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STATUS OF *COLLETOTRICHUM* SPECIES INFECTING CHILLI GERMPLASM PROCESSED FOR PATHOGEN-FREE CONSERVATION IN NATIONAL GENE BANK, INDIA

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Abstract

Seed health testing of 384 accessions of chilli (*Capsicum annuum* L.) representing eight states of India revealed the prevelance of *Colletotrichum capsici* (Syd.) Butler & Bisby in three states, namely Andhra Pradesh, Meghalaya and Uttarakhand which is the causal agent of anthracnose (= fruit-rot and die-back). Out of four *Colletotrichum* species reported on chilli from different parts of the world, only *C. capsici* was observed in accessions tested. Occurrence of *C. capsici* was recorded maximum from Uttarakhand (7.4%). The observations also revealed the occurrence of the pathogen in seed samples up to 5.0 per cent infection index indicating its wide spread in India. Further, correlation analysis revealed that there is highly significant negative correlation between level of infection and reduction in seed germination with r value 0.90 and R^2 value of 0.76. The present study confirms the anthracnose of chilli in India is mainly due to *C. capsici* and the infected seeds are mainly responsible for the long distance spread of the pathogen in absence of effective domestic quarantine.

Introduction

It is well established that chilli (*Capsicum annuum* L.) seeds are carrier of several destructive pathogens which cause considerable yield losses (CABI 2007). Among these pathogens, *Colletotrichum* spp. causing anthracnose (= ripe fruit-rot and die-back) is one of the most destructive fungal pathogens of this crop (Akhtar and Singh 2007). Four species of *Colletotrichum viz., C. acutatum, C. capsici, C. coccodes* and *C. gloeosporioides* have been reported to cause chilli anthracnose (Gopinath *et al*. 2006, Than *et al*. 2008). However, *C. capsici* is the most frequently cited causal organism of chilli anthracnose in India (Sharma *et al.* 2005). The fruit-rot/anthracnose pathogen is reported to be seed-borne (Richardson 1990) and is known to survive in and on seeds as acervuli and microsclerotia. Podaganur and Naik (1991) reported externally seed-borne nature of *C. capsici*. Manandhar *et al*. (1995) also reported survival of mycelium and stroma in colonized chilli seed. Siddiqui *et al*. (1984) reported the anthracnose fungus to be viable for over eight years in chilli seeds stored at 5°C. Survival of *C. capsici* has also been reported on cryo-preserved chilli seeds for more than ten years (Dev *et al*. 2012). Seed-borne infection of *C. capsici* affects germination and early seedling growth. Moreover, Gopinath *et al.* (2006) and Akhtar and Singh (2007) reported that anthracnose of chilli has been a serious problem for chilli cultivation in India. Therefore, keeping in view the importance of the disease, an experiment was conducted to detect and identify the prominent fungal species associated with chilli seeds received from different states causing anthracnose in India.

Materials and Methods

An experiment was conducted at the Division of Plant Quarantine, ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi to detect and identify the fungal pathogen(s) associated with chilli seeds. The seed samples of 384 accessions of chilli from different states of

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India viz., Andhra Pradesh (148), Assam (2), Goa (2), Kerala (1), Meghalaya (80), Odisha (1), Uttarakhand (135) and West Bengal (15) representing different agro-ecological zones of the country were received through Division of Germplasm Conservation, ICAR-NBPGR, New Delhi for seed health testing. The seed samples were first examined visually and later, diseased or suspected unhealthy seeds were subjected to blotter test (Mathur and Kongdal 2003). The seeds were surface sterilized by immersing in 4.0% sodium hypo chlorite solution for 30 seconds, subsequent rinsing three times in sterilized distilled water in aseptic conditions under laminar air flow. Twenty-five seeds per Petri plate (110 mm) were placed in such a manner that one seed in the centre, 9 seeds in middle and 15 seeds at periphery at equidistance on three layers of sterilized moist blotting papers in sterilized Petri plates and labeled with accession number and the date of observation to be taken. Unsterilized seeds of each accession were similarly plated as control. Three plates of each sterilized and unsterilized seeds of each accession were then incubated for 7 days at 22 ± 1°C under alternating cycles of 12 hrs light and darkness in incubation room. On the 8th day, the incubated seeds were observed for detection of fungal fructification(s) associated with the seeds under stereozoom microscope (Nikon - SMZ 1500) at different levels of magnification from 0.75× to 11.25×, if any, and mounts using lactophenol-cotton blue stain were prepared for identification of fungus under compound microscope (Nikon - Eclipse 80i) followed by micrometry. Further, to establish the identity of causal fungus, pure cultures of the associated fungus were obtained on potato dextrose agar (PDA) medium plate using handmade sterilized needle for transferring single acervulus from superficial growth of the fungus on seeds under laminar air flow (Akhtar *et al*. 2014). The observations on frequency of fungal infection and seed germination were recorded and correlation analysis was done.

