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Research Paper

Resistance in rice against Brown planthopper, *Nilaparvata lugens* (Homoptera: Delphacidae) and White backed planthopper, *Sogatella furcifera* (Horvath) (Homoptera: Delphacidae): Role of new sources of resistance, BPH populations and screening methodology

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ABSTRACT

Rice (*Oryza sativa* L.) is an important cereal crop in Asia. In order to address food security and strategy to enhance rice production under shrinking resources of arable land and soil quality and water availability, hybrid rice is being cultivated in many countries to increase rice yield to feed the ever-increasing human population. Hybrid rice gives an advantage of 15 to 20% increment of grain yield over inbred cultivars developed by various public sector organizations. Hybrid rice has certainly the potential to boost the stagnant yield of inbred rice varieties, thus, providing a clear-cut advantage of grain yield increment. However, hybrid rice has also increased the input cost of the farmers by purchasing pesticides to control various biotic stresses due to its extra attractiveness to various insect pests. Among the notorious pests of rice, the Brown planthopper (BPH), *Nilaparvata lugens,* Stal (Homoptera: Delphacidae) and the white backed planthopper (WBPH), *Sogatella furcifera*, Hovarth (Homoptera; Delphacidae) are the most dreaded insect pests of rice. Recently, pests have caused huge losses to farmers, particularly, after the adoption of hybrid rice. The pesticide application on hybrid rice to control these sucking pests has not yielded the desired results due to various reasons including the development of resistance against the most potent insecticide chemistries such as Imidacloprids. Among the other control measures, genetic resistance in rice has been advocated by various workers to be one of the alternative pest control tactics on rice because of its being carried in the rice seed, its effectiveness from seedling to harvest, environmentally safe, socially acceptable and economically feasible. The current paper describes the methods to development of some new sources of resistance using the methodology, which deviates, but complements the one developed and used by various public and private ventures. Experiments have been carried out to characterize resistance in the new sources of resistance by infestation by the standard methodologies as well as, by the ones developed and used in this paper. Several sources of resistance characterized by various workers to map resistance genes against BPH were found either susceptible or varied in their resistance at seedling stages and flowering stages. The new sources of resistance identified herein have been shown to display high level of resistance not only at different crop stages but also against 13 populations of BPH collected from various rice agro-ecosystems of India. The identified sources of resistance showed a good level of resistance against WBPH at seedling and flowering stages of the crop. The sources of resistance have been utilized very effectively to breed a rice hybrid AZ8433 DT with anti-xenosis type of resistance against BPH. The implications of using new sources of resistance in providing protection to the hybrids against BPH and WBPH under choice and no-choice situations have been discussed.

Harish Kumar

Bayer Seeds, Hyderabad, India.

E-mail: harishkumar_sharma@hotmail.com

Key words: *Nilaparvata lugens, Sogatella furcifera*, planthoppers, rice breeding, BPH, WBPH, *Oryza sativa*, Homoptera.

INTRODUCTION

Rice (*Oryza sativa L.*) is the world's most important food crop and a primary source of food for more than half of the world's population. More than 90% of the world's rice is grown and consumed in Asia where 60% of the earth's

people live (Khush, 1992). The world will have to produce 40% more rice by 2025 from less land, less water and less labor else we will have to face disastrous consequences of biodiversity and water sheds (Khush, 1992; FAO, 2009). In order to meet the challenges, we need rice varieties with higher yield and greater yield stability (FAO, 2009). The concept of Super rice initiated in China (Tang et al., 2017) can be strengthened by further incorporating resistance genes against various stresses.

Hybrid rice offers an opportunity to achieve this target. Using hybrid rice with high technology precision farming along with approaches pertaining to integrated crop management practices, the crop yield can be increased several folds without expanding agricultural area. There is need to promote sustainable and climate smart rice production.

The higher yielding potentials of rice hybrids has also been linked with their higher susceptibility to pests like brown planthopper (BPH*), Nilaparvata lugens* Stal and White backed planthopper (WBPH), *Sogatella furcifera* (Kumar et al., 2016). The susceptibility is linked to the CMS parental line rather than the restorer parent (Horgan and Crisol, 2013). Growing hybrid rice in environments where the use of green revolution technologies such as high yielding inbred varieties, synthetic fertilizer and huge amounts of pesticides by the Asian farmers have transformed planthoppers into dangerous and destructive pests of rice and it is a big challenge achieving the desired and expected yield increments. The author's personal observations in rice field of India revealed that the hybrid rice get higher infestations, particularly by WBPH, as compared to inbred varieties growing in close proximity.

BPH is dimorphic, with fully winged 'macropterous' and truncate-winged 'brachypterous' forms. The macropterous forms are potentially migrants and are responsible for colonizing new fields. After settling down on the rice plants, they produce the next generation in which most of the female insects develop as brachypters and males as macropters. The combined effects of the two types make BPH an internationally explosive and devastating pest of rice.

The yield advantage associated with hybrid rice is delimited by pest attack, particularly, BPH. The private seed companies which have big stakes to sell hybrid rice seeds, often get pushed back and suffer loss of clients and prestige as hybrids suffer heavy losses due to pests attack and all solutions, particularly, the pesticides hardly offer any protection to hybrids.

The causes for such failures of pesticides to control BPH are several folds. Among these, the application timing, the dossier and application methods to apply the pesticides at the target site on the plants are very important (Bass et al., 2015; Shun et al., 2018). The constraints further bring about other problems of pesticide usage like pollution, resistance in pests and pest resurgence.

In recent years, BPH infestations have intensified across Asia, causing significant yield losses (Normile, 2008; Sogawa, 2015). BPH not only causes direct damage to the rice crop by sucking plant sap, often resulting in "hopper burn," but it can also cause indirect damage by transmitting virus diseases such as rice grassy stunt and ragged stunt (Cabauatan et al., 2009).

Brown planthopper infestations have destroyed rice crop from time immemorial. In 1732, Japan reported famine – death of about 1 million people due to severe attack of BPH on rice crop (Suenaga and Nakatsuka, 1958). In 1973 to 1974, almost 50,000 ha of rice were severely damaged by brown planthopper, and 8,000 ha of rice crop totally wiped out by the insect in the Kerala state of India. In 2005, China reported a loss of 2.7 mt of rice due to direct damage by BPH. Almost 0.5 mt of rice in Vietnam was damaged due to indirect losses by viruses transmitted through BPH (Brar et al., 2009). In 2017, rice crops in nearly 1, 78, 932 ha was affected by BPH menace in nine districts in Odisha state in India. Standing crops in 8,211 villages of 92 blocks and 19 urban local bodies were affected by the BPH in Bargarh, Sambalpur, Nuapada, Sonepur, Balangir, Ganjam, Kalahandi and Koraput districts in the state of Odisha of India. Estimated crop losses have been 33 to 50% in nearly 1.10 lakh hectares of land.

The white backed planthopper (WBPH), *S. furcifera* is a sporadic pest of rice in India. WBPH has been reported to possess a lower rate of population growth than BPH (Kuno, 1979; Sogawa, 2015). Compared to BPH, WBPH therefore has a different type of population dynamics.

The frequency of outbreaks of WBPH has been reported to increase with the corresponding spread of hybrid rice area in the 1980 to 1990s in South China (Sogawa, 2015; Tang et al., 2017). Particularly, WBPH increased unusually and became the most predominant insect pest of hybrid rice (Liu et al., 2015; Sogawa et al., 2003). In India, the outbreaks of WBPH have been sporadic on the inbred varieties though trend similar to China have already been observed on hybrid rice.

The two species of BPH and WBPH occur simultaneously in the paddy fields following colonization by the macropterous immigrants. Generally, the density of BPH immigrants is low and these initial colonists produce offsprings, which moult primarily into brachypterous forms, which are more fecund than their macropterous counterparts. Thus, rapid population growth occurs in the paddy fields and within 2 to 3 generations BPH can cause 'hopper burn' on the susceptible plants of a genotype, thus, affecting yield seriously (Kisimoto, 1965; Kuno, 1968). As the paddy crop matures, more and more macropterous forms of BPH which migrate to colonize new paddy fields are produced.

Unlike BPH, damage by WBPH on rice is uniquely different than that caused by BPH. Generally, it has been observed that after completing one to two generations on rice, WBPH gets converted into swarms of adults migrating from one plant to another and feeding indiscriminately on

rice leaves and soft panicles alike. As a result of feeding by the WBPH adults, the panicles get transformed into brown ears of rice plants, black – cracked rice kernels and rusty rice kernels (Noda, 1986; Sogawa et al., 2009; Kumar, year). Therefore, it is important to breed hybrid rice for resistance against not only BPH but also against WBPH.

Host plant resistance in plants to insects is a method of crop protection, which is environmentally safe, economically viable and socially acceptable (Kumar and Mihm, 1996; Kumar, 1997). The introduction of Bt crops has put some kind of limitations/constraints of using environmental safety, and economic viability of these crops. The Bt crops have provided foolproof method to control lepidopterous pests despite debatable environmental and economic constraints of using such crops. The use of Bt crops has pushed the conventional breeding approaches on the back seat because of the ease of transferring Bt genes in the elite commercial varieties and hybrids and the effectiveness of the toxins to control the target pests.

However, equally short is the list of resistance genes transferred from the natural sources/wild relatives into the commercial varieties and hybrids. The hybrid rice has been commercialized in several countries including China and India but there have been reports of widespread BPH attacks on hybrids and varieties in China, India, Japan, Vietnam Thailand and Indonesia.

Little attention has been given to improving hybrids for resistance against various pests like BPH and WBPH though considerable work has been done for improving hybrid for diseases like bacterial leaf blight. Similarly, hybrid rice has also been associated with high susceptibility to stem borers. It is therefore very important to develop rice hybrids with decreased susceptibility to various pests.

In view of the aforementioned, the private seed companies have started various programs for developing hybrids with genetic resistance against BPH and Gall Midge through the use of conventional plant breeding efforts. The first and foremost step in this direction is to identify the sources of resistance against BPH. To date, 22 major BPH resistance genes have been reported. Among these, 14 major effective BPH resistance genes have been assigned to chromosomes in indica cultivars.

Planthopper resistance in rice was first reported in the landrace Mudgo in 1969 (Pathak and Khush, 1979; Ahtwal et al., 1971; Jena and Kim, 2010; Fujita et al., 2013; Jing et al., 2017). Since then, almost 33 resistance genes have been reported from the native as well as, from the wild rice (Brar et al., 2009; Jena and Kim, 2010; Jing et al., 2017; Prahalada et al., 2017). Among these genes, fifteen (*Bph1-9, Bph17, Bph19, Bph25-26, Bph28 and Bph32*) were discovered in traditional indica varieties of rice while sixteen (*Bph10-16, Bph18, Bph20-24, Bph27, bph29, and bph30*) were discovered from seven wild species of rice (*O. australiensis, O. eichingeri, O. glaberrima, O. latifolia, O. minuta, O. officinalis and O. rufipogon*) (Myint et al., 2012; Fujita et al., 2013; Jing et al., 2017). These different genes have been located and mapped on different rice chromosomes in the forms of clusters. Almost 80% of the resistance genes have been mapped in four major clusters on rice chromosomes 3, 4, 6, and 12.

Bph1, bph2, bph7, Bph9, Bph10, Bph18, Bph21, and *Bph26* are clustered on long arm of chromosome 12. Short arm of chromosome 4 harbors *Bph12, Bph15, Bph17, Bph20 and bph22,* while long arm of chromosome 4 harbors *Bph6, bph16, and Bph27* genes. Five resistance genes (*Bph3, bph4, Bph25, bph29 and Bph32*) are clustered on short arm of chromosome 6 (Du et al., 2009; Fujita et al., 2013; Hu et al., 2016; Jing et al., 2017). *Bph11, Bph13, Bph14 and Bph19* were mapped on chromosome 3. Recently, Naik et al. (2018) elucidated Bph33 (t) gene in another rice line RP2068-18-3-5, a line derived from the landrace Velluthacheera. These different resistance genes might be distinct but tightly linked or may represent different alleles at the same locus and could be allelotypes (Zhao et al., 2016).

In spite of such a rich knowledge about the diversity of BPH resistance genes, there is hardly any information about the utilization of resistance genes for developing commercial rice hybrids though BPH resistant inbred cultivars have been developed and commercialized successfully in some countries such as Philippines and Indonesia (Brar et al., 2010). For example, the International Rice Research Institute (IRRI) developed the first BPH resistant variety IR26 using *Bph1* in 1973. The resistance of IR26 broke down rapidly in 2 to 3 years time with the evolution of BPH biotype 2 in the Philippines which was able to feed on the variety with Bph1 resistance gene (Botrell and Schoenly, 2012).

Subsequently, another resistant variety was released replacing the *Bph1* gene with *bph2*. In 1981 and another biotype of BPH was detected in the laboratory, which was able to feed on rice variety with *bph2* resistance gene, though its existence in the rice field was seldom demonstrated. Thereafter, rice varieties, such as IR36 with *Bph3* gene were released. Resistance of IR36 has remained durable in some countries except India where it continue to show susceptibility against Indian BPH populations. The resistance of germplasm with *Bph18* resistance gene has also remained inconsistent.

It is noteworthy that most of the resistant inbred cultivars seldom showed resistance against BPH populations from India, though the cultivars were predominantly used for agronomic improvement. In view of this, the control of BPH and WBPH on rice largely remained dependent on insecticides.

The rice ecosystems are slowly evolving towards high input agricultural systems with the introduction of hybrid rice, high doses of fertilizers and high rates of pesticide applications. These transformations in the rice ecosystems are also creating optimum conditions for pest – proliferation, thus, increasing the magnitude of crop losses for pest damage. Unlike inbred varieties of rice, there is

hardly any information on the development of hybrids resistant to BPH. The first and foremost step towards the development of BPH resistant hybrids is to characterize the BPH populations for their virulence against known sources of resistance as well as, new germplasm lines.

