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Seedling Growth Performance of Selected Rootstock Almond Genotypes and Their Nematode Resistance

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Abstract
The aim of the study was to determine the germination and seedling growth performances of 25 rootstock candidates of almond genotypes and their tolerance to *Meloidogyne incognita* and *Meloidogyne javanica* nematode species. It was found that genotypes 29, 57, 58, 76, and 156 showed a germination output of over 90% in the years 2017 and 2018 and they stood out in terms of seed germination. We determined that genotypes 29, 68, 133, and 196 showed less than 5% variation in terms of the coefficients of variation in the seed diameter in both years. In terms of seedling size variation, genotypes 101, 161, and 183 came to the fore with a variation of less than 5% in both years. Although it was determined that all genotypes reached the thickness that can be grafted at a high rate in the same year, genotypes 29 and 161 stood out with the rate of seedlings with a diameter of over 7 mm in both years. Generally, the gall ratio of *Meloidogyne incognita* and the resulting population in the soil were higher than those of *Meloidogyne javanica*. According to the gall ratios in the roots, the gall ratio values in genotypes 29, 66, 80, 121, 127, 134, 143, 161, and 163 were 2.0 and below, and they stood out as promising genotypes for resistance. Genotype 29 was noteworthy in terms of both its seedling growth performance and nematode tolerance characteristics.

Keywords Biotic stress · *Meloidogyne spp.* · Rootstock · Seed germination

Introduction

In most fruit species, seedling production maintains its importance thanks to being free from virus diseases, being easily adaptable to unfavorable soil and climatic conditions, and the high drought resistance in areas under arid and semi-arid climatic conditions. In addition, the fact that it is easy to obtain, to transport, and to store seeds makes seedling production advantageous (Özyurt and Akça 2017).

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Today, seedling rootstocks are used intensively in production because of the lack of vegetatively growing rootstocks, their high costs and low resistance to drought, and their diseases and pests as compared to the seedlings, especially in hard-shelled fruit species (Sousa and Pereira 1994; Yıldırım and Koyuncu 2005; Küden et al. 2014; Kızıltan et al. 2016; Karlıdağ et al. 2018). It has also been reported that seedlings can be propagated more easily than clonal rootstocks and do not show incompatibility with almond varieties (Janick and Moore 1996). In fact, Rahmani et al. (2006) emphasized that the deep roots, narrow leaves, and thorny branches of almond seedlings increase their adaptability to adverse environmental conditions, especially to drought. Some studies have reported that the wide variation of almond seedlings is an important feature that can facilitate the emergence of seedling candidates that can adapt to different conditions in the orchard; additionally, it is very important to use the seeds of a variety or genotype that give homogeneous seedlings in production (Sousa and Pereira 1994; Lansari et al. 1998; Küden et al. 2014). It has been reported that the seeds of the 'Atocha,' 'Garrigues,' and 'Desmayo Rojo' varieties in European countries, the seeds of the 'Texas

variety' in the United States, and the seeds of the 'Chelleston' and 'Nonpareil' varieties in Australia are widely used as seed sources in the production of seedling rootstocks due to their homogeneous development (Ramos 1976; Akça 2000; Orero et al. 2004). Although heterozygous expansion is seen in seed rootstocks, the seeds of some varieties produce plants with a remarkably homogeneous development and they are preferred in production (Rubio-Cabetas et al. 2017). At the same time, the previously cited studies emphasized that a modern cultivation with rootstocks and varieties that grow healthy and strong and that develop in a way that tolerates drought, diseases, and pests would prevent significant losses in yield and quality (Kaşka et al. 1998; Balta et al. 2003; Akçay and Tosun 2005). Grauke and Thomson (2003) emphasized that almond seedlings in arid and calcareous soils, peach seedlings in irrigated areas, 'Nemaguard' seedlings in nematode-contaminated soils, and 'Marianna' plums in heavy textured soils could be used as the rootstocks in almond cultivation.

