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Studies on inheritance and allelic relationship of Gene (S) governing resistance to brown planthopper (*Nilaparvata Lugens* Stal.) in some donors of rice

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Abstract
Mode of inheritance and allelic relationships of genes conforming resistance to brown plant hopper (*NILAPARVATA LUGENS* STAL.) was investigated in three rice (*Oryza sativa* L.) genotypes viz. R1723-1413-3-357-1, R1519-773-3-581-1 and R1243-1224-578-1. Eight-ten days old seedlings were infested with first- and second-instar nymphs of brown planthopper and seedling injury was recorded 7 to 10 days after infestation. Inheritance studies revealed presence of a single dominant gene for resistance in R1723-1413-3-357-1 and R1243-1224-578-1. Allelic tests revealed that gene for brown plant hopper resistance present in R1519-773-3-581-1 was non-allelic to the genes *bph*5 (ARC10550), *bph7* (T12), *Bph1* (MTU15) and B*ph6* (Swarnalata). The cross of resistant donor R1723-1413-3-357-1 with MTU15 showed no segregation for BPH resistance in the F1 and F3 generations, indicating that the genes conferring resistance are common. The dominant gene control of BPH resistance of resistant donors can be used for easy incorporation of BPH resistance into susceptible high yielding rice varieties.

Keywords: Brown planthopper, Nilaparvata lugens Stal., Genetics, Insect resistance, allelic relationships, *Oryza sativa* L.

Introduction
Rice (*Oryza sativa* L.) is a primary source of nutrition for over half of the world's population (Nagadhara *et al*., 2003) [23]. The widespread damage caused by insect pests is one of the main biotic constraints on rice production. More than 100 species of insects are pests of rice but only about 20 of them are of major economic significance (Pathak and Khush, 1979) [26]. Out of these, a few are widely distributed with great potential to create havoc to the paddy growers and among them brown plant hopper, stem borer, gall midge, leaf folder and rice caseworm are of major occurance. Brown planthopper (BPH) is one of the most destructive rice pests, causing considerable losses to this important crop worldwide (Jena and Mackill, 2008; Krishnaiah and Varma, 2011) [12, 18]. The phloem sucking feeding activity of this insect causes considerable physiological damage to the rice plants by removing nutrients and disrupting physiological processes, and consequently affects the growth and development of the plant (Sogawa 1982; Watanabe and Kitagawa 2000) [30, 32]. Moreover, feeding by large numbers of BPH results in drying of the leaves and wilting of the tillers, which is referred to as a hopper-burn condition. BPH also causes indirect damage by transmitting several viral diseases such as rice grassy stunt (Alam and Cohen 1998) [1] and rugged stunt (Athwal *et al*., 1971) [3].
The interaction between rice and brown plant hopper should prove an excellent model system for studying the genetic basis of plant defence against phloem-feeding insects. To develop a sustainable pest management system, it is important to find out the right balance between breeding and management strategies to reduce the ecological fitness of brown plant hopper and

to keep the pest under economic threshold levels (Bosque-Perez and Buddenhagen, 1992) [4]. Brown plant hopper has assumed importance in recent times because of emergence of new biotypes. It was eventually determined that, the principal cause of the outbreaks was over-use of broad-spectrum insecticides, which disrupt biological control of BPH by predators and parasitoids (Kenmore *et al.*, 1984) [15] and at sublethal doses, can actually stimulate BPH reproduction (Heinrichs and Mochida, 1984) [6]. To solve the problem, naturally evolved resistance systems would provide a promising and readily acceptable means of control. Utilization of host plant resistance has been recognized as one of the most economic and effective measures for BPH management. Many donors of BPH resistance have been identified and genetics of resistance have been investigated. Knowledge on the genetics of BPH resistance is useful for breeders to decide the breeding strategies to be adopted to reduce the damage caused by BPH and increase rice productivity. Consequently, breeding for rice cultivars with insect resistance is a priority in crop improvement programs. Resistant rice varieties often interact additively or synergistically with biological control and can also provide important "insurance" against BPH outbreaks caused by factors outside of farmer's control, such as unusual weather patterns or insecticide over-use in neighboring fields. The logical approach to BPH control would be to use host-plant resistance as part of an integrated pest-management program. Cultivated as well as wild species of rice are known to carry resistant genes against brown plant hopper which can be transferred into cultivated species. In order to efficiently come across the threat of emergence of new biotypes, it is essential to identify as many non-allelic sources of resistance as may be possible and use them under various combinations as gene pyramids whenever necessary. So, considering the importance of resistant varieties and of diverse resistant sources for controlling BPH, the present study was undertaken to investigate the inheritance of resistance and allellic relationships of gene (s) governing resistance to this pest in some donors.

