ISSN: 2230-9926

*Available online at http://www.journalijdr.com*

IJDR

*International Journal of Development Research*
*Vol. 5, Issue, 05, pp. 4412-4417, May, 2015*

*International Journal of*
**DEVELOPMENT RESEARCH**

## *Full Length Research Article*

# EVALUATION OF TWO FORMS OF *SPORISORIUM SCITAMINEA* SYDOW INOCULUM IN SUGARCANE

**[1]Cui-Feng Yang, [2]Yi-Jing Gao, [2]Cui-Fang Yang, [1]Yu-Xin Huang, [2]Bao-Qing Zhang, [2]Shan Zhou, [1]Li-Tao Yang, *[2]Ge-Min Zhang and *[2]Yang-Rui Li**

[1]Agricultural College, State Key Laboratory of Conservation and Utilization of Subtropical Agro-Bioresources, Guangxi University, Nanning 530005, China;
[2]Key Laboratory of Sugarcane Biotechnology and Genetic Improvement (Guangxi), Ministry of Agriculture, Guangxi Key Laboratory of Sugarcane Genetic Improvement, Sugarcane Research Center, Chinese Academy of Agricultural Sciences – Sugarcane Research Institute, Guangxi Academy of Agricultural Science, Nanning 530007, China

**ARTICLE INFO**

***Article History:***
Received 19th February, 2015
Received in revised form
23rd March, 2015
Accepted 05th April, 2015
Published online 31st May, 2015

***Key words:***
Sugarcane;
Smut;
Inoculation;
Resistance.

**ABSTRACT**

Smut, caused by *Sporisorium scitaminea* Sydow, is one of the main destructive diseases in sugarcane (*Saccharum* spp.). To assess the resistance of sugarcane to smut, two different isolates of *S. scitaminea* were inoculated to sugarcane plants by means of pin-prick dipping in nodal buds. Data on the disease latent period and disease incidents per population were recorded and analyzed. Results showed the inoculum used in the present study was the *S. scitaminea* race 2 according to the resistance levels of standard control varieties F134 and NCo310. While the variety NCo376 used to be immune to smut became susceptible, inferring the pathogenicity of *S. scitaminea* in our study was stronger than that reported previously, or it had generated virulence mutations. The "+" and "-" mating type sporidia mixture (1:1) as inoculum gave better results than teliospores suspension inoculation, indicating the sporidia suspension by pin-prick dipping inoculation is more appropriate for identifying primary sources of resistance. Six sugarcane clones (GT36, GT37, YT93-159, GXS85-30, GXS145 and GXS222) were found to have higher levels of resistance to smut than others, so they could be useful resources to improve smut resistance in sugarcane breeding.

*Copyright © 2015 Cui-Feng Yang et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.*

## INTRODUCTION

Smut caused by the fungus *Sporisorium scitaminea* Sydow is a major disease in sugarcane that occurs in all sugarcane-producing countries except Papua New Guinea, which can be responsible for serious yield losses (Su *et al*., 2013). *S. scitaminea* belongs to the Basidiomycetes, and the spores produced by the whip are diploid teliospores. This pathogen produces two opposite mating types of sporidia, "+" and "-", which are able to work together to form dikaryotic hyphae (Alexander *et al*., 1978; Croft *et al*., 2006).

***Corresponding author: Ge-Min Zhang and Yang-Rui Li***
*Key Laboratory of Sugarcane Biotechnology and Genetic Improvement (Guangxi), Ministry of Agriculture, Guangxi Key Laboratory of Sugarcane Genetic Improvement, Sugarcane Research Center, Chinese Academy of Agricultural Sciences – Sugarcane Research Institute, Guangxi Academy of Agricultural Science, Nanning 530007, China*

