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Antixenosis and antibiosis mechanism of resistance in selected rice entries against brown planthopper, *Nilaparvata lugens* (Stal)

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ABSTRACT

Six different rice entries along with susceptible and resistant check varieties were evaluated under glasshouse conditions for different parameters of antixenosis and antibiosis resistance against brown planthopper (BPH, *Nilaparvata lugens* Stål). In antixenosis studies, proportion of insects settled on test entries in relation to the susceptible control TN1 was recorded, with average lower proportion of nymphs settled on N22-CC-DTM-893 and Ptb 33 in relation to those on TN1. In antibiosis studies adult population, adult longevity and population build-up were recorded. N22-CC-DTM-893 and Ptb 33 displayed significantly better performance as compared to other test entries in these parameters studied and did not differ from each other. These results helped in relative quantification of BPH resistance levels in different test entries and N22-CC-DTM-893 show substantial levels of antixenosis and antibiosis effects on BPH and it is considered as new effective source of BPH resistance and can be used in resistance breeding after tagging of resistant genes/QTLs linked to brown planthopper resistance with selectable molecular markers.

Key words: Antixenosis, Antibiosis resistance, Molecular markers, *Nilaparvata lugens*, Resistance breeding, Rice.

Introduction

Rice (*Oryza sativa* L.) is cultivated under different ecosystems of tropical and sub-tropical regions of the world. With the projected increase in world population may be expected to 9–10 billion by 2050 it is a great challenge to meet the food requirements of this population. Various biotic factors are constraints for rice production. Among various biotic

factors insect pests are of prime importance (Heong and Hardy, 2009). More than 100 species of insects are reported as pests of this crop, 20 are of major economic importance (Prakash *et al.*, 2007). The brown planthopper (BPH), *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae) is one of the economically important pest and frequent occurrence of this insect attack has been identified as the key for the low rice productivity. It is a typical phloem sap

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feeder that has emerged as the threat to rice crop and that causes significant yield losses in most of the rice cultivars in Asia (Prasannakumar *et al.*, 2013). BPH has the capability of damaging rice crop from vegetative stage to reproductive stage.

Both nymphs and adults of BPH suck sap from the lower portion of the plant, which results in yellowing of leaves, reducing number of tillers, plant height and increasing in unfilled grains. Feeding also causes the reduction in chlorophyll and rate of photosynthesis and in case of severe infestation it causes extensive plant mortality referred as 'hopper burn' symptom (Watanabe and Kitagawa, 2000 and Vanitha *et al.*, 2011). BPH also act as vector of grassy stunt and ragged stunt viral diseases thus causing severe losses to rice crop (Bottrell and Schoenly 2012). Regular monitoring of rice fields is crucial for timely detection of its incidence and helps in effective pest management. Many conventional insecticides are recommended for manage this pest but they detrimentally affect the delicate balance between BPH and natural enemies in rice ecosystem (Yang *et al.*, 2017) development of biotypes, developed resistance against insecticides and thus aggravating the planthopper problem.

Cultivation of resistant varieties is the most economical and environmentally safe strategy for effective management of this insect pest. Such varieties were useful to minimize the pesticide applications (Sarao *et al.*, 2016). Resistant and moderately resistant cultivars keep the pest densities below the economic threshold levels and also useful to increase natural enemies population (Gurr *et al.*, 2011). So there is a need to identify new sources for resistance. Hence, breeding programme for development of BPH resistant varieties with different mode of host plant resistance is extremely important. Screening rice germplasm at global level and breeding BPH resistant rice varieties were initiated during 1970s and several resistant varieties have been released for cultivation (Kumar and Tiwari, 2010; Li *et al.*, 2011, Sarao and Bentur, 2016). Host plant resistant studies are useful for characterize the relationship between insect populations and rice varieties (Padgham and Woodhead, 1988) and also useful in resistance gene/QTL tagging and mapping (Harini *et al.*, 2013; Ali and Chowdhury, 2014). Hence the current work was undertaken to study antixenosis and antibiosis levels in selected rice entries.

Materials and Methods

Insects

The source BPH population was collected from unsprayed rice fields of paddy breeding station (PBS), Coimbatore. Insects were collected and continuously reared under greenhouse conditions on 30-45 day-old TN1 rice plants as per the protocol of Heinrichs *et al.* (1985).

