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Donors for Resistance to Brown Planthopper *Nilaparvata lugens* (Stål) from Wild Rice Species



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Abstract: Out of 1 989 wild accessions sown in seed boxes for screening, only 1 003 wild accessions with good germination were screened against brown planthopper (BPH), *Nilaparvata lugens* (Stål) under greenhouse conditions. The collection comprised of accessions from 11 wild species and African cultivated rice. The germplasm was screened for BPH following standard seed box screening technique in the greenhouse. As many as 159 accessions were identified as resistant during the year 2012 based on one year screening. A selected set of BPH resistant accessions were screened again during 2013. Based on the two years screening, seven accessions of *O. nivara* (AA), one accession of *O. officinalis* (CC), seven accessions of *O. australiensis* (EE), five accessions of *O. punctata* (BB and BBCC) and nine accessions of *O. latifolia* (CCDD) were confirmed to be resistant to BPH. So far no BPH resistance genes have been identified and designated from *O. nivara* and *O. punctata*, hence these may act as new sources of resistance.

Key words: brown planthopper; *O. nivara*; *O. officinalis*; *O. australiensis*; *O. punctata*; resistance; rice

The genus *Oryza* of Gramineae family has 22 wild species and two cultivated ones, *Oryza sativa* L. and *O. glaberrima* Steud distributed worldwide (Brar and Khush, 1997). *O. sativa* L. is cultivated extensively in the most diverse ecosystems of tropical and sub-tropical regions of the world. It is the staple food for people in 39 countries, which include 2.70 billion people in Asia alone. Among various biotic constraints of rice production, the insect pests are of prime importance and warm humid environment of the crop is also conducive for their survival and proliferation. Among them, brown planthopper, *Nilaparvata lugens* (Stål) (BPH), is a typical phloem sap feeder and one of the most serious and destructive pests of rice throughout Asia (Normile, 2008; Heong and Hardy, 2009). It causes yield loss amounting to as high as 60% under epidemic conditions (Srivastava et al, 2009; Kumar et al, 2012). It is difficult to monitor this pest and by the time plant damage become evident, significant loss in yield is inevitable. Only way possible for the management of this pest is the regular monitoring of the crop. Both nymphs and adults suck sap from the leaves and leaf sheaths, which

results in yellowing of leaves, reduced tillering, reduced plant height and increase in number of unfilled grains. BPH also causes the reduction in chlorophyll, protein content of leaves and photosynthetic rate, whereas severe attack of BPH causes ‘hopper burn’ symptoms (Liu et al, 2008; Horgan, 2009; Vanitha et al, 2011). It also transmits virus diseases like grassy stunt, ragged stunt (Ling et al, 1978) and wilted stunt (Chen et al, 1978).

Though many chemicals were recommended for the control of this pest (Sarao, 2015), due to its feeding behaviour at the base of the plant, the farmers are unable to control this pest effectively. As a result, farmers resort to blanket application of insecticides which often disrupts the ecological balance of rice ecosystem due to which this pest has already developed resistance against many insecticides in different Asian countries (Gorman et al, 2008; Matsumura et al, 2009). The use of genetic resistance is the most effective measure for BPH management. Cultivation of resistant varieties is an economical, efficient and environmentally sound strategy for population management of insect-pests. These varieties provide pest control at essentially no cost to the

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farmers and it is extremely important as today's rising input cost has severely eroded the farmer's profit margin. Varieties with various levels of resistance can be deployed for insect control in combination with other components of pest management. It also helps in conservation of natural enemies and minimizing the number of pesticide applications. Resistant and moderately resistant varieties keep the pest densities below the economic threshold levels and best in combination with natural enemies (Gurr et al, 2011). Restless behavior of insects on the resistant varieties also increases their vulnerability to the natural enemies. The use of these combinations brings the unrelated mortality factors and provides the density-independent mortality of insect-pests (Gould et al, 1991).

