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## Screening of capsicum germplasm for resistance against *Phytophthora capsici* causing leaf blight and root rot

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### Abstract

Eighty six lines of capsicum were screened for resistance against leaf blight and root rot disease caused by *Phytophthora capsici* during 2018 by adopting detached leaf and seedling inoculation method, respectively. Out of 86 lines, six lines namely KTC-148, KTC-149, KTPL-19, Chilli Local, Pant Chilli and PBC-631 were found resistant by evaluating 14 days old pepper seedlings grown in poly trays containing peat mixture. Whereasthree lines viz., KTC-144, KTC-148 and PBC-631 were found resistant, when evaluated with detached leaf inoculation method while Chilli Local, KTPL-19 and Chilli Pant were found to be moderately resistant.

**Keywords:** resistance, *Phytophthora capsici*, capsicum

### Introduction

Pepper (*Capsicum annum*) is an economically important crop grown in different areas of Himachal Pradesh and used as a fresh vegetable and as a processed food product. *Phytophthora capsica* (Leon.) poses a serious threat to pepper production across the globe (Bosland and Lindsey 1991) [1]. It can infect pepper plants at any growth stage, resulting in damping off, root rot, stem rot, collar rot, fruit rot, and foliar blight. Under warm (25–28 °C) and humid environmental conditions (Foster and Hausbeck 2010) [4]. *P. capsici* root rot (PcRR) is causing up to 100% yield losses. The pathogen attack the plant roots or stem region and causing water-soaked lesion and stem girdling which lead to wilting and death of the plant. The broad host range, as well as its soil-borne and random mating nature, makes it very difficult to control. Use of cultural practices and chemical control measures for PcRR have proven to be ineffective and unsafe (Lamour and Hausbeck 2000) [6]. So, the use of host resistance to PcRR represents the best control measure method. Therefore, the identification of resistant sources will useful in developing resistant varieties.

### Material and Methods

#### Pathogen inoculum production

#### Collection of fungal isolates

Different capsicum growing areas of Himachal Pradesh in Kullu, Mandi, Bilaspur, Hamirpur, Kangra and Solan districts were visited during cropping season 2016-17 for the collection of *Phytophthora* root rot, leaf blight and fruit rot samples of capsicum/chilli infected by *Phytophthora capsici*. The plants showing the symptoms of *Phytophthora* root rot, leaf blight and fruit rot were collected, placed in paper bags and brought to laboratory for isolation purpose. Isolation from infected samples collected from different locations was taken in order to ascertain the associated pathogens with the disease. Pathogen was purified through hyphal tip method.

#### Isolation, purification, maintenance and multiplication of the pathogen

The fungal cultures were isolated from diseased tissues using standard methodology on PDA. The diseased samples were surface sterilized by dipping in mercuric chloride (0.1%) solution for 10-15 sec followed by 3-4 times subsequent washing in sterilized distilled water and finally putting the bits on sterilized filter papers to remove the excess moisture.

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The bits were then transferred to PDA medium slants under aseptic conditions and incubated in BOD incubator at  $25\pm 1$  °C. Precautions were taken to avoid contamination of culture from time to time and were purified by hyphal tip method. For this, culture was allowed to grow in Petri plates on sterilized water agar (2%) under aseptic conditions and incubated at  $25\pm 1$  °C. The single well isolated hyphal tip growing in water agar media (after 72 hrs of growth) as located under microscope (10X) and marked with fine tip pen then area was cut with cork borer (5 mm disc) and transferred to PDA medium slants using inoculation needle. Fungal colony arising from single hypha of each isolate was multiplied on PDA medium and used for further studies. All the isolates were transferred to live hosts after 3 sub-culturing to avoid loss of virulence.

### Preparation of inoculum

Seven days old fungal culture of *P. capsici* was used for preparing the sporangial suspension. Mycelia bits of 10 mm in diameter of fungal culture (agar plugs) were cut and transferred into a Petri plates containing 1%  $\text{KNO}_3$  (potassium nitrate) solution using inoculation needle under aseptic conditions in laminar air flow and incubated at  $25\pm 1$  °C for 4 days. The sporangia thus formed were observed under microscope (10X) and placed at 10 °C for 45-90 minutes to induce zoospore release. After the cold treatment, the Petri plates were returned to  $25\pm 1$  °C for 30 minutes and then checked for zoospore release (Plate 1; A, B). When the number of zoospores released apparent to be abundant, the agar plugs were washed through a double layer cheese cloth with distilled water. Inoculum was adjusted with haemocytometer to  $4 \times 10^4$  zoospores per ml. This concentration was used as standard for carrying out different inoculation studies.

