INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
17F–025/2017/19–4–880–886
DOI: 10.17957/IJAB/15.0384
http://www.fspublishers.org

Full Length Article

Analysis of the Resistance to Rice Blast and False Smut of 18 Varieties of Hybrid Rice in Sichuan Province, China

Yan Li[1†], Jun Shi[2†], Zufen Xiang[2], Rongping Hu[3], Shoupei Shi[2], Tao Peng[2], Dingyou Liu[2], Tingyou Huang[2*]
[1]*Ecological Security and Protection Key Laboratory of Sichuan Province/Mianyang Normal University, Mianyang, Sichuan 621000, China*
[2]*Mianyang Academy of Agricultural Sciences, Mianyang, Sichuan 621023, China*
[3]*Institute of Plant Protection, Sichuan Academy of Agricultural Sciences/MOA Laboratory of Integrated Management of Pests on Crops in Southwest China, Chengdu, Sichuan 610066, China*
[*]For correspondence: htysca@126.com
[†]These authors contributed equally to this work

Abstract

Rice blast and false smut are severe diseases of rice reducing not only rice yield but also affect grain quality. The most effective and economical mean of controlling diseases is to breed disease-resistant rice cultivars. Here, we used 18 hybrid rice varieties for identifying their functional rice blast resistance genes, and for estimating their sensitivities to false smut disease. This experiment was conducted in Sichuan Province, China in 2012–2014. The results showed that the Pi2 and Pik loci have strong rice blast resistance. Rice varieties i.e. CNY445, CNYHZ, and YXY2115 expressed the *Pi2*, while CFY188 and LY908 expressed *Pikm.* Varieties R18/2348, CGY2348, GY66, YLY973 and N5Y828 demonstrated strong resistance to rice false smut. No correlation in the resistance between rice blast and false smut was observed among the 18 hybrid rice varieties. These results can provide guidance for rational distribution of hybrid rice varieties.

Keywords: Rice blast; Rice false smut; Hybrid rice; Resistance

Introduction

Rice blast, caused by the fungus *Magnaporthe oryzae,* is one of the most severe diseases of rice (Kush and Jena, 2009; Liu *et al*., 2010), and can cause considerable yield losses in rice production. Rice false smut has also become a serious disease of rice in recent years (Ladhalakshmi *et al*., 2012). The pathogen responsible for this disease is an ascomycete fungus, which possesses an anamorphic state named *Ustilaginoidea virens* (Cooke) Tak. (Uv) and a teleomorphic state called *Villosiclava virens* (Tanaka *et al*., 2008). False smut not only reduces the rice grain yield but also affects grain quality. Moreover, ustiloxins produced by the pathogen are harmful to both humans and animals (Koiso *et al*., 1994; Nakamura *et al*., 1994). Various sources responsible for the spread of this disease and their management using various fungicides have been identified (Cartwright *et al.,* 2004; PrasannaKumar *et al.,* 2011, 2012; Singh and Sunder, 2015).

To date, more than 100 rice blast resistant genes (R genes) have been mapped, and 23 R genes have been cloned (Fath *et al.,* 2014; Shi *et al*., 2015; Vasudevan *et al.,* 2015). However, rice false smut R genes have not yet been identified. Sichuan Province in China is a highly productive region for growing rice, with an area of ~2,000,000 ha used for rice production per year. It therefore plays an important role in China's food security. Because of the potentially serious effects of rice blast and false smut on rice production in Sichuan Province, it is important to identify resistance mechanisms for the two diseases.

Materials and Methods

Plant Materials

The rice blast susceptible *japonica* variety of rice, Lijiangxin Tuan Heigu (LTH), which is considered to carry no R genes (Shi *et al*., 2015), was used as a negative control for rice blast evaluation. PuJiang 6 (PJ6), a cultivar highly susceptible to rice false smut, was used as a negative control for rice false smut evaluation. We also evaluated the following 18 hybrid rice varieties: Rong18/2348 (R18/2348), ChuanGuYou2348 (CGY2348), QuanYou357 (QY357), FuYiYou188 (FYY188), ChengFengYou188 (CFY188),

To cite this paper: Li, Y., J. Shi, Z. Xiang, R. Hu, S. Shi, T. Peng, D. Liu, T. Huang, 2017. Analysis of the resistance to rice blast and false smut of 18 varieties of hybrid rice in sichuan province, China. *Int. J. Agric. Biol.*, 19: 880–886

ChuanGuYou7329 (CGY7329), LuXiangYou177 (LXY177), LuYou908 (LY908), ChuanNongYouHuaZhan (CNUHZ), HuaXiangYou1 (HXY1), GuangYou66 (GY66), YLiangYou973 (YLY973), HuaYou1199 (HY1199), RongYou1808 (RY1808), DeYou4727 (DY4727), ChuanNongYou445 (CNY445), Nei5You828 (N5Y828), and YiXiangYou2115 (YXY2115). LTH monogenic lines, each of these lines carries a single resistance gene developed in the genetic background of Japonica-type variety Lijiangxintuanheigu (LTH) (Table 1).

