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Response of the citrus rootstock genotypes against artificial inoculation of *Phytophthora nicotianae*

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ABSTRACT

Effect of *Phytophthora nicotianae* Breda de Haan pathogen on different rootstock genotypes including sour orange, Troyer citrange and six variants of *C. jambhiri* Lush, viz. RLC-5, RLC-6, RLC-7, Grambhiri, rough lemon and Italian rough lemon were studied. Of the various rootstocks, *Phytophthora* inoculation tended to show significantly maximum plant height in Grambhiri (30.45cm). Significantly highest leaf numbers/plant (30.45), fresh root mass (4.13g) and dry root mass (2.47g) were recorded in the infected plants of RLC-5, however, it was statistically similar with sour orange, Italian rough lemon and Troyer citrange for leaf numbers, RLC-6 and Grambhiri for fresh root mass and RLC-6 and Italian rough lemon for stem diameter. RLC-6 had the highest stem diameter (3.62 cm) without having significant difference with RLC-5, RLC-7 and Italian rough lemon. After fungal inoculation, Troyer citrange showed the highest feeder root volume (2.45 cm^3) followed by sour orange. These two rootstocks also prove superiority to have the highest photosynthetic rate (*A*) and transpiration rate (*E*) over other infected genotypes of citrus rootstocks tested. Except Italian rough lemon and rough lemon, all the rootstocks were found similar statistically in respect of stomatal conductance (g_s). Overall,Troyer citrange proved best, and showed the lowest reduction in growth and physiological attributes after fungal inoculation. Being a *Phytophthora* tolerant rootstock, Troyer citrange was considered the standard rootstock to study the per cent change in disease incidence and feeder root infection in other genotypes, which indicated sour orange to be the next best rootstock. Of the six variants of Italian rough lemon, RLC-5 and RLC-6 tended to show the lower root infection than rest of the variants studied.

Key words: Leaf gas exchange, Rough lemon variants, Sour orange, Troyer citrange

Phytophthora species are considered one of the most important soil borne problems leading to considerable losses to citrus fruits worldwide. Some *Phytophthora* species are more important than others, in terms of distribution, host range and impacts on crop productivity. *P. nicotianae* Breda de Haan stands out among *Phytophthora* spp. because it is responsible for heavy losses on a particularly high number of host plants. Within the list of the "top 10 oomycete pathogens" recently established, *P. nicotianae* was ranked 8th on the basis of scientific and economic importance (Kamoun *et al.* 2015).There are two main economically important *Phytophthora* species, viz. *P. nicotianae* (syn. *P. parasitica*) and *P. citrophthora*, causing serious losses in citrus industry throughout the world (Cacciola and di San Lio 2008). The most serious disease caused by *Phytophthora* spp. is foot rot, characterized by necrosis and gum exudation, and is also called gummosis. The cambium and inner bark are damaged and lesions spread around the circumference of the trunk, girdling the cambium and killing the tree (Graham and Feichtenberger 2015).

Resistant or tolerant rootstocks are the most ecofriendly approach to manage the *Phytophthora* caused root rot and gummosis diseases. No systematic efforts have been made to evaluate indigenous variability to find out desirable *Phytophthora* tolerant citrus rootstocks. In the continuation to the systematic rootstock improvement programme on going at Division of Fruits and Horticultural Technology, IARI, New Delhi, the present study was attempted to screen the citrus rootstock genotype to identify the resistant rootstock (s) against *Phytophthora nicotianae*.

MATERIALS AND METHODS

The present experiment was conducted set at the experimental orchard of the Division of Fruits and Horticultural Technology, IARI, New Delhi. The seeds of six variants of *Citrus jambhiri* Lush. (RLC-5, RLC-6, RLC-7, Grambhiri, Italian rough lemon and Rough lemon), sour orange (*Citrus aurantium* L.) and Troyer citrange (*C. sinesnsis* (L.) Osbeck × *Poncirus trifoliata* (L.) Raf.)

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rootstocks were sown in the first week of December 2015, and mulched with polyethylene sheet till the end of January 2015. The potting mixture (2 part soil: 1 part farmyard manure) was sterilized with 1% formalin solution under a transparent polyethylene cover for 24 h. Seven months old nucellar seedlings of citrus rootstocks were planted in September 2015 in the pots (12”) containing 8 kg sterilized soil mixture, which was inoculated with *Phytophthora nicotianae* through infected roots. Pure culture of *P. nicotianae* was procured from Central Citrus Research Institute, Nagpur (India) and maintained on Potato Dextrose Agar medium at 4^0C for further use. The roots of citrus seedlings were inoculated with the pure culture of *P. nicotianae* by spore suspension method. Soil inoculation was done using a suspension of seven days old culture, for this purpose suspension of culture was prepared at the rate of 10^5 spores/ml. This suspension was added to the soil. The inoculated seedlings were periodically checked for *Phytophthora* infection through isolation of same pathogen from root of the seedlings. The pathogen was identified by sending the culture to national culture collection centre, Indian Type Culture Collection, New Delhi for the confirmation of its identity.The roots of these established diseased seedlings were used as the source of disease inoculum. One month after planting, 10 g mixture of urea, single super phosphate and potassium sulfate in the ratio of 1:1:1 was applied. The data were recorded after 6 weeks of disease inoculation.

Observations on seedling height, number of leaves, stem diameter, feeder root volume, and fresh and dry root masses were recorded. The feeder root volume was recorded by water displacement method.

Net rate of photosynthesis (*A*), stomatal conductance (g_s) and the rate of transpiration (*E*) of fully expanded leaves were measured between 12.00 – 14.00 h during the season using an LCi-SDUltra Compact Photosynthesis System.

Feeder root infection (number of feeder roots infected/Total number of roots × 100) and root rot severity (sum of numerical ratings/Number of replicate × highest rating × 100) were recorded, and further, the feeder root infection and disease severity were calculated as per cent change over Troyer citrange rootstock.

The experiment was laidout in a completely randomized design (CRD) with four replications, having ten plants per replication. Data were analysed using the SAS package (9.3 SAS Institute, Inc, USA) to calculate F values followed by Tukey's honest significance test. P values ≤ 0.05 were considered as significant.

RESULTS AND DISCUSSION

The seedling growth of citrus rootstock genotypes was significantly influenced by the inoculation of *P. nicotianae* (Table 1).The seedling height, number of leaves, stem diameter, feeder root length, and feeder root volume, fresh root mass and dry root mass were lower in the *Phytophthora* inoculated plants as compared to their respective control.The lowest plant height was recorded in RLC-6 inoculated with *Phytophthora* (25.71cm) having similarity statistically with

Table 1 Influence of *P. nicotianae* on plant growth of citrus rootstocks

Treatment	Plant height (cm)	Leaves/plant	Stem diameter (cm)	Fresh root mass (g)	Dry root mass (g)	Feeder root volume (cm^3)
Non-inoculated						
RLC- 5	39.50a	35.50b	3.81a	4.76a	2.85a	1.95cb
RLC- 6	32.50bc	25.05ef	3.84a	4.61ba	2.73ba	1.80cbd
RLC-7	31.50bdc	28.40ed	3.80ba	3.50edc	2.11de	1.90cb
Sour orange	30.39edc	31.50cd	2.44h	2.80egf	1.68feg	2.05b
Grambhiri	39.42a	35.26cd	3.36egdfc	4.46ba	2.23dc	1.85cbd
Italian rough	32.50bc	36.13b	3.70bac	4.42ba	2.65bac	1.95cb
Rough lemon	34.75bac	44.28a	3.84a	3.49edc	1.58fhg	1.65ced
Troyer citrange	35.50ba	28.35ed	3.16egf	1.79hi	1.04i	2.60a
Phytophthora inoculated						
RLC- 5	32.19bc	30.45bcd	3.51ebdac	4.13bac	2.47bdac	1.70cd
RLC- 6	25.71e	22.00f	3.62bdac	3.88bdc	2.32bdc	1.70cd
RLC-7	26.78ed	16.63g	3.23egdf	2.52hg	1.51hg	1.55ed
Sour orange	26.75ed	27.88d	2.32h	2.66gf	1.59fhg	1.85cbd
Grambhiri	35.37ba	16.00g	3.02gf	3.49edc	1.75feg	1.55ed
Italian rough	26.69ed	27.70ed	3.39ebdfc	3.34edf	2.00fde	1.75cbd
Rough lemon	26.64e	24.81ef	3.16egf	2.33hgi	1.17ih	1.35e
Troyer citrange	34.44bc	27.70ed	2.95g	1.65i	1.01i	2.45a
LSD (P ≤ 0.05)	4.84	3.90	0.42	0.75	0.47	0.31

