Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(6): 939-944
Received: 17-09-2020
Accepted: 21-10-2020

Thiyagarajan Thulasinathan
Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, TNAU, Coimbatore, Tamil Nadu, India

Jagadeeshselvam Nallathambi
Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, TNAU, Coimbatore, Tamil Nadu, India

Hifzur Rahman
Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, TNAU, Coimbatore, Tamil Nadu, India

Rohit Kambale
Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, TNAU, Coimbatore, Tamil Nadu, India

Bharathi Ayyenar
Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, TNAU, Coimbatore, Tamil Nadu, India

Balasubramani Venkatasamy
Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, TNAU, Coimbatore, Tamil Nadu, India

Raveendran Muthurajan
Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, TNAU, Coimbatore, Tamil Nadu, India

Corresponding Author:
Raveendran Muthurajan
Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, TNAU, Coimbatore, Tamil Nadu, India

Marker assisted introgression and validation of resistance genes *Bph20* and *Bph21* for brown plant hopper (*Nilaparvata lugens* sta1) into a popular rice variety of CO51

Thiyagarajan Thulasinathan, Jagadeeshselvam Nallathambi, Hifzur Rahman, Rohit Kambale, Bharathi Ayyenar, Balasubramani Venkatasamy and Raveendran Muthurajan

Abstract
Rice production needs to be doubled by 2050 because rice is the stable food for half of the world's population. Rice productivity is greatly affected by various biotic stresses. Among biotic stresses the brown plant hopper is one of the most serious constraints and devastating insect pest of rice in most rice growing areas of the world which causes significant yield loss in rice production. Developing resistance in host plant is important and it is the most effective method to control brown plant hopper damage. The host plant resistance can be developed through pyramiding of more than one gene in a single plant, because the single gene resistance can be suddenly broken by the emergence of new BPH biotypes. In order to develop improved version in a popular rice variety of CO51 against BPH resistance. The advanced early generation BC_1F_3 (BILs) mapping population lines were developed by cross between CO51 and donor IR71033-121-15B.The SSR markers namely RM16556 and RM3331 were employed for foreground selection of *Bph20* and *Bph21* resistance genes respectively. The homozygous positive *Bph20* and *Bph21* pyramided lines were used for screening against Brown plant hopper, the phenotypic screening work was carried out under glass house condition. The BILs 12-35-6-3, 12-35-7-16 and 12-35-13-1 were recorded with moderate resistance to BPH.

Keywords: Gene pyramiding, Rice, CO51, BPH resistance genes, *Bph20*, *Bph21*

Introduction
Rice is an important cereal food crop in the Asia-pacific region of the world. More than 52% of world rice production is affected by various biotic stresses, in which 21% of rice production lost due to insect pest damage (Brookes and Barfoot, 2003) [6]. Insect pests are major problem in Rice production system, BPH is one of the most serious and devastating pest of rice. Brown plant hopper *Nilaparvata lugens* Sta1 sucks the sap from phloem of the plants and causes hopper burn (Watanabe and kitagawa, 2000) [32]; and more infestation results in the lodging of the crops with yield loss upto 10-70%. BPH acts as a vector system for carrying viruses like grassy stunt virus and ragged stunt virus results in the further yield loss in rice production (Brar *et al.*,2009; Cha *et al.*, 2008) [5, 7]. Less infestations by the BPH causes reduction in plant height, vigour, number of productive tillers and grain filling. Likewise severe infestations by the BPH causes ''hopper burn'' symptoms which includes death of the plants and complete drying.

The BPH biotypes are widely present in South and Southeast Asia. The biotype 4 is the most devastating biotype present in South Asia, and it is widely occur over the Indian subcontinent (Heinrichs, 1985) [10]. The resistant varieties against BPH has been developed by using some of the identified effective BPH resistance genes (Suh *et al*., 2011) [30]. However some of the resistant varieties which are harboring single BPH resistance gene were prone to rapidly broken down within a short period of time due to the rapid adaptation of BPH or emergence of new biotypes (Jena and Kim, 2010) [16]. For BPH management in rice ecosystem several methods have been followed which includes both chemical and biological control measures (Normile, 2008) [23]. But compare to conventional chemical control measure, developing host plant resistance by BPH resistance genes has been considered as the most effective and economical approach for controlling the BPH (Matsumura *et al*., 2009) [22].