Successful infection is only achieved after teliospores land on a sugarcane bud, germinate and produce the dikaryotic infection hyphae. The infection causes the growing point mutation that produces black whips eventually (Fontaniella *et al*., 2002; Shamsul *et al*., 2012). The most efficient and cost-effective way of controlling the disease is use of resistant varieties. An important strategy for controlling smut disease on a broad scale is selection and planting of resistant sugarcane cultivars. To assess smut reaction, researchers typically use a dip inoculation assay in which nodal buds are immersed briefly in a suspension of mixed teliospores and then planted in the field (Wang *et al*., 2010; Huo *et al*., 2013; Shen *et al*., 2011, 2014). The use of teliospore suspension have already been demonstrated as a wide inoculum for detection of sugarcane resistance to *S. scitaminea* disease, but its complexity like collecting teliospores in the field, unstable field results due to lack of uniform inoculum, impossibility in identifying the physiological race, etc. made it unappealing to investigators.

To fungal disease, identifying physiological races of pathogen is the key for disease resistance breeding because they are the result of pathogenic virulence differentiation. The sporidia suspension of *S. scitaminea* as inoculum was proved having many advantages viz. no need to collect teliospores before planting, using the same strains for a series of experiments or repeat tests, etc. (Chen *et al*., 2013). In addition, it has the benefits of short incubation period, high incidence, fully identifying the resistance levels of host varieties and assessing the virulence strength of inoculum (Shen *et al*., 2014). To date, little has been done in using sporidia suspension inoculum to assess sugarcane resistance to smut. It is necessary to have an effective inoculation method that is practical, rapid, sensitive and reliable in identifying sugarcane resistance to smut. The objective of this work was to assess the feasibility of the sporidia mixture of "+" and "-" mating types (1:1) and teliospores suspension of *S. scitaminea* by means of pin-prick dipping inoculation, in order to explore a novel method for identification of sugarcane resistance to smut by skipping bud morphological obstacle, and to evaluate the resistance to smut of commercial sugarcane cultivars and *S. spontaneum* accessions.

## MATERIALS AND METHODS

### Plant Materials

Twelve sugarcane cultivars GT29, GT32, GT36, GT37, GT40, GT42, GT43, ROC10, ROC16, ROC22, YT93-159 and YT94-128, six *S. spontaneum* materials GXS87-16, GXS85-30, GXS145, GXS165, GXS222 and GXS238, and four standard control varieties: F134 (resistant to *S. scitaminea* race 1 but susceptible to race 2), NCo310 (resistant to *S. scitaminea* race 2 but susceptible to race 1), NCo376 (immune to *S. scitaminea* races 1 and 2) and YC71-374 (susceptible to *S. scitaminea* races 1 and 2) were used as the plant materials. "GT" is short for "Gui Tang", and GT varieties are bred by Sugarcane Research Institute, Guangxi Academy of Agricultural Sciences/Sugarcane Research Center, Chinese Academy of Agricultural Sciences; "ROC" varieties are bred by Taiwan Sugar Research Institute; "YT" is short for "Yue Tang", and YT varieties are bred by Guangzhou Sugarcane Industry Research Institute; "YC" is short for "Ya Cheng", and YC varieties are bred by Hainan Sugarcane Breeding Station; "NCo" varieties are bred by South Africa; *S. spontaneum* accessions (GXS) were collected from Guangxi region, China by Sugarcane Research Institute, Guangxi Academy of Agricultural Sciences/Sugarcane Research Center, Chinese Academy of Agricultural Sciences.

### Teliospores Collection, Preservation and Viability Determination

Whips from smut-infected plants were collected from the host sugarcane variety F134 cultivated in Sugarcane Research Institute, Guangxi Academy of Agricultural Sciences/ Sugarcane Research Center, Chinese Academy of Agricultural Sciences, Nanning, China, of which the teliospores were put in paper bags and stored at 4$^{o}$C until naturally dried. Teliospores were spread on yeast extract peptone sugar (YEPS) medium containing 50 μg/mL ampicillin (Shenggong, Shanghai, China) after they were fully mixed in sterile water, cultured for 2-3 days at 28$^{o}$C, and then teliospores germination rate and hyphae growth were detected with microscope.

