

# Screening of peach rootstock hybrids for resistance to root-knot nematode

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## ABSTRACT

Two peach genotypes 'Sharbati' and 'Flordaguard' were crossed to develop F, hybrid rootstocks and subsequently tested for resistance against root-knot nematode . Minimum reduction in plant height after 120 days of nematode inoculation was recorded in Flordaguard (11.07%), it was followed by Sharbati × Flordaguard (16.73%). Sharbati × Flordaguard seedlings inoculated with nematode juveniles had high plant height, root length, leaf chroma, 'b' values, hue angle, leaf size and leaf area. Flordaguard seedlings had higher shoot dry weight, 'a' value, spad values, petiole length and leaf number but, lower plant height, leaf area, leaf size, chroma values, hue angle. Hybrid seedlings from both the cross combinations had dark red coloured young leaves, a trait from Flordaguard. Number of juveniles penetrated the root system was negatively correlated with shoot dry weight, internodal length, leaf number, petiole length, spad values, 'a' value shoot dry weight and positively correlated with 'L'value, 'b' value, chroma and hue angle. The highest number of juvenile penetration per root system was recorded in Sharbati with (38 juvenile per roots system) followed by seedlings of Sharbati × Flordaguard (15 juvenile per root system) and Flordaguard × Sharbati (11 juveniles per root system). Shoot dry weight, internodal length, petiole length, spad values, 'a' value, and hue angle vary among the hybrid seedlings with lower nematode population and root penetration whereas higher nematode population and root penetration were abserved among the seedling population. These characters can be used to assess nematode tolerance/ resistance in peach hybrid seedlings.

**Key words**: Nematode resistance, rootstock breeding, peach, morphological markers

## INTRODUCTION

In India, the area under cultivation of peach is19,000 ha with annual production of 1,14,000 metric tonnes (NHB, 7). Medium and high chilling peach varieties are cultivated in the Himalayan states having temperate climate. Punjab Agricultural University, Ludhiana has pioneered in the introduction of low chill peach, nectarine and plum in India. Apart from Punjab, these low chill varieties have been recommended in the sub-tropical regions of Jammu and Kashmir, Himachal Pradesh and North and East India due to high productivity (17-22 tons/ ha), precocity, regularity, early maturity and good economic returns. One of the major problems in peach industry, especially in the warm subtropics is short tree life which results in early decline of the orchards with average life of 10-12 years or less. It is caused by multiple factor like nematode, bacterial canker, iron chlorosis and variety of non-specific secondary pathogens (Liu et al., 5). Rootstock which provides root system to the composite grafted peach plants is the major contributor to tree performance and

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longevity as it determines tolerance to various biotic and abiotic stresses. Root-knot nematode (RKN) species Meloidogyne incognita (Kofoid & White) Chitwood and *M. javanica* (Treub) are the predominant species causing damage to peach (Nyczepir et al., 8 and Singh et al., 13). Use of nematicides have been banned due to environmental issues hence, peach rootstocks with durable resistance to root-knot nematodes are needed. Several rootstock breeding programs using interspecific hybridization among Prunus species has been initiated in the developed nations but, most of them are in private domain. 'Sharbati' variety of peach is used as rootstock in sub-tropical regions of India, because of its wide adaptability to warm climatic conditions and alkaline soils, but it is susceptible to root-knot nematode. 'Flordaguard' rootstock is inter-specific cross of Chico 11 × Prunus davidiana. It has been found to be resistant against the root-knot nematodes viz. M. javanica, M. incognita and M. floridensis (Singh et al., 13 and Rubio-Cabetas, 10). However, 'Flordaguard' is susceptible to severe iron deficiency under alkaline conditions (Egilla and Byrne, 2); and shows very poor seedling growth under subtropical regions. There is a need to develop new peach rootstocks having root-knot nematode resistance along with

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better growth characters. Hence, the present studies were conducted with an objective of developing and screening new low chill candidate peach rootstocks having resistance to root-knot nematode.