### Pathogenicity test

Bell pepper cultivar "California Wonder" serves as a test plant. Seedlings of "California Wonder" were raised on small pots (6" dia.) filled with the mixture of soil and FYM (3:1) which was sterilized with 5% formalin for 15 days. Surface sterilization of pots was also done with formalin (5%) before a day of sowing. After one month, seedlings were transferred to pots (15cm) and allowed to grow up to 5-6 leaf stage. After 6 weeks, 5-6 leaf stage seedlings (5 seedlings/ pot) were drenched with inoculum (10ml/ plant). Three pots were kept for inoculation with each isolate and one kept as check. After inoculation with *P. capsici* isolates, the pots were maintained at saturated condition for 36 hrs and thereafter watered three times daily.

The symptoms and incidence of root rot was observed on regular intervals (7 and 14 days after inoculation) in both the sets. Pathogen was then re-isolated and cultured by methods discussed previously. The characteristics of pathogen culture thus obtained were compared with that of corresponding inoculated isolates of the pathogen to prove the pathogenicity. Pathogenic cultures were maintained for further studies.

### Evaluation of resistant sources

Seventy five genotypes/ lines of capsicum (bell pepper and chilli) procured from Department of Vegetable Science CSK HPKV, Palampur, nine genotypes from IARI Research Station Katrain, one farmer grown variety Chilli Local from Solan and Pant Chilli were screened to evaluate resistance against *P. capsici* at seedling and adult stage for root rot and

leaf blight infection respectively. The seeds of each genotype were multiplied in polyhouse during 2018.

### Screening for root rot

A total of 86 bell pepper genotypes were screened at seedling stage for root rot infection by creating artificial conditions under greenhouse conditions in Department of Plant Pathology during 2017. Seeds from each variety were sown in plastic trays containing peat mixture (Coco peat, Perlite and Vermiculite in 2:1:1 ratio) and drainage holes. After sowing, the peat mixture was watered immediately and as often as needed, usually once a day. The trays were placed on greenhouse bench where air temperature was maintained at  $28\pm 2$  °C during the day and  $15\pm 2$  °C at night. Inoculum was prepared using the procedure described above. Inoculum was adjusted with haemocytometer to 2000 zoospores per millilitre (Bosland and Lindsey 1991) [1].

A Plastic tray without drainage holes was filled with tap water. The poly tray with drainage holes containing the 14 days old pepper seedlings was put into water filled tray to saturate plant roots. Each cell was infested with 5 ml of inoculum, giving a final concentration of 10,000 zoospores per cell. The roots were maintained in the flooded conditions for initial 48 hrs. The plant trays with drainage holes were removed from the water filled tray and placed on a greenhouse bench. The plants were irrigated three times daily and disease severity was scored after 7-14 days.

The plants were evaluated based on a 10-point scale according to Bosland and Lindsey (1991) [1]: 0 = no response, vigorous, healthy; 3 = brown roots, slight stunting, very small lesions on stems; 5 = brown roots, small lesions on stems, lower leaves wilted, stunted plants; 7 = brown roots, large lesions on stems, girdling, whole plant wilted, and stunted; 9 = death. A disease index value of 2 or less was considered resistant and a value greater than 2 was susceptible.

### Screening for leaf blight

Eighty six bell pepper genotypes were also evaluated against *P. capsici* using detached leaf method. Three leaves were placed in 11 cm diameter plastic Petri plates lined with moist blotting sheets. Sporangial suspension of *P. capsici* ( $9 \times 10^4$  sporangia/ ml) was inoculated on the centre of inverted leaves. Petri plates were incubated at  $25\pm 1$  °C. Data on disease development were recorded for 7 days following 1-5 scale of Verma (1997) [8] as described below:

Category	Disease severity (%)	Reaction
1	0-5	Resistant
2	5.1-10.0	Moderately Resistant
3	10.1-25.0	Moderately Susceptible
4	25.1-50.0	Susceptible
5	More than 50.0	Highly Susceptible

## Results and Discussion

### Evaluation of resistance sources

#### Screening for root rot

Eighty-six genotypes were screened for disease reaction against root rot of capsicum. Data on categorization of genotypes into different resistant category have been presented in table 1. Root rot symptoms were appeared after 14 days of inoculation. The lines were categorized on 10 point scale (Bosland and Lindsey 1991) [1]. Majority of the germplasm was found susceptible to the pathogen. Out of 86 screened accessions, six lines namely, KTC-144, KTC-148, KTPL-19, Chilli Local, Pant Chilli and PBC-631 were found

resistant. Rest of the lines were found highly susceptible against the pathogen.

