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FREE AMINO ACIDS IN THE PHLOEM SAP FROM OATS AND BARLEY RESISTANT TO *RHOPALOSIPHUM PADI*

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Key Word Index—*Hordeum*; *Avena*; Gramineae; free amino acids; phloem sap; *Rhopalosiphum padi*; plant resistance.

Abstract—The concentration and composition of free amino acids were determined in the phloem sap of barley and oat cultivars, breeding lines and wild species previously selected for resistance against the aphid *Rhopalosiphum padi* (L). No relationship was found between degree of aphid resistance and the total concentration of free amino acids in plant sap. In all investigated accessions aspartic acid, glutamic acid, glutamine, and serine dominated the amino acid composition. The concentration of glutamic acid was significantly higher in resistant plants than in susceptible cultivars. Sap from most resistant accessions contained less asparagine than that of the controls. The content of γ-aminobutyric acid, glycine, histidine, methionine, and tryptophan was generally low in the tested plants. The oat accessions had significantly more methionine in their phloem sap than the barley accessions. These results are discussed in light of previous work on plant resistance to aphids and host plant nutritional quality.

INTRODUCTION

Allelochemicals studied in relation to plant resistance to insects have mainly been secondary compounds such as alkaloids [1–3], terpenoids [4], hydroxamic acids [5, 6], flavonoids [7], and others. The effects of composition and variation of free amino acids on plant resistance to insects, especially to aphids, have not been extensively investigated. A classical study of resistance to *Acyrthosiphon pisum* (Harr.) [8] suggested a possible role of free amino acid levels. Resistance in *Vicia* to *Aphis fabae* (Scop.) and *A. pisum* with respect to both protein and non-protein amino acids is only circumstantial [9], although studies of *V. faba* (L.) cultivars [10] have indicated that free amino acids are partial determinants of resistance. Significant negative correlations between contents of proline and γ-aminobutyric acid (GABA) and relative growth rates of *Myzus persicae* (Sultz). were demonstrated in Brussels sprouts [11]. The relative growth rates of *R. padi* closely followed the phenological changes of free amino acids in phloem sap as oat and barley plants were ageing [12]. This result was in accordance with predictions based on theoretical simulations relating plant resistance characters to population development in this polyphagous species [13]. It was therefore suggested that variations in the concentration or composition of free amino acids might influence host resistance to this aphid pest in oats and barley.

In the present study the total content of free amino acids as well as the composition of individual amino acids were determined in phloem sap from various oat and barley accessions. Only protein amino acids were considered. The accessions were cultivars, breeding lines, and wild species previously selected for *R. padi* resistance on the basis of screening experiments [14, 15].

RESULTS AND DISCUSSION

There was no obvious relationship between the degree of resistance to *R. padi* and the total free amino acid concentration in the phloem sap of the investigated accessions. Two breeding lines, however, Cl 1470 (barley) and Obee (oats), differed from the rest in having significantly higher amounts of free amino acids in the phloem sap (Table 1). In both accessions much of this difference was attributable to higher concentrations of glutamic acid (Table 2). Also, in Cl 1470 the amino acids isoleucine, leucine, valine, and lysine were present in higher

Table 1. Total sample volumes and mean total free amino acid content (per cent) (s.e.) of phloem sap from the various accessions

Accession	No. of samples	Sample volume (nl)	Free amino acids (%)(s.e.)
Barleys			
cv Tellus	11	84.5	15.58 (1.70)
Cl 16145	11	93.4	15.83 (2.80)
Cl 1470	11	101.8	26.65 (2.41)
H. bogdani	8	37.0	14.76 (1.91)
Oats			
cv Selma	6	31.5	16.66 (0.99)
Obee	11	74.9	22.45 (2.21)
A. barbata	5	26.5	14.19 (0.96)
A. macrostachya	3	71.5	6.86 (0.77)*

*Only 10 amino acids quantified.