Rice materials

Six different rice entries includes IR71033-121-15 (*Bph20* and *Bph21*), N22 (short duration aus rice), their mutants N22-CC-DTM-893, N22-MG-145, N22-MG-491, N22-MG-516 along with susceptible and resistant check varieties (TN1 and Ptb 33). The pre-germinated seeds of the test entries were sown in pots or trays depending on the experiment, containing well puddled soil. All the test plants were raised in an insect-proof greenhouse.

Antixenosis studies

Settling behavior of nymphs

In this experiment, pre-germinated seeds of the test entries were sown in random rows, 3 cm apart, in a seed box (60 cm × 40 cm × 12 cm). Each row contained 12-14 seeds. The susceptible control TN1 was sown in two border rows and resistant control was sown in centre of the box. The tray was kept in dark place to enhance seedling growth. The 7-day-old seedlings were infested with the 2nd-3rd instar hopper nymphs with 6-8 nymphs per seedling.

The tray was covered with light-transmitting nylon mesh to prevent escape of nymphs. The number of nymphs settled on each seedling was counted at 1, 2 and 3 days after infestation. The seedlings were disturbed after each count for reorientation of the hopper nymphs.

Adult population: sex ratio

To study sex ratio 10 early instar nymphs were released on 30-day-old plants of the test entries and covered with mylar cages. The number of nymphs that reached adulthood were counted and their sex ratio were calculated.

Adult longevity

To study adult longevity five freshly emerged male and female insects were released on potted rice plants and covered with mylar cages. The caged

adults were daily observed for mortality. Survival male and female insects were recorded daily till the hoppers died. The period between the release of insects and the insect mortality was recorded as the adult longevity.

Population development of BPH on different rice entries

For this experiment three pairs of newly emerged adult insects were released on uninfested potted rice plants and covered with mylar cages. Adults were removed after 5 days. Plants were observed daily for nymphal emergence. Nymphs emerged from plants were counted daily and removed. The total number of nymphs was recorded.

Results and Discussion

Antixenosis studies

Significant differences in the settling response of *Nilaparvata lugens* nymphs on different rice entries were evident at different hours after release when allowed in free choice experiment (Table 1) and it ranges between 4.05- 8.06. 24 hours after release least number of nymphs settled on N22-CC-DTM-893 (4.05) followed by Ptb 33 (4.92). Similarly, 48 hours after release the least number of nymphs settled on Ptb 33 (3.06) followed by N22-CC-DTM-893 (3.31). Likewise, 72 hours after release least number of nymphs settled on N22-CC-DTM-893 (2.83) followed by Ptb 33 (2.84). Highest number of nymphs observed on TN1 in all the observed days.

Ptb 33 harboured minimum nymphal population

(Table 1). Earlier reports also suggested that Ptb 33 had strong antixenosis effect to brown planthopper infestation (Alagar *et al.*, 2007). Several reports suggested that higher number of BPH nymphs settled on susceptible genotypes as compared to resistant ones (He *et al.*, 2013).

Our findings are supported by the findings of Alagar *et al.*, (2007) and Gangaram *et al.*, (2019) as they observed that the settling response of nymphs was more apparent at 24 hours after infestation on all the tested rice entries and also supported by Madurangi *et al.* (2013) as they reported that over the time insects preferred susceptible plants compared to resistant plants. So on Ptb 33 and N22-CC-DTM-893 there is decrease in nymphal population at 48 and 72 hrs of observation as compared to 24 hr (Figure 1).

Time series of observations suggest that nymphs or adults tend to move to the susceptible plants with increasing exposure. Thus, it appears that antixenosis is more guided by feeding response rather than olfactory or tactile stimuli (Sarao and Bentur, 2016).

