

## **Gramine and Resistance of Barley to Aphids : Analysis of EDTA Exudates from Barley Leaves**

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Young leaves of barley contain a large amount of gramine, which is one of the factors involved in the resistance of barley against aphids. Using stylectomy by laser beam and EDTA-exudate method, we tried to determine if gramine exists in phloem sap which aphids ingest mainly. Phloem sap was not obtained by laser stylectomy using aphids feeding on young leaves. Components of exudates from cut leaves of barley in EDTA solution are known to be very similar to phloem sap. The time course of sucrose and gramine content of EDTA exudates from barley leaves suggested the existence of gramine in phloem sap.

**Key words:** Barley, Resistance to aphids, Gramine, EDTA,  
Localization

### **INTRODUCTION**

Aphids severely damage crops by transmission of plant viruses, chlorosis or necrosis of plant tissue with their feeding, and reduce productivity of crops by ingestion of photosynthetic products. Breeding crops with resistance to aphids is an effective strategy to prevent damage by aphids. It is very important to know the mechanisms of resistance and factors involved, for breeding resistant crops.

Some secondary metabolites of plants are known as resistance factors against insect pests (Corcuera 1993). We have reported that the content of gramine, an indole alkaloid and secondary metabolite of barley, is related to resistance of barley to aphids in the fields. The gramine content was high in leaves of resistant lines of barley, but low in leaves of susceptible lines

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(Kanehisa *et al.* 1990).

However, although seedlings of barley generally contain a very large amount of gramine and the gramine content decreases rapidly as plants grow in the field, aphids can grow very well on seedlings or young barley plants. This suggests that resistance in each stage of barley to aphids cannot be explained by gramine concentration in the leaves, alone.

To investigate further the relationship between gramine concentration and resistance, the gramine concentration in the phloem sap which aphids ingest must be examined. We collected phloem sap from seedlings of barley and analyzed the gramine concentration, and discussed about the role of gramine in resistance to aphids.

## MATERIALS AND METHODS

### 1. *Plants*

Young plants of Kikai Hadaka, a barley cultivar, grown in vermiculite at 20°C under a photoperiod of 16L : 8D for 1 week were used.

### 2. *Stylectomy*

A young barley plant was covered with a long prismatic tube, like drinking straw, made by transparent plastic sheet commercially available for an over head projector. One or several apterous adult aphids, *Rhopalosiphum maidis*, were put in the area covered by the tube. After aphids start ingestion from phloem of barley, a stylet was cut with an infrared beam from a YAG LASER apparatus (a welding machine, model SL443, NEC).

### 3. *Collection of exudate from barley by EDTA method*

Young plants of barley were cut just above the vermiculite. One gram of the cut leaves in fresh weight, about 40 leaves, was put into a tube containing 1 ml of three kinds of testing solutions for respective purpose, as the cut ends were dipped in the solution. Five mM EDTA (ethylenediaminetetraacetic acid), 10 mM CaCl<sub>2</sub>, or DDW were used as testing solution. The test tubes were covered with parafilm to avoid evaporation and exudates were collected at room temperature. The solutions were exchanged periodically to obtain data of exudation in time course. The solutions containing exudate were used to determine gramine and sugar concentration.

### 4. *Determination of gramine*

Gramine was extracted three times with the same volume of chloroform

after a drop of 3-fold diluted ammonia water was added into the sample solution. Gramine in the concentrated chloroform phase was determined by HPLC analysis (Kanehisa *et al.* 1990).

### 5. Determination of sugar

The water phase of the above extraction was added by a 3-fold volume of ethanol. After removing precipitates by centrifugation, the total sugar content in the concentrated sample was determined by the anthrone-sulfuric acid method. The sugar content was calculated as glucose.

## RESULTS

### 1. Stylectomy

Laser beam of 70 micrometers in width cut stylets of aphid, but no solution came out from the cut end of the stylet on the barley leaf. Therefore, we could not determine the gramine contents in sap. The reasons why nothing came out might be that the width of the laser beam was too large or that pressure of phloem sap was insufficient. Further studies in rearing plant materials will be needed.

