Contents available at: Sri Lanka Journals Online

Tropical Agricultural Research

Journal Home Page: https://tar.sljol.info

Wild Rice Species in Sri Lanka as Genetic Resources for Breeding for Brown Plant Hopper (*Nilaparvata lugens* (Stål)) Resistance in Rice

A.V.C. Abhayagunasekara[1,2], G.D.S.N. Chandrasena[3], D.M.O.K.B. Dissanayake[3], J.M.S.M. Jayasundara[4], D.K.N.G. Pushpakumara[5], W.L.G. Samarasinghe[6] and P.C.G. Bandaranayake[4*]

[1]*Postgraduate Institute of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka.*
[2]*Fruit Crops Research and Development Station, Gannoruwa, Peradeniya, Peradeniya, Sri Lanka.*
[3]*Rice Research and Development Institute, Batalagoda, Sri Lanka.*
[4]*Agricultural Biotechnology Centre, Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka.*
[5]*Department of Crop Science, Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka.*
[6]*Ministry of Agriculture, 80/5, "Govijana Mandiraya", Battaramulla, Sri Lanka.*

ARTICLE INFO

Article history:
Received: 23 August 2021
Revised version received: 21 December 2021
Accepted: 22 January 2022
Available online: 1 April 2022

Keywords:
Backcross breeding
Crop wild relatives
Endemic species
Honeydew test
"Hopperburn"

Citation:
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. and 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.

DOI: http://doi.org/10.4038/tar.v33i2.8550

Abhayagunasekara, A.V.C.
https://orcid.org/0000-0001-5040-8010

CC BY

**Corresponding author: pradeepag@pdn.ac.lk, pradeepag@agri.pdn.ac.lk*

ABSTRACT

Brown planthopper (BPH) *Nilaparvata lugens* (Stål.) is considered the most destructive rice pest in rice-growing countries including Sri Lanka. Resistance breeding considered the most economical management strategy, starts by identifying new genetic resources for BPH resistance and comparative analysis with existing donors. So far, scientists have identified 38 genes/QTLs responsible for BPH resistance. This study explored the BPH resistance in five wild rice species in Sri Lanka, including the endemic species *Oryza rhizomatis*. One recommended rice variety; Bg 352, fourteen *O. rhizomatis* accessions, and one accession from *Oryza nivara*, *Oryza rufipogon*, *Oryza granulata* and *Oryza eichingeri* screened by the honeydew test. The experiment was arranged in a complete randomized design with fifteen replicates with Bg 380 and Ptb 33 as susceptible and resistant checks respectively. The lower amount of honeydew excretion recorded in all tested *O. rhizomatis* accessions, *O. nivara*, *O. granulata*, and *O. eichingeri* accessions suggested BPH resistance. Both Ptb 33 and *O. rufipogon* showed a similar level of resistance with low honeydew excretion. The varieties Bg 352 and Bg 380 showed a high amount of honeydew excretions confirming higher susceptibility to the BPH. Therefore, all fourteen accessions of *O. rhizomatis* and the selected accessions of *O. nivara* and *O. eichingeri* are potential donors for rice breeding programs. Further, the coding sequences (CDS) of known BPH genes of *O. sativa* were used as queries to search similar genes in wild rice genomes. The CDS coverage and phylogenetic analysis suggest, of known BPH resistance genes, at least BPH6, BPH9, BPH14-1, and BPH18 are present in *O. rhizomatis*, *O. eichingeri*, *O. nivara*, and *O. rufipogon* genomes. This information will assist in marker-assisted breeding attempts.

INTRODUCTION

Rice productivity is affected by many biotic and abiotic stresses. Among the biotic stresses, the brown planthopper (BPH), *Nilaparvata lugens* (Stål) is considered as one of the most devastating pests in rice farming (Dyck and Thomas, 1979). The BPH infestations have been reported across Asia, causing significant yield losses (Normile, 2008). In Sri Lanka, the BPH population and frequency of outbreaks have steadily increased (Sivasubramaniam *et al.*, 2018).

The BPH damage is common in rainfed and irrigated wetland environments and the areas with high humidity (greater than 80%) and temperatures of about 25 to 32 ^{o}C. Factors such as high plant density, a closed canopy, continuous submerged conditions in the field, excessive use of nitrogen fertilizer, and spraying of insecticides early in the season favor BPH and further destroy their natural enemies (Rashid *et al*., 2017).

The BPH attacks the rice plants between the booting and heading stages, though the damage does not appear until the crop reaches the milk or dough stages. The crop infestation occurs after the anthesis, in reducing the number of grains per panicle and percentages of ripened grains (Chen *et al.*, 1979). The BPH is primarily a phloem feeder, which sucks photosynthetic assimilates by piercing into the parenchymal cells of the phloem tissue. The pest sucks the rice sap with their stylets and blocks xylem and phloem by laying egg masses in midribs of leaf sheaths and leaf blades. The affected plants show symptoms such as chlorosis of stems, wilting of leaves, low productivity, and ultimately death of the entire plant which is named as 'hopperburn' (Du e*t al.*, 2009). These hopperburn patches can spread rapidly as the planthoppers move from dying plants to neighboring plants (Sogawa and Cheng, 1979; Krishnaiah, 2014). A single female adult discharges approximately 13 µL or more honeydew per day during sustained feeding, and honeydew becomes a medium for the sooty mold fungus (Sogawa, 1982). Besides that, the BPH can transmit both rice grassy stunt (RGSV) and rice ragged stunt (RRSV) viruses (Dyck and Thomas, 1979; Cabauatan *et al*., 2009).