Results and Discussion

During visual examination, seeds of certain accessions *viz.* EC345641 (EC stands for exotic collection), IC338795 (IC for indigenous collection), IC383150, IC469836, IC538010, IC538026, IC538030 and IC538085 had tiny black spots on seed surface. Seeds of 384 accessions of chilli examined revealed 19 accessions infected with pathogen showing acervuli on seed surface. These 19 infected accessions belonged to States of Andhra Pradesh, Meghalaya and Uttarakhand (Table 1). Acervuli were sub-epidermal emerging by disrupting outer epidermal cell walls of the seed. Setae were dark brown, rigid, swollen at the base, slightly tapered to the paler acute apex, 1 to 5-septate, 250 × 6 µm (Fig. 1). Conidia were hyaline, falcate with acute apex and narrow truncate base, aseptate, uninucleate, 18 - 23 × 3 - 5 µm (Fig. 2). Mycelial growth of the associated culture on PDA was initially white which later converted to dark-grey cottony growth and acervuli with abundant dark setae (Fig. 3).

Other characteristics of the fungus were also recorded as described by several workers (Mathur and Kongsdal 2003, CABI 2007). Based on characteristics observed pertaining to acervuli, setae, colony growth on seed and colony characteristics such as growth, shape, margin, colour, texture and zonation on PDA as well as conidial morphology, the fungus was identified as *Colletotrichum capsici* (Syd.) Butler & Bisby, which causes anthracnose in chilli. No other species of *Colletotrichum* were observed from any accession tested.

Altogether 365 accessions comprising from Andhra Pradesh (144), Assam (2), Goa (2), Kerala (1), Meghalaya (75), Odisha (1), Uttarakhand (125) and West Bengal (15) did not produce any fungal growth on seeds in incubation test showing no infection. However, the disease is prevalent throughout India but is reported to be more common and aggressive in Assam, Bihar, Aandhra Pradesh and Uttar Pradesh (Sharma *et al*. 2005, Gopinath *et al.* 2006, Akhtar and Singh 2007). But, in the present study, the accessions collected from Assam, Goa, Kerala, Odisha and West Bengal did not show any seed-borne infection or symptoms which could possibly be due to

very less number of accessions tested or resistance in the host and or use of protective chemicals for the management of the disease in field.

Table 1. Infection level of *Colletotrichum capsici* and its effect on chilli seed germination.

Accession	Source	*C. capsici**	Infection (%)	Germination (%)
BPB/KR/HDS-243	Meghalaya	+	10.0 (18.4)**	100.0 (90.0)
BPB/IW/SN225	"	+	10.0 (18.4)	70.0 (56.8)
BPB/KR/HDS-219	"	+	10.0 (18.4)	70.0 (56.8)
BPB/IW/SN221	"	+	10.0 (18.4)	60.0 (50.8)
BPB/IW/SN224	"	+	10.0 (18.4)	60.0 (50.8)
IC19973	Andhra Pradesh	+	20.0 (26.5)	60.0 (50.8)
IC19943	"	+	10.0 (18.4)	100.0 (90.0)
IC538029	"	+	70.0 (56.8)	20.0 (26.6)
IC426565	"	+	80.0 (63.4)	0.0 (0.0)
IC23886	Uttarakhand	+	80.0 (63.4)	30.0 (33.0)
EC345641	"	+	90.0 (71.8)	0.0 (0.0)
IC538038	"	+	80.0 (63.4)	10.0 (18.4)
IC538010	"	+	90.0 (71.8)	50.0 (45.0)
IC538026	"	+	90.0 (71.8)	20.0 (26.6)
IC338795	"	+	90.0 (71.8)	20.0 (26.6)
IC469836	"	+	90.0 (71.8)	0.0 (0.0)
IC538030	"	+	100.0(90.0)	20.0 (26.6)
IC538085	"	+	100.0 (90.0)	10.0 (18.4)
IC383150	"	+	100.0 (90.0)	0.0 (0.0)
CD (p-0/05)			3.1	5.1
SE(d)			1.5	2.5
SE(m)			1.1	1.8
CV			3.5	8.9

**C. acutatum, C. coccodes* and *C. gloeosporioides* were not detected in present investigation. ***Figures in parentheses are angular transformed values.

