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Relations between Brown Planthopper (BPH) Resistance and Sucking Inhibitors of BPH in Rice

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ABSTSTRACT : This study was conducted to investigate the relations between brown planthopper (BPH, *Nilapavata lugens*) resistance and specific organic acids (oxalic acid, silicic acid, and *trans*-aconitic acid) known as BPH sucking inhibitors on different rice varieties and/or lines. There were no specific relations between BPH resistance and the contents of oxalic and silicic acids in the rice plant tissues. However, the stronger the BPH resistance was occurred, the higher the content of *trans*-aconitic acid was contained in the rice plants. The relations between the injury rate of rice plant by BPH and the content of *trans*-aconitic acid in the rice plants were negatively correlated, which were -0.84 and -0.82 at 30 and 60 days after seeding, respectively. Therefore, the content of *trans*-aconitic acid in rice plant tissues might be utilized as an index for improving BPH resistance of rice varieties.

Keywords: brown planthopper (BPH) resistance, sucking inhibitor, oxalic acid, trans-aconitic acid, silicic acid

Brown planthopper is widely distributed in rice cultivation areas. BPH inhabits around rice leaf sheaths by sucking insect pest. BPH has been considered a threat to rice production because it causes considerable damages to rice plant. For example, it causes the growth inhibition and hopperburn of rice plants (Ling, 1977; Kisimoto, 1977; Aguiero & Ling, 1977; Sogawa *et al*., 1979; Kim, 1993). Since the 1970's BPH has been also a one of the most harmful insects to rice plants in Korea due to extending the cultivation of a high-yielding rice variety such as Tongil-byeo, an early transplantation of rice seedlings, high density of rice plantings, increase of chemical fertilizer applications, and changes in the methods for controlling diseases and insect pests. For these reasons, a severe outbreak of BPH has been appeared; BPH has been infested in approximately 25% of total rice production area in Korea. For controlling BPH, excess amounts of insecticide have been used, so that it causes high rice production expenses as well as harmful effects to farmers and rice plants. However, if BPH is not controlled, it leads to decrease in approximately 8 % of rice production (Chang, 1992).

Development of BPH resistant rice varieties has been considered as an efficient way to control BPH without the application of insecticides. A research to develop BPH resistant rice varieties was first conducted at International Rice Research Institute (IRRI) in 1967 (IRRI, 1967). Since then, this type of research was propagated through worldwide. From the 1970's, Korean Government also pursued to develop BPH resistant rice plants using high-yield rice varieties such as Milyang 30 and Tongil lines. However, farmers have avoided cultivating the high-yield rice varieties because of rice taste problems. Therefore, this type of research was also accomplished by using Japonica varieties because most of farmers cultivated only the Japonica varieties. Only Japonica variety Hwacheungbyeo, moderately resistant to BPH, has been supplied to Korean farmers; thus, the development of various rice varieties strongly resisted to BPH is considered as an urgent subject (Kim, 1993).

On the other hand, reduced or no BPH damage has been observed as cultivated BPH resistant rice varieties. Sogawa and Pathak (1970) reported BPH resistance mechanism; the resistance to BPH is due to the presence of a sucking inhibitor and/or the absence of a sucking stimulator in the phloem sap because BPH excretes little honeydew after sucking the resistant cultivars. Existence of the sucking inhibitor has been reported by several researchers: BPH damages have not been appeared in barnyard grass unlike in rice plants (Kim *et al*., 1975) because *trans*-aconitic acid, a BPH sucking inhibitor, existed in the barnyard grass (*Echinochloa crus-galli*) acted as a BPH antifeedent (Kim *et al*., 1976). Yoshihara *et al*.(1979) reported when silicic acid was treated in 0.01 mg Si L^{-1} level to rice plants, a strong BPH sucking inhibition was observed. In addition, they also found that oxalic acid worked as a BPH sucking inhibitor in Mudgo, the most resistant rice cultivar to BPH, while malonic acid, succinic acid, and glutaric acid that have similar chemical structures as comparing with oxalic acid did not show any BPH sucking inhibition (Yoshihara *et al*., 1980).

