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SHORT COMMUNICATION

INTROGRESSION OF A RESISTANCE GENE TO BROWN PLANT HOPPER FROM *Oryza rufipogon* TO CULTIVARS

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INTRODUCTION

Mekong Delta is the biggest granary of Vietnam. It produces more than 50% of rice in the country. Intensive cultivation of rice in the delta has led to increased threat due to continuous changing disease races and insect biotypes. Moreover, a large part of the delta is severely affected by the acid sulfate soil conditions. Thus, pests and disease stresses lower rice productivity in Mekong Delta. Breeding brow plant hopper resistant genotypes using marker assisted selection in rice have reported (Lang et al. 1999). One of the main objectives of plant breeders is to improve existing cultivars which are deficient in one or more traits by crossing such cultivars with lines which possess the desired trait. A conventional breeding program thus involves crossing whole genomes followed by selection of the superior recombinants from among the several segregation products. Such a procedure is laborious and time consuming, involving several crosses, several generations, and careful phenotypic selection. Also, tight linkage of the desired loci with undesired loci may make it difficult to achieve the desired objective. Recombinant DNA methodology can help to overcome a few limitations, but genetic engineering approaches are also limited by the lack of sufficient number of cloned genes and the lack of availability of standardized transformation protocols in many crop species. Moreover, polygenic traits are difficult to manipulate by genetic engineering procedures. To overcome these constraints limiting rice production in Vietnam, there is an urgent need to widen the gene pool of rice cultivars cultivated in Mekong Delta. Fortunately, wild species of rice are an important reservoir of useful genes to meet these challenges. This report elaborates on the applications of DNA marker technology in the genetic analysis and the markers, which can be employed in MAS for breeding varieties and hybrids with durable bacterial blight resistance, blast resistance in rice.

MATERIALS AND METHODS

DNA isolation

A crude DNA preparation suitable for PCR analysis was prepared using a simplified miniscale procedure (Lang 2002). A single piece of healthy young leaf was harvested and placed in a labeled 1.5 ml centrifuge tube in ice. The leaf sample was macerated using thick glass rod after adding 400 μl of extraction buffer (50 mM Tris-HCl, pH 8.0, 2.5 mM EDTA, 300 mM NaCl and 1% SDS). The leaf was grounded until the buffer turned into green color. After grinding, another 400 μl of extraction buffer was added and mixed by pipetting. The contents were centrifuged at12,000 g in micro centrifuge for 10min. Nearly 400 μl of lysate was extracted with 400 μl of chloroform. The top aqueous supernatant was transferred to another 1.5 ml tube and DNA was precipitated with absolute ethanol. The contents were centrifuged for 3 min at full speed and the supernatants were discarded. The pellet was washed with 70% ethanol. The DNA was air dried and resuspended in 50 μl of TE buffer (10mMTris-HCl, pH 8.0, 1 mM EDTA, pH 8.0). One ml of aliquot was used for PCR analysis and the remaining solution was stored at –20°C for any further use.

Polymerase chain reaction (PCR amplification)

A series of optimization experiments using parents and isogenic samples was carried out in which concentrations of template DNA, primers, dNTPs and *Taq* polymerase were varied to determine which conditions The PCR reaction mixture of 20 μl contained 25–50 ng template DNA, 50 ng of each primer obtained from CLRRI, 0.05 mM dNTPs, 1X PCR buffer (10 mMTris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$ and 0.01% gelatin) and 1 unit of *Taq* polymerase obtained from Lang, Genome Mapping Laboratory, CLRRI. The template DNA was initially denatured at 94°C for 5 min followed by 35 cycles of PCR amplification under the following parameters: 1 min denaturation at 94°C, 1 min primer annealing at 55°C and 1.5–2.0 min primer extension at 72°C. A final 7 min incubation at 72°C was allowed for completion of primer extension on thermalcycler. Following amplification, the samples were run on a 1% agarose in 1X TBE buffer stained with 0.1μg/ml of ethidium bromide and visualized under ultraviolet light. For PCR-RFLP, initially 5 μl of PCR product was used for gel electrophoresis to determine successful of amplification. Seven to eight μl of the PCR product was then used for restriction enzyme digestion if the amplification was successful. The total reaction volume was usually 20 μl. The reaction mixture consisted of 11.4 μl of double distilled water, 2μl of 10 X restriction buffer, 0.6 μl restriction enzyme (10 U/μl) and 6–8 μl of PCR products. The reaction mixture was incubated at 37°C for 6–8 h. The DNA fragments digested by restriction enzymes were separated on 1.5% agarose gel and visualized under UV light after staining with ethidium bromide.