† Mean values in each column and for each grapefruit cultivar, rootstock, or cultivar-rootstock combination followed by different lower-case letters were significantly different at P ≤ 0.05 by Tukey’s HSD test.

inoculated plants of RLC-7, sour orange, Italian rough and rough lemon. The fungal inoculated plants of Grambhiri were significantly tallest (35.37 cm) having similarity statistically with RLC-5 and Troyer citrange. Overall, lowest reduction in plant height (2.98%) over initial height was registered in Troyer citrange, while it was highest in rough lemon (23.34%) due to inoculation of *P. nicotianae.*

Fungal inoculation significantly reduced the number of leaves, and Grambhiri could retain the lowest number of leaves (16.00) having non-significant difference with the seedling of RLC-7. RLC-5, Italian rough lemon, sour orange and Troyer citrange showed the higher number of leaves per plant (27.70-30.45%) than rest of the genotypes tested against the fungal inoculation. The most severe defoliation due to *Phytophthora* inoculation was noticed in Grambhiri (54.62%) followed by rough lemon (43.97%) and RLC-7 (41.44%). The lowest reduction in leaves was exhibited by Troyer citrange (2.29%).

There was the significant impact of *Phytophthora* inoculation on stem diameter, which retarded the stem growth as compared to their respective control. The highest stem diameter was noticed in RLC-6 (3.62cm) without any significant difference with those of RLC-5, RLC-7, Italian rough and rough lemon, while it was lowest in Troyer citrange (2.95 cm).The reduction in stem diameter due to *Phytophthora* inoculation was highest in rough lemon (17.71%), while it was quite lower in RLC-6, sour orange and Troyer citrange (4.91-6.64%).

Of the various rootstock genotypes, RLC-5 exhibited the highest mass of fresh (4.13g) and dry (2.47g) roots, which was lowest in Troyer citrange (1.65g and 1.01g, respectively) having similarity statistically with rough lemon following fungal inoculation. *Phytophthora* inoculation tended to show the reduced root masses, and the highest reduction in fresh (28.00- 33.24%) and dry (25.95-28.44%) roots were recorded in RLC-7 and rough lemon genotypes than the rest of rootstock genotypes tested. The reduction in fresh (5.00-7.82%) and dry (2.88-5.36%) root masses were rather lower in sour orange and Troyer citrange than the other genotypes tested.

The feeder root volume was significantly highest in Troyer citrange (2.45 cm^3) followed by sour orange (1.85 cm^3) in the fungal inoculated seedlings of rootstock genotypes. Although root volume following *Phytophthora* inoculation reduced in respect to their non-inoculated seedlings, however, the reduction was rather higher in RLC-7 and rough lemon (18.18-18.42%) and the lower in Troyer citrange and sour orange (5.76-9.76%) that the rest of the rootstock genotypes evaluated.

