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DISTRIBUTION OF GRAMINE AND HYDROXAMIC ACIDS IN BARLEY AND WHEAT LEAVES

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Key Word Index—*Hordeum*; *Triticum*; *Schizaphis graminum*; greenbug; aphids; gramine; DIMBOA.

Abstract—The first leaf of 12-day-old barley seedlings, contained 1.3 μmol/g fr. wt gramine while the concentrations were 2.2, 2.0 and 3.1 μmol/g fr. wt in lower and upper epidermis and mesophyll parenchyma, respectively. Gramine was not detected in the vascular bundles, nor in xylem exudates or guttation drops. Thus, about 70% of total gramine in the leaf was found in the mesophyll parenchyma and 30% in epidermal tissue. The content of hydroxamic acids was 3.0 μmol/g fr. wt in the first leaf of 12-day-old wheat plants, while the concentrations were 6.8 and 4.1 μmol/g fresh wt in the vascular bundle and mesophyll parenchyma, respectively. These compounds were not detected in epidermis, xylem exudates and guttation drops. About 50% of total hydroxamic acids were found in the vascular bundles. The significance of these results in plant protection against aphids is discussed.

INTRODUCTION

Secondary metabolites may protect plants against insects and pathogens [1]. Quinolizidine alkaloids from the Fabaceae may be involved in plant resistance to the broom aphid *Aphis cytosorum* [2] and in allelopathy [3]. The concentration of these compounds varies among tissues of the plant with marked diurnal fluctuations [4, 5]. Dhurrin is located in the epidermal layers of the leaf blade of sorghum [6]. Thus, to assign a defensive role to a compound, the location and concentration of the compound in the plant should be considered, in addition to the toxic and feeding deterrent properties of that compound. Gramine, a simple indole alkaloid, is a constituent of barley leaves [7]. The major secondary compounds in wheat are hydroxamic acids [8]. It has been suggested that gramine and hydroxamic acids are involved respectively in resistance of barley and wheat to aphids [9–12]. In this paper we study the distribution of these compounds in tissues of barley and wheat leaves.

RESULTS AND DISCUSSION

Barley leaves were analysed at various ages for gramine content. Younger leaves always had a higher concentration of this compound (Table 1). Gramine in barley and hydroxamic acids in wheat were heterogeneously distributed among leaf tissues (Table 2). Since only 33% of total gramine present in a barley leaf was in the epidermal tissues and it was not detected in the vascular bundles, the rest should be found in the parenchyma cells of the mesophyll. For hydroxamic acids about 50% were present in the vascular bundles and were not detected in the epidermis. Thus, the other 50% should be found in the parenchyma cells of the mesophyll. To test this hypothesis, mesophyll protoplasts were obtained from wheat and barley. These protoplasts were then analysed for gramine and hydroxamic acids. As expected, both gramine and hydroxamic acids were found in high concentrations in mesophyll protoplasts of barley and wheat, respectively (Table 3).

Gramine has been reported to have toxic and feeding deterrent properties against aphids [12]. Since gramine is not present in the vascular bundles, a preferred feeding site of *Schizaphis graminum*, its effects in protecting barley plants against this aphid may be a consequence of the deterrent properties of the compound during penetration of the leaf by the stylet and subsequent probing before reaching phloem. On the other hand, hydroxamic acids are present in the parenchyma cells of the mesophyll and in the vascular bundles, and it is more likely that the aphids would ingest these compounds. The presence of gramine in epidermal tissues may be relevant to protection of barley against other insects and plant pathogens.

Table 1. Gramine concentration in barley leaves at various plant ages

	Gramine (mmol/kg fr. wt)		
Leaf	9-day-old	15-day-old	30-day-old
First	1.10 ± 0.05	0.90 ± 0.08	0.20 ± 0.05
Second	—	1.60 ± 0.06	0.30 ± 0.04
Third	—	—	0.30 ± 0.04

Barley var. × 81-T-1030 was grown at 25° with a 12 hr photoperiod. Leaves are ordered from the oldest (first to appear) to the youngest. Values represent the mean ± s.e. of 3 samples.