Data on state wise occurrence of the fungus indicated that out of 135 accessions tested from Uttarakhand, only 10 accessions (7.4%) showed infection of *C. capsici*. Out of 148 accessions from Andhra Pradesh, only 4 accessions showed infection of *C. capsici* with occurrence of 2.7%. Similarly, occurrence of *C. capsici* was recorded only in 5 out of 80 accessions from Meghalaya (6.3%). Overall, the occurrence of *C. capsici* as seed-borne infection was 5.0% which is high, thereby showing wide spread in India.

Frequency recorded from infected seeds revealed that level of infection ranged between 10.0 and 100.0%. The highest level of infection ranging from 80.0 to 100.0% was recorded in 10 accessions from Uttarakhand. Relatively low level of infection ranging from 40.0 to 80.0% was observed in 4 accessions from Andhra Pradesh in comparison to Uttarakhand. Whereas, 5 accessions representing Meghalaya showed relatively very low level of infection (10.0%). Data pertaining to seed germination ranged from 0.0 to 100% (Table 1).

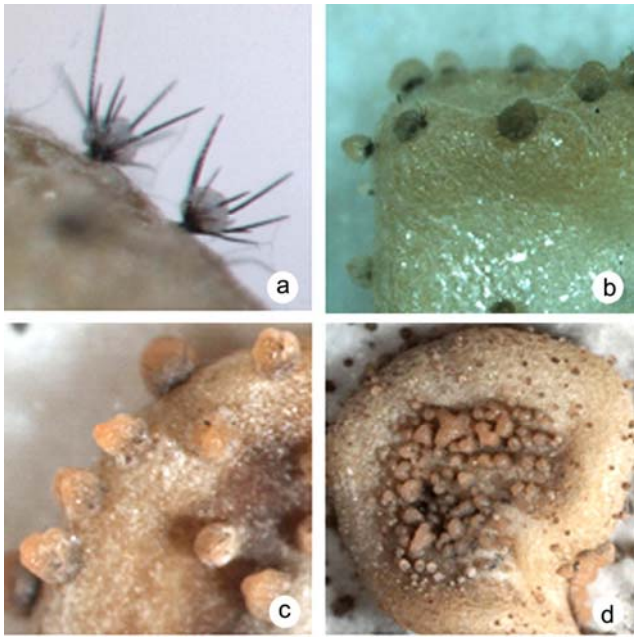

Fig. 1. Growth characteristics of *Colletotrichum capsici* on seed of some chilli accessions: a- IC538026, b- IC538030, c- IC538085; d- IC338795.

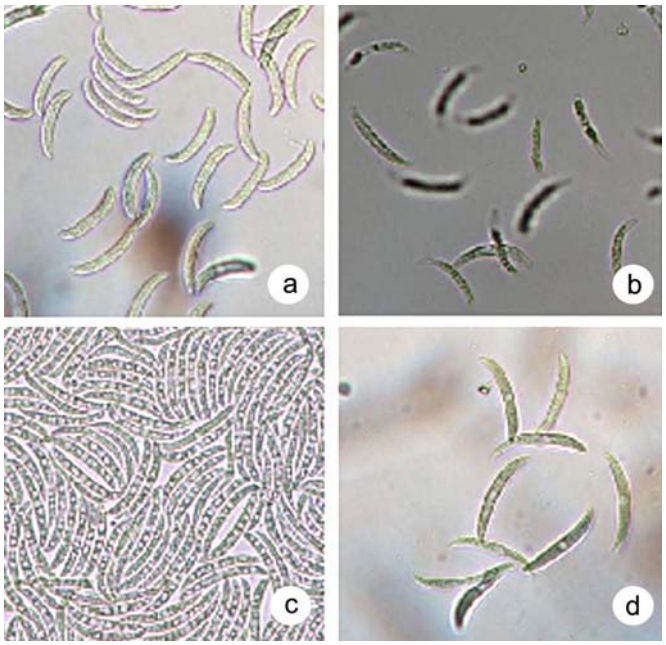

Fig. 2. Conidial morphology of *Colletotrichum capsici* isolated and purified on PDA from four accessions: a- IC538026, b- IC538030, c- IC538085, d- IC338795.