Candole and Conner (2010) [3] screened 2301 accessions of *C. annuum* with 6 Georgia isolates of *P. capsici* and identified 77 accessions as resistant to root rot. They also found two accessions i.e., PI-201237 and PI-640532 demonstrated high levels of resistance to root rot. Foster and Hausbeck (2010) [4] observed pepper lines CM 334, NYO7-8001, NYO7-8007 and NYO7-8006 resistant to the isolate tested. Rodriguez *et al.* (2016) studied differential response of pepper lines to *P. capsici* isolates and found Serrano pepper lines 41-1, 41-2, 41-6 and 55-2 with a resistance response to all *P. capsici* isolates followed by Huacle pepper lines 33-3, 35-3 and 34-3, which were only susceptible to one isolate.

### Screening for leaf blight

Eighty-six genotypes of capsicum were evaluated for resistance against *P. capsica* under laboratory conditions using detached leaf method of inoculation. Leaf blight

symptoms were observed after 14 days of inoculation (Plate 1). These lines were categorized on 1-5 disease scale (Verma 1997). Data on disease rating, disease severity and reaction type have been presented in table 2. Majority of the germplasm was found susceptible to the pathogen. Out of 86 screened accessions, three lines namely KTC-144, KTC-148 and PBC-631 were found resistant, whereas, 3 lines namely Chilli Local, KTPL-19 and Chilli Pant were found to be moderately resistant. Seven germplasm lines were found moderately susceptible and 41 were found susceptible. Rest of lines was highly susceptible against the pathogen for leaf blight.

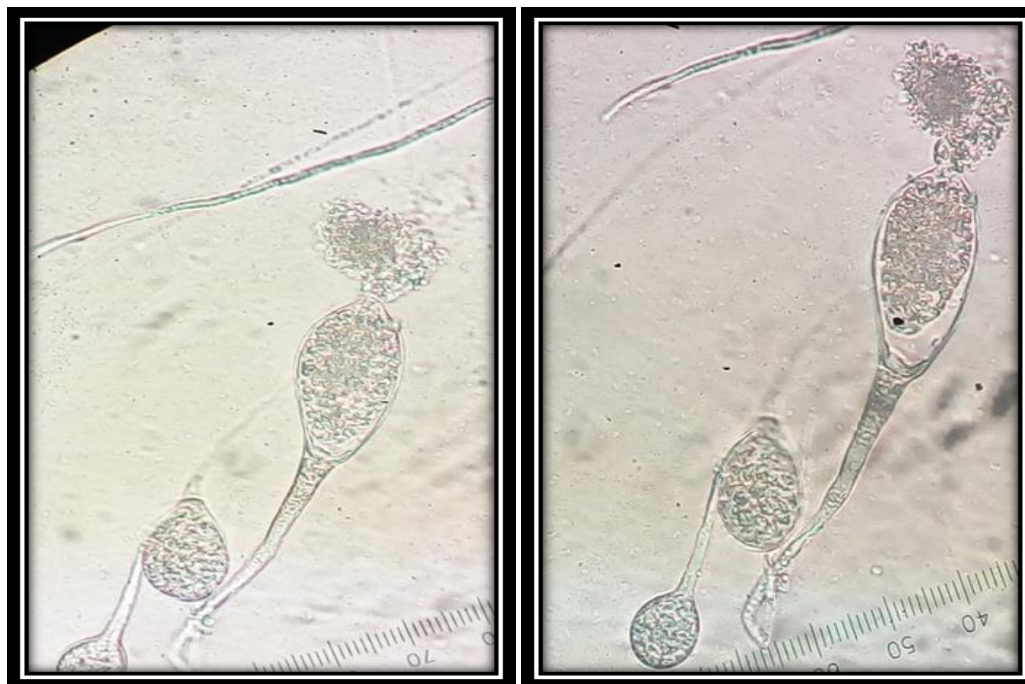
Our findings on host resistance are in agreement with several workers in India and abroad. Saleem *et al* 1999 reported sweet peppers to be susceptible and California Wonder as most susceptible. Candole *et al.* (2012) [2] screened 700 accessions of *C. annuum* and evaluated that 69 per cent of accessions were resistant to stem blight and 71 per cent were resistant to foliar blight.

