

DIBOA in wild Poaceae: Sources of resistance to the Russian wheat aphid (*Diuraphis noxia*) and the greenbug (*Schizaphis graminum*)

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Summary

Hydroxamic acids are secondary metabolites typical of Poaceae that play a role in cereal resistance against pests and pathogens. The content of the hydroxamic acid aglucones DIBOA (2,4-dihydroxy-1,4-benzoxazin-3-one) and DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) was evaluated in wild Poaceae belonging to the tribes Triticeae (genera *Hordeum* and *Elymus*) and Aveneae (genera *Deschampsia* and *Phalaris*). The concentration of DIBOA in seedling extracts of the wild barleys *Hordeum chilense*, *H. brevisubulatum* subsp. *violaceum* and *H. bulbosum* was negatively correlated with parameters related to performance of the cereal aphids *Schizaphis graminum* and *Diuraphis noxia*. The relevance of the results obtained for breeding programs aimed at increasing cereal resistance against aphids is discussed.

Introduction

Hydroxamic acids (Hx) are a family of plant secondary metabolites (1,4-benzoxazin-3-ones) present in wild and cultivated Poaceae, mainly found within the tribe Triticeae (Niemeyer, 1988; Niemeyer et al., 1992). Hx confers resistance against a wide range of natural enemies, including chewing and sap-sucking insects as well as bacterial and fungal diseases (Niemeyer & Pérez, 1995). The main Hx-aglucones found in extracts of cereals are DIBOA (2,4-dihydroxy-1,4-benzoxazin-3-one) and DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) (Niemeyer, 1988). Distribution of DIBOA and DIMBOA within cultivated Poaceae is uneven. Thus, wheat (*Triticum aestivum* L.) possesses both compounds although DIMBOA levels are much higher, rye (*Secale cereale* L.) contains only DIBOA, whereas barley (*Hordeum vulgare* L.) lacks both. Finally, the man-made wheat/rye hybrid triticale contains both DIBOA and DIMBOA (Niemeyer et al., 1992).

Breeding maize for increased concentrations of DIMBOA led to enhanced resistance against the European corn borer (Grombacher et al., 1989). Likewise,

inverse relationships between performance of cereal aphids on wheat and DIMBOA concentration, found both in bioassays (Bohidar et al., 1986; Thackray et al., 1990) and field trials (Gianoli et al., 1996), support the proposal of increasing DIMBOA levels in wheat plants in order to obtain increased resistance against aphids (Escobar & Niemeyer, 1993). With regard to DIBOA, its toxicity and feeding detergency in artificial diets against the cereal aphid *Schizaphis graminum* (Rondani) was similar to that of DIMBOA (Zúñiga et al., 1983). Moreover, it was shown that despite DIBOA's absence from cultivated barley, it occurs in wild *Hordeum* L. species and its concentration correlated negatively with fecundity of the cereal aphid *Rhopalosiphum padi* (L.) (Barría et al., 1992).

Two species of cereal aphids have proved particularly difficult to control, the greenbug *S. graminum* and the Russian wheat aphid *Diuraphis noxia* (Mordvilko). While the first species readily generates virulent biotypes colonizing new hosts and possessing enhanced resistance towards deployed control strategies (Puterka & Burton, 1991), the second produces in the plant a curling of the leaves which makes it inaccessible to contact pesticides and to natural enemies (Burd et

al., 1993). Hence, there is a continuous need for new sources of host plant resistance against these aphids. Earlier reports found resistance against cereal aphids in wild *Hordeum* species (Weibull, 1987; Kindler & Springer, 1991; Castro et al., 1994). On the other hand, negative effects of DIMBOA in wheat on the performance of *S. graminum* (Argandoña et al., 1981) and *D. noxia* (Givovich & Niemeyer, 1996) have been described. In this study we present results of the relationship between DIBOA in wild *Hordeum* species and the performance of both aphid species. In addition, we continue on previous reports (Zúñiga et al., 1983; Copaja et al., 1991; Niemeyer et al., 1992) addressing DIBOA and DIMBOA levels in seedlings of wild Poaceae as useful germplasm for breeding programs aimed at increasing host plant resistance. Thus, we analyze species of *Elymus* L., a Triticeae genus shown to possess interesting levels of Hx (Copaja et al., 1991), and also include in the analysis species of *Deschampsia* Beauv. and *Phalaris* L., genera from the tribe Aveneae, in the context of breeding programs devoted to oat, a cereal lacking Hx and belonging to the same tribe.

