Changes in Hydroxamic Acid Levels of Wheat Plants Induced by Aphid Feeding

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Abstract—Seedlings of four wheat cultivars were infested with Metopolophium dirhodum nymphs. After aphids had fed for 40 hr on the plants, the levels of the defense metabolite 2,4-dihydroxy-7-methoxy-1,4-benzoxin-3-one had changed. The changes depended on the cultivar and the portion of the leaf examined. The increase was greater in cultivars Naofen (45.0% at the tip of the leaf, 96.2% at the base where aphids were feeding) and Quilafen (14.7 and 35.8% respectively). The increase was not significant in cultivars Huenufen and Sonka. A simple and sensitive high performance liquid chromatographic method is described for the quantitation of the above benzoxinone and its demethoxylated analogue using small amounts of plant tissue.

Introduction

Hydroxamic acids (Hx) are constitutive secondary metabolites in several Gramineae [1], including the cereals wheat, maize and rye [2]. The main acid in wheat and maize is 2,4-dihydroxy-7-methoxy-1,4-benzoxin-3-one, DIMBOA [1, 3], while in rye it is the demethoxylated analogue DIBOA [4]. These compounds have been associated with the resistance of the plant to pathogenic fungi [5-8] and bacteria [9], and to insects such as the European corn borer Ostrinia nubilalis [10] and cereal aphids [11-14].

The level of hydroxamic acids in a plant varies with the age of the plant [12], and, within a plant, with the age of the plant part concerned [15]. It is also affected by growth conditions such as light intensity [16], photoperiod [17], temperature [17], nitrogen fertilization [18, 19] and availability of iron in the soil [20]. We now report that Hx levels may also vary upon infestation by aphids. Hx were quantified by a new high performance liquid chromatographic (HPLC) method suitable for fast analysis using small amounts of plant tissue. Using this technique, we have studied the effects of aphid feeding on the concentration of hydroxamic acids locally, using an empty aphid cage as a control.

Results and Discussion

Analysis of hydroxamic acids

Recovery of hydroxamic acids by the method described is quantitative (Fig. 1). This, coupled with the reproducibility of retention times (see Experimental), shows it to be a reliable method for quantifying individual hydroxamic acids.
has been shown however, that this reaction is not quantitative, its yield depends on pH, temperature and composition of the reaction medium [25, 26]. Other methods described using gas chromatography [3], thin layer chromatography [1] or high performance liquid chromatography [27] require extensive manipulation of the sample. While this work was in progress [28], another method employing HPLC was reported [29]. While glucosides of hydroxamic acids as well as aglucones could be analysed, this method still involved considerable sample manipulation. Colorimetric methods based on the formation of blue complexes between hydroxamic acids and ferric chloride [30, 31] has been extensively used, but distinction is not possible between different hydroxamic acids in an extract. The method described herein allows a fast and reliable quantitation of different hydroxamic acids in small amounts of plant tissue.

In agreement with earlier reports [1, 32], only DIMBOA could be found in wheat plants. Additionally, two rye cultivars were analysed. As expected [33], only DIBOA was found, the levels differing markedly for the two cultivars: 6.8 mmol/kg fr. wt in Permontra and 0.5 in Merkator. It would be interesting to obtain data for the relative pest resistance of these cultivars.

**Hydroxamic acid levels and aphid infestation**

The results from the analysis of four different cultivars under non-infestation and infestation conditions are given in Table 1. Two cultivars, Huenufen and Sonka, did not show statistically significant changes in DIMBOA levels upon caging or aphid infestation. Cultivar Naofen, although unaffected by caging, showed substantial increases in DIMBOA levels upon aphid infestation. The increases differed between the infested area (base of the leaf, 96.2% increase) and the area above it, which remained uninfested (tip of the leaf, 45.0% increase). Cultivar Quilafen showed a behaviour similar to Naofen, the increases in DIMBOA levels being in this case smaller (35.8% at the base and 14.7% at the tip of the leaf). Additionally, all cultivars had substantially higher DIMBOA levels in the tip of the leaf than in the base, the differences between tip and base depending on the cultivar.