The application of pesticides is the most common practice to control BPH. Other than the cost and environmental impact, the application of pesticides has led to a resurgence of BPH and affected natural BPH predators (Gallagher *et al.*, 1994; Tanaka *et al*., 2000; Matsumura *et al.*, 2008). Therefore, cultivating resistant rice varieties is considered the most economical, environmentally sound, and effective BPH management approach (Ramkumar *et al.,* 2016). To date, above 38 genes/QTLs that control BPH resistance have been established through *indica* cultivars and wild species of *Oryza* (Haliru *et al.,* 2020).

Pathak *et al.* (1979) first reported the BPH resistance in rice. The resistant varieties suppress the weight gain of nymphs and maintain low BPH populations across multiple generations (Alam and Cohen, 1998; Jung and Im, 2005). In general, resistant plants exhibit two strategies against BPH: antixenosis and antibiosis. Antixenosis affects insect settling, colonization, or oviposition, while antibiosis reduces insect feeding (Jena and Kim, 2010).

The honeydew excretion is widely used to assess the feeding activities of the BPH. It helps develop a reliable index for resistance or susceptibility of a crop variety to homopteran pests (Auclair, 1959; Liu *et al*., 1994). Accordingly, responses of the test varieties for BPH were determined based on the honeydew excretion by the pest after feeding on the test plants.

However, the pest can rapidly adapt to the host plant resistance leading to enhanced pest virulence against host defense mechanisms (Horgan, 2009). The evolution of new biotypes is a serious threat, and four biotypes have been identified so far (Ikeda and Vaughan, 2009). Therefore, the presence of at least moderate resistance to BPH is a compulsory breeding objective of the crop improvement programs of the Department of Agriculture, Sri Lanka. Since most of the cultivated germplasm is susceptible to BPH, new sources of strong resistance are urgently needed. The current research was conducted to identify BPH resistance in wild *Oryza* species in Sri Lanka including the endemic species *O. rhizomatis* and to assess the availability of known BPH resistant genes in those species.

METHODOLOGY

We screened one recommended popular rice variety; Bg 352, fourteen accessions of endemic wild rice species *O. rhizomatis*, and one accession from each of four other wild rice species *O. nivara*, *O. rufipogon*, *O. granulata*, and *O. eichingeri* by honeydew test under greenhouse conditions at the Rice Research and Development Institute of Batalagoda. Varieties Bg 380 and Ptb 33 were used as susceptible and resistant check varieties, respectively.

Plant Material

Plant materials were collected from the North-Central and North-Western provinces, and Yala national park of Sri Lanka (Table 01), and maintained at the planthouse of the Agricultural Biotechnology Centre, Faculty of Agriculture, University of Peradeniya.

Table 01: Collection sites, features, and GPS coordinates of test entries

Species	Genome	Test line	Collected location		Special features
			N	E	
O. Sativa	AA	Bg 352	Gene bank RRDI, Bg		Cultivating variety
O. Sativa	AA	Bg 380	Gene bank RRDI, Bg		Susceptible check variety
O. Sativa	AA	Ptb33	Gene bank RRDI, Bg		Resistant check variety
O. rhizomatis	CC	INRC 1	79.82	79.94	Endemic WR species
O. rhizomatis	CC	INRC 2	8.05	79.93	Endemic WR species
O. rhizomatis	CC	INRC 3	8.12	79.86	Endemic WR species
O. nivara	AA	INRC 4	8.24	79.86	WR species
O. rhizomatis	CC	INRC 5	7.98	79.91	Endemic WR species
O. rhizomatis	CC	INRC 6	8.31	80.32	Endemic WR species
O. rhizomatis	CC	INRC 7	8.42	80.28	Endemic WR species
O. granulata	GG	INRC 12	7.23	80.32	WR species
O. eichingeri	CC	INRC 13	7.94	80.75	WR species
O. rhizomatis	CC	INRC 15	6.39	81.4	Endemic WR species
O. rhizomatis	CC	INRC 16	6.4	81.43	Endemic WR species
O. rhizomatis	CC	INRC 17	6.49	81.46	Endemic WR species
O. rhizomatis	CC	INRC 18	6.41	81.46	Endemic WR species
O. rhizomatis	CC	INRC 19	6.39	81.47	Endemic WR species
O. rhizomatis	CC	INRC 20	6.42	81.44	Endemic WR species
O. rhizomatis	CC	INRC 21	6.34	81.65	Endemic WR species
O. rhizomatis	CC	INRC 22	6.28	81.37	Endemic WR species
O. rufipogon	AA	INRC 23	6.59	79.96	WR species

N - North of the equator E - East of the prime meridian WR – wild rice
RRDI, Bg – Rice Research and Development Institute, Batalagoda

Honeydew Test

Since the initiation of the resistance breeding program for BPH in 1974, *Ptb 33* has been used as the donor parent while *Bg 380* has been used as the susceptible check variety (Fernando *et al.*, 1979; Kudagamage and Nugaliyadde, 1995). Seedlings of selected accessions were grown along with the resistant check and susceptible variety individually in 15 cm diameter and 20 cm height pots (Kumar, 2019) (Figure 1).

