Selection of genotypes of peach rootstock resistant to *Meloidogyne incognita*

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ABSTRACT

The aim of this study was to evaluate the genotypes developed by the Peach Breeding Program at the Federal University of Viçosa, as regards to resistance to *M. incognita*. Six rootstocks genotypes propagated by cuttings (713-07, 713-13, 913-3, 913-6, 913-11 and 913-17) and two rootstocks propagated by seeds ('Okinawa' and hybrid between scion cultivars Aurora 2 x Aurora 1), were evaluated. The experimental design was randomized block design with five replicates and one plant per experimental units. After establishing the plants in pot, maintained in a greenhouse, this were inoculated with 11.000 juveniles + eggs of *M. incognita*. Evaluations were performed at 140 days after inoculation. The roots were evaluated and the number of galls and egg mass in the roots were determined. The eggs were extracted from each plant for quantification and determination of the Reproduction Factor (RF) of the nematode. The peach genotypes 913-3, 913-6, 913-11, 913-17 and 713-7 showed an immune reaction to *M. incognita*. Genotype 713-13 showed susceptible reaction to *M. incognita*. The hybrid between scion cultivars Aurora 2 x Aurora 1 confirmed susceptible.

Keywords: *Prunus persica*; breeding; resistance; root-knot nematode.

INTRODUCTION

Peach [*Prunus persica* (L.) Batsch] is considered a fruit of importance economically, with elevated consumption in worldwide. World production, in the year 2019, was approximately 25.7 million tons, the Brazil production being 183.132 tons; considered insufficient for consumption demand brazilian, generating imports, mainly from Chile, Argentina and Spain (FAO, 2021). In Brazil, the South region stands out as the largest producer, however, the limited area for the expansion has provided the migration to the Southeast region, presenting this region favorable conditions for economical exploitation of fruit trees of temperate climate, with areas of milder climate, mainly in high altitude regions (Ramos & Leonel, 2008).

The cultivation of peach tree has evolved in regions with subtropical climate and mild winter (Penso *et al*., 2020), due to the obtaining of new cultivars with as low chilling requeriments, which present favorable agronomic traits, associated the technologies that allow the development of culture such as irrigation (Leonel *et al*., 2011).

However, with the expansion of crops there is still a need to solve problems related to the incidence of different diseases and pests in cultivation, with emphasis on phytonematoids, mainly, due to implantation of the peach tree in previously used areas with susceptible crops causing reduction in the production.

The nematodes that cause greater losses in peach trees are *Meloidogyne incognita*, *M. javanica* and *M. arenaria*, being highly polyphagous and reproduce by mitotic parthenogenesis (Khallouk *et al*., 2013), promoting the formation of galls on the roots.

There are some management alternatives in order to minimize the damage caused by nematodes, such as the

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adoption of nematicides, but these are highly toxic and their use has been banned in some countries (Abawi & Widmer, 2000). The use of resistant or tolerant rootstocks is one of the main alternatives (Ye *et al*., 2009), since it is considered of low cust and environmentally friendly. In Brazil, most cultivars of peach rootstocks are obtained seeds, taken fruits processed by the industry, originated from scion cultivars with late maturation, as predominates in the south region (Fachinello, 2000), resulting in obtaining rootstocks without guarantee of genetic identity causing plant unevenness and different plant reactions to soil pathogens and abiotic stresses (Picolotto *et al*., 2010; Timm *et al*., 2015).

Hussain *et al*. (2013) and Gullo *et al*. (2014) reported the importance of choosing the rootstock due to its influence on the vigor of the plant, quality of the fruit and productivity of the orchard. In the Southeast region of Brazil, the most used peach rootstock is the cultivar Okinawa, obtained by the genetic breeding program of the University of Florida in 1953 (Sharpe, 1957; Sharpe *et al*., 1969) and introduced by the Instituto Agronômico de Campinas in 1969 (Ojima *et al*., 1999), possessing resistance to *Meloidogyne* nematodes (Sharpe, 1957; Malo, 1967).

With the prevalence of *Meloidogyne* spp. in a large part of the agricultural areas of Brazil and the increases in the cultivated area with the Okinawa rootstock, the resistance to these nematodes can be overcome. For these reasons, there is a need to select new genotypes that are more adapted to the edaphoclimatic conditions of the Southeast region and that have resistance genes to the root-knot nematodes.

