CHARACTERIZATION OF LOCAL GENETIC RESOURCE FOR BACTERIAL LEAF STREAK RESISTANCE IN RICE

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Received date: 27.12.2016 **Accepted date: 28.04.2017**

ABSTRACT

Bacterial leaf streak (BLS) disease in rice, caused by *Xanthomonas oryzae* pv*. oryzicola* (*Xoc*), has become a more common and serious disease in tropical regions along with bacterial leaf blight. Breeding new rice varieties by introducing resistance genes is considered one of the most effective and eco-friendly ways to control the disease. With a high diversity of resistance genes, local rice varieties play an important role in developing durable resistant varieties against diseases. In this study, 50 local rice accessions provided by Center for Conservation and Development of Crop Genetic Resources (CCD-CGR) were used to evaluate the reaction pattern with two isolates *Xoc*, TB4 and TN158. Lesion lengths measured after artificial inoculation showed 3 highly resistant (HR) accessions and 15 resistant (R) accessions to both TB4 and TN158 isolates. Four pairs of flanking SSR markers, RM587, RM510, RM153, and RM159, were used to detect the *bls1* and *qBlsr5a* genes. The results of genotyping revealed 6 accessions containing the *bls1* gene and 6 accessions containing *qBlsr5a*, of which, accession 11189 contained both genes. Based on the correlation analysis between reaction patterns and genotypes, the resistance ability of the *bls1* gene was highly effective to *Xoc,* while the *qBlsr5a* gene did not show clear resistance. With our results, accessions that were HR or R, and carried the *bls1* gene could be used as donors for breeding new rice varieties resistant to BLS.

Keywords: Bacterial leaf streak, resistance gene, SSR marker, *Xanthomonas oryzae* pv. *oryzicola*.

Khảo sát khả năng kháng bệnh đốm sọc vi khuẩn trong nguồn gen lúa địa phương

TÓM TẮT

Bệnh đốm sọc vi khuẩn (BLS) trên cây lúa do vi khuẩn *Xanthomonas oryzae* pv*. oryzicola* (*Xoc*) gây ra, cùng với bệnh bạc lá (BLB) đã trở thành dịch hại phổ biến và nghiêm trọng ở các vùng nhiệt đới. Phát triển các giống lúa mang các gen kháng là một chiến lược hiệu quả và thân thiện nhất với môi trường. Các giống lúa địa phương sở hữu nguồn gen kháng đa dạng và phong phú, giữ vai trò quan trọng trong việc tạo ra các giống lúa chống chịu tốt. Tiến hành khảo sát tính kháng nhiễm với vi khuẩn *Xoc* bằng phương pháp lây nhiễm nhân tạo trên 50 mẫu giống lúa địa phương được cung cấp bởi Trung tâm bảo tồn và phát triển nguồn gen cây trồng (CCD-CGR). Kết quả xác định được có 3 mẫu giống có khả năng kháng cao(HR) và 15 mẫu giống thể hiện khả năng kháng (R) với cả hai isolate TB4 và TN158. Đồng thời, 4 cặp chỉ thị SSR đặc hiệu RM587, RM510, RM153 và RM159 được sử dụng để xác định 2 gene kháng là*bls1* và *qBlsr5a*. Kết quả khảo sát xác định được 6 mẫu giống mang gen *bls1* và 6 mẫu giống mang gen *qBlsr5a*, đặc biệt mẫu giống 11189 mang đồng thời cả hai gen. Kết quả đánh giá mối tương quan giữa khả năng kháng của các mẫu giống và kết quả xác định kiểu gen đã chỉ ra tính kháng hữu hiệu của gen *bls1*, trong khi gen *qBlsr5a* không thể hiện rõ tính kháng. Với kết quả này, các mẫu giống có khả năng kháng và kháng cao đồng thời mang gen *bls1* có thể sử dụng như là nguồn gene trong công tác chọn tạo giống lúa mới kháng bệnh đốm sọc.

Từ khóa: Bệnh đốm sọc vi khuẩn, chỉ thị SSR, gen kháng, vi khuẩn *Xanthomonas oryzae* pv. *oryzicola*.

