Rice Science, 2010, 17(4): −

DOI:

High Temperature Modifies Resistance Performances of Rice Varieties to Brown Planthopper, *Nilaparvata lugens* (Stål)

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Abstract: To investigate the effect of temperature on the resistance characteristics of rice varieties with different resistance genes to brown planthopper (BPH), *Nilaparvata lugens* (Stål), the resistances of IR26 (*Bph*1) and IR36 (*bph*2) to BPH population in Hangzhou, China were monitored in greenhouse during September in 2007 and 2008 by using the Standard Seedling Screening Techniques (SSST) developed by International Rice research institute (IRRI). Furthermore, soluble sugar and oxalic acid contents in 25-day-old rice plants of susceptible variety TN1 and resistant varieties IR26 and IR36 were detected at five temperatures (22ºC, 25ºC, 28ºC, 31ºC and 34ºC). The results showed that IR26 completely lost resistance both in greenhouse and at five tested temperatures, IR36 still had medium resistance at natural temperature, but its resistance decreased gradually from 25ºC to 34ºC, while IR36 fully lost its resistance at 31ºC and 34ºC. The highest durable resistance of IR26 and IR36 were recorded at 25ºC. The soluble sugar content in plants of the three tested rice varieties increased with temperature increase, and the oxalic acid content increased with the temperature increase at first, maximized at 25ºC, and then declined. Two-way ANOVA indicated significant effects of temperature and rice variety on contents of soluble sugar and oxalic acid in rice plant.
Key words: brown planthopper; temperature; rice; resistant characteristics

The brown planthopper (BPH), *Nilaparvata lungens* Stål, characterized by its seasonal migration and r-strategy life pattern, was just a secondary insect pest in most rice-growing countries of Asia before 1970s. With the changes in the cultivation system, the adaptation of high-yield rice varieties, the improvement of fertilizer and water conditions and the excessive use of chemical insecticides, BPH has become one of the most serious insect pests of rice in China and other rice grown Asian countries (Cheng et al, 2003; Cheng et al, 2008; Peng et al, 2009) . At present, the control of BPH mainly depends on the use of chemical insecticides, resulting in many ecological problems caused by long-term unreasonable application of these chemicals (Jiang et al, 2005). It has been solidly evidenced that the utilization of resistance rice varieties is the most economical and effective method to suppress the BPH population (Chen et al, 2009). More and more attention has been paid to the breeding of the resistance rice varieties in most Asian countries since 1960s, and a series of resistant rice varieties such as IR26 (with resistant gene *Bph1*) and IR36 (with resistant gene *bph2*) were bred successfully by the International Rice Research Institute (IRRI), and has been applied widely in the Southeast Asian countries for effective and economical control of BPH population (Lu et al, 2002).

Eleven of the top 12 warmest years since 1850 have occurred between 1995 and 2006. The linear warming trend over the 50 years from 1956 to 2005 is nearly twice that for the 100 years from 1906 to 2005 (IPCC, 2007). The simulation results obtained by the modified climate models also showed that the increasing emission of greenhouse gases was predicted to trigger a significant global warming (Peng et al, 1995). Agricultural ecosystem is greatly sensitive to environmental factors, and will be impacted by global warming. It was predicted that the increasing temperature about 2ºC to 3ºC would result in the potential rice yield loss by 5% to 10% in China, if no proper countermeasure was adopted between 2030 and 2050. Climate changes, including the rising emission of greenhouse gases, enhanced ultraviolet-B radiation and increasing global surface temperature, will induce the outbreak of BPH (Peng et al, 1995; Alam and Cohen, 1998). The frequently outbreaks of BPH in China has caused an unprecedented yield losses since 2005 (Zheng et al, 2009). Some studies indicated that the temperature obviously influenced the biological characteristics of BPH (Feng et al, 2001; Liu et al, 2004), however, little focused on the effects of temperature on the insect-resistance of rice varieties. The purpose of this paper is to determine the relationship of temperature and varieties with rice BPH resistance and to provide the theoretical basis for cultivation and reasonable application of rice varieties.