There are plant diseases and pests that often reduce the yield and cause significant economic losses during the production season. Root-knot nematodes (*Meloidogyne* spp.; Netscher and Sikora 1990; Whitehead and Turner 1998), which spread around the world and cause huge economic losses not only in vegetable but also in perennial fruit cultivations, are the leading ones. The root-knot nematodes were first identified by Berkeley in 1855 and are microscopic creatures causing galls in the roots of the host plant that they feed on (Whitehead and Turner 1998). It has been reported in studies conducted in different regions of Turkey (Mediterranean, Marmara, Aegean, Black Sea, Southeastern Anatolia, Central and Eastern Anatolia) that *Meloidogyne javanica* and *M. incognita* are the most common and most economically important root-knot nematode species in different plant species (vegetable, banana, hard-shelled fruit, some soft and hard-core fruit trees), while *M. arenaria* and *M. hapla* are rare species (Yüksel 1974; Ağdacı 1978; Elekcioğlu and Uygun 1994; Mennan and Ecevit 1996). Among these, some of the wild species used as almond rootstocks are resistant to root-knot nematodes of bitter almonds (*Prunus amygdalus* BATSCH var. Amara DC) and some are sensitive to root-knot nematodes of both bitter and sweet seeds (*P. amygdalus* BATSCH var. Dulcis DC; Wachtel 1984). In addition, it has been reported that some seedlings and clone rootstocks used in almond production (peach, plum, and almond–peach hybrids) are particularly susceptible to the root-knot nematodes of *M. incognita* and *M. javanica* (Gradziel 2009; Soylu 2012). In addition to direct damage, root-knot nematodes also enable the soil-borne microorganisms (fungal and bacterial) that enter the wounds opened by capillary roots to cause disease in the plant (Stirling and West 1991). The roots of plants contaminated with nematodes cannot provide enough water and plant nutrients, as well as causing chlorosis in the leaves, resulting in a decrease in the photosynthesis capacity and, ultimately, in growth retardation in plants (Thorne 1961). The fight against nematodes in perennial woody plants such as fruit trees over a period of 2–70 years reveals once again how important it is to fight nematodes with cultural practices (Dowler and Van Gundy 1984; Kızıltan et al. 2016). To this end, the studies of obtaining resistant rootstocks are preferred because they decrease or completely prevent the reproduction of nematodes, they do not require special application techniques and equipment, their costs are lower than other methods, and they are environmentally friendly (Cook and Evans 1987; Boerma and Hussey 1992; Lopez-Pereza et al. 2006).

In this study, we aimed to determine the tolerance of some almond genotypes due to their rootstock characteristics (especially high emergence rates and homogeneous seedling development) selected by Yıldırım (2007) against *M. incognita* and *M. javanica*, which cause great damage in the cultivation.

Materials and Methods

The seeds of some genotypes selected by Yıldırım (2007) in the Isparta region were collected from the parent plants in August 2016 and 2017. The fruits taken during these 2 years were brought to the laboratory, and the seeds were dried in a shaded place for 15 days after they being separated from their outer shells. The dried seeds were kept in a cool and dry place until the time of stratification. The seeds to be used for stratification in both years were kept in water for 24 h before stratification in order to remove the germination inhibitors present in the seed coat and to absorb enough water into the seeds. Then, the seeds were placed in the folding boxes containing perlite and placed in cold storage for 75 days at +4 °C and 90–95% humidity. The seeds and stratification boxes were treated with a fungicide before the stratification. During folding, the crates were checked for humidity from time to time and moistened when necessary. At the end of the stratification period, the emergence rates of the seeds were determined. The seeds obtained from the stratification were transferred to the six-hole polyethylene bags of 20 cm × 30 cm in size, containing the mortar at a ratio of 1:1:1 (sand:soil:peat) during these 2 years.

The polyethylene bags were kept outside and the necessary maintenance (irrigation, fertilization, weed, spraying, etc.) operations were carried out. In the experiment, the seeds belonging to the genotypes were planted in three replications with 30 seeds per replication. The emergence started approximately 14–16 days after the seeds were planted in the polyethylene bags, and the emergence rates (%) were determined by counting the seedlings that had

Fig. 1 Almond roots removed from the pots and galls on the roots. **a** The roots of genotype 29, **b** the roots of genotype 68

completed their emergence in the following days. At the end of the vegetation period, the plants were removed from the polyethylene bags to evaluate their seedling growth performance. The seedling diameter (Gönüleşen et al. 1985; Öylek et al. 2013), seedling length (Gönüleşen et al. 1985; Abay 1985), seedling size, seed diameter uniformity in the genotypes included in the study (Düzgüneş et al. 1983) and the rate of inclusion of seedlings to grafting (Martinez-Gomez and Dicenta 2001) were determined.