Material and methods

Origin of plant material

Hordeum chilense (Roem. et Schult.) accessions were collected in different locations of Central and Southern Chile, and were provided by Dr. Diego Rubiales, Instituto de Agricultura Sostenible, Córdoba, Spain. Accessions of *H. brevisubulatum* Link subsp. *violaceum* Tzvelev and *H. bulbosum* L. were provided by Mr. Harold E. Bockelman, National Small Grains Collection, USDA-ARS, Aberdeen, Idaho, USA. Accessions of *Elymus agropyroides* J. Presl, *E. arizonicus* Gould, *E. breviaristatus* Löve, *E. magellanicus* Löve, *E. sibiricus* L., *E. vaillantianus* Phil., *E. virginicus* L., *Deschampsia caepistosa* Beauv., *D. flexuosa* Trin., *Phalaris amethystina* Trin., *P. angusta* Nees, and *P. minor* Retz. were provided by Mr. David M. Stout, Western Regional PI Station, USDA-ARS, Pacific West Area, Washington, USA. Accession numbers are listed in Table 1.

Quantitation of DIBOA

Seeds were germinated in individual plastic pots (25 ml) filled with soil. Plants developed in a growth chamber at 15 °C and a photoperiod of 12:12 (L:D).

Aerial tissue (20–40 mg fr. wt) from seedlings in decimal growth stage 12, i.e. primary leaf fully unfolded and secondary leaf visible (Zadoks et al., 1974) was macerated with 3 × 0.33 ml H₂O using mortar and pestle and allowed to stand at room temperature for 15 min. The extract was taken to pH 3 with 0.1 ml 0.1 N H₃PO₄ and centrifuged at 10,000 g for 15 min. An aliquot (50 µl) of the supernatant was directly examined by HPLC as described elsewhere (Gianoli & Niemeyer, 1997).

Relationship between DIBOA and aphid performance

Values of DIBOA obtained for *Hordeum* species were contrasted with already published parameters of aphid performance on either species by performing linear regressions. Fecundity of *Schizaphis graminum* on *H. chilense* was taken from Castro et al. (1994), and performance of *Diuraphis noxia* on *H. brevisubulatum* subsp. *violaceum* and *H. bulbosum* was taken from Kindler & Springer (1991).

Results and discussion

The DIBOA and DIMBOA levels in seedlings of wild Poaceae analyzed are given in Table 1. There was substantial variation among them in both DIBOA and DIMBOA levels. Unlike what is found for cultivated Poaceae (Escobar & Niemeyer, 1993), a considerable intraspecific variability for these traits was observed as well. This is a general characteristic of wild species for the concentration of secondary metabolites. In accordance with previous work (Copaja et al., 1991; Barría et al., 1992), the three *Hordeum* species analyzed showed only DIBOA and in concentrations laying close to the range reported previously. Since variation in DIBOA within *Hordeum* species, i.e. inter-accessions variation, was as large as that among species it is suggested that environment is an important factor in the accumulation of this hydroxamic acid (see Gianoli & Niemeyer, 1997). Although belonging to the same subtribe of Triticeae as *Hordeum* (Hordeinae), the genus *Elymus* showed the presence of both DIBOA and DIMBOA. An interesting species within this genus is *E. arizonicus* whose level of DIBOA (28 mmol/kg fresh weight) is among the highest ever reported for Poaceae and is only comparable to those found in some accessions of *Secale* L. species (Copaja et al., 1991). This is the first report on Poaceae genera belonging to the tribe Aveneae (*Deschampsia*, *Phalaris*) addressing its content of

Table 1. DIBOA and DIMBOA content (mmol/kg fresh weight, Mean \pm SE) in wild Poaceae^a

