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Full Length Article

Identification of Three *Sporisorium scitamineum* Pathogenic Races in Mainland China

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Abstract

Sugarcane smut, caused by *Sporisorium scitamineum*, is one of the most severe sugarcane diseases around the world, particularly in mainland China. Understanding its pathogenicity is important in developing resistant cultivars; however, current available information is limited. In 2015 and 2016, the pathogenicity of isolates derived from three different *S. scitamineum* clusters from mainland China was assessed using ISSR and SCoT markers data were determined using injection inoculation. We observed highly significant differences in pathogenicity among the isolates based on the incidence of smut in 10 sugarcane domestic and overseas cultivars. Based on genetic variation and pathogenicity differentiation of the isolates, three pathogenic races of *S. scitamineum* were identified. This is the first identification of pathogenic *S. scitamineum* races from mainland China. This finding may be utilized in smut resistance breeding and quarantine of imported sugarcane varieties. © 2018 Friends Science Publishers

Keywords: Sugarcane smut; *Sporisorium scitamineum*; Pathogenic differentiation; Race

Introduction

Sugarcane (*Saccharum* spp. hybrids) is a major commercial crop that is used in sugar production. It is propagated asexually and is readily infected by pathogens. *Sporisorium scitamineum*, formerly called *Ustilago scitaminea*, causes sugarcane smut disease and occurs in all sugarcane-growing regions around the world except for Papua New Guinea and Fiji (Raboin *et al*., 2007). Sugarcane smut is a global disease that causes severe economic losses (Comstock, 2000; Magarey *et al.*, 2010). Smut was first reported in 1932 in Guangzhou, China (Antoine, 1961; Presley, 1978). During the past two decades, smut has developed into a major sugarcane disease, causing severe economic losses that have been estimated at 8–10 billion dollars per year in mainland China (Shen *et al.*, 2013, 2016a). Currently, almost all the main sugarcane cultivars in mainland China are susceptible to smut disease. For example, ROC22 is a major sugarcane cultivar that is propagated in > 60% of the total sugarcane growing area, and the average field incidence of smut stools in ROC22 is 10−15% in plant cane and 15−25% in ratoon cane (Shen *et al.*, 2013).

The most efficient and economical method to control sugarcane smut is to use resistant cultivars (Shen *et al.*, 2014b; Dou *et al.*, 2017). Identification of variations in virulence among different pathogen populations is thus important in the development of resistant cultivars and disease management strategies. The differentiation of pathogenic races of *S. scitamineum* has been reported in some countries or regions (Grisham, 2001; Bhuiyan and Croft, 2015). For example, there are three races in Taiwan (Lee *et al.*, 1999), three races in Hawaii (Schenck, 2003), two in Brazil (Da Silva and Sanguino, 1978), and five in Pakistan (Muhammad and Kausar, 1962). In mainland China, the loss of smut resistance in sugarcane has been reported, thereby suggesting the presence of pathogenicity differentiation involving *S. scitamineum* (Shen *et al.*, 2013, 2014). However, the systematic identification of *S. scitamineum* races from mainland China has not been conducted to date. The objective of the present study was to determine the pathogenicity of selected representative isolates from mainland China, identify pathogenicity or physiological race differentiation of *S. scitamineum*, and provide the basis for sugarcane breeding resistance to smut.

Materials and Methods

In the summer of 2014, 90 compatible positive and negative mating-type isolates of *S. scitamineum* derived from 90 single-whips (sori) of sugarcane smut containing at least 27

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different sugarcane genotypes were collected from the five main sugarcane-producing provinces (Guangxi, Yunnan, Guangdong, Hainan, and Jiangxi) in mainland China (Shen *et al.*, 2016b; Xu *et al.*, 2017). The genetic diversities and structures of the 90 positive mating-type isolates of *S. scitamineum* were assessed using a combination of SCoT and ISSR molecular markers. The 90 positive mating-type isolates were clustered using unweighted pair-group method with arithmetic mean (UPGMA) and principal component analysis (PCA) based on their similarity, and they were divided into three main groups (G_1, G_2 and G_3 based on UPGMA, Fig. 1; A, B and C based on PCA, Fig. 2) using Jaccard`s similarity coefficient of 0.755 as criterion. The results of UPGMA and PCA showed similar clustering patterns. The genetic diversity of *S. scitamineum* was associated with the geographic origin of the isolates, with no evidence of co-evolution between the host and *S. scitamineum*.

