

Identification of brown rust resistance in the field and detection of the *Bru1* gene in sugarcane varieties

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Rong-Yue Zhang¹, Hong-Li Shan¹, Kun Yang¹, Xiao-Yan Wang¹,
Xiao-Yan Cang¹, Chang-Mi Wang¹, Jiong Yin¹, Zhi-Ming Luo¹,
Wen-Feng Li¹ and Ying-Kun Huang^{1*}

Abstract: *The aim of this study was to assess brown rust resistance in 60 new sugarcane varieties bred by the China Sugarcane System in recent years and in 34 major varieties cultivated in sugarcane growing areas in China. The resistance of these sugarcane varieties to brown rust was investigated in the field, and molecular markers were used to detect the brown rust-resistance gene Bru1. The results of the field survey showed that 66 (70.21%) of the 94 sugarcane varieties were highly resistant to moderately resistant, and 28 (29.79%) were susceptible to highly susceptible. The Bru1 gene was detected in 54 (57.45%) of the 94 sugarcane varieties. Seven highly resistant varieties and five resistant varieties did not carry Bru1, suggesting that they carry other genes associated with brown rust resistance. This study provides a scientific basis and identifies disease-resistant germplasm for selection of plants for sugarcane production.*

Keywords: *Sugarcane, brown rust, resistance gene Bru1, natural disease resistance.*

INTRODUCTION

Brown rust of sugarcane (*Saccharum officinarum* L.) is caused by *Puccinia melanocephala* H. Sydow and P. Sydow, which is an obligate parasite (Martin et al. 1982). This fungal disease attacks sugarcane leaves (Huang et al. 2018, Chaulagain et al. 2019a). Brown rust was first reported in Java in 1890 (Martin et al. 1982) and is now widely distributed in various sugarcane growing countries and regions across the globe. There have been many brown rust epidemics, which have led to considerable economic losses worldwide (Hoy and Hollier 2009, Garcés et al. 2014, Sanjel et al. 2019). In mainland China, Ruan et al. (1983) first reported brown rust in the Yunnan sugarcane area in 1982. Subsequent reports came from Fujian, Guangdong, Sichuan, Jiangxi, Guangxi, Hainan, and other sugarcane growing provinces (regions) (Liu et al. 2008, Wei et al. 2010, Li et al. 2017c).

Brown rust is currently one of the most widespread and destructive sugarcane diseases in China (Huang and Li 2016, Li et al. 2017a, Li et al. 2018a). In recent years, the growing of susceptible varieties under climatic conditions that favor the fungus (ample rainfall and high humidity) has led to large-scale brown rust outbreaks in sugarcane growing areas such as Banna, Puer, Lincang, and Dehong, and other sugarcane areas in Yunnan Province. A growing trend of

***Corresponding author:**

E-mail: huangyk64@163.com

 [ORCID: 0000-0001-6598-3587](https://orcid.org/0000-0001-6598-3587)

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¹ Sugarcane Research Institute, Yunnan Academy of Agricultural Sciences, Yunnan Key Laboratory of Sugarcane Genetic Improvement, Kaiyuan 66.169-9, China

serious damage to sugarcane crops is evident, and a number of current major varieties will be eliminated due to their susceptibility to brown rust. Therefore, this disease poses a great threat to the sustainable and stable development of the Chinese sugar industry (Cang et al. 2017, Li et al. 2017a, Huang et al. 2018).

Timely understanding of brown rust disease resistance and the distribution of disease resistance genes in existing varieties is of great significance for the sugar industry. This information will aid in breeding disease resistant varieties and in effective prevention and control of sugarcane diseases. Global research has shown that different sugarcane varieties have different levels of resistance to brown rust (Shan et al. 2014, Neuber et al. 2017, Avellaneda et al. 2018, Maria et al. 2018). Selecting resistant varieties is the most economical and effective way to control brown rust. Most countries have effectively controlled brown rust through cultivation and selection of disease resistant varieties and early monitoring and prevention of the disease (Shan et al. 2014, Chaulagain et al. 2019b). With the development of molecular marker technology, markers that are closely linked to disease resistance genes can be used to effectively track them.

