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SCREENING OF CITRUS ROOTSTOCKS AND COMPARATIVE ANALYSIS OF DIFFERENT SCREENING METHOD AGAINST FOOT ROT OF KINNOW MANDARIN

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ABSTRACT
Phytophthora nicotianae var. *parasitica* is major pathogen causing foot rot/gummosis in Kinnow mandarin in Punjab conditions. Eleven rootstocks were tested against the pathogen by inoculation of spore suspension as well as leaf baiting technique. Among all rootstocks Cleopatra (45.80%) and Rough lemon (44.93%) showed maximum reduction in number of leaves, while Citrumelo (26.02%) exhibited minimum. In another parameters like seedling height, feeder root volume, feeder root length, tap root length and root weight, Citrumelo and X639 showed minimum reduction (>25%). Minimum leaf injury was observed in Citrumelo with 1.4 cm up to 120 hours and maximum for Rough lemon (2.4cm). In leaf baiting with injury, Cleopatra had maximum lesion (4 cm) at 120 hr. Comparison with number of sporangia on leaf disc not found fruitful because after 48 hours number of sporangia on leaf edge were not differed significantly. In this study Cleoapatra and Rough lemon found to be most susceptible while Citrumelo and X639 showed tolerant reaction.

INTRODUCTION

Phytophthora nicotianae var. *parasitica* is one of the most important soil-borne pathogen of citrus which causes mortality of trees (Verniere *et al.*, 2004). It remains a threat and a persistent problem wherever, citrus is grown that can result in substantial tree loss particularly trees on susceptible rootstock. Gummosis is responsible for 10 to 30 per cent of losses in citrus grown around the world (Timmer *et al.*, 2000). Das (2009) stated that more than 20 per cent plants die due to this pathogen in citrus nurseries of Central India where 7-8 million citrus plants are being propagated every year. The disease causes heavy destruction of the Kinnow plantations and also reduces the life expectancy, quality and yield potential of the trees in Punjab (Thind and Sharma., 1996). Use of unidentified strains of Rough lemon (*Citrus jamhiri* Lush) and Rangpur lime (*C. limonia*) that are highly susceptible to *Phytophthora* spp. in almost all the citrus nurseries in India makes the situation further grave (Kaur *et al.*, 2013). The purpose of present study was to screen citrus rootstocks against pathogen as well as different methods of evaluation to develop rapid and reproducible screening method for testing tolerance to *Phytophthora* root rot.

MATERIALS AND METHODS

Isolation and production of spore suspension of pathogen

The experiments were conducted during 2012-13 and 2013-14 at Department of Fruit Science, Punjab Agricultural University and Ludhiana. Pathogen was isolated (Fig.1) from root zone soil of infected plant on selective PARPH media (Naqvi., 1994) by using soil plating method (Kannwischer and Mitchell., 1978). Multiplication of pathogen was done on sorghum seeds (Fig. 2) as described by Kaur *et al.*, (2013). The autoclaved sorghum seed media was inoculated with 5 mycelial disks (5mm) of pathogen from fresh culture. These flasks were incubated at 25+ 1°C. After three days of incubation, growing mycelium upon seed was dispersed by shaking. Pathogen attained full growth after 15 days of incubation.

Spore suspension was made as described by Naqvi (2004). Forty seeds covered with mycelial growth of the pathogen in Petri plates having 20 ml of sterilized deionized water. These plates were incubated at 25+ 1°C. Water was replaced with fresh water for first two days. Abundant sporangia were formed in 3-4 days from mycelium.

Raising of citrus rootstocks

The various rootstocks *viz.* Pectinifera (*Citrus depressa* Hyata), Karna khatta (*Citrus karna* Raf.), Cleopatra (*Citrus reshni* Tanaka), Rough lemon (*Citrus jambhiri* Lush), Volkameriana (*C. volkameriana* Teng and Pasq.), Carrizo (*C. sinensis* × *Poncirus trifoliata*), Troyer (*Poncirus trifoliate* × *C. sinensis*), Sour orange (*Citrus aurantium* L.), Citrumelo (*C. paradise* × *C. trifoliata*), Rangpur lime (*Citrus limonia* Osbeck), X639 (*Cleopatra mandarin* × *Poncirus trifoliata*) were sown in nursery beds in screen house in 2012-13 and 2013-14 during October-November. After one and half month, seedlings were transplanted in pot mixture under screen house conditions. Raising of rootstocks and transplanting is followed as

mentioned by Kaur *et al.*, (2013).

