Evaluation of BC1 and BC2 from the crossing Erianthus arundinaceus with Saccharum for resistance to sugarcane smut caused by Sporisorium scitamineum

Wan-Kuan Shen¹, Hai-Hua Deng², Qi-Wei Li², Zhan-Duan Yang² & Zi-De Jiang³

¹College of Agronomy, South China Agricultural University/Scientific Observing and Experimental Station of Crop Cultivation in South China, Ministry of Agriculture, 510642, Guangdong, Guangzhou, China; ²Guangzhou Sugarcane Industry Research Institute /Guangdong Key Laboratory of Sugarcane Improvement and Biorefinery, 510316, Guangdong, Guangzhou, China; ³Department of Plant Pathology, South China Agricultural University/Guangdong Key Laboratory of Microbial Signals and Disease Control, 510642, Guangdong, Guangzhou, China

Author for correspondence: Zide Jiang, e-mail: wkshen69@126.com

ABSTRACT
Sugarcane smut disease caused by the fungus Sporisorium scitamineum is one of the important fungal diseases affecting sugarcane yield and sucrose content around the world. Cultivar resistance is the most appropriate control method for this disease. In this study, 37 BC1 lines chosen from the crossing YC96-40 (F1 of Erianthus arundinaceus) × CP84-1198 (commercial sugarcane cultivar) and 42 BC2 lines chosen from the crossing YCE01-116 (BC1 of E. arundinaceus) × Neijiang57-416 (commercial sugarcane cultivar) were evaluated for smut resistance using artificial inoculation. The results showed that of 79 tested BC1 and BC2 lines of E. arundinaceus, 10 (12.7%) were highly to moderately resistant to smut. BC1 of E. arundinaceus had more resistant lines than BC2 of E. arundinaceus. Of the 37 tested BC1 lines of E. arundinaceus, seven (18.9%) were highly to moderately resistant, while three (7.1%) of the 42 tested BC2 lines of E. arundinaceus were highly to moderately resistant to smut. The resistant lines identified in this study could be used as sources of smut resistance in sugarcane breeding programs.

Key words: Erianthus arundinaceus, Saccharum officinarum, Sporisorium scitamineum, backcross progenies, evaluation of resistance.

INTRODUCTION
Sugarcane (Saccharum hybrid species) is an important economic crop for sugar and ethanol production. Mainland China is currently the third largest producer of sugarcane in the world, following Brazil and India. Southern China, including Guangxi Zhuang autonomous region, Yunnan and Guangdong Provinces, is the major sugarcane-producing region in mainland China (Chen & Yuan, 2010). Sugarcane smut caused by the fungus Sporisorium scitamineum, formerly called Ustilago scitaminea (Stoll et al., 2003), is an important disease worldwide (Comstock, 2000). It was reported for the first time in the world in 1877 when it was found in Natal, South Africa (McMartin, 1945), and numerous outbreaks were noted in Africa and Asia in the following decades. Smut remained confined to the Eastern hemisphere until it was found in Argentina in 1940 (Comstock, 2000). In China, smut was found in 1932 in Guangzhou for the first time (Antoine, 1961; Presley, 1978). During the past 20 years, smut has developed into a major disease and caused serious yield loss in sugarcane production in mainland China (Que et al., 2012; Shen et al., 2013).

The most efficient and economic method for disease control, including sugarcane smut, is the use of resistant cultivars (Wada, 2003; Shen et al., 2014). However, the development of resistant sugarcane cultivars requires elite sources of resistance to smut. Modern sugarcane cultivars are derived from a relatively few interspecific hybrids between Saccharum officinarum L. and S. spontaneum L., resulting in a narrow germplasm base (Berding & Roach, 1987). To increase this restricted genetic base, breeders have been interested in the introgression of genes from wild species. Erianthus arundinaceus is an important closely related wild species of S. officinarum. This species has great potential as a germplasm source for modifying the ratoning ability, vigour, tolerance to environmental stresses, and disease resistance of sugarcane (George et al., 2000; Fukuhara et al., 2013). E. arundinaceus was first hybridized with sugarcane in 1885 (Deng et al., 2004). However, further progress was not made until the 1990s, because of the sterility of hybrids and the difficulty in identifying genuine progenies (Shen, 2002). In recent years, great progress has been made in the use of E. arundinaceus, and some promising BC1 and BC2 lines have been obtained from crossing E. arundinaceus with Saccharum (Deng et al., 2004). Several studies on physiological and biochemical characteristics or chromosome transmission in backcross...
progenies of *E. arundinaceus* have been conducted (Chen et al., 2006; Deng et al., 2007; Deng et al., 2009). However, there have been no reports on the assessment of BC, and BC, of *E. arundinaceus* for resistance to sugarcane smut. The objective of this study was to evaluate smut resistance in BC, and BC, lines of *E. arundinaceus*.

