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Influence of rootstocks on white root rot (*Dematophora necatrix*) resistance in apple (*Malus baccata*)

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ABSTRACT

An experiment was conducted under polyhouse and field conditions during 2013, 2014 and 2015 to evaluate the level of resistance of less known apple rootstock, i.e. *Paron* (*Malus baccata* var. *himalaica*) and some popular rootstocks of the region (MM 106, MM 111, M 9 and Srinagar crab) against white root rot disease. All the test rootstocks were grafted with CITH Lodh Apple-1 before their use in field conditions. *Paron* showed high resistance against *D. necatrix* by exhibiting least mortality (15.33%) at highest test dose (10 g/kg soil) of inoculum in pot culture under polyhouse, whereas other test rootstocks showed 100 percent mortality at this dose of inoculum. In the field, *Paron* showed no wilting symptoms on scion cultivar even after 60 days of inoculation, it showed least bronzing and inward cupping of leaves (3.25%), defoliation (3.50%), necrosis on bark (21 mm), wood (17 mm) and vascular tissues (23 mm). It was rated as resistant against *D. necatrix* as compared to other test rootstocks on the basis of significantly lowest disease severity (4.35%) and least effect of test pathogen inoculation on its above and below ground parts. Srinagar crab was rated as highly susceptible in comparison as, it exhibited significantly highest disease severity (69.80%). From this study, it can be inferred that the rootstock of *Paron* should be used for shaping production of apple in Kumaon region of Uttarakhand.

Key words: Apple, *Dematophora necatrix*, *Malus baccata* var. *Himalaica*, Resistance

Kumaon hills in the state of Uttarakhand in India are well known for apple production. Apple (*M. domestica* Borkh) is an important fruit crop of temperate regions of India and occupies an area of 2,46,600 ha. It is predominantly grown in Jammu and Kashmir, Himachal Pradesh and Uttarakhand in India. Productivity of apple is only 6–7 MT per ha in India in comparison to more than 40 MT per ha in countries like Belgium, Denmark, Netherlands, New Zealand and USA (Raj and Sharma 2009).

There are a number of biotic and abiotic limiting factors affecting the productivity and quality of apple. Among the biotic factors, white root rot caused by *Dematophora necatrix* is one of the most important diseases of apple affecting the plants both in the nurseries as well as in the orchards (Bharat and Bhardwaj 2001, Gupta and Verma 1978 and McKinney 1923). In India, this disease was first observed in Uttar Pradesh hills (now in Uttarakhand) (Singh 1943). The earliest estimated loss due to this single disease was about ₹ 1.3 million (Agarwala and Sharma 1966), which is certainly expected to be much more as the disease has now been reported to occur in almost all apple-growing regions of the country (Sharma *et al*. 2013). Affected seedlings are killed within three weeks, whereas adult plants survive for two to three seasons. Different chemicals and integrated approaches have been tried for the management of the disease but it continues to affect a large number of saplings in the nurseries and plants in the orchards (Gupta and Gupta 1992, Rana *et al*. 2010 and Raj and Sharma 2009). Research works carried out also showed that none of the available apple rootstocks exhibit immune reaction against white root rot disease of apple (Sharma and Sharma 2008). Hosts express symptoms on above ground parts at very advanced stage of infection and most of the time it becomes difficult to control the disease. Presently, there is no quick detection method for this disease at the early stage of infection or before it express symptoms on above ground parts. In view of the seriousness of the disease and importance of apple crop in Kumaon hills of Uttarakhand, it was thought worthwhile to evaluate the resistance level of locally available crab apple (*Paron*) along with some popular rootstocks of the region against soil borne pathogen *D. necatrix*.

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MATERIALS AND METHODS

Periodic surveys of different apple growing localities in Nainital district of Uttarakhand were conducted for recording the incidence of white root rot of apple in nursery as well as in orchards. Percent disease incidence was calculated with the formula:

$$\text{Percent disease incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

Dematophora necatrix was isolated on Potato Dextrose Agar (PDA) medium from infected roots of seedlings of apple collected from nursery of ICAR-Central Institute of Temperate Horticulture, Regional Station, Mukteshwar, Nainital, (Uttarakhand). On the above ground parts, infected plants showed characteristic symptoms of disease, i.e. wilting, bronzing and inward cupping of leaves, and defoliation types of symptoms, whereas on the below ground parts, lateral roots turned dark brown and were covered with white mycelial mat.