According to the patterns of infestation and damage occurring on rice in the field conditions, the most appropriate stage of rice plant for screening against BPH is the early tillering stages when the adults invade the crop and initiate population build up. However, contrary to this requirement, rice germplasm in almost all the studies has been characterized for BPH resistance by infesting the plants at 7 to 15 days after sowing mostly with second instar nymphs of BPH by the widely used IRRI's methodology of standard seed box screening technique (SSST) at seedling stages of the crop (Brar et al., 2009). The methodology of standard seed box screening technique (SSST) consists of growing germplasm in a seedbed in a tray and infesting the plants at 7 to 10 days after sowing in choice situations. The rice workers have followed this methodology strictly over the years to identify sources of resistance for breeding new varieties.

There is hardly any report, which describes the resistance characterization of rice at different phonologies of rice. In the present work, an attempt has been made to elucidate resistance of certain germplasm materials at different crop phenologies. Recently, the rice varieties characterized for resistance against BPH by SSST methodology have been reported to suffer extensive damage in the panicle stages of rice (Jairin et al., 2017).

In rice growing countries like India and China, where rice is grown in different ecologies, it is also very important to determine the resistance of germplasm against BPH or WBPH populations prevalent in different regions so that the developed resistant varieties could be deployed in different rice ecologies as per virulent nature of BPH or WBPH populations. In this paper, an attempt was made to characterize resistance of rice genotypes against BPH populations from diverse rice growing ecologies.

In recent times, the damage by the White-backed planthopper on rice has also increased. Resistance would be required in rice hybrids not only against BPH but also against WBPH.

A reference to the literature shows a great deal of synonymy of gene loci identified by various workers (Fujita et al., 2013) which could be due to variability in infestation methodology or by the nature of BPH population used for screening or crop phenology used for gene tagging. In the present study, an attempt was made to characterize germplasm by (1) different BPH populations, (2) different crop phenology, (3) screening in choice and no-choice situation and (4) by infesting genotypes in single - row vs. multi-row plots. The work could help the molecular breeders to standardize the methodology for screening germplasm for resistance against planthoppers in order to further streamline the gene nomenclature.

MATERIALS AND METHODS

The research work described in this paper was carried out in the green houses and laboratories of Bayer Seeds Tolichowki, Hyderabad, India from 2004 to 2013. The methodology used in this paper was described by Kumar (in press) and Heinrichs et al. (1985). The BPH populations used in this work were collected from the following geographical regions within India where the planthoppers cause consistently high damage on rice and are considered as the potential "hotspots" for BPH infestations. Geographical regions from which the BPH populations used in this work were collected include the following:

1) Andhra Pradesh -the West Godavari;
2) Punjab;
3) Haryana state - Dhantori 1. Population collected in 2003;
4) Haryana state - Dhantori 2. Population collected in 2007;
5) West Bengal - 24 Parganas South;
6) Chhattisgarh - Dhamtari;
7) Chhattisgarh - Janjgir – Champa;
8) Odisha - Cuttack in 2007;
9) Karnataka - Mysore region;
10) Telangana - Warangal;
11) Uttarakhand - Pantnagar;
12) Telangana - Karimnagar;
13) Kerala - Monkompu;
14) Andhra Pradesh - East Godavari.

The BPH was mostly collected as adults from the light source erected near the rice fields. The collected adults were stored on rice seedlings in a plastic box (20 cm × 15 cm diameter) closed with a tightly closed lid fitted with nylon - net. The box containing BPH was transported to the research station. The insects were released on fresh rice plants (50 to 60 days old) kept inside a rearing cage (90 cm high, 85 cm wide and 70 cm deep). The field - collected insects were examined for the presence of any predator and parasite. The unwanted insects were removed and destroyed.

For oviposition, 7 to 8 weeks old rice plants of the susceptible variety, TN1 were used. The plants were grown in pots (20 cm high × 15 cm diameter). The plants in a pot were enclosed inside a polycarbonate cylinder (henceforth, will be called oviposition cylinder) and 100 adults of BPH (60 females and 40 males) were transferred from rearing cage to the cylinder with the help of an aspirator.

The oviposition cylinder was made of 0.5 mm thick polycarbonate sheet, rolled into cylinder (80 cm high and 12 cm diameter). The bottom - less oviposition cylinder was provided with a nylon net (40 mesh/cm^2) top. Each cylinder was provided with two windows fitted with nylon net (40 mesh/cm^2) for aeration at 45 and 65 cm from the bottom, the upper one being 8 cm diameter and the lower one of 10 cm diameter. On one side, the cylinder was provided with a 15 cm diameter, window fitted with a

Table 1: Germplasm used for various experiments.

Genotype (accession number)	Origin	Resistance gene
Mudgo (ac. 6663)	India	*Bph1*
ASD7 (ac. 6303)	India	*Bph2*
PTB33 (ac. 19325)	India	*Bph2, Bph3*
Rathuheenathi (ac. 11730)	Sri Lanka	*Bph3, Bph17*
Babawee (ac. 6730)	Sri Lanka	*bph4*
ARC10550 (ac. 12507)	India	*bph5*
Swarnalatha (ac. 33964)	Bangladesh	*Bph6*
T-12	Bangladesh	*bph7*
Chinsaba (ac.33016)	Myanmar	*bph8*
Velluthacheera	Sri Lanka	*unknown*
Pokkali (ac. 15602)	Sri Lanka	*Bph9*
G7253 (identified in 2007)	India	*unknown*
G4267 (identified in 2009)	India	*unknown*
G4267P (restorer line)	India	*unknown*
F4 line of cross G1198* x G4267P)	India	*unknown*
F4 line of cross G7186** x G7253)	India	*unknown*
TN1	Taiwan	Susceptible check
BOO2	India	Maintainer line – susceptible to BPH

*G1198 was a restorer line without gene for Bacterial leaf blight (BLB); **G7186 was restorer line with BLB gene for producing rice hybrid Arize 6444 Gold.

nylon net sleeve through which the adults of BPH were introduced onto the plants for egg laying. Thereafter, the sleeve was tightly closed by fastening a knot in the cloth.

For egg laying, 7 to 8 weeks old potted plants were used. Plants were cleaned of the dry leaf sheaths and predators, if any, prior to fixing the cylinders. The cylinders were then placed over the plant. At 7 to 10 days after their release, the adults were removed from the cylinder and transferred to a fresh plant enclosed inside a new oviposition cylinder. The process was repeated severally.

Production of BPH neonates for infesting rice germplasm

In order to obtain neonates, 8 to 9 weeks old rice plants of a susceptible genotype TN1 were enclosed singly inside oviposition cylinders (70 cm high and 15 cm diameter) whose top was formed by a fine nylon net and cylinder provided with two nylon net windows for aeration.

Almost 100 adults were introduced on each plant through the nylon net sleeve for 7 days. Thereafter, the adults were removed from the plants and the latter were kept inside the oviposition cylinders for hatching. Eight to ten days later, the eggs laid by BPH hatched and the plants got covered with small whitish neonates of BPH, mostly confined to the basal regions of the rice stalks.

Upon hatching, the nymphs were either used for further multiplication of the insect colony or for infesting rice germplasm seedling for elucidating resistance against BPH. The adults begin to appear in the wooden cages at 20 to 22 days after egg hatching inside the wooden cages.

Germplasm used

For various experiments, the following germplasm was used. The germplasm material was retrieved from the Bayer's germplasm bank based on their history of resistance status against BPH in the literature (Brar et al., 2009) (Table 1). Some new sources of resistance were also identified during the germplasm evaluation for resistance against BPH and WBPH at Bayer Seeds, India.

For various experiments, the rice plants were grown in plastic tubs (60 cm × 40 cm × 10 cm) or fiber trays (170 cm × 100 cm × 10 cm) or directly in the soil bed of the greenhouse. The experiments were conducted in choice or no-choice situations.

For experiments with plastic tubs, the trays were filled with black soil up to half and soil was nicely puddled. Thereafter, the soil was marked for 10 rows with the help of a marker. Fifteen to twenty seeds were sown in each of the 10 rows. Each row represented a germplasm entry, which was different from the row following or preceding it.

Experiment 1: Resistance/susceptibility of 20 rice differentials against 6 populations of Brown Plant hopper on 20 rice differentials in relation to crop phenology at infestation

For this trial, 20 rice genotypes were used as earlier described. The plants were grown in plastic tubs; the trays containing the experimental plants were arranged inside the fiber trays filled with water with four tubs per fiber tray. The trays were then enclosed inside a nylon net cage (180 cm long, 70 cm broad and 90 cm high).

The plants were infested with six different BPH populations, for example, Dhantori, Pant Nagar, West Bengal, Mysore, Chhattisgarh and West Godavari at three growth stages, that is, 10, 15 and 20 days after sowing (DAS) with neonates of BPH at 20 to 25 nymphs per plant in as many different nylon net cages, replicated twice. The trays containing plants were enclosed inside a nylon net chamber. When the plants of the susceptible check suffered 90% mortality, the experiment was sprayed with an insecticide (imidacloprid at 0.5 ml/L) and data on plant mortality and damage caused by BPH were recorded. Data were subjected to factorial ANOVA with crop phenology, BPH populations and genotype as the factors. Means were separated by LSD test at 0.05.

Experiment 2: Resistance in genotypes infested with BPH adults in 4-row and single-row plots

For the experiment, 16 genotypes (BPH differentials) with varying resistance genes against BPH were used. For this experiment, two new genotypes (G7253 and Velluthacheera) identified for resistance against BPH in 2007 were also included. The differentials were grown in two sets on November, 4th 2008.

In the first set, each genotype was grown in 4 rows of 20 plants per row in a galvanized iron tray measuring 180 cm × 70 cm × 10 cm. The tray accommodated 64 rows of 4 rows/genotype, while in the second set each genotype was grown in single row plots of 20 plants/row in a galvanized iron tray measuring 180 cm × 70 cm × 10 cm. The experiment was replicated 4 times. The experimental design used was a Randomized Complete Block Design (RCBD).

At 36 days after sowing (December, 10th 2008), the plants in each set were infested with 1 week old adults of BPH from Chhattisgarh. The plants were infested at 2 females + 1 male per plant and the following data recorded:

1) Number of adults counted in the middle two rows of 4 – row plot at 10 days after the adult release;
2) The nymphal density of each row at 3 weeks after adult release in single and multi row plots (0 to 10 with 0 representing no nymphal population and 10 signifying 50 to 60 nymphs per plant);
3) Percentage of plants dead in each row (computed on the basis of total number of plants and the number dead ones);
4) Damage scores of all the plants in a row (0 to 9 with 0 representing no damage and 9 signifying dead plants).

The environmental conditions inside the greenhouse were maintained by fan-pad assembly. The temperature ranged from 25 to 32°C, while the humidity ranged between 50 to 80%.

Data were subjected to factorial ANOVA with BPH population as the main factor and the genotypes being the sub-factor. The effect of the main factor was significant indicating differences in the virulence of BPH populations on rice differentials. The genotypic effect was highly significant ($p < 0.001$) indicating a strong difference in the damage suffered by genotypes as a result of BPH infestations. BPH population × genotypic effect was not significant indicating that the genotypic differences were consistent over BPH populations and vice versa. Hence, LSD tests were performed on mean values combined over BPH populations and genotypes.

Experiment 3: Resistance in rice differentials for nymphs feeding resistance by 13 BPH populations in choice situation in 2009 and 2011

Experiment in 2009

For this trial, 22 rice genotypes were used and the plants grown in plastic tubs as earlier described. The trays containing the experimental plants were arranged inside the fiber trays filled with water with four tubs per fiber tray (Figure 16). The trays were then enclosed inside a nylon net cage (180 cm long, 70 cm broad and 90 cm high), as explained in experiment 1.

The plants were infested with 9 different BPH populations, for example, Dhantori, Pant Nagar, West Bengal, Mysore, Chhattisgarh and West Godavari 17 days after sowing with neonates of BPH at 20 to 25 nymphs per plant in as many different nylon net cages, replicated twice. The trays containing plants were enclosed inside a nylon net chamber. When the plants of the susceptible check suffered 90% mortality, the experiment was sprayed with an insecticide (imidacloprid at 0.5 ml/L) and data on plant mortality and damage caused by BPH recorded.

Data were subjected to factorial ANOVA with BPH population as the main factor and the genotypes being the sub-factor. The effect of the main factor was significant indicating differences in the virulence of BPH populations on rice differentials. The genotypic effect was highly significant ($p < 0.001$) indicating strong differences in the damage suffered by genotypes as a result of BPH infestations. BPH population × genotypic effect was not significant indicating that the genotypic differences were consistent over BPH populations and vice versa.

Hence, LSD tests were performed on mean values combined over BPH populations and genotypes. Data show that Tanaku and Mysore populations of BPH caused a significantly higher damage on rice differentials than other BPH populations, though the damage scores ranged between 6 to 8.

The genotypic differences were clearly visible and the rice differentials could be split into 3 clear categories in terms of damage caused by BPH populations. The genotypes PTB33, Velluthacheera, RP2068 displayed high level of resistance across all BPH populations; the genotypes T-12 and ARC 10550 were resistant to BPH populations, while Rathuheenathi and CR-MR1523 displayed a moderate level of resistance across all BPH populations. The remaining genotypes showed either moderate susceptible to high susceptible reaction across all BPH populations.

Experiment in 2011

The seeds were sown in large fiber trays (170 cm long, 80 cm wide and 15 cm depth), kept inside a GI pipe frame (185 cm long, 80 cm wide and 100 cm high) which was supported on 4 GI pipe legs, each kept in a petri dish filled with water to prevent the entry of crawling insects. The trays were filled with black soil to the depth of 8 cm and thoroughly puddled after incorporating urea, FYM and potash. In the tray, 64 rows were used.

