ANTHRACNOSE OF CHILLI CAUSED BY COLLETOTRICHUM CAPSICI

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ABSTRACT

Anthracnose disease is one of the major economic constraints to chilli production worldwide, especially in tropical and subtropical regions. Accurate taxonomic information is necessary for effective disease control management. In the Colletotrichum patho-system, different Colletotrichum species can be associated with anthracnose of the same host. Little information is known concerning the interactions of the species associated with the chilli anthracnose although several Colletotrichum species have been reported as causal agents of chilli anthracnose disease worldwide. The ambiguous taxonomic status of Colletotrichum species has resulted in inaccurate identification which may cause practical problems in plant breeding and disease management. Although the management and control of anthracnose disease are still being extensively researched, commercial cultivars of Capsicum annuum that are resistant to the pathogens that cause chilli anthracnose have not yet been developed. This paper reviews the causal agents of chilli anthracnose, the disease cycle, conventional methods in identification of the pathogen and molecular approaches that have been used for the identification of Colletotrichum species. Pathogenetic variation and population structure of the causal agents of chilli anthracnose along with the current taxonomic status of Colletotrichum species are discussed. Future developments leading to the disease management strategies are suggested.

INTRODUCTION

Introduction

Plants survive with a variety of strategies to defend themselves against pathogen. Plants are major target of microbe seeking a source of nutrition a complex array of interactions between plants and microbes evolved that reflects both the nutrient a acquisition strategies of microbes and defense strategies of plants. Part of plant defense strategies includes an active offense against invading microbes using an array of antimicrobial gene products within the context of the overall plant microbe interaction, we attempt here to emphasize the role of antimicrobial proteins and peptides in plant defense.

The majority of plant microbe encounters don't results in disease. Preformed factors including constitutively expressed waxes cell wall components., antimicrobial peptides, proteins and non proteinaceous secondary metabolites that deter invasion have been proposed

to contribute significantly to the host range of pathogens. The importance of preformed defenses has been inferred from the observation that plants can be rendered susceptible by a deficiency in the production of these secondary metabolites or by the abilities of pathogens to degrade them.

Inducible Defenses Require detection of pathogens by lost surveillance. The sentinels

Plants defense responses are induced by microbial products in non host and host resistance plant defense systems are also in induced by microbial products in compatible and incompatible plant microbe interaction.

Specific host- pathogen interaction models describing induced defense responses in plants have been greatly influenced in recent years gene for gene interaction originally reported by floor (1956) in these specific host pathogen interactions resistance to a particular pathogen is conditional on the presence of specific Avr (avirulence)gene of the pathogen and specific (R) resistance gene in plant host wide spread interest in gone for gene interaction resulted for recognizing the resistance was usually controlled by single dominant genes making genetic analysis very detectable.

Antimicrobial proteins and peptides are important components of innate immunity

A common feature of the innate immune system of taxonomically diverse organism i.e. plant is the ability to marshal the accumulation of antimicrobial proteins and peptides in response to an invasive challenge by foreign organism more than 500 different antimicrobial proteins and peptides encoded within the genomes of many organism including plants. Genes encoding these protein peptides have an important role in host-pathogen interaction. Much less certain is specific function of each in individual pathogen-plant interaction.

Materials and Methods

Plant material and Collection of seeds

The plant material was taken chilli plants .The collection of seeds were done from Uttaranchal Technical University

The plant materials were obtained by germinating the seeds of chilly. The healthy and non diseased seeds were taken.

Sterilization of Seeds

The seeds were sterilized with sterile distilled water and then kept in 0.1% $Hgcl_2$ for 30 seconds washed repeatedly with sterile distilled water and kept in petriplates layered with moist cotton and also sterilized seeds were sown on soil in pots.

DETECTION OF PATHOGEN FROM SEEDS

Colletotrichum capsici is both internally and externally seed borne such effected seed and infected seeding act as primary sources of inoculum.

Blotter methods

Seed borne *colletotrichum capsci* can be detected by subjecting chilli seed samples to the standard blotter test. Individual samples of ten seeds are plated in petriplates.. Plates were placed in petriplates. Plates were placed on three layered moist blotter paper plates. On the fifth day of the seeds were observed for the presence of fungal growth with the help of microscope. *Colletotrichum capsici is* identified based on morphological characters of acervuli and conidia growing on the blotter paper.

