岡大資生研報 (Bull. Res. Inst. Bioresour. Okayama Univ.) 4 : 49-58 (1996)

Relationship between Gramine Concentration and Cereal Aphid Populations in Seedling and Maturation Stages in Barley Lines

Saeid MOHARRAMIPOUR, Shin-ichi MURATA, Katsuo KANEHISA*
and Hisaaki TSUMUKI

The relationship between the indole alkaloid gramine concentration and aphid population was examined at seedling and maturation stages in 14 barley lines of *Hordeum spontaneum* and *H. vulgare*.

The density of *Schizaphis graminum* did not differ significantly with the gramine concentration in the seedling in the greenhouse. However, the population of *Rhopalosiphum padi* sometimes differed with the seedling.

The plant resistance to the natural infestation of cereal aphids was obvious at the heading stage. There was a negative correlation between the high population density of aphids and gramine concentration. The gramine concentration was high in matured resistant lines, especially wild lines, as compared with susceptible lines due to higher biodegradation activity.

Key words: Barley, Resistance, Gramine, Cereal aphids

INTRODUCTION

Cereal aphids are serious insect pests on cereal plants and grasses (Vickerman and Wratten 1979, Carter *et al.* 1980). Barley plants are damaged by their sap withdrawal, injection of toxic saliva, transmission of plant virus diseases, especially barley yellow dwarf virus (BYDV), and excretion of honeydew. Gramine indole alkaloid is considered to play an important role in barley resistance to aphids both in laboratory and field experiments (Zuniga *et al.* 1985, Kanehisa *et al.* 1990, Rustamani *et al.* 1992, Kanehisa *et al.* 1993). The life history of *S. graminum* is affected by gramine added to the artificial diets (Zuniga *et al.* 1988, Kawada *et al.* 1989), leading to reduction of fecundity and longevity, and change in feeding behavior.

Research Institute for Bioresources, Okayama University, Kurashiki 710, Japan
Received December 6, 1995
*Emeritus professor, Okayama University

Gramine content in barley leaves is decreased with age (Zuniga *et al.* 1985) due to biodegradation by a pathway which starts by the oxidative elimination of its side chain (Ghini *et al.* 1990, 1991) and is higher in resistant lines than in susceptible lines (Kanehisa *et al.* 1990, Rustamani *et al.* 1992, Kanehisa *et al.* 1993). In spite of the higher concentration of gramine at seedling stage, aphids increased successfully. This suggests that the parasitic properties are somewhat different between the seedling and maturation stages. Herein, we describe these properties.

MATERIALS AND METHODS

1. *Plant materials*

Barley seeds were obtained from the Barley Germplasm Center of our institute. Table 1 shows the names and accession numbers of selected lines, partly described by Takahashi *et al.* (1983).

The plants were kept in a greenhouse during the seedling stage and in the field during the maturation stage. Seedlings were cultured in pots (8 diam. ×7 cm depth or 12 diam. ×12 cm depth) at 25±2°C. Seeds of each line were sown in a single row of ca. 80 cm in length in mid-November and harvested in early June. Each plant was grown ca. 8 cm apart in rows spaced ca. 90 cm apart.

Table 1. Names of barley lines and accession numbers

Line name	OU Access. NO.[1]
Hordeum spontaneum :	
H. sp. 2258	H603
H. sp. 4969	H689
Hordeum vulgare :	
Jomson-2	N673
Nejire 2	J091
Kashimamugi	J536
f_9-gl_3-*Hs-k*	L117
Kumt-239 (gl_5)	M294
Aizu 6	J628
Goseshikoku-1 hen	J314
Hood-gl_3	L055
Kirin Choku 1	J511
Nihon-ichi	J172
Goseshikoku sai-1	J634
Shinriki	J780

[1]: Okayama University Accession Number

2. Assessment of aphid populations

Aphid species *S. graminum* and *R. padi* were reared in our laboratory on the seedling of barley line Kikaihadaka and wheat line Shirasagi respectively.