**MATERIALS AND METHODS**

Materials and experimental site

Seventy-nine backcross progenies of *E. arundinaceus*, including 37 BC, and 42 BC, lines, and their parents, YC96-40 (F, of *E. arundinaceus*), CP84-1198 (commercial sugarcane cultivar), YCE01-116 (BC, of *E. arundinaceus*) and Neijiang57-416 (commercial sugarcane cultivar), were kindly provided by Hainan Sugarcane Hybridization Station, Guangzhou Sugarcane Industry Research Institute, Guangzhou, China. Seventy-nine BC, and BC, lines of *E. arundinaceus*, YC96-40 and YCE01-116 have been identified as true hybrids of *E. arundinaceus* by molecular approaches (He et al., 2008). This study was carried out in June of 2008 at Guangzhou Sugarcane Industry Research Institute, China.

Preparation of planting sets

Sugarcane stalks from a 7-month-old plantation were cut and the leaves detached to expose the buds. These were then cut into one-budded sets ready for inoculation.

Inoculation and planting of prepared planting sets

For screening resistance in the field, teliospores of *S. scitamineum* were collected from mature unopened sori produced on canes in field at Zhanjiang sugarcane production areas, Guangdong Province, China. Spore germination was determined under a compound microscope (Olympus, Model BH-2) at 100× using a micro-counter as described by Bhuiyan et al. (2012). Two gram smut spores were mixed with one liter of distilled water as per standard screening practices (Shen & Deng, 2011). The spore suspension is prepared in a 50 liter tank giving a concentration of approximately 4-5 million spores per milliliter. One-budded sets of the tested BC, BC, lines of *E. arundinaceus* and their parents were dipped into smut spore suspension for 30 min as described by Shen and Deng (2011). The inoculated sets were then incubated in wet jute gunny bags overnight and planted in plastic buckets (35 cm diameter, 30 cm depth) filled with a steam-sterilized mixture of soil and organic matter (3:1 v/v). A total of 30 plants of each test material were treated according to a completely randomized experimental design including three replicates of individual bucket containing 10 plants. Plants were grown in greenhouse at 28-30°C.

Investigation of incidence and resistance classification

Approximately 4-5 weeks after inoculation, surveys of disease incidence were initiated and carried out every 15 days until the disease incidence was stable (six months). The date of inoculation, number of total stools, number of diseased stools were recorded. Disease reactions of the tested materials for *S. scitamineum* were rated on a scale from 1 to 9 based on the percentage of diseased stools (Shen et al., 2014), where 0-3% was scored as grade 1 (highly resistant), 4-6% as grade 2 (resistant), 7-9% as grade 3 (resistant), 10-12% as grade 4 (moderately resistant), 13-25% as grade 5 (moderately susceptible), 26-35% as grade 6 (susceptible), 36-50% as grade 7 (susceptible), 51-75% as grade 8 (highly susceptible), and 76-100% as grade 9 (highly susceptible).

**RESULTS**

From a total of 79 BC, and BC, lines of *E. arundinaceus*, resistance to smut ranging from grade 1 (highly resistant) to grade 4 (moderately resistant) was detected in 12.7% (10 out of 79) lines (Table 1). The percentage of resistant lines in BC, of *E. arundinaceus* (18.9%, seven out of 37) was higher than that of BC, (7.1%, three out of 42). In BC, of *E. arundinaceus*, five (13.5%) of the 37 tested BC, lines were highly resistant to smut. Resistant was found in 5.4% (two out of 37) of BC, lines, and 81.1% (30 out of 37) of BC, lines were susceptible to smut, ranging from grade 5 (moderately susceptible) to grade 9 (highly susceptible). Of the BC, lines of *E. arundinaceus*, one line was scored as highly resistant (grade 1), counting for 2.4% (1 out of 42), two lines exhibited resistance (grade 3) to smut, and 92.9% (39 out of 42) lines were susceptible to smut, ranging from grade 5 (moderately susceptible) to grade 9 (highly susceptible). The female parent YC96-40 (F, of *E. arundinaceus*) and the male parent CP84-1198 (commercial sugarcane cultivar) of BC, were both susceptible to smut, while the female parent YCE 01-116 (BC, of *E. arundinaceus*) and male parent Neijiang57-416 (commercial sugarcane cultivar) of BC, were both highly susceptible to smut.

