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COMPARATIVE STUDIES ON ISOATES OF COLLETOTRICHUM LINDEMUTHIANUM, CAUSAL AGENT OF COWPEA ANTHRACNOSE COLLECTED FROM TWO DIFFERENT PLACE

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Abstract— **Isolates of anthracnose disease infested cowpea plant parts were collected from GKVK (University of Agricultural Science) and IIHR (Indian Institute of Horticultural Research), Bangalore, India for screening of the differences between conidia and setae(the two characteristic features) of *Colletotrichum gloeosporoides.* It was observed that the dimensions of conidia and setae of the causal agent of same anthracnose disease of cowpea grown in two different fields are different and are not of the same race of *C. gloeosporoides.* Results showed that average conidial length of GKVK isolate is 11.1 µm and IIHR isolate is 13.32 µm and average conidial width of GKVK isolate is 7.25 µm and IIHR isolate is 6.36 µm. Again, average size of seta of GKVK isolate is 64.44 µm and IIHR isolate is 54.99 µm. But, there is no significant differences observed between acervuli of isolates of two different places. Further, the strains were found to vary morphologically between the isolates under the study. Conidia of GKVK isolates are cylindrical and narrow in shape whereas conidia of IIHR isolates are ovoid.**

KEY WORDS: **Cowpea, *Colletotrichum gloeosporoides*, conidia, setae, sizes.**

I. INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp or *Vigna sinensis*], widely known as Lobia, belongs to the bean family Leguminosae (Papilionaceae) is one of the major pulses in the sub-humid and humid tropics which has the ability to grow in any type of soil condition and it can fix atmospheric nitrogen into the soil. Cowpea suffers from various diseases caused by different pathogenic groups. Anthracnose is one of the devastating worldwide fungal diseases that affect the above ground parts. This disease is induced by hemibiotroph Deuteromycetous (fungi imperfecti) fungus called *Colletotrichum* (seed borne and soil borne). The genus was established by Corda in 1831. The teleomorph stage of many *Colletotrichum* species is *Glomerella* and both stages are widely prevalent in hot and humid climates. This genus includes more than 900 species. The taxonomy of *Colletotrichum* species has been developed through classical descriptive criteria such as the shape and size of conidia, setae and appressoria coupled with knowledge of the identity of the host plant.

B. B. Singh (1997) mentioned in his book that the pathogen of cowpea anthracnose was regarded as a form of *C. lindemuthianum* (Sacc. and Magn.) Briosi and Cav., the pathogen of anthracnose on *Phaseolus* beans. However, Bailey and associates (Bailey et al. 1990; O' Connell et al.1992; Pain et al. 1992) have raised important questions about the taxonomic status of the cowpea anthracnose pathogen. On the basis of the molecular, morphological and antigenic differences that exists between the anthracnose pathogens of cowpea and *Phaseolus* beans, it was suggested by this group of researchers that the cowpea anthracnose pathogen should be regarded as a species that is distinct from *C. lindemuthianum*, probably a form of *C. gloeosporoides*. The name *Colletotrichum gloeosporioides* was first proposed in Penzig (1882), based on *Vermicularia gloeosporioides*, the type specimen of which was collected from *Citrus* in Italy. Much of the early literature used this name to refer to fungi associated with various diseases of *Citrus*, with other species established for morphologically similar fungi from other hosts. However, several early research works discussed the morphological similarity between many of the *Colletotrichum* spp. that had been described on the basis of host preference, and used inoculation tests to question whether or not the species were distinct.

Colletotrichum destructivum was described from red clover (*Fabaceae*) by O'Gara (1915) and has been confused with several species including *C. gloeosporioides*, *C. lindemuthianum* and *C. truncatum*. In reviewing the genus, von Arx (1957) maintained *C. destructivum* as a distinct species. Sutton (1980) did not consider *C. destructivum* an acceptable species.

Eighty five isolates of the pathogen *C. lindemuthianum* were collected from the various kidney shaped beans (*Phaseolus vulgaris*) from North Western Himalayan Region

of India for variability analysis and the evaluation of resistance in the host. On the basis of their reaction types on international and CIAT differentials 12 races were found, viz. Alpha-Brazil, Beta, Gamma and Ind I to Ind IX (Sharma et al., 2008).

The pathogenic and genetic diversity of *Colletotrichum lindemuthianum* has been studied and characterized on isolates collected from a total of 10 Central and South American, European and African countries, using common bean differential cultivar pathogenicity tests and amplified fragment length polymorphism (AFLP) analysis. On the basis of pathogenicity tests, 74 isolates were attributed to 30 different pathogenic races using the CIAT-defined binary race-classification system. Twenty-one races were restricted geographically, being exclusive to different countries. Race 9 was the most widespread, being detected in four different countries. (Ansari et al. 2004).

In this study, disease survey has been carried out in UAS, GKVK campus, Bangalore and IIHR campus in Hesserghatta. Isolates of infected stems, leaves and pods have been collected from the disease invested fields. Variability test of the pathogen has been carried out from the collected diseased samples.

II. MATERIALS AND METHODS

Culture media Potato Dextrose Agar (PDA) was prepared and pH was adjusted to 5.6 for getting optimum growth of the fungus.