1. INTRODUCTION

Bacterial leaf streak (BLS) in rice, caused by *Xanthomonas oryzae* pv. *oryzicola (Xoc)*, is a destructive bacterial disease. BLS was first discovered in the Philippines in 1918 and it has become a serious disease of emerging importance that constrains rice production in certain rice growing regions in China and South/Southeast Asia. *Xoc* infection occurs in the pollination period, leading to reduced photosynthetic capacity of leaves and null-panicles. BLS can damage yield loss up to 32% under favorable conditions (He *et al.,* 2012).

Utilization of resistance genes was one of the best solutions for managing this disease in rice. In Vietnam, some varieties were released by introducing resistance gene elite cultivars, for example BT7 carrying the *Xa21* gene, and DCG84 carrying the *Xa7* and *Xa21* genes are resistant to bacterial leaf blight (BLB) disease. However, there were limited results on BLS disease. Around the world, there have been several discoveries of resistance genes/QTL to *Xoc*: Chen *et al.*, (2006) detected one QTL named as *qBLSR-11-1* on chromosome 11 and flanking markers were developed RM120 và RM441; Han *et al.* (2008) identified another gene, *qBlsr5a*, on chromosome 5 with a genetic distance of about 2.4 cM to flanking markers RM153 and RM159 and this gene shared the same locus with the *xa5* gene; He *et al.* (2012) detected the *bls1* gene on chromosome 6 in wild rice, *Oryza* rufipogon, and flanking markers RM587 and RM510 were developed with a distance of about 4 cM.

Recently, a research group at the Department of Molecular Biology and Applied Biotechnology, Vietnam National University of Agriculture (VNUA) conducted the first study on isolating and identifying *Xoc* bacteria (Vu Huy Minh *et al.*, 2014) and evaluating the genetic diversity of 23 isolates of *Xoc* from several provinces of Northern Vietnam (Nguyen Quoc Trung *et al.,* 2015). We have also preliminary data evaluating resistance to BLS disease of blight resistance the genes *Xa4*, *xa5*, *Xa7*, *Xa10*, and *Xa21* (Nguyen Quoc Trung *et al.*, 2016).

In Vietnam, the epidemiological factors and pathotypes of BLS are rarely found in independent studies on because BLS is always combined with BLB. By screening genetic resources for BLS disease resistance with artificial inoculation and DNA markers, this study will facilitate the conservation of local varieties and a breeding program for resistant rice varieties against BLS in Vietnam.

2. MATERIALS AND METHODS

2.1. Materials

Fifty accessions were provided by the Center for Conservation and Development of Crop Genetic Resources (CCD-CGR), VNUA (Table 1).

BLS pathogens were provided by the Laboratory of Plant Breeding, Center of International Plant Research Vietnam and Japan (CIPR), VNUA, and included 2 isolates that had high virulence: TB4 and TN158, collected in Thai Binh and Thai Nguyen in the autumn season, 2014 (Nguyen Quoc Trung *et al.*, 2015).

Table 1. List of the 50 accessions used for characterizing BLS resistance

Accession code	Accessions name	Origin place
10244	CCD1	CCD-CGR
10252	CCD2	CCD-CGR
11342	CCD3	CCD-CGR
11512	CCD4	CCD-CGR
11521	CCD5	CCD-CGR
11541	CCD6	CCD-CGR
11610	CCD7	CCD-CGR

