SHORT NON-REFEREED PAPER

TAWNY RUST: AN UPDATE ON THE NEW SPECIES OF RUST INFECTING SUGARCANE IN SOUTHERN AFRICA

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Abstract

A new species of rust, originally referred to as African sugarcane rust, but recently renamed tawny rust, was first reported in Swaziland in 2008. This rust has since spread to most cane growing areas in South Africa, and more recently to sugarcane fields in Mozambique and Zimbabwe. It has been observed on a number of South African sugarcane varieties grown under varying environmental conditions. Infected sugarcane leaves display typical rust symptoms, with brown lesions developing parallel to the leaf midrib and profuse bright orange urediniospores released from the uredinia. Molecular phylogenetic analyses together with morphological examination confirmed that this rust is a new, previously undescribed species. A full taxonomic description has been developed and the name proposed for this newly discovered disease of sugarcane is tawny rust, caused by *Puccinia fulva* sp. nov. This short paper serves as an update and brief overview of the tawny rust research currently under way at the South African Sugarcane Research Institute (SASRI), which includes: (a) a thorough investigation into the phylogenetic placement of this species within a broader context of the Pucciniales and the overall Basidiomycota based on a portion of the rDNA (including 5.8S, ITS2 and 28S nuclear large subunit (nLSU) genes), (b) the development of a molecular diagnostic tool for rapid identification of the pathogen using polymerase chain reaction, (c) laboratory and field-based studies to determine the optimum environmental conditions for spore germination and rust development and (d) fungicide trials to determine the effect of this disease on sugarcane yield and future management strategies.

Keywords: African sugarcane rust, tawny rust, *Puccinia fulva* sp. nov., Pucciniales, diagnostic PCR, yield

Introduction

Prior to 2008, sugarcane grown around the world was commonly infected by two rust types, namely brown rust caused by *Puccinia melanocephala* (H&P Sydow) and orange rust caused by *Puccinia kuehnii* (EJ Butler) (Egan, 1964). Brown rust has been prevalent in the South African sugar industry since the mid-1970s (Bailey, 1979; Bailey, 1995) and causes yield losses in the region of 12-26% on susceptible varieties (McFarlane *et al*., 2006). Orange rust, although prevalent in the eastern and western hemispheres, has not been observed on sugarcane in South Africa to date; however, it was reported in western and central Africa in

2010 (Saumtally *et al*., 2011). Since the discovery of a new rust on sugarcane in southern Africa in 2008, research has focused on describing and classifying this new sugarcane pathogen, now referred to as tawny rust, and understanding its epidemiology. Trials investigating yield effects and efficacy of fungicides were established.

Methods

Molecular sequence data and phylogenetic analyses were used to place this rust in context with the other sugarcane rusts and within the order Pucciniales. Phylogenetic analyses initially focused on the 5.8S and 28S nuclear large subunit (nLSU) genes with a limited number of other rust species included in the tree. A more computationally rigorous phylogenetic analysis which is currently underway, includes the full ITS2 region within the nLSU gene, and approximately 70 fungal taxa have been added to the original dataset.

A specific PCR diagnostic assay for tawny rust is being developed to assist with the identification of this rust. Although it is possible to distinguish brown, orange and tawny rust microscopically based on spore morphology, a rapid diagnostic assay using specific primers is required to accurately and definitively identify tawny rust. This method, once fully established, will be particularly useful for researchers in other sugarcane growing regions who require a rapid diagnostic method for the identification of tawny rust. The fungal ribosomal DNA (rDNA) gene, including the ITS2 and 28S regions, was the general target and PCR primers, AR-F2 (5' GAC TTC TAA ACA CGC AAT A 3') and AR-R1 (5' CCT TGG GGT CCC ACC TTA TTA 3'), were designed based on conserved regions, specific to tawny rust, within alignments of rust sequences accessioned in the National Centre for Biotechnology Information (NCBI) Genbank database. These primers amplified a 660 base pair product from tawny rust genomic DNA. PCR reactions consisted of a final volume of 30 µL using the KAPA2G Robust PCR kit (Kapa Biosystems (Pty) Ltd, SA) containing the following: 6 µL 5X KAPA2G Buffer A, 6 µL 5X KAPA Enhancer 1, 0.6 µL dNTP mix (10 mM each), 15 µM of each primer (AR-F2 and AR-R1), 0.75 U KAPA2G Robust HotStart DNA Polymerase and 10 ng of template DNA. The PCR was performed using a touchdown annealing approach (annealing temperature decreasing from 68-58°C in 20 cycles) for improved primer specificity to the target DNA. The thermocycling profile consisted of initial denaturation at 94°C for 5 min, followed by 20 cycles of 94°C for 30 s, 68-58°C for 45 s, 72°C for 30 s, followed by 15 cycles of 94°C for 30 s, 58°C for 30 s, 72°C for 30 s, a final extension of 72°C for 7 min, and a holding temperature of 4°C. These primers were tested against 14 other fungal species, including the other two sugarcane rusts to confirm exclusive specificity to tawny rust. Since genomic DNA of *P. kuehnii* was not available at the time of testing, *P. kuehnii* plasmid DNA was used instead. The plasmid DNA was generated using the primers Rust2Inv (Aime, 2006) and LR6 (Vilgalys and Hester, 1990) which flank upstream and downstream of the AR-F2 and AR-R1 primer regions respectively. *P. kuehnii* genomic DNA is currently being sent from Argentina and Australia and upon receipt will be tested accordingly. PCR using general fungal primers ITS4 and ITS5 (White *et al*., 1990) was run in parallel to the tawny rust specific PCR to verify fungal DNA quality and to ensure the extracted DNA was amplifiable.

