Identification and characterization of *Fusarium oxysporum* gx3 causing sugarcane pokkah boeng in China

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**Abstract**  Pokkah boeng is one of the most serious and devastating diseases of sugarcane, resulting in significant damage and yield loss in China. Ten fungal isolates were obtained from sugarcane leaves collected during a disease survey of 24 sugarcane varieties in Guangxi Province. The leaves showed symptoms of chlorosis, with lens or rhomboid-shaped holes and a malformed top or stalk. Based on phylogenetic analyses, morphological observations and pathogenicity tests, the isolate *F. oxysporum* gx3 was shown to cause the top rot symptoms characteristic of sugarcane pokkah boeng. Compared to our previous reported causal agents *F. verticillioides* gx1 and *F. proliferatum* gx2, *F. oxysporum* gx3 caused top rot symptoms to develop in a shorter time and eventually resulted in the death of sugarcane plant. The results also confirmed that mycelial growth of *F. oxysporum* gx3 was optimal at pH 6.0 and a temperature range of 20 to 25°C. *In vitro* assays showed that, of the nine fungicides tested, carbendazim was most effective in suppressing the radial growth of the fungus. The findings of this study will provide basic information for sugarcane pokkah boeng management in the field.

**Key words**  Pathogenicity tests, fungicides, disease management

**INTRODUCTION**

Pokkah boeng is one of the most serious and devastating diseases of sugarcane, resulting in significant damage and yield loss in China. The disease is caused by a *Fusarium* species complex, namely *F. verticillioides* (Fv), *F. proliferatum* (Fp) and *F. sacchari* (Hsuan *et al*. 2011). Fv and Fp have been identified as the causal pathogens of sugarcane pokkah boeng in China (Lin *et al*. 2014); however, there are no reports on *F. oxysporum* (Fo) causing this disease.

Here, we analyze fungal isolates obtained from sugarcane leaves collected during a disease survey of 24 sugarcane varieties in Guangxi Province to determine the status of pokkah boeng.

**METHODOLOGY AND RESULTS**

During a disease survey of 24 sugarcane varieties in August 2015 in Baise (106°62'E, 23°91'N) Guangxi, sugarcane leaves with chlorotic tissue, lens or rhomboid-shaped holes and a malformed or damaged top rot or stalk were collected (Fig. 1). Leaf pieces (5×5 mm) were cut from the margins of diseased sections and surface-sterilized by immersing in 0.1% HgCl₂ for 30 s, 70% ethanol for 1 min, and then rinsed three times in sterile water. After air-drying on sterile paper, 15 pieces were placed on potato dextrose agar (PDA) supplemented with 0.1 g/mL of ampicillin (Sigma-Aldrich, MO) and incubated in darkness at 28 °C for 2-3 days.

Fungal growth initiated with white mycelium which subsequently turned pale violet (Fig. 2). Single spore cultures were derived and maintained on PDA and their identity was confirmed as described previously (Lin *et al*. 2014). Ten isolates were obtained and a single colony of isolate gx3 was selected for further characterization. The mycelia were floccose, sparse or abundant. The microconidia were oval, elliptical or kidney shaped and with no septa, while the macroconidia usually had three septa. The apical cell was tapered and the basal cell was foot-shaped (Fig. 1). The morphological features and sporulation pattern were consistent with the description of Fo (Leslie *et al*. 2006).

DNA from the cultured fungal isolate gx3 was extracted using an SDS protocol (Lin *et al*. 2014) and amplified with the conserved rDNA-ITS, the *exo* polygalacturonase (pgx4) and the transcription elongation factor 1 alpha (tef) gene (Hirano *et al*. 2006). The pairwise alignment and phylogenetic tree based on the three gene regions (rDNA-ITS, GenBank...
Accession No. KU863663; pgx4, KU863663; tef, KU933831) and other reference sequences from GenBank showed that isolate gx3 matched Fo and was closely related to Fv and Fp.

**Fig. 1.** (A) Top rot symptoms of sugarcane pokkah boeng in the field; (B) the pathogenicity test was conducted on sugarcane seedlings with *F. oxysporum* gx3, which showed decay and necrosis symptoms; (C) no symptoms were observed on the healthy control plants inoculated with water.

**Fig. 2.** Morphological characteristics of assorted *F. oxysporum* gx3 isolates from BS2-6. Seven-day-old colonies growing on the PDA medium (A, top view and B, bottom view); C: septate hyphae; D: macroconidia are slightly sickle-shaped and the apical cell was tapered and basal cell was foot-shaped; E: Oval- to kidney-shaped microconidia.
To complete Koch’s postulates, pathogenicity test was conducted on 1-month old sugarcane plants of the same cultivar (ROC22), susceptible to pokkah boeng. Five-day-old fungal cultures grown in potato dextrose water (PDW) were filtered to eliminate the mycelium and to collect spores. The spores were washed using sterile water and diluted to 10⁶ spores/mL. The conidial suspension was stab-inoculated into young shoots of sugarcane seedlings with 10 replicates using a 1 mL sterile syringe. Sterile water was injected into control plants. The inoculated plants were placed in a growth chamber at 26°C to 28°C with a 16 h day/8 h night photoperiod. The inoculated plants became chlorotic and developed irregular necrotic areas after one week (Fig. 1), while the water-inoculated plants remained asymptomatic (Fig. 1). Five isolates recovered from the fungi-inoculated plants showed the same characteristics in culture and sequences were identical to the previous isolates. Compared to Fv and Fp, F. oxysporum gx3 caused the top rot in a shorter time, which resulted in the death of sugarcane plant. To the best of our knowledge, this is the first report of Fo causing pokkah boeng disease in China.