Kaur *et al*. (2013) also recorded the high reduction in the leaf number (-32.72%), seedling height (-21.82%), feeder root length (-23.32%) and feeder root volume (-63.45%) in *Phytophthora* inoculated rough lemon seedlings than Carrizo citrange. Dhakad *et al*. (2014) found rough lemon to be the highly susceptible rootstock as it showed the maximum reduction in leaves, seedling height, feeder root volume, feeder root length, tap root length and root weight, while lowest reductions in these attributes was observed in Citrumelo rootstock. The similar findings were also reported by Fleischmann *et al*. (2005) who reported the huge reduction in the fine root lengths (30%) as well as the number of root tips of all infected plants (50%) in beech saplings following *Phytophthora* inoculations. They found the reduction in the nutrient uptake in plants having severe root infection. Besides, blocked carbohydrate allocation from leaves into roots due to root infection might be the possible cause of reduced plant growth in infected plants (Labanauskas *et al*. 1976).

The photosynthetic rate (A), stomatal conductance (g_s) and transpiration rate (E) of citrus rootstock genotypes were significantly influenced by *Phytophthora* inoculation (Table 2). Of the fungal inoculated rootstock seedlings, the highest A (5.76 μmol/m^2/s) was maintained by sour orange followed by Troyer citrange (5.52 μmol/m^2/s). The lowest value of A was noticed in rough lemon (2.99 μmol/m^2/s) without showing any significant difference with those of RLC-7. Although A was reduced in fungal inoculated seedlings in all the rootstock to their respective control, however, the highest reduction (51.77%) was noticed in rough lemon rootstock. Similarly, sour orange and Troyer citrange along

Table 2 Influence of *P. nicotianae* on leaf gas exchange parameters in citrus rootstocks

Treatment	Photosynthetic rate (A) (μmol/m^2/s)	Stomatal conductance (gs) (mol/m^2/s)	Transpiration rate (E) (mol/m^2/s)
Non-inoculated			
P0 × RLC- 5	5.66bcd	0.033cb	0.97a
RLC- 6	5.32efd	0.033cb	0.64cd
RLC-7	4.86gf	0.028cbd	0.67cd
Sour orange	6.63a	0.053a	1.03a
Grambhiri	4.32ghi	0.028cbd	0.63cd
Italian rough lemon	5.48ecd	0.033cb	0.95a
Rough lemon	6.20ba	0.038b	0.66cd
Troyer citrange	5.98bc	0.038b	0.95a
Phytophthora inoculated			
RLC- 5	5.06ef	0.025cbd	0.75cb
RLC- 6	3.28kj	0.025cbd	0.48ef
RLC-7	3.84ji	0.023cd	0.46ef
Sour orange	5.76bcd	0.035cb	0.82b
Grambhiri	3.97hi	0.023cd	0.46ef
Italian rough lemon	4.47gh	0.015d	0.58ed
Rough lemon	2.99k	0.015d	0.37f
Troyer citrange	5.52ecd	0.033cb	0.73cb
LSD (P ≤ 0.05)	0.56	0.014	0.13

† Mean values in each column and for each grapefruit cultivar, rootstock, or cultivar-rootstock combination followed by different lower-case letters were significantly different at P ≤ 0.05 by Tukey's HSD test.

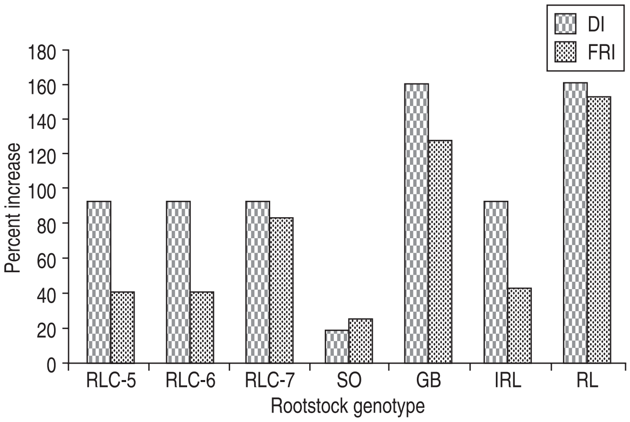

Fig 1 Per cent increase in the disease incidence (DI) and feeder root infection (FRI) in different rootstock genotypes over Troyer citrange due to *Phytophthora* inoculation (SO- Sour orange; GB- Grambhiri, IRL- Italian rough lemon; RL- Rough lemon).