Nymphal preference is also influenced by mechanical barrier to penetrate for probing, difference in colour, hairiness or presence of anti-feedant compounds (Horgan, 2009). These differences were reflected due to genetics of the genotypes. Genetic basis of antixenosis is just emerging. Only several QTLs linked to different parameters of antixenosis against both BPH and WBPH are reported in rice (Fujita *et al.*, 2013). Qiu *et al.* (2013) reported a QTL*Qbph8* along with the major gene *Bph6* in rice

Table 1. Preference of *Nilaparvata lugens* nymphs for settling on selected rice entries

| Rice Entry | 24 HAI | 48 HAI | 72 HAI | Avg. No. of nymphs on test entries |
|------------------|---------------------------|--------------------------|----------------------------|------------------------------------|
| IR 71033-121-15B | 5.21 ^{cd} (2.49) | 4.86 ^c (2.42) | 4.62 ^{cd} (2.37) | 4.90 ^{de} (2.43) |
| N22 | 6.95 ^{ab} (2.82) | 6.07 ^b (2.66) | 7.07 ^b (2.83) | 6.70 ^b (2.77) |
| N22-CC-DTM-893 | 4.05 ^e (2.24) | 3.31 ^e (2.07) | 2.83 ^e (1.96) | 3.39 ^f (2.10) |
| N22-CC-MG-145 | 5.02 ^{cd} (2.45) | 4.09 ^d (2.26) | 4.55 ^d (2.35) | 4.55 ^e (2.36) |
| N22-CC-MG-491 | 4.93 ^{de} (2.43) | 5.29 ^c (2.51) | 5.90 ^{bc} (2.63) | 5.37 ^{cd} (2.52) |
| N22-CC-MG-516 | 6.12 ^{bc} (2.67) | 4.95 ^c (2.44) | 5.54 ^{bcd} (2.55) | 5.54 ^c (2.55) |
| Ptb 33 | 4.92 ^{de} (2.43) | 3.06 ^e (2.01) | 2.84 ^e (1.96) | 3.61 ^f (2.15) |
| TN1 | 8.06 ^a (3.01) | 8.19 ^a (3.03) | 8.68 ^{cd} (3.11) | 8.31 ^a (3.05) |
| SEd | 0.10 | 0.07 | 0.12 | 0.05 |
| CD(0.05) | 0.21 | 0.15 | 0.27 | 0.11 |

Table 1: Figures in parenthesis are square root transformed values. Different letters represent significant difference at 0.05% level.

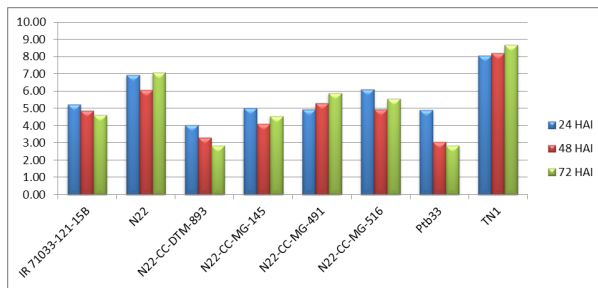


Fig. 1. Number of brown planthopper nymphs settled on different rice entries

variety Swarnalata accounting for antixenosis in BPH.

Adult population: Sex ratio

From total survival of adults female emergence differed significantly among different test entries and it is in the range between 40.00-83.33%. Fewer females emerged from Ptb33 (40.00%) followed by those from N22-CC-DTM-893 (46.67%) and highest population was on TN1 (83.33%) (Table 2). Another influence of plant resistance was seen in the distorted sex ratio reported for BPH by Lakra (2016). In the present investigation, emergence of females was more than male insects on all test entries except Ptb33, N22-CC-DTM-893, N22-CC-MG-516 and IR71033-121-15. Our results are supported by Sarao and Bentur (2018) as they reported that higher percentage of males were produced on the resistant genotypes than on the susceptible ones.

Adult longevity

The longevity of females was shorter than that of

males in all test entries of 45 day old plants (Table 2). The female longevity was minimum in Ptb 33 (7.64 days) followed by N22-CC-DTM-516 (8.28 days). The maximum female longevity was observed on susceptible check, TN1 (14.72 days). The male longevity was found to be minimum in N22-CC-DTM-516 (5.68 days) followed by Ptb 33 (5.84 days) and maximum longevity was observed on N22 (9.80 days).

In general the female especially if mated live longer than the males of same age. The plant age had significant influence on the longevity of males of Delfacid insects on all the genotypes earlier reported by Rath and Mishra, (1998) and Sable *et al.*, (2014). Our results are supported by Kumar *et al.*, (2012), as they reported that reduced longevity of adults on resistant genotypes. Reduction in the longevity may be due to the forced feeding on resistant genotypes containing either toxic chemicals or deficient nutrients or low amino acids. Sometimes longevity is reduced due to competition for feeding. The plant chemicals influenced the physiology of phytophagous insects in various ways and nutritional effects on growth and development.