In previous studies, researchers found and mapped the major brown rust-resistance gene, *Bru1*, in the sugarcane cultivar R570 (Daugrois et al. 1996, Asnaghi et al. 2004). Two molecular markers, R12H16 and 9O20-F4, closely related to *Bru1* were developed to detect resistance resources (Costet et al. 2012, Li et al. 2017c, Li et al. 2018a). *Bru1* is a major gene for brown rust resistance; however, resistance to brown rust in the sugarcane cultivar disappears following the mutation of *P. melanocephala* concurrent with the selection of host resistance genes. In recent years, researchers have shown that the *Bru1* gene is present in some susceptible sugarcane varieties (Parco et al. 2014). The China Sugarcane System has supported joint efforts for the development of several new elite sugarcane varieties across the country. However, the resistance of these sugarcane varieties to brown rust was unknown. Timely detection and evaluation of brown rust resistance will help to screen for disease-resistant varieties and germplasm. The use of resistant varieties in production can effectively prevent outbreaks of sugarcane brown rust.

The objectives of the current study were to assess brown rust resistance in 60 new sugarcane varieties and 34 major cultivars. For this purpose, the resistance of these varieties to brown rust was investigated in the field, and molecular markers were used to detect the brown rust-resistance gene *Bru1* among the varieties. The level of brown rust resistance of each variety and the distribution of the *Bru1* gene were determined to provide a scientific basis and identify disease-resistant germplasm for the selection of varieties for sugarcane production.

MATERIAL AND METHODS

Plant materials

A total of 60 new elite sugarcane varieties bred in China in recent years (Table 2) and 34 major varieties cultivated in Yunnan and Guangxi sugarcane fields (Table 3) were used in this study.

Investigation of brown rust infection severity and natural resistance of varieties in the field

All new elite sugarcane varieties were planted at regional experimental stations in Kaiyuan and Lincang, Yunnan Province, China, in December 2016. Cultivars R570, ROC 1, and ROC22 carrying the *Bru1* gene were used as brown rust-resistant control varieties. Yuetang 60 was used as a susceptible control variety. For this regional experiment, a randomized complete block design was used with three replicates. Each plot consisted of five rows (6 m long and 5 m wide, total area of 30 m²), with 1 m spacing between rows. To maximize infection, the cultivar Yuetang 60, which is highly susceptible to brown rust, was planted along the borders surrounding the trial fields. Two rows of Yuetang 60 were also planted between every other elite variety. The natural resistance to brown rust of the newly planted varieties and of the ratoon cane of each major cultivar was evaluated in Lincang, Puer, and Yuxi of Yunnan Province and in Yizhou of Guangxi Province.

When brown rust had full expression in the susceptible control variety in September 2017 and October 2018, the natural brown rust resistance of the newly planted varieties and ratoon cane test materials was evaluated. A disease severity score was attributed based on the percentage of the visible area of upper fully expanded leaves exhibiting a reaction. Resistance was scored as described in Table 1. For each variety, incidence of the disease was recorded in three replicates, and 100 successive plants were investigated per replicate (a total of 300 plants per variety). The score

representing the largest number of leaves is the resistance score of the variety. In the event of differences in the resistance score of a specific variety in different places or in different years, the highest score was determined as the resistance score of the variety.

Molecular detection of the brown rust-resistance gene *Bru1*

The PCR markers R12H16 and 9O20-F4 of the sugarcane brown rust-resistance gene *Bru1* were amplified according to the methods described by Costet et al. (2012). The Shanghai biological engineering company was commissioned to design the primers. The expected lengths of the amplification products of R12H16 and 9O20-F4 were 570 bp and 200 bp, respectively.

The first fully expanded leaf from each variety was collected, and total DNA was extracted using an Easy Pure Plant Genomic DNA Kit (TransGen Biotech Co., Ltd., Beijing, China). The total extracted DNA was used as the template for PCR amplification detection, according to the method of Li et al. (2015).

