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Short Communication

Comparative study on early detection of sugarcane smut (*Sporisorium scitamineum)* by polymerase chain reaction (PCR) and microscopy

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Sugarcane smut caused by *Sporisorium scitamineum* has the potential to result in substantial tonnage loss and significant reduction in sucrose recovery. As early and precise diagnosis is an integral component in the successful management of sugarcane smut, the present study was undertaken to accurately determine the presence of pathogen employing PCR-based methods supplemented with microscopy. Healthy sets of sugarcane cultivars *viz.,* Co 96007 (Susceptible) and Co 6806 (Resistant) were challenge inoculated by hypodermal injection with teliospore suspension of *S. scitamineum* (containing 1 x 10^6 teliospores/ml) and planted in sterile soil with appropriate uninoculated controls. Actively growing meristem of the plantlets was sampled at different time points for examination with microscopy and PCR using primers *bE4* and *bE8* of mating type genes. The whole experiment was conducted for eight weeks and meristem tissue was sampled weekly starting from three weeks post inoculation. Our results show that the PCR assay is more sensitive in early detection of the pathogen (3^{rd} week) in both susceptible and resistant cultivars as compared to microscopic observations of the meristem samples stained with lactophenol cotton blue. However, the pathogen could not be detected from the 4th week onwards in resistant variety Co 6806. In microscopy assay, mycelial colonization was evident only from the 5^{th} week onwards in the susceptible cultivar Co 96007, but not in the resistant cultivar Co 6806 at any of the time intervals until 8 weeks post inoculation. Results of this study suggest that, for the early and precise detection of smut pathogen in sugarcane, the PCR-based assay should be considered as a suitable diagnostic tool rather than microscopy. This could add to effective sugarcane quarantine and successful management of sugarcane smut.

Key words: Smut pathogen, *Saccharum officinarum*, cultivars, pathogen detection, light microscopy, host resistance.

INTRODUCTION

Sugarcane smut is caused by the basidiomycete fungus *Sporisorium scitamineum* (syd.) (Piepenbring et al., 2002 (Syn: *Ustilago scitaminea* H. and *P.* Sydow)) that belongs to the fungal sub-class *Ustilaginaceae*. *S. scitamineum*

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was first noted in 1877, in the Natal region of South Africa (Martin, et al., 1961) and has been a serious threat for sugarcane cultivation in India and other parts of the world for many years. It can devastate susceptible sugarcane varieties by quick spreading and considerable reduction in yield (Fletcher, 2013). In the 1930's, *S. scitamineum* caused severe problems in India and since then it became widespread in most of the sugarcane growing states in the country (Viswanathan et al., 2009).

The life cycles of the smut fungi are similar for all species and involve transitions between three cell types. Diploid teliospores are the resting cell type and are disseminated mainly by wind or rain splashes. They germinate by forming a probasidium on which, following meiosis, four sporidia emerge. The haploid sporidia represent the second cell type. They grow by budding, and compatible (opposite mating-types/plus and minus) sporidia fuse to give rise to the dikaryotic pathogenic third cell type which exhibits mycelial growth (Alexopoulos, 1962). Karyogamy takes place in the dikaryotic mycelium and diploid teliospores are formed within the host tissues (Bakkeren and Kronstad, 1993). The life cycle is regulated by the *a* and *b* mating-type loci within the sporidia. *a* has two alleles which encode a pheromone and a receptor whilst *b* is multiallelic and appears to control pathogenicity and sexual development (Bakkeren et al., 1992). With the use of primers based on the *U. maydis b*E mating-type gene, (Albert and Schenck, 1996) the corresponding gene was sequenced in *U. scitaminea*. Molecular detection of the smut pathogen in sugarcane has since become possible by using PCR to amplify the *b*E mating-type gene of *U. scitaminea*

To control the disease, sugarcane seeds are treated with hot water and breeding for resistance is perfomed; all of which increases the costs for production. Hence, early and accurate diagnosis of plant disease is a crucial component of *S. scitamineum*-sugarcane as well as other pathogen-management systems. To detect meristem colonization of sugarcane with *S. scitamineum,* previous studies performed histopathological examinations of the infected stalk (Alexander and Ramakrishan, 1980; Waller, 1970). However, this has implications on the accuracy of the prediction as microscopy is insufficient to distinguish between different fungal pathogen species. In the recent years, molecular biology techniques like PCR involving specific primers is aiding significantly in early detection and evaluation of plant diseases. The objective of this study was to evaluate the accuracy and sensitivity of the diagnostic tools - PCR with pathogen-specific primers and microscopy to detect smut.