For this, 22 rice genotypes were used with known and unknown status of resistance/susceptibility against BPH. Each of the 22 genotypes was sown in single row plots of 20 plants per row. The 22 row plots were replicated thrice. The sowing was done in 13 different trays as earlier explained. The plants in each tray were infested with neonates of a particular BPH population. In this way, plants in 13 trays were infested with 13 different BPH populations. After every 15 rows, a susceptible check, BOO2 was sown.

At 17 days after sowing, the plants were infested with neonates of BPH population from West Godavari at 20 to 25 nymphs per plant. When the plants of the susceptible check suffered 90% mortality, the experiment was sprayed with an insecticide (imidacloprid at 0.5 ml/L) and data on plant mortality and damage caused by BPH were recorded on row basis. For this, each row was assigned a damage score (0 to 9 scale) and mean values were computed from the data recorded for different replicates. Data were subjected to the statistical analyses and means separated by Least Significant Difference test (LSD).

Experiment 4: Egg-laying, egg-hatching and damage among rice infested at 7 WAS (5 WAT) with adults of Brown plant hopper in the greenhouse field

For the experiment, we used the rice genotypes with differential resistance genes. The genotypes used were: Pokkali, Manoharsali, Swarnalatha, Mudgo, ARC10550, BOO2 (the susceptible parent of hybrid A6444), ARC5984, Chaitanya, T_{12}, ARC6650, ASD7, Chinsaba, Rathuheenathi, PTB33, Velluthacheera, G7253, G4267S (source for male 4267), G4267M (the Male parent derived from 4267S), G4267-F4 (F_4 line derived from G1198 × G4267M). Each genotype (seed lot) was sown in plastic trays on 20-01-2012 inside the green house.

At 15 days after sowing, the plants were transplanted in well prepared and puddled field inside the greenhouse in 1 m^2 mini-plots (Figure 18). Each genotype was transplanted in a square meter plot having 50 plants in 5 rows of 10 plants each. There were 20 of such plots corresponding to 20 genotypes, as earlier explained. Plots were distributed randomly in the field.

At 7 weeks after sowing (WAS), 50 plants in each plot were infested with 200 males and 200 females of BPH collected from the West Godavari district of Andhra Pradesh in India. For infestation, required adults collected from the rearing cages were transferred into specimen tubes (75 mm × 20 mm) with help of an aspirator and slowly released uniformly over the 50 plants of each plot. Thus, all the 20 plots had uniform infestation by BPH. Since the experiment was conducted inside the green house, infestation by BPH from natural population was completely absent. The plants were monitored daily for any incidence of predators such as ants. The adults of BPH were thus given optimum conditions for egg laying and egg hatching for 15 days.

At 15 days after the adult release, the nymphal population of BPH appearing on the plants of each genotype was assessed visually on a score of 0 to 10 with 0 indicating no nymphs and 10 indicating high population of 1000 BPH per plant. For assessing BPH population, plants in each row of a mini-plot were assigned a population score. This was done twice for each genotype; one in the morning and the other in the evening before sun set. The mean value for 10 rows of the mini-plot was computed.

Damage caused by BPH feeding on the plants was assessed on a scale of 0 to 9 as earlier mentioned. On the basis of the number of plants showing hopper burn in each mini plot and the total number of plants, the percentage of the plants suffering hopper burn was also computed.

Experiment 5: A comparison of rice hybrids and their parents for resistance against BPH in choice and no-choice situation

For this, the resistant source G7253 was crossed with a restorer line "R" and selfed for 6 generations. The progeny of each selfed material was infested with BPH nymphs and the resistant families advanced to the next level. From the F_6 progeny, four parents were selected and designated as P_1, P_2, P_3 and P_4. All the four male parents were crossed

with a CMS female line, "CMS line" to yield four hybrids H_1, H_2, H_3 and H_4. The four hybrids along with their resistant male parents, the CMS line, the source of resistance G7253, a commercial hybrid VNR Laxmi, a popular commercial variety in the state of Andhra Pradesh and Telangana, MTU1010 and the susceptible check TN1 were evaluated for resistance in a choice and no-choice situation using neonates of BPH. For a choice situation, the test genotypes were grown in single row plots of 20 plants per row replicated four times.

For no-choice tests, each genotype was grown as a block of 200 plants of 10 rows × 20 plants per row. Each genotype was completely isolated from the other by placing transparent polycarbonate sheets 30 cm × 20 cm between different plots. Thus, no movement of BPH was possible from one plot to the other. Plants in both trials were infested with neonates of BPH at 20 to 25 nymphs per plant. The plants were enclosed in a nylon net cage as earlier explained. When the plants of the susceptible check suffered 100% mortality, the data were recorded as the percentage of plants dead for each genotype.

Experiment 6: Resistance in rice differentials for nymphs feeding by WBPH in choice situation

The 19 rice differentials with BPH resistance genes were sown in two sets, 5 days apart in plastic tubs (60 cm × 40 cm × 10 cm). When the plants in the two sets were 10 and 15 days old, respectively, the trays containing the plants of both sets were arranged inside a large fiber tray containing water to a depth of almost 2 inch. The trays were then covered with a nylon net enclosure, as previously described.

The plants were infested with freshly hatched nymphs of White backed planthopper (WBPH) at 15 to 20 nymphs per plant in the early hours of morning between 8 a.m. and 9 a.m. Thereafter, the enclosure was tightly closed from all sides to prevent the escape of WBPH nymphs.

When the plants of TN1 suffered 80 to 90% mortality, the plants in the experiment were sprayed with confidor to kill the hoppers. Each plant was assessed for damage by WBPH on a damage rating scale of 0 to 9, as earlier explained. Mean values were computed for each genotype separately (Figure 19).

Experiment 7: Egg hatching and damage by WBPH on selected rice differentials in no-choice situation

For this, we used the following rice differentials: TN1, G7253; PTB 33, T_{12}, Rathuheenathi, and ARC10550. Each genotype was grown in a plastic tray in 10 rows of 15 plants each. When the plants were 35 days old, each tray was separately enclosed inside a nylon net chamber. Each genotype was infested with 100 males and 100 females of 5-day old WBPH. The adults were uniformly released on each genotype. The chamber was tightly closed on all the sides. The adults were allowed to lay eggs, while the plants were examined daily for predator/parasite, if any.

In an interval of two weeks, the egg hatching occurred on the susceptible check TN1. At 2 weeks after the adult release, the plants were evaluated for WBPH nymphal density on a scale of 0 to 10 with 0 indicating no insect on the plant and 10 indicating high insect population (1000 or more nymphs).

At 3 weeks after the adult release, the plants of each genotype were examined for the number of plants dead in each row. On the basis of total number of plants in the row, the percentage of those dead was computed. The plants were also scored for damage by WBPH on a rating scale of 0 to 9.

Data were subjected to statistical analyses. The correlation coefficients were computed between the nymphal density on the genotypes and the percentage of plants dead and between the nymphal density and the WBPH damage scores on the genotypes.

RESULTS AND DISCUSSION

Experiment 1: Resistance/susceptibility of 20 rice differentials against 6 populations of Brown Plant hopper on 20 rice differentials in relation to crop phenology at infestation

ANOVA (Table 2) for percentage of plant mortality showed that there was no significant interaction among phenology × BPH populations × Genotype ($F = 0.7$; $df = 190, 359$; $P > 0.05$). Crop phenologies differ significantly in terms of plant mortality. The plants infested at 10 days after sowing suffered a greater plant mortality followed by those infested at 15 and 20 days after sowing ($F= 7.02$; $df = 2, 359$, $P < 0.01$). BPH populations differed significantly for causing plant mortality of different genotypes ($F = 2.47$, $df = 5, 359$; $P< 0.05$). The plants of different genotypes suffered the highest mortality by BPH from Pant Nagar followed by Mysore, Dhantori, West Godavari, Chhattisgarh and West Bengal ($F = 2.42$; $df = 5, 359$; $P <0.05$). The genotypes differ significantly under infestation with six BPH populations ($F = 32.49$; $df = 19, 359$; $P < 0.01$). The genotypes PTB33, T12, ARC10550 suffered the lowest plant mortality followed by Manoharsali, Rathuheenathi, ARC6605 and Chaitanya. The genotypes TN1, BPT2053, Mudgo and ASD7 suffered the highest degree of plant mortality under infestation with different BPH populations.

When damage was assessed on the surviving plants (Table 2), ANOVA showed that damage scores by six BPH populations were the same at three phenology stages of the plants ($F= 0.6$, $df = 2, 359$; $P > 0.05$). BPH populations differ significantly in terms of damage caused on different genotypes ($F = 2.91$; $df = 5, 359$; $P < 0.05$). The BPH

Table 2: Factorial ANOVA for the plant mortality and damage scores by six populations of BPH on 20 rice genotypes at 3 different phenologies.

Source	df	Mean squares for percent plant mortality	Mean squares for damage scores
Replication	1	4217	4
Factor A (Crop phenology)	2	6826**	0.04
Factor B (BPH population)	5	2397**	3.5*
A × B	10	1629	4.4*
Factor C (Genotypes)	19	31598**	74.9**
A × C	38	3536*	4.4*
B × C	95	1030NS	1.4
A × B × C	190	652NS	0.8
Error	359	972	1.2

** = p< 0.01; * = p<0.05; NS = not significant.

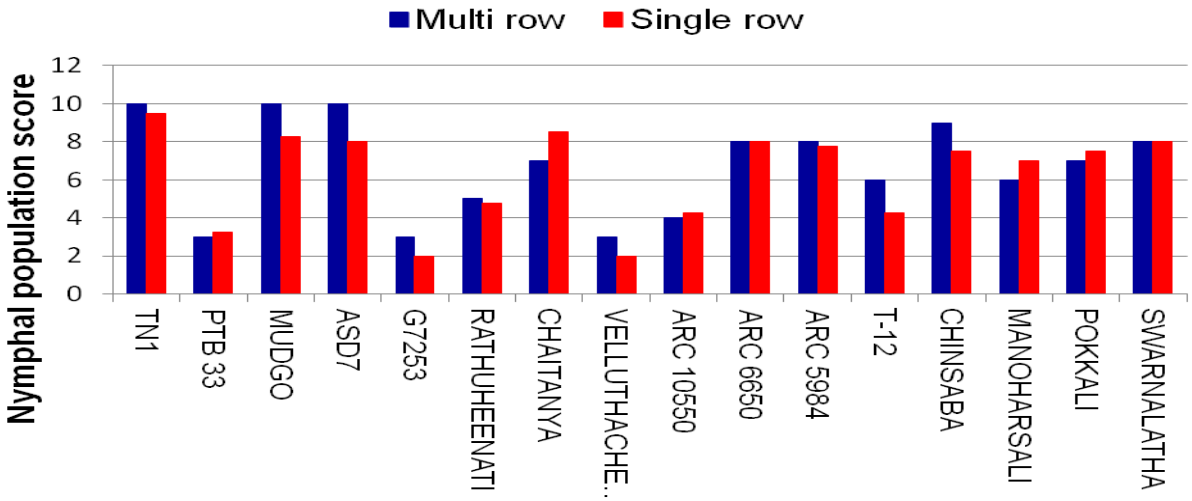

Figure 1: Population build up by the ovipositing adults of BPH on certain rice genotypes at tillering stages in choice situation in the green house when grown in multi-row and single row plots.

population from Pant Nagar, Chhattisgarh, Mysore and Dhantori were equally virulent and the populations from West Godavari and West Bengal were significantly lesser virulent as indicated by the damage scores on various genotypes. The genotypes differ significantly in terms of damage suffered by six BPH populations (F = 32.49, df = 19, 359; P < 0.01). The surviving plants of PTB33 suffered the least damage by six BPH populations while the remaining plants had almost similar damage scores.

The methodology adopted here show that artificial infestation of rice genotypes clearly distinguished the resistant and susceptible genotypes. The BPH populations were equally virulent though damaged by Pantnagar population was high on genotypes. The younger plants suffered a greater damage by BPH than those sown 5 to 10 days later. The differences between resistant and susceptible genotypes were manifested clearly despite different phenologies at infestation. Thus, for distinguishing the resistant and susceptible genotypes, infestation by BPH could be accomplished between 10 to 20 days after sowing. The crop phenology of 15 to 20 DAS is also quite ideal because at these stages, the most preferred feeding sites on the rice plants, that is, the basal culm gets prominent and BPH could establish itself at the preferred feeding sites. Infestations at 7 to 10 days, mostly exposes the insects to the foliar parts of the plant where BPH is seldom established for feeding.