Brown plant hopper screening

To evaluated BPH resistance in germplasm, all genetic resource materials were sown in a 20 cm diameter plastic pot with a hole in the bottom. 15 days after sown, the seedlings were infested with BPH in screening boxes. Scoring of BPH reaction is followed by IRRI standard as 0, 1, 3, 5, 7 and 9.

The criteria for brown plant hopper resistance-scoring used in this study

0: None of the leaves shrank and the plant was healthy
1: One leaf was yellowing
3: One to two leaves were yellowing or one leaf shrank
5: One to two leaves shrank or one leaf shriveled
7: Three to four leaves shrank or two to four leaves shriveled, the plant was still alive
9: The plant died

On the other hand plant hopper with RGSV and RRSV, each parent and population was used to evaluate reaction to virus by inoculating brown plant hopper's nymphs

Statistical analysis

Chi square tests were performed to examine the goodness of fit between expected Mendelian ratio and SSRs and phenotype data for analysis.

RESULTS AND DISCUSSION

Reaction of brown plant hopper (BPH) to *O. rufipogon* and *O. officinalis*

Several wild species with lush degree of resistance to pests have been identified at IRRI. Similarly, *O. rufipogon*, wild rice lushly tolerant to acid sulfate soil occurs in Dong thap Muoi, Vietnam. The wild species thus offer great potential to transfer genes for tolerance to biotic and abiotic stresses into rice cultivars. CLRRI has generated a series of hybrids, and introgression lines from the crosses of elite breeding lines of rice with several wild species. Genes for resistance to brown plant hopper (BPH), bacterial leaf blight, blast and new sources of CMS (cytoplasmic male sterility) have been transpassed from several wild species into rice. Some of the BPH resistant lines from *O. sativa / O. rufipogon* have been released as commercial varieties for cultivation in Mekong Delta such as AS996. Some of genes introgressed from wild species have been tagged by molecular markers. IRRI and CLRRI have strong on-going collaboration on the evaluation and utilization of wide cross progenies.Under the Rockefeller Foundation (RF) support, tagging of BPH resistance loci was conducted with microsatellite markers at Texas A&M University from IR50 / *Oryza officinalis*. It showed that the genes for BPH resistance (biotype 4) are linked with RM18 of chromosome 7 at a distance of 1.3 cM, and RM168 of chromosome 3

at a distance of 1.9 cM. (Buu et al. 1997)

We evaluated BPH response in germplasm including 75 accessions with *O. rufipogon* and *O. officinalis* populations. The results indicated that *O. officinalis* populations exhibited highly resistant traits to BPH (table 1)

Table 1: Reaction patterns of wild rices to BPH in Vietnam

No.	Designation	Origin	Acc	Score	Damage index (%)	Response
01	*O. rufipogon*	Dong Cat State Farm, DTM	001	5	53.33	M
02	*O. rufipogon*	Dong Cat State Farm, DTM	009	5	33.33	M
03	*O. rufipogon*	Dong Cat State Farm, DTM	010	5	46.67	M
04	*O. rufipogon*	Dong Cat State Farm, DTM	011	3	31.11	R
05	*O. rufipogon*	Dong Cat State Farm, DTM	012	7	37.78	S
06	*O. rufipogon*	Hau Giang	013	3	24.44	R
07	*O. rufipogon*	Hau Giang	014	3	31.11	R
08	*O. rufipogon*	Hau Giang	015	9	10.00	S
09	*O. rufipogon*	Dong Thap Muoi	080	3	4.44	R
10	*O. rufipogon*	Dong Thap Muoi	081	5	5.78	M
11	*O. rufipogon*	Dong Thap Muoi	090	5	7.78	M
12	*O. rufipogon*	Hau Giang	101	3	3.11	R
13	*O. rufipogon*	Hau Giang	111	3	1.33	R
14	*O. rufipogon*	Kien Giang	151	3	2.22	R
15	*O. rufipogon*	Kien Giang	152	3	24.44	R
16	*O. rufipogon*	Kien Giang	156	3	28.89	R
17	*O. rufipogon*	Kien Giang	159	3	28.89	R
18	*O. rufipogon*	Kien Giang	161	3	24.44	R
19	*O. rufipogon*	Kien Giang	162	3	24.44	R
20	*O. rufipogon*	Kien Giang	166	3	40.00	R
21	*O. rufipogon*	Kien Giang	167	3	20.00	R
22	*O. rufipogon*	Kien Giang	160	3	53.33	R
23	*O. rufipogon*	Vinh Long	346	1	13.33	R
24	*O. rufipogon*	Vinh Long	347	1	15.56	R
25	*O. rufipogon*	Ca Mau	352	3	55.56	R
26	*O. rufipogon*	Ca Mau	355	5	95.56	M
27	*O. rufipogon*	Long An	300	3	26.67	R
28	*O. rufipogon*	Long An	301	1	13.33	R
29	*O. rufipogon*	Ca Mau	356	3	51.11	R
30	*O. rufipogon*	Kien Giang	339	5	68.89	M
31	*O. rufipogon*	Kien Giang	172	3	55.56	R
32	*O. rufipogon*	Kien Giang	177	3	20.00	R
33	*O. rufipogon*	Dong Thap Muoi	063	3	35.56	R
34	*O. officinalis*	Dong Nai	193	1	12.22	R
35	*O. officinalis*	Dong Nai	194	1	11.11	R
36	*O. officinalis*	Tay Ninh	195	1	5.56	R
37	*O. officinalis*	Tay Ninh	196	1	4.44	R
38	*O. officinalis*	Tay Ninh	197	1	11.11	R
39	*O. officinali*	Dam Doi, Ca Mau	198	1	6.67	R

