Received: 20 December 2019 | Accepted: 19 January 2021

DOI: 10.1002/csc2.20468

Crop Science

ORIGINAL RESEARCH ARTICLE

Plant Genetic Resources

Evaluation of Guangxi common wild rice for resistance to brown planthopper using a new stem evaluation method

Liang Luo[1,3,#] | **Xuan Wang**[1,3,#] | **Xinying Guo**[1,3,#] | **Taian Lu**[1,3] | **Ruizhi Yuan**[1,3] | **Shanyue Liao**[1,3] | **Suosheng Huang**[4] | **Yue Han**[1,3] | **Babar Usman**[1,3] | **Baoxiang Qin**[1,3] | **Jijing Luo**[2,3] | **Fang Liu**[1,3] | **Fengkuang Huang**[4] | **Rongbai Li**[1,3]

[1] College of Agriculture, Guangxi Univ., Nanning 530004, P.R. China

[2] College of Life Science and Technology, Guangxi Univ., Nanning 530004, P.R. China

[3] State Key Lab. for Conservation and Utilization of Subtropical Agro-Bioresources, Guangxi Univ., Nanning 530004, P.R. China

[4] Plant Protection Institute, Guangxi Academy of Agricultural Sciences, Nanning 530007, P.R. China

Correspondence
Rongbai Li, State Key Lab. for Conservation and Utilization of Subtropical Agro-Bioresources, Guangxi Univ., Nanning 530004, P.R. China
Email: lirongbai@126.com

[#]These authors contributed equally to this work.

Assigned to Associate Editor Georgia Eizenga.

Abstract

Brown planthopper [BPH, *Nilaparvata lugens* (Stål)] is considered one of the most important pests of rice (*Oryza sativa* L.), which poses a serious threat to rice production. Identifying resistant *Oryza* germplasm can provide reliable accessions for breeding BPH resistant rice cultivars. In this study, the stem evaluation method (SEM) was first applied to identify the BPH resistance of 1,221 accessions of common wild rice (*O. rufipogon* Griff.) collected from three different regions of Guangxi Province, China. From this screening, 58 BPH resistant accessions were screened a second time, with 33 accessions ultimately identified as stable, highly resistant germplasm as confirmed by a third identification at the adult-plant stage. The distribution of the 58 BPH-resistant common wild rice accessions varies significantly from region to region. Genotypic analyses based on 42 simple sequence repeat (SSR) markers revealed that these 58 BPH-resistant accessions were genetically diverse, reflecting the rich genetic diversity reported in Guangxi common wild rice. Furthermore, results verified that the SEM is efficient for rapid and accurate screening of BPH-resistant germplasm, especially when a limited number of seeds are available or elite breeding lines need to be screened immediately. Also, SEM is the best method for evaluating BPH resistance at the adult stage because fewer insects are needed, and it is possible to repeat the evaluation in the same crop season. The 33 resistant rice accessions are a potential source of novel BPH resistance genes for developing cultivars with improved BPH resistance.

1 | INTRODUCTION

Rice (*Oryza sativa* L.) is a staple food that feeds more than half of the world's population, but rice production is severely threatened by different abiotic and biotic stresses (Liu et al., 2014). Brown planthopper [BPH, *Nilaparvata lugens* (Stål)] is one of the most harmful pests in Asian rice production areas. Brown planthopper is a migratory and destructive pest that is distributed in many countries and severely threatens

Abbreviations: BPH, brown planthopper; IRRI, International Rice Research Institute; MAS, marker-assisted selection; PCoA, principal coordinates analysis; PIC, polymorphic information content; SEM, stem evaluation method; SSR, simple sequence repeat; SSST, standard seedbox screening technique; TN1, Taichung Native 1; UPGMA, unweighted pair-group method with arithmetic means

rice production (Way & Heong, 1994). In recent years, BPH has become more widespread across Asia, sharply reducing rice production (Normile, 2008). It not only damages rice directly by sucking the juice but also indirectly by transmitting viruses including the *Rice ragged stunt virus* (RSV) and *Rice grassy stunt virus* (GSV) (Cha et al., 2008). After BPH infestation, seed set significantly decreases and the plants dry up, referred to as "BPH burn," leading to complete plant death (Sogawa, 1982).

Chemical control has long been a major measure to prevent BPH, but it is expensive and environmentally unsafe. Insecticides are highly destructive to BPH, but they also enhance its chemical resistance and contributed to strong resurgence (Tanaka et al., 2000). Research and practice showed that the most economical and environmentally friendly measure to control BPH is breeding resistant cultivars (Khush, 2001; Ramkumar et al., 2016). The key to breeding resistant cultivars and diversifying single resistant germplasm is to provide the basic building blocks for the development of stress-resistant cultivars. As an example, in the 1970s at the International Rice Research Institute (IRRI) in the Philippines, screening for BPH-resistant germplasm identified 44,335 *O. sativa* accessions resistant to BPH biotype 1, 10,553 accessions resistant to BPH biotype 2, and 13,021 accessions resistant to BPH biotype 3 (Jackson, 1997). Also, Sarao et al. (2016) screened 1003 *Oryza* accessions representing 12 different species, which were obtained from the IRRI and Indian collections, and initially identifed 159 accessions resistant to BPH.

In recent years, genetic and genomics tools have accelerated the rice molecular breeding program, and significant progress has been made towards the identification of BPH resistance sources from diverse germplasm against all the four prevalent BPH biotypes. Many advanced technologies including marker-assisted selection (MAS), genomics, proteomics, metabolomics, and transcriptomics have been used to explore and utilize the BPH germplasm (Kumar et al, 2018; Li et al., 2017; Peng et al., 2016; Sangha et al., 2013).

Wild species of *Oryza* are reservoirs of useful genes and quantitative trait loci (QTLs) for rice improvement and to broaden the gene pool of rice. Several genes and QTLs governing agronomic traits have been transferred from wild species into rice, and a few of these were tagged with molecular markers and used in MAS. The resistant germplasm from the ancestral rice wild species *O. rufipogon*, which has an AA genome like cultivated Asian rice (*O. sativa*), is more easily crossed with *O. sativa* than non-AA genome resistant germplasm such as *O. officinalis* Wall. (Barbier et al., 1991; Brar & Khush, 2018; Khush, 1997; Oka, 1988; Tang et al., 2008). Common wild rice, *O. rufipogon*, harbors abundant genetic diversity after extensive natural selection that can be used for rice improvement through breeding techniques (Chen et al., 2010; Fuller et al., 2010; Liu et al., 2013). Guangxi Province is one of the main natural habitats of wild rice in China and is also considered the center of genetic diversity for China's common wild and cultivated rice (Huang et al., 2012). Guangxi common wild rice is a rich source of many valuable accessions, which carry genes for resistance to diseases and pests (Chen et al., 2017; Guo et al., 2017; Qin et al., 2014; Zhu et al., 2016), thus providing new sources of resistance for rice breeding programs. Qin et al. (2004) screened 198 Guangxi medicinal wild rice for BPH resistance and identified 195 (98.5%) resistant accessions. Li et al. (2001) identified the resistance of Guangxi common wild rice line 94-42-5-1 to BPH and discovered the line that had broad-spectrum resistance to BPH and showed high resistance (HR). Later, they used multiple BPH biotypes to identify resistance in >1,200 Guangxi common wild rice accessions and successfully obtained 30 resistant accessions, six with broad-spectrum and HR to BPH biotypes (Li et al., 2006). At present, among 40 reported BPH-resistant genes, nine resistance genes—namely, bph19(t), bph21(t), bph22(t), bph23(t), Bph24(t), Bph27 [originally named bph18(t)], bph29[originally named bph20(t)], Bph35, and Bph36—were derived from Guangxi common wild rice (Chen et al., 2009, 2010; Huang et al., 2013; Li et al., 2006, 2019; Yang et al., 2012; Zhang et al., 2019; Zhou et al., 2015).

Molecular markers play an important role in the identification and characterization of new germplasm (O'Neill et al., 2003), and simple sequence repeat (SSR) markers are often used to analyze the genetic diversity of rice. The ability of SSR markers to differentiate rice accessions genotypically is documented by the fact that a "fingerprint marker" panel consisting of 12 SSR markers differentiated a diverse subset of nearly 2,000 *O. sativa* accessions from the U.S. germplasm collection, and with two additional SSR markers, the identity of nearly 95 U.S. varieties that are closely related was ascertained (McClung et al., 2020). The rich genetic diversity found in common wild rice (*O. rufipogon*) accessions from Southeast Asia, mainly China, has been documented in several

Core Ideas

- We used a new stem evaluation method (SEM) to evaluate rice accessions for resistance to BPH.
- The discovery of these resistant resources will provide more basic resources for further study.
- Genetic diversity of BPH-resistant accessions in rice deserves further study.
- Discovery of resistant resources should focus on sampling from the lower reaches of Xijiang.
- Guangxi *Oryza rufipogon* was taken as experimental accessions, which shows superiority.