Experiment 2: Resistance in genotypes infested with BPH adults in 4-row and single-row plots

When the genotypes were grown in 4-row and single – row plots, the egg hatching on different genotypes varied (Figure 1). The population of BPH nymphs was the lowest on PTB33, G7253 and Velluthacheera followed by

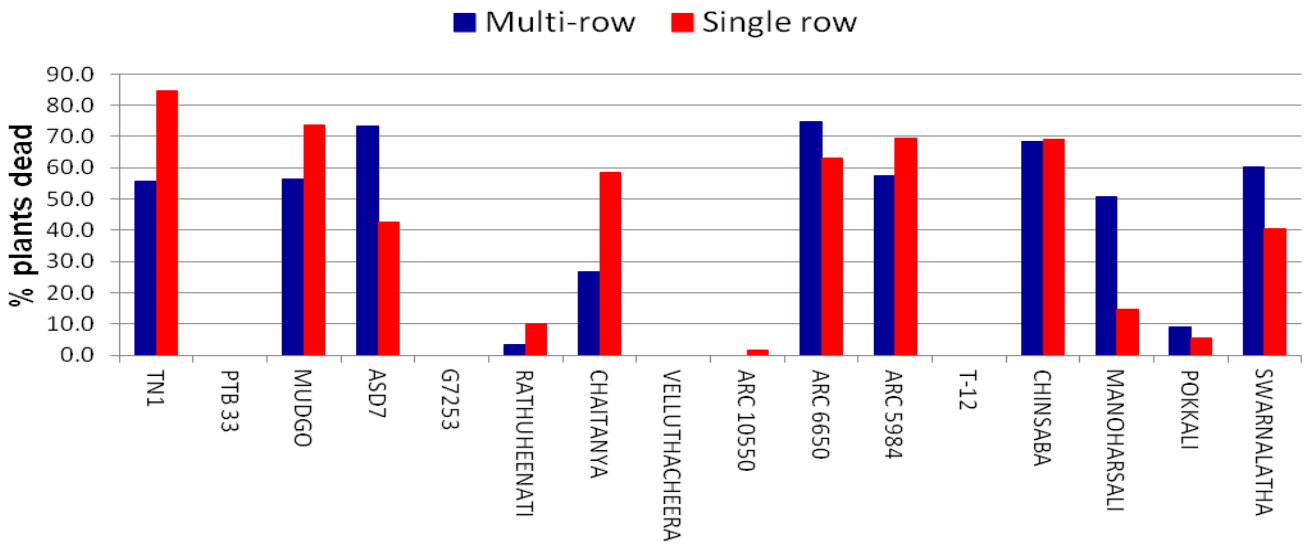

Figure 2: Plant mortality suffered by various rice genotypes in multi-row and single-row plots at 21 days after infestation with BPH adults at tillering stages in choice situation in the green house.

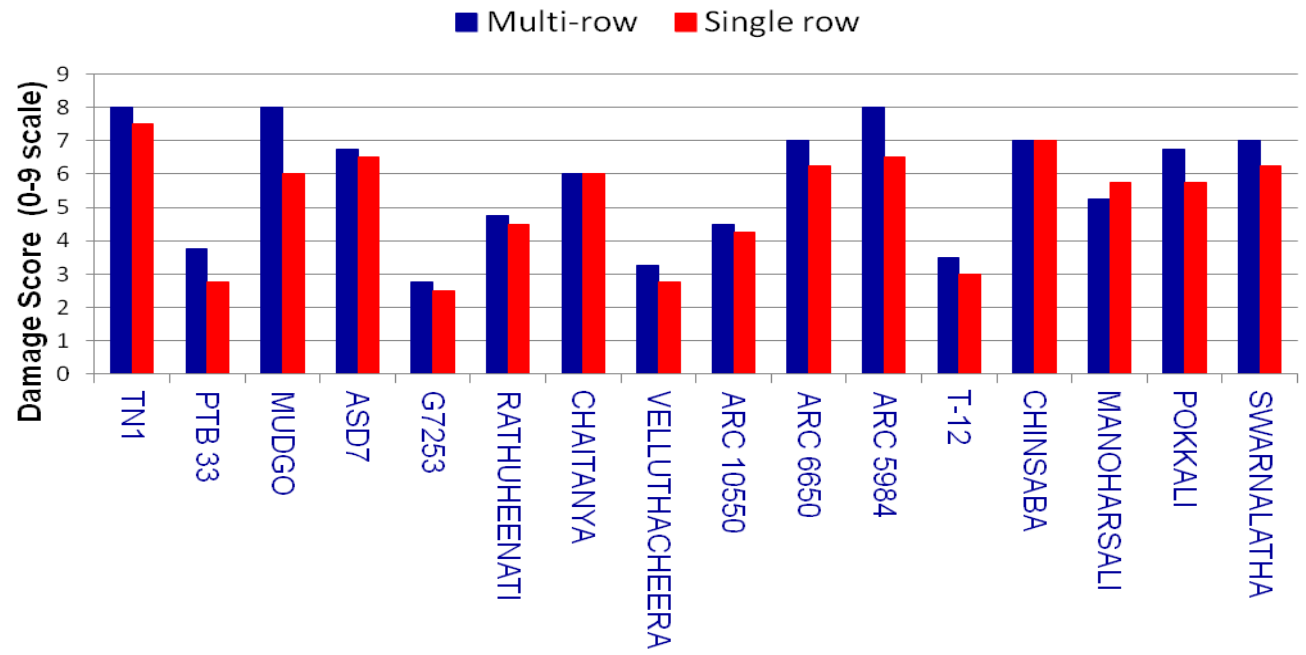

Figure 3: Damage scores (0-9 scale) on various rice genotypes in multi-row and single-row plots at 21 days after infestation with BPH adults at tillering stages in choice situation in the green house.

ARC10550 and T_{12}. The egg hatching as indicated by the nymph population was almost equally high on the remaining genotypes. It is also observed that the egg hatching as indicated by the population of BPH nymphs was higher on multi-row plots than the single –row plots. It is assumed that in single row plots, BPH adults left the resistant genotype and established on the neighboring ones within 24 h after the release. On four – row plots, on the other hand, the BPH adults did not show a different genotype in the neighbor but found the same on leaving one row. BPH adults were thus forced to deposit eggs on resistant plants in multi-row plots. It is therefore assumed that screening should be carried out in multi-row plots rather than single row plots of different genotypes (Figure 2).

The damage caused by BPH on various genotypes also displayed almost the same pattern as for the population build up ($F= 26.56$; $df= 15, 15$; $P < 0.001$) (Figures 2 and 3).

Table 3: ANOVA for the resistance of 22 rice genotypes against 13 populations of brown planthopper in India.

K-Value	Source	Degrees of freedom	Sum of squares	Mean square	K-Value	Probability
1	Replication	3	3.214	1.071	1.212	0.3041
2	BPH Populations (A)	12	56.025	4.669	5.283	0.000
4	Genotype (B)	21	6311.041	300.526	340.101	0.000
5	AB	252	260.944	1.035	1.171	0.054
-7	Error	855	755.508	0.884		
	Total	1143	7386.731			

Coefficient of variation: 13.94%.

The genotype G7253 suffered the lowest damage by BPH followed by Velluthacheera, T_{12}, PTB 33 and ARC10550. The genotype Rathuheenathi had a moderate level of resistance as indicated by the damage scores by BPH. The remaining genotypes had damage scores greater than 7 and hence, were considered as susceptible to BPH implying that invading BPH adults would colonize the genotype to build damaging level of populations (Figure 3).

When various genotypes harboring resistance genes against BPH were infested in a 4-row plots and single – row plots with BPH adults, the population build-up as indicated by the nymphal populations density was high on genotypes grown in 4-row plots as compared to genotypes grown and infested in single-row plots. In the single row plots, the BPH adults encountered genotypes of varying susceptibility/resistance in close proximities and adults were easily attracted towards the genotype with desirable sensory stimuli and not towards the one emanating undesirable signals. That is why, large number of BPH settled on the genotypes, which produced strong sensory stimuli and not towards the plants whose sensory signals were weak or did not attract the insects due to non-preference type of resistance mechanism operating within them.

In 4-row plots, on the other hand, the BPH landing in the midst of the plot perceived the sensory stimuli only from one genotype from all sides and had no choice but to settle there and deposit eggs on the genotype. Hence, the whole egg load was released right on the genotype encountered soon after their release on the plants. In single row plots, BPH had the option of choosing the preferred genotype emanating the desirable sensory signal.

In view of the aforementioned, the egg load of the BPH adults was split between preferred and non-preferred host in single row plots but such a split of egg – load did not occur in the 4-row plots. Consequently, egg hatching on the genotypes grown in 4-row plots was higher than those grown in single-row plots. The genotypes grown in 4-row plots suffered a higher damage by BPH than those grown in single –row plots. Notwithstanding the single or 4-row plots, the genotypes PTB33, G7253, Velluthacheera, T_{12} and ARC10550 showed resistance as indicated by the low population build up and damage scores by BPH relative to remaining genotypes, for example, Pokkali, TN1, Manoharsali, Chinsaba, ARC5984, ARC6650 (Figures 1 to 3). The genotype Rathuheenathi displayed a moderate level of resistance for egg laying and egg hatching by BPH. Such a phenomenon is very important for testing genotypes for resistance against BPH/WBPH for the development of commercial varieties resistant to BPH.

Experiment 3: Resistance in rice differentials for nymphs feeding by 13 BPH populations in choice situation in 2009 and 2011

Experiment 2009

The results show that when the 22 rice genotypes were infested with 13 population of BPH from India, the effect of BPH differ significantly across 13 populations ($F= 5.28$, $df= 12, 855$; $p < 0.05$) (Table 3). The genotypes differed significantly from one another across 13 BPH populations ($F= 340.1$; $df = 21, 855$; $p< 0.010$). BPH population × genotype interaction was not significant ($F= 1.1$; $df = 252, 855$; $p > 0.05$). Data combined over different genotypes showed that the virulence of most BPH populations was equally high except that of BPH population from Mysore which caused a significantly high damage to rice genotypes than the remaining BPH populations (Table 4).

The rice genotypes having varied resistance genes also suffered a varying degree of damage by the BPH populations (Table 5). The genotypes Mudgo, ASD7, Swarnalatha, Pokkali, Chaitanya, Chinsaba and ARC6650 were susceptible to 13 populations of BPH, while the genotype T_{12} and ARC 10550 showed a moderate level of resistance against all the 13 BPH populations. The newly discovered genotypes, G4267, G7253 and Velluthacheera displayed a high level of resistance across all the 13 BPH populations. These three genotypes had lower damage levels than the PTB33.

These results show that certain rice genotypes such as Rathuheenathi, Swarnalatha and Pokkali which have shown resistance to BPH populations from Southeast Asia and whose resistance genes have been studied widely, failed to show desirable resistance against BPH populations from India both at the seedling stages against nymphal feeding tests as well as, for egg laying/hatching resistance against

Table 4: Mean damage scores by BPH populations combined over rice differentials during 2009 (22 Geno × 13 BPH POP × 4 Repetition).

Genotype	Damage scores
West Godavari-2	7.8[a]
Mysore	7.2[ab]
West Godavari-1	6.9[ab]
Champa	6.9[ab]
Pant Nagar	6.8[ab]
Dhantori-2	6.8[ab]
Chhattisgarh-Raipur	6.8[ab]
Warangal	6.7[ab]
Karim nagar	6.7[ab]
Dhantori-1	6.7[ab]
Monkompu	6.6[ab]
Punjab	6.4[b]
West Bengal-24 P	6.3[b]

Mean values followed by the same letters are not significantly different at P=0.05 (F = 05.28; DF = 12, 855; P < 0.001); LSD = 1.305.

Table 5: Mean damage scores of rice differentials under infestations combined over 13 BPH populations during 2009 (F = 340.1; DF = 21, 855; P < 0.0001).

Genotype	Damage scores	Reaction
BPT 5205	9[a]	High susceptibility
TN1	8.97[a]	
TN1	8.9[a]	
TN1	8.88[a]	
ARC 5984	8.71[a]	
Panchami	8.65[a]	
Chinsaba	8.49[a]	
ASD-7	8.39[a]	
Chaitanya	8.36[a]	
Mudgo	8.27[a]	
ARC 6650	8.23	
Manohar Sali	8.11[a]	
Pokkali	7.59[ab]	Moderate susceptibility
Swarnalatha	7.4[ab]	
Rathuheenathi	6.15	Moderate resistance
CR-MR 1523	5.25	
ARC 10550	4.67	Resistant
T-12	4.36	
PTB 33	3.15	High resistance
PTB 33	3.14	
Velluthacheera	2.93	
G7253	2.71	

The mean values followed by the same letters are not significantly different at P=0.05 (F= 340.1; df = 21, 855; p< 0.001; LSD value= 1.705).

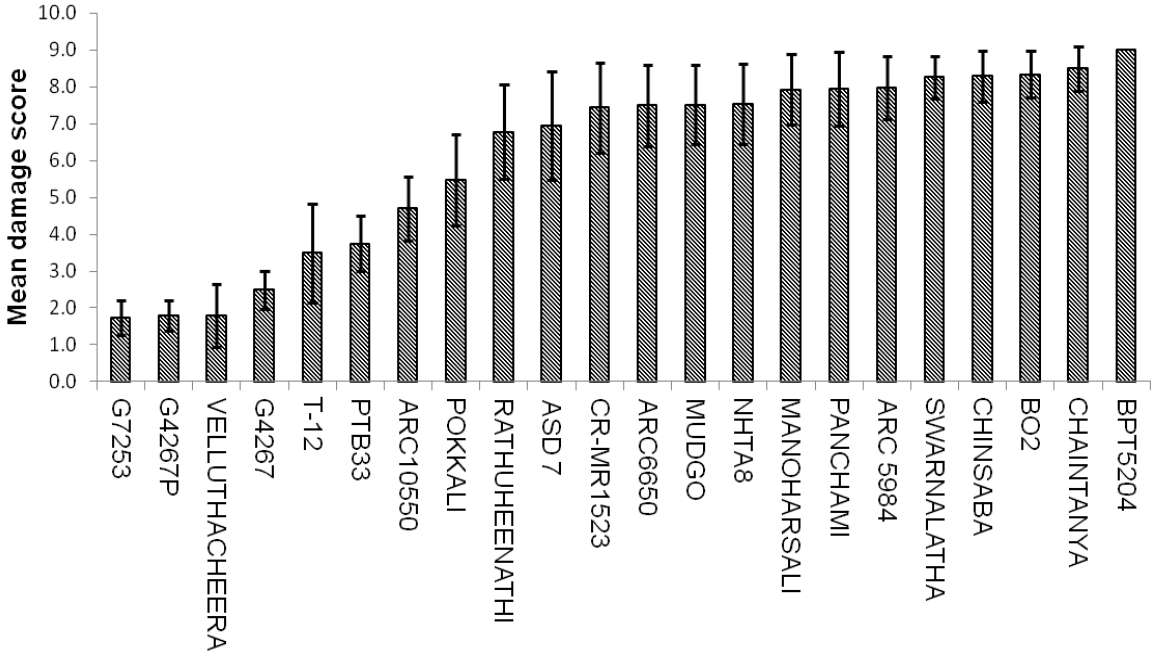

Figure 4: Mean damage scores (Mean ± SD) for 22 rice genotypes combined over infestations by 13 BPH populations at seedling stages under a choice situation in the green house in 2011 using fiber trays[a]. [a]22 genotypes were infested at 17 days after sowing with neonates of BPH in fiber glass trays separately for each of the 13 BPH populations. Data were recorded when plants of the susceptible genotype BOO2 suffered 100% mortality.