No.	Designation	Origin	Acc	Score	Damage index (%)	Response
40	*O. officinalis*	Dam Doi, Ca Mau	199	1	13.13	R
41	*O. officinalis*	Dam Doi, Ca Mau	200	1	5.56	R
42	*O. officinalis*	Dam Doi, Ca Mau	202	1	7.78	R
43	*O. officinalis*	Dam Doi, Ca Mau	203	1	7.78	R
44	*O. officinalis*	Dam Doi, Ca Mau	204	1	8.89	R
45	*O. officinalis*	Dam Doi, Ca Mau	205	1	3.33	R
46	*O. officinalis*	Dam Doi, Ca Mau	206	1	5.56	R
47	*O. officinalis*	Dam Doi, Ca Mau	207	1	2.22	R
48	*O. officinalis*	Dam Doi, Ca Mau	208	1	4.44	R
49	*O. officinalis*	Dam Doi, Ca Mau	209	3	27.78	R
50	*O. officinalis*	Dam Doi, Ca Mau	210	1	14.44	R
51	*O. officinalis*	Dam Doi, Ca Mau	211	1	6.68	R
52	*O. officinalis*	Dam Doi, Ca Mau	212	1	5.56	R
53	*O. officinalis*	Dam Doi, Ca Mau	213	1	7.78	R
54	*O. officinalis*	Dam Doi, Ca Mau	214	1	6.67	R
55	*O. officinalis*	Dam Doi, Ca Mau	215	1	4.44	R
56	*O. officinalis*	Dam Doi, Ca Mau	216	1	3.33	R
57	*O. officinalis*	Dam Doi, Ca Mau	217	1	5.56	R
58	*O. officinalis*	Dam Doi, Ca Mau	218	1	15.56	R
59	*O. officinalis*	Dam Doi, Ca Mau	220	1	10.00	R
60	*O. officinalis*	Dam Doi, Ca Mau	221	1	11.11	R
61	*O. officinalis*	Dam Doi, Ca Mau	222	1	2.22	R
62	*O. officinalis*	Dam Doi, Ca Mau	223	1	5.56	R
63	*O. officinalis*	Dam Doi, Ca Mau	224	1	8.89	R
64	*O. officinalis*	Dam Doi, Ca Mau	225	1	11.11	R
65	*O. officinalis*	Dam Doi, Ca Mau	226	1	6.67	R
66	*O. officinalis*	Dam Doi, Ca Mau	227	1	13.33	R
67	*O. officinalis*	Dam Doi, Ca Mau	228	3	21.11	R
68	*O. officinalis*	Dam Doi, Ca Mau	229	1	3.33	R
69	*O. officinalis*	Dam Doi, Ca Mau	230	1	1.11	R
70	*O. officinalis*	Dam Doi, Ca Mau	231	1	14.44	R
71	*O. officinalis*	Dam Doi, Ca Mau	232	1	5.56	R
72	*O. officinalis*	Dam Doi, Ca Mau	233	1	6.67	R
73	*O. officinalis*	Dam Doi, Ca Mau	234	1	4.44	R
74	*O. officinalis*	Dam Doi, Ca Mau	235	1	7.78	R
75	*O. officinalis*	Dam Doi, Ca Mau	236	1	16.67	R
76	PTB33	India	200	3	44.20	M
77	TN1	IRRI	200	9		S
78	IR64	IRRI	228	3	24.11	M

DNA survey by SSRs

Evaluated genotypes by SSR markers conducted to analyze the germplasm.

