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Evaluation of screening methods for anthracnose disease in chilli

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ABSTRACT: Experiments were conducted to evaluate the existing methods of screening for resistance to chilli anthracnose. In fruit puncture method, conidia of *Colletotrichum capsici* germinated and differentiated into appresoria on fruit surfaces in both green and ripened red fruits. However, the lesion area was more in green fruits compared to red fruits, which is not common under field conditions. Further, the green fruits which were tolerant towards anthracnose were certainly tolerant at ripened red stage, whereas if fruits were found susceptible at green stage, ripened red fruits were found to be either tolerant or susceptible. *Glomeralla*, which is sexual stage of *Colletotrichum* sp. is known to produce more appresoria on the surface of immature fruits than on fully ripened fruits. In contrast, ripened red fruits were found to be more susceptible with large lesion areas under field conditions, while green fruits were showing initial symptoms only at the colour changing stage. Moreover, the antifungal activity may become inactive after the detachment of the fruit from the live plant system. The percentage incidence of anthracnose affected fruits under field conditions employing the spray inoculation is found to be the ideal method to identify resistant hybrids. Further, while evaluating disease intensity under field conditions, it was felt necessary to note down the phenotypic characters, which perhaps correlate with the disease incidence in the field. The anthracnose susceptible chilli hybrids exhibited more fruit number, more fruit weight and less fruit thickness as compared to the tolerant hybrids. Our findings indicate some useful traits in chilli that have been linked with resistance to anthracnose and can be considered as basis for breeding resistant varieties or perhaps used as markers in Marker Assisted Selection (MAS) breeding of chilli varieties.

Key words: Chilli, anthracnose, *Colletotrichum capsici*, screening

INTRODUCTION

Chilli is an important spice and vegetable crop of India. India accounts for 1.2 million tonnes of production annually, and is the largest producer in terms of international trade, exporting 25 per cent of its total production (FAO, 2010). However, the average productivity of chilli is low (1 ton/ha) as compared to China, Taiwan, and Mexico where it yields 3 tons/ha of dry chilli (Peter, 1998). The main reason for the low productivity in India is the cultivation of open pollinated varieties which do not have the genetic capacity to break the yield barriers (Kaur *et al*, 2011). Moreover, the local cultivars are prone to various diseases which account for significant reduction in productivity. Chilli anthracnose, the most important fungal disease, drastically reduces yield, deteriorates the quality of fruit, and hence gives low returns to the farmers. Thus, it is one of the major pests of economic importance to chilli production in India and it has been reported that pre-harvest and post harvest losses account for more than 50 percent in severe cases (Pakdeevaraporn *et al.*, 2005). Many studies have indicated that disease management practices are often inadequate to control the diseases. Moreover, pesticide residue has become the major constraint in an approach to meet the stringent requirements of the importing countries. Hence, most economical way to minimize the crop losses is to cultivate resistant varieties/hybrids. However, there is no reliable method to identify anthracnose resistant varieties/hybrids of chilli. Currently, resistance of chilli variety/hybrid against anthracnose pathogen is measured employing fruit puncture method and spray inoculation methods, at lab and field conditions, respectively, without considering the mode of anthracnose development on fruit surfaces. Therefore, development of an effective ideal screening method is an important requirement to minimize crop losses by identifying the anthracnose resistant chilli varieties/ hybrids.

MATERIALS AND METHODS

Fully grown green fruits and matured ripened red fruits of four commercial chilli hybrids were chosen for this study. Further, a strong pathogenic fungus (*Colletotrichum capsici*) was preferred as a screening agent based on its previous record as the most predominant species in the major chilli growing states namely Karnataka and Andhra Pradesh in India (Ramachandran *et al.*, 2008).

Isolation of pathogen

Anthracnose affected chilli fruits were collected from the farmer's fields in the different locations in the Guntur District, Andhra Pradesh, India. Pathogen was isolated from anthracnose lesions of disease affected fruits cultured on Potato Dextrose Agar (PDA) at 25°C for 7 days. Ten ml of sterile distilled water was added to the culture to obtain conidial suspension by scraping the conidial mass from the plate using a sterile glass slide. The conidial suspension was filtered through double layered cheese cloth to remove mycelia and cultural debris. The conidial concentration is adjusted with sterile, distilled water using a haemocytometer.

Pathogenecity of all the isolates was tested by employing fruit puncture and spray inoculation methods under laboratory and field conditions, respectively.

Laboratory studies

The detached healthy green and ripened red fruits (30 each), harvested from the chilli plants were used for determining the resistance against the pathogen. Chilli fruits were surface sterilized in 1% sodium hypochlorite for 3 min, washed in sterile distilled water three times and placed on the sterile paper towel and then air dried. It has been reported that suitable concentration of conidia in inoculum drop is 5 x 10^5 conidia per ml of water (Rajapakse, 1998). Therefore suspension of conidia (5 x 10^5 conidia ml^{-1} water) of pathogen isolate was prepared and drops of conidial suspension was placed at proximal and distal ends on each fruit after puncturing the fruit (0.6 mm diameter × 1.2 mm depth) using a syringe needle. Sterile distilled water was used as control instead of the conidial suspension (or treatment of puncturing alone). After inoculation, the chilli fruits were placed in a plastic container (30 × 20 × 7 cm3) lined with four layers of paper towel moistened with sterile distilled water to produce a humid environment and later sealed with a plastic sheet. Symptoms on the chilli fruit were examined and evaluated after a week by measuring the area of the lesion for disease development. Data were analysed by ANOVA through Completely randomised design.

Field Studies

Chilli hybrids were screened under field conditions in a randomized block design with three replications separately for green and ripened red fruits. Mycelium of *C. capsici* was grounded in mortar and mixed with sterilized water to make up a liquid mixture. Spray inoculation was done by spraying suspension of conidia (5 x 10^5 conidia ml^{-1} water) evenly onto the chilli plants bearing green and red fruits separately in respective plots using hand sprayer. Un-inoculated plots served as controls. Then, canopy of all plants was watered from next day morning up to 1 week period by spraying water, two times per day at morning and evening using Knapsack sprayer to stimulate conidial differentiation on fruit surfaces. Generally, under dry conditions with low relative humidity when chilli is cultivated with irrigation, occurrence of anthracnose frequency is low. Therefore, water spraying from next day after artificial inoculation is an important requirement for conidial differentiation into infective structure on fruit surface. Finally, the number of anthracnose affected fruits was counted and the percentage incidences of anthracnose in each plot were then calculated. Data was analysed by ANOVA through randomised blocked design. The hybrids were rated as resistant and susceptible based on the range of lesion area (mm) or of the disease incidence (%) given in Table 1.

Table 1. Resistance/susceptibility with respect to range of the disease incidence

Range (%)	Category
0	Immune
0.1-5.0	Highly Resistant
5.1-10.0	Resistant
10.1-50.0	Tolerant
50.1-90.0	Susceptible
>90.0	Highly Susceptible

Phenotypical Characters

To see the correlation between the disease intensity under field conditions and the phenotypic characters, the following observations were made.

i. Plant Height (cm): At maturity, the height was measured form the ground level to the highest bud point.

ii. Branches/Plant: The number of primary branches was counted from five plants of each replication and each genotype at the time of last picking.

iii. Fruits/Plant: The fruits from each harvesting were added after last harvesting to get the total number of fruits per plant.

iv. Fruit Length (cm): Ten fruits from each treatment/genotype of each replication were used for measuring fruit length and width. The fruit length was measured with the help of a scale after removing the pedicel.

v. Fruit thickness (cm): Fruit thickness was measured with vernier caliper at the maximum width of the fruit.

vi. Fruit weight (kg): The average weight of fresh mature fruits harvested from five plants of each replication and genotype (all harvesting) was taken as total yield/ plant.

RESULTS AND DISCUSSION

Fruit Puncture Method under Laboratory Conditions

In fruit puncture method, 30 fruits each from four chilli hybrids were inoculated with conidial suspension with 8-10 day-old culture of the pathogen at proximal and distal ends. The lesion development was assessed as an area of lesion by taking average of proximal and distal ends of 30 fruits, after 10 days of inoculation. Significant difference in the lesion development was found among the fruits with different maturity stages. Lesion development occurred at both immature green stages as well as in matured red stage. However, the highest lesion development was observed on immature green fruits compared to red fruits, which is not common under field conditions. CHB-3 was showing the highest lesion area at green fruit stage (74.2 mm) and red fruit stage (16.6 mm) (Table 2). Further, the green fruits which were tolerant towards anthracnose viz., CHB-1 (17.5 mm) & CHB-4 (14.1 mm) were certainly resistant at ripened red stage (9.2 & 8.6 mm), whereas if fruits were found susceptible at green stage (CHB-2: 61.3 mm & CHB-3: 74.2 mm), ripened red fruits were found to be tolerant (CHB-2: 11.3 & CHB-3: 16.6). *Glomeralla*, which is sexual stage of *Colletotrichum* sp. is known to produce more appresoria on the surface of immature fruits than on fully ripened fruits (Adikaram *et al.*, 1983).

Pathogen behaviour on fruit surface

Colletotrichum species utilize diverse strategies for invading host tissues, which vary from intracellular hemibiotrophy to subcuticular intramural necrotrophy (Bailey and Jeger, 1992). *Colletotrichum* species produce a series of specialized infection structures such as germ tubes, appressoria, intracellular hyphae, and secondary necrotrophic hyphae (Perfect *et al.*, 1999). These pathogens infect plants by either intramural colonization of subcuticular tissues or through intracellular establishment. The preinfection stages of the both are very similar, in which conidia adhere to and germinate on the plant surface, producing germ tubes that form appressoria which in turn penetrate the cuticle directly (Bailey and Jeger, 1992). However, if wounding is readily available for the pathogen, penetration becomes easy and it affects the fruit whether it is green or red. Although the mechanisms developed by *Colletotrichum* species appear similar in pre-penetration events, there are differences between species in the later mechanisms such as spore adhesion, melanization and cutinization in penetration of the plant cuticle by the appressoria (Po Po Than *et al.*, 2008).

Spray inoculation method under field conditions

Variation in anthracnose development on chilli fruits at various maturity stages when artificial inoculation was practiced under field conditions was given in Table 2. Significantly higher percentages of anthracnose lesions were observed at red ripe stage over green fruits. Though inoculation was done at green fruit stage too, the expression of the symptom was observed at colour turning stage only. CHB-3 was showing high disease incidence of 67.8 percent and 21.3 percent at red fruit and green fruit stages, respectively. It was followed by CHB-2 (52.3%) at red fruit stage. However, its incidence at green fruit stage was low (17.8%).

Table 2. Areas of necrotic symptom (mm) on Chilli fruits under Laboratory Conditions and Disease Incidence (%) on Chilli Plants under Field Conditions

Hybrid	Lab (Area of lesion mm)		Field (Disease Incidence %)	
	Red	Green	Red	Green
CHB-1	9.2	17.5	41.3	18.9
CHB-2	11.3	61.3	52.3	17.8
CHB-3	16.6	74.2	67.8	21.3
CHB-4	8.6	14.1	30.1	8.2
CD (0.05)	2.5	14.0	8.2	4.0

Correlation Analysis

Correlation analysis of disease occurrence was done for the red fruits and green fruits under laboratory and field conditions employing fruit puncture method and spray inoculation method, respectively. The results indicated that there is a positive correlation between disease occurrences on red fruits observed under laboratory and field conditions for different methods of inoculation (Table 3). This indicates that preliminary screening may be done on red fruits under laboratory conditions using fruit puncture method for large scale germplasm.

Table 3. Correlation of disease occurrence for red fruits and green fruits under laboratory and field conditions

Parameter	Lab Red	Lab Green	Field Red	Field Green
Lab Red	1			
Lab Green	0.894	1		
Field Red	0.954*	0.941	1	
Field Green	0.681	0.671	0.84	1

* Significant at 5% level

In contrast to the laboratory findings, ripened red fruits were found to be more susceptible with large lesion areas under field conditions, while green fruits were showing initial symptoms only at the colour changing stage. These contradictory findings may be attributed to the failure of detached fruit puncture method in giving the accurate results.

The reason may be due to the development of sclerenchyma like cells with thickened cell walls were proliferated around the wounding sites, which were partially dissolved by delayed wounding inoculation, probably leading to some disease development (Sang Gyu Kim *et al.*, 2008). Moreover, the antifungal activity may become inactive after the detachment of the fruit from the live plant system. Hence, the resistance of the hybrids are needed to be confirmed under field conditions employing spray inoculation method.

Further, different *Colletotrichum* species may also play an important role in different diseases of mature stages of chilli fruit as well. For example, *C. capsici* is widespread in red chilli fruits, whereas *C. acutatum* and *C. gloeosporioides* have been reported to be more prevalent on both young and mature green fruits (Hong and Hwang, 1998; Kim *et al.*, 1999) under natural conditions. Hence, screening hybrids at immature stage with *C. capsici* is not recommended whose infection is very uncommon on green fruits under natural field conditions.

Pathogen infection on fruits

Initial infection by *Colletotrichum* species involves a series of processes including the attachment of conidia to plant surfaces, germination of conidia, production of adhesive appressoria, penetration of plant epidermis, growth and colonization of plant tissue and production of acervuli and sporulation (Bailey and Jeger, 1992; Prusky *et al.*, 2000). Anthracnose is mainly a problem on mature fruits, causing both pre- and post-harvest fruit decay resulting severe economic losses (Hadden and Black, 1989; Bosland and Votava, 2003). Appressoria that formed on immature fruits may remain quiescent until the fruits mature or ripen. Many post-harvest diseases of fruit exhibit the phenomenon of quiescence in which symptoms do not develop until the fruit ripens. *Colletotrichum* species are the most important pathogens that cause latent infection (Jeffries *et al.*, 1990). Appressoria are known to form adhesive disks that adhere to plant surfaces and remain latent until physiological changes occur in fruits (Bailey and Jeger, 1992).

Table 4. Means of Phenotypic Characters and Disease Incidence in Chilli Hybrids

Hybrid	DI (%)	Plant height (cm)	Branches/ plant	Fruit Length (cm)	Fruit thickness (cm)	Fruit no./ plant	Fruit wt./ plant (Kg)
CHB-1	41.3	98	7.9	7.2	1.5	163	1.1
CHB-2	52.3	125	9.2	13.6	0.8	180	1.26
CHB-3	67.8	135	8.9	8.8	0.5	240	1.45
CHB-4	30.1	150	7.2	9.2	1.9	110	1.05
CD (0.05)	8.2	26.337	NS	3.903	0.896	53.25	NS

Table 5. Correlation between disease intensity (%) observed under field conditions and the phenotypic characters

Parameters	DI (%)	Plant Height (Cm)	Branches /Plant	Fruit Length (Cm)	Fruit Thickness (Cm)	Fruit No./ Plant	Fruit Weight/ Plant (Gm)
DI (%)	1						
Plant Height (Cm)	-0.064	1					
Branches/Plant	0.859	-0.177	1				
Fruit Length (Cm)	0.186	0.256	0.598	1			
Fruit Thickness (Cm)	-0.975*	0.11	-0.951	-0.373	1		
Fruit No./Plant	0.987*	-0.181	0.81	0.053	-0.946	1	
Fruit Weight/Plant (Gm)	0.984*	0.112	0.821	0.225	-0.95	0.952	1

*Significant at 5% level of significance

Appressoria that formed on immature fruits may remain quiescent until ontogenic changes occur in the fruits (Prusky and Plumbley, 1992).

Phenotypic Characters

While evaluating disease intensity under field conditions, it was felt necessary to note down the phenotypic characters, which perhaps correlate with the disease incidence in the field. Hence the 7 phenotypic parameters were noted down to correlate and analyse with the disease intensity.

There were significant amount of variations in phenotypic characters present in chilli hybrids for resistance to anthracnose. Analysis of seven phenotypic characters revealed that the anthracnose susceptible chilli hybrids exhibited more fruit number, more fruit weight and less fruit thickness as compared to the tolerant hybrids (Table 4).

Among the hybrids tested, the number of branches, plant height and fruit length had showed a wide range of genetic variability in chilli hybrids in the present study. However, analysis on fruit thickness of chilli hybrids revealed that CHB-3 (0.5 cm) followed by CHB-2 (0.8 cm) showed high disease incidence of 67.8 and 52.3%, respectively (Table 4). Further, the hybrids with more fruit numbers per plant such as CHB-3 (240) and CHB-2 (180) expressed more disease intensity of 67.8 & 52.3 respectively (Table 4). CHB-4 bearing less number of fruits (110) has shown less DI of (30.1%) and this can be explained on the basis that pathogen dissemination in the field is more efficient in prolific genotypes. Kaur *et al*, 2011, also reported that genotypes: EC 21667 and IC 11670 with more number of fruits per plant showed more fruit rot symptoms. There was a significant and positive correlation between fruit weight and fruit rot (Table 5). The minimum fruit weight per plant was observed in tolerant hybrids: CHB-4 (1.05) and CHB-1 (1.1) (Table 4). However, the phenotypic characters such as plant height, branches per plant and fruit length had not shown any correlation with disease intensity. Hybrids with less fruit thickness had more disease intensity, whereas number of fruits and fruit weight per plant had showed a positive correlation with disease intensity (Table 5).

Our experimental findings indicate that some traits in chilli have link with resistance to anthracnose and can be considered as a basis for resistance in breeding resistant varieties or perhaps used as markers in MAS breeding of chilli varieties. However, these results are needed to be strengthened by increasing the number of hybrids to be tested. Although the hybrids have been subjected to different inoculation methods under different conditions in relation to phenotypic parameters, there remain many gaps in the knowledge of the disease process and understanding of the complex relationships between the species involved. Hence screening against multiple species is also suggested in breeding studies for resistance.

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