M. oryzae Pathogen Strains

M. oryzae strains used in this study were obtained using a single-spore isolation method from diseased rice plants in Sichuan Province, China (Gong *et al*., 2010).

Evaluation of Resistance to Rice False Smut and Rice Blast Diseases

Rice varieties for rice false smut field identification were planted in a nursery garden in Mianyang (E104.45, N31.10; northwest Sichuan Province, China) in 2015. The plants were sown in three stages on April 18 (stage I), April 25 (stage II), and May 2 (stage III). For each variety from each planting stage, 30 plants at three lines, with 25 cm of interline space and 15 cm of intraline space, were evaluated at yellow ripening stage for natural infection of rice false smut disease. The evaluation of disease scale and disease index of rice false smut was performed as previously described (Wang *et al*., 2013; Huang *et al*., 2016).

Rice blast resistance of LTH monogenic lines was evaluated in Ya'an (E103.0, N29.98; southwest Sichuan Province, China) in 2014, and was repeated in Pujiang (E103.29, N30.20; central Sichuan Province, China) in 2015. Both areas are commonly affected with rice blast. For each monogenic lines planted in Mianyang or Pujiang, 30 plants at three lines, with 25 cm of interline space and 15 cm of intraline space. Lesion type was scored on a scale of 1 (resistant) to 9 (susceptible) in accordance with the standard evaluation method of the IRRI (International Rice Research Institute, 1996).

For rice blast resistance identification under controlled conditions, three-week-old rice seedlings were prepared for spray inoculation in the greenhouse facilities at Mianyang Academy of Agricultural Sciences as previously described (Liu *et al*., 2002). Approximately 100 single-spore *M. oryzae* strains were used.

Gene Expression Analysis

Three-week-old seedlings were prepared for spray inoculation in the greenhouse facilities at Mianyang Academy of Agricultural Sciences. For the expression analysis of blast R genes, they were inoculated with mixed *M. oryzae* strains as previously described (Li *et al*., 2014). Inoculated leaves were harvested at 0, 12, 24 and 48 h post inoculation and were immediately frozen in liquid nitrogen. The remaining plants were kept for disease symptom observation for 7–10 days after inoculation.

Total RNA was isolated and cDNA was got from mix leaves of the 18 hybrid rice varieties 0, 12, 24 and 48 h after inoculation using ReverTra Ace qPCR RT Master Mix with gDNA Remover (Osaka, Japan) according to the manufacturer's instructions. cDNA which was used as a template for RT-PCR. Rice *OsActin* was used as the reference gene. RT-PCR conditions were as follows: 94°C for 3 min, followed by 30 cycles of 94°C for 20 s, 58°C for 20 s and 72°C for 20 s, with a final extension at 72°C for 5 min. PCR products were resolved on 1.5% agarose gels.

Sequencing of Pi2 and Pik Locus Genes

DNA was extracted from varieties leaves using the CTAB method (Doyle and Doyle, 1990). The full length of Pi2 and Pik loci genes were cloned and PCR products were directly sequenced in Sangon Biotech (Shanghai, China). The primers used in this study are shown in Supplementary Table 1.

Results

Identification of Rice Blast Resistance in the Field using LTH Monogenic Lines

After 2 years of investigating resistance to rice blast in LTH monogenic lines LTH was highly susceptible to rice blast, so it was used as a control, given a resistance frequency of 0 (Table 2).

The Pi2 locus includes the cloned alleles *Pi2*, *Pi9*, and *Pizt*, which showed resistance frequencies of 96.63%, 90.57% and 68.97%, respectively following artificial inoculation. Natural disease investigation in the field revealed average leaf blast rates of 0.58%, 0.60% and 56.48%, respectively, resistance levels of 2, 1, and 9, and disease indices of 0.10, 0.07 and 23.60, respectively. This suggests that *Pi2* and *Pi9* have maintained their strong blast resistance, so should be used in resistance breeding, but that *Pizt* is gradually losing its resistance.