Materials and Methods

The experimental material comprised of three resistant donors viz. R1723-1413-3-357-1, R1519-773-3-581-1 and R1243-1224-578-1, their F_1, F_2 and F_3, populations of crosses made between resistant donors (whose sources of resistance were unknown) with known resistant parents and resistant donors with susceptible parents. The known resistant donors were ARC10550, T12, MTU15, and Swarnalata. Susceptible parents used in the investigation were Danteshwari and Mahamaya. The experiment was carried out in the Department of Genetics and Plant Breeding, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh during Kharif 2009-2011. Crosses were made and F_1, F_2 and F_3 populations generated were tested along with the parents for their reaction against BPH under greenhouse condition as per methodology suggested by Kalode *et al*. (1979) [14] in consequent summer and Kharif seasons. The rice genotypes and their reactions to the brown planthopper are listed in Table 1.

Table 1: Reaction of donor or parental genotypes to brown planthopper under greenhouse conditions

S. No.	Parents	Parentage	Plant damage score during Kharif 2009	Reaction to BPH
1	R1723-1413-3-357-1	Nidhee/IR36		R
2	R1519-773-3-581-1	BR240-47/Charder		R
3	R1243-1224-578-1	Mahamaya/MTC-4		R
4	Swarnalata	N/A		HR
5	ARC10550	N/A		R
6	T12	N/A		R
7	MTU15	N/A		R
8	Danteshwari	Samridhi/IR8608-298		S
9	Mahamaya	Asha/Kranti		S

a: HR – Highly Resistant; R - Resistant; S - Susceptible; N/A - Not available

To get the steady supply of insect for various studies, the brown plant hopper, initially, collected from field and its culture being maintained throughout the year in the air-cooled glass house, Department of Entomology, College of Agriculture, IGKV, Raipur at 30 ^{0}C $\pm$ 5 ^{0}C on the potted TN1 variety. BPH were reared on 40 to 45 days old potted TN1 plant inside a rearing cage of 75 x 75 x 75 cm size consisting of wooden furniture frame with small window on front side and fine wire mesh on top and other sides. Cages were mounted on cemented platform having a water level of 7.5 cm. Female BPH insects were placed inside rearing cages for egg laying on the susceptible variety TN1. Twenty-five adults were released on a single pot. After 2 to 3 days, the females start egg laying inside the leaf sheath of paddy plants. For transferring the adult females an aspirator was used which works on the principle of suction of air. Nymphs emerged within 5 to 8 days from the eggs. Later on, after emergence of nymphs from plants, released BPH pairs were transferred to another TN1 pot with the help of aspirator for egg laying. Second instar nymphs were collected and used to infest the test materials for genotype screening.

The F_1, F_2 and F_3 population of the crosses were generated and screened against the brown plant hopper population for inheritance studies, while only F_1 and F_2 populations of the crosses were screened against the brown plant hopper population in allelic studies for classification of the plants/progenies to fit the appropriate genetic ratios. Screening of rice genotypes were conducted, under controlled conditions of glass house, as per methodology suggested by Kalode *et al*. (1979) [14]. The test material was pre-germinated in petridishes and these germinated seeds were sown in rows in wooden boxes of size 60 x 40 x 10 cm, containing well mixed homogeneous soil. Each seed box contained twenty four test lines with 20 seedlings of each including two middle rows of resistant check (Ptb33) and susceptible check (TN1) and four border rows of susceptible check (TN1). Eight to ten days old seedlings were infested with 1st and 2nd instar nymphs of brown planthopper. The seedlings were infested uniformly by distribution of 6 to 8 nymphs per seedling throughout the seed box. Observations on the plant reaction were recorded on single plant basis 7-10 days after releasing insects, when about 95 per cent of the susceptible check i.e.,