### Single Spore Isolation and Identification of Mating Type

Fresh teliospores from sugarcane variety F134 was used for gradient dilution with sterile water and plated onto YEPS medium containing μg/mL ampicillin. The plates were incubated for 2 days at 28$^{o}$C for sporulation. A single colony was transferred onto the new YEPS medium and cultured at 28$^{o}$C for 2 days. A number of "yeast-like" single colonies were randomly picked up and placed in 400 μL YEPS liquid medium, respectively, to make hybridization on another new YEPS medium after they were shaken with 150 rpm for 1 day at 28$^{o}$C, and cultured for 3 days at 28$^{o}$C. If the colony is Fuzz-like, the two strains are opposite (+, -) mating types of haploid sporidium; if it is yeast-like, the two strains are the same (+, + or -, -) mating type haploid sporidium. The concentration of spores was adjusted to $5\times10^{6}$ spores/mL using a hemacytometer (Nikon, Tokyo, Japan).

### Infection Experiments

Pin-prick dip inoculation was used to inoculate the "+" and "-" mating types mixture of sporidia (sporidia suspension) of *S. scitaminea* in sugarcane. Twenty six double bud seedcane setts were taken from each tested material. For inoculation, seedcane setts were needled 6 times around each bud using insect pin, and soaked in a mixture of sporidia suspension ($5\times10^{6}$ spores/mL) for 10 min. The inoculated seedcane setts were kept in greenhouse with moisture for 48 h at 28$^{o}$C before planted in the field. A completely randomized block design was used with single line of 5.0 m in length, 1.0 m in space, and two replications. Each plot had total 26 buds.

### Field Investigation and Disease Incidence Grading

Emergence number, smut whip originating date, cumulative diseased stalks, total stalks, cumulative diseased clumps and cumulative incidence were investigated since inoculation. The investigations were conducted every 7 days at primary infection stage, and every 15 days afterwards till the end of smut whip generation. Each diseased plant or clump was labeled in each investigation. The sugarcane smut response was measured according to cumulative incidence using a 1–9 rating scale: 1 = 0–3 % (highly resistant), 2 = 4–6 % (resistant 1), 3 = 7–9 % (resistant 2), 4 = 10–12 % (moderately resistant), 5 = 13–25 % (moderately susceptible), 6 = 26–35 % (susceptible 1), 7 = 36–50 % (susceptible 2), 8 = 51–75 % (highly susceptible 1), and 9 = 76–100 % (highly susceptible 2).