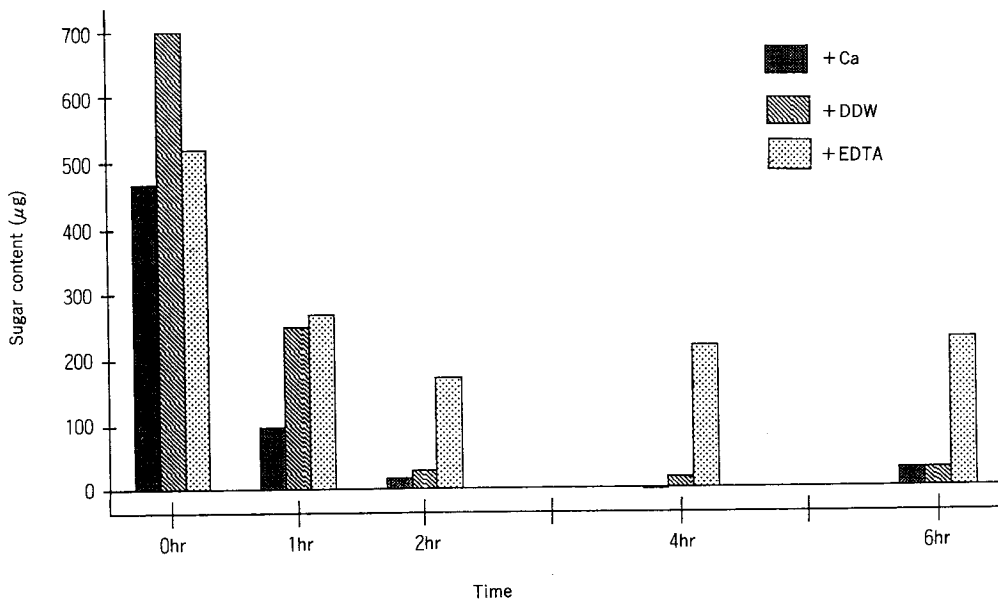


Fig. 1. Time course of sugar content in exudates. Sugar content of exudates collected after 0, 1, 2, 4, 6 hrs was determined as described in Materials and Methods. The vertical axis means  $\mu\text{g}$  of sugar.

## 2. Analysis of phloem sap by EDTA method

When cut plant parts were put into water at the cut end, phloem sap exuded. The addition of EDTA in water enhanced the rate of exudation, owing to the binding of the  $\text{Ca}^{2+}$  which was normally involved in callose formation. Using barley and oat, Weibull et al. (1990) reported by comparing amino acid compositions between leaf exudate collected in a 5mM EDTA-solution (pH7.0) and phloem sap obtained by stylectomy that the exudate technique holds great promise as interesting alternative to stylet-cutting technique.

Figs. 1 and 2 show the gramine and sugar content in exudates from barley leaves with time, respectively. Gramine and sugar were present in the EDTA exudate at 6 hrs, but, both gramine and sugar contents became very low after 2 hrs in exudates from barley dipped in DDW or  $\text{Ca}^{2+}$  solution. Fig. 2 shows that EDTA enhanced the rate of exudation of phloem sap by binding of the  $\text{Ca}^{2+}$ . Fig. 3 shows the time-course patterns of gramine and sugar contents. Each pattern coincided very well both in EDTA exudates and in  $\text{Ca}^{2+}$  exudates.

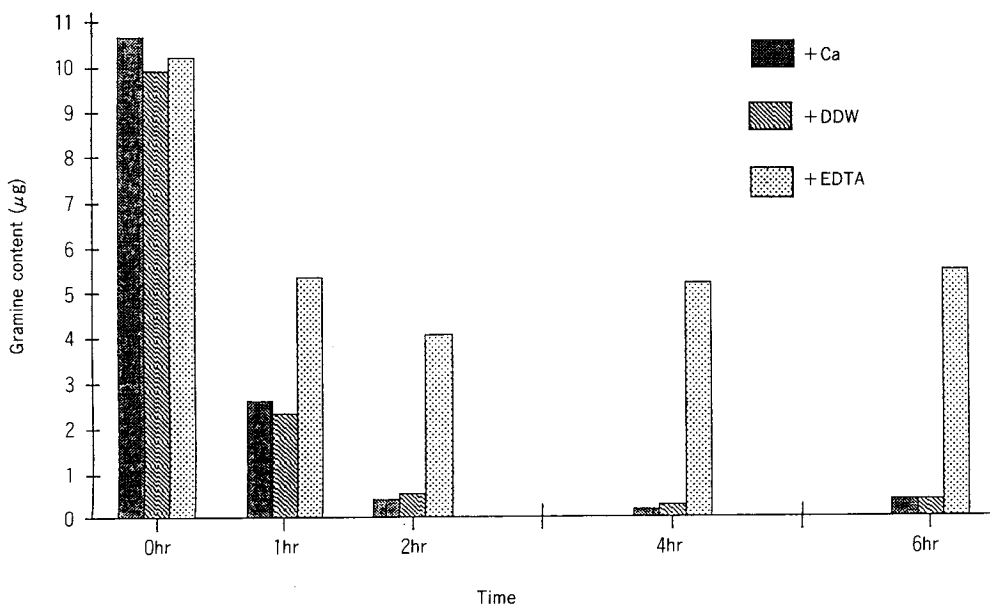


Fig. 2. Gramine content in exudates with time. Gramine content of the same samples as in Fig. 1 was determined as described in Materials and Methods. The vertical axis means  $\mu\text{g}$  of gramine.