The *Pikm* locus is composed of two closely linked NBS-LRR genes (i.e. *Pikm1-TS* and *Pikm2-TS*) and both are required for the resistance function (Ashikawa *et al*., 2008). The Pik locus includes the cloned alleles *Pi1*, *Pikm*, *Pik* and *Pikp*. The resistance frequency of *Pi1* was 28.74% following artificial inoculation, while natural disease investigation in the field detected an average leaf blast rate and disease index of 4.66% and 0.59, respectively. The resistance level was 2. In general, *Pi1*

Table 1: LTH-monogenic lines each possessing a single blast resistance gene

No.	Monogenic lines	Carrying gene	No.	Monogenic lines	Carrying gene
IR2	IRBLa-C	*Pia*	IR16	IRBLsh-S	*Pish*
IR6	IRBLk-Ka	*Pik*	IR18	IRBL1-CL	*Pi1*
IR7	IRBLkp-K60	*Pikp*	IR20	IRBL5-M	*Pi5*
IR10	IRBLz5-CA	*Pi2*	IR22	IRBL9-W	*Pi9*
IR11	IRBLzt-T	*Pizt*	IR25	IRBLkm-Ts	*Pikm*
IR14	IRBLb-B	*Pib*	IR29	IRBLta-CP1	*Pita*
IR15	IRBLt-K59	*Pit*			

Table 2: LTH-monogenic lines of blast resistance genes and donors with artificial inoculation and naturalinfection

Material name	R gene	Number of resistant strains	Artificial inoculation			Field natural resistance		
			Number of susceptible strains	Strains	Resistance frequency (%)	Average rate of leaf blast (%)	Resistance level	The average disease index
IRBLa-C	*Pia*	1	93	94	1.06	94.17	9	64.50
IRBLk-Ka	*Pik*	31	48	79	39.24	90.91	9	66.11
IRBLkp-60	*Pikp*	5	85	90	5.56	69.24	9	37.39
IRBLz5-A	*Pi2*	86	3	89	96.63	0.58	2	0.10
IRBLzt-T	*Pizt*	60	27	87	68.97	56.48	9	23.60
IRBLb-B	*Pib*	3	89	92	3.26	100.00	9	88.75
IRBLt-K59	*Pit*	2	78	80	2.5	95.48	9	74.64
IRBLsh-S	*Pish*	9	79	88	10.23	85.38	9	58.93
IRBL1-CL	*Pi1*	25	62	87	28.74	4.66	2	0.59
IRBL5-M	*Pi5*	19	63	82	23.17	65.41	9	36.03
IRBL9-W	*Pi9*	77	8	85	90.59	0.60	1	0.07
IRBLkm-s	*Pikm*	76	5	81	93.83	0.55	2	0.08
IRBLta-CP1	*Pita*	8	83	91	8.79	57.69	9	28.27
LTH	None	0	94	94	0	100.00	9	92.17

Table 3: The result of the resistance evaluation of varieties about rice false smut

Varieties	stage I			stage II			stage III		
	diseased panicle rate (%)	disease index	Resistance evaluation	diseased panicle rate (%)	disease index	Resistance evaluation	diseased panicle rate (%)	disease index	Resistance evaluation
PJ6	10.07a	3.07a	MS	19.86a	7.98a	S	29.27a	9.99a	HS
R18/2348	0	0	R	0.58i	0.26kl	R	3.16i	1.05hi	MR
CGY2348	0	0	R	0.90h	0.37k	R	1.33j	0.27k	R
QY357	0.56e	0.06e	R	9.81d	2.79f	MS	13.78c	4.93d	MS
FYY188	0.28f	0.03e	R	14.11c	4.28c	MS	8.33f	2.16f	MS
CFY188	1.76b	0.59b	R	19.53a	5.75b	S	6.23g	1.75g	MR
CGY7329	0.59e	0.07e	R	16.72b	4.24c	S	13.88c	5.57c	S
LXY177	1.17c	0.46c	R	3.86g	1.07j	MR	1.30j	0.36k	R
LY908	0	0	R	8.87e	3.02e	MS	18.75b	6.10b	S
CNYHZ	0	0	R	9.41de	2.83f	MS	4.47h	1.26h	MR
HXY1	0	0	R	9.51de	3.30d	MS	10.14e	2.16f	MS
GY66	0	0	R	0	0	R	0	0	R
YLY973	0	0	R	0	0	R	2.87i	0.57jk	R
HY1199	0.90d	0.23d	R	9.09de	2.57g	MS	5.78g	2.21f	MR
RY1808	0.30f	0.03e	R	0.87h	0.16l	R	17.97b	2.98e	S
DY4727	0.89d	0.30d	R	7.34f	1.82i	MS	10.51de	2.37f	MS
CNY445	0	0	R	6.95f	2.02h	MR	11.25d	1.99fg	MS
N5Y828	0	0	R	0	0	R	2.91i	0.76ij	MR
YXY2115	0	0	R	0	0	R	8.52f	1.65g	MS

Different lowercase letters within a column indicate significant differences based on one-way analysis of variance in SPSS 13.0 followed by the least significant difference test ($P < 0.05$). R, resistant; S, susceptible; MR, middle resistance; MS, middle susceptible

shows considerably strong resistance, suggesting that we can use it for marker-assisted breeding. The resistance frequency of *Pikm* was measured as 93.83% following artificial inoculation, while the average leaf blast rate and disease index were 0.55% and 0.08, respectively. Thus, *Pikm* appears to be a good R gene for breeding. *Pikp* and *pik* showed little resistance, appear not to be useful for breeding.