To determine the population increase of aphids in the greenhouse, a newly emerged nymph was put on each stem of the 30-days-old potted seedlings. This procedure was repeated three times for *S. graminum* and four to seven times for *R. padi*. Nymphs and adults were counted 12, 17, 22 and 8, 15, 22 days after infestation in two experiments.

Aphids were counted weekly in the field from the beginning of April until harvesting time. The number of aphids per stem was calculated. Weekly counted aphids per stem from about the middle of April to end of May were summed up and used as an aphid index to characterize the susceptibility in the field. The lines with more than 20, between 5–20 and lower than 5 indices were defined as susceptible, intermediate and resistant respectively.

3. Extraction of gramine

Each line was sampled for gramine measurement in five replications at the middle of April. Gramine was extracted from second leaves of barley lines and measured as described by Hahlbrock and Conn (1971) and Kanehisa *et al.* (1990). Three leaves from individual plants were taken and stored at −20°C until used. The leaves were ground in liquid nitrogen and then homogenized in 20 ml of 1% ammoniacal methanol. The supernatant was evaporated in a metal block EMG-2 below 40°C and dissolved in 10 ml of 0.1 M HCl solution. After adjustment to pH 9, the solution was fractionated two times with an equal volume of chloroform. The chloroform layers were confined, evaporated and used for measuring gramine by high performance liquid chromatography (Kanehisa *et al.* 1990).

4. Thin layer chromatography

Silica gel (Merck 60 G) was used as the absorbent. The methanol, chloroform and 1/2 concentration of NH_4OH (50 : 50 : 1) were used as a developing solvent. Urk Salkowsky reagent (Ehmann 1977) was used to detect the compounds. Plates sprayed with the reagent were heated at 110°C for 10 minutes. Gramine was stained blue and other relatives with different colors.

RESULTS

Tables 2 and 3 show the populations of *S. graminum* and *R. padi* on the barley seedlings in the greenhouse. The population density of *R. padi* differed significantly with the line but not that of *S. graminum*. The population grew slower on H603 and J628, but higher on M294 than on the others (Table 2). The population density of *R. padi* eight days after infestation was lower on resistant lines (H603, H689 and J172) than the others (Table 3). Table 4 shows the dynamic population of cereal aphids. Aphid populations counted in field evaluations contained *R. maidis* (L.) and *R. padi* (F.) as a dominant species in April and May, respectively, while *S. graminum* (Rondani) and *Sitobion akebiae* (Shinji) were observed intermittently in small colonies. The aphids appeared from the second week of April and increased gradually on all lines until the first week of May. Thereafter, the population decreased due to heavy rainfall. After rainfall, the population increased slowly again until the end of May. Differences with the barley line were seen from April 25 to May 2 when the aphid population was the highest. The average aphid index was 24.8, 12.9 and 1.7 in susceptible, intermediate and resistant lines, respectively (Table 5).

Table 2. Comparison of barley seedling lines infested with *Schizaphis graminum* and *Rhopalosiphum padi* in greenhouse test

	No. of aphids per stem					
	S. graminum[1)]			*R. padi*[2)]		
Entries	12[3)]	17	22	12	17	22
H603	0.9	11.7	83.3	2.5	6.6	21.2
H689	5.7	21.7	103.3	3.4	13.5	45.3
N673	3.5	23.4	69.8	1.8	14.1	40.0
J091	2.8	20.6	146.7	4.0	10.1	32.5
J536	3.1	21.7	190.0	4.2	11.1	41.6
L117	5.9	25.3	250.0	2.4	9.6	24.4
M294	5.1	29.8	203.3	2.8	13.2	64.4
J628	3.0	19.1	148.0	3.1	3.7	20.0
J314	2.0	13.2	103.3	0.7	5.4	43.1
F-Value	2.04	0.82	1.08	1.01	2.51*	4.52**
P-Value	0.10	0.60	0.42	0.45	0.04	0.00