To determine the resistance of almond seedlings against *M. incognita* and *M. javanica*, the mass production of root-knot nematodes was carried out in the 'Rio Grande,' 'Troy,' and 'Panda' tomato varieties that are sensitive to the nematodes. The responsive tomato seedlings were transplanted into the pots with 500 g of soil containing 70% autoclaved sandy soil, after the peats were cleaned. Approximately 1 week later, one egg pack from both root-knot nematode species was placed into Eppendorf tubes and inoculated into 2-cm-deep holes opened near the root collar of the plants. The pure culture populations to be used in the experiments were used in the mass production of nematodes by removing the tomato plants approximately 3 months later. Approximately 10 egg packs extracted from each sample of pure cultured root-knot nematodes under binocular microscope was placed into Eppendorf tubes, and was inoculated into the soil at approximately 2 cm root depth near the plant root collar. The holes made after inoculation were covered with sterile soil. Pure culture mass production of nematodes was carried out at 25 ± 1 °C and under controlled climate room conditions with 60 ± 5% humidity. In determining the resistant/susceptible host reactions of the seedlings belonging to the almond genotypes, two root-knot nematode eggs were inoculated per 1 g of soil. The plants where the mass production of the nematodes was made were dismantled after about 12 weeks and washed in tap water and divided into pieces of approximately 1 cm length; 200 mL 0.5% NaOCl was added in a beaker and shaken for 3–5 min by closing its cover. After this process, the NaOCl solution in the beaker was poured on the sieve set with 90 µm, 50 µm, 38 µm, and 20 µm diameters. Nematode eggs and second-stage juveniles in the sieve system were washed sufficiently in clean tap water to remove NaOCl in the environment. After the washing process was completed, the nematode eggs collected on a 20-µm sieve were taken into a 100-mL measuring cylinder and allowed to settle. Then they were placed in 15-mL centrifuge tubes and stored in a cooled incubator (+15 °C) for use when necessary (Hussey and Barker 1973). The nematode eggs in the centrifuge tubes were counted under a light microscope and prepared for inoculation with two eggs per 1 g of soil. The almond seedlings that were displaced in the pots were kept in pots in an open area for 30 days, after which the root-knot nematode inoculations were made in each pot and the experiments were carried out in the controlled climate rooms. For the experiments, suitable dark-colored plastic pots that can take 3000 g of soil with a mixture of 13.3% clay, 18.4% silt, and 68.3% sandy sterile soil were used.

The nematodes, the density of which was prepared as two eggs per 1 g of soil, were inoculated into the 2-cm-deep holes opened around the root collar. A total of 6000 *M. incognita* and *M. javanica* eggs were inoculated per pot. After 12 weeks of incubation, the plants were scored and

Table 1 The 0–5 egg sac number and gall number index of *M. incognita* and *M. Javanica* nematode species

Number of egg sac	Gall number index	Sensitivity condition
0	No egg sac or gall formation on the root	Resistant
1	1–2 egg sac and gall formation on the root	Resistant
2	3–10 egg sac and gall formation on the root	Resistant
3	11–30 egg sac and gall formation on the root	Sensitive
4	31–100 egg sac and gall formation on the root	Sensitive
5	More than 100 egg sacs and gall formation on the root	Sensitive

evaluated. For this purpose, the rate of gall formation in the plant roots and the counts of active larvae of the second period of root-knot nematodes in the pots were determined (Fig. 1).

The resistances of almond rootstocks used against root-knot nematodes included in the experiment were determined according to the 0–5 egg sac and gall number index as described by Hartman and Sasser (1985). The rootstocks with 0–2 gall index value in the almond roots were evaluated as durable, while the rootstocks with 3–5 gall index value were evaluated as sensitive (Table 1).