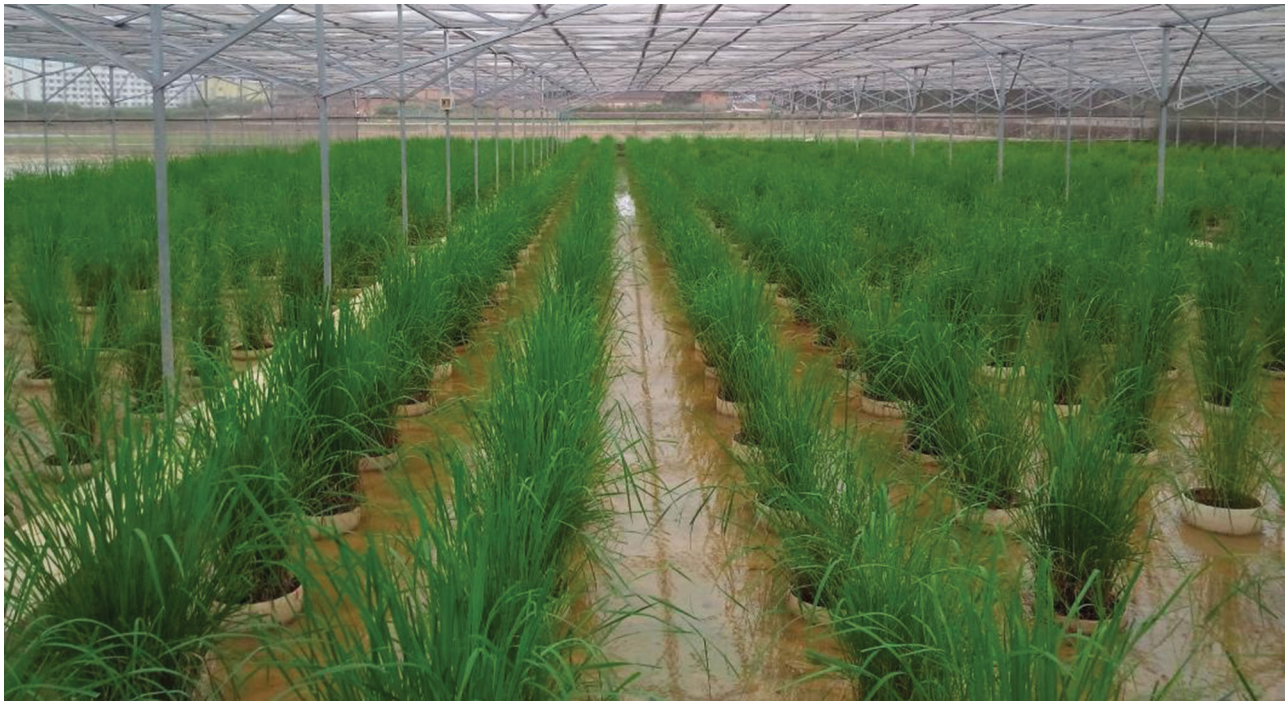

FIGURE 1 The wild rice nursery at Guangxi University. The collected common wild rice was planted in pots at the wild rice nursery. From the 20th day after transplanting through the tillering stage, stems can be taken repeatedly from mother plants to screen for brown planthopper (BPH) resistance when there is an adequate number of BPH nymphs

studies involving 90–4,173 accessions genotyped with 24–46 SSR markers (Li et al., 2010; Pan et al., 2018; Sun & Yang, 2009; Xue et al., 2016), confirming the ability of SSR markers to document the genetic diversity in this ancestral wild species.

The main objective of this study was to identify new sources of resistance to BPH in a collection of *O. rufipogon* accessions native to Fusui, Yongning, and Pingnan Counties in Guangxi Province, China. To screen this collection for resistance to BPH, we (a) developed a stem evaluation method for identification of BPH resistant germplasm, (b) screened 1,221 accessions in the collection, (c) verified the resistance germplasm by repetitive screening, and a third identification at the adult-plant stage, (d) analyzed the regional distribution of resistance accessions, and (e) analyzed the genotypic diversity of resistance accessions with SSR markers.

2 | MATERIALS AND METHODS

2.1 | Experimental materials

A total of 1,221 accessions of common wild rice (*O. rufipogon*) were collected from three representative regions, one region in Fusui County (22°34′ N, 107°95′ E), one in Yongning (22°70′ N, 108°35′ E), and one in Pingnan (23°30′ N, 110°21′ E), Guangxi Province, P.R. China, with 297 accessions originating from Yongning County, Nanning City; 331 from Pingnan County, Guigang City; and 593 from Fusui County, Chongzuo City. The accessions were provided by the Wild Rice Germplasm Nursery of State Key Laboratory of Conservation and Utilization of Subtropical Agro-bioresources, Guangxi University, Nanning, P.R. China, as tillers (Figure 1) and are available for research and breeding purposes to researchers within China. The susceptible control cultivar, Taichung Native 1 (TN1, IRGC 038845), was obtained from IRRI.

The BPH insects used in the experiment were mixed field biotypes collected from rice fields in Nanning. The BPH biotype 2 accounted for >70%, the Bangladesh biotype for about 20%, and biotype 1 for less than 10% according to Liu et al. (2011). The insects were raised on susceptible cultivar TN1 for one or two generations to obtain an adequate number of nymphs to infest the plants.

2.2 | Stem evaluation method

Most rice is screened for BPH resistance using the standard seedbox screening technique (SSST) developed by IRRI (IRRI, 1996). Briefly, the SSST involves sowing >30 seeds in a plastic box; after 5 d, the plants are thinned to about 20–30 seedlings, and at the two- to the three-leaf stage, the seedlings are infested with the second or third-instar BPH nymphs at a density of 10–12 nymphs per seedling. When all the plants of susceptible TN1 have died, the seedlings are rated as 0 (no

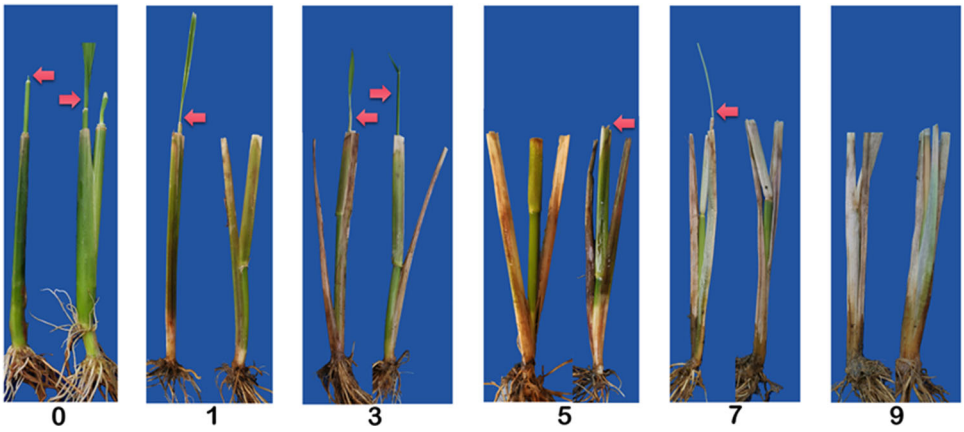

FIGURE 2 Resistance scale of the stem evaluation method (SEM). The rating scale is 0 (immune, I) = free from damage; 1 (highly resistant, HR) = free from damage and slightly brown in the upper part of the outer leaf sheath; 3 (resistant, R) = outer leaf sheath brown and inner two leaf sheaths are alive, with new leaves emerging from the center (arrow); 5 (moderately resistant, MR) = only one inner leaf sheath is alive with new leaves emerging (arrow); 7 (susceptible, S) = inner leaf sheaths are turning brown with weak growth potential for new leaves (arrow); and 9 (highly susceptible, HS) = the whole plant is brown and dead. Arrows identify the new leaves

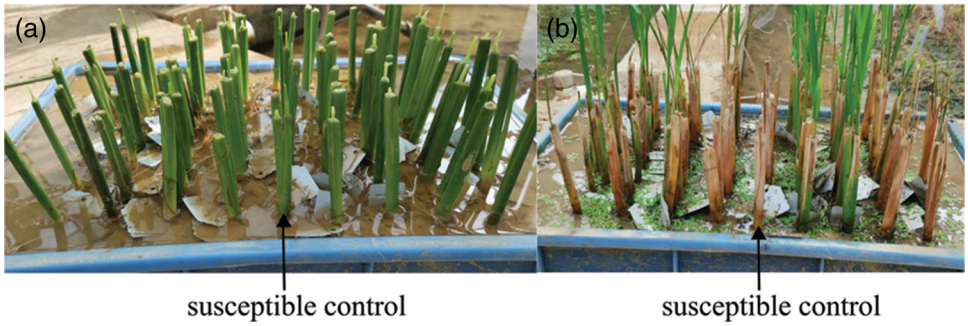

FIGURE 3 Images illustrating planting and rating stages for the stem evaluation method (SEM), which evaluates resistance to brown planthopper (BPH). (a) Rice stems (tillers) planted for a screening. Rice stems were planted in the plastic tray, with susceptible control in one row (arrow), and one stem from each accession was used to identify the level of resistance. (b) Results from ~7 d after BPH infection. The plants were rated at this stage when the susceptible control had wilted