### MATERIALS AND METHODS

This experiment was conducted at Fruit Research Farm of the Department of Fruit Science, Punjab Agricultural University, Ludhiana from the year 2016 to 2018. The reciprocal crosses were made between varieties 'Sharbati' and 'Flordaguard' and the seeds of parents and the F<sub>1</sub> hybrids were germinated after summer stratification under controlled conditions as described in Singh et al., (14). The new F<sub>1</sub> hybrids along with the parents were tested against the most prevalent root-knot nematode species Meloidogyne incognita. Pure culture of M. incognita was developed on susceptible eggplant (Solanum melongena L.) variety 'Punjab Sadabahar'. At 3-4 leaf stage the plants were inoculated with *M. incognita* egg masses collected from the previous culture maintained on the brinjal plants at nematology sick plot of Department of Vegetable Science, Punjab Agricultural University, Ludhiana. The egg mass were collected from the roots of infected brinjal plants manually with the help of forceps and were kept for hatching at 37°C in the incubator on a tissue paper supported by a wire mesh placed on a Petri-plate containing fresh water. Freshly hatched second stage larvae (J<sub>2</sub>) were collected for inoculations. Sixty days old seedlings of parents Sharbati and Flordaguard; and their F, hybrids viz. Sharbati × Flordaguard and Flordaguard × Sharbati with uniform growth grown in root trainers containing 300 cc soil in each cell were infested with 2<sup>nd</sup> stage juveniles of *M. incognita* @ 2.0 J<sub>2</sub>/g of media by making holes with the help of a glass rod near the roots of the plant. Plants were watered regularly and properly maintained under polycarbonate roof HDPE shade net (50%) house. The nematode penetration was recorded at 7<sup>th</sup>. 14<sup>th</sup> and 35<sup>th</sup> days after inoculation. After uprooting the seedlings, roots were washed gently under tap water to remove soil or other adhering materials for recording number of nematode juveniles penetrated per root system. Roots were then disinfected with 2.5% sodium hypochlorite solution and washed again 3 to 4 times in running water. Then roots were stained with Acid Fuschin stain (0.1%) and observed under stereo-binocular microscope for estimating the number of juveniles penetrated per gram of root system. Lignification and hardening of roots after 35 DAI prevented the proper staining and estimation of juvenile penetration. Soil samples were also analysed for estimation of root knot nematode population. Washing of the individual soil sample

was excuted using Cobb's sieving and decanting technique (Cobb, 1 and Schnidler, 11).

The data regarding the plant height, plant girth, internodal length, leaf number, petiole length, leaf length, leaf breadth and leaf length breadth (L/B) ratio and leaf size was recorded at 60, 90 and 120 days after inoculation. The leaf colour and leaf spad values were recorded also recorded at 120 days after inoculation. The 'L', 'a' and 'b' chromacity values were recorded for the leaves with Colour difference meter (ColorFlex®, EZ, USA). L ranges from 0 (black) to 100 (white) which represents the lightness of the colour. Chromacity 'a' represents redness (+a) or greenness (-a) and 'b' depicts yellow (+b) or blue (-b) colour. The chroma (C) was calculated as C=  $(a^2 + b^2)^{\frac{1}{2}}$ which shows the intensity of colour saturation from dull to vivid colour depicted by low to high values, respectively. The hue angle (h°) was calculated by equation tan<sup>-1</sup> b/a; represents red at 0° or 360°, yellow at 90°, green at 180° and blue at 270°. SPAD values were recorded using a SPAD meter (SPAD 502 plus Konica Minolta Sensing, Europe B.V.). The data were subjected to analysis of variation (ANOVA) using statistical software SAS (V 9.3 SAS Institute Inc., USA). The mean separation was done using least significant difference (Fisher's LSD) at  $P \leq 0.05$  following significant F test. The experiment was replicated four times with 24 rootstock seedlings of each genotype (n=96). The Principal component analysis was done using XLSTAT Trial (v 2018 Trial, Addinsoft, USA).

## **RESULTS AND DISCUSION**

The inoculation of *Meloidogyne incognita* resulted in significant reduction in plant height at 60, 90 and 120 days after inoculation, irrespective to the genotypes (Table1a). At 120 days after inoculation, maximum seedling height (25.6 cm) was found in Sharbati which was at par with all the other three genotypes. The plant height of 22.72cm was recorded in the seedlings of Flordaguard. At 120 days after inoculation, 18.73% decrease in plant height was recorded in the inoculated seedlings of Sharbati in comparison to uninoculated ones. Minimum reduction in plant height (11.08%) was recorded in Flordaguard seedlings. While this reduction in the seedlings of Sharbati × Flordaguard was 16.73% and 19.90% in the Seedlings of Flordaguard × Sharbati. The Meloidogyne incognita infection resulted in reduction in plant girth at different growth stages, with significant reduction in plant girth at 120 DAI irrespective of the genotypes (Table1b). After 120 days of inoculation, highest average plant girth (3.13 mm), was recorded in the hybrid seedlings of Sharbati × Flordaguard which did not differ significantly from the plant girth

