

Hydroxamic acid content of *triticum* species

Hermann M. Niemeyer

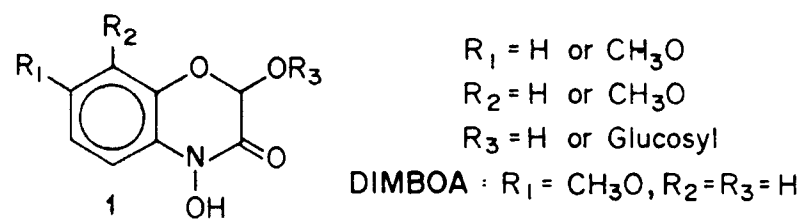
Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile

Received 13 October 1986; accepted in revised form 23 March 1987

Key words: *Triticum*, hydroxamic acids, 1,4-benzoxazin-3-ones, plant resistance, wheat breeding

Summary

Fifty-five accessions of *Triticum* species were analyzed for content of hydroxamic acids (Hx), a natural resistance factor against various organisms. Hx were found in all accessions analyzed. Extreme values were found in wild diploid species: highest in *T. speltoides* (16.0 mmol/kg fr. wt) and lowest in *T. tauschii* (0.21). Modern polyploid wheats sharing the same genome did not show substantial variations in Hx levels. The data suggest possible sources of high Hx levels for wheat breeding programs.



Introduction

Host plant resistance plays a key role in the control of pest populations in crops. Extracts of cereals such as wheat, maize and rye contain hydroxamic acids (Hx, 1) which are claimed to be involved in resistance of wheat to stem rust, *Puccinia graminis* (Elnaghy & Linko, 1962), and to the aphids *Metopolophium dirhodum* (Argandoña et al., 1980, 1981), *Schizaphis graminum* (Argandoña et al., 1983) and *Sitobion avenae* (Bohidar et al., 1986) and to contribute to resistance of maize to fungal disease (Bemiller & Pappelis, 1965; Long et al., 1975; Toth Toldi, 1984), and to insect attack (Klun et al., 1967; Long et al., 1977; Beck et al., 1983).

In maize, breeding for high Hx levels has been suggested (Klun et al., 1970; Cabulea et al., 1977; Gahukar, 1979; Kostandi et al., 1981), and the inheritance of Hx and sources of suitable parental

material have been studied (Dunn et al., 1981; Simcox & Weber, 1985).

We herein report Hx levels of several *Triticum* species and suggest sources of high Hx levels for wheat breeding programs.

Materials and methods

Plant material

Seed samples were obtained from USDA-Beltsville and from INIA-Chile. Plants were grown in a greenhouse under permanent light at ca. 26° with an 8° range, and harvested at different times according to the experiment.

Quantitation of hydroxamic acids

The method employed was essentially as described (Bohidar et al., 1986). Plant tissue (0.2 to 1.5 g) was macerated with mortar and pestle in water (3 x 2 ml), filtered through cheesecloth and left 15 min at room temperature. The extract was adjusted to pH

3 with 1 M HCl and centrifuged at 10,000 g for 10 min. The supernatant was extracted with diethylether Et₂O (3 x 6 ml) and the organic phases evaporated to dryness. Ferric chloride reagent was added (3 ml) and absorbance was measured at 590 nm. Hx concentration was obtained by comparison with a standard curve made with DIM-BOA 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one. Values reported are averages of at least 3 replicate determinations which agreed within 10 per cent.

Hx levels determined by this method have been shown to closely correspond to the sum of individual hydroxamic acids determined by specific methods (Woodward et al., Zuñiga et al., 1983).

Results

Table 1 shows Hx content data for the accessions analyzed. Typical determinations of Hx levels are shown in Table 2 for *T. compactum*.

Hx levels in wheat vary with the developmental

stage of the plant (Argandoña et al., 1981). The variation of Hx levels at 2 plant ages was determined for a few of the accessions studied (Table 3). The results show that decrease of Hx levels was comparable in all the accessions studied, with the exception of *T. monococcum*. For the comparisons in Tables 1 and 2, ten-day-old seedlings were analyzed, since at that age variation of Hx with age is smaller than in younger seedlings, but still reasonable amounts of tissue for analysis may be readily obtained.

Discussion

Development of maize varieties with increased host plant resistance based on breeding for higher Hx levels has been suggested (Klun et al., 1970; Cabulea et al., 1977; Gahukar, 1979; Kostandi et al., 1981). Similar suggestions may be put forward for wheat resistance. In this case, availability of suitable germplasm must first be evaluated.