Screening of rootstocks by seedling inoculation

Six month old citrus seedlings were inoculated with 60 ml of spore suspension of *P. nicotianae* var. *parasitica* (4×10^4 per ml) by making 5-6 cm deep and 2 cm diameter holes in the pot mixture around the root zone of seedlings (Kaur *et al.*, 2013). Pots were watered regularly to maintain the moisture for pathogen development. Simultaneously, control pots of each rootstock were also maintained in which no inoculum was added. After eight weeks of inoculation, the seedlings were uprooted carefully by removing the pot mixture and minimum disturbance to roots. Observations were recorded for number of leaves, seedling height, feeder root length, feeder root volume, tap root length and fresh root weight and feeder root rot index. The feeder root rot rating of each rootstock was recorded using scale (1-5) given by Grimm and Hutchinson (1973).

Root rot scale (1-5): 1 = No visible symptoms, 2 = A few roots with symptoms (1-25%), 3 = Majority of roots with symptoms (26-50%), 4 = All roots infected, cortex sloughed from major roots (51-75% rotted), 5 = Majority roots dead or missing (>76% rotted).

Screening of citrus rootstocks without injury to leaf

Twenty ml of the spore suspension was taken and mixed with 80 ml of water in a beaker. Healthy leaves from six months old seedlings were taken as baits. These were disinfected by washing with 95 per cent alcohol before baiting upon spore suspension. No injury was made on leaves surface before baiting. Lesion size on leaves was observed after 48 hours up to 120 hours.

Screening of citrus rootstocks with injury to leaf

Twenty mL spore suspension was mixed with 80 mL of water in a beaker and fresh leaves were taken from six months old seedlings of each rootstock. Injury to the leaves was made before baiting by sterilized needle. Lesion size on leaves was observed after 48 hours up to 120 hours.

Screening of citrus rootstocks by leaf disc baiting method

Twenty ml of spore suspension was taken in a Petri plate for leaf disc baiting of each rootstock. Five leaf discs were taken for each rootstock per replication and three replications were taken. Number of sporangia were counted on leaf disc after 48 hours up to 120 hours of incubation.

RESULTS

The data presented in Table 1 revealed that number of leaves, seedling height, feeder root length, feeder root volume, tap root length and root weight decreased in the *Phytophthora* inoculated plants as compared to the control. The minimum

Table 1: Effect of Phytophthora on plant growth parameters of citrus rootstocks

Rootstock	Increase/decrease over healthy (%)*						
	Number of leaves	Seedling height	Feeder root volume	Feeder root length	Tap root length	Root weight	Feeder root rot index
Carrizo	22.93 (28.57)	33.20 (35.16)	34.09(35.70)	31.27(33.98)	31.32(34.01)	39.82(39.11)	2.4
Citrumelo	19.29 (26.02)	11.73(19.99)	22.40(28.23)	20.71(27.05)	13.56(21.58)	20.31(26.76)	1.6
Cleopatra	51.43 (45.80)	45.18(42.21)	71.42(57.66)	48.73(44.25)	50.29(45.15)	48.52(44.13)	3.2
Karna khatta	25.38 (30.23)	31.42(34.06)	41.75(40.23)	39.10(38.69)	37.33(37.64)	35.36(36.46)	2.5
Pectinifera	21.9(27.88)	12.43(20.60)	21.32(27.48)	13.65(21.66)	12.58(20.75)	22.44(28.25)	1.2
Rangpur lime	32.17 (34.53)	28.31(32.12)	48.26(43.98)	31.46(34.10)	38.69(38.44)	36.43(37.11)	2.6
Rough lemon	49.91 (44.93)	40.25(39.36)	50.57(45.30)	48.78(44.28)	41.19(39.91)	46.52(42.98)	3.0
Sour orange	21.67 (27.73)	12.02(20.25)	26.33(30.84)	16.88(24.23)	33.55(35.37)	16.76(24.13)	1.2
Troyer	37.96 (38.01)	26.94(31.22)	47.47(43.53)	38.94(38.59)	13.48(21.51)	42.25(40.52)	2.7
Volkameriana	31.41 (34.06)	38.06(38.07)	42.13(40.45)	38.79(38.50)	45.91(42.63)	40.32(39.40)	2.9
X639	19.56(26.23)	16.49(23.92)	15.63(23.24)	18.58(25.51)	20.41(26.83)	27.50(31.61)	1.4
CD (p=.05)	2.13	0.59	1.4	0.92	1.8	1.8	0.2

