IJANS
International Journal of Agricultural and Natural Sciences
Uluslararası Tarım ve Doğa Bilimleri Dergisi
E-ISSN: 2651-3617 1(2): 137-141, 2018

Evaluation of Some Stone Fruit Rootstocks Against Resistance to Root Knot Nematode (*Meloidogyne incognita*)

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Abstract
Root nematodes have a very broad host range, causing economic losses in many plant species. The clonal rootstocks used in the grafted-stone fruit seedling production were tested for resistance to root nematodes. The seedlings were transplanted into plastic pots with 5 replications. Soil structure used in pots was 80% sand, 5% clay and 15% top soil, and disinfected by autoclaving prior to testing. The study was conducted under 25 ± 1 °C temperature and 60 ± 10% relative humidity conditions. Nematod inoculation was carried out when the rootstocks reached about 15-25 cm in length. Approximately 1500 second-stage larvae per plant were placed at 2cm soil depth near the root region. Three months after nematod inoculation, root galling index of rootstocks were determined. The results of galling index in the roots was determined as resistant (0-2) or susceptible (3-5 scale) plants. In this study, Patrones Arda, Garnem, Cadaman, Patrones Toro, Mariana GF 8-1, Myrobalan 29-C rootstocks were identified to be resistant to M. incognita while Myrobalan B and GF677 rootstocks were detected as sensitive. Patrones Arda and Patrones Toro rootstocks, developed by Betafidan, were resistant to M. incognita the first time in the present study.

Keywords: Root knot nematode, fruit rootstocks, resistant

INTRODUCTION

Turkey is the homeland for several plant species due to ecological factors such as soil and the climate. However, diseases and pests restrict economic orchards [1]. Root-knot nematodes (*Meloidogyne* spp.) are obligate plant parasites that lead to economic damage in several plant species such as vegetables, stone fruits, pome fruits, industrial and ornamental plants[2;3]. In Mediterranean regions, the RKN M. arenaria, M. incognita and M. javanica represent one of the major problems of the Prunus crops (peach, almond, apricot, plum and cherry) [4;5]. The root-knot nematodes consist the most significant nematode threat. *Meloidogyne* Goldi 1887 genus root-knot nematodes are also obligate plant parasites that feed on roots of about all vascular plant species [6]. Nematode damage cause global losses up to 100 billion dollars and *Meleidogyne* spp. are responsible for most of these losses [7]. Meloidogyne genus includes over 100 identified species that include the most harmful species such as *M. arenaria, M. incognita, M. javanica, M. hapla, M. fallax, M. chitwoodi* and *M. enterolobii* [8]. It was reported that the nematodes that are prevalent over 95% were *M. incognita, M. javanica, M. arenaria, M. chitwoodi*, *M. fallax* and *M. hapla* and these feed on more than 5500 plant species (Trudgill & Blok, 2001). *M. incognita, M. javanica*, and *M. arenaria* are prevalent in tropical regions, while *M. chitwoodi, M. fallax* and M. hapla are found in cooler regions[9]. Studies on Turkey reported that the most prevalent species in vegetable agriculture in Turkey with the most significant economic impact were *M. incognita*, *M. arenaria* and *M. javanica* [10;11;12;13]. Reported populations of Meloidogyne ethiopica in Europe identified as Meloidogyne luci in Turkey[14]. Made so far in Turkey in different regions and different cultures M. arenaria, M. artiellia, M. chitwoodi, M. luci, M. exigua, M. hapla, M. incognita, M. javanica, M. thamesi a total of 9 species were identified [14;15;16;17;18;19;20]

Resistance of plants to root-knot nematode can be divided into three stages; front penetration, penetration and penetration[21]. There is limited information about Prunus's resistance mechanisms to the root-knot nematode, especially at the pre-penetration and penetration stages. Root dissemination and root structural organization are factors that may affect nematode infection[22;23;24]. The studies on resistance against nematodes to completely inhibit or minimize the propagation of the nematode are preferred due to the lack of special application technique or equipment requirements, low costs and the environment-friendly nature of the method[25;26].

Wild plum species in our country have a very rich variety in their natural environment and some are their homeland. There are Prunus domestica, Prunus spinosa, Prunus cerasifera and Prunus divaricata in the natural environment of our country. This rich diversity offers significant differences in selection rehabilitation work[27]. The present study aimed to determine whether Myrobalan 29-C, Mariana GF 8-1, Myrobalan B, Garnem, Cadaman, Patrones Arda, Patrones Toro and GF677 rootstocks were resistant against the root-knot nematode (*M. incognita*).

