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ORIGINAL ARTICLE

Assessment of the Sequence-Based Haplotype-Variants in Selected DNA Marker Loci for the Molecular Breeding of Resistant Rice Varieties to Brown Plant Hopper

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Abstract

Brown plant hopper (BPH) is the most devastating insect pest of rice. The BPH damage is trivial that farmers may end up with zero or below average yields. The application of insecticide would only lead to the emergence of virulent biotypes, thus marker assisted breeding of BPH resistant rice varieties is the only feasible solution. Although, the molecular genetics of BPH resistance and the linked DNA makers are known; the breeders struggle to use them in marker assisted breeding due to the limited ability to identify length-based allele polymorphisms in gel electrophoresis. Therefore, the present study was conducted to assess the employability of haplotype variants of the DNA markers linked to BPH resistant genes using a set of resistant and moderately resistant rice cultivars. The BPH resistant landraces Murungakayan302, PtB33, Sulai and moderately resistant Bg300, and Bw367 cultivars in Sri Lanka were assessed using six DNA makers linked to Bph resistant genes. The band length polymorphism using 2.5% agarose gel electrophoresis and haplotype variants using 3× sequence-reads of the PCR products of DNA markers were assessed. The DNA markers RM246 and C3-14 provide Murungakayan302 and Sulai specific haplotypes respectively. The DNA markers assessed yielded polymorphic bands that are closely positioned in agarose gels making the band scoring cumbersome and ambiguous. The sequences of PCR products provided distinct haplotypes for the rice cultivars. Thus, the introgression of BPH resistance or moderate resistance into rice cultivars can be undertaken using the sequence based haplotypes in place of the band polymorphisms.

Keywords: *BPH, Bph2, Bph3, Bph4, Murungakayan 302, PtB33, Sulai,*

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1. Introduction

Rice productivity is affected by many biotic and abiotic stresses. Attack by Brown Planthopper (BPH), *Nilaparvata lugens* Stål can be considered as one of the most devastating biotic stresses in rice farming (Dyck and Thomas 1979). BPH sucks an enormous amount of photosynthetic assimilates of the rice plant by piercing into the parenchymal cells of the phloem tissue (Sōgawa 1982). Mature BPH infested rice plants show chlorosis of stems, wilting of leaves, lowered productivity and ultimate death of the entire plant (Du et al. 2009). Extensive removal of the phloem sap and the deposition of ammonia from the salivary secretions of BPH result in the circular patches leading to severe 'hopperburns' (Sōgawa and Cheng 1979; Krishnaiah 2014). Functional damage done by BPH with respect to the rice plant physiology has given rise to a greater reduction in grain yield (Nagadhara et al. 2003). Furthermore, BPH acts as a vector of two viruses; Ragged Stunt Virus and Grassy Stunt Virus (Ling 1972; Du et al. 2009). These viral diseases also account for a considerable loss of rice yields (Cabauatan et al. 2009).

BPH can rapidly adapt to the host plant resistance, an enhancement of the pest virulence against host defence mechanism (Horgan 2009). Four discrete BPH strains (Biotypes) that are virulent to the host resistant genes have been identified (Jena and Kim 2010). Local BPH populations of Sri Lanka have recorded with different virulence patterns and closer adaptations to the rice varieties from which they were collected (Claridge and Hollander 1980). Moreover, outbreaks of BPH due to rigorous destruction of rice plants via large-scale pest occurrences have been recorded especially in the South Western and Eastern Sri Lanka resulting in huge disparities in rice production (Fernando et al. 1979; Horgan 2009). The outbreaks have been more persistent along with the introduction of high yielding rice cultivars and improved agronomic practices (Sōgawa and Cheng 1979).

Extensive damage caused by BPH outbreaks can be eliminated by applying insecticides and planting resistant varieties. However, insecticides have also been identified to induce the outbreaks, which result in detrimental effects on rice farming (Gallagher et al. 1994). Outbreaks have a strong correlation with the development of resistance in BPH towards particular insecticides. Insecticide such as Imidacloprid was earlier used as an effective solution against the outbreaks; however, later it became the major contributory factor promoting the BPH outbreaks due to the development of resistance (Matsumura et al. 2008). Hence, the strong correlation of outbreaks with the resistance of BPH towards the insecticides has left the rice growers with the solitary option of cultivating the resistant rice varieties.