Broad-spectrum resistant gene, *Pi5*, showed a resistance frequency of 23.17% following artificial

inoculation. However, natural disease investigation in the field indicated an average left blast rate and disease index of 65.41% and 36.03, respectively. The resistance level was 9, and we observed it to lose blast resistance in Sichuan, suggesting that it is unsuitable for breeding.

Pia, *Pib*, *Pit* and *Pish* showed average leaf blast rates and disease indices that exceeded 85% and 58, respectively, while resistance levels were 9, because all lost blast resistance in Sichuan. Therefore, none of these genes have any value in breeding. *Pita* so also appear not to be useful for breeding. Finally, the resistance frequency of *Pid2*, *Pid3*, *Pid3-A4*, *Pi25*, *Pi21*, *Pb1*, *Pi36*, *Pi37*, *Pi54* and *Pi56*, were not examined, because no LTH monogenic lines possessing these genes were available currently.

Expression Profiling of Rice Blast R Genes

RT-PCR was carried out to detect the expression levels of reported blast R genes in the 18 rice varieties (Fig. 1). Strong blast resistance was detected in rice varieties CNYHZ, CNY445 and YXY2115, which are LTH monogenic lines carrying the Pi2 locus. LTH monogenic lines carrying the Pik locus also showed strong blast resistance in varieties CFY188, LY908 and LTH. *Pi5* was detected in varieties HXY1 and CFY188, while *Pia* was detected in all varieties. As mentioned above, both genes are losing their blast resistance. *Pid2* was detected in all varieties, except for CNYHZ, while *Pb1* was only detected in HXY1, *Pi36* in LTH and CFY188, *Pi37* and *Pid3* in LTH. Since LTH is considered to carry no functional blast R genes, detection of some R genes in LTH by RT-PCR indicated that those genes should be the susceptible alleles.

To identify functional genes detected fragment of Pi2 and Pik loci came from the resistant allele or susceptible allele, we amplified the full length of Pi2 loci resistance genes from gDNA of CNYHZ, CNY445 and YXY2115. Also full length of Pik loci resistance genes from gDNA of CFY188 and LY908. Sequence analysis showed the amplified fragment contained the full length of *Pi2* and *Pikm*, respectively, which were identical to the resistance genes reported previously (Zhou *et al*., 2006, Ashikawa *et al*., 2008).

Identification of False Smut Resistance in the Field

The diseased panicle rate, disease index, and resistance evaluation were investigated in rice varieties sown at different stages until the yellow ripening stage (Table 3). The diseased panicle rate was measured as 10.07, 19.86 and 29.27%, and the resistance evaluation was up to mid-level susceptible (MS), susceptible (S), and highly susceptible (HS), respectively. This indicates that the identification results are reliable. As shown in Table 3, the diseased panicle rate and disease index showed significant differences among the 18 rice varieties. The differences between diseased panicle rates were greater than those of the disease index, while varieties sown at different stages showed more differences in resistance evaluation. In general, varieties sown in stage I were less susceptible to disease. Some varieties sown at the same stage showed different levels of resistance. Varieties LXY177, R18/2348, CGY2348, GY66, YLY973 and N5Y828 all demonstrated high resistance in all three stages, while GY66 demonstrated the most strongly high resisted false smut during all three stages because no one showed rice false ball. YXY2115 was resistant in stage I and stage II, but was MS in stage III.