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**Table 1.** Effect of *M. incognita* on (A) plant height (B) plant girth (C) internodal length and (D) leaf number of the peach rootstocks and their hybrids. 'S  $\times$  FG' Sharbati  $\times$  Flordaguard; 'FG  $\times$  S' Flordaguard  $\times$  Sharbati. The values with the same letter are not significant.

			(A)	) Plant heig	ght (cm)				
Genotype	60 DAI			90 DAI			120 DAI		
	Control	Inoculated	Mean	Control	Inoculated	Mean	Control	Inoculated	Mean
Sharbati (A)	17.4	13.4	15.4ª	22.1ª	20.4 <sup>ab</sup>	21.2ª	31.5ª	25.6ª	28.6ª
Flordaguard (B)	16.4	14.7	15.5ª	18.8 <sup>bc</sup>	17.6 <sup>cd</sup>	18.2 <sup>♭</sup>	25.6ª	22.7ª	24.1°
S×FG	16.0	13.6	14.8 <sup>ab</sup>	19.3 <sup>bc</sup>	16.0 <sup>d</sup>	17.6 <sup>bc</sup>	30.5ª	25.4ª	27.9 <sup>ab</sup>
FG×S	14.7	12.4	13.6	20.2 <sup>ab</sup>	16.4°	18.3°	29.3ª	23.5ª	26.4 <sup>b</sup>
Mean	16.1ª	13.5 <sup>⊳</sup>		20.1ª	17.6 <sup>b</sup>		29.2ª	24.3 <sup>b</sup>	
LSD <sub>0.05</sub>	Genotype (A): 1.3 Nematode (B): 0.9 A×B: NS			Genotype (A): 1.7 Nematode (B): 1.2 A×B: 2.43			Genotype (A): 2.1 Nematode (B): 1.5 A×B: NS		
			(B	) Plant girt	h (mm)				
Genotype	60 DAI			90 DAI			120 DAI		
	Control	Inoculated	Mean	Control	Inoculated	Mean	Control	Inoculated	Mean
Sharbati (A)	2.6	2.2	2.4ª	2.9ª	2.6 <sup>b</sup>	2.7 ª	3.2ª	2.9ª	3.0ª
Flordaguard (B)	2.3	2.2	2.3 <sup>ab</sup>	2.7 <sup>ab</sup>	2.6 <sup>b</sup>	2.6 ª	3.2ª	2.9ª	3.0ª
S×FG	2.5	2.3	2.4ª	2.8 <sup>ab</sup>	2.5 <sup>bc</sup>	2.6 ª	3.3ª	3.1ª	3.2ª
FG×S	2.3	2.1	2.2 <sup>b</sup>	2.5 <sup>bc</sup>	2.3°	2.4 <sup>b</sup>	2.9ª	2.6ª	2.7 <sup>b</sup>
Mean	2.4ª	2.2ª		2.7ª	2.5ª		3.1ª	2.9ª	
LSD <sub>0.05</sub>	A: 0.2	2; B: NS; A×E	B: NS	A: 0.2	2; B: NS; A×E	3: 0.3	A: 0.2	B: 0.14; A×I	B: NS
			(C) lı	nternodal le	ength (cm)				
Genotype	60 DAI			90 DAI			120 DAI		
	Control	Inoculated	Mean	Control	Inoculated	Mean	Control	Inoculated	Mean
Sharbati	0.95°	0.97°	0.96 <sup>b</sup>	1.02°	1.08 <sup>bc</sup>	1.05 <sup>b</sup>	1.38 <sup>d</sup>	1.42 <sup>cd</sup>	1.40 <sup>b</sup>
Flordaguard	1.08 <sup>ab</sup>	0.81 <sup>d</sup>	0.95 <sup>b</sup>	1.23ª	1.04 <sup>c</sup>	1.14 <sup>a</sup>	1.49 <sup>cd</sup>	1.65 <sup>ab</sup>	1.57ª
S×FG	1.13ª	1.02 <sup>bc</sup>	1.08ª	1.19 <sup>ab</sup>	1.20ª	1.20ª	1.67 <sup>ab</sup>	1.55 <sup>bc</sup>	1.61ª
FG×S	0.98 <sup>bc</sup>	0.71°	0.85°	1.22ª	0.86 <sup>d</sup>	1.04 <sup>b</sup>	1.70ª	1.42 <sup>cd</sup>	1.56ª
Mean	1.04ª	0.88 <sup>b</sup>		1.17ª	1.05 <sup>b</sup>		1.56ª	1.51ª	
LSD <sub>0.05</sub>	A: 0.7; B: 0.05; A×B: 0.09			A: 0.8; B: 0.5; A×B: 0.10			A: 0.07: B:NS; A×B: 0.14		
				(D) Leaf nu	umber				
Genotype	60 DAI			90 DAI			120 DAI		
	Control	Inoculated	Mean	Control	Inoculated	Mean	Control	Inoculated	Mean
Sharbati	22.2	18.7	20.5ª	21.0 <sup>cd</sup>	17.7 <sup>ef</sup>	19.3°	27.1 <sup>de</sup>	24.8°	26.0°
Flordaguard	24.9	21.5	23.2ª	28.1ª	25. <sup>b</sup>	26.6ª	31.1 <sup>bc</sup>	30.2 <sup>bcd</sup>	30.7 <sup>b</sup>
S×FG	22.8	19.2	21.0ª	22.8 <sup>bc</sup>	19.6 <sup>de</sup>	21.2 <sup>⊳</sup>	32.4 <sup>ab</sup>	27.4 <sup>cde</sup>	29.9 <sup>b</sup>
FG×S	26.2	18.9	22.6ª	25.3 <sup>b</sup>	17.0 <sup>f</sup>	21.2 <sup>⊳</sup>	35.5ª	32.0 <sup>ab</sup>	33.8ª
Mean	24.0ª	19.6 <sup>b</sup>		24.3ª	<b>19.8</b> ⁵		31.5ª	28.6ª	
LSD <sub>0.05</sub>	A: NA; B: 0.9; A×B: NS			A:2.5; B: 1.3; A×B: 1.8			A: NS: B: 1.9; A×B: 2.7		