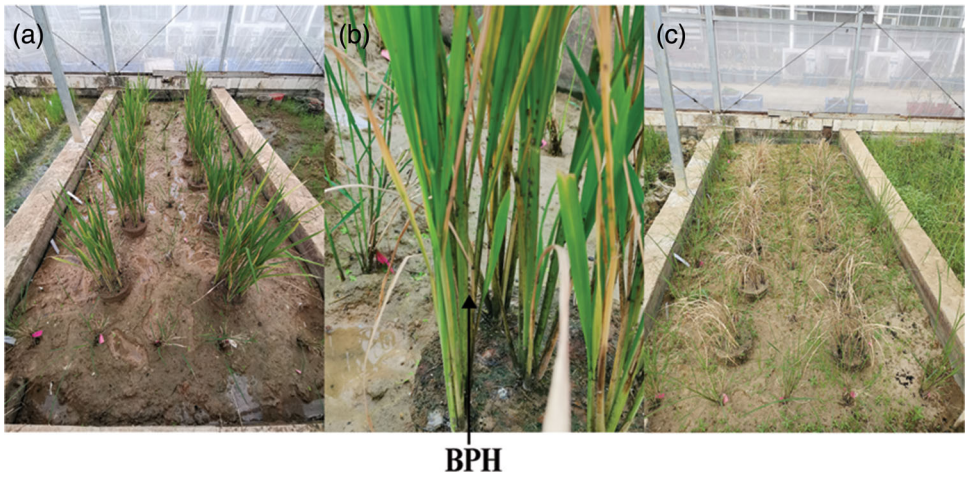

FIGURE 4 Confirmation of the brown planthopper (BPH)-resistant accessions at the adult plant stage. (a) Images illustrating planting of resistant accessions and insect-attractant TN1 adult plants; (b) the insect-attractant TN1 adult plants infested with adult BPH (arrow); and (c) results after all the insect-attractant TN1 plants wilted, at which time the potentially resistant accessions were rated for BPH resistance. Arrows point to BPH

TABLE 1 Results from the preliminary screening of Guangxi common wild rice (*O. rufipogon*) accessions for resistance to the brown planthopper (BPH)

Resistance scale	No. of materials	Percentage of materials
1	5	0.41
3	28	2.29
5	25	2.05
7	10	0.82
9	1,152	94.35
Total	1,221	100

damage) to 9 (dead) according to the standard evaluation system (SES) of IRRI (Hu et al., 2016; Li et al., 2019; Zhang et al., 2019).

Because the *O. rufipogon* accessions produce limited seed, it is not feasible to use the SSST, thus the stem evaluation method (SEM) was designed to screen the *O. rufipogon* accessions. For this method, the stem of a single tiller represents one *O. rufipogon* accession and is used to evaluate the accession for resistance. The tillers were removed from the *O. rufipogon* plants growing in the wild rice nursery (Figure 1) at the tillering stage over 10 mo. During the preliminary screening, one stem with the root was extracted from each rice plant, and its 16-cm stem base was taken for stem identification. This step was repeated three times. The stems with leaf sheaths were washed and planted in rows in a plastic tray filled with wet soil, 4 cm deep, with the row and plant spacing of 5 × 3 cm. As the control, TN1 stems were planted in the middle row. From the same day to the fifth day after transplanting, stems were infested with second or third instar nymphs. To shorten the identification period and obtain accurate resistance ratings, each stem was generally infested with more than 50 BPH nymphs. Subsequently, the stems were covered by an insect-proof net, placed in a greenhouse or a net-house with rain-proof facilities maintained at 26–30 ˚C, and shallow water. About 10 d after insect infestation, when all plants of the control cultivar TN1 wilted, the plants were rated using the following scale: 0 (immune, I) = free from damage; 1 (highly resistant, HR) = free from damage and slightly brown in the upper part of out sheath; 3 (resistant, R) = out leaf sheath brown and inner two leaf sheaths alive, with new leaves growing out from the center (indicated by the arrow); 5 (moderately resistant, MR) = only one inner leaf sheath alive, with new leaves (indicated by the arrow); 7 (susceptible, S) = inner leaf sheaths turning brown (indicated by the arrow), with weak growth potential; and 9 (highly susceptible, HS) = whole plant brown (Figure 2). For the resistant accessions obtained from the preliminary screening, the same method was used for two additional evaluations in the current season. The average resistance value from these three independent values was calculated to confirm the resistant germplasm.

2.3 | Evaluation of the *O. rufipogon* accessions for resistance to BPH

Initially, the 1,221 accessions of common wild rice were screened for BPH in the greenhouse during the 2018 spring season. The accessions with the scores 1 (highly resistant, HR), 3 (resistant, R), or 5 (moderately resistant, MR) were evaluated in a second screening in the 2018 spring season. Subsequently, the accessions that scored 1 or 3 after this second screening were subjected to a third evaluation at adult-plant stage in the same season to verify resistance. In the greenhouse, we used a cement pool as the "identification pool," because it had convenient drainage and irrigation and was not affected by confounding diseases and insect pests. The wild rice resistant accessions selected from the second screening were sparsely planted in the identification pool in the greenhouse. After about 15 d, when the stems were growing well and developing some tillers, the susceptible cultivar TN1 was planted intermittently between the rows to attract and raise insects.

After each TN1 plant was infested with 10 mature oviposition adult BPHs, the identification pool was covered and wrapped by an insect-proof net to prevent the adults from escaping but allowing the BPHs to move around freely. The plants were irrigated with a shallow flood (Figure 4). Thousands of nymphs reproduced on the TN1 plants after the adults laid eggs and the eggs hatched; thus, each wild rice stem was generally infested with more than 50 second- to third-instar BPH. The number of insects on each stem was far more than used for the SSST BPH resistance screening (IRRI, 1996), thus mimicking an excessive infestation.

When the insect-attractant TN1 cultivar completely wilted, the results of the BPH resistance identification of the resistant accessions were rated. The rating standard at this adult-plant stage was 0 (immune, I; free from damage), 1 (highly resistant, HR; free from damage); 3 (resistant, R; base leaves are slightly yellowing); 5 (moderately resistant, MR; base leaves are yellowing), with weak growth potential; 7 (susceptible, S; upper leaf sheaths are yellowing or wilting); and 9 (highly susceptible, HS; all plants wilting).

2.4 | Geographic origin and distribution analysis of resistant accessions

ArcGIS (ArcGIS for Developers, https://developers.arcgis.com/) was used to draw the regional distribution map of resis-

TABLE 2 Geographic sources and resistance frequency of common wild rice (*Oryza rufipogon*)

Distribution region	No. of accessions identified	Resistant accessions							
		Scale 1 (HR)		Scale 3 (R)		Scale 5 (MR)		Total	% of total
		No.	%	No.	%	No.	%		
Pingnan	331	2	0.60	16	4.23	11	3.32	27	8.16
Yongning	297	3	1.01	8	2.69	9	3.03	20	6.73
Fusui	593	0	0	6	1.01	5	0.84	11	1.85

Note. HR, highly resistant; R, resistant; MR, moderately resistant.

tant germplasm. Also, the distribution frequencies of high resistance (HR), resistance (R), and moderate resistance (MR) of the materials in each region were calculated using ArcGIS.

2.5 | Genetic diversity analysis of resistant accessions

For genotyping, genomic DNA was extracted using the cetyl trimethylammonium bromide (CTAB) method reported by Chen and Ronald (1999). Based on the SSR markers designed by Li et al. (2015), 42 highly polymorphic SSRs were selected based on PIC (polymorphic information content) values, and covering all 12 chromosomes were selected.

The SSR primer pairs (Supplemental Table S1) were synthesized by Guangzhou IGE Biotechnology. The polymerase chain reaction (PCR) amplification procedure was pre-denaturing at 95 ˚C for 5 min, denaturing at 95 ˚C for the 30 s, annealing at 58 ˚C for 30 s, extending at 72 ˚C for 45 s, final extending at 72 ˚Cfor 10 min, and maintaining at 16 ˚C for 16 min, and Steps 2–4 were repeated 34 times. Polymerase chain reaction amplification products were detected on 7% nondenaturing polyacrylamide gel (Sanguinetti et al., 1994). Lastly, the gel bands for each marker were converted to alleles and the corresponding data matrix was established.

For genetic diversity analysis, alleles for each marker were recorded according to the position of amplified bands on the gel. Popgene 32 (Yeh & Yang, 1999) and PIC-CALC (Nagy et al., 2012) were used to calculate the average number of alleles (N_a), the effective number of alleles (N_e), Shannon's information index (I), actually observed heterozygosity (H_o), expected observed heterozygosity (H_e), the diversity index (Nei), and the polymorphism information content (PIC). MEGA-X (Tamura et al., 2007) was applied for cluster analysis; the online website ImageGP (http://www.ehbio.com/imagegp/index.php/home/index/index.html) was used for principal coordinate analysis (PCoA) analysis, and the Structure 2.3.4 (Pritchard et al., 2000) was adopted to analyze the population structure of wild rice accessions.

3 | RESULTS

3.1 | Screening of Guangxi common wild rice for BPH resistant accessions

The BPH resistance of 1,221 accessions of Guangxi common wild rice was preliminarily screened with SEM (Figure 3). The identification results (Table 1) indicated that the susceptible cultivar TN1 scored 9 (highly susceptible, HS), and 58 accessions (4.75%) were resistant to BPH (score 1–5), with only five accessions (0.04%) rated as highly resistant (score 1).

To confirm these results, stems of the same plants used for the preliminary screening were taken from the 58 BPH-resistant accessions and evaluated a second time using the SEM. The results of this screening were similar to those of the preliminary screening, showing five accessions were resistant (score 1), 28 (score 3), and 25 (score 5), verifying the accuracy of the preliminary screening results (Table 1, Supplemental Table S2).

Based on the second screening, the 33 BPH resistant accessions (score 1 and 3) were considered as potential sources of resistance and transplanted to the identification pool to validate their resistance to the BPH at the adult stage by using an excessive BPH infestation. Fifteen days after transplanting, the plants with a few new tillers were infested with BPH nymphs carried out for re-identification (Figure 4a and 4b). The results showed that at the time all the insect-attractant TN1 plants completely died, the 33 resistant accessions still showed stable high resistance (Figure 4c). Therefore, these 33 resistance accessions were confirmed to be potential sources of novel BPH resistance genes.

