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High levels of silicon provided as a nutrient in hydroponic culture enhances rice plant resistance to brown planthopper

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ABSTRACT

Silicon plays a significant role in the resistance of rice plants to multiple stresses including diseases and pests. However, there has been little research on the effect of silicon application on plant resistance to brown planthopper (BPH), *Nilaparvata lugens* Stål, (Homoptera: Delphacidae), in rice (*Oryza sativa* L.). In the present work, experiments were conducted in greenhouses to study the impact of rice plants grown in hydroponic culture and fed with high concentrations of silicon (240 mg/L SiO_2) as a macronutrient on the performance of the phloem-feeding insect, BPH. It appeared that a high silicon concentration had no significant effects on the phenotypic performance of two rice lines. However, the BPH survival rate and settled insect number on one of the rice lines treated with high silicon concentrations decreased significantly from 8 and 4 day points, respectively, after their release. A similar downward trend was observed on the other rice line treated with a high concentration silicon solution from the 24 h time point. Furthermore, BPH insects on both rice lines treated with high silicon concentrations were significantly less fertile and excreted less honeydew compared to controls. In conclusion, high silicon had significant antibiotic and antixenotic effects on BPH in the two rice lines when compared to controls. We conclude that silicon is one of the main factors that restrict BPH performance in rice-BPH interactions, and it is potentially of great benefit for non-pesticide BPH management.

1. Introduction

Rice (*Oryza sativa* L. Poaceae), one of the world's most important food crops, is cultivated in a special type of artificial wetland. Unfortunately, many epidemic diseases and insect pests attack various parts of the rice plant resulting in severe losses of rice production (Litsinger et al., 2011). In particular, the brown planthopper [BPH, *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae)], which is one of the serious insect pests of cultivated rice, has become a major threat since farmers widely adopted green revolution technologies in the 1960s, and continued to play a major limiting role in agricultural production (Bottrell and Schoenly, 2012). The control of BPH using traditional insecticides raised serious concerns about food safety, environmental quality and insecticide resistance, which would result in resurgence of the BPH because of their harmful effects on natural enemies (Heinrichs et al., 1982; Tanaka et al., 2000). On the other hand, breeding and cultivation of resistant rice varieties have been considered to be an effective and economical way to manage this insect pest (Alam and Cohen, 1998; Renganayaki et al., 2002). However, up to now there are still a few BPH-resistance rice varieties developed and cultivated widely in the rice cultivation area (Fujita et al., 2013). Therefore, more effective and ecological methods should be detected to improve the integrated pest prevention and control systems.

It is known that application of chemical fertilizers could affect the tolerance or resistance of rice plants to BPH. For example, high concentrations of nitrogen fertilizer applied to rice, especially hybrid rice, can increase the risk for BPH outbreaks (Lu et al., 2005; Bottrell and Schoenly, 2012). Silicon is one of the most abundant elements in the earth's crust, and was reported to have a beneficial effect on growth and production in various crops including rice, wheat, barley, sugarcane, potato and cucumber (Epstein, 1999; Ma et al., 2007; Gomes et al., 2009). In particular, the rice plant was a typical silicon-accumulator and silicon could make up to 10% of the

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shoot dry weight, which was several fold higher than other essential macronutrients (Ma and Takahashi, 2002). Previous studies have shown that silicon in rice plants could enhance resistance to disease and insect damage by stimulating some defense reaction mechanism(s) (Fauteux et al., 2005; Reynolds et al., 2009; Ye et al., 2013). . However, little research has been performed on the effect of silicon administration on the resistance of rice to BPH. In the present study, experiments were conducted in a greenhouse in order to study the impact of rice plants fed through the root in hydroponic culture with solutions containing high concentrations of silicon on the performance of the phloem-feeding insect, BPH.

2. Materials and methods

2.1. Plant material and BPH insects

Two rice lines, 9311 and BPHR96, were used. 9311, an *indica* rice, is known to be highly susceptible to BPH (Qiu et al., 2010). BPHR96, introgressed from *Oryza rufipogon* Griff, has been reported to carry the BPH resistance gene *Bph24*(*t*) and has been shown to be as highly resistant to BPH (Chen et al., 2009).

BPH insects were collected from rice fields in 2008 and 2013 in Nanning (22°49' N, 108°19' E), China, and maintained at the Rice Research Institute, Guangxi University, on Taichung Native 1 (TN1) plants. Second to forth-instars and adult insects were used for experiments.

