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Short Communication

Report of a Quarantine Sugarcane Leaf Scald Disease in Guangxi, China

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Abstract

In 2016, sugarcane plants were observed in Beihai, Laibin, and Baise in Guangxi province, China for leaf scaled disease. To identify the pathogen, DNA was extracted and PCR was performed with specific primer XAF1/XAR1 for *Xanthomonas allbilineans* on 62 suspected leaf scald samples collected from Beihai, Laibin and Baise. The PCR results showed that about 600 bp PCR products were obtained from all the samples. Twenty-one PCR products were randomly selected for sequencing. Subsequent sequence analysis revealed that all nucleotide sequences of this study were identical and shared 100% sequence similarity with the nucleotide sequence from the *raxB1* gene of *X. albilineans* (GenBank accession no. FP565176), and were clustered with *X. albilineans* sequences in one branch of a phylogenetic tree. Field surveys showed that sugarcane cultivar 'Guitang46' and 'Guitang06-2081' were highly susceptible with disease incidences of 18-100%. Leaf scald can cause huge losses in sugarcane yield and sugar content because severely infected plants show wilting, abnormal development of side shoots, and white-striped leaves on side shoots. Leaf scald disease can be easily transmitted by vegetative propagation in sugarcane, so it is necessary to take appropriate prevention and management measures to prevent the spread of leaf scald from onset.

Keywords: China; Guangxi; PCR detection; Sugarcane leaf scald disease; Sequence analysis; *Xanthomonas albilineans*

Introduction

Sugarcane leaf scald, caused by *Xanthomonas albilineans* (Ashby) Dowson, is widespread and outbreaks in some cane-growing countries, and caused huge economic losses to the local sugarcane and sugar industries (Rott and Davis, 2000; Saumtally and Dookun-Saumtally, 2004).

Sugarcane leaf scald is a bacterial vascular disease (Mensi *et al.*, 2014). It can easily spreaded long distances by infected seedlings. Because the disease has a strong capacity to spread (Daugrois *et al.*, 2012), it is listed as an important quarantine object for plants imported into China (Li and Huang, 2012).

The conventional phenotypic diagnostic method has limitations because of the latent infections characteristics of *X. albilineans*. With the development of molecular biology, scholars have consecutively reported specific PCR techniques to detect and identify *X. albilineans* (Pan *et al.*, 1999; Wang *et al.*, 1999; Alvez *et al.*, 2016), and *X. albilineans* isolates have been identified in many countries. In 2016, plants of having leaf scald were observed in Beihai, Laibin, and Baise in Guangxi province in China. To identify the pathogen, we assayed leaf scald samples collected from Beihai, Laibin, and Baise using PCR.

Materials and Methods

Collection of Diseased Samples

In 2016, 62 leaf scald samples were collected from Beihai, Laibin, and Baise of Guangxi province in China (Table 1). The positive control was *X. albilineans* DNA which is got from Yunnan key laboratory of sugarcane genetic improvement in Kaiyuan, China. The negative control was the DNA of a bacterium-free healthy sugarcane plant.
Sterile deionized water served as a blank control.

Total DNA Extraction

For each tested sample, a total of 2 mL of sugarcane juice was centrifuged for 10 min, and the Bacterial Genomic DNA Purification Kit (Sangon Biotech Co., Ltd, Shanghai, China) was applied to extract total DNA from the pellet according to the manufacturer's instructions.

PCR Detection

Primer design and synthesis: According to the report by Wang *et al.* (1999) to designe the *X. albilineans* specific

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PCR primers, XAF1 (5'-CCTGGTGATGACGCTGGGTT-3') and XAR1(5'-CGATCAGCGATGCACGCAGT-3'), for amplifying a 600 bp region of the *raxB1* gene. Primers were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China).

PCR amplification: PCR method described by Wang *et al.* (1999) for detection of *X. albilineans* was used to amplify the DNA from each tested sample. PCR was carried out in a 25 μL reaction mixture contained 8.5 μL ddH_2O, 1.0 μL of each primer (20 μg/ μL) 2.0 μL DNA template, and 12.5 μL 2×*Easy* Taq PCR SuperMix (TransGen Biotech Co., Ltd., Beijing, China). The PCR thermocycling procedure was as follow: pre-denaturation at 95ºC for 5 min followed by 10 cycles of 94ºC for 45 s, 65ºC for 1 min, and 72ºC for 1 min; subsequently 10 cycles of 94ºC for 45 s, 65ºC for 1 min, and 72ºC for 2 min; then 10 cycles of 94ºC for 45 s, 65ºC for 1 min, and 72ºC for 3 min; and a last extension at 72ºC for 10 min. The PCR products were electrophoresed through a 1.5% agarose gels.

