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Screening and Inheritance Study of F_1, F_2 and F_3 Population for Brown Planthopper Resistant in Rice (*Oryza sativa* L.)

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A B S T R A C T

Observations on parent lines, F_2 and F_3 lines were recorded when the susceptible check, TN1 shown complete susceptibility to BPH. Scoring for BPH reaction was done following the guidelines of Standard Evaluation System for Rice (IRRI, 1998). The male parent IR64 showed resistance to BPH with score 1.33 where as the female parent CG Zn Rice I showed susceptibility with a score of 9.0, the female parent Muskan showed susceptibility with a score of 6.7 and another female IET22290 showed susceptibility with a score of 7.0 under glasshouse conditions. The resistant check, PTB33 showed complete resistance with a score of 0, and susceptible check, TN1 exhibited complete susceptibility with 9 as score. Genetics of BPH resistance in F_2 and F_3 population derived from CG Zn Rice I x IR64 for BPH resistance show Mendelian segregation. They show 3:1 and 1:2:1 segregation ratio in F_2 and F_3 respectively possess only single dominant gene for resistance which is indicated by 3:1 (3 resistant: 1 susceptible) segregation observed in F_2 generation. This is also supported by F_1 showing resistance and classification of F_3 progenies in the ratio of 1: 2: 1 (1 breeding true for resistance: 2 segregating for resistance and susceptibility: 1 breeding true for susceptibility).This confirmed the inheritance of a single dominant gene present in these resistant parent IR64.

Introduction

Rice (*Oryza sativa* L.) is the food source for billions of people in the world (Normile, 2008), which rely on this crop for more than 20% of daily calorie intake (IRRI, Africa Rice and CIAT, 2010). To guarantee global food security for continuing population expansion it is crucial to control the different insect pests that harm rice crop (Normile, 2008) leading to influential and unpredictable decrease of yield (Jairin *et al.*, 2007). The Brown planthopper (BPH), *Nilaparvata lugens* Stâl, is one of the most important devastating insects in Asia where rice is widely produced (Hu *et al.*, 2014). The BPH obtains the nutrients from the phloem sap of rice plant through its stylet mouth parts (Huang *et al.*, 2001). So the heavy infestation of BPH causes complete drying of plants to the field known as "hopperburn", whereas the light infestation reduces growth vigor, plant weight and number of productive tillers (Sogawa, 1982). Popular varieties are almost susceptible to BPH and control methods are dependent on insecticides, which is expensive in terms of demanding more labor, money and unfavorable environmental effects (Tanaka, 2000; Heinrichs *et al.*, 1982). Several sprayings upset natural balance between the BPH and its natural enemies enhancing, in the other side, its resistance to insecticides, which lead to BPH resurgence (Heinrichs and Mochida,1984).To grow genetically rice resistant variety is seen as the most economical and affective method for controlling the BPH.

IR64 is a semi dwarf indica rice variety, with average mature plant height of approximately 100 cm in the Philippines. It is a relatively early duration variety, with total growth duration of about 117 days (Khush and Virk, 2005). It inherits the same semidwarf*sd1* allele as other IRRI semi dwarf varieties, ultimately derived from Dee-geo-woo-gen.

According to Wei *et al.*, (2016) it has the loss of function alleles for Hd1 and *Ehd1*, which confer earlier duration and insensitivity to photoperiod. At the time of its release, IRRI (1986) listed the valuable traits as resistance to brown planthopper (BPH) biotypes 1 and 3. IR64 has relatively durable resistance to BPH, and it is known to carry the major gene *Bph1*. However, it is reported to have better resistance than other varieties carrying *Bph1* and has good field resistance to the pest, exhibiting antiobiosis, antixenosis and tolerance (Cohen *et al.*, 1997). This is partly attributed to its possessing additional QTLs controlling BPH resistance which confer greater durability of the resistance (Alam and Cohen, 1998). It is also relatively sensitive to Zn deficiency (Impa *et al.*, 2013). The excellent grain quality of IR64 has become the standard for rice quality requirements in a number of countries. Because of its popularity with farmers, IR64 has been used widely as a parent in rice breeding, as a recipient of new genes through marker-assisted backcrossing and genetic transformation and as a standard check for basic studies by many rice researchers.

