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MECHANISMOF RESISTANCE IN RICE GENOTYPES AGAINST BROWN PLANTHOPPER, *NILAPARVATA LUGENS* (STAL) FOR EVALUATION OF RESISTANCE DONOR

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ABSTRACT

The brown plant hopper (BPH), *Nilaparvata lugens* (Stal) is one of the serious insect pestof rice production. In Chhattisgarh also, this pest has assumed as a major pests of rice. The present investigation on identify the source of resistance against BPH in rice, among 101 genotypes were screened with resistant (PTB33) and susceptible check (TN1). The result indicated that among all genotypes, only four lines IC no. 205837, 205162, 209035 and 247986 had shown least damage and categorized resistant lines on the basis of seed box techniques, honey dew excretion test, probing marks test and nymphal survival to the infestation of BPH. In screening, the rice genotypes IC no. 209035 was observed minimum damage with a score 1.7 followed by other as highly resistant. Results of honeydew excretion test, genotype IC no. 205837 had the lowest honeydew excretion value (16.33 mm2) in 24 hrs per two female which obtained as resistant. Genotype IC no. 209035 had the highest (32.33) average probing marks which shown resistant donor and finally carried outin nymphal survival, IC no. 209035 had the lower developmental period value (14.67 days) which shown resistant followed by others.

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INTRODUCTION

Rice, *Oryza sativa* L. is one of the most important cereal crops of the world and serves as the primary source of staple food for more than half of the global population (Emani *et al.*, 2008). In Chhattisgarh state, rice is important crop which acreage 3982.2 thousand hectare with a total production and productivity 11772.6 thousand tones and 1970kg/ha, respectively (Anonymous, 2013). It is one of the important cereal crops of the world and forms the staple food for more than 50 per cent of population. Even though, there are many constraints in rice production, insects' pests remain a constant problem in all the rice growing regions (Manikandan *et al.*, 2014). An approximate 52% of the global production of rice is lost annually owing to the damage caused by various biotic factors, of which ~21% is attributed to the attack of insect pests (Brookes and Barfoot, 2003). Rice is infested by more than hundred species of insects and about twenty of them are considered serious pests as they cause significant damage to rice crop. Among them brown plant hopper (BPH), *Nilaparvata lugens* (Homoptera: Delphacidae) is one of the most destructive insect pests causing significant yield loss in most of the rice cultivars of Asia. It is a phloem-sap sucking insect pests of tropical and temperate rice in Asia feeds on the rice phloem sap using its piercing-sucking mouthparts, which affects the growth of rice plants and results in 'hopper burn'(Watanabe and Kitagawa, 2000). BPH is also a vector, transmitting viral diseases such as Grassy stunt, rugged stunt and associated diseases (Khush *et al.*, 1985). Control of brown planthopper with chemical pesticides has given rise to many problems, including elimination of natural predators, environmental pollution, resurgence and outbreak (Balakrishna and Satyanarayana, 2013). The use of resistant rice varieties is the most economical and efficient method for controlling the BPH (Alam and Cohen, 1998) therefore it is the most important approaches to identify BPH resistance genes from diverse sources and incorporate them into rice cultivars. Identification and deployment of new genes for BPH resistance in rice varieties by host plant resistance mechanisms is the important strategy to reduce the damage caused by BPH to rice crop (Kumar and Tiwari, 2010). Joshi *et al.* (2015) also screened among 94 rice genotypes in same place under lab condition which findings that four entries showed the resistance to rice BPH and recorded low damaging score. The present studies were conducted to identify the sources of resistance against BPH in rice. Among 101 rice genotypes were screened along with resistant (PTB33) and susceptible checks (TN1) during 2015 in glass house at department of Entomology, IGKV, Raipur using standard seed box technique.

MATERIALS AND METHODS

The present investigation, 'Mechanism of resistant in rice genotypes against brown planthopper, *Nilaparvata lugens* (Stal) for evaluation of resistance donor' was conducted under glass house of Department of Entomology, Indira GandhiKrishiVishwavidyalaya, Raipur (C.G.) during 2014-15.

Plant material

The experimental material consists of one hundred one genotypes of rice with TN1 and PTB33 were used as susceptible and resistant checks, respectively. They screened against BPH in the glasshouse throudh standard seed box screening test and studies for their levels of antibiosis and tolerance.

Rearing and maintenance of BPH

Brown planthoppers initially collected from field were maintained throughout the year in the air cooled glasshouses at 30 ± 5ºC on forty day old TN-1 seedlings in clay plots. The pots were placed inside rearing cages of 75x75x75 cubic centimeter which consist with of an iron frame with glass panels and small window on front side and fine wire mesh on top and other sides. Adult insects 3 to 4 pair (male and Female) per hill were placed inside rearing cages for egg laying. After 2-3 days, the females started egg laying inside the leaf sheath of paddy plant. Later on, when adults emerged they were transferred to another pot for egg laying. For transferring theadults, an aspirator is used which works on the suction of the air principle. Nymphs emerged out within 5 to 6 days from the eggs and reached second instar; they were collected and used to infest the test materials. Above the following methodology was used to mass rearing and maintaining of BPH in Glasshouse condition during investigation.