MATERIALS AND METHODS

Plant material was provided by Betafidan. Myrobalan 29-C, Mariana GF 8-1, Myrobalan B, Garnem, Cadaman, Patrones Arda, Patrones Toro and GF677 rootstock were used in the present study. These rootstocks were planted in plastic pot with a diameter of 11 cm and a volume of 1000 cm^3. Soil structure where the plants were grown included 80% sand, 5% silt and 15% soil and was disinfected by autoclaving at 121ºC for 1 hour before the test. The experiements were conducted under 25 ± 1ºC temperature, 60 ± 10% humidity, 16 hours of light and 8 hours of darkness. The plants were not fertilized one week before and one week after nematode inoculation. In other periods, routine balanced fertilization was implemented. The root-knot nematode (*M. incognita*) used in the experiments was propagated in a variety of long pepper lines that are known to be sensitive to nematodes. Second stage larvae were obtained from the roots of the plants where the root-knot nematodes were cultured by collecting the egg masses under a stereo binocular according to the Baermann-funnel method. The experiments were conducted in 6 repetitions based on random blocks design when the length of the rootstocks reached about 15 cm. An average of 1500 second stage larvae were inoculated in each pot at a depth of 2 cm on four sides of each rootstock. The tests were assessed 80 days after root-knot nematode inoculation based on the root knotting rate. The seedling root gall index scale were analyzed based on the egg mass and the gall number index between 0 and 5 as described and presented in Table 1. Thus, the varieties within the 0-2 scale were determined to be resistant and those within the 3-5 scale as sensitive [28].

Table 1. The gall index values used in the varieties seedlings (Hartman and Sasser 1985)

İndex Values for t he number galls and Egg masses	**Root galling and egg mass indices were assessed on 0 to 5 scale**
There is no egg masses or galls in the root	0
There are 1–2 egg masses and galls in the root	1
There are 3–10 egg masses and galls in the root	2
There are 11–30 egg masses and galls in the root	3
There are 31–100 egg masses and galls in the root	4
There are more than 100 egg masses and galls in the root	5

Variance analysis was conducted with SPSS 18.0 (SPSS Inc., Chicago, IL, USA) software and the averages were made according to Duncan's at a significance level of 0.05.

RESULTS AND DISCUSSION

Myrobalan 29-C, Mariana GF 8-1, Garnem, Cadaman, Patrones Arda, Patrones Toro, Myrobalan B and GF677 rootstocks were used in rootstock resistance tests. The rate of galling occurred in tested rootstock roots due to M. incognita are presented in Table 2. Based on the findings, it was determined that there was no galling in Myrobalan 29-C, Mariana GF 8-1, Garnem, Cadaman, Patrones Arda and Patrones Toro rootstock roots at zero level. These seedling rootstocks were considered resistant to *M. incognita*. In Myrobalan B and GF677 rootstocks, it was determined that the rate of root galling was 5 and these seedling rootstocks were identified as sensitive (Table 2, Figure 1). It was determined that there was a statistically significant difference between these rootstocks based on the rate of galling. It was also found that the Patrones Arda and Patrones Toro rootstocks, developed by Betafidan, were resistant to *M. incognita* the first time in the present study.

Table 2. The root galling indexes that *M. incognita* formed in the roots of different varieties

Varieties	**Gall Index Values**	**Resistance Rate**
Myrobalan B	5.0 ± 0.0b	S
Myrobalan 29-C	0 ± 0a	R
Mariana GF 8-1	0 ± 0a	R
Garnem	0 ± 0a	R
Cadaman	0 ± 0a	R
Patrones Toro	0 ± 0a	R
Patrones Arda	0 ± 0a	R
GF677	5.0 ± 0.0b	S

R Resistant, S Suscebtible

*The differences among the averages indicated are statistically significant at the level of 5%

Figure 1. At the roots of Myrobalan B, the egg masses and galling index of M. incognita are visible.

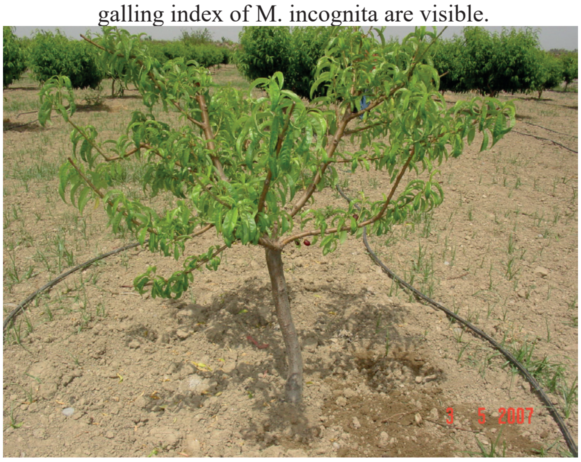

Figure 2. The shoot development of the nectarine plant seems to have stopped

Figure 3. At the roots of Gf-677, the egg masses and galling index of M. incognita are visible.

The root of the root knot nematode is damaged and the plant can not get water and nutrients as the root system of the end result is damaged. The root nematodes in the GF 677 host are large and small gallings. So the exiles of the plant have stopped and the plant is not growing. Choosing the wrong rootstock in the areas where the root nematode is located causes great economic loss(Figure 2;Figure 3).

