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Full Length Article

Evaluation of BC_3F_1 Lines from Intergeneric Cross between *Erianthus arundinaceus* and *Saccharum* for Resistance to Sugarcane Smut Caused by *Sporisorium scitamineum*

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Abstract

Sugar cane smut disease caused by the fungus *Sporisorium scitamineum* is one of the major fungal diseases affecting sugar cane yield and sucrose content worldwide. Cultivar resistance is the most appropriate method to control this disease. In sugar cane breeding program, broadening the genetic basis of smut resistance using wild germ plasm resources is valuable. *Erianthus arundinaceus* is closely related wild species of *Saccharum* genus. In the present study, 80 BC_3F_1 lines with *E. arundinaceus* ancestry identified by a molecular method from an intergeneric crossing YC73-226 (*Saccharum* inter specific hybrid) × YCE 06-111 (BC_3 of *E. arundinaceus*) were evaluated for the first time for smut resistance using artificial inoculation. The results showed that of the 80 BC_3F_1 lines tested with *E. arundinaceus* ancestry, 31 lines (38.75%) were highly-to-moderately resistant to smut, among which, 26 lines (83.87%) showed high resistance. On the other hand, 49 lines (61.25%) were moderately-to-highly susceptible to smut, of which, 29 (59.18%), 17 (34.69%), and 3 lines (6.12%) were moderately susceptible, susceptible, and highly susceptible to smut, respectively. The resistant lines identified here could be used as parents for breeding smut resistant cultivars in sugar cane breeding programs or even directly breeding smut resistant cultivars with *E. arundinaceus* ancestry. Also, the potential value of *E. arundinaceus* in sugar cane smut resistance breeding was investigated.

Keywords: *Saccharum*; *Erianthus arundinaceus*; Back cross and hybrid progenies; *Sporisorium scitamineum*; Resistance; Susceptibility

Introduction

Sugarcane (*Saccharum* hybrids spp.) cultivated in tropical and subtropical regions is a vital economic crop for sugar and ethanol production. Currently, mainland China is the third largest producer of sugarcane in the world, followed by Brazil and India. Southwest and Southern China, including Guangxi Zhuang autonomous region, Yunnan and Guangdong Province, are the major sugarcane-producing regions in mainland China (Li and Yang, 2015). Owing to its vegetative mode of propagation, sugarcane is prone to infection by systemic pathogens. Sugarcane smut caused by the fungus *Sporisorium scitamineum*, formerly known as *Ustilago scitaminea* (Stoll *et al*., 2003), is a common disease worldwide (Comstock, 2000). It was found in Natal, South Africa and reported for the first time in 1877 (McMartin, 1945). 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), and now it is presented in all the sugarcane-producing regions of the world except Papua New Guinea and Fiji. In China, smut was found in 1932 in Guangzhou for the first time (Antoine, 1961; Presley, 1978). During the past two decades, smut has developed into a major sugarcane disease, causing severe economic losses, estimated at 8–10 billion dollars per year in mainland China (Shen *et al*., 2013, 2016a).

The most efficient and economical method to control sugarcane smut is to use resistant cultivars (Wada, 2003; Shen *et al*., 2014). A robust genetic control of resistance suggests that rapid progress could be made in developing resistant cultivars through breeding and selection (Burner *et al*., 1993). However, the development of resistant sugarcane cultivars needs elite sources of resistance to smut. Modern sugarcane varieties are derived from a relatively few inter specific hybrids between *Saccharum officinarum* L. and *S. spontaneum* L., resulting in a narrow germ plasm base (Berding and Roach, 1987). Thus in order to increase this limited genetic base, breeders have been interested in the introgression of genes from wild species.