Screening of rice genotypes

The pre-germinated seeds of the rice varieties were sown 3 cm apart in a seed box filled with mud soil. Each test variety was sown in three replications in a row across the width of the seed box with at least 20 plants per row. At seven days after sowing, the seedlings were infested with second and third instar nymphs of BPH at the rate of eight to ten nymphs per seedling. After infestation each seed box was covered with a wire mesh cage to prevent any escape and to prevent entry of natural enemies. The test varieties were observed daily for the damage by the BPH. Damage rating of test varieties was observed when 90 percent of the seedlings in TN1 were killed (Anonymous, 1996). The reactions were recorded on a 0-9 scale suggested by Heinrichs (1986).

Probing mark test

Probing mark test was carried out according to methodology suggested by Natio (1964). For this purpose, seeds of identified resistant rice genotypes and check varieties i.e. TN1 and PTB33 were germinated separately in petridishes. Germinated seeds were sown in wooden trays containing well puddled soil. After seven days, the seedling of each variety was removed from trays and washed thoroughly with water and then transferred individually into 15 cm long test tubes containing a few drops of water. One female (two days old) was introduced individually into each test tube and test tubes were plugged with sterilized cotton swab. The female was allowed to make punctures on the seedling for overnight (12 hrs). Thereafter, the seedlings were taken for staining in another tube containing 1.0 per cent erythrosine dye aqueous solution. Insect probing marks stained thereby counted visually after 30 minutes of staining. Three replicates were maintained for each rice genotypes and each replicate contains one seedling.

Honeydew excretion test

Honeydew excretion test was suggested by Sogawa and Pathak (1970). For this White whatman No. 1 filter papers (10 cm diameter) were dipped in a solution of bromocresol green (2mg/ml ethanol) indicator and allowed to dry in sunlight thereby filter paper turned to yellowish orange colour as suggested by (Pathak and Heinrichs, 1982). The treated filter papers were placed on an inverted petridish (10 cm dia) at the base of each plant through a slit made in centre. Thereafter, each plant was covered with inverted glass funnel (75 mm) along with two days old female which allowed feeding on leaf sheath for 24 hours. The insects were starved for three hours prior to release for the test. Feeding activity was investigated at 30 days old potted plants. Three replicates were used for each genotype and each replication contains two females on one tiller of plant. Immediately, upon contact with honeydew secreted by female, blue spots appeared on the treated filter papers. As the concentration of the honeydew increased, the spots turned white in the center with the blue edges. The spots were traced on transparency and later on measured by keeping on millimeter square graph.

Nymphal survival

The well germinated seeds of selected rice genotypes were sown in 500 ml earthen pots filled with fertilizer enriched puddled soil. After 30 days, the plants were covered by the Mylar tube with ventilating windows. Then 10 nymphs (First and second instar) were released in such tubes then the open end of the tube covered by the muslin cloth and tied with rubber band. For each variety tree replications were kept. The plants were observed daily for the emergence of the adult. These emerged adults were removed from the tubes and observation on total average no. of days which require to development of nymphs to adults.

RESULTS AND DISCUSSION

In all one hundred one rice genotypes were used to screening for the identification of resistant source against BPH in the seed box screen test varying moderately resistance to highly susceptible. The results indicated thatfour lines were least damage to the infestation of this insect and categorized resistance (1-3) on the basis of 0 to 9 scale viz. genotypes IC no.205837, 208162, 209035 and 247986 but other ten, twenty five and sixty two lines were found moderately resistant,

Table 1: Standard evaluation system for rating damage by BPH

Score	Criteria	Reaction
0	Plant healthy or no Visible damage	Immune
1	Partially yellowing first leaf	Highly Resistance
3	First and second leaves partially yellowing	Resistance
5	Pronounced yellowing and Stunting growth	Moderately resistance
7	The plant is still alive	Moderately Susceptible
9	The plant is dead	Highly susceptible

moderately susceptible and highly susceptible to the infestation of BPH respectively (Table 2). The rice genotypes IC no. 209035 with a score 1.7 shown minimum infestation followed by other as highly resistant to BPH. The rice genotypes with resistant check PTB33 (2.11) displayed resistant reaction and susceptible check (9.00) displayed susceptible to BPH. The resistant genotypes were further used for probing mark test, honey dew excretion test and nymphal survival test to check the resistant source. (Table 3)