The research was planned according to the random plot trial pattern. The results obtained were subjected to variance analysis using the Minitab 17 package program (MINITAB LTD., Coventry, UK). Significant differences between the means were determined with the Tukey test ($p \leq 0.05$) and are shown with different letters.

Results and Discussion

The seedling emergence rates of the genotypes were determined every 5 days and the results are presented in Table 2.

Table 2 Seedling emergence rates of the genotypes (%) for the year 2017

Genotype	10.05.2017	15.05.2017	20.05.2017	25.05.2017	30.05.2017
29	16.0	74.7	92.0	94.7	96.0
57	4.0	40.0	82.7	88.0	92.0
58	0.0	64.0	92.0	93.3	96.0
66	0.0	45.3	77.3	82.7	85.3
68	0.0	37.3	62.7	68.0	72.0
76	6.7	53.3	76.0	84.0	92.0
80	6.7	49.3	54.7	58.7	60.0
84	12.0	56.0	77.3	81.3	82.7
101	6.7	65.3	77.3	81.3	84.0
102	17.3	85.3	89.3	88.0	88.0
121	2.7	44.0	78.7	85.3	88.0
127	0.0	4.0	64.0	81.3	84.0
129	0.0	48.0	65.3	65.3	65.3
132	6.7	62.7	76.0	77.3	78.7
133	2.7	38.7	76.0	80.0	80.0
134	2.7	65.3	84.0	86.7	86.7
143	4.0	50.7	73.3	74.7	77.3
156	4.0	76.0	92.0	94.7	94.7
161	0.0	44.0	77.3	81.3	84.0
163	1.3	44.0	81.3	84.0	88.0
176	0.0	52.0	81.3	84.0	86.7
183	0.0	0.0	0.0	17.3	26.7
196	10.7	60.0	70.7	76.0	77.3
231	6.7	84.0	97.3	97.3	97.3
241	6.7	58.7	65.3	66.7	70.7

The highest emergence rate in 2017 was determined in genotype 231 with 97.3%. This genotype was followed by genotypes 29 (96.0%), 58 (96.0%), and 156 (94.7%). The lowest emergence rate was found in genotype 183 with 26.7%.

In 2018, the highest emergence rate was determined in genotype 143 with 95.0%. This genotype was followed by genotype 156 with 94.7%, and genotypes 29, 132, and 196 with 93.3% each. The lowest emergence rate was detected in genotype 231 with 53.3% (Table 3). Genotype 29 had a high emergence rate in both years. The germination rates of the seeds in almonds are also related to the cooling time. Despite the application of chilling, problems of low seed germination may be encountered in some genotypes. In this case, it is possible to increase the germination rate by using in vitro seed germination or embryo culture techniques (San and Yildirim 2009). It is reported that genotypes with low seed power can also be reproduced with this method.

The average seedling diameter, seedling size, and the coefficients of variation for the seedling size and seedling diameter of the almond genotypes examined in 2017 are presented in Table 4. When the seedling diameter and seedling

Table 3 Seedling emergence rates of the genotypes (%) for the year 2018

Genotype	14.05.2018	17.05.2018	01.05.2018	25.05.2018	28.05.2018
29	81.6	86.6	91.6	93.3	93.3
57	78.3	81.6	86.6	90.0	90.0
58	55.0	73.3	80.0	90.0	91.6
66	48.3	55.0	58.3	60.0	61.6
68	61.6	65.0	73.3	73.3	75.0
76	6.7	53.3	76.0	84.0	92.0
80	6.7	38.3	55.7	60.0	63.0
84	57.1	71.4	77.1	77.1	77.1
101	43.3	51.2	60.0	60.0	60.0
102	17.3	75.3	78.3	85.0	85.0
121	48.1	65.3	65.3	65.3	67.3
127	10.0	18.3	52.0	78.0	78.0
129	83.3	88.3	88.3	91.6	91.6
132	86.6	90.0	90.0	90.0	93.3
133	65.0	73.3	75.0	76.6	78.3
134	25.7	42.1	68.3	74.7	79.8
143	83.3	86.6	86.6	86.6	95.0
156	24.0	49.5	92.0	94.7	94.7
161	46.6	58.3	58.3	60.0	60.0
163	38.3	56.6	58.3	65.0	73.3
176	71.6	85.0	85.0	88.3	90.0
183	46.6	55.0	60.0	65.0	65.0
196	80.0	86.6	90.0	93.3	93.3
231	33.3	40.0	45.0	50.0	53.3
241	61.6	75.0	75.0	81.6	83.3