TN1 in seed box was damaged. At this stage resistant seedlings exhibited little or no feeding damage. The scoring was done as per 0-9 scale (0=no visible damage, 1= First leaf is yellow, 3= One or two leaves are yellow or one leaf shrank, 5= One or two leaves shrank or one leaf shriveled, 7=3-4 leaves shrank or 2-4 leaves shrivel and the plant is still alive, 9= The plant is dead) of Standard Evaluation System (SES) for rice (IRRI, 1996). Fixed genotypes showing score of 0 were rated as highly resistant (HR), 1 as resistant (R), 3 as moderately resistant (MR), 5 as moderately susceptible (MS), 7 as susceptible (S) and 9 as highly susceptible (HS), (IRRI, 1996). For the genetic studies plants with score 0-3 (0, 1, 3) were pooled as resistant and those with scores 5-9 (5, 7, 9) were pooled as susceptible. In F_1 generation, all the individual plants were scored for BPH damage whereas, in F_2 population of different crosses, score for BPH damage was recorded on 500 plants. Each F_2 seedling was classified as resistant or susceptible. The F_3 lines were classified as either homozygous resistant, segregating or homozygous susceptible. The X^2 test was applied to test the goodness of fit of genetic ratios (Panse and Sukhatme, 2000) [25].

Results

The resistant donors R1723-1413-3-357-1 and R1243-1224-578-1 were studied for inheritance of brown plant hopper resistance. The reaction of F_1, F_2 and F_3 progenies from the crosses of susceptible parents and resistant donors are given in Table 2. The F_1 populations of the crosses of R1723-1413-3-357-1 and R1243-1224-578-1 with Danteshwari and Mahamaya showed resistant reaction against the brown plant hopper population, indicating that dominant genes govern their resistance. The F_2 populations of the crosses R1723-1413-3-357-1 and R1243-1224-578-1 with their susceptible parents was observed in a frequency of three resistant: one susceptible (3R: 1S) and indicating the presence of single dominant gene in these resistant donors. The χ^2 value for the 3:1 ratio in these cross combinations varied from 0.467 to 2.02 (Table 2). Further, the F_3 progenies of these crosses for each resistant parent were also analyzed for segregation pattern. All of these F_3 lines could be classified as homozygous resistant, segregating and homozygous susceptible. The segregation gave a good fit to a ratio of 1 resistant: 2 segregating: 1 susceptible in all crosses, thus confirming monogenic control of resistance in the donors.

The allelic relationship studies enable breeders to know if the resistance imparting gene present in two different resistant parents is the same or different. Identification of non allelic source of resistance against any insect pest is necessary for increasing the durability of resistance. Test for allelic relationship is made by crossing resistant cultivars among themselves. Allelic relationship studies of gene for brown plant hopper resistance are presented in Table 3. To study the allelic relationship regarding BPH resistance genes, the unknown resistant donors R1723-1413-3-357-1 and R1519-773-3-581-1 were crossed with resistant parents viz, ARC10550 (*bph5*), T12 (*bph7*), MTU15 (*Bph1*) and Swarnalata (*Bph6)* having known genes for resistance for brown plant hopper. Data recorded on BPH reaction revealed that, the F_1 of these crosses showed resistant reaction against brown plant hopper. The segregation behavior of the F_2 populations of the crosses of resistant donors R1723-1413-3-357-1 and R1519-773-3-581-1 with resistant parents ARC10550 and T12 showed a ratio of 13 resistant: 3 susceptible plants. The F_2 populations of the crosses of resistant donors R1723-1413-3-357-1 and R1519-773-3-581-1 with resistant parent Swarnalata and also R1519-773-3-581-1 with resistant parent MTU15 were classified in 15 resistant: 1 susceptible segregation ratio. Such ratios are obtained when two independently dominant genes are involved for resistance. The resistance to rice brown plant hopper involving parent R1723-1413-3-357-1 found to be possess one independent dominant gene and did not segregate in F_2 population of its crosses with parent MTU15 having only one resistant gene (*Bph1*). It indicated the presence of same genetic constitution of both the parents (R1723-1413-3-357-1 and MTU15) for resistance.

