Crop Protection 110 (2018) 77–82

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Identification of resistance to *Sugarcane streak mosaic virus* (SCSMV) and *Sorghum mosaic virus* (SrMV) in new elite sugarcane varieties/clones in China

Wen-Feng Li[1], Hong-Li Shan[1], Rong-Yue Zhang, Xiao-Yan Wang, Kun Yang, Zhi-Ming Luo, Jiong Yin, Xiao-Yan Cang, Jie Li, Ying-Kun Huang*

Sugarcane Research Institute, Yunnan Academy of Agricultural Sciences, Yunnan Key Laboratory of Sugarcane Genetic Improvement, Kaiyuan, 661699, China

ARTICLE INFO

Keywords:
Sugarcane
New elite varieties/clones
Sugarcane streak mosaic virus
Sorghum mosaic virus
Resistance identification

ABSTRACT

Sugarcane mosaic disease is one of the most serious and prevalent viral diseases of sugarcane in China. The use of resistant varieties is the most economical and effective measures for controlling this disease. *Sugarcane streak mosaic virus* (SCSMV) and *Sorghum mosaic virus* (SrMV) are the two predominant pathogens of mosaic disease in the cane-growing regions of China. In 2015, 2016, double resistance to SCSMV and SrMV was identified once a year using a combination of artificial stalk-cutting inoculation and RT-PCR detection in 71 new elite sugarcane varieties/clones. Of the 71 new elite sugarcane varieties/clones, 24 were highly (Grade 1) to moderately resistant (Grade 3), and 47 were susceptible (Grade 4) to highly susceptible (Grade 5) to SCSMV. Furthermore, 27 were highly (Grade 1) to moderately resistant (Grade 3), and 44 were susceptible (Grade 4) to highly susceptible (Grade 5) to SrMV. Fifteen new elite sugarcane varieties/clones were highly resistant (Grade 1) to resistant (Grade 2) to SCSMV and SrMV. While, five new elite sugarcane varieties/clones (Yuegan 34, Yuetang 55, Yunzhe 03–258, Yunzhe 05–51, Yunzhe 06–80) were highly resistant (Grade 1) to SCSMV and SrMV. We defined the resistance of 71 new elite sugarcane varieties/clones to the two main pathogens of mosaic disease and identified 15 resistant to both SCSMV and SrMV. These results provide an elite resistance resource for effective prevention and control of mosaic disease and may serve as a reference for commercial varieties.

1. Introduction

Mosaic disease caused by a kind of virus is an important global sugarcane disease (Yang and Mirkov, 1997; Alegria et al., 2003; Huang and Li, 2016). Yield losses are generally between 30 and 40%, but can reach 60–80% in badly affected areas in South America and North America (Koike et al., 1989), and the reduction was 10–50% in South Africa (Wang et al., 2009). Currently, sugarcane mosaic disease become one of the most serious and prevalent diseases of sugarcane in China and has resulted in losses of over US$30 million annually and poses a serious threat to the stability and sustainability of the sugar industry in China (Huang et al., 2007; Huang and Li, 2016).

Sugarcane mosaic disease is mainly caused by the *Sugarcane mosaic virus* (SCMV), *Sorghum mosaic virus* (SrMV) and *Sugarcane streak mosaic virus* (SCSMV). SCMV and SrMV belong to *Potyvirus* in the family *Potyviridae,* while SCSMV belongs to *Poacevirus* of the family *Potyviridae*. SCSMV is a new pathogen that has been ascertained in recent years. SCSMV was reported for its devastating effects in many cane-growing areas, such as India, Pakistan, Sri Lanka, Thailand, Vietnam and Indonesia, and has caused huge losses to the local sugarcane and sugar industries (Chatenet et al., 2005; Damayanti and Putra, 2011; Parameswari et al., 2013; Putra et al., 2014). Li et al. (2011) first detected SCSMV in 2011 in Yunnan, and the virus has spread rapidly with increasing pathogenicity. In resent years, SCSMV has become the predominant pathogen of mosaic disease of 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 the Yunnan, Guangxi, Guangdong, Hainan, and Fujian provinces(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 pathogens causing sugarcane mosaic disease in China cane-growing regions (Jiang et al., 2009; Li et al., 2011; He et al., 2014).