The honeydew test was conducted in a complete randomized design with 15 replicates for all tested lines under greenhouse conditions at the RRDI, Batalagoda. Plants at the first tiller stage (40 days old) were planted individually. A Whatman no. 2 filter paper stained with the pigment bromocresol green (2 mg/1 mL of 70 % ethanol, orange in color) was kept on the top of the cup (5 cm diameter x 5 cm height). Thereafter each plant was covered by an inverted cup of similar size with a small hole at the bottom to make a feeding chamber. The upper portion of the test plant was

slipped through the hole of the upper cup to make a feeding chamber, illustrated in Figure 1 a. A pair of gravid females of BPH previously starved for 5 hrs were placed in the feeding chamber and allowed to feed for 24 hrs, and subsequently, the filter papers were collected.

Figure 01: a. The feeding chamber installation for a single plant; b. The layout of the experiment.

Bromocresol green indicates phloem-based honeydew as blue-rimmed spots (indicate susceptible plants) and xylem-based honeydew as transparent spots (indicate resistant plants). The spots were traced on tracing paper, and squares were counted over a millimeter-square graph paper to calculate area. The resulting area was proportional to the honeydew production and for BPH feeding (Heinrichs *et al.,* 1985).

Statistical Analysis

The honeydew excretion data were analyzed using ANOVA and the mean separation was done following Duncan Multiple Range Test using the statistical software SAS® version 9.1 software (SAS Institute, 2011).

Gene alignments and CDS coverage

The annotated genomes of *O. rhizomatis*, *O. eichingeri*, *O. nivara*, *O. rufipogon*, and *O. granulata* are not available so far. Therefore, coding sequences (CDS) for known BPH-resistant genes are not readily available for these species. Therefore, a read mapping and consensus generating approach was applied to obtain the orthologs. Available CDS for known BPH resistance genes, *BPH3* (Rathnayake *et al.*, 2019), *BPH4* (Rathnayake *et al.*, 2019), *BPH6* (Guo *et al.*, 2018), *BPH9* (Zhao *et al.*, 2016), *BPH14* (Du *et al.*, 2009), and *BPH18* (Ji *et al.*, 2016) in *O. sativa* were downloaded from National Center for Biotechnology Information (NCBI). The lengths of *BPH3* (MK879827) and *BPH4* (MK879837) cDNA sequences are 75bp and 176bp, respectively. Since this length is less than the insert sizes of selected whole-genome sequencing (WGS) datasets, the read aligner can misalign the reads from other similar genomic sites, creating problems in constructing ortholog sequences. Hence, BPH3 and BPH4 were not included in the analysis. The *BPH6* (KX818198), *BPH9* (KU216221), *BPH14* (FJ941067), and *BPH18* (KJ850252) were considered for the downstream analysis. *O. rhizomatis* (DRR054692 and DRR055281), *O. eichingeri* (DRR057205, DRR057208, and DRR054687), *O. nivara* (SRR1450138, SRR11070145, and SRR1743122), *O. rufipogon*, (DRR226060 and SRR12481866), and *O. granulata* (SRR6175405 and DRR056667) WGS datasets were retrieved from NCBI Sequence Read Archive (SRA).

We used Bowtie2 (v2.3) (Langmead and Salzberg, 2012) short read aligner to align cDNA reads to CDS of selected genes. The horizontal coverage, defined as the number of total bases of considered CDS covered by the mapped read, was calculated using Geneious Prime software (v2020.1.2). Then the consensus sequences were generated using Geneious Prime software, and multiple sequence alignments were performed using MAFFT (v7.450) (Katoh and Standley, 2013). The longest aligning region within the coding region was selected for further analysis by omitting the zero coverage regions in each alignment. As the coverage was low for three out of four genes considered, *O. granulata* (Table 02) was not considered in further analysis. Due to low coverage, SRR1450138 and DRR057205 were also not included in the alignments of BPH6 and BPH18, respectively. By normalizing the base pair difference by the CDS lengths, the percentage variable sites compared to *O. sativa* were computed (Table 03).

Gene phylogenies

The phylogenetic analysis was conducted using MEGA X (Kumar *et al.*, 2018) and the maximum likelihood trees were constructed using the Tamura–Nei (Tamura and Nei, 1993) genetic distance model with 1000 bootstrap replicates.

RESULTS AND DISCUSSION

The honeydew test results among the wild and cultivated rice varieties in Sri Lanka were significantly different (α = 0.05). There was no honeydew produced on all tested *O. rhizomatis* accessions (Figure 02 and 03), suggesting strong resistance against BPH (mean = 0.0 c). Honeydew production by BPH on *Bg 380,* the susceptible check was significantly higher (mean = 37.0, F = 24.5, P < 0.0001) than *Ptb 33*. The promising variety, *Bg 352*, resulted in significantly higher honeydew production confirming its susceptibility to the pest (mean=23.0 b). All the other tested accessions of *O. nivara, O. granulata, O. eichingeri* showed similar resistance levels with means of 0.3 c. The resistant check, *Ptb 33,* and *O. rufipogon* resulted in similar levels of resistance (mean=3.0 c) with little honeydew excretions. These results suggest that the *O. rhizomatis* accessions have significantly higher negative effects on the feeding behavior of BPH females over the other species and varieties tested. Duncan Vaughan (1990), the first reporter of the endemic wild rice species of *O. rhizomatis* suggested possible BPH resistance though not validated to date.