We observed in vitro mycelia growth of F. oxysporum under a wide range of temperatures (5°C to 35°C) and pH (4 to 8). The optimum temperature for mycelial growth of F. oxysporum gx3 was between 20°C and 25°C, while the optimum pH was 6.0.

Nine compounds were tested for their ability to inhibit mycelial growth of F. oxysporum at three concentrations (100 ppm, 50 ppm and 10 ppm). Among the tested compounds, carbendazim was the most effective in inhibiting radial growth. Mycelial growth was completely inhibited at 100 ppm and 50 ppm, and 93.15% of growth was inhibited at 10 ppm. Five fungicides, mancozeb, carbendazim, thiophanate-methyl, triadimefon and myclobutanil, inhibited mycelial growth by more than 50 % at all tested concentrations.

CONCLUSIONS

Isolation, colony morphology, microscopic observations, pathogenicity tests, pairwise alignment and a phylogenetic tree based on the three gene regions and other reference sequences from GenBank showed that F. oxysporum was associated with pokkah boeng in China. To our knowledge, this is the first time that this pathogen has been associated with this disease in China.

Carbendazim was the most inhibitory fungicide to F. oxysporum. The findings will be important to manage the disease in China.

REFERENCES


Identification et caractérisation de Fusarium oxysporum gx3 agent causal du pokkah boeng en Chine

Résumé. Le pokkah boeng est l’une des maladies de la canne à sucre les plus sévères et dévastatrices se traduisant par des dommages significatifs et des pertes de rendement en Chine. Dix isolats de champignon ont été obtenus à partir de feuilles de canne à sucre collectées durant une enquête de maladie sur 24 variétés de canne à sucre dans la Province du Guangxi. Les plantes présentaient des symptômes de chlorose foliaire, avec des tirs de forme lenticulaire ou rhomboïde ainsi que des malformations du sommet ou de la tige. Des analyses phylogénétiques, des observations morphologiques et des tests de pouvoir pathogène ont montré que l’isolat F. oxysporum gx3 causait les symptômes de pourriture sommitale caractéristiques du pokkah boeng de la canne à sucre. En comparaison avec les agents caux que nous avons précédemment signalés, F. verticillioides gx1 et F. proliferatum gx2, F. oxysporum gx3 a provoqué plus rapidement le développement de pourriture sommitale, laquelle a fini par occasionner la mort des plants de canne à sucre. Les résultats ont aussi confirmé que la croissance du mycelium de F. oxysporum gx3 était optimale à pH 6,0 et dans une gamme de température de 20 à 25°C. Des tests in vitro ont montré que, sur les neuf fungicides testés, le carbendazime était le plus efficace pour inhiber la croissance radiale du champignon. Les résultats de cette étude fourniront des informations de base pour la gestion du pokkah boeng de la canne à sucre au champ.

Mots-clés: Tests de pouvoir pathogène, fungicides, gestion de maladie
Identificación y caracterización de *Fusarium oxysporum* gx3 causante del pokkah boeng de la caña de azúcar en China

**Resumen.** El cogollo retorcido ‘pokkah boen’ es una de las enfermedades más graves y devastadoras de la caña de azúcar, resultando en daños y en pérdidas de rendimiento significativas en China. Se obtuvieron diez aislamientos a partir de hojas de caña de azúcar colectadas durante un muestreo de la enfermedad en 24 variedades de caña de azúcar en la provincia de Guangxi. Las hojas mostraron síntomas de clorosis, con agujeros en forma de lente o de rombo y una malformación apical del tallo. Basado en los análisis filogenéticos, observaciones morfológicas y pruebas de patogenicidad, el aislamiento *F. oxysporum* gx3, causó los síntomas característicos de pudrición apical del cogollo retorcido de la caña de azúcar. En comparación con la información de agentes causales previos *F. veticilloides* gx1 y *F. proliferatum* gx2; *F. oxysporum* causó síntomas de pudrición en un tiempo más corto y, eventualmente, resultó en la muerte de la planta. Los resultados también confirmaron que el crecimiento del micelio de *F. oxysporum* gx3 fue óptimo a un pH 6.0 y un intervalo de temperatura de 20 a 25°C. Los ensayos in vitro mostraron que de los nueve fungicidas probados, carbendazim fue más eficaz en la supresión del crecimiento radial de los hongos. Los resultados de este estudio proporcionarán información básica para el manejo del pokkah boeng de la caña de azúcar en el campo.

**Palabras clave:** Pruebas de patogenicidad, fungicidas, manejo de enfermedades