Video Article

Preference Assessment of White-backed Planthopper Feeding on Rice

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Abstract

Exploiting insect-resistant rice germplasm resources and related genes is the primary need for breeding insect-resistant varieties, but the accuracy of the identification of insect-resistant phenotypes of rice is a major difficulty. It is urgent to develop a new method or improve existing methods to screen rice for insect resistance. This article describes a simple and feasible method to assess nonpreference-type resistance of rice to the white-backed planthopper (WBPH), *Sogatella furcifera*, in the laboratory. The preference of adult WBPHs feeding or inhabiting on maturing rice plants is continuously analyzed by pairwise comparison. The dynamic changes of WBPHs on rice plants are recorded and compared as an index of resistance identification. The current method is simply operable and easily observable and has a short cycle. The use of this method could be extended to investigate the feeding and oviposition preference of similar hemipterans, such as the brown planthopper (BPH), *Nilaparvata lugens*(Stål).

Introduction

Rice is a staple food for over one-third of the world's population, and more than 90% of rice is produced and consumed in Asia[1,2]. The WBPH and BPH are the most destructive pests of rice and a substantial threat to rice production[3]. From the perspective of cost and environment, the breeding and application of insect-resistant rice is the most effective approach to control the damage caused by planthoppers[4,5,6]. Accordingly, the screening of resistant rice germplasm resources is a key prerequisite for breeding insect-resistant rice. The accuracy in the identification of rice-resistant phenotype is helpful for fine mapping and further functional research of target genes. However, phenotypic identification has become a major difficulty due to the complexity of the resistance mechanism. Rice's resistance to pests can be divided into three types, namely antibiosis, tolerance, and nonpreference[7]. Each type reflects a different aspect of the resistance mechanism of rice to pests. At present, the most widely used method of screening for resistance to planthoppers is the standard seedbox screening technique (SSST) which can be used to quickly identify the phenotypic resistance of a large number of rice plants and to obtain candidate resistant germplasm lines in a short time[8].

However, the SSST method only reflects the resistance of rice at the seedling stage and is more effective in assessing tolerance-type resistance mechanisms. Rice's resistance to insects is also reflected in antibioses, such as nymph survival rate, nymph duration, and egg hatching rate, and in nonpreference, such as habitat, feeding, and oviposition preference[9]. In addition, the performance of rice seedlings for resistance is often not very stable. With the growth of plants, resistance tends to become more stable. Therefore, the SSST method cannot completely reflect the resistance level of rice. Moreover, rice's resistance to pests varies at different growth stages, and there are obvious differences in resistance mechanisms between seedling and maturing plants. Studies have shown that maturing rice plants can release volatile secondary metabolites to avoid infestation by insect pests, which are manifested by the insect's nonselectivity in feeding or oviposition on the rice plant[10,11]. This is also a very critical kind of resistance mechanism, which plays an important role in preventing insect pests and ensuring rice yield at maturity.

At present, the identification of rice's resistance by nonpreference is still a challenge. In this case, two main approaches are currently used. On the one hand, planthoppers and rice plants are put in a square nylon net cage[12]. Although this approach is considered to be relatively efficient for carrying out experiments on multiple rice lines simultaneously, it requires a larger experimental space and, thus, causes some difficulties in observation and counting due to nontransparent nylon net materials. On the other hand, the Y-tube olfactometer method is used in insect selection experiments according to the difference in volatile substances released from rice. This method facilitates easy observation because of its glass container[14]. One of the major limiting factors of this method is that it can only judge volatile smell, and it also has a strict requirement on the tightness of the experimental devices and takes a long time.

Herein, we describe an improved method for evaluating the nonpreference-type resistance of the rice plant to WBPHs, which is simple to operate and easy for observation. This method can also be used to study the habitat, feeding, and oviposition preference behavior of BPHs and other hemipterous pests.

Protocol

1. Preparation of planthoppers, rice plants, and the polyvinyl chloride cage

1. **Planthoppers**

1. Rear WBPHs on tillers of a susceptible rice variety called Taichung Native 1 (TN1) in insect-proof cages and let them reproduce naturally for generations. Choose long-winged, newly emerged female adults for further experiments.
   NOTE: WBPHs were provided by the Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences.