Thirty BPH resistance genes had been identified from rice cultivars (Fujita et al, 2013). The BPH resistance genes from *Bph1* to *Bph9* are from *O. sativa* as gene source, whereas from *Bph10* to *Bph30* gene source are from wild rice species (except for *Bph17*, *Bph25*, *Bph26*, *Bph27* and *Bph28*). Among different methods evaluated to find BPH resistance in rice (Myint et al, 2009; Nanthakumar et al, 2012), seedling screening is the most popular and high throughput method due to its rapid and efficient screening of rice lines (Li et al, 2010; Fujita et al, 2013). Majority of the genes identified from *O. sativa* and other wild species are ineffective against the BPH biotype(s) prevalent in North Western India, thus requiring identification of new sources of resistance. In the present study, we evaluated 1 003 accessions of wild species of rice for resistance to BPH and here we report the resistant accessions. We also discussed the possibilities of transferring these to cultivated rice. This will also help in broadening of genetic base, which is vital and integral part of germplasm improvement.

MATERIALS AND METHODS

Rearing of BPH colony

The colony of BPH biotype 4 was maintained on 30-day-old

TN1 rice plants under greenhouse conditions at $28 \text{ }^{\circ}\text{C} \pm 2 \text{ }^{\circ}\text{C}$, $75\% \pm 5\%$ relative humidity and 14 h light and 10 h dark photoperiod conditions positioned at Ludhiana, India ($30^{\circ}54' \text{ N}$ and $75^{\circ}48' \text{ E}$) in India according to Heinrichs et al (1985). Such cages of insect culture were maintained throughout the studies to obtain sufficient number of insects for conducting the experiment. For obtaining the 2nd to 3rd instar nymphs, newly emerged male and female adults were paired and released on 30-day-old TN1 plants for oviposition.

Raising test genotypes

Seeds of 1 989 wild rice accessions were received primarily through International Rice Research Institute, Los Baños, the Philippine and Central Rice Research Institute, Cuttack, India. After sowing all the accessions in seed boxes successively, the 1 003 accessions with good germination were finally evaluated during 2012. These accessions consist of *O. glaberrima* Steud., *O. barthii* A. Chev., *O. nivara* Sharma et Shastry, *O. rufipogon* Griff., *O. longistaminata* A., *O. meridionalis* Ng, *O. glumaepatula* Steud., *O. officinalis* Wall ex. Watt, *O. australiensis* Domin., *O. punctata* Kotschy ex Steud., *O. minuta* J.S. Presl. ex C.B. Presl. and *O. latifolia* Desv. (Table 1). During 2013 crop season, only the selected set of BPH resistant accessions were reevaluated. The seeds of these wild accessions and TN1 (susceptible check) were soaked in separate petri dishes as per sowing schedule and decanted after one day during wet seasons 2012 and 2013. The pre-germinated seeds of the test accessions were sown in seed boxes (45 cm \times 35 cm \times 10 cm) containing well puddled soil in rows at spacing of 3.5 cm apart. The sprouted seeds of susceptible check TN1 were sown in two border rows and in half of the middle row. The accessions were sown with the maximum of 10 seeds in each line depending upon availability of seed. All the test plant trays were raised in an insect-proof greenhouse at $30 \text{ }^{\circ}\text{C} \pm 2 \text{ }^{\circ}\text{C}$, $80\% \pm 5\%$ relative humidity.

Screening of genotypes

Screening of the genotypes was done according to standard

Table 1. Number of accessions of *Oryza* species evaluated against brown planthopper in 2012.

