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Synergistic association between pathotypes Cf 07 and Cf 08 of *Colletotrichum falcatum* Went. in causing red rot of sugarcane

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Sixteen sugarcane genotypes were inoculated with *Colletotrichum falcatum* Went pathotypes Cf 07 and Cf 08 by plug method either separately or in combination for development of red rot disease. The pathotypes expressed differential reactions among the sugarcane genotypes. *C. falcatum* pathotype Cf 08 was relatively more virulent to Cf 07 in seven genotypes and Cf 07 was more virulent to Cf 08 in two genotypes. Two pathotypes when combined in equal volume for inoculation, it showed synergistic relationship in seven genotypes, by expressing higher red rot disease score over any single pathotype. The results partially explain the reasons for sudden breakdown of resistance in popular resistant cultivars of sugarcane to develop an epidemic. On the other hand, screening of pre-release sugarcane genotypes with combined pathotypes of *C. falcatum* may eliminate some of the non-deserving entries because of artificial enhancement of virulence of the red rot pathogen.

Key words: Brix, genetic resistance, *Glomerella tucumanesis, Saccharum* sp. (hybrid)

INTRODUCTION

Red rot, caused by *Colletotrichum falcatum* Went (teleomorphic phase *Glomerella tucumanensis* (Speg.) Arx & Müller), is the most devastating disease of sugarcane, the second most important commercial crop in India, The disease has been fiest described from Java (Indonesia) and the causal fungas was named *Colletotrichum falcatum* (Went, 1893). In India, the disease has been first reported by Barber (1901) in cultivar Red Mauritius in Samalkot region of East Godavari district in coastal Andhra Pradesh. Butler (1906) has conducted extensive studies on this disease, particularly on causal organism, source and mode of infection and proposed red rot as common name. He has first surveyed the sugarcane disease of Bengal. Red rot is prevalent in this region since pre-historic times (Duttamajumder, 2008). Old sugarcane varieties are low yielding but are tolerant to red rot. After the introduction of hybrid canes in this country since 1950, many epidemics of red rot has wiped out important sugarcane varieties in India, such as, Co 313, Co 419, Co 527, Co 997, Co 1008 and Co 1148 from West Bengal (1955-1985). So far, no fungicide is available that can effectively eradicate internal infection of red rot from seed cane (setts). Entire sugarcane breeding in India is now geared around red rot and no sugarcane variety is released for general cultivation without resistance to the prevalent pathotypes of red rot of this zone. For the development of red rot resistant varieties an in depth knowledge is needed on pathogenic variability in red rot fungus and resistance in varieties as the fungus exist with number of strains (Chattopadhyay and Sarkar, 1957). Variation in *C. falcatum* has been reported from India and abroad. Abbott (1935, 1938) has found that failure of variety POJ 213 in USA in 1930-31 is due to a change from the previously existing dark type strain to the more virulent light strain. This is first authentic report of pathogenic variability. Later, pathogenic variability is studied based on differential reaction on a common set of differential varieties. Alexander *et al.* (1985) have classified *C. falcatum* isolates in India into five major pathotypes. Beniwal *et al.* (1989) have also reported three pathotypes 1,2 and 3 based on their differential reactions on varieties Co 7717 and Co 1148 by plug and nodal method of inoculation. Under All India Coordinated Research project on Sugarcane [AICRP(S)] thirteen differentials are identified and using these differentials, Satyavir *et al.* (2001) have reported ten pathotypes from all over India and designated them as Cf 01 to Cf 10.

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Development of new virulent races from existing pathotypes in nature is the primary reason for breaking the red rot resistance in sugarcane cultivars. Even long preservation of *C.falcatum* culture develops black mycelium from white with altered virulence and the cultures are needed to be passed through a susceptible host at regular intervals to maintain virulence. Often combined infection of *C. falcatum* with wilt pathogens *Fusarium moniliforme* (Biswas and Samajpati, 1991), *F. sacchari, Acremonium furcatum* or sett rot pathogen *Ceratocystis paradoxa* (Dade) Moreau. were associated with enhanced disease development. But whether association of more than one pathotypes of *C.falcatum* has any synergistic effect on development of red rot in sugarcane is not well understood. Singh *et al.* (1965) have observed that mixed culture of *C.falcatum* isolated from Co 419 and Co 997 produced lesser lesion length than individual culture on 4 sugarcane varieties. Satyanarayana and Achutaramarao (1982) have inoculated 65 sugarcane varieties with *C.falcatum* pathotypes isolated from sugarcane cv. Co 419 and Co 997 separately and in mixture (1:1) and observed divergent red rot reactions. The present study has been undertaken to evaluate the effect of C. falcatum pathotypes Cf 07 and Cf 08 separately or in combination on developing red rot in sugarcane and on reducing the brix content in sugarcane juice. Cf 07 abd Cf 08 (source sugarcane cv. Co 1148 and Co 7717 respectively) are the designated pathotypes for screening new sugarcane entries of North central & Eastern zone (comprising Eastern Uttar Pradesh, Bihar, West Bengal and Assam) under AICRP(S).