in the seedlings of Sharbati and Flordaguard. The lowest plant girth was recorded in hybrid seedlings of Flordaguard × Sharbati (2.55 mm). The data also shows that minimum decrease in plant girth (5.15%) after 120 days of RKN infection over the control seedlings was recorded in Sharbati × Flordaguard. The inoculation of *M. incognita* also resulted in significant decrease in the internodal length in all the genotypes at all the intervals (Table 1c). After 120 days of inoculation, maximum internodal length was recorded in Flordaguard (1.65 cm) which did not differ significantly from the internodal length recorded in seedlings of Sharbati × Flordaguard seedlings. However, minimum internodal length (1.42 cm) was observed in seedlings of Sharbati. The lesser reduction in growth characteristics of Sharbati × Flordaguard and Flordaguard × Sharbati seedlings over the Sharbati seedlings might be due to higher resistance of hybrid seedlings to the nematodes. Singh et al. (14) has reported Sharbati peach rootstock to be highly susceptible to root knot nematodes. The resistance to M. incognita in Prunus rootstocks is controlled by two dominant genes (Mi and Mij) and to M. javanica by a single dominant gene (Mij) Lu et al. (6). Flordaguard has been found to be resistant to M. javanica, M. incognita and M. floridensis (Rubio-Cabetas, 10). Nyczepir et al. (8) found Flordaguard to be resistant or nonhost to Meloidogyne mayaguensis. Flordaguard is also resistant to M. floridensis which even attacks nematode resistant rootstock 'Nemaguard'.

The inoculation of *M. incognita* resulted in significant decrease in leaf number, leaf blade length, width and ratio at all the intervals (Table 1d & Figure 1). After 120 days of inoculation, highest leaf number (32.03) was recorded in Flordaguard × Sharbati which did not differ significantly from the leaf number recorded in Flordaguard seedlings. It was followed by leaf number (27.37) in Sharbati × Flordaguard which was also at par with the leaf number in Flordaguard. The lowest leaf number was recorded in Sharbati (24.83). At 120 days after inoculation, maximum leaf length (Figure 2a) were observed in seedlings of Sharbati (7.28 cm) followed by leaf length in hybrid seedlings of Sharbati × Flordaguard (5.94 cm). The leaf length Sharbati × Flordaguard did not differ significantly from leaf length in Flordaguard and Flordaguard × Sharbati. Similar, results were recorded for leaf width (Figure 1b). At 120 days after inoculation highest leaf blade width (1.58 cm) was recorded in seedlings of Sharbati. There was no significant difference in leaf width of among the hybrid seedlings of Flordaguard × Sharbati, Sharbati × Flordaguard and Flordaguard. At 120 days after inoculation, highest leaf blade ratio (4.57 cm) was recorded