BPH adults at the tillering stages of the crop.

The genotypes G7253 and Velluthacheera are typically used as sources of resistance against rice gall midge (Kalode et al., 1977). However, when these genotypes were infested with BPH in 2007, both showed excellent levels of resistance against all the 13 BPH populations. Numerous tests conducted over the years from 2007 to 2018 showed that the plants of these two genotypes did not suffer any hopper burn even under high infestations of BPH (Figure 15). The resistance of the genotype G7253 has also been very successfully utilized to develop the first ever commercial rice hybrid AZ8433 DT harboring resistance derived from G7253.

Experiment 2011

The results show that when the plants of 22 genotypes were infested with the neonate nymphs of 13 different BPH populations from India in large fiber trays, the genotype G7253, G4267 and Velluthacheera continued to display a high level of resistance against all the BPH populations (Table 6). The genotypes PTB33, T12 and ARC 10550 showed resistance reaction, while Pokkali showed moderate resistance to 13 BPH populations. The remaining rice differentials continue to show susceptibility against different populations of BPH at the seedling stages of the plants. Figure 4 shows the resistance reactions of various rice genotypes against 13 BPH populations. The profiles show that the new sources of resistance G7253, G4267 and Velluthacheera were the only genotypes which displayed resistance across 13 populations of BPH collected from different rice ecologies of India. The other two genotypes such as ARC10550 and T_{12} were also resistant against majority of BPH populations but the resistance levels were lower than G7253, G4267 and Velluthacheera. The genotype Rathuheenathi, which has been reported to show resistance against BPH from South East Asia, was not consistent for its resistance against different BPH populations of India. The genotype Pokkali also showed susceptibility against BPH population at seedling stages of infestations. The remaining genotypes were also susceptible (Figure 4).

Incidentally, several resistance genes including those from Rathuheenathi, Pokkali, and Swarnalatha have been identified through map-based cloning approach providing a means for understanding the molecular basis of BPH-host interactions (Jing et al., 2017). The resistance gene of Rathuheenathi has been reported to be a cluster of three genes encoding lectin receptor kinases (*OsLecRK1* – *OsLecRK3*). Similarly, the gene, *Bph9*, from Pokkali has been cloned and is reported to encode a coiled – coil, nucleotide – binding, and leucine – rich repeat (CC-NB-LRR) protein. Most of these genes have been reported to be expressed in the vascular tissues of the rice plants conferring antixenosis towards the insects. In our study, the genotypes Rathuheenathi and Pokkali hardly showed any resistance at the seedling stages of the crop typical of the seed box

Table 6: Damage scores (0-9 scale) by 13 populations of BPH on 22 rice genotypes infested at 17 days after sowing with neonates 20-25 nymphs per plant in the green house in fiber trays in 2011.

Genotype	Resistance gene	WG	WB-24P	RP-CG	DHAN	MYS	CMP-CG	WGL-TL	OD	PUN	PNT	BURD-WB	KRM-TL	MON-KL
BO2	None	9.0	7.7	7.3	8.0	9.0	7.3	8.3	9.0	9.0	9.0	8.0	8.3	8.3
ARC6650		8.7	6.7	5.7	7.0	9.0	6.0	8.3	7.7	9.0	8.3	6.7	7.3	7.0
ASD 7	*bph2*	8.3	5.0	4.3	7.7	9.0	6.3	8.0	7.3	9.0	6.3	5.3	6.7	7.0
ARC 5984		9.0	8.0	6.0	8.0	9.0	8.0	8.0	8.3	9.0	8.3	7.3	7.0	7.7
Mudgo	*bph1*	8.7	7.3	6.0	7.3	9.0	7.7	7.3	7.0	8.3	9.0	7.3	5.3	7.3
ARC10550	*bph5*	5.0	4.3	4.3	4.3	6.0	4.0	4.0	4.3	6.0	4.3	3.7	4.3	6.0
Chinsaba		8.7	7.0	7.0	8.3	9.0	8.0	8.0	8.3	9.0	9.0	8.0	8.3	9.0
Swarnalatha	*Bph6*	8.3	7.3	7.3	8.0	9.0	8.0	8.0	8.7	9.0	9.0	8.3	8.0	8.3
Rathuheenathi	*Bph3*	6.0	6.7	6.3	4.7	7.0	5.3	6.3	8.3	8.3	8.7	8.0	5.3	7.0
Pokkali	*Bph9*	5.0	5.0	5.7	3.7	4.7	4.3	5.7	6.7	8.0	7.0	4.0	5.3	6.0
T-12	*Bph7*	2.3	4.0	3.7	3.7	2.3	2.7	4.3	3.0	6.0	2.3	2.0	3.0	5.3
Manoharsali		7.7	8.0	8.7	6.7	9.0	7.7	5.7	8.7	8.7	9.0	7.7	7.7	8.0
Chaitanya		8.7	9.0	8.7	8.3	9.0	8.0	8.7	9.0	8.7	9.0	7.7	7.0	8.7
NHTA8		9.0	7.7	7.0	7.0	8.7	5.7	8.0	7.0	8.3	9.0	7.3	5.7	7.7
PTB33	*Bph3*	3.3	4.3	3.0	3.3	4.7	2.7	4.0	4.0	3.3	4.0	2.7	4.3	5.0
Panchami	Not known	8.0	8.7	8.3	8.3	8.7	5.3	7.0	8.7	8.0	9.0	8.7	7.3	7.3
91090830028	Not known	2.7	2.7	3.3	2.7	3.3	2.3	2.3	2.3	2.7	1.7	1.7	2.0	2.7
BPT5204	None	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0
91090830030	Not known	1.3	1.3	2.0	2.0	1.7	1.0	2.0	1.7	2.7	1.7	2.0	2.0	2.0
CR-MR1523	Not known	6.7	6.3	8.0	9.0	9.0	6.3	8.0	7.7	6.7	9.0	8.3	5.3	6.3
G7253	Not known	1.0	1.0	2.0	2.0	1.3	2.0	2.0	1.7	2.7	2.0	2.0	1.3	1.7
Velluthacheera	Not known	1.0	1.0	2.0	2.0	1.3	1.0	1.7	2.0	4.0	1.7	1.0	2.0	2.7

Note: WG= West Godavari; WB-24P= West Bengal 24-Parganas; RP-CG= Raipur-Chhattisgarh; Dhan= Dhantori-Haryana; MYS= Mysore; CMP-CG= Champa –Chhattisgarh; WGL-TL= Warangal-Telangana; OD= Odisha; PUN=Punjab; PNT= Pant Nagar; BURD-WB= Burdwan- West Bengal; KRM-TL= Karim Nagar-Telangana; MON-KL= Monkompu- Kerala. Resistance categories: 1- 5 = Resistant (shown in green); 5 - 6 = Moderately resistant; (in blue) 6 - 9 = Susceptible (in red).

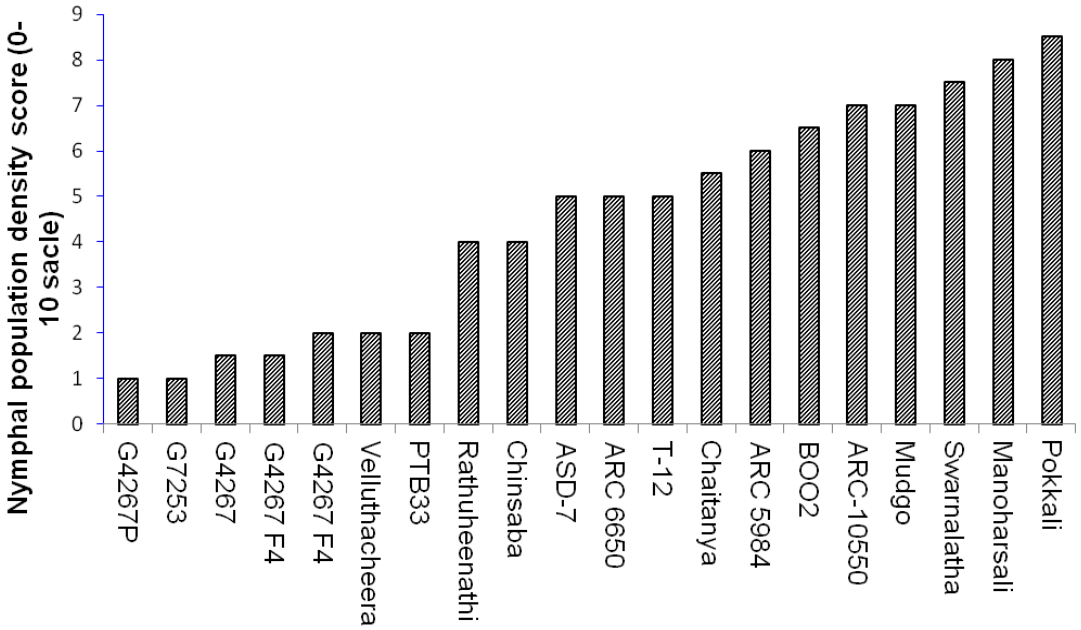

Figure 5: Nymphal population build - up on certain genotypes grown directly in the soil inside the green house at 21 days after infestation with adults of BPH.

screening methodology. However, when infested at the tillering stages with BPH adults, the two genotypes did exhibit some level of resistance against both BPH as well as, WBPH. Perhaps, the two types of immune systems reported for plants resistant to BPH (Jones and Dangl, 2006; Jing et al., 2017) get activated at the tillering stages when plants are infested with few BPH adults at 5 to 10 BPH/plant. Feeding by these BPH adults did activate the immune systems to strengthen the plant defense system, which responded well when the egg hatching occurred on the infested plants to counter the insect attack. At the seedling stages, perhaps, the nymphal feeding by the high number of nymphs (20 to 25 nymphs/plant) could not activate the plant immune system well on time and the plants suffered mortality.

In the genotypes G7253 and G4267, the major genes seems to be constitutive in nature as plant defense mechanisms were equally operative during seedling stages and tillering stages. More detailed investigations are required to elucidate the complex interaction of BPH with rice plants.

The data also show that BPH populations collected from different rice ecologies within India did not differ in terms of virulence against different resistant genotypes. Such a reaction of BPH populations was expected because of its wing dimorphism (macropterous and brachypteous adults) and the capability to migrate long distances to exploit the rice host grown in different rice ecologies at different time of the year. The long distance migration of BPH has also been reported across different countries but still reaction of resistant genotypes such as Rathuheenathi, T_{12} and Pokkali differ across different continents of Asia (Fujita et al., 2013).

Experiment 4: Egg-laying, egg-hatching and damage among rice genotypes infested at 7 weeks after sowing with adults of BPH in the greenhouse field

When the 20 genotypes grown in mini-plots inside the green house were infested with BPH adults, the populations build up by the adults varied from one genotype to another. The population build up, as indicated by the Nymphal population density scores was very low on G4267 P, G4267 S, and F_4 lines of G4267, G7253, Velluthacheera and PTB33, moderate on Rathuheenathi and Chinsaba and high on ASD7, ARC6650, T_{12} and Chaitanya (Figure 5). The population build up was very high on the genotyoes ARC 5984, BOO2, ARC10550, Mudgo, Swarnalatha, Manoharsali and Pokkali (Figure 5).

The patterns of damage caused by the BPH populations on different genotypes varied at 25, 30 and 31 days after the infestation (DAI) (Figure 6). The degree of damage generally ought to increase temporally from 25 DAI to 31 DAI. The expected trend was generally true for genotypes ARC10550, Mudgo, Chailtanya, BOO2, Swarnalatha, Chinsaba, ASD7, Manoharsali ARC5984 ARC6650 and Pokkali. However, such a progression of damage with time was not observed for genotypes G4267 and its related material, G7253, Velluthacheera, T_{12} and Rathuheentahi. These results suggest that G4267 and G7253 are the durable sources of resistance whose resistance remained robust after artificial infestation with BPH adults. It is assumed that these two genotypes possess plant characters

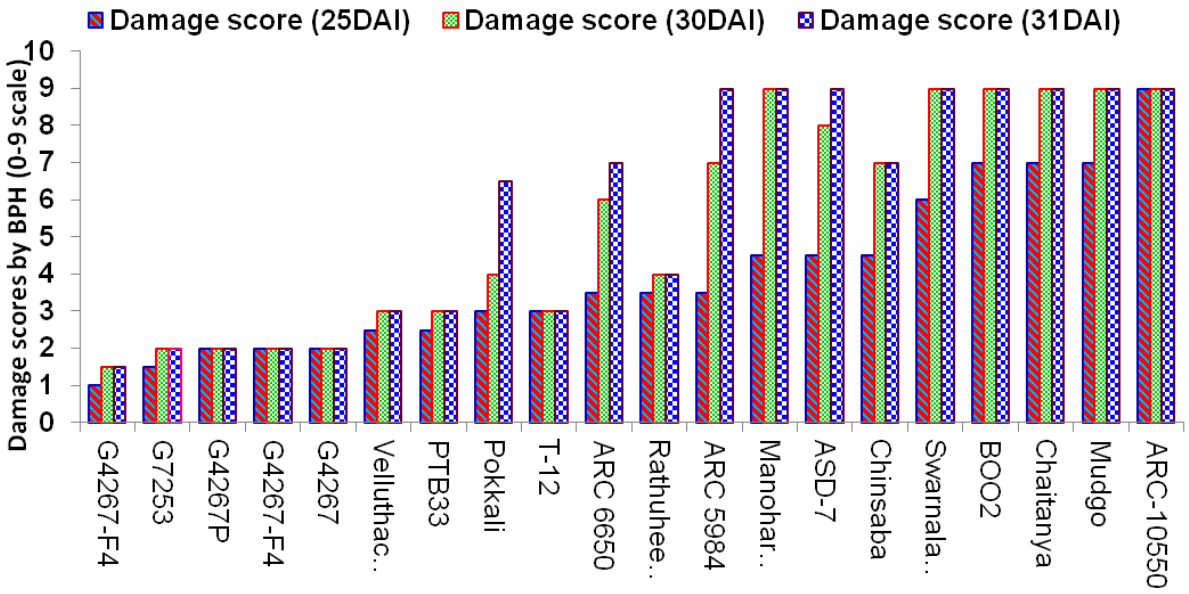

Figure 6: Damage caused by BPH population build- up on certain genotypes at three different periods after infestation with ovipositing adults on the plants grown directly in the soil inside the green house.