BPH genes/loci in rice genome for the resistance to diverse BPH biotypes have been characterized (Hou et al. 2011; Yang et al. 2012; Cheng et al. 2013; Hu et al. 2016; Jing et al. 2017). The linked DNA markers have also been reported for each gene (Chang-Chao et al. 2006; Jairin et al. 2007). However, utilization of these markers in country specific or regional breeding programs is tricky as the expected length polymorphisms are depending on the platform of gel electrophoresis, running conditions and concentration of the gel material (Szoke et al. 1999; Lee et al. 2012). For the exact band size determination for DNA markers such as microsatellites, 6% or higher denaturing polyacrylamide gel electrophoresis with silver staining is required. Manufacturing and utilization of large vertical gel units are currently not practiced and the only option is to use higher % (e.g.: 2.5% or more) agarose gel electrophoresis or sequencing of the PCR products of the DNA markers to select the specific haplotypes for marker assisted breeding (Wang et al. 2003; Salmaso et al. 2005). Therefore, the present study was conducted as a ground-breaking attempt to see the applicability of BPH resistant specific haplotype variants in marker loci in comparison to the band lengths in agarose gel electrophoresis using a panel of BPH resistant and moderately resistant rice cultivars.

2. Materials and Methods

2.1 Plant material and DNA extraction

Three rice landraces that were confirmed to contain BPH resistant gene/s were selected. Two newly improved rice varieties; Bg 300 and Bw 367 (reported as moderately resistant to BPH) were also selected (Table I) [Rice Research and Development Institute (RRDI), Bathalagoda, Sri Lanka)]. Henceforth, the landraces and varieties are referred to as cultivars. The breeder seeds of the cultivars were collected from RRDI. The seeds were germinated, and the seedlings were maintained for two weeks to obtain immature leaf material for DNA extraction. The collected leaves were ground into fine powder samples in liquid nitrogen using mortar and pestle. The genomic DNA was isolated using DNeasy Plant Mini Kit (Qiagen, Solna, Sweden).

Table 1: BPH resistant and sensitive rice cultivars assessed for microsatellite polymorphism

Variety	Type	Age (Months)	BPH resistant gene/s present	Remarks
Murungakayan302	Landrace (Sri Lanka)	4	*Bph2* (Lakshminarayana and Kush 1977; Kaneda et al.1981)	Susceptible to BPH biotype 3. (Kaneda et al.1981) Resistant to BPH biotype 2. (Kaneda et al.1981)
PtB33	Landrace. (*Pathambi*, India)	4	*Bph2* (Angeles et al.1986) *Bph3* (Sidhu and Khush 1978; Jairin et al. 2007; Horgan et al. 2015)	Highly resistant to BPH populations in many Asian countries. (Seshu and Kauffmann 1980) Resistant to BPH and Green Leaf Hopper (Nugaliyadde et al. 2000)
Sulai	Landrace (Sri Lanka)	4-4.5	*Bph4* (Hu et al. 2016)	Red rice Moderate Resistant to BPH Prone to lodging
Bg 300	Newly improved variety (Bg 367-7//IR 841/Bg 276-5)	3	PtB33 has been used to give BPH resistance to Bg 300 (Nugaliyadde et al. 2001 and 2004)	Resistant to gold midge, blast and bacterial blight Moderately resistant to BPH Intermediate bold grain shape with white colour pericarp
Bw 367	Newly improved variety (Bw 361/ Bg 358)	3.5	Bg 379-2 has been used to provide resistance to Bw 367	Moderately susceptible or moderately resistant to gold midge and bacterial blight resistant or moderately resistant to blast and BPH Short round grain shape with white colour pericarp

2.2 PCR, DNA sequencing and data analysis

PCR was conducted with a panel of DNA markers that are linked to BPH resistant genes (Table II). PCR conditions were provided by using a Thermal Cycler (Takara, Japan) with following criteria; initial denaturation at 94 °C for 5 mins followed by 35 cycles of denaturation at 94 °C for 30 sec, primer annealing at temperature (Ta) (Table

2) for 1 min, extension at 72 °C for 2 mins and final extension at 72 °C for 10 mins. PCR products were size separated in 2.5% agarose gel electrophoresis.

