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Technique for screening of apple and pear germplasm against white root rot (*Dematophora necatrix*)

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Abstract

A screening technique for the identification of resistant germplasm of apple (*Malus* spp.) and pear (*Pyrus* spp.) to white root rot (*Dematophora necatrix* Hartig) was standardized under field conditions. Rooted suckers were planted in the field during late dormancy stage (February). These were challenge inoculated when plants had established in the field and attained new growth (during the month of July) by introducing fungus inoculum multiplied on wheat grain in the rhizosphere (50 grains/ sucker). Soil moisture was maintained at the field capacity. Different species exhibited differential reaction to disease development parameters *viz.*, mycelial colonization of the infected plant parts, rate of wilting, defoliation, necrosis on the bark, wood and vascular region. Necrosis on the leaves was observed as first symptom of disease expression which took 10 days after the inoculation in susceptible species whereas incubation period was extended in resistant species. Most of the population of different susceptible host species collapsed within 30 days of inoculation. The mortality rate varied with species. The technique evolved is easy, quick, reliable and capable of creating required disease pressure under field conditions for screening rootstocks against white root rot disease.

Key words: *Malus, Pyrus,* grain inoculum, rhizosphere, rooted sucker, differential reaction, white root rot, *Dematophora necatrix*

Introduction

White root rot (*Dematophora necatrix* Hartig) is a serious disease of temperate fruits in India (Agarwala and Sharma, 1966) with a wide host range among cultivated and wild plants in temperate and sub temperate region of the world (Behdad, 1975; Sztejnberg and Madar, 1980; Teixeira *et al.,* 1995). Continuous efforts are being made in almost all the temperate fruit growing countries throughout the world to identify resistant sources to combat the disease more effectively (Sztejnberg *et al*., 1987). This calls for an easy, rapid and reliable field screening technique. In the techniques reported so far, screening has been done either in sterilized soils in pots or boxes inoculated with the mycelium of the fungus cultured on rice/ artificial media. Field screening has been conducted to a limited extent on naturally infested orchard soils (Gupta and Verma, 1978; Ram, 1982).

Screening techniques reported by different workers require naturally infested orchards soil which lack quantification of the disease pressure. Similarly an introduction of mycelium as inoculum in pots/ boxes is cumbersome and difficult to apply uniform inoculum quantity. The technique used involved inoculation of mycelium after making injury to the host with the help of a cork borer which does not permit host to express its true defence potential against the disease. The techniques used by Gupta and Verma (1978) and Ram (1982) took very long periods (500 days to six years) for disease expression. The method of wooden chips made of disposable chopsticks is cumbersome in which individual host plant are inoculated by fixing the chopsticks with parafilm tape (Negishi *et al*., 2011). Therefore, an attempt was made to standardize a rapid and reliable screening technique that would ensure uniform inoculum density to compare disease reaction of germplasm and host pathogen interaction in field plots.

Materials and methods

Rooted suckers of different *Malus* species/ rootstocks of Malling and Malling Merton series, *Pyrus pashia* var *kumaonii* and *Cydonia oblonga* were obtained from mound layers of pure somatic clones of various species maintained in the nursery block of the research station. These layers were planted during late dormancy stage (February) in an isolated field plot. Seven rooted suckers of each species were planted in one row and two replication were maintained. Before planting the rooted suckers, fields were prepared to proper tilth and well rotten FYM @ 1 kg /m^2 was added in the furrows.

The white root rot fungus does not sporulate on artificial nutrient media. To facilitate the application of measured inoculum quantity, a grain inoculum was prepared by using wheat grains. For this purpose, bold and unbroken grains were soaked in water overnight, boiled for 20-30 minutes until becoming soft but with intact seed coat. Excess water was drained out before filling 100 g boiled grains in the conical flask/ high density propylene bags and autoclaved for 20 minutes at 15 lbs. These were inoculated aseptically with pure mother culture of white root rot fungus *D. necatrix*. The flasks were incubated at 25 ^{o}C for 20 days. In order to avoid clumping of grains, the flasks were shaken on alternate days during the incubation period. The different *Malus species* and rootstocks were challenge inoculated during the month of July when the suckers were well established in the field and attained new growth. Un-inoculated checks were also maintained for comparative observation. Grain inocula (50 grains/sucker) were placed in the rhizosphere, by opening furrows on both sides of the row. The field was irrigated and soil moisture was maintained at field capacity. Observations on disease syndrome *viz.*, leaf chlorosis, wilting and rate of defoliation were recorded. The

rootstocks identified as tolerant to root rot disease were further observed for the mortality rate at variable disease pressure created by varying the grain inocula number *viz.,* 5, 10, 25, 50 and 100 grains per plant to find out optimum disease pressure required to evaluate the resistant sources on large scale under field conditions.

In order to confirm the mycelial colonization on the test plants, rooted suckers were uprooted from the inoculated fields after 30 days of inoculation and washed in running tap water. After removing the bark, these were wrapped in moist filter paper sheets and incubated in an environment chamber maintained at 25 ± 5 °C and 95 % RH for 40 h. Mycelial growths visible on the surface of colonized areas were measured. Observations were also recorded on the extent of necrosis on bark wood and vascular region. Mortality was recorded at the end of the growing season and mortality rate in different species and rootstocks were compared.