Supplementary Table S1: Primers for RT-PCR analysis

Primers	Sequence (5'-3')
RTPita-F	TACATCTTCACCAGCATCCC
RTPita-R	AGACCCGAACCCCTCATT
RTPid2-F	GCCTGAGAATGTTCTACTTGACG
RTPid2-R	GCTCTTCCTCCCACCGA
RTPi2-loc-F	ATCACGACCTGGGGGCTGAA
RTPi2-loc-R	TTCGTCGTCAACGTGATCA
RTPid3-loc-F	CCTGCTCTGTCCAAACCTG
RTPid3-loc-R	CACCATTTCTGATGAACCCA
RTPia-RGA4-F	AGACGTTGATAGTGTAATGGAGG
RTPia-RGA4-R	CAGCAGGAGACATCTGAAAGC
RTPia-RGA5-F	TGAACTCTGCCTTGCTTTTATG
RTPia-RGA5-R	TGCTTGTTGACAGTTTCCTTG
RTPikml-loc-F	TCCTCATCAATGCTGGGTAT
RTPikml-loc-R	CGATCTTGGGTTTCCTCTTC
RTPikm2-loc-F	GGATCAGGACATAATAAAGGACA
RTPikm2-loc-R	CTCACGGAGATTTTCAAGGA
RTPi36-F	ATGTTCGGTTCCTAAAAGATGC
RTPi36-R	TGGACGGTTGGGATGGC
RTPi37-F	ATCTCACAGTTTCGCGTCC
RTPi37-R	CCTGGTGGTGACCTCATTTC
RTPit-F	AAGGAAGCAACATCGTTTACC
RTPit-R	CAGCATTTACACCCACCGT
RTPi5-1F	AGAAATGCGACAACACTCCC
RTPi5-1R	AGGAACCAGGCTAACGGAC
RTPi5-2F	AATAGACTACTCCCGTCCTCCC
RTPi5-2R	TTCCTTGATAACCAATGTGCTGT
RTPish-F	AGGTTTCAAAGTTCCAGGGTT
RTPish-R	AGATGTTATGTTGGGGCAGTC
OsActin-F	CCTCGTCTCGACCTTGCTGGG
OsActin-R	GAGAACAAGCAGGAGGACGGC
Pi2-loc-F	GAACGAGTCCA TGGCGGAGA
Pi2-loc-R	AGATCGTCAGCCAGCTTGAG
Pik-1-loc-F	ATGGAGGCGGCTGCCATGGC
Pik-1-loc-R	CTAGCTAGTAGTTTCTGTTTGAATTTCAATAT
Pik-2-loc-F	ATGGAGTTGGTGGTAGGTGCTTC
Pik-2-loc-R	TCATGCAGTGACGATGCCATCAAC

Discussion

Sequence analysis has been used as a great tool in the genetic analysis of plant species under control or disease conditions (Alberts *et al.,* 2002; Prida *et al.,* 2012; Qin *et al.,* 2014; Das *et al.,* 2017). The hybrid rice breeding system has contributed greatly to the production of rice, which is one of the most important grain crops

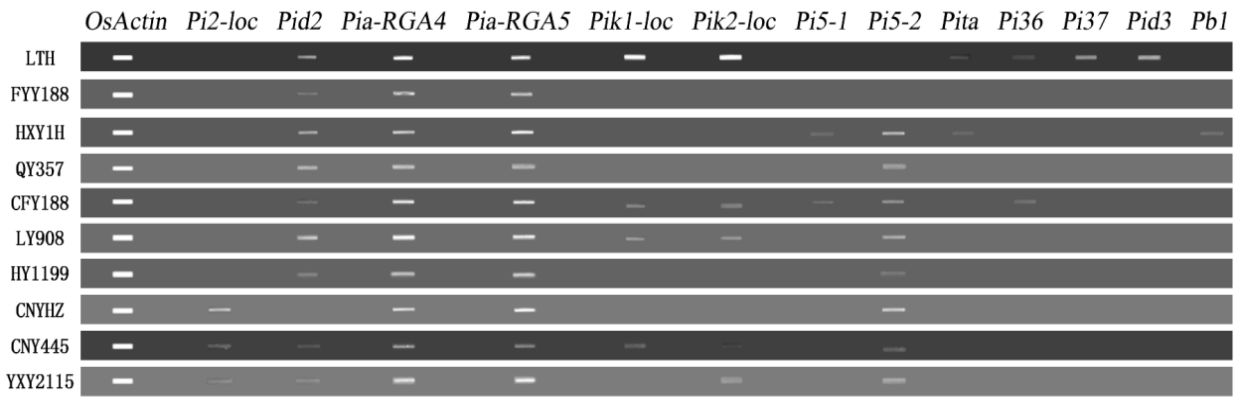

Fig. 1: Expression profiling of blast resistance genes in some varieties

worldwide. However, many pathogenic microorganisms cause important diseases in rice, leading to significant yield and quality losses that threaten global food security. Rice blast and false smut now represent the most serious pathogens for rice production, so it is important to identify rice genotypes with durable resistance to such diseases (Kumar *et al.,* 2003; PrasannaKumar *et al.,* 2011, 2012; Singh and Sunder, 2015).