SCSMV and SrMV are mainly transmitted by mechanically rubbing

* Corresponding author. Sugarcane Research Institute, Yunnan Academy of Agricultural Sciences, Yunnan Key Laboratory of Sugarcane Genetic Improvement, Kaiyuan, 661699, China.
E-mail address: huangyk64@163.com (Y.-K. Huang).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.cropro.2018.04.002
Received 25 November 2017; Received in revised form 29 March 2018; Accepted 6 April 2018
Available online 11 April 2018

and infected seed-cane. The researcher reported that SCSMV was not transmitted by aphids and mites, while a variety of aphids were the vectors of SrMV, such as *Aphis craccivora*, *Myzus persicae* and *Rhopalsiphum maidis* (Gillaspie et al., 1978; Hema et al., 2001). The natural hosts of SCSMV are poaceae plants, including sugarcane, maize, sorghum, johnsongrass and sudan grass (Singh and Rao, 2010; Srinivas et al., 2010).

In Indonesia, SCSMV spread rapidly with the promotion and planting of highly susceptible variety PS 864, and the incidence increased from 0.44% to 86.75% in 2007–2011, and all sugarcane-growing areas were infected. Infection of SCSMV caused a significant reduction up to 20% on cane yields (Putra et al., 2015). In Thailand, the incidence of SCSMV in germplasm collection fields was higher than the incidence in sugarcane growing areas, up to 100%, and the UT sugarcane variety groups were found to be more frequently infected with SCSMV (Kasemsin et al., 2016).

Promotion and planting of susceptible cultivars is the primary cause of mosaic disease. Screening, breeding and planting resistant cultivars may be the most economical and effective measure for control of sugarcane mosaic disease (Zhou et al., 1989; Matsuoka et al., 1990). However, few varieties exist with resistance to multiple mosaic viruses, so it is important to explore new resistant germplasm to effectively control sugarcane mosaic disease.

Previously, only single resistance to SrMV or SCMV has been identified and evaluated in sugarcane germplasm resources and varieties/clones (Zhou et al., 1989; Grisham et al., 1992; Li et al., 2013, 2014a). Single resistance to SCSMV or double resistance to SCSMV and SrMV has not been reported in China.

The aim of this study was to determine the resistance of 71 new elite sugarcane varieties/clones to the two main pathogens of mosaic disease.

2. Materials and methods

2.1. Tested materials

A total of 71 new elite sugarcane varieties/clones bred by the China Sugarcane System were studied (Table 1). Cultivars ROC 22 and Yunzhe 89–151 were used as a susceptible control and Mintang 70–611 as a resistant control.

2.2. Artificial inoculation identification

For each variety/clone, healthy plants confirmed to be free from SCMV, SrMV and SCSMV by RT-PCR were planted once a year in the greenhouse at the Sugarcane Research Institute, Yunnan Academy of Agricultural Science (YSRI) (Kaiyuan, Yunnan province, China) in March of 2015 and 2016 according the method described by Li et al. (2013, 2014b). Each cane stalk was cut into 2-eye setts which were then soaked in cold running water for 48 h and then heated at 50 ± 0.5 °C for 2 h. Subsequently, the setts were immersed in a mixture of 800 × Dichlorvos, 80% EC and 800 × Carbendazim, 50% WP for 10 min. Thereafter, setts were planted in plastic pots (35 cm diameter, 30 cm depth) and filled with a 3:1 mixture (v/v) of steam-sterilized soil and organic matter. 20 plants of each test material used for SCSMV or SrMV identification were treated according to a completely randomized experimental design including 4 replicates of individual pots containing 5 plants. Plants were grown in a pest-proof greenhouse at 20–30 °C. During the experiment, the plants were frequently applied with 70% Thiamethoxam to avoid any presence of aphid vectors, and regularly monitored aphid to avoid crossed contamination.