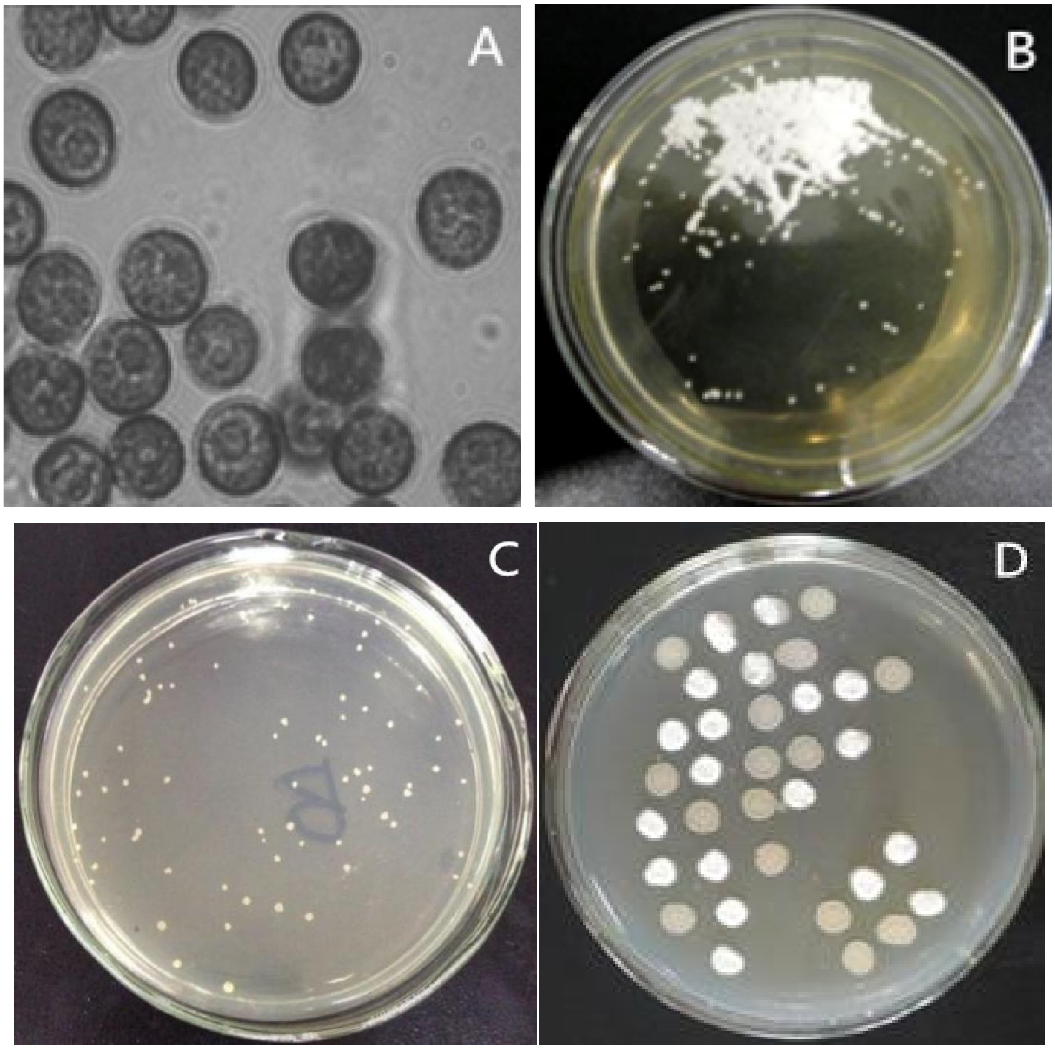
## RESULTS

### Isolation of Single Spore and Identification of Mating Type

In the present study, we successfully isolated the sporidia from sugarcane smut whips and obtained the opposite mating types of sporidia. The chlamydospores were nearly round and sepia under optical microscope (Fig. 1 A), and they are dormant teliospores that can germinate and produce white woolly single colonies on YEPS medium (Fig. 1 B).

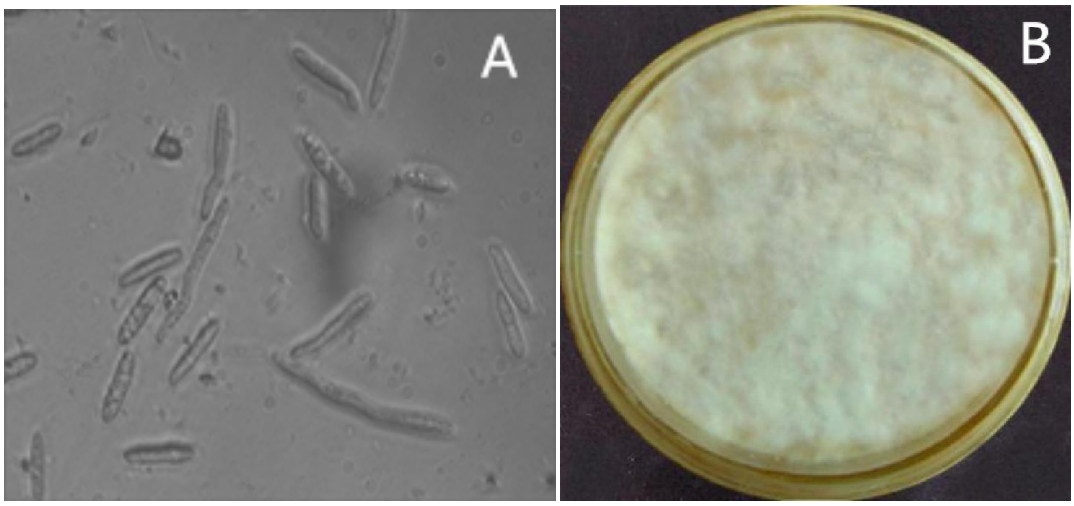
**Table 1. Smut incidence, latent period and resistance evaluation levels of the tested sugarcane genotypes**

