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Mechanism of resistance in rice varieties showing differential reaction to brown planthopper

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Abstract. A total of 1070 rice varieties, mainly from Assam Rice Collection, were evaluated to identify better sources of resistance to brown planthopper, *Nilaparvata lugens* (Stål). In mass screening replicated tests 17 varieties were identified as resistant. Moderate resistance was observed in 73 varieties.

All the resistant and moderately resistant varieties were relatively less preferred by nymphs and there was a positive correlation between the number of nymphs settled and the damage score. Test varieties non-preferred by adult insects for feeding and shelter were also less suitable for oviposition with the exception of ARC 13854, and ARC 14766A. On resistant varieties the nymphal survival was much lower (18·5–28·4%) and nymphal duration was prolonged by 5–7 days as against those on the susceptible check. Results of probing behaviour tests indicated that resistant varieties received more number of probing punctures (80–121) than the susceptible check (31). Further, insects caged on resistant varieties quickly lost their body weight while those on the susceptible check registered gain in weight. Honey dew excretion by brown planthopper adults on resistant varieties was 6·6 to 11·9 times less than that on susceptible T(N)1. Selected varieties showing moderate damage reaction (ARC 5918, ARC 10443, ARC 13984, ARC 14529 and ARC 14864) exhibited more feeding marks, greater amounts of excretion, and higher gain in body weight of the insects, thus confirming a moderate degree of resistance. Based on various parameters, ARC 5780, ARC 5988 and ARC 14394 were comparable to resistant check, Ptb 33 in level of resistance. No association of Lemma and Palea colour with brown planthopper resistance was observed in the rice varieties tested.

Keywords. Varietal resistance; rice varieties; *Nilaparvata lugens*.

1. Introduction

In India the brown planthopper (*Nilaparvata lugens* (Stål)) has assumed greater importance since its outbreak in Kerala during 1973–74 and subsequently in many other parts of the country. Host plant resistance as a component of pest management programme is being successfully utilized in Philippines and Indonesia in controlling this pest. In view of the more virulent biotype in India, screening for resistance to brown planthopper was carried out at various research institutes and the number of resistant donors identified have been reported (Kalode and Krishna 1979; Kalode *et al* 1983; Kalode 1983). Though there are reports from India about nymphal non-preference for certain rice varieties, no detailed information is available on adult preference. Investigations were, therefore, undertaken at the AICRIP, Hyderabad to study the reaction of selected varieties/cultures to both nymphs and adults of brown planthopper (BPH) as well as to understand the mechanism manifesting different degrees of resistance, so that better varieties (donors) with desirable characters could be utilized effectively in resistance breeding programme.

2. Material and methods

2.1 *Mass rearing and varietal screening*

BPH was reared on 30 day old T(N)1 plants inside the green house provided with coolers to maintain the temperature at $30 \pm 5^{\circ}C$ to ensure uniform and steady supply of insects. The rearing cages ($70 \times 62 \times 75$ cm) were provided with glass panels with a small window on one side and fine nylon wire mesh on the other sides. Pre-mated gravid females were allowed to oviposit on plants for two days and the emerging nymphs were further maintained to get age specific insects for different experiments.

ARC cultivars (1000), 20 varieties from IRRI and another 50 cultures were screened by adopting the modified mass screening layout (Kalode *et al* 1975). Pre-germinated seeds were sown in rows in wooden flats along with susceptible and resistant check, which were then transferred to galvanized iron trays filled with water to maintain adequate humidity and to prevent ants. Seven day old seedlings were infested with a large number of 1–2 instar nymphs so as to get 5 to 10 insects/seedling and were scored for damage reaction on a 0–5 scale when more than 90% of T(N)1 seedlings were killed. Test varieties showing damage score up to 2·5 in a preliminary test were retested, replicated 3 times to confirm their reaction.

2.2 *Studies on preference/non-preference mechanism on selected rice varieties*

2.2a *Response of nymphs*: During the retest, the number of nymphs settled on each seedling was counted at different intervals *viz.*, 1 day, 3 days, 5 days and 7 days after infestation to assess the nymphal preference for different varieties.