Our data revealed that *Pi2, Pi9, Pi1* and *Pikm* showed strong resistance in rice grown in Sichuan Province, suggesting that these genes can be exploited in hybridization and molecular breeding programs to develop resistant varieties of rice. *Pizt, Pi5, Pita* and *Pikp* demonstrate mid-range resistance that is gradually being lost, indicating that they would be less useful for breeding purposes. The Pi2 locus demonstrated strong expression in CNYHZ, CNY445, and YXY2115, while the Pik locus was strongly expressed in CFY188 and LY908 varieties.

The Pi2 locus is a complicated nucleotide binding site leucine-rich repeat R gene family. It contains several R genes, of which *Pi2*, *Pi9* and *Pizt* have been cloned, and some genes have been mapped in the region. Our field resistance investigation showed that the Pi2 locus was strongly resistant to most rice blast populations in Sichuan. Sequence analysis revealed *Pi2* is the resistant functional gene in CNYHZ, CNY445 and YXY2115. While *Pikm* is the resistant functional gene in CFY188 and LY908.

Feng *et al*. (2013) reported that most rice parents in Sichuan lacked *Pi2* and *Pi9* R genes, and detected Pik locus R genes in some parents. In a recent research, Shi *et al*. (2015) confirmed that *Pi2* is the major R gene in the hybrid rice restorer line Yahui2115, while Chen *et al*. (2015) also confirmed this in the restorer line Huazhan. Yahui2115 and Huazhan lines are the male parents of hybrid rice varieties YXY2115 and CNYHZ, respectively, and our present study detected strong expression of the *Pi2* and got the full length in YXY2115 and CNYHZ, in line with their research.

Because of the complex disease cycle process of rice false smut, few studies have investigated its pathology compared with rice blast. As a result, we do not fully understand the infection process or how false smut balls are released. Moreover, no false smut R genes have been cloned yet. However, in recent years, rice false smut disease has become more serious in Sichuan and throughout China. Fundamental research has therefore been undertaken, such as time-course microscopy, transcriptional studies, and investigations of the fungal–plant pathosystem (Andargie and Li, 2016; Fan *et al*., 2015; Han *et al*., 2015).

We investigated the false smut resistance of 18 rice varieties in the field. Our data showed that R18/2348, CGY2348, GY66, YLY973 and N5Y828 varieties presented with the strongest natural resistance, followed by LXY177 and YXY2115. The notable differences in resistance among varieties indicate the possible existence of different R genes, although differences in sowing time may also have influenced the resistance level. For example, no false smut balls were detected in variety YXY2115 sown in the first two stages, but MS to false smut was detected in the last stage. However, we largely observed improved resistance in plants grown in the first stage. Additionally, the temperature and humidity can also affect the growth of false smut, as seen for rice blast.

Our findings suggest that there is no correlation between resistance to blast and false smut among the 18 varieties of rice studied. These findings will be useful in rice production and the development of new resistant varieties, as well as the distribution of rice varieties worldwide.

Conclusion

Our findings suggest that *Pi2* locus and *Pik* locus still have strong rice blast resistance in Sichuan province. Three tested varieties expressed functional *Pi2*, two expressed functional *Pikm*. Meanwhile, five showed

strong resistance to rice false smut. Especially, the resistance between rice blast and rice false smut had no correlation among the 18 hybrid rice varieties. These findings will be useful in rice production and the development of new resistant varieties, as well as the distribution of rice varieties worldwide.

Acknowledgments

The research was supported by the Science and Technology Department of Sichuan Provice (2017FZ0022), Mianyang science and technology bureau (14N-01-1) and Research Fund of Mianyang Normal University (QD2014A001).

References

Alberts, B., A. Johnson, J. Lewis, M. Raff, K. Roberts and P. Walter, 2002. *Molecular Biology of the Cell*, 4th edition. Gerland Science, New York, USA

Andargie, M. and J. Li, 2016. Arabidopsis thaliana: a model host plant to study plant–pathogen interaction using rice false smut isolates *Ofustilaginoidea virens*. *Front. Plant Sci.,* 7: e91391

Ashikawa, I., N. Hayashi, H. Yamane, H. Kanamori, J. Wu, T. Matsumoto, K. Ono and M. Yano, 2008. Two adjacent nucleotide-binding site-leucine-rich repeat class genes are required to confer pikm-specific rice blast resistance. *Genetics,* 180: 2267–2276

Cartwright, R.D., K.B. Watkins, C.E. Parsons, E.A. Sutton, J. Allen and C.E. Wilson, 2004. Effect of preventative fungicide application on rice yield, milling quality, and return. *B.R. Wells Rice Res. Stud. AAES Res. Ser.*, 529: 1–85

