Crop Protection 60 (2014) 44–47

Contents lists available at ScienceDirect

Crop Protection

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

Incidence of sugarcane ratoon stunting disease in the major cane-growing regions of China

Wen-Feng Li, Ke Shen, Ying-Kun Huang*, Xiao-Yan Wang, Jiong Yin, Zhi-Ming Luo, Rong-Yue Zhang, Hong-Li Shan

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

ARTICLE INFO

Article history:
Received 8 July 2013
Received in revised form
4 December 2013
Accepted 25 February 2014

Keywords:
Sugarcane
Ratoon stunting disease
Pathogen detection
Disease assay
Resistant germplasm

ABSTRACT

Ratoon stunting disease (RSD), caused by *Leifsonia xyli* subsp. *xyli* (Lxx), is one of the most important diseases that limits sugarcane production worldwide. A scientific understanding of the distribution, occurrence, and damage of RSD in cane-growing areas will provide basal information for the application of effective RSD control strategies. In the present study, the occurrence and distribution of RSD were surveyed in the 21 cane-growing regions of Yunnan and Guangxi Provinces of China from 1270 samples using a PCR-based assay. The results showed that 949 samples (74.7% out of 1270) were positive for the presence of RSD. In Yunnan and Guangxi provinces, RSD was detected in all 21 cane-growing areas at rates of 65.5–88% and in the 33 main cultivars at rates of 48.9–100%. The results also showed that plant crop and ratoons from both irrigated and rainfed fields were infected with RSD. Thus, RSD has become an established disease that seriously restricts the development of the cane-sugar industry in China due to cane yield loss, a shortened ratoon period, and cultivar degeneration. Effective control of RSD presents a major challenge to the further development of the sugarcane industry in China. The results of our survey indicated that under the field condition, the main cultivars grown over large areas, including Guitang 94-119, Yuetang 93-159, Yuetang 00-236, and Guitang 11 showed high RSD incidence rates, suggesting that the focus on these cultivars should be the production, propagation, application and extension of healthy, bacteria-free seedlings. Relatively low RSD incidence rates were found in the cultivars Liucheng 03-1137, Liucheng 05-136, Yuanlin 1, ROC22, and F95-8899. Further research is required to determine if these cultivars are resistant and can be used to reduce the incidence of the disease and for breeding RSD resistant sugarcane cultivars. Sequencing of 100 PCR products selected randomly from sugarcane samples that tested positive for RSD showed that all 100 sequences were identical and highly homologous to the previously published Lxx 16S–23S spacer region in GenBank (99.54–100% similarity).

1. Introduction

Ratoon stunting disease (RSD) is an important disease of sugarcane that is found in all cane-growing areas of the world. Since it was first reported in Queensland, Australia in 1944 and 1945, RSD has been reported in many countries and districts including the USA, South Africa, Mauritius, India, and Brazil, and is now present in all cane-growing areas worldwide (Martin et al., 1982; Bailey and Tough, 1991; James, 1996; Hoy et al., 1999; Duttamajumder, 2001; Rago et al., 2004; James et al., 1995). In China, RSD was reported in Taiwan in 1954, and its presence on the mainland was confirmed in 1986 (Wu and Huang, 1986). Thereafter, using phase contrast microscopy to assay samples, an infected field rate of 86.5% and an infected plant rate of 49% were shown in partial cane-growing areas of Guangdong in 1987 (Huang and Xiao, 1987). Similarly, an infected field rate of 57.1% and an infected plant rate of 29.17% were detected in partial cane-growing areas in Fujian from 1995 to 1997 (Zheng and Gan, 1998). During the period 2001–2003, 100% of sampled cultivars (clones) from partial cane-growing areas in Guangxi were positive for RSD infection by phase contrast microscopy and PCR (Deng et al., 2004). In 2004, 2005, samples from partial cane-growing areas of Guangdong and Hainan were assayed for RSD by PCR, and positive rates of 99% and 60% were found in samples from Guangdong and Hainan, respectively (Zhou and Zhou, 2005). In 2006, dot blot enzyme linked immunoassay (DB-EIA) was used to assay samples for RSD from Zhanjiang of

* Corresponding author. Tel.: +86 873 7227017; fax: +86 873 7222489.
E-mail address: huangyk64@163.com (Y.-K. Huang).

http://dx.doi.org/10.1016/j.cropro.2014.02.011
0261-2194/

Guangdong, and a positive infection rate of 62.84% was found (Shen et al., 2007). In 2004, 2005, and 2006, Huang et al. (2007) used electron microscopy with negative staining and indirect enzyme-linked immunosorbent assay (I-ELISA) to detect RSD in samples from partial cane-growing areas of Yunnan Province, and found a positive infection rate of 69.05%.

RSD is caused by the bacterium *Leifsonia xyli* subsp. *xyli* (Lxx) invading vascular bundles, and is spread mainly by infected seedlings and cutting tools used to harvest and cut sets with high transmissibility (Martin et al., 1982; Lopes and Damann, 1993; James, 1997). As a vascular disease, RSD is difficult to eliminate by conventional physical or chemical measures. Effective measures for RSD control include production, propagation, and extension of bacteria-free, healthy canes, which have been applied for many years and in many countries such as Australia, the USA, Brazil, Cuba, South Africa, Philippines, and Taiwan (Martin et al., 1982; Davis et al., 1980; You et al., 2001). Ascertaining the distribution and incidence of RSD is critical to the scientific extension of healthy seedlings and the effective control of RSD. Taking up over 85% of cane-growing areas in China, Guangxi and Yunnan are the two major cane-growing areas in China with the highest potential for continued future development. However, recent improvements in sugarcane cultivars and the rapid increase in sugarcane production, along with lacking of comprehensive investigations on the distribution and disease incidence of RSD in these regions have made it difficult to apply appropriate methods for control of this important disease. Therefore, we collected samples and surveyed the distribution and incidence of RSD using a PCR-based assay in 2009, 2010, 2011, and 2012 in 21 major cane-growing regions of Yunnan Province and Guangxi Province. The objectives were to determine the distribution, incidence of RSD and the extent of infection present in the major cultivars in the main cane-growing areas of China, and to provide a sound scientific basis for extension of bacteria-free seedlings for effective control of RSD.