To determine the pathogenicity of *S. scitamineum* isolates, using sterile distilled water as control, inoculation tests were performed for the selected representative isolates Ss16, Ss24 and Ss89, which were derived from different geographical origins based on the above three different cluster groups (Fig. 1 and 2), respectively. The information on the three representative isolates is listed in Table 1. The representative isolates were mixed with their own compatible negative mating-type isolates using the same concentration (5×10^5 basidiospores/mL) and volume, and inoculated by injection (100 μL/plant) of young seedlings (growth points or nearby) of different sugarcane cultivars that were bred locally or abroad (Table 2) as described by Shen *et al.* (2014a). The pathogenecity experiment was designed using the randomized complete block with three replicates. Each of the cane stalks was cut into one-eye setts, soaked in cold running water for 48 h, and then treated at 50 ± 0.5°C for 2 h. Thereafter, the setts were planted in a steam-sterilized mixture of soil and organic matter (V:V = 3:1). Twenty sugarcane seedlings were inoculated per replicate, resulting in a total of 60 seedlings in each treatment per isolate. The inoculation experiment was performed twice, once in October 2015 and once in May 2016 in a greenhouse (temperature: 25−30°C, relative humidity: 60−70%), which was located in the experimental base of South China Agricultural University.

Approximately 4–5 weeks after inoculation, surveys of the disease incidence were initiated and carried out every 10 days until stability was achieved (about 4 months). The date of inoculation, the total number of stools, and the number of diseased stools were recorded. The following equation was used to calculate disease incidence.

Smut incidence (%) = (Number of smut stools/Total number of stools evaluated) × 100

Mean smut incidence of October 2015 and May 2016 was calculated and analyzed for statistical significance.

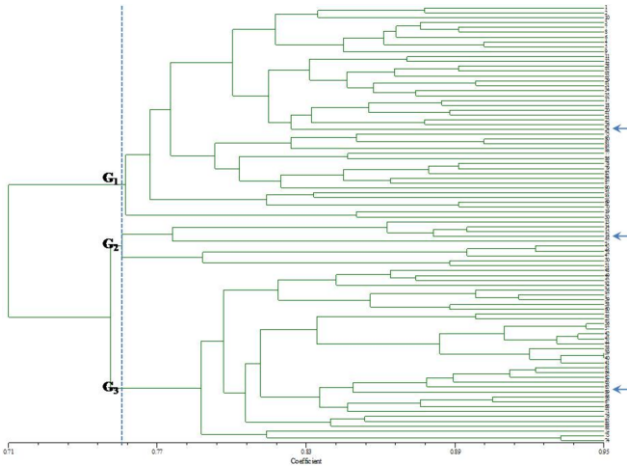

Fig. 1: UPGMA dendrogram of cluster analysis of the 90 mating-type isolates of *S. scitanmineum* collected from Mainland China based on SCoT and ISSR maker data
Note: Blue arrows point to selected representative isolates in this study

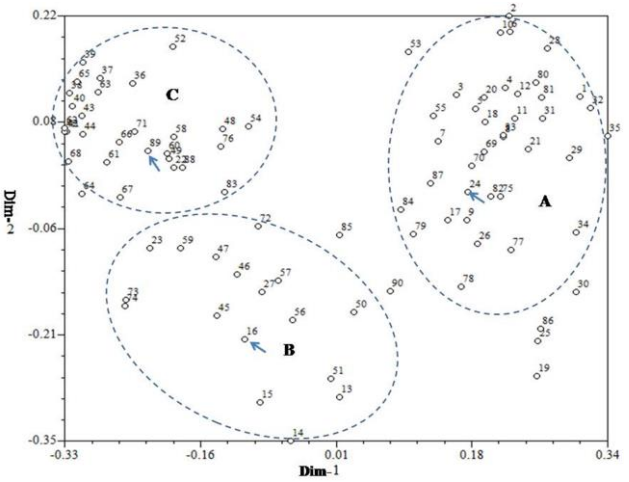

Fig. 2: Principal component analysis of the 90 isolates of *S. scitanmineum* collected from Mainland China based on SCoT and ISSR marker data
Note: Blue arrows point to selected representative isolates in this study

Results

Among the 10 varieties examined, the incidence of smut stools in isolate Ss16 was significantly higher than that of the other two isolates Ss89 and Ss24. Except for YT96-835 and GT05-136, the incidence of smut stools in isolate Ss89 was significantly higher than that of isolate Ss24. No smut plants were observed among the controls (Table 3). Based on the genetic diversities and pathogenic differentiation of the *S. scitamineum* isolates, three distinct races were identified in mainland China. Isolate Ss16 represents a highly pathogenic race, isolate Ss89 is a moderately pathogenic race, and isolate Ss24 is a weak pathogenic race.