RESULTS

Natural resistance of new varieties in the field

The results of investigation of natural brown rust infection in the field confirmed the high resistance of the resistant control varieties R570 ROC 1 and ROC 22. Additionally, the susceptible control variety, Yuetang 60, was highly susceptible in all three replicates of newly planted and ratoon cane in the field in Lincang. The brown rust resistance of these varieties did not shift between resistant and susceptible scores in the different locations and crop years. Of the new varieties, 41 (68.33%) were highly resistant to moderately resistant, and 19 (31.67%) were moderately susceptible to highly susceptible. In all, 15 new varieties (25%) were classified as highly resistant, 16 new varieties (26.67%) as resistant, and 10 new varieties (16.67%) as moderately resistant. In contrast, 4 new varieties (6.67%) were moderately susceptible, 11 new varieties (18.33%) were susceptible, and 4 new varieties (6.67%) were highly susceptible (Table 2).

Natural resistance of major cultivars in the field

The resistance of the newly planted sugarcane and ratoon cane of each of the 34 major cultivars and control varieties in the field was stable and consistent (Table 3). Of these 34 major cultivars, 25 (73.53%) were highly resistant to moderately resistant, and 9 (26.47%) were moderately susceptible to highly susceptible. The resistant major cultivars included 16 (47.06%) classified as highly resistant, 5 (14.71%) classified as resistant, and 4 (11.76%) classified as moderately resistant. In terms of susceptibility of the major cultivars, 2 (5.88%) were moderately susceptible, 3 (8.82%) were susceptible, and 4 (11.76%) were highly susceptible.

Molecular detection of the brown rust-resistance gene *Bru1*

The brown rust-resistance gene *Bru1* was detected in the test material if a band of approximately 570 bp was amplified using the R12H16 primers and a band of approximately 200 bp, resulting from digestion of the PCR product, was amplified using 9O20-F4 primers. If the two bands were not amplified, the *Bru1* gene was not detected. The PCR test results showed that the two targeted bands were present in the PCR products of the *Bru1*-carrying resistant cultivars ROC 1 and R570. The bands were absent in the susceptible control cultivar Yuetang 60.

Bru1 was detected in 36 resistant varieties out of the 60 new varieties tested (54%); it was not detected in the other 5 resistant new varieties or the 19 susceptible new varieties (Table 2, Figure 1). *Bru1* was detected in 18 resistant varieties out of the 34 major cultivars tested (52.94%). The gene was not detected in the other 7 resistant major cultivars or the 9 susceptible major cultivars (Table 3, Figure 2).

Table 1. Identification standard for sugarcane resistance to brown rust disease

Score	Resistance	Diseased area of leaf tissue
1	Highly resistant (HR)	No symptoms
2	Resistant (R)	<10%
3	Moderately resistant (MR)	11–25%
4	Moderately susceptible (MS)	26–35%
5	Susceptible 1 (S1)	36–50%
6	Susceptible 2 (S2)	51–60%
7	Susceptible 3 (S3)	61–75%
8	Highly susceptible 1 (HS1)	76–90%
9	Highly susceptible 2 (HS2)	91–100%

Table 2. PCR detection of *Bru1* and field evaluation of resistance to *Puccinia melanocephala* in new sugarcane varieties