Purified culture of *D. necatrix* was mass multiplied on wheat seeds, which were soaked for 12 hr in 250 ml flask filled with sterilized distilled water. The flasks, each containing 100 ml of seed, were subsequently autoclaved after excess water had been drained off. After sterilization three fungal disks of 9 mm size from 2 weeks old culture of *D. necatrix* grown on PDA were placed aseptically in each flask. Flasks were then incubated at 25°C for 2 weeks and shaken every 2-3 days to avoid clustering of the seeds. The inoculum in each flask was then macerated under sterile conditions and utilized for the experimental use (Sztejnberg and Katan 1986).

Experiment on effect of different inoculum doses of *D. necatrix* on the test plants was carried out under polyhouse in a completely randomized design (CRD) with six treatments and twenty replications. In the 2nd fortnight of February one year old seedlings/rooted suckers of MM 106, MM 111, M 9, *Paron* (Jolepokhra) and Srinagar crab were transplanted in pots containing autoclaved substrate, composed of one part good orchard soil, one part sand and one part farmyard manure and they were maintained in the hi-tech polyhouse. Test doses of inoculum, *viz*. 2, 4, 6, 8, and 10 g/kg soil were added in the rhizosphere/root zone of the pot plants after three and half months to see the effect of different inoculum doses on the host plants. Pot plants without inoculum were kept as control. Plants were watered regularly and soil moisture was maintained at the field capacity. Each treatment was replicated 6 times and single pot plant was considered as one replication. Percent mortality of host plants was calculated 60 days after inoculation by using formula:

$$\text{Percent morality} = \frac{\text{Total number of dead plants}}{\text{Total number of healthy plants}} \times 100$$

Disease expression on different test plants against white root rot disease of apple was studied and it was performed in the field in a Randomized Block Design (RBD) with five treatments and three replications. Under this experiment apple plants were raised by grafting the most popular cultivar CITH Lodh Apple-1 on one year old seedlings/rooted suckers of MM 106, MM 111, M 9, *Paron* and Srinagar crab apple rootstocks and maintained under recommended management practices at experimental farm of ICAR-Central Institute of Temperate Horticulture, Regional Station, Mukteshwar, Nainital, (Uttarakhand). Plants were trained to a single shoot, and such single shooted plants with almost of equal height and thickness were preferred under this study. Test plants were planted at 1 × 1 m^2 spacing in raised nursery beds of 2 × 2 m^2 size and transplanted to naturally infested soil in the nursery area in the last week of February. In the nursery area, inoculum build up was observed so high that even 100 percent mortality of one year old apple grafted plants was recorded in the previous years. The reason for this was probably the non-rotation of nursery site for the last one decade. Additional inoculum pressure was generated on the plants by adding 10 g/plant inoculum of *D. necatrix* in the rhizosphere/root zone of the plants in second fortnight of June. This means, sufficient time was given to all the rootstocks to establish in soil before artificial inoculation. Plants were watered regularly and soil moisture was maintained at the field capacity. Each treatment was replicated thrice and a single nursery bed was treated as one replication. Observations of above ground symptoms (wilting, bronzing and inward cupping of leaves and defoliation) and below ground symptoms were recorded within 60 days after artificial inoculation. Different rootstocks were also categorized into different reaction classes on the basis of percent disease severity against *D. necatrix* causing white root rot of apple. Disease severity was calculated after 60 days of artificial inoculation by scoring individual plant on 0–6 scales where 0 denoted Healthy, 1 meant 1–5% root rot, 2 meant 6–25% root rot, 3 meant 26–50% root rot, 4 meant 51–60% root rot; 5 denoted 61–70% root rot and 6 meant >70%. Percent disease severity was calculated according to the formula suggested by McKinney (1923):

$$\text{Percent disease severity} = \frac{\text{Sum of all disease ratings}}{\text{Total number of ratings} \times \text{Maximum disease grade}} \times 100$$

The pooled data of the years 2013, 2014 and 2015 were subjected to statistical analysis using SPSS 16.0 software and comparison among means were made using Duncan's multiple range test.