Materials and Methods

Identification and monitoring of functional resistance genes over the years

Three different crosses are made between 1. CG Zn Rice I x IR64, 2. Muskanx IR64 and 3.IET2290 x IR64.Total 11 crosses were made *viz.*, 5 crosses from CG Zn Rice I x IR64, 3 crosses from Muskan x IR64 and 3 crosses IET2290 x IR64. 2105 plants taken from F_1 population and advanced to F_2 population. A set of 2105progenieswere selected in F_3 generation.

The crosses were made between CG Zn Rice I and IR64 where CG Zn Rice I is as female parent and IR64 is used as a male parent. IR64 shows the resistant character in Chhattisgarh region while CG Zn Rice I shows susceptibility. Susceptible checks *viz.*, TN1, CG Zn Rice I, IET22290 and Muskan along with two resistant checks *viz.*, PTB33 and IR64 were screened against brown plant hopper population in glass house condition of the Department of Entomology, College of Agriculture, Raipur during *Kharif* 2018, 2019 and 2020. Variation in the reactions of these populations over the years was expected to give an insight to the stability of resistance possessed.

Inheritance studies

All the F_1 seeds of the crosses made during *Kharif* 2017 were used for advancing the generation from F_1 to F_2. All the F_2 seeds of each cross obtained from individual F_1 plants were grown in *Kharif*2018 for advancing the generation. The number of progenies tested for each cross is given in Table.1. Out of 2105 plants of F_2 generation, 2105 plants F_3panicles were harvested in two sets for future study purpose. Seeds from single panicle were used in glasshouse for screening purpose in two seasons (2019 and 2020) and same panicle seeds were used in field for sowing purpose to know the morphological characters as well as for molecular study in the laboratory. The F_2 and F_3 seeds were screened against the brown plant hopper during Summer 2019 and 2020respectively and genetic ratio was worked out on F_2 and F_3 data. The 2105 plants of F_2 and 2105 plants of F_3 generation were screened in glass house is given in Table 1. In F_3generation each plant was screened against BPH and confirmation of the genetic ratio obtained in F_2 was ascertained. For morphological and molecular purpose CG Zn Rice I x IR64 cross plants were studied.

Screening procedure

Insect rearing

In the study standard seed box technique was used as described by IRRI (Pathak and Khush, 1977) to rear the BPH. The source insects were collected from the field and continuously reared in greenhouse for screening purpose that infested cultivated variety of rice in the field in Raipur (CG). The insects were reared on 40 to 50 day sold rice plants (susceptible variety TN1) inside a 0.5 × 0.5 × 1.0 m cage. This cage consisted of a steel frame covered with a fine mesh wire screen. The cage bottom was open and setting in water. Potted plants were changed as needed. Each cage could accommodate several potted plants that could support 2,000 to 3,000 late-instar BPH nymphs. The original colony per cage was started by 30–40 gravid adults. Eggs of about the same day age were obtained by placing the plants in a cage with gravid adults for two days. Screening for resistance to the BPH was conducted at the seedling stage in the greenhouse. The screening procedures standardized at IRRI and described by Heinrichs *et al.,* (1985) were adopted in this study. A row of the susceptible check variety (TN1) and a resistant check variety (PTB33) was planted in a proper sequence in the seed boxes. At the sixth day after seeding, plants were thinned to 20 to 30 seedlings per row. The seed boxes were placed upon water inside a screened room in the glasshouse. To provide suitable humidity for insect survival and avoid the disturbance of watering on the tested insects, we maintained a depth of about 5 cm standing water in the tray. Screening of rice lines were conducted, under controlled conditions of glass house, as per methodology suggested by Kalode and Krishna (1979). The test and check varieties were pre germinated in petri dishes and these germinated seeds were transferred to wooden boxes of size 60 x 40 x 10 cm, containing well mixed homogeneous soil. Each seed box contained 24 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). The boxes were covered so as to enhance seedling growth. After sowing the seed boxes were placed on cemented platform with 6-8 cm border and 3-4 inches water level to provide adequate humidity for the insects and protection against ants.