Table 4 Seedling diameter (mm), seedling size (cm), and coefficients of variation (%) of the genotypes for the year 2017

Genotype	Seedling diameter (mm)	Seedling diameter variation coefficient (%)	Seedling size (cm)	Coefficient of variation of seedling size (%)
29	7.29 a	2.98	71.93 abc	2.85
57	4.56 g	0.95	29.97 ı	6.81
58	7.17 ab	17.67	67.61 abcd	2.26
66	6.19 abcdefg	10.49	61.08 bcdef	7.26
68	4.97 fg	1.17	52.60 efg	8.43
76	6.99 abcd	6.69	56.18 defg	2.02
80	6.31 abcdefg	10.38	58.50 bcdef	10.98
84	4.71 g	9.42	42.39 ghi	6.00
101	6.23 abcdefg	3.86	69.87 abcd	2.06
102	5.29 bdefg	2.95	51.23 efgh	9.79
121	5.93 abcdefg	6.78	57.41 cdef	8.72
127	6.71 abcdef	5.46	60.51 bcdef	11.25
129	5.19 cdefg	15.41	47.73 fgh	7.78
132	5.45 abcdefg	5.64	48.08 fgh	7.30
133	5.00 efg	3.25	47.30 fgh	9.42
134	5.94 abcdefg	9.99	73.08 ab	10.63
143	5.46 abcdefg	9.76	50.20 efgh	16.53
156	6.11 abcdefg	8.57	68.67 abcd	9.50
161	7.14 abc	2.18	78.82 a	4.25
163	6.96 abcde	7.59	57.11 cdefg	11.46
176	5.33 bcdefg	10.45	36.38 hi	0.87
183	5.05 defg	13.56	37.33 hi	0.96
196	6.30 abcdefg	0.97	61.42 bcdef	6.55
231	5.97 abcdefg	5.00	55.31 defg	7.53
241	5.04 defg	36.65	63.93 abcde	11.79

CV coefficient of variation

*The differences between the means in the same column indicate statistical difference at the $p < 0.05$ significance level according to the Tukey test

size were examined, a significant difference was found between the genotypes at $p < 0.05$.

Among the genotypes, the highest seedling diameter development was obtained in genotype 29 with 7.29 mm. This was followed by genotype 58 with 7.17 mm and genotype 161 with 7.14 mm. The lowest seedling diameter value was measured in genotype 57 with 4.56 mm. In the study, the highest seedling size was obtained in genotype 161 with 78.82 cm. This was followed by genotype 134 with 73.08 cm. The shortest seedling length was found in genotype 57 with 29.97 cm.

The differences between the genotypes were determined for 2017 according to the coefficient of variation. The highest variation determined in the diameters of the seedlings was observed in genotype 241 with 36.65%, which was followed by genotypes 58 with 17.67% and 129 with 15.41%. The lowest variation was obtained in genotype 57 with 0.95%. When the seedling size was examined, the highest variation was found in genotype 143 with 16.53%, followed by genotypes 241 with 11.79% and 163 with 11.46%. The lowest variation was determined in genotype 176 with 0.87%.

The seedling diameter, seedling size, and the coefficients of variation for the average seedling diameter and size for 2018 are presented in Table 5. When the seedling diameter and seedling size were examined, statistically significant differences were determined between the genotypes at $p < 0.05$. Among the genotypes, the highest seedling diameter development was obtained in genotype 156 with 7.26 mm. This was followed by genotype 66 with 6.32 mm and genotype 241 with 6.03 mm. The lowest seedling diameter was obtained in genotype 80 with 4.83 mm. The highest seedling size was obtained in genotype 66 with 84.26 cm. This was followed by genotype 121 with 79.55 cm. The shortest seedling length was determined in genotype 133 with 56.69 cm.