PCR products were purified using a QIAquick® PCR purification kit (Catalog No: 28104, Qiagen, Hilden, Germany) and subjected to 3× DNA sequencing in Macrogen Inc., South Korea. The sequences obtained were used to carry out a reference sequence search in NCBI Nucleotide Blast Tool. Then, the sequences obtained for each marker was aligned with the reference sequences retrieved *via* Clustal W algorithm in Mega 7 (Kumar et al. 2016). Sequences were manually inspected for any inaccuracies in automated sequencing. Next, forward and reverse end noise was eliminated, and consensus sequences were set. Ambiguous regions were eradicated, and our sequences alone were aligned using the Clustal W algorithm to detect the unique haplotypes of the five varieties affecting the BPH resistance and susceptibility.

Table 2: DNA markers assessed for identification of the haplotypes linked to BPH resistance

Resistant Gene	Marker	Primer Sequences (5'→3')	T_a (°C)	Reference
Bph2	*RM463*	TTCCCCTCCTTTTATGGTGC TGTTCTCCTCAGTCACTGCG	55	Li-Hong et al. (2006)
Bph2	*RM7102*	TTGAGAGCGTTTTTAGGATG TCGGTTTACTTGGTTACTCG	55	Li-Hong et al. (2006)
Bph2	*RM1246*	CTCGATCCCCTAGCTCTC TCACCTCGTTCTCGATCC	55	Li-Hong et al. (2006)
Bph3	*RM589*	ATCATGGTCGGTGGCTTAAC CAGGTTCCAACCAGACACTG	55	Liu et al. (2016)
Bph4	*RM217*	ATCGCAGCAATGCCTCGT GGGTGTGAACAAAGACAC	55	Kawaguchi et al. (2001)
Bph4	*C3-14*	GGCAAAATTAGACGGCACG GAATATGCATTTTGTTTGGAG	55	Hu et al. (2015)

3. Results and Discussion

3.1 Band-length polymorphisms of the DNA markers for selecting BPH resistance in breeding

The resistant cultivar *Murungakayan302*, Ptb33 and *Sulai*, and the moderately resistant cultivars Bg 300 and Bw 367 contain two different bands (i.e. alleles) for the *Bph2* linked markers *RM1246*, *RM463* and *RM7102*. For the *Bph3* linked marker *RM589* and *Bph4* linked markers *RM217* and *C3-14* contains three alleles (Fig. 1). The specific band found in *Sulai* for the marker *C3-14* can be used to introgress the *Sulai* specific allele to the rice cultivars. Also, the other bands detected for the six markers can be used to check the introgression of BPH resistant alleles to the rice varieties in the future breeding programs. However, even at 2.5% agarose gel electrophoresis, the bands were less resolved in to close positions making the scoring difficult.

