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Short Communication

Genetic analysis of sugarcane brown rust resistance genes in wild sugarcane germplasm *Erianthus rockii* 'Yundian 95-19' and *Erianthus rockii* 'Yundian 95-20'

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Abstract

We assessed inheritance of resistance to sugarcane brown rust (*Puccinia melanocephala*) in selfing F_1 populations of wild sugarcane germplasm *Erianthus rockii* 'Yundian 95-19' and *E. rockii* 'Yundian 95-20'. We tested parent and selfing F_1 individuals for the brown rust resistance gene, *Bru*1, that has been shown to confer resistance to brown rust in sugarcane. The *Bru*1 gene was not detected in *E. rockii* 'Yundian 95-19', *E. rockii* 'Yundian 95-20' or their selfing F_1 individuals, and we found there was segregation of resistance in the two selfing F_1 populations (segregation ratio: 3:1). The results confirmed resistance in *E. rockii* 'Yundian 95-19' and *E. rockii* 'Yundian 95-20' to sugarcane brown rust is controlled by a novel, single dominant gene.

Keywords: *Bru*1 gene, selfing population, inheritance, single dominant gene

Introduction

Sugarcane brown rust, which is a globally widespread, economically important disease of sugarcane caused by *Puccinia melanocephala* H. Sydow & P. Sydow (Hoy and Hollier, 2009). Currently, the disease tends to occur in China where sugarcane cultivation is predominant, and has become one of the most common and most important crop diseases in the country causing degeneration of germplasm and reduction in yield that threaten the sustainable development of the sucrose industry (Huang and Li, 2011).

Large-scale planting of susceptible varieties of sugarcane is an important factor in the epidemic occurrence of the brown rust (Huang and Li, 2011), and field-scale control using large-scale applications of pesticides tends to be ineffective. Therefore, breeding and cultivating varieties that are resistant to the disease represent the most economical and effective control measures. In this study, we analysed the inheritance of resistance to sugarcane brown rust in two-wild germplasm of resistant sugarcane to improve understanding of genetic disease resistance that is vital for sustainable sugarcane breeding programs.

Experimental

In March 2017, about 700 selfing seeds (F_1) of *Erianthus rockii* 'Yundian 95-19' and about 800 selfing seeds (F_1) of *E. rockii* 'Yundian 95-20' were sown in seedling pots and in July 2017, we transplanted 400 of the raised seedlings from

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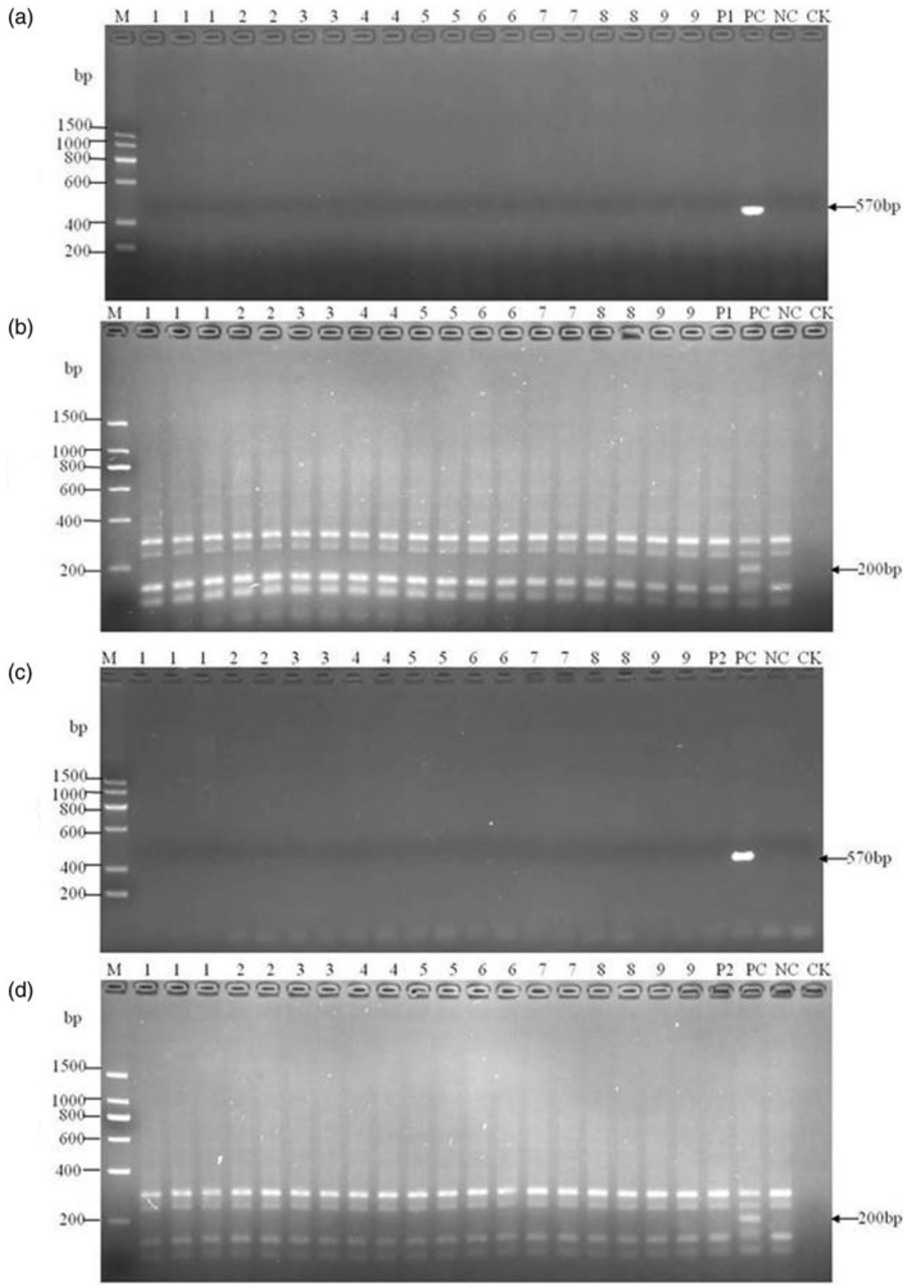