The differences were determined between the genotypes according to the coefficient of variation. The highest variation in the diameter of the seedlings was found in genotype 129 with 18.18%, followed by genotypes 127 with 15.06% and 231 with 14.06%. The lowest variation was obtained

Table 5 Seedling diameter (mm), seedling size (cm), and coefficients of variation (%) of the genotypes for the year 2018

Genotype	Seedling diameter (mm)	Seedling diameter variation coefficient (%)	Seedling size (cm)	Coefficient of variation of seedling size (%)
29	5.94 ab	2.06	77.94 abc	7.66
57	5.74 ab	10.95	69.62 bcdefgh	3.62
58	5.59 b	8.75	58.23 i	7.05
66	6.32 ab	8.81	84.26 a	4.71
68	5.31 b	3.12	66.67 defghi	8.29
76	5.98 ab	1.75	63.33 ghi	8.98
80	4.83 b	10.78	56.76 i	5.78
84	5.81 ab	4.64	67.14 cdefghi	4.16
101	5.49 b	10.40	75.34 abcdef	1.38
102	5.33 b	10.68	76.33 abcde	4.60
121	5.71 ab	2.65	79.55 ab	3.99
127	5.31 b	15.06	63.57 ghi	2.59
129	5.10 b	18.18	58.87 hi	1.57
132	5.59 b	2.96	69.41 bcdefgh	5.88
133	5.55 b	3.36	56.69 i	1.83
134	5.78 ab	10.00	62.67 ghi	3.25
143	5.56 b	13.12	70.76 bcdefg	2.62
156	7.26 a	10.55	71.50 bcdefg	3.20
161	5.50 b	8.48	73.11 bcdefg	3.76
163	5.32 b	0.44	67.35 cdefghi	2.64
176	5.79 ab	4.00	63.51 ghi	5.56
183	5.11 b	12.08	62.17 ghi	4.68
196	5.24 b	1.64	66.29 efghi	1.63
231	5.10 b	14.06	64.89 fghi	6.35
241	6.03 ab	2.74	77.74 abcd	8.72

in genotype 163 with 0.44%. When the seedling size was examined, the highest variation was detected in genotype 76 with 8.98%, followed by genotypes 241 with 8.72% and 68 with 8.29%. The lowest variation was determined in genotype 101 with 1.38%. One of the most important features in the seedling rootstocks is the low coefficient of variation in the seedling diameter and length, which is an indicator of seedling homogeneity.

When the coefficients of variation in seedling diameter were evaluated, it was determined that genotypes 29, 68, 133, and 196 had low values in both years and formed a homogeneous stem diameter (Tables 4 and 5).

The seeds of almond varieties such as 'Texas,' 'Chelleston,' and 'Nonpareil,' which have the property of giving homogeneous seedlings, are used extensively in the production of seedling rootstocks in nurseries (Sousa and Pereira 1994). In addition, it has been reported that the seedlings belonging to species such as *Prunus hauskonetchii* and *P. dulcis* stand out in terms of rootstock characteristics compared to the seedlings of other species and they have a greater potential to be used as rootstocks (Rahemi et al. 2011; Atlı et al. 2011).

It is accepted that grafting can be administered in the seedlings with a diameter between 4 and 7 mm (Öylek et al. 2013). In our study, it was determined that the seedling diameters in 2017 varied between 4.56 mm (genotype 57) and 7.29 mm (genotype 29; Table 4). Therefore, the seedlings belonging to the genotypes examined here reached the sufficient graft thickness.

However, considering that the retention rates of the graftings made on the rootstocks with a seedling diameter of 7 mm and over are higher, it was determined that 72.74% of the seedlings of genotype 29 in 2017 had a diameter development of 7 mm and over. This was followed by genotype 76 with 52.23%, genotype 163 with 51.39%, and genotype 161 with 51.28% (Table 6). In 2018, it was determined that the seedling diameters varied between 4.83 mm (genotype 80) and 7.26 mm (genotype 156; Table 5). Accordingly, it was determined that the seedlings belonging to the genotypes examined in 2018 also reached the sufficient grafting thickness. Most of the seedlings belonging to the genotypes were in the 5–5.9-mm group in 2018. However, it was determined that the majority of the seedlings in genotypes 29 and 156 reached a diameter of 7 mm or more (Table 7). In 2018, it was determined that 62.50% of the seedlings of