1) : Average of three replications
2) : Average of four replications
3) : Days after infestation

Table 3. Comparison of barley seedling lines infested with *Schizaphis graminum* and *Rhopalosiphum padi* in greenhouse test

	No. of aphids per stem					
	S. graminum[1]			*R. padi*[2]		
Entries	8[3]	15	22	8	15	22
H603	7.5	28.6	90.6	4.9	15.8	26.2
H689	9.1	31.3	83.8	6.8	31.5	59.4
L117	19.4	36.0	72.8	16.9	34.6	82.7
L055	9.7	38.5	99.9	14.2	47.6	86.9
J511	26.6	69.4	143.8	18.0	64.5	66.8
J628	8.6	29.6	85.9	7.4	25.0	71.9
J172	4.6	24.4	93.9	3.4	33.1	71.0
F-Value	1.30	1.39	0.63	3.07**	1.21	0.53
P-Value	0.32	0.28	0.71	0.01	0.32	0.78

1) : Average of three replications
2) : Average of seven replications
3) : Days after infestation

Table 4. Dynamic population of cereal aphids on different barley lines in the field

	No. of aphids per stem							
Line	Apr. 13	Apr. 18	Apr. 25	May. 2	May. 9	May. 16	May. 23	May. 30
H603	0.0	0.0	0.2	1.8	0.0	0.0	0.1	0.3
H689	0.0	0.1	0.2	0.4	0.1	0.0	0.1	0.2
N673	0.0	0.2	0.3	3.1	1.8	0.2	3.8	1.2
J091	0.2	0.3	0.4	2.9	5.9	2.1	4.8	3.7
J536	0.3	0.4	1.7	3.3	3.8	3.0	3.3	1.1
L117	0.5	5.3	6.9	9.5	2.9	0.2	1.7	3.7
M294	0.1	0.1	1.5	11.6	10.4	1.0	2.8	0.8
J628	0.1	1.4	2.0	5.0	2.7	3.4	3.5	3.1
J314	0.1	0.2	0.4	3.8	2.3	1.5	1.4	2.8
L055	1.0	5.8	7.1	3.9	0.5	0.1	0.8	4.8
J511	0.6	2.3	2.3	2.7	0.6	0.7	1.0	1.3
J172	0.0	0.0	0.1	0.3	0.1	0.1	0.4	0.4

The amount of gramine extracted from matured plant leaves was considerably high (14.5 μg/gf.w.) in the resistant lines and nearly zero in the susceptible lines. The value averaged 6.8 μg/gf.w. in the intermediate lines (Table 5). The aphid density on barley lines was inversely correlated with the gramine concentration in the leaves (Fig. 1). According to TLC experiments, all lines contained gramine more or less in seedling stage, but only resistant

Table 5. Relationship between cereal aphid index and gramine concentration in various barley lines

Line	Aphid index	Reaction to aphids[1)]	Gramine concentration (μg/gf.w.) Seedling[2)]	Maturation[3)]
H603	2.4	R	75.0	14.0
H689	1.1	R	130.0	18.2
N673	10.6	I		0.5
J091	20.2	S		4.1
J536	16.9	I		18.4
L117	30.7	S		0.0
M294	28.2	S		0.0
J628	21.1	S	9.8	0.0
J314	12.5	I		8.2
L055	23.9	S	13.0	0.0
J511	11.5	I		0.1
J172	1.5	R	8.0	11.4
Average:	1.7	R		14.5
	12.9	I		6.8
	24.8	S		0.8

[1)]: R, I and S indicate Resistant, Intermediate and Susceptible, respectively
[2)]: Measured on Jan. 26
[3)]: Measured on Apr. 18