Comprehensive resistance response	Sample number	Variety	PCR detection results of <i>Bru1</i>	Comprehensive resistance response	Sample number	Variety	PCR detection results of <i>Bru1</i>
HR	1	Yuegan 48	<i>Bru1</i>	MR	32	Yuegan 53	<i>Bru1</i>
	2	Funong 09-2201	<i>Bru1</i>		33	Funong 07-3206	<i>Bru1</i>
	3	Funong 09-7111	N		34	Funong 09-4095	<i>Bru1</i>
	4	Funong 11-2907	N		35	Funong 10-0574	<i>Bru1</i>
	5	Guitang 08-120	<i>Bru1</i>		36	Mintang 06-1405	<i>Bru1</i>
	6	Guitang 08-1533	<i>Bru1</i>		37	Guitang 06-2081	<i>Bru1</i>
	7	Guitang 11-1076	<i>Bru1</i>		38	Guitang 08-1589	<i>Bru1</i>
	8	Liucheng 09-15	<i>Bru1</i>		39	Zhongzhe 10	N
	9	Zhongzhe 1	<i>Bru1</i>		40	Yunrui 11-450	N
	10	Yunzhe 08-1095	<i>Bru1</i>		41	Zhontang 1202	N
	11	Yunzhe 08-1609	<i>Bru1</i>		42	Yuegan 47	N
	12	Yunzhe 11-1204	<i>Bru1</i>		43	Funong 10-14405	N
	13	Yunzhe 11-3898	<i>Bru1</i>		44	Haizhe 22	N
	14	Yunrui 10-187	<i>Bru1</i>		45	Zhontang 1301	N
	15	Dezhe 12-88	<i>Bru1</i>		46	Yuegan 52	N
	16	Yuegan 50	<i>Bru1</i>	47	Mintang 07-2005	N	
	17	Yuegan 51	<i>Bru1</i>	48	Liucheng 07-150	N	
	18	Funong 08-3214	<i>Bru1</i>	49	Yunrui 12-263	N	
	19	Funong 09-6201	<i>Bru1</i>	50	Haizhe 28	N	
	20	Funong 09-12206	<i>Bru1</i>	51	Guitang 44	N	
	21	Mintang 12-1404	<i>Bru1</i>	52	Liucheng 07-506	N	
	22	Guitang 06-1492	<i>Bru1</i>	53	Yuegan 46	N	
	R	23	Guitang 08-1180	<i>Bru1</i>	54	Mintang 11-610	N
		24	Liucheng 09-19	<i>Bru1</i>	55	Guitang 40	N
		25	Zhongzhe 6	<i>Bru1</i>	56	Dezhe 07-36	N
		26	Zhongzhe 13	<i>Bru1</i>	57	Guitang 13-386	N
		27	Yunzhe 11-1074	<i>Bru1</i>	58	Yuegan 43	N
		28	Yunzhe 11-3208	<i>Bru1</i>	59	Yuegan 49	N
		29	Yunrui 10-701	<i>Bru1</i>	60	Yunzhe 09-1601	N
		30	Dezhe 09-78	<i>Bru1</i>			
		31	Zhontang 1201	<i>Bru1</i>			

HR: highly resistant, R: resistant, MR: moderately resistant, MS: moderately susceptible, S1: susceptible 1, S2: susceptible 2, S3: susceptible 3, HS1: highly susceptible 1, HS2: highly susceptible 2; N: no *Bru1* detected.

DISCUSSION

In this study, resistance to brown rust was evaluated in the field, and tests were conducted to detect the brown rust-resistance gene *Bru1* in 60 new sugarcane varieties and 34 major cultivars. In all, 54 new varieties and major cultivars were identified as carrying the *Bru1* gene; this finding provides the scientific basis and elite disease-resistant germplasm for the selection of varieties for production, breeding, and effective control of sugarcane brown rust. The frequency of *Bru1* in resistant new varieties (87.8%) was higher than the frequency of *Bru1* in the resistant major cultivars (72%). The increased frequency of *Bru1* in the resistant new varieties indicated that reliance on *Bru1* for resistance is increasing. The reason may be that *Bru1* is so effective that the breeding program has unintentionally repeatedly selected it. *Bru1* appears to be broadly adapted and durable, but it would be advisable to avoid overreliance on one resistance gene. The selective introduction and use of new resistance genes in brown rust-resistance breeding programs could help avoid potential breakdown of *Bru1* resistance. Brown rust is an important epidemic fungal disease that damages sugarcane leaves. Breeding and growing resistant varieties is the most economical and effective way of controlling this disease (Shan et al. 2014, Huang et al. 2018). As sugarcane brown rust has become an important disease that seriously affects development of a high-quality sugarcane industry in China, brown rust resistance should be regarded as one of the main economic indexes for sugarcane variety breeding programs. The introduction and use of other types of resistant