A laboratory based study was conducted to determine the environmental conditions favouring tawny rust spore germination. Spores were placed onto microwell slides using a dissecting needle, and these slides were placed in petri dishes containing filter paper pre-wetted with 1 ml sterile deionised water. To determine whether spores require free water to germinate, 10 µL of sterile deionised water was placed onto each microwell on the slide representing wet

conditions, whereas the slides representing dry conditions were left dry. The petri dishes were incubated at temperatures ranging from 12-35°C at 100% humidity for 18 hours. Thereafter, the number of germinated spores was recorded using a light microscope at 200X magnification.

In a rainfed trial at Bruyns Hill (Midlands North), plots (10 m x 5 rows) of tawny rust-infected N16 were sprayed weekly with mancozeb (a non-growth promoting fungicide) from October through November 2013 to determine the effect of the disease on yield. Paired plots were left unsprayed and the trial was replicated six times. The trial was harvested at 20 months. A further trial was established in Pongola under irrigated conditions to test the efficacy of three fungicides against tawny rust infecting N25. The trial was sprayed with the three fungicides on 18 April and 16 May 2013, and mancozeb was applied weekly for two months over this period to determine yield effects. The trial was replicated four times.

Results and Discussion

Initial phylogenetic analyses revealed that the causal organism of tawny rust, proposed name *Puccinia fulva* sp. nov., is most closely related to *P. physalidis*, a rust species that infects *Physalis* species, a genus of flowering plants in the nightshade family (Solanaceae). The next closest relative, *P. sparganioides*, is known as ash rust and infects *Fraxinus* species, several species of cordgrass (*Spartina* species) and a marsh grass (*Distichlis spicata*) (Gillman, 2011). Although *P. fulva* sp. nov. is genetically distinct from both *P. melanocephala* and *P. kuehnii*, these three sugarcane rust species group neatly into the *Pucciniaceae* family clade. A more comprehensive analysis is currently underway that will give a broader perspective of the placement of this new rust species within the Phylum Basidiomycota and will provide further insights to the ancestral lineage of *P. fulva* sp. nov.

The specific tawny rust PCR generated fragments of rDNA of the expected length from *P. fulva* sp. nov. using primers AR-F2 and AR-R1. To verify that the amplified fragment was the target of interest, direct sequencing was carried out and the resultant sequences were subjected to BLAST searching using the NCBI database. Resultant sequences matched the tawny rust accessioned sequences (JX036026, JX036026, JX036028) on Genbank with a 100% identity match. When the primers were tested against other members of the *Puccinia* genera and a number of fungal species commonly associated with sugarcane to confirm specificity to *P. fulva* sp. nov. (Table 1), primers AR-F2 and AR-R1 only amplified *P. fulva* sp. nov.

Laboratory-based spore germination studies showed that tawny rust spores were able to germinate under both wet and dry conditions at 100% humidity, unlike brown rust spores that require free moisture for germination (Purdy *et al*., 1983; Raid and Comstock, 2000; Ramouthar, 2009). Conditions for the germination of tawny rust spores were, however, optimal when free moisture was available and at temperatures between 18 and 22°C (brown rust 20-25°C), (Hsieh *et al*., 1977; Ramouthar, 2009), with highest germination rates recorded at 19°C. Field observations support these findings with tawny rust infections tending to begin and end earlier in the spring/summer season than brown rust.

In the yield loss trial at Bruyns Hill the mean cane yield in the sprayed plots was 9.7% higher than the unsprayed plots, but this difference was not significant. Tawny rust infection in the Pongola trial was inconsistent but generally mild in the plant crop and no differences in yield were recorded. While tawny rust did not have a substantial impact on yield in these two trials,

general field observations suggest that losses may be higher in more severely infected fields and determination of the full impact of tawny rust on cane and sugar yield is ongoing.

Research on this pathogen is continuing, with the more comprehensive phylogeny currently being finalised, and ongoing yield loss and variety screening trials are underway.

Table 1. Rusts and other fungal species used to test the tawny rust specific primers developed in this study. General fungal primers ITS4 and ITS5 were used as a standard to confirm DNA quality.

Rusts and other fungal species	Primary host plant common name	PCR primers tested	
		***P. fulva* sp. nov. AR-F2 and AR-R1**	**General fungal ITS4 and ITS5**
P. fulva sp. nov.	sugarcane	+	+
Puccinia melanocephala	sugarcane	-	+
*Puccinia kuehnii**	sugarcane	-	NR
Puccinia graminis f. sp. *tritici* (UVPgt50)	wheat	-	+
Puccinia graminis f. sp. *tritici* (UVPgt55)	wheat	-	+
Puccinia striiformis	wheat	-	+
Puccinia triticana (UVPt5)	wheat	-	+
Puccinia triticana (UVPt10)	wheat	-	+
Puccinia striiformoides	cock's-foot	-	+
Puccinia coronata	oats and barley	-	+
Phakopsora pachyrhizi	soybean	-	+
Beauvaria bassiana	various	-	+
Fusarium sacchari	various	-	+
Penicillium species	various	-	+
Sporisorium scitamineum	sugarcane	-	+

*plasmid DNA, NR = No result

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