

Cross Infectivity Studies of *Colletotrichum* spp., causing Anthracnose in Different Beans

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ABSTRACT

Colletotrichum isolates from naturally infected beans were isolated and tested for cross-infective potential in different beans. All isolates readily infected their native hosts resulting in anthracnose/leaf spots. Cross infectivity revealed that *Colletotrichum* isolates CI 5 from horsegram and CI 4 from cowpea were most virulent and infect in all the test bean crops, except pole type beans like yard longbean, wingedbean and swordbean. Among the test plants, cowpea, dolichosbean, French bean, urdbean and horsegram were susceptible to most of the *Colletotrichum* isolates, while clusterbean expressed different responses to different *Colletotrichum* isolates. Pillipesara was highly prone to anthracnose disease incidence (55.50%) followed by cowpea (53.50%) and low disease incidence (37.50%) was recorded in horsegram, when inoculated with their respective *Colletotrichum* isolates.

Keywords: Anthracnose, Beans, *Colletotrichum* spp., and Cross infectivity.

Beans are cultivated throughout year continuously or intercropped or rotated with other crops. Beans are excellent source of proteins, vitamins and minerals particularly calcium, phosphorus, iron and zinc for human diet. They are highly nutritious and used as green vegetables or green shelled seeds or dry seeds as pulse, based on the stage at which they are harvested (Braughton *et al.*, 2003). They are subjected to one or more infection of various species of *Colletotrichum*, since different types of beans are available throughout the year.

Colletotrichum species are reported to cause anthracnose in more than 121 plant genera from 45 different families, including gymnosperms, angiosperms, ornamentals, fruit plants, vegetables, field crops or even grasses (Farr *et al.*, 2016). Most crops grown throughout the world are susceptible to one or multiple species of *Colletotrichum* (Weir *et al.*, 2012). Many species of *Colletotrichum* may be found on a single host or single *Colletotrichum* species may be able to infect different hosts (Freemann *et al.*, 2013). Of 900 *Colletotrichum* species, over 100 species cause anthracnose disease (Cannon *et al.*, 2012) and about nine species were recorded on legume crops worldwide (Lenne, 1992).

Hence, the present study was undertaken to know the cross infectivity potential of different *Colletotrichum* spp., infecting beans, which help to know the survival of the pathogen during the off season.

MATERIAL AND METHODS

Cross inoculation studies of *Colletotrichum* spp., were carried out using selected bean crop species *viz.*, dolichosbean, french bean, cowpea, mungbean, urdbean, soybean, horsegram, clusterbean, swordbean, wingedbean, yard longbean and pillipesara.

Isolation of *Colletotrichum* spp.,

The above mentioned bean crop species showing the typical symptoms of anthracnose were collected from different areas of Andhra Pradesh, College farms of Agricultural College, Bapatla and College of Horticulture, Venkataramannagudem. Standard isolation technique was followed for isolation of pathogen from the anthracnose infected leaves, pods and stem.

Preparation of standard spore suspension

A concentration of 5×10^6 spores ml⁻¹ was used as standard inoculum for carrying out cross inoculation studies. Spore suspension was prepared as per the method described by Pathania *et al.* (2004). Tween 20 (polyoxyethylene sorbitan monolaurate), a surfactant and dispersing agent, was added to the spore suspension @ 0.1 per cent to enable uniform spread of inoculum on the leaves as well as on stem.

Cross inoculation studies in pot culture

Cross infectivity of *Colletotrichum* spp., on different beans was tested by inoculating the spore suspension to the seedlings by spray inoculation

method. Ten seeds of each plant species were sown into each of three 30 cm earthen pots containing 2:1:1(v/v/v) mixture of soil, sand and peat moss. Seedlings were thinned to five per pot and placed in the greenhouse. Two pots were selected for cross inoculation, while one pot was meant for control by spraying with sterilised distilled water. Ten days old healthy plant species were inoculated with freshly prepared spore suspension of *Colletotrichum* spp. Each bean crop was inoculated with respective isolate and *vice versa*.

Inoculation to the seedlings by spraying of spore suspension

Ten days old seedlings (2-4 trifoliate leaf stage) were sprayed with standard spore suspension using hand atomizer after pricking the leaves with sterile needle on both dorsal and ventral side of leaves. Such inoculated plants were kept in growth chambers at 25±1°C for 10-15 days and sufficient humidity was provided for three days by covering the inoculated plants with moist transparent polythene bags. Disease severity was recorded by five point scale suggested by Mayee and Datar (1986). Alcohol washed hand atomizer was used for spraying of spore suspension for each *Colletotrichum* isolate. Cross infectivity potential of each *Colletotrichum* isolate was assessed by recording data on infection parameters like disease incidence (DI) and disease severity (DS) at 35 days after sowing.