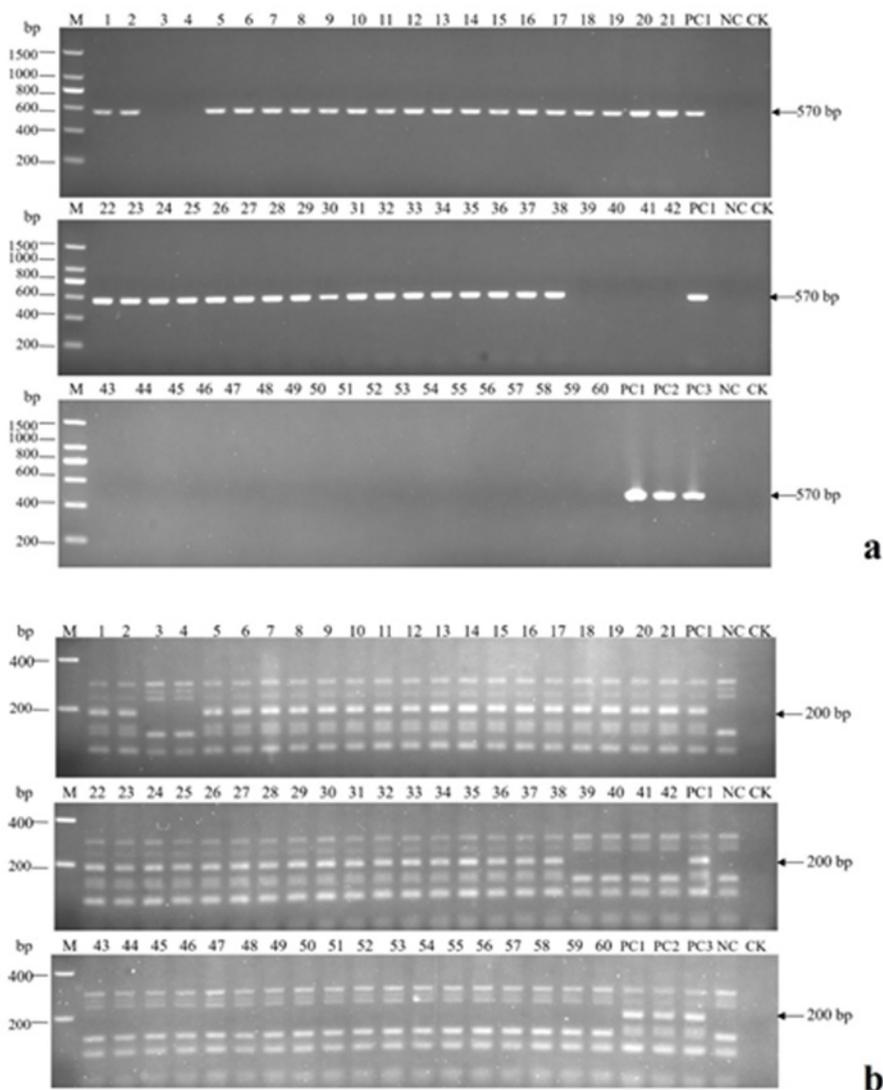


Figure 1. PCR detection of the *Bru1* gene in new sugarcane varieties. a) R12H16-PCR marker; b) 9O20-F4-PCR-*Rsa* I marker; 1-60: sample number of new sugarcane varieties; PC1: resistant control 1 (R570); PC2: resistant control 2 (ROC 1); PC3: resistant control 3 (ROC22); NC: susceptible control (Yuetang 60); CK: blank control.

genes in brown rust-resistance breeding programs should be strengthened in the future. The resistance of sugarcane varieties to brown rust should be evaluated by combining studies on artificial inoculation and natural disease in the field. In addition, disease-resistant resources should be explored and used, the breeding of intermediate material should be improved, and varieties with different sources of resistance should be grown in alternation with varieties with resistance through *Bru1*. This will aid in the selection and breeding of new resistant varieties for application and commercialization in sugarcane production.

Yunnan Province is an important distribution center for wild sugarcane resources in China and one of the global origins of wild sugarcane (Chen et al. 2001, Fan et al. 2001, Zhang et al. 2019). Wild sugarcane resources are an important source of resistance genes in modern sugarcane breeding. Some studies have shown that the wild germplasm resources preserved in the National Nursery of Sugarcane Germplasm Resources in China contain elite disease resistance genes, which is promising for the breeding of disease-resistant sugarcane varieties (Huang et al. 2012, Li et al. 2013, Xu et al.