2.2. Hydroponic culture and phenotypic evaluation

The hydroponic culture experiment was conducted in a greenhouse with an average day/night temperature of 32 °C/26 °C, 75% relative humidity and natural daylight at Guangxi University, during the period of September to December in 2011 and May to September in 2013. Following studies by Yoshida et al. (1972, International Rice Research Institute) and Mao (2004), a modified solution (designated as a control) was prepared with deionized H_2O and formulated in our laboratory for the two rice lines, 9311 and BPHR96. As for the control hydroponic culture, the macronutrient concentrations were 40 mg/L N, as NH_4NO_3; 10 mg/L P, as $NaH_2PO_4 \bullet 2H_2O$; 40 mg/L K, as K_2SO_4; 40 mg/L Ca, as $CaCl_2$; 40 mg/L Mg, as $MgSO_4 \bullet 7H_2O$ and 120 mg/L SiO_2, as $Na_2SiO_3 \bullet 9H_2O$. The micronutrient concentrations were 0.5 mg/L Mn, as $MnCl_2 \bullet 4H_2O$; 0.05 mg/L Mo, as $(NH_4)_6Mo_7O_{24} \bullet 4H_2O$; 2.0 mg/L Fe, as EDTA-Fe; 0.2 mg/L B, as H_3BO_3; 0.01 mg/L Zn, as $ZnSO_4 \bullet 7H_2O$ and 0.01 mg/L Cu, as $CuSO_4 \bullet 5H_2O$. Based on the control components, a high silicon solution (240 mg/L SiO_2, as $Na_2SiO_3 \bullet 9H_2O$) was applied into the hydroponic culture experiment. Ten-day old seedlings grown in coarse sand were transferred into 60-cm-long, 20-cm-wide and 10-cm-height plastic box with 20 plants per box. The box contained either control or high silicon solution. The solution (pH 5.0–6.0) was replaced every 7 days, and deionized water was used throughout the experiment.

By the thirtieth day after treatment, five traits of phenotypic performance, tiller number (TN), plant height (cm, PH), root length (cm, RL), aboveground fresh weight (g, AFW) and root fresh weight (g, RFW) were measured to determine plant response to silicon application (Li et al., 2009). Ten replicates were conducted for each treatment.

2.3. BPH performance on rice plants

To detect BPH fertility and emergence (growing up wing) on 9311 and BPHR96 plants, ten-day old seedlings were transferred onto individual 0.7 L plastic cups that contained either control or high silicon concentration solution. After ten days, each cup/plant was infested with a female with swollen abdomens which was allowed to oviposit onto the plant freely. Then seven days after BPH release, the hatched BPH instars on each plant were counted and removed every day until no insects were detected. To count the BPH emergence number, each plant was treated with five second-instars and counted every two days for a total 14 days after five days of infestation. The tests were performed twice and each treatment contained 10 replicates. The solution used in the tests was replaced every 7 days.

To measure BPH survival and growth on 9311 and BPHR96 plants, the seedlings were treated following the method conducted in fertility and emergence tests. After ten days, each cup/plant infested with 10 second-instar nymphs was covered with a same size light-transmitting cup. The number of surviving BPH in each cup/plant was recorded at 1, 2, 3, 4, 5, 6, 7, 8 and 9 h after release. Each treatment for BPH survival and growth contained five to eight replicates.

BPH growth was measured using ten pre-weighed, second-instar nymphs. Five to eight 20-day-old seedlings of the two rice lines were treated with a solution containing a high concentration of silicon. After 120 h of infestation, the surviving BPH were re-weighed. The growth rate of surviving BPH was calculated as the per nymph difference between the initial mean weight of BPH (W_{orig}) and the final mean weight of surviving BPH in the cup/plant (W_{final}):

$$\text{BPH weight increase } (\%) = \frac{W_{final} - W_{orig}}{W_{orig}} \times 100\%$$

2.4. BPH host choice behavior and honeydew excretion

To measure BPH host choice behavior on 9311 and BPHR96 plants, each seedling (10 days old) was fixed on the top of the individual 0.2 L plastic cup using cystosepiment. After ten days, two similar cups/plants of same rice line from control and a high silicon treatment were covered with fine, light-transmitting nylon mesh bags. Ten cups/plants were used for each pair of silicon treatments. To observe the settling BPH, 20 adult insects randomly selected were placed in each mesh bag and allowed to choose host plants (20 days old) on which to feed and reproduce over a 120-h period. The insect numbers of BPH settling on each plant were counted at 12, 24, 48, 72, 96 and 120 h after release. 9311 and BPHR96 seedlings were tested at the same time.

BPH honeydew excretion on the plants was surveyed using a parafilm sachet (Pathak et al., 1982; Smith et al., 1994). Rice plants were treated as the BPH performance experiments and used at the four-leaf stage (approximately 21 days old). An individual male emergent BPH previously starved for approximately 2 h was placed in a folded parafilm sachet, and then fastened onto the plant shoot. Afterwards, the insect was allowed to feed for 48 h, and then the parafilm sachets without the insects were collected and measured in an electronic balance with a sensitivity of 1/10,000 g. This allowed the net weight of honeydew excretion to be obtained. The feeding chambers were arranged in a randomized complete block design with each plant serving as a replicate. The experiment was performed twice and each treatment was replicated 15 times.

2.5. Statistical analysis

Data of honeydew excretion was analyzed by Mann–Whitney U measurement of nonparametric test for 2 independent samples. BPH host choice test was analyzed by repeated measure-ANOVA. The other data were analyzed with one-way ANOVA and