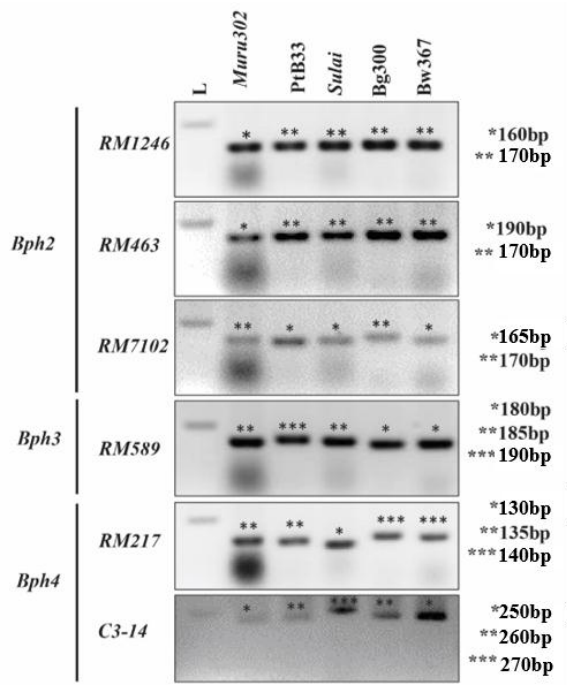

Figure 1: The polymorphism of BPH resistance linked DNA markers. The corresponding Bph resistant gene (*Bph2*, *Bph3*, and *Bph4*) and marker names are given on the left side and variety names are given on the top. The approximate band size is denoted on the right side. The number of star marks (*) represent the polymorphic bands separately in the increasing order of their sizes given in base pairs (bps).

3.2 Haplotype sequence-variants of marker loci for selecting BPH resistance in breeding

For *RM1246* marker, *Murungakayan302* and Ptb33 shared unique haplotypes whereas *Sulai*, Bg 300 and Bw 367 shared a common haplotype. For *RM463*, three haplotypes were identified in which *Murungakayan302* contains a unique haplotype. For *RM7102, Murungakayan302*, *Sulai* and Bg 300 contains unique haplotypes whereas Ptb33, Bw 367 share a common haplotype. For *Bph3* linked marker *RM589*, *Murungakayan302*, Ptb33, and *Sulai* possess unique haplotypes whereas Bg 300 and Bw 367 share a common haplotype. *RM217*, *Bph4* linked marker, provides unique haplotypes for *Murungakayan302* and Ptb33. However, *Sulai* haplotype is also seen in Bg 300 and Bw 367. For *C3-14* marker, which is linked to *Bph4*, unique haplotypes identified for the *Murungakayan302*, Ptb33, and *Sulai*. However, shared haplotype was observed in Bg 300 and Bw 367 (Figure 2; Table III).

The introgression of the alleles of *Bph* resistance genes from the rice landraces to improved cultivars is the only viable solution to combat against BPH (Jena and Kim 2010). The marker assisted backcross breeding is the most pragmatic approach to introgress the favourable alleles from landraces to the cultivated germplasm (Frisch and Melchinger 2001; Bouchez et al. 2002). However, the DNA markers available in the public domain cannot be directly used in breeding programs. The length polymorphisms are ideal for the selection (Yang et al. 1994) however, the length difference among marker bands are too small to detect with limited facilities available in developing countries. In the present study, with 2.5% agarose gel electrophoresis, the polymorphic bands were observed, however, their close positions on the gels made it difficult to call the exact band sizes. This demands difficult and time-consuming polyacrylamide gel electrophoresis for the resolution of alleles in breeding programs that often have many individuals to be genotyped for marker assisted selection. In addition to the tediousness of the process of electrophoresis; the laboratory chemicals, equipment and conditions greatly affect the detection of 1-20 bp band length differences accurately. Therefore, the most feasible solution is, if 2.5% or higher percentage agarose gel electrophoresis is not capable of detecting the maker allelic status, the sequencing of PCR products of the marker loci and select based on the sequence haplotypes (Varshney et al. 2009). As the sequencing cost is getting low, DNA sequencing facilities both locally and internationally are now greatly facilitating the large-scale sequencing of populations as a routine tool in breeding (He et al. 2014).