**DISCUSSION**

In modern sugarcane breeding, the screening, identification and evaluation of systemic resistance in source materials is critical due to the importance of wild sugarcane resources as a source of resistance genes. Subsequent characterization and utilization of wild resistance genes can be used to broaden the genetic base of sugarcane resistance against disease and has important significance for screening and breeding of resistant cultivars (Li et al., 2013). Sugarcane smut has been the major sugarcane disease in mainland China in recent years. In this study, a total of 79 backcross progenies (BC, and BC,) of *E. arundinaceus* were screened for resistance to smut using artificial inoculation method. Seven BC, and three BC, lines of *E. arundinaceus* were identified as highly to moderately resistant germplasms, which could provide an elite array of resistance sources for effective breeding of sugarcane cultivars against smut.
TABLE 1 - Identification of smut resistance in BC₁ and BC₂ lines from the crossing *Erianthus arundinaceus × Saccharum* by artificial inoculation.

| Line | Type | Latent period (days)¹ | Incidence (%) | Grade | Resistance response² |
|------|------|-----------------------|---------------|-------|----------------------|
| 78   | BC₂  | 174                   | 13            | 5     | MS                   |
| 373  | BC₂  | 81                    | 100           | 9     | HS                   |
| 135  | BC₂  | 81                    | 62            | 8     | HS                   |
| 393  | BC₂  | 65                    | 43            | 7     | S                    |
| 163  | BC₂  | 124                   | 29            | 6     | S                    |
| 221  | BC₂  | 174                   | 14            | 5     | MS                   |
| 325  | BC₂  | 65                    | 63            | 8     | HS                   |
| 226  | BC₂  | 124                   | 23            | 5     | MS                   |
| 69   | BC₂  | 124                   | 36            | 7     | S                    |
| 75   | BC₂  | 81                    | 79            | 9     | HS                   |
| 323  | BC₂  | 65                    | 62            | 8     | HS                   |
| 79   | BC₂  | 109                   | 85            | 9     | HS                   |
| 385  | BC₂  | 81                    | 62            | 8     | HS                   |
| 116  | BC₂  | 81                    | 87            | 9     | HS                   |
| 37   | BC₂  | 81                    | 47            | 7     | S                    |
| 250  | BC₂  | 174                   | 8             | 3     | R                    |
| 327  | BC₂  | 81                    | 55            | 8     | HS                   |
| 218  | BC₂  | 81                    | 47            | 7     | S                    |
| 356  | BC₂  | 65                    | 92            | 9     | HS                   |
| 277  | BC₂  | 81                    | 42            | 7     | S                    |
| 105  | BC₂  | 81                    | 43            | 7     | S                    |
| 349  | BC₂  | 81                    | 46            | 7     | S                    |
| 333  | BC₂  | 81                    | 39            | 7     | S                    |
| 41   | BC₂  | n.a.                  | 0             | 1     | HR                   |
| 138  | BC₂  | 65                    | 50            | 7     | S                    |
| 94   | BC₂  | 124                   | 8             | 3     | R                    |
| 220  | BC₂  | 65                    | 80            | 9     | HS                   |
| 20   | BC₂  | 81                    | 58            | 8     | HS                   |
| 16   | BC₂  | 81                    | 100           | 9     | HS                   |
| 381  | BC₂  | 81                    | 75            | 8     | HS                   |
| 300  | BC₂  | 81                    | 67            | 8     | HS                   |
| 150  | BC₂  | 65                    | 83            | 9     | HS                   |
| 279  | BC₂  | 124                   | 23            | 5     | MS                   |
| 231  | BC₂  | 81                    | 31            | 6     | S                    |
| 313  | BC₂  | 124                   | 21            | 5     | MS                   |
| 104  | BC₂  | 109                   | 60            | 8     | HS                   |