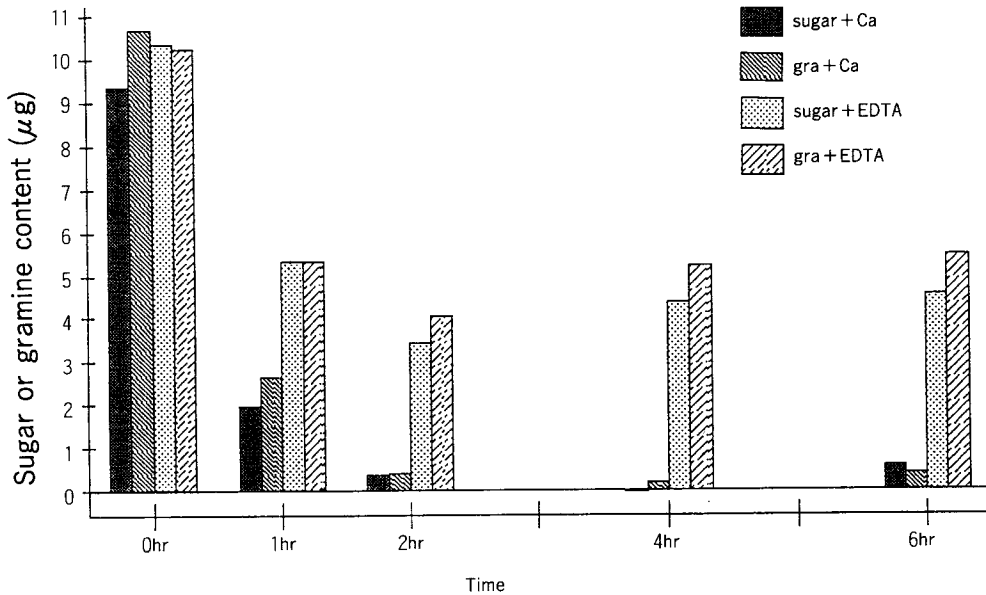


Fig. 3. Comparison of pattern of sugar and gramine content. Data shown in Fig. 1 and Fig. 2 were re-drawn to compare the time-course change of sugar and gramine concentrations in exudates. The scale for the vertical axis is different from one in Fig. 1 and Fig. 2.

## DISCUSSION

These findings strongly suggest that gramine exists in phloem sap. Almost all sugar in phloem sap is sucrose and the concentration is from 5% to 20% (weight/volume). If gramine exuded from phloem of cut leaves, the gramine concentration in phloem sap of barley leaves may be estimated as nearly 0.1% (w/v). Although a small amount of gramine is released from the leaf surfaces (Yoshida *et al.* 1993), it is difficult to explain the high gramine concentration detected in the EDTA exudate by the surface gramine. Because it is not clear that only phloem sap exudes into the EDTA solution, it is also possible that origins of gramine in the EDTA exudate may be any tissues other than phloem and that  $\text{Ca}^{2+}$  is involved in the mechanism of exudation of gramine from the tissue. Gramine has been found in mesophyll parenchyma and in epidermis, but has not been detected in vascular bundles of barley (Argandoña *et al.* 1987). Because information about detection limits of gramine and the amount of a collected vascular bundles is not reported (Argandoña *et al.* 1987), we cannot discuss about these discrepancies further. Another possibility is that "unloading" gramine from mesophyll-parenchymal cell to phloem is enhanced by EDTA. Aphids ingest

phloem sap, therefore it will be important to make clear the location of toxic gramine in barley tissues in the near future.

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## オオムギのアブラムシ抵抗性とグラミン：EDTA 法による解析

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オオムギのアブラムシ抵抗性要因物質の一つ、グラミンは幼苗に多量に存在している。アブラムシ口針切断法と EDTA 法を用いて、アブラムシが吸汁するオオムギの師管液にグラミンが存在しているかどうか検討した。幼苗に寄生しているアブラムシの口針をレーザー光線を用いて切断したが、師管液は得られなかった。オオムギ葉を切断し EDTA 溶液に浸し、切り口から液中に浸潤してくる成分は師管液に非常に類似したものといわれている。この EDTA 法により得られた浸潤液のグラミンと糖の含量を時間を追って測定したところ、グラミンが師管液中に存在する可能性を示唆する結果を得た。

**キーワード：**オオムギ、アブラムシ抵抗性、グラミン、EDTA、局在