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RESEARCH ARTICLE

Identification and Evaluation of Resistance to *Sugarcane Streak Mosaic Virus* (SCSMV) and *Sorghum Mosaic Virus* (SrMV) in Excellent Sugarcane Innovation Germplasms in China

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Abstract Sugarcane mosaic disease is one of the most serious and prevalent viral diseases of sugarcane in China. Resistant varieties are the most economical and effective measures for controlling this disease. Resistance against Sugarcane streak mosaic virus (SCSMV) and Sorghum mosaic virus (SrMV) in 41 excellent sugarcane innovation germplasms and their parents (*Saccharum officinarum* L. "Ludashi" × *Erianthus rockii* Keng "Yundian 95-19") was checked by using a stalk inoculation and RT-PCR detection during 2015 and 2016. Results indicated that among the 41 excellent sugarcane innovation germplasms and parents, 23 were highly (Grade 1) to moderately resistant (Grade 3) to SCSMV, meanwhile, 31 were highly (Grade 1) to moderately resistant (Grade 3) to SrMV. Ten germplasms were highly resistant (Grade 1) to resistant (Grade 2) to both SCSMV and SrMV. Among these, six germplasms (Yun 09-604, Yun 09-607, Yun 09-619, Yun 09-633, Yun 09-656, Yundian 95-19) were highly resistant (Grade 1) to both SCSMV and SrMV, accounting for 13.95% of the total germplasm materials. These results may provide resistance resource available for the breeding program of sugarcane cultivars against the major vrius associated with mosaic disease in China.

Keywords Sugarcane · Germplasms · Resistance · *Sugarcane streak mosaic virus* · *Sorghum mosaic virus*

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Introduction

Sugarcane mosaic is an important and prevalent viral disease in China and has wide impacts (Huang and Li 2016). Sugarcane mosaic disease causes substantial economic losses and severely constrains the sustained and steady development of Chinese sugarcane industry (Huang et al. 2007; Huang and Li 2016). Previous researches in China showed that sugarcane mosaic disease is mainly caused by *Sugarcane mosaic virus* (SCMV), *Sorghum mosaic virus* (SrMV) and *Sugarcane streak mosaic virus* (SCSMV). Li et al. (2011) first detected SCSMV in 2011 in Yunnan, and the virus has spread rapidly with increasing pathogenicity. In recent years, SCSMV has become the predominant pathogen of mosaic disease in Yunnan cane-growing regions (Huang and Li 2016). SrMV is widely distributed over the sugarcane area in the world and is the main causal agent of mosaic disease in all the major cane-growing regions of China including Yunnan, Guangxi, Guangdong, Hainan and Fujian (Chen and Chen 2002; Zhou and Xu 2005; Li et al. 2007; Xiong et al. 2011). Currently, SCSMV and SrMV have become the two main virus pathogens causing sugarcane mosaic disease in China cane-growing regions (Jiang et al. 2009; Li et al. 2011; He et al. 2014).

Planting susceptible cultivars on a large scale is the important epidemiological reason for mosaic disease. Breeding resistant cultivars are the most cost-effective control strategies (Zhou et al. 1989; Matsuoka et al. 1990). But, as resistant variety has become limited, it is of great significance to explore new resistant germplasm sources to effectively control sugarcane mosaic disease.

Wild forms of sugarcane and near relatives (Grisham et al. 1992) and some commercial cultivars (Zhou et al. 1989) have been identified the resistance to SCMV by artificial inoculation. Li et al. (2009, 2013, 2014b)

identified SrMV resistance among breeding cultivars and wild germplasm resources by the stem-cutting inoculation. Previously, only single resistance to SrMV or SCMV has been identified and evaluated in sugarcane germplasm resources and varieties/clones (Zhou et al. 1989; Li et al. 2009, 2013, 2014b). Single resistance to SCSMV or double resistance to SCSMV and SrMV has not been reported in China.