RESULTS AND DISCUSSION

Disease survey and pathogen identification: White root rot is a major soil borne disease of apple in different growing areas of Kumaon hills of Uttarakhand, India. During survey of Nainital district, mean disease incidence in apple nurseries (52.90%) was recorded higher as compared to apple orchards (28.00%). Among the localities visited, apple nurseries and orchards in Dhanachuli area were adversely affected by disease with average disease incidence of 70.33

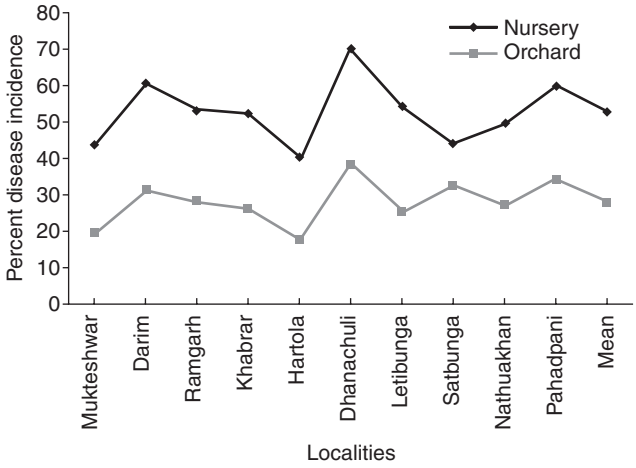

Fig 1 Survey of major apple growing areas of Nainital district of Uttarakhand for incidence of white root rot disease.

and 38.25 percent respectively (Fig 1). *D. necatrix* was identified as the causal organism of white root rot of apple on the basis of pear shaped swelling at the septum (Fig 2) of fungal hyphae (Kumar *et al*. 2014 and Schena *et al*. 2002).

Disease expression on the rootstocks under polyhouse conditions: It is evident from the data presented in Table 1 that rootstocks were killed by the test pathogen in varied proportions which, determined by inoculums doses used. *Paron* showed highest level of tolerance against *D. necatrix* at all the test inoculum concentrations. It registered least mortality (15.33%) of test plants at the highest test inoculums dose (10 g/kg soil) as compared to other test rootstocks. At this dose, all the other rootstocks were found dead on 60th day after inoculation. It was considered as complete resistance breakdown in MM 106, MM 111, M 9 and Srinagar crab which might led them to their 100 percent mortality under pot culture. *Paron* showed no mortality at inoculum doses of 2 and 4 g/kg soil (Table 1). M 111 and M 9 showed similar results but only at lowest inoculum dose (2 g/kg soil). Previously, similar work was performed on mortality rate of different *Mallus* species, viz. *M. baccata* (Kashmir), *M. purpurea*, *M. prunifolia* (var. *ringoasami*) and *M. baccata* (Shillong) to *D. necatrix* at variable disease pressure (Sharma *et al*. 2013). They inoculated the apple plants with different number of inoculum grains (inoculated wheat grains), *viz*. 5, 10, 25, 50 and 100 and found that all the test rootstocks showed high degree of tolerance at lowest inoculum level, i.e. 5 grains, however tolerance decreased with increased inoculum level.

Disease expression on the rootstocks under field conditions: It was observed that white root rot pathogen first infected the roots of rootstocks and during the course of infection pathogen also produced different types of symptoms on the above ground parts of plants also which, encompasses wilting, bronzing and inward cupping of leaves and defoliation. Wilting was observed as the first symptom being produced by *D. necatrix* on the above ground parts. However, *D. necatrix* produced no wilting symptoms on *Paron*. This might be due to its resistance power against the test pathogen and long tap root system which enable it to draw the nutrition from distant and deep places in soil. No doubt, Srinagar Crab had also got deep tap root system, however no resistance power was observed which might have forced the rootstock to express wilting symptoms at the earliest and in highest percentage (58.15%). In case of clonal rootstocks, low resistance level and shallow root system was observed as the important factors in the development of wilting symptoms. The soils in Kumaon hills are

Fig 2 Disease symptoms, growth on PDA medium, mycelial characteristics and mass multiplication of *D. necatrix*.

