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RESEARCH ARTICLE

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

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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 BC_1 lines chosen from the crossing YC96-40 (F_1 of *Erianthus arundinaceus*) × CP84-1198 (commercial sugarcane cultivar) and 42 BC_2 lines chosen from the crossing YCE01-116 (BC_1 of *E. arundinaceus*) × Neijiang57-416 (commercial sugarcane cultivar) were evaluated for smut resistance using artificial inoculation. The results showed that of 79 tested BC_1 and BC_2 lines of *E. arundinaceus*, 10 (12.7%) were highly to moderately resistant to smut. BC_1 of *E. arundinaceus* had more resistant lines than BC_2 of *E. arundinaceus*. Of the 37 tested BC_1 lines of *E. arundinaceus*, seven (18.9%) were highly to moderately resistant, while three (7.1%) of the 42 tested BC_2 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 ratooning 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 BC_1 and BC_2 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_1 and BC_2 of *E. arundinaceus* for resistance to sugarcane smut. The objective of this study was to evaluate smut resistance in BC_1 and BC_2 lines of *E. arundinaceus.*

MATERIALS AND METHODS

Materials and experimental site

Seventy-nine backcross progenies of *E. arundinaceus*, including 37 BC_1 lines and 42 BC_2 lines, and their parents, YC96-40 (F_1 of *E. arundinaceus*), CP84-1198 (commercial sugarcane cultivar), YCE01-116 (BC_1 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_1 and BC_2 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 dettached to expose the buds. These were then cut into one-budded setts 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_1, BC_2 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_1 and BC_2 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_1 of *E. arundinaceus* (18.9%, seven out of 37) was higher than that of BC_2 (7.1%, three out of 42). In BC_1 of *E. arundinaceus*, five (13.5%) of the 37 tested BC_1 lines were highly resistant to smut. Resistant was found in 5.4% (two out of 37) of BC_1 lines, and 81.1% (30 out of 37) of BC_1 lines were susceptible to smut, ranging from grade 5 (moderately susceptible) to grade 9 (highly susceptible). Of the BC_2 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_1 of *E. arundinaceus*) and the male parent CP84-1198 (commercial sugarcane cultivar) of BC_1 were both susceptible to smut, while the female parent YCE 01-116 (BC_1 of *E. arundinaceus*) and male parent Neijiang57-416 (commercial sugarcane cultivar) of BC_2 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_1 and BC_2) of *E. arundinaceus* were screened for resistance to smut using artificial inoculation method. Seven BC_1 and three BC_2 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 BC_1 and BC_2 lines from the crossing *Erianthus arundinaceus* × *Saccharum* by artificial inoculation.

Line	Type	Latent period (days)[1]	Incidence (%)	Grade	Resistance response[2]
78	BC_2	174	13	5	MS
373	BC_2	81	100	9	HS
135	BC_2	81	62	8	HS
393	BC_2	65	43	7	S
163	BC_2	124	29	6	S
221	BC_2	174	14	5	MS
325	BC_2	65	63	8	HS
226	BC_2	124	23	5	MS
69	BC_2	124	36	7	S
75	BC_2	81	79	9	HS
323	BC_2	65	62	8	HS
79	BC_2	109	85	9	HS
385	BC_2	81	62	8	HS
116	BC_2	81	87	9	HS
37	BC_2	81	47	7	S
250	BC_2	174	8	3	R
327	BC_2	81	55	8	HS
218	BC_2	81	47	7	S
356	BC_2	65	92	9	HS
277	BC_2	81	42	7	S
105	BC_2	81	43	7	S
349	BC_2	81	46	7	S
333	BC_2	81	39	7	S
41	BC_2	n.a.	0	1	HR
138	BC_2	65	50	7	S
94	BC_2	124	8	3	R
220	BC_2	65	80	9	HS
20	BC_2	81	58	8	HS
16	BC_2	81	100	9	HS
381	BC_2	81	75	8	HS
300	BC_2	81	67	8	HS
150	BC_2	65	83	9	HS
279	BC_2	124	23	5	MS
231	BC_2	81	31	6	S
313	BC_2	124	21	5	MS
104	BC_2	109	60	8	HS

Cont.

Line	Type	Latent period (days)[1]	Incidence (%)	Grade	Resistance response[2]
53	BC_2	174	20	5	MS
11	BC_2	81	38	7	S
53	BC_2	174	20	5	MS
11	BC_2	81	38	7	S
14	BC_2	174	29	6	S
145	BC_2	81	23	5	MS
265	BC_1	n.a.	0	1	HR
46	BC_1	81	43	7	S
49	BC_1	n.a.	0	1	HR
15	BC_1	65	42	7	S
30	BC_1	81	67	8	HS
28	BC_1	65	89	9	HS
372	BC_1	65	64	8	HS
9	BC_1	81	40	7	S
25	BC_1	81	44	7	S
151	BC_1	174	50	7	S
204	BC_1	65	70	8	HS
366	BC_1	81	40	7	S
4	BC_1	81	56	8	HS
1	BC_1	81	50	7	S
121	BC_1	124	29	6	S
240	BC_1	124	71	8	HS
182	BC_1	n.a.	0	1	HR
64	BC_1	65	69	8	HS
126	BC_1	81	60	8	HS
189	BC_1	65	82	9	HS
56	BC_1	81	50	7	S
358	BC_1	174	9	3	R
282	BC_1	109	20	5	MS
396	BC_1	65	100	9	HS
352	BC_1	174	9	3	R
390	BC_1	81	43	7	S
24	BC_1	174	15	5	MS
302	BC_1	81	40	7	S
179	BC_1	81	27	6	S
374	BC_1	50	91	9	HS
22	BC_1	81	57	8	HS

Cont.

Line	Type	Latent period (days)[1]	Incidence (%)	Grade	Resistance response[2]
154	BC_1	124	33	6	S
74	BC_1	81	63	8	HS
6	BC_1	n.a.	0	1	HR
296	BC_1	81	50	7	S
158	BC_1	n.a.	0	1	HR
100	BC_1	81	50	7	S
CP84-1198 (Male parent, cultivar)		124	30	6	S
YC96-40 (Female parent, F_1)		109	28	6	S
YCE01-116 (Female parent, BC_1)		124	57	8	HS
Neijiang57-416 (Male parent, cultivar)		65	54	8	HS

[1]n.a., not applicable.
[2]Resistance response: HR, highly resistant; R, resistant; MR, moderately resistant; MS, moderately susceptible; S, susceptible; HS, highly susceptible.

In this study, backcross progenies of *E. arundinaceus* with *Saccharum* showed no stronger resistance ability to smut, leading to only 18.9 % of BC_1 lines and 7.1% of BC_2 lines with highly to moderate resistance. The main reason was that the backcross progenies derived from susceptible crossings: BC_1 lines from a susceptible vs. susceptible crossing, and BC_2 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, BC_1 and BC_2 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 BC_1 lines of *E. arundinaceus* the number of chromosomes ranged from 21 to 30, while in the BC_2 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 BC_1 and BC_2 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.

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