Phytochemistry, Vol. 30, No. 5, pp. 1531–1534, 1991
Printed in Great Britain.

0031-9422/91 $3.00 + 0.00

HYDROXAMIC ACID CONTENT OF PERENNIAL TRITICEAE

SYLVIA V. COPAJA, BERNARDITA N. BARRIA and HERMANN M. NIEMEYER

Departamento de Química, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile

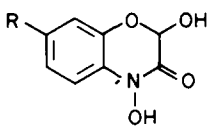
(*Received* 5 *September* 1990)

Key Word Index—Gramineae; Triticeae; wheat breeding; hydroxamic acids; DIMBOA; DIBOA.

Abstract—Ninety-four accessions of perennial Triticeae were examined for content of the hydroxamic acids DIBOA and DIMBOA. Levels of DIBOA (up to 44 mmol kg^{-1} fr. wt) were higher than those of DIMBOA (up to 4.5 mmol kg^{-1} fr.wt). The genera *Hordeum*, *Psathyrostachys* and *Secale* did not contain DIMBOA. Some accessions represented potentially useful germplasm for producing wheat with high levels of hydroxamic acids through wide hybridization.

INTRODUCTION

Hydroxamic acids (Hx) derived from 4-hydroxy-1,4-benzoxazin-3-one have been isolated from extracts of cereals such as wheat, maize and rye [1]. The main Hx in wheat and maize is 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (**1**) (DIMBOA) [2, 3] while in rye it is the demethoxylated analogue (**2**) DIBOA [4]. This family of compounds has been associated with resistance of plants to bacteria [5], fungi [6] and insects. Among the best studied cases of Hx involvement in insect resistance, are maize resistance to the European corn borer, *Ostrinia nubilalis* [7] and wheat resistance to cereal aphids [8, 9]. Hydroxamic acids are deleterious to aphids through antibiosis [10, 11] and antixenosis [12]. This latter property has been invoked in the decrease of aphid transmission of barley yellow dwarf virus to wheat plants containing high levels of Hx [13]. Hydroxamic acids play also an important role in detoxification of atrazine-derived herbicides [14] and in allelopathic effects in cereals [15, 16].



1 DIMBOA R = OMe
2 DIBOA R = H

Breeding for high Hx levels in maize has been suggested in order to obtain resistance towards leaf feeding by the first brood of the European corn borer [17–20]. The inheritance of Hx in maize has been studied [21] and suitable germplasm for breeding has been described [17]. Potential parental material for breeding wheat for high Hx levels has been screened in accessions of the genus *Triticum* [22], and in other genera of the Gramineae [2]. We report here on DIBOA and DIMBOA levels in seedlings of perennial Triticeae.

RESULTS AND DISCUSSION

The DIBOA and DIMBOA levels in accessions from 10 genera of the Triticeae containing mostly perennial species are given in Table 1. Classification and naming of the Triticeae is under debate [23–27]; the genomic system proposed by Dewey [23] has been used in this work.

Hx levels vary considerably with plant age: Hx begin to accumulate upon germination of the seed, which lacks Hx [10], reaching a maximum level after a few days and then decreasing [2, 8, 10]. In order to produce comparable results, the levels of DIBOA and DIMBOA were followed in all accessions for 15 days after germination. Within this period, Hx levels reached a maximum, which occurred between 4 and 8 days, depending upon the accession studied. Values reported in Table 1 represent Hx levels at the plant age where the maximum occurred.

Hx levels have also been shown to be influenced by growing conditions such as light intensity [28], photoperiod [29], temperature [30] and mineral and water availability [31]. Seedlings in the experiments described were subjected to comparable external conditions. The sensitivity of different accessions to particular environmental conditions may, however, be different.

Different accessions showed considerable differences in the proportion of water in the tissue. The results are presented in terms of fresh weight, as this variable is best related to the concentration to which a pest may be exposed. It should be borne in mind, however, that compartmentation of Hx within the plant may influence the relationship between the plant and the attacking organism.

There is substantial variation among perennial Triticeae in DIBOA and DIMBOA levels (Table 1). With only a few exceptions, levels of DIBOA were higher than of DIMBOA, particularly in *Critesion*, *Elymus* and *Pseudoroegneria*. Moreover, DIMBOA was not detected in any of the accessions examined in the genera *Hordeum*, *Secale* and *Psathyrostachys*. Only in *Agropyron*, *Thinopyrum* and *Elytrigia* were DIBOA and DIMBOA levels similar.

Agropyron (genome P), *Pseudoroegneria* (genome S) and *Elytrigia* (genome SX) show low levels of both