The identification of BPH resistance germplasm resources from various varieties and Introgressing such BPH resistance genes into a elite rice cultivar has emerged as important component in breeding programs (Pathak *et al*., 1969; Alam and Cohen, 1998a) [24, 1].

Pyramiding multiple resistance genes is a sustainable and eco-friendly strategy to develop durable resistant varieties against BPH. Molecular marker assisted selection is used as an efficient and rapid method for introgression and pyramiding BPH resistance genes (Qiu *et al*., 2010) [26]. So far 34 BPH-resistance loci have been identified from indica and wild rice (Hu *et al*., 2018; Kumar *et al*., 2018) [13, 20]. Out of 33, 22 QTLs or genes were fine mapped (jairin *et al*., 2007; Rahman *et al*., 2009) [15, 28]; and remaining genes were identified and isolated by map based cloning (Du *et al*., 2009) [9]. Most of the identified genes are reported as dominant and few were recessive genes such as *bph4*, *bph5*, *bph7*, *bph8*, *bph19*, *bph25* and *bph29*. Apart from this some previous research paper reported that, *Bph13* was present on chromosome 2, *Bph11*, *Bph13*, *Bph14*, and *Bph19* genes were present on chromosome 3, *Bph12*, *Bph15*, *Bph17*, and *Bph20* genes were present on chromosome 4 (Rahman *et al*., 2009) [28]. *bph4* genes were present on chromosome 6 (Kawaguchi *et al*., 2001) [19]. *Bph6* was located on chromosome 11 (Jena *et al*., 2002) [17]. *Bph1*, *bph2*, *Bph9*, *Bph10*, *Bph18*, and *Bph21* genes were located on chromosome 12 (Jena *et al*., 2006; Sharma *et al*., 2004) [18, 29]. *Bph33* and *Bph34* genes provides resistance for BPH (Hu *et al*., 2018; Kumar *et al*., 2018) [13, 20].

The previous research demonstrated that the varieties were developed with *Bph3* resistant gene have been used in cultivation for over 30 years in the phillipines, they still shows resistance effect against BPH (Penalver Cruz *et al*., 2011) [25]. Myint and his coworkers found that BPH resistance level of NILs harboring resistance genes *Bph25* and *Bph26* was significantly higher than either *Bph25*-NILs or *Bph26*-NILs (Myint *et al*., 2012) [21]. An additive effect was observed after introgression of three dominant BPH resistance genes such as *Bph14*, *Bph15* and *Bph18* into elite indica rice variety 93-11 than the double gene lines or monogenic lines (Hu *et al*., 2013) [12].

The identification of two BPH resistance genes such as *BPh20* and *Bph21* which are present on the Chromosome 4 and Chromosome 12 respectively from the wild species of *O. minuta* (2n=48, BBCC genome) belongs to a *O. officinalis* complex used as resistant sources for BPH. IR71033-121-15B is a cross derivative line between *O. sativa* and *O. minuta* carries the *Bph20* and *Bph21* resistance genes, and it has shown higher resistance to biotypes of BPH. The present study was carried out to evaluate advanced early generation BC_1F_3 (BILs) lines harboring *Bph20* and *Bph21* resistance genes in the background of popular elite cultivar CO51 for BPH resistance.

Materials and Methods

The elite popular rice cultivar CO51 has high yield potential and fine rice grain type, which is suitable for cultivation during all rice seasons in Tamil Nadu was used as the recurrent parent. The CO51 was crossed with the donor IR71033-121-15B which harbors the resistance genes *Bph20* and *Bph21*.The BC_1F_1 mapping population was developed with the help of marker assisted backcross breeding (MABB) approach in Department of Plant Biotechnology, TNAU Coimbatore. The foreground selection was carried out in BC_1F_1 to identify the positive plants with heterozygous loci by using tightly linked SSR markers namely RM16556 and RM3331 for *Bph20* and *Bph21* genes respectively. The identified positive plants were selfed and advanced to BC_1F_2 and BC_1F_3 generation. Foreground selection was carried out for all the plants to identify the positive plants with homozygous loci for the *Bph20* and *Bph21*.The BC_1F_3 homozygous positive plants for double gene and single gene were selected and utilized for evaluation of BPH resistance along with recurrent parent CO51, donor parent IR71033-121-15B, resistant check PTB33 and susceptible check Taichung native1 (TN1).

