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Detection of chilli anthracnose seed borne inoculum by Scanning Electron Microscopy

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Anthracnose of chilli caused by *Colletotrichum capsici* (Sydow) Butler and Bisby is an economically important disease of chilli affecting both fruit and seed quality. In chilli seed samples collected from different chilli growing districts of Tamil Nadu *Colletotrichum capsici* was the most predominant fungus encountered (78.89 %).SEM analysis of the seed samples revealed the seed borne nature of *C. capsici.* The presence of fungal mycelium along with conidia and structures such as acervuli and the acervuli on the endosperm emerged just beneath the endosperm and testa.

Key words: Chilli, anthracnose, Scanning Electron Microscopy, detection

INTRODUCTION

Chilli (*Capsicum annum*) is the fourth most important vegetable crops in the world and first in Asia, with world production approximately 122.34 million tonnes of fresh chilli and 2.8 tonnes of dry chilli in 2010 (Indian Horticultural Database). Chilli is a very remunerative spice crop of the Indian subcontinent (Sharma *et al.,* 2005) and occupies an area of about 0.81 million ha (Suthin Raj and Christopher, 2009) which accounts for 25% of the world production (Chandra Nayaka *et al.*, 2009). In Tamil Nadu, chilli is cultivated on 49.0 thousand hectares with 31.8 thousand tonnes of production. Chilli not only meets domestic consumption but also helps in earning foreign exchange. Unlike other chilli-producing countries, about 90 per cent of the production (estimated over 10 lakh tonnes of chilli) in India is absorbed by the huge domestic market. India exports only about 1.5 lakh tonnes of chilli out of the total production of 7.5 lakh tonnes (Anon, 2008).

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Chilli is attacked by several fungal, bacterial and viral diseases among them, anthracnose and powdery mildew are found to be the major diseases incurring heavy losses, if not cared. Anthracnose (fruit rot and die back) caused by *Colletotrichum capsici* (Syd. Butler and Bisby) is prevalent throughout the chilli growing areas of India. (Jeyalakshmi, 1996). It is a wide spread problem limiting the profitable 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 vigour to a greater extent (Perane and Joi, 1988, Mesta, 1996, Asalmol *et al.,* 2001). Bansal and Grover (1969) report losses due to anthracnose ranged from 10-25 per cent in 1966 and 20-60 per cent in 1967. Thind and Jhooty (1985) report losses due to anthracnose of chilli varied between 66-84 per cent.

Previously many works have been done on seed borne nature of *C. gloeosporioides* and *C. truncatum* on soybean seeds by blotter paper test and potato dextrose agar to find infection and severity by pathogen and several studies have been carried indicating the presence of mycelium

in the inner and outerlayer of chilli seeds by histopathology. The present study has been undertaken to study the seed borne nature of *C.capsici* and location of pathogen on the internal parts of the seed which are giving higher economic losses to chilli crops.

MATERIALS AND METHODS

Survey for the incidence of anthracnose of chilli in Tamil Nadu

An intensive survey was conducted to assess the severity of the anthracnose disease and to collect different isolates of *Colletotrichum capsici* causing anthracnose in chilli. The diseased fruits were collected from the different districts of Tamil Nadu. Twenty five fruits were randomly selected and Per cent Disease Index was worked out

Detection of seed borne infection of C.capsici

Ten seed samples were collected from Coimbatore districts during survey and used in this study. The seed borne infection was assessed by Standard blotter method (ISTA, 1993). The seeds were incubated in plastic Petri dishes containing three layers of Whatman No.1 filter paper moistened with sterile water. The chilli seeds were placed at equidistance in each Petri plate @ 20 seeds per plate and were incubated at 28 ± 2°C under alternate cycles of 12 h NUV light and 12 h darkness for 7 days. For each treatment four replications were maintained and 100 seeds formed one replication. Then the seeds were examined on eighth day under scanning electron microscope.

Sample Preparation for SEM

All samples of an appropriate size to fit in the specimen chamber and are mounted rigidly on a specimen holder called a specimen stub. For taking images of samples, 2 mm seed sample was placed on the carbon conducting tape. Then the tap was mounted on sample stage and the images were taken in 24,000X magnification and 20 KV using FEI SEM Model "QUANTA 250" (Singh and Khare, 2004)

RESULTS AND DISCUSSION

Field surveys were conducted in 12 districts of Tamil Nadu. A total of 20 fruit sample were collected from the above districts. The Per cent Disease Index was worked out for each sample and shown in (Table 1) The results revealed that the PDI ranged from 8.88 – 78.89. Angadi (1999) carried out survey for the incidence of anthracnose of chilli caused by *C. capsici* in Raichur, Dharwad and Gadag districts. The disease was more prevalent in Raichur district than in Dharwad and Gadag districts. Sanathkumar (1999) during his survey in and around Bangalore district observed that, the chilli varieties Chikkaballapur, Gauribidanur, Byadagi Kaddi and Pant C-2 showed anthracnose infection of 25, 35, 30 and 25 per cent, respectively

The seed samples collected during survey were subjected to Standard Blotter tests to document the fungi present in the seeds. The results indicated that *C.capsici* was predominantly present, followed by *C. gloeosporioides*. (Table 2) the seed borne nature of these pathogens was also reported by several workers in *C.capsici* (Sariah and Nik, 1988; Sariah, 1992) and *C.gloeosporioides* (Ammari *et al.*, 1997).