Population buildup

Significant variation in the population build-up was observed in the test entries (Table 2). The number of nymphs emerging on test plants ranged from 61.20 to 240.40 nymphs. The lowest population was observed on resistant check Ptb 33 (61.2) followed by N22-CC-DTM-893 (73.00) highest population was observed on susceptible check TN1 (240.4). This population indicates the antibiosis in these entries.

Table 2. Reaction of different rice entries to *Nilaparvata lugens* feeding

| Rice Entry | Adult population (%)* | | Adult longevity (days)** | | Population buildup (No.)** |
|-----------------|------------------------------|------------------------------|----------------------------|----------------------------|------------------------------|
| | Female | Male | Female | Male | |
| IR71033-121-15B | 48.00 ^{cd} (43.83) | 52.00 ^{abc} (46.13) | 11.04 ^{bc} (3.47) | 7.00 ^b (2.83) | 110.60 ^{bc} (10.54) |
| N22 | 78.25 ^{ab} (62.71) | 21.75 ^{de} (27.25) | 11.76 ^{ab} (3.57) | 9.60 ^a (3.24) | 145.60 ^b (12.01) |
| N22-CC-DTM-893 | 46.67 ^{de} (43.03) | 53.33 ^{ab} (46.93) | 8.72 ^d (3.12) | 6.56 ^b (2.75) | 73.00 ^{de} (8.56) |
| N22-CC-MG-145 | 62.10 ^{bcd} (52.00) | 37.90 ^{bcd} (37.96) | 11.08 ^{bc} (3.47) | 9.12 ^a \ (3.18) | 102.40 ^{cd} (10.04) |
| N22-CC-MG-491 | 67.38 ^{abc} (55.23) | 32.62 ^{cde} (34.73) | 11.60 ^{ab} (3.54) | 9.72 ^a (3.27) | 123.80 ^{bc} (11.12) |
| N22-CC-MG-516 | 48.43 ^{cd} (43.96) | 51.57 ^{abc} (46.01) | 8.28 ^{cd} (3.04) | 6.28 ^b (2.69) | 111.40 ^{bc} (10.57) |
| Ptb 33 | 40.00 ^e (35.99) | 60.00 ^a (53.99) | 7.64 ^d (2.94) | 5.92 ^b (2.63) | 61.20 ^e (7.84) |
| TN1 | 83.33 ^a (66.17) | 16.67 ^e (23.79) | 14.72 (3.96) ^a | 8.60 ^a (3.10) | 240.40 ^a (15.53) |
| SE (d) | 6.09 | 6.10 | 0.09 | 0.10 | 0.74 |
| CD (0.05) | 12.47 | 12.48 | 0.19 | 0.21 | 1.52 |

*Figures in parenthesis are arc-sine transformed values.

** Figures in parenthesis are square root transformed values

Different letters represent significant difference at 0.05% level.

The preferred genotypes may have favoured more feeding, increased fecundity and quicker life cycle (Painter, 1958). Painter (1951) reported that the resistant or moderately resistant genotypes generally permit very low population build-up. However, Kim *et al.* (1982) reported that the resistant accessions could support high population build up of BPH and decline in population during the plant maturity.

Different antibiosis experiments the population build-up is the most practical one (Panda and Heinrichs, 1983). Population increase represents the combined effects of feeding rate, nutritional value of food, ovipositional rate and adult emergence (Heinrichs and Rapusas, 1983). Our results are in agreement with the findings of Alagar *et al.* (2007) and Boopathi and Bharathi (2008) which reported that the population of first generation nymphs were lowest on resistant genotypes.

From these experiments it might be concluded that since the resistant genotypes are less preferred, the insects would have fed less on them which in turn resulted in lesser plant damage than in susceptible TN1.N22-CC-DTM-893 displayed high levels of antixenosis and antibiosis levels to BPH feeding as like resistant check. This will provide better option for plant breeders and biotechnologists to develop suitable varieties to combat BPH. It is apparent from our study that development of resistant varieties which can disrupt the settling and feeding of BPH could play a pivotal role in pest management strategies.

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