Table 2: Inheritance pattern of F_1, F_2 and F_3 populations of crosses resistant parents with susceptible parents in rice for BPH resistance

S. No.		Reaction of F_1 plants	Reaction of F_2 plants						Reaction of F_3 progenies						
			No. of plants			Expected ratio	χ^2 value	Table value	No. of progenies				Expected ratio	χ^2 value	Table value
			R	S	Total	R : S			R	Sg	S	Total	R:Sg:S		
1.	R1723-1413-3-357-1 x Danteshwari	R	407	156	563	3:1	2.202	3.841*-6.635**	29	77	35	141	1:2:1	1.699	5.991*-9.210**
2.	R1723-1413-3-357-1 x Mahamaya	R	389	118	507	3:1	0.805	3.841*-6.635**	35	68	26	129	1:2:1	1.635	5.991*-9.210**
3.	R1243-1224-578-1 x Danteshwari	R	369	132	501	3:1	0.467	3.841*-6.635**	33	78	28	139	1:2:1	2.420	5.991*-9.210**
4.	R1243-1224-578-1 x Mahamaya	R	381	139	520	3:1	0.831	3.841*-6.635**	35	68	31	134	1:2:1	0.201	5.991*-9.210**

Note: R - Resistance, S - Susceptible, Sg - Segregating
**1% level of significance *5% level of significance

Table 3: Segregation pattern of F_1 and F_2 populations using resistant donors for allelic studies in rice for BPH resistance

S. No.	Cross combination	Reaction of F_1 plants	Reaction of F_2 plants					
			No. of plants			Expected ratio	χ^2 value	Table value
			R	S	Total	R : S		
1.	R1723-1413-3-357-1 x ARC10550	R	398	86	484	13:3	0.3059	3.841*-6.635**
2.	R1723-1413-3-357-1 x T12	R	426	106	532	13:3	0.4819	3.841*-6.635**
3.	R1723-1413-3-357-1 x MTU15	R	457	-	457	-	-	3.841*-6.635**
4.	R1723-1413-3-357-1 x Swarnalata	R	456	37	493	15:1	1.293	3.841*-6.635**
5.	R1519-773-3-581-1 x ARC10550	R	468	93	561	13:3	1.719	3.841*-6.635**
6.	R1519-773-3-581-1 x T12	R	415	84	499	13:3	1.192	3.841*-6.635**
7.	R1519-773-3-581-1 x MTU15	R	435	26	461	15:1	0.291	3.841*-6.635**
8.	R1519-773-3-581-1 x Swarnalata	R	552	38	590	15:1	0.041	3.841*-6.635**

** 1% level of significance * 5% level of significance

Discussion

The intention of inheritance studies is to know the mode of inheritance of the trait. This includes finding out number of genes responsible for the trait, their nature of interaction (dominant or recessive) among the genes involved and their expression. The F_{1s} involving cross combination of resistant donors R1723-1413-3-357-1 and R1243-1224-578-1 with susceptible parents were found resistant, indicating involvement of dominant genes for resistance in donors R1723-1413-3-357-1 and R1243-1224-578-1. The 3R: IS ratio of F_2 population of these crosses suggested that the resistance to brown plant hopper in R1723-1413-3-357-1 and R1243-1224-578-1 is controlled by a single dominant gene. This is also supported by the classification of F_3 progenies in the ratio of 1 resistant: 2 segregating: 1 susceptible. Resistance to brown plant hopper governed by a single dominant gene was also reported by Pongprasert and Weerapat, 1979; Nemoto *et al.*, 1989; Soni *et al.*, 2005 and Rana *et al.*, 2009 [27, 24, 31, 28]