Two major sugarcane mosaic pathogens in Chinese sugarcane growing regions, *Sorghum mosaic virus* (SrMV-HH) (Li et al. 2007) and *Sugarcane streak mosaic virus* (SCSMV-JP1) (Li et al. 2011), were used as inocula to screen the resistance. We identified the SCSMV and SrMV double resistance in 41 excellent sugarcane germplasms of *Saccharum officinarum* L. "Ludashi" × *Erianthus rockii* Keng "Yundian 95-19" and parents by stem-cutting inoculation and RT-PCR detection in 2015 and 2016. The aim was to explore new resistance germplasm and provide resistance resources against sugarcane mosaic disease for effective breeding of improved sugarcane cultivars.

Materials and Methods

Test Materials

Forty-one excellent sugarcane germplasms of F1 hybrids of *S. officinarum* "Ludashi" × *E. rockii* "Yundian 95-19" and their parents (Table 1) were tested in the present study for their resistance against both the test virus. Cultivar Mintang 70-611 was served as a resistant control, and Yunzhe 89-151 as a susceptible control.

Method of Artificial Inoculation

Inoculation trials were conducted in March of 2015 and 2016, respectively, at the Yunnan Sugarcane Research Institute (YSRI) (Kaiyuan, Yunnan, China). Tested materials were planted and treated as described by Li et al. (2013).

Virus inocula were, respectively, obtained from young symptomatic leaves of susceptible cultivar Yunzhe 06-407 infected with SCSMV-JP1 (GenBank Acc. no. JF488064) (Li et al. 2011) and from young symptomatic leaves of susceptible cultivar Yunzhe 89-151 infected with SrMV-HH (DQ530434) (Li et al. 2007), and prepared as described by Li et al. (2014b).

The stem-cutting inoculation method of Li et al. (2008) was used, respectively, for SCSMV inoculation and SrMV inoculation at 4–5 months after planting. Twenty plants of each tested germplasm were inoculated independently with SCSMV and SrMV inocula. Each cane stem was cut just above ground level using a sharp knife or pruning scissors; 50 μL inocula was dropped on the cut portion of stem; and the inoculated plants were covered with paper for 24 h.

Twenty days after the inoculation, disease incidence was checked by observing any leaf symptoms on tested varieties until steady symptoms were appeared on susceptible control. Disease incidence was recorded. Disease response on the tested materials against SCSMV and SrMV was graded on 1–5 scale (Li et al. 2013, 2014a). After the last incidence survey, leaves were collected immediately to analyze the presence/absence of SCSMV and SrMV by RT-PCR assays.

RT-PCR Detection

Specific PCR primers for SCSMV detection (SCSMV-F, 5′ACAAGGAACG CAGCCACCT3′ and SCSMV-R, 5′ACTAAGCGGTCAGGCAAC3′) were used to amplify a 939 bp region of the SCSMV coat protein (CP) gene as described by He et al. (2014). The specific PCR primers for SrMV detection (SrMV-F, 5′CATCARGCAGGRGG CGGYAC3′ and SrMV-R, 5′TTTCATCTGCATGTGG GCCTC3′) were used to amplify an 828 bp region of SrMV CP gene as described by Jiang et al. (2009).

Total RNA was extracted from 0.2 g fresh leaf tissue using *TransZol* Plant Kit (TransGen, Beijing, China). TransScript One-Step gDNA Removal and cDNA synthesis SuperMix Kit (TransGen, Beijing, China) was used to synthesize the first-strand cDNA with the template total RNA. SCSMV-F/SCSMV-R or SrMV-F/SrMV-R PCR primers were used for the amplification of cDNA. The amplified products were analyzed by electrophoresis on a 1.5% agarose gels stained with Goldview.

Results

The result of artificial inoculation showed that 23 of the 43 tested materials were rated as highly resistant (Grade 1) to moderately resistant (Grade 3) to SCSMV, the remaining 20 were rated as susceptible (Grade 4) to highly susceptible (Grade 5) to SCSMV; 31 of the 43 tested materials were rated as highly resistant (Grade 1) to moderately resistant (Grade 3) to SrMV, and the remaining 12 were rated as susceptible (Grade 4) to highly susceptible (Grade 5) to SrMV. Comprehensive analysis showed 10 of the 43 tested materials were rated from highly resistant (Grade 1) to resistant (Grade 2) to SCSMV and SrMV, including Yun 09-603, Yun 09-604, Yun 09-607, Yun 09-608, Yun 09-619, Yun 09-622, Yun 09-633, Yun 09-635, Yun 09-656, Yundian 95-19. Of these, six germplasms (13.95%) including Yun 09-604, Yun 09-607, Yun 09-619, Yun 09-633, Yun 09-656, Yundian 95-19 were rated as