Species	Genome	No. of accessions screened	No. of susceptible accessions	No. of resistant accessions
<i>O. glaberrima</i>	AA	44	44	0
<i>O. barthii</i>	AA	29	29	0
<i>O. nivara</i>	AA	451	391	60
<i>O. rufipogon</i>	AA	326	279	47
<i>O. longistaminata</i>	AA	51	38	13
<i>O. meridionalis</i>	AA	16	16	0
<i>O. glumaepatula</i>	AA	12	11	1
<i>O. officinalis</i>	CC	7	1	6
<i>O. australiensis</i>	EE	12	3	9
<i>O. punctata</i>	BB, BBCC	29	22	7
<i>O. minuta</i>	BBCC	2	0	2
<i>O. latifolia</i>	CCDD	24	10	14
Total		1003	844	159

seedbox screening technique developed by Heinrichs et al (1985). There were three replications for each accession and these were infested at 10–12 d old with the 2nd to 3rd instar hopper nymphs @ 6–8 nymphs per seedling. Damage score on each line was recorded on 0–9 scale when more than 90% plants of TN1 were killed following the Standard Evaluation System (SES) of rice (IRRI, 1996). Each accession was scored on the individual plant basis as 0 (no visible damage), 1 (partial yellowing of first leaf), 3 (first and second leaves of most of plants partially yellow), 5 (pronounced yellowing and stunting or about half of the plants wilted or dead), 7 (more than half plants wilted or dead and remaining plants severely stunted), 9 (all plants wilted and dead) (IRRI, 1996). The interpretation of results of each accession had been based on standard evaluation system in which accessions with a mean rating of 0–3.49, 3.50–5.49 and 5.50–9.00 were designated as resistant, moderately resistant and susceptible, respectively (Heinrichs et al, 1985).

RESULTS AND DISCUSSION

During 2012 season, as many as 159 accessions were found to be resistant to BPH (Table 1). The BPH resistant accessions were identified in *O. nivara* (60), *O. rufipogon* (47), *O. longistaminata* (13), *O. glumaepatula* (1), *O. officinalis* (6), *O. australiensis* (9), *O. punctata* (7), *O. minuta* (2), and *O. latifolia* (14) (Table 1). Of the 159 accessions that had shown resistant reaction during 2012, 31 accessions were again screened during 2013. Seven *O. nivara* accessions were resistant over the two years, while one accession was moderately resistant during 2013 (Table 2). Similarly, *O. longistaminata* accession IRGC81967, which was resistant during 2012, showed moderate resistance during 2013. The performance of moderately resistant lines may be due to relative position of these lines in the screening trays over the years. The remaining 22 accessions comprising of *O. officinalis*, *O. australiensis*, *O. punctata* and *O. latifolia* showed resistant reaction during both the two years (Table 2). Thus a total of 29 accessions showed resistance to BPH over the two years.

The wild species of *Oryza* genus contains different species having genes of economic importance for resistance to biotic stress. Several reports in the literature indicates that wild species viz., *O. nivara*, *O. punctata*, *O. longistaminata*, *O. barthii*, *O. rufipogon*, *O. officinalis*, *O. australiensis*, *O. minuta*, *O. latifolia* and *O. glaberrima* are important source of planthopper resistance genes (Jena, 2010; Fujita et al, 2013), but in our studies none out of the 44 accessions of *O. glaberrima* were resistant to BPH (Table 1). This variation in resistance reaction may be due to difference in BPH biotype used for screening. Seven accessions of *O. nivara* were consistently resistant to BPH for the two years. This species

considered being an ecotype or sibling species of *O. rufipogon* and the closest progenitor of *O. sativa* having AA genome and these species are cross compatible and show homologous chromosome pairing. It is easy to transfer valuable genes from AA genome wild species into cultivated rice by conventional breeding methods. The genetic base of this species will be helpful in using these accessions to develop pre-breeding lines resistant to BPH so as to unravel *denovo* variability in germplasm (Mui and Bong, 1999; Jena, 2010; Madurangi et al, 2011). Some scientists have transferred the BPH resistance and grassy stunt gene from *O. rufipogon* (Chen et al, 2010; Deen et al, 2010) and grassy stunt gene from *O. nivara* (acc. 101508) (Ling et al, 1970; Brar and Khush, 1997) into elite breeding lines of indica background (Brar and Khush, 1997).