In mixed fungal infections of seeds, fungal hyphae compete for colonization, showing antagonistic or synergistic behavior to each other which affect the extent of invasion in the seed tissues (Kunwar *et al.*, 1985; Sariah, 1992; Singh and Mathur, 2004). Mesta (1996) also studied the seed mycoflora of chilli by standard blotter method and reported the presence of *C. capsici* and species of *Alternaria, Cercospora, Fusarium, Curvularia*. Solanke *et al.* (2001) reported the presence of *C. capsici*, *Fusaruim moniliformae*, *Aspergillus niger*, *Aspergillus flavus*, *Alternaria alternata* and *Curvularia lunata* from chilli seed samples.

In the present study two species of *Alternaria* and one species each of *Fusarium* , *Curvularia, Penicillium* and *Aspergillus* were recorded and this is in accordance with the above findings. Bhale *et al.* (2000) and Asalmol *et al.*, (2001) also reported standard blotter method is better than agar method in detecting the seed borne fungi on chilli. Singh and Khare (2004) reported standard blotter method as best for the detection of *C. dematium* from chilli seeds compared to other methods like visual inspection of dry seeds, seeds washing test, agar plate method with PDA, agar plate with malt yeast and 2, 4-D blotter method.

In the present study, seed lots were also used for Scanning Electron Microscopy studies. The images from Fig. 1 exhibited the collapse of epider-

Table 1 : Survey for the incidence of chilli anthracnose disease in different districts of Tamil Nadu

Sample No.	District	Location	Per cent disease index (PDI)
C1	Madurai	Chittampatti	78.89 (62.58) [a]
C2	Virudhunagar	Arupukottai	51.76 (45.97) [b]
C3	Tirunelveli	Narnapuram	26.65 (31.05) [f]
C4	Salem	Karupur	40.00 (39.23) [c]
C5	Virudhunagar	Sankarankovil	38.86 (38.53) [d]
C6	Virudunagar	Rajapalayam	53.12 (46.78) [b]
C7	Tuticorin	Kovilpatti	28.84 (32.46) [f]
C8	Salem	Omalur	64.41 (53.37) [b]
C9	Erode	Sakthi	22.89 (28.52) [h]
C10	Coimbatore	Perur	76.53 (61.00) [a]
C11	Nagapattinam	Mayiladuthurai	45.00 (42.13) [c]
C12	Pondicherry	Karaikal	39.42 (38.88) [d]
C13	Coimbatore	Othakalmandabam	46.70 (43.11) [c]
C14	Thirunelveli	Menachipuram	25.55 (30.33) [g]
C15	Dindugal	Palani	50.00 (45.00) [b]
C16	Coimbatore	Mathampatti	39.49 (38.65) [bc]
C17	Coimbatore	TNAU	32.73 (34.88) [e]
C18	Nagapattinam	Nagore	21.11 (27.27) [g]
C19	Coimbatore	Lakshmipuram	48.25 (43.97) [c]
C 20	Nagapattinam	Nagore	8.88 (17.26) [f]

*Mean of three replications
Values in parentheses are arcsine-transformed values In a column, means followed by a common letter are not significantly different at the 5% level by DMRT

mal cells and disruption of epidermis. In addition, it revealed the presence of structures such as acervuli, conidia and emergence of fungal mycelia from testa. The presence of acervuli on endosperm just beneath the testa was also seen. The present findings are in line with the works of the others. During maturation , the acervulus from the seeds ruptured

Table 2 : Seed borne microflora of chilli seeds

Fungus	Per cent seed infection*
Colletotrichum capsici	30.98[a] (34.44)
C. gloeosporioides	20.78[b] (27.12)
Fusarium sp	5.75[f] (15.06)
Alternaria alternata	7.33[d] (16.78)
A. capsici	2.67[h] (11.04)
Curvularia sp	4.00[g] (14.18)
Helminthosporium sp	1.67[i] (7.42)
Penicillium sp	6.78 [e] (15.00)
Aspergillus sp	10.14[c] (18.53)

*Values are the mean of four replications.
The values in parentheses are arcsine transformation. Means followed by a common letter are not significantly different at the 5 % level by DMRT

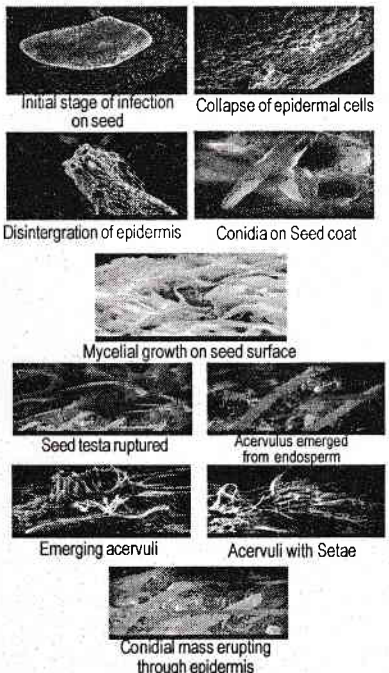

Fig. 1 : Scanning electron microscopy of anthracnose infected chilli seeds

and conidia were dispersed in the form of ooze and serve as a primary inoculums source for the spread of the disease (Sariah and Nik, 1988). The inter and intra cellular dark mature mycelia of *C. truncatum* were found in all layers of the seed coat, cotyledon and embryo and could remain dormant as a latent infection for a prolonged period without visible symptoms (Sinclair, 1991).

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