3.2 | Geographic origin and distribution of BPH resistant accessions

The 1,221 common wild rice accessions evaluated for resistance were collected from three major wild rice distribution regions located in Pingnan, Yongning, and Fusui Counties

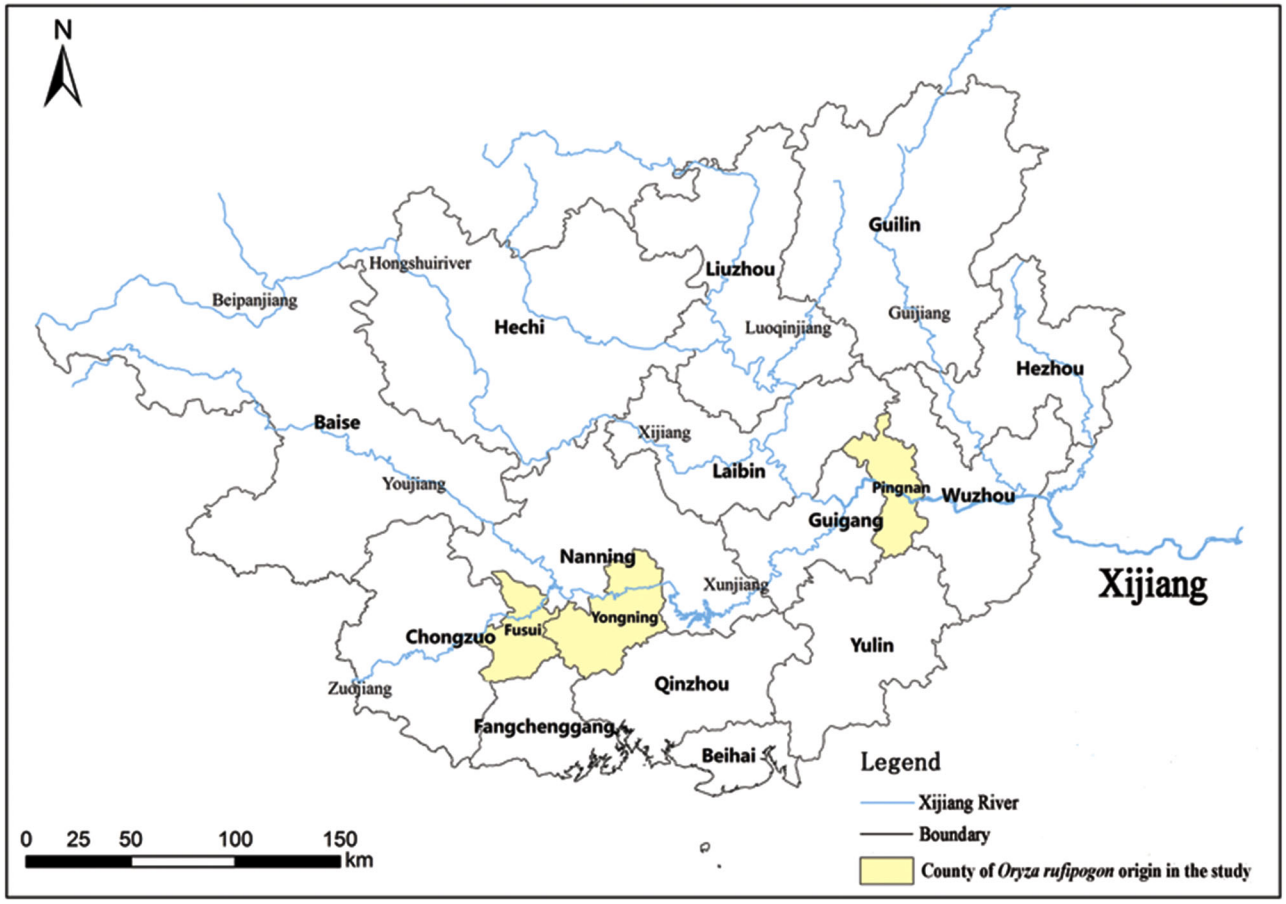

FIGURE 5 Regional distribution of resistant accessions of common wild rice (*O. rufipogon*). The map of the Guangxi Region of China shows three common wild rice distribution regions Pingnan, Yongning, and Fusui Counties (yellow) located along the Xijiang River (blue), which is the main branch of the Zhujiang River in South China

(Figure 5). Based on the BPH screening, 27 of the 331 (8.16%) accessions collected from Pingnan were resistant, 20 of the 297 (6.73%) accessions collected from Yongning were resistant, and 11 of the 593 (1.85%) accessions collected from Fusui were resistant (Table 2). Results revealed that the common wild rice accessions collected from the Pingnan distribution region showed the highest resistance to BPH, followed by the ones from Yongning, and those from Fusui showed the lowest resistance.

Further analysis revealed that the three major wild rice distribution regions of Pingnan, Yongning, and Fusui are exactly on a line in the Xijiang River basin. Pingnan is located at the lower reaches, Yongning at the middle reaches, and Fusui at the upper reaches of the Xijiang River. The distribution of BPH-resistant gene set in common wild rice was the highest in Pingnan, followed by Yongning, and the lowest in Fusui, indicating that there was a significant correlation between the distribution frequency of resistance and the regions (Table 2, Figure 5).

The results of the study on the imbalance of the frequency of regional distribution of BPH-resistant resources have important guiding significance for future exploitation, indicating that the discovery of BPH-resistant germplasm should focus on sampling from the wild rice distribution regions in the lower reaches of the Xijiang River which had the highest number of BPH resistant accessions.

TABLE 3 Regional differences in genetic diversity of common wild rice (*Oryza rufipogon*)

Region	N_a	N_e	H_o	H_e	Nei	I	PIC
Pingnan	3.167	2.616	0.333	0.602	0.456	0.997	0.516
Yongning	3.167	2.703	0.319	0.615	0.600	1.020	0.527
Fusui	3.048	2.467	0.320	0.573	0.547	0.928	0.480

Note. N_a, actual number of alleles; N_e, expected number of alleles; H_o, actual observation heterozygosity; H_e, expected observed heterozygosity; Nei, diversity index; I, Shannon's Information index; PIC, polymorphic information content.

3.3 | Genetic diversity of BPH-resistant accessions

The 58 BPH-resistant accessions were genotyped with 42 SSR polymorphic markers (Supplemental Table S3). Across the markers, the mean number of alleles (N_a) was 3.170, ranging from 2 to 5; mean expected number of alleles (N_e) was 2.780, ranging from 1.420 (RM525) to 4.640 (RM257); actual observation heterozygosity (H_o) was 0.326, ranging from 0.069 (RM489) to 0.690 (RM402); the expected observed heterozygosity (H_e) was 0.614, ranging from 0.299 (RM525) to 0.792 (RM257); and the polymorphic information content (PIC) was 0.536, ranging from 0.253 (RM525) to 0.750 (RM257). The diversity index (Nei) was 0.608, ranging from 0.297 (RM525) to 0.785 (RM257), indicating the 58 accessions are genetically divergent from each other. The Shannon's Information index (I) was 1.035, ranging from 0.473 (RM525) to 1.572 (RM257), suggesting a high degree of genetic polymorphism among these resistant accessions. These analyses indicate that the 58 resistant accessions have rich genetic diversity, which should be protected and utilized as a source of BPH resistance. The high genetic diversity parameters detected for RM286 (chromosome 11; 0.38 Mb), RM257 (chromosome 9; 17.72 Mb) and RM276 (chromosome 6; 6.23 Mb) suggest that these genomic regions are regions of high-frequency variation in the common wild rice in Guangxi (Supplemental Table S3).

Evaluation of the genetic diversity across the three regions (Table 3) using the same parameters (N_a, N_e, H_o, H_e, Nei, I, and PIC) revealed the highest diversity index was in Yongning County, followed by Pingnan County. The PIC value of these two regions was >0.5, indicating that the BPH-resistant accessions were rich in genetic diversity. Fusui County had the lowest genetic diversity index, with a PIC value of <0.5. However, the number of BPH-resistant accessions was relatively small (only 11 accessions), but the PIC value was still 0.48, which was close to 0.5. Therefore, the genetic diversity of BPH resistant accessions in Fusui County was also high.