Casual Organism

Colletotrichum capsici Scientific

classification Kingdom

Fungi Phylum

Ascomycota

Class Sordariomycetes Order

Glomerellalels Family

Glomerellaceae Genus

Colletotrichum Species

capsici

Colletotrichum is one of the most important plant pathogens world wide causing the economically important disease anthracnose in a wide range of hosts including cereals, legumes, vegetables, perennial crops and tree fruit. Among these hosts, chilli, an important economic crop worldwide is severely infected by anthracnose which may cause yield losses of up to 50%.

In the *colletotrichum* pathosystem, different *colletotrichum* species can be associated anthracnose of the same host. Different species cause disease of different organs of the chilli plant.

For example – *C. acutatum* and *C. gloeosporides* infect chilli fruits at all developmental stages but usually not leaves or stems. Stems or leaves are damaged

by *C. coccodes* and *C. dematium*. Leaf anthracnose of chilli seeding caused by *C.coccodes* was first reported in chilli growing in a field in chungam province of Korea in 1988. Different *colletotrichum* species may also play an important role in different disease of mature stages of chilli fruit.

For example- *C. capsici* is wide spread in red chilli fruits whereas *C.acutatum , C. gloeosporioides* have been reported to be more prevalent both young and mature green fruits.

Pathogen isolation, culture, maintenance

The anthracnose disease causing Pathogen *colletotrichum capsici* was isolated from a diseased seed of chilly which was provided by Department of Plant pathology, UTU University, Dehradun.

For isolation, culture and maintenance of desired pathogen, Potato Dextrose Agar (PDA) media was used and the media was prepared as per as the indication given on the containers, in aseptic conditions with sterilized glass waves & inoculation loops.

POTATO DEXTROSE AGAR Medium

Component	Amount
Peeled potato	250gm
Glucose	20.00gm
Agar	15gm
Distilled water	1000ml
pH	6.0-6.6

The seeds were first washed with sterile distilled water and then surface sterilized with 0.1% $Hgdl_2$ for 30 seconds followed by washing sterile distilled water in three times. Then the

leaves were placed on the PDA plated with aseptic condition in laminar airflow and incubated at 28^0C for 48 hours. After 48 hours of incubation the plates were observed to be containing the colonies of fungal mycelia on the PDA plates showing the typical colony morphology & growth symmetry. All the process of isolation, culture and maintenance were done in aseptic condition.

RESULT AND DISCUSSION

Identification

Colletotrichum sp. was identified based on reports of Kulshrestha *et al*. (1976), Ahmed (1986) and Desai and Prasad (1955).

Colony character

On potato sucrose agar the fungal colony was fluffy white, thick with branched mycelium later becoming blackish brown.

(i) Acervuli

Acervuli were disc shaped, waxy, sub epidermal, typically with dark needle like septate setae.

(ii) Setae

Setae were dark brown to black in colour erect and tapering towards the apex.

(iii) Conidia

Conidia were sickle shaped, single celled, hyline, smooth walled with a central oil globule.

Symptomatology

Anthracnose diseases occur on leaves, stems, and both pre- and post-harvest fruits. The fungus infects the leaves, branches, green as well as ripe fruit. On the leaves, small circular spots appear. Severely infected leaves fall off leading to defoliation. The infection of growing tips leads necrosis of branches which progress back ward on the diseased branches, a large no. of dots represent the presence of acervuli. The die back symptom is severe and it may kill the whole plant. (Plate-3)