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Erianthus arundinaceus, a species of *Erianthus* genus, is a closely related wild species of *Saccharum* genus, which exhibits high potential as a germ plasm source for modifying the ratooning ability, tolerance to environmental stresses, and disease resistance of sugarcane (Cai *et al*., 2012; Fukuhara *et al*., 2013). *E. arundinaceus* was first hybridized with *Saccharum* in 1885 (Deng *et al*., 2004). However, further progress was not made until the 1990s, owing to the sterility of hybrids, and the difficulty in identifying genuine progenies (Shen, 2002). In recent 15 years, remarkable progress has been made in the use of *E. arundinaceus*, and some promising lines of BC_1 and BC_2 have been produced and evaluated from crossing *E. arundinaceus* with *Saccharum* (Deng *et al*., 2007; Deng *et al*., 2010; Piperidis *et al*., 2010). In addition, we have innovated some BC_3F_1 lines from intergeneric crossing BC_3 lines of *E. arundinaceus* with *Saccharum* interspecific hybrids for the first time. Therefore, it is necessary to identify and evaluate these lines, especially for their smut resistance. To the best of our knowledge, these are the off springs of the highest generation that contain *E. arundinaceus* ancestry worldwide. The objective of the present study was to evaluate the BC_3F_1 lines of *E. arundinaceus* in order to obtain some smut resistant lines or cultivars with *E. arundinaceus* ancestry and broaden the genetic basis of smut resistance in sugarcane breeding.

Materials and Methods

Seedlings Arising, Planting, and Selection

Seeds from the cross, YC 73-226 (*Saccharum* inter specific hybrid) × YCE 06-111 (BC_3 of *E. arundinaceus*), were kindly provided by Hainan Sugarcane Breeding Station, Guangzhou Sugarcane Industry Research Institute, China, in March 2015. The seedlings were raised and planted from April–December 2015 in the sugarcane breeding experiment field of South China Agricultural University (SCAU). The superior off springs were selected according to the agronomic traits and Brix in January 2016.

E. arundinaceus Ancestry Identification of Hybrid Progenies

In order to obtain true hybrid progenies with *E. arundinaceus* ancestry, the screened off spring were identified using *Erianthus*-specific PCR primer pair AGRP 80/81-based PCR (Alix *et al*., 1999). Briefly, genomic DNA from young leaves was extracted using the cetyl trimethyl ammonium bromide method (Zhang *et al*., 2006). *Erianthus*-specific PCR primer pair AGRP 80=5'-GGGTTGTCYTTGCCATCATA-3', AGRP 81=5'-GAGYAGCRCAGAGGTTACGA-3' was used. Primer synthesis, reaction system, amplification procedures and product detection were referred to Shen *et al*. (2016b).

Preparation of Planting Setts

Sugarcane stalks of true hybrid progenies with *E. arundinaceus* ancestry, from a 9-month-old seedling nursery, were cut and the leaves detached to expose the buds, in March and September of 2016, respectively. These were then cut into one-budded setts ready for inoculation.

Inoculation and Planting of Prepared Planting Setts

For screening resistance in the field, teliospores of *S. scitamineum* were collected from mature unopened sori produced on canes in the field at Zhan jiang sugarcane production areas, Guangdong Province, China in June 2015 and preserved in paper bags (10 g/bag) at 4°C for further usage. Spore germination was determined by a compound microscope (Olympus, Model BH-2) at ×100 using a micro-counter (Bhuiyan *et al*., 2012). 2 g smut spores were mixed with 1 L distilled water as per standard screening practices (Shen and Deng, 2011). The spore suspension was prepared in a 50 L tank at a final concentration of approximately 4–5 million spores/mL. One-budded sett of the tested BC_3F_1 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 setts were then incubated in wet jute gunny bags overnight at 28°C.

Then, the inoculated setts were planted in steam sterilized soil in Randomized Complete Block Design (RCBD) with three replications in a greenhouse at 20–30℃, relative humidity 50–70% in the crop experimental field of SCAU. The plot size was two furrows, each of 2 m length; 10 setts of each test material were planted per plot, and a total of 30 setts of each test material were planted. The inoculation test was repeated two times in March and September of 2016, respectively.

