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SHORT COMMUNICATION

Detached non-wounded fruit inoculation technique for pathogenicity of *Colletotrichum capsici* on chilli

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ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR) has a mandate to carry out activities concerning collection, characterization, evaluation, conservation, exchange, quarantine and documentation of diverse plant genetic resources (PGR) and their wild relatives to ensure their availability for use over the time to breeders and other researchers. PGR is essential basics for any crop improvement programme such as development of new varieties/ hybrids designed to combine with certain characters like high yield potential with superior quality, resistance to diseases and pests or better adaptation to abiotic stresses. India is a major chilli (*Capsicum annuum*) growing country and biotic stresses especially anthracnose/ fruit-rot disease has major impact on chilli production, as most of the commercial varieties are considered susceptible to *Colletotrichum capsici*. This disease alone resulted in yield losses up to 50 per cent (1) and gradual decrease in ascorbic acid, an important component in chilli fruit, is also reported due to fruit-rot. Anthracnose disease complex of chilli is caused by more than one *Colletotrichum* species. Occurrence of different *Colletotrichum* species such as *C. acutatum* (Simmonds), *C. capsici* (Syd.) Butler & Bisby, *C. coccodes* (Wallr.) Hughes, *C. dematium* (Pers.) Grove, *C. gloeosporioides* (Penz.) Penz. and Sacc., and *C. nigrum* (Wallr.) Hughes in chilli in various parts of the world has been reported (2), but, in India the disease is caused by *C. capsici* (3). Existence of 15 pathotypes of *C. capsici* from Himachal Pradesh in Northern India based on the symptoms developed has been reported (4) while three pathotypes of *C. capsici* are reported in Thailand on the basis of quantitative infection on chilli (5). In addition to its wide distribution, there are reports of existence of variability in the pathogen which makes it a pest of quarantine significance to India. Considering the economic importance of the disease, a quick and reliable technique is required to test the pathogenicity of large number of isolates for further studies. Proving the pathogenicity/ virulence of a large number of isolates in limited period of time is also a challenging task. Keeping these points in a view, an inoculation technique was developed for proving pathogenicity or virulence analysis of large number of isolates of the pathogen on detached non-wounded chilli fruit.

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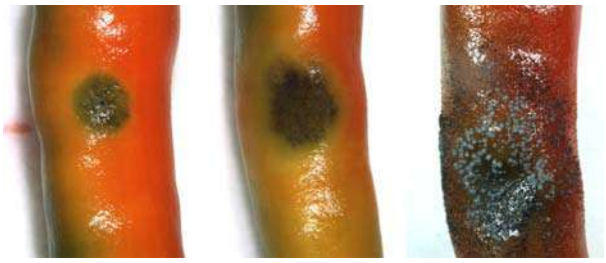
While doing seed health testing of indigenous chilli germplasm, *C. capsici* was detected and pure cultures were obtained using standard isolation technique (6). The experiment was conducted with 20 each red and green chilli fruits procured from local market (New Delhi). The healthy fruits of green and red chillies were first surface sterilized in $HgCl_2$ solution (1:1000) for 30 sec. followed by three thorough rinsing in sterilized distilled water. The chilli fruits (2 fruits per Petri plate) were placed on pre-sterilized blotting paper to remove excess moisture. The surface sterilized fruits were aseptically transferred on triple layer of moist blotters in Petri plates (110 mm). The non-wounded (without any pin pricking or injury on skin) fruits were spot inoculated by placing 20 µl conidial suspension (3-4 conidia/ µl) of *C. capsici* in water gelatine (2.0%) using micropipette. Gelatin was used to avoid the over spreading of the conidial droplets. Two chilli fruits per Petri plate were inoculated with equal volume of sterilized distilled gelatin suspension which served as control. Petri plates were incubated at 22±1°C. Incubated fruits were observed daily for 2 weeks under stereobinocular microscope to record infection and symptom development. The symptoms developed were assessed as lesion area (mm^2) and incubation period (day) by taking average of 10 fruits each from both types of chilli. Re-isolation was made on potato dextrose agar for further identification of the pathogen to compare with original culture used for inoculation.

The fungus, *C. capsici* proved pathogenic on detached non-wounded fruits of green and red chilli cultivars procured from local market. While lesions started appearing on green chilli fruits 4 days after inoculation (DAI), and on red chilli fruits they appeared 7 DAI. Fruit rot symptoms fully developed at 12 DAI on green chilli fruits, whereas, it took 14 DAI on red chilli fruits. The inoculated fruits showed fungal colony with dark pin head acervuli which were sub-epidermal emerging by disrupting outer epidermal cell walls of green and red chilli fruits (Fig. 1, 2). The fruits of green chilli cultivar showed relatively larger lesion size ranging from 11×7 mm^2 to 15×10 mm^2, whereas, the lesion size on red chilli fruits ranged from 8x5 mm^2 to 10x6 mm^2.

The result clearly indicated that there were differences in the lesion areas as well as incubation

(a) (b) (c)

Fig. 1. Fruits of green chilli (local) showing infection of *Colletotrichum capsici* at (a) 4DAI, (b) 7 DAI and (c) 12 DAI



(a) (b) (c)

Fig. 2. Fruits of red chilli (local) showing infection of *Colletotrichum capsici* at 7 DAI (a), 10 DAI (b) and (c) 14 DAI

periods between green and red chilli fruits. The fungus after re-isolation from both types of fruits was identified and found identical with that of original one i.e. *C. capsici* based on the specific characters. Setae were dark brown, rigid, swollen at the base, slightly tapered to the paler acute apex, 1 to 5-septate, 250 × 6 µm. Conidia were hyaline, falcate with acute apex and narrow truncate base, aseptate, uninucleate, 18-23 × 3-5 µm.

Detached fruit technique has been used by several workers where pin prick method was employed for testing pathogenicity (7). Detached wounded fruits are more prone to infection caused by the pathogens. The present study reports a modified technique which is more suitable for proving pathogenicity as well as virulence of *C. capsici* using non-wounded detached chilli fruits.

Infection process involves a chain of events starting with attachment and germination of conidia, appressorial formation, penetration of plant epidermis, growth and colonization of plant tissue and production of acervuli and sporulation (8). In the present study, longer incubation period as well as smaller lesion area in red chilli fruits may be due to either slow conidial germination or appresorial formation followed by penetration and colonization of chilli fruits, which is a kind of quiescence of appressoria exhibited by immature chilli fruits (9) or because of capsanthin, a carotenoid present in red chilli which indirectly underlies the expression of disease-resistance mechanisms (10) leading to delayed and smaller symptom development.

An *in vitro* technique for proving the pathogenicity of *C. capsici* on detached non-wounded green and red chilli fruits was developed within a minimum possible time. In this technique, recovery of the test pathogen from inoculated fruits was 100% without any contamination. The method developed in the present study could be useful in ascertaining the pathogenicity and for virulence analysis of a large number of isolates of the pathogen on fruits of several cultivars of chillies within a short period of time under laboratory conditions.

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