11612	CCD8	CCD-CGR
11651	CCD9	CCD-CGR
11703	CCD10	CCD-CGR
11807	CCD11	CCD-CGR
11989	CCD12	CCD-CGR
10255-2	CCD13	CCD-CGR
11267-1	CCD14	CCD-CGR
11298-2	CCD15	CCD-CGR
HYT102	Advanced cultivar	CCD-CGR
10983	Khẩu tan pỏm	Tuan Giao, Dien Bien
11059	Pe lón	Tuan Giao, Dien Bien
11187	Nếp Thơm	Lai Chau
10095	Nếp Be Lanh2	Muong Lay, Lai Chau
11048	Không tên	Than Uyen, Lai Chau
11049	Nhạ Páo	Than Uyen, Lai Chau
10546	Cai Xanh	Lao Bao, Nghe An
10281	Khai Vai Ruong	Tam Nong, Phu Tho
11087	Ble chùa	Phong Lai, Son La
11088	Ble tớ đớ	Phong Lai, Son La
11091	Tớ li a	Phong Lai, Son La
11094	Nếp Tủa Chùa dạng 1	Phong Lai, Son La
11098	Nếp Tủa Chùa dạng 2	Phong Lai, Son La
11189	Chiêm Sành Cẩm Khê	Phu Yen, Son La
11191	Tẻ cẩm dạng 1	Phu Yen, Son La
11195	Nếp cẩm	Phu Yen, Son La
11203	Tẻ Râu 2	Phu Yen, Son La
11204	Tẻ Râu 3	Phu Yen, Son La
11215	Kháu cẩm pị 1	Phu Yen, Son La
11216	Kháu cẩm pị 2	Phu Yen, Son La
11227	Nếp cẩm	Phu Yen, Son La
11080-1	Nếp Mai Sơn	Tuan Giao, Son La
11092	Tẻ nương 64	Tuan Giao, Son La
11095-2	Ble Chùa	Tuan Giao, Son La
10689	Tẻ Thơm	Thuan Chau, Son La
11063	Dạng khác của Plệnh đỏ	Thuan Chau, Son La
11071	Dạng khác của pelạnh mèo	Thuan Chau, Son La
11073	Pe lạnh mèo	Thuan Chau, Son La
11076	Nếp cẩm	Thuan Chau, Son La
11080	Nếp Mai Sơn	Thuan Chau, Son La
11082	Nếp cẩm	Thuan Chau, Son La
11084	Nếp cẩm	Thuan Chau, Son La
11180-2	Nếp cẩm	Thuan Chau, Son La
10134-2	Lúa Tiên ưu	Yen Binh, Yen Bai

2.2. Methods

2.2.1. Field layout

The experiment was carried out in a randomized complete block design. Seeds were soaked for 48 hours at 30ºC and incubated for 24 hours at 30ºC before sowing on a tray. Seedlings were transplanted at a rate of 10 plants per accession. The plants were transplanted at a distance of 20 cm between plants in a row and the rows were 25 cm apart in the field layout.

2.2.2. Inoculation methods

Rice plants were artificially inoculated at the maximum tillering stage; inoculation was carried out using a syringe (Reimers and Leach, 1991; with slight modification by Trung N.Q *et al.*, 2016). *Xoc* was cultured on PeSA medium with (in grams per litter) sucrose 10, sodium glutamate 1, peptone 10, and agar 20, at pH 7.0 at 28ºC for 3 days. *Xoc* colonies were diluted in distilled water at standardized concentrations (OD600nm = 0.5). Inoculum was injected on the underside of the leaf using a 1 *ml* syringe containing 0.25 *ml* of fluid. Two leaves at same age from each plant were inoculated at 3 sites per leaf (Figure 1).

In order to evaluate the resistant pattern to *Xoc*, lesion length on the leaves was measured at 13 days post inoculation.

2.2.3. DNA extraction

Leaves were collected 30 days after transplanting and free-dried before extracting DNA using the potassium acetate protocol (Dellaporta *et al.*, 1983). Leaves were cut into 0.5-1.0 cm pieces in each well of the plate. Samples were ground by the Multil-Beads Shocker at 1800 rpm for 60 seconds, and replicated 2 times. Extraction buffer (600 µl, at 65ºC) was added and then each tube was incubated at 65°C for 45 minutes in a water bath. Potassium acetate 5M (200 µl) was added, then each tube was put on ice for 30 minutes. The tubes were centrifuged at 9000 rpm for 15 minutes at 4ºC and supernatant was transfered (about 400 µl) into new a tube. Isopropanol was added with same volume and centrifuged at 9000 rpm for 30 minutes at 4ºC. Supernatant was gently poured off and the DNA pellets were lightly dried. Pellets were washed with 70% ethanol, dried thoroughly, and redissolved in 50 µl TE 0.1x.