Results and discussion

Mycelium recovery from wheat grain inocula in culture medium was up to six months. Grain inoculum could be easily stored at room temperature with out loosing the viability till six months. However when stored in refrigerated condition recovery was found for more than two years. The viability of *Rosellinia necatrix* on PDA slant stored at 4 °C has been reported upto 10 years (Hoopen and Krauss, 2006).

Mycelium colonization on the root was first recorded after seven days of inoculation. The first symptom on the leaves was noticed as chlorosis on the leaf margin and interveinal area at 10 days after inoculation. Gradually the affected leaves turned brown, rolled upwards and finally defoliated. Time (days) taken for expression of various disease symptoms *i.e.* leaf chlorosis, wilting, defoliation and mortality varied with different species (Table 1).

The suckers were uprooted 30 days after inoculation from the inoculated fields and incubated for confirming the colonization of the inoculated fungus on the host tissues. The colonized area measured as the length of rooted suckers possessing mycelial growth showed large variation (125 to 202mm) amongst different host species (Table 1) indicating variable reaction of host species.

Comparison of mortality rate revealed that white root rot severity decreased as the inoculum was reduced from hundred grains to five grains per plant (Table 2). Mortality rate varied with the host species indicating the suitability of the inoculation technique for evaluating germplasm for their threshold level and efficiency of white root rot management techniques.

The present study revealed that all the three Malling and Malling Merton series rootstock were found susceptible (Table 1). It is evident from the results that under low inoculum level, species like Saishi-E (*M. prunifolia* var *ringo* Asami) and *M. purpuria, M. baccata* Kashmir and *M. baccata* Shillong can be utilized as tolerant rootstock for apple (Table 2) along with other root rot management practices in white root rot endemic areas (Sztejnberg *et al*., 1987; Katan, 1987). In case of wild pear *Pyrus pashia var Kumaonii* and *Cydonia oblonga* exhibited a tolerant reaction under high inoculum level and could be utilized as rootstock for pear.

Table 1. Time taken (days) for expression of white root rot symptoms by different rootstocks

Species/rootstock	Symptoms/Syndrome						Mycelial growth (mm)	Mortality (%)
	Chlorosis		Wilting		Defoliation			
	Days	Population (%)	Days	Population (%)	Days	Population (%)		
M 9	10	85.7	12	75.7	20	92.8	202	100.0
M.M 106	11	55.7	12	21.4	20	14.3	181	87.5
M. 7	10	84.4	12	74.9	20	100	118	100.0
M. baccata (Shillong)	12	57.1	13	14.3	20	16.2	169	88.8
M. baccata (Kashmir)	12	28.6	13	14.3	20	7.1	161	88.8
M. purpuria	18	57.1	18	14.3	20	0	179	88.8
M. prunifolia var Ringo asami	18	35.7	18	0	20	0	159	44.4
Cydonia oblonga	18	65.7	18	18.6	20	12.5	144	40.5
Pyrus pashia var kumaonii	18	85.7	18	50.0	20	14.3	125	44.0

Table 2. Mortality rate (%) of different rootstocks at variable disease pressure of *D. necatrix*

Number of grain inocula	Mortality (%) of different rootstocks					
	M. baccata Shillong	*M. baccata* Kashmir	*M. purpuria*	*M. prunifolia* var *ringo* Asami	*Pyrus pashia* var kumaonii	*Cydonia oblonga*
100	100.0	100.0	100.0	88.8	66.6	66.6
50	100.0	100.0	88.8	44.4	16.6	50.0
25	88.8	88.8	55.5	22.2	0.0	16.6
10	66.6	66.6	22.2	0.0	0.0	0.0
5	0.0	0.0	0.0	0.0	0.0	0.0
Check	0.0	0.0	0.0	0.0	0.0	0.0

It emanates from these studies that the reaction of a host species might have been related to its ability to tolerate the toxic effects of metabolites produced by the fungus, which require further investigation. Toxin produced by this fungus is thermostable and its production was found maximum from 7th to 11th day of fungus growth and its concentration was more in woody parts than diseased roots and bark (Gupta and Gohain, 1982; Kanematsu *et al*., 1997). The present studies also revealed that disease symptom expression initiated 10th day onward and delayed appearance of the symptoms exhibited by different species could be related with the tolerance of the host species.

These results revealed that the technique is ideal for screening the germplasm against white root rot under field conditions. Some of the advantages of this technique are that the technique imitates the disease development under natural conditions. Host is allowed to attain normal growth to avoid transplanting shock. Little or no injury is given to the root system, which permits the host to express its true resistance potential. The inoculated fungus has to compete with the natural inhabitants and antagonists of the soil. The reserve food in the grain ensures nourishment to the growing mycelium initially before the fungus colonises substrates including the host roots. The use of grain spawn also ensures quantification of the inoculum. Varying the number of spawn grains could regulate need based disease pressure. Disease gradient can be created to find out the threshold value of different host species as well as at variable age groups. The technique is rapid and germplasm evaluation results could be obtained within 35 to 40 days of inoculation than 500 days to six years reported by Gupta and Verma (1978) and Ram (1982). Inoculum delivery system with grain spawn is easy, quick and mechanization could be developed to create a sick plot by application of grain spawn than a cumbersome method of inoculating each host with chopsticks reported by Negishi *et al*. (2011).

Screening techniques reported by different workers require naturally infested orchards soil which lack quantification of the disease pressure. The technique evolved is easy, quick, reliable and capable of creating required disease pressure under field conditions for screening rootstocks against white root rot disease.

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