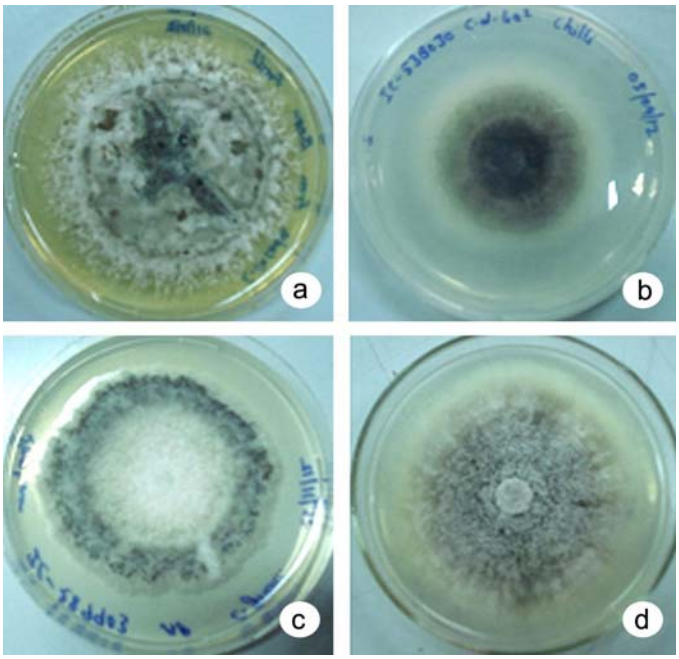

Fig. 3. Colony characteristics of *Colletotrichum capsici* isolated and purified on PDA from four accessions: a- IC538026, b- IC538030, c- IC538085, d- IC338795.

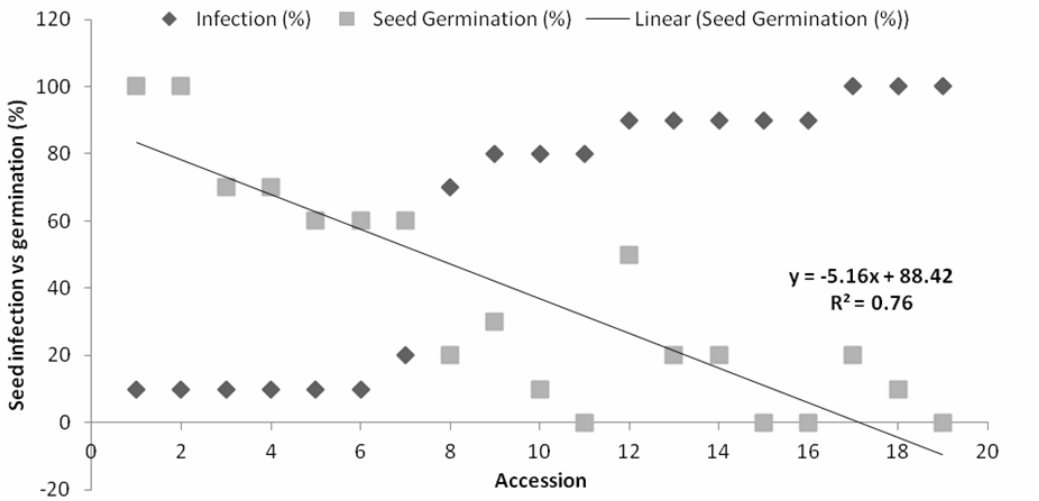

Fig. 4. Correlation between infection of *Colletotrichum capsici* and germination of chilli seed.

Further, correlation analysis revealed that there was highly significant negative correlation between level of infection and reduction in germination of seed, with r value 0.90 and R^2 value of 0.76 (Fig. 4). With the increase in infection level, there was corresponding decrease in germination of seed. This result clearly indicates that the chilli seed multiplied at the location(s) in Uttarakhand is certainly a hot spot location for anthracnose disease of chilli. Though the occurrence of infection

is about 6.0% in Meghalaya, but its infection level is very low which indicates that the disease is prevalent there in endemic form on the cultivars grown there.

In previous report, Smith and Crossan (1958) had observed 48.0% infection of *C. capsici* on chilli seeds. Our observations on loss of germination due to *C. capsici* infection is justified as histopathological observations have already proved pathogen's transmission from endosperm to hypocotyls and radicals (Lee *et al.* 1995).

However, a number of other *Colletotrichum* species such as *C. capsici, C. gloeosporioides, C. graminicola, C. acutatum* (Machenahalli *et al.* 2014, Tanwar *et al.* 2015) and *C. atramentarium* (Selvakumar 2007) have been found associated with the disease in different geographical areas. However, the detection of *C. capsici* in chilli in the present study clearly indicates that it is the main cause of anthracnose of chilli in India and its transmission through seeds carrying up to 5.0% infection index may be responsible for spread of the disease as seed is the main source of long distance spread of pathogen in absence of strict domestic quarantine. Keeping in view that chilli is an important commodity for export, the present finding may prove significant in formulating a suitable management strategy of this devastating disease in India.

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