2. **Rice plants**
   1. Soak seeds of each rice line in water and put them into a climate-controlled room with parameters set to 28 °C, 75%-80% relative humidity (RH), and cycles of 14 h light/10 h dark for 2 days until germination.
   2. Sow 30 germinated seeds of each tested rice line evenly in a plastic seedbox (20 cm [length] x 15 cm [width] x 10 cm [height]) which is filled with paddy soil to a depth of 3-4 cm.
   3. Cover the seeds with a thin layer of fine dry soil; then, wet the dry soil with water.
   4. Put the seedbox in a 200 mesh insect-proof cage (75 cm [length] x 75 cm [width] x 75 cm [height]) at 28 °C, with 75%-80% RH and a 14 h light/10 h dark cycle treatment in a climate-controlled room. Water every day to keep the soil moist. Continue growing the plants for 7 days, till they have reached the two- to three-leaf stage.
   5. Choose 20 seedlings with similar growth potential, transplant the seedlings into 10 cm-diameter plastic seed pots (one seedling per pot) with a hole at the bottom.
   6. Place the pots in a 200 mesh insect-proof cage (75 cm [length] x 75 cm [width] x 75 cm [height]) at 28 °C, with 75%-80% RH and a 14 h light/10 h dark cycle treatment in a climate-controlled room, with water at the bottom of the tray, for about 30 days of growing till they reach the tillering stage with one or two tillers.
   7. Trim the rice plants to one tiller 48 h before starting the experiment.

3. **Cylindrical polyvinyl chloride cage**
   1. Obtain transparent polyvinyl chloride (PVC) with dimensions of 120 cm x 90 cm and a thickness of 0.5 mm.
   2. Make it into a cylindrical structure with a height of 90 cm and a diameter of 35 cm.
   3. Use a stapler to fix the overlap area at both ends of the cylinder. Ensure the overlap area is about 90 cm in length and 10 cm in width.
   4. Seal the entire overlap area from the periphery of the cylinder with pressure-sensitive tape.
      NOTE: Ensure that the cylindrical cage can be placed vertical to the ground and there is no obvious gap between the cage and the ground.
   5. Cut 200 mesh nylon nets, each with dimensions of 50 cm x 50 cm; prepare enough for the subsequent steps.
   6. Get adequate rubber bands; make sure the diameter is about 1.5 mm and the circumference is at least 32 cm when the band is contracted.

2. Insect and rice treatment

1. Place a round plastic tray with a diameter of 28 cm and a height of 10 cm on flat concrete ground in a climate-controlled room with parameter settings as described in step 1.2.6.
   NOTE: If the greenhouse floor is soil, find an as flat as possible surface to ensure that the tray is laid flat.
2. Choose two pots of different rice lines (from step 1.2.7) and put them into the tray, side by side, and fill the plastic tray with enough water.
3. Cover the two test rice pots with the cylindrical cage made in step 1.3.4.
4. Put one piece of nylon net (from step 1.3.5) on top of the cage.
   NOTE: The two rice pots in the cage can be used as a group; repeat 15 sets of each group. Place the rice pots randomly in position and direction, but try to ensure that the leaves of the two rice plants do not touch.
5. Use a handmade suction trap to collect 40 newly emerged female WBPH adults (refer to section 1.1).
6. Put the WBPH adults into a glass tube (with a diameter of 2 cm and a height of 15 cm) and cover it with a sponge stopper.
7. Lift up a corner of the nylon net (see step 2.4).
8. Remove the sponge stopper from the glass tube and put the tube in the middle part of the cage to release all WBPHs.
9. Cover the nylon net quickly and use a rubber band to seal it to prevent the WBPHs from escaping (**Figure 1**).

3. Recording and observation

1. Observe the distribution of WBPHs on each rice plant at 3, 6, 24, 48, 72, 96, and 120 h after the infestation.
2. Record the number of WBPHs on different rice plants including leaf sheath and leaf from all directions through the transparent cage.
   NOTE: Be gentle during the observation process so as not to disturb the WBPHs.

Representative Results

There were three test rice lines used in this study. Rice line FY01 is WBPH susceptible and used as control group. Rice line HZ08 and HZ06 were transgenic lines in which the potential WBPH resistant X1 gene and X5 gene were introduced, respectively, based on the background of FY01. Therefore, a rice resistance comparison between HZ08/HZ06 and FY01 could reveal whether the corresponding inserted gene had a potential resistance function. In this study, the resistance of two rice plants in terms of feeding or inhabiting was compared, thus revealing whether these two genes were potentially involved in the mechanism of nonpreference-type resistance.

The number of WBPHs on experimental rice plants at different time points was recorded. The ratio of WBPHs was obtained by dividing the number of WBPHs on each plant by the total number of WBPHs on the two rice plants in the cage, excluding the WBPHs located elsewhere, such as on the ground and cage walls. A commercial software was used for further statistical data analysis. One-way analysis of variance (ANOVA) was applied, followed by Tukey's multiple range test, to assess the significant difference between the compared groups. Percentage data were transformed to arcsine square root for ANOVA. The results showed that for the comparison experiment between rice HZ08 and FY01, the number of WBPHs on both rice lines were relatively small at the initial stage from 0 to 6 h (**Figure 2A**). Most of the WBPHs did not show feeding activities and might have been in an adaptive stage. However, both the number and the ratio of WBPHs on the FY01 line were significantly different from those on the HZ08 line (**Figure 2A** and **Figure 3A**). After 24 h, the number of WBPHs on the FY01 line increased gradually. Nevertheless, the number of WBPHs on the HZ08 line remained low from the beginning to the end of the experiment after 120 h (**Figure 2A**), and the ratio showed the same result (**Figure 3A**), which was indicating an obvious preference for inhabiting and feeding on FY01. This also implied that the corresponding inserted X1 gene is a candidate gene for rice resistance and may be associated with the nonpreference resistance mechanism.