Table 2. Tissular distribution of gramine in barley and hydroxamic acids in wheat

Plant (compound)	Compounds: mmol kg fr. wt	mmol kg dry wt	amount per leaf (mmol)
Barley (gramine)			
Complete leaf	1.3 ± 0.1	14.1 ± 0.1	1.5×10^{-4}
Lower epidermis	2.2 ± 0.1	44.6 ± 0.8	2.7×10^{-5}
Upper epidermis	2.0 ± 0.1	39.2 ± 0.8	2.3×10^{-5}
Vascular bundles	N.D.	N.D.	
Wheat (hydroxamic acids)			
Complete leaf	3.0 ± 0.5	37.6 ± 0.1	3.4×10^{-4}
Lower epidermis	N.D.	N.D.	—
Vascular bundles	6.8 ± 0.4	40.7 + 5.0	1.7×10^{-4}
Guttation drops	N.D.	N.D.	—
Xylem exudates	N.D.	N.D.	—

Barley (var. × 81-T-1027) and wheat (var. SNA-3) leaves of 12-day-old plant grown at 25° were used for the analyses as described in the methods section. Data are the mean of four replicates ± s.e. The total amount of compound present in tissues of one leaf was obtained from the concentration of the compound based on fr. wt and the total wt of the tissue in one leaf. N.D., not detected.

Table 3. Gramine and hydroxamic acids concentration in mesophyll protoplasts of barley and wheat

	Gramine (mmol/kg fr. wt)	Hydroxamic acids (mmol/kg fr. wt)
Complete leaf	1.3 + 0.1	3.7 ± 0.3
Mesophyll protoplast	3.1 ± 0.2	4.1 ± 0.5

Leaves of 12-day-old barley (var.× 81-T-1027) and wheat (var. SNA-3) were used to isolate protoplasts as described in methods. About 10^6 protoplasts were used to analyse for gramine in barley or hydroxamic acids in wheat. Values are the mean of four replicates ± s.e.

EXPERIMENTAL

Separation of tissues. Plants grown at 25° with a 14 hr photoperiod were used to isolate leaf tissues. Vascular bundles were separated by using carborundum as described in ref. [13] and then by mechanical separation with a dissection needle under a microscope. The lower epidermis was obtained by storing the leaves at −15° for 5 min, two times with an interval of 3 min. Then, the leaves were put on a cold glass with the upper epidermis on the glass surface. The lower epidermis was moistened with H_2O using a soft brush and mechanically separated. Upper epidermis was obtained from the same leaf after removing lower epidermis, veins and parenchyma cells with a dissection needle and a fine brush. The upper epidermis remained on the glass.

Protoplast isolation. Protoplasts were prepared from the mesophyll of primary leaves of barley seedlings (*Hordeum vulgare* var. × 81-T-1027) and wheat seedlings (*Triticum aestivum* L var. SNA-3) grown for 12 days in soil. The excised surface-sterilized leaves (4 g) were cut in pieces (about 1 mm) and then incubated in 40 ml of medium containing 1.5% cellulase (Calbiochem), 0.55 M mannitol (pH 5.6) at 28° and gently shaken for 3 hr. Leaf residues were removed through an 80 μm nylon net and protoplasts were purified as described in ref. [14]. These protoplasts were used for quantification of gramine and hydroxamic acid in barley and wheat, respectively.

Quantitation of hydroxamic acids. Tissues were ground in water using a mortar and pestle, filtered through cheese cloth and left for 15 min at room temp. The extract was adjusted to pH 3 with M HCl and centrifuged at 5500 *g* for 15 min. The supernatant was extracted into Et_2O (2:1) and the organic phase was evaporated to dryness. Hydroxamic acids form a blue complex upon addition of $FeCl_3$ reagent (50 g $FeCl_3$ $6H_2O$; 500 ml of 95% EtOH and 5 ml of 11 M HCl). The concentration of hydroxamic acids was determined by comparing the *A* of the extracts with a standard curve made with DIMBOA, the main hydroxamic acid from wheat ($\varepsilon_{590} = 1315$). Thus, the values represent DIMBOA equivalents [8, 15].

Quantitation of gramine. Plant tissues were frozen, homogenized in a mixture of MeOH–NH_4OH (100:1) filtered and evaporated to dryness. This extract was solubilized with 0.1 M HCl. After adjusting to pH 9, the alkaloids were extracted with $CHCl_3$ (1:2). This extract was used for quantitation of gramine with Ehmann's reagent for indoles, as described in refs [11, 16]. Gramine was the only compound detected in the extract with Ehmann's reagent.

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