Species	Accession ^b	N	DIBOA	DIMBOA
<i>Deschampsia caepistosa</i>	PI 223364	9	0.33 \pm 0.50	2.23 \pm 0.41
<i>D. flexuosa</i>	PI253191	9	6.05 \pm 0.65	2.31 \pm 0.25
<i>Elymus agropyroides</i>	PI 269646	9	5.97 \pm 1.22	2.79 \pm 1.31
<i>E. arizonicus</i>	PI 531558	8	28.01 \pm 8.72	1.01 \pm 0.58
<i>E. breviaristatus</i>	PI 504445	12	1.68 \pm 0.39	1.35 \pm 0.21
<i>E. magellanicus</i>	PI 531634	10	1.56 \pm 0.18	5.28 \pm 2.19
<i>E. sibiricus</i>	PI 406465	10	6.69 \pm 2.79	0.03 \pm 0.01
<i>E. vaillantianus</i>	PI531549	8	5.00 \pm 1.47	3.94 \pm 0.47
<i>E. virginicus</i>	PI 531706	9	3.76 \pm 1.50	4.16 \pm 1.27
<i>Hordeum brevisubulatum</i> subsp. <i>violaceum</i>				
	PI 401380	5	3.93 \pm 0.47	ND
	PI401381	5	3.50 \pm 0.33	ND
	PI 401389	4	0.56 \pm 0.09	ND
<i>Hordeum bulbosum</i>				
	PI 204579	5	0.52 \pm 0.09	ND
	PI207770	4	1.51 \pm 0.18	ND
	PI 240161	5	3.43 \pm 0.31	ND
	PI 249747	5	0.57 \pm 0.11	ND
	PI 250930	4	2.47 \pm 0.28	ND
<i>Hordeum chilense</i>				
	H8	5	1.65 \pm 0.41	ND
	H13	4	1.38 \pm 0.22	ND
	H16	4	0.55 \pm 0.08	ND
	H39	5	0.99 \pm 0.08	ND
	H46	5	0.58 \pm 0.07	ND
	H55	4	0.43 \pm 0.07	ND
	H60	5	0.77 \pm 0.11	ND
	H93	5	0.97 \pm 0.15	ND
<i>Phalaris amethystina</i>	PI 286171	10	0.63 \pm 0.24	0.84 \pm 0.05
<i>P. angusta</i>	PI 310292	6	0.95 \pm 0.30	0.88 \pm 0.24
<i>P. minor</i>	PI 231045	10	0.51 \pm 0.09	0.73 \pm 0.13

^a ND = not detected.^b See Material and methods for place of deposit.

hydroxamic acids. An earlier study had failed to detect these secondary metabolites in *Phalaris* (Zúñiga et al., 1983). Nevertheless, the analytical technique used in the present work is more sensitive than that used previously.

With regard to the relationship between DIBOA in accessions of wild *Hordeum* species and aphid performance, Figure 1 summarizes the results obtained. DIBOA content of the seedlings of *H. brevisubulatum* subsp. *violaceum* and *H. bulbosum* analyzed correlated negatively with population size of *Diuraphis noxia* ($r = -0.88$, $p = 0.004$). Likewise, DIBOA level of *H. chilense* seedlings correlated negatively with *Schizaphis graminum* fecundity ($r = -0.67$, $p = 0.067$). The

inverse relationships found for DIBOA levels in these *Hordeum* species and performance of the greenbug *S. graminum* and the Russian wheat aphid *D. noxia* add support to the role of hydroxamic acids in resistance against aphids. Similar negative correlations have been reported between populations of the bird cherry-oat aphid *Rhopalosiphum padi* growing in a wide range of *Hordeum* species and DIBOA content in them (Barría et al., 1992). These results suggest that DIBOA may be used as a chemical marker for aphid resistance in wild *Hordeum*.