Control of a trait by a dominant gene is considered to be an advantage to the breeder as it makes identification of the resistant plants easier, which is also expressed, in heterozygous condition. The incorporation and selection of single dominant genes are easier in breeding population. The simple inheritance nature of brown plant hopper resistance against the prevailing biotype thus indicates that an effective breeding programme can be undertaken to breed brown plant hopper resistant varieties. Athwal *et al.* 1971 [3] identified the dominant gene *Bph1* in donor MTU 15 and recessive resistant gene *bph2* in donor H 105 governing resistance to brown plant hopper. Later on, the existence of *Bph1* gene was confirmed by Fernando *et al.* (1971) [5] in Ptb33; Martinez and Khush (1974) [22] in TKM-6, Ikeda and Kaneda (1982) [9] in Andragahwewa and the existence of *bph2* gene also repoted by Martinez and Khush (1974) [22] in IR1154-243; Ikeda and Kaneda (1982) [9] in Ptb34. The dominant resistant gene *Bph6* in Swarnalata and recessive resistant gene *bph5* in ARC10550 and *bph7* in T12 was identified for brown plant hopper resistance by Kabir and Khush, 1988 [13].

The allelic study exploits the possibility of identifying new gene(s) for resistance against any insect pest is valuable to the breeders especially it is necessary for increasing the durability of resistance and to overcome the threat possessed by emergence of new biotypes of the insect. Cross between donor R1723-1413-3-357-1 and MTU15 (*Bph1*) did not segregate in F_2 generation for brown plant hopper resistance, therefore genetic constitution of both the parents are the same (*Bph1*) for resistance. The segregation behavior of the F_2 populations of the crosses between R1723-1413-3-357-1 with other resistant parents ARC10550, T12 and Swarnalata have clearly showed that the resistance imparting gene present in R1723-1413-3-357-1 are different from the parents ARC10550, T12 and Swarnalata. Martinez and Khush (1974) [22] also reported that IR747 B_2-6 had dominant gene for resistance was allelic to *Bph1*. Lin (1981) reported resistance gene in Taichung Senyu 223 was allelic to *Bph1*. Further, Ikeda and Kaneda (1982) [9] reported that, resistance of Andragahwewa to be controlled by *Bph1*.

The F_2 populations of the crosses between resistant parent R1519-773-3-581-1 with MTU15 (*Bph1*) and Swarnalata (*Bph6*) segregated in a ratio of 15 resistant: 1 susceptible demonstrating that two independently dominant gene were involved in each of these crosses. The gene present in R1519-773-3-581-1 was inherited independently and non-allelic to the gene present in MTU15 (*Bph1*) and Swarnalata (*Bph6*). Reddy and Pasalu (2004) [29] also reported the duplicate dominant gene governing resistance to brown plant hopper in crosses of Manohar Sali with Rathu Heenati and Velluthacheera with Rathu Heenati. The F_2 population from the crosses of R1519-773-3-581-1 with ARC10550 and T12 segregated in a ratio of 13 resistant: 3 susceptible indicating that resistance is governed by two genes i.e. one dominant gene present in R1519-773-3-581-1 and a recessive gene present in ARC10550 (*bph5*) and T12 (*bph7*). Thus, it may be concluded that resistance carried by R1519-773-3-581-1 is due to a dominant gene, which is non-allelic to ARC10550 (*bph5*) and T12 (*bph7*). Lakshminarayana and Khush (1977) [19] in donors Rathu Heenati and Babawee; Ikeda and Kaneda (1985) [9] in Andragahwewa and Ptb34; Marotirao (2002) [21] in Suraksha and Nagarjun also reported the similar results. The allelic information helps in identifying donors to be selected in breeding programmes so that, the breeders may not incorporate the same resistant gene in improved breeding lines although they may be using two different donors. The sources of resistance to brown plant hopper as identified in the present study would prove to be promising to combat the brown plant hopper menace and to obtain stable resistance.

Conclusion

The present studies have resulted in the identification of a new gene in rice for resistance to rice brown planthopper. A new duplicate dominant gene non allelic to *bph5* (ARC10550), *bph7* (T12), *Bph1* (MTU15) and Bp*h6* (Swarnalata) appears to be present in R1519-773-3-581-1, which needs conformation by a study of F3 progenies. Similarly, R1243-1224-578-1 has dominant gene for resistance to brown planthopper of which allelic relationship is to be ascertained. The dominant resistant gene of R1723-1413-3-357-1 is allelic to the gene carried by MTU15 (*Bph1*).

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