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Identification of field resistance and molecular detection of the brown rust resistance gene *Bru*1 in new elite sugarcane varieties in China

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A B S T R A C T

Brown rust, caused by *Puccinia melanocephala* has become a serious problem for sugarcane production in China. Therefore, 50 new elite varieties bred by the Chinese Sugarcane System were screened for brown rust resistance and the presence of a major resistance gene, *Bru*1. Identification of field resistance and molecular detection of the brown rust resistance gene *Bru*1 in the 50 new elite varieties and 2 main cultivated varieties were carried out in 2014 and 2015 at regional experimental stations of the China Sugarcane System in Dehong and Baoshan, Yunnan Province, where the incidence of brown rust is particularly high. Resistance evaluations based on ratings of symptom severity resulting from field infection showed that 32 (64%) of the 50 new elite varieties were highly resistant (n = 13, 26%), resistant (n = 16, 32%) and moderately resistant (n = 3, 6%). Molecular detection identified *Bru*1 in 27 (54%) of these resistant varieties suggesting that brown rust resistance in these new elite varieties is primarily controlled by *Bru*1. The absence of *Bru*1 in the remaining five resistant suggested the presence of additional brown rust resistance genes. Two main cultivated varieties were highly resistant and *Bru*1-positive varieties. Different series differed in the frequencies of resistant and *Bru*1-positive varieties. Yunzhe series varieties had the highest frequency of resistant varieties (87.5%) and the highest frequency of *Bru*1-positive varieties (81.3%); In contrast, the Yuetang series varieties had the lowest frequency of resistant varieties (40%) and the lowest frequency of *Bru*1-positive varieties (30%). In this study, we defined the field resistance of 50 new elite varieties to brown rust, and determined the distribution of brown rust resistant gene *Bru*1 in different series varieties. These results will provide new options for commercial production with effective resistance to brown rust. Moreover, these new elite resistant varieties can be used for subsequent breeding and selection to produce agronomically superior varieties with effective brown rust resistance.

1. Introduction

Brown rust disease caused by *Puccinia melanocephala* H. Sydow & P. Sydow is one of the most important diseases of sugarcane, resulting in extensive losses worldwide (Ryan and Egan, 1989; Raid and Comstock, 2000; CSIRO, 2005; Hoy and Hollier, 2009). Although first reported in Java in 1890, epidemics have increased in frequency since 1949. Since the 1970s, brown rust has been particularly pervasive and outbreaks occurred across important cane-growing countries including Cuba, Jamaica, Australia, the USA, Mexico, India, Thailand and the Mauritius, resulting in the abandonment of previously high-yielding high sugar-content cultivars such as Co475, T34362 and CP78-1247 (Chen, 1982; Comstock, 1992; Comstock et al., 1992b; Ma, 1995; CSIRO, 2005; Raid, 2006). In mainland China, brown rust was first recorded in Yunnan Province in 1982 (Ruan et al., 1983), with subsequent reports in Fujian, Guangdong, Jiangxi, Sichuan, Guangxi and Hainan. It is now one of the most prevalent and damaging sugarcane diseases in China (Ruan et al., 1983; The important sugarcane diseases research cooperation group, 1991, Liu et al., 2008; Wei et al., 2010). Yield losses are generally between 15 and 30%, but can reach 40% in badly

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affected areas, resulting in a loss in the sucrose yield by 10–36% (Huang and Li, 1998, 2011). Sugarcane cultivars that produce the highest yields and sucrose contents currently grown in China are Guitang 15, Guitang 17, Guiying 9, P44, and F 86–1626, and the popular varieties Yuetang 60, Dezhe 03–83, Liucheng 03–1137, and Funong 1110 will soon be abandoned due to their high susceptibility to brown rust. The stability and sustainability of the sugar industry in China is under serious threat (Huang and Li, 2011). Thus, breeding and screening of new resistant varieties to brown rust disease is essential for replacement of the susceptible varieties. With the development of molecular marker technology, molecular markers that are closely linked to the resistance gene can be used to effectively track the resistance genes. *Bru*1 has been identified as a major gene that is associated with brown rust resistance in sugarcane and has been genetically mapped in the cultivar 'R570' (Daugrois et al., 1996; Asnaghi et al., 2004). Furthermore, *Bru*1 has been shown to confer resistance to various brown rust isolates originating from different countries (Asnaghi et al., 2001). Two molecular markers that are closely linked to *Bru*1, R12H16 and 9O20-F4, had been developed (Costet et al., 2012) and used in marker-assisted selecting for brown rust-resistant germplasm, and improved the efficiency of breeding and screening for brown rust-resistant varieties.

Resistance to brown rust disease differs among sugarcane varieties. Understanding resistance in existing varieties is therefore important for effective control of sugarcane brown rust disease and rational distribution of resistant varieties (Comstock et al., 1992a; Hogarth et al., 1993; Ramdoyal et al., 2000). In recent years, with support from the China Sugarcane System, a batch of new elite sugarcane varieties have been obtained through a national joint research effort; however, they have yet to be evaluated for brown rust resistance. In this study, field resistance to brown rust was examined in 50 of these new elite sugarcane varieties and two main cultivated varieties, and molecular detection of the brown rust resistant gene *Bru*1 was carried out. The results will help provide a reference and elite resistance resources for breeding and selection of resistant varieties aimed at effective control of brown rust disease in sugarcane.