MATERIALS AND METHODS

Rice seeds

All seeds, TN1 susceptible to BPH, IR26 and IR36

Received: 4 March 2010; **Accepted:** 23 June 2010
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resistant to BPH, were provided by the Genetic Resource Center (GRC), International Rice Research Institute, Los Baños, Laguna, Philippines.

Rice plants

Four germinating seeds of one variety were sown separately in a clay pot (Φ=9 cm). Five pots for each variety were prepared and individually placed into five incubators with 22±1ºC, 25±1ºC, 28±1ºC, 31±1ºC and 34±1ºC, respectively, and 12L: 12D, normal water and fertilizer management. Three replications were set up for each variety at each temperature. The whole plants were sampled after 25 days and preserved at -20ºC for the determination of soluble sugar and oxalic acid contents.

Insects

Adults of BPH were collected at rice fields in Hangzhou, Zhejiang province, China, and were maintained on susceptible rice variety TN1 at 26±1ºC and photoperiods of 12L: 12D.

Resistance of IR26 and IR36 to BPH under natural condition

Damaged grades of rice seedlings by BPH nymphs were evaluated with the standard seedling screening techniques (SSST) developed by IRRI (2002), and the resistance performances of IR26 and IR36 to BPH population under natural conditions were tested during July and August in 2007 and 2008. Germinating seeds were sown in plastic tray (45 cm×30 cm×10 cm) filled with soil for 4 cm in depth. The tray was divided equally into 9 rows, 15 germinating seeds of one tested rice variety were sown in one row, and three rows were arranged by an array of TN1, IR26 and IR36, respectively. Three replications were established for each rice variety in a tray. There were a total of 10 trays or 30 replications for each rice variety in the experiment. All trays were under the natural temperature during July and August and received normal water and fertilizer management. Ten stronger seedlings were remained on each row at the two full leaf stages, and the 2^{nd} or 3^{rd} instar BPH nymphs were introduced into the tray with an average density of 6 to 8 BPH nymphs on one rice seedling. Based on the SSST, the survival seedlings of IR26 and IR36 were recorded daily when susceptible variety TN1 reached grade 7, and their resistant performances were assessed by their mortalities while TN1 reached at grade 9.

Resistance of IR26 and IR36 to BPH under different temperatures

Damage grades of rice seedlings under different temperatures were also evaluated by SSST. The similar trays (40cm×30cm×8.5cm) were maintained at five different temperatures (22±1ºC, 25±1ºC, 28±1ºC, 31±1ºC and 34±1ºC), respectively, and two trays were set up for each temperature. After TN1 reaching grade 9, the survival seedlings of IR26 and IR36 were recorded daily until more than 90% IR26 and IR36 seedlings died, and the durations for durable resistance of IR26 and IR36 were computed.

Determination of soluble sugar content in rice plants

Colorimetric analysis was performed by the anthrone reagent for determining the content of soluble sugar in rice plants (Wang et al, 2008). All plant samples kept at -20ºC were dried at 80ºC to constant weight, respectively, and 0.1 g of each sample was collected randomly and put into a test tube with 15ml distilled water. Then the tubes were cooled after being heated in boiling water bath for 20 min and the distilled water was added into the tubes with the filtered extraction up to 100 mL. The tube with mixture of 1.0 ml extraction solution and 5.0 mL anthrone reagent was well-distributed and heated in boiling water bath for 10min. The value of OD_{620} was determined with spectrophotometer after being cooled. In addition, the standard curve of pure anhydrous glucose was made to calculate the content of soluble sugar in the samples.

Determination of oxalic acid content in rice plants

Contents of oxalic acid in the rice plants were determined by Titanium-Trichloride-Spectrophotometry method (Xu, 2006). One gram of each sample was fully grinded and put into 50 ml triangular flask with 20ml ultrapure water. Active carbon was added into the flask for 1/3 volume of mixture. Then the mixture was decolorized for 30 min until it turned into colourless or oyster white. After 2 mL of mixture supernatant was centrifuged at 3000 r/min for 15 min under 4ºC, 0.5 mL of the supernatant was mixed with 40 µL 3% titanium trichloride solution, and the volume was adjusted to 1 mL with ultrapure water. The OD_{400} value of 300 mL solution was determined with spectrophotometer. The standard curve of pure oxalic acid was used to calculate the content of oxalic acid in the samples.

