SHORT COMMUNICATION

A REPORT OF *USTILAGO CYNODONTIS* INFECTING THE BERMUDA GRASS - *CYNODON DACTYLON* IN COIMBATORE, TAMIL NADU

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Abstract

Cynodon dactylon (Bermuda grass) plants were predominantly found to be infected by smut in discontinuous stretches alongside the sugarcane experimental fields. Frequency of smutted inflorescence was relatively higher in plants that were in close proximity to the border rows of sugarcane. This invoked our interest on the identity of the weed smut pathogen and if *C. dactylon* could be an alternate host that can harbor the sugarcane smut pathogen - *Sporisorium scitamineum*. Interestingly, PCR amplification with *S. scitamineum* (*bE*) gene specific primers yielded consistent negative results, indicating that the smut fungus infecting *C. dactylon* is not *S. scitamineum.* Scanning Electronic Microscopic (SEM) examination did not reveal any distinguishable difference between the teliospores of sugarcane smut and the weed smut fungi. PCR amplification involving ITS primers 1 and 4 indicated that the PCR amplicon from the weed smut fungus shared 99% identity with *Ustilago cynodontis* sequences. It was further established that *U. cynodontis* infecting *C. dactylon* is not pathogenic to sugarcane for the present. Besides, in view of the fact that Bermuda grass shares a common ecological niche with sugarcane, the possibility of *U. cynodontis* switching over to sugarcane as a pathogen is foreseen.

Key Words: Sugarcane, smut, *Sporisorium scitamineum,* Bermuda grass, *Cynodon dactlyon, Ustilago cynodontis,* inflorescence, teliospores, ITS

Cynodon dactylon (L.) Pers. is a perennial grass with slender, apparently prostrate stems that spreads laterally by rhizomes and branching stolons. It is cultivated as a turf grass in landscape designs and is also known for a wide range of medicinal and health benefits (Auddy et al. 2003). However, it is regarded as one of the most invasive and competitive weeds, especially in sugarcane ecosystem, as it can rapidly proliferate and invade cultivated lands. The weed can cause serious yield loss (Beltrao et al. 1978) and is a potential host capable of harboring various pathogens and pests (Bogdan 1977; Marley 1995). *Ustilago cynodontis* (Pass.) Henn. is an obligate fungus belonging to Ustilaginales family, causing smut in *C. dactylon*. The infection results in complete modification of awn-bearing spikelets to sori with teliospores, and reduction in dry matter production and growth rate of stolons in *C. dactylon*. Since the inflorescence of infected grass is completely modified into sori bearing teliospores, no viable seeds are produced (Garcýìa-Guzmán and Burdon 1997). *C. dactylon* and sugarcane belong to the same clade (PACMAD) of Poaceae family and the genetic relatedness between *U. cynodontis* and *Sporisorium scitamineum* (sugarcane smut pathogen) is also established (Stoll et al. 2003).

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During October-November 2014, smutted *C. dactylon* plants were found in discontinuous stretches alongside border rows, in the vicinity of sugarcane experimental fields of Sugarcane Breeding Institute, Coimbatore, Tamil Nadu, India (Fig.1a). Climatic conditions were relatively dry and warm, favoring opulent growth of *C. dactylon*. Smutted inflorescences were distorted, usually failed to develop fully and were covered with brown-black teliospores (Fig. 1b). Teliospores collected from the smutted inflorescences were examined under light microscope (Fig. 1c) and Scanning Electron Microscope (SEM) (Fig. 1d). Teliospores (n=30) were globose to sub-globose, smooth-walled and echinate, ranging from 5.2 to 6.8 µm x 4.8 to 6.5 µm. Minor differences in size, shape and echination were observed between the teliospores of *C. dactylon* smut fungus and *S. scitamineum*. However, microscopic examination of the teliospore morphology did not yield any conclusive information on the identity of the grass smut fungus.

For subsequent germination assay and molecular analyses, teliospores from smutted inflorescence of *C. dactylon* were surface sterilized with 1% sodium hypochlorite, rinsed with distilled water and plated on potato dextrose agar (PDA) medium. After incubation at 25°C for 5 days, teliospores germinated giving rise to four-celled basidia, followed by lateral and/or terminal emergence of basidiospores, which were almost ellipsoidal in shape (Fig. 1e). Genomic DNA was extracted from the resultant single sporidial and mycelial colonies. Reports of *C. dactylon* being an alternate host for *Sporisorium*