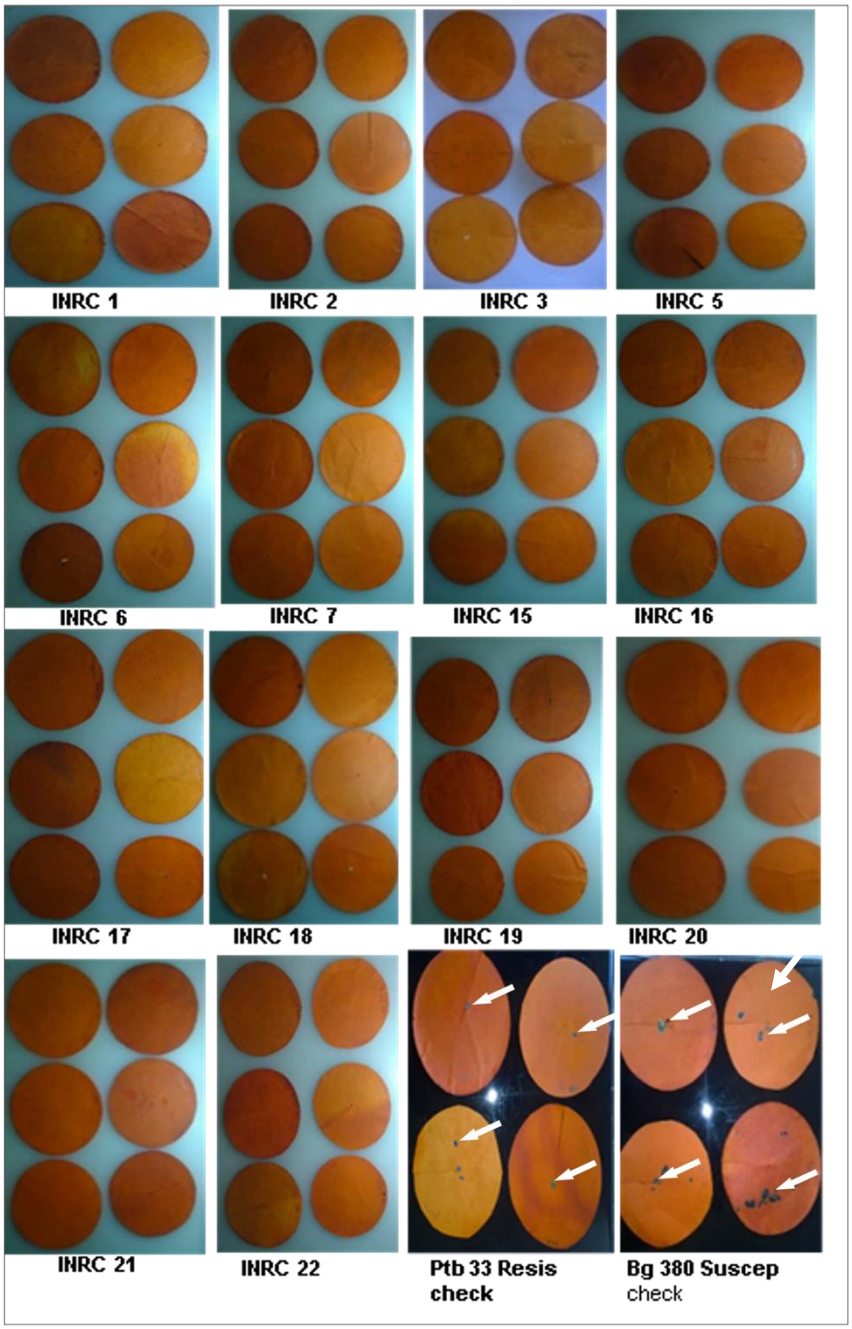

Figure 02: Honeydew test against the *O. rhizomatis* accessions and the standard check varieties.
Arrows mark the presence of honeydew spots on the filter papers

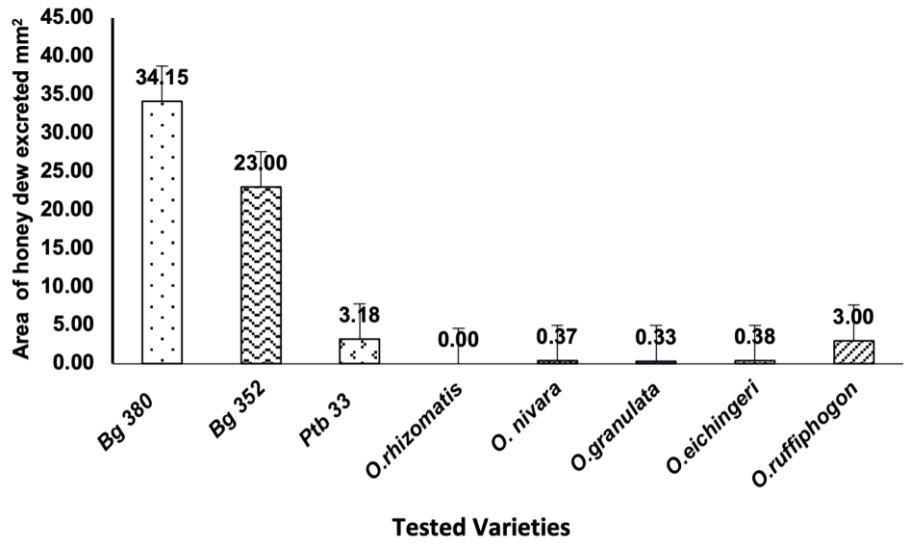

Figure 03: Mean area (mm²) of honeydew excreted by previously starved BPH females when fed on tested varieties.