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Therefore, the objectives of this study were to determine the contents of selected organic acids, oxalic acid, silicic acid, and *trans*-aconitic acid, known as BPH sucking inhibitors, in different rice varieties and/or lines distinguished by BPH resistant responses, and to investigate the relations between BPH resistance and the contents of organic acids in the rice plants to utilize as an index for improving BPH resistance of rice varieties.

MATERIALS AND METHODS

Rice plants

Three different rice varieties, Jangseongbyeo (JSB), Hwacheongbyeo (HCB), and Dongjinbyeo (DJB) were selected on the basis of different resistant responses to brown planthopper (BPH). JSB and HCB varieties were strongly resistant and moderately resistant to BPH, respectively, but DJB variety was susceptible to BPH (Kim, *et al.*, 2006). Also, eight lines of rice plants that being developed in Honam Agricultural Research Institute, Republic of Korea were selected by the same manner described above: HR86006, HR86011, HR86147, and HR86153 were strongly resistant, HR86079 and HR86085 were moderately resistant, and HR86176 and HR86185 were susceptible to BPH. Experiment to investigate the BPH response of the rice plants was conducted using a method described by Kim *et al.* (2006), and the rice plants used in this study were finally selected after re-examination at 5 leaf stage.

Preparation for rice plant samples

Rice plant samples were collected at 30 and 60 days after seeding (DAS). Leaf blades and sheaths of the rice plants were separated, and then oven-dried at 80°C for 24 h. The dried samples were ground to pass through 0.42 mm sieve. The ground samples were stored in a desiccator at room temperature by the time of analysis.

Determination of oxalic acid

The procedures to determine the content of oxalic acid in the rice plant samples was modified from a method described by Wu *et al.* (1998) and Xu and Zhang (2000). Triplicate 2g samples were placed into 250 mL flasks, and 100 mL of distilled water was added to the each flask. The mixture in the flask was adjusted to pH 2.0 with concentrated hydrochloric acid, and it was settled down for 30 min. The mixture was boiled at 90°C for 10 min and then cooled to room temperature. The mixture was filtered using a Whatman No 2 filter paper, and the filtrate was diluted to 150 mL mark with distilled water. Ten milliliter water-extracted solution was transferred into 50 mL flask with adding 2.0×10^{-5} mol L^{-1} of bromophenol blue, 3.6×10^{-2} mol L^{-1} of sulfuric acid, and 3.2×10^{-3} mol L^{-1} of potassium dichromate in order. The reagent treated solution was thoroughly mixed and reacted for 10 min. After 10 min of the reaction, it was quenched by adding 1.0 mL 2.0 mol L^{-1} sodium hydroxide solution. The concentrations of oxalic acid were measured using a spectrophotometer at 600 nm of wavelength.

Determination of *trans*-aconitic acid

The content of *trans*-aconitic acid in the rice plant samples was determined using a method modified from the procedures described by Poe and Barrentine (1968). Triplicate 10 g ground samples were transferred into 200 mL flask with 50 mL of distilled water. The mixture was shaken for 30 min, and filtered using a Hyflo super-cel aid with Whatman No.2 filter paper. Residues in the filter paper were shaken again and filtered as described above. The second filtrate was adjusted to pH 1.3 with concentrated sulfuric acid solution. The pH adjusted filtrate was fractioned twice with adding 20 mL of saturated sodium hydroxide and 100 (50 + 50) mL of 2-butanone. An aliquot of 2-butanone layer was hydrated with 20g of sodium sulfate anhydrate, and it was transferred into a 250 mL concentration flask to elute at 40°C. The eluate was thoroughly mixed with 10 mL of acetic anhydride and 0.01 mL of pyridine. After 45 min, color was read to determine the concentration of *trans*-aconitic acid using a spectrophotometer at 550 nm wavelength.