Investigation of Incidence and Resistance Classification

Approximately, 4–5 weeks after inoculation, surveys of the disease incidence were initiated and carried out every 10 days until stability was achieved (about 6 months). The date of inoculation, the number of total stools, and the number of diseased stools were recorded. The disease reactions of the tested lines for *S. scitamineum* were rated on a Grade 1–9 based on the percentage of diseased stools, 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), 76–100% as Grade 9 (highly susceptible).

Results

E. arundinaceus Ancestry Identification of Hybrid Progenies

Primer pair AGRP 80/81 was an *Erianthus*-specific PCR primer pair, which could amplify a 270 bp target fragment, indicating that an *Erianthus* ancestry off spring. In this study, a total 80 hybrid off springs (BC_3F_1 lines) from 89 screened off springs (BC_3F_1 lines) by the crossing of *E. arundinaceus* with *Saccharum* could amplify a 270 bp target. This indicated that 90% of the off springs (BC_3F_1lines) have *E. arundinaceus* ancestry (true hybrids or self-bred lines). The electrophoresis of some samples was shown in Fig. 1.

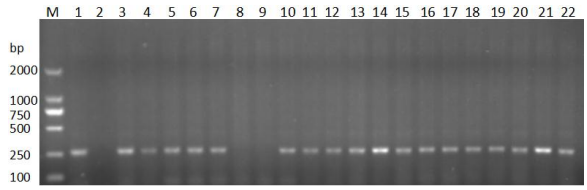

Fig. 1: Gel electrophoresis of some samples
Note: Lane M: DNA molecular standards with length (bp) in left. Lane 1: YCE06-111; Lane 2: YC73-226; Lanes 3–22: 20 hybrid offspring samples

Reactions of Hybrid off Spring to Sugarcane Smut by Artificial Inoculation in the Field

In total, 80 true BC_3F_1 lines of *E. arundinaceus*, resistant to smut ranging from Grade 1 (highly resistant) to Grade 4 (moderately resistant) were detected in 38.75% (31/80) of all the tested BC_3F_1 lines of *E. arundinaceus*, among which, 26 lines (83.87%) were highly resistant to smut, 3 lines were resistant (Grade 3), and 2 lines were moderately resistant (Grade 4) (Table 1).

Resistance to smut ranging from Grade 5 (moderately susceptible) to Grade 9 (highly susceptible) was calculated as 61.25% (49/80) of all the 80 tested BC_3F_1 lines of *E. arundinaceus*, among which, 29 lines (59.18%) were scored as moderately susceptible (Grade 5) to smut, 17 exhibited as susceptible (Grade 6 or Grade 7), and only 6.12% (3/49) lines were highly susceptible (Grade 8 or Grade 9) (Table 1). The female parent YC 73-226 (*Saccharum* interspecific hybrid) was susceptible to smut, while the male parent YCE 06-111 (BC_3 of *E. arundinaceus*) was resistant to smut.

Discussion

In modern sugarcane breeding, screening, identification, and evaluation of systemic resistance in the source materials are critical as wild sugarcane is the 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 is significant for screening and breeding of resistant cultivars (Li *et al.*,

Table 1: Identification of smut-resistance in BC_3F_1 lines from an intergeneric cross between *Erianthus arundinaceus* with *Saccharum* by artificial inoculation