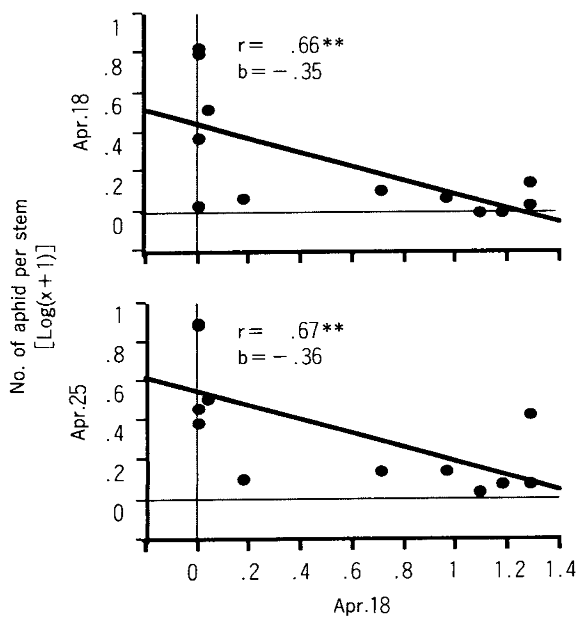

Fig. 1. Correlation between cereal aphid populations and gramine concentration in berley lines at maturation stage.