Table 1 Effect of inoculum dose of *D. necatrix* on the mortality rate of different apple rootstocks under polyhouse conditions

Inoculum dose (g/ kg soil)*	Mortality (%)				
	MM 106	MM 111	M 9	Paron	Srinagar Crab
10	100.00e	100.00e	100.00e	15.33d	100.00e
8	100.00e	72.33d	65.25d	9.50c	100.00e
6	82.66d	54.57c	50.25c	5.25b	84.57d
4	26.25c	20.57b	18.33b	2.50ab	48.66c
2	10.25b	5.33a	2.66a	0.00a	15.66b
Control	0.00a	0.00a	0.00a	0.00a	0.00a

*Means followed by the same letter within a column are not significantly different at 5% level based on Duncan's multiple range test

mostly sandy and have poor water holding capacity. This could also be a major problem for shallow rooted apple plants particularly during water scarcity conditions in summer. Such situations become hazardous for the survival of the crops, if plants are also attacked by the soil borne pathogens like *D. necatrix* simultaneously. Generally, during summer season, Kumaon hills experience scanty rainfall which makes the apple plants nutritionally weak and highly prone to the infection of most of the plant diseases. Rainy season is a favorable time for the development of white root rot disease of apple and this problem could aggravate more when the apple germplasm is nutritionally poor and disease susceptible. During the present studies, *Paron* showed significantly lowest bronzing and inward cupping of leaves and defoliation as compared to all the treatments (Table 2). It showed least mean bronzing and inward cupping of leaves (3.25%) and defoliation (3.50%) as compared to all the test rootstocks. *D. necatrix* is soil borne pathogen so first it attacks the below ground parts of the host. It produces symptoms on above ground part after long time of infection at below ground parts. At the initial stages of infection, it is difficult to detect the infection of *D. necatrix*. Under the present study, on below ground parts significantly lowest necrosis on bark (21.00 mm), wood (17.00 mm) and vascular tissues (23.00 mm) was recorded in *Paron* among all the rootstocks (Table 3). *Paron* showed significantly lowest disease severity (4.35%) as compared to all other test plants, whereas Srinagar Crab showed significantly highest disease severity (Table 4). The present findings are in consensus with the work of Sharma *et al*. (2013), who found that different *Malus* species register differential disease reaction on exposure to the inoculum of white root rot pathogen, which might show the ability of host plant to nullify the effect of thermostable toxin produced by *D. necatrix* (Gupta and Gohain 1982). In the light of available literature, none of the available apple rootstocks were categorized resistant against *D. necatrix* (Sharma *et al*. 2013), thus present findings constitute the first report on resistant rootstock against white root rot of apple (*D. necatrix*).

It can be concluded from this study that for effective management of white root rot and growth of apple plants, *Paron* (*Malus baccata* var. *Himalaica*) should be used as rootstock. It will provide environmentally safer disease management strategy and definitely reduce the use of fungicides like carbendazim, which are extensively used for the management of white root rot of apple in India (Kumar *et al*. 2015, Kumar *et al*. 2014, Rana *et al*. 2010, Raj and Sharma 2009 and Gupta 1977). The present findings will be helpful in managing this disease particularly, under organic cultivation. *Paron* can be further utilized in various rootstock breeding programmes. This valuable/important germplasm *Paron* (*Malus baccata* var. *Himalaica*) has been registered (IC- 0614799) for its high level resistance against soil borne pathogen *D. necatrix* and consumer acceptability in National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India.

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Table 2 Disease expression on the above ground parts of different test plants after inoculation with *D. necatrix*

Test plant/Apple rootstock*	Wilting (%)	Bronzing and inward cupping of leaves (%)	Defoliation (%)
MM 106	54.15c	45.25d	46.67d
MM 111	29.77b	16.85c	22.50c
M 9	22.55b	13.45b	15.90b
Paron	0.00a	3.25a	3.50a
Srinagar Crab	58.15c	58.15e	54.82e

*Means followed by the same letter within a column are not significantly different at 5% level based on Duncan's multiple range test.

Table 3 Disease expression on the below ground parts of different test plants after inoculation with *D. necatrix*

Test plant/Apple rootstock*	Necrosis on stem (mm)	Necrosis on wood (mm)	Necrosis on vascular tissues (mm)
MM 106	156.00d	165.00d	195.00d
MM 111	128.00c	131.00c	151.00c
M 9	107.00b	116.00b	136.00b
Paron	21.00a	17.00a	23.00a
Srinagar Crab	170.00e	181.00e	211.00e

*Means followed by the same letter within a column are not significantly different at 5% level based on Duncan's multiple range test.