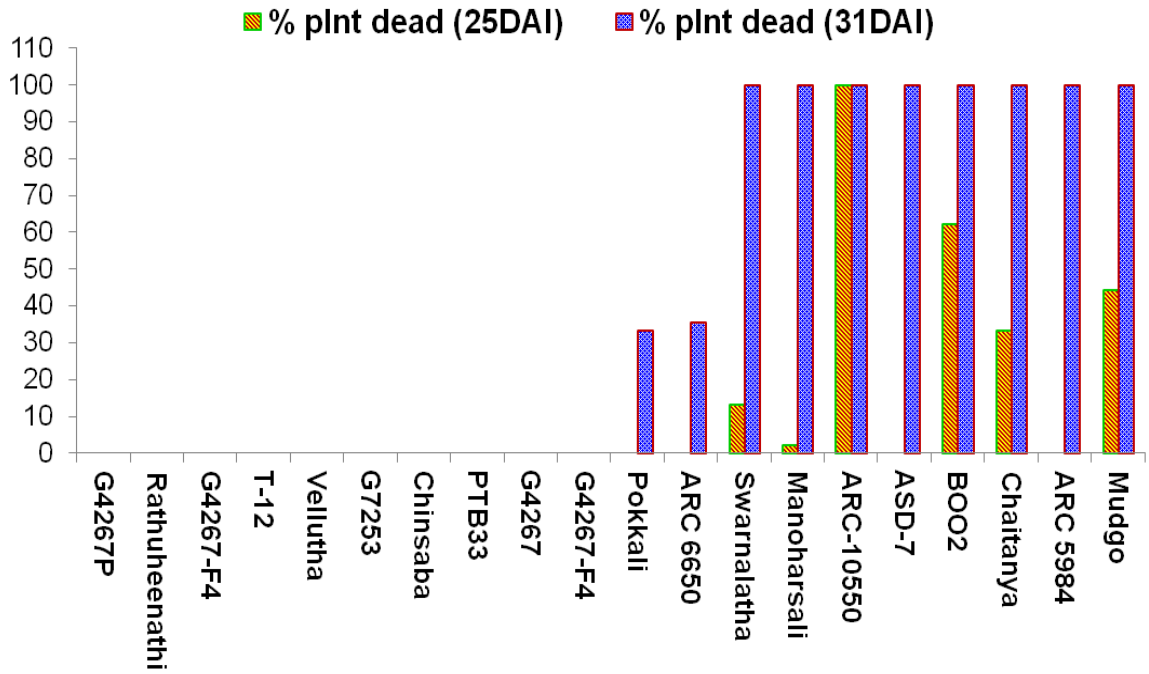

Figure 7: Percentage of plants suffering hopper burn by BPH population build- up on certain genotypes at 25 and 31 days after infestation with ovipositing adults on the plants grown directly in the soil inside the green house.

which impart anti-xenosis type of resistance against BPH (Kumar; unpublished data). BOO2 suffered hopper burn within 25 days of infestation with nymphal population reaching 7 to 8 score. At the back of BOO2 seen is ARC 5984 which showed susceptibility against BPH at early stages of plant growth but showed tolerance against BPH infestation at tillering stages.

The observations earlier mentioned suggest that certain differentials like Pokkali, Rathuheenathi and ARC5984 displayed high population build up by BPH but still suffered no hopper burn (Figure 7). Thus, these genotypes have tolerance as basis of resistance at later growth stages of the crop. The same genotypes suffer complete plant mortality at pre-tillering infestation by BPH.

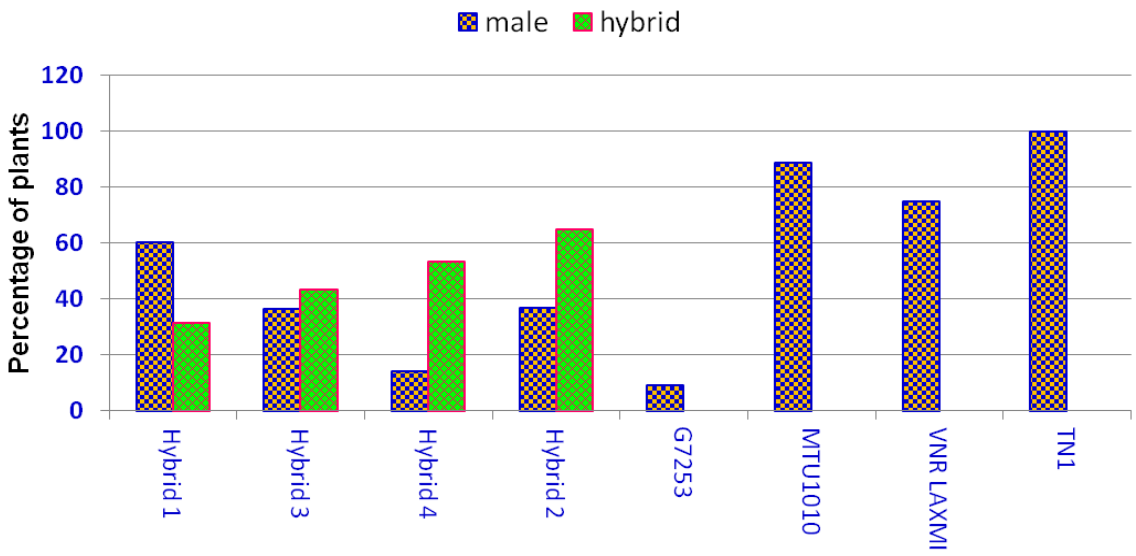

Figure 8: Percentage of plants dead at 10 days after the infestation in multi row plots in a no-choice situation (Data based on 150-200 plants of each genotype).

Pokkali showed susceptibility at early stages of plant growth against BPH but did not suffer hopper burn in spite of the fact that nymphal population build up by BPH was the highest among all the genotypes infested with adults. Results show that Pokkali has "tolerance" against BPH. Howewer, the high population buiid up on Pokkali later migrated towards the neck region of the panicles where soft tissue of the panicle region was colonized by the individuals of BPH. As a result of extensive feeding on the panicle, the plant collapsed and suffered mortality (Figure 17). Thus, the tolerance type of resistance in the vegetative parts of the plants was not sufficient at the flowering regions of the plant. It is therefore suggested that stacks of resistance genes from Pokkali can be used as a source of tolerance mechanism along with resistance genes from sources like G4267 and G7253 by reducing the population at the vegetative stages of the crop and small populatione eventually reaching the panicles from the vegetative parts. However, on the basis of my experimental work not reported here, it is assumed that even stacked genes may not be able to prevent the collapse of Pokkali due to BPH feeding at the panicles. It is therefore suggested that an insecticide spray at flowering stage of the crop would be needed to make the resistance genes of Pokkali or any other resistant genotype durable during the grain forming stages of the crop.

A reference to the literature show that certain plant factors of a few genotypes display ovicidal effects against planthoppers, particularly, WBPH (Yamasaki et al., 1999). Our preliminary data did not show any egg mortality by BPH on the resistant sources G7253 and G4267 (Kumar, personal observation). Feeding non-preference/antixenosis and oviposition non-preference seems to be the primary mechanism of resistance of these two genotypes.

Experiment 5: A comparison of rice hybrids and their parents for resistance against BPH in choice and no-choice situation

When the plants of four hybrids and their parents were infested at 15 days after sowing in a no-choice situation, the percentage of plants that suffered mortality varied from one hybrid to another (Figure 8). Among the test entries, the donor G7253 suffered the lowest (9%) hopper burn damage followed by the male parent of hybrid 4 (15%). Among the male parents of hybrids 2 and 3, almost 35 to 40% plants suffered hopper burn by BPH. The male parent of hybrid 1 suffered the highest (60%) hopper burn damage by BPH.

Among the four hybrids under no-choice situation, the hopper burn damage was lowest on hybrid 1 followed by hybrids 3, 4 and 2. Thus, contrary to the damage suffered by the male parents, the hybrids showed a different pattern of hopper burn damage by BPH. The damage patterns on the male parent and the hybrid did not match. This is as a result of segregation for resistance in the male parents and random fusion of male and female gametes from the segregating pollen load. Under no-choice situation, the susceptible TN1 was completely wiped off as 100% plants suffered hopper burn. The commercial variety MTU1010 suffered almost 89% hopper burn, while VNR Laxmi had 70% plants with hopper burn (Figure 8).

Under no choice situation, the magnitude of damage as indicated by the damage scores was very low on the donor parent G7253. None of the male parents could match the resistance levels of the donor parent though the male parent of the hybrid 4 suffered lower damage than the other three male parents of the 3 hybrids (Figure 9). These results suggest that the resistance factors of the donor

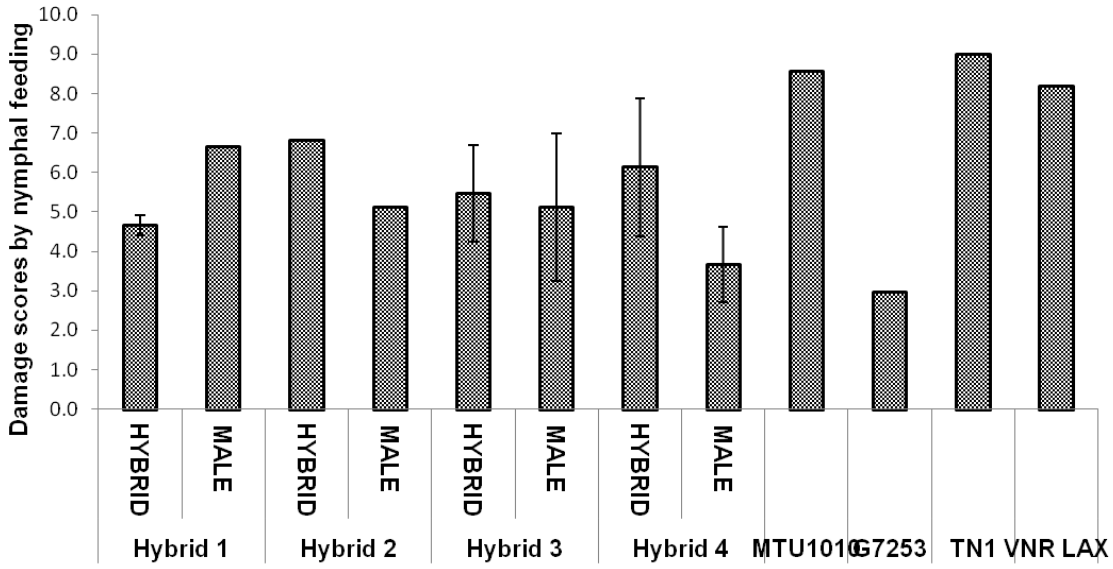

Figure 9: Mean damage score (Mean ± SD) AT 10 DAI in no choice test conducted at seedling stage.

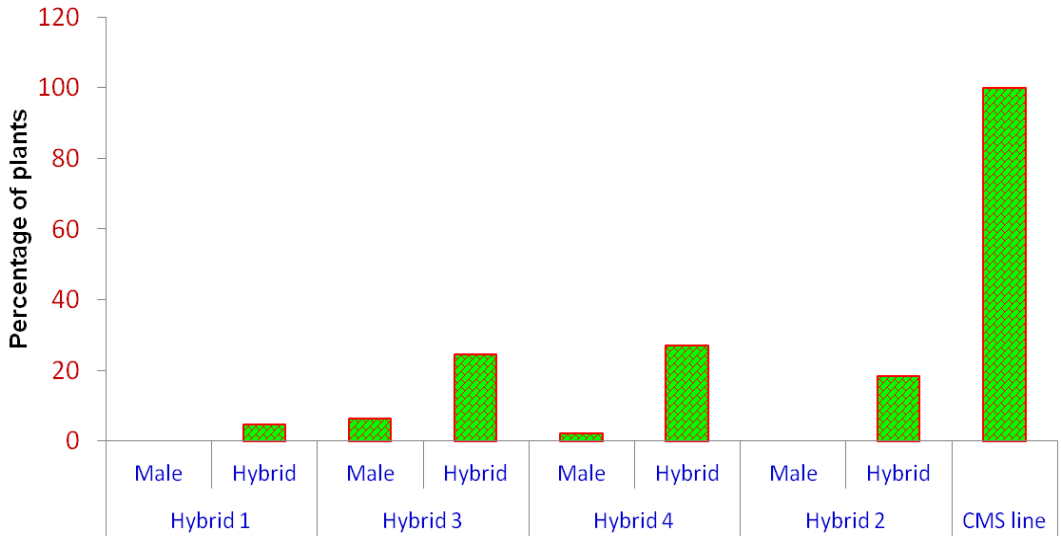

Figure 10: Percentage of plants dead (Mean_2 rep at 10 DAI in single row plots infested at 15 DAS with BPH nymphs) in choice situation.

parent were not inherited completely by the males of 4 hybrids. This could be due to the fact that the four male parents were selected for their resistance only through phenotypic reactions of the resistance and certain recessive factors of the plants might be playing their role in the overall resistance of the donor parent. Such recessive factors could not be selected through phenotypic selections under artificial infestations. A detailed marker assisted breeding approach would perhaps help capture all the resistance factors from the donor parent.

