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Variability In Red Rot Pathogen *Colletotrichum falcatum* Went

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ABSTRACT

Five isolates of *Colletotrichum falcatum* Went collected from five different sugarcane growing areas showed cultural-morphological and pathogenic variations. Each isolate differed from the other at least in one or more character(s). The isolate RSM was distinctly different from the rest four because of its extreme white colour and lack of acervuli and setae. The study shows that two morphologically distinct types of isolates of *C. falcatum;* the light and dark type exist in Bangladesh. The light type isolates TSM & RSM proved to be more pathogenic to three test sugarcane clones, Isd-2/54, I-156/66 and Isd-5/55 than the dark type isolates RJSM, SRTI and MKSM.

INTRODUCTION

The existence of variability in the red rot pathogen *Colletotrichum falcatum* Went is the most important factor due to which some sugarcane varieties are unstable in their resistance to the disease (Abbot, 1953). Often a new variety resistant to prevailing isolates at the time of its release may suddenly succumb to infection later on by the appearence of a new isolate which is virulent towards that variety (Chona and Padwick, 1942; Kirtikar, *et. al.* ,1965). Abbot (1938 and 1946) in seeking the cause of the red rot epidemic in Louisiana in 1930-31 discovered a sudden development of a light coloured highly virulent strain in addition to the existing dark coloured less virulent one. Singh *et. al.* (1984) found a constant development of newer isolates in nature showing a promise of greater virulence or pathogenecity on a number of host varieties.

In Bangladesh the sugarcane varieties Isd- 5/55 and Co-1158 found to be resistant to red rot at the time of their release but later on due to the appearance of new isolates of *C. falcatum*, both the varieties became susceptible and hence they have been droped from cultivation (SRTI Annual Report, 1983). On the otherhand same sugarcane variety shows variable reaction to red rot in different cane growing areas of Bangladesh which supports the existance of variable isolates of *C. falcatum* in the country. So, for testing the new strain of sugarcane to different isolates of *C. falcatum* necessitated this study.

With a view to identifying the various isolates of *C. falcatum*, their systematic studies were done and presented in this paper.

MATERIALS AND METHODS

The red rot affected diseased samples of different cane varieties were collected during the month of September 1983, from Thakurgaon Sugar Mills (TSM), Rangpur Sugar Mills (RSM), Rajshahi Sugar Mills (RJSM),Sugarcane Research & Training Institute (SRTI) farm and Mobarakganj Sugar Mills (MKSM) areas and the naming of the isolates were made accordingly.

The isolates TSM and RJSM were obtained from naturally infected and heavily damaged canes of the variety Isd-5/55, RSM and MKSM from Co. 1158 and SRTI from NCO-339. The pathogen from the collected samples were isolated in the pure form and the stock cultures were maintained on oat meal agar slants at 4±1°C. To obtain pure cultures of the isolates,single spore culture and hyphal tip techniques were followed . For studying cultural and morphological characteristics of the isolates, petriplates (8 cm dia) containing 15 ml. of sterilized oat meal agar were inoculated with single spore. Ten plates were used for each isolates. five plates were inocubated at 25±1° C (Ahmed and Divinagracia,1977) for 15 days to study the sporulation and five plates at 28±1°C for 12 days for mycelial growth studies. The colony diameter were thus measured at an interval of 2 days upto12 days. The growth rate per day per colony was measured and then the average was calculated. During culturing of the individual isolates. the colour and texture of the colonies as well as the mycelial characters were observed. To assess the varying degree and capacity of sporulation of different isolates. their 15 days old cultures were mixed with 100ml distilled water together with a drop of tween-20. The mixtures were then subjected to a thorough stirring in a warring blender for 3 minutes to obtain a homogenous suspension and the spores were counted by means of haemacyto-meter. Simultaneously the size and shape of conidia of each isolate were also noted.

For studying pathogenic variation among the isolates, three sugarcane varieties from the categories of resistant intermediate and susceptible groups were chosen and planted in the month of November 1982 in three plots separately, each with 5 rows of 3 meter length. The variety Isd-2/54 was selected from resistant group, I-156/66 from intermediate and Isd-5/55 from susceptible group (SRTI Annual Report, 1979). Twenty canes of each variety were randomly inoculated with 14 days old cultures of each isolate in the 1st week of October/ 1983 by standard plug method (Chona, 1954).