Table 3. PCR detection of *Bru1* and field evaluation of resistance to *Puccinia melanocephala* in major sugarcane varieties

Comprehensive resistance response	Sample number	Variety	PCR detection results of <i>Bru1</i>	Comprehensive resistance response	Sample number	Variety	PCR detection results of <i>Bru1</i>	
HR	1	ROC 1	<i>Bru1</i>	R	20	Yuetang 93-159	N	
	2	ROC 10	<i>Bru1</i>		21	Yuetang 00-236	N	
	3	ROC 16	<i>Bru1</i>		MR	22	Yunzhe 05-51	<i>Bru1</i>
	4	ROC 20	<i>Bru1</i>			23	Yunyin 10	<i>Bru1</i>
	5	ROC 22	<i>Bru1</i>			24	Guitang 11	<i>Bru1</i>
	6	ROC 25	<i>Bru1</i>			25	Guitang 36	<i>Bru1</i>
	7	Yuetang 86-368	<i>Bru1</i>	MS	26	Guitang 42	N	
	8	Liucheng 03-182	<i>Bru1</i>		27	Liucheng 05-136	N	
	9	Guitang 21	<i>Bru1</i>		S1	28	Guitang 29	N
	10	Yunyin 3	<i>Bru1</i>	29		Guitang 44	N	
	11	Mintang 69-421	<i>Bru1</i>	S3	30	Dezhe 03-83	N	
	12	Yuetang 79-177	N		HS1	31	Liucheng 03-1137	N
	13	Yuetang 83-88	N	HS2		32	Yuetang 60	N
	14	Yingyu 91-59	N		33	Baxi 45	N	
	15	Chuantang 61-408	N	34	Guitang 46	N		
	R	16	Yunyin 58	N				
17		Yunzhe 03-258	<i>Bru1</i>					
18		Yunzhe 05-49	<i>Bru1</i>					
19		Chuantang 79-15	<i>Bru1</i>					

HR: highly resistant, R: resistant, MR: moderately resistant, MS: moderately susceptible, S1: susceptible 1, S2: susceptible 2, S3: susceptible 3, HS1: highly susceptible 1, HS2: highly susceptible 2; N: no *Bru1* detected.

2014, Li et al. 2015, Li et al. 2017b, Li et al. 2018b). The results of this study are consistent with the results of Li et al. (2015) in indicating that both new varieties bred in China in recent years and the major cultivars contain the brown rust-resistance gene *Bru1*. The brown rust-resistant varieties identified can potentially be used as materials for breeding sugarcane varieties with brown rust resistance. The heritability of the resistance of these varieties should be analyzed, and a gene bank of disease-resistant germplasm should be established to further select new brown rust-resistance varieties for application in production.

Researchers in many countries have carried out *Bru1* gene detection studies in wild germplasms and new varieties. Results have shown that *Bru1* is the predominant source of brown rust resistance in breeding materials and most modern sugarcane varieties (Glynn et al. 2013, Josefina et al. 2013, Molina et al. 2013, Li et al. 2015, Li et al. 2018a). Interspecific hybrid populations under recurrent selection for resistance to brown rust based on natural infection ratings have unintentionally increased the frequency of *Bru1* (Asnaghi et al. 2004, Glynn et al. 2013) and it has been suggested that this frequency has resulted in a potentially risky dependence on *Bru1* for disease resistance worldwide (Costet et al. 2012, Glynn et al. 2013). Therefore, there is a potential major threat from breakdown of the resistance provided by *Bru1* because of variation in the pathogenic race. Further studies are therefore needed to explore these new genes, which could help overcome the problems associated with reliance on the *Bru1* gene to provide disease resistance. In this study, *Bru1* was not detected in twelve brown rust-resistant varieties (five new varieties and seven major cultivars), which implies that these varieties may carry brown rust-resistance-associated genes other than *Bru1*. The use of varieties containing new resistance genes may decrease the selection pressure for pathogen mutants and maintain the resistance of the *Bru1* gene.