RESULTS AND DISCUSSION

Colletotrichum sp., isolated from dolichosbean was inoculated to other beans and *vice versa* for cross infectivity potential of each isolate. The data presented in Tables 1 and 2 revealed that *Colletotrichum* isolates expressed differences in terms of disease incidence and disease severity on their original host as well as other bean crop species.

Based on cultural and morphological characters, *Colletotrichum* spp., infecting the different bean species were identified. *Colletotrichum lindemuthianum* infected dolichosbean, french bean, urdbean, cowpea and horsegram, while *Colletotrichum truncatum* caused disease on mungbean, pillipesara, soybean, yard longbean, wingedbean and swordbean and *Colletotrichum capsici* f. sp. *cyamopsicola* occurred on clusterbean.

Disease parameters

Significant difference between *Colletotrichum* isolates in terms of disease incidence (DI) and disease severity (DS) was observed in original hosts and other bean hosts. *Colletotrichum* isolates from beans when inoculated, pillipesara was found highly

susceptible to anthracnose disease with an incidence of 55.50% followed by mungbean (53.50%) and least disease incidence of 37.50% in horsegram (Table 1), while disease severity was high on seedlings of urdbean (7.25%) followed by dolichosbean, cowpea and mungbean (7.00%) and the least was on yard longbean (3.50%) (Table 2). These results are in accordance with the findings of Mackenzie *et al.* (2009) who reported that the highest incidence and the biggest lesions were observed in the original host.

Potential of different isolates of *Colletotrichum* in original host and other bean species

Colletotrichum isolate (CI 1) from the dolichosbean readily infected dolichosbean, french bean, urdbean and cowpea, while others test plants were disease free. The disease incidence and disease severity varied significantly from 31.50 to 48.50 per cent and 4.75 to 7.00 per cent, respectively, with the lowest in urdbean and the highest in dolichosbean (Tables 1 and 2). Disease incidence and disease severity of urdbean, cowpea and french bean are at par with each other at 35 days after sowing. Same trend was noticed in french bean isolate (CI 2) but varied with disease parameters like disease incidence (28.50 to 49.00%) and disease severity (4.5 to 6.5%) with the highest readings in French bean and the lowest in cowpea.

The disease incidence varied from 18.50 to 47.50 per cent and disease severity ranged from 4.00 to 7.25 per cent when the CI 3 isolate from urdbean inoculated to other beans. The lowest disease incidence (18.50%) was recorded in pillipesara which was at par with horsegram (19.00%) and dolichosbean (21.00%). But disease severity was the lowest in french bean and horsegram (4.00%), which was at par with cowpea (5.5%) and pillipesara (5.75%). These results are in conformity with the findings of Rana and Kaushal (2004) who reported urdbean and horsegram to be susceptible to all the isolates derived from different hosts.

Colletotrichum isolate (CI 5) from horsegram expressed the significant variations in terms of disease incidence (DI) and disease severity (DS) in other bean crops. CI 5 when inoculated on seedlings of different bean crop species produced typical symptoms of anthracnose in all the crops except pole type beans and clusterbean. The lowest disease incidence was noticed on pillipesara (17.50%) followed by dolichosbean (18.15%), urdbean (20.00%), french bean (21.50%), cowpea (24.00%) and soybean (25.00%) and were at par with each other, where as the highest disease incidence was observed on horsegram (37.50%) (Table 1). The lowest disease severity was noticed on urdbean (2.00%) but was at

Table 1. Cross infective potential of *Colletotrichum* isolates in different beans with respect to disease incidence at 35 DAS.