Nitrogen translocation towards the site of aphid feeding has been demonstrated in the cases of *Sitobion avenae* [34] and *Rhopalosiphum padi* [35] in wheat. Furthermore, when colonies of the aphid *Aphis fabae* fed on *Vicia faba*, aminoacids including tryptophan accumulated in the phloem exudates [36]. The process resulted mainly from enhanced synthesis, although at later stages induced protein breakdown was demonstrated [36]. This effect may be related to our observation of accumulation of hydroxamic acids upon aphid feeding, since hydroxamic acids and tryptophan share a considerable portion of their biosynthetic pathway [37, 38].

Abnormal growth of plant tissue associated with aphid feeding has been related to the plant hormone indoleacetic acid [39], originating either from the plant or from the insect [40]. It is interesting to note that this phenomenon may also be associated with the capacity of aphids to divert the plant enzyme system to produce tryptophan and tryptophan-related metabolites such as hydroxamic acids and indoleacetic acid. The effect may also be related to the known capacity of hydroxamic acids to bind to auxin receptors [41]. Further work will be needed to unravel the nature of these plausible relationships.

**EXPERIMENTAL**

Plants. Seeds of *Triticum durum* cv. Quilafen and *T. aestivum* cvs Huenufen, Naofen and Sonka were obtained from INIA, Chile, and germinated in a greenhouse at 20 ± 3°C with a 16 light: 8 dark cycle. The plants were grown under these conditions for 3 weeks before being transferred to individual pots for further growth.

**Table 1.** DIMBOA levels in infested and uninfested wheat seedlings

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Leaf portion</th>
<th>Control</th>
<th>Cage only</th>
<th>Cage with aphids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Huenufen</td>
<td>Tip</td>
<td>0.31 ± 0.07</td>
<td>0.74 ± 0.05</td>
<td>0.71 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>Base</td>
<td>0.43 ± 0.04</td>
<td>0.45 ± 0.04</td>
<td>0.43 ± 0.03</td>
</tr>
<tr>
<td>Sonka</td>
<td>Tip</td>
<td>0.74 ± 0.14</td>
<td>0.74 ± 0.03</td>
<td>0.68 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>Base</td>
<td>0.49 ± 0.03</td>
<td>0.45 ± 0.04</td>
<td>0.44 ± 0.05</td>
</tr>
<tr>
<td>Naofen</td>
<td>Tip</td>
<td>1.23 ± 0.08</td>
<td>1.31 ± 0.12</td>
<td>1.90 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>Base</td>
<td>0.68 ± 0.06</td>
<td>0.53 ± 0.05</td>
<td>1.04 ± 0.07</td>
</tr>
<tr>
<td>Quilafen</td>
<td>Tip</td>
<td>3.80 ± 0.10</td>
<td>3.88 ± 0.15</td>
<td>4.45 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>Base</td>
<td>3.01 ± 0.26</td>
<td>2.88 ± 0.11</td>
<td>3.91 ± 0.12</td>
</tr>
</tbody>
</table>

*Tip refers to the uppermost portion (2 to 2.5 cm long) of the leaf. Base refers to the following 3 cm sector of the same leaf, where the cage was clipped. The aerial parts of the seedlings were 8 to 9 cm long.

†Value reported are the mean of 5 replicates. Standard deviations are given.
dark photoperiod. 8-day-old seedlings at growth stage 10 [42] were used. They were kept between 8 and 9 cm tall.

Aphids. Colonies of Metopolophium dirhodum (Walk.) were kept on barley plants, a Hx-lacking cereal [12]. Adult females were allowed to deliver nymphs on barley plants for a period of 24 hr. After this period, adults were withdrawn and nymphs left to develop until the third or fourth instar.

Infestation experiments. Plants were subjected to three different treatments: (a) undisturbed, (b) with an empty clip cage [43] attached to it 2 to 2.5 cm below the tip of the leaf, and (c) with a clip cage in the position mentioned containing a cohort of 40 aphids.

Reference compounds. DIBOA was isolated from extracts of Zea mays L. cv. T129, as described [44]. DIBOA was synthesized essentially as described [45].

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REFERENCES