Data analysis

The data were transformed properly and then analyzed by SPSS 13.0. The differences of contents of soluble sugar and oxalic acid in rice plants among different temperatures and rice varieties were analyzed by two-way analysis of variance (ANVOA) and Turkey's multiple comparisons.

RESULTS

Resistance of IR26 and IR36 under natural conditions

Based on the results in 2007 and 2008 (Fig. 1), the resistance score of IR26 to BPH population was the same as the susceptible rice variety TN1 in both 2007 and 2008, implying that IR26 has fully lost its resistance to BHP. Furthermore, the resistance scores of IR36 were 5 and 3, respectively, indicating

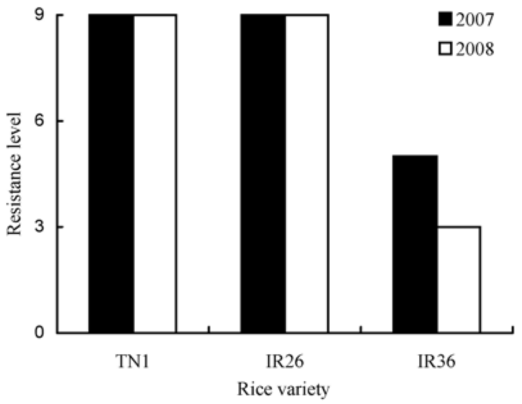

Fig. 1. Resistance of TN1, IR26 and IR36 to BPH under natural temperature.

the medium and high resistance of IR36 to BPH.

Resistance of IR26 and IR36 to BPH under different temperatures

The resistance scores of IR26 and IR36 increased with elevated temperature between 25ºC and 34ºC, which suggested that the resistance of these two varieties decreased (Table 1). Both of the resistance level of IR26 and IR36 reached grade 9 at 31ºC and 34ºC, indicating that the two varieties had lost the resistance completely. The resistance of IR36 was higher than IR26 at 25ºC and 28ºC, respectively. Moreover, IR36 also lost the resistance to BPH at 22ºC, although it has medium resistance at 25ºC and 28ºC.

Content of soluble sugar at different temperatures

An obvious increasing trend of soluble sugar contents was recorded in all three tested rice varieties with the elevated temperature. There were significant differences in soluble sugar content of rice plants between different temperatures (P<0.001) and rice varieties (P=0.006). However, no statistical difference was found in the interaction of temperatures and rice varieties (P=0.495) (Table 2). Soluble sugar content in TN1 and IR26 were obviously higher than that in IR36, but not significantly (Fig. 2).

Content of Oxalic acid at different temperatures

The oxalic acid contents in all three test rice plants obviously decreased with the increase of temperature. Oxalic acid contents in rice plants at 22ºC were markedly lower than those at 25ºC, but similar with those at 28ºC and 31ºC (Fig. 3). Significant differences were found in the oxalic acid contents between different rice varieties (P<0.05), different temperatures (P<0.05), and the interaction of rice varieties and temperatures (P<0.05) (Table 2).

DISSCUSSION

The unprecedented large-scale outbreak of brown planthopper population in China caused great 'hopper-burn' and massive grain losses in 2005, about 1.2 million tones of rice lost due to the damage of BPH in Zhejiang Province, China (Lu and Huang, 2006). The fact of BPH population kept in

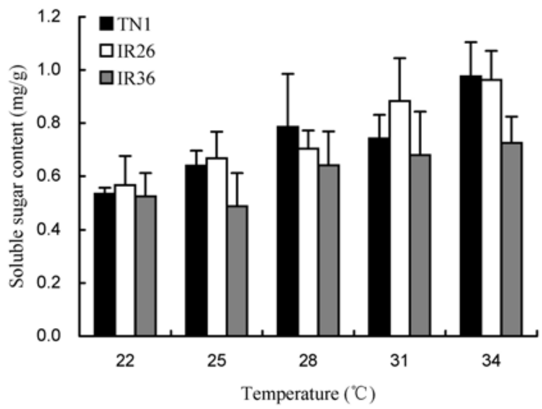

Fig. 2. Soluble sugar content in rice plants at different temperatures.