| Genotype | Pin-prick dipping of sporidia suspension | | | | Pin-prick dipping of teliospores suspension | | | |
|---|---|---|---|---|---|---|---|---|
| | Latent period (d) | Disease incidence (%) | Resistance grade | Resistance types | Latent period (d) | Disease incidence (%) | Resistance grade | Resistance type |
| GT29 | 47 | 24.05 | 5 | MS | 61 | 19.26 | 5 | MS |
| GT32 | 40 | 32.69 | 6 | S1 | 82 | 18.06 | 5 | MS |
| GT36 | 82 | 3.13 | 1 | HR | 124 | 2.78 | 1 | HR |
| GT37 | 82 | 3.85 | 2 | R1 | - | 0.00 | 1 | HR |
| GT40 | 75 | 14.76 | 5 | MS | 110 | 2.00 | 1 | HR |
| GT42 | 68 | 34.14 | 6 | S1 | 68 | 15.19 | 5 | MS |
| GT43 | 47 | 32.46 | 6 | S1 | 61 | 17.09 | 5 | MS |
| ROC10 | 54 | 16.67 | 5 | MS | 89 | 13.25 | 5 | MS |
| ROC16 | 54 | 27.78 | 6 | S1 | 82 | 13.89 | 5 | MS |
| ROC22 | 54 | 18.97 | 5 | MS | 82 | 18.11 | 5 | MS |
| YT93-159 | 75 | 9.09 | 3 | R2 | - | 0.00 | 1 | HR |
| YT94-128 | 54 | 18.43 | 5 | MS | 54 | 18.68 | 5 | MS |
| F134 | 61 | 63.33 | 8 | HS1 | 61 | 50.88 | 8 | HS1 |
| NCo310 | - | 0.00 | 1 | HR | - | 0.00 | 1 | HR |
| NCo376 | 61 | 30.67 | 6 | S1 | 68 | 23.33 | 5 | MS |
| YC71-374 | 68 | 46.11 | 7 | S2 | 68 | 42.43 | 7 | S2 |
| GXS87-16 | 68 | 23.53 | 5 | MS | 82 | 16.67 | 5 | MS |
| GXS85-30 | - | 0.00 | 1 | HR | - | 0.00 | 1 | HR |
| GXS145 | 82 | 3.57 | 2 | R1 | 89 | 2.78 | 1 | HR |
| GXS165 | 75 | 22.22 | 5 | MS | 75 | 23.70 | 5 | MS |
| GXS222 | - | 0.00 | 1 | HR | - | 0.00 | 1 | HR |
| GXS238 | 61 | 15.29 | 5 | MS | 68 | 13.21 | 5 | MS |




A: Teliospores of *S. scitaminea* Sydow (1000×); B: Woolly monocolony; C: Yeast-like monocolony; D: Sexual mating of monosporidia. White woolly colonies are "+" and "-"mating type strains

**Fig. 1. Monosporidial isolation and sexual mating of *Sporisorium scitaminea* Sydow**




A: The haploid sporidia derived from teliospore germination of *S. scitaminea* (1000×); B: Woolly dikaryon hyphae derived from teliospore germination of *S. scitaminea*

**Fig. 2. Viability determination for teliospores of *Sporisorium scitaminea* Sydow**

Sexual mating of yeast like single colonies (Fig. 1 C) could be used to identify the mating type. If a yeast-like colony generates, it means that the two sporidia are the same mating type, and if a white woolly colony produces, it means that the two sporidia were the opposite mating types (Fig. 1 D).

**Viability Detection for Teliospores**

The teliospores (chlamydospores) cultured on YEPS medium generated dikaryotic infection hyphae (Fig. 2 A, B). Test results showed 90 % of the teliospores were active, which were qualified enough for inoculation.