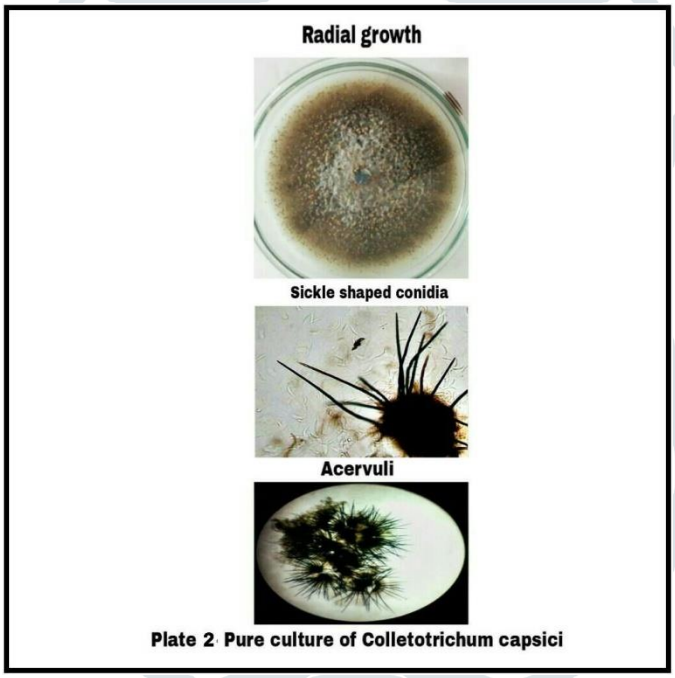

Plate 2 Pure culture of Colletotrichum capsici

a. On leaves

The symptoms were produced in the form of numerous necrotic spots on leaves. These spots are brown later they become dark brown surrounded by a dark margin. The spots at first are small and dark or water soaked. As they enlarge, they become irregular in shape, variable in size and give a scorched appearance. The diseased portion become papery and drop off from the leaves producing shot holes. The infected area spreads to any direction giving irregular shape. Defoliation occurred at later stages of infection. The infected foliage becomes dark brown to black particularly during the period of continuous rains and cloudy weather.

b. On stem

On stem the fungus produces dark brown cankerous spots or lesions. Later, the fungus develops acervuli. These structures are clearly evident by naked eye. The twigs are water soaked to brown, becoming grayish white or straw coloured, in advance stage of the disease. Large numbers of acervuli are found scattered all over the necrotic surface of the affected twigs. The necrotic area is separated from the healthy area by dark brown or black band.

c. On fruit

Typical fruit symptoms were found to be circular or angular sunken lesions, with concentric rings of acervuli that are wet and produce pink to orange conidial masses. Under severe disease pressure, lesions coalesced. Conidial masses sometimes also occur scattered or in concentric rings on the lesions.

: Pathogenicity test of collected isolated anthracnose pathogen

Colletotrichum capsici.

S.No	Inoculated site	Disease incidence (%)*
1.	Leaf	43.60

2.	Ripe fruit	66.50
3.	Green fruit	82.10
SEm±		19.065
C. D. at 5%		5.404

* Mean of three replications.

. Evaluation of different media on growth of *Colletotrichum capsici.*

Name of Medium	Radial growth of fungal mycelium in (mm) after days	
	5 DAI	**7 DAI**
Corn meal agar media	42.8	56.3
Starch agar media	37.8	51.5
Nutrient agar media	33.5	44.1
Ashby agar media	29.0	44.8
Czapak's-Dox agar media	44.8	62.0
Pikovaskara agar media	33.1	51.0
Potato dextrose agar media	46.8	62.6
SE(m) ±	**0.856**	**1.112**
C.D. at 5 %	**2.622**	**3.404**

SUMMARY AND CONCLUSION

Colletotrichum spp. are highly specialized pathogens that cause anthracnose disease in a wide range of commercially important fruits and vegetables. Anthracnose or ripe fruit rot caused by *Colletotrichum capsici* (Sydow), Butler and Bisby is a wide spread problem limiting the cultivation and seed production throughout the major chilli growing regions of India. The disease is both seed borne and air borne and affects seed germination and plant vigor to a greater extent (Perane and Joi, 1988, Mesta, 1996; Asalmol *et al.*, 2001). Losses due to anthracnose of chilli accounts to a range of 66-84% (Thind and Jhooty, 1985).

Accurate identification, characterization, infection processes, disease cycle, pathogenicity and genetic diversity of *C. capsici* are essential for the effective disease management and breeding of resistant cultivars. Extensive research, to identify and characterize *C. capsici* accurately, and combat the disease by various methods of control measures have been carried out at both national and international laboratories. Therefore, an attempt has been made in the present work to find out the status of seed borne incidence of anthracnose of chilli across Karnataka, by screening different chilli cultivars, evaluating the site of infection and accurate detection of pathogen including its characterization through morphological, biochemical and molecular techniques. In this direction, the present research work was undertaken with the following objectives:

1. Isolation, identification and molecular characterization of *C. capsici*.

2. Host-pathogen interaction studies.

3. Development of suitable management strategies to control anthracnose disease in chilli.

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