When the same four hybrids and their male parents were infested with BPH nymphs in a choice situation, all the hybrids, and their male parents displayed high resistance by the donor parent, as indicated by the low level of plant suffering mortality as well as, equally low levels of damage suffered by all the resistant materials (Figures 10 and 11).

The two tests earlier described under no-choice and choice situation have important implications for various studies in rice resistance to planthoppers. The choice tests seem to be a highly insensitive test for characterizing various factors responsible for the resistance of a genotype to planthoppers. In choice tests, even the smallest effects of a QTL may sound big for plant resistance and it may not be possible to segregate QTLs with varying levels of contributions in the overall resistance of the plant against insects. Therefore, for separating the contributions of

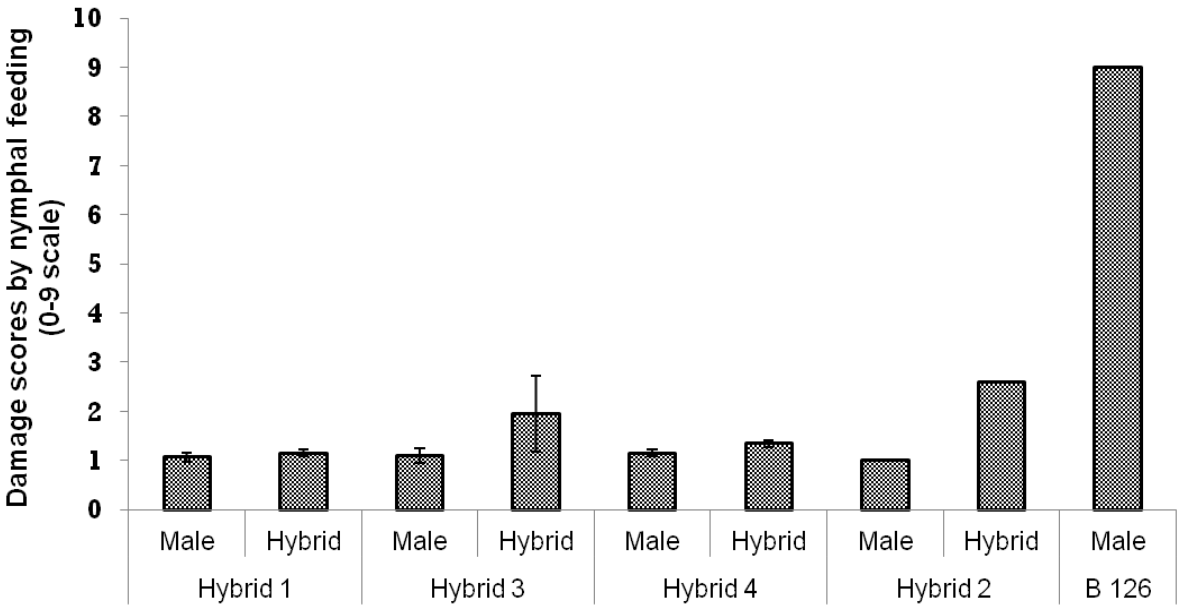

Figure 11: Damage score (mean+SD) at 10 DAI in single row plots in choice situation.

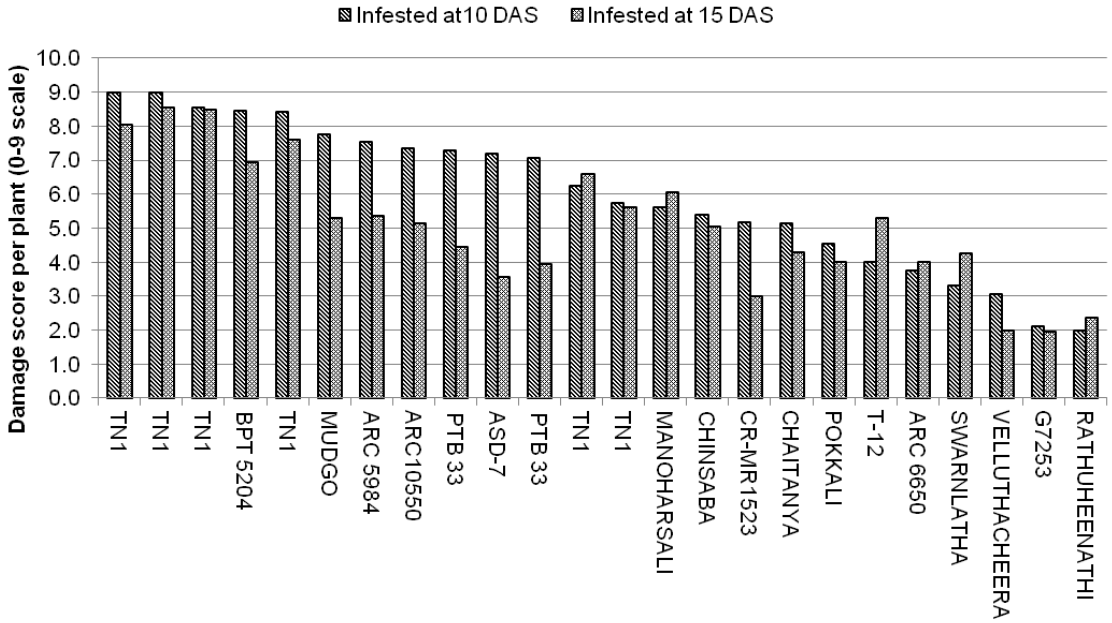

Figure 12: WBPH damage on certain rice genotypes infested at 10 and 15 days after sowing with neonates. Mean values based on 20 plants.

various QTLs in the overall resistance of a genotype, no-choice tests would be compulsory and ideal.

Experiment 6: Resistance in rice differentials for nymphs feeding by WBPH in choice situation

Figure 12 shows that when the 19 genotypes were infested with WBPH at 10 and 15 days after sowing, the genotype ASD7 with *bph2* gene and Manohar –sali, found susceptible against BPH showed resistance against WBPH. Similarly, Swarnalatha with resistance gene *Bph6* was also resistant against WBPH. The genotype Chinsaba with resistance gene bph8 had a moderate level of resistance against WBPH. Mudgo with resistance gene *Bph1* was moderately susceptible against WBPH. The genotype Pokkali (*Bph9*), Chaitanya and ARC6650 were moderately resistant against WBPH.

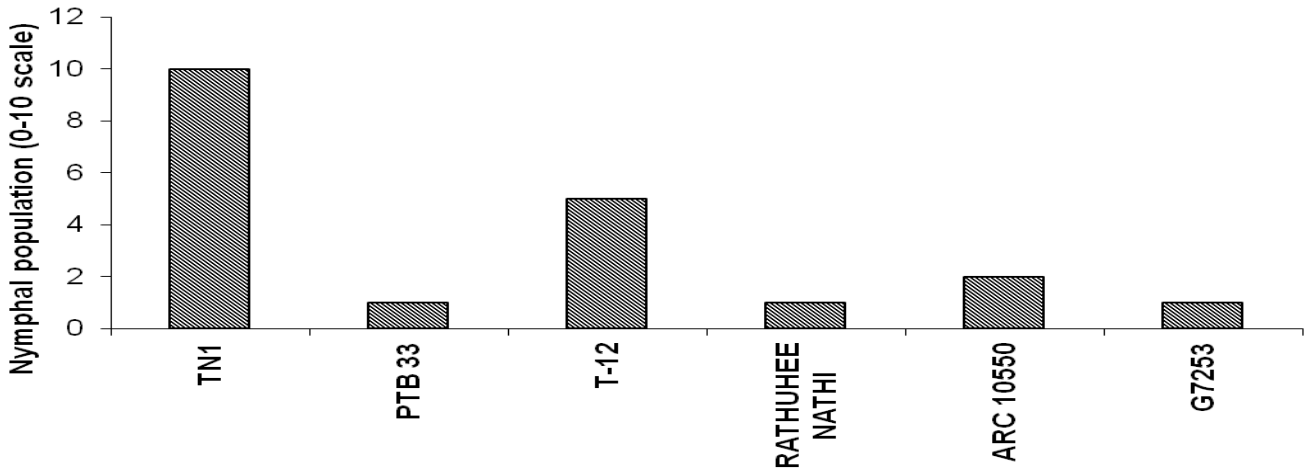

Figure 13: WBPH nymphal population build up on certain rice genotypes infested with adults in a no-choice situation in the green house.

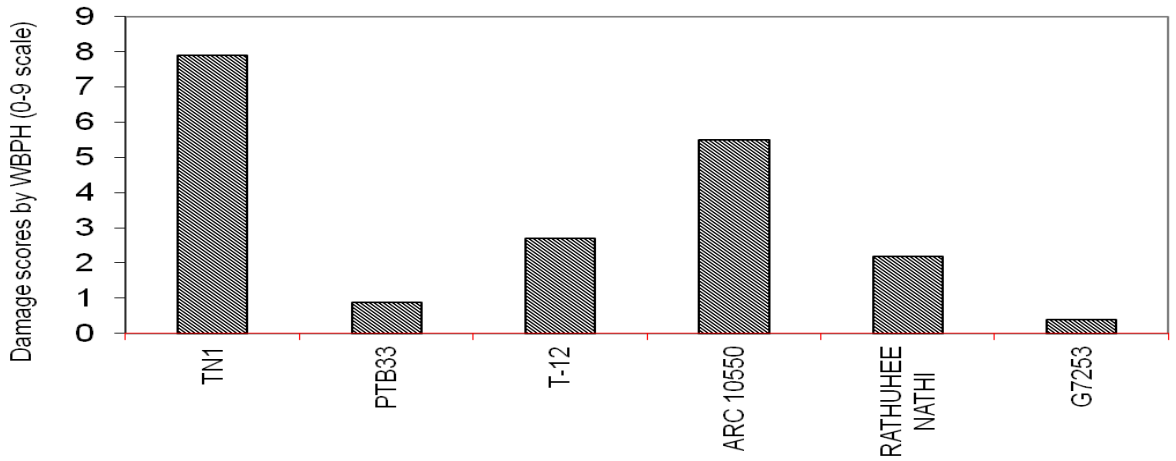

Figure 14: WBPH damage on certain rice genotypes infested at tillering stages with adults in a no-choice situation.

The genotypes G7253, Rathuheenathi and Veluthacheera showed a high level of resistance against WBPH. Incidentally, the genotype Rathuheenathi and G7253 also showed high level of resistance against WBPH at tillering stages as earlier explained in experiment 2 (Figure 12). The genotypes suffered slightly greater damage by WBPH in the 10 day old crop as compared to the 15 days old crop. It will be ideal to screen rice germplasm for resistance against WBPH on 10 - day old crop. Thus, genotype MUDGO with *Bph1* and ASD7 with *bph2* resistance genes did not suffer damage by WBPH even though these genotypes are killed under BPH infestation. The BPH populations have already overcome the resistance factor of ASD7 and Mudgo because of widespread cultivation of rice varieties possessing resistance genes, particularly, *Bph1* in whole south and Southeast Asia in the early seventies. Resistance in these cultivars did not evolve against WBPH as this pest was largely absent from the rice fields in the early seventies. In view of the fact that resistance genes against WBPH and BPH hold a great deal of similarity, the present work also show that genotypes found resistant against BPH also show resistance against WBPH resistance.

On the basis of results aforementioned, the genotype G7253 and Rathuheenathi would be the ideal source of resistance for breeding rice crop for resistance against WBPH.

Experiment 7: Egg hatching and damage by WBPH on selected rice differentials in no-choice situation

Data (Figure 13) show that nymphal density was very high on the susceptible TN1; moderate on T_{12} and low on PTB33, RP2068, G7253, Rathuheenathi and ARC 10550. A high nymphal density was also strongly reflected by the corresponding high percentage of plant mortality and the high damage scores by WBPH (Figure 14). On genotype CR-MR1523, no nymphal population was recorded.

Figure 15: The resistant genotype G7253 and the susceptible parent BOO2 after infestation with BPH nymphs. The one half of a row containing G7253 (Right) completely green and healthy while the other half containing plants of BOO2 suffered complete hopper burn.

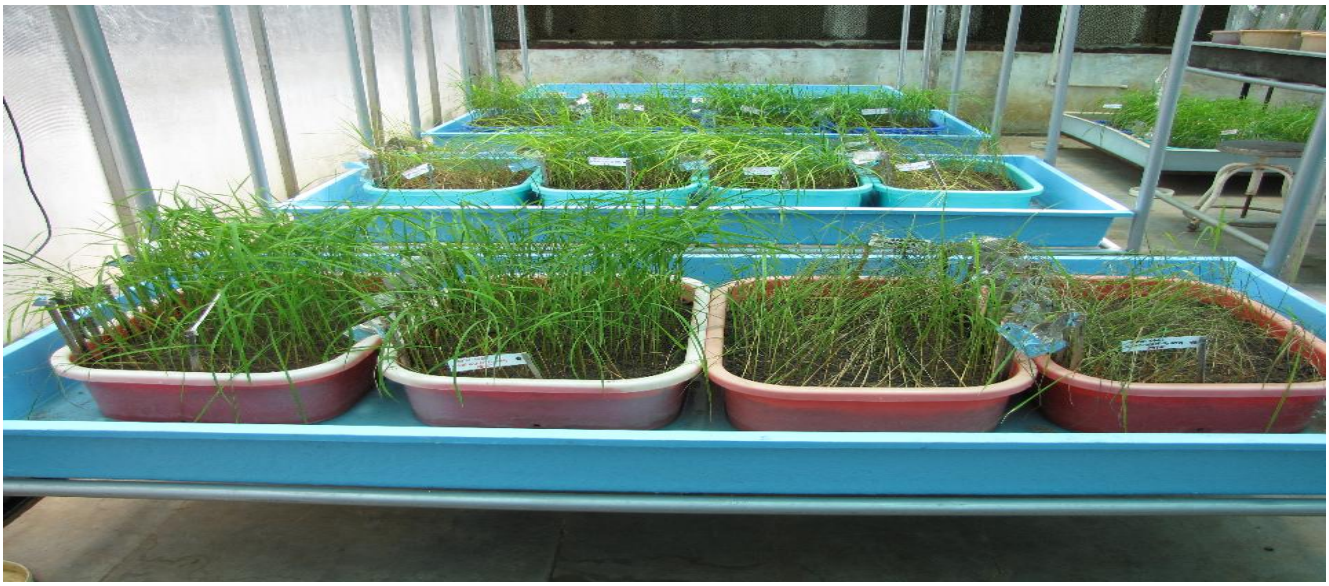

Figure 16: Four plastic tubs used to grow rice differentials per BPH population for resistance study in the green house.