Table 2. Content (nmol/mg sample fresh weight) of individual amino acids in phloem sap of tested accessions

	Accession							
	cv Tellus	Cl 16145	Cl 1470	*H. bogdani*	cv Selma	Obee	*A. barbata*	*A. macr.*
Ala	50.0	80.4	66.3	50.0	51.9	44.9	59.2	14.8
Arg	32.8	32.3	81.3	26.8	60.8	48.9	17.0	4.6
Asn	70.9	18.5	59.9	10.4	95.1	40.0	36.9	12.0
Asp	145.9	110.3	174.0	168.5	79.0	174.5	155.7	51.6
GAB	3.3	0.7	1.1	16.6	4.6	4.8	3.4	+
Gln	196.9	223.1	89.6	130.9	191.2	172.7	154.4	28.2
Glu	254.9	286.3	566.5	372.4	191.4	422.6	338.9	230.3
Gly	7.6	3.3	0.9	3.5	7.8	1.9	2.2	4.5
His	6.3	2.8	22.6	26.4	6.3	14.3	—	+
Ile	22.0	19.5	82.2	18.2	45.4	53.7	7.6	+
Leu	23.5	19.5	101.9	22.5	43.9	62.0	5.9	+
Lys	24.9	21.1	76.0	17.5	70.5	51.4	8.9	+
Met	0.5	0.2	4.6	1.0	3.2	12.6	5.3	+
Phe	18.1	16.5	65.7	17.8	27.7	40.3	7.9	+
Scr	148.8	215.0	189.0	100.7	147.1	235.9	170.1	110.8
Thr	55.6	70.9	113.5	58.9	50.9	144.1	52.2	26.0
Trp	5.7	3.9	25.0	8.4	14.6	13.6	1.6	—
Tyr	13.1	10.9	39.8	9.3	17.9	20.7	7.4	—
Val	58.2	60.1	161.9	45.9	75.3	88.4	29.3	+
Total	1104.1	1195.3	1903.1	1079.6	1174.0	1599.7	1015.2	

+ Detected, not quantified.
— Not detected.

amounts, whereas in Obee serine and threonine were comparatively more prevalent.

In *A. macrostachya* it was difficult to collect phloem sap in amounts sufficient for quantification. Almost immediately after the droplet appeared at the tip of the cut stylet the plant juice began to crystallize, making it impossible to collect the sap in a microcap. Therefore, only three samples could be quantitatively analysed and in these the total concentration of free amino acids was comparatively low (Table 1). No statistical analysis was made on these values.

As there was no obvious relationship between resistance level and total amino acid concentration (see above) the data were normalized to relative values prior to multivariate analysis. This procedure introduces covariance between high and low variance variables. Therefore a second normalization was done as relative values against the content of serine + threonine (both acids having rather low coefficients of variation) to test the multivariate model stability. For both types of normalizations three sets of data (descriptors) were modelled (see Table 3).

Together, aspartic acid, glutamic acid, glutamine, and serine constituted from 51.2% (cv Selma) to 77.0% (*A. barbata*) of the total amino acid composition. Similarly, these four amino acids dominate in other grass species such as wheat [16, 17] maize [18], rice [19], and millet [20; *nota bene* xylem exudate]. Some of these data may, however, be questioned in light of sample instability, as discussed elsewhere [21], and detection methods. The first multivariate PLS model on the complete descriptor set gave two significant components explaining 54% of the variation in aphid resistance (Table 3). Obviously the structure of the oat amino acid data was better than that of the barley data, since in the oat PLS model as much as 81% of the variation in resistance was explained. Of the 19 amino acids included in the models aspartic and glutamic acid gave the strongest positive contribution (in the multidimensional space) whereas phenylalanine, isoleucine, leucine, and lysine contributed most negatively. For the second significant PLS component glutamine was found to give a strong negative orientational influence. Subsequent PLS analyses using the serine + threonine type of normalization led to a slight improvement in per cent explained variance, primarily for barley (Table 3). Phenylalanine, isoleucine, leucine, and lysine again influenced the projection negatively, and GABA contributed most to the positive projection of the first component. The second significant component was

Table 3. Multivariate analyses of aphid resistance data

Type of normalization	Data/descriptor set	No. of comp.*	% explained variance of aphid resistance
% of sum	Oats + barley	2	54
	Oats	3	81
	Barley	2	51
% of ser + thr	Oats + barley	2	57
	Oats	3	84
	Barley	3	66

*Only significant components included

influenced positively by glutamic acid and threonine, and negatively by serine.

It is thus important to point out that the variation in amino acid composition alone is sufficient to separate levels of resistance, expressed as population growth, between cultivars. This supports the hypothesis that nutritional compounds play a role in determining plant resistance in oats and barley [12]. It also conforms to the suggestion that a substrate effect is more important in this insect/plant system than either positive or negative allelochemical signals [13]. All accessions were very low in their content of GABA, glycine, histidine, methionine and tryptophan, again accordance with the refs above.