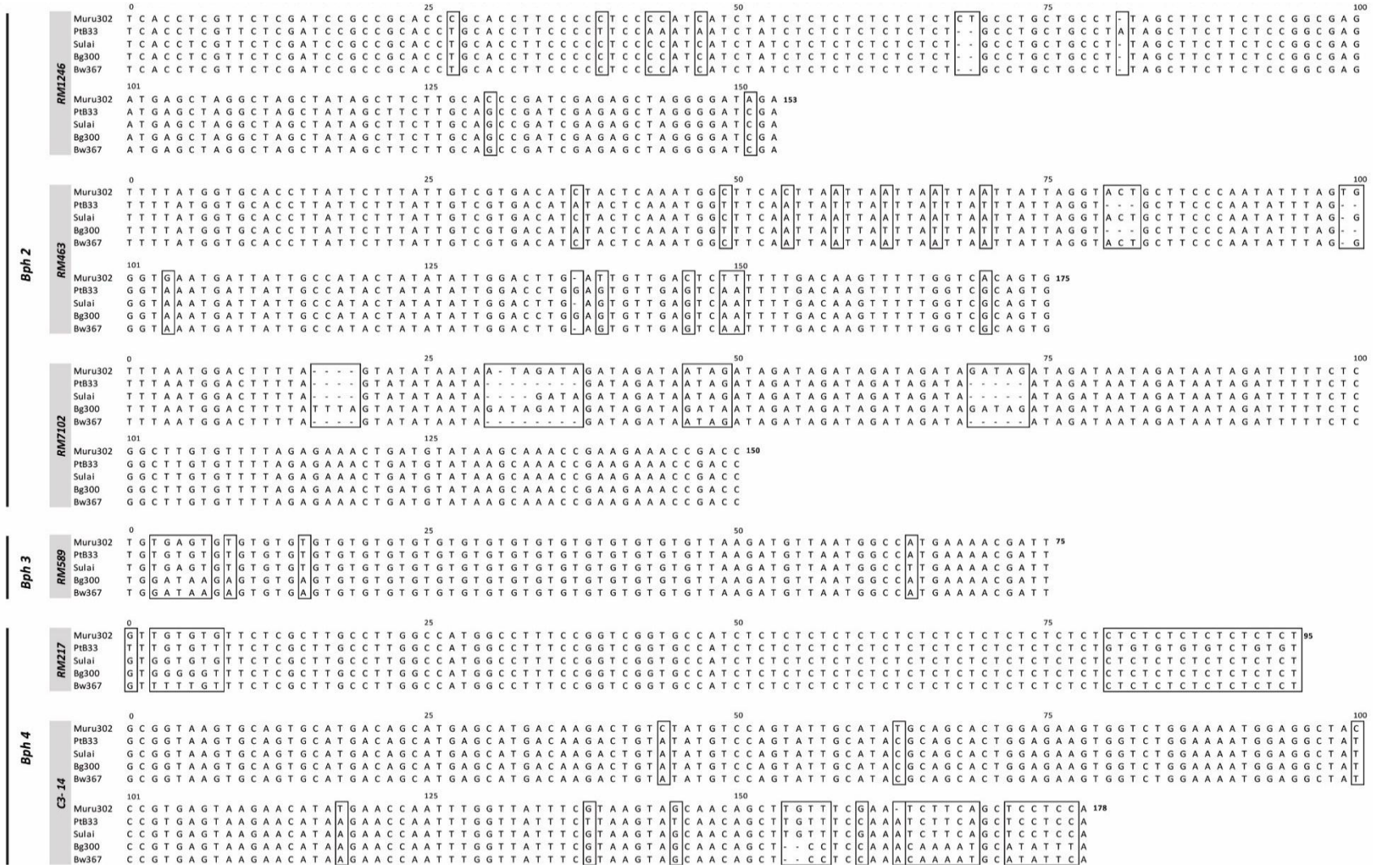

Figure 2: The sequence alignments of six maker loci for five rice cultivars. The marker names and linked BPH resistant genus are shown in the left. The primer regions were trimmed off from the alignment. The relative base positions (in 25-base intervals) are given above the sequence and total length of each alignment is given at the end just after the last base of each sequence. The useful polymorphic sites for marker assisted breeding of BPH resistance are shown within boxes drawn over the alignments.