The results for the rice lines HZ06 and FY01 were different than the results for HZ08. The number of WBPHs inhabiting the rice lines HZ06 and FY01 were relatively large at the beginning, but there was no obvious difference between the number and ratio of WBPHs on line HZ06 and those on line FY01 (**Figure 2B** and **Figure 3B**). As the experiment went on, the number and ratio of WBPHs on each rice line changed slightly, but the differences were not significant. This result showed that WBPHs had no feeding or inhabiting preference between the two rice lines, and also indirectly indicated that the inserted gene X5 did not participate in the mechanism of nonpreference resistance.

These results indicated that this method could be used for the identification of rice's resistance to WBPHs. Rice line HZ08 was successfully identified as resulting in a nonpreference by WBPHs for feeding or inhabiting when compared to the control line FY01, thus showing a certain resistance to WBPHs. On the other hand, compared with the control group, rice line HZ06 showed no nonpreference resistance to WBPHs.

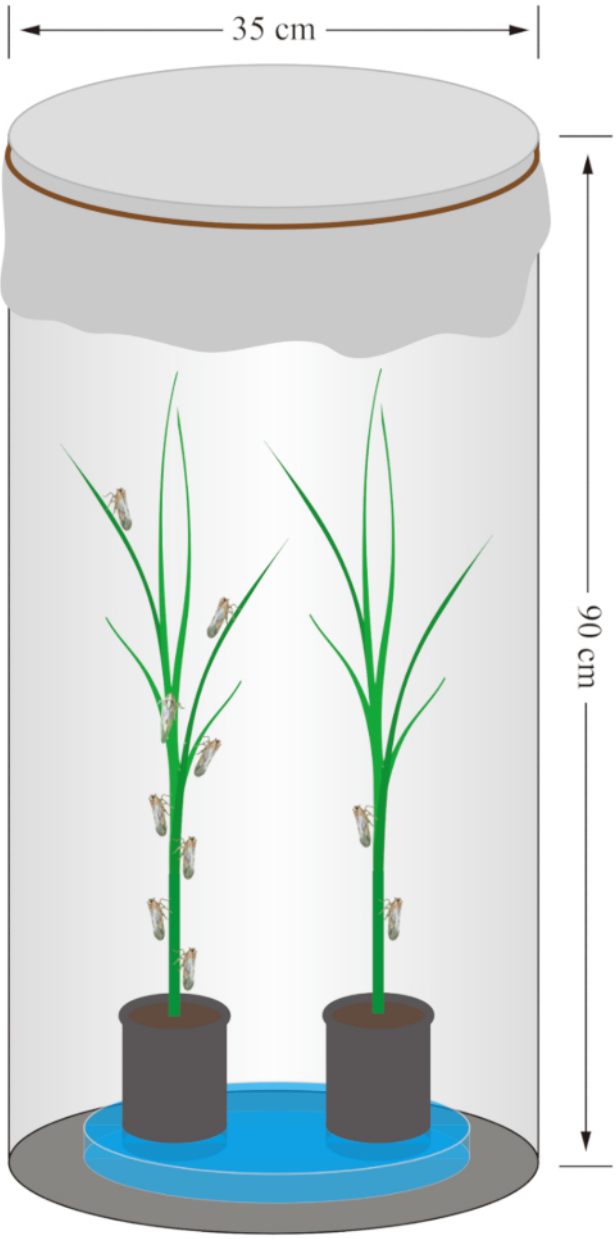

Figure 1: Diagram of the nonpreference experiment of WBPHs feeding on rice in this study. Please click here to view a larger version of this figure.

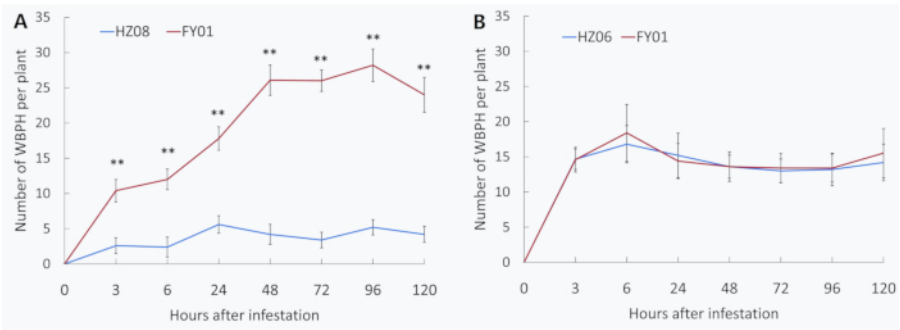

Figure 2: Dynamic changes of WBPH populations on experimental rice lines. (**A**) WBPH populations on single seedlings of HZ08 and FY01. (**B**) WBPH populations on single seedlings of HZ06 and FY01. Error bars, mean ± standard error (SE), by Tukey's multiple range test (n = 15, $^{**}P < 0.01$). Please click here to view a larger version of this figure.