3.4 | Cluster analysis of BPH resistant accessions

Based on the abovementioned genotyping with 42 SSR markers and Nei's genetic distance, unweighted pair-group method with arithmetic means (UPGMA) cluster analysis was conducted (Tamura et al., 2007), which divided the 58 BPH-resistant accessions into four clusters (Figure 6). "Cluster 1" had the most genetic diversity, with 15 BPH resistant accessions, including GXU (Guangxi University) 1329, 1305, 1323, 1324, 1136, 1096, 304, 306, 436, 895, 920, 1157,

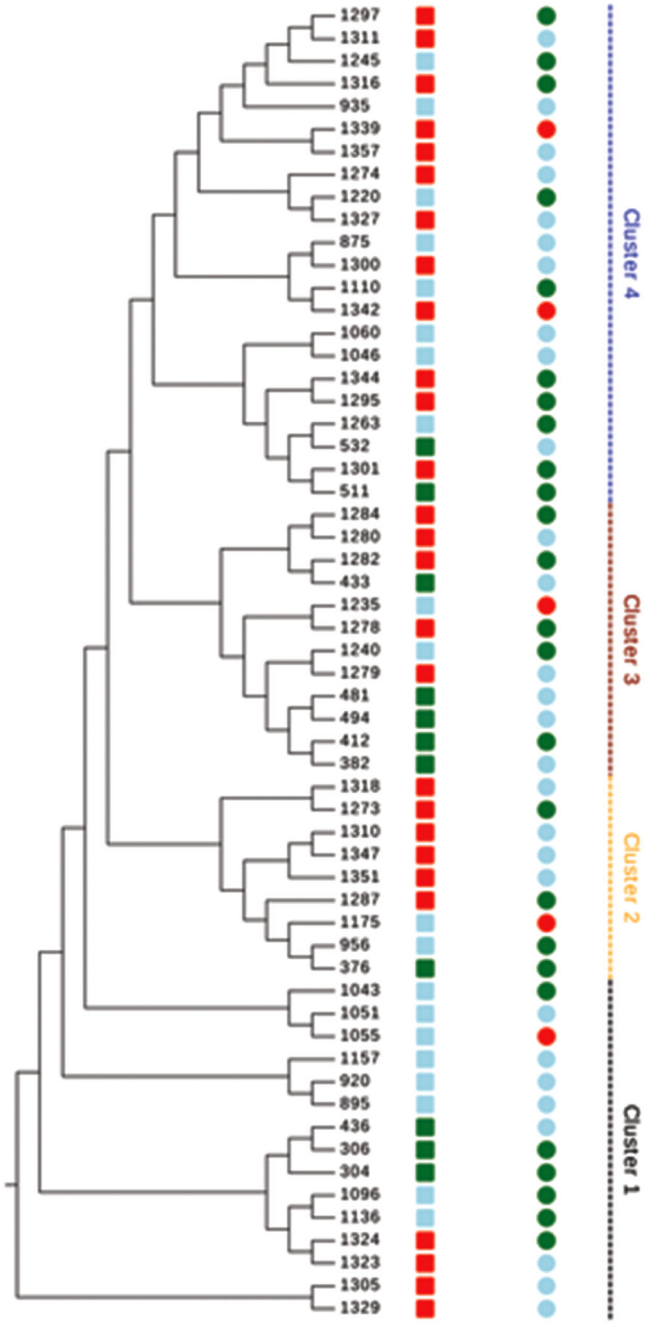

FIGURE 6 Unweighted pair-group method with arithmetic means (UPGMA) cluster analysis of 58 brown planthopper (BPH)-resistant accessions from common wild rice (*O. rufipogon*). The numbers for the 58 accessions are GXU (Guangxi University) accession numbers of common wild rice of Guangxi University. Red squares = Pingnan; blue squares = Yongning; green squares = Fusui. Red circles = scale 1 (highly resistant); blue circles = scale 3 (resistant); and green circles = scale 5 (moderately resistant)

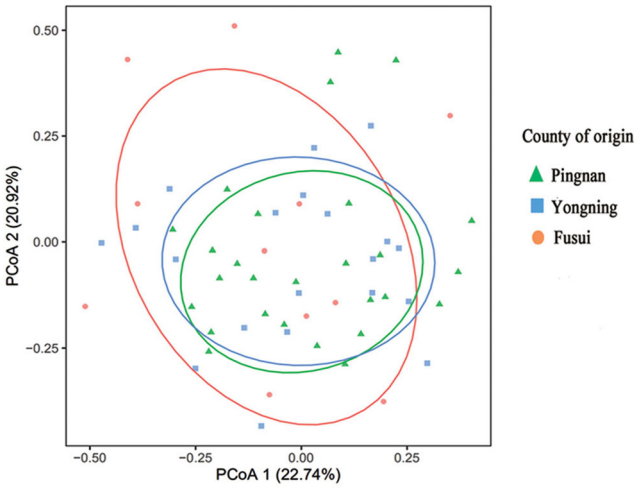

FIGURE 7 Principal coordinate analysis (PCoA) of 58 brown planthopper (BPH)-resistant accessions of common wild rice (*Oryza rufipogon*)

1055, 1051, and 1043. Clusters 2, 3, and 4 contained 9, 12, and 22 resistant accessions, respectively. In all four clusters, the accessions from the three regions were intermingled and did not form independent clusters representing the three regions but were both intersecting and independent clusters. The results also showed that accessions at different resistance levels were distributed across the four clusters and appeared to cross with each other (Figure 6).

3.5 | PCoA analysis of BPH-resistant accessions

To investigate the genetic relationships between the 58 resistant accessions from the three regions, a PCoA analysis was conducted. The scatter plots of the first and second principal components showed the same genetic pattern of an intermingling of these accessions from the three regions, indicating that their genetic distance was large and the level of genetic diversity was high. These results indicate that the individual BPH-resistant accessions in each region did not tend to cluster together and crossed each other without significant regional differentiation (Figure 7).

3.6 | Population structure analysis of BPH resistant accessions

Based on the Structure 2.3.4 model (Pritchard et al., 2000), the population number (K) was set to 1–10, and the population genetic structure of the tested accessions were analyzed three times. Figure 8a shows the log-likelihood ln$P(D)$ increased as the K value increased, and there was no obvious maximum inflection point. Therefore, it was necessary to use the ln$P(D)$ value to calculate the ΔK value, and based on this analysis (Figure 8b), when $K = 2$, the maximum inflection point appeared. Thus, it appears the 58 accessions divide into two stable subpopulations, based on the K value as shown in Figure 8c. Observing the geographic origin and the subpopulation of the accession again showed intermingling of the accessions and no clear differentiation by geographical origin. The genetic relationship between some of the accessions from different geographical regions was relatively close, indicating that the population structure had become diversified and the genetic basis was broad. These results imply that conservation efforts should focus on protecting and utilizing this set of BPH-resistant accessions to identify novel BPH resistance genes.

4 | DISCUSSION

Currently, SSST (IRRI, 1996) is the primary method used to screen BPH resistance (Li et al., 2001; Sarao et al., 2016). This method requires bulked seed to be sown in a box to produce a large number of plants, which then requires a large population of insects. Also, SSST is hindered by the excessive BPH infestation required before the plants are rated, which takes about 2 or 3 wk, and the ratings are often inaccurate; thus, SSST is not ideal for large-scale screening to identify resistant germplasm. As an alternative, the SEM is fast, convenient, and accurate and can be used in large-scale screenings for BPH resistance. A single rice accession is represented by a single tiller (stem) taken from a single plant, which is easy to collect and transplant to a tray. Since there is one tiller from each accession in a single tray (or infestation pool), significantly fewer insects are needed to screen a large number of accessions. Also, the SEM can be used to evaluate BPH resistance not only at the vegetative stage but also at the reproductive stage, which is not possible with the SSST. In summary, the SEM has the following advantages: (a) the same plants can be evaluated repeatedly because tillers can be taken from about the 20th day after transplanting, which is the tillering stage in cultivated rice (*O. sativa*), until about 10 mo later for wild rice (*O. rufipogon*) thus improving the reliability and accuracy of the ratings; (b) it is easier to synchronize the development periods of the rice plants and BPH insects for better infestations and ratings; (c) there is less stem biomass as compared with the whole plant, and thus the wilting period of susceptible rice cultivars is shortened, as well as the evaluation period; and (d) at the adult stage, synchronization of the evaluation period and BPH insect development cycle results in excessive BPH infestation and more accurate ratings.

For the SEM, the process of evaluating a negative control may be important for a case study on BPH resistance; however, because insects can move from plant to plant, it is