Table 3: SNPs and INDEL based haplotypes of the marker loci for the selection for BPH resistance in breeding

BPH resistant gene	Marker	Position (bp)*	*Muru302*	PtB33	*Sulai*	Bg300	Bw367
Bph2	***RM1246***	27	C	T	T	T	T
		39	C	T	C	C	C
		43-44	CC	AA	CC	CC	CC
		47	C	A	C	C	C
		68-69	CT	--	--	--	--
		81	-	A	-	-	-
		130	C	G	G	G	G
		151	A	C	C	C	C
	RM463	37	C	A	C	A	C
		49	C	T	C	T	C
		54	C	A	A	A	A
		58	A	T	A	T	A
		62	A	T	A	T	A
		66	A	T	A	T	A
		70	A	T	A	T	A
		80-82	ACT	---	ACT	---	ACT
		99-100	TG	--	-G	--	-G
		104	G	A	A	A	A
		138	-	G	-	G	-
		140	T	G	G	G	G
		146	C	G	G	G	G
		149-150	TT	AA	AA	AA	AA
		170	A	G	G	G	G
	RM7102	16-19	----	----	----	TTTA	----
		30-37	A-TAGATA	--------	----GATA	GATAGATA	--------
		46-49	ATAG	ATAG	ATAG	GATA	ATAG
		69-73	GATAG	-----	-----	GATAG	-----
Bph3	***RM589***	3-7	TGAGT	TGTGT	TGAGT	GATAA	GATAA
		9	T	T	T	A	A
		15	T	T	T	A	A
		64	A	A	T	A	A
Bph4	***RM217***	1	G	T	G	G	G

	3-8	TGTGTG	TGTGTT	GGT	GGGGGT	TTTTG
				GTG		T
	80-95	(CT)8	(GT)5(CT)1(GT)2	(CT)8	(CT)8	(CT)8
C3-14	44	C	A	A	A	A
	63	T	C	C	C	C
	100	C	T	T	T	T
	118	T	A	A	A	A
	138	G	T	G	G	G
	145	G	T	G	G	G
	154-157	TGTT	TGTT	TGT	--CC	--CC
				T		
	160	G	C	G	C	C
	163	-	A	A	A	A
	164-169	TCTTCA	TCTTCA	TCT	CAAAAT	CAAAA
				TCA		T
	172-177	TCCTCC	TCCTCC	TCC	ATATTT	ATATT
				TCC		C

*Position (bp) as in the alignment shown in Fig 2

4. Conclusion

The present study proved that the DNA markers linked to BPH resistant genes provide polymorphic bands that are closely positioned in high concentration agarose gel electrophoresis. This makes the band scoring difficult which hinders the transferability of polymorphic band data across different laboratories. However, the sequencing of PCR products of the assessed markers provided clearly distinguishable haplotypes for the studied resistant and moderately resistant cultivars. Therefore, in marker assisted breeding for BPH resistance of rice, the introgression of BPH resistance or moderate resistance into novel cultivars can be swiftly practiced using the sequence based haplotypes rather than using the band polymorphisms detected in gel electrophoresis.

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6. References

Angeles, E R, Khush G S, Heinrichs E A. (1986). Inheritance of resistance to planthoppers and leafhopper in rice. In: IRRI (Ed.) Rice Genetics, IRRI, Los Baños, Philippines, pp537–549.

Bouchez A, Causse M, Gallais A, Charcosset A (2002). Marker-assisted introgression of favourable alleles at quantitative trait loci between maize elite lines. Genetics 162(4): 1945-1959.

Cabauatan, P Q, Cabunagan, R C, Choi I R (2009). Rice viruses transmitted by the brown planthopper Nilaparvata lugens Stål. In: Planthoppers: New threats to the sustainability of intensive rice production systems in Asia, Heong KL, Hardy B (Eds.) International Rice Research Institute, Los Banos, Philippines, pp357-368.

Chang-Chao Su, Hu-Qu Zhai, Chun-Ming Wang, Li-Hong Sun, Jian-Min Wan (2006) SSR mapping of brown planthopper resistance gene *Bph9* in *Kaharamana*, an *indica* rice (*Oryza sativa* L.). Acta Genetica Sinica 33(3): 262-268

Cheng X, Zhu L, He G (2013) Towards understanding of molecular interactions between rice and the brown planthopper. Mol Plant 6(3):621-634.

Claridge M F, Hollander J D (1980) The ―biotypes of the rice brown planthopper, *Nilaparvata lugens*. Entomol Exp Appl 27(1):23-30.