Genomic DNA was extracted from all the plants by using modified cetyl trimethyl ammonium bromide protocol (Ausubel F.M. 1994) [3]. DNA was measured by using Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). PCR was carried out in 15 µl reactions containing 50-100 ng of DNA template, 8.0 µl of sterile water, 1.5 µl of Assay buffer (10 X), 0.50 µl of 2.5 mM dNTPs, 0.20 µl (3 U/µl) of *Taq* DNA polymerase and 1.00 µl each of 10 µM forward primer and reverse primer. PCR amplification was done with a profile of 35 cycles at 94 ^{0}C for 5 min initial denaturation, 94 ^{0}C for 1 min denaturation, 50 ˚C/55 ˚C for 1 min annealing, 72 ^{0}C for 1 min extension, 72 ˚C for 10 min final extension and 4 ^{0}C for hold. The PCR products were resolved by 3.5% agarose gel electrophoresis in 1X TBE buffer and bands were observed after Ethidium Bromide staining and documented using a gel documentation system (BIO-RAD, USA) and banding pattern were scored.

Brown plant hopper used for bioassays were collected from paddy fields and maintained on the TN1 (Susceptible cultivar) in the greenhouse of Entomology at Department of Rice, TNAU Coimbatore. The bioassay experiment was conducted at relative humidity of 70-80% and the ambient temperature of 28-30 ^{o}C by standard seed box screening technique proposed at IRRI (Heinrichs 1985) [10]. The seeds were presoaked in water one day prior for sowing and soaked seeds were sown in rows along with the recurrent parent CO51, donor parent IR71033-121-15B, resistant check PTB33, few other rice genotypes, which includes Improved White Ponni (IWP) and CBMAS14065 (a RIL of IWP x APO) and susceptible check TN1 (Taichung native 1) in the seed box of size 60x45x10 cm, 10-15 seedlings were maintained per rows. Seven to ten days old seedlings were infested with the BPH at the rate of 15-20 per seedlings, one week after infestation ‘’hopperburn’’ symptoms were appeared and observed on the seedlings. When more than 90% of the susceptible check TN1 shows drying and wilting symptoms, the seedlings were individually scored based on the scoring system of International Rice Research Institute (IRRI, 1996) [14]. (Fig.4) and (Table-1).

Table 1: IRRI Standard evaluation system for BPH resistance

Scale	Damage	Resistance level
0	No damage	Immune
1	Very slight damage	Highly resistant
3	First and second leaves of most plants partially yellowing	Resistant
5	Pronounced yellowing and stunting or about 10 to 25% of the plants severely stunted or dying	Moderately resistant
7	More than half of the plants dead	Moderately susceptible
9	All plants dead	Susceptible

Results and Discussion

Introgression of *Bph20* and *Bph21* into a elite popular rice cultivar CO51

CO51 is a short duration (110-115 days), high yielding and fine grain rice variety. It is one of the most widely cultivated elite rice variety in Tamil Nadu and 14 other states in India. Even though it is popular rice but it is moderately resistant to BPH. In order to enhance BPH resistance in CO51 variety.

Backcross inbreed lines (BILs) were developed for BPH resistance using the recurrent parent CO51 and the donor parent IR71033-121-15B. Breeding scheme was shown in (Fig.1)

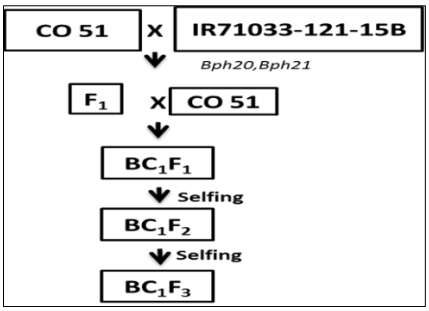

Fig 1: Marker assisted backcross breeding scheme for development of Backcross inbred lines (BILs) of CO 51 x IR71033-121-15B

Foreground Selection for screening of *Bph20* and *Bph21* resistance genes in advanced backcrossed population

For fore ground selection, 13 linked SSR markers were used to identify *Bph20* resistance gene such as RM16547, RM16548, RM16550, RM16553, RM16554, RM16555, RM16556, RM16557, RM16558, RM16560, RM16562, RM16563 and RM16564 from which RM16556 is found polymorphic between the recurrent and donor parents and used as foreground marker for further identification of positive plants for *Bph20* resistance gene. Likewise for *Bph21* resistance gene four linked SSR markers such as RM244, RM3331, RM2854 and RM6615 were used, out of these four markers RM3331 is able to clearly differentiate the CO51 from the donor IR71033-121-15B allele, which is further utilized for the identification of positive plants for *Bph21* resistance genes for all the generation. Primer sequence of the markers used in the study is given in (Table- 2).

Table 2: SSR markers primer sequence used in the study

S. No	Resistance Genes	SSR markers	Forward Primer	Reverse Primer
1.	*Bph20*	RM16556	TTGGACCAGGAGATCAATGAAGG	GTGCGCACACTCTTCTATGTGC
2.	*Bph21*	RM3331	CCTCCTCCATGAGCTAATGC	AGGAGGAGCGGATTTCTCTC

In BC_1F_1 population, four and three plants were heterozygous for *Bph20* and *Bph21* resistance gene alone respectively. Only three plants were found to be positive with both *Bph20* and *Bph21* resistance genes (Fig.2). From which, single plant was forwarded to BC_1F_3 generation through selfing. The foreground selection results in BC_1F_3 population showed that forty nine and thirty two plants were found to be homozygous for *Bph20* and *Bph21* resistance gene alone respectively. Totally thirty two plants were found to be homozygous for both *Bph20* and *Bph21* resistance genes (Fig.3 and Fig.4).

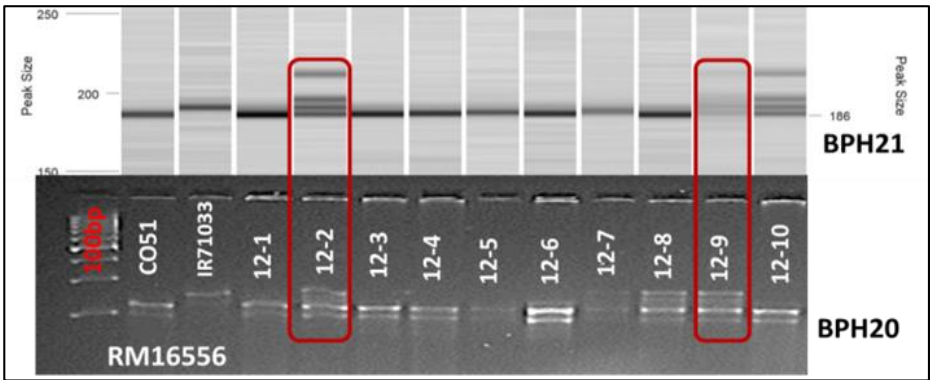

Fig 2: Foreground selection of BC_1F_1 plants of CO51 x IR71033-121-15B for *Bph20* and *Bph21* by using RM16556 and RM3331 markers respectively

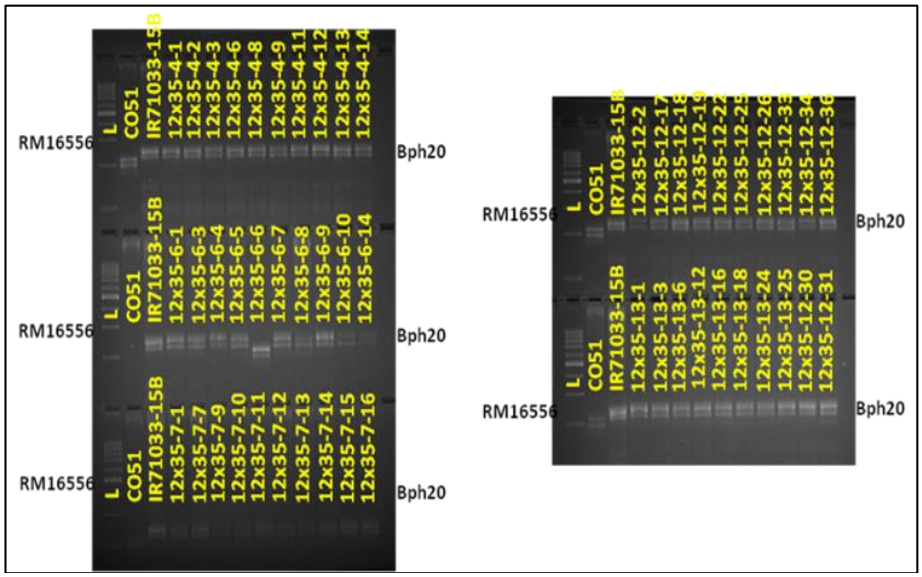

Fig 3: Foreground selection of BC_1F_3 plants of CO51 x IR71033-121-15B for *Bph20* by using RM16556 marker

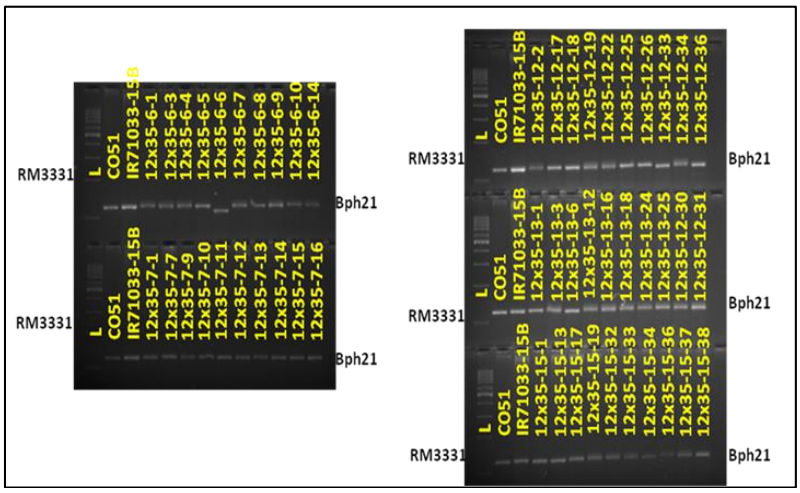

Fig 4: Foreground selection of BC_1F_3 plants of CO51 x IR71033-121-15B for *Bph21* by using RM3331 marker

Evaluation of Backcross inbreed lines (BILs) for BPH resistance through Standard Seed box Screening Technique (SSST)

Backcross inbreed lines (BILs) of CO51 harbouring two BPH resistant gene combinations *Bph20* and *Bph21* were used for bioassay study responses against brown plant hopper (BPH), *Nilaparvata lugens* (Sta l) along with the recipient parent CO51, donor IR71033-121-15B, resistant check (PTB 33) and susceptible check (TN 1).The donor parent IR71033-121-15B and BILs # 12-35-6-3, 12-35-7-16 and 12-35-13-1 (containing both *Bph20* and *Bph21)* were shown to be moderately resistant (SES score, 5) to BPH. Resistant check PTB33 was shown to exhibit a resistant reaction of resistance with a SES score of 3 and susceptible check, TN 1 was found to be highly susceptible (HS) with a score of 9 (Fig.5) (Table-3).

The resistant varieties with single gene may easily breakdown of BPH resistance (Cohen *et al*., 1997; Alam and Cohen 1998b) [8, 2]. Therefore with the help of new and effective BPH resistance genes, pyramiding two or more BPH resistance genes can able to develop durable resistance rice varieties. The double gene pyramided lines with *Bph6* and *Bph12* resistance genes have shown additive effect against BPH, compared with introgression of single gene either with *Bph6* or *Bph12* alone (Qiu *et al*., 2012) [27]. Likewise pyramiding of *Bph14* and *Bph15* resistance genes introgressed lines have shown enhanced or increased resistance than single introgression lines either with *Bph14* or *Bph15* resistance gene alone (Hu *et al*., 2012) [11]. The different test entries/cultivar with specific resistance genes are known for resistance to BPH, but it had shown diverse reaction to Coimbatore (India) biotype of brown plant hopper (Thamarai and soundararajan, 2017) [31]. For example the entries such as Rathuheenathi and PTB-33 carries *Bph3* gene had shown resistant reaction in seed box screening technique. The donor parent IR71033-121-15B used in the present study was shown as moderate resistant reaction to *Nilaparvata lugens* Stal population of Andhra Pradesh (Bhanu *et al*., 2014) [4]. In our present study we pyramided two BPH resistance genes (*Bph20* and *Bph21*) into a popular rice variety CO51 by using marker assisted backcross breeding (MABB) approach. The double gene pyramided lines with BPH resistance genes (*Bph20* and *Bph21*) have shown significant improvement in resistance for brown plant hopper in the bioassay study. BILs # 12-35-6-3, 12-35-7-16 and 12-35-13-1 (containing both *Bph20* and *Bph21)* were recorded as moderate resistant (MR) reaction (SES score, 5) to BPH, will give durable resistance than the CO51 (recurrent parent) for Coimbatore biotypes BPH. The development of BPH resistance line into a different rice cultivar or variety can be used as promising alternative sources for BPH resistance in breeding durable resistant varieties.

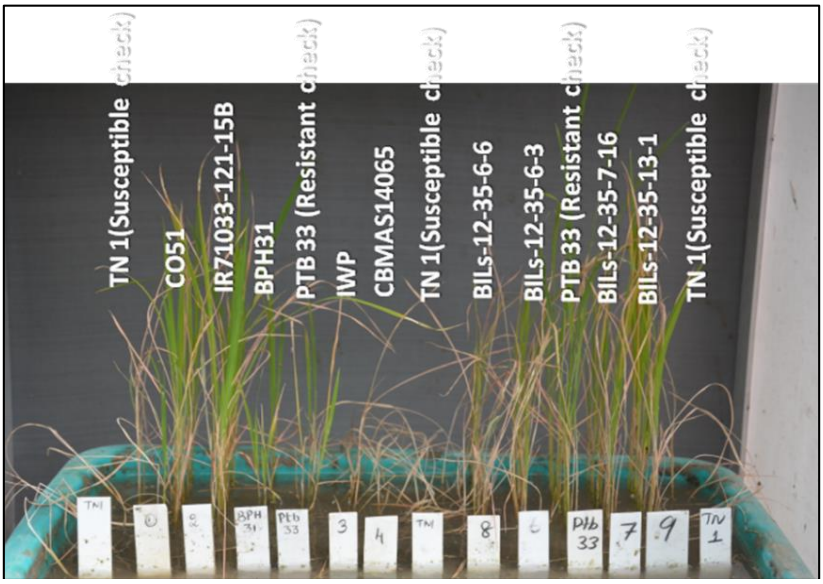

Fig 5: BPH bioassay screening of rice genotypes including with BILs

Table 3: Responses of rice genotypes including NILs against *N. lugens*

S. No	Genotypes	Score	IRRI Scale
1.	12-35-6-6 (Negative for both *Bph20* &*Bph21*)	7	MS
2.	12-35-6-3 (Positive for both *Bph20* &*Bph21*)	5	MR
3.	12-35-7-16 (Positive for both *Bph20* &*Bph21*)	5	MR
4.	12-35-13-1 (Positive for both *Bph20* &*Bph21*)	5	MR
5.	Improved White Ponni (IWP)	9	S
6.	CBMAS14065 (a RIL of IWPxAPO)	9	S
7.	BPH31	5	MR
8.	CO 51 (Recurrent Parent)	7	MS
9.	IR71033-121-15B (Donor Parent)	5	MR
10.	PTB 33(Resistant check)	3	R
10.	Taichung Native 1(TN1)	9	S

S: Susceptible; MS: Moderately Susceptible; R: Resistant; MR: Moderately Resistant

Acknowledgement
The financial support given for the project by Department of Biotechnology (DBT), Govt. of India, New Delhi (BT/PR13454/COE/34/43/2015) is greatly acknowledged.

References

1. Alam SN, Cohen MB. Detection and analysis of QTLs for resistance to the brown planthopper, *Nilaparvata lugens*, in a doubled-haploid rice population. Theor Appl Genet 1998a;97:1370-1379.
2. Alam SN, Cohen MB. Durability of brown planthopper, *Nilaparvata lugens*, resistance in rice variety IR64 in greenhouse selection studies. Entomol Exp Appl 1998b;89:71-78.
3. Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K. (ed.) Current Protocols in Molecular Biology. New York: John Wiley and Sons 1994,1.
4. Bhanu KV, Jhansilakshmi V, Katti G, Vishnuvardhan Reddy A. Antibiosis and tolerance mechanism of resistance in rice varieties carrying brown plant hopper resistance gene. Asian J Biol. Life Sci 2014;5(1):32-37.
5. Brar D, Virk P, Jena K, Khush G. Breeding for resistance to planthoppers in rice, Planthoppers: new threats to the sustainability of intensive rice production systems in Asia 2009,401-409.
6. Brookes G, Barfoot P. *GM Rice:* Will This Lead the Way for Global Acceptance of GM Crop Technology, ISAAA Los Banos, Philippines: ISAAA Los Banos, Philippines 2003.
7. Cha Y, Ji H, Yun D, Ahn B, Lee M, Suh S *et al*. Fine mapping of the rice Bph1 gene which confers resistance to brown planthopper and development of EST markers for marker assisted selection 2008;26:146-151.
8. Cohen MB, Alam SN, Medina EB, Bernal CC. Brown plant hopper, *Nilaparvata lugens*, resistance in rice cultivar IR64: mechanism and role in successful *N. lugens* management in Central Luzon, Philippines. Entomol Exp Appl 1997;85:221-229.
9. Du B, Zhang W, Liu B, Hu J, Wei Z, Shi Z *et al*. Identification and characterization of Bph14, agene conferring resistance to brown planthopper in rice. Proc. Natl. Acad. Sci 2009;106(52):22163-22168.
10. Heinrichs E. Genetic evaluation for insect resistance in rice. Los Banos, the Philippines: International Rice Research Institute: Los Banos, the Philippines: International Rice Research Institute 1985,71-170.
11. Hu J, Li X, Wu CJ, Yang CJ, Hua HX, Gao GJ *et al*. Pyramiding and evaluation of the brown planthopper resistance genes *Bph14* and *Bph15* in hybrid rice. Mol Breeding 2012;29:61-69.
12. Hu J, Cheng MX, Gao GJ, Zhang QL, Xiao JH, He YQ. Pyramiding and evaluation of three dominant brown planthopper resistance genes in the elite indica rice 9311 and its hybrids. Pest Manag Sci 2013;69:802-808.
13. Hu J, Chang XY, Zou L, Tang WQ, Wu WR. Identification and fine mapping of *Bph33*, a new brown planthopper resistance gene in rice (*Oryza sativa* L.). Rice 2018;11:55.
14. IRRI. Standard Evaluation System for Rice. IRRI, Manila, Philippines 1996.
15. Jairin J, Teangdeerith S, Leelagud P, Phengrat K, Vanavichit A, Toojinda T. Detection of brown planthopper resistance genes from different rice mapping populations in the same genomic location. Sci. Asia 2007;33:347-352.
16. Jena KK, Kim SM. Current status of brown planthopper (BPH) resistance and genetics. Rice 2010;3:161-171.
17. Jena KK, Pasalu IC, Rao YK, Varalaxmi Y, Krishnaiah K, Khush GS *et al*. Molecular tagging of a gene for resistance to brown planthopper in rice (*Oryza sativa* L.). Euphytica 2002;129:81-88.
18. Jena KK, Jeung JU, Lee JH, Choi HC, Brar DS. High-resolution mapping of a new brown planthopper (BPH) resistance gene, Bph18(t), and marker-assisted selection for BPH resistance in rice (*Oryza sativa* L.). Theor Appl Genet 2006;112:288-297.
19. Kawaguchi M, Murata K, Ishii T, Takumi S, Mori N, Nakamura C. Assignment of a brown planthopper (*Nilaparvata lugens* Stål) resistance gene bph4 to the rice chromosome 6. Breed Sci 2001;51:13-18.
20. Kumar K, Sarao PS, Bhatia D, Neelam K, Kaur A, Mangat GS *et al*. High resolution genetic mapping of a novel brown planthopper resistance locus, *Bph34* in *Oryza sativa* L.×*Oryza nivara*(Sharma & Shastry) derived interspecific F2population. Theor ApplGenet 2018;131(5):1163-1171.
21. Myint KK, Fujita D, Matsumura M, Sonoda T, Yoshimura A, Yasui H. Mapping and pyramiding of two major genes for resistance to the brown planthopper ([Stål]) in the rice cultivar ADR52. Theor Appl Genet 2012;124(3):495–504.
22. Matsumura M, Takeuchi H, Satoh M *et al*. Current status of insecticide resistance in rice planthoppers in Asia. In: Heong KL, Hardy B (eds) Planthoppers: new threats to the sustainability of intensive rice production systems in Asia. International Rice Research Institute, Los Baños (Philippines) 2009,233-244.
23. Normile D. Agricultural research. Reinventing rice to feed the world. Science 2008;321(5887):330-333.
24. Pathak MD, Cheng CH, Fortuno ME. Resistance to Nephotettix impicticeps and *Nilaparvata lugens* in varieties of rice. Nature 1969;223:502-504.
25. Penalver Cruz A, Arida A, Heong KL, Horgan FG. Aspects of brown planthopper adaptation to resistant rice varieties with the Bph3 gene. Entomologia Experimentalis et Applicata 2011;141(3):245-257.
26. Qiu YF, Guo JP, Jing SL, Zhu LL, He GC. High-resolution mapping of the brown planthopper resistance gene Bph6 in rice and characterizing its resistance in the 93-11 and Nipponbare near isogenic backgrounds. Theor Appl Genet 2010;121:1601-1611.

27. Qiu YF, Guo JP, Jing SG, Zhu LL, He GC. Development and haracterization of japonica rice lines carrying the brown planthopper-resistance genes *Bph12* and *Bph6*. Theor Appl Genet 2012;124:485-494.
28. Rahman ML, Jiang WZ, Chu SH, Qiao YL, Ham TH, Woo MO *et al*. High-resolution mapping of two rice brown planthopper resistance genes, *Bph20*(t) and *Bph21*(t) originating from *Oryza minuta*. Theor Appl Genet 2009;119(7):1237-1246.
29. Sharma PN, Torii A, Takumi S, Mori N, Nakamura C. Marker-assisted pyramiding of brown planthopper (*Nilaparvata lugens* Stål) resistance genes *Bph1* and *Bph2* on rice chromosome 12.Hereditas. 2004;136:39-43.
30. Suh JP, Yang SJ, Jeung JU, Pamplona A, Kim JJ, Lee JH *et al*. Development of elite breeding lines conferring *Bph18* gene-derived resistance to brown planthopper (BPH) by marker-assisted selection and genome-wide background analysis in japonica rice (*Oryza sativa* L.). Field Crops Res 2011;120:215-222.
31. Thamarai M, Soundararajan RP. Reaction of rice genotypes against specific population of brown planthopper, *Nilaparvata lugens* (Stal). Annals of Plants Protection sciences 2017;25(1):74-77.
32. Watanabe T, Kitagawa H. Photosynthesis and translocation of assimilates in rice plants following phloem feeding by the planthopper *Nilaparvata lugens* (Homoptera: Delphacidae). J Econ. Entomol 2000;93(4):1192-1198.