*Observations based on fifteen rootstock seedling; Figures in parentheses are arc sine transformed value

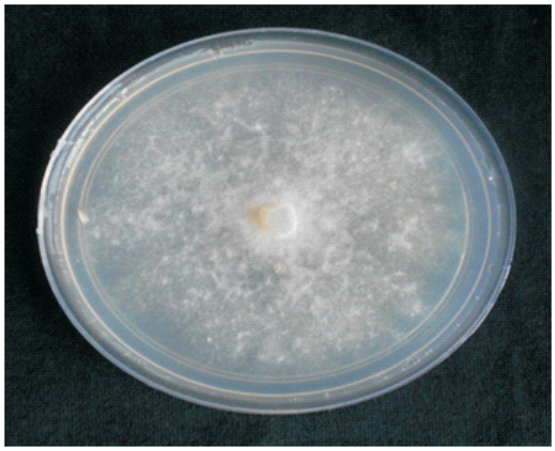

Figure 1: Culture of *Phytophthora nicotianae* var. *parasitica*

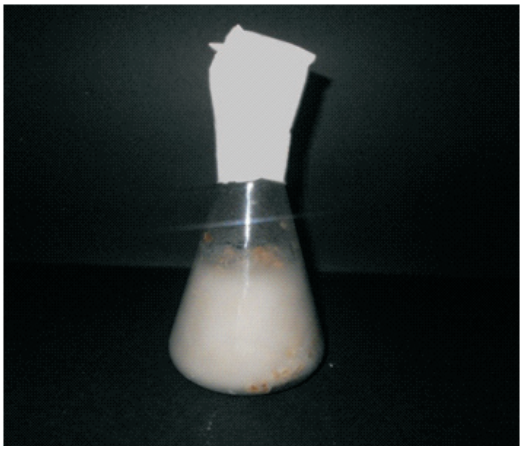

Figure 2: Mass culturing of pathogen on sorghum seeds

per cent decrease in the number of leaves (19.29%) was recorded for Citrumelo (Fig. 3) while maximum per cent decrease in the number of leaves (51.43%) was in Cleopatra (Fig. 4).

Cleopatra showed maximum per cent decrease in height (45.18%) while Citrumelo showed minimum decrease (11.73%). The minimum per cent decrease (15.63%) in feeder root volume was observed for X639 and Pectinifera (21.32%) exhibiting tolerant reaction against pathogen. The maximum per cent decrease was observed for Cleopatra and Rough lemon with 71.42 and 50.57 per cent respectively, exhibiting susceptible reaction. The observation based on the feeder root length revealed that minimum per cent decrease (13.65%) in feeder root length was observed for Pectinifera while Rough lemon (48.78%) and Cleopatra (48.73%) showed susceptible response with maximum per cent decrease. The minimum per cent decrease in tap root length was observed for Pectinifera (12.58%) exhibited most tolerant reaction against pathogen Cleopatra (50.29%) showed the maximum per cent decrease in tap root length. The minimum per cent decrease in root weight was observed in Sour orange (16.76%) and Citrumelo (20.31%) showed tolerant reaction followed by Cleopatra (48.52%), exhibiting susceptible response against pathogen.

The feeder root index was observed highest for Cleopatra (3.2) followed by Rough lemon (3.0), Volkameriana (2.9), Rangpur lime (2.6) and Troyer (2.7). Minimum root rot rating was 1.2 which was observed for rootstocks *viz*. Sour orange and Pectinifera.

Among the eleven rootstocks screened, out of all the eight rootstocks screened, Cleopatra and Rough lemon showed highly susceptible reaction while X639, Citrumelo and Pectinifera found to be tolerant against pathogen.