Cloning and sequencing of PCR products and sequence analysis: The DNA agarose gel Purification kit (Tiangen Biotech Co., Ltd., Beijing, China) was used to purify the PCR products. The purified PCR products were cloned with *pEASY-T5* Zero Cloning Kit (TransGen Biotech Co., Ltd., Beijing, China) and six positive clones per sample were sequenced. When a BLAST search have finished in GenBank, a phylogenetic tree was constructed by the neighbour-joining method and Kimura's two-parameter model as implemented in the software MEGA, version 4.1 (Tamura *et al*., 2013). The bootstrap values was 1000 replicates.

Occurrence and Damage Investigation in the Field

The occurrence and damage caused by sugarcane leaf scald was investigated in 2016. A three-point sampling method was used to investigated diseased plants. For each disease cultivar, diseased plants was recorded from each 100 successive plants of three sampled points, involving a total of 300 plants. The following equation was used to calculate disease incidence:

Disease incidence (%) = (number of diseased plants/total number plants evaluated) × 100.

Results

The Damage and Symptoms Characteristics of Sugarcane leaf Scald

The occurrence of sugarcane leaf scald within a 200 ha area was observed in cane-growing fields in Beihai, Laibin, and Baise in Guangxi province. The main symptoms of disease varied from a single, white, anrrow, sharply defined stripe to complete wilting and necrosis of infected leaves, finally leading to death of entire plants. Seriously infected plants exhibited shorter internodes, smaller leaves, abnormal development of the side shoots on the stalk, and the leaves on the side shoots had similar white stripes to those on the main stalk (Fig. 1).

Fig. 1: Symptoms of leaf scald-infected sugarcane plants in the field. **A**, a view of a diseased field; **B**, **C**, typical diseased leaves showing white stripes; **D**, diseased leaves of side shoots showing white stripes; **E**, wilting and drying of leaves (seedling stage); **F**, wilting and drying of leaves (mature stage)

Sugarcane cultivar 'Guitang 46' and 'Guitang 06-2081' were highly susceptible to leaf scald, with a disease incidence of 18-100% and 50-96%, respectively. Cultivar 'Funong 41' was mildly susceptible, with a disease incidence of 5-15%, and the remaining three sugarcane cultivars, 'Guitang 42', 'Liucheng 05-136', Yunzhe08-1095', 'Yuegan 47', 'Mintang 06-1405', 'Liucheng 07-150', 'Haizhe 22', 'Guitang 08-1180', 'Funong 09-7111', 'Funong 09-2201', 'Dezhe 07-36' , and 'ROC 22' were resistant to leaf scald, with no symptoms in the field (Table 1).

The PCR Results from the Sugarcane Leaf Scald Samples

The 600-bp target DNA fragment was present in all the 62 suspected samples and in the positive control, and no amplifcation product was obtained from the negative and blank controls (Table 2 and Fig. 2).

Sequence Analysis

Twenty one PCR products were randomly selected from the leaf scald-positive samples and sequenced them. The results showed that all nucleotide sequences obtained in this study were 608-bp length and identical (GenBank accession numbers KY315183 to KY315203). BLAST results indicated that our nucleotide sequences shared 100% sequence similarity with the nucleotide sequence of the

Table 1: Results of a field survey for sugarcane leaf scald disease in Guangxi Province, China in 2016

Sugarcane region	Experiment name	Variety	Disease incidence (%)	Resistance response
Laibin, Guangxi	Integrated demonstration of new varieties	Guitang 42	0	Resistant
		Funong 41	5~10	Mildly susceptible
		Liucheng 05-136	0	Resistant
		Guitang 46	18~65	Highly susceptible
		ROC 22	0	Resistant
Beihai, Guangxi	Breeding demonstration of new varieties	Guitang 42	0	Resistant
		Funong 41	8~15	Mildly susceptible
		Liucheng 05-136	0	Resistant
		Guitang 46	45~100	Highly susceptible
		Guitang 49	0	Resistant
		ROC 22	0	Resistant
Baise, Guangxi	The 11th round national regional experiment	Yunzhe 08-1095	0	Resistant
		Yuegan 47	0	Resistant
		Mintang 06-1405	0	Resistant
		Liucheng 07-150	0	Resistant
		Haizhe 22	0	Resistant
		Guitang 08-1180	0	Resistant
		Guitang 06-2081	50~96	Highly susceptible
		Funong 09-7111	0	Resistant
		Funong 09-2201	0	Resistant
		Dezhe 07-36	0	Resistant
		ROC 22	0	Resistant