Table 1 Resistance identification of elite sugarcane innovative germplasms to SCSMV and SrMV

Sample number	Germplasms	Provenance	SCSMV				SrMV			
			Disease incidence[a] (%)	Grade[b]	Detection by RT-PCR	Resistance response	Disease incidence[a] (%)	Grade[b]	Detection by RT-PCR	Resistance response
N1	Yun 09-601	Yunnan	52.94	4	+	S	75.00	5	+	HS
N2	Yun 09-602	Yunnan	30.00	3	+	MR	0	1	−	HR
N3	Yun 09-603	Yunnan	10	2	+	R	0	1	−	HR
N4	Yun 09-604	Yunnan	0	1	−	HR	0	1	−	HR
N5	Yun 09-607	Yunnan	0	1	−	HR	0	1	−	HR
N6	Yun 09-608	Yunnan	10.0	2	+	R	0	1	−	HR
N7	Yun 09-610	Yunnan	50.00	4	+	S	0	1	−	HR
N8	Yun 09-611	Yunnan	41.67	4	+	S	0	1	−	HR
N9	Yun 09-612	Yunnan	14.29	3	+	MR	0	1	−	HR
N10	Yun 09-613	Yunnan	100	5	+	HS	0	1	−	HR
N11	Yun 09-614	Yunnan	20.00	3	+	MR	0	1	−	HR
N12	Yun 09-615	Yunnan	100	5	+	HS	0	1	−	HR
N13	Yun 09-616	Yunnan	100	5	+	HS	100	5	+	HS
N14	Yun 09-618	Yunnan	50.00	4	+	S	80.00	5	+	HS
N15	Yun 09-619	Yunnan	0	1	−	HR	0	1	−	HR
N16	Yun 09-621	Yunnan	25.00	3	+	MR	0	1	−	HR
N17	Yun 09-622	Yunnan	10.00	2	+	R	0	1	−	HR
N18	Yun 09-624	Yunnan	44.44	4	+	S	100	5	+	HS
N19	Yun 09-625	Yunnan	29.41	3	+	MR	0	1	−	HR
N20	Yun 09-629	Yunnan	57.14	4	+	S	0	1	−	HR
N21	Yun 09-630	Yunnan	100	5	+	HS	80.00	5	+	HS
N22	Yun 09-631	Yunnan	53.33	4	+	S	0	1	−	HR
N23	Yun 09-633	Yunnan	0	1	−	HR	0	1	−	HR
N24	Yun 09-634	Yunnan	0	1	−	HR	44.44	4	+	S
N25	Yun 09-635	Yunnan	0	1	−	HR	10.0	2	+	R
N26	Yun 09-636	Yunnan	31.58	3	+	MR	0	1	−	HR
N27	Yun 09-639	Yunnan	36.36	4	+	S	0	1	−	HR
N28	Yun 09-643	Yunnan	58.33	4	+	S	0	1	−	HR
N29	Yun 09-644	Yunnan	42.31	4	+	S	0	1	−	HR
N30	Yun 09-648	Yunnan	78.57	5	+	HS	0	1	−	HR
N31	Yun 09-651	Yunnan	33.33	4	+	S	0	1	−	HR
N32	Yun 09-653	Yunnan	81.82	5	+	HS	0	1	−	HR
N33	Yun 09-654	Yunnan	0	1	−	HR	57.14	4	+	S
N34	Yun 09-655	Yunnan	50.00	4	+	S	0	1	−	HR
N35	Yun 09-656	Yunnan	0	1	−	HR	0	1	−	HR
N636	Yun 09-657	Yunnan	100	5	+	HS	0	1	−	HR
N37	Yun 09-658	Yunnan	0	1	−	HR	36.36	4	+	S
N38	Yun 09-659	Yunnan	18.18	3	+	MR	81.82	5	+	HS
N39	Yun 09-660	Yunnan	0	1	−	HR	50.00	4	+	S
N40	Yun 09-661	Yunnan	0	1	−	HR	58.33	4	+	S
N41	Yun 09-662	Yunnan	0	1	−	HR	42.31	4	+	S
N42 (female parent)	Ludashi	Yunnan	100	5	+	HS	0	1	−	HR
N43 (male parent)	Yundian 95-19	Yunnan	0	1	−	HR	0	1	−	HR
Susceptible cultivar	Yunzhe 89-151	Yunnan	100	5	+	HS	100%	5	+	HS
Resistant cultivar	Minting 70-611	Fujian	0	1	−	HR	0	1	−	HR