Table 2: Screening of citrus rootstocks without injury to the leaf bait

Rootstock	Lesion size on leaf (cm)			
	48 Hours	72 Hours	96 Hours	120 Hours
Carrizo	0.9	1.4	2.0	2.3
Citrumelo	0.6	0.8	1.1	1.4
Cleopatra	1.3	1.5	2.0	2.2
Karna khatta	1.0	1.2	1.4	1.6
Pectinifera	0.5	0.9	1.3	1.8
Rangpur lime	1.1	1.4	1.6	2.0
Rough lemon	1.2	1.8	2.1	2.4
Sour orange	0.6	1.3	1.7	2.3
Troyer	1.0	1.6	2.1	2.3
Volkameriana	1.1	1.4	1.9	2.3
X639	0.8	1.0	1.2	1.5
LSD(p=.05)	0.36	0.30	0.28	0.20

Table 3: Screening of citrus rootstocks with injury to the leaf bait

Rootstock	Lesion size on leaf (cm)			
	48 Hours	72 Hours	96 Hours	120 Hours
Carrizo	1.6	2.0	2.2	2.3
Citrumelo	2.0	2.5	2.8	3.0
Cleopatra	2.7	3.0	3.3	4.0
Karna khatta	2.0	2.2	2.4	2.6
Pectinifera	1.6	2.1	2.5	3.0
Rangpur lime	2.1	2.5	2.7	3.1
Rough lemon	2.5	2.7	3.2	3.8
Sour orange	1.5	2.2	2.4	2.5
Troyer	1.8	2.1	2.2	2.4
Volkameriana	2.2	2.6	3.1	3.4
X639	1.9	2.1	2.2	2.3
LSD (p=.05)	0.75	0.63	0.64	0.47

Screening of citrus rootstock without injury to leaf bait

The data presented in Table 2 revealed that minimum lesion size (0.5 cm) was observed in Pectinifera followed by Sour orange (0.6 cm), Citrumelo (0.6 cm) and X639 (0.8 cm). Maximum lesion size was observed in Rough lemon (1.2 cm) and Cleopatra (1.3cm) after 48 hours of incubation. After 72 hours incubation, the minimum lesion size (0.8cm) was recorded in Citrumelo while it was maximum in Rough lemon (1.8 cm). The moderate lesion size was obsereved in Karna khatta (1.2 cm), Carrizo (1.4 cm) and Troyer (1.6 cm).

Screening of citrus rootstocks with injury to leaf bait

The data presented in Table 3 showed that Sour orange had a minimum lesion size of 1.5 cm whereas Rough lemon (2.5 cm) and Cleopatra (2.7 cm) after 48 hour of incubation had maximum lesion size. Other rootstock *viz*. Rangpur lime (2.1cm), Volkameriana (2.2 cm) and Rangpur lime (2.1 cm) showed moderate lesion size. The lesion size exceeded up to 3.0 cm after 72 hour incubation for Cleopatra and 2.1cm for X639. Pectinifera (0.9 cm) and X639 (1.0 cm) showed minimum lesion size after 72 hour of incubation. The rootstock X639 showed minimum lesion size (1.2 cm) after 96 and 120 hours of incubation. The data showed that minimum lesion size

Table 4: Screening of citrus rootstocks by comparing number of sporangia on leaf bait

Rootstock	Number of sporangia on leaf disc				LSD(p=.05)
	48 Hours	72 Hours	96 Hours	120 Hours	
Carrizo	388	195	102	56	47.73
Citrumelo	246	132	67	29	43.66
Cleopatra	470	241	115	53	29.90
Karna khatta	263	132	74	35	28.52
Pectinifera	229	120	61	34	34.95
Rangpur lime	329	153	72	36	52.93
Rough lemon	531	258	130	66	30.09
Sour orange	233	124	66	32	52.35
Troyer	392	180	84	37	29.59
Volkameriana	256	125	71	42	23.85
X639	310	160	74	39	31.17
LSD(p=.05)	63.46	NS	NS	NS	