Thus, this study aimed to select genotypes belonging to the Peach Breeding Program at the Federal University of Viçosa (UFV) regarding resistance to *Meloidogyne incognita*.

MATERIAL AND METHODS

Genotypes 713-07, 713-13, 913-03, 913-6, 913-11 and 913-17, all belonging to the Peach Breeding Program of the UFV, were selected to evaluate resistance to *M. incognita* (Figure 1), for presenting excellent rooting of herbaceous cuttings according to the results obtained by Oliveira *et al*. (2018) and Oliveira *et al*. (2020). In addition, the Okinawa rootstock was used as resistance pattern and the hybrid between scion cultivars Aurora 2 x Aurora 1 (Aur2 x Aur1) as a susceptibility pattern to *M. incognita*.

Nursery trees of genotypes 713-07, 713-13, 913-03, 913-6, 913-11 and 913-17 were obtained by herbaceous cuttings treated with indolbutyric acid at a concentration of 3000 mg L^{-1} per 5 seconds, according to the methodology proposed by Oliveira *et al.* (2020). Soon after the treatment, cuttings were accommodated in plastic boxes containing sterelize sand and stored in a greenhouse under daytime fogging activated every 5 min for 10 seconds (Oliveira *et al*., 2020), by a period of 60 days. The seedlings of the Okinawa cultivar and Aur2 x Aur1 hybrid were multiplied via semiferous propagation, with stratification in a chamber cold at 5 º C during 60 days.

After this periods, the cuttings plants that showed roots and seedlings ('Okinawa' and hybrid) were transplanted to plastic pots with a capacity of 11 L containing a mixture of soil + sand in a 1:1 (v/v) ratio, previously submitted to biofumigation with mustard oil at a dose of 60 mL m^{-3} of soil (Aguiar, 2008) ensuring that there was no contamination with other types of nematodes. The seedlings were maintained in a greenhouse, irrigated and fertilized as required by the plants.

The *M. incognita* population used in this study was obtained from roots of carrots collected in Rio Paranaíba, Minas Gerais state, Brazil. From this field population, it was settled down pure population of *M. incognita*. For this, tomato 'Santa Clara' seedlings with two to three pairs of final leaves were transplanted into 2 L plastic pot containing a 1:1 mixture of soil + sand previously treated with mustard oil at a dose of 60 ml m^{-3} of soil (Aguiar, 2008). After 20 days of transplanting, each pot was infested with a single egg mass removed from the infected tissue of carrot. (Coyne & Ross, 2014). The seedlings were maintained in a greenhouse and after 60 days the infected roots were collected, washed in taping water and used for the extraction of eggs and females for multiplication of the inoculum and for identification, respectively. The population identity was determined using the isoenzyme electrophoresis technique, according to methodology proposed by Ornstein (1964) and Davis (1964).

For extraction of eggs, the infected tomato roots were washed in beakers with taping water, chopped into pieces of approximately 1 to 2 cm and crushed in a blender with 0.5% NaOCl solution, for 20 seconds (Boneti & Ferraz, 1981). The resulting suspension was poured through a set of 200 mesh (75 µm) and 500 mesh (25 µm) sieves and the eggs collected in the 500 mesh sieve. The extracted eggs were counted in a Peters chamber with the aid of a light microscope, the concentration of the suspension was adjusted and used to multiply the inoculum used in the experiment. For this, tomato 'Santa Clara' seedlings were inoculated with 2.000 eggs pl^{-1}, maintained in a greenhouse for approximately 60 days. After this period, the infected roots were collected, the eggs extracted according to Boneti & Ferraz (1981), followed by the assembly of an hatching chamber (Cliff & Hirschmann, 1985) and incubation for 3 days, at 25 °C in BOD to obtain juvenile stage 2 (J2) of *M. incognita*. The suspension was calibrated with the aid

of a Peters chamber under a light microscope and then used in the inoculation of peach cultivars, according described below.