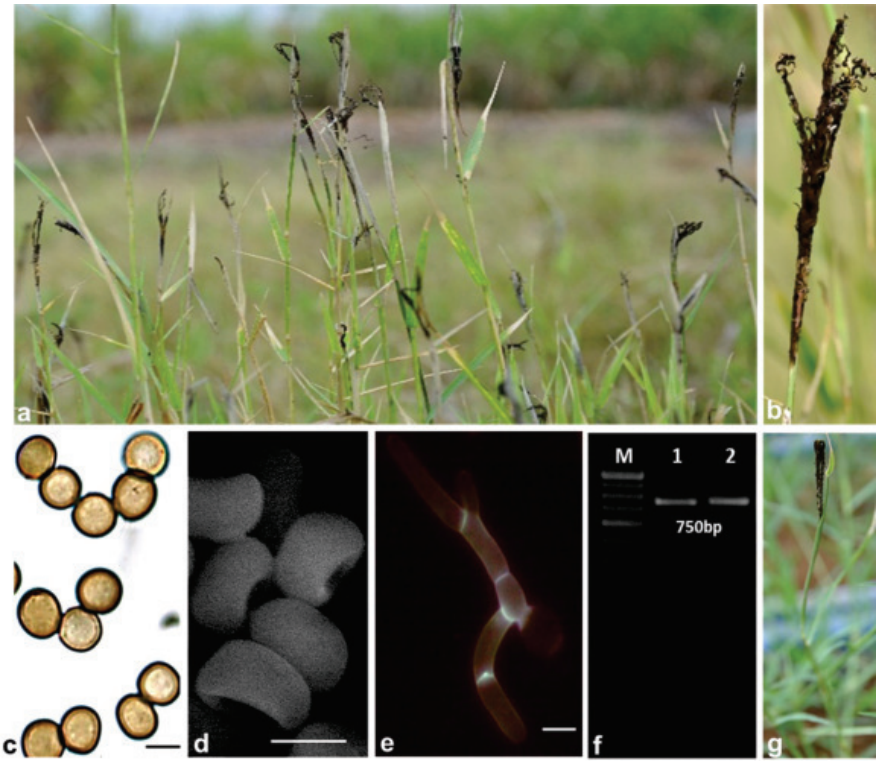

Fig.1 a. Prevalence of smut infected *C. dactylon* at close proximities to sugarcane; b. smutted inflorescence of *C. dactylon*; c. teliospores under light microscope (100x); d. teliospores under SEM (12,000x); e. emergence of basidiospores from germinated teliospores stained with Calcofluor white. (Scale bar represents 5µM); f. ITS region amplified using ITS1/4 from *U. cynodontis* genomic DNA; g. proving Koch's postulates in glass house

sorghi - the pathogen causing cover smut in sorghum (Marley 1995) necessitated us to verify, if this weed grass harbors the sugarcane smut pathogen. PCR amplification with *S. scitamineum* (*bE*) gene specific primers (Albert and Schenck 1996) yielded consistent negative results, indicating that the smut fungus in *C. dactylon* was not *S. scitamineum.* The identity of the grass smut pathogen was confirmed by amplifying the ITS region using ITS 1 (TCCGTAGGTGAACCTGCGG) and 4 (TCCTCCGCTTATTGATATGC) primers (White et al.1990). PCR amplification was carried out in 25 µl reactions, each reaction mixture consisting of 50ng of genomic DNA template, 40 micro moles of ITS 1 and 4 primers, 2µl of 2.5mM dNTPs, 2.5µl of 10X Taq buffer and 0.33µl of Taq Polymerase; the final volume was made up to 25 µl with Milli Q water. Following were the PCR conditions used: initial denaturation at 94ºC for 4min, 35 cycles of denaturation at 94ºC for 30s, annealing at 54 ºC for 45s, extension at 72ºC for 30s and final extension at 72ºC for 8min.The resultant amplicons (750bp) (Fig. 1f) were sequenced and deposited in GenBank under accessions KP834589 and KP834590. BLAST search and alignment of these sequences against the *U. cynodontis* sequences submitted previously in GenBank (AY740168.1, KM213625.1 and HM143013.1) indicated 99% identity. Alignment of *U. cynodontis* (KP834589) and *S. scitamineum* (KP893340) ITS sequences indicated 80% similarity, which in turn highlighted that these two smut fungi are phylogenetically closer. An ITS-based report on the phylogeny of *Ustilago* and *Sporisorium* spp. by Stoll et al. (2003) further substantiated the genetic relatedness between these two smut fungi.

To validate Koch's postulates, healthy stolons of *C. dactylon* were inoculated by immersing in a suspension consisting of *U. cynodontis* teliospores ($5x10^6$ per ml) collected from smutted inflorescences of infected plants. The inoculated stolons along with respective mock inoculated controls were planted under green-house condition for observation. Characteristic symptoms of smutted inflorescence were observed in all inoculated plants at 15dpi and no symptoms were observed in control plantlets thus confirming the Koch's postulates (Fig. 1g). In addition, cross-infectivity of *U. cynodontis* was examined on smut susceptible sugarcane cultivar (Co 96007), which did not result in any phenotypic symptoms associated with smut. Also, *S. scitamineum* inoculation on *C. dactylon* did not result in any smut symptoms. This indicated that *U. cynodontis* infecting *C. dactylon* is not pathogenic to sugarcane for the present. However, smut incidence in *C. dactylon* exclusively at close proximities to sugarcane is intriguing. Taking into cognizance the genetic relatedness of the two smut fungi, phylogenetic relatedness between the grasses and the selection pressure imposed by weed eradication programs, a possibility of *U. cynodontis* evolving and adapting to infect sugarcane is foreseen. Phylogenetic proximity of the hosts and identical nutritional/physiological requirements of the pathogens are the major reasons attributed for host range expansion. There are several reports on the shift of host range, notably in a basidiomycetes fungus *Microbotryum* sp. (anther-smut pathogen of *Caryophyllaceae*) (de Vienne et al. 2009). Interestingly, long-range host jumps from monocot to dicot plants have also been evidenced in *Melanopsichium pennsylvanicum* (gall smut pathogen of *Persicaria* species). Based on comparative genome analyses of *M. pennsylvanicum* with other related smut fungi, viz. *U. maydis, S. reilianum, and U. hordei*, it was concluded that loss of genes is required for such broad range host expansions, rather than gain of genes (Sharma et al. 2014). Giraud et al. (2010) has reviewed the impact of various extrinsic and intrinsic factors on host range expansion and has

elaborated on the congruence of host-range expansion and emergence of new plant diseases. To our knowledge, this is the first report of *U. cynodontis* infecting *C. dactylon* in Coimbatore, India and this further gains importance by the very fact that Bermuda grass shares a common ecological niche with sugarcane vis-à-vis the possibility of *U. cynodontis* switching over to sugarcane as a pathogen.

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