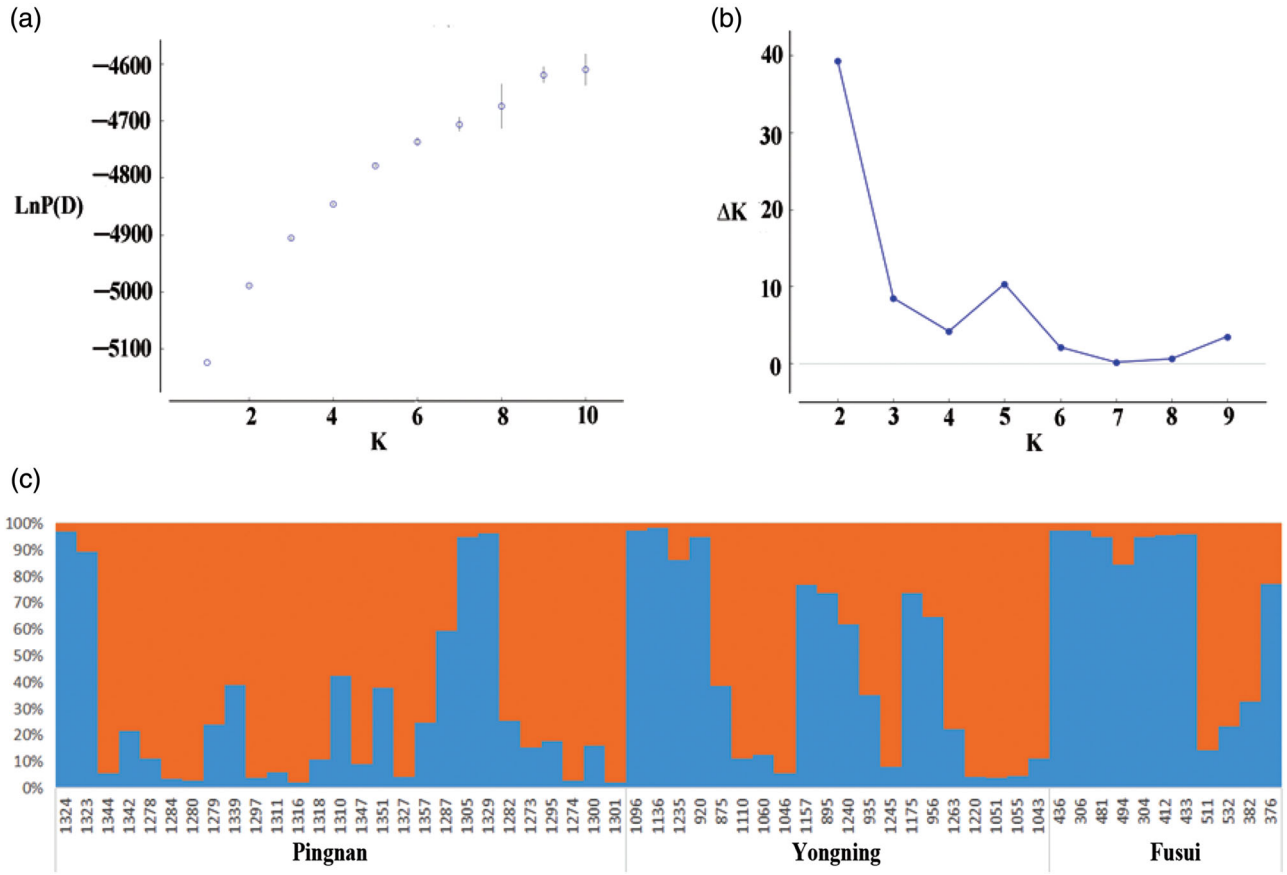

FIGURE 8 Population structure analysis of 58 brown planthopper (BPH)-resistant accessions of common wild rice (*O. rufipogon*): (a) *k* value analysis of the BPH resistant accessions; (b) ΔK value analysis of the BPH-resistant accessions; and (c) population structure chart of the BPH-resistant accessions based on $\Delta K = 2$ and arranged by county of origin. Blue squares = Subpopulation 1; orange squares = Subpopulation 2

difficult to include a negative control. A moderate resistant or resistant control would be better to include as a comparison. Even though our screening did not include a resistant control, the results are accurate because the resistant accessions were identified under high BPH pressure with an overabundance of insects.

The limited number of BPH-resistant rice cultivars available is one of the main reasons for the annual BPH epidemic. Despite both public and private breeding efforts, the lack of BPH-resistant germplasm has hampered the development of BPH-resistant cultivars. From 2016 to 2018, of the 513 rice cultivars approved by the Ministry of Agriculture and Rural Affairs of China, only four were BPH-resistant hybrid rice cultivars, including one three-line hybrid rice, Gyou 429, and three two-line hybrids, Aolongyou 282, Deliangyouhuazhan, and Guangliangyou 990, and their resistance was only classified as moderately resistant (National Rice Data Center, China Rice Variety and Pedigree Database, http://www.ricedata.cn/variety/). To address this unfavorable situation, the discovery of BPH-resistant accessions with novel BPH resistance genes is necessary so that breeding programs can develop improved BPH-resistant cultivars.

At present, the BPH-resistant genes [*bph18(t)* (currently name *Bph27*), *bph19(t)*, *bph20(t)* (currently name *bph29*),

TABLE 4 Comparison of genetic diversity index of common wild rice

Study	No. accessions	No. SSR markers	N_a	H_o	H_e	*I*	Nei
Current study	58	42	3.170	0.326	0.614	1.035	0.608
Xue et al. (2016)	623	24	4.750	0.175	0.638	1.180	0.638
Pan et al. (2018)	4,173	46	18.44	0.509	0.719	1.737	0.719
Li et al. (2010)	90	24	2.180	0.606	0.503	0.880	0.503

Note. SSR, simple sequence repeat; N_a, actual number of alleles; N_e, expected number of alleles; H_o, actual observation heterozygosity; H_e, expected observed heterozygosity; Nei, diversity index; *I*, Shannon's Information index; PIC, polymorphic information content.

bph21(t), and *Bph24(t)*] obtained from Guangxi common wild rice have been successively pyramided into elite cultivars to develop cultivars with BPH resistance (Zhao et al., 2013), as summarized by Chen et al (2009) and Liu et al. (2011). Specifically, Zhao et al. (2013) pyramided *bph20(t)* and *bph21(t)* into the elite maintainer line BoIIIB and subsequently developed five additional resistant lines. New double-resistance maintainer and sterile lines were bred by transferring *bph18(t)*, *bph19(t)*, *bph20(t)*, *bph21(t)*, and *Bph24(t)* into these hybrid rice parents (Chen et al., 2010). Lastly, Liu et al. (2011) introduced *Bph24(t)* and *Bph3* from *O. sativa* into five hybrid rice restorer lines, resulting in 13 *Bph3Bph24(t)* elite lines. Of the *Bph* genes from common wild rice, *Bph24(t)* has been used the most in cultivar development. Most recently, rice strain 209B was crossed with Guangxi *O. rufipogon* accession RBPH96 carrying *Bph24(t)*, and the offspring were screened using the SEM to identify the maintainer line 18B with high BPH resistance, which was then transferred into CMS line 18A. Three new hybrid rice cultivars (18A/R223, 18A/R217, and 18A/F028) obtained from crosses with CMS line 18A are currently being evaluated in regional trials.

A total of nine BPH resistance genes were mined from 30 Guangxi wild rice accessions collected 14 yr ago (Li et al., 2006). In this study, 1,221 accessions of Guangxi common wild rice were screened for BPH resistance, and 33 highly resistant (HR) and resistant (R) BPH-resistant accessions were identified (Supplemental Table S2). Because the biotypes of BPH evolve gradually, it is particularly important to identify additional resistance accessions that may contain novel resistance genes. The discovery of these additional resistant accessions is a powerful supplement to the currently available BPH resistant germplasm and potentially provides accessions for further exploration of BPH resistant genes, development of additional resistant cultivars through breeding, and germplasm for conducting the related theoretical research on BPH resistance.

Genetic diversity is necessary for crop improvement through breeding and cultivar development programs (Swarup et al., 2020). Utilization of crop wild relatives is one important aspect of increasing the available genetic diversity. In China, one fundamental question still being argued is the geographic origin of *O. sativa*. The germplasm of both O. sativa and its wild ancestor, *O. rufipogon* are abundant with their extremely wide distribution in China, over centuries of domestication and evolution. *Oryza sativa* is cultivated in >20 provinces, whereas *O. rufipogon* grows in seven provinces: Fujian, Hunan, Jiangxi, Yunnan, Guangdong, Guangxi, and Hainan, with a higher concentration in the last three provinces (Wang et al., 2020). According to the previous studies, Guangxi Province and the lower region of Yangtze River was proposed as the center of origin of *O. sativa* based on evidence from the distribution of *O. rufipogon*, the rich germplasm of *O. sativa* and the discovery of rice phytoliths (Fuller et al., 2009). Guangxi is an important distribution region of common wild rice in China and is considered the center of evolution and origin of cultivated rice. After *O. rufipogon* around the middle area of the Pearl River in southern China (Guangxi Province) was domesticated into *japonica* rice, it was spread to Southeast and South Asia. Subsequently, *indica* rice was bred by crossing between *japonica* rice and local wild rice (Huang et al., 2012; Wei et al., 2012). Kim et al (2016) used genotyping-by-sequencing and Sanger sequencing to analyze the nuclear variation of 286 *O. rufipogon* Species Complex (ORSC). The results showed that ORSC samples from Guangxi and Guangdong in southern China are related to both *japonica* rice and *indica* rice and supports rice domestication in this region.

Sun and Yang (2009) used 24 SSR markers to compare the genetic diversity among 282 common wild rice plants representing 15 populations including China (10 populations), Laos (1), Vietnam (2), and Cambodia (2). The results showed that the genetic diversity of common wild rice is richest in China, followed by Laos and the lowest in Vietnam and Cambodia, and that the common wild rice from Wuxuan and Hezhou Counties in Guangxi Province is closely related to the three Southeast Asian countries. Thus, the utilization value of common wild rice in Guangxi is high, and this confirms the importance of the accessions evaluated in this study, which were collected from representative regions in Fusui, Yongning, and Pingnan Counties with dense distributions of common wild rice habitats.

In recent years, many studies have been conducted to determine the genetic diversity of common wild rice in Guangxi and its surrounding provinces. In Table 4, we compare the diversity indices (N_a, H_o, H_e, I, and Nei) reported in this study with previous studies of more accessions. These studies include Xue et al. (2016), who used 24 SSR markers to analyze the genetic diversity of 623 Guangxi common wild rice accessions; Pan et al. (2018), who used 46 SSR markers to analyze the genetic diversity and population structure of 4,173 Guangxi common wild rice accessions; and Li et al. (2010), who used 24 SSR markers to analyze the genetic diversity of 90 common rice accessions from Guangdong, which borders Guangxi on the west. These results confirm that common wild rice from this region is rich in genetic diversity and that the 42 SSR markers used to analyze the genetic diversity of 58 BPH-resistant accessions in this study are similar to previous reports. Thus, even though only 58 accessions were genotyped, based on the genetic diversity analyses, these resistant accessions represent the rich genetic diversity of Guangxi common wild rice. Based on this diversity, these accessions are likely to harbor novel BPH resistance genes, which could be used to develop rice cultivars with improved BPH resistance, further documenting the potential importance and value of Guangxi common wild rice.