Cont.
Evaluation of BC1 and BC2 from the crossing *Erianthus arundinaceus* with *Saccharum* for resistance...

| Line | Type | Latent period (days) | Incidence (%) | Grade | Resistance response |
|------|------|----------------------|---------------|-------|---------------------|
| 53   | BC2  | 174                  | 20            | 5     | MS                  |
| 11   | BC2  | 81                   | 38            | 7     | S                   |
| 53   | BC2  | 174                  | 20            | 5     | MS                  |
| 11   | BC2  | 81                   | 38            | 7     | S                   |
| 14   | BC2  | 174                  | 29            | 6     | S                   |
| 145  | BC2  | 81                   | 23            | 5     | MS                  |
| 265  | BC1  | n.a.                 | 0             | 1     | HR                  |
| 46   | BC1  | 81                   | 43            | 7     | S                   |
| 49   | BC1  | n.a.                 | 0             | 1     | HR                  |
| 15   | BC1  | 65                   | 42            | 7     | S                   |
| 30   | BC1  | 81                   | 67            | 8     | HS                  |
| 28   | BC1  | 65                   | 89            | 9     | HS                  |
| 372  | BC1  | 65                   | 64            | 8     | HS                  |
| 9    | BC1  | 81                   | 40            | 7     | S                   |
| 25   | BC1  | 81                   | 44            | 7     | S                   |
| 151  | BC1  | 174                  | 50            | 7     | S                   |
| 204  | BC1  | 65                   | 70            | 8     | HS                  |
| 366  | BC1  | 81                   | 40            | 7     | S                   |
| 4    | BC1  | 81                   | 56            | 8     | HS                  |
| 1    | BC1  | 81                   | 50            | 7     | S                   |
| 121  | BC1  | 124                  | 29            | 6     | S                   |
| 240  | BC1  | 124                  | 71            | 8     | HS                  |
| 182  | BC1  | n.a.                 | 0             | 1     | HR                  |
| 64   | BC1  | 65                   | 69            | 8     | HS                  |
| 126  | BC1  | 81                   | 60            | 8     | HS                  |
| 189  | BC1  | 65                   | 82            | 9     | HS                  |
| 56   | BC1  | 81                   | 50            | 7     | S                   |
| 358  | BC1  | 174                  | 9             | 3     | R                   |
| 282  | BC1  | 109                  | 20            | 5     | MS                  |
| 396  | BC1  | 65                   | 100           | 9     | HS                  |
| 352  | BC1  | 174                  | 9             | 3     | R                   |
| 390  | BC1  | 81                   | 43            | 7     | S                   |
| 24   | BC1  | 174                  | 15            | 5     | MS                  |
| 302  | BC1  | 81                   | 40            | 7     | S                   |
| 179  | BC1  | 81                   | 27            | 6     | S                   |
| 374  | BC1  | 50                   | 91            | 9     | HS                  |
| 22   | BC1  | 81                   | 57            | 8     | HS                  |

Cont.
In this study, backcross progenies of *E. arundinaceus* with *Saccharum* showed no stronger resistance ability to smut, leading to only 18.9% of BC₁ lines and 7.1% of BC₂ lines with highly to moderate resistance. The main reason was that the backcross progenies derived from susceptible crossings: BC₁ lines from a susceptible vs. susceptible crossing, and BC₂ lines from a highly susceptible vs. highly susceptible crossing. The heritability of sugarcane smut resistance is moderate (Wu et al., 1977, 1983; Comstock, 1983; Chao et al., 1990) therefore the resistance level of parental combinations affected the resistance ability of the offspring. On the other hand, BC₁ and BC₂ plants of *E. arundinaceus* have larger buds with smaller or no sprout wings, which are morphological features that may be beneficial to germination and infection of *S. scitamineum* (Muthusamay, 1974; Padmanaban et al., 1988a, 1988b) and thus may also have affected the resistance backcross progenies of *E. arundinaceus* to smut. Piperidis et al. (2010) reported that in the BC₁ lines of *E. arundinaceus* the number of chromosomes ranged from 21 to 30, while in the BC₂ lines the number ranged from 14 to 15, revealing cases of chromosome loss. Therefore, it is possible that resistance genes were lost in backcross progenies of *E. arundinaceus*, which may have lead to hybrid offspring without stronger resistance against smut.