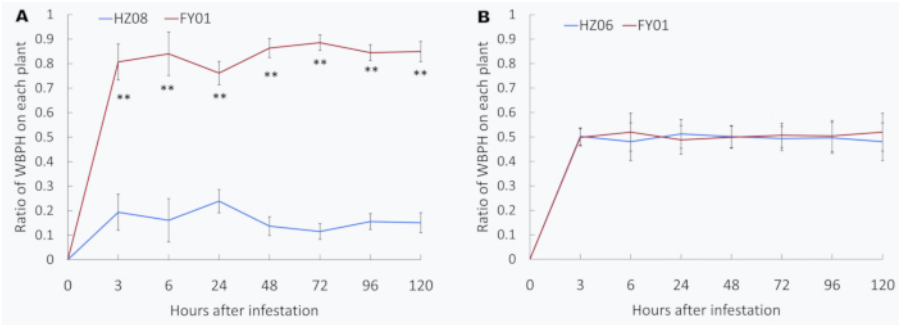

Figure 3: Dynamic changes of WBPH on different experimental rice lines. (**A**) Ratio of WBPHs on single rice plants of HZ08 and FY01. (**B**) Ratio of WBPHs on single rice plants of HZ06 and FY01. Error bars, means ± SE, by Tukey's multiple range test (n = 15, $^{**}P < 0.01$). Please click here to view a larger version of this figure.

Discussion

Maturing rice plants release volatile secondary metabolites to control insect pests or reduce the mating capacity of these pests (such as in WBPHs) via a special physical structure on the leaf sheath surface, which is a key resistance mechanism[13]. In rice plants, the nonpreference is not only related to feeding but also associated with habitat and mating. However, current studies have focused on the nonpreference of nymphs[4,12], and a new method needs to be developed for the identification of adult nonpreference. The present study mainly described a method to identify nonpreference-type resistance of the maturing rice plant to adult WBPHs. The method requires a few experimental materials and the experimental procedures are simple. However, the preliminary preparation is very important since a large amount of WBPHs are needed during the experiment. To ensure the accuracy and persuasiveness of the experimental results, it is necessary to use WBPHs at similar growing stages. Considering the long growth duration of rice, it is necessary to plan the experiment well in advance to ensure that there is a sufficient source of planthoppers when the rice plants are ready. In addition, several strains of each rice line should be planted as backup. It must be noted that when performing step 2.8, do not release WBPH on the rice plant, but try to ensure that the planthoppers are evenly distributed in the cage at the beginning.

This method is more suitable for the nonpreference-type resistance identification of flying pests, especially for those insects whose feeding or habitat behavior is attracted by volatile emissions. Therefore, it is not suitable for the study of nymphs or short-winged morphs, who cannot fly. The cylindrical cage in this experiment is made up of PVC, and the physical structure will be unstable if the cage diameter is enlarged as PVC material is soft. Therefore, the current cage size is more suitable for the comparison of two rice pots at the same time, and it is difficult to accommodate three or more rice plants in one experimental group. In addition, the method is only suitable for the study of rice plants with a single tiller or two tillers, which excludes matured rice plants since they have many tillers which occupy a large volume and will be inconvenient for insect pest counting. Moreover, the observation time is limited to a certain extent because the insects cannot be observed at night.

A traditional raising container for planthopper experiments is usually made up of a 200 mesh nylon net cage with zippers, usually large in size[14]. Due to the low transparency of nylon net material, it is necessary to unzip the zipper when observing the insects, and they can usually only be seen from one direction. The method described in this study uses transparent PVC as the experimental container, which has the advantage of omnidirectional observation without disturbing the pests. Nylon net is used to cover the cylindrical cage, which can make up for the shortcomings of PVC's impermeability. At the same time, high transparent PVC material ensures that the rice plants and pests receive sufficient light, which is very similar to natural conditions. In addition, this method effectively reduces the space needed for the experiment and facilitates more than ten groups of experiments at the same time. By using this method, the observation of oviposition preference of female WBPHs on maturing rice plants is also possible, which could be considered as another resistance evaluation index. Overall, the method provides the basis for further effective behavioral studies of various pests, such as the population competition relationship between WBPHs and BPHs.

Disclosures

The authors have nothing to disclose.

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