MATERIALS AND METHODS

Sugarcane genotypes Co 0419 and Co 0420 were received from Indian Institute of Sugarcane Research, Regional Centre, Motipur 843111, Muzaffarpur, Bihar; BO 150, CoP 04181, CoP 04182, CoP 05436, CoP 05437, CoP 06436 and CoP 06437 from Sugarcane Reasearch Institute, Rajendra Agricultural University, Pusa 848125, Bihar; CoSe 01423, CoSe 05452, CoSe 06456 from G.S. Sugarcane Breeding & Research Institute, Seorahi, P.O. Tamkuhi, Distt. Kushinagar 274407, U.P.; CoBln 05501, CoBln 04174 and CoBln 05502 from Sugarcane Research Station, Assam Agricultural University, Buralikson, P.O. Baruabamungaon 785618, Golaghat, Asom and CoB 99161 from Sugarcane Research Station, Bethuadahari, West Bengal. The genotypes have distinct genology and are either early (300 days) or mid-late (360 days) duration type. These genotypes were maintained disease free in the field by following the guidelines of Seeds Act 1961 for Breeders Seed (Misra and Singh, 2003). Planting of these sugarcane genotypes was done during February using three-node-stalk cutting (setts) which were soaked in water for six hrs and sterilized in a Moist Hot Air Treatment Plant (MHAT) at 52°C for 2 hrs (Singh *et al.*,1980)followed by sett treatment with 2.5% MEMC (w/v) in water for 15 minutes and air-dried. Normal package of practices were adopted for conducting field experiments.

Cultures of sugarcane red rot pathogen *C. falcatum* pathotypes Cf 07 and Cf 08 were received from the Division of Crop Protection, Sugarcane Breeding Institute, Coimbatore. The cultures were multiplied in oat meal agar (OMA) medium in Petri dishes at 32°C for 7 days under darkness in BOD incubator. From freshly sporulating, 7-day-old culture, spore mass was washed with 100 ml sterile water and collected in a flask. Conidial suspension at a spore concentration of 10^6 conidia/ml (Prakasham *et al.*, 1971) was prepared for inoculation. Sugarcane stalks were inoculated in the field during August by plug method of inoculation. Two canes in each of 20 clumps were inoculated by making a hole in the middle of third exposed internode from bottom without causing any damage or cracking of the cane, two drops of the spore suspension was injected with a large syringe in each cane and sealed with modelling clay to avoid any contamination due to stagnation of water during the rains. This method is rather rigorous by breaching of morphological resistance, but more consistent in grading genotypes, as physiological resistance was considered more important to flight against red rot. The sugarcane genotypes were inoculated with either Cf 07, Cf 08 or with their mixture (1:1, v/v).

The disease evaluation was done by splitting the canes open longitudinally 60 days after inoculation along the point of inoculation. The disease severity was graded on the international scale of 0-9 (Srinivasan and Bhat, 1961; modified by Duttamajumder and Singh, 1999, and accepted by AICRP(S) for evaluation of sugarcane by plug

method for red rot resistance). The scale comprises four variables: (1) Condition of the top assigned the score 0 [Green (G)] or 1 [Yellow (Y) / Dry(D)], (2) Lesion width above to inoculated internode is assigned the score 1(1/3rd), 2 (2/3rd) or 3 (>2/3rd) (Prasadrao *et al.*, 1977), (3) white spot externally on the node is assigned the score of 1 (restricted) or 2 (progressive) and (4) Nodal Transgression (lesion lenght, based on number of nodes crossed above the inoculated internode) given the scores 1 (one node crossed), 2 (two nodes crossed), 3 (three or more nodes crossed). Average score was calculated by dividing total score by number of canes evaluated. Disease reaction in 0-9 scale was 0.0 to 2.0 – R (resistant), 2.1 to 4.0 – MR (moderately resistant), 4.1 to 6.0 – MS (moderately susceptible), 6.1 to 8.0 –S (susceptible) and above 8.0 –HS (highly susceptible). Entries which tested MR to red rot are recommended for release.

