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RESEARCH ARTICLE

MTU IJ 206-7-4-1 (BM 71), A NEW BROWN PLANTHOPPER RESISTANT DONOR WITH HIGH LEVELS OF ANTIXENOSIS AND ANTIBIOSIS EFFECTS

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Abstract:

MTU IJ 206-7-4-1, a highly resistant brown planthopper resistant (BPH) donor developed at Andhra Pradesh Rice Research Institute and Regional Agricultural Research Station, Maruteru, Andhra Pradesh, India was tested along with other eleven resistant rice cultures for their antixenosis and antibiosis effects on BPH. Among the tested rice cultures, MTU IJ 206-7-4-1 exhibited high level of antixenosis for colonization of BPH (3.22 nymphs/seedling and 1.12 adults/ten plants). It recorded significantly lowest number of eggs (79.67) and more number of feeding marks (112.80) indicated that it exhibited high level of antixenosis. MTU IJ 206-7-4-1 recorded significantly prolonged development period of nymphs (26.00 days), lower growth index (3.07) than the susceptible check TN1 (8.48). MTU IJ 206-7-4-1 was the least preferred rice culture for BPH feeding with less amount of honeydew excretion (25.60 mm^2) compared to the susceptible check, TN1 (450.40 mm^2). It also recorded significantly lowest female population (20.0 %), lowest population numbers of first generation nymphs (41.60) and high level of antibiosis index (0.88).

Key Words: Rice cultures, Brown planthopper, *Nipalparvata lugens*, Mechanisms of resistance, Antixenosis, Antibiosis.

Introduction

The brown planthopper (BPH), *Nilaparvata lugens* (Stal) (Homoptera: Delphacidae) is one of the most destructive monophagous insect pests of rice throughout the rice growing countries in Asia (Park *et al*., 2008). The BPH damages the rice plant directly by sucking the phloem sap and plugging the xylem and phloem with their feeding sheaths, which causes serious yield reductions (Sogawa, 1982). BPH also serves as a vector for transmission of viruses that cause grassy stunt (Rivera *et al*., 1966) and ragged stunt (Ling *et al*., 1978) viral diseases in rice. Though insecticide application is providing immediate control, ill effects like resurgence, secondary out break and development of resistance to insecticides are most common with BPH. Hence, cultivation of resistant rice varieties is the most economical and efficient method for the management of BPH (Renganayaki *et al*., 2002). So, there is a recurring need to identify the varieties resistant to BPH. It is also necessary to understand the mechanisms of resistance that are responsible for manifesting the resistance among the plants and is essential for the development of varieties with durable resistance. Generally, the insect resistance types with regard to the physiological function were classified into antixenosis (non-preference), antibiosis and tolerance (Painter, 1951). In general, resistant rice plants exhibit two strategies against BPH: antixenosis and antibiosis. The present study was conducted with an aim to identify the antixenosis and antibiosis affects of certain identified resistant rice cultures on BPH. The studies include antixenosis for nymphal settlement, antixenosis of adults for feeding and oviposition; antibiosis effects on nymphal development, growth, population buildup and feeding rate etc.

Materials and methods

Twelve rice cultures identified as highly resistant to moderately resistant in field and glasshouse screening were used to study the antixenosis and antibiosis affects on BPH along with resistant check (Ptb 33) and susceptible

check (TN1). Studies were carried out in the glasshouse as pot culture experiments under controlled conditions at Andhra Pradesh Rice Research Institute and Regional Agricultural Research Institute, Maruteru, West Godavari district, Andhra Pradesh during the period 2010 to 2011. The rice cultures under study were NLR 3090, NLR 3093, MTU 1075, WGL 401, WGL II 218-5-1, MTU PLA 99-1-3-1-2, NLR 20131, BPT 2404, RDR 34, RGL 7001, RGL 7002 and MTU IJ 206-7-4-1. BPH reared on the susceptible TN1 plants were used for the studies (Heinrichs *et al*., 1985).