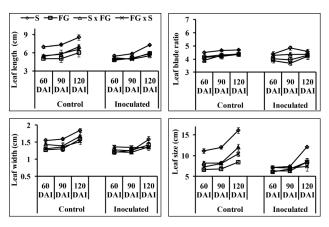


Fig. 1. Effect of *M. incognita* on the leaf length, leaf width, leaf blade ratio and leaf size of the peach rootstocks and their hybrids. The vertical bars represent standard error.

in seedlings of Sharbati followed by seedlings of Sharbati × Flordaguard (4.37 cm) which did not differ significantly from leaf blade ratio in Flordaguard and Flordaguard  $\times$  Sharbati. The inoculation of *M*. incognita resulted in significant decrease in the leaf size at all the growth stages (Figure 1d). At 120 days after inoculation, maximum leaf size (12.07 cm<sup>2</sup>) was recorded in seedlings of Sharbati followed by seedlings of Flordaguard (8.47 cm<sup>2</sup>) and Sharbati × Flordaguard (8.44 cm<sup>2</sup>). While, the minimum leaf size was recorded in seedlings of Flordaguard × Sharbati (7.41 cm<sup>2</sup>). Meloidogyne incognita also resulted in significant reduction in petiole length at different growth stages (Figure 2a). At 120 days of inoculation maximum seedling petiole length (5.59 mm) was observed in seedlings of Sharbati × Flordaquard followed by in seedling of Flordaguard × Sharbati (5.15 mm). Petiole length of Flordaguard seedlings did not significant differs from the seedlings of Flordaguard × Sharbati. The lowest petiole length of (4.78 mm) Sharbati seedlings was at par with remaining three genotypes. In view of vigorous growth habit the peach hybrid seedlings have to be transferred to field on the onset of winters. The non-significant variation in leaf length, width, length-breadth ratio and leaf size following nematode inoculation might be due to short period available for growing the seedlings in root trainers due to approaching dormancy period which is the time for transplanting the seedlings in field. Hence, leaf size can't be a biomarker for nematode tolerance or resistance for peach as peach cannot be grown in pots for long.

There was no significant effect of inoculation of *M. incognita* on root and shoot length of the four genotypes (Figure 2c). The highest root length was recorded in Sharbati (18.69 cm) which

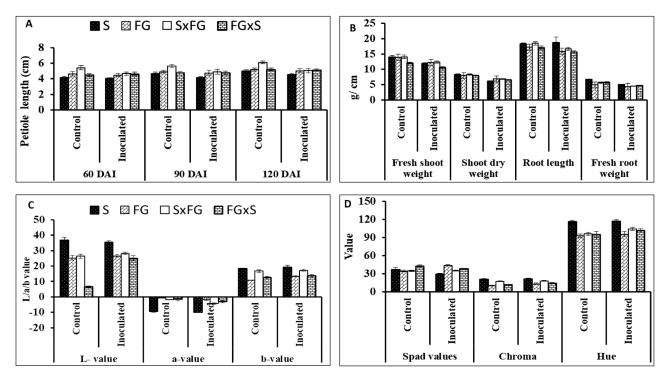


Fig. 2. Effect of *M. incognita* on the (A) petiole length (B) shoot fresh and fry weight (g); root length (cm) and fresh weight (g) (C) leaf colour (L, a, b values) (D) Spad values, Chroma and Hue of the peach rootstocks and their hybrids. The vertical bars represent standard error.

did not differ significantly among the other three genotypes. The highest shoot length was recorded in Sharbati × Flordaguard (47.83 cm) which did not differ significantly from shoot length in all the other genotypes. The maximum fresh root weight was recorded in Sharbati (5.86 g) which was followed by fresh root weight in Flordaguard × Sharbati (5.19g). The fresh root weight in Flordaguard × Sharbati was at par with Sharbati × Flordaguard. The maximum fresh shoot weight was found in Sharbati × Flordaguard (13.20g) which was at par with of fresh shoot weight in all the other genotypes. The highest shoot dry weight was recorded in Sharbati × Flordaguard (7.60g) and it did not differ significantly from shoot dry weight in other genotypes. The no significant differences in the root and shoot length might be due to shorter period of time available up to the winter season for growing the seedlings in root trainers before their transfer to soil.