There are different mechanisms proposed to explain the BPH resistance in rice. While some of them are physical barriers, others are enzymatic reactions. Low honeydew excretion by BPH females indicated the existence of the antibiosis mechanism that reduces insect feeding. The resistant genotypes against BPH of rice have a higher content of soluble phenolics, peroxidases, and polyphenol oxidases (Sing *et al*., 2017). The feeding habits of insects affect the leaf surface lipids and the ability of the insect to select suitable feeding sites on the plant (Denno and Roderick, 1990). Further, the host choice of BPH for feeding and ovipositing is positively associated with the nitrogen concentration of plant tissues, while negatively correlated with silicon (Si), but not with phosphorus (P) and potassium (K) (Denno and Roderick, 1990). The biochemical composition of the tissues is associated with the BPH host preference and regulates how fast and where they settle on a rice plant (Rashid *et al.,* 2017). The phenolic biochemicals negatively correlated with the phloem-feeding habit of the planthoppers, and they avoid many allelochemicals that are tagged in nonvascular tissues (Sogawa, 1982). Interestingly, the mean protein, carbohydrate, and fat content of *O. rhizomatis;* is higher than that of the cultivated rice variety, *Bg 352* (Rajkumar *et al*, 2014). Therefore, the presence of higher nutrient contents along with other biochemical compounds (Punithavalli *et al.*, 2013; Singh *et al.*, 2017) may be responsible for the less preferred feeding of BPH on *O. rhizomatis.*

Up to date, BPH resistance has been reported from wild rice species, *O. officinalis* (Hirabayashi, 1998), *O. australiensis* (Ishii *et al.*, 1994), and *O. eichingeri* (Liu *et al.*, 2001). Our results on the tested accession of *O. eichingeri* were also in agreement with the previous results. The presence of the *BPH2* gene has already been confirmed in *O. nivara* (Madurangi *et al.*, 2013). Identification of genetic resistance is important for successful breeding programs. While the different genes and mechanisms of resistance may be present in wild species, the knowledge of the presence of known resistance genes for BPH may be helpful in marker-assisted breeding programs in rice. Therefore, to assess the presence of those genes, we calculated the horizontal coverage of coding sequences of known genes, *BPH6, BPH9, BPH14-1,* and *BPH18* (Table 02).

Interestingly, all the CDS considered are present in both accessions of *O. rhizomatis with some sequence data.* The *BPH14-1* gene is present in all five species with over 70% coverage to the *O. sativa* sequence. The other three genes are present with more than 70% coverage in all the accessions of considered species except for both accessions of *O. granulata and* one accession of *O. eichingeri* (Table 02). Poor coverage may be due to the absence of those genes on *O. granulata* or the low availability of data. Even though the coding sequences of considered genes were present in four species, there is a considerable difference in the percentage of variable sites compared to the coding sequence of *O. sativa* (Table 03).

Table 02: Horizontal coverage of CDS regions of respective BPH resistance genes

Oryza sp.	ID	NCBI SRA ID	Horizontal coverage of CDS region %			
			BPH6	***BPH9***	***BPH14-1***	***BPH18***
O. rhizomatis	*O. rhizomatis 1*	DRR054692	96.7	74.4	93.8	98.4
	O. rhizomatis 2	DRR055281	93.1	79.8	96.0	98.9
O. eichingeri	*O. eichingeri 1*	DRR057205	73.9	54.3	92.4	41.1
	O. eichingeri 2	DRR057208	83.5	78.5	98.2	99.3
	O. eichingeri 3	DRR054687	85.0	80.5	95.2	99.5
O. nivara	*O. nivara 1*	SRR1450138	41.9	93.5	98.0	100.0
	O. nivara 2	SRR11070145	99.4	83.5	95.6	100.0
	O. nivara 3	SRR1743122	99.1	85.3	95.2	99.9
O. rufipogon	*O. rufipogon 1*	DRR226060	96.9	80.3	94.9	99.5
	O. rufipogon 2	SRR12481866	100.0	75.3	94.9	100.0
O. granulata	*O. granulata 1*	SRR6175405	19.8	27.9	77.4	39.8
	O. granulata 2	DRR056667	10.4	21.8	71.9	38.4

Gene phylogenies were assessed with non-rooted trees to evaluate the intraspecies diversity and relatedness among species. There is considerable intraspecies diversity in CDS of considered genes. For example, one *O. nivara* accession is always grouped with other species but not with the accessions of the same species (Figure 04). Interestingly, *O. rhizomatis* always clustered with *O. eichingeri,* suggesting the closeness of DNA sequences of considered genes. The close relationship of these two species has previously considered proposed as a single species in some literature (Bautista *et al.,* 2006; Zhang and Ge, 2007; Zang *et al.*, 2011). *O. sativa* sequence grouped with *O. nivara* and *O. rufipogon* in all three cases except in *BPH14*. Even though the known genes are present in the wild species with some degree of similarity to *O. sativa* sequences, these species may also consist of different genes and mechanisms of resistance to BPH and other biotic and abiotic stresses.

