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Establishment of *Colletotrichum capsici* during early hours of infection in seedlings of a tolerant and a susceptible cultivar of chilli

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The production of chilli in India is greatly impacted by the fungal pathogen *Colletotrichum capsici.* This fungus is often seed borne, leading to severe infections during the early seedling stage. In this study, we focused on studying the early interactions between *C. capsici* and one week-old chilli seedlings of a tolerant cultivar (IR8) and a susceptible cultivar (Bullet) and establishment of the pathogen in early stages of infection. The disease caused by *C. capsici* appeared as discolored necrotic lesions on the young cotyledonary leaves, with symptoms being more severe and the disease progressing rapidly in the susceptible Bullet cultivar compared to the tolerant IR8 cultivar. The disease index, based on the extent of necrotic lesions on the infected cotyledonary leaves, was significantly lower in IR8 than in Bullet. Additionally, the percentage of cell necrosis was also less in IR8 compared to Bullet. The pathogen displayed different growth patterns on the cotyledonary leaves of the two host genotypes, showing denser hyphal growth on the susceptible host than on the tolerant one. Study of anthracnose blight in the seedling stage is integral to understanding ways to prevent diseases caused by this soil-borne and often seed-borne pathogen.

Keywords : Chilli, blight, anthracnose, *Colletotrichum capsici*, disease index, host-pathogen interaction, hyphal density, necrotic lesion

INTRODUCTION

Chilli is an important spice crop cultivated worldwide in the tropical and subtropical regions (Saxena *et al*. 2016). India is the largest producer, consumer and exporter of chilli (Reddy *et al.* 2023). Total yield of green chillies in India was recorded to be approximately 45.83 lakh metric tonnes in the year 2022-2023 (Prasad *et al.* 2025). But due to various diseases in chilli, the total yield is reduced and it negatively impacts the economy of the country (Naveen *et al.* 2020). *Colletotrichum capsici* is one of the major phytopathogens of chilli casing anthracnose, die back or blight disease, which constrains chilli cultivation (Mishra *et al.* 2017).

Investigation of the different aspects of interactions of economically important plants with filamentous phytopathogens forms an active area of current research (Mishra *et al.* 2017). Anthracnose, die back or blight disease has been reported to be caused by the conidia of *Colletotrichum sp.* and the high humidity in the tropics favours its prevalence (Joshi *et al.* 2023). The fungus *C. capsici* is found almost all over India (Saxena *et al.* 2016). Various species of *Colletotrichum* like *C. truncatum* (Singh *et al.* 2021), *C. lindemuthianum* (Yesuf *et al.* 2007), *C. gloeosporioides* (Thomas *et al.* 2003), and *C. capsici* (Choudhary *et al.* 2013; Bijali *et al.* 2021) have been reported to be soil-borne and also seed-borne in important crop plants like soybean, common bean, chilli etc. *C. capsici* causes necrosis in both green and mature chilli fruits (Saini *et al.* 2023). Besides fruits, the phytopathogen also attacks all other aerial parts of the plant such as stems and leaves (Saxena *et al.* 2016). In advanced stages of disease, concentric rings of acervuli form that produce pinkish-orange conidial masses (Joshi *et al.* 2023).

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Although anthracnose is an economically significant plant disease, there is a lack of study on the early phases of interaction between *C. capsici* and chilli plants, especially in seedling stage. Despite previous research work on chilli and *Colletotrichum sp.* there is a gap in knowledge about the first few hours of host-pathogen interaction. In this study, we have compared disease progression between two commonly grown Indian chilli cultivars (IR8 and Bullet) infected with *C. capsici* in order to get an insight into the early stages of establishment of the pathogen in the seedling stage of host.

MATERIALS AND METHODS

Fungal Material

Pure culture of *Colletotrichum capsici* (Syd.) Butler & Bisby was obtained from MTCC (Collection Acc. No. 9691).

Culture and maintenance of fungus

C. capsici was grown on potato dextrose agar (PDA) medium and incubated at 28^{0}C in incubator. Sub-culturing was done to maintain the culture under similar conditions and stored at 4^{0}C.

Plant material and growth conditions

The seeds of *Capsicum annuum* of cultivars IR8 and Bullet were obtained from Sutton Pvt. Ltd., India. The seeds were vernalised at 4^{0}C for 48 hours and sowed in commercial Soilrite (Irish peat moss: vermiculite: perlite in 1:1:1 ratio) potting mixture. The seedlings were maintained at 25±2 ^{0}C in a plant growth chamber at ~ 80% humidity.