Breeding programs aiming at raising the content of hydroxamic acids have been successfully implemented in maize (Grombacher et al., 1989). These compounds

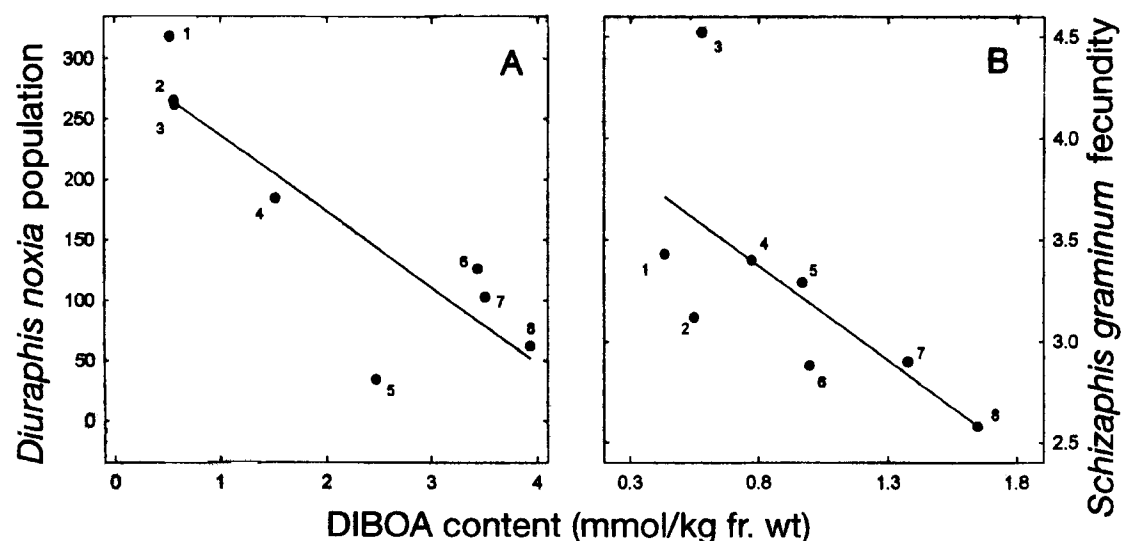


Figure 1. Correlations of DIBOA content of seedlings of *Hordeum* species and measures of performance of the cereal aphids *Diuraphis noxia* (A) and *Schizaphis graminum* (B). The points refer to the following accessions: A: *H. brevisubulatum* subsp. *violaceum* PI 401389 (2), 401381 (7) and 401380 (8) and *H. bulbosum* PI 204579 (1), 249747 (3), 207770 (4), 250930 (5), 240161 (6); B: *H. chilense* H55 (1), H16 (2), H46 (3), H60 (4), H93 (5), H39 (6), H13 (7), H8 (8). Aphid performance data were taken from Kindler & Springer 1991 (A), and Castro et al. 1994 (B).

are absent from cultivated barley, a fact reflecting the erosion of genetic variability and resistance mechanisms in modern crops (Harlan, 1976). Hence, breeding barley to accumulate DIBOA requires the transfer of genetic material from its wild relatives. Although interspecific hybrids between cultivated barley and *H. bulbosum* have been achieved and, moreover, shown to be resistant to the Russian wheat aphid (Mornhinweg et al., 1990), it is generally considered that wild *Hordeum* species hybridize only with difficulty with cultivated barley (Bothmer et al., 1991). However, the wild and weedy subspecies of barley, usually designated *H. spontaneum* (Harlan, 1976), may alternatively be used as a bridge between wild *Hordeum* spp. and cultivated barleys (Weibull, 1991). In this respect, *Hordeum chilense* appears as an interesting species for breeding issues. Thus, on one hand it possesses resistance traits against aphids (Castro et al., 1994, and this study) and fungal diseases (Rubiales et al., 1993). On the other hand, it shows high crossability with *Hordeum*, *Triticum* and *Secale* (Martín & Cubero, 1981). Furthermore, *H. chilense* × *Triticum* spp. amphiploids (tritordeum) is considered as a potential new crop (Alvarez et al., 1992).

Regarding the other genera analyzed here, *Elymus* is the most promising genus for breeding programs since it harbors significant variability for DIBOA and DIMBOA levels, and is suitable for crosses with wheat (Sharma & Gill, 1983), rye (Lapitan et al., 1987), and barley, where resistance to the Russian wheat aphid was found in intergeneric hybrids (Aung, 1991). Finally, the finding of hydroxamic acids in genera belong-

ing to the tribe Aveneae could suggest the inclusion of oat (*Avena sativa* L.), a cultivated Poaceae lacking hydroxamic acids, in breeding programs similar to those outlined above for barley.

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