Table 1. Seedling resistance performances of rice varieties to BPH at different temperatures.

Temperature (ºC)	TN1 mortality (%)	IR26			IR36		
		Mortality (%)	Resistance score	Durable resistance (d)	Mortality (%)	Resistance score	Durable resistance (d)
34	100	93.3	9	0	96.7	9	0
31	100	88.5	9	1	89.2	9	1
28	100	67.8	7	2	50.6	5	6
25	100	52.4	7	9	20.0	3	11
22	100	85.7	9	3	84.6	9	4

Table 2. ANOVA of soluble sugar and oxalic acid content in rice varieties at different temperatures.

Treatment	Soluble sugar content			Oxalic acid content		
	MS	F	P	MS	F	P
Temperature	0.126	8.090	<0.001	0.039	30.956	<0.001
Rice variety	0.097	6.257	0.006	0.005	3.650	0.034
Temperature×Variety	0.006	0.949	0.495	0.0161	13.123	<0.001

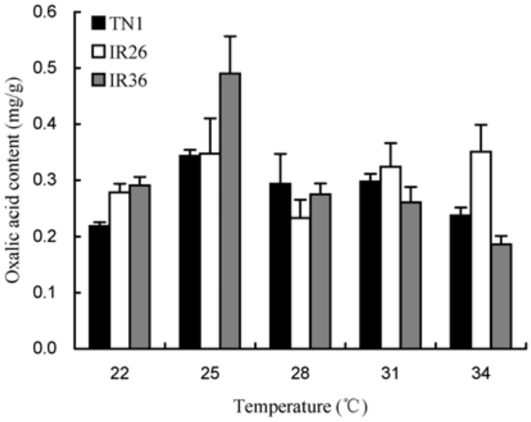

Fig. 3. Oxalic acid content of rice plants at different temperatures.

higher risk level from 2006 to 2009 has verified that the intensive use of pesticides in fact led to the lower control efficiency and the rapid development of resistance of BPH (Cheng et al, 2008). It resulted in the more regard in breeding and utilizing resistance rice varieties for suppressing BPH population (Cheng et al, 2008).

Global warming, especially the local climate changing with the tendency of warmer autumn and cooler summer, will accelerate the development of BPH nymphs, and improve the potential fecundity of BPH female adults. The outbreak of BPH population will be strongly promoted (Cheng et al, 2008). Changes of temperature influence the relationship among rice varieties, insect pests and natural enemies, even the whole agro-ecosystem, and substantially bring on the resurgence of insect pests. Our results showed that the changes of temperature affected the resistance performance of rice varieties. The seedling resistance of IR26 and IR36 to BPH decreased with a rise of temperature between 25ºC and 34ºC, while IR36 lost fully its resistance at 31ºC and 34ºC, although it still had medium resistance at natural temperature. It is imaginable that increase of global temperature resulting from the changing climate will cause obvious decrease in resistance ability of rice varieties to BPH. Furthermore, the same trend was found in the extreme low temperature, however, the proper mechanism needs to be further studied. Breeders should pay more attention to coordinate the resistance performance at high temperature with the high yield characteristics of rice.

Soluble sugar content in rice plants under different temperatures significantly increased with the elevated temperature. The main reason may be that the activities of rubisco activase and RuBP carboxylase, the two key enzymes in rice leaves for photosynthesis, increased with the increasing temperature, and photosynthesis was accelerated and photosynthetic daily assimilation amounts increased. It was reported that the feeding behavior of BPH was related closely to the content of oxalic acid in rice plants, and the feeding amount of BPH decreased with the increase of oxalic acid content in rice plants (Yoshihara et al, 1980). Our results showed that over-high temperature might cause the decreasing resistance of rice varieties to BPH due to the reduction of oxalic acid content in rice plants. On the other hand, the low temperature might reduce the photosynthesis capacity of rice plant. Influence process of the changes in temperature on physiology and resistant performances of rice plants is very complicated, and further study is needed.