Table 03: Base pair difference and percentage of variable sites with compared to *O. sativa* sequence

ID	% Variable sites			
	***BPH6* (727bp)**	***BPH9* (600bp)**	***BPH14*-1 (837bp)**	***BPH18* (1044bp)**
O. rhizomatis 1	8.4	9.2	11.4	7.3
O. rhizomatis 2	8.2	9.2	9.2	7.7
O. eichingeri 1	10.3	7.8	9.7	*
O. eichingeri 2	10.3	7.8	8.1	5.8
O. eichingeri 3	9.4	7.8	8	5.8
O. nivara 1	*	6.6	10	3.5
O. nivara 2	6.2	6.8	10.9	3.6
O. nivara 3	3.2	6.2	10	0.6
O. rufipogon 1	3.3	5.8	9.4	0.6
O. rufipogon 2	1.7	6.2	9.6	0.6

***** Did not include in the analysis due to low horizontal coverage (Table 02)

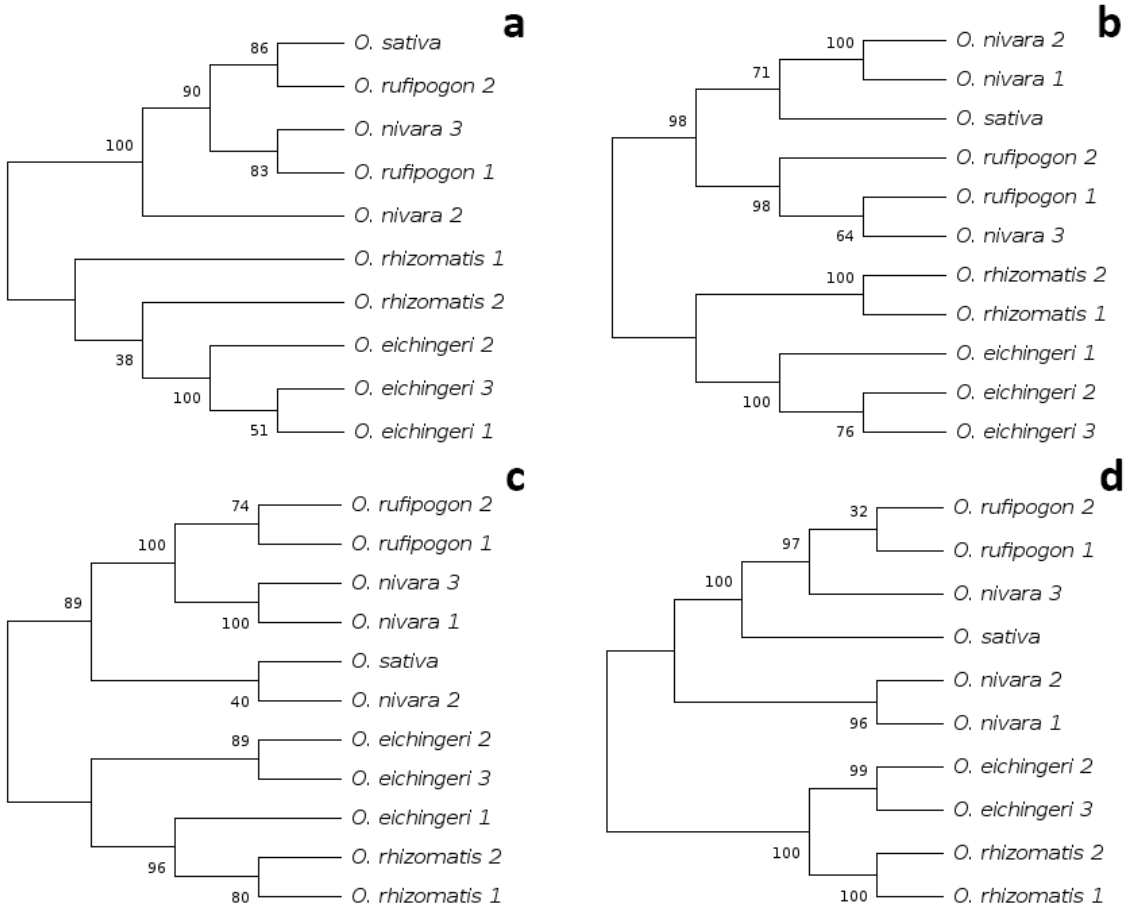

Figure 04: Maximum likelihood trees (unrooted) for considered genes.
a. ***BPH6*** **b.** ***BPH9*** **c.** ***BPH14-1*** **d.** ***BPH18*** (Values on branches represent bootstrap values)

CONCLUSIONS

All fourteen *O. rhizomatis* accessions showed strong resistance against BPH under greenhouse conditions. Similarly, selected accessions of four wild rice species, *O. nivara, O. granulata, O. eichingeri,* and *O. rufipogon* showed resistance to the BPH. The analysis suggests the presence of orthologous sequences of known resistant genes, *BPH6*, *BPH9*, *BPH14-1,* and *BPH18* in *O. rhizomatis, O. eichingeri, O. nivara, and O. rufipogon*. However, clear inter and intraspecies variability exists in considered gene regions. Therefore, further studies are proposed to identify genes and QTLs in wild species. The resistant germplasm identified can be used in future breeding programs for BPH resistance.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support of the International Relations Office [grant IRC/RG/13/01] of the University of Peradeniya. The authors express sincere gratitude to the staff of the Entomology Division, Rice Research and Development Institute, Batalagoda, and the Agricultural Biotechnology Center, the University of Peradeniya for their support for the successful completion of the research.