2.2b *Response of adults for settling and oviposition*: About 30 selected varieties including resistant and susceptible checks were grown randomly (8 cm apart) in polythene sheet lined wooden flats. Each variety was replicated four times with seven seedlings per replication. Thirty days after sowing, each wooden flat was transferred to a suitable cage and a large number of adults were released. The counts of adult insects settled on each seedling were taken at 12, 24 and 48 hr after release. The plants were then cut as close to the base as possible and the number of eggs laid per seedling was recorded by staining in 1% erythrocin dye in an aqueous solution as suggested by Naito (1964) under a binocular microscope.

2.3 *Studies on antibiosis mechanism*

2.3a *Survival and development of nymphs*: Thirty six resistant and moderately resistant varieties were included along with resistant and susceptible checks Ptb 33 and T(N)1, respectively. Seeds of each variety were sown in earthen pots and each variety was replicated 6 times. Thirty days after sowing each plant was caged with 10 freshly hatched nymphs in mylar film cages (5×45 cm) the open end of which was closed with fine muslin cloth. The counts of surviving nymphs were taken, 24 hr after infestation and thereafter once in five days till 20 days.

2.3b *Feeding response of adult brown planthopper*: Thirty varieties which were identified as resistant and moderately resistant in mass screening test were included for various investigations. The varieties were grown in wooden flats along with the resistant and susceptible checks.

2.3b(i) *Attempts of feeding—Probing marks*: Seven days after germination, the seedlings of each variety were removed from the flats and washed and then transferred individually into test tubes (2 × 17 cm) containing water. Two gravid females were released in each tube. Twelve hours later, the seedlings were transferred into 70% ethyl alcohol. These were then stained and the probing marks counted as described earlier.

2.3b(ii) *Amount of feeding—change in body weight*: Five adult insects per replication were first weighed in a small vial and then starved for 3 hr. The insects were then allowed to feed on 15-day old seedlings in a test tube for double the time of starvation i.e. 6 hr. The insects were again weighed to assess gain or loss in body weight. Each variety was replicated 5 times.

2.3b(iii) *Amount of feeding—honey dew excretion*: Each variety was replicated five times with single thirty day old plant per pot. The plant was drawn through a wooden plank which rested on the rim of the pot. Whatman No. 1 filter paper was placed on the plank by drawing the plant through a slit made in the centre. Then each plant was caged with an inverted glass funnel along with ten pre-starved adult insects (figure 1). The

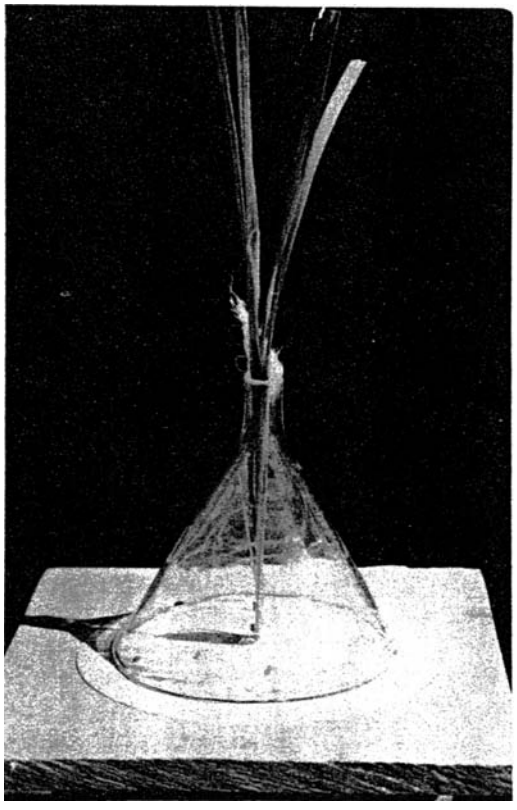

Figure 1. A set-up for honey dew collection.

adults were allowed to feed for 24 hr. The filter papers were removed, dried and sprayed with 0·2% ninhydrin solution, which turned the areas of honey dew excretion to pinkish violet. The coloured portions were dissolved in 80% ethanol and the amount of honey dew excreted was determined by reading the concentration in the spectrophotometer at 540 mμ.