The inoculated canes were harvested after three months following inoculation and the spread of the disease in the form of internal lesions (length and width) was measured after splitting the cane longitudinally. Average reading of 10 number of canes of 20 inoculated ones in each

treatment was calculated out for lesions length and width. length and width of inoculated internode to determine the index of virulence which was determined by using the following formula (Abbott, 1938):

$$\text{Index of virulence} = \frac{\text{Lesion's length}}{\text{Length of inoculated internode}} \times \frac{\text{Lesion's length}}{\text{Width of inoculated internode}}$$

The disease ratings as recommended by Hutchinson (1968) was used in determining the resistance/susceptibility.

RESULTS AND DISCUSSION

Observation on mycelial characters and colours as well as spore formation and setae characters of five isolates grown on oat meal agar at 25±1°C for 15 days are presented in Table 1. From the data it is clear that the isolate RSM was distinctly different from all other isolates due to

Table 1. Colour and mycelial characters as well as spore formation and setae characters of different isolates of *Colletotrichum falcatum* on oat meal agar at 25 ± 1°C.

Isolate	Place and source of isolation	Colour of colony and nature of mycelium	Spore formation and setae characters.
TSM	Thakurgaon Sugar Mills area (Isd-5/55).	Light type, mycelium-cottony white and loose fluffy, later turned slightly greyish.	Abundant spores formed in prominent round pinkish masses with abundant smaller sized acervuli and setae.
RSM	Rangpur Sugar Mills area (Co. 1158).	Light type, mycelium-extremely white, later turned slightly greyish, sparse and loose.	Spores formed in slimy pinkish masses without any acervuli and setae.
SRTI	Sugarcane Research & Training Institute area (NCO-339).	Dark type, mycelium-light greyish, sparse, cottony and loose, later turned dark greyish.	Spores formed in tiny inconspicuous dots. Abundant larger sized acervuli and setae present.
RJSM	Rajshahi Sugar Mills area (Isd-5/55)	Dark type, mycelium-greyish,sparse, cottony and loose, later turned dark greyish.	Spores formed scattered in a conspicuous masses with large and long sized abundant acervuli and setae.
MKSM	Mobarakganj Sugar Mills area (Co. 1158).	Dark type, mycelium grey to olivacious black, dense and felty.	Spores formed in a slimy pale pink layer, Abundant short sized acervuli and setae present.

Table 2. Radial growth rate of five isolates of *Colletotrichum falcatum* on oat meal agar at 28 ± 1°C in the dark.

Name of isolate	Colony diameter (mm).												Average growth per day
	2nd day		4th day		6 th day		8th day		10th day		12th day		
	Total growth	Mean	Total growth	Mean	Total growth	Mean	Total growth	Mean	Total growth	Mean	Total growth	Mean	
TSM	3.5	1.75	8.0	2.0	12.0	2.0	17.0	2.12	24.0	2.4	38.6	3.21	2.24
RSM	5.0	2.5	11.0	2.75	17.5	2.91	24.0	3.0	33.4	3.34	46.0	3.83	3.05
SRTI	3.5	1.75	8.0	2.0	12.0	2.0	18.5	2.31	24.0	2.4	32.4	2.7	2.19
RJSM	6.5	3.25	17.0	4.25	27.0	4.5	38.0	4.75	49.0	4.9	59.4	4.95	4.43
MKSM	3.5	1.75	8.0	2.0	12.0	2.0	17.0	2.12	24.0	2.4	31.0	2.58	2.14

it's extreme white colour and absence of acervulus and seta in it. Isolate MKSM was also distinctly different because of its dense black olivacious mycelium. The isolates also differed in their mycelial characters and sporulation. Loose and fluffy mycelia were noticed in TSM, while MKSM showed dense and felty mycelia. On the basis of colour and texture of the mucelium the isolates can be grouped into two distinct types namely, light type and dark type. The observation coincided with the findings obtained by Chona and Srivastava (1960). Though the acervulus and seta are the most important characters of *Colletotrichum falcatum*, the isolate RSM did not produce any acervulus and seta. This is in agreement with the observation of Angihotri (1983) and Chona and Srivastava (1960).

Maximum growth rate per day was recorded in isolate RJSM followed by RSM. No marked difference in growth rate was noticed among the isolates SRTI, TSM and MKSM (Table 2). Data indicate that the isolate RJSM had the radial growth of 17.0 mm in 4 days, whereas the same amount of growth was recorded by isolate TSM, SRTI and MKSM in 8 days.