T. speltoides showed the highest Hx level among

Table 1. Hydroxamic acid content of *Triticum* species.^a

Species	n ^b	Range of Hx	Mean Hx
		(mmol/kg fr · wt)	
<i>T. aestivum</i>	4	-0.69-2.0	1.4
<i>T. araraticum</i>	2	2.1-3.5	2.8
<i>T. boeoticum</i>	1	0.52	0.52
<i>T. carthlicum</i>	3	3.5-4.5	3.8
<i>T. compactum</i>	5	1.2-2.8	1.9
<i>T. dicoccum</i>	4	4.1-6.1	5.1
<i>T. durum</i>	2	3.6-4.9	4.2
<i>T. macha</i>	2	1.6-2.5	2.0
<i>T. monococcum</i>	4	0.26-1.7	0.62
<i>T. polonicum</i>	3	3.8-6.2	5.3
<i>T. spelta</i>	5	1.6-3.4	2.5
<i>T. speltoides</i>	5	12.4-16.0	14.3
<i>T. sphaerococcum</i>	4	2.6-3.4	2.9
<i>T. tauschii</i>	3	0.21-0.46	0.37
<i>T. timopheevi</i>	4	1.3-2.9	2.5
<i>T. turgidum</i>	4	2.6-6.4	4.7
<i>T. zhukovskyi</i>	1	1.8	1.8

^aTen-day old seedlings were analyzed.

^bNumber of accessions studied.

the accessions analyzed (Table 1). Interestingly, resistance towards biotype E greenbug (*S. graminum*) in wheat streak mosaic virus-resistant wheat lines was claimed to be derived from *T. speltoides* (Tyler et al., 1985). Furthermore, persistence of Hx in older plants of *T. monococcum* (Table 1) may be related to the higher resistance to the aphids *M. dirhodum* and *S. avenae* reported, as compared with accessions of *T. dicoccum*, *T. spelta* and *T. aestivum* (Sotherton & Van Emden, 1982).

Modern wheats are amphipolyploids containing different combinations of genomes G, A, B, and D, as shown in Fig. 1 (Schmidt, 1974; Briggie, 1980). It may be suggested from Fig. 1 that the unknown wild diploid species contributing genome B may contain high Hx levels. It would be desirable

to test this prediction on *T. searsii*, recently claimed as the B genome donor (Thompson & Nath, 1986). Additionally, the present results indicate that substantial increases in Hx levels are unlikely to be attained by hybridization within a set of wheats sharing the same genomes. Furthermore, they show that the largest differences in Hx levels occur upon changes of ploidy. It would seem desirable to produce amphipolyploidy utilizing the wild species possessing the G and B genomes (Fig. 1).

Wild relatives of wheat have been extensively used as genetic sources for resistance towards various organisms through wide hybridization (Sharma & Gill, 1983). Hence, it would be desirable to search for sources of high Hx levels in species of the subtribe Triticinae, in which intergeneric hybrid-

Table 2. Hx levels in 10-day-old seedlings of 5 accessions of *T. compactum*.

Accession	Origin	n ^a	Hydroxamic acids ^b
			(mmol/kg fr · wt)
B 237	Turkey	4	1.72 ± 0.13
K192Q x AL	Kenya	4	2.77 ± 0.13
Montsec	Spain	3	1.21 ± 0.12
Compactoide 5	Switzerland	3	2.17 ± 0.11
Vardenin 9	USSR	4	1.44 ± 0.08

^aNumber of replicates.

^bMean ± standard deviation.

Table 3. Variation of Hx content with plant age in *Triticum* species.

Species	PI number	Hydroxamic acids (mmol/kg fr · wt)		Decrease in Hx (%)
		Plant age		
		6-day	10-day	
<i>T. carthlicum</i>	94754	6.50	3.24	50
<i>T. compactum</i>	352288	5.77	2.23	61
<i>T. macha</i>	428179	5.54	1.95	65
<i>T. monococcum</i>	168804	0.72 ^a	0.58 ^a	19
<i>T. polonicum</i>	290512	7.87	4.80	39
<i>T. spelta</i>	348474	6.74	3.77	44
<i>T. sphaerococcum</i>	4531-0	4.74	2.26	52
<i>T. turgidum</i>	91673	5.36	2.35	56

^aNumbers do not differ significantly (P= 0.05).