It was reported that *in vitro* grown Myrobalan 29C Cadaman and Garnem clones were resistant to *M. incognita* and *M. javanica* [1]. GN 15 and GN 9 rootstocks were resistant to M. incognita nematodes based on the gall index figures. They reported that the GF-677 rootstock exhibited the highest value (170 pieces) and classified as sensitive[29;30]. Marull et al. (1991) inoculated various Prunus rootstocks with M. incognita nematode species and determined that GN1, GN3 and GN9 rootstocks were resistant[31]. Genotype 9 and 31 as tolerant to *M. javanica*, while genotype 54 was classified as resistant to *M. incognita*[32]. there were no galling in Adesoto 101, Bruce, Ishtara, AC-952, Garnem, and Cadamen rootstocks and no nematode reproduction was recorded in the Adesoto 101, Adara, Myro-10, Constanti, AD 105, and Cadaman rootstocks, and Gx N No: 17 exhibited no galling[33]. Floride isolates resulted in galling in GF.557 rootstock. *M. arenaria, M. incognita*, and *M. javanica* led to no galling in Nemaguard, Nemared, G × N no. 15, and G × N no. 22. The Prunophora rootstocks, P.2175, P.1079, P.2980, and Myro 29C, were not galled by any of the four isolates. It was determined that Myrobalan (*Prunus cerasifera*) P.2175, P.1079 and P.2980 clones were highly resistant to root-knot nematodes [34]. Myrobalan genotypes, which are plum rootstocks, against *M. arenaria* (1 pop.), *M. incognita* (2 pop.) and *M. javanica* (1 pop) populations and determined that P. 1079 and P. 2175 genotypes exhibited resistance at low and high temperatures[35]. In a study conducted, it was reported that GF 677 was sensitive to root-knot nematodes, while Nemared, GXN no. 22, Marianna GF 8-1 and Myrobalan 29C persisted their resistance even at high temperatures[36]. The almond, nectarine and peach rootstock GF 677 was sensitive to *M. arenaria, M. incognita* and *M. javanica*, while Nemared, Garnem (GXN 15, GXN 22) were resistant, and the plum rootstock myrobalan 29C was also resistant[37]. Tested 10 peach rootstocks against *M. javanica* and found that they were resistant, and GF 677 rootstock was sensitive[38]. *M. javanica* is very pathogenic to peaches in Italian populations and may cause severe crop losses in infected areas[39]. The host suitability to RKN in Myrobalan plum material ranges from susceptible clones (P.2646, P.16.5 and P.2032) to highly resistant clones (P.2175, P.1079 and P.2980). Resistance to M. arenaria is monogenic and completely dominant in P.2175 (gene Ma1, heterozygous) and in P.1079 (gene Ma2, homozygous) [40]. The differences in resistance between rootstocks are not tied to differences related to the effects of root spread, but to the different structural organizations of root ends. The epidermal structure of Tsukuba-4 and Tsukuba-5 completely prevented the penetration of *M. incognita*'s second stage jüveniles [41]. In a study, 1 year old SP-1, SP-2, SP-3 (*Prunus spinosa*), DO-1, DO-2 and DO-3 (*Prunus domestica*) rootstocks were all resistant against root-knot nematodes *M. incognita* race 1 and *M. javanica* race-1. The present study findings were consistent with the results of previous studies[42].

Patrones Arda is bred by Beta Fidancılık and a hybrid of (Prunus dulcis x Prunus persica) x Prunus amygdalus. It is a dwarf, red leafed rootstock. It has good grafting compatibility with peach, nectarine, almond and some Japanese plum varieties. It is tolerant to calcareous and heavy textured soils. It is suitable for intense plantations. It is more suitable than Garnem and GF 677 rootstocks for modern intense plantation techniques because it is dwarf. It is a dwarf rootstock which causes the grafted variety to fructify early and give more yields from unit area.(http://www.betafidan.com.tr/patrones-arda/). Patrones Toro® is bred by Beta Fidancılık and a hybrid of (Prunus saliciana x Prunus cerasifera) x Prunus pisardi. It is a dwarf rootstock. It fructifies the grafted plum varieties early. It grows very well under negative soil conditions such as heavy textured, calcareous, high pH soils. It is tolerant to most of the soil based fungus and root asphyxiation. Compatibility with apricot varieties is still being observed. It enables to install intensive plum orchards. It increases fruit quality (http://www.betafidan.com.tr/patrones-toro/).

CONCLUSION

In conclusion, in vegetable cultivation fields, rootstocks that are resistant to root-knot nematodes should be selected during allotment. Since it was determined that Myrobalan 29-C, Mariana GF 8-1, Garnem, Cadaman, Patrones Arda and Patrones Toro rootstocks were resistant to *M. incognita*, these rootstocks should be recommended to producers. It was found that Patrones Arda and Patrones Toro rootstocks, developed by Betafidan in Turkey, were resistant to nematodes. These rootstocks are important for their resistance against nematodes and other characteristics in nurseries and garden allotment. In areas where the greenhouses are common, the gardens have to be dismantled due to the selection of wrong rootstock such as GF 677 rootstock, which is sensitive to root-knot nematodes. Establishment of the gardens are expensive; therefore, in areas with nematode problems, the producers should be advised to use resistant rootstocks.

Acknowlesgement

The authors thank Betafidan, who provided support in the realization of the subject matter mentioned in this article

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