At 150 days after transplanting, peach plants were inoculated with a suspension containing 11.000 J2 of *M. incognita*, deposited in four equidistant holes 2 cm depth. Tomato 'Santa Cruz' plants were inoculated with 2.000 J2 pl^{-1} to prove the viability of the inoculum. The experimental design used was a randomized block with eight treatments (genotypes 713-07; 713-13; 913-03; 913-6; 913-11; 913-17; Okinawa and hybrid Aur2 x Aur1) and five replications, with one plant per experimental units.

After 140 days of inoculation, the roots were separated from the shoot and washed. Then, root segments were removed at random, totaling 100 g of moist matter, in which they were made as evaluations, what constituted in the count of the number of galls, the number of egg masses and the number of eggs present in the roots.

To count the number of egg masses, the roots were submitted to staining with phloxin B to facilitate their visualization and counting (Taylor & Sasser, 1978), with an adaptation. For this, the roots were submerged for approximately 20 min in solution containing 150 mg of Phloxin B L^{-1} of water. Soon after this time, the roots were washed to remove excess dye, and the egg masses now stained red, were counted with the aid of a table magnifying glass.

After counting the number of galls and egg mass the root the nematode eggs were extracted from the roots (Hussey & Barker, 1973), processing an sample of 100 g of roots was stirred in plastic containers for 4 min to extract the eggs. The extracted eggs were counted in a Peters chamber under a light microscope and used to determine the nematode's Reproduction Factor (RF) in the different genotypes, considering RF = final population/initial population, where the reaction of each genotype was provided based on the RF value, and plants with RF = 0 were considered immune; resistant, RF <1; and susceptible, RF > 1 (Oostenbrink, 1966).

Posteriorly extracting the eggs, the roots of each plot (evaluated sample + remaining roots) were dried at 60 ºC in an oven with forced air circulation until constant mass. The variables number of galls, number of eggs and number of eggs masses were calculated as a function of the total dry mass of the roots, obtaning Number of Galls/Dry Root Mass (g), Number of Eggs/Dry Root Mass (g) and Number of Egg Masses/Dry Root Mass (g).

The data were analyzed by descriptive statistics, using the program R (R development core team, 2010), introducing himself mean with the confidence intervals of 97.5%.

RESULTS AND DISCUSSION

Meloidogyne incognita was the only species found in roots carrot this study, with esterase profile typical of the specie, due present two very obvious main bands (Figure 2 A).

The resistance of a plant refers to its ability to prevent or delay the development or multiplication of the nematode in its tissues (Trudgill, 1991; Roberts, 2002). In the case of the interaction plants *Meloidogyne* spp., this attribute is often measured by the nematode reproduction factor in the plant tissues (Oostenbrink, 1966), but when there is evidence of a high correlation between reproduction and symptoms, other variables can be used such as number of galls (Roberts, 2002).

The hybrid Aur2 x Aur1, was susceptible to *M. incognita* (RF = 45.93) (Table 1), with elevated number of galls (Figure 2 B). In this hybrid there was a greater severity of symptoms (Figure 3 A) and a higher reproduction rate (Figures 3 B; 3 C) of the nematode in its roots, when compared with the other genotypes tested. The cultivar Aurora-1 originates from the crossing of the cultivars Tutu x Colombina (Figure 1), with 'Tutu' being the full sib of the cultivar Talismã and descendant of the cultivar Rei da Conserva, both reported as susceptible by Menten *et al.* (1977) when evaluating the reaction of peach rootstocks to *Meloidogyne* spp., from a mixed population of *M. arenaria* and *M. incognita* in São Paulo-Brazil.