Lines	Type	Latent period/d	Incidence/ %	Grade	Resistance response
HE15-27	BC_3F_1	96	15	5	MS
HE15-64	BC_3F_1	103	24	5	MS
HE15-03	BC_3F_1	103	33	6	S
HE15-63	BC_3F_1	96	44	7	S
HE15-08	BC_3F_1	96	25	5	MS
HE15-48	BC_3F_1	110	36	7	S
HE15-15	BC_3F_1	89	17	5	MS
HE15-87	BC_3F_1	/	0	1	HR
HE15-86	BC_3F_1	89	15	5	MS
HE15-24	BC_3F_1	96	33	6	S
HE15-33	BC_3F_1	/	0	1	HR
HE15-54	BC_3F_1	96	50	7	S
HE15-67	BC_3F_1	96	25	5	MS
HE15-62	BC_3F_1	/	0	1	HR
HE15-25	BC_3F_1	/	0	1	HR
HE15-17	BC_3F_1	/	0	1	HR
HE15-78	BC_3F_1	96	25	5	MS
HE15-74	BC_3F_1	89	50	7	S
HE15-28	BC_3F_1	/	0	1	HR
HE15-83	BC_3F_1	103	17	5	MS
HE15-57	BC_3F_1	/	0	1	HR
HE15-70	BC_3F_1	89	20	5	MS
HE15-32	BC_3F_1	/	0	1	HR
HE15-36	BC_3F_1	96	25	5	MS
HE15-37	BC_3F_1	/	0	1	HR
HE15-12	BC_3F_1	/	0	1	HR
HE15-35	BC_3F_1	/	0	1	HR
HE15-73	BC_3F_1	89	29	6	S
HE15-50	BC_3F_1	110	100	9	HS
HE15-72	BC_3F_1	/	0	1	HR
HE15-71	BC_3F_1	103	16	5	MS
HE15-47	BC_3F_1	89	25	5	MS
HE15-59	BC_3F_1	89	19	5	MS
HE15-11	BC_3F_1	96	14	5	MS
HE15-14	BC_3F_1	/	0	1	HR
HE15-10	BC_3F_1	110	55	8	HS
HE15-80	BC_3F_1	96	45	7	S
HE15-19	BC_3F_1	/	0	1	HR
HE15-84	BC_3F_1	96	20	5	MS
HE15-81	BC_3F_1	/	0	1	HR
HE15-39	BC_3F_1	110	13	5	MS
HE15-56	BC_3F_1	/	0	1	HR
HE15-49	BC_3F_1	89	17	5	MS
HE15-82	BC_3F_1	/	0	1	HR
HE15-22	BC_3F_1	124	17	5	MS
HE15-55	BC_3F_1	110	7	3	R
HE15-34	BC_3F_1	138	17	5	MS
HE15-26	BC_3F_1	96	17	5	MS
HE15-41	BC_3F_1	103	36	7	S
HE15-85	BC_3F_1	89	31	6	S
HE15-66	BC_3F_1	/	0	1	HR
HE15-68	BC_3F_1	96	60	8	HS
HE15-30	BC_3F_1	/	0	1	HR
HE15-16	BC_3F_1	/	0	1	HR
HE15-51	BC_3F_1	103	12	4	MR
HE15-02	BC_3F_1	/	0	1	HR
HE15-21	BC_3F_1	138	13	5	MS
HE15-20	BC_3F_1	96	18	5	MS
HE15-18	BC_3F_1	96	33	6	S
HE15-44	BC_3F_1	103	13	5	MS
HE15-01	BC_3F_1	103	33	6	S
HE15-43	BC_3F_1	103	29	6	S
HE15-38	BC_3F_1	89	12	4	MR

HE15-23	BC_3F_1	/	0	1	HR
HE15-46	BC_3F_1	/	0	1	HR
HE15-05	BC_3F_1	124	33	6	S
HE15-65	BC_3F_1	103	23	5	MS
HE15-45	BC_3F_1	103	14	5	MS
HE15-13	BC_3F_1	89	14	5	MS
HE15-53	BC_3F_1	/	0	1	HR
HE15-61	BC_3F_1	103	25	5	MS
HE15-04	BC_3F_1	103	33	6	S
HE15-10	BC_3F_1	110	33	6	S
HE15-09	BC_3F_1	/	0	1	HR
HE15-31	BC_3F_1	89	38	7	S
HE15-75	BC_3F_1	110	9	3	R
HE15-52	BC_3F_1	89	19	5	MS
HE15-77	BC_3F_1	110	13	5	MS
HE15-69	BC_3F_1	/	0	1	HR
HE15-60	BC_3F_1	103	8	3	R
YC73-226 (Female parent)		124	30	6	S
YCE06-111 (Male parent)		124	8	3	R

Resistance response: HR-highly resistant; R-resistant; MR-moderately resistant; MS-moderately susceptible; S: susceptible; HS-highly susceptible

2013). Sugarcane smut is the major sugarcane disease in mainland China in recent 20 years. Nowadays, the main sugarcane cultivars, such as ROC 22, ROC 16, and Yuetang 93-159, lack resistance to smut in sugarcane production regions in mainland China (Wu *et al*., 2013; Shen *et al*., 2016a). Therefore, it is extremely necessary to strengthen the screening of smut resistance source for breeding resistant cultivars against smut. In this study, a total 80 BC_3F_1 lines with *E. arundinaceus* ancestry were screened for resistance to smut using artificial inoculation method in a green house. 31 lines were identified as high-to-moderate smut resistant lines, which could provide an elite array of resistance sources for effective breeding of sugarcane cultivars against smut. BC_3F_1 lines were the fifth generation of *E. arundinaceus*, and the agronomic characters and sucrose contents of several lines were similar to that of the cultivars according to our preliminary field investigation. Therefore, directly breeding elite smut resistance cultivars with *E. arundinaceus* ancestry from these BC_3F_1 lines is possible.

Till date, there are only a few reports about smut resistance evaluation of *E. arundinaceus* and its hybrid or back cross progenies from intergeneric crosses between *E. arundinaceus* with *Saccharum*. Burner *et al*. (1993) evaluated the resistance of 102 clones of sugarcane relatives to sugarcane smut. The results showed that clones of *E. arundinaceus* were the most resistant, clones of *S. officinarum* and *S. robustum* were the most susceptible to smut. Wu *et al*. (2013) evaluated the resistance of 11 F_1, BC_1, BC_2, or BC_3 lines of *E. arundinaceus* to smut, out of which 5 lines of high smut resistance were identified. Shen *et al*. (2014) assessed the smut resistance of back cross progenies from cross *E. arundinaceus* with *Saccharum*. The results showed that only 18.9% of BC_1 lines and 7.1% of BC_2 lines were highly-to-moderately resistant to smut, which suggested that obtaining a resistant progeny was not straight forward. In this study, 38.75% (31/80) of BC_3F_1 lines of *E. arundinaceus* were identified as highly-to-moderately resistant to smut; only 3.75% (3/80) lines were highly susceptible to smut, which indicated that these BC_3F_1 lines had stronger resistance to sugarcane smut. The resistance evaluation results of sugarcane smut are associated with inoculation methods (Burner *et al*., 1993; Lwin *et al*., 2012; Bhuiyan *et al*., 2013; Bhuiyan *et al*., 2015). The above assessment of the resistance ability of *E. arundinaceus* and its off spring to smut was not consistent, which may be attributed to different inoculation methods.

The inheritance of sugarcane smut resistance is moderately heritable (Wu *et al*., 1977, 1983; Comstock *et al*., 1983; Chao *et al*., 1990). Therefore, the resistance level of parental combinations affected the resistance ability of off spring to smut. In this study, BC_3F_1 lines were derived from a susceptible (female parent) vs. resistant (male parent) cross (Table 1). Thus, further studies are essential to objectively evaluate the resistance ability of BC_3F_1 lines with *E. arundinaceus* ancestry to smut from resistant vs. resistant crosses or highly resistant vs. highly resistant crosses.

It would be useful to obtain more promising resistance parents or even cultivars against sugarcane smut disease and further reveal the prospect of *E. arundinaceus* in cultivar breeding for resistance to smut.

Conclusion

In conclusion, this study, for the first time, has identified 31 BC_3F_1 lines with *E. arundinaceus* ancestry against sugarcane smut disease from 80 tested lines derived from a susceptible vs. resistant intergeneric cross between *E. arundinaceus* and *Saccharum*. This would enrich the resistance resources of sugarcane smut, or may even directly breed new resistant cultivars with *E. arundinaceus* ancestry against smut and broaden the genetic basis of smut resistance in sugarcane breeding.

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