of these features are desirable characteristics of xylem specialist bacteria that might be developed for biological control or biotechnological uses in crop plants. Once such bacteria are detected by microscopy, they can be concentrated from xylem sap collections by centrifugation and mechanically re-inoculated into additional plants to increase the numbers of colonized plants. Culture media and methods could then be developed to attempt to culture the bacterium. This method will not apply to bacteria that do not meet the three criteria listed above or that cannot colonize plants via mechanical inoculation.

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Study on interactions between *Nilaparvata lugens* Stål (Hemiptera: Delphacidae) and mutants of IR64, a commercial cultivar of rice (*Oryza sativa* L.): a step to discover insect defense genes

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The brown planthopper (BPH), *Nilaparvata lugens* Stål is an important pest of rice in Asia. It causes severe hopper burn and susceptible rice cultivars can suffer up to 60% yield loss from its attack (Panda and Khush, 1995). Many varieties have been developed carrying different genes for *N. lugens* resistance but the resistance does not appear to be durable. *N. lugens* can quickly evolve resistance to these genes. There is thus need to identify new sources for durable resistance and transfer genes/ QTLs to develop varieties resistant to *N. lugens*.

IR64, a popular cultivated rice variety, has moderate resistance to BPH. One major gene and several QTLs contributing to resistance in IR64 has been identified (Alam and Cohen, 1998). But this variety does not tolerate high *N. lugens* infestation. Enhancing resistance of this variety will be useful for sustainable crop production. The IR64 mutant collection has been developed at IRRI through physical and chemical mutagenesis and is being used to understand defense pathways in rice (Leach et al. 2001). The mutants with higher insect resistance and good agronomic performance can be isolated and used for testing the practical effectiveness of candidate defense genes.

In order to understand interactions between *N. lugens* and host plants and to identify genes responsible for insect resistance, we initiated research on two kinds of mutants (a) gain of resistance (GOR) and (b) loss of resistance (LOR). We studied how *N. lugens* responds on mutants and IR64, and what are the protein expression differences between mutants and IR64 under stress induced conditions.

Methods and Materials

The plant material consisted of IR64, a GOR mutant and a LOR mutant. All the experiments were conducted using the *N. lugens* colony reared on a susceptible variety, Taichung Native-1 (TN1) at the IRRI greenhouse. To test if *N. lugens* shows differences in preference to settle and oviposit on test entries, a free-choice experiment was conducted. Thirty days after sowing, 3 potted plants of each entry were arranged in a circle in a replication. Six *N. lugens* females per plant were released in the centre. The females were counted on the plants 24, 48 and 72 hours after infestation. We also measured *N. lugens* performance based on honeydew production in parafilm sachets, nymphs per adult, development and survival of nymphs and adult weight on different entries. Tolerance was measured based on dry weight biomass (Dixon et al. 1990) and recovery of plants for grain yield after 15 days of stress at seedling stage. We used a randomized complete block design (RCBD) with 15 replications for all experiments.

Two dimensional gel electrophoresis was used to determine whether protein expression profiles from the leaf sheaths of resistant and susceptible lines differed in response to *N. lugens* feeding. Sampling was done at various points of *N. lugens* development on rice plants to characterize the timing for constitutive and induced expression of defense genes.

Results

N. lugens females avoided settling and egg laying on the GOR seedlings. Significantly fewer *N. lugens* were recorded on the GOR after 24, 48 and 72 hours of infestation. The *N. lugens* fecundity was also low on GOR. The amount of honeydew excreted by *N. lugens* on GOR seedlings was lower than on other entries. The number of nymphs per female recorded on GOR was significantly lower than on LOR and IR64. Development and survival of nymphs and weight of *N. lugens* adults on both GOR and LOR mutants did not show significant differences from IR64. The GOR mutant was more tolerant to *N. lugens* stress, as its tolerance index value was significantly lower than LOR and IR64. It also produced significantly higher biomass and grain yield than IR64.

Protein profiles of the two mutants and IR64 from 2D gels showed a number of both constitutive and stress-induced differences that were reproducible. One constitutively expressed protein in the GOR line had a lower molecular weight and slightly higher pH value than its original form in IR64. This indicates that some amino acids have been removed from the pure form of the protein. The differences in induced expressions of proteins were found only after emergence of second generation nymphs, i.e., 23 days after infestation with parent *N. lugens*. Three induced proteins were over-expressed in GOR and down-regulated in LOR as compared with IR64. These proteins are currently being sequenced to identify candidate genes involved with resistance and to study their role in mechanism of resistance.

Discussion

Non-preference shown by insects as well as low antibiosis and tolerance of plants to insect feeding are considered key components for durable resistance. This type of resistance will have little selection pressure on the insect population. Such mechanisms of resistance in rice may involve presence of certain toxic chemicals (Grayer et al. 1994; Zhang et al. 1999). Low content of amino acids have also been reported as a cause for *N. lugens* resistance in rice (Sogawa and Pathak, 1970). The GOR mutant exhibits resistance to *N. lugens* either due to the presence of these deterrents or a nutritional deficiency in certain amino acids for the insect's diet. This could explain avoidance by *N. lugens* to settle, oviposit and feed. GOR is also morphologically similar to IR64. The pattern of F3 segregation suggests that resistance in GOR is controlled by a single dominant gene. Thus it is a valuable elite germplasm for developing durable resistance to *N. lugens*.

From above studies, the mutants seem useful for understanding defense pathways and to find candidate genes. Mutations of genes in IR64 might have resulted in gain and loss of resistance to *N. lugens*. Sequencing of up-regulated or down-regulated proteins will help to identify candidate defense genes and understand defense pathways involved with *N. lugens* stress. Once the genes for resistance are identified, they may be used in improving the rice germplasm to protect the crop from *N. lugens* damage.

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