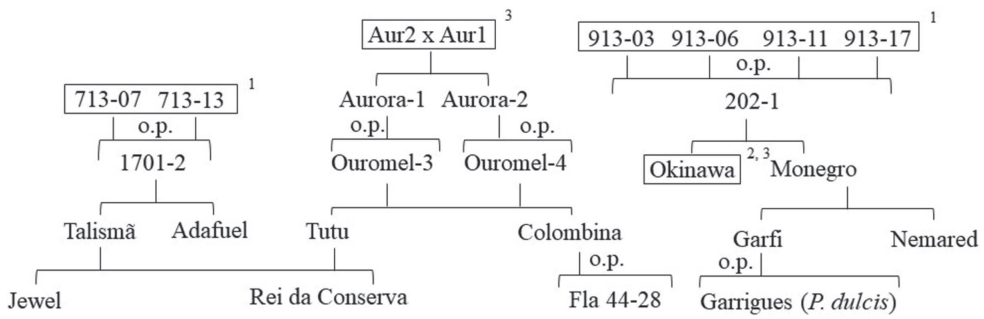

Figure 1: Genealogy of the *Prunus persica* genotypes used in the experiment. [1]Peach breeding Program at the Federal University of Viçosa, propagated by herbaceous cuttings [2]Cultivar made available by the Instituto Agronômico de Campinas. [3]Propagated by seeds. Genotypes inserted in a box are to be taken in the experiment. o.p. – open-pollination.

The tested peach genotypes behaved differently regarding the reaction to *M. incognita*, as can be seen in Figure 3 and Table 1. *M. incognita* was not able to induce symptoms (Figure 3 A) or reproduce (Figures 3 B; C; Table 1) in genotypes 713-7, 913-3, 913-6, 913-11 and 913-17, behaving in the same way as the resistant cultivar Okinawa. The Okinawa rootstock is resistant to *M. arenaria, M. incognita* and some populations of *M. javanica* (Fachinello *et al*., 2000; Mayer *et al*., 2005; Saucet *et al*., 2016) e a *M. enterolobii* (Souza *et al*., 2014).

There is evidence in the literature that resistance to *Meloidogyne* spp. found in *Prunus* subgenus *Amygdalus* (which includes peach and almond) is controlled by a dominant resistance (R) gene (Sharpe *et al*., 1969; Esmenjaud *et al*., 1997; 2009; Gillen & Bliss, 2005; Saucet *et al*., 2016). In peach trees, this resistance is attributed to the R gene identified as RMia, which confers resistance to *M. arenaria* and *M. incognita*, present for example in Nemared and Nemaguard rootstocks (Esmenjaud *et al*., 2009; Duval *et al*., 2014). However, a single R gene has been hypothesized in 'Okinawa', but not precisely located in linker group 2 (LG2) (Gillen & Bliss, 2005), or suppose that the R gene is not allelic with RMia (Duval *et al*., 2014). In almonds, this resistance is attributed to the RMja gene, which confers specific resistance to *M. javanica* and possibly *M. arenaria* (Esmenjaud *et al*., 2009; Van Ghelder *et al*., 2010).

In general, the resistance mechanism attributed by these R genes involves a hypersensitivity response that leads to the isolation and collapse of giant cells, which are vital to the nutrition, development and reproduction of *Meloidogyne* spp. (Saucet *et al*., 2016). Thus, the resistance conferred by the R gene causes cell necrosis (Khallouk *et al*., 2011), causing the inhibition of the reproduction of these nematodes in the tissues of *Prunus* spp. that carry such R genes, resulting in the effective suppression of these nematoids.

Genotypes 913-3, 913-6, 913-11 and 913-17, immune to *M. incognita* (RF = 0), come from open pollination possibly self-pollination, of the UFV 202-1 genotype, which in turn was obtained by crossing 'Okinawa' with 'Monegro' (Figure 1), both resistant to *M. incognita*. Cultivar Monegro also has resistance to *M. arenaria, M. hapla, M. hispanica* and M. *javanica* (Felipe, 2009). Although the UFV 202-1 genotype has not been evaluated for its resistance to *M. incognita*, it can be assumed to be resistant, since its parents are resistant and there was no segregation in the 913 progeny (Table 1). Although the 913 progeny was generated by open pollination, it can be considered an F2