A large number of distantly related wild species were reported as novel sources of resistance to insect-pests (Heinrichs et al, 1985). The transfer of useful genes from these wild species into elite cultivated rice species is difficult due to non-compatibility but the advances in the biotechnology in recent years has provided an opportunity to generate inter specific hybrids by embryo rescue methods (Jena and Khush, 1989). In case of introgression from CC genome, useful genes for resistance to BPH like *Bph11(t)*, *Bph12(t)*, *Bph13(t)*, *Bph14* and *Bph15* have been transferred from *O. officinalis* into elite breeding lines by different scientists (Hirabayashi et al, 1998, 1999; Renganayaki et al, 2002; Yang et al, 2004; Du et al, 2009). In our studies, *O. officinalis* accession IR106399 showed resistance to BPH over the two years. Similarly, Huang et al (2001) transferred strong BPH resistant gene into rice line Zhenshan 97B from *O. officinalis*.

Of the CCDD genome species, we found nine accessions of *O. latifolia* showed resistance to BPH over the years (Tables 1 and 2). Qiu et al (2012) also reported that advanced lines derived from the introgression of *Bph12* gene from *O. latifolia* had shown resistance to BPH. In case of *O. australiensis* screening, seven accessions had showed resistance to BPH over the years (Table 2). Earlier also two dominant genes, *Bph10* and *Bph18*, had been introgressed from *O. australiensis* which is an EE genome species (Ishii et al, 1994; Jena et al, 2006).

Many valuable genes for BPH resistance were also identified and tagged with molecular markers from secondary and tertiary gene pools like *O. officinalis* (acc. 100914), *O. australiensis* (acc. 100882), *O. minuta* (acc. 101141) and *O. latifolia* (acc. 100914) (Hirabayashi et al, 1998, 2004; Jena et al, 2002, 2006; Renganayaki et al, 2002; Yang et al, 2004; Rahman et al, 2009; Ram et al, 2010; Jena and Kim, 2010; Yang et al, 2012). BPH resistance genes from *O. minuta* and *O. australiensis* had been fine mapped using sequence information of *O. sativa* cv Nipponbare (Jena et al, 2006; Jena and Kim, 2010), whereas *Bph14* gene derived from *O. officinalis* conferring resistance to

Table 2. Reaction of wild *Oryza* species accessions against BPH over the years.