Preparation of conidial suspension and inoculation of plants

Conidial suspension was prepared from 10 day old *C. capsici* culture following our previously published protocol by Ray *et al.* (2015). The final concentration of the suspension was adjusted to $4x10^6$ conidia/ml using a haemocytometer. Cotyledonary leaves of healthy 1 week-old chilli seedlings of IR8 and Bullet cultivars were inoculated with the conidial suspension and incubated for 24, 48 and 72 hours post inoculation (hpi).

Comparison of disease progression and disease index calculation

The appearance of necrotic lesions was observed and the disease index was calculated in accordance to our published protocol (Basu *et al.* 2016).

Cell necrosis Assay

Trypan blue staining was used to detect cell necrosis in the inoculated cotyledonary leaves following our previously published protocol (Koley *et al.* 2022). The infected cotyledonary leaves were soaked overnight in aceto-ethanol (acetic acid: ethanol=1:3, v/v) to remove chlorophyll. The decolourised leaves were stained with lactophenol-trypan blue solution (0.5% trypan blue, lactic acid: phenol: H_2O 1:1:1 v/v/v) and kept overnight. The excess stain was removed by washing in distilled water. The samples were photographed and percentage of affected cotyledonary leaf area was calculated using Image J software.

Comparison of growth of C. capsici hyphae on tolerant and susceptible chilli leaf surface

This was carried out according to (Basu *et al.* 2016). Hyphal growth on inoculated cotyledonary leaves of both IR8 and Bullet were observed at 24hpi, 48hpi and 72hpi using compound microscope (Zeiss Winkel 161971) and photographed.

Statistical analyses

All experimental data were statistically analysed according to our previous publication (Chowdhury *et al.* 2017). All experiments consisted of three independent sets with at least three replicates in each and the values were represented as the mean ± SD.

RESULTS AND DISCUSSION

Comparison of disease symptoms of seedling blight caused by C. capsici in cotyledonary leaves of tolerant and susceptible 7-day-old chilli seedlings

Appearance of discoloured lesions on cotyledonary leaves of 7-day-old chilli seedlings infected with *C. capsici* conidial suspension was monitored at 24, 48 and 72 hpi (Fig. 1A). Affected areas in tolerant IR8 exhibited smaller lesions compared to susceptible Bullet, which displayed significantly larger lesions. The lesions continued to increase in size till the end of the experiment. At 72 hpi, the necrotic lesions covered almost entire surface of the cotyledonary leaf in Bullet cultivar, whereas in IR8, lesion formation was limited to relatively smaller areas. Consequently, the disease index at the young 7-day-old seedling of the tolerant cultivar was significantly lower than the susceptible cultivar seedling and at 72 hpi, the disease index in Bullet was 1.32 fold higher than IR8 (Fig. 1B).

Distinct genotypic differences between hosts affect their ability to defend against phytopathogens, leading to variation in disease progression rates and their overall severity (Pan *et al.* 2018). Some cultivars are more tolerant to certain pathogens compared to other cultivars of the same species of crops. Our findings can be correlated with previous studies from our laboratory on other pathosystems. Comparison of disease severity between two rice varieties infected with *Rhizoctonia solani* (Basu *et al.* 2016) and two tomato varieties infected with *Alternaria solani* (Ray *et al.* 2015) demonstrated significantly higher disease index in the susceptible hosts.

Assessment of cell necrosis in cotyledonary leaves of tolerant and susceptible chilli seedlings caused by C. capsici infection