In respect to the genotypes, significant differences were was recorded in leaf colour among the different genotypes at seven month after inoculation (Figure 2b&d). The maximum lightness (L value 35.51), yellowness (b value 19.32), chroma (21.72) and hue angle (116.97) and minimum greenness/ redness (a value -9.57) were recorded in Sharbati. It was followed by Sharbati × Flordaguard (L value 28.28),

greenness (a value -4.42), yellowness (b value 17.25), chroma (17.93) and Hue angle (104.11). The seedlings of Flordaguard × Sharbati (L value 25.23), greenness (a value -3.02), yellowness (b value 13.77), chroma (14.21) and hue angle (95.11). In Flordaguard lightness (L value 26.62), greenness (a value -1.38), yellowness (b value 13.45), Chroma (13.63) and Hue angle (95.53). The leaf colour values of both hybrids were similar to Flordaguard. The red leaf colour of Flordaguard was transfered to both the hybrids Sharbati × Flordaguard and Flordaguard × Sharbati. Leaf colour in peach is a qualitative trait with red leaf colour dominant over green. The presence of dark red colour in young leaves in the seedlings from the crosses Sharbati × Flordaguard and Flordaguard × Sharbati may be due to the dominance of red leaf colour over green in Flordaguard (Pinochet et al., 9). The highest chlorophyll content in terms of SPAD units (44.28) was recorded in seedlings of Flordaguard followed by Flordaguard × Sharbati (38.2) and Sharbati × Flordaguard (34.98) (Figure 2d). However, least SPAD units were recorded in seedlings of Sharbati (29.83). Higher SPAD values depict potential tolerance of rootstocks to high pH soils (Jimenez et al., 4). It suggests that leaf colour and SPAD value can used as a biomarker to assess nematode tolerance/ resistance in peach rootstocks.

The maximum penetration of juvenile penetration per root system was recorded in Sharbati with 38 juvenile per root system followed by seedlings of Sharbati × Flordaguard with 15 juvenile per root system and Flordaguard × Sharbati (11 juveniles per root system). However, minimum number of juveniles was observed in the root system of Flordaguard (8 juveniles) at 35<sup>th</sup> days after inoculation, respectively (Figure 3). The maximum numbers of nematode juveniles were recorded in the root zone soil of Sharbati (615 juveniles) followed by Flordaguard (530 juveniles) and Sharbati × Flordaguard (460 juveniles). However, minimum nematode population per sample was recorded in Flordaguard × Sharbati (350 juveniles) per 300cc soil samples. Flordaguard has been found to be resistant to *M. javanica*, *M.* incognita and M. floridensis (Rubio-Cabetas, 10). The lower penetration of RKN nematode juveniles in the roots of the Sharbati × Flordaguard and Flordaguard × Sharbati seedlings in the presence of high population of nematodes in the root zone depicts the resistance of the hybrid seedlings to the nematodes. Though, there were significant differences in root nematode penetration however, no nodulation was observed in the seedlings till the time of transplanting in soil. Shaltout et al. (12) evaluated almond and peach hybrid seedlings after 50 days of inoculation and found Line no. 6 (Okinawa × Om El-fahm) highly resistant to nematodes since neither galls nor J2 juveniles were detected in soil or root tissues at the end of evaluation. The differences in the Prunus rootstocks were due to different structural organizations at the root tips (Hang et al., 3). In the immune *Prunus persica* rootstock varieties, the epidermal structure prevented the penetration of J2 juveniles of *M. incognita*. While in resistant variety Nanking cherry (P. tomentosa) there was a reduction in penetration of J2 juveniles and delay in the development of J2 to female stage. In the present

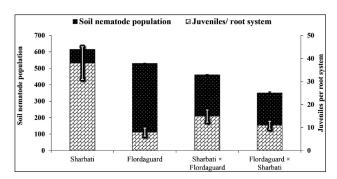


Fig. 3. Juvenile population and penetration in the roots of peach rootstocks and their hybrids. The vertical bars represent standard error.

investigation, the better growth characteristics of the hybrids (Sharbati × Flordaguard and Flordaguard × Sharbati) even after inoculation with *M. incognita* may be due to the transfer of dominant genes Mi and Mij genes to the progeny from Flordaguard which might have provided the resistance to the hybrid seedlings.