Antixenosis for nymphal settlement: Settlement of nymphs on seedlings of selected resistant rice cultures was assessed by using conventional seed boxes (Heinrichs *et al*., 1985). Pre germinated seeds of the test cultures were sown 3 cm apart in rows in a galvanized iron tray filled with mud soil. Each test culture was replicated three times. Seven days after sowing, the seedlings in each tray were thinned to have 20 seedlings per row and per each rice culture. At ten days after sowing, second instar nymphs of BPH were released on the seedlings at the rate to have at least 6-8 nymphs per seedling. The trays were covered with fine mesh screen to prevent migration. The number of nymphs settled on each seedling was counted at 24, 48 and 72 hours after infestation and their mean was arrived. The seedlings were disturbed after each count for reorientation of nymphs on seedlings. Preference of settling was noted based on the average number of nymphs settled on each culture and analysis of variance test was adopted to compare the cultures.

Antixenosis of adults for colonization and oviposition: Antixenosis of adults for oviposition on different tested rice cultures was studied by growing the cultures in galvanized iron trays along with resistant and susceptible checks and replicated three times. Ten days after sowing, the seedlings in each tray were thinned to have 20 seedlings per row and allowed to grow up to 30 days and the trays were covered with a fine mesh screen. At 30 days removed the dried or yellowing outer leaf sheaths and thinned the plants to have two tillers per hill and infested the plants of each test culture in seed box at the rate of ten adult female hoppers/hill and the trays were covered with a fine mesh screen to prevent hoppers from escaping. The adult hoppers settled on each test culture were counted at 24, 48 and 72 hours after infestation and their mean was arrived. The plants were dissected 72 hours after infestation and counted the eggs under a microscope to study the antixenosis of adults for oviposition among cultures (Heinrichs *et al*., 1985).

Antixenosis of adults for feeding: The antixenosis of adults for feeding was studied by enclosing two adult female hoppers in a parafilm sachet attached to the leaf sheaths of each test culture along with the resistant and susceptible checks. Each test culture was replicated ten times. The insects were allowed to feed for 24 hours. After that the portion of the leaf sheath where the hoppers fed was cut and dipped in the 0.1% erythrosine dye for 10-15 minutes. The portion of leaf sheath was observed under the microscope and counted the number of feeding marks that stained pink per each test culture. The feeding preference was determined on the basis of average number of stylet sheaths/plant among the cultures (Heinrichs *et al*., 1985).

Nymphal development period: Nymphal development period on selected rice cultures along with resistant and susceptible checks was studied by releasing five first instar BPH nymphs on 30-days old Mylar film caged plants. From 9th day onwards, nymphs on each culture were observed daily for ecdysis and recorded the number of days taken for the nymphs to reach adult stage on each rice culture (Pongprasert and Weeraput, 1979).

Growth Index (GI): Growth index of BPH on each selected rice culture and the resistant and susceptible checks was computed by using the data obtained from the experiments on nymphal survival and development period (Panda and Heinrichs, 1983) as follows:

$$\text{Growth Index} = \frac{\text{\% nymphs survived on test culture}}{\text{Development period of nymphs on test culture}}$$

Feeding rate: The preference of BPH for each selected rice culture was assessed by estimating the amount of honeydew excreted by the adult hoppers as an indication of the feeding preference. Whatman No.1 filter paper was dipped in a 0.02% bromocresol green solution in ethanol and allowed to dry for one hour and dipped again till the filter paper turned yellowish orange. The treated paper was then placed on the wooden plank kept at the base of 30-days old plants. A plastic cup was placed over the filter paper and five freshly emerged female hoppers, pre-starved for four hours and were released into the feeding chamber having bromocresol green treated filter paper (Pathak and Heinrichs, 1982). The BPH adults were allowed to feed for 24 hours at the base of the stem. The honeydew droplets excreted by the adults when came in contact with the filter paper turned into blue spots. The area blue spots appeared on filter paper as a result of honeydew excretion was measured by graph method. The antibiosis effect on feeding among the test cultures were determined by comparing the average area of honeydew excreted in mm^2.