A. Isolation and purification of isolates

Several disease isolates have been collected from experimental fields of GKVK and IIHR in Bangalore, Karnataka. Isolated plant parts have been cut into small bits and surface sterilised with 0.1% mercuric chloride ($HgCl_2$) solution for about one minute and washed repeatedly thrice in sterile distilled water before transferring them to sterile petriplates containing PDA media (5mm) under LF (Laminar Flow Chamber). Excess water has been wiped off by sterile tissue paper. Petriplates containing diseased plant parts of GKVK variety and IIHR variety were kept in room temperature for 7-10 days under aseptic condition and observed at regular intervals for the growth of the pathogens.

After the incubation period, fungal pathogens were observed under compound microscope.

For purification of the cultures, single spore isolation technique has been followed (by Streaking technique and Spore suspension dilution technique). Following these methods pure culture has been prepared for getting species purity. Subcultures have been prepared in media slants for conducting experiments without disturbing pure culture. Slants of pure culture have been made in the test tubes tightly plugged with nonabsorbent cotton plug and kept in refrigerator at 5◦C for storage. Sub- culturing was done subsequently at an interval of 30 days in order to maintain virulence of culture purposes.

Antibiotic streptomycin was always used in growth media to suppress the bacterial growth.

III. RESULTS AND DISCUSSION

The isolates of diseased plant parts taken from two different places have shown differences in setae and conidia under low power. The following tables showcase these result.

Measurement of Conidial length under occular micrometer are as follows:

Observation No.	GKVK	IIHR
1	1	1
2	1	1.5
3	1	1.5
4	1	1
5	1	1
6	1	1
7	1	1.5
8	1	1
9	1	1.5
10	1	1

Table 1. Conidial length

Average conidia size of GKVK isolate = 10/10 = 1Occular div.

Average conidia size of IIHR isolate = 12/10 = 1.2 Occular div.

Considering 1 stage div = 10 µm and 10 stage divisions equaling to 9 Occular div., i.e 1 occular =100/9 µm

Hence the average size of conidia of GKVK isolate is 11.1µm and IIHR isolate is 13.3 µm.

Measurement of conidial width under ocular micrometer are as follows:

Observation no.	GKVK	IIHR
1	0.65	0.57
2	0.65	0.57
3	0.6	0.58
4	0.65	0.58
5	0.6	0.57
6	0.65	0.5
7	0.65	0.57
8	0.65	0.57
9	0.66	0.5
10	0.65	0.57

Table 2. Conidial width

Hence, the average width of conidia of GKVK isolate is 7.25µm and IIHR isolate is 6.36µm.

Measurement of seta (length) under ocular micrometer are as follows:

Observation no.	GKVK	IIHR
1	4	5
2	5	4.5
3	6	5
4	6	5
5	5	5
6	5	5
7	5	5
8	8	5
9	8	5
10	6	5

Table 3. Length of Seta

Hence, the average size of seta of GKVK isolate is 64.44 µm and IIHR isolate is 54.99 µm.

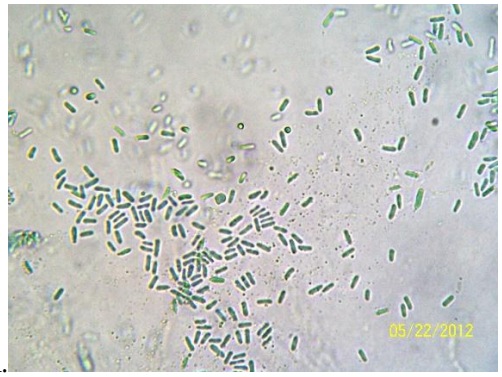
A. 

Fig. A. Conidia of GKVK isolate

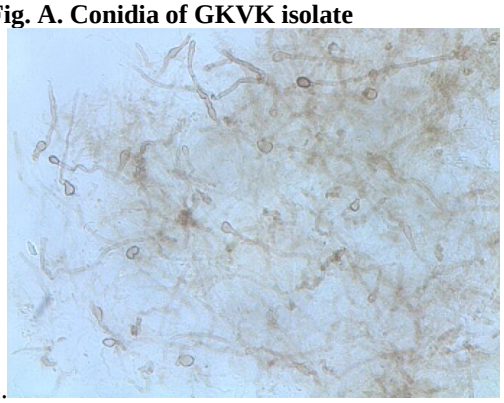
B. 

Fig.B. Conidia of IIHR isolate

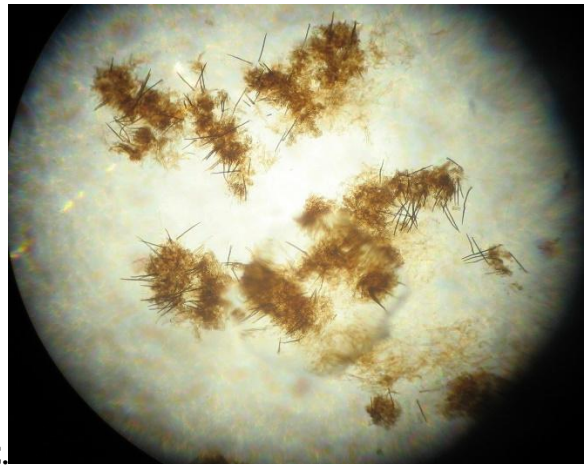
C. 