ACKNOWLEDGEMENTS

The authors thank GRC of IRRI for providing rice seeds. This study was supported by the National Basic Research Program of China (973 Program) (Grant No. 2010CB126202), the Agro-Industry R&D Special Fund of China (Grant No. 200803003) and Zhejiang Provincial Natural Science Foundation of China (Grant No. Z3080437).

REFERENCES

Alam S N, Cohen M B. 1998. Durability of brown planthopper, *Nilaparvata lugens*, in rice variety IR64 in greenhouse selection studies. *Entomol Exp Appl*, **89**: 71–78.

Chen F, Fu Q, Luo J, Lai F X, Gui L Y. 2009. Adult stage resistances to brown planthopper, *Nilaparvata lugens* of rice varieties with different seedling resistances. *Chin J Rice Sci*, **23**(2): 201–206. (in Chinese with English abstract)Cheng J A, Zhu J L, Zhu Z R, Zhang L G. 2008. Rice planthopper outbreak and environment regulation. *J Environ Entomol*, **30**(2): 176–182. (in Chinese with English abstract)

Cheng X N, Wu J C, Ma F. 2003. Brown planthopper: Occurrence and Control. Beijing: China Agriculture Press. (in Chinese)

Feng C J, Dai G H, Wu S W. 2001. Stress response of *Nilaparvata lugens* (Stål) at high temperature and activities of its protective enzyme systems. *Chin J Appl Ecol*, **12**(3): 409–413. (in Chinese with English abstract)

IPCC. 2007. Observed changes in climate and their effects. *In*: Pachauri and Reisinger (Eds.). Climate change 2007: Synthesis report. Cambridge, UK: Cambridge University Press: 8–15.

IRRI. 2002. Standard Evaluation System for Rice. Los Bamos, Philippines: International Rice Research Institute: 56.

Jiang H, Lin R H, Liu L, Qu W G, Tao C J. 2005. Planthoppers damage to rice and the resurgence mechanism. *Chin Bull Entomol*, **42**(6): 612–615. (in Chinese with English abstract)

Liu Z W, Han Z J, Wang Y C, Zhang H W. 2004. Effect of temperature on population growth of susceptible and resistant strains of *Nilaparvata lugens* to imidacloprid. *Entomol Knowl*, **1**: 47–50. (in Chinese with English abstract)

Lu J F, Huang G Y. 2006. Reason and control countermeasures on the disaster brought by brown planthoppers *Nilaparvata lugens* (Stål) in Zhejiang province. *Pestic Sci Admin*, **27**(1): 42–44. (in Chinese with English abstract)

Lu Z X, Yu X P, Tao L Y, Wu G R, Chen J M, Zheng X S, Xu H X. 2002. Resistance evaluation of newly-bred rice varieties (lines) to brown planthopper *Nilaparvata lugens* (Stål) in China. *Sci Agric Sin*, **35**(2): 225–229. (in Chinese with English abstract)

Peng S, Ingram K T, Neue H U, Ziska L H. 1995. Climate change and rice. Manila, Philippines: International Rice Research Institute: 1–374.

Peng S, Tang Q, Zou Y. 2009. Current status and challenge of rice production in China. *Plant Prod Sci*, **12**(1): 3–8. (in Chinese with English abstract)

Wang Q S, Zhen R H, Ding Y F, Wang S H. 2008. Strong stem effect and physiological characteristics of rice plant under rice & duck farming. *Chin J Appl Ecol*, **19**(12): 2661–2665. (in Chinese with English abstract)

Xu D H. 2006. Determination of oxalic acid in beer. *China Brew*, **4**: 102–104. (in Chinese with English abstract)

Yoshihara T, Sogawa K, Pathak M D, Juliano B, Sakamura S. 1980. Oxlic acid as a sucking inhibitor of the brown planthopper in rice (Delphacidae, Homoptera). *Entomol Exp Appl*, **27**: 149–155.

Zheng X S, Chen G H, Xu H X, L u Z X. 2009. Interactive effects of temperature and nitrogen fertilizer on the survival, development, and reproduction of brown planthopper *Nilaparvata lugens* (Stål). *Chin J Appl Ecol*, **20**(5): 1171–1175. (in Chinese with English abstract)