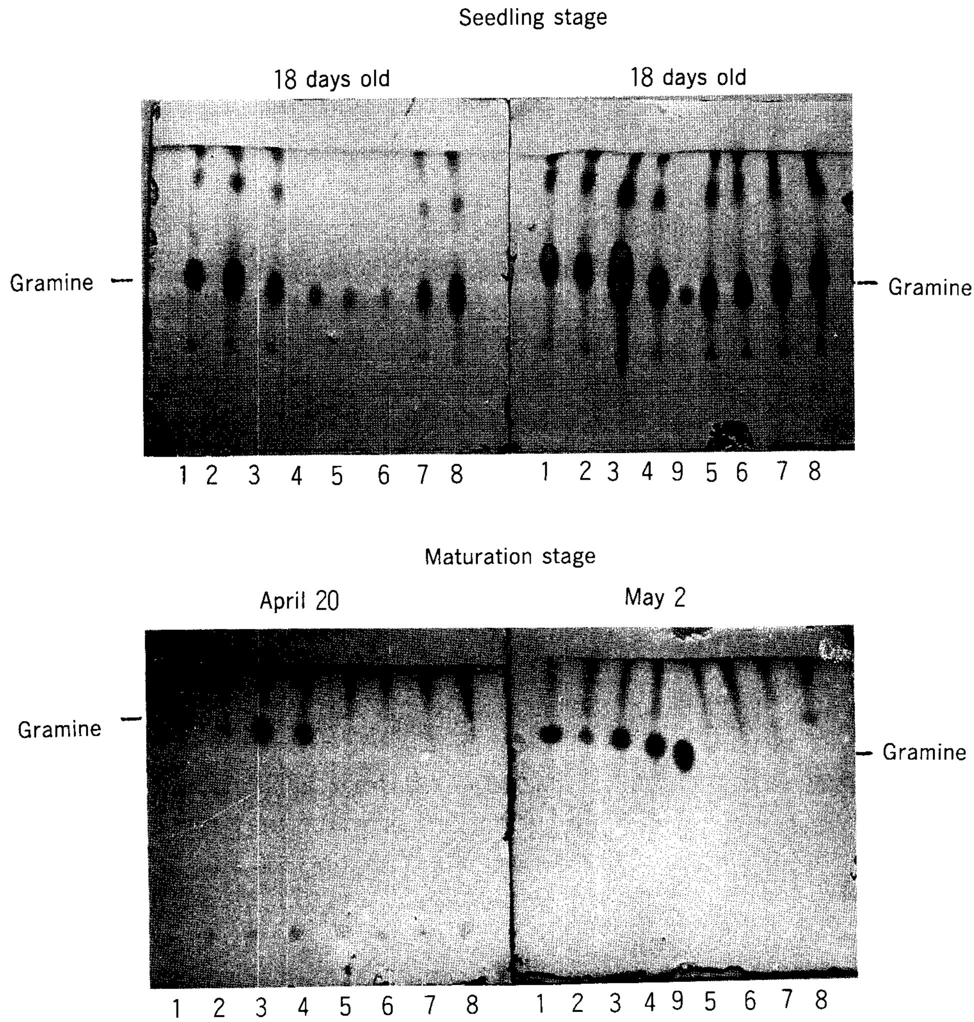

Fig. 2. Thin layer chromatograms of gramine indole alkaloid in barley lines at seedling and maturation stages. Absorbent : Silica gel G ; Solvent : Methanol-Chloroform-1/2 NH_4OH (50 : 50 : 1) ; Spray reagent : Urk-Salkowsky ; 1. J780, 2. N673, 3. H603, 4. H689, 5. M294, 6. J628, 7. L117, 8. J634, 9. Gramine standard.

lines contained gramine in maturation stage (Fig. 2). High biodegradation of gramine in susceptible lines was evident in this stage. At the maturation stage, a few indole spots derived from gramine decomposed compounds were observed.

DISCUSSION

Gramine is thought to be an important anti-aphid substance in barley (Corcuera 1984), especially in field conditions (Kanehisa *et al.* 1990). The gramine concentration differed considerably with the barley lines at the seedling stage (Table 5), but aphids grew on all the lines. Consequently, the difference between susceptible and resistant lines was obscure. Aphids are phloem sap feeders while gramine is localized at the epidermis (Yoshida *et al.* 1993) and mesophyl parenchyma (Argandona *et al.* 1987), and might be in vacuole which contained many kinds of secondary metabolized substances. Gramine may act as an interference substance on the way of stylet penetration until vascular bundle. The susceptibility of seedlings may be due to soft structure and short distance from surface to phloem sap. However, the resistance in maturing stage may be due to the hard structure and long distance. Another point might be the occupation of enlarged vacuole in the cell which increase the chance to contact gramine by aphids during intracellular penetration.

One more important point, at the maturation stage, in susceptibility to aphids is related to aphid increasing time and simultaneous gramine decreasing due to biodegradation (Ghini *et al.* 1991). Resistant lines could maintain higher amounts of gramine, and further, highly resistant lines could increase gramine synthesizing activity during aphid increasing time (unpublished data). Moreover, in resistant lines, some unknown antibiotic substances with or without synergism effects on toxicity of gramine may increase and play a role in resistance at growing stage. Other factors such as amount of leaf surface wax confer resistance to aphids (Tsumuki *et al.* 1989, Moharramipour *et al.* submitted).

REFERENCES

Argandona, V.H., Zuniga, G.E. and Corcuera, L.J. 1987. Distribution of gramine and hydroxamic acids in barley and wheat leaves. Phytochemistry 26 : 1917–1918.

Carter, N., Mclean, I.F.G., Watt, A.D. and Dixon, A.F.G. 1980. Cereal aphids : a case study and review. Ann. Appl. Biol. 5 : 271–348.

Corcuera, L.E. 1984. Effects of indole alkaloids from graminae on aphids. Phytochemistry 23 : 539–541.

Ehmann, A. 1977. The van urk-salkowski reagent-A sensitive and specific chromogenic reagent for silica gel thin layer chromatographic detection and identification of indole derivatives. J. Chromatogr. 132 : 267–276.

Ghini, A.A., Burton, G. and Gros, E.G. 1990. Catabolism of gramine in *Hordeum vulgare*. Phytochemistry 29 : 1781–1783.

Ghini, A.A., Burton, G. and Gros, E.G. 1991. Biodegradation of the indolic system of gramine in *Hordeum vulgare*. Phytochemistry 30 : 779-784.