5 | CONCLUSIONS

The SEM is a convenient, highly efficient, and accurate method to screen for BPH-resistant rice germplasm. This method is especially useful when seeds are limited or not available, when screening needs to be done immediately, or when evaluating plants at the reproductive (adult) stage because fewer insects are needed due to tillers (stems) being screened and not entire plants. This method can be used to evaluate a collection of rice accessions as demonstrated in this study, screen breeding lines as part of a cultivar development program, or access the resistance of the individual lines composing a segregating mapping population BPH gene identification. Future studies will focus on determining if there are novel BPH resistance genes in the 33 resistance accessions identified in this study. If novel genes are identified, marker-assisted selection methods will be used to incorporate these resistance genes into elite rice cultivars.

ACKNOWLEDGMENTS

We would like to thank Gul Nawaz, Neng Zhao, Mohsin Niaz, Mudassar Abbas, and Umair Khalid for the helpful discussion and invaluable comments to make this research meaningful.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING

This study was funded by Guangxi Innovation-Driven Development Special Project (Guike AA17204070), Guangxi Key R & D Project (Guike AB16380093), and the National Natural Science Foundation of China (31560423).

REFERENCES

Barbier, P., Morishima, H., & Ishihama, A. (1991). Phylogenetic relationships of annual and perennial wild rice: Probing by direct DNA sequencing. *Theoretical and Applied Genetics*, *81*, 693–702. https://doi.org/10.1007/BF00226739

Brar, D. S., & Khush, G. S. (2018). Wild relatives of rice: A valuable genetic resource for genomics and breeding research. In *The wild oryza genomes* (pp. 1–25). Springer. https://doi.org/10.1007/978-3-319-71997-9_1

Cha, Y. S., Ji, H., Yun, D. W., Ahn, B. O., Lee, M. C., Suh, S. C.,… Eun M.-Y. (2008). Fine mapping of the rice Bph1 gene, which confers resistance to the brown planthopper (*Nilaparvata lugens* Stål), and development of STS markers for marker-assisted selection. *Molecules and Cells*, *26*, 146–151. https://doi.org/10.0000/PMID18612237

Chen, C., Guo, H., Zhang, X. L., Liu, B. L., Qin, X. Y., & Feng, R. (2017). Blast resistance identification for wild rice from Guangxi and geographical distribution of resistant resources. (In Chinese.) *Journal of Southern Agriculture*, *48*, 1999–2003. https://doi.org/10.3969/j.issn.2095-1191.2017.11.12

Chen, D. H., & Ronald, P. C. (1999). A rapid DNA minipreparation method suitable for AFLP and other PCR applications. *Plant Molecular Biology Reporter*, *17*, 53–57. https://doi.org/10.1023/A:1007585532036

Chen, Y. Z., Wei, Y. P., Li, X. Q., Chen, Q., Sun, R. K., Yang, L., … Li, R. B. (2009). Evaluation and utilization of resistant sources to brown planthopper in rice. (In Chinese.) *Southwest China Journal of Agricultural Science*, *22*, 1581–1588. https://doi.org/10.16213/j.cnki.scjas.2009.06.008

Chen, Y. Z., Chen, Q., Yang, L., Huang, F. K., Huang, D. H., Wei, S. M., … Li, R. B. (2010). Improvement of rice resistance to brown planthoppers. (In Chinese.) *Southwest China Journal of Agricultural Science*, *23*, 1099–1106. https://doi.org/10.16213/j.cnki.scjas.2010.04.012

Fuller, D. Q., Qin, L., Zheng, Y., Zhao, Z., Chen, X., Hosoya, L. A., & Sun, G. P. (2009). The domestication process and domestication rate in rice: Spikelet bases from the lower Yangtze. *Science*, *323*, 1607–1610. https://doi.org/10.1126/science.1166605

Fuller, D. Q., Sato, Y. I., Castillo, C., Qin, L., Weisskopf, A. R., Kingwell-Banham, E. J., … van Etten J. (2010). Consilience of genetics and archaeobotany in the entangled history of rice. *Archaeological and Anthropological Sciences*, *2*, 115–131. https://doi.org/10.1007/s12520-010-0035-y

Guo, H., Chen, C., Zhang, X. L., Qin, X. Y., & Feng, R. (2017). Study on cold tolerance identification and genetic homozygosity of wild rice in Guangxi. (In Chinese.) *Southwest China Journal of Agricultural Science*, *30*, 1245–1250. https://doi.org/10.16213/j.cnki.scjas.2017.6.001

Hu, W., Xiao, H. X., Kan Hu, H., Jiang, Y. J., & Zhang, Y. (2016). Application of marker-assisted backcross to introgress *Bph3*, *Bph14* and *Bph15* into an elite *indica* rice variety for improving its resistance to brown planthopper. *Plant Breeding*, *135*, 291–300. https://doi.org/10.1111/pbr.12364

Huang, D., Qiu, Y., Zhang, Y., Huang, F., Meng, J., Wei, S., Li R., & Chen B. (2013). Fine mapping and characterization ofBPH27, a brown planthopper resistance gene from wild rice (*Oryza rufipogon* Griff.). *Theoretical and Applied Genetics*, *126*, 219–229. https://doi.org/10.1007/s00122-012-1975-7

Huang, X., Kurata, N., Wei, X., Wang, Z.-X., Wang, A., Zhao, Q., Zhao, Y., Liu, K., Lu, H., Li, W., Guo, Y., Lu, Y., Zhou, C., Fan, D., Weng, Q., Zhu, C., Huang, T., Zhang, L., Wang, Y., … Han, B. (2012). A map of rice genome variation reveals the origin of cultivated rice. *Nature*, *490*, 497–501. https://doi.org/10.1038/nature11532

IRRI. (1996). *Standard evaluation system for rice*. Manila: International Rice Research Institute.

Jackson, M. T. (1997). Conservation of rice genetic resources:The role of the International Rice Genebank at IRRI. *Plant Molecular Biology*, *35*, 61–67.

Khush, G. S. (1997). Origin, dispersal, cultivation and variation of rice. *Plant Molecular Biology*, *35*, 25–34. https://doi.org/10.1023/A:1005810616885

Khush, G. (2001). Green revolution: The way forward. *Nature Reviews Genetics*, *2*, 815–822. https://doi.org/10.1038/35093585

Kim, H. J., Jung, J., Singh, N., Greenberg, A., Doyle, J. J., Tyagi, W., Chung J.-W., Kimball J., Hamilton R. S., & McCouch S. R. (2016). Population dynamics among six major groups of the oryza rufipogon species complex, wild relative of cultivated asian rice. *Rice*, *9*, 1–15. https://doi.org/10.1186/s12284-016-0119-0

Kumar, K., Sarao, P. S., Bhatia, D., Neelam, K., Kaur, A., Mangat, G. S., & Singh, K. (2018). High-resolution genetic mapping of a novel brown planthopper resistance locus, *Bph34* in *Oryza sativa* L. X *Oryza nivara* (Sharma & Shastry) derived interspecific F_2 population. *Theoretical Applied Genetics*, *131*, 1163–1171. https://doi.org/10.1007/s00122-018-3069-7

Li, C., Luo, C., Zhou, Z., Wang, R., Ling, F., Xiao, L., & Chen, H. (2017). Gene expression and plant hormone levels in two contrasting rice genotypes responding to brown planthopper infestation. *BMC Plant Biology*, *17*, 57. https://doi.org/10.1186/s12870-017-1005-7

Li, M. B., Wang, H., & Cao, L. M. (2015). Evaluation of population structure, genetic diversity and origin of northeast asia weedy rice based on simple sequence repeat markers. *Rice Science*, *22*, 180–188. https://doi.org/10.1016/j.rsci.2015.02.001

Li, R. B., Li, L. S., Wei, S. M., Wei, Y. P., Chen, Y. Z., Bai, D. L., … Wei Y. (2006). The evaluation and utilization of new genes for brown planthopper resistance in common wild rice (*Oryza rufipogon* Griff.). *Molecular Plant Breeding*, *4*, 365–371. https://doi.org/10.5376/me.2010.01.0001

Li, R. B., Qin, X. Y., Wei, S. M., Pandey, M. P., Pathak, P. K., Huang, F. H., … Luo, S. Y. (2001). Inheritance of resistance to brown planthopper in an *Oryza rufipogon* (Griff.)-derived line in rice. *Current Science*, *80*,1421–1423.

