**Fusarium** species associated with sugarcane pokkah boeng in China

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**Abstract**
Pokkah boeng is one of the major fungal diseases of sugarcane in China and the disease is considered to be more serious in Guangxi than other sugarcane-producing provinces. Before implementing a screening and breeding programme for resistance to pokkah boeng, it was necessary to identify the **Fusarium** species associated with the disease and determine the dominant species. More than 500 sugarcane leaf samples showing symptoms of pokkah boeng were collected from different sugarcane-producing areas in China, including Guangxi, Guangdong, Yunnan, Hainan and Fujian provinces, and more than 800 **Fusarium** isolates were obtained. Forty-three isolates have been identified based on morphological and molecular characteristics. Four **Fusarium** species (**F. sacchari**, **F. proliferatum**, **F. verticillioides** and **F. andiyazi**) were found associated with sugarcane in China. **Fusarium sacchari** was the dominant species.

**Key words**
Sugarcane, pokkah boeng, **Fusarium**

**INTRODUCTION**

Pokkah boeng is a major fungal disease of sugarcane, and is prevalent in a number of countries including China, Iran, Malaysia and South Africa (Siti Nordahlia Wate *et al*. 2008; Govender *et al*. 2010; Mohammadi *et al*. 2012; Lin *et al*. 2014). Pokkah boeng can result in yield losses of 40.8-64.5% in susceptible varieties (Dohare *et al*. 2003). A key step to developing disease management strategies is identifying the causal agent and determining the population composition. At least five **Fusarium** species have been identified as a causal agent of pokkah boeng in sugarcane, namely **F. sacchari**, **F. verticillioides**, **F. proliferatum**, **F. subglutinans** and **F. andiyazi** (Siti Nordahlia Wate *et al*. 2008; Govender *et al*. 2010; Zakaria *et al*. 2011; Mohammadi *et al*. 2012; Khani *et al*. 2013). While **F. verticillioides** and **F. proliferatum** were previously reported to occur in China (Lin *et al*. 2014), the causal agent of pokkah boeng is still unclear.

The aim of our study was to identify the **Fusarium** species associated with pokkah boeng of sugarcane in China to assist with the formulation of management strategies.

**MATERIALS AND METHODS**

**Sample collection and isolation**

Sugarcane leaf samples with symptoms of pokkah boeng were collected from different sugarcane-producing areas in China, including Guangxi, Guangdong, Yunnan, Hainan and Fujian provinces. **Fusarium** species were isolated with PDA medium (Sigma Co. Ltd, USA) from the leaves by the tissue isolation method and maintained as single-spore isolates (Lin *et al*. 2011).

**Pathogenicity tests**

Five tissue-culture plantlets (cv. GT42) with 4-5 leaves and approximately 30 cm in height were used for the pathogenicity tests. We injected 1 mL of spore suspension (1 × 10⁶ conidia/mL of sterilized distilled water) of one representative isolate for each **Fusarium** species using a pipette and incubated the plant for the development of symptoms. An equal number of plantlets were inoculated with sterilized distilled water as controls.
Morphological identification

The single-spore isolates were cultured on PDA medium at 25°C for 4 days and the morphological characteristics of the colony were recorded (Leslie and Summerell 2006). Conidial characteristics were observed under a light microscope.

Molecular identification

Fusarium total DNA was extracted using the Cetyltrimethyl Ammonium Bromide (CTAB) method (Guo et al. 2007). Translation elongation factor-1α (TEF) primer pairs were used for amplification. Sequences of the isolates from sugarcane and other related Fusarium sequences sourced from the GenBank database were analyzed in the NCBI website.

RESULTS AND DISCUSSION

Pathogenicity tests

Our results showed that four representative Fusarium species isolates caused infection on sugarcane tissue plantlets. The symptoms on the inoculated sugarcane leaves or tip were quite similar to those in the field (Fig. 1). The re-isolated fungal cultures from the artificially infected lesion showed the same morphological characteristics as each inoculum, fulfilling Koch’s postulates.