Table 4 Disease reaction of different test plants of apple after inoculation with *D.necatrix*

Test plant/Apple rootstock*	Disease severity (%)	Disease reaction**
MM 106	59.75d	S
MM 111	18.30c	MR
M 9	14.84b	MR
Paron	4.35a	R
Srinagar Crab	69.80e	HS

*Means followed by the same letter within a column are not significantly different at 5% level based on Duncan's multiple range test. **Based on disease severity level where; R-Resistant (0-5%), MR-Moderately resistant (6-20%), MS-Moderately susceptible (21-40%), S-Susceptible (41-60%) and HS-Highly susceptible (>60%).

REFERENCES

Agarwala R K and Sharma V C. 1966. White root rot disease in Himachal Pradesh. *Indian Phytopathology* **19**: 82–6.

Bharat N K and Bhardwaj L N. 2001. Interactions between VA-mycorrhiza and *Dematophora necatrix* and their effect on apple seedlings. *Indian Journal of Plant Pathology* **19**: 47–51.

Gupta V K. 1977. Possible use of carbendazim in the control of root rot (*Dematophora necatrix*) of apple. *Indian Phytopathology* **30**: 527–31.

Gupta V K and Gupta S K. 1992. Management of white root rot of apple with fungicide drenching. *Indian Phytopathology* **45**: 239–40.

Gupta V K and Gohain B N. 1982. *In vitro* production of toxic compounds by *Dematophora necatrix*, *Contemporary Trends in Plant Sciences*, pp. 103–10. Verma S S, (Eds). Kalyani Publishers, New Delhi.

Gupta V K and Verma K D. 1978. Comparative susceptibility to apple rootstocks *Dematophora necatrix*. *Indian Phytopathology* **31**: 377–78.

Kumar A, Attri B L, Kishor A, Narayan R, Debnath S and Ahmed N. 2015. Integrated management of white root rot of apple caused by *Dematophora necatrix* Hartig. (*In*) *National Conference of Temperate Fruits and Nuts- A Way Forward for Enhancing Productivity and Quality*. The Horticulture Society of India, New Delhi, p 194.

Kumar A, Attri B L, Raj Narayan, Kishor A, Debnath S, Ahmed N and Mer M S. 2014. Identification and management of *Dematophora necatrix* Hartig causing white root rot of apple. *Souvenir of 6th Indian Horticulture Congress-2014*, 6-7th November, 2014, Coimbatore, Tamil Nadu, pp 395–6.

McKinney H H. 1923. Influence of soil temperature and moisture on infection of wheat seedlings by *Helminthosporium sativum*. *Journal of Agriculture Research* **26**: 195–207.

Raj H and Sharma S D. 2009. Integration of soil solarization and chemical sterilization with beneficial microorganisms for the control of white root rot and growth of nursery apple. *Scientia Horticulturae* **119**: 126–31.

Rana S, Sharma S K and Sharma J N. 2010. Integration of cultural, chemical and biocontrol methods in management of white root rot of apple. *Indian Phytopathology* **63**(2): 207–11.

Schena L, Nigro F and Ippolito A. 2002. Identification and detection of *Rosellinia necatrix* by conventional and real time scorpion PCR. *European Journal of Plant Pathology* **108**: 355–66.

Sharma Y P, Pramanick S K, Sharma S K and Kashyap P. 2013. Disease reaction of apple germplasm to white root rot (*Dematophora necatrix*). *Indian Journal of Horticulture* **70**(1): 130–4.

Sharma J N and Sharma R C. 2008. Soil born diseases of temperate fruits and their management. *Advances in Soil Born Plant Diseases,* pp. 219–13. Naik M K and Devika Rani G S, (Eds). New India Publishing Agency, New Delhi.

Singh U B. 1943. Control of fruit diseases in Kumaon. *Indian Farm* **4**: 411–12.

Sztejnberg A and Katan J. 1986. Control of *Rosellinia necatrix* in soil and in apple orchard by solarization and *Trichoderma harzianum*. *Plant Disease* **71**: 365-9.