Determination of silicic acid

Triplicate 10 g ground samples were digested in concentrated HNO_3 and 30% H_2O_2 (Jones, 1991). The digests were evaporated to near dryness at 80°C and transferred to a 20 mL scintillation vial with 0.1 *M* nitric acid solution and brought to 10 mL of final volume. The sample solution was used to determine the concentration of silicic acid using an inductively coupled plasma emission spectroscopy (ICPS-7500, Shimadzu Corp.).

RESULTS

Resistance to BPH in different rice plants

In a previous paper (Kim *et al.*, 2006), it was reported that the most resistance to BPH was observed in Jangseongbyeo (JSB) and moderate resistance to BPH was in Hwacheongbyeo (HCB), but Dongjinbyeo (DJB) was susceptible to

Table 1. Difference in brown planthopper resistance among rice varieties and lines at 5-leaf stage employed in this study.

Rice variety or line	Injury rating (0-9)†	Resistance level‡
Jangseongbyeo	0	R
HR86006	0	R
HR86011	0	R
HR86147	1	R
HR86153	1	R
Hwacheongbyeo	4	MR
HR86076	4	MR
HR86085	5	MR
HR86179	7	MS
HR86185	7	MS
Dongjinbyeo	9	S

†Injury rating(n=30): 0=No injury, 9=Completely killed
‡R=Resistance, MR=Moderately resistance, MS=Moderately susceptible, S = Susceptible

BPH as determined by the resistant responses at 5 leaf stage. In the mean time, among the various rice lines being developed for BPH resistant rice plants, HR86006 and HR86011 were strongly resistant to BPH as much as JSB, HR86147 and HR 86153 showed strong BPH resistance, BPH resistance of HR 86079 and HR86085 was ranged between that of HCB and DJB, and HR86179 and HR86158 were moderately resistant or moderately susceptible to BPH, respectively (Table 1).

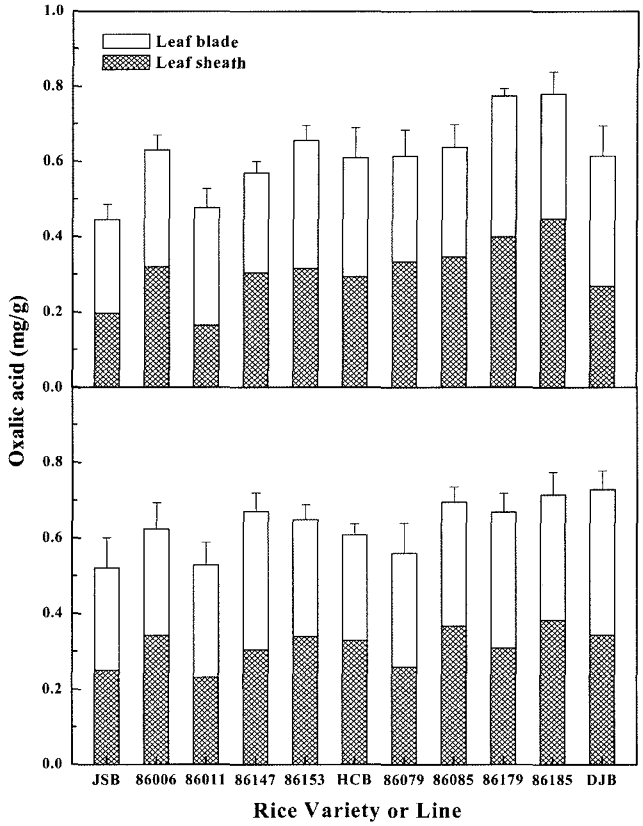

Fig. 1. Contents of oxalic acid in leaf blade and sheath of rice varieties and/or lines at 30 (Top) and 60 days after seeding (Bottom). JSB = Jangseongbyeo, HCB = Hwacheongbyeo, and DJB = Dongjinbyeo. Error bars are standard deviations of three replications.