On T_{12}, the moderate nymphal density did not cause any plant mortality and the damage scores were also low. This indicates that T_{12} will not inhibit egg - laying by WBPH but will certainly resist damage by the nymphal population emerging on it. On ARC 10550, a low nymphal density of WBPH led to high damage scores on the plant, though plant

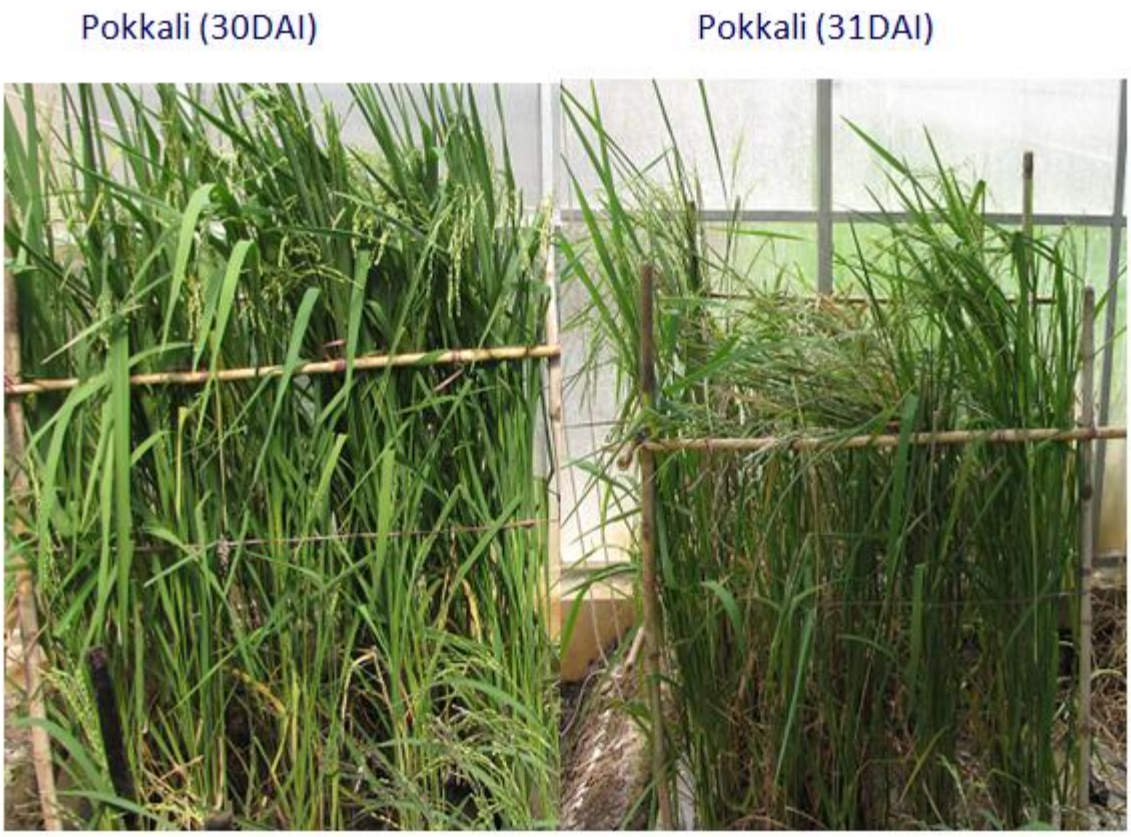

Figure 17: The tolerant genotype Pokkali succumbed to BPH population migrated to the panicle neck and panicle at 31 days after the infestation with BPH adults.

Figure 18: WBPH Damage on TN1 (left) and G7253 (Right) after infestation with adults at tillering stages in the green house.

Figure 19: A mini plot of 50 plants of a genotype for screening for egg laying and egg hatching resistance at tillering stages of the crop in the green house field.

mortality was not observed.

The genotypes PTB33, RP2068 and Rathuheenathi displayed egg laying/egg hatching resistance as well as, nymphal feeding resistance against WBPH as indicated by the low to very low nymphal population as well as, low plant mortality. The genotype CR-MR1523 did not suffer any plant damage by WBPH even though we infested the plants twice with a total of 220 males and 220 females.

The regression of nymphal density on damage scores across genotypes ($y = 1.022 + 0.66x$; $r = 0.81$; $p < 0.05$) or regression of nymphal density on percentage of plant mortality ($y = -5.95 + 3.83x$; $r = 0.89$; $p < 0.05$) was significant though the correlation was not a complete fit.

Conclusion

The work presented in this paper deviates from the traditional methodology of IRRI's seedbox for screening germplasm for resistance against brown planthopper (BPH) and Whitebacked planthopper (WBPH). Firstly, we used 15 to 20 days old plants for screening germplasm for BPH resistance and secondly, used several populations of BPH, in particular, to identify the most robust and durable sources of resistance against BPH and WBPH across different agro-ecological zones in India. The identified sources and the available sources of resistance were further validated for their resistance against the planthopppers in no choice situations under conditions very close to those prevailing in the natural field conditions as shown in our tests in the greenhouse fields. Under natural conditions, the infestation by BPH begins by the arrival of the immigrant adults and building population by egg laying and hatching. We simulated similar field conditions in the greenhouse field by releasing the macropterous adults of BPH and WBPH on different sources of resistance to validate resistances. Thus, the sources of resistance identified in this work were selected not only for nymphal feeding but also for adult feeding, egg laying, egg - hatching and growth and development of newly emerged nymphs along with damage done on the plants.

Using the aforementioned profiles for resistance validation, we hereby report two new sources of resistance against BPH and WBPH: G7253 and G4267. G7253 has already been successfully utilized to develop the first ever BPH resistant hybrid AZ8433 DT, which is being grown widely by the rice farmers in India. The work shows that the resistance of G7253 is durable against several BPH populations of India. The feeding and oviposition non-preference are the principal mechanisms of resistance operating within these resistant line (Figure 15). The work also shows that G7253 displayed an acceptable level of resistance against White Backed planthopper (WBPH) at both the seedling stages as well as, the tillering stages.

Overall, G7253 had a negative effect on the overall population dynamics of BPH and WBPH.

The second source of resistance G4267 is also very robust and the plants of this genotype did not suffer mortality under high artificial infestation with BPH. The genotype showed an antixenotic reaction towards BPH for inhibiting nymphal feeding and population build up. Data presented in the paper suggest that likewise G7253, the G4267 was resistant to BPH under different categories of testing protocols described in this paper. The population dynamics of BPH was negatively affected by this new source of resistance.

Although, no allelic studies were conducted, it is assumed that the resistance genes of these two new resistant lines seems to be different from the 32 mapped genes on different chromosomes (Jing et al., 2017). The preliminary gene mapping studies conducted revealed that resistance genes/OTL,s might be located on chromosomes 4, 6 and 12.

The resistance seems to be governed by the constitutive expression of major genes in G7253, G4267 and Velluthacheera. The two branched innate immune system, for example, pathogen-associated molecular patterns triggered immunity and effector triggered immunity operating through lectin receptor kinases and coiled-coil, nucleotide-binding and leucine – rich protein (CC-NB-LRR), respectively, operating within genotypes harboring Bph14, Bph3/32 or Bph26, could also be operating within these new sources of resistance. However, there is no experimental evidence yet for these two types of immunity operating within genotypes as a result of BPH feeding. The resistance of G7253 and G4267 has seldom increased with the advance in the age of plants after infestation; rather the resistances of the two genotypes remained manifested within the first 24 h after the infestation. It is observed that damage on these two genotypes does increase by the population built up by the first generation of BPH. Resistance seldom increased after the infestations.

We hereby report resistant hybrid, which is practically farmer's friendly, environmentally safe and sustainable for BPH management in the farmer's field. The resistance is manifested effectively in the parental male line and the commercially viable rice hybrid for the benefit of farmers in India. The resistance is likely to be durable because the paddy cultivation area for hybrids is still 7 to 10% and there is likely to be plenty of commercial varieties available for inter-mating of BPH from the resistant hybrids and those from varieties without BPH resistance genes. BPH being highly migratory pest, this inter-mating will dilute the selection of virulent BPH individuals.

Host plant resistance in plants to insects is a method of crop protection, which is environmentally safe, economically viable and socially acceptable. The introduction of Bt crops has put some kind of limitations/constraints of using environmental safety, and economic viability of these crops.

The Bt crops provided foolproof method to control lepidopterous pests despite debatable environmental and economic constraints of using such crops. The use of Bt crops has literally pushed the conventional breeding approaches towards the back seat because of the ease of transferring Bt genes in the elite commercial varieties and hybrids and the effectiveness of the toxins to control the target pests without any adverse effects on plant agronomy.

The present study shows that certain BPH resistance genotypes found highly resistant against BPH were found susceptible. For example, the genotype like Rathuheenthi found resistant was not holding resistance in the present study against any of the 13 BPH populations. This could be due to a difference in the BPH population composition of the different regions. These different populations have also been designated as different biotypes by certain authors. However, because of highly migratory nature of BPH, the likelihood of their population segregated into biotypes seems to be highly doubtful (Claridge and Hollander, 1983; Kobayashi, 2016). The existence of pure population in different rice ecologies seems highly unlikely. In view of the aforementioned, the differences could be due to different screening methodology adopted by various authors in comparison to the current paper. Most authors have followed the age-old screening techniques developed at IRRI whereby BPH nymphs, mostly 2^{nd} to 3^{rd} instars are released on 7 to 10 days old seedlings. Under natural conditions, infestation on rice commences either by neonates, that is, after egg hatching or by eggs laid by the emigrating BPH adults. The methodology followed by various authors does not consider any of these parameters. Under these circumstances, the BPH establishment on the seedlings is unlikely to occur in the conventional manner. It is therefore very important that the conventional system of host plant selection by the Brown planthopper should be followed to get the desired results.

In the present study, efforts have been made to follow the conventional protocols used by the insect to select and establish its population on rice plants. All screening plants for feeding resistance was done using the neonates of BPH. Various genotypes have also been screened by infesting the plants with BPH adults so that the complete profile used by the insect for host plant selection is observed, that is, adult establishment, oviposition, hatching and damage done by the hatched nymphs or gravid adults of BPH.

In most studies conducted, rice seedlings have been screened in a choice situation by growing the resistant and susceptible entries in an alternating fashion. Under these conditions, BPH have the tendency to select the most susceptible genotype over genotype having any level of resistance. BPH tends to congregate on the most susceptible genotypes, thus, causing high damage. Under such conditions, the data recorded gets skewed and biased. In the present study, efforts have been made to evaluate rice genotypes under both choice and no-choice situation.

Jena and Kim (2010) reported that Mudgo, ASD7, Rathuheenathi, Babawee, ARC10550, Swarnalata, T_{12}, Chin

Saba and Balamawee are resistant donors. The present study showed that those varieties had no resistance to the BPH at the seedling stage in greenhouse screening. This study suggests that the Bangladesh BPH population could be a new biotype with high virulence.

The rice genotypes were the same as earlier described in previous experiments except we included one more germplasm namely G4267. We used its parent G4267P as well as, G4267 F_4 line derived from the resistant parent.

It is also imperative to note that resistant rice hybrid developed through using resistance genes of native rice varieties cannot provide complete immunity against BPH in the farmer's field. It will be dangerous and risky for the farmers to rely solely on host plant resistance in their cultivars to prevent yield losses by pests such as BPH and WBPH. The resistant rice hybrids are capable of containing the pest outbreaks up to certain limits temporally and spatially. Therefore, right from the beginning of cultivation of resistant hybrids, it will be highly desirable to use resistant cultivars in a package of IPM practices involving plant resistance, insecticides and natural enemies. While plant resistance and insecticides can readily complement each other's effects in the farmer's field, the use of natural enemies is still not very common among the farmers and this area still need a lot of efforts to convince the farmer's community for its benefits. One area where resistant hybrid can be directly useful among the farmers is to reduce the applications of pesticides for BPH control, thus, directly reducing the magnitude of insecticide applications on rice crop and providing direct profit to the farmers in terms of cost saving.

In India, the rice farmers usually use 3 to 4 insecticidal sprays to manage this destructive pest and on many occasions, the pest outbreak still occurs forcing the farmers to use sometimes 7 to 8 sprays. Such a situation makes the insecticides highly vulnerable to lose their efficacy through the development of virulent pests (Shun et al., 2018). It is suggested that the insecticidal sprays can easily but effectively complement the genetic resistance of rice hybrids to manage the most dangerous pest, BPH. The resistant hybrids also provide a convenient method of pest management to the farmers because the pest control solution lies within the seeds and the farmers easily get rid of the inconvenience of applying insecticides after raising the healthy crop.

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