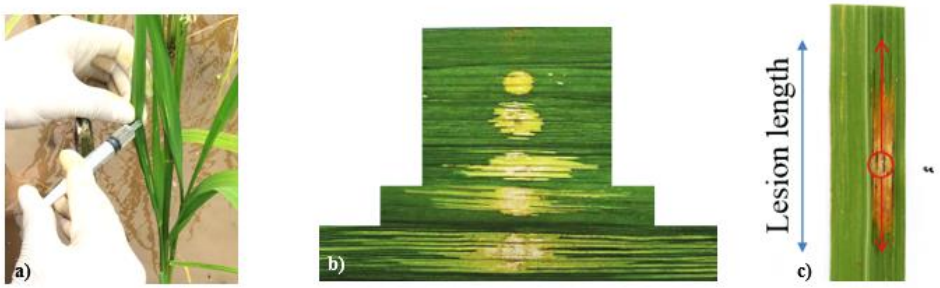

Figure 1. a)Inoculum was injected on the underside of the leaf; b) Levels of resistance/susceptibility to *Xoc* on leaves; c) The measurement of a lesion length

Table 2. Level of resistance/susceptibility to Xoc

Level	Length lesion
Highly resistant (HR)	0 - 0.5 cm
Resistant (R)	>0.5 - 1.0 cm
Moderately resistant (MR)	> 1.0 - 2.0 cm
Moderately susceptible (MS)	> 2.0 - 3.0 cm
Susceptible (S)	> 3.0 cm

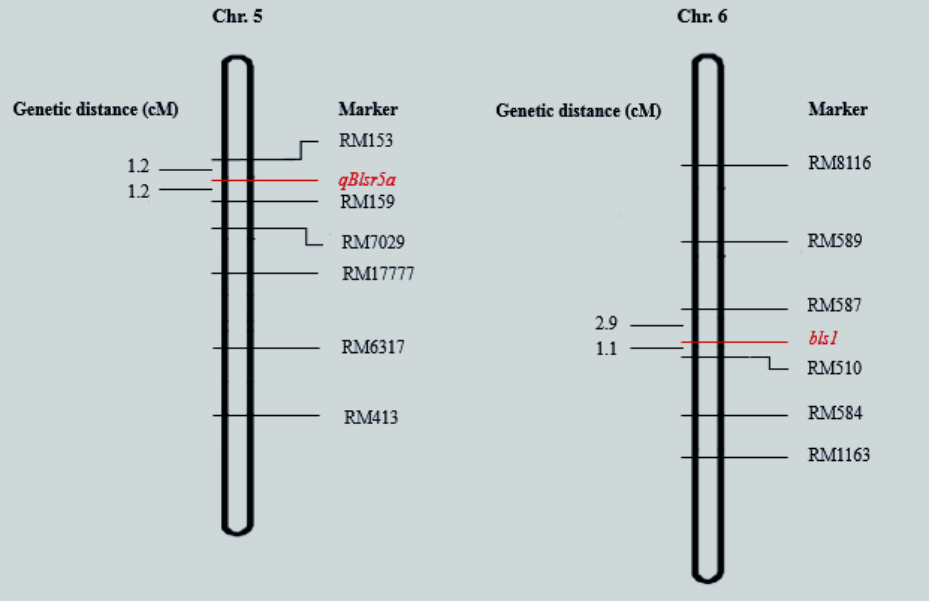

Figure 2. Position of the qBlsr5a and bls1 genes and the linked SSR markers on the short arms of chromosome 5 and 6 following Han *et al.*, 2008 and He *et al.*, 2012, respectively

Table 2. List of markers linked to resistance genes

Marker name	Sequence	Annealing temperature	Product size
RM587	Forward: ACGCGAACAAATTAACAGCC Reverse: CTTTGCTACCAGTAGATCCAGC	55°C	217 bp
RM510	Forward: AACCGGATTAGTTTCTCGCC Reverse: TGAGGACGACGAGCAGATTC	55°C	122 bp
RM153	Forward: GCCTCGAGCATCATCATCAG Reverse: ATCAACCTGCACTTGCCTGG	55°C	201 bp
RM159	Forward: GGGGCACTGGCAAGGGTGAAGG Reverse: GCTTGTGCTTCTCTCTCTCTCTCTCTCTC	55°C	248 bp

2.2.4. PCR conditions

PCR technique was conducted to detect resistance genes with specific markers as in Table 2. We used two pairs of primers, RM587-RM510, to detect the bls1 gene on chromosome 6, and two pairs of primers, RM153-RM159, to detect the qBlsr5a gene on chromosome 5 (Figure 2). All SSR primer sequences were created according to Mc Couch *et al.* (2002).