Table 6 Classification of the seedling diameter development of the genotypes for the year 2017

Genotype	Seedling diameter development (%)			
	4–4.9 mm	5–5.9 mm	6–6.9 mm	≥ 7 mm
29	5.68	4.54	17.04	72.74
57	79.73	18.92	1.35	0.00
58	7.00	36.00	35.00	22.00
66	14.93	32.84	32.84	19.40
68	66.00	22.00	2.00	10.00
76	2.22	23.33	22.22	52.23
80	16.67	18.75	35.42	29.16
84	71.25	15.00	12.50	1.25
101	16.92	13.85	32.31	36.92
102	36.11	48.61	11.11	4.17
121	21.33	32.00	20.00	26.67
127	16.67	13.64	31.82	37.87
129	47.73	25.00	20.45	6.82
132	32.26	43.01	17.2	7.53
133	64.29	21.43	5.71	8.57
134	20.51	30.77	33.33	15.39
143	38.57	40.00	12.86	8.57
156	8.45	50.70	23.94	16.91
161	7.69	12.82	28.21	51.28
163	8.33	26.39	13.89	51.39
176	45.56	33.33	15.56	5.55
183	60.00	15.00	22.50	2.50
196	4.29	40.00	38.57	17.14
231	6.12	54.08	32.65	7.15
241	37.5	26.79	21.43	14.28

Table 7 Classification of the seedling diameter development of the genotypes for the year 2018

Genotype	Seedling diameter development (%)			
	4–4.9 mm	5–5.9 mm	6–6.9 mm	≥ 7 mm
29	6.25	16.25	35.00	42.50
57	22.00	46.80	15.60	15.60
58	19.40	47.90	25.50	7.00
66	8.90	25.00	39.30	26.80
68	30.00	50.00	17.50	2.50
76	0.00	100.00	0.00	0.00
80	56.00	33.30	10.40	0.00
84	8.69	47.83	43.48	0.00
101	26.47	38.23	32.35	2.95
102	53.30	40.00	6.70	0.00
121	16.00	48.20	35.80	0.00
127	50.00	29.41	17.64	2.95
129	43.80	34.60	17.30	4.30
132	17.30	54.00	27.50	1.20
133	25.39	36.58	34.94	3.17
134	20.80	29.00	37.50	12.70
143	24.44	36.67	30.00	8.89
156	12.50	12.50	12.50	62.50
161	6.46	25.81	41.93	25.80
163	37.00	40.70	18.50	3.80
176	15.30	44.20	32.60	7.90
183	39.47	39.47	18.42	2.64
196	42.20	30.00	18.80	9.00
231	52.44	29.27	15.85	2.44
241	7.10	38.00	46.40	8.50

the genotype 156 had a diameter development of 7 mm and above. This was followed by genotype 29 with 42.50%, genotype 66 with 26.80, and genotype 161 with 25.80% (Table 7).

When the seedlings of the genotypes were evaluated in terms of their grafting status, we found that genotype 29 stood out in both years.

Reproductive power was determined by proportioning the resulting populations of active second-stage larvae in 1 g soil in pots to the initial populations. Reproductive powers were found to be over 1 in both nematode species. In the pot experiment in which *M. incognita* was applied, the highest reproductive power was determined as 3.8 in genotype 196, but the lowest reproductive power was found in genotype 80 as 1.8. These results show that, as in the root-knot scale in the genotypes, *M. incognita* feeds on these species and females grow and reproduce in the roots. In the pot experiment in which *M. java*nica was applied, the highest reproductive power was obtained in genotype 183 with 3.0 but the lowest reproductive power was obtained in genotypes 127 (1.6), 29 (1.9), 66 (1.9), 80 (1.9), and 134 (1.9). These results show that as in the root-knot scale in the genotypes, *M. javanica* feeds on these species and females grow and reproduce in the roots. Both root-knot nematode species developed in all almond genotypes used. Generally, the gall ratios of *M. incognita* and the resulting population in the soil were higher than those of *M. javanica*. According to the gall ratios in the roots, the gall ratio values in genotypes 29, 66, 80, 121, 127, 134, 143, 161, and 163 were 2.0 and below, and they stood out as the promising genotypes for resistance (Table 8).