The inoculum of SCSMV-JP1 (GenBank accession number JF488064) (Li et al., 2011) and SrMV-HH (GenBank accession number DQ530434) (Li et al., 2013, 2014b) were propagated on susceptible cultivars ROC22 and Yunzhe 89–151, respectively. Before inoculation, RT-PCR test has been carried out to confirm purity of viruses' inoculum sources. Viral inoculum was prepared as described by Li et al. (2013, 2014b).

At 4–5 months old, 20 plants of each variety/clone were inoculated independently with SCSMV or SrMV using the stem-cutting method as described by Li et al. (2008). Each cane stem was cut just above ground level using a sharp knife or pruning scissors; 50 μL inoculum was dropped on the section of stem; and the inoculated plants were covered with newspaper for 24 h.

Twenty days after inoculation, disease incidence surveys were initiated by observe leaf symptoms every 15 days until moderate symptoms were observed on susceptible control and disease incidence was stable. Disease incidence was recorded. The means of the last disease incidence in October 2015 and 2016 were calculated. Disease response of the tested varieties/clones to SrMV and SCSMV was graded1-5 based on the means of the last disease incidence (according to Li et al., 2013, 2014b), where 0 was scored as Grade 1 (highly resistant), 1–10% as Grade 2 (resistant), 11–33% as Grade 3 (moderately resistant), 34–66% as Grade 4 (susceptible), and 67–100% as Grade 5 (highly susceptible). Immediately after the last survey, a piece of young leaf was collected from one inoculated plants, a total of 20 young leaves per variety. The medium part of 20 young leaves were cut into fine pieces with sterile scissors and mixed. 0.2 g mixed leaves were taken for RNA extractions. The RT-PCR testing of SCSMV and SrMV was replicated three times for confirming the presence/absence of SCSMV and SrMV mixed infections and ensuring the authenticity of the resistance of each tested varieties/clones.

2.3. RT-PCR detection

2.3.1. Primer design and synthesis

The specific PCR primers for SCSMV and SrMV amplification were designed as described by He et al. (2014) and Jiang et al. (2009), respectively (Table 2), and synthesized by Sangon Biotech Co., Ltd (Shanghai, China).

2.3.2. Total RNA extraction

A piece of young leaf was collected from one inoculated plants, a total of 20 young leaves per variety. The medium part of 20 young leaves were cut into fine pieces with sterile scissors and mixed. 0.2g mixed leaves were taken for RNA extractions. Total RNA was extracted using a *TransZol* Plant Kit (TransGen Biotech Co., Ltd., Beijing, China) according to the manufacturer's instructions.

2.3.3. cDNA synthesis

Total RNA was used as the template and the first strand of cDNA was synthesized using TransScript One-Step gDNA Removal and cDNA synthesis SuperMix Kit (TransGen Biotech Co., Ltd., Beijing, China) according to the manufacturer's instructions. The reverse transcription was performed in a 10 μL reaction mixture contained 1.5 μL ddH_2O, 5.0 μL 2 × TS reaction mix, 0.5 μL 0.5 μg/μL Oligod $(T)_{18}$, 0.5 μL RT/RI enzyme mix, 0.5 μL gDNA remover and 2.0 μL RNA template. The reverse transcription conditions were as follows: 30 min at 42 °C, and 5 s at 85 °C.

2.3.4. PCR amplification

The cDNA was amplified by PCR using SCSMV or SrMV specific primers (Table 2). PCR amplification of SCSMV was performed in a 25 μL reaction mixture contained 9.5 μL ddH_2O, 12.5 μL 2 × *Easy* Taq PCR SuperMix (TransGen Biotech Co., Ltd.), 2.0 μL cDNA template and 0.5 μL of each primer (20 μg/μL). The thermal cycling conditions were as follows: 5 min at 94 °C followed by 35 cycles for 30 s at 94 °C, 30 s at 50 °C and 1 min at 72 °C, with a final extension for 10 min at 72 °C. PCR amplification of SrMV was performed in a 20 μL reaction mixture contained 7.2 μL ddH_2O, 10.0 μL 2 × *Easy* Taq PCR SuperMix (TransGen Biotech Co., Ltd.), 2.0 μL cDNA template and 0.4 μL of each primer (20 μg/μL). The thermal cycling conditions were as follows: