Research Paper

Differential Responses of *Rathu Heenati* Accessions Available in Sri Lanka for Brown Planthopper [*Nilaparvata Lugens* (Stål)] Resistance

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Abstract

Brown Planthopper, BPH [*Nilaparvata lugens* (Stål)] is one of the most devastating insect pests reported in rice, which accounts for considerable yield losses in paddy throughout the world. The Sri Lankan traditional rice cultivar *Rathu Heenati* is known to carry a strong BPH resistance and it is used as a donor parent in rice breeding programmes of many countries. Therefore, this study was carried out to determine the BPH resistance in *Rathu Heenati* accessions conserved at PGRC using phenotypic and molecular characterization. Cultivar screening was done using Standard Seed box Screening Test (SSST), different morphological characters and gene specific molecular markers at the Rice Research and Development Institute, Batalagoda during 2021/2022 *Maha* season. Morphological analysis showed that there is high diversity among the tested accessions. Phenotypic screening revealed that accessions 005579 and 004992 showed high resistance, and the accession 003390 showed moderate resistance to BPH. Molecular marker SSR and STS studies showed that the accessions 005579 and 004992 contain *Bph2, Bph3, Bph10* and *Bph13* resistant genes while 005486, 003888, 002080 identified as susceptible accessions containing different combinations of *Bph2, Bph3* and *Bph13* genes except *Bph10*. These information can be utilized for efficient introgression of resistant genes, *Bph2, Bph3, Bph10* and *Bph13* into elite rice cultivars using marker-assisted selection to generate long lasting and a durable BPH resistance in rice.

Keywords: **BPH Resistance, Molecular characterization, Phenotyping, SSR, STS**

Introduction

Rice (*Oryza sativa* L.) is the staple crop consumed by 3.5 billion people of the world population (Tuyen *et al*., 2012). Rice provides 21% of global human per capita energy and 15% of per capita protein (Gnanamanickam, 2009). In Sri Lanka rice is cultivated extensively, except a few locations, during two cropping seasons, *Yala* and *Maha*. In 2020 the average rice productivity was 4.8 Mt ha^{-1} (Department of Census and Statistics, 2020). Most of the new improved rice varieties cultivated in Sri Lanka carries the yield potential of 8-9 mt/ha. However, the actual paddy yields are lower than the potential yields and pest and diseases account for low yields to a considerable extent. Among pests reported, Brown Plant Hopper, (BPH) (*Nilarparvata lugens* Stål)(Hemiptera: Delphacide) is known to be one of the most damaging insect pests of rice worldwide. BPH feeds on phloem sap of the rice plant. Under conducive environmental conditions, BPH spread very fast in paddy fields and heavily infected plants turn into brown colour, completely dry up and die. This condition known as "hopper burn" cannot be reversed with pest management options (Cagampang *et al*., 1974; Du *et al*., 2009). To control BPH application of pesticides is the most common practice and the application of pesticides may affect natural BPH predators and lead to a resurgence of BPH while hazarding to the environment. (Abhayagunasekara *et al*., 2022; Gallagher *et al*., 1994; Tanaka *et al*., 2000; Matsumura *et al*., 2008). Sivasubramaniam & Imthiyas (2018) stated that BPH population and frequency of outbreaks have steadily increased In Sri Lanka. Sarathchandra *et al*. (2018) stated that the var. Bg 379-2 show resistant/moderate resistant BPH reaction and var. Bg 403, Bg 369, Bg 366, Bg 359, Bg 357, Bg 310, Bg 304, Bg 300 moderate resistance to BPH in Sri Lanka. Traditional rice cultivar "*Rathu Heenati*" has been reported to exhibit a strong resistance to BPH and is widely used as a BPH resistant donor parent in rice breeding programs in many countries (Kusumawati *et al*., 2018). The microarray analysis indicated that BPH resistance in RHT is probably controlled by a series of resistance-related genes (Yubing *et al*., 2012). *Rathu Heenati* was confirmed to carry the dominant resistance gene *Bph3* (Lakshminarayana and Khush 1977; Jairin *et al*., 2007). The *Bph4* locus has been mapped to the same region on chromosome 6 with *Bph3* between the flanking markers RM589 and RM586 (Jairin *et al*., 2010). Yan *et al*. (2002) mapped the Bph3 gene on chromosome 4 using RFLP markers and BAC-FISH technology and it was named subsequently as a new resistance gene, Bph15 (Yang *et al*. 2004). Sun *et al*. (2005) assigned three QTLs on chromosomes 3, 4 and 10 and the major QTL on the short arm of chromosome 4 was tentatively designated *Bph17* in RHT (Sun *et al*., 2005). In Sri Lanka presence of several *Rathu Heenati* accessions is reported. Therefore, this study was carried out to find whether these "*Rathu Heenati*" accessions exhibits phenotypic differences and possess the same level of resistance against BPH populations in Sri Lanka and molecular confirmation of BPH resistant genes available in "*Rathu Heenati*" accessions.

Materials and Methods

Planting materials

Seeds of eight *Rathu Heenati* accessions; 004992, 002080, 003390, 003888, 005486, 005579, 011184, and 011545 were obtained from the Plant Genetic Resources Centre (PGRC), Gannoruwa Sri Lanka. Seeds of Ptb 33, and Bg 380 varieties, which are BPH resistant and susceptible, respectively were obtained from the Rice Research and Development Institute (RRDI), Batalagoda, Sri Lanka for the study.

Screening of *Rathu Heenati* accessions for BPH resistance

During 2021/22 *Maha*, above mentioned *Rathu Heenati* accessions along with Ptb 33 and Bg 380 varieties were screened for BPH resistance using the Standard Seed Box Screening Technique (SSST) as described by Sani *et al*. (2020) in a Completely Randomized Design with three replicates. Hundred seeds from each *Rathu Heenati* accession, Ptb 33 and rice variety Bg 380 were sown with a row spacing of 5 cm in six standard seed boxes (trays) made of galvanized (60 x 45 x 10 cm^3) filled with sterilized soil (depth 5 cm). Eight (8) days post sowing of the seeds, first and second instar nymphs of BPH were introduced to seedling at the at the rate of 8 to 10 insects per the seedling and seedlings were allowed to be fed by BPH. Hopper burn symptom development was observed after about one month period of the introduction of BPH. The same experiment was also carried out taking daily counts on hopper burn symptoms for thirty days. When more than 90% of susceptible check showed wilting with drying, the plants were scored individually based on the scoring system proposed by the International Rice Research Institute (IRRI, 1996) (Table 01).

Molecular evaluation of *Rathu Heenati* accessions for the presence of different BPH resistant genes

DNA extraction

Two weeks after seed germination, fresh composite leaf samples (100 mg) were collected from six *Rathu Heenati* accessions (accessions; 004992, 002080, 003390, 003888, 005486 and 005579) and the var. Ptb 33 separately and total genomic DNA was extracted using the CTAB method (Murray & Thompson, 1980). Since two of the accessions did not germinate DNA extraction was done form seeds of the accession 011184 and 011545. For this, seeds of two *Rathu Heenati* accessions were soaked overnight and the rice husk was removed.

Table 1. Scoring system proposed by the International Rice Research Institute (IRRI, 1996) for BPH resistance evaluation in rice

Reaction interpretation	Seedling score	Interpretation
No visible damage	0	Highly resistant (HR)
Partial yellowing of the first leaf	1	Resistant (R)
First and second leaves partially yellowing	3	Moderately resistant (MR)
Pronounced yellowing or some stunted or drying,	5	Moderately susceptible (MS)
More than half of the plants die	7	Susceptible (S)
All plants dead	9	Highly susceptible (HS)

Rice embryos and endosperms were separated using sterile forceps. The rice endosperm was used for DNA extraction using modified CTAB method. Final DNA pellets were dissolved in 50 *µl* of nucleases free water separately and stored at -20 °C for further use. Agarose gel electrophoresis was done to check the quality of the DNA.

Polymerase Chain Reaction (PCR) amplification

For SSR and STS primers (Table 2) the amplification was carried out in 15 *µl* reaction volume, consisting of 7.5 *µl* Go Taq® Green Master Mix (Promega, USA), 1 *µl* of forward (10 mM) and reverse primer (10 mM) each, 3 *µl* of genomic DNA and 2.5 *µl* of nucleases free water. Amplification was done using a thermal cycler (My Cycler, BioRaD, USA) programmed with initial denaturation at 94 °C for 5 min, followed by 40 cycles of 30 s denaturation at 94 °C, 1 min annealing at 50-65 °C and 1 min extension at 72 °C followed by the final extension of 7 min at 72 °C and 4 °C. The PCR products were run on 1% agarose gel for the confirmation of PCR products.

Phenotypic characterization of *Rathu Heenati* accessions

Morphological diversity of tested *Rathu Heenati* accessions were analysed at field of RRDI. Thirty seeds of eight *Rathu Heenati* accessions were soaked for 24 h. Then, seeds were kept on tissue paper laid in a Petri dish for germination. Eight days after germination, seedlings were separately placed into holes of a parachute tray spread with mud and the seedlings were maintained there for 18 days and seedlings were transplanted in the field with the spacing of 5 cm. Crop maintenance was done according to the recommendations of the Department of Agriculture. Phenotypic data recorded were, initial flowering (days), days to 50% flowering (days), culm length (cm), no. of tillers, panicle length (cm), flag leaf length and width (cm), shattering %, thousand grain weight (g), filled grain % and weight of ten panicles were collected. The data were analysed using SAS Software package (version 9.1). Pest and diseases were controlled as per the recommendations of the Department of Agriculture.

Results and Discussion

Resistance level of *Rathu Heenati* accessions to BPH

Results of BPH hopper burn symptoms observed 30 days post infestation in standard seed box screening test done according to Sani *et al*. (2020) are indicated in the table 2. Ptb 33, accession 5579, accession 4992 showed highly resistant reaction to BPH and the accession 3390 showed moderate resistance to BPH. Bg 380 and the rest of the accessions showed highly susceptible reactions to BPH (Table 03).

The results of the second experiment done using the standard seed box screening test with daily reading on dead plant % with hopper burn symptoms due to BPH feeding are indicated in the figure 1. Variety Ptb 33 showed highest level of resistance to BPH. The acc. 005579 and 004992 showed a higher level of resistance to BPH while acc. 003390 showed moderately resistance up to 30 days and these results also confirms the results observed in the previous study.

Table 2. SSR and STS forward and reverse primers used in this study

Primer	Forward Primer (5´-3´)	Reverse Primer (5´-3´)	AT	Detectable BPH gene	References
RM 463 (SSR)	TTCCCCTCCT-TTTATGGTGC	TGTTCTCCTC-AGTCACTGCG	50 °C	Bph2	Meshram *et al*., 2018
RM 589 (SSR)	TCATGGTCGG-TGGCTTAAC	CAGGTTCCAA-CCAGACACTG	59.7 °C	Bph3	Meshram *et al*., 2018
RG 457 (STS)	GCAGTGGCAG-ATGGGATCGT	GCTCCGAAAT-CCCAAGCGAT	64.8 °C	Bph10	Madurangi *et al*., 2011
AJ 096 (STS)	TCGACCTAGAA-AGGCCTGTGT	CACTGGAAAT-TTGAGCGAGAA	59.7 °C	Bph13	Madurangi *et al*., 2011

SSR = Simple Sequence Repeat, STS = Sequence Tagged Sites, AT= Annealing Temperature

Table 3. Reaction of *Rathu Heenati* accessions in standard seed box screening test

Variety/Accessions	BPH damage according to the IRRI standard on thirty days
Ptb 33	3 (HR)
Acc 3390	3 -5 (MR)
Acc 5486	9 (HS)
Acc 5579	3 (HR)
Acc 3888	9 (HS)
Acc 2080	9 (HS)
Acc 4992	3 (HR)
Acc 11184	Not Germinated
Acc 11545	Not Germinated
Bg 380	9 (HS)

Acc. = accession, HR = Highly Resistant, MR = Moderately Resistant, HS = Highly Susceptible

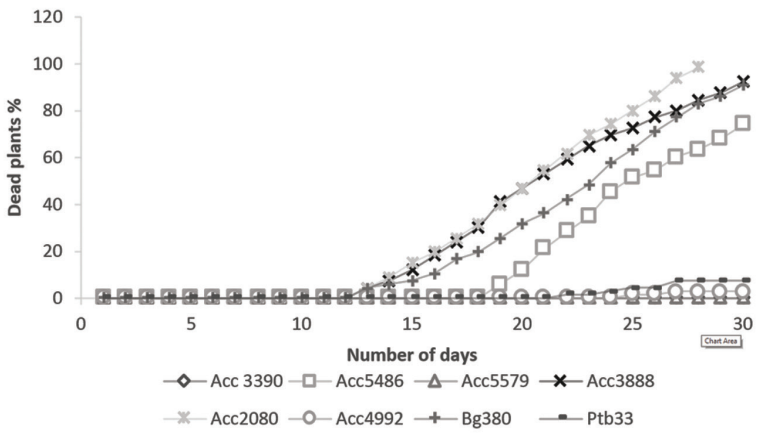

Figure 1. Dead plant percentage due to the BPH infestation with time in standard seed box screening test

Acc. 005579 & 004992 showed the minimum number of dead plant percentage. All plants of the accession 002080 died after 28 days due to BPH feeding. Accessions 002080 and 003888 showed higher susceptibility to BPH, which was more than that Bg 380, which was used as the susceptible control in this study. Though the acc. 005486 showed higher susceptibility to BPH, it's susceptibility level is lower than the susceptible check variety Bg 380. Two accessions (011184 and 011545) could not be evaluated in standard seed box screening test as the seeds of these two accessions did not germinate.

PCR confirmation on the presence of BPH resistant genes in *Rathu Heenati* accessions

PCR results showed the presence of different BPH resistant genes in the tested *Rathu Heenati* accessions. Var. Ptb 33 is known to contain BPH resistant genes *Bph2*, *Bph3* and QTLs (Sidhu and Khush, 1978; Jairin *et al*., 2007, Yadavalli *et al*., 2012). SSR and STS based PCR markers revealed the presence of *Bph2*, *Bph3*, *Bph10* and *Bph13* genes in var. Ptb 33. SSR marker, RM 589 amplified with the DNA of eight *Rathu Heenati* accessions showed the presence of *Bph3* gene in all of the tested accessions. SSR marker, RM 463 showed the presence of *Bph2* gene in all the accessions except the accession 011184. STS marker RG 457 showed the presence of *Bph10* gene in the accessions 004992 and 005579. The STS marker AJ 096 confirmed the presence of *Bph13* gene only in the accessions, 002080, 003888, 004992, 005579, 011184 and 011545 (Table 4, Figure 2).

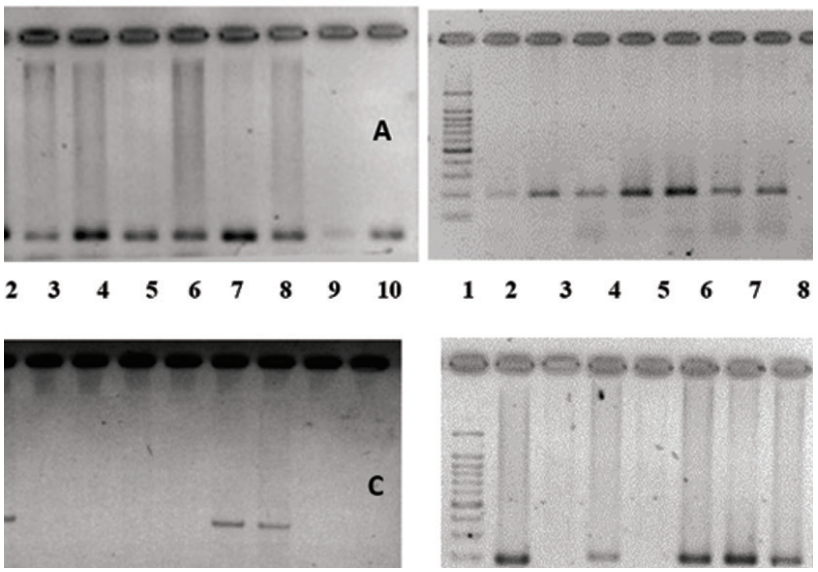

Figure 2. A = PCR with SSR primer RM 589, B = PCR with SSR primer RM 463, C = PCR with STS primer RG 457, D = PCR with STS primer AJ09, 1= 100 Kb ladder, Lane 2 = Ptb 33, Lane 3 = 005486, Lane 4 = 002080, Lane 5 = 003390, Lane 6 = 003888, Lane 7 = 004992, Lane 8 = 005579, Lane 9 = 011184, Lane 10 = 011545.

Table 4. Confirmation of the presence of BPH resistant genes in *Rathu Heenati* accessions and var. Ptb 33 with SSR and STS markers

Accession No	Bph2	Bph3	Bph10	Bph13
005486	+	+	-	-
002080	+	+	-	+
003390	+	+	-	-
003888	+	+	-	+
004992	+	+	+	+
005579	+	+	+	+
011184	-	+	-	+
011545	+	+	-	+
Ptb 33	+	+	+	+

+ = presence of the relevant band in agarose gel electrophoresis, - = absence of the relevant band in agarose gel electrophoresis

Morphological characterization of *Rathu Heenati* accessions

Different morphological characteristics could be identified amongst the six *Rathu Heenati* accessions (004992, 002080, 005486, 003390, 005579, 003888) in the field (Table 5). Initial flowering (days), days to 50% flowering (days), culm length (cm), no. of tillers, panicle length (cm), flag leaf length and width (cm), shattering %, thousand grain weight (g), filled grain % and weight of ten panicles were found to be varying with the accessions. The earliest initial flowering was observed with the accession 002080 (54 days) and the highest number of days (77 days) to initial flowering was reported from the accessions 003390 and 005579. The earliest days to 50% flowering (57 days) was found with the accession 002080 and the lengthiest days to 50% flowering (84 days) was observed with the accession 003390. According to the 50% flowering data (Table 4) these *Rathu Heenati* accessions belong to different age groups. The highest culm lengths were observed in the accession 005579 (143.00cm) and the accession 003390 (142.00cm) while the shortest culm length was observed in the accession 004992 (76.8cm). The maximum number of tillers was observed in Acc. 003888 (19.6) and the minimum number of tillers was observed in the accessions 005579 (7.4). The lengthiest panicle was observed in the accession 005579 (44.4 cm) and the shortest from the acc. 003888 (23.8 cm). The lengthiest flag leaf was observed in the accessions, 003390 (51.0 cm), and 005579 (50.4 cm) while the shortest was in the accession 003888 (30.4 cm). The highest flag leaf width was observed in the accession 003888 (1.46 cm) and the lowest flag leaf width was observed in the accession 002080 (1.04 cm). The highest shattering percentage was observed in the accessions 005486 (143.86 %), and the lowest shattering percentage was observed in the accession 003888 (6.46 %). The highest and lowest thousand grain weights were reported from the accessions 002080 (56.84 g) and 005486 (24.84 g), respectively (Table 5).

Kumari *et al*. (2010) mentioned that *Rathu Heenati* is found to be resistant to all biotypes of brown planthopper. Jena and Kim (2010) mentioned that twenty-one major genes for BPH resistance have been identified from diverse genetic resources such as land races, cultivars and wild species of *Oryza* by using standard evaluation methods developed at the International Rice Research Institute (IRRI) to distinguish resistance or susceptibility of rice genotypes to BPH biotypes/ populations.

Table 5. Different morphological characters (mean values) observed in different *Rathu Heenati* accessions

PGRC Accession No	Initial Flowering (Days)	50 % Flowering (Days)	Culm Length (cm)	No of tillers	Panicle length (cm)	Flag Leaf length (cm)	Flag Leaf width (cm)	Shattering %	Thousand grain weight (g)	Filled grain %*	Weight of 10 panicles* (g)
004992	56 ^{d}	61 ^{e}	76.8 ^{d}	12.6 ^{b}	25.2 ^{c}	34.6 ^{b}	1.06 ^{c}	10.03 ^{ab}	26.72 ^{ab}	58.44 ^{a}	20.61 ^{a}
002080	54 ^{e}	57 ^{f}	95.4 ^{c}	10.6 ^{b}	24.6 ^{c}	31.0 ^{b}	1.04 ^{c}	9.18 ^{ab}	26.84 ^{a}	51.41 ^{a}	22.46 ^{a}
005486	57 ^{c}	64 ^{d}	99.0 ^{cb}	10.8 ^{b}	24.0 ^{c}	34.2 ^{b}	1.26 ^{b}	13.86 ^{a}	24.84 ^{b}	50.89 ^{a}	22.06 ^{a}
003390	77 ^{a}	84 ^{a}	142.0 ^{a}	11.0 ^{b}	40.8 ^{b}	51.0 ^{a}	1.14c ^{b}	13.2 ^{a}	25.2 ^{ab}	48.47 ^{a}	21.03 ^{a}
005579	77 ^{a}	81 ^{b}	143.0 ^{a}	7.4 ^{b}	44.4 ^{a}	50.4 ^{a}	1.06 ^{c}	9.85 ^{ab}	25.96 ^{ab}	62.698 ^{a}	22.65 ^{a}
003888	62 ^{b}	66 ^{c}	104.6 ^{b}	19.6 ^{a}	23.8 ^{c}	30.4 ^{b}	1.46 ^{a}	6.46 ^{b}	25.56 ^{ab}	55.90 ^{a}	22.54 ^{a}

In this study the results based on standard evaluation methods developed at the International Rice Research Institute (IRRI) revealed that *Rathu Heenati* accessions, 005579 and 004992 and Ptb 33 showed resistant/ moderately resistant while accessions 003390 showed a moderate level of resistance to BPH. In this study a mixed population of BPH, collected from different parts of the country, was used to check the level of resistance to BPH. Therefore, the resistant/ moderate resistance shown by the accession 005579, 004992 and Ptb 33 can be applicable in managing BPH throughout the country. Interestingly, molecular studies showed that the accessions 005579, 004992 and Ptb 33, which also, contain *Bph2*, *Bph3*, *Bph10*, and *Bph13* resistant genes. The accession 003390, which showed the moderate level of resistance to BPH contains only *Bph2* and *Bph3* genes. However, the accessions, 005486 and 003888, which were found to posses with the same BPH resistant genes, *Bph2* and *Bph3* showed susceptibility to BPH suggest the possibility of involving some other genes in BPH resistance. The accession11545, which contains *Bph2*, *Bph3* and *Bph13* resistant genes and the accession 011184, which contains the *Bph3* and *Bph13* genes also show susceptibility reaction to BPH. The present results suggests that the presence of *Bph10* with *Bph2*, *Bph3* and *Bph13* resistance genes or *Bph10* resistance gene alone play an important role in determining the resistance of *Rathu Heenati* accessions to a BPH mixed population found in Sri Lanka. Jena and Kim (2010) also mentioned that 18 major BPH resistant

genes are localized on specific region of six rice chromosomes and some of these resistance genes are clustered together such as *Bph1, Bph2, Bph9, Bph10, Bph18*, and *Bph21* on the long arm of chromosome 12; *Bph12*, *Bph15*, *Bph17* and *Bph20* on the short arm of chromosome 4; *Bph11* and *Bph14* on the long arm of chromosome *3* and *Bph13* (t) and *Bph19* on the short arm of chromosome 3. Apart from these major resistant genes, some associated with BPH resistance have also been identified on eight chromosome. Only the *Bph2, 3, 10 and 13* genes were considered. Interestingly the BPH resistant *Rathu Heenati* accessions also showed variations in morphological characters such as days to 50% flowering, culm length, panicle length, flag leaf length, flag leaf length and % of shattering. These observations reveal that the *Rathu Heenati* accessions represent significantly different populations. This may be mainly due to the farmers naming of their seed collection as Rathu Hennati. Therefore, further morphological and phenotypic screening are needed. Efficient introgression of BPH resistant genes, *Bph2*, *Bph3, Bph10*, and *Bph13* into elite rice cultivars or developing rice lines using marker-assisted selection will be useful to generate a long lasting and wide BPH resistance in rice crop of Sri Lanka. It will help to increase rice productivity in the country reducing the crop losses due to BPH while minimizing use of pesticide and incurred cost for the application of pesticides in rice cultivation to control BPH.

Conclusion

The study reveals that the presence of *Bph10* gene alone or with *Bph2*, *Bph3* and *Bph13* genes play an important role in determining the resistance of *Rathu Heenati* accessions to a mixed BPH population. Different *Rathu Heenati* accessions show different phenotypic characters suggesting that they do not belong to the same population. This information is also important in understanding different levels of BPH resistance observed in different *Rathu Heenati* accessions. *Rathu Heenati* accessions; 004992 and 005579 that contain *Bph2*, *Bph3*, *Bph10*, and *Bph13* genes may be used to generate BPH resistance in developing rice varieties.

References

Abhayagunasekara, A. V. C., Chandrasena, G. D. S. N., Dissanayake, D. M. O. K. B., Jayasundara, J. M. S. M., Pushpakumara, D. K. N. G., Samarasinghe, W. L. G., & Bandaranayake, P. C. G. (2022). Wild Rice Species in Sri Lanka as Genetic Resources for Breeding for Brown Plant Hopper (*Nilaparvata lugens* (Stål)) Resistance in Rice. *Tropical Agricultural Research*, *33*(2), 102-112.

Cagampang, G. B., Pathak, M. D., & Juliano, B. O. (1974). Metabolic changes in the rice plant during infestation by the brown planthopper, *Nilaparvata lugens* Stål (Hemiptera: Delphacidae). *Applied Entomology and Zoology, 9*(3), 174-184.

DCS (2020). Department of Census and Statistics, Sri Lanka

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. *Proceedings of the National Academy of Sciences, 106*(52), 22163-22168.

Gnanamanickam, S, S., (2009). Biological control of rice disease, Home Book, https://links.springer.com > book:

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. In: R.F. Denno, T.J. Perfect (Eds), *Planthoppers*. (pp. 599-614). Springer, Boston, MA. https://doi.org/10.1007/978-1-4615-2395-6_18

IRRI (1996). Standard Evaluation System for Rice. International Rice Research Institute, Manila, Philippines.

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. *Molecular Breeding, 19*, 35-44.

Jairin, J., Sansen, K., Wongboon, W., & Kothcharerk, J. (2010). Detection of a brown planthopper resistance gene *Bph4* at the same chromosomal position of *Bph3* using two different genetic backgrounds of rice. *Breeding Science, 60*(1), 71-75.

Jena, K. K., & Kim, S. M. (2010). Current status of brown planthopper (BPH) resistance and genetics. *Rice, 3*(2), 161-171.

Kumari, S., M, Sheba J., Marappan, M., Ponnuswamy, S., Seetharaman, S., Pothi, N., Subbarayalu, M., Muthurajan, R and Natesan, S., (2010). Screening of IR50 x Rathu Heenati F7 RILs and identification of SSR markers linked to brown planthopper (*Nilaparvata lugens* Stål) resistance in rice (*Oryza sativa* L.). *Molecular Biotechnology, 46*(1), 63-71.

Kusumawati, L., Chumwong, P., Jamboonsri, W., Wanchana, S., Siangliw, J. L., Siangliw, M., Khanthong, S., Vanavichit, A., Kamolsukyeunyong, W. and Toojinda, T. (2018). Candidate genes and molecular markers associated with brown planthopper (*Nilaparvata lugens* Stål) resistance in rice cultivar *Rathu Heenati. Molecular Breeding, 38*(7), 1-1

Laksminarayana, A., & Khush, G. S. (1977). New Genes for Resistance to the Brown Planthopper in Rice 1. *Crop Science, 17*(1), 96-100.

Madurangi, S.A.P., Samarasinghe, W. L. G., Senanayake, S. G. J. N., Hemachandra, P. V., & Ratnasekera, D. (2011). Resistance of *Oryza nivara* and *Oryza eichingeri* derived lines to brown planthopper, *Nilaparvata lugens* (Stal). *J Natl Sci Found Sri Lanka, 39*(2), 175-181.

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 Management Science, 64*(11), 1115-1121.

Meshram, P., Bhandarkar, S., Banerjee, S., Nair, S. K., Rana D. K and Sarawgi A. K., (2018). Screening and validation of known DNA markers for BPH resistance in Germplasm of rice (*Oryza sativa* L.) International journal of chemical studies, Department of Genetics and Plant Breeding, College of Agriculture, IGKV, Raipur, Chhattisgarh.

Min, Y. H., Rui, Q. I. N., Wei, J. W., Cun, H. G., & Chun, S. Y. (2002). Comparative physical mapping of *Bph3* with BAC-FISH in *Oryza officinalis* and *O. sativa*. *Journal of Integrative Plant Biology, 44*(5), 583.

Murray, M. G., & Thompson, W. (1980). Rapid isolation of high molecular weight plant DNA. *Nucleic acids research, 8*(19), 4321-4326.

Sani Haliru, B., Rafii, M. Y., Mazlan, N., Ramlee, S. I., Muhammad, I. I., Silas Akos, I., Halidu, J., Swaray, S., & Rini Bashir, Y. (2020). Recent strategies for detection and improvement of brown planthopper resistance genes in rice: A review. *Plants, 9*(9), 1202.

Sarathchandra, S. R., Chandrasena, G.D.S.N. Rathnayake., R. M. U. K Bandara, R. M. U. S., Sudheera, N. L., Konara., K. M. A. S., Kahawatta, U. C. U. Bandaranayake, M. A. B. R. P., Jayasinghe., J. T. C Liyanage, H. L. K., Semasinghe, S. B. S. K and L. Nugaliyadde (2018) Outbreak of Rice Brown Planthopper (Nilaparvata lugens) and White-backed Planthopper (Sogatella furcifera) in Maha 2017/18 in Kurunegala District: Lesson Learned. *Tropical Agriculturist, 166*(2), 29-40.

Sidhu, G. S., & Khush, G. S. (1978). Genetic analysis of brown planthopper resistance in twenty varieties of rice, Oryza saliva L. *Theoretical and Applied Genetics, 53*, 199-203.

Sivasubramaniam, N., & Imthiyas, M. S. M. (2018). Evaluation of the major factors associated in controlling of brown planthopper (Nilaparvata lugens) in paddy cultivation during its outbreak in selected divisional secretariats of the Ampara District of Sri Lanka. *Scholars Journal of Agriculture and Veterinary Sciences, 5*(11), 595-600.

Sun, L., Su, C., Wang, C., Zhai, H., & Wan, J. (2005). Mapping of a major resistance gene to the brown planthopper in the rice cultivar Rathu Heenati. *Breeding Science, 55*(4), 391-396.

Tanaka, K., Endo, S., & Kazano, H. (2000). Toxicity of insecticides to predators of rice planthoppers: spiders, the mirid bug and the dryinid wasp. *Applied Entomology and Zoology, 35*(1), 177-187.

Tuyen, L. Q., Liu, Y., Jiang, L., Wang, B., Wang, Q., Hanh, T. T. T., & Wan, J. (2012). Identification of quantitative trait loci associated with small brown planthopper (Laodelphax striatellus Fallén) resistance in rice (Oryza sativa L.). *Hereditas, 149*(1), 16-23.

Wang, Y., Li, H., Si, Y., Zhang, H., Guo, H., & Miao, X. (2012). Microarray analysis of broad-spectrum resistance derived from an indica cultivar *Rathu Heenati. Planta, 235*, 829-840.

Yadavalli, V., Narwane, G. P., Krishna, M. S. R., Pothi, N., & Muthusamy, B. (2012). Tagging of Brown Planthopper Resistance Genes in F2s of IR50× Ptb 33 of Rice by Using Bulked Segregant Analysis. *Rice Science, 19*(1), 70-74.

Yang, H., You, A., Yang, Z., Zhang, F., He, R., Zhu, L., & He, G. (2004). High-resolution genetic mapping at the *Bph15* locus for brown planthopper resistance in rice (Oryza sativa L.). *Theoretical and Applied Genetics, 110*, 182-191.