**Latent Period and Incidence**

Disease latent period is an important indicator to evaluate the ability of host defense against the invasion of pathogens. Smut latent period in the 22 tested sugarcane clones were 40-82 days after sporidia inoculation, while 54-124 days in teliospores inoculation (Table 1). Among them were 5 genotypes showing the same smut latent period in two inoculation tests, 17 genotypes showing 7-42 days shorter in sporidia inoculation than in teliospore inoculation, and nine showing more than 14 days shorter which accounted for 40.91% of the tested genotypes. Smut incidence in the 22 sugarcane clones were between 0 and 63.33 %, and we observed that those having a longer latent period showed lower smut incidence, while those having a shorter latent period recorded a higher smut incidence.

**Identification of Sugarcane Smut Resistance**

By means of sporidia inoculation, the standard control sugarcane varieties F134, NCo310, NCo376 and YC71-374 showed highly susceptible 1, highly resistant, susceptible 1 and susceptible 2 to smut, respectively. The accessions of GT36, GXS85-30 and GXS222 had high level of resistance against smut; GT37, YT93-159 and GXS145 showed resistance to smut; GT29, GT40, ROC10, ROC22, YT94-128, GXS87-16, GXS165 and GXS238 had moderate susceptibility to smut accounting for 50 % of the tested genotypes; and the rests, GT32, GT42, GT43 and ROC16, showed susceptible 1 to smut. That is, 66.67% of the tested genotypes (except 4 standard control varieties) were susceptible to smut. The results of teliospore inoculation shown that the standard control varieties F134, Nco310, Nco376 and YC71-374 had levels of highly susceptible 1, highly resistant, moderately susceptible and susceptible 2 to smut, respectively. Seven genotypes (account for 38.89%) viz. GT36, GT37, GT40, YT93-159, GXS85-30, GXS145 and GXS222 were highly resistant against smut, while the other 11 genotypes were all moderately susceptible to smut, account for 61.11% of the tested genotypes. Comparing the effects of the two forms of *S. scitaminea* inoculum in sugarcane, 13 of the tested genotypes had the same resistance level to smut in the present study, while the other genotypes showed 1 to 4 levels of lower resistance against smut in sporidia inoculation than in teliospore inoculation. The biggest change was observed in GT40, which was highly resistant (level 1) to smut in teliospore inoculation but became moderately susceptible (level 5) to smut in sporidia inoculation.

## DISCUSSION

F134, NCo310 and NCo376 are hosts varieties of differential *S. scitaminea* races in China (Hisieh and Lee, 1978; Xu, 2000). The smut incidence occurred in the four standard control varieties, which was an indicator of a successful inoculation. In the present study, we inferred the pathogen used for inoculation was *S. scitaminea* race 2 according to the performance of F134 (resistant to race 1 but susceptible to race 2) and NCo310 (resistant to race 2 but susceptible to race 1). But smut incidence of NCo376 (immune to race 1 and race 2) were 30.67% and 23.33% (Table 1), respectively, indicating that the pathogenicity of *S. scitaminea* in this study was stronger than that previously reported, or it had generated virulence mutations (Shen *et al*., 2011, 2013, 2014), which made NCo376 susceptible to smut. An effective program in sugarcane breeding for resistance to smut, caused by *S. scitaminea*, requires an inoculation method that is practical,

rapid and reliable. Furthermore, the inoculation process must result in sufficiently high levels of disease to discriminate between susceptible and resistant genotypes. Previous studies have proven that smut disease resistance is closely associated with the structure characteristics of sugarcane buds, like bud size, groove depth, bud-scale compact degree, bud hole location etc. (Glória *et al*., 1995; Gong *et al*., 1996). The pin-prick dipping inoculation used in this study excluded the morphological obstacles of cane buds and imported the pathogen directly into the bud tissue, in order to detect the smut reaction directly at physiological and biochemical level. Gao *et al*. (2013) proved this method is effective than dip inoculation by inoculating teliospores suspension in 8 sugarcane varieties. The dipping inoculation with sporidia suspension of *S. scitaminea* in sugarcane was studied by Chen *et al*. (2013), the same inoculation but using teliospores suspension was studied by Shen *et al*. (2011), and the results showed 5/6 of the same tested sugarcane clones had the uniform level of smut resistance.