Population buildup: The population buildup of BPH on different test cultures along with the resistant and susceptible checks was studied by releasing ten first instar nymphs on 30-days old Mylar film caged plants. Each

culture was replicated five times. The emerging adult hoppers were observed for their sex. Further, the surviving adults were then allowed to feed and reproduce on the same plant. The number of nymphs emerged after hatching was recorded as first generation nymphs (Heinrichs *et al*., 1985).

Antibiosis Index: To study the level of antibiosis in selected rice cultures, 30-days old Mylar film caged plants of each rice culture were used and released on to each plant 50 numbers of fourth to fifth instar nymphs of BPH. A control plant without insect was maintained for each test culture. When the plants started to wilt, the hoppers on the potted plants of each test culture were collected, oven dried for 48 hours and weighed. Similarly the BPH dry weight on TN1 was recorded. Tolerance and antibiosis index were calculated based on BPH dry weight produced on each of the test cultures and TN1 (Panda and Heinrichs, 1983).

$$\text{Tolerance Index} = \frac{\text{BPH dry weight on test culture}}{\text{BPH dry weight on TN1}}$$

Antibiosis index= 1-Tolerance index

The data obtained from the experiments were subjected to ANOVA in simple RBD analysis after transforming the data into suitable transformations and the mean values were compared (Gomez and Gomez, 1984).

Results and Discussion

Antixenosis for nymphal settlement: Among the rice cultures tested for nymphal preference, the highly resistant rice culture, MTU IJ 206-7-4-1 (3.22 numbers) recorded significantly low nymphal settlement per seedling and was followed by other resistant rice cultures (Table 1). The susceptible check, TN1 recorded higher number of nymphs' settlement (12.01numbers).

Antixenosis of adults for colonization and oviposition: All the resistant and moderately resistant rice cultures recorded significantly lowest number of BPH adults settled per ten seedlings and were ranged from 1.12 to 7.67 with a maximum of 20.56 numbers in susceptible check, TN1. Among the rice cultures tested, MTU IJ 206-7-4-1 (1.12 numbers) recorded significantly low adults' settlement per ten seedlings and was on par with resistant check, Ptb 33 (1.78 numbers). These were followed by other rice cultures (Table 1).

The mean number of eggs laid per 10 plants ranged from 79.67 to 608.00. MTU IJ 206-7-4-1 recorded significantly lowest number of eggs per ten seedlings (79.67) and on par with resistant check, Ptb 33 (109.33). These were followed by MTU 1075, RGL 7001 and RGL 7002. The rice cultures which recorded relatively higher number of eggs to other resistant rice cultures were WGL II 218-5-1, NLR 3090, NLR 3093, WGL 401, MTU PLA 99-1-3-1-2, BPT 2404, NLR 20131 and RDR 34 (Table 1).

Antixenosis of adults for feeding: The data from table 1 indicated that the feeding marks on leaf sheath differed significantly among the cultures. The number of feeding marks ranged from 12.50 to 128.10. The feeding marks were observed to be significantly more in rice cultures MTU 1075 (128.10), MTU IJ 206-7-4-1 (112.80), MTU PLA 99-1-3-1-2 (110.20) and were higher than the resistant check, Ptb 33 (61.50). The rice cultures *viz*., NLR 3090, WGL II 218-5-1, BPT 2404, WGL 401, NLR 20131, RGL 7001, NLR 3093 and RDR 34 recorded moderate number of feeding marks among the rice cultures tested. The rice culture, RGL 7002 (12.50) recorded significantly lower number of feeding marks than the susceptible Check, TN1 (24.30).

Nymphal development period and growth index: Nymphal development period was significantly prolonged on the resistant and moderately resistant cultures (Table 2). Nymphal period ranged between 14.60 to 26.00 days in the resistant and moderately resistant rice cultures as compared to 11.80 days in the susceptible check, TN1. Significantly prolonged development period was observed in rice culture MTU IJ 206-7-4-1 (26.0 days) and was followed by MTU PLA 99-1-3-1-2 (23.0 days). These two cultures took more days for the development even than the resistant check, Ptb 33 (20.8 days).