2. Materials and methods

2.1. Plant materials

A total of 50 new elite sugarcane varieties bred by the China Sugarcane System were studied (Table 1). Two main cultivated varieties, 'ROC 16' and 'ROC 22', were used as the control varieties of regional experiment. The *Bru*1-containing cultivar 'R570' was used as a brown rust-resistant control and 'Xuanzhe 3' as a susceptible control in regional experiment.

2.2. Natural brown rust infection severity in the field

The 50 new elite sugarcane varieties were planted for brown rust resistance evaluation, under natural infection conditions following a randomized complete block design with three replicates at regional experimental stations in Dehong and Baoshan, Yunnan Province, China, in February 2014. Both regions are considered high incidence areas of brown rust disease. Each plot consisted of five rows 6 m in length and 5.5 m wide, with1.1 m spacing between rows, and covering a total area of 33 m^2. Cultivar 'Xuezhe 3' which is highly susceptible to brown rust, was planted along the borders surrounding the trial fields as well as repeatedly two rows between every other elite variety plot to maximize infection. Severity of newly planted cane and ratoon cane of tested materials was investigated in September 2014 and 2015 respectively, the period during which rust fully develops in the susceptible control variety.

For each variety, disease severity was recorded from three replicates, involving a total of 60 plants. The diseased state of the leaves and percentage of leaf infection area were determined in all of the fully emerged leaves as determined by visual inspection. Resistance to brown rust was rated on a scale of rating 1 to 9 as follows: Rating 1, Highly resistant (HR), no symptoms; Rating 2, resistant (R), necrotic spots on leaves and a diseased leaf area <10%; Rating 3, moderately resistant (MR), very few sporulating lesions visible on the plant and a diseased leaf area of 11–25%; Rating 4, moderately susceptible (MS), very few sporulating lesions on 1–3 upper leaves, but many sporulating lesions on lower leaves and a diseased leaf area of 26–35%; Rating 5, susceptible 1 (S1), numerous sporulating lesions on 1–3 upper leaves and few necrotic spots on lower leaves, a diseased leaf area of 36–50%; Rating 6, susceptible 2 (S2), abundant sporulating lesions on 1–3 upper leaves and increased necrotic spots on lower leaves, a diseased leaf area of 51–60%; Rating 7, susceptible 3 (S3), abundant sporulating lesions on 1–3 upper leaves and necrotic lower leaves, a diseased leaf area of 61–75%; Rating 8, highly susceptible 1 (HS1), necrotic spots on 1–3 upper leaves and a diseased leaf area of 76–90%; Rating 9, highly susceptible 2 (HS2), necrotic leaves and plants close to death, a diseased leaf area of 91–100% (Li et al., 2013).

2.3. Molecular detection of the brown rust resistance gene Bru1

PCR primers for two molecular markers (R12H16 and 9O20-F4) linked to the sugarcane brown rust resistance gene *Bru*1 were designed as described by Costet et al. (2012) and synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). R12H16 was used to amplify a 570-bp fragment with the primer pair 5′-CTACGATGAAACTACACCCTTGTC-3′ (forward) and 5′-CTTATGTTAGCGTGACCTATGGTC-3′ (reverse).

Moreover, 9O20-F4 was used to perform PCR amplification with the primer pair 5′-TACATAATTTTAGTGGCACTCAGC-3′ (forward) and 5′-ACCATAATTCAATTCTGCAGGTAC-3′ (reverse), and then 9O20-F4 PCR products was digested with the restriction enzyme *Rsa* I to obtain a 200-bp specific fragment.

A fully expanded first young leaf from each variety was collected and total DNA extracted using the *Easy Pure*™ plant Genomic DNA Kit (TransGen Biotech Co., Ltd., Beijing, China) according to the manufacturer's instructions. The quality of the extracted DNA was assessed using an AG 22331 protein and nucleic acid analyzer (Eppendorf, Germany).

Using the total DNA as a template, PCR was then performed as described by Costet et al. (2012).

3. Results

3.1. Field resistance

Analysis of natural brown rust infection revealed that resistant control cultivar 'R570' was highly consistently resistant, and susceptible control cultivar 'Xuanzhe 3' was highly consistently susceptible in each plot of Baoshan and Dehong (for two seasons). Among the 50 new elite varieties, the disease severity was different in five varieties, including Yuegan 35, Funong 40, Funong 07–2020, Liucheng 03–1137, and Dezhe 03–83. Their disease severities were S2, S1, HS1, HS1, and S3, respectively in Dehong; however in Baoshan, their disease severities were S3, S2, S3, S3 and S2, respectively. The disease severity of all the other varieties was the same for all four experiments. Under field infection conditions, 32 of the 50 new elite varieties (64%) were highly to moderately