S. No	Test plant	CI 1*	CI 2*	CI 3*	CI 4*	CI 5*	CT 1*	CT 2*	CT 3*	CT 4*	CT 5*	CT 6*	CC 1*
1	Dolichosbean	48.50	34.00	21.00	24.00	18.15	0.00	0.00	23.50	0.00	0.00	0.00	0.00
2	French bean	32.50	49.00	37.65	31.50	21.50	0.00	0.00	23.00	0.00	0.00	0.00	0.00
3	Urdbean	31.50	32.00	47.50	31.00	20.00	0.00	0.00	24.50	0.00	0.00	0.00	0.00
4	Cowpea	32.00	28.50	32.90	53.50	24.00	0.00	0.00	33.50	33.50	0.00	0.00	0.00
5	Horsegram	0.00	0.00	19.00	24.00	37.50	38.50	26.50	21.50	0.00	0.00	0.00	0.00
6	Pillipesara	0.00	0.00	18.50	22.00	17.50	55.50	0.00	25.00	0.00	0.00	0.00	0.00
7	Soybean	0.00	0.00	0.00	0.00	25.00	0.00	42.50	0.00	0.00	0.00	0.00	0.00
8	Mungbean	0.00	0.00	25.00	34.00	28.50	34.00	0.00	52.50	0.00	0.00	0.00	0.00
9	Yard long bean	0.00	0.00	0.00	36.00	0.00	0.00	0.00	0.00	44.00	31.50	20.00	0.00
10	Winged bean	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	27.50	40.50	31.50	0.00
11	Sword bean	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	31.50	28.00	44.00	0.00
12	Clusterbean	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	39.00
	C.D. at 5%	4.61	3.11	5.92	5.73	8.48	4.09	2.95	6.54	2.55	1.79	2.21	3.18
	SE(m)±	1.46	0.99	1.88	1.82	2.69	1.30	0.94	2.08	0.81	0.57	0.70	1.01
	C.V. (%)	17.19	11.68	15.83	12.06	23.78	17.22	23.06	17.31	13.36	7.23	12.47	43.97

* CI-1- *Colletotrichum lindemuthianum* isolate -Dolichosbean* CI-2- *Colletotrichum lindemuthianum* isolate - French bean* CI-3- *Colletotrichum lindemuthianum* isolate - Urdbean* CI-4- *Colletotrichum lindemuthianum* isolate- Cowpea* CI-5- *Colletotrichum lindemuthianum* isolate- Horsegram* CC-1- *Colletotrichum capsici* isolate- Clusterbean* CT-1- *Colletotrichum truncatum* isolate-Pillipesara* CT-2- *Colletotrichum truncatum* isolate- Soybean* CT-3 - *Colletotrichum truncatum* isolate-Mungbean* CT-4- *Colletotrichum truncatum* isolate-Yardlongbean* CT-5- *Colletotrichum truncatum* isolate- Wingedbean* CT-6- *Colletotrichum truncatum* isolate- Swordbean

Table 2. Cross infective potential of *Colletotrichum* isolates in different beans with respect to disease severity at 35 DAS

S. No	Test plant	CI 1*	CI 2*	CI 3*	CI 4*	CI 5*	CT 1*	CT 2*	CT 3*	CT 4*	CT 5*	CT 6*	CC 1*
1	Dolichosbean	7.00	6.00	6.00	4.50	3.00	0.00	0.00	3.00	0.00	0.00	0.00	0.00
2	French bean	5.50	6.50	4.00	5.50	4.25	0.00	0.00	4.00	0.00	0.00	0.00	0.00
3	Urdbean	4.75	5.50	7.25	6.50	2.00	0.00	0.00	5.00	0.00	0.00	0.00	0.00
4	Cowpea	5.00	4.50	5.50	7.00	3.50	0.00	0.00	4.50	3.50	0.00	0.00	0.00
5	Horsegram	0.00	0.00	4.00	5.00	4.50	4.00	2.75	4.00	0.00	0.00	0.00	0.00
6	Pillipesara	0.00	0.00	5.75	4.50	4.50	4.50	0.00	3.00	0.00	0.00	0.00	0.00
7	Soybean	0.00	0.00	0.00	0.00	3.50	0.00	4.00	0.00	0.00	0.00	0.00	0.00
8	Mungbean	0.00	0.00	6.50	3.00	3.50	4.00	0.00	7.00	0.00	0.00	0.00	0.00
9	Yard long bean	0.00	0.00	0.00	5.00	0.00	0.00	0.00	0.00	3.50	3.00	2.25	0.00
10	Wingedbean	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.75	4.50	3.25	0.00
11	Swordbean	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.00	3.50	4.25	0.00
12	Clusterbean	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.50
	C.D. at 5%	1.36	1.42	1.79	2.31	2.03	1.13	0.53	1.48	1.43	1.13	0.36	0.91
	SE(m)±	0.43	0.45	0.57	0.73	0.64	0.36	0.01	0.47	0.45	0.36	0.11	0.29
	C.V. (%)	32.99	33.95	24.69	30.36	37.99	48.72	42.02	26.08	60.41	55.37	19.68	75.30