All the rice cultures registered 1.31 to 6.37 time's lower growth index than the susceptible check TN1 (8.48). Significantly lowest growth index was observed in the rice culture WGL II 218-5-1 (1.33) and was on par with resistant check Ptb 33 (1.91). These were followed by other rice cultures *viz*., NLR 20131 (2.64), RGL 7001 (2.74) and MTU IJ 206-7-4-1 (3.07) (Table 2).

Feeding rate: The amount of honeydew excreted in the present study ranged from 25.60 mm^2 to 456.40 mm^2. The data from table 2 indicated that all the resistant rice cultures showed significantly less amount of honeydew excretion as compared to susceptible check TN1 (456.40 mm^2). Among the resistant rice cultures, MTU IJ 206-7-4-1 recorded lowest honeydew excreted area of 25.60 mm^2 and followed by resistant check, Ptb 33 (58.80 mm^2), NLR 3093 (65.60 mm^2) and RGL 7002 (87.80 mm^2).

Population buildup of BPH

Female population: The sex of emerged adults from the nymphs developed on tested rice cultures along with the resistant and susceptible checks was presented in table 2. The per cent female emergence ranged from 20.0 to 64.0.

Significantly lowest female population of 20.0 per cent was observed in MTU IJ 206-7-4-1 which was 24.0 and 44.0 per cent lower than the resistant check, Ptb 33 (44.0) and susceptible check, TN1 (64.0) respectively. It was on par with RGL 7001 (32.0 per cent) and WGL II 218-5-1 (32.0 per cent). These were followed by other rice cultures.

Emergence of first generation nymphs: The number of first generation nymphs emerged on the tested rice cultures were presented in table 2. The number of first generation nymphs varied from 32.40 to 450.80. Significantly lowest numbers of first generation nymphs was observed on WGL II 218-5-1 (32.40) and was on par with WGL 401 (40.80), MTU IJ 206-7-4-1 (41.60), resistant check, Ptb 33 (46.00). These were followed by other rice cultures. Significantly highest population of first generation nymphs was observed on BPT 2404 (438.00) and was on par with susceptible check, TN1 (450.80).

Antibiosis index: MTU IJ 206-7-4-1 (0.96), NLR 3093 (0.89), WGL II 218-5-1 (0.87) and RDR 34 (0.87) recorded significantly high level of antibiosis index and were on par with resistant check, Ptb 33 (0.89). These were followed by NLR 20131 (0.86). Significantly low level of antibiosis index was observed in RGL 7001 (0.02).

In the present investigation settling of fewer nymphs and gravid females on resistant cultures than on the susceptible check TN1 indicating that antixenosis may be one of the mechanisms for resistance in those rice cultures that deter or reduce the BPH nymphs or adults colonizing on them. The highly resistant culture, MTU IJ 206-7-4-1 recorded lower number of nymphs and adults settled and also eggs per ten plants than resistant check, Ptb 33. These results are in concurrence with the concept of Painter's 'Non-preference' or 'antixenosis' concept (Painter, 1951). Several workers reported that the BPH settling on the resistant and susceptible varieties significantly differs with higher numbers of insects settling on the susceptible varieties (Senguttuvan *et al*., 1991; Velusamy *et al*., 1995). Kalode *et al*. (1978) reported that Ptb 33, Ptb 21, Leb Mue Nahng, ARC 6650 and CR-57-MR-1523 had less number of BPH nymphs as compared to TN1 and suggested the presence of some attractants in the susceptible variety and absence of these in the resistant ones.

Sogawa and Pathak (1976) reported that the non-preference response of BPH was due to gustatory rather than olfactory or visual stimuli, as the hoppers discriminated between the varieties only after feeding. In the present investigation, the distinct differences with significantly higher number of BPH nymphs and adults on susceptible TN1 and less number on the resistant cultures were indicative for the response of BPH to gustatory stimuli. According to Karim (1975), lower number of egg deposition on resistant varieties was due to insufficient feeding. The resistant varieties did not sustain prolonged feeding due to the presence of certain feeding deterrents or toxic chemicals present in their sap. Therefore, the insect had to make more feeding marks on the resistant genotypes to locate feeding sites (Sogawa, 1982). In the present investigation, some of the rice cultures *viz*., MTU 1075, MTU IJ 206-7-4-1 and MTU PLA 99-1-3-1-2 recorded more number of feeding marks compared to resistant check, Ptb 33 indicating that these cultures were least preferred for feeding.