The seedlings were infested at the one to two leaf stage (about 7 days after seeding) by uniformly scattering a large number of 2^{nd} to 3^{rd} instar BPH nymphs on them. The seed boxes were covered with nylon nets after infestation. An average of 5–7 insects per seedling constituted an optimum population to differentiate the resistant level of tested lines. The damage rating was taken when about 90% of the plants of the susceptible check variety were killed, usually about 5 to 7 days after infestation. The varieties were rated using the standard evaluation system for rice (IRRI 1988). We first conducted an initial evaluation of 2105 F_2plants. The 2105 plants whose resistance fell into grade 0 to 5 as well as 5 to 9 were selected for further evaluation, using the same technique. All of the screening was conducted in Raipur (CG) during the period 2019 and 2020 Summer season.

Recording of observations

During the process of slowly moving the potted plants over the boxes, the dropped nymphs were visually estimated to drop approximately 8–10 nymphs onto each seedling. Thereafter, the boxes were returned to the cages individually. Observations were recorded 7-10 days after releasing insects, when 90% of the plants in the susceptible check line TN1 were killed. The entries were scored for damage following the criteria for scoring the damage of individual plants. When the TN1 seedlings in a box had become completely wilted due to plant hopper feeding, the tests were terminated and the damage to all seedlings in a box was scored according to Horgan *et al.,* (2015) (Table.2), where higher scores indicated greater susceptibility to BPH.

Analysis and interpretation of results

Plants showing score of 0 were rated as immune, 1 as resistant (HR), 3 as resistant (R), 5 as moderately resistant (MR), 7 as susceptible (MS) and 9 as highly susceptible (S), (IRRI, 1996). In F_1 and F_2, plants were individually scored. The F_3 progenies were classified as breeding true for resistance (all plants in the line being resistant), segregating (both resistant and susceptible occurring) or breeding true for susceptibility (all plants in the line being susceptible). The reaction of F_1 indicated the dominance or recessive nature of the resistance gene(s) involved in resistant parent IR 64.

Results and Discussion

Phenotyping of Parents along with advanced population F_2 and F_3

In this study, 2105 F_2 plants along with parents and two checks *i.e.,*TN1 (susceptible) and PTB33 (resistant) were evaluated for BPH reaction under glasshouse condition at IGKV, Raipur during 2018-2019. F_2population of three crosses were made. Cross first that was made between CG Zn Rice I x IR64 had 753 plants, Cross second that was made between Muskan x IR64 had 563 plants and cross third that was made between IET22290 x IR64 had 789 plants. From 2105 F_2plants, two panicles were harvested and kept in two individual packets. The seeds of individual panicles were used for the screening and molecular purpose. So the 2105 plants along with parents (CG Zn Rice I and IR64) and two checks *i.e.,* TN1 (susceptible) and PTB33 (resistant) were evaluated for BPH reaction under glasshouse condition at IGKV, Raipur during 2019-2020. For phenotypic screening all 2105 lines of F_3 populations of three crosses were used to screen against know the BPH resistance.

Scoring of BPH resistance

Observations on parent lines, F_2 and F_3 lines were recorded when the susceptible check, TN1 shown complete susceptibility to BPH. Scoring for BPH reaction was done following the guidelines of Standard Evaluation System for Rice (IRRI, 1998). The male parent IR64 showed resistance to BPH with score 1.33 whereas the female parent CG Zn Rice I showed susceptibility with a score of 9.0, the female parent Muskan showed susceptibility with a score of 6.7 and another female IET22290 showed susceptibility with a score of 7.0 under glasshouse conditions. The resistant check, PTB33 showed complete resistance with a score of 0, and susceptible check TN1 exhibited complete susceptibility with 9 as score.

Classification of the 2105 F_2 individuals of cross I (CG Zn Rice I x IR64), based on BPH reaction indicated that 552 fell into the resistant class and 201 plants were in the susceptible class (Fig. 1). Classification of the 2105 F_2 individuals of cross II (Muskan x IR64), based on BPH reaction indicated that 414 fell into the resistant class and 149 plants were in the susceptible class (Fig. 2). Classification of the 2105 F_2 individuals of cross III (IET22290 x IR64), based on BPH reaction indicated that 602 fell into the resistant class and 187 plants were in the susceptible class (Fig. 3). Classification of the 2105 F_3 individuals of cross I (CG Zn Rice I x IR64), based on BPH reaction indicated that 289 fell into the resistant class, 370 fell in to segregating and 94 plants were in the susceptible class (Fig. 4). Classification of the 2105 F_3 individuals of cross II (Muskan x IR64), based on BPH reaction indicated that 225 fell into the resistant class, 256 fell in to segregating and 82 plants were in the susceptible class (Fig. 5). Classification of the 2105 F_3 individuals of cross III (IET22290 x IR64), based on BPH reaction indicated that 230 fell into the resistant class, 367 fell in to segregating and 192 plants were in the susceptible class (Fig. 6). Several studies reported the presence of strong quantitative resistance and involvement of polygenes for BPH resistance in rice (Soundararajan *et al.*, 2004). All the observations suggest that BPH resistance in this population was qualitative and involve the polygenes.