The contents of oxalic acid in the rice plants

In the different rice plants observed BPH resistance, there were no specific correlations between BPH resistance and the contents of oxalic acid in the rice plants (Fig. 1). The content of oxalic acid in JSB, the most resistant cultivar to BPH in this study, was 0.45 mg g^{-1} at 30 days after seeding (DAS), whereas the oxalic acid contents in HCB and DJB at 30 DAS were 0.61 and 0.62 mg g^{-1}, respectively, so that there was no correlation between BPH resistance and the contents of oxalic acids in the rice plants. Moreover, at 60 DAS the oxalic acid content in JSB was 0.52 mg g^{-1}, and the contents of oxalic acid in HCB and DJB were 0.61 and 0.73 mg g^{-1}, respectively.

In addition, there is no correlation between BPH resistance and oxalic acid contents in the rice lines studied. The contents of oxalic acid in HR86006, HR86011, HR86147, and HR 86153, strongly resistant or resistant to BPH, were ranged from 0.47 to 0.65 mg g^{-1}, and those in other rice lines were ranged between 0.61 and 0.78 mg g^{-1} at 30 DAS. That is, the contents of oxalic acid were higher in the moderately resistant or moderately susceptible rice plants than in the strongly resistant rice plants. Also, it tended to be similar at 60 DAS. Furthermore, the contents of oxalic acid in the leaf blades or sheaths of the rice varieties and/or lines studied were not reliably correlated to BPH resistance.

The contents of *trans*-aconitic acid in the rice plants

The content of *trans*-aconitic acid in JSB was considerably higher than that in DJB, whereas the *trans*-aconitic acid content in HCB was ranged between the contents in JSB and DJB (Fig. 2). At 30 and 60 DAS, the contents of *trans*-aconitic acid in JSB, DJB, and HCB were 9.59 and 9.17 mg kg^{-1}, 5.64 and 6.27 mg kg^{-1}, and 9.29 and 7.98 mg kg^{-1}, respectively. Also, the contents in the leaf blades or sheaths of the rice plants studied were shown in similar trends as compared to the total contents of *trans*-aconitic acid in the shoots of them presented above. Thus, the total contents of *trans*-aconitic acid in the rice plants would not be affected by a specific part of the rice plant leaves.

The contents of *trans*-aconitic acid in the strongly resis-

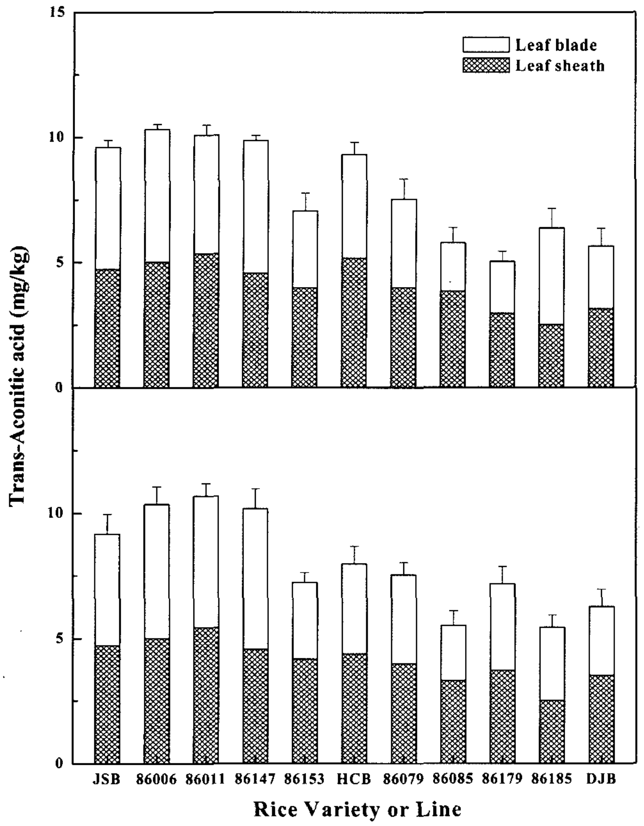

Fig. 2. Contents of trans-aconitic acid in leaf blade and sheath of rice varieties and/or lines at 30 (Top) and 60 days after seeding (Bottom). JSB = Jangseongbyeo, HCB = Hwacheongbyeo, and DJB = Dongjinbyeo. Error bars are standard deviations of three replications.