2.4 *Association of Lemma and Palea colour with resistance*

Lemma and Palea colour of the grains of all the varieties (1070) was noted and statistically analysed by the χ^2 test to know the association of Lemma and Palea colour with BPH resistance.

3. Results and discussion

3.1 *Mass screening*

Of the 1070 varieties evaluated in the preliminary tests, 152 varieties showed damage grade up to 2·5 on a 0–5 scale. These were retested in replicated test for confirmation of reaction. ARC 5754, 5757, 5764, 5780, 5838, 5973, 5981, 5500, 5988, 13507, 12864, 13854, 13966, 14394, 14539, 14766(A) and 14903 were resistant (damage score up to 1·5) and 73 varieties indicated moderate degree of resistance recording damage score of 1·6 to 3. The rest of the entries recorded higher damage reaction indicating that these varieties might have escaped the damage in the preliminary test. About 24–36 varieties showing different degrees of resistance were selected for various tests.

3.2 *Studies on preference/non-preference mechanisms*

3.2a *Response of nymphs*: It is evident from table 1 that all the resistant and moderately resistant varieties were relatively less preferred as compared to susceptible check T(N)1. The nymphs could locate the feeding site within 24 hr and no distinct variation was observed between the varieties after 24 hr of release. However, on majority of the resistant varieties there was a decreasing trend in the number of nymphal population settled between 1 day and subsequent observations while on the susceptible variety T(N)1 more number of nymphs were noted. On an average, 4·2–6·5 and 4·5–7·2 nymphs were recorded on resistant and moderately resistant varieties respectively as against 12·7 nymphs on the susceptible check T(N)1. Non-preference mechanism was reported to be a factor of resistance in BPH as early as 1969 (IRRI, 1969). Kalode and Krishna (1979) and 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 T(N)1 and suggested the possibility of some attractants in the susceptible variety. Absence of feeding stimulants or presence of feeding deterrants/repellents could be other possible reasons for non-preference. In the present investigation, a positive correlation was observed with regard to the number of nymphs and damage grade. Higher the number of nymphs settled greater was the damage and *vice-versa*. It appears that non-preference has a definite role in the manifestation of resistance in some of the varieties tested.

Table 1. Preferential response of *N. lugens* (Stål) nymphs and adults on selected rice varieties.

ARC No.	Reaction to nymphs			Reaction to adults		
	Damage score	Average of nymphs settled after days*		No. of adults/seedling after hours		Av. no. of eggs per seedling
		1	7	12	36	
Resistant (R)						
5780	1·1	4·2	4·2	1·3	1·0	17·7
5973	1·1	5·7	4·7	2·2	2·2	33·2
14539	1·4	5·1	4·9	2·1	1·3	32·7
13854	1·4	5·1	4·8	3·0	4·3	66·3
5838	1·5	6·7	6·1	1·7	1·2	20·1
5981	1·5	6·6	5·7	1·7	1·8	28·0
5754	1·5	7·3	6·5	1·0	1·6	22·1
14394	1·5	4·4	4·1	1·3	1·0	34·1
14766A	1·5	5·1	4·9	1·7	2·9	41·7
13507	1·5	5·1	5·1	3·3	1·7	36·3
5988	1·5	5·3	4·7	1·4	1·6	23·7
Moderately resistant (MR)						
5913	1·6	7·3	6·3	2·2	2·2	26·8
14426	1·7	4·5	4·5	2·9	3·8	63·2
15381	1·7	5·3	5·3	2·9	3·9	38·2
5916	1·7	5·7	5·3	1·6	2·1	30·7
5912	1·8	6·2	6·0	2·5	3·0	44·7
13522	1·9	5·4	5·2	1·8	1·0	29·5
5906	2·0	8·1	7·2	1·6	2·0	39·8
5924	2·0	6·0	5·7	1·6	2·5	40·2
5918	2·0	6·1	5·6	1·8	2·8	42·3
Ptb 21	2·1	5·5	5·2	2·3	1·3	28·0
14864	2·1	5·8	5·6	1·9	2·5	38·0
13984	2·2	5·7	5·0	2·0	1·0	25·0
10443	2·2	6·0	5·7	2·7	3·3	44·1
Ptb 33 (Resistant check)	1·2	5·0	4·2	1·3 to 1·5	0·9 to 1·1	16.5 to 25·9
T(N)1 (Susceptible check)	5·0	10·4	12·7	6·0 to 8·9	8·5 to 9·1	186·9 to 210·3

*Average of 4 observations.

3.2b *Response of adults*: Marked differences were observed in the preference of adult BPH after 36 hr, although some differences were apparent even after 12 hr of their release (table 1). ARC 5780 was least preferred by adults followed by resistant check Ptb 33 and ARC 5838, ARC 5754, ARC 5988 for settling and oviposition. Even on moderately resistant varieties the number of adults settled (1–3·9) and the number of eggs laid (25–63·2) were comparatively lower as compared to the susceptible check T(N)1 on which 8·5–9 adults settled and 186·9–210·3 eggs were laid. However, on some of the resistant varieties (ARC 13854 and ARC 14766A) comparatively more number of eggs

were deposited as compared to other resistant varieties. Choi *et al* (1979) reported that resistant varieties which were non-preferred for feeding did not exhibit the same trend towards oviposition also. The reasons could be inconsistent feeding on resistant varieties. In the present investigation varieties which were non-preferred for feeding and shelter were also non-preferred for egg laying with the exception of ARC 13854 and ARC 14766A. The ovipositional preference for these two resistant varieties, may be due to the presence of ovipository stimulants which needs further investigation.

3.3 *Studies on antibiosis mechanism*

3.3a *Survival and development of nymphs*: Antibiosis studies carried out with thirty six varieties indicated that some resistant varieties had adverse effects on BPH nymphs resulting in low survival of the insects (figure 2) as evident with resistant check Ptb 33 (16·5 %), ARC 5780 (18·5 %), ARC 5988 (22·5 %), ARC 5838 (25·6 %), ARC 5981 (26·5 %), ARC 5973 (27·5 %), ARC 5782 (28·5 %), ARC 14766A (26·7 %) and ARC 14394 (28·4 %) as against 90–93·4 % on T(N)1. These varieties also adversely affected the development where the nymphal period was delayed by 3–7 days as compared to that on T(N)1. BPH nymphs took 23 days on resistant check Ptb 33 and ARC 14394; 22 days on ARC 5780, ARC 14766A and ARC 13854.

It was observed that from the 6th to the 21st day there was a gradual decrease in the survival of nymphs. This might be due to nutritional deficiency in the test varieties. However, some varieties *viz.*, ARC 5780, ARC 5988, ARC 5838 had shown relatively higher antibiosis effects. Karim (1975), reported that the survival of the nymphs on resistant varieties *x*B5, Ptb 20 and Mudgo ranged from 8–17 % just 3 days after caging and only 1 and 2 % of the caged nymphs on Ptb 20 and *x*B5 reached the adult stage.

In the present study, a high level of nymphal mortality was not observed as evident by only 19·5–30 % mortality up to the sixth day. This may be either due to the ability of the

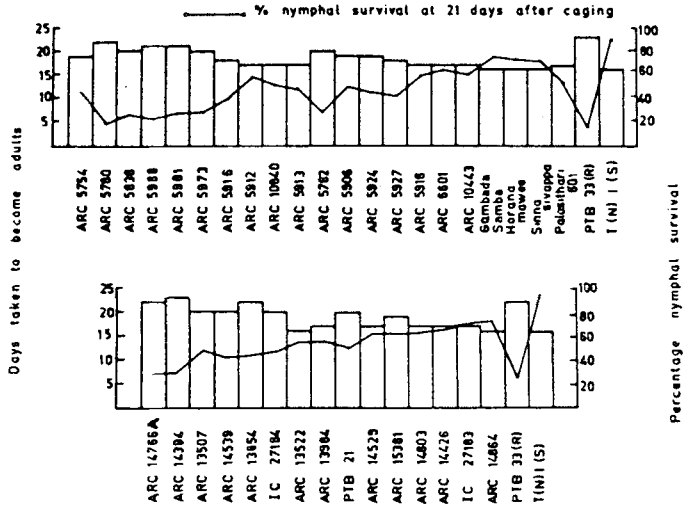

Figure 2. Rate of development and percentage survival of *N. lugens* nymphs on selected rice varieties.

biotype involved in the present study to tolerate antibiosis effects in earlier stages of development or the varieties involved might not be having such high concentrations of toxic components required to induce a high mortality in a short period. However, the resistant varieties could confirm their resistance by virtual low survival of BPH on them, while some moderately resistant varieties had relatively higher survival of BPH nymphs and had relatively low antibiosis effects on them. This indirectly suggests that these varieties might be tolerant to the BPH.

3.3b *Feeding response of adult BPH*

3.3b(i) *Attempts of feeding—Probing marks*: The results of the probing behaviour indicated that the resistant varieties received more number of probing punctures than the susceptible ones. T(N)1 the susceptible check recorded the least number of probing punctures (31·2) whereas a greater number of probing punctures was observed on resistant varieties (figure 3). Resistant varieties *viz.*, ARC 14394 (121), ARC 14766A (119·4), ARC 13507 (115·6), ARC 5780 (100·2), ARC 5838 (94·6), ARC 5754 (83·6), ARC 5988 (83), ARC 5973 (80) and moderately resistant variety ARC 14803 (100·2) received the maximum number of punctures. On the other hand, varieties *viz.*, Mudgo and ASD 7 reported to be resistant to biotype 2 in Philippines, received less number of probing marks compared to T(N)1 in the present investigation indicating that these varieties were suitable for feeding by the test insect.

It was also observed that the percentage of probing marks on the leaf blade was more on the resistant varieties than on the susceptible varieties. The results indirectly revealed that non-preference of BPH to certain varieties may be gustatory rather than olfactory or visual.

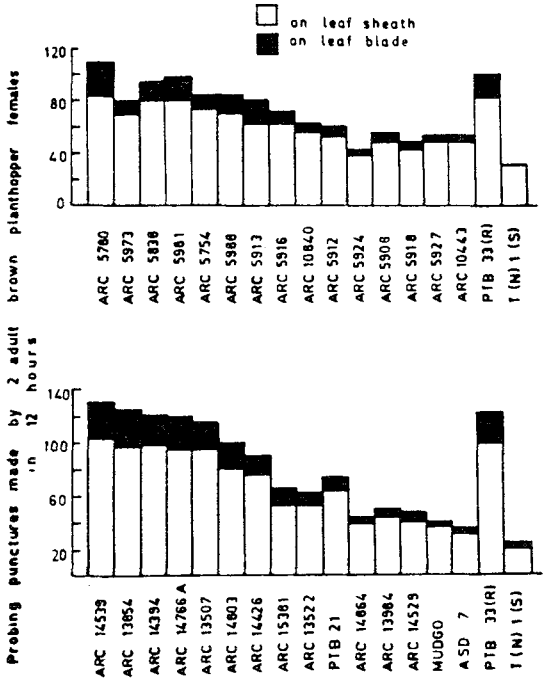

Figure 3. Probing punctures made by *N. lugens* adults on selected rice varieties.

Karim (1975) reported that the varieties *x*B5 and HR 12 received significantly higher number of punctures (50·9 and 48·2 respectively) which were about 2–10 times more than that received by other resistant varieties.

3.3b(ii) *Amount of feeding—change in body weight*: In earlier experiments it was observed that the insects suffered high mortality and made more feeding marks when caged on resistant varieties. In order to know whether the insects had actually fed on the test varieties or not, experiments were carried out on the amount of feeding done by the insects. The results showed that the insects caged on the resistant varieties lost their body weight; while on some of the moderately resistant varieties and susceptible check they gained weight (figure 4). Insects lost their body weight up to a maximum of over 33·3% on ARC 5780 followed by 30% on ARC 13854 and ARC 13507. However, insects fed on ARC 10443, ARC 6601, ARC 5918, ARC 14864, ARC 13984 and ARC 14529 gained some body weight (5·7 to 13·9%) as compared to 27·6% on the susceptible check.

Sogawa (1982) reported that the reduced concentration of phagostimulant amino acids might be the reason for less intake of sap and loss in body weight of the insects which fed on resistant varieties.

3.3b(iii) *Amount of feeding—honey dew excretion*: Amount of feeding by the insects was judged by the honey dew deposited when they were allowed to feed for specified period of time on selected varieties. The results indicated that the BPH adults fed very little on resistant varieties and in turn excreted honey dew in traces (table 2). On resistant varieties ARC 5780, ARC 5973, ARC 5858, ARC 5754, ARC 14539, ARC 13854, ARC 14394, ARC 14766A and ARC 13507, the hoppers excreted as little as 6·6–11·9 times less than that on susceptible T(N)1. As observed in the previous experiments, there was a higher nymphal mortality and loss in body weight on these varieties. These varieties had also received higher number of probing punctures. The less honey dew excretion while

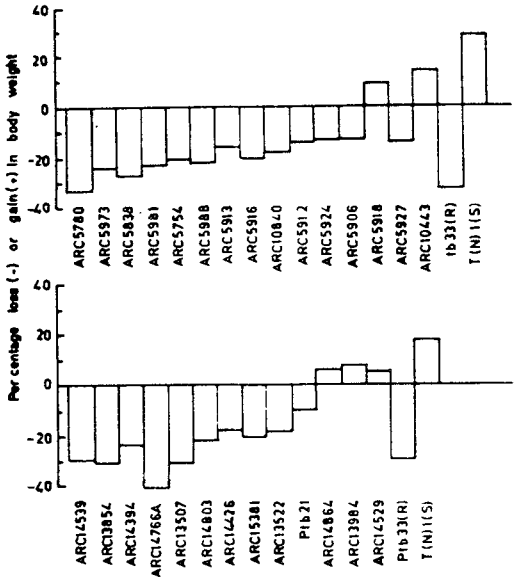

Figure 4. Gain or loss in body weight of *N. lugens* adults on selected rice varieties.

Table 2. Amount of honey dew excreted by brown planthopper adults caged on selected rice varieties for 24 hr.*

Variety	Average	Ratio
Resistant (R)		
ARC 5780	0·20	10·10
ARC 5973	0·29	6·52
ARC 5838	0·17	11·88
ARC 5981	0·23	7·83
ARC 5754	0·28	6·75
ARC 5988	0·22	9·18
ARC 14539	0·34	6·47
ARC 13854	0·28	7·88
ARC 14394	0·20	9·45
ARC 14766A	0·18	10·80
ARC 13507	0·13	6·66
Moderately resistant (MR)		
ARC 5913	0·66	2·86
ARC 5916	0·29	6·52
ARC 10840	0·56	3·38
ARC 5912	0·33	6·12
ARC 5924	0·65	2·91
ARC 5906	0·83	2·43
ARC 5918	0·87	2·32
ARC 5927	0·58	3·30
ARC 10443	0·73	2·60
ARC 14803	0·56	3·93
ARC 14426	0·49	4·49
ARC 15381	0·73	3·01
ARC 13522	0·81	2·72
Ptb 21	0·78	2·82
ARC 14864	0·84	2·62
ARC 13984	1·03	2·13
ARC 14529	1·05	2·10
Ptb 33 (Resistant check)	0·14–0·16	11·78–14·42
T(N)1 (Susceptible check)	1·89–2·20	1·00

*Ten insects per plant; five replications per variety.

feeding on resistant variety may be due to the presence of feeding repellants and/or feeding deterrants or lack of feeding stimulants. It was also observed that the amount of honey dew excreted was in accordance with the degree of resistance, *i.e.*, the insects excreted comparatively little honey dew on resistant varieties than on moderately resistant varieties and susceptible check T(N)1.