Genetical studies

Three crosses were attempted to analyze the inheritance study of the genes involved in the resistant parents. The F_2 and F_3 population of the crosses were generated and screened against the brown plant hopper population for inheritance studies, for classification of the plants/progenies to fit the appropriate genetic ratios.

Inheritance studies

Inheritance studies of BPH resistance was studied on variety IR64 by carrying it with susceptible F_3 plants of CG Zn Rice I, Muskan and IET22290. The donor IR64 was crossed with three susceptible varieties *i.e.* CG Zn Rice I, Muskan and IET22290. Reaction of F_1, F_2 and F_3 population of above generated crosses are presented in Table.3.

Table.1 List of crosses made, $F_1$and F_2 plants harvested and populations of F_2 and F_3 screened in glass house for inheritance studies

S.No.	Cross combinations	Screening in glass house		
		F_1 Plants harvested	No. of F_2 Plants	No. of F_3 progenies
1.	CG Zn Rice I x IR64	5	753	753
2.	Muskan x IR64	3	563	563
3.	IET 22290 x IR64	3	789	789
	Total	11	2105	2105

Table.2 Evaluation standard for rice resistance to plant hoppers based on seedling mortality (adapted from Horgan *et al.*, 2015)

Score	Rice damage	Resistance level
0	No damage	Immune
1	Slight damage to a few plants within a row	Highly resistant
3	First and second leaves of each plant partially yellowing	Resistant
5	Pronounced yellowing or stunting of plants, or 10–25% of plants wilted within a row	Moderately resistant
7	More than 50% of plants wilted or dead and the remaining plants severely stunted or dying	Moderately susceptible
9	All plants wilted or dead	Susceptible

Table.3 Distribution of BPH resistance among the F_2 and F_3 plants (Including cross I, II and III)

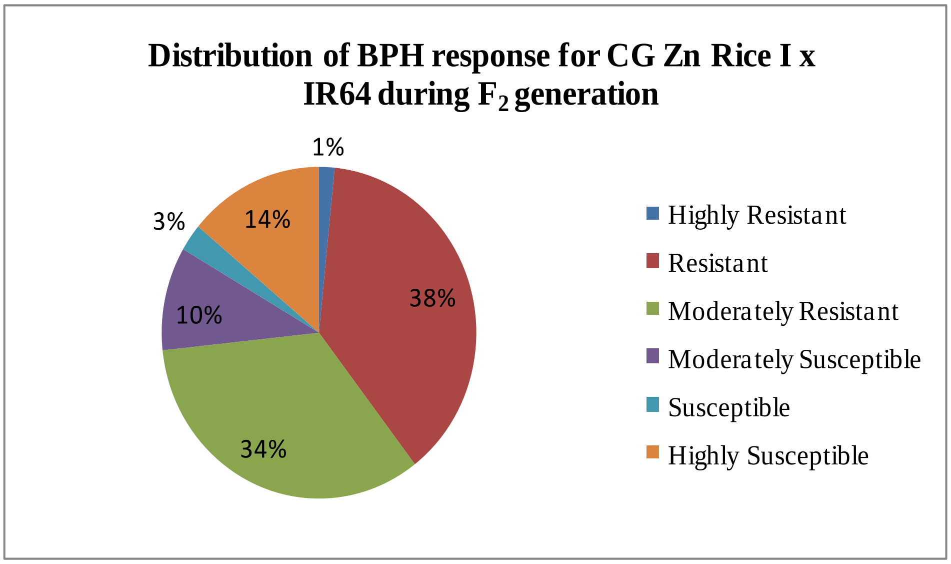
Phenotypic class (Score)	CG Zn Rice I x IR64		Muskan x IR64		IET22290 x IR64	
	No. of F_2 plants	No. of F_3 plants	No. of F_2 plants	No. of F_3 plants	No. of F_2 plants	No. of F_3 plants
Highly Resistant (1)	12	6	5	12	15	10
Resistant (1-3)	286	283	211	213	351	220
Moderately Resistant (>3-5)	254	270	160	186	236	339
Moderately Susceptible (>5-7)	76	100	67	70	68	20
Susceptible (7-9)	20	57	53	27	43	87
Highly Susceptible (9)	105	37	67	55	76	105
Total	753	753	563	563	789	789