- * CI-1- *Colletotrichum lindemuthianum* isolate -Dolichosbean * CT-2- *Colletotrichum truncatum* isolate- Soybean
 * CI-2- *Colletotrichum lindemuthianum* isolate -French bean * CT-3 - *Colletotrichum truncatum* isolate-Mungbean
 * CI-3- *Colletotrichum lindemuthianum* isolate - Urdbean * CT-4- *Colletotrichum truncatum* isolate-Yardlongbean
 * CI-4- *Colletotrichum lindemuthianum* isolate-Cowpea * CT-5- *Colletotrichum truncatum* isolate- Wingedbean
 * CI-5- *Colletotrichum lindemuthianum* isolate- Horsegram * CT-6- *Colletotrichum truncatum* isolate- Swordbean
 * CT-1- *Colletotrichum truncatum* isolate-Pillipesara * CC-1- *Colletotrichum capsici* isolate- Clusterbean

par with dolichosbean (3.00%), cowpea, soybean and mungbean (3.50%), while french bean (4.25%) and pillipesara (4.50%) were at par with original host *i.e* horsegram (4.50%) at 35 days after sowing (Table 2). In general, horsegram is naturally infected by several species of *Colletotrichum*, including *C. lindemuthianum*, *C. dematium* and *C. truncatum*. In India, *C. lindemuthianum* (Sharma, 1976), *C. capsici* (Pangtey and Sinha, 1980) *C. dematium* f. sp. *truncatum* (Bharadwaj and Singh, 1986), *C. dematium* (Saharan, 1979) were identified as pathogens responsible for anthracnose of horsegram. Similarly, in the present study horsegram was infected by the *Colletotrichum* isolates from urdbean, cowpea, mungbean, pillipesara, soybean and horsegram.

Colletotrichum isolate-4 from cowpea didn't infect soybean, wingedbean, swordbean and clusterbean. The isolates of CI 3 and CT 3 from urdbean and mungbean, respectively, infected six beans *viz.*, dolichosbean, french bean, cowpea, pillipesara, mungbean and horsegram in addition the original host. This study indicates that among all twelve *Colletotrichum* isolates, CC 1 from clusterbean as least virulent and CI 4 and CI 5 to be most virulent. Gopalakrishnan and Prakasam (2007) reported cross pathological variations in isolates of *C. gloeosporioides* on different legumes. These results are in line with the findings of Wijeratnam *et al.* (2008) who reported that, isolates of *C. gloeosporioides* produced larger lesions on their original host when compared with the collateral hosts.

Colletotrichum isolates from three pole type beans like yard longbean, swordbean and winged bean cross infected each other, and isolate from yard longbean cross infected the cowpea in addition to other pole types (Tables 1 and 2).

Host preference and host specificity of beans to different isolates of *Colletotrichum*

Cowpea cultivar PKM 1 showed positive reaction to most of the isolates of *Colletotrichum* isolated from dolichosbean, French bean, uredbean, mungbean, horsegram and yard longbean and negative reaction to pillipesara, soybean, wingedbean, swordbean and clusterbean (Tables 1 and 2).

Differences in cross infectivity of *Colletotrichum* isolates can be attributed to the existence of different pathogenic variants of *Colletotrichum* spp., in nature and their specific adaptation to the host species and also selection pressure on the pathogen by the commercial cultivation of resistant varieties might have lead to develop pathogenically variable forms. These results are in conformity by the Thomazella *et al.* (2002) who

reported the existence of more than 25 races of *C. lindemuthianum*.

CONCLUSION

It can be concluded that *Colletotrichum* isolates tested were able to infect different beans, but with different degree of reactions (positive/negative). Several studies have demonstrated, non-host specific nature of *Colletotrichum* species (Peng *et al.*, 2013) in which the same species can be associated with different hosts or one host can be infected by different species (Freeman *et al.*, 2013). In nature, inoculum may be dispersed from one crop to other crops. Further, such ability of the pathogen may provide an opportunity to the pathogen to survive during adverse periods in crops. Host specificity was noticed in clusterbean, soybean and pole type beans but, some of the beans *viz.*, cowpea, dolichosbean, French bean, urdbean and horsegram could readily infected from the *Colletotrichum* isolates from other hosts.

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