Figure 3: Citrumelo exhibited tolerant reaction

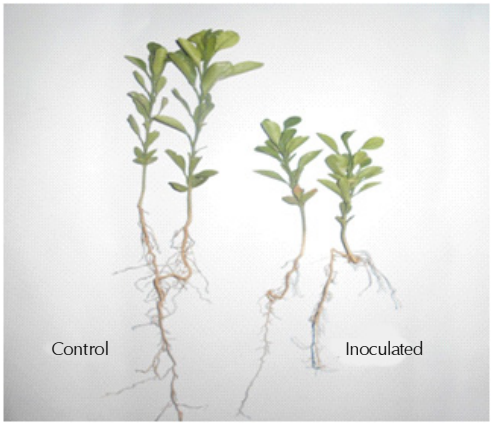

Figure 4: Cleopatra exhibited susceptible reaction

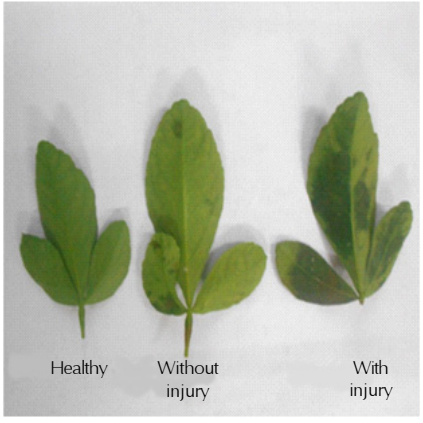

Figure 5: Screening by leaf baits with and without injury in rootstock X639

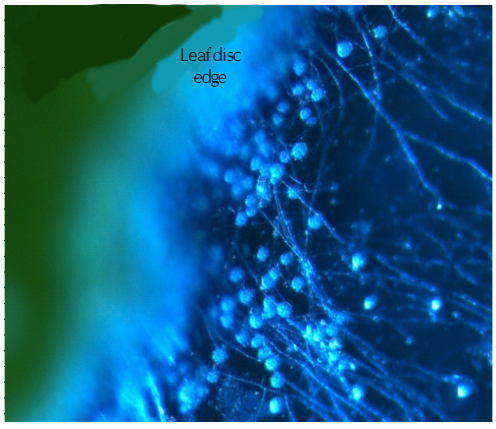

Figure 6: Abundant sporangia on the edge of leaf disc

observed for Pectinifera, Sour orange and X639 (Fig. 5) whereas Cleopatra and Rough lemon showed maximum lesion size.

Screening of citrus rootstocks by leaf disc baiting method

Data presented in Table 4 showed that leaf discs of each rootstock attracted large number of sporangia (Fig. 6) which decreased with time interval due to germination of sporangia into mycelium on leaf disc edge. The minimum number of sporangia after 48 hours was observed on Pectinifera (229), Sour orange (233) and Citrumelo (246). The maximum number of sporangia after 48 hour were observed on leaf disc of Rough lemon (531) and Cleopetra (470).

Comparison of resistance or susceptibility by counting sporangia was not seems familiar because all baits were attacked by abundant sporangia.

DISCUSSION

Kaur *et al*. (2013) observed reduction in all growth parameters after inoculation with spore suspension and observed that Pectinifera was tolerant rootstock while Cleopatra was susceptible followed by Rough lemon. The present findings are in agreement with Vanderweyen (1973) who reported that Sour orange and trifoliate orange were more resistant whereas Volkameriana was least susceptible rootstocks against *Phytophthora parasitica*. Citrus rootstocks *viz*. Sour orange and trifoliate orange exhibited tolerant reaction against the pathogen (Broadbent *et al*., 1971).

Gade (2012) tested three rootstocks against foot rot and found that Cleopatra and Rough lemon more susceptible than Rangpur lime. Similarily, Armarkar. (2011) found Cleopatra and Rough lemon most susceptible in his findings. Graham and Timmer (2007) also reported that Citrumelo was normally resistant to foot rot, whereas Cleopatra mandarin was prone to attack. Volkameriana showed intermediate reaction between the two groups.

Rogers *et al*. (1996) reported that Cleopatra and Carrizo as susceptible whereas Citrumelo as tolerant rootstocks against pathogen. Naqvi (2002) also reported that X639 and Citrumelo were tolerant against pathogen whereas Cleopatra was highly susceptible. Rough lemon and Rangpur lime are highly susceptible rootstocks which are mainly used in Indian conditions. Cheema *et al*. (1990) observed the degree of tolerance/ resistance to *Phytophthora* spp. of different rootstocks and arranged them as Kinnow (least resistant) <rough lemons < grapefruit < sweet oranges < sour oranges.

In India, 80% of citrus plantation are budded on rough lemon rootstock and Rangpur lime which are ranked highly susceptible to Phytophthora root rot and root rot (Naqvi., 2002)

Present investigation showed that, leaf baiting technique provides quick and similar results as inoculation of young seedling. It can be used as substitute method for screening citrus rootstock. Leaf baiting of citrus rootstocks with injury found to be better than without injury to leaf because comparison of lesion size was more accurate in this method. Leaf baiting method of screening can be utilized for reaction against pathogen, but final consideration of rootstock reaction can be made by inoculation of seedling by spore suspension.

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