Table.4 Inheritance pattern of F_1, F_2 and F_3populations of crosses resistant parents with susceptible parents in rice for BPH resistance

S.No.	Cross Name	Reaction of F_1 plants	Reaction of F_2 plants						Reaction of F_3 Progenies						
			No. of Plants			Expected Ratio	Chi Sq. value	Table value	No. of Progenies				Expected Ratio	Chi Sq. value	Table value
			R	S	Total	R:S			R	Sg	S	Total	R:Sg:S		
1	CG Zn Rice I x IR64	R	552	201	753	3:1	1.197	3.841*-6.635**	289	370	94	753	1:2:1	0.2568	5.991*-9.210**
2	Muskan x IR64	R	414	149	563	3:1	0.6055	3.841*-6.635**	225	256	82	563	1:2:1	1.1448	5.991*-9.210**
3	IET22290 x IR64	R	602	187	789	3:1	0.6765	3.841*-6.635**	230	367	192	789	1:2:1	1.5519	5.991*-9.210**

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

Fig.1 Distribution pattern of BPH response for cross of CG Zn Rice I x IR64 during F_2 generation



*BPH reaction score as per SES, IRRI, 1996

Fig.2 Distribution pattern of BPH response for cross of Muskan x IR64 during F_2 generation

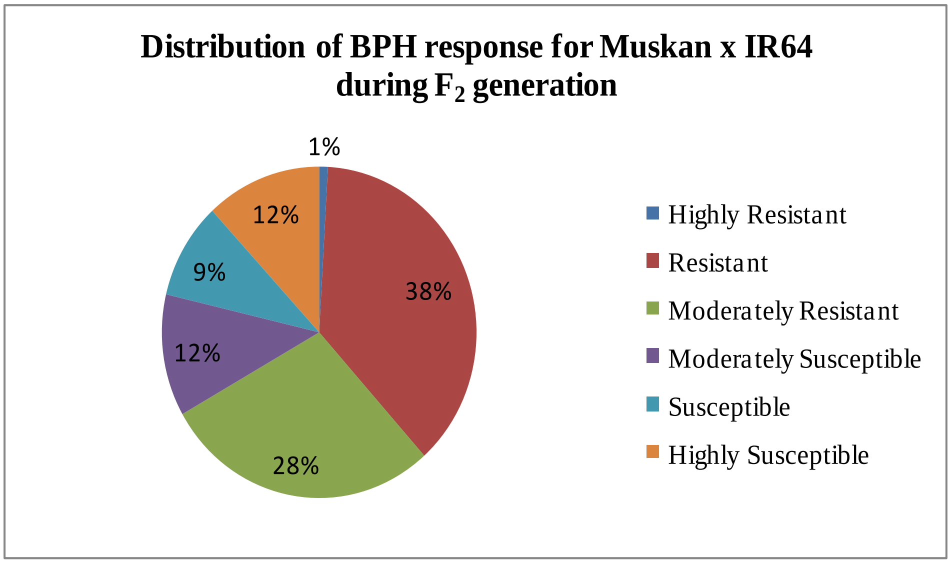


Fig.3 Distribution pattern of BPH response for cross of IET22290 x IR64 during F_2 generation