Table 1: List of selected representative isolates of *S. scitamineum* used in this study

Code of isolate	Genetic group		Host(sugarcane varieties)	Geographical origin
	UPGMA	PCA		
Ss16	G2	B	YN93-204	Wenyuan, Guangdong, China
Ss89	G3	C	MT69-421	Yuxi, Yunnan, China
Ss24	G1	A	ROC22	Danzhou, Hainan, China

Table 2: Sugarcane varieties and origins

Code	Variety[a]	Origin[b]
1	ROC22	TSRI/China
2	Q171	BSES/Australia
3	ROC5	TSRI/China
4	YT96-86	GSIRI/China
5	YT96-835	GSIRI/China
6	TT89-1626	TSRI/China
7	CP72-1210	CP/USA
8	GT05-136	GSRI/China
9	F134	TSRI/China
10	TT7929	TSRI/China

Notes: [a]TT=Taitang; YT=Yuetang; GT=Guitang
[b]TSRI: Taiwan Sugar Research Institute; GSIRI: Guangzhou Sugarcane Industry Research Institute; GSRI: Guangxi Sugarcane Research Institute; BSES: Bureau of sugar experiment station; CP: Canal Point

Table 3: Analysis of incidence rate of smut stools in this study

Code of isolate	Incidence of smut stools[a]									
	ROC22	Q171	ROC5	YT96-86	YT96-835	TT89-1626	CP72-1210	GT05-136	F134	TT7929
Ss16	70.0 A[b]	30.0 A	60.0A	50.0A	60.0A	100.0A	50.0A	100.0A	45.0A	35.0A
Ss89	45.0 B	13.3 B	25.0B	25.0B	0.0B	15.0B	25.0B	0.0B	15.0B	13.3B
Ss24	10.0 C	0.0C	0.0C	1.6C	0.0B	1.6C	3.3C	0.0B	0.0C	0.0C
CK	0.0 D	0.0C	0.0C	0.0C	0.0B	0.0C	0.0C	0.0B	0.0C	0.0C

Notes: [a]Incidences of smut stools were the average values of the twice experiments
[b]Different capital letters represent significant differences at the level of 0.01 in multiple comparisons

To our knowledge, this is the first identification of three *S. scitamineum* races from mainland China. This finding is helpful for smut resistance breeding and quarantine of imported sugarcane varieties.

Discussion

The present study aimed to determine whether there is a correlation between genetic diversity based on molecular makers and pathogenicity differentiation in *S. scitamineum.* We identified three *S. scitamineum* races with distinct differences in pathogenicity that were derived from three different *S. scitamineum* isolates from mainland China using ISSR and SCoT marker data. These findings indicate that differences in pathogenicity among various isolates may be due to genetic variations. Environmental heterogeneity is the main driving force of genetic variation in *S. scitamineum* (Que *et al.*, 2012; Xu *et al.*, 2014; Shen *et al.*, 2016b; Xu *et al.*, 2017). In the present study, three representative *S. scitamineum* isolates were collected from three extremely different ecological environments. For example, isolate Ss16 was collected from Wenyuan County, Guangdong Province (located in the northern edge of the subtropical frost mountain area), isolate Ss89 originated from Yuxi City, Yunnan Province (subtropical plateau inland climate), and isolate Ss24 was obtained from Danzhou City, Hunan Province (tropical marine climate). Studies investigating whether environmental heterogeneity is the main driving force of pathogenicity differentiation in *S. scitamineum* are warranted. Previous study by Schenck *et al*. (2005) observed high genetic similarity among isolates from old and new *S. scitamineum* races, and cluster analysis of 35 *S. scitamineum* isolates from Hawaii using AFLP molecular markers did not clearly discriminate the new from the old races. To further understand the pathogenicity differentiation in *S. scitamineum*, it is essential to increase the number of isolates that would be tested in terms of pathogenicity, particularly those showing minimal to no genetic variations.

The present study has identified different pathogenic races of *S. scitamineum* in mainland China. It is thus important to quarantine *S. scitamineum* in future outbreaks to prevent further spreading to other sugarcane materials in different regions of mainland China or abroad. There is also a need to define the distribution range and dominant pathogenic races of *S. scitamineum*, which may facilitate in the development of disease-resistant cultivars.

Conclusion

Based on genetic variation and pathogenicity differentiation of the isolates, three pathogenic races of *S. scitamineum* were identified. This is the first identification of pathogenic *S. scitamineum* races from mainland China. This finding may be utilized in smut resistance breeding and quarantine of imported sugarcane varieties.

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