used hybrids originating from peach. Nicotra and Moser (1978) used 2000 ppm IBA to incite rooting in plum rootstocks and reached a rooting rate of 54.7%, which was higher than that in the present study. These results indicate that high concentrations of IBA trigger root development in almond.

Table 4. Grafting and rooting rates of the individual hybrids.

Crosses	Rooting rates (%)		Grafting ates (%)	General appearance (seedling vigor) (1–5 points)	Seedling growth rates	
	(500 ppm IBA)	(1000 ppm IBA)			Diameter (mm)	Hybrid vigor
FC1	8.3	16.7	100	4	45.22	Strong
FC2	25.0	33.3	83.3	5	36.60	Strong
FC3	25.0	25.0	100	5	33.87	Med. strong
FC4	33.3	33.3	100	5	28.99	Medium
FC5	8.3	16.7	100	3	30.57	Medium
FC6	0	8.3	100	5	23.71	Weak
FC7	16.7	16.7	100	4	24.95	Weak
FC9	25.0	33.3	100	3	26.32	Medium
FC10	25.0	16.7	100	5	27.90	Medium
FS1	16.7	8.3	100	4	27.62	Medium
FS2	25.0	33.3	100	5	28.43	Medium
FS3	8.3	16.7	83.3	4	32.35	Med. strong
FS4	0	8.3	100	4	21.51	Weak
FS5	25.0	25.0	100	4	33.27	Med. strong
FS6	8.3	16.7	100	4	33.73	Med. strong
FS9	8.3	16.7	50.0	3	34.72	Med. strong
FS10	16.7	16.7	100	4	41.60	Strong
FS11	0	8.3	83.3	4	33.64	Med. strong
FS12	8.3	16.7	100	3	36.42	Strong
FS16	33.3	33.3	100	4	28.45	Medium
FS17	25.0	33.3	100	3	23.43	Weak
FS19	25.0	33.3	100	5	29.48	Medium
FS21	16.7	33.3	100	5	36.07	Strong
FS22	16.7	25.0	100	5	26.14	Medium
FS23	16.7	25.0	100	5	37.29	Strong
CA2	41.7	50.0	100	5	24.79	Weak

3.4. Grafting compatibility of the hybrid individuals

The grafting compatibility of 10 F1 plants was determined according to rooting, shoot development, health, and strong growth (Table 4). The microscopic investigation of the sections taken 12 months following grafting was conducted by examining connections among the tissues, integrity, compatibility, interruption, abnormality or sloping, existence of necrotic areas, and barrier formation of necrotic lesions within the tissues. Among the grafts, 3 groups were formed, as given below.

Among the hybrids created in this study, 3 different groups were constructed. Necrotic areas that occurred around the grafting point were reported to decrease in highly-compatible combinations (Hepaksoy, 1994). In this study, S-2/Ferragnes, FS-19/Ferragnes, and FS-22/Ferragnes were identified as highly compatible.

Hence, Seferoğlu (1991), Errea et al. (1994), and Hepaksoy (1994) reported better development of the cambium and floem tissues in good compatible grafting, while deformation was observed in the xylem formation. They also reported a slight entrance of the floem into the xylem tissue in most of the grafting combinations.

FS-23/Ferragnes and FC-4/Ferragnes were defined as good compatible combinations, despite light bark and grafting point thickening.

The bark formation at the rootstock-scion union was thicker in grafting exhibiting incompatibility than those of the compatible combinations (Özçağıran, 1974).

Table 5. Analyzed characters and balanced points of the hybrids.

Hybrids	Nematode resistance	Balanced points	Cutting propagation	Balanced points	Graft compatibility	Balanced points	Total points
FC2	Resistant	360	33.3	180	Not	30	570
FC3	Resistant	360	25.0	180	Not	30	570
FC4	Resistant	360	33.3	180	Good	180	720
FC10	Resistant	360	16.7	30	Not	30	420
FS2	Resistant	360	33.3	180	Very good	270	810
FS19	Resistant	360	33.3	180	Very good	270	810
FS21	Resistant	360	33.3	180	Not	30	570
FS22	Resistant	360	25.0	180	Very good	270	780
FS23	Resistant	360	25.0	180	Good	180	720
CA2	Resistant	360	50.0	270	Not	30	660

Cytological staining indicates high starch deposition in these tissues supporting graft incompatibility (Herrero, 1951; Demirsoy and Bilgener, 2006). The present study defined FS-21/Ferragnes, CA-2/Ferragnes, FC-2/Ferragnes, FC-3/Ferragnes, and FC-10/Ferragnes as risky combinations for incompatibility defined through cytological staining, bark thickening, formation of old callus, and necrotic xylem tissues between the rootstock and scion.

3.5. Plant growth values of the hybrid individuals

Growth parameters were between 21.51 mm–45.22 mm. According to the data obtained, classifications are given in Table 4. Of the hybrids, 6 were determined as strong, 6 were medium-strong, 9 were medium, and 5 exhibited weak growth.

3.6. Data evaluation

The data was analyzed according to the modified balanced point method (Düzgüneş, 1963) and evaluations were conducted according to marker-assisted selection for nematode resistance that was published previously (Bay Türkoğlu et al., 2015), propagation through rooting, and graft compatibility (Table 5). Of the hybrids, 4 were Ferragnes × *P. nigra* (FS) and 1 was Ferragnes × Myrobalan (FC). The highest point was valued to the FS2, FS19, FS22, FS23, and FC4 hybrids, which were defined as nematode-resistant, clonal almond rootstock candidates. Among them, FS23 exhibited strong growth and the others were medium. Weak developing rootstock was not obtained in this study and further studies should be conducted using weak parents to determine rootstock with poor growth parameters.

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