Chen, S., S.U. Jing, L.X. Hua, W.J. Wang, C.Y. Wang, J.Y. Yang, L.X. Zeng and X.Y. Zhu, 2015. Genetic analysis and gene identification of restorer line huazhan against rice blast. *Acta Phytopathol. Sin*., 45: 598–605

Das, G., J.K. Patra and K.-H. Baek, 2017. Insight into MAS: A molecular tool for development of stress resistant and quality of rice through gene stacking. *Front. Plant Sci.,* 8: 985

Doyle, J.J. and J.L. Doyle, 1990. Isolation of plant DNA from fresh tissue. *Focus,* 12: 13–15

Fan, J., X.Y. Guo, L. Li, F. Huang, W.X. Sun, Y. Li, Y.Y. Huang, Y. Xu, J. Shi, Y. Lei, A.P. Zheng and W.M. Wang, 2015. Infection of *Ustilaginoidea virens* intercepts rice seed formation but activates grain-filling-related genes. *J. Integr. Plant Biol*., 57: 577–590

Feng, H., C.M. Yang, X.B. Wu, Y.S. Liu and Y.L. Peng, 2013. Detection and analysis of rice blast gene in some hybrid rice parents and 32 resistance meterials of Sichuan province. *Southeast Chin. J. Agric. Sci*., 26: 987–993

Fatah, T., M.Y. Rafii, H.A. Rahim, S. Meon, M. Azhar and M.A. Latif, 2014. Cloning and analysis of QTL linked to blast disease resistance in Malaysian rice variety Pongsu Seribu 2. *Int. J. Agric. Biol*., 16: 395–400

Gong, G.S., Q. Xu, M. Zhang, J.Z. Yang, H.B. Chen, S.A. Shen and T.F. Tang, 2010. A simple method for single fungal spore isolation. *J. Maize Sci*., 18: 126–127

Han, Y.Q., K. Zhang, J. Yang, N. Zhang, A.F. Fang, Y. Zhang, Y.F. Liu, Z.Y. Chen, T. Hsiang and W.X. Sun, 2015. Differential expression profiling of the early response to *Ustilaginoidea virens* between false smut resistant and susceptible rice varieties. *BMC Genomics*, 16: 955

Huang. F., Y. Li, J. Shi, D.Q. Li, Fan J, Xu Y.J. and W.M. Wang, 2016. Screening and polymorphism analysis of rice germplasms for resistance to false smut disease in Sichuan Privince. *Acta Phytopathol. Sin*., 46: 247–257

International Rice Research Institute, 1996. *Standard Evaluation System for Rice*, 4th edition. International Rice Research Institute, Manila, Philippines

Koiso, Y, Y. Li, S. Iwasaki, K. Hanaoka, T. Kobayashi, R. Sonoda, Y. Fujita, H. Yaegashi and Z. Sato, 1994. Ustiloxins, antimitotic cyclic peptides from false smut balls on rice panicles caused by Ustilaginoidea virens. *J. Antibiotics*, 47: 765–773

Kumar, A., R.Singh and B.L. Jalali, 2003. Management of stem rot of rice with resistance including chemicals and fungicides. Ind. Phytopath., 56: 266–269

Kush, G.S. and K.K. Jena, 2009. Current status and future prospects for research on blast resistance in rice (*Oryza sativa* L.). *In: Advances in Genetics, Genomics and Control of Rice Blast Disease*, pp: 1–10. Wang, G.L. and B. Valent (eds.). Springer, The Netherlands

Ladhalakshmi, D., G.S. Laha, R. Singh, A. Karthikeyan, S.K. Mangrauthia, R.M. Sundaram, P. Thukkaiyannan and B.C. Viraktamath, 2012. Isolation and characterization of *Ustilaginoidea virens* and survey of false smut disease of rice in India. *Phytoparasitica*, 40: 171–176

Li, Y., Y.G. Lu, Y. Shi, L. Wu, Y.J. Xu, F. Huang, X.Y. Guo, Y. Zhang, J. Fan, J.Q. Zhao, H.Y. Zhang, P.Z. Xu, J.M. Zhou, X.J. Wu, P.R. Wang and W.M. Wang, 2014. Multiple Rice microRNAs are involved in immunity against the blast fungus *Magnaporthe oryzae. Plant Physiol*., 164: 1077–1092

Liu, G., G. Lu, L. Zeng and G.L. Wang, 2002. Two broadspectrum blast resistance genes, *Pi9*(*t*) and *Pi2*(*t*), are physically linked on rice chromosome 6. *Mol. Genet. Genomics*, 267: 472–480