In the future, further studies are needed to objectively evaluate the resistance ability of backcross progenies of *E. arundinaceus* to smut from resistant vs. resistant crossings or highly resistant vs. highly resistant crossings. It would be useful to get more promising resistance sources against sugarcane smut disease and reveal prospect of *E. arundinaceus* in breeding for resistance to smut.

In conclusion, this study has identified ten BC₁ and BC₂ lines of *E. arundinaceus* with resistance against sugarcane smut disease out of 79 tested lines, broadening the genetic basis of smut resistance in sugarcane breeding.

**ACKNOWLEDGEMENTS**

This work was supported by grants from the Earmarked Fund for Key Agriculture Project of Guangdong Province, China (2010B020302001) and the Earmarked Fund for President Project of South China Agricultural University, China (K13009).

**REFERENCES**

Antoine R (1961) Smut. In: Martin JP, Abbott EV, Hughes CG (Eds.) Sugarcane Diseases of the World. Amsterdam, The Netherlands. Elsevier. pp. 327-354.

Berding N, Roach BT (1987) Germplasm collection, maintenance, and use. In: Heinz DJ (Ed.) Sugarcane Improvement Through Breeding. Amsterdam, The Netherlands. Elsevier. pp. 143-210.

Bhuivan SA, Croft BJ, James RS, Cox MC (2012) Laboratory and field evaluation of fungicides for the management of sugarcane smut caused by *Sporisorium scitamineum* in seedcane. Australasian Plant Pathology 41:591-599.

Chao CP, Hoy JW, Martin FA (1990) Heritability of resistance and repeatability of clone reactions to sugarcane smut in Louisiana. Phytopathology 80:622-626.

Chen RK, Yuan ZN (2010) Sugarcane production and research in China. International Sugar Journal 112:452-457.

Chen YS, Deng HH, Liang JN, Li QW, Tan ZW (2006) Differences of physiological and biochemical characters among the progenies of *Erianthus arundinaceus*. Journal of Huazhong Agricultural University 25:598-602.

Comstock JC (2000) Smut. In: Rott P, Bailey RA, Comstock JC, Tropical Plant Pathology 39 (5) September - October 2014

| Line      | Type   | Latent period (days)¹ | Incidence (%) | Grade | Resistance response² |
|-----------|--------|------------------------|---------------|-------|-----------------------|
| 154       | BC₁    | 124                    | 33            | 6     | S                     |
| 74        | BC₁    | 81                     | 63            | 8     | HS                    |
| 6         | BC₁    | n.a.                   | 0             | 1     | HR                    |
| 296       | BC₁    | 81                     | 50            | 7     | S                     |
| 158       | BC₁    | n.a.                   | 0             | 1     | HR                    |
| 100       | BC₁    | 81                     | 50            | 7     | S                     |
| CP84-1198 (Male parent, cultivar) | 124 | 30                     | 6     | S                     |
| YC96-40 (Female parent, F₁) | 109 | 28                     | 6     | S                     |
| YCE01-116 (Female parent, BC₁) | 124 | 57                     | 8     | HS                    |
| Neijiang57-416 (Male parent, cultivar) | 65 | 54                     | 8     | HS                    |

¹n.a., not applicable.
²Resistance response: HR, highly resistant; R, resistant; MR, moderately resistant; MS, moderately susceptible; S, susceptible; HS, highly susceptible.
Evaluation of BC1 and BC2 from the crossing *Erianthus arundinaceus* with *Saccharum* for resistance...

Croft BJ, Saumtally AS (Eds.) A guide to sugarcane diseases. Montpellier, France. CIRAD and ISSCT. pp. 181-185.

Comstock K JC, Ferreira SA, Tew TL (1983) Hawaii’s approach to control sugarcane smut. Plant Disease 67:452-457.

Deng HH, Fu C, Chen B, Yu ZL, Tan ZW, Li QW, Chen PS, Liang JN (2007) Differences of physiological and biochemical characters of leaves between BC2 lines of *Erianthus arundinaceus* and their parents. Journal of Huazhong Agricultural University 26:766-771.