Hahlbrock, K. and Conn, E.E. 1971. Evidence of the formation of linmarin and lotaustralin in flax seedlings by the same glucosyl transferase. Phytochemistry 10 : 1019-1023.

Kanehisa, K., Tsumuki, H., Kawada, K. and Rustamani, M.A. 1990. Relations of gramine contents and aphid populations on barley lines. Appl. Entomol. Zool. 25 : 251-259.

Kanehisa, K., Rustamani, M.A., Tsumuki, H., Kasahara, T. and Shiraga, T. 1993. Changes of gramine contents as an aphid resistant substance with barley growth. Bull. Res. Inst. Bioresour. Okayama Univ. 1 : 105-112.

Kawada, K. and Lohar, M.K. 1989. Effect of gramine on the fecundity, longevity and probing behavior of the greenbug, *Schizaphis graminum* (Rondani). Ber. Ohara Inst. Landwirt. Biol. Okayama Univ. 19 : 199-204.

Moharramipour, S., Tsumuki, H., Sato, K., Murata, S. and Kanehisa, K. Effects of leaf color, epicuticular wax amount and gramine content in barley hybrids on cereal aphid populations. Appl. Entomol. Zool. Submitted.

Rustamani, M.A., Kanehisa, K., Tsumuki, H. and Shiraga, T. 1992. Additional observations on aphid densities and gramine contents in barley lines. Appl. Entomol. Zool. 27 : 151-153.

Takahashi, R., Yasuda, S., Hayashi, J., Fukuyama, T., Moriya, I. and Konishi, T. 1983. Catalogue of barley germplasm preserved in Okayama University. Inst. Agric. Biol. Okayama Univ., Kurashiki, pp. 1-217.

Tsumuki, H., Kanehisa, K., Kawada. K. and Shiraga, T. 1989. Leaf surface wax as a possible resistance factor of barley to cereal aphids. Appl. Entomol. Zool. 24 : 275-281.

Vickerman, G.P. and Wratten, S.D. 1979. The biology and pest status of cereal aphids (Hemiptera : Aphididae) in Europe : a review. Bull. Entomol. Res. 69 : 1-32.

Yoshida, H., Tsumuki, H., Kanehisa, K. and Corcuera, L.J. 1993. Release of gramine from barley leaf surface. Phytochemistry. 34 : 1011-1013.

Zuniga, G.E., Salgado, M.S. and Corcuera, L.J. 1985. Role of an indole alkaloid in the resistance of barley seedlings to aphids. Phytochemistry 24 : 945-947.

Zuniga, G.E., Varanda, E.M. and Corcuera, L.J. 1988. Effect of gramine on the feeding behavior of the aphids *Schizaphis graminum* and *Rhopalosiphum padi*. Entomol. Exp. Appl. 47 : 161-165.

幼苗期と成熟期のオオムギ系統間における禾穀類アブラムシの密度とグラミン含量の関係

MOHARRAMIPOUR, Saeid・村田眞一・兼久勝夫・積木久明

オオムギ（*Hordeum spontaneum* 及び *H. vulgare*）14系統の幼苗期と成熟期における，ア

ブラムシの密度とインドールアルカロイド化合物であるグラミン含量の関係を調べた.

温室内の幼苗では，グラミン含量に関わらずムギミドリアブラムシ（*Schizaphis graminum*）の寄生密度に有意差は認められなかった．一方，ムギクビレアブラムシ（*Rhopalosiphum padi*）の密度は，幼苗間で異なる場合もあった．

圃場におけるアブラムシの寄生に対して，抵抗性は出穂期に顕著に現れた．アブラムシの寄生密度とグラミン含量の間には負の相関が認められた．成熟期のオオムギでは，グラミンの分解活性の高い感受性系統と比較して，抵抗性系統のグラミン含量は多く，とくに野生系統では著しかった．

キーワード：オオムギ，抵抗性，グラミン，禾穀類アブラムシ