**Table 1:** Screening of capsicum genotypes for Phytophthora root rot

Disease Rating	Reaction Type	Genotypes
0	Resistant	KTC-144, KTC-148, Chilli Local, Pant Chilli, KTPL-19, PBC-631
3	Moderately Resistant	-
5	Moderately Susceptible	-
7	Susceptible	DPCH-2015-74, DPCH-2015-46, DPCH-2015-66, DPCH-27, KTC-133, KTC-143, KTC-148, Sweet pepper, DPCH-2015-27, DPCH-2015-55, Cap-2, DPCH-2015-53, DPCH-2015-12, Paprika-1, PusaSadabahar, DPCH-2-3-5-1, DPCH-2015-16, DPCH-2015-7, DPCH-2015-4, DPCH-2015-47, DPCH-2015-10, C-1, DPCH-2015-58, DPCH-19-1, DPCH-36, DPCH-71, DPCH-11-2-3-1, Surajmukhi, Yolo Wonder, Russian Yellow, KTC-152, KTC-153, DPCH-41(PP), DPCH-38-2-1, DPCH-17-2, DPCH-38-1-1, DPCH-6-1-3, DPCH-17-1-1, DPCH-29, DPCH-17-3, DPCH-32-3, DPCH-4, DPCH-5, DPCH-6, DPCH-6-1-2, DPCH-22-2, DPCH-22-1-2, DPCH-14-1(PP)
9	Highly Susceptible	California Wonder, DPCH-2015-8, DPCH-7, Chilli-1, Chilli-2, Chillilocal Solan, DPCH-2015-1, DPCH-2015-3, Cap-R-5, DPCH-2015-77, DPCH-2015-39, DPCH-2015-36, DPCH-32-2-6, DPCH-32-2-1, DPCH-38-2, DPCH-26-1-2, DPCH-22-1, DPCH-26-2, DPCH-35, DPCH-12-1, DPCH-29-1, DPCH-10(PP), DPCH-22, DPCH-38-2-2, DPCH-62, DPCH-32-1-2, DPCH-29-2, DPCH-12-2, DPCH-57(PP), DPCH-9, DPCH-31, DPCH-21

**Table 2:** Screening of capsicum genotypes by detached leaf method to Phytophthora blight

Disease Rating	Disease Severity	Reaction Type	Genotypes
1	0-5%	Resistant	KTC-144, KTC-148, PBC-631
2	5.1-10.0%	Moderately Resistant	Chilli Local, Pant Chilli, KTPL-19
3	10.1-25.0%	Moderately Susceptible	DPCH-2015-74, DPCH-2015-46, DPCH-2015-66, DPCH-27, KTC-133, KTC-143, KTC-148,
4	25.1-50.0%	Susceptible	Sweet pepper, DPCH-2015-27, DPCH-2015-55, Cap-2, DPCH-2015-53, DPCH-2015-12, Paprika-1, PusaSadabahar, DPCH-2-3-5-1, DPCH-2015-16, DPCH-2015-7, DPCH-2015-4, DPCH-2015-47, DPCH-2015-10, C-1, DPCH-2015-58, DPCH-19-1, DPCH-36, DPCH-71, DPCH-11-2-3-1, Surajmukhi, Yolo Wonder, Russian Yellow, KTC-152, KTC-153, DPCH-41(PP), DPCH-38-2-1, DPCH-17-2, DPCH-38-1-1, DPCH-6-1-3, DPCH-17-1-1, DPCH-29, DPCH-17-3, DPCH-32-3, DPCH-4, DPCH-5, DPCH-6, DPCH-6-1-2, DPCH-22-2, DPCH-22-1-2, DPCH-14-1(PP)
5	>50%	Highly Susceptible	California Wonder, DPCH-2015-8, DPCH-7, Chilli-1, Chilli-2, Chillilocal Solan, DPCH-2015-1, DPCH-2015-3, Cap-R-5, DPCH-2015-77, DPCH-2015-39, DPCH-2015-36, DPCH-32-2-6, DPCH-32-2-1, DPCH-38-2, DPCH-26-1-2, DPCH-22-1, DPCH-26-2, DPCH-35, DPCH-12-1, DPCH-29-1, DPCH-10(PP), DPCH-22, DPCH-38-2-2, DPCH-62, DPCH-32-1-2, DPCH-29-2, DPCH-12-2, DPCH-57(PP), DPCH-9, DPCH-31, DPCH-21



A.

B.

**Plate 1:** Zoospore release of *P. capsici* (A, B); Screening of capsicum lines for *Phytophthora* leaf**Conclusion**

Out of 86 genotypes/lines, six lines namely KTC-144, KTC-148, KTPL-19, Chillilocal, Pant Chilli and PBC-631 were found to be resistant to *Phytophthora* root rot and *Phytophthora* blight. Other lines were found to be moderately susceptible, susceptible and highly susceptible.

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