Brix % (percentage of total solid in sugarcane juice) of the inoculated stalks was determined at the time of recording red rot score (230 days after planting). Sugarcane juice was collected from the middle internode of the stalk with the help of a pouch piercer and brix % was determined in the field using a hand held refractometer (ERMA, Japan). The experiment was laid out in Factorial RBD design taking sugarcane genotype as main plot and *C.falcatum* pathotype as sub-plot. For collection of data, single sugarcane stalk was taken as plot with 5 replicates.

RESULTS AND DISCUSSION

Differential reaction was observed among sugarcane genotypes against the *C.falcatum* pathotypes Cf 07 and Cf 08. Genotypes CoBln 05501 and CoB 99161 were relatively more susceptible to Cf 07, wherea, Co 0420, BO 150, CoP 04181, CoP 05436, CoP 06436, CoP 06437 and CoBln 04174 were more susceptible top Cf 08. Overall, Cf 08 was more virulent pathotype. The disease score of combined inoculation of Cf 07 and Cf 08 was higher than any of the single pathotype in respect of sugarcane genotypes Co 0419, Co 0420, CoB 99161, CoSe 05452, CoP 06437, CoBln 04174 and CoBln 05502. This indicated synergistic relationship between the two pathotypes to develop red rot disease in sugarcane (Table 1).

Table 1 : Effect of *Colletotrichum falcatum* pathotypes on development of red rot in sugarcane

Sugarcane genotype	Red rot developed by *C. falcatum* pathotypes Score (Grade)		
	Cf 07	Cf 08	Cf 07 +Cf 08
Early (300 days)			
Co 0419	2.0 (R)	2.0 (R)	4.2 (MS)
Co 0420	1.2 (R)	2.4 (MR)	4.6 (MS)
BO 150	1.4 (R)	2.4 (MR)	2.8 (MR)
CoP 04181	0.6 (R)	2.0 (MR)	3.6 (MR)
CoP 05436	0.8 (R)	2.6 (MR)	3.2 (R)
CoBln 05501	4.2 (MS)	3.2 (MR)	4.0 (MR)
CoB 99161	4.0 (MR)	3.0 (MR)	6.2 (MS)
Mid-late (360 days)			
CoSe 01423	2.6 (MR)	3.2 (MR)	3.8 (MR)
CoSe 05452	1.4 (R)	2.0 (R)	4.2 (MR)
CoSe 06456	1.6 (R)	2.0 (R)	2.0 (R)
CoP 04182	2.0 (R)	2.2 (MR)	2.6 (MR)
CoP 05437	2.0 (R)	2.2 (MR)	1.8 (R)
CoP 06436	1.0 (R)	4.4 (MS)	1.8 (MR)
CoP 06437	0.6 (R)	1.8 (MR)	2.6 (MR)
CoBln 04174	1.8 (R)	3.0 (MR)	4.2 (MS)
CoBln 05502	1.6 (R)	2.2 (MR)	3.2 (MR)
CD (P = 0.05)			
C. falcatum pathotype	0.8		
Sugarcane genotype	NS		
Interaction	NS		

For confirmation of the result further investigations are needed involving 14 sugarcane differentials of AICRP(S) viz. *Baragua* (*S. officinarum*), *Khakai* (*S. sinense*), SES 594 (*S. spontaneum*), CoS 767, BO 91, CoC 671, Co 7717, Co 997, CoJ 64, Co 1148, Co 419, Co 62399, Co 975 and CoS 8436 and involving more *C. falcatum* pathotypes. This result has far reaching implications because plug method of inoculation is already the most rigourous testing method for red rot resistance in sugarcane. Use of combined pathotypes for inoculation may increase disease pressure due to synergistic effect resulting in the elimination of more number of entries than needed.

The inoculation of *C. falcatum* pathotypes had no effect on brix% of sugarcane juice (Table 2) because the data were recorded at pre-mature stage (230 days after planting) when disease development just started.

Table 2 : Effect of *Colletotrichum falcatum* pathotypes on brix % sugarcane juice

Sugarcane genotype	Juice brix(%) of sugarcane inoculated with *C. falcatum* pathotypes			
	Cf 07	Cf 08	Cf 07+Cf 8	Control
Early (300 days)				
Co 0419	17.1	18.2	16.7	15.5
Co 0420	16.0	18.5	14.9	18.0
BO 150	19.4	19.7	17.9	19.6
CoP 04181	18.3	19.6	19.3	15.9
CoP 05436	16.1	17.9	18.7	18.8
CoBl 05501	15.6	17.4	17.1	17.8
CoB 99161	12.4	16.3	16.6	18.4
Mid-Late (360 days)				
CoSe 01423	18.9	19.9	18.0	18.4
CoSe 05452	15.3	17.3	16.8	14.9
CoSe 06456	18.0	19.8	20.9	18.8
CoP 04182	20.3	17.6	20.0	20.7
CoP 05437	12.4	12.8	15.5	16.0
CoP 06436	18.8	16.0	18.5	19.2
CoP 06437	15.8	16.8	16.8	14.2
CoBln 04174	18.0	17.0	16.8	17.2
CoBln 05502	16.8	17.5	17.6	13.7
CD (P = 0.05)				
C. falcatum pathotype	NS			
Sugarcane genotype	0.6			
Interaction	NS			