The prolonged development period was observed in MTU IJ 206-7-4-1 (26 days) and the lowest growth index of BPH in the rice culture WGL II 218-5-1 (1.33) indicated that these two cultures exhibited high level of antibiosis among the cultures tested and not suitable for BPH development. In the present investigation, the resistant culture MTU IJ 206-7-4-1 (25.60 mm^2) recorded lowest honeydew excreted area than the resistant check, Ptb 33 (58.80 mm^2). Sogawa and Pathak (1976) reported that the difference in the amount of honeydew excretion is mainly because of differences in the relative amount of sap intake. The little sap intake on resistant varieties, despite successful stylet penetration into the vascular bundle indicates the occurrence of certain undesirable gustatory factors that block the sustained sucking by the insect. In the present investigation, except BPT 2404, the other rice cultures tested recorded lowest number of first generation nymphs than the susceptible check, TN1 is probably due to the reduced rate of reproduction. These results are in agreement with the observations of Kalode *et al*. (1978) who reported that population build up was adversely affected to varying degrees on Ptb 33, Ptb 21, ARC 6650 and CR-57-MR-1523.

Table 1 Antixenosis effects of MTU IJ 206-7-4-1 (BM 71) on brown planthopper

Rice culture No.	Field reaction to BPH	Mean no. of			
		Nymphs settled/ seedling	BPH adults settled/ 10 plants	Eggs/10 plants	Feeding marks
NLR 3090	MR	5.12 (2.26) [b]	3.89 (1.96) [b]	207.33 (14.39) [c]	105.40 (10.05) [e]
NLR 3093	MR	5.71 (2.39) [b]	3.33 (1.81) [b]	207.67 (14.39) [c]	48.80 (6.83) [cd]
MTU 1075	R	5.01 (2.23) [b]	4.01 (1.97) [b]	119.33 (10.92) [b]	128.10 (11.27) [g]
WGL 401	MR	6.09 (2.46) [b]	3.89 (1.96) [b]	211.00 (14.52) [c]	62.00 (7.67) [cd]
WGL II 218-5-1	MR	5.40 (2.32) [b]	3.00 (1.73) [b]	192.33 (13.86) [c]	97.10 (9.68) [e]
MTU PLA 99-1-3-1-2	MR	5.87 (2.41) [b]	3.22 (1.77) [b]	217.00 (14.69) [c]	110.20 (10.46) [fg]
NLR 20131	MR	5.83 (2.40) [b]	7.67 (2.75) [c]	291.67 (17.01) [d]	56.80 (7.32) [cd]
BPT 2404	MR	5.83 (2.41) [b]	4.22 (2.04) [bc]	234.33 (15.26) [c]	91.80 (9.45) [e]
RDR 34	MR	6.13 (2.47) [b]	3.33 (1.82) [b]	320.00 (17.88) [d]	39.40 (6.24) [bc]
RGL 7001	R	5.70 (2.38) [b]	2.56 (1.60) [b]	161.33 (12.62) [bc]	51.30 (7.05) [cd]
RGL 7002	R	5.83 (2.41) [b]	5.89 (2.41) [c]	162.00 (12.72) [bc]	12.50 (3.37) [a]
MTU IJ 206-7-4-1	HR	3.22 (1.78) [a]	1.12 (1.01) [a]	79.67 (8.90) [a]	112.80 (10.20) [efg]
Ptb 33	HR	6.07 (2.46) [b]	1.78 (1.31) [ab]	109.33 (10.43) [ab]	61.50 (7.77) [cd]
TN 1	HS	12.01 (3.46) [c]	20.56 (4.52) [d]	608.00 (24.63) [e]	24.30 (4.90) [b]