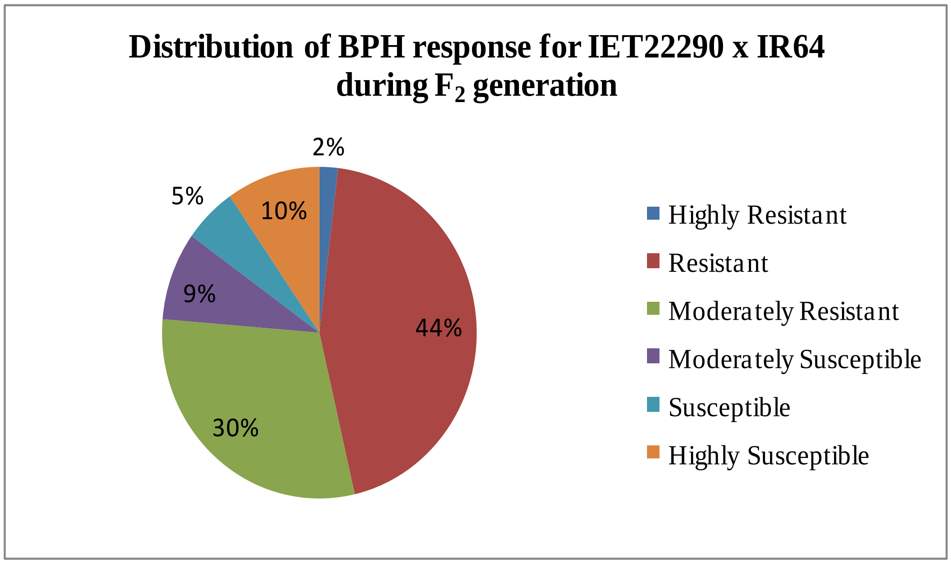


Fig.4 Distribution pattern of BPH response for cross of CG Zn Rice I x IR64 during F_3 generation

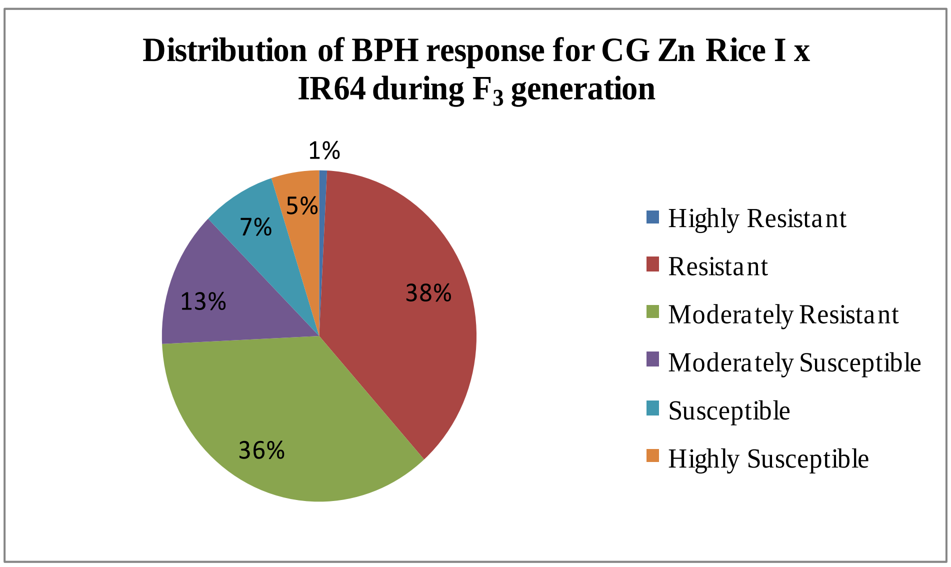


Fig.5 Distribution pattern of BPH response for cross of Muskan x IR64 during F_3 generation