In case of Honeydew excretion test, All the selected resistant genotypes exhibited average honeydew excretion values varied from 16.33 to 27.33 mm2 per two female in 24 hrs, which was significantly lower than the susceptible check TN1 (65.67). Resistant check PTB33showed honeydew excretion value of 18.00 mm2 which was lower than all resistant rice genotypes tested and also than the susceptible check TN1. The genotype IC no. 205837 had the lowest honeydew excretion value (16.33 mm2) in 24 hrs per two female followed by IC no. 209035 (19.67 mm2), 208162 (21.83 mm2) and 247986 (27.33 mm2).(Table 3)

The entries showing damage score up to 3 were further evaluated for confirming their resistance to probing mark test. Statistically numbers of probes received by all resistant genotypes tested were significantly high as compared to susceptible check TN1, resistant varieties receive higher number of probes than that of susceptible variety TN1. Piercing the stylets and holding it for long time depend upon the nutritional fulfillment of sap being drained by insect through stylets (Velusamy and Heinrich, 1986). Female of BPH made more probing marks on resistant variety than on susceptible (Bagui, 1989).In all the selected resistant rice genotypes, the average probing marks values per seedling were ranged from 25.00 to 32.33. Although, in resistant check PTB33, the probe marks was 34.33 per seedling per female. The resistant genotype IC no. 209035 had the highest (32.33) average probing marks, which have the mean honeydew excretion value of 19.67 mm2 followed by IC no. 205837 (30.67), 247986 (28.67) and 208162 (25.00). The average probing mark per seedling in resistant check PTB 33 was found 18.67 which was significantly higher than four genotypes, but significantly lower than the genotypes TN1 (11.00). (Table 3)

Table 2: BPH reaction of rice genotypes

S.N.	Score	Genotypes/ lines
01.	0	Nil
02.	1	Nil
03.	3	Four lines
04.	5	Ten lines
05.	7	Twenty five lines
06.	9	Sixty two lines

It is very clear that susceptible host has received less probe marks and excrete more honeydew because of the presence of required nutritional value or the absence of harmful biochemicals in the plant itself, whereas, in resistant host the more probe marks and less honeydew excretion might be the indication of unsuitability of nutrition in the plant or presence of certain plant biochemicals which checks the feeding and proves the presence of antibiosis mechanism of resistance and those plants are indicate resistance lines have antibiosis and antixenosis properties.

Among rice genotypes, four resistant genotypes including standard checks were selected to study the nymphal survival of BPH as the antibiosis parameter. The nymphal survival test was carried out on 30 days old plant. Among all the resistant genotypes tested, the genotype IC no. 209035 had the lower developmental period value (14.67 days) followed by IC no. 247986 (16.00 days), 208162 (15.00 days)and 205837 (17.33 days), the nymphal survival of PTB33 with 18.67 days but it was significantly higher than the susceptible check TN1 (10.33 days). (Table 3)

In the present investigation, One hundred sixteen rice genotypes were screened against *Nilaparvata lugens,* out of these, 4 genotypes were categorized as resistant, 10 as moderately resistant, 25 moderately susceptible and 62 genotypes indicated highly susceptible to this pest. Among all genotypes,screened the genotypes IC no. 209035 shows the best resistance with least plant damage score 2.17. In depth studies on selected resistance genotypes were confirmed by honey dew excretion, probing marks along with period of nymphal survival.

Similarly, Varma *et al.* (2014) One hundred sixty seven rice genotypes were screened against BPH, in the Glass House, IGKV, Raipur (C.G.) which found that among the screened material, 39 genotypes were categorized as resistant, whereas 24 as moderately resistant, 12 as moderately susceptible and 92 as susceptible to BPH. Bhanu *et al.* (2014) screened 15 rice varieties in greenhouse, out of this the results shown that one is highly resistance and five were moderately resistance. The rice varieties, T12 and PTB33 showed high level of antibiosis and tolerance to BPH. Joshi *et al.* (2015) also screened 94 rice genotypes in greenhouse along with resistant and susceptible checks viz., PTB 33 and TN1 respectively during 2014-15. Among the 94 genotypes four was shown resistant (IC numbers 145651, 145824, 145828 and 145846).

Table 3: Average plant damage score, honey dew excretion and average probing marks on moderately resistance on rice genotypes

S.N.	IC numbers of genotypes	Average plant damage score	Honeydew (mm2/ 24 hrs)	Average probing marks/seedlings	Nymphal development period (days)
01.	205837	2.50	16.33	30.67	17.33
02.	208162	2.23	21.83	25.00	15.00
03.	209035	1.74	19.67	32.33	14.67
04.	247986	2.46	27.33	28.67	16.00
05.	PTB33 (R.check)	2.11	18.00	34.33	18.67
06.	TN1 (S.check)	9.00	65.67	11.00	10.33
	SEm ±		2.07	1.84	1.97
	CD (0.05)		6.63	5.895	6.09

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