Liu, J.L., X.J. Wang, M. Thomas, Y.J. Hu, X.L. Liu, L.Y. Dai and G.L. Wang, 2010. Recent progress andunderstanding of the molecular mechanisms of the rice-*Magnaporthe oryzae* interaction. *Mol. Plant Pathol*., 11: 419–427

Nakamura, K., N. Izumiyama, K. Ohtsubo, Y. Koiso, S. Iwasaki, R. Sonoda, Y. Fujita, H. Yaegashi and Z. Sato, 1994. "Lupinosis"-like lesions in mice caused by ustiloxin, produced by *Ustilaginoieda virens*: a morphological study. *Nat. Toxins*, 2: 22–28

Qin, C., C. Yu, Y. Shen, X. Fang, L. Chen, J. Min, J. Cheng, S. Zhao, M. Xu, Y. Luo, Y. Yang, Z. Wu, L. Mao, H. Wu, C. Ling-Hu, H. Zhou, H. Lin, S. González-Morales, D.L.Trejo-Saavedra, H. Tian, X. Tang, M. Zhao, Z. Huang, A. Zhou, X. Yao, J. Cui, W. Li, Z. Chen, Y. Feng, Y. Niu, S. Bi, X. Yang, W. Li, H. Cai, X. Luo, S. Montes-Hernández, M.A. Leyva-González, Z. Xiong, X. He, L. Bai, S. Tan, X. Tang, D. Liu, J. Liu, S. Zhang, M. Chen, L. Zhang, L. Zhang, Y. Zhang, W. Liao, Y. Zhang, M. Wang, X. Lv, B. Wen, H. Liu, H. Luan, Y. Zhang, S. Yang, X. Wang, J. Xu, X. Li, S. Li, J. Wang, A. Palloix, P.W. Bosland, Y. Li, A. Krogh, R.F. Rivera-Bustamante, L. Herrera-Estrella, Y. Yin, J. Yu, K. Hu and Z. Zhang, 2014. Whole-genome sequencing of cultivated and wild peppers provides insights into *Capsicum* domestication and specialization. *PNAS*, 111: 5135–5140

Parida, S.K., M. Mukerji, A.K. Singh, N.K. Singh and T. Mohapatra, 2012. SNPs in stress-responsive rice genes: validation, genotyping, functional relevance and population structure. *BMC Genomics*, 13: 426

PrasannaKumar, M.K., D.K. Siddegowda, N. Kiran Kumar, H.M. Atheekur Rehaman, S.C. Chandrashekhar, K.T. Pandurange Gowda, G.K. Sudarshan, S.K. Biswas, 2011. Comparative efficacy of new fungicide groups against paddy sheath blight. *Pestology*, 35: 39–44

PrasannaKumar, M.K., D.K. Siddegowda, K.T. Pandurange Gowda, and K. Vishwanath, 2012. A new carboxnilide group fungicide against paddy sheath blight. *Res. J. Agric. Sci.,* 3: 500-505

Shi, J., D.Q. Li, Y. Li, X.Y. Li, X.Y. Guo, Y.W. Luo, Y.G. Lu, Q. Zhang, Y.J. Xu, J. Fan, F. Huang and W.M. Wang, 2015. Identification of rice blast resistance genes in an elite hybrid rice restorer line Yahui2115. *Genome*, 58: 91–97

Singh, R. and S. Sunder. 2015. Identification of sources of resistance to blast and false smut of rice and their management with fungicides *J. Mycol. Plant Pathol.,* 45: 55-59

Tanaka, E., T. Ashizawa, R. Sonoda and C. Tanaka, 2008. Villosiclava virens gen. nov., comb. nov., teleomorph of Ustilaginoidea virens, the causal agent of rice false smut. *Mycotaxon*, 106: 491–501

Vasudevan, K., W. Gruissem and N.K. Bhullar, 2015. Identification of novel alleles of the rice blast resistance gene *Pi54*. Sci. Rep., 5: 15678

Wang, L., J. Shi, D.Q. Li, Q. Zhang, X.Y. Li, Y.W. Luo, Q.S. Jiang, D.M. Zhao and F. Huang, 2013. Studies on rice false smut resistance of yixiang hybrid varieties. *J. Sichuan Agric. Univ.*, 31: 365–369

Zhou, B., S. Qu, G. Liu, M. Dolan, H. Sakai, G. Lu, M. Bellizzi and G.L. Wang, 2006. The eight amino-acid differences within three leucine rich repeats between *Pi2* and *Piz-t* resistance proteins determine the resistance specificity to *Magnaporthe grisea*. *Mol. Plant Microbe Interact*, 19: 1216–1228

(Received 17 April 2017; Accepted 26 April 2017)