HR= Highly resistant; R=Resistant; MR= Moderately resistant; HS= Highly susceptible

Figures in parenthesis are square root transformed values

Mean with same letter are not significantly different at 5 % level by Duncan's Multiple Range test

Table 2. Antibiosis effects of MTU IJ 206-7-4-1 (BM 71) on brown planthopper

Rice culture No.	Nymphal period (days)	Growth index	Honeydew excreted (mm^2)	1st generation nymphs	Female (%)	Antibiosis index
NLR 3090	17.00 (4.12) ^{e}	5.78 (2.40) ^{d}	218.40 (14.63) ^{d}	174.20 (13.14) ^{cd}	48.00 (43.85) ^{ab}	0.83 ^{bc}
NLR 3093	16.80 (4.10) ^{e}	5.96 (2.44) ^{d}	65.60 (8.06) ^{b}	319.80 (17.77) ^{f}	36.00 (36.70) ^{ab}	0.89 ^{a}
MTU 1075	17.80 (4.22) ^{de}	4.38 (2.09) ^{e}	160.80 (12.58) ^{cd}	285.60 (16.82) ^{e}	36.00 (36.70) ^{ab}	0.48 ^{d}
WGL 401	15.80 (3.97) ^{f}	6.34 (2.52) ^{d}	260.80 (16.05) ^{de}	40.80 (6.21) ^{ab}	52.00 (43.85) ^{ab}	0.81 ^{bc}
WGL II 218-5-1	21.00 (4.58) ^{c}	1.33 (1.15) ^{a}	168.20 (12.93) ^{cd}	32.40 (5.66) ^{a}	32.00 (34.16) ^{abc}	0.87 ^{ab}
MTU PLA 99-1-3-1-2	23.00 (4.79) ^{b}	4.01 (2.00) ^{c}	200.80 (13.97) ^{d}	73.40 (8.39) ^{b}	44.00 (41.54) ^{ab}	0.83 ^{bc}
NLR 20131	20.40 (4.51) ^{c}	2.64 (1.62) ^{b}	164.00 (12.75) ^{cd}	142.00 (11.82) ^{c}	48.00 ^{ab} (43.85)	0.86 ^{b}
BPT 2404	14.60 (3.82) ^{g}	6.46 (2.54) ^{d}	340.60 (18.29) ^{e}	438.00 (20.86) ^{g}	44.00 (41.31) ^{ab}	0.74 ^{a}
RDR 34	14.60 (3.82) ^{g}	4.26 (2.05) ^{c}	184.80 (13.48) ^{cd}	186.80 (13.55) ^{d}	44.00 (41.54) ^{ab}	0.87 ^{ab}
RGL 7001	18.20 (4.26) ^{d}	2.74 (1.63) ^{b}	133.20 (11.49) ^{c}	60.40 (7.75) ^{b}	32.00 (33.94) ^{bc}	0.02 ^{e}
RGL 7002	18.80 (4.33) ^{d}	4.89 (2.21) ^{cd}	87.80 (9.33) ^{b}	72.20 (8.45) ^{b}	40.00 (39.23) ^{ab}	0.80 ^{bc}
MTU IJ 206-7-4-1	26.00 (5.10) ^{a}	3.07 (1.74) ^{b}	25.60 (4.89) ^{a}	41.60 (6.13) ^{ab}	20.00 (26.56) ^{c}	0.96 ^{a}
Ptb 33	20.80 (4.56) ^{c}	1.91 (1.36) ^{a}	58.80 (7.62) ^{b}	46.00 (6.67) ^{ab}	44.00 (41.54) ^{ab}	0.89 ^{a}
TN 1	11.80 (3.43) ^{h}	8.48 (2.91) ^{e}	456.40 (21.63) ^{f}	450.80 (21.20) ^{g}	64.00 (53.30) ^{a}	0.00 ^{e}

Figures in parenthesis are square root transformed values

Mean with same letter are not significantly different at 5 % level by Duncan's Multiple Range test

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