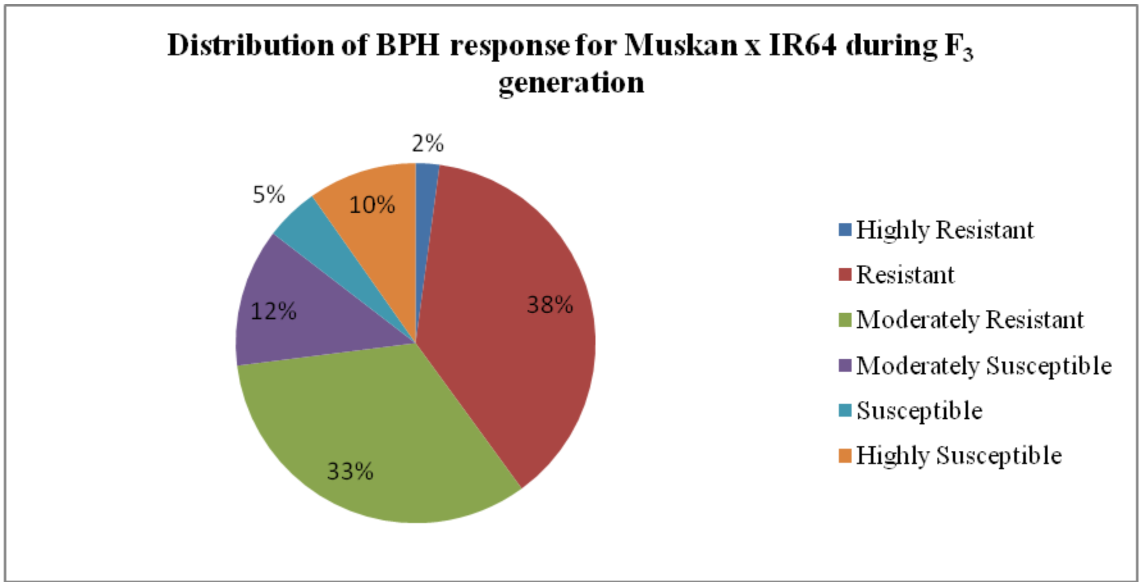
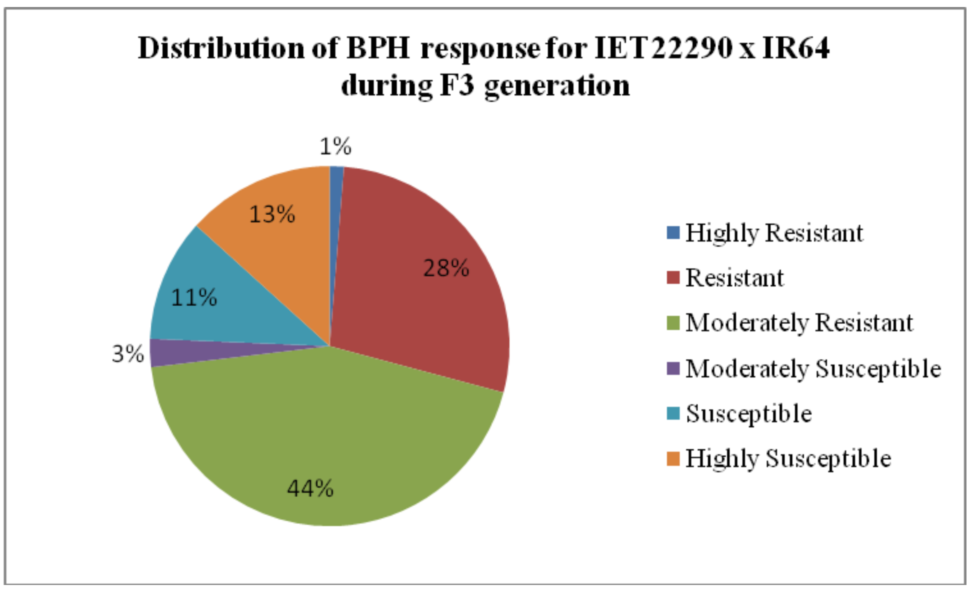


Fig.6 Distribution pattern of BPH response for cross of IET22290 x IR64 during F_3 generation



The F_1 populations of the crosses CG Zn Rice I x IR64, Muskan x IR64 and IET22290 x IR64 showed resistant reaction against the brown plant hopper population and shows presence of a single dominant gene for resistance in donor IR64. The reaction of BPH evaluated for segregation in F_2 population of the crosses CG Zn Rice I x IR64, Muskan x IR64 and IET22290 x IR64 with their respective susceptible parents was observed in a frequency of three resistant plants : one susceptible plant (3R: 1S) confirms the presence of single dominant gene in the resistant parent IR64. Further, the F_3 progenies of these crosses for each resistant parent were also analyzed for segregation pattern. Data reveals that, a segregation pattern of one homozygous resistant: two segregating (heterozygous): one homozygous susceptible, (1R: 2Sg: 1S) was observed for these crosses as expected in simple Mendelian inheritance pattern. This confirmed the inheritance of a single dominant gene present in this resistant parent IR64.

These results suggested that there was Mendelian segregation for BPH resistance in the F_2 and F_3 population. Resistance to BPH in the population appeared to be qualitative as indicated by frequency distribution of phenotypic values of F_2 and F_3 population (Ram *et al.,* 2010).

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