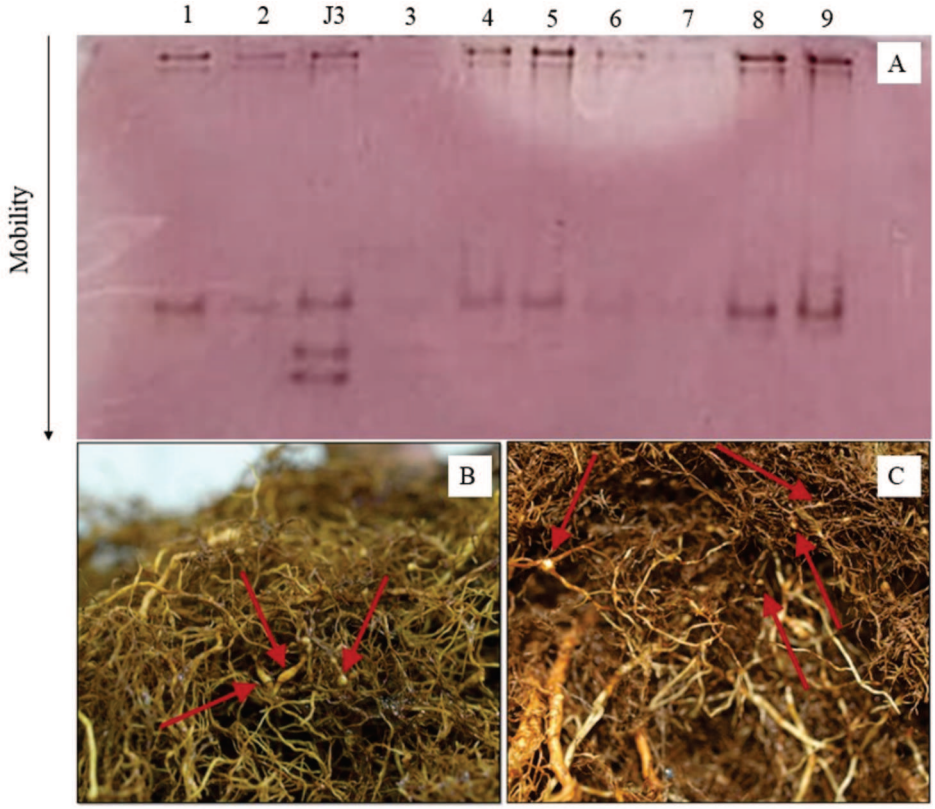

Figure 2: Esterase phenotypes of *Meloidogyne incognita* populations from plants carrots collected in Rio Paranaíba, state of Minas Gerais, Brazil (Female - 1 to 9 and J3 - *M. javanica* esterase phenotype used as comparison standard) (A). Roots of peach genotypes with galls. Arrows indicate as galls. (B) Hybrid Aur2xAur1; (C) genotype 713-13.

generation of 'Okinawa' x 'Monegro', given that the peach tree is considered an autogamous plant with a negligible crossing rate (Ojima *et al*., 1983).

The genotypes 713 segregated for resistance to *M. incognita*, with 713-07 being immune (RF = 0) and 713-13 susceptible (RF = 8.85) (Figure 2 C; Figure 3; Table 1). These genotypes were obtained by open pollination of genotype 1701-2, which is the result of the crossing between the rootstocks Talismã and Adafuel (Table 1).

Menten *et al*. (1977) verified the susceptibility of 'Talismã' x 'Rei da Conserva', being the last parent of 'Talismã'. The cultivar Adafuel, on the other hand, is a rootstock of Spanish origin, which despite being selected for its vigor and rooting superiority, is susceptible to *Meloidogyne* species (Cambra, 1990). Considering that 'Talismã' and 'Adafuel' are susceptible to *Meloidogyne* spp., there is doubt about the origin of the allele responsible for resistance in genotype 713-07, supposing the