Du B, Zhang W, Liu B, Hu J, Wei Z, Shi Z, He R, Zhu L, Chen R, Han B, He G (2009) Identification and characterization of *Bph14*, a gene conferring resistance to brown planthopper in rice. Proc. Natl. Acad. Sci. U.S.A. 106(52):22163-22168.

Dyck V A, Thomas B (1979) The brown planthopper problem. In: Brown planthopper: threat to rice production in Asia. International Rice Research Institute, Los Banos, Philippines pp 3-17.

Fernando H, Senadhera D, Elikawela Y, De Alwis H M, Kudagamage C (1979) Varietal resistance to the brown planthopper in Sri Lanka. In: Brown Planthopper: Threat to Rice Production in Asia. International Rice Research Institute, Los Banos, Philippines pp241-249.

Frisch M. and Melchinger A E. (2001). Marker-assisted backcrossing for introgression of a recessive gene. Crop science 41(5):1485-1494.

Gallagher, K D Kenmore, P E Sogawa, K (1994) Judicial use of insecticides deter planthopper outbreaks and extend the life of resistant varieties in Southeast Asian rice. Planthoppers pp599-614.

He J, Zhao X, Laroche A, Lu Z X, Liu H, Li Z (2014). Genotyping-by-sequencing (GBS), an ultimate marker-assisted selection (MAS) tool to accelerate plant breeding. Frontiers in plant science 5: 484

Horgan F (2009). Mechanisms of resistance: a major gap in understanding planthopper-rice interactions. Planthoppers: New threats to the sustainability of intensive rice, p62.

Horgan F G, Ramal A F, Bentur J S, Kumar R, Bhanu KV, Sarao P S, Iswanto E H, Van Chien H, Phyu M H, Bernal C C, Almazan M L P (2015) Virulence of brown planthopper (*Nilaparvata lugens*) populations from South and South East Asia against resistant rice varieties. Crop Prot 78:222-231.

Hou Ly, Yu P, Xu Q, Yuan Xp, Yu Hy, Wang Yp, Wang Ch, Wan G, Tang Sx, Peng St (2011) Genetic analysis and preliminary mapping of two recessive resistance genes to brown planthopper, *Nilaparvata lugens* Stål in rice. Rice Sci 18:238-242.

Hu J, Xiao C, Cheng M, Gao G, Zhang Q, He Y (2015) Fine mapping and pyramiding of brown planthopper resistance genes *QBph3* and *QBph4* in an introgression line from wild rice *O. officinalis*. Mol Breed 35(1):3.

Hu J, Xiao C, He Y (2016) Recent progress on the genetics and molecular breeding of brown planthopper resistance in rice. Rice 9(1):1-12.

Jairin J, Phengrat K, Teangdeerith S, Vanavichit A. Toojinda T (2007) Mapping of a broad-spectrum brown planthopper resistance gene, *Bph3*, on rice chromosome 6. Mol Breed 19(1): 35-44.

Jena K K, Kim S M (2010) Current status of brown planthopper (BPH) resistance and genetics. Rice (3):161-171.

Jing S, Zhao Y, Du B, Chen R, Zhu L, He G (2017) Genomics of interaction between the brown planthopper and rice. Curr Opin Insect Sci 19**:**82-87.

Kaneda C, Ito K, Ikeda R (1981) Screening of rice cultivars for resistance to the brown planthopper, *Nilaparvata lugens* Stål., by three biotypes. Jpn J Breed 31(2):141-151.

Kawaguchi M, Murata K, Ishii T, Takumi S, Mori N, Nakamura C (2001) Assignment of a brown planthopper (*Nilaparvata lugens* Stål) resistance gene *bph4* to the rice chromosome 6. Breeding Sci 51(1):13-18.

Krishnaiah N V (2014) A global perspective of rice brown planthopper management I--crop-climatic requirement. Int J Mol Zool 4(2): 45-55.

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol Biol Evol 33(7):1870-1874.

Lakshminarayana A, Khush G S (1977) New genes for resistance to brown planthopper in rice. Crop Sci 17: 96-100.

Lee P Y, Costumbrado J, Hsu C Y, Kim Y H (2012) Agarose gel electrophoresis for the separation of DNA fragments. J Vis Exp 62:3923.