Table 1. Hydroxamic acid content in the perennial genera of the Triticeae

Genus	Species	Number of accessions studied	Hydroxamic acids (mmol kg^{-1} fr.wt) Concentration range DIBOA	DIMBOA	Mean concentration for genus DIBOA	DIMBOA
Agropyron	*cristatum*	9	1.27–0.08	0.77–0.05	0.40	0.30
	c. puberulum	1	N. D.	0.17		
	desertorum	3	0.20–0.17	0.37–0.07		
	fragile	2	0.50–0.12	0.26–0.05		
Critesion	*bogdanii*	3	6.68–0.22	0.43–ND	3.46	0.37
	brevisubulatum	4	11.56–2.80	0.48–ND		
	b. iranicum	4	5.80–1.45	1.04–ND		
	b. violaceum	1	1.56	0.13		
	californicum	2	2.21–1.66	0.51–0.22		
	capense	1	2.17	0.56		
	chilense	2	5.25–1.25	0.40–0.19		
	lecheri	1	6.90	0.05		
	stenostachys	1	1.26	0.42		
Elymus	*alatavicus*	1	5.41	1.33	5.71	0.67
	canadensis	1	16.96	ND		
	dahuricus	1	ND	0.12		
	drobovii	1	2.87	1.15		
	elymoides	1	4.88	0.80		
	lanceolatus	2	6.41–2.41	0.20–ND		
	mutabilis	1	5.56	ND		
	patagonicus	1	9.24	0.09		
	scabriglumis	1	3.37	ND		
Elytrigia	*elongatiformis*	2	0.16–0.071	0.25–0.26	0.11	0.26
Hordeum	*bulbosum*	1	1.40	ND	1.25	ND
	depresum	1	0.82	ND		
	intercedum	1	1.20	ND		
	murinum	1	0.23	ND		
	procerum	1	2.58	ND		
Leymus	*arenarius*	1	8.61	0.53	2.86	1.22
	angustus	2	2.08–1.23	3.05–0.70		
	cinereus	2	9.90–4.66	0.41–ND		
	karelinii	2	1.60–0.44	4.47–1.0		
	multicaulis	1	2.01	0.87		
	racemosus	2	2.69–0.64	0.87–1.27		
	triticoides	1	0.44	0.89		
Secale	*montanum*	1	31.66	ND	31.14	ND
	m. chaldicum	1	18.08	ND		
	m. kuprijanovi	1	43.67	ND		
Thinopyrum	*caespitosum*	2	1.20–0.71	1.22–0.40	0.70	1.23
	intermedum	3	2.67–0.82	2.38–1.72		
	junceum	2	0.16–0.07	0.81–0.33		
	nodosum	1	0.55	0.21		
	podperae	2	0.66–0.24	1.58–1.40		
	ponticum	2	0.11–0.07	2.84–1.87		
	p. turcicum	1	0.29	0.44		
Pascopyrum	*smithii*	3	0.18–0.09	1.53–0.79	0.14	1.13
Psathyrostachys	*juncea*	1	9.54	ND	14.45	ND
	j. bozoisky	1	12.99	ND		
	j. vinall	1	10.67	ND		
	fragilis	1	24.59	ND		
Pseudoroegneria	*libanotica*	4	1.53–0.57	0.36–0.05	0.93	0.26
	spicata	2	0.88–0.76	ND		
	spicata-inermis	1	1.24	ND		
	stipifolia	3	1.20–0.28	0.37–ND		

hydroxamic acids. These three genera have traditionally been grouped together under *Agropyron* [23, 27]. Also showing low levels of both acids is *Thinopyrum* (genome J). This genus has been placed together with *Pseudoroegneria* and *Elytrigia* in the conventional treatment of Tzvelev [32].

Psathyrostachys (genome N) is taxonomically one of the least controversial genera in the Triticeae and has only DIBOA. *Critesion* (genome H) on the other hand, is genomically heterogeneous and has been the subject of long controversy. Traditionally it has been a part of *Hordeum* (genome I). The two genera can, however, be separated on the basis of hydroxamic acid levels: while *Hordeum* contained only DIBOA and in relatively low levels, *Critesion* contained both acids, DIBOA being present in higher concentration and, in some accessions, in rather high absolute levels.

Analysis of *Leymus* (genome JN) and *Elymus* (genome SHY) showed the presence of both acids, with DIBOA predominating over DIMBOA in both genera. Interestingly, *Leymus* is part of *Elymus* in traditional treatments of the Triticeae [33].

The genus *Secale* contains a single perennial species, *S. montanum*. It contained only DIBOA, in unusually high levels. Analysis of cultivated rye also included a cultivar with unusually high level of DIBOA [34].

The genetic variability of cultivated wheats has suffered a continuous decrease in recent years [35]. Genetic diversity is a necessary prerequisite for the production of cultivars with pest and disease resistance, wide adaptation and increased yields. The restoration of variability in the genetic material of cultivated wheats may be accomplished by exploiting the genetic resources of some of its wild relatives.

Crosses have been produced between wheat and all the perennial genera of the Triticeae, with the exception of *Psathyrostachys*, thus permitting the transfer of useful characters [25, 36–39]. The results in Table 1 show that high levels of hydroxamic acids may also be transferred into wheat through wide hybridization with the perennial Triticeae.

EXPERIMENTAL

Plants. Seeds were grown under a 12L:12D photoperiod at 22° with a 3° range.

Reference compounds. DIMBOA was isolated from ethereal extracts of *Zea mays* cv T129, as described previously [40]. DIBOA was synthesized essentially as described in ref. [41].

Analytical method. Aerial parts of the seedlings (20–50 mg fr. wt) were macerated successively with 3 × 0.33 ml H_2O, using a mortar and pestle. The aq. extract was left at room temp. for 15 min, taken to pH 3 with 0.1 N H_3PO_4 and centrifuged at 10000 rpm for 10 min. Aliquots of the supernatant (50 μl) were filtered (0.45 mμ) and then injected into a high performance liquid chromatograph. An RP-100 Lichrosfer-C18 column was used with a constant solvent flow of 1.5 ml min^{-1} and the following linear gradients between solvents A (MeOH) and B (0.5 ml H_3PO_4 in 1 l H_2O): 0–9.5 min, 30–50% A; 9.5–10 min, 50–30% A; 10–13 min, constant at 30% A. Detection was carried out at 263 nm. R_ts were 4.5 ± 0.3 min for DIMBOA and 3.5 ± 0.3 min for DIBOA.

Acknowledgements—The financial support from the International Program in the Chemical Sciences, the International Foundation for Science, the Agency for International Development and FONDECYT are gratefully acknowledged. We are deeply indebted to Dr. D. R. Dewey (USDA-ARS Crops Research Laboratory, Logan, Utah, USA) for the provision of plant material, and to Ms Claudia Ocares for able technical assistance.

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