Li, X. X., Liu, Y., Duan, Y. H., Wang, S. H., Zhang, Q. C., Sun, G. H., & Gao, L. Z. (2010). Estimation of mating system in natural *Oryza rufipogon* popolation by SSR markers. (In Chinese.) *Chinese Journal of Rice Science*, *24*, 601–607. https://doi.org/10.3969/j.issn.1001-7216.2010.06.007

Li, Z. H., Xue, Y. X., Zhou, H. L., Li, Y., Usman, B., Jiao, X. Z., Wang X., Liu F., Qin B., Li R., & Qiu Y. (2019). High-resolution mapping and breeding application of a novel brown planthopper resistance gene derived from wild rice (*Oryza. rufipogon* Griff). *Rice*, *12*(41), 1–13. https://doi.org/10.1186/s12284-019-0289-7

Liu, K. Y., Lu, S. N., Qiu, J. L., Li, X. Q., Ma, Z. F., Chen, Q., … Li, R. B. (2011). Development of brown planthopper resistance gene-inserted and accumulated lines of rice restorers. (In Chinese.) *Molecular Plant Breeding*, *9*, 410–417. https://doi.org/10.3969/mpb.009.000410

Liu, K. Y., Zhang, Y. X., Liu, F., Qiu, Y. F., Feng, J. X., Huang, F. K., … Li, R. B. (2013). Resistant performance of bacterial blight and brown planthopper resistance gene-pyramided lines in rice. *Southwest China Journal of Agricultural Science*, *26*, 1852–1857. https://doi.org/10.16213/j.cnki.scjas.2013.05.041

Liu, Y., Wu, H., Chen, H., Liu, Y., He, J., Kang, H., … Wan, J. (2014). A gene cluster encoding lectin receptor kinases confers broad-spectrum and durable insect resistance in rice. *Nature Biotechnology*, *33*, 301–305. https://doi.org/10.1038/nbt.3069

McClung, A. M., Edwards, J. D., Jia, M. H., Huggins, T. D., Bockelman, H. E., Ali, M. L., & Eizenga, G. C. (2020). Enhancing the searchability, breeding utility, and efficient management of germplasm accessions in the USDA-ARS rice collection. *Crop Science*, *60*, 3191–3211. https://doi.org/10.1002/csc220468.20256

Nagy, S., Poczai, P., Cernák, I., Gorji, A. M., Hegedűs, G., & Taller, J. (2012). PICcalc: An online program to calculate polymorphic information content for molecular genetic studies. *Biochemical Genetics*, *50*, 9–10. https://doi.org/10.1007/s10528-012-9509-1

Normile, D. (2008). Agricultural research. Reinventing rice to feed the world. *Science*, *321*(5887), 330–333. https://doi.org/10.1126/science.321.5887.330

Oka, H. I. (1988). *Origin of cultivated rice*. Japan Scientific Societies Press.

O'Neill, R., Snowdon, R., & Kohler, W. (2003). Population genetics: Aspects of biodiversity. *Progress in Botany*, *64*, 115–137. https://doi.org/10.1007/978-3-642-55819-1_7

Pan, Y. H., Xu, Z. J., & Liang, Y. T. (2018). Genetic structure and core collection of common wild rice (*Oryza rufipogon* Griff.) in Guangxi. *Journal of Plant Genetic Resources*, *19*, 498–509. https://doi.org/10.13430/j.cnki.jpgr.2018.03.015

Peng, L., Zhao, Y., Wang, H., Zhang, J., Song, C., Shangguan, X., & He, G. .(2016). Comparative metabolomics of the interaction between rice and the brown planthopper. *Metabolomics*, *1*, 28746–28764. https://doi.org/10.1007/s11306-016-1077-7

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*, 945–959.

Qin, B. X., Liu, C., & Jiao, X. Z. (2014). Identification and evaluation of Guangxi wild rice (*Oryza rufipogon* Griff.) resources with wide spectrum resistance to bacterial blight. *Journal of Southern Agriculture*, *45*, 1527–1531.

Qin, X. Y., Zhu, R. C., Wei, S. M., Wu, B., Huang, F. K., Li, D. Y., & Tang, J. H. (2004). Technique for utilization and identification of resistance to brown planthopper in*Oryza officinalis*. (In Chinese.) *Chinese Journal of Rice Science*, *18*, 573–576. https://doi.org/10.16819/j.1001-7216.2004.06.019

Ramkumar, G., Prahalada, G. D., Hechanova, S. L., Kim, S. R., & Jena, K. K. (2016). Exploring genetic diversity of rice cultivars for the presence of brown planthopper (BPH) resistance genes and development of SNP marker for *Bph18*. *Plant Breeding*, *135*, 301–308. https://doi.org/10.1111/pbr.12365

Sangha, J. S., Yolanda, H. C. , Kaur, J. , Khan, W. , Abduljaleel, Z., Alanazi, M. S., & Leung, H. (2013). Proteome analysis of rice (*Oryza sativa* L.) mutants reveals differentially induced proteins during brown planthopper (*Nilaparvata lugens*) infestation. *International Journal of Molecular Sciences*, *14*, 3921–3945. https://doi.org/10.3390/ijms14023921

Sanguinetti, C. J., Dias, N. E., & Simpson, A. J. (1994). Rapid silver staining and recovery of PCR products separated on polyacrylamide gels. *Biotech*, *17*, 915–919.

Sarao, P. S., Sahi, G. K., Neelam, K., Mangat, G. S., Patra, B. C., & Singh, K. (2016). Donors for resistance to brown planthopper *Nilaparvata lugens* (Stål) from wild rice species. *Rice Science*, *23*, 219–224. https://doi.org/10.1016/j.rsci.2016.06.005

Sogawa, K. (1982). The rice brown planthopper: Feeding physiology and host plant interactions. *Annual Review of Entomology*, *27*, 49–73. https://doi.org/10.1146/annurev.en.27.010182.000405Sogawa

Sun, X. P., & Yang, Q. W. (2009). Comparative study on genetic diversity of wild rice (*Oryza rufipogon* Griff.) in China and three countries in southeast Asia. *Acta Agronomica Sinica*, *35*, 679–684. https://doi.org/10.3724/SP.J.1006.2009.00679

Swarup, S., Cargill, E. J., Crosby, K., Flagel, L., Kniskern, J., & Glenn, K. C. (2020). Genetic diversity is indispensable for plant breeding to improve crops. *Crop Science*. https://doi.org/10.1002/csc220468.20377 (in press).

Tamura, K., Dudley, J., Nei, M., & Kumar, S. (2007). MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*, *24*, 1596–1599. https://doi.org/10.1093/molbev/msm092

Tanaka, K., Endo, S., & Kazano, H. (2000). Toxicity of insecticides to predators of rice planthoppers: Spiders, the mirid bug and the dryinid wasp. *Appled Entomology and Zoology*, *35*, 177–187. https://doi.org/10.1303/aez.2000.177

Tang, S. X., Wei, X. H., & Xu, Q. (2008). Progress of evaluation and utilization of wild rice resources abroad. *Journal of Plant Genetic Resources*, *9*, 223–229. https://doi.org/10.3724/SP.J.1005.2008.01083

Wang, J., Shi, J., Liu, S., Sun, X., Huang, J., Qiao, W., … Yang, Q. (2020). Conservation recommendations for *Oryza rufipogon* Griff. in China based on genetic diversity analysis. *Scientific Reports*, *10*, 14375. https://doi.org/10.1038/s41598-020-70989-w

Way, M. J., & Heong, K. L. (1994). The role of biodiversity in the dynamics and management of insect pests of tropical irrigated rice—A review. *Bulletin of Entomological Research*, *84*, 567–587. https://doi.org/10.1017/s000748530003282x

Wei, X., Qiao, W. H., Chen, Y. T., Wang, R. S., Cao, L. R., Zhang, W. X., … Yang, Q. W. (2012). Domestication and geographic origin of *Oryza sativa* in China: Insights from multilocus analysis of nucleotide variation of *O. sativa* and *O. rufipogon*. *Molecular Ecology*, *21*, 5073–5087. https://doi.org/10.1111/j.1365-294X.2012.05748.x

Xue, Y. X., Liang, Y. L., Feng, X., Huang, J. Y., Liu, F., Qin, B. X., … Li, R. B. (2016). Center of genetic diversity and core collection of common wild rice, *Oryza rufipogon* Griff., in Guangxi. *Journal of South China Agricultural University*, *37*, 24–30. https://doi.org/10.7671/j.Issn.1001-411X.2016.05.005

Yang, L., Li, R. B., Li, Y. R., Huang, F. K., Chen, Y. Z. … Jiang J. J. (2012). Genetic mapping of *bph20(t)* and *bph21(t)* loci conferring brown planthopper resistance to*Nilaparvata lugens* Stål in rice (*Oryza sativa* L.). *Euphytica*, *183*, 161–171. https://doi.org/10.1007/s10681-011-0437-7

Yeh, F. C., & Yang, R. C. (1999). *POPGENE Version 1.31 Microsoft Window-based freeware for Population Genetic Analysis quick user guide*. University of Alberta.

Zhang, Y. X., Qin, G., Ma, Q. Q., Wei, M. Y., Yang, X. H., … Li, R. (2019). Identification of a major locus *Bph35 r*esisting to brown planthopper in rice (*Oryza sativa* L.). *Rice Science*, *27*, 237–245. https://doi.org/10.1016/j.rsci.2020.04.006

Zhao, P., Feng, R. R., Xiao, Q. Z., Yang, P. Z., Liu, P. Q., & Li, R. B. (2013). Pyramiding brown planthopper genes,*bph20(t)*and*bph21(t)*,and rice blast resistant gene *Pi9* in rice (*Oryza sativa* L.). *Journal of Southern Agriculture*, *44*, 885–892.

Zhou, T., Du, L., Wang, L., Wang, Y., Gao, C., … Zhou, Y. (2015). Genetic analysis and molecular mapping of QTLs for resistance to rice black-streaked dwarf disease in rice. *Scientific Reports*, *5*, 10509. https://doi.org/10.1038/srep10509

Zhu, Y., Xu, A. L., Ji, L., He, W. A., Li, R. B., & Qiu, Y. F. (2016). Identification of common wild rice's resistance to white-backed planthopper (*Sogatella furcifera*) in Guangxi. *Journal of Southern Agriculture*, *47*, 1693–1697.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Luo L, Wang X, Guo X, et al. Evaluation of Guangxi common wild rice for resistance to brown planthopper using a new stem evaluation method. *Crop Science*. 2021;1–14. https://doi.org/10.1002/csc2.20468