Concentrations of glutamic acid were generally higher in the phloem sap of resistant accessions than in that of susceptible ones (Table 2). Both control cvs contained significantly less glutamic acid in comparison with all other accessions, except for *A. macrostachya*. The sap from this latter species was comparatively low in all detected amino acids except for glutamic acid and serine, for which the concentrations were similar to those in the other plants. Gustatory experiments with *M. persicae* [22] have demonstrated a slightly deterrent effect of glutamic acid in a 20% sucrose solution, even in a buffered solution (pH 7.2). *R. padi* appears to have a slight alkaline pH-optimum around 8.0–8.2 (preliminary observations). It might therefore be argued that glutamic acid is of importance in the host plant selection process of *R. padi*. Resistant *Hordeum* accessions also contained significantly lower asparagine concentrations than the corresponding control. In *Avena* the content of arginine was higher in the control cv than in the other oat accessions. Intergeneric differences were only found for methionine (*t*-test; $p < 0.001$; Table 2) where the amounts in *Avena* spp. were higher. Methionine acts as a feeding stimulant for various aphid species [23–25] and omission of this acid from a synthetic diet causes a decrease in the feeding rate. In choice-experiments with oats and barley (unpublished data) *R. padi* has been found to preferentially settle on oats. That no marked difference in total amino acid concentration exists between the two cereals [12] suggests that the methionine content could influence selection behaviour.

It can thus be concluded that *R. padi* resistance in accessions of oats and barley in part relates to the amino acid composition of phloem sap and not to the total concentration of free amino acids. These results will be further evaluated through *in vitro* tests.

EXPERIMENTAL

Plant and insect material. Phloem sap samples were collected from three accessions of spring barley (*Hordeum vulgare* L. cv Tellus and breeding lines CI 16145 and CI 1470), *H. bogdani* (Wil.; acc. no. H 240), two accessions of spring oats (*Avena sativa* L. cv Selma and breeding line Obee), *A. barbata* (Pott ex Link; acc. no. CAV 6331/32) and *A. macrostachya* (Bal., ex Coss. et Dur.; acc. no. CAV 5264). In CI 16145, resistance has been demonstrated to *Sitobion avenae* F. [26, 27] and, together with CI 1470, also to *Rhopalosiphum padi* (Hanson, R., personal communication). Other accessions are referred to elsewhere [14, 15]. The two cvs Tellus and Selma were used as susceptible controls.

All plants used for sap collection were grown in plastic pots (12 cm diam; 3 plants per pot) in a Fisons growth cabinent under a 16:8 hr photoperiod. Day and night temps were 19 and 12°, respectively, and relative humidity ranged from 60 to 85%. The plants did not receive any supplementary N.

Aphids were cultured on a mixture of oats (*A. sativa* cv Selma) and barley (*H. vulgare* cv Tellus). The plants were discarded after use.

Sap collection and analysis. High frequency microcautery was used to cut the aphids' stylets. Extensive descriptions of the procedures for collection and analysis of the sap are given elsewhere [12, 21]. However, due to an unavoidable reallocation of personnel before completion of the analyses the sap samples of *A. macrostachya* had to be analysed differently. Thus, fluorescent derivatives were obtained using 15 mM 9-fluorenylmethyl chloroformate (FMOC-Cl) [28], separated on a 250 × 4.6 mm i.d. ODS-Hypersil (5 μm) column, and detected with a Shimadzu Model RF 530 fluorescence detector (exc. 260 nm, em. 313 nm). For buffers, solvents, flows, and gradients, see Näsholm *et al.* [29].

Quantitative analyses of honeydew using the above method gave results similar to those previously obtained with *o*-phthaldehyde (OPA; unpublished data) and the method was therefore considered to be adequate. The amounts of phloem sap sampled from the various accessions are given in Table 1.

Data on total free amino acid content were evaluated by means of analysis of variance using the SAS-package (Statistical Analysis System Inst. Inc., N. C.). Variations in individual amino acids or groups of these were evaluated using a *t*-statistic for unpaired samples, and partial least squares regression (PLS) [30] on normalized values using the individual resistance ranks (from 1 to 4) as dummy variables.

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