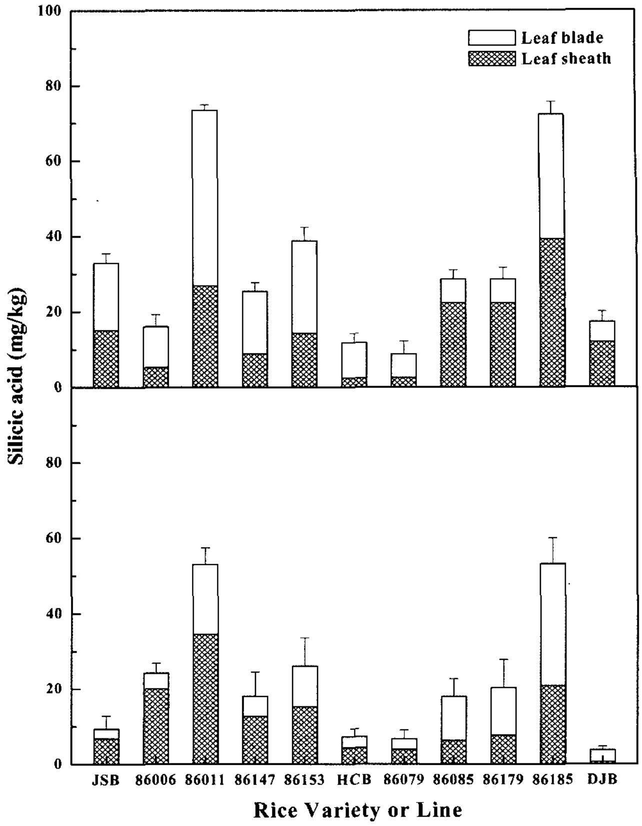

Fig. 3. Contents of silicic acid in leaf blade and sheath of rice varieties and/or lines at 30 (Top) and 60 days after seeding (Bottom). JSB = Jangseongbyeo, HCB = Hwacheongbyeo, and DJB = Dongjinbyeo. Error bars are standard deviations of three replications.

tant rice lines to BPH, except in HR86153, were ranged between 9.88 and 10.32 mg kg^{-1} at 30 DAS, which were even slightly higher than the content of *trans*-aconitic acid in JSB. Also, the contents were markedly higher than 7.53 mg kg^{-1} contained in HR86079 that was the highest content of *trans*-aconitic acid among the contents in the moderately resistant or moderately susceptible rice lines to BPH. These trends were continuously observed at 60 DAS. Therefore, the stronger the BPH resistance was occurred, the higher the content of *trans*-aconitic acid was contained in the rice plants.

The contents of silicic acid in the rice plants

Correlation between BPH resistance and the contents of silicic acid in 3 varieties and 8 genotypes of rice plants was not found (Fig. 3). Among the different rice varieties and/or lines studied, the highest content of silicic acid was obtained from HR8611, a strongly resistant rice line to BPH, which was 73.4 mg kg^{-1}, and then the second highest content of silicic acid, 72.2 mg kg^{-1}, was found in HR86185 that was a moderately susceptible one. However, the lowest content of silicic acid, 8.7 mg kg^{-1}, was determined from HR86079, a moderately resistant line. The contents of silicic acid in the rice plants decreased with increasing the growth stages, which is that the content of silicic acid in the plants was lower at 60 DAS than at 30 DAS.

DISCUSSION

We found some of BPH resistant rice lines among the various rice lines that obtained during the developing process of BPH resistant rice varieties. Among the BPH resistant rice lines we identified, BPH resistances of HR86006 and HR86011 were as much as the resistance of JSB that is the most resistant rice varieties to BPH. HR86147 and HR86153 were relatively strong resistant to BPH, although they were weaker than JSB. HR86079 and HR86085 were moderately resistant, and HR86179 and HR86185 were moderately susceptible to BPH (Table 1).