PCR conditions: 95°C for 3 minutes, and 35 cycles of 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds and 72°C for 7 minutes. PCR products were analyzed by electrophoresis with agarose gel 4% mixed with ethilium bromide 0.5 µg/ml, at 250V for 45 minutes and observed under UV light.

3. RESULTS AND DISCUSSION

3.1. Results

3.1.1. Evaluation of BLS resistance

The results of artificial inoculation with 2 isolates, TB4 and TN158, after 13 days is shown in Figure 3.

Results of artificial inoculation with distilled water as a control showed that all of the wounds did not have any lesions (Figure 4). The reaction pattern of IR24 (known as a sensitive variety) to *Xoc* showed that IR24 was S with both isolates. The average length of the lesion with isolate TB4 was about 3.71 cm and about 3.26 cm with isolate TN158. The range of the lesion lengths with isolate TB4 was distributed from 0.4 cm to 3.96 cm, and

from 0.33 cm to 3.45 cm with isolate TN158. These results showed that the virulence of TB4 was stronger than the virulence of TN158.

Reaction patterns of the 50 accessions inoculated with isolate TB4 showed that 3 were HR, 9 R, and 27 MR. Inoculation with isolate TN158 revealed 3 accessions were HR, 17 R, and 20 MR. Of these, accessions 11227, 11267-1, and 11298-2 were HR, 15 R, and 22 MR, respectively (Table 3).

3.1.2. Characterization of the resistance gene with SSR makers

Rice accessions containing the *bls1* resistance gene were defined simultaneously by two pairs of primers RM587-RM510, and the PCR products had specific bands sized 217 bp and 122 bp, respectively (Figure 5).

There were 6 accessions containing the *bls1* gene: 10255-2, 11204, 11267-1, 11189, 11298-2, and 11216. In the 6 accessions above, 3 accessions, 11204, 11189, and 11216, were collected from Phu Yen, Son La.

Rice accessions containing the *qBlsr5a* gene were identified by two pairs of primers RM153 and RM159. Specific amplification by PCR using RM159 was not successful. Based on the results of PCR using RM153, there were 6 accessions containing the *qBlsr5a* gene (Figure 6).

There were 6 accessions containing the *qBlsr5a* gene: 11088, 11203, 11082, 11049, 11189, and 11094. Of these, 5 accessions, 11088, 11203, 11082, 11189, and 11094, were collected from Son La province, and accession 11049 was collected in Lai Chau province.

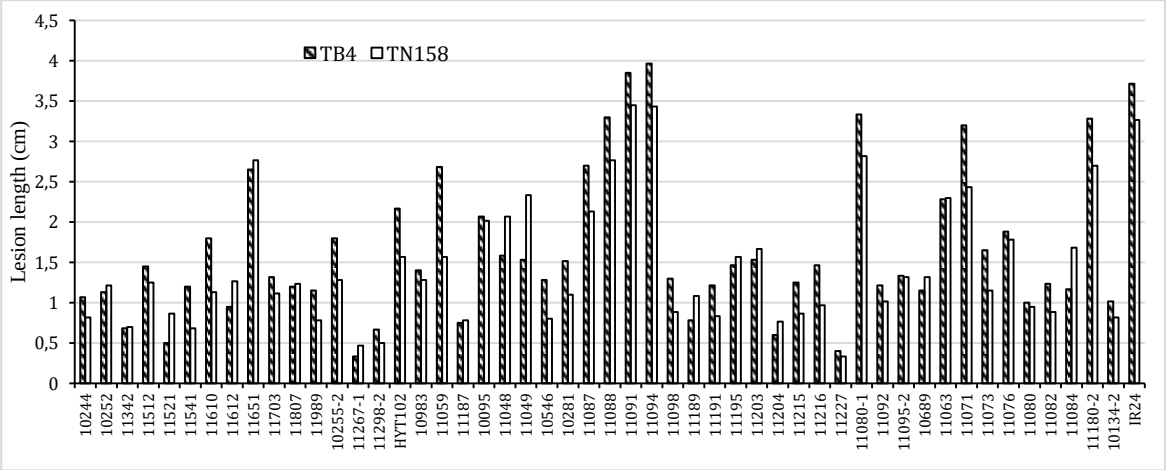

Figure 3. Chart showing the length of lesions measured at 13 days after inoculation (mm)