Staining infected cotyledonary leaves with trypan blue revealed blue patches corresponding to the areas of cell necrosis. Tolerant IR8 showed significantly less necrosis than susceptible Bullet in response to *C. capsici* infection (Fig. 2A). In IR8, the cell necrosis was quantified to be almost 30% less than that in Bullet at 72 hpi (Fig. 2B).
Necrosis of host cell is a classic symptom of fungal infection (Doehlemann *et al.* 2017). Ability to induce cell necrosis in host plants have been reported when infected by *Magnaporthe oryzae* (Wengler *et al.* 2025), *R. solani* (Koley *et al.* 2021), *C. acutatum* (Ali *et al.* 2020), *C. gloeosporioides* (Wang *et al.* 2016) and *A. solani* (Ray *et al.* 2015). Such fungi lyse the cell wall of host using an array of cell wall degrading enzymes (CWDEs) like pectinase, cutinase and cellulase (Ma *et al.* 2019; Wang *et al.* 2016). This penetration disrupts host cell homeostasis, which is closely followed by cellular leakage and cell death (Arya *et al.* 2022). Such hemibiotrophic or necrotrophic fungi derive essential nutrients from the necrotising tissues and proliferate rapidly within the host (Lutzoni *et al.* 2018). Greater rates of cell necrosis are therefore extremely beneficial for fungal growth within the host (Ma *et al.* 2019). In the present study, we report relatively higher cell necrosis in the cotyledonary leaf of the Bullet chilli cultivar induced by *C. capsici* that can be correlated with the aforementioned higher disease index compared to IR8. Weaker immunity of Bullet cultivar was beneficial for *C. capsici* as the latter induced greater cell necrosis and derived nutrients therefrom. Our findings are consistent with observations on other pathosystems, such as sesame-*M. phaseolina* (Chowdhury *et al.* 2017) and tomato-*A. solani* (Ray *et al.* 2015).

Comparison of hyphal density of C. capsici on the cotyledonary leaf surface of tolerant and susceptible chilli cultivars in the early establishment phase

Growth of *C. capsici* hyphae on 7-day-old cotyledonary leaf was observed under a compound microscope during the early establishment phase of the pathogen (24, 48 and 72 hpi). Profuse hyphal growth was observed in susceptible Bullet, whereas tolerant IR8 significantly restricted hyphal proliferation (Fig. 3A). At 24 hpi the hyphal density in Bullet was almost 2.5 folds compared to IR8 but the gap was less at 72 hpi where hyphal density was approximately 1.17 fold higher in Bullet in comparison to IR8 (Fig. 3B).

Fungal behaviour is subjective, depending largely on host tolerance level, as demonstrated by previous works from our laboratory (Koley *et al.* 2021; Chowdhury *et al.* 2017). We found *C. capsici* hyphae growing in more abundance on the susceptible host surface than the tolerant surface, which corroborates observations made by Basu *et al.* in 2016 in rice-*R. solani* interactions. Variations in the amount of fungal biomass in hosts of tolerant and susceptible genotypes may be explained by the differences

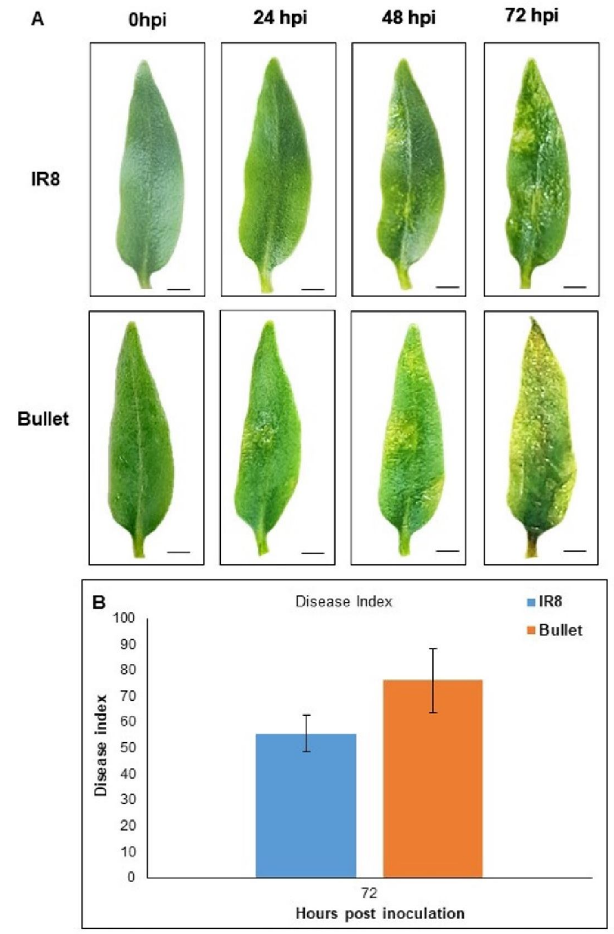

Fig. 1: Assay of disease progression and disease index in seedling blight caused by *Colletotrichum capsici* in cotyledonary leaf of tolerant and susceptible chilli cultivars. (A) Formation of necrotic lesions in infected cotyledonary leaves of tolerant IR8 and susceptible Bullet chilli. (B) Disease index based on disease progression assay. Bars represent mean ± SD of three independent experiments with three replicates. Scale=1mm.

in their leaf surface chemistry. Previous research in this regard revealed that surface structures, diffusible anti-fungal secondary metabolites on the leaf surface and other host defense factors are some of the crucial parameters that determine fungal growth rates in different crop cultivars (Arya *et al.* 2021).