One of the BPH resistance mechanisms can be inferred from the damaging methods of BPH to rice plants. BPH feeds by sucking saps from phloem of rice plants (Sogawa, 1973a); thus, if sucking inhibitors are present in the phloem tissues of certain rice varieties, BPH resistance of the rice varieties might be accounted for the sucking inhibitors (Sogawa and Pathak, 1970). This fact was confirmed with *trans*-aconitic acid in the rice plants studied. In this study, we identified the stronger the resistance to BPH was in the rice varieties and/or lines, the higher the content of *trans*-aconitic acid was in the rice plants, which was that the contents of *trans*-aconitic acid were much higher in the strong resistant rice plants, such as JSB, HR86006, and HR86011, while the contents were low or relatively lower in the moderately resistant or susceptible rice plants, such as HR86176, HR86185, and DJB (Fig. 2). Therefore, the relations between the injury rates by BPH and the contents of *trans*-aconitic acid in the rice plants were negatively correlated, and the correlation coefficients were -0.84 at 30 DAS and -0.82 at 60 DAS. Kim *et al.*(1976) reported that difference between BPH feeding habits on rice plant and on barnyard grass was existed because rice plant did not contain the *trans*-aconitic acid, but it was in barnyard grass. However, Yoshihara *et al.*(1980) said that the existence of *trans*-aconitic acid was determined from Indica cultivar Mudgo, and the *trans*-aconitic acid reduced sucking activity of BPH. Also, Nagata and Hayakawa (1998) reported that the difference of BPH feeding habits speculated by the existence of *trans*-aconitic acid in barnyard grass was not clear; furthermore, *trans*-aconitic acid existed in rice plant markedly reduced BPH sucking activity. Therefore, these results suggested that the different resistances to BPH in rice plants are obviously influenced by the different contents of *trans*-aconitic acid rather than only the existence of the organic acid.

However, the different contents of oxalic acid in various rice varieties and/or lines studied were not correlated with BPH resistance (Fig. 1 and Table 2) even though oxalic acid was reported as a BPH sucking inhibitor (Yoshihara *et al.*, 1979; Nagata and Hayakawa, 1998). These results might be attributed either that the contents of oxalic acid in the rice plants were out of ranges to cause BPH resistance or that the BPH resistance is dependent upon the amounts existed in phloem sap only rather than the contents in whole rice plant bodies, or both. These propositions might be justified by followings; when 0.1% of oxalic acid was directly applied to BPH, less than 5% of BPH was survived at 2 days after treatment (Nagata and Hayakawa, 1998), and also in this study, we found that DJB, susceptible to BPH, contained 0.62% of oxalic acid. Therefore, the propositions about oxalic acid related to BPH would be true, which is the contents of oxalic acid in whole rice plant bodies are not involved in BPH sucking inhibition.

Table 2. Correlation coefficients between injury rating of the rice varieties and/or lines and content of organic acids determined in this study.

Organic acid	Correlation coefficient	
	30 DAS†	60 DAS
Oxalic acid	0.66	0.69
trans-Aconitic acid	-0.84**	-0.82**
Silicic acid	-0.10	-0.16

†DAS = Days after seeding
**means the significant differences at $P<0.01$.

Silicic acid in plants is present in outside of the phloem (Yoshihara *et al.*, 1979). However, BPH is sucking the sap from phloem tissues only, which is not sucking the sap from other tissues (Sogawa, 1973b). Therefore, silicic acid may work as a physical barrier to prevent sucking up the sap from phloem by BPH. Nonetheless, in this study, the relations between BPH resistance and the contents of silicic acid in the rice plants selected were not correlated (Fig. 3 and Table 2). Thus, the contents of silicic acid existed in the rice varieties and/or lines studied might not be enough to work as the physical barrier to prevent sucking the sap by BPH.

In conclusion, only the *trans*-aconitic acid inhibited BPH sucking activity even though oxalic acid and silicic acid as well as *trans*-aconitic acid are known as BPH sucking inhibitor. Therefore, the results obtained from this study can be utilized for developing BPH resistant rice varieties, especially using the negative correlations between the injury rates of rice plants by BPH and the contents of *trans*-aconitic acid in rice plants.

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