There was no relation between the growth rate of the isolates and their sporulation. Maximum radial growth occured in isolate RJSM followed by RSM but they occupied the 2nd and 5th position in sporulation respectively. There were also variation in shape and size of the conidia. The conidia of isolates TSM and RJSM Looked more or less same with one end more pointed and curved. Isolate SRTI were pointed at both ends while in RSM both ends were more or less flat and less curved. As regards length, the lowest length of conidia (20μ) was observed in isolate RJSM and the highest (28μ) in TSM. The average conidial width in all the isolates were more or less same (Table 3). The findings are more or less similar to those obtained by Abbott (1938) and Went (1893) who found average length 25.1μ and 25μ respectively.

The results obtained from inoculation tests are presented in Table 4. The reaction of different varieties to the five isolates varied. Isd-5/55 was susceptible to all the isolates and Isd-2/54 resistant to all isolates but they varied in their degree of susceptibility or resistance; while I-156/66 showed mixed reaction, being resistant to isolates RJSM and MKSM, moderately resistant to RSM, intermediate to SRTI and susceptible to TSM.

The colour and texture of the mycelium and the nature and degree of sporulation appeared to be interrelated to some extent with the virulence of the isolates. For example, among the five isolates, TSM being a light type and highly sporulating one showed higher degree of virulence. On the contrary, the isolates MKSM and RJSM were dark type and highly sporulating but less virulent. This corresponds to the observation of Rafay and Singh (1957), Abbott (1938) and Chona and Padwick (1942). However, the low sporulating dark type isolate SRTI also showed much less virulence in the present study.

From the above discussion it is clear that each isolate differs from the other at least in some character (s). Based on colour and texture, the isolates may be grouped into two distinct types, light type and dark type. Based on acervulus and seta formation they may again be classified into two groups: those which form acervulus and those without acervulus and seta. From the inoculation trial it has become apparent that each isolates has a distinct parasitic relationship with each variety. For example, the isolate MKSM proved least virulent against Isd-2/54 but on I-156/66 it was the third virulent type. Similarly, the isolate SRTI occupied fourth position against Isd-2/54 but on I-156/66 it became second in virulence. This phenomenon thus proved that an isolate pathogenic to one variety may not act in the same way in case of another variety. Thus the results authenticated that the isolates had different host specificity as regards parasitism. The phenomenon supports the findings of Singh *et. al.* (1984), Khirbat *et. al.* (1980) and Kirtikar (1959).

Table 3. Sporulation and conidial characters of different isolates of *Colletotrichum falcatum* on oat meal agar at 25± 1 °C.

Isolate	Average no. of spores / plate[x]	* Sizes of conidia	Shape of conidia
TSM	$32X10^7$	25—28μX5—6μ (27μX5.5μ)	One end of the conidia more curved and pointed.
RSM	$2.2X10^7$	23—27μX5—6μ (24μX5.5μ)	Conidia are less curved and less pointed at both ends.
SRTI	$4.4X10^7$	23—28μX5—6μ (26.2μX5.4μ)	Conidia more curved and more pointed at both ends.
RJSM	$9.24X10^7$	20—26μX5—6μ (25.2μX5.5μ)	One end of the conidia more pointed and curved.
MKSM	$6X10^7$	24—27μX5—6μ (26.0μX5.4μ)	One end of the conidia more flat and curved.

[x] Values are the means of six aquous aliquot samples from each three replications per treatment.
* Figures in the parenthesis are the averages.

Table 4. Index of varulence of the five isolates of *Colletotricihum falcatum* on three varieties of sugarcane.

Variety	Isolates					
	TSM	RSM	SRTI	RJSM	MKSM	Average
Isd-2/54	1.87(R)	1.51 (HR)	1.05 (HR)	1.57 (R)	0.7 (VHR)	1.34
I-156/66	3.94(S)	2.06 (MR)	3.0 (INT)	1.8 (R)	2.0 (R)	2.56
Isd-5/55	5.2 (VHS)	6.97 (VHS)	4.28 (HS)	5.4 (VHS)	4.37 (HS)	5.24
Average	3.67	3.51	2.78	2.92	2.36	

C. D. at 1% level : Variety-0.38 ; Isolate-0.49 ; Interaction-varietiesXIsolates-0.85.

From this study it may be suggested that the isolates of red rot pathogen with differential pathogenicity exist in Bangladesh. It is, therefore, necessary to exercise great care in the selection of *C. falcatum* isolates for varietal resistance tests. The most virulent isolate must be picked up in a preliminary screening test on the host. It is also necessary to make regular collection of prevailing isolates of red rot pathogen in nature for determining the existence of its physiologic races in the country.

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