The large-scale growing of susceptible varieties combined with rain and high humidity is the main reason for outbreaks of sugarcane brown rust. The results of this study showed that seven major varieties cultivated over large areas, including Guitang 29, Guitang 44, Dezhe 03-83, Liucheng 03-1137, Yuetang 60, Baxi 45, and Guitang 46, were highly susceptible to brown rust. In contrast, 31 new elite varieties bred in recent years displayed strong brown rust resistance. Thus, in the sugarcane growing areas with high incidence of brown rust and wet and rainy climates, more effort should be made to eliminate the major susceptible varieties and to promote the growing of new resistant varieties. This will help to achieve

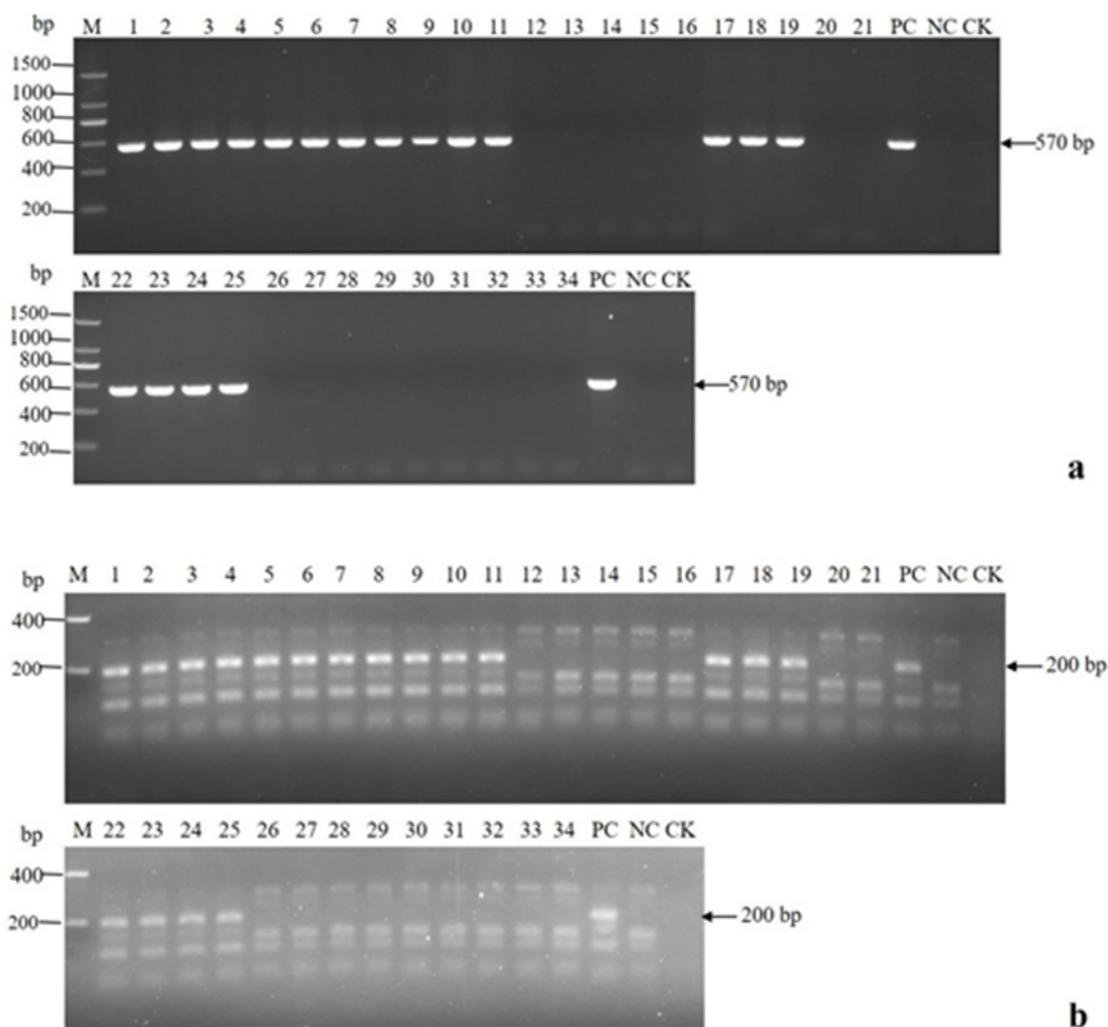


Figure 2. PCR detection of the *Bru1* gene in major cultivars. a) R12H16-PCR marker; b) 9O20-F4-PCR-*Rsa* I marker; 1-34: sample number of major cultivars; PC: resistant control (ROC22); NC: susceptible control (Yuetang 60); CK: blank control.

a reasonable distribution of varieties, fundamentally control the outbreak of disease in sugarcane growing areas, and provide security in development of the high-quality sugarcane industry in China in the future.

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