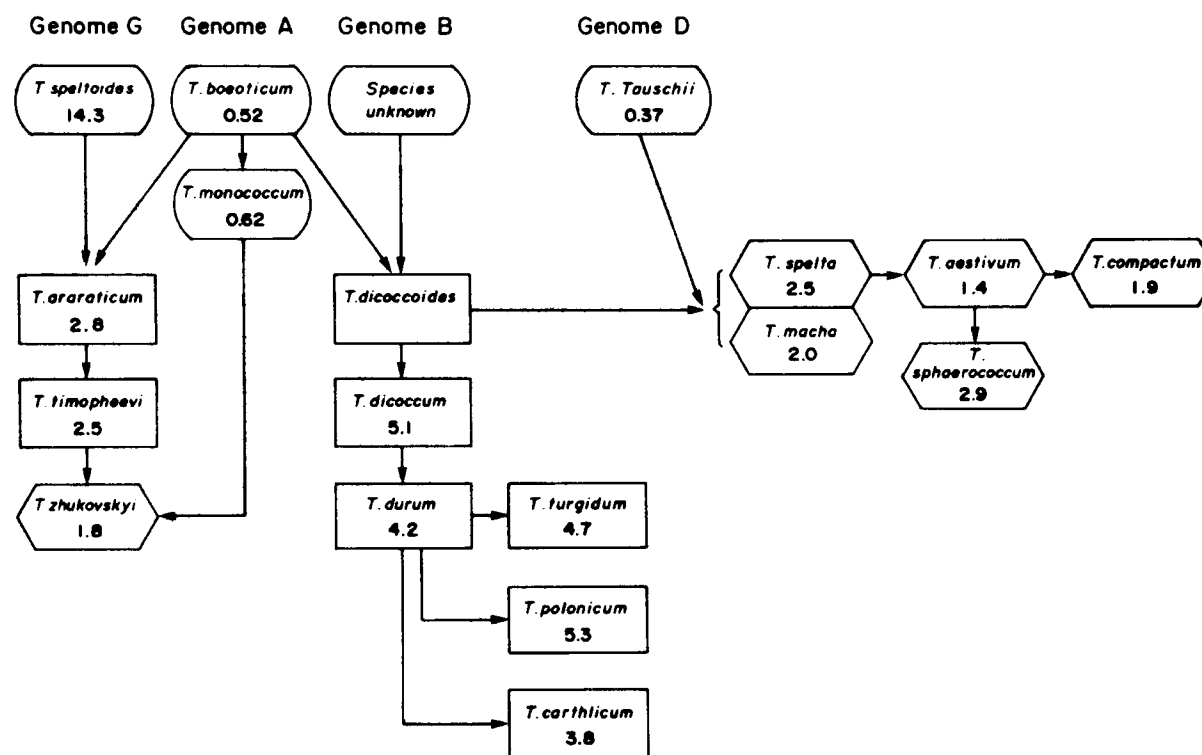


Fig. 1. Mean hydroxamic acid levels along the evolutionary development of wheat. Hexagons = hexaploid wheats; rectangles = tetraploid; truncated circles = diploid.

ization with wheat is readily achieved (Dewey, 1984). This approach has already shown promise: a screening of several wild Gramineae showed that *Elymus gayanus* contains relatively high Hx levels (Zungiga et al., 1983).

Hydroxamic acids are involved in the detoxification of atrazine-derived herbicides (Hamilton, 1964). Hence, in addition to increasing host plant resistance to fungal and insect attack, an increase in Hx levels could bring increased differential tolerance to these herbicides.

Finally, it should be pointed out that an increase in the level of toxic hydroxamic acids in wheat poses no threat to human consumption, since Hx are not present in the grain (Argandoña et al., 1981).

Acknowledgements

The author wishes to express his gratitude to Dr. D.H. Smith, Jr. of USDA-Beltsville for providing most of the seeds used in this study, and to Mrs. Cristina Muñoz for able technical assistance. Financial support from Agency for International De-

velopment, International Seminar in Chemistry (Uppsala University), Universidad de Chile and FONDECYT are gratefully acknowledged.