REFERENCES

Alam, S. N., & Cohen, M. B. (1998). Detection and analysis of QTLs for resistance to the brown planthopper, *Nilaparvata lugens*, in a doubled haploid rice population. Theor Appl Genet *97,* 1370-1379.

Auclair, J. L. (1959). Feeding and excretion by the pea aphid, *Acyrthosiphon pisum* (Harr.) (Homoptera: Aphididae), reared on different varieties of peas. *Entomologia experimentalis et applicata*, *2*(4), 279-286.

Bautista, N. S., Vaughan, D., Jayasuriya, A. H. M., Liyanage, A. S. U., Kaga, A., & Tomooka, N. (2006). Genetic diversity in AA and CC genome Oryza species in southern South Asia. *Genetic Resources and Crop Evolution*, *53*(3), 631-640.

Cabauatan, P. Q., Cabunagan, R. C., & Choi, I. R. (2009). Rice viruses transmitted by the brown

planthopper *Nilaparvata lugens* Stål. *Planthoppers: New threats to the sustainability of intensive rice production systems in Asia*, 357-368.

Chen, R. C., Cheng, S. N., & Yan, L. M. (1979). The ovarian development of the brown planthopper (*Nilaparvata lugens*) and its relation to migration. Acta Entomol. Sinica. *22,* 280-286.

Denno, R. F., & Roderick, G. K. (1990). Population Biology of Planthoppers. Ann. Revi. entomo*, 35,* 489–520*.*

Du, B., Zhang, W., Liu, B., Hu, J., Wei, Z., Shi, Z., ... & 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.

Dyck, V. A., & Thomas, B. (1979). The brown planthopper problem. In: Brown planthopper: a 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. *Brown Planthopper: Threat to Rice Production in Asia*, 241-249.

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: Denno R.F., Perfect T.J. (eds) Planthoppers. Springer, Boston, MA. pp. 599-614.

Guo, J., Xu, C., Wu, D., Zhao, Y., Qiu, Y., Wang, X., ... & He, G. (2018). Bph6 encodes an exocyst-localized protein and confers broad resistance to planthoppers in rice. *Nature genetics*, *50*(2), 297-306.

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

Heinrichs, E. A., Medrano, F. G., & Rapusas H. R. (1985). Genetic Evaluation for Insect Resistance in Rice. Int. Rice Res. Inst.*1*. 356.

Hirabayashi, H. (1998). Identification of brown planthopper resistance gene derived from *O. officinalis* using molecular markers in rice., Breed. Sci*.* 48*(1)*, 82.

Horgan, F. G., (2009). Mechanisms of resistance: a major gap in understanding planthopper-rice interactions. In: Planthoppers: new threats to the sustainability of intensive rice production systems in Asia. Heong, K. L, Hardy, B. (Eds.) International Rice Research Institute, Los Baños, Philippines. pp 281-302.

Ikeda, R., & Vaughan, D. A. (2009). The distribution of resistance genes to the brown planthopper in rice germplasm. Advances in Agronomy. *8*, 2 – 4.

IshiI, T., brar, D. S., Multani, D. S., & Khush, G. S. (1994). Molecular tagging of genes for brown planthopper resistance and earliness introgressed from *Oryza australiensis* into cultivated rice, *O*. *sativa*. Genome, *37*(*2*), 17-22.

Jena, K. K., & Kim, S. M. (2010). Current Status of Brown Planthopper (BPH) Resistance and Genetics. Rice *3*(*2*),161–171.

Ji, H., Kim, S. R., Kim, Y. H., Suh, J. P., Park, H. M., Sreenivasulu, N., ... & Jena, K. K. (2016). Map-based cloning and characterization of the BPH18 gene from wild rice conferring resistance to brown planthopper (BPH) insect pests. *Scientific Reports*, *6*(1), 1-14.

Jung, J. K., & Im, D. J. (2005). Feeding inhibition of the brown planthopper, *Nilaparvata lugens* (Homoptera: *Delphacidae*) on a resistant rice variety. J. Asia-Pac. Entomol.*8* (*3*), 301–308.

Katoh, K., & Standley, D. M. (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Molecular Biology and Evolution, 30(*4*), 772–780.

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

Kudagamage, C., & Nugaliyadde, L. (1995). Present status and future direction of insect pest management in rice. In (Amarasiri K. and Nagarajah B.M.K eds) Rice congress: 1990. pp 39-50.

Kumar, P., Kumar, M., Singh, H., Sengar, R., & Sirohi, A. (2019). Evaluation of resistance in rice genotypes against the brown planthopper

(Nilaparvata lugens Stal). Inter. Jor. of chemi. Studies. 7(*4*). 3015-3017.

Kumar, S., Stecher, G., Li, M., Knyaz, C., and Tamura, K. (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular biology and evolution, *35*(*6*), 1547 -1549.

Langmead, B. and Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2, Nature Methods. 9(*4*), 357–359.