But between the two inoculums done by means of pin-prick dipping inoculation assay in the present study, the sporidia inoculation produced symptoms in a shorter latent time, and gave a higher incidence and a shorter period for identification compared to teliospores inoculation. The effects of sporidia suspension of *S. scitaminea* were in accordance with Shen *et al*. (2014) in which injection inoculation had been done in sugarcane seedlings. This study showed that the sporidia inoculum by a pin-prick dipping inoculation is more appropriate for identifying primary sources of resistance. It took shorter time for disease development using sporidia since inoculation in plants as compared to teliospores inoculation. In addition, this method may be used in some instances especially in genetic mapping studies of smut resistance. Of the 18 genotypes evaluated, GT36, GT37, YT93-159, GXS85-30, GXS145 and GXS222 were resistant or even highly resistant to smut, which can be used as resistance sources for breeding smut resistant cultivars. In this study, 66.67 % of the tested genotypes were susceptible to smut, indicating smut is a widespread sugarcane disease in China.

Some of the smut-resistant varieties may have already lost resistance and become susceptible to smut for some reasons. For instance, ROC10 used to be highly resistant to smut showed susceptible in the present study. The data on GT29 that moderately susceptible to smut was not accordant to the practical performance in commercial production (Zhang *et al*., 2011), which showed high resistance to smut in fields. ROC22 is the main sugarcane cultivar in mainland China which is smut-susceptible (Su *et al*., 2013), and even though application of smut pathogen free healthy seedcanes could not get ideal results, but it was identified as moderately susceptible. These may probably due to the influences of environmental factors and new physiological races (Gao *et al*. 2013), and crop seasons and years (Wu *et al*., 1983; Olweny *et al*., 2008) and pathotypes of isolates could also influence the development of smut. It is urgent to screen a set of new sugarcane hosts for smut physiological races identification, and select resistant sources for application in smut-resistant breeding (Shen *et al*. 2013). In addition, molecular marker-assisted breeding, molecular genetic map and QTL mapping on smut pathogenicity may be helpful to improve the breeding efficiency.

**Acknowledgements**

This work was supported in part by China National Program for International Scientific Exchange and Cooperation project (2013DFA31600), National Natural Science Foundation of China (31301382), Guangxi Special Funds for Bagui Scholars and Distinguished Experts (2013), Guangxi R & D Research Program projects (Gui Ke He 1347004-2, Gui Ke Gong 1222009-1B, Gui Ke Neng 14121008-2-1), Funds of GXAAS (Gui Nong Ke 2013YT06, Gui Nong Ke 2014JQ02).

## REFERENCES

Alexander, K.C. and R.K. Krishna, 1978. Studies on smut disease (*Ustilago scitaminea*) of sugarcane: Longevity and viability of teliospores. *Indian Journal of Sugarcane Technology* 1:47-49.

Chen, J.W., W.K. Shen, Z.D. Yang, Z.H. Chen, R. Liu, Y.S. Chen and H.H. Deng, 2013. Evaluation of some important sugarcane varieties for smut resistance using the inoculation method basing on plus and minus mating type sporidia. *Sugarcane and Canesugar* 1: 23-26.

Croft B.J. and K.S. Braithwaite, 2006. Management of an incursion of sugarcane smut in Australia. *Australasian Plant Pathology* 35: 113–122.

Fontaniella, B., A. Márquez, C.W. Rodríguez, D. Piñón, M.T. Solas, C. Vicente and M.E. Legaz, 2002. A role for sugarcane glycoproteins in the resistance of sugarcane to *Ustilago scitaminea*. *Plant Physiol. Biochem.* 40:881–889.

Gao, Y.J., G.M. Zhang, R.H. Zhang, H.Z. Song, T. Luo, W.X. Duan, W. Xian, J.X. Liao, H. Zhou and J.H. You, 2013. Evaluation of resistance to smut disease in new sugarcane varieties and breeding lines. *Sugar Crop of China* 2: 25-28.