Li-Hong S, Chun-Ming W, Chang-Chao S, Yu-Qiang L, Hu-Qu Z, Jian-Min W (2006) Mapping and marker-assisted selection of a brown planthopper resistance gene *bph2* in rice (*Oryza sativa* L.). Acta Genetica Sinica 33(8):717-723.

Ling K C (1972) Rice virus diseases, International Rice Research. Institute., Los Banos, Philippines pp78-102.

Liu Y, Chen L, Liu Y, Dai H, He J, Kang H, Pan G, Huang J, Qiu Z, Wang Q, Hu J (2016). Marker assisted pyramiding of two brown planthopper

resistance genes, *Bph3* and *Bph27(t)*, into elite rice cultivars. *Rice* **9**(1): 27.

Matsumura M, Takeuchi H, Satoh M, Sanada-Morimura S, Otuka A, Watanabe T, Van Thanh D (2008) Species-specific insecticide resistance to imidacloprid and fipronil in the rice planthoppers *Nilaparvata lugens* and *Sogatella furcifera* in East and South-east Asia. Pest Manag Sci 64(11):1115-1121.

Nagadhara D, Ramesh S, Pasalu I C, Rao Y K, Krishnaiah N V, Sarma N P, Bown D P, Gatehouse J A, Reddy V D, Rao K V (2003) Transgenic *indica* rice resistant to sap-sucking insects. Plant Biotechnol J 1(3):231-240.

Nugaliyadde L, Des Abeysiriwardena D S, Samanmalee L G A, Pathirana R, Wilkins R M (2004). Inheritance of resistance in rice to brown planthopper: its implications on rice varietal improvement in Sri Lanka.

Nugaliyadde L, Dissanayake N, Mitrasena J (2000) Advances of pest and disease management of rice in Sri Lanka: A review. Annual Symposium of the Department of Agriculture, Sri Lanka pp 402-422.

Salmaso M, Faes G, Segala C, Stefanini M, Salakhutdinov I, Zyprian E, Toepfer R, Grando M S, Velasco R (2005) Genome diversity and gene haplotypes in the grapevine (*Vitis vinifera* L.), as revealed by single nucleotide polymorphisms. Mol Breed 14(4):385-395.

Seshu D V, Kauffmann H E (1980) Differential response of rice varieties to the brown planthopper in international screening tests. International Rice Research Institute, Los Banos, Laguna Philippines.

Sidhu G S, Khush G S (1978) Genetic analysis of brown planthopper resistance in twenty varieties of rice, *Oryza sativa* L. Theor Appl Genet 53(5): 199-203.

Sōgawa K (1982) The rice brown planthopper: feeding physiology and host plant interactions. Annu Rev Entomol 27(1):49-73.

Sōgawa K, Cheng C H, (1979). Economic thresholds, nature of damage, and losses caused by the brown planthopper. Brown planthopper: Threat to rice production in Asia pp125-142.

Szoke M, Sasvari-Szekely M, Guttman A (1999) Ultra-thin-layer agarose gel electrophoresis: I. Effect of the gel concentration and temperature on the separation of DNA fragments. J Chromatogr A 830(2):465-471.

Varshney R K, Nayak S N, May G D, Jackson S A (2009). Next-generation sequencing technologies and their implications for crop genetics and breeding. Trends in biotechnology 27(9): 522-530.

Wang D, Shi J, Carlson S R, Cregan P B, Ward R W, Diers B W (2003) A low-cost, high-throughput polyacrylamide gel electrophoresis system for genotyping with microsatellite DNA markers. Crop sci 43(5):828-1832.

Yang G P, Maroof M S, Xu C G, Zhang Q, Biyashev R M (1994). Comparative analysis of microsatellite DNA polymorphism in landraces and cultivars of rice. Molecular and General Genetics 245(2): 187-194.

Yang L, Li R B, Li Y R, Huang F K, Chen Y Z, Huang S S, Huang L F, Liu C, Ma Z F, Huang D H, 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(2): 161-171.