2. Materials and methods

2.1. RSD survey and sample collection

A total of 1270 samples composed of 21 batches were collected from mature sugarcane in 2009, 2010, 2011, and 2012 in 21 main cane-growing regions of China (Table 1) to determine the distribution and incidence of RSD. Samples were collected from the representative main cultivars and classified based on cultivar and crop cycle. A five-point sampling method was used to collect samples. At each of the five sampled points, two plants were randomly selected and cut down. Then a basal internode in length of 7-cm was removed from each of the 10 plants. These ten basal internodes composed a sample. Each internode was split longitudinally into four parts. A total of 25 mL of cane juice was extracted from the 10 internodes using pincers, and the samples were mixed and placed into 50 mL centrifuge tubes. The samples were then stored at −20 °C. After collecting each sample, the sampling tools were washed with running water and then disinfected with 75% ethyl alcohol.

2.2. PCR detection of RSD

2.2.1. Total DNA extraction from cane juice

An improved CTAB procedure was used to extract total DNA from sugarcane juice. A total of 2 mL of each mixed juice sample was transferred to a 2.0 mL tube and centrifuged at 12,000 rpm for 10 min. The pellet was resuspended in 300 μL sterile distilled water. Then 600 μL of 2% CTAB buffer (pre-warmed to 65 °C) was added. The tubes were incubated at 65 °C for 1 h (with gentle mixing every 20 min). One volume (600 μL) of chloroform/isoamyl alcohol (24:1) was added, and the samples were mixed thoroughly for 30 s and then centrifuged for 10 min at 12,000 rpm. Next, 700 μL of supernatant was transferred to a 1.5 mL tube and mixed gently with 700 μL of chloroform/isoamyl alcohol (24:1). The sample was then centrifuged at 12,000 rpm for 10 min. A total of 650 μL of the supernatant was transferred to a new 1.5 mL tube and mixed with two-thirds volume of isopropanol. After incubation at −20 °C for 4 h or overnight, the samples were centrifuged at 12,000 rpm for 10 min at 4 °C to recover the nucleic acids. The pellets were washed twice with 70% ethanol, dried, resuspended in 30 μL of sterile water, and stored at −20 °C.

Table 1
Incidence of RSD in the cane-growing areas of China.

Cane-growing area	Number of samples	Number of positive samples	Percentage of positive samples (%)
Kaiyuan, Yunnan Province	100	76	76.0
Mile, Yunnan Province	80	61	76.3
Honghe, Yunnan Province	42	29	69.1
Yunxin, Yunnan Province	50	44	88.0
Nanen,Yunnan Province	50	44	88.0
Yuanjiang,Yunnan Province	58	38	65.5
Gengma,Yunnan Province	97	73	75.3
Yunxian, Yunnan Province	60	51	85.0
Shuangjiang, Yunnan Province	60	51	85.0
Fengqing, Yunnan Province	58	39	67.2
Menghai, Yunnan Province	54	42	77.8
Shidian, Yunnan Province	51	35	68.6
Changning, Yunnan Province	63	42	66.7
Yingjiang, Yunnan Province	60	48	80.0
Yizhou, Guangxi Province	52	37	71.2
Huaiyuan, Guangxi Province	45	33	73.3
Shibie, Guangxi Province	47	32	68.1
Bodong, Guangxi Province	42	34	81.0
Wuxuan, Guangxi Province	100	69	69.0
Boxuan, Guangxi Province	51	36	70.6
Xiangzhou, Guangxi Province	50	34	68.0
Total	1270	949	74.7

2.2.2. PCR amplification

Primers were designed to amplify the 438-bp internal transcribed spacer region of the *L. xyli* subsp. *xyli* 16S–23S rDNA as reported by Pan et al. (1998). Primer sequences were Lxx1: 5′-CCGAAGTGAGCAGATTGACC-3′ and Lxx2: 5′-ACCCTGTGTTG TTTTCAACG-3′. The primers were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China).

For PCR detection, Lxx strain Mintang69-421 obtained from the Yunnan Key Laboratory of Sugarcane Genetic Improvement (Kaiyuan, China), which 16S–23S rDNA internal transcribed spacer region were 100% identical to two isolates deposited in GenBank from Brazil and Australia, was used as a positive control, and sugarcane plants free of RSD bacteria treated with hot water at 50 ± 0.5 °C for 2 h were used as a negative control; sterile deionized water was used as the blank.

Using total DNA as a template, PCR amplifications were conducted on a C1000 Thermal Cycler Chassis (BIO-RAD, U.S.A.). Each PCR reaction contained 8 μL 2X Taq PCR MasterMix (Tiangen Biotech Co., Beijing, China), 500 ng DNA template, 0.2 μL of each primer (20 μg/μL), and a $dddH_2O$ to a final volume of 20 μL. The thermal cycling conditions were as follows: 5 min at 95 °C, followed by 35 cycles of denaturation for 30 s at 94 °C, annealing for 30 s at 56 °C, and extension for 1 min at 72 °C, followed by a final extension for 5 min at 72 °C.

2.2.3. Detection of amplified products

Samples of each amplification reaction (10 μL) were analyzed by electrophoresis on 1.5% agarose gels at 140 V for 20 min using 0.5×