CONCLUSION

The present work demonstrates that the chilli cultivar Bullet is more susceptible compared to IR8 to the pathogen *C. capsici* causing seedling blight. The disease index was found to be nearly 30% more in Bullet compared to IR8, showing significantly larger lesions on the cotyledonary leaves. The tolerant host IR8 is better able to restrict pathogen proliferation on the surface of

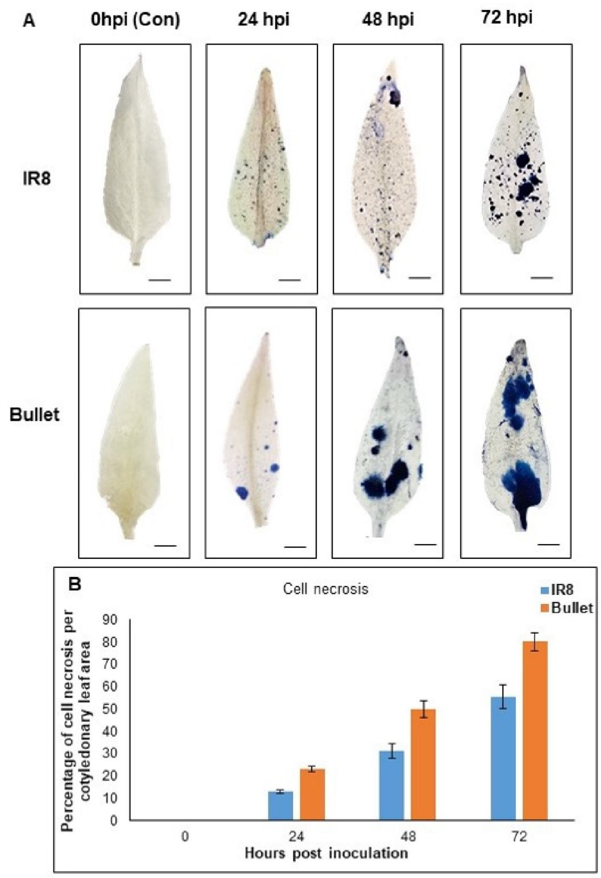

Fig. 2: Assay of cell necrosis in cotyledonary leaves of tolerant and susceptible chilli cultivars infected with *C. capsici* during seedling blight. (A) Visualisation of areas with cell necrosis appearing as blue patches after staining with trypan blue in IR8 and Bullet chilli cultivars. (B) Comparison of percentage of cell necrosis per cotyledonary leaf area. Bars represent mean ± SD of three independent experiments with three replicates. Scale=1mm.

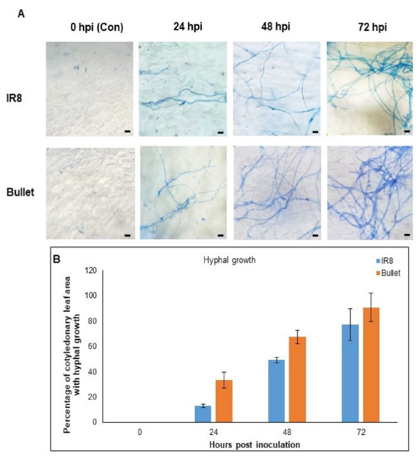

Fig.3: Comparison of hyphal proliferation of *C. capsici* on cotyledonary leaf surface of IR8 and Bullet in the early establishment phase. (A) Lesser hyphal density on IR8 in contrast to relatively denser hyphal growth on Bullet at 24, 48 and 72 hours post inoculation. (B) Comparison of percentage of hyphal density of *C. capsici* on infected cotyledonary leaves. Bars represent mean ± SD of three independent experiments with three replicates. Scale=10μm.

the cotyledonary leaves compared to the susceptible variety Bullet and exhibited lower disease severity than the susceptible host, during early establishment phase of the disease. This study thus provides useful insights into the dynamics of disease development and establishment of the pathogen in early phases of infection in chilli seedlings.

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DECLARATION

Conflict of interest. Authors declare no conflict of interest.

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