The principal components (PC1 and PC2) accounted for the 84.80% of the total variability observed for the different vegetative characters of the four genotypes (parents and reciprocal hybrids). The components, PC1 and PC2 accounted for 63.43 and 21.36% of the total variability, respectively. The first two principal components were plotted to categorize the variables and to observe the relationship between clusters (Figure 4a). Each genotype was plotted in accordance to the score of its principal components for the first two principal components. Plant height was positively correlated to leaf size, leaf area, leaf length, leaf breadth, root weight, root length, shoot dry weight and 'b value. The plants of Sharbati had higher plant height, leaf area, leaf size, leaf L/B ratio. However, Sharbati × Flordaguard seedlings showed higher root length, 'L' value, shoot weight and plant girth. The Flordaguard seedlings had higher petiole length, 'a' value, spad values, internodal lenght and leaf number. Besides, the Flordaguard seedlings had lower plant height, leaf area, leaf size, root weight, chroma values, hue angle. The Sharbati seedlings had lower internodal length, 'a' values, leaf number, spad values and petiole length. The PCA of the inoculated seedlings showed that 90.60% of the total observed variability for the various vegetative characters of the four genotypes (parents and reciprocal hybrids) was explained by the first two components i.e., PC1 and PC2 which accounted for 73.72 and 16.89% of the total variability, respectively (Figure 4b). Plant height was positively correlated to plant girth, root length, leaf size, leaf area, leaf length and leaf breadth. The biplot shows that the plants of Sharbati × Flordaguard had higher plant height, root length, leaf chroma, 'b' values, hue angle, leaf size and leaf area. Besides, Sharbati × Flordaguard seedlings also showed lower penetration of nematode juveniles in root system under high soil nematode conditions over Sharbati seedlings. The Flordaguard seedlings had higher shoot dry weight, 'a' value, spad values, petiole length and leaf number. The Flordaguard seedlings had lower plant height, leaf area, leaf size, chroma values, hue angle, soil nematode population and nematode penetration in root system. The Sharbati seedlings showed highest nematode penetration in root system, hue values, plant height, leaf size and leaf area. Besides, Sharbati seedlings showed lower shoot dry weight, internodal length, 'a' values, spad

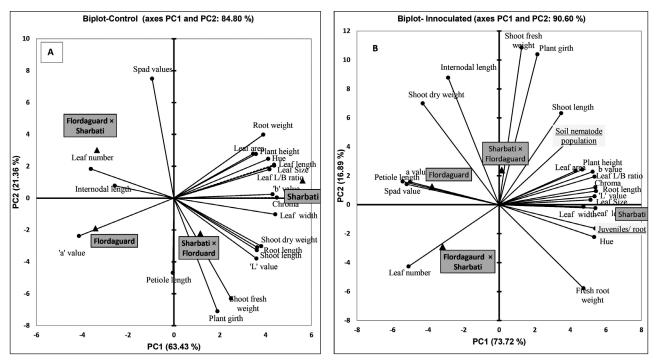


Fig. 4. Principal component analysis for vegetative characters of parents and hybrids (A) without inoculation with nematode juveniles; Control (B) with inoculation with nematode juveniles.

values and petiole length. The number of juveniles penetrated the root system was negatively correlated with shoot dry weight, internodal length, leaf number, petiole length, spad values, 'a' value shoot dry weight and positively correlated with 'L'value, 'b' value, chroma and hue angle. The vegetative characters like shoot dry weight, internodal length, petiole length, spad values, 'a' value, and hue angle were reliable in distinguishing the seedlings with lower nematode population and nematode penetration from the seedlings with higher nematode population and penetration in root system.

# **AUTHORS' CONTRIBUTION**

Conceptualization of research (AT and DP); Designing of the experiments and methodology (AT, HS, SK, RK, DP); Execution of field/lab experiments (PR, AT, JS, SK, RK, DP); Statistical analyses and interpretation (AT, JS); Manuscript writing (PR, AT, JS, HS).

# DECLARATION

The authors declare that they have no conflict of interest.

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