Species (Accession number) ^a	Genome	Country of origin	2012		2013	
			BPH score ^b	BPH reaction	BPH score ^b	BPH reaction
<i>O. nivara</i> (IRGC81859)	AA	India	1.50	R	1.64	R
<i>O. nivara</i> (IRGC92945)	AA	Cambodia	2.00	R	1.13	R
<i>O. nivara</i> (IRGC92960)	AA	Cambodia	1.50	R	2.50	R
<i>O. nivara</i> (IRGC93092)	AA	Cambodia	2.14	R	1.66	R
<i>O. nivara</i> (IRGC93198)	AA	Nepal	1.00	R	1.00	R
<i>O. nivara</i> (CR100204)	AA	India	2.80	R	3.70	MR
<i>O. nivara</i> (CR100313A)	AA	India	1.80	R	2.41	R
<i>O. nivara</i> (IRGC104646)	AA	Thailand	1.50	R	1.20	R
<i>O. longistaminata</i> (IRGC81967)	AA	Botswana	3.22	R	5.00	MR
<i>O. officinalis</i> (IRGC106399)	CC	Myanmar	1.25	R	1.00	R
<i>O. australiensis</i> (IRGC86527)	EE	Australia	1.00	R	1.00	R
<i>O. australiensis</i> (IRGC101397)	EE	Australia	1.28	R	1.00	R
<i>O. australiensis</i> (IRGC103318)	EE	Australia	1.40	R	1.00	R
<i>O. australiensis</i> (IRGC105267)	EE	Australia	1.00	R	1.00	R
<i>O. australiensis</i> (IRGC105270)	EE	Australia	1.00	R	2.06	R
<i>O. australiensis</i> (IRGC105275)	EE	Australia	1.67	R	1.50	R
<i>O. australiensis</i> (IRGC105278)	EE	Australia	1.00	R	1.13	R
<i>O. punctata</i> (IRGC99576)	BB, BBCC	Tanzania	2.25	R	1.00	R
<i>O. punctata</i> (IRGC99577)	BB, BBCC	Tanzania	2.33	R	1.00	R
<i>O. punctata</i> (IRGC105122)	BB, BBCC	Malaysia	1.00	R	1.80	R
<i>O. punctata</i> (IRGC105129)	BB, BBCC	Philippines	1.80	R	1.00	R
<i>O. punctata</i> (IRGC105980)	BB, BBCC	Cameroon	1.55	R	1.00	R
<i>O. latifolia</i> (IRGC99583)	CCDD	Costa Rica	3.20	R	2.20	R
<i>O. latifolia</i> (IRGC99584)	CCDD	Costa Rica	1.80	R	1.33	R
<i>O. latifolia</i> (IRGC99588)	CCDD	Costa Rica	3.00	R	3.03	R
<i>O. latifolia</i> (IRGC99590)	CCDD	Costa Rica	1.46	R	2.23	R
<i>O. latifolia</i> (IRGC99592)	CCDD	Costa Rica	1.00	R	1.20	R
<i>O. latifolia</i> (IRGC100963)	CCDD	Guatemala	1.30	R	1.00	R
<i>O. latifolia</i> (IRGC100964)	CCDD	Guatemala	1.30	R	1.39	R
<i>O. latifolia</i> (IRGC100965)	CCDD	Costa Rica	1.40	R	2.06	R
<i>O. latifolia</i> (IRGC103787)	CCDD	Surinam	1.00	R	1.60	R

^aIRGC and CR denote accessions from International Rice Genetic Resources Centre, International Rice Research Institute, the Philippines and Central Rice Research Institute (CRRI), Cuttack, India, respectively. ^b0 to 3.49 are considered as resistant (R), 3.50 to 5.49 are considered as moderately resistant (MR) and 5.50 to 9.00 are considered as susceptible (S).

BPH population of China had been cloned and found to encode CC-NBS-LRR proteins (Du et al, 2009). The lines derived with wild rice introgression include *O. minuta* (6 loci), *O. officinalis* (6 loci), *O. australiensis* (1 locus), *O. rufipogon* (4 loci), *O. latifolia* (1 locus), *O. glaberrima* (1 locus) and *O. eichingeri* (1 locus) (Fujita et al, 2013). One locus, *Bph10*, is thought to occur in both *O. australiensis* and *O. officinalis* (Ishii et al, 1994; Lang and Buu, 2003).

CONCLUSION

The wild species of rice are the precious genetic resources for crop improvement against biotic stress. Out of 30 designated BPH resistance genes, *Bph10* and *Bph18* have been transferred from *O. australiensis*; *bph11*, *Bph13*, *Bph14* and *Bph15* from *O. officinalis*; *Bph12* from *O. latifolia*; *Bph20(t)*, *Bph21(t)* and *Bph23(t)* from *O. minuta* and *Bph24(t)*, *Bph29* and *Bph30* from *O. rufipogon*. So far no BPH resistance gene had been identified in *O. nivara* (AA) or *O. punctata* (BB). These two species thus may represent new source for resistance to BPH.

The seven resistant accessions of *O. nivara* originates from India, Nepal and Cambodia and may have different genes for BPH resistance. We have already initiated the transfer and mapping of BPH resistance genes from *O. nivara* and *O. punctata*, which have not been reported so far.

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