Fig. C. Seta of GKVK isolate

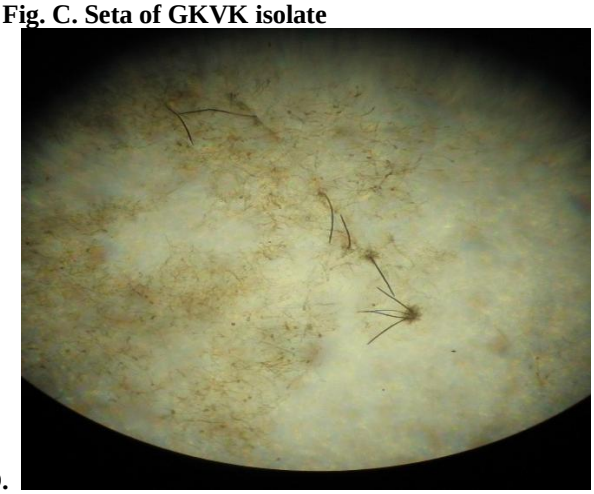
D. 

Fig. D. Seta of IIHR isolate

Fig. Magnification under low power of compound microscope (45x)

Maziah, Z. and Bailey, J. A., 2000 (13), in their studies found morphological and cultural variation among *Colletotrichum* isolates obtained from tropical forest nurseries. Preliminary morphological studies suggested that most of the isolates produced ovoid/straight conidia with rounded apices typical of *C. gloeosporioides.* Morphological analysis of the other isolates illustrated that most of them showed variation in their conidial size, appressorial shape and size, suggesting the existence of different forms of *C. gloeosporioides.* The results also revealed distinct appressorial shapes. They concluded that morphological study alone has many limitations in trying to distinguish species of *Colletotrichum.*

Thangamani, *et al.* (23) in their studies on morphological and physiological Characterization of *Colletotrichum musae* the causal organism of Banana anthracnose found that all the sixteen isolates have hyaline and short conidiophores bear single hyaline cylindrical conidia.The conidia measured 14.7 µm x 7.1 µm with a centrally placed oil globule. These characters agreed with the original descriptions given by Lemme and Sonoda (12) and Sutton (21). Das Gupta (6) also reported the variation in the spore size (17.36-21.8 µm x 2.66-2.88 µm) among the isolates of *C. capsici* causing anthracnose of betelvine. The average size of the spores however, did not

vary among the isolates. Chakrabarty *et al*. (5) reported that in *C. lindemuthianum* also the average size of the spores did not vary much among the isolates. Nandinidevi (14) reported that the conidiophores were hyaline and septate having ovoid to cylindrical conidia measuring 22.5 x 10 µm, which were one celled with one or two oil globules. Quimio and Quimio (15) found differences in the degree of virulence of eleven *C. gloeosporioides* isolates of mango and reported that the conidial size was 12.0- 17.0 x 3.5-6.0 µm. *C. gloeosporioides* isolates obtained from apple, peach, pecan and other hosts varied greatly in their growth, virulence and conidial size (4).

IV. CONCLUSION

Considering above literature discussions and comparing the results obtained from the current study, it is concluded that there is subtle morphological differences in the size of conidia and seta obtained from GKVK and IIHR isolates. Von Arx (1970) and Sutton (1980) distinguished the *C. gloeosporioides* group using conidial shape and size. In this study conidia of GKVK is much narrow and cylindrical than IIHR isolates, whereas conidia of IIHR isolate is ovoid in shape under microscope. Again, it was found that the length of seta under microscope present in acervuli of GKVK isolates are longer than IIHR isolates. So, it is evident that anthracnose disease showing typical symptoms of dark-brown, sunken lesions with pink masses of spores in the centre occurred in cowpea plants grown in different fields are not affected by same races of *Colletotrichum gloeosporioides.* Weir *et al*., 2012 (25) stated in their studies that the availability of DNA sequence data, taxonomic concepts within *Colletotrichum* were based on features such as host species, substrate, conidial size and shape, shape of appressoria, growth rate in culture, colour of cultures, presence or absence of setae, whether or not the teleomorph develops, etc. Some studies have found characters such as these useful for distinguishing groups within *C. gloeosporioides* (*e.g.* Higgins 1926, Gorter 1956, Hindorf 1973, and Johnston & Jones 1997). However, problems arise because many of these morphological features change under different conditions of growth (dependent upon growth media, temperature, light regime, etc.), or can be lost or change with repeated subculturing. The same disease can be caused by genetically distinct sets of isolates, the shared pathogenicity presumably independently evolved, *e.g.* the bitter rot disease of apple is caused by members of both the *C. acutatum* and *C. gloeosporioides* species complexes (Johnston *et al.* 2005).

So, the reason of differences in the size of conidia and seta in same pathogen causing anthracnose disease showing same charecteristic symptoms but isolated from different places remain to be studied.

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