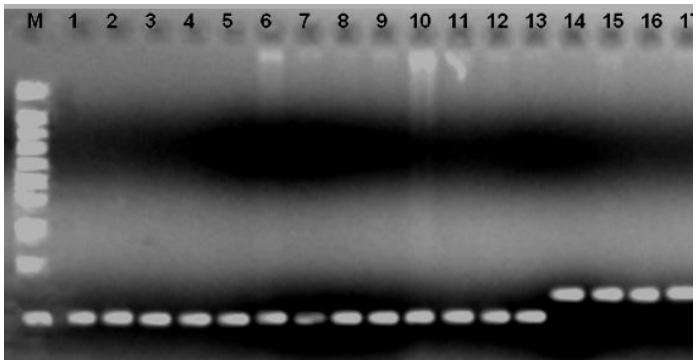

Fig 1. Diagnosis of individual lines carrying BPH genes at locus RM270
lane M: marker, lanes 1-17: BC_2F_1 of IR 64 / *O. rufipogon*

Genetic analysis of BPH resistance

Two sets of introgression lines each carrying wild and cultivated improvements have been developed from IR64 / *O.* r*ufipogon.*

For IR64 / *O.* r*ufipogon*, the 226 F_2 individuals showed a discrete distribution, segregating into 170 resistant : 56 susceptible ones. The segregation ratio fitted to a 3:1 ratio, indicating that the BPH resistance introgressed from *O. rufipogon* was controlled by a single dominant gene (table 2).

To detect BPH resistance gene, the BC_2F_1 population of IR64 / *Oryza rufipogon* was analyzed through polymorphic survey using RM270. The BC_2F_1 individuals derived from a single resistant BC_2F_1 plant were analyzed using SSR markers (Lang et al. 2005) for linkage analysis. The phenotype score for screening at 7 days infested with BPH. Ninety one plants out of BC_2F_1 plants showed resistance to BPH. These carried a common segment from *O. rufipogon* in the short arm of chromosome 12 based on SSR marker (RM270) analysis. The 121 BC_2F_1 individuals showed a discrete distribution, segregating into 91 resistant : 30 susceptible plants.

Table 2: Reaction patterns of BC_2F_1 lines to BPH from Vietnam

	Bph		RGSV		RRSV	
	Numbers of susceptible plants	Numbers of resistant plants	Numbers of susceptible plants	Numbers of resistant plants	Numbers of susceptible plants	Numbers of resistant plants
IR64 / *O.* r*ufipogon*	56	170	26	226	0	226

Brown plant hopper causes direct damage by sucking plant sap, and it also transmits several viral diseases such as rice grassy stunt virus (RGSV) (Rivera et al. 1966) and ragged stunt virus (RRSV) (Ling et al.1978). The genetic materials included 121 plants from IR64 / *O. officinalis* exhibited resistance to RGSV and RRSV, while IR64 / Nang thom Cho Dao and IR 64 / *O. rufipogon* (table 2) became susceptible.

CONCLUSION

The efficiency in selection for desirable traits in rice will be enhanced by application of marker-assisted selection technique (MAS). MAS is a tool that holds promise in raising selection efficiency and adapted to our current situation. PCR-based markers such as SSR, STS will be useful to detect

the target genes which control the resistance of BPH. Direct selection for resistance to BPH is difficult due to complicate phenotyping. Polymorphism of candidate gene marker RM270 indicated its level of polymorphism lower than RM13 for resistance to BPH. However, RM270 detected very well the polymorphism among germplasm, especially *O. rufipogon* and *O. officinalis* populations.

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Khai thác gen kháng rầy nâu từ nguồn lúa hoang *Oryza rufipogon*

Nguồn kháng rầy nâu từ hai lòai hoang dại *Oryza rufipogon, O. officinalis* đã được điều tra bằng chỉ thị phân tử. Đây là những quần thể lúa hoang đã được thu thập ở Nam Bộ. Hai chỉ thị phân tử RM13 và RM270 thể hiện hiệu quả cao trong tìm kiếm đa hình. Riêng *Oryza officinalis* có genome CC khác với lúa trồng AA, nên phải cần thời gian tạo dòng dẫn xuất (derivatives) để tiến hành lai tạo thuận lợi hơn.