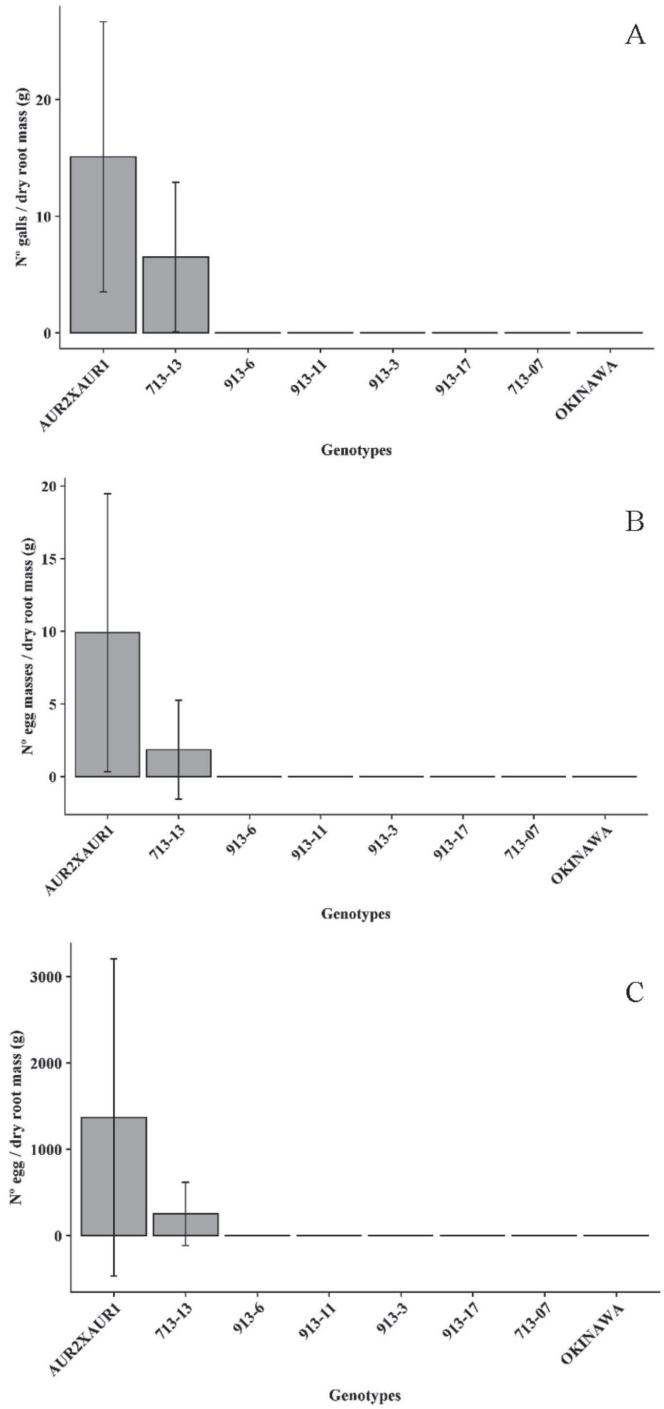

Figure 3: *M. incognita* symptoms and reproduction induced in peach genotypes. Number of galls g^{-1} of dry root (A), Number of egg mass g^{-1} of dry root (B) and Number of eggs g^{-1} of dry root (C), presenting the means with the respective confidence intervals at 97.5%.

Table 1: Reaction of peach genotypes to *Meloidogyne incognita*

Genotypes	RF[1]	Reaction[2]
713-07	0	I
713-13	8.85	S
913-3	0	I
913-6	0	I
913-11	0	I
913-17	0	I
Aur2xAur1	45.93	S
OKINAWA	0	I

[1]Reproduction factor value (n = 5) (RF = final population / 11.000 J2 of *M. incognita*); [2]Genotype reaction classification according to Oostenbrink (1966): I = Immune; S = Susceptible.

possibility of some crossing and the progeny 713 not being self-fertilization of the 1701-2 plant, although the crossing rate is negligible.

The absence of galls (Figure 3 A) and the suppression of the multiplication of *M. incognita* (Figures 3 B; 3 C) in the roots of the tested genotypes show that the resistance mechanism involves the induction of hypersensitivity and collapse of giant cells, preventing the nematode be reproduce. However, histopathological studies are necessary to confirm this.

The results presented here show that the genotypes for peach rootstocks 913-3, 913-6, 913-11, 913-17 and 713-7 are promising for the management of *M. incognita*, being an alternative use with orchards infested. However, these genotypes need to be challenged against other populations of *M. incognita* and also to *M. javanica* and *M. arenaria* in order to test the hypothesis that they would also be resistant to these species, considering the resistance information of their parents. In addition, other traits of these genotypes, such as vigor, size, dwarfing effect, precocity, compatibility with scion cultivars, need to be determined.

CONCLUSIONS

The peach genotypes 913-3, 913-6, 913-11, 913-17 and 713-7 are immune to *M. incognita*, a reaction characterized by the complete suppression of the nematode's reproduction in its roots;

Genotype 713-13 and the hybrid between scion cultivars Aurora 2 x Aurora 1 are susceptible to *M. incognita*, not be selected as rootstock.

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