Table 2: PCR detection of 62 sugarcane leaf scald disease samples

Sampling site	Number	Sample numbers	Variety	Positive numbers
Laibin, Guangxi	LB1-LB12	12	Guitang 46	12
Beihai, Guangxi	BH1-BH26	26	Guitang 46	26
	BH27-BH28	2	Funong 41	2
Baise, Guangxi	BS1-BS22	22	Guitang 06-2081	22

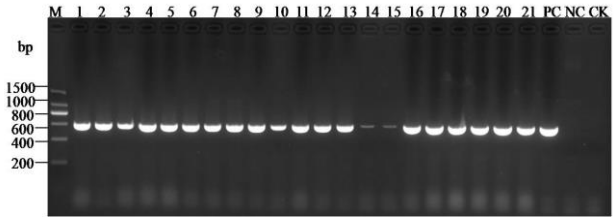

Fig. 2: PCR-based assay for detection of *Xanthomonas albilineans* in 21 sugarcane leaf scald samples by electrophoresis on 1.5% agarose gel. M: Molecular weight marker; Lanes 1–21: sugarcane leaf scald samples; PC: positive control; NC: negative control; CK: blank control

raxB1 gene of *X. albilineans* strain GPE PC73 (accession number FP565176) from the island of Guadeloupe. Phylogenetic analysis (Fig. 3) revealed that the sequences obtained in this study were clustered in one branch with *X. albilineans* (GenBank accession no. FP565176) and have a close genetic relationship. Moreover, the other known species of *Xanthomonas* were clustered into other branch.

Discussion

According to the result of symptom diagnosis in the field and molecular biological identification, the sugarcane disease occurred in Beihai, Laibin and Baise was confirmed to be leaf scald caused by *X. albilineans*, which was consistent with the reported by Pieretti *et al.* (2009). This result will lay a foundation for further research and scientific and effective control of sugarcane leaf scald in China. Sugarcane leaf scald is a dangerous disease which can be easily transmitted by vegetative propagation. Therefore, in order to control the spread of leaf scald from the source, it is necessary to enhance quarantine and monitoring of sugarcane seedlings from propagation bases and for imported/exported sugarcane varieties/materials.

Breeding and planting of resistant cultivars to manage leaf scald is highly economical and effective (Daugrois *et al.*, 2012). The qPCR can quantitatively detect *X. albilineans*. Resently, some reseachers evaluated and screened leaf–scald-resistant varieties by using natural resistance survey, artificial inoculation and qPCR detection (Viveros, 2014; Garces *et al.*, 2014; Gutierrez *et al.*, 2016). Sugarcane leaf scald disease is a potential threat to the sugarcane development in China, the resistant cultivar breeding strategy of sugarcane leaf scald should strengthen the screening and utilizling of the *X. albilineans* resistant germplasms.

Conclusion

The PCR detection and sequence analysis on 62 diseased samples verified that the pathogen of the leaf scald disease

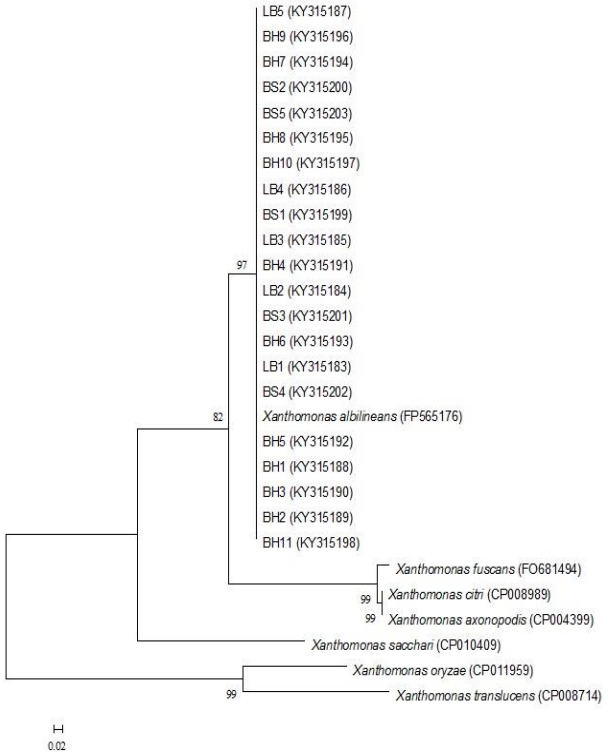

Fig. 3: Phylogenetic tree constructed with MEGA version 4.1 using the nucleotide sequences obtained in this study and from different species of *Xanthomonas* retrieved from GenBank. Numbers on branches indicate bootstrap values based on 1000 replicates

symptom appeared in Beihai, Laibin and Baise of Guangxi province was *Xanthomonas albilineans* (Ashby) Dowson. Sugarcane cultivar 'Guitang 46' and 'Guitang 06-2081' were highly susceptible to leaf scald.

Acknowledgements

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