![Fig. 1. Symptoms on sugarcane inoculated with four Fusarium species. A: F. sacchari; B: F. proliferatum; C: F. verticillioides; D: F. andiyazi.](image)

![Fig. 2. Colonies, fructification and conidia of Fusarium species isolated from sugarcane leaves with pokkah boeng symptoms. E-L, scale bar=25 μm. A, E, I: F. sacchari; B, F, J: F. proliferatum; C, G, K: F. verticillioides; D, H, L: F. andiyazi.](image)
Morphological identification

Colonies on PDA showed floccose to powdery mycelium with light-violet pigmentation. Reverse pigmentation of colonies varied from pale to dark purple 5 days after inoculation, but these characteristics were not suitable for discriminating among the different the *Fusarium* species. Four species were identified based on the size and shape of the conidia (Fig. 2), and *F. sacchari* was found to be the dominant species.

Molecular identification

An expected band of approximately 700 bp of PCR amplification product was acquired in agarose gel. BLASTn analysis of the TEF amplicon obtained with similar sequences available in GenBank database revealed 99-100% sequence identity to *Fusarium*. Sequences obtained were used for construction of a phylogenetic tree with MEGA 5.0 software (Fig. 3).

We identified four *Fusarium* species (*F. sacchari*, *F. proliferatum*, *F. verticillioides* and *F. andiyazi*) based on morphological and molecular characterization. Of the 43 isolates, 20 isolates were *F. sacchari*, 9 were *F. proliferatum*, 10 were *F.verticillioides* and 4 were *F. andiyazi*. The results indicated that *F. sacchari* was one of the most important pathogen causing sugarcane pokkah boeng in China.

![Phylogenetic tree](image)

*Fig. 3.* Phylogenetic tree based on TEF sequences of *Fusarium* species causing pokkah boeng.
CONCLUSION

Pokkah boeng is an important fungal disease of sugarcane in China. Four Fusarium species were identified as pathogens causing pokkah boeng on sugarcane in China, and F. sacchari was the dominant species.

REFERENCES


Les espèces de Fusarium associées au pokkah boeng en Chine

Résumé. Le pokkah boeng est l’une des maladies fongiques majeures de la canne à sucre en Chine, et il est considéré comme plus grave dans le Guangxi que dans les autres provinces productrices de canne à sucre. Avant de mettre en œuvre un programme de criblage et de création variétale pour la résistance au pokkah boeng, il était nécessaire d’identifier les espèces de Fusarium associées à la maladie et de déterminer la ou les espèces dominantes. Plus de 500 échantillons de feuilles de canne à sucre présentant des symptômes de pokkah boeng ont été collectés dans différentes régions de Chine productrices de canne à sucre, à savoir les provinces du Guangxi, du Guangdong, du Yunnan, de Hainan et du Fujian, et plus de 800 isolats de Fusarium ont été obtenus. Quarante-trois isolats ont été identifiés sur la base des caractéristiques morphologiques et moléculaires. Quatre espèces de Fusarium (F. sacchari, F. proliferatum, F. verticillioides et F. andiyazi) étaient associées à la canne à sucre en Chine. Fusarium sacchari était l’espèce dominante.

Mots-clés: Canne à sucre, pokkah boeng, Fusarium

Especies de Fusarium asociadas con el pokkah boeng de la caña de azúcar en China

Resumen. El pokkah boeng es una de las enfermedades fungosas más importantes de la caña de azúcar en China, y comparada con otras provincias productoras de caña de azúcar es considerada una de las más serias en la provincia de Guangxi. Antes de establecer un método de evaluación y un programa de mejoramiento para resistencia a pokkah boeng, fue necesario identificar las especies de Fusarium asociadas con la enfermedad y determinar la especie dominante. Más de 500 muestras de hoja de caña con síntomas de pokkah boeng se colectaron en diferentes zonas productoras de caña de azúcar de China, incluyendo las provincias de Guangxi, Guangdong, Yunnan, Hainan y Fujian, obteniéndose más de 800 especies de Fusarium. Basados en características morfológicas y moleculares, se identificaron cuarenta y tres aislamientos. En China se encontraron asociadas a caña de azúcar cuatro especies de Fusarium (F. sacchari, F. proliferatum, F. verticillioides y F. andiyazi), siendo Fusarium sacchari la especie dominante.

Palabras clave: Caña de azúcar, pokkah boeng, Fusarium