Fig. 1. Detection of *Bru*1 in *E. rockii* 'Yundian 95-19', *E. rockii* 'Yundian 95-20' and their selfing F_1 individuals. (a) *E. rockii* 'Yundian 95-19' (R12H16); (b) *E. rockii* 'Yundian 95-19' (9O20-F4); (c) *E. rockii* 'Yundian 95-20' (R12H16) and (d) *E. rockii* 'Yundian 95-20' (9O20-F4). Grades 1–9 indicate the degree of resistance; P1: *E. rockii* 'Yundian 95-19'; P2: *E. rockii* 'Yundian 95-20'; PC: positive control; NC: negative control and CK: blank control.

each selfing into the field. For each population, we planted 80 seedlings and one parent in five rows that were 1 m apart; seedlings were managed following local conventional production methods. One week later, we replaced any seedlings that had died. In October 2017 and September 2018, sugarcane leaves with severe disease were collected and soaked in a plastic pot that was two-thirds filled with water, then the leaves were rubbed by hand, and the filtrate was filtered through two layers of gauze to create a 10×10^4/ml spore. The inoculation was applied as a spray twice, with an interval of 2 d, and after 4 weeks, we evaluated sugarcane brown rust resistance according to the method described by Li *et al.* (2017), using a scale of 1 to 9, where grades 1 to 3 were resistant, and grades 4 to 9 were

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Table 1. Segregation ratios of resistance to susceptibility in selfing F_1 populations of *E. rockii* 'Yundian 95-19' and *E. rockii* 'Yundian 95-20'

Parents and selfing	2017				2018			
	R	*S*	Expected ratio	χ^2	*R*	*S*	Expected ratio	χ^2
E. rockii 'Yundian 95-19'	5	0			5	0		
E. rockii 'Yundian 95-20'	5	0			5	0		
E. rockii 'Yundian 95-19' × *E. rockii* 'Yundian 95-19'	290	110	3*R*:1*S*	1.203	304	96	3*R*:1*S*	0.163
E. rockii 'Yundian 95-20' × *E. rockii* 'Yundian 95-20'	287	113	3*R*:1*S*	2.083	303	97	3*R*:1*S*	0.083

R, resistant; *S*, susceptible; $\chi^2_{0.05} = 3.84$.

susceptible. We tested the segregation ratios of resistant (*R*) to susceptible (*S*) individuals using Chi-squared.

For each population, we randomly selected sugarcane leaves graded from 1 to 9 that represented varying levels of resistance, and used polymerase chain reaction (PCR) primers R12H16 and 9O20-F4 to detect the *Bru*1 gene following the method reported by Li *et al*. (2015).

Discussion

The PCR results showed that the *Bru*1 gene was not detected in *E. rockii* 'Yundian 95-19', *E. rockii* 'Yundian 95-20', or the selfing F_1 individuals (Fig. 1). In 2017, there were no brown rust spores on any leaves of five *E. rockii* 'Yundian 95-19', and we found 290 selfing F_1 individuals were resistant (grades 1–3), while 110 individuals were susceptible (grades 4–9) (expected segregation ratio: 3:1; $\chi^2{}_{0.05} = 1.203$) (Table 1). In 2018, there were no spores on any *E. rockii* 'Yundian 95-19' leaves; 304 selfing F_1 individuals were resistant (grades 1–3) and 96 individuals were susceptible (grades 4–9) (expected segregation ratio of 3:1; $\chi^2{}_{0.05} = 0.163$) (Table 1). In 2017, we found that there were no spores on any leaves of five *E. rockii* 'Yundian 95-20', and 287 individuals were resistant (grades 1–3), while 113 individuals were susceptible (grades 4–9) (expected segregation ratio: 3:1; $\chi^2{}_{0.05} = 2.083$) (Table 1). In 2018, we found no spores any *E. rockii* 'Yundian 95-20' leaves, and 303 individuals were resistant (grades 1–3), while 97 were susceptible (grades 4–9) (expected segregation ratio: 3:1; $\chi^2{}_{0.05} = 0.083$) (Table 1).

E. rockii is a rare wild germplasm of sugarcane with distribution limited to Yunnan, Sichuan and Tibet in China (Wang *et al*., 2008). The species exhibits resistance to cold, drought and disease, especially to sugarcane brown rust (Li *et al*., 2005). In this two-year study, we found resistance of diseased F_1 individuals of *E. rockii* 'Yundian 95-19' and *E. rockii* 'Yundian 95-20' tended to be grade 2 to 4, with few individuals graded as highly susceptible, and we found no spores on leaves of *E. rockii* 'Yundian 95-19' and *E. rockii* 'Yundian 95-20'. However, we found there was a range of disease resistance in the selfing F_1 populations, where segregation ratios were consistent with the 3:1 ratio of single gene dominant inheritance, indicating disease resistance in the F_1 populations had diverged and resistance to sugarcane brown rust was controlled by a heterozygous dominant gene. The next step in this research is to look for molecular markers linked to these two resistance genes. In this study, we confirmed a novel major sugarcane brown rust resistance gene in *E. rockii* 'Yundian 95-19' and *E. rockii* 'Yundian 95-20' that may improve the diversity of resistance genes used in sugarcane breeding.

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

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