Liu, G., Saxena, R.C., and Wilkins, R.M. (1994). Behavioral responses of the white-backed planthopper, *Sogatella furcifera* (Homoptera: *Delphacidae*) on rice plants whose odors have been masked. J. Insect Behav. *7*, 343-353.

Liu, G., Yan, H., Fu, Q., Qian, Q., Zhang, Z., Zhai, W., and Zhu, L. (2001) Mapping of a new gene for brown planthopper resistance in cultivated rice introgressed from *Oryza eichingeri*. Chinese Science Bulletin. 46(17),1459–1462.

Madurangi, S., Ratnasekara, D., Senanayake, S., Samarasingha, W. and Hemachandra, P. (2013). Antixenosis and antibiosis effects of Oryza nivara accessions harboring *bph2* gene on brown planthopper (*Nilaparvata lugens* (Stal)). National Science Foundation of Sri Lanka, 41(*2*), 147-154.

Matsumura, M., Takeuchi, H., Satoh, M., Morimura, S. S., Otuka, A., Watanabeb, T. and Thanh, D.V. (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 Mana. Sci. 64(*11*),1115–1121.

Muduli, L., Pradhan, S. K., Mishra, A., Bastia, D. N., Samal, K. C., Agrawal, P. K., and Dash, M. (2021). Understanding Brown Planthopper Resistance in Rice: Genetics, Biochemical, and Molecular Breeding Approaches. Rice Science. *28*, (*6*),532-546.

Normile, D. (2008). Reinventing rice to feed the world. Science. *321,* 330–333.

Punithavalli, M., Muthukrishnan N.M., Balaji M. and Rajkuma, (2013). Defensive Responses of Rice Genotypes for Resistance Against Rice Leaffolder *Cnaphalocrocis medinalis*, Rice Science, *20 (5),* 363-370.

Rajkumar, G. (2014). Comparison of the general nutritional composition of wild rice *Oryza rhizomatis* D. A. Vaughan and the commercial variety *Bg 352*. Tropical plant research*. 1*. 8-10.

Ramkumar, G, Prahalada, G. D, Hechanova, S. L., Kim, S. R., & Jena. K. K. (2016). Exploring genetic diversity of rice cultivars for the presence of brown planthopper (BPH) resistance genes and development of SNP marker for *Bph18*. Plant Breed *10*.1111-.12365.

Rashid, M. M., Jahan, M., & Islam, K. S. (2017). Effects of Nitrogen, Phosphorous, and Potassium on Host-Choice Behavior of Brown Planthopper, *Nilaparvata lugens* (Stål) on Rice Cultivar. J Insect Behav. *30* (*1*), 1–15.

Rathnayake, S., Rathnayake, R., Jayarathne, H., Nizam, R., Ranaweera, T., Kannangara, S., Udawela, U., Weebadde, C., & Sooriyapathirana, S. (2019). Assessment of the Sequence-Based Haplotype-Variants in Selected DNA Marker Loci for the Molecular Breeding of Resistant Rice Varieties to Brown Plant Hopper. *1*. 2-15.

SAS Institute Inc., SAS 9.1.3 Help and Documentation, Cary, NC: SAS Institute Inc., 2002-2004.

Shi, L., Dong, M., Lian, L., Zhang, J., Zhu, Y., Kong, W., ... & Jiang, Z. (2021). Genome-Wide Association Study Reveals a New Quantitative Trait Locus in Rice Related to Resistance to Brown Planthopper Nilaparvata lugens (Stål). *Insects*, *12*(9), 836.

Singh, I., Sarao, P. S., & Sharma, N. (2017). Antibiosis components and antioxidant defense of rice as mechanism of resistance to brown planthopper, *Nilaparvata lugens* (Stål). *Cereal Research Communications*, *45*(2), 284-295.

Sivasubramaniam, N., & Imthiyas, 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. Jour. of Agri. and Vete. Sci. *5.* 595-600.

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

Sōgawa, K., & Cheng, C H, (1979). Economic thresholds, nature of the damage, and losses caused by the brown planthopper. Brown

planthopper: Threat to rice production in Asia. pp 125-142.

Tamura, K., & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molec. Biol. and Evol. 10(*3*), 512–526.

Tanaka, K., Endo, S., & Kazano, H. (2000). Toxicity of insecticides to predators of rice planthoppers: spiders, the mired bug, and the *dryinid* wasp. Appl. Entomo. Zool*. 35*(*1*), 177–187.

Vaughan, D. A. 1990. A new rhizomatous *Oryza* species (Poaceae) from Sri Lanka, Bota Jour*.* 103:*2*. 159–163.

Zang, L. L., Zou, X. H., Zhang, F. M., Yang, Z. & Ge, S (2011). Phylogeny and species delimitation of the C-genome diploid species in *Oryza*. Jour. of Syst. and Evolu. 49(*5*), 386-395.

Zhang, L. B., & Ge, S. (2007). Multilocus analysis of nucleotide variation and speciation in Oryza officinalis and its close relatives. *Molecular Biology and Evolution*, *24*(3), 769-783.

Zhao, Y., Huang, J., Wang, Z., Jing, S., Wang, Y., Ouyang, Y., ... & He, G. (2016). Allelic diversity in an NLR gene BPH9 enables rice to combat planthopper variation. *Proceedings of the National Academy of Sciences*, *113*(45), 12850-12855.