MATERIALS AND METHODS

Healthy plants of susceptible Co 96007 and resistant Co 6806 cultivars were placed for three to four days in moistened gunny bags for sprouting. Sprouted buds were de-scaled and subjected to hypodermal syringe inoculation with *S. scitamineum* teliospore suspension containing 1 x 10^6 spores/ml, without damaging the buds. Inoculated buds were planted in pots along with un-inoculated sprouted buds (syringe inoculated with sterile water) which served as mock-control and the pots were maintained under ideal glass house conditions. After germination, meristem from the control and inoculated plantlets were sampled at weekly intervals from three to eight weeks post inoculation (wpi) and were subjected to the PCR based assay and microscopic examination. DNA was extracted from the meristem of the plantlets and subjected to PCR using *bE4* and *bE8* primers (Albert and Schenck, 1996). The reaction mixture also contained 0.2 mM dNTPs, 0.5 mM Mg Cl_2 and 1.5 unit μL^{-1} Taq DNA polymerase. The reaction was run for 30 cycles of denaturation at 94°C for 30 s, annealing at 57°C for 30 s and extension at 72°C for 1 min (Ali Moosawi-Jorf and Mahin, 2007). For the microscopic study, meristem was longitudinally cut into ultra-thin sections and fixed with formalin acetic acid (FAA), mounted on a glass slide, stained with 0.1% lacto phenol cotton blue (Lloyd and Naidoo, 1981) examined microscopically and photographed under low (100x) and high (1000x).

RESULTS AND DISCUSSION

In the PCR based method, specific amplicons (459 bp) were observed in all the six intervals (from 3 to 8 wpi) in susceptible variety Co 96007 (Figure 1). In contrast, the resistant variety Co 6806 revealed *bE* amplicons only for 3 wpi. This may result from the restriction of pathogen colonization probably by the host resistance mechanism. The non-inoculated plant samples from both cultivars revealed no PCR products in all the six intervals. In the microscopic study, pathogen colonization in the susceptible variety Co 96007 was examined from 3 wpi. However, evident colonization was observed only from 5 wpi. Interestingly, the presence of the pathogen in the resistant variety Co 6806 could not be detected in any of the intervals (Figure 2). In confirmation with the PCR approach non-inoculated controls were also negative in the microscopic study. Table 1 depicts the results obtained with both diagnostic tools. The accuracy of the microscopic examination is limited and time-consuming. As discussed by Ali Moosawi-Jorf and Mahin (2007), microscopic detection of the sugarcane smut fungus may not be accurate. Moreover, detection and discrimination become difficult at early stages of plant colonization both in field and laboratory conditions, because in the infected tissues smut hypha cannot be discriminated morphologically from other fungal hypha.

Results of this study suggest that the PCR based assay is more sensitive, rapid and accurate compared to microscopic examination of infected plant tissue. The *bE* mating-type gene used in this study for detection is specific for *S. scitamineum*, and the results of PCR were validated using appropriate positive controls from DNA sourced out from the dikaryotic mycelia confirming our results obtained by PCR amplification. Similar study was observed by Toth (1998) the mating-type (*bE*) gene to detect the smut pathogen is more specific and extremely sensitive in deduction of pathogen. Dalvi et al. (2012) finding similar observation during field and PCR screening to evaluate the clones to confirm smut infections whip production is the most reliable symptom of smut disease