Honey dew excretion was considered to be directly proportional to food intake by the insects (Maxwell and Painter 1959; Sogawa and Pathak 1970). Sogawa (1982) opined that reduced concentration of phagostimulant amino acids might be one of the reasons for the less amount of excretion of honey dew on resistant varieties. Kalode and

Table 3. Overall performance of selected resistant and moderately resistant varieties to *N. lugens* (Stål).

Variety	Mass screening	Preference		Survival of nymphs	Probing puncture	Gain/loss in body weight	Honey dew excreted	Resistance index
		Nymphs	adults					
ARC 5780	RR	RR	RR	RR	RR	RR	RR	14
ARC 5988	RR	RR	RR	RR	R	RR	RR	13
ARC 14394	RR	RR	RR	R	RR	RR	RR	13
ARC 5838	RR	R	RR	RR	R	RR	RR	12
ARC 14539	RR	RR	RR	R	RR	RR	R	12
ARC 14766A	RR	RR	R	R	RR	RR	RR	12
ARC 13507	RR	R	RR	R	RR	RR	R	11
ARC 5981	RR	R	RR	R	R	RR	R	10
ARC 5754	RR	R	RR	R	R	RR	R	10
ARC 5973	RR	RR	R	R	R	RR	R	10
ARC 13854	RR	RR	S	R	RR	RR	R	10
ARC 14803	R	RR	R	R	RR	RR	R	10
ARC 14426	R	RR	R	S	R	R	R	7
Ptb 21	R	R	RR	R	R	R	S	7
ARC 13522	R	R	RR	S	R	R	S	6
ARC 10443	R	R	R	S	R	S	S	4
ARC 13984	R	R	RR	S	S	S	S	4
ARC 14864	R	R	R	S	S	S	S	3
Ptb 33	RR	RR	RR	RR	RR	RR	RR	14
T(N)1	S	S	S	S	S	S	S	0
				Grading basis				
RR	upto 1·5	upto 5 nymphs	upto 2 adults	upto 25% survival	above 100	above 20% loss	upto 0·25	
R	1·6–3	5·1–10	2·1–5	25·1–50%	51–100	upto 20% loss	0·26–0·60	
S	above 3	above 10	above 5	above 50	upto 50	gain in body weight	above 0·60	

Krishna (1979) reported that resistant cultivars (Ptb 33, Ptb 21, MR 1523 and ARC 6650) restricted insect feeding and only a little amount of honey dew was excreted by the insects during feeding on these varieties. They further stated that on Leb Mue Nahng and T(N)1 the insects had excreted heavily.

3.4 *Association of Lemma and Palea colour with brown planthopper resistance*

Lemma and Palea colour of 1070 rice varieties noted during the preliminary mass screening test was statistically analysed. The results were non-significant indicating that there was no association of Lemma and Palea colour with BPH resistance.

The overall performance of selected rice varieties based on various parameters to understand the mechanism of resistance as summarised in table 3 indicated that insects fed little on the resistant varieties *viz.*, ARC 5780, ARC 5988, ARC 14394, ARC 5838, ARC 14539, ARC 14766A, ARC 13507, ARC 5981, ARC 5754, ARC 5973, ARC 13854, ARC 14803 and lost their body weight. The nymphs also exhibited high mortality on these varieties, and their development period was also prolonged. The mechanism in manifestation of resistance to BPH appeared to be due to non-preference and antibiosis. Most of the varieties which were less preferred by nymphs were also less preferred by adults for settling and oviposition.

However, in some of the moderately resistant varieties like ARC 5918, ARC 10443, ARC 13984, ARC 14529 and ARC 14864 relatively less number of feeding marks, higher amount of honey dew excretion and gain in body weight were observed as compared to the resistant varieties indicating intermediate reaction to the pest. It is evident that the number of probing punctures, amount of honey dew excretion and gain or loss in body weight are all inter-related to the degree of resistance. Based on these parameters ARC 5780, ARC 5988 and ARC 14394 were comparable to resistant check, Ptb 33 in level of resistance.

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