HR highly resistant, *R* resistant, *MR* moderately resistant, *S* susceptible, *HS* highly susceptible

+, SCSMV or SrMV detected by RT-PCR; −, SCSMV or SrMV not detected by RT-PCR

[a]Disease incidence under artificial inoculation is the mean of the last evaluation in October 2015 and 2016

[b]Resistance to SCSMV or SrMV was graded 1–5 as per disease incidence. A grade of 1–3 indicates highly resistant, resistant and moderately resistant varieties with 0%, 0.01–10.00% and 10.01–33.00% disease incidence, respectively. A grade of 4–5 was considered susceptible and highly susceptible with 33.01–66.00% and 66.01–100% disease incidence, respectively

highly resistance (Grade 1) against SCSMV and SrMV (Table 1).

RT-PCR results showed that the 939 bp expected fragment of the SCSMV CP gene was not detected in the 13 tested materials which were highly resistant (Grade 1) to SCSMV-JP1, and the 828 bp expected fragment of the SrMV CP gene was not detected in the 30 tested materials which were highly resistant (Grade 1) to SrMV-HH. These expected fragments were present in all susceptible to highly susceptible (Grade 2 to Grade 5) tested materials (Table 1). RT-PCR test confirmed the results of artificial inoculation resistance.

Discussion

Sugarcane mosaic disease is a systemic disease that can be transmitted by infected seed cane. Cultivars vary in the level of resistance to sugarcane mosaic virus. Breeding disease-resistant cultivars is the most cost-effective strategy for controlling mosaic disease. However, there are few cultivars that have resistance to multiple mosaic viruses. Developing and utilizing resistance germplasms is the basis and key for the breeding programs of resistant varieties (Li et al. 2009, 2013, 2014b). In the present study, resistance to both SCSMV and SrMV was evaluated in 41 excellent sugarcane innovation germplasms of *S. officinarum* × *E. rockii* and their parents using stalk inoculation and RT-PCR test in 2015 and 2016. The resistance of 41 excellent sugarcane germplasms and their parents to SCSMV and SrMV was determined. Ten germplasms were found resistant to both SCSMV and SrMV and offered a promising resource of resistance in mosaic resistant sugarcane cultivars breeding.

At present, three viruses, SCMV, SrMV and SCSMV, cause mosaic disease in the cane-growing regions of China. Symptoms caused by each of the three viruses are hard to differentiate visually. The RT-PCR test of virus for artificial inoculation materials can improve the scientificity and reliability of the identification results of artificial inoculation. In view of the fact that SCSMV has become the major pathogen of mosaic disease in the cane-growing regions of China (Li et al. 2011; He et al. 2014; Wang et al. 2017), the resistant cultivar breeding strategy of sugarcane mosaic disease should utilize the SCSMV and SrMV double-resistance germplasms.

Wild sugarcane resources are an important source of resistance genes. *E. rockii* is an importance resource of sugarcane-related genera and has some elite characteristics, such as rich genetic diversity, drought tolerance, barren tolerance, good rationing ability and high rust resistance (Li et al. 2005; Xu et al. 2014), which is of great value in the development of sugarcane germplasm and the creation of new diseases resistant varieties. These research results suggest that excellent sugarcane innovation germplasms resulting from the hybridization of *E. rockii* contain valuable resistance genes for SCSMV and SrMV and are a source of resistance in breeding mosaic resistant cultivar. These new excellent resistant germplasms could be crossed with common parents, to evaluate the heritability of resistance, and establish a resistant germplasm gene bank. Moreover, these germplasms may further contribute to SCSMV and SrMV double-resistance breeding for commercial application.

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