with RLC-5 proved their superiority to have higher *E* (0.73-0.82 µmol/m^2/s) significantly than other rootstocks. Except Italian rough and rough lemon, the value of g_s did not exhibit the significant difference among rest of the rootstock genotypes tested in the present study. Similar to *A*, g_s and *E* also followed the same trend, and highest reduction in g_s (60.53%) and *E* (43.94%) was registered in rough lemon rootstock. The lowest reduction in g_s and *E* was recorded in the *Phytophthora* inoculation seedlings of Troyer citrange (13.16%) and sour orange (20.39%), respectively.

Rootstock influences the photosynthetic capacity of leaves and carbohydrates allocation from source leaves to reproductive or vegetative sinks (Davies *et al*. 2012). In addition, rootstock-specific hydraulic conductance is positively correlated with g_s and *E*, influencing the water relations, leaf gas exchange, and nutrient and hormone status of plants (Cohen and Naor 2002, Saliendra *et al*. 1995). The increase in ABA concentration in infected roots by *P. cinnamomi*, plays an important role in the regulation of g_s of plants suffering drought stress (Cahill *et al*. 1986, Davies and Zhang 1991). *Phytophthora* infection causes a decrease in g_s and *E* occurring at the early stages of infection. In *Castanea sativa* saplings, decrease in g_s has been found to be correlated with the proportion of roots infected by *P. cinnamomi* (Maurel *et al*. 2001). The holm oaks infected with *P. cinnamomi*, which had lost about 67% of their root system, g_s and water potential were strongly reduced, indicating that water relations were more strongly impaired than photosynthesis. The study on the effects of *P. plurivora* on physiological parameters of infected beech seedlings showed that net photosynthesis rates decreased about 2 days after inoculation in comparison to controls (Fleischmann *et al*. 2005). Crombie and Tippett (1990) also observed the lower g_s of trees infected by *P. cinnamomi* significantly than healthy trees.

The disease incidence and feeder root infection were calculated, and expressed as per cent change over Troyer citrange (Fig 1), which showed the highest disease incidence in rough lemon and Grambhiri (160.00%) followed by RLC-5, RLC-6 and Italian rough lemon (93.79%), while it was lowest in sour orange (20.00%). Similarly, the per cent change in feeder root infection over Troyer citrange was highest in rough lemon (153.96%) followed by Grambhiri (127.27%), RLC-7 (83.91%), Italian rough lemon (43.07%), RLC-5 (42.56%), RLC-6 (39.34%) and sour orange (26.68%).

Troyer citrange and Sacaton citrumelo (*C. sinensis* × *C. paradisi*) showed a good degree of resistance similar to sour orange, whereas rough lemon (*C. jambhiri*) and Rangpur lime (*C. limonia* Osb) had high sensibility against *Phytophthora* infection (Mohammed *et al*. 2015). Cheema *et al*. (1990) said that the degree of tolerance/resistance to *Phytophthora* spp. of different rootstocks could be arranged as Kinnow (least resistant) <rough lemons < grapefruit < sweet oranges < sour oranges. The mean root weight resulting from inoculation with *P. citrophthora* had been reported 27 to 96 % lower than the comparable control treatment (Matheron *et al*. 1988). In India, 80% of citrus plantation is budded on rough lemon rootstock and Rangpur lime, which are ranked highly susceptible to *Phytophthora* root rot and foot rot (Graham and Menge 1999). Similarly, Graham (1990) also reported that the disease severity at higher inoculum densities of *P. parasitica* was significantly greater for susceptible rootstocks than for the tolerant rootstock.

Certain rootstocks are considered resistant because roots become infected but do not rot, while others are classified as tolerant because they generate new roots to maintain root mass density in *Phytophthora* infested soil (Graham 1995).Young fibrous roots of most rootstocks support equally high populations of *Phytophthora* spp. However, as roots of resistant or tolerant rootstocks age, the pathogen population declines in rhizosphere soil, while this sustains on susceptible rootstocks (Graham 1995).

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