Glória B.A., M.C. Capote-Albernaz and L. Amorim, 1995. Structural characteristics of buds of sugarcane cultivars with different levels for resistance to smut. *Zeitschrift fur Pflanzenkrankheiten and Pflanzenschutz* 102: 502-508.

Gong, D.M., Y.Q. Lin and R.K. Chen, 1996. Sugarcane smut resistance breeding research III. Relationship between anti-smut with cane buds characteristics. *Acta Agronomica Sinica* 22(3): 362-364.

Hisieh, W.H. and C.S. Lee, 1978. Compatibikity and pathogenicity of two races of *Ustiliago scitaminea* Sydow in Taiwan. *Taiwan Sugar* 25: 46-48.

Huo, X.J., C.S. Li and R.S. Lu, 2013. Evaluation of several sugarcane varieties for smut resistance in Guangxi. *Sugar Crop of China* 4: 29-31.

Olweny, C., N. Kahiu, H. Nzioki and S.M. Githiri, 2008. Evaluation of smut inoculation techniques in sugarcane seedlings. *Sugar Tech* 10(4): 341-345.

Shamsul, A.B., J.C. Barry, S.J. Rebecca, and C.C. Mike. 2012. Laboratory and field evaluation of fungicides for the management of sugarcane smut caused by *Sporisorium scitamineumin* seedcane. *Australasian Plant Pathol.* 41: 591–599.

Shen, W.K., and H.H. Deng. 2011. Analysis of results from smut resistant identification in sugarcane varieties introduced. *Chinese Agricultural Science Bulletin* 27(19): 234-238.

Shen, W.K., Z.D. Jiang, H.H. Deng, and R. Liu. 2013. Research progress on sugarcane smut disease and *Sporisorium scitaminea*. *Chinese journal of Tropical Crops* 34(10): 2063-2068.

Shen, W.K., Z.D. Jiang, R. Liu, J.W. Chen, and H.H. Deng. 2014. A new method of identification sugarcane smut resistance and resistant evaluation of varieties. *Journal of Huazhong Agricultural University* 2(33):51-56.

Shen, W.K., Z.D. Yang, and F.Y. Liu. 2014. Identification and evaluation for sugarcane varieties (lines) of smut resistance. *Journal of Huazhong Agricultural University* 5(33): 40-44.

Su, Y.C., L.P. Xu, B.T. Xue, Q.B. Wu, J.L. Guo, L.G. Wu, and Y.X. Que. 2013. Molecular cloning and characterization of two pathogenesis-related β-1,3-glucanase genes *ScGluA1* and *ScGluD1* from sugarcane infected by *Sporisorium scitamineum*. *Plant Cell Rep.* 32:1503–1519.

Su, Y.C., S.S. Wang, J.L. Guo, B.T. Xue, L.P. Xu, and Y.X. Que. 2013. A TaqMan Real-Time PCR Assay for Detection and Quantification of *Sporisorium scitamineum* in Sugarcane. *The Scientific World Journal* 1-9.

Wang, W.Z., H. Hong, Q.Z. Zhu, D.F. Huang, Y.W. Luo, J.L. Xie, and T. Liang. 2010. Evaluation of some new introduced sugarcane varieties for smut resistance. *Chinese Agricultural Science Bulletin* 26(15): 285-288.

Wu, K.K., D.J. Heinz, and H.K. Meyer. 1983. Heritability of sugarcane smut resistance and correlation between smut grade and yield components. *Crop Science* 23(1): 54-56.

Xu. 2000. Construction of smut resistant or susceptible pools and molecular marker for resistance gene in sugarcane. *Fujian Agricultural and Forestry University.* 1-3.

Zhang, R.H., H. He, G.M. Zhang, H.B. Liu, Y.R. Li, F.X. Fang, H.Z. Song, W.K. Fang, and S.L. Bi. 2011. Breeding of new sugarcane variety Guitang 29 with high ra toon ability. *Sugar Crop of China* 1: 1-4.

*******