Short Communication

Sterols and Asparagine in the Rice Plant, Endogenous Factors Related to Resistance against the Brown Planthopper (*Nilaparvata lugens*)

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The brown planthopper (*Nilaparvata lugens*, abbreviated as BPH) is one of the most harmful insects to rice plants in Asia. From the research into protecting rice plants from BPH, several rice cultivars with marked resistance to the insect have been found among *indica* rice plants. The *Bph 1* gene, which is responsible for BPH-resistance in *indica* rice, has been introduced into *japonica* rice from "Mudgo,"[1] the most resistant one, to develop several resistant *japonica* rice cultivars in Japan. BPH cannot normally live upon those cultivars and this is supposed to be due to the presence of a sucking inhibitor(s) and/or the absence of a sucking stimulator(s) in the phloem sap, because BPH excretes little honeydew when fed on resistant cultivars.[2] We attempted to identify the active principles controlling the resistance to BPH in rice plants and have identified asparagine as a sucking stimulator and β-sitosterol as a sucking inhibitor. The preliminary results are reported here.

For plant materials, a pair of isogenic lines, F_{20}-80 (abb., 80R) and F_{20}-74 (abb., 74S), resistant and susceptible to BPH respectively, were used to compare the endogenous active principles responsible for BPH-resistance. They were bred after the repeated selection of heterogeneous plants of F_{11} through F_{19} from the cross (Hoyoku × Mudgo) F_2 × Kochikaze. Three kinds of materials from the rice plants, namely, phloem sap, honeydew and extracts from the aerial parts of the plants, were used to identify the active principles responsible for BPH-resistance.

The collection of honeydew and phloem sap from both cultivars was achieved by using BPH of *biotype 2*,[3] which has an adaptability to rice plants with *Bph 1*. The honeydew was collected by extraction from filter paper on which the honeydew was excreted. Phloem sap was collected by the method of Kawabe *et al.*[4] A stylet of the BPH sucking phloem sap was cut off with a laser beam and the phloem sap exuding through the stylet was collected. About 0.3 μl of phloem sap was obtained from one stylet. The extract from the aerial part was obtained by extracting shoots with methanol using a blender.

The bioassay for sucking stimulators and inhibitors was carried out as follows. BPH was fed on a 15% sucrose solution containing samples and riboflavin (100 ppm) through Parafilm.[5] The honeydew was collected on filter paper and detected as fluorescent spots under an ultra-violet light. The sucking-inhibitory or stimulative activity was roughly evaluated by the number of spots and the intensity of fluorescence. A more precise quantification of the honeydew was done by a quantitative analysis of sugar.

Since there is a report concerning the effect of amino acids on the sucking behavior of BPH,[6] amino acids in the phloem sap collected at several growth stages of the rice plants were analyzed. A remarkable difference in asparagine content between the phloem saps of 80R and 74S was found, as shown in Table I, its content in the phloem sap from 80R being much lower than that from 74S.

Although phloem sap is considered to be the most suitable material in this study, it is very

TABLE I. ASPARAGINE CONTENTS IN THE PHLOEM SAPS OF 80R AND 74S ($\mu g/\mu l$)

Stage	80R	74S
2nd-Leaf	1.21	5.26
6th-Leaf	3.55	5.07
8th~9th-Leaf	5.74	13.41
Ripening	0.75	18.32
Average	2.81	10.52

TABLE II. CONTENTS OF STEROLS IN THE PHLOEM SAPS OF 80R AND 74S AT THE 8TH~10TH LEAF STAGE ($ng/\mu l$)

Determination was carried out for three samples collected separately.

Sterol	80R	74S
β-Sitosterol	1.8, 2.2, 3.0	0.3, 0.7, 0.8
Stigmasterol	* 0.9, 1.0	* 0.4, 0.3
Campesterol	* — —	* — —

* Not determined; —, not detected.

difficult to obtain a large quantity of phloem sap in the manner described above. Consequently, extracts from the aerial part and honeydew were used for an extensive survey of the active compounds. After solvent partitioning and fractionation by high performance liquid chromatography, several sucking-inhibitory fractions were found but no marked difference was observed between the samples from 80R and 74S. This may be partly due to the occurrence of "general inhibitors"[7] in the materials. Furthermore, although a similar inhibitory pattern was observed in the extracts from the aerial parts of 80R and 74S as well as in the honeydews from both cultivars, it is possible that a difference may exist between the contents of inhibitors in the phloem saps of the two cultivars. Because of this, we proceeded with experiments to identify the inhibitors in phloem sap in the following way.

Firstly, we looked for inhibitors common to both honeydew and the extract from the aerial part of 80R in the hope that those inhibitors may be also contained in phloem sap. The common inhibitors were found in the fraction obtained by thin layer chromatography (silica gel) and subsequent high performance liquid chromatography (Nucleosil $5C_{18}$) of an ethyl acetate-soluble neutral fraction. The active fraction from the extract of the aerial part was shown to contain β-sitosterol, stigmasterol and campesterol by GC-MS analysis after trimethylsilylation. On the other hand, cholesterol and β-sitosterol were detected in the corresponding fraction from the honeydew. These sterols showed a marked sucking-inhibitory effect. The solution containing 50 ppm of β-sitosterol and 15% sucrose caused an almost perfect inhibition in sucking. The other three sterols also showed a similar activity. This activity was much stronger than that of oxalic acid[8] or *trans*-aconitic acid,[9] which were identified as sucking inhibitors in the rice plant and barnyard grass, respectively.

Next, identification of sterols in the phloem saps of 80R and 74S was attempted. About 1 μl of phloem sap from the rice plant at the eighth- to tenth-leaf stage was extracted with ethyl acetate. The extract was analyzed by combined gas-liquid chromatography-selected ion monitoring (GC-SIM) after trimethylsilylation. As shown in Table II, β-sitosterol was identified as a main sterol and its content in the phloem sap of 80R was several times that of 74S.

However, at those concentration in the phloem sap of 80R, β-sitosterol could not effect sucking-inhibition in our bioassay. This might be because BPH would respond less sensitively to β-sitosterol in our bioassay system than in the natural condition or some synergetic substances could exist in the phloem sap. It has been reported that amino acids exceeding 4% in sucrose solution cause sucking-inhibition in a bioassay.[10] Since the total amino acid content was determined to be 3~7% in the phloem sap, it is quite possible that sterols in the low level may exhibit an inhibitory activity on BPH by a synergetic effect of the amino acids.

The addition of asparagine to a 15% sucrose

solution caused an increase in the amount of honeydew; namely, 150% with 0.2% asparagine and 200% with 1.0% asparagine. This result was compatible with the effect of asparagine reported by Sōgawa *et al.*[6] The lower asparagine content in the phloem sap of 80R than that of 74S, together with the sucking-stimulating activity of asparagine suggests that the difference of asparagine content in phloem sap between 80R and 74S can be related to the host selection of BPH. The antagonistic effect of asparagine and the synergetic effect of amino acids existing at relatively high concentrations on the activity of sterols are now under investigation.

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