In all other genotypes, both *M. incognita* and *M. javanica* roots were found to be susceptible to these two root-knot nematodes by causing galling of more than 2.0. In parallel to the galling of all roots, the development of root-knot nematode egg packs was also observed. Accordingly, the resulting populations obtained by counting the active juveniles in the soil were also higher than the initial population and they showed that both root-knot nematode species developed in all genotypes in direct proportion to the gall ratios in the roots.

Some researchers have conducted similar studies to determine the rootstocks that are resistant to the root-knot nematodes and *Pratylenchus* species in different fruit species,

Table 8 The root galling indexes that *M. javanica* and *M. incognita* formed in the roots of different almond genotypes

Genotype	Gall index values			
	M. Javanica	Tolerance rate	*M. incognita*	Tolerance rate
29	1.9	R	2.1	MR
57	2.5	MR	2.3	MR
58	2.6	MR	3.2	S
66	1.9	R	2.2	MR
68	2.3	MR	2.6	MR
76	2.2	MR	2.7	MR
80	1.9	R	1.8	R
84	2.1	MR	2.9	MR
101	2.1	MR	2.5	MR
102	2.4	MR	3.3	S
121	2.1	MR	2.0	MR
127	1.6	R	2.4	MR
129	2.2	MR	2.7	MR
132	2.5	MR	2.7	MR
133	2.4	MR	3.4	S
134	1.9	R	3.3	S
143	2.0	MR	2.4	MR
156	2.2	MR	2.9	MR
161	2.0	MR	2.3	MR
163	2.0	MR	3.2	S
176	2.9	MR	3.6	S
183	3.0	S	2.9	MR
196	2.6	MR	3.8	S
231	2.7	MR	3.0	S
241	2.5	MR	3.1	S

R resistant, *MR* moderately resistant, *S* sensitive

and they used the gall indexes in the roots to establish the resistance status of root-knot nematodes in particular. Previous studies, similar to this study, generally used Hartman and Sasser's scale and determined the rootstocks with a gall index value of over 2 as susceptible to root-knot nematodes (Marull et al. 1991; Esmanjaud et al. 1994; Fernandez et al. 1994; Lu et al. 1998).

The basic principle in combating root-knot nematodes is that the areas where orchards are to be established are not contaminated with root-knot nematodes. This is because combating root-knot nematodes in perennial orchards is more difficult and costly than in areas with annual plants. The control of root-knot nematodes in orchards using cultural practices can be succesful, but it can taking many years (Dowler and Van Gundy 1984). For this reason, it is recommended to establish an orchard in areas free from root-knot nematodes by performing soil analyses in the first orchard facilities. After establishing orchards in clean areas, taking care of cultural precautions should be considered as a basic principle to prevent root-knot nematodes from infecting the orchard.

However, establishing orchards with resistant genotypes is important in terms of the economical use of orchards for many years by protecting the trees that will have long-term yields in the case of contamination with root-knot nematodes. Therefore, the detection and commercialization of the genotypes resistant to root-knot nematodes are of great importance in combating root-knot nematodes.

Conclusion

Our results showed that genotypes 29, 68, 133, and 196 were promising in terms of seedling growth performance and the coefficient of variation in the diameter development. Genotypes 29, 66, 80, 121, 127, 134, 143, 161, and 163 were noteworthy for their tolerance to *M. javanica* nematode species. It is planned to evaluate the promising genotypes with detailed studies focusing on other maternal characteristics. It is predicted that these genotypes have a high potential to be used for the production of seedling rootstocks for almonds, especially genotype 29 with its characteristics of being resistant to nematodes and the low coefficient of variation in the diameter development.

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Conflict of interest A.N. YıldırımX, U. Gözel, F. Yıldırım, B. Şan, C. Çelik, B. Bayar, H. Özgönen Özkaya and Y. Karakurt declare that they have no competing interests.

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