However, variability was observed in brix content of juice among the genotypes indicating their genetic divergence in respect of accumulation of sucrose and maturity.

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REFERENCES

Abbott, E.V. 1935. Physiological specialization in *Colletotrichum falcatum* Went. *Proceedings of International Society for Sugar Cane Technologist* (ISSCT), **5:** 730-736

Abbot, E.V. 1938. Red rot of sugarcane. *U.S. Department of Agriculture, Technical Bulletin.* 641 : 96.

Alexander, K.C., Rao, M.M. and Mohanraj, D. 1985. Disease Reaction-Catalogue on genetic Resources II, *Sugarcane Breeding Institute, Coimbatore.* pp 226.

Barber, C.A. 1901. Sugarcane disease in Godavari and Ganjam districts. *Madras Department Land Record of Agriculture Bulletin.* **43:** 181-194.

Beniwal, M.S., Satyavir and Virk, K.S. 1989. Pathogenic variability in *Colletotrichum falcatum* incitant of red rot of sugarcane. *Indian Phytopathology.* **42:** 95-98.

Biswas, A. and Samajpati, N. 1991. Study of ecological relationship of *Colletotrichum falcatum* with other fungi in sugarcane stalk. *Journal of Mycopathological Research.* **29(1) :** 67-75.

Butler, E.J. 1906. Fungal diseases of sugarcane in Bengal. Memoirs of Department of Agriculture, *India, Botanical Series.* **1:** 2-24.

Chattopadhyay, S.B. and Sarkar, A. 1957. Strains of *Colletotrichum falcatum* Went in West Bengal. *Indian Journal of Mycological Research.* **3 :** 25-28.

Duttamajumder, S.K. 2008. Red rot of sugarcane. *Indian Institute of Sugarcane Research, Lucknow,* India.

Duttamajumder, S.K. and Singh, R.P. 1999. Received criteria for grading resistance against red rot disease of sugarcane. *Indian Phytopathology,* **52:** 157-159.

Misra, S.R. and Singh, P.K. 2003. Sugarcane Nucleus and Breeder Seed Production Manual. *Indian Institute of Sugarcane Research, Lucknow.*

Prakasam, P., Appalanarasiah, P. and Satyanarayana, Y. 1971. Note on an improved method of screening sugarcane varieties against red rot disease. *Indian Journal of Agricultural Sciences.* **41 :** 1131-1133.

Prasadrao, K.K., Sarma, M.N., Satyanarayana, Y. and Achutaramarao, M. 1977. Discriminant function as reliable guide for assessing varietal reaction to red rot of sugarcane. *Proceedings of ISSCT.* **16 :** 295-301.

Satyavir, Singh, N., Virk, K.S., Nageswararao, GV., Singh, H. and Mishra, S.R. 2001. Pathogenic variability in sugarcane red rot system. In : *Proceedings of National Symposium on Role of Resistance in Intensive agricultute (EDS., Nagarajan,* S. and Singh, O.P.). pp. 109-114. Kalyani Publishers, Ludhiana.

Satyanarayana, Y. and Achutaramarao, M. 1982. Studies on the reaction of different cane varieties to light race isolates of red rot fungus from CO 419 and CO 997 individually and in combination. *Maharashtra Sugar.* **7(10):** 27-30.

Singh,K., Misra, S.R., Shukla, U.S. and Singh, R.P. 1980. Moisthot air therapy of sugarcane : control of sett-borne infections of GSD, smut and red rot. *Sugar Journal* **43:** 26-28.

Singh, R.K., Srivastava, R.N.P. and Thakur, M. 1965. Efficacy of pure and mixed inocula of *Colletotrichum falcatum* Went. *Indian Sugar.* **9 :** 123-124.

Srinivasan, K.V. and Bhat, N.R. 1961. Red rot of sugarcane-Criteria for grading resistance. *Journal of Indian Botanical Society,* **40** : 566-577.

Went, F.A.F.C. 1983. Het Root Snot. *Arch. Java Suikerindustrie.* **1:** 265-282.