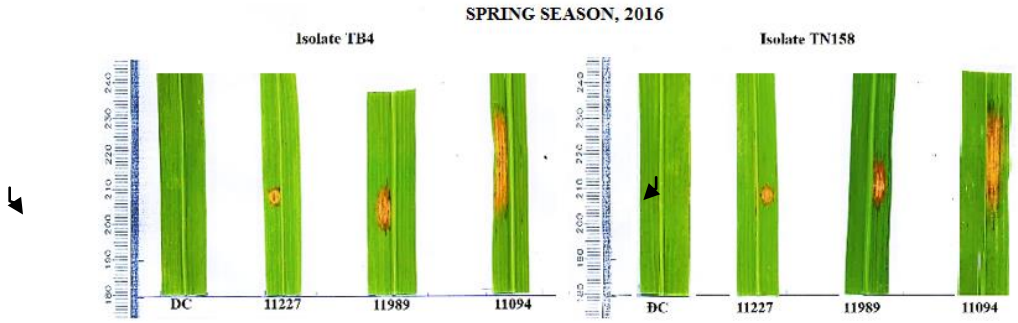

Figure 4. Lesion lengths of some accessions in the Spring season, 2015 with H_2O injection as control

Note: ĐC: Control; 11227: Short lesion; 11989: Medium lesion; 11094: Long lesion

Table 3. Distribution of lesion lengths on leaves of the 50 accessions with isolate TB4, isolate TN158, and both isolates

Level of BLS resistance	Isolate TB4	Isolate TN158	Both
Highly resistant (HR)	3	3	3
Resistant (R)	9	17	15
Moderately resistant (MR)	27	20	22
Moderately susceptible (MS)	5	8	7
Susceptible (S)	6	2	3

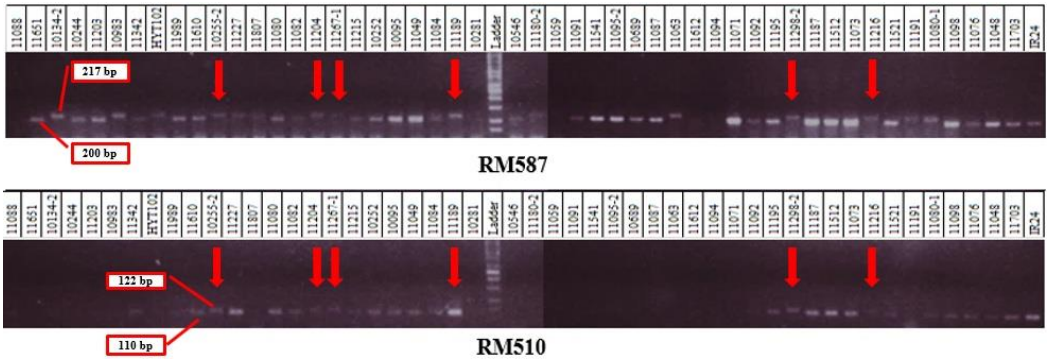

Figure 5. Results of PCR amplification of the 50 accessions with RM587-RM510 markers with IR24 as the negative control

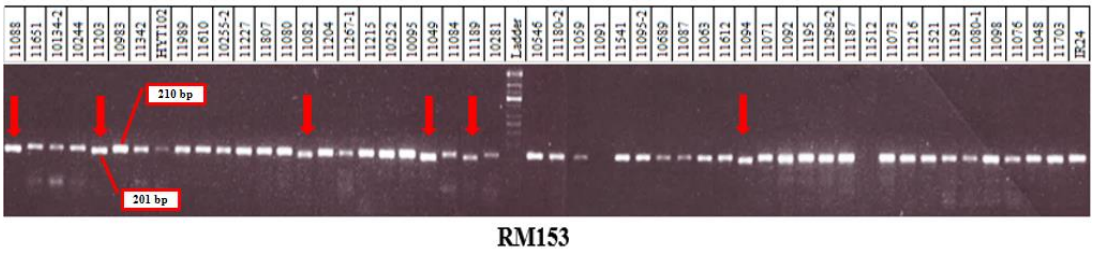

Figure 6. Results of PCR amplification of the 50 accessions with IR24 as the negative control

3.2. Discussion

To analyze the correlation between the reaction patterns and genotypes, the reaction patterns and genotypes of the *bls1* and *qBlsr5a* loci of the 50 accessions were compared, as shown in Table 4.