Deng HH, Hu C, Li QW, Liao ZZ, Chen XW, Liang JN, Zhang CM, Tan ZW (2004) Studies on fertile *S. officinarum × E. arundinaceus* hybrids and their BC1 performance. Chinese Journal of Tropical Crops 25:97-101.

Deng ZH, Zhang MQ, Lin WL, Fu C, Zhang CM, Li YC, Lai LP, Lin YQ, Chen RK (2010) Analysis of disequilibrium hybridization in hybrid and backcross progenies of *Saccharum officinarum × Erianthus arundinaceus*. Agricultural Sciences in China 9:1271-1277.

Fukuhara S, Terajima Y, Irei S, Sakaigaichi T, Ujihara K, Sugimoto A, Matsuoka M (2013) Identification and characterization of intergeneric hybrid of commercial sugarcane (*Saccharum* spp. hybrid) and *Erianthus arundinaceus* (Retz.) Jeswiet. Euphytica 189:321-327.

George P, Mandy J, Christopher B, Carroll J, Berding N, D’Hont A (2000) Molecular contribution to selection of intergeneric hybrids between sugarcane and the wild species *Erianthus arundinaceus*. Genome 43:1033-1037.

He HY, Lao FY, Liu R, Chen JW (2008) Molecular marker analysis of the progenies derived from intergeneric cross of *Saccharum* with *Erianthus arundinaceus*. Journal of Huazhong Agricultural University 27:573-577.

Li WF, Wang XY, Huang YK, Shan HL, Luo ZM, Ying XM, Zhang RY, Shen K, Yin J (2013) Screening sugarcane germplasm resistant to *Sorghum mosaic virus*. Crop Protection 43:27-30.

McMartin A (1945) Sugarcane smut: Reappearance in Natal. South African Journal of Sugar 29:55-57.

Muthusamy S (1974) Varietal susceptibility to smut in relation to bud characters. Proceedings of the International Society of Sugar Cane Technologists 22:737-749.

Padmanaban P, Alexander KC, Shanmugan N (1988a) Mechanism of smut resistance in sugarcane. Sugar Cane 6:14-16.

Padmanaban P, Alexander KC, Shanmugan N (1988b) Studies on certain characters associated with smut resistance in sugarcane. Indian Phytopathology 41:594-598.

Piperidis N, Chen JW, Deng HH, Wang LP, Jackson P, Piperidis G (2010) GISH characterization of *Erianthus arundinaceus* chromosomes in three generations of sugarcane intergeneric hybrids. Genome 53:331-336.

Presley J (1978) The culmnicolous smut of sugarcane. Sugar 73:34-39.

Que YX, Xu LP, Lin JW, Chen RK, Grisham MP (2012) Molecular variation of *Sporisorium scitamineum* in Mainland China revealed by RAPD and SRAP markers. Plant Disease 96:1519-1525.

Shen WK (2002) Discussion of the value of intergeneric crosses of *Saccharum × Erianthus*. Sugar Cane 9:1-5.

Shen WK, Deng HH (2011) Analysis of results from smut resistant identification in sugarcane varieties introduced. Chinese Agricultural Sciences Bulletin 27:234-238.

Shen WK, Jiang ZD, Deng HH, Liu R (2013) Research progress on sugarcane smut disease and *Sporisorium scitamineum*. Chinese Journal of Tropical Crops 34:2063-2068.

Shen WK, Jiang ZD, Yang ZD, Liu R, Chen JW, Deng HH (2014) New resistance identification method and resistance evaluation of sugarcane varieties to smut disease. Journal of Huazhong Agricultural University 33:51-56.

Stoll M, Piepenbring M, Begerow D, Oberwinkler F (2003) Molecular phylogeny of *Ustilago* and *Sporisorium* species (Basidiomycota, Ustilaginiales) based on internal transcribed spacer (ITS) sequences. Canadian Journal of Botany 81:976-984.

Wada AC (2003) Control of sugarcane smut disease in Nigeria with fungicides. Crop Protection 22:45-49.

Wu KK, Heinz DJ, Meyer HK (1983) Heritability of sugarcane smut resistance and correlation between smut grade and yield components. Crop Science 43:54-56.

Wu KK, Ladd SL, Meyer HK (1977) Combining ability analysis in sugarcane smut resistance. Sugarcane Breed Newsletter 39:59-62.