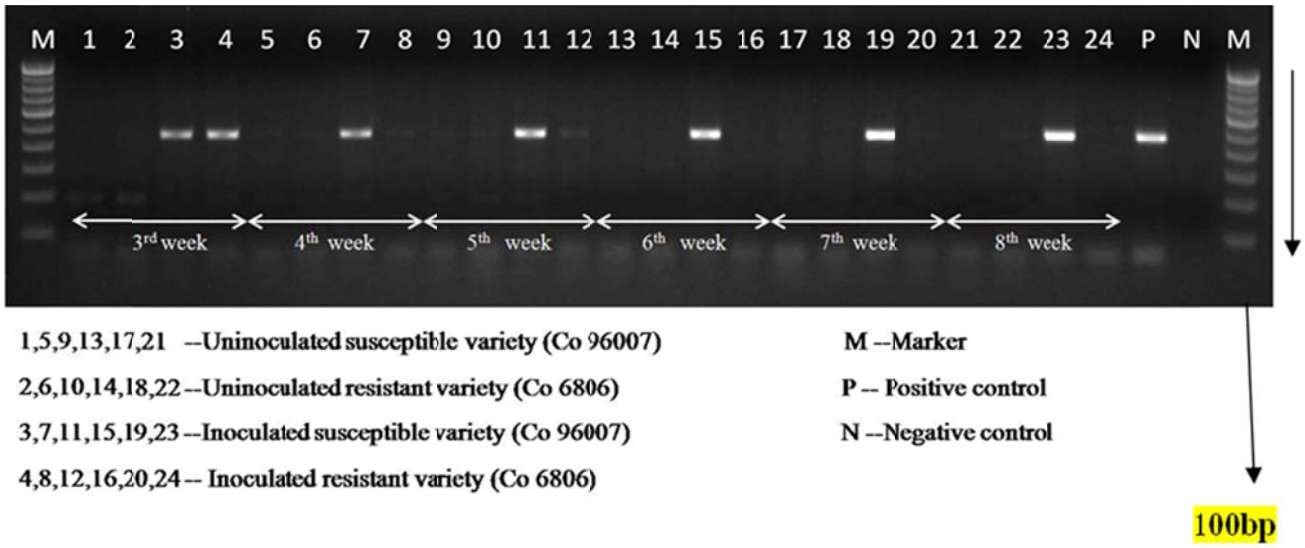

Figure 1. Profile of the detection of *Sporisorium scitamineum* by PCR 100 bp.

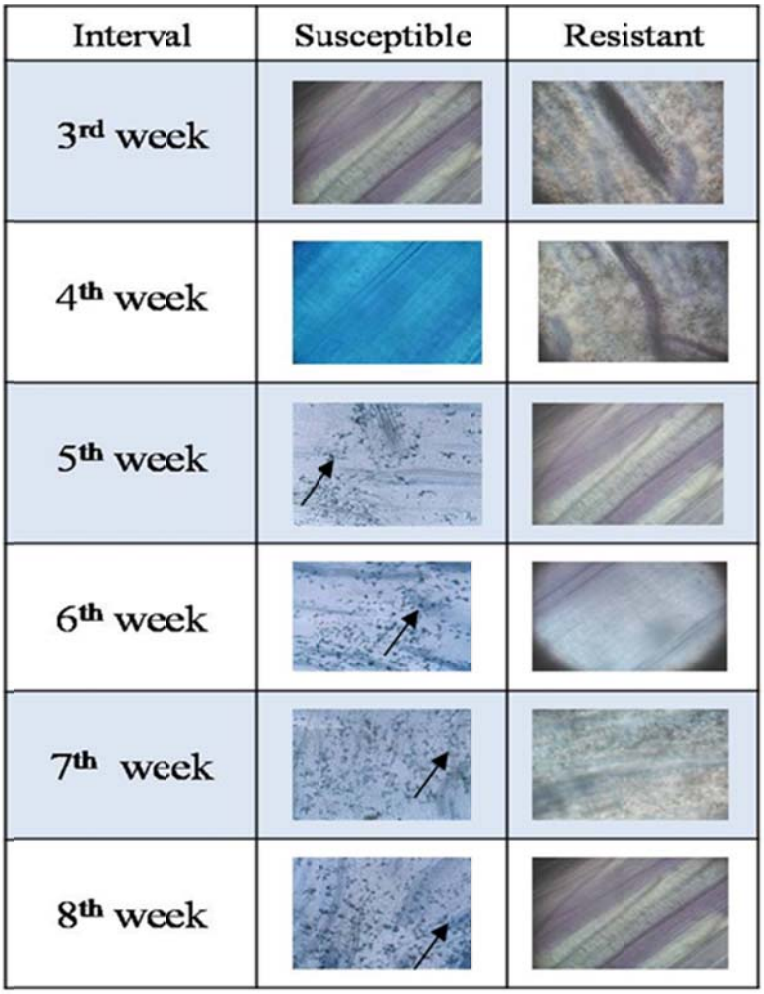

Figure 2. Detection of *Sporisorium scitamineum* by Light Microscopy.
→ - indicates the pathogen colonization and intensity.

Table 1. Detection of *Sporisorium scitamineum* by PCR and microscopic study.

Variety	Diagnostic technique	Result at different time intervals					
		3rd week	4th week	5th week	6th week	7th week	8th week
Co 96007	PCR	+ve	+ve	+ve	+ve	+ve	+ve
(Sensitive)	Microscopy	-ve	-ve	+ve	+ve	+ve	+ve
Co 6806	PCR	+ve	-ve	-ve	-ve	-ve	-ve
(Resistant)	Microscopy	-ve	-ve	-ve	-ve	-ve	-ve

in sugarcane but when there is no whip expression due to environmental conditions, the infested sugarcane plants, often tiller profusely with the shoots being more spindly and the leaves being more upright and narrow emerging from the shoots following infection. Similar study was reported by Singh et al. (2004).

Conflict of Interests

The author(s) have not declared any conflict of interests.

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