In the 6 accessions containing the *bls1* gene, 2 showed HR, 3 R, 1 MR, and there were no accessions showing S. In contrast, in the 6 accessions containing the *qBlsr5a* gene, 2 showed R, 2 MR, 2 S, and there were no accessions showing HR. We found that accession 11189 showed R and contained both genes.

Based on the correlation analysis between the reaction patterns and genotypes, it was revealed that the *bls1* gene had effective resistance to *Xoc*. The research of He *et al.* (2012) also indicated that rice lines containing the *bls1* gene were highly resistant to *Xoc*. In contrast, the *qBlsr5a* gene did not show clear resistance ability, while Han *et al.* (2008) showed that *qBlsr5a* had the largest effect on the resistance to *Xoc*. This might be due to the difference between the BLS pathogens in Vietnam and China.

Table 4. Correlation between reaction patterns and genotypes

Code	*bls1*	*qBlsr5a*	TB4	TN158	No.	Code	*bls1*	*qBlsr5a*	TB4	TN158
11267-1	+	-	HR	HR	26	11195	-	-	MR	MR
11227	-	-	HR	HR	27	11073	-	-	MR	MR
11298-2	+	-	HR	HR	28	11098	-	-	MR	R
11189	+	+	R	MR	29	11076	-	-	MR	MR
11204	+	-	R	R	30	11048	-	-	MR	MS
11342	-	-	R	R	31	11703	-	-	MR	MR
11080	-	-	R	R	32	10244	-	-	MR	R
11187	-	-	R	R	33	11989	-	-	MR	R
11521	-	-	R	R	34	10546	-	-	MR	R
11612	nd	-	R	MR	35	10689	-	-	MR	MR
11216	+	-	MR	R	36	11512	-	nd	MR	MR
11082	-	+	MR	R	37	11807	nd	-	MR	MR
10255-2	+	-	MR	MR	38	10281	nd	-	MR	MR
11203	-	+	MR	MR	39	HYT102	-	-	MS	MR
11049	-	+	MR	MS	40	11063	-	-	MS	MS
11092	-	-	MR	MR	41	10095	-	-	MS	MS
10134-2	-	-	MR	R	42	11651	-	-	MS	MS
10983	-	-	MR	MR	43	11087	-	-	MS	MS
11191	-	-	MR	R	44	11059	nd	-	MS	MR
11610	-	-	MR	MR	45	11080-1	-	-	S	MS
11215	-	-	MR	R	46	11071	-	-	S	MS
10252	-	-	MR	MR	47	11088	nd	+	S	MS
11084	-	-	MR	MR	48	11094	nd	+	S	S
11541	-	-	MR	R	49	11091	-	nd	S	S
11095-2	-	-	MR	MR	50	11180-2	nd	-	S	MS

Note: (+) containing resistance gene. (-) not containing resistance gene. (nd) no data

There were several accessions shown to be HR to Xoc but those did not contain either gene, and could be explained by the existence of other resistance genes. These genetic resources have the high potential for BLS resistance and need to studied further for mapping novel resistance genes.

4. CONCLUSION

Characterization of the reaction pattern to *Xoc* in 50 local rice accessions with two isolates revealed that 3 accessions were HR and15 R.

Results of genotyping the resistance genes showed that 6 accessions were found containing the *bls1* gene, and 6 accessions were found containing the *qBlsr5a*. Of these, accession 11189, collected from Phu Yen, Son La, contained both genes.

The resistance ability of the *bls1* gene was highly effective against isolates of *Xoc* in Vietnam. Therefore, these two closely linked markers, RM587 and RM510, may be used for marker assisted selection (MAS) of BLS resistant lines in rice breeding.

Acknowledgements

We would like to express our sincere thanks to Prof. Phan Huu Ton, MSc. Tong Van Hai, and the Center for Conservation and Development

of Crop Genetic Resources, Vietnam National University of Agriculture for providing us rice accessions for this research.

We would like to thank the Monsanto-VNUA scholarship, Vietnam National University of Agriculture for providing funding to complete project SV2016-12-13MST.

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