References

- Argandoña, V.H., J.G. Luza, H.M. Niemeyer & L.J. Corcuera, 1980. Role of hydroxamic acids in the resistance of cereals to aphids. *Phytochemistry* 19: 1665-1668.
- Argandoña, V.H., H.M. Niemeyer & L.J. Corcuera, 1981. Effect of content and distribution of hydroxamic acids in wheat on infestation by the aphid *Schizaphis graminum*. *Phytochemistry* 20: 673-676.
- Argandoña, V.H., L.J. Corcuera, H.M. Niemeyer & B.C. Campbell, 1983. Toxicity and feeding deterrence of hydroxamic acids from Gramineae in synthetic diets against the greenbug, *Schizaphis graminum*. *Entomol exp & appl* 34: 134-138.
- Beck, S.D., G.M. Dunn, D.G. Routley & J.S. Bowman, 1983. Biochemical basis of resistance in corn to the corn leaf aphid. *Crop Sci* 23: 995-998.
- Bemiller, J.N. & A.J. Pappelis, 1965. 2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-one glucoside in corn. I. Relation of water-soluble, 1-butanol-soluble glycoside fraction content of pith cores and stalk rot resistance. *Phytopath* 55: 1237-1240.
- Bohidar, K., S.D. Wratten & H.M. Niemeyer, 1986. Effect of hydroxamic acids in the resistance of wheat to the aphid *Sitobion avenae*. *Ann Appl Biol*, 109: 193-198.

- Briggle, L.W., 1980. Origin and Botany of Wheat. In 'Wheat', Documenta Ciba-Geigy, pp 6-13.
- Cabulea, I., D. Mustea, P. Ardelean, A.W. Agbary & M. Terbea, 1977. Studies of the genetics of resistance to the European corn borer (*Ostrinia nubilalis* Hbn.) in maize. *Prob Gen Teor Apl* 9: 155-171.
- Dewey, D.R., 1984. The genomic system of classification as a guide to intergeneric hybridization with the perennial Triticeae, in Gustafson JP (ed.): 'Gene Manipulation in Plant Improvement', Plenum Press, New York, pp 209-279.
- Dunn, G.M., B.J. Long & D.G. Routley, 1981. Inheritance of cyclic hydroxamates in *Zea mays* L.. *Can J Plant Sci* 61: 583-593.
- Elnaghy, M.A. & P. Linko, 1962. The role of 4-O-Glucosyl-2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one in resistance of wheat to stem rust. *Physiol Plant* 15: 764-771.
- Gahukar, R.T., 1979. Comportement alimentaire et prise de nourriture des chenilles d'*Ostrinia nubilalis* (Lep. Pyraustidae) en présence de 'DIMBOA', *Ann Soc Entomol Fr (NS)* 15: 649-657.
- Hamilton, R.H., 1964. Tolerance of several grass species to 2-chloro-s-triazine herbicides in relation to degradation and content of benzoxazinone derivatives. *J Agric Food Chem* 12: 14-17.
- Klun, J.A., C.L. Tipton & T.A. Brindley, 1967. 2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), an active agent in the resistance of maize to the European corn borer. *J Econ Entomol* 60: 1529-1533.
- Klun, J.A., W.D. Guthrie, A.R. Hallauer & W.A. Russell, 1970. Genetic nature of the concentration of 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one and resistance of the European corn borer in a diallel set of eleven maize inbreds. *Crop Sci* 10: 87-90.
- Kostandi, S.F., Y.S. Koraiem, A. Kamara & M.A. Omar, 1981. Effect of phenols in host-plant interaction of maize (*Zea mays* L.) - *Cephalosporium maydis* - system. *Agrochim* 25: 367-375.
- Long, B.J., G.M. Dunn & D.G. Routley, 1975. Relationship of hydroxamic acid content in maize and resistance to the Northern corn leaf blight, *Crop Sci* 15: 333-335.
- Long, B.J., G.M. Dunn, J.S. Bowman & D.G. Routley, 1977. Relationship of hydroxamic acid content in corn and resistance to the corn leaf aphid. *Crop Sci* 17: 55-58.
- Schmidt, J.W., 1974. Breeding and Genetics. In 'Wheat Production and Utilization', Avi Publishing Co., Westport, Conn.
- Sharma, H.C. & B.S. Gill, 1983. Current status of wide hybridization in wheat. *Euphytica* 32: 17-31.
- Simcox, K.D. & D.F. Weber, 1985. Location of the benzoxazinless (bx) locus in maize by monosomic and B-A translocation analysis. *Crop Sci* 25: 827-830.
- Sotherton, N.W. & H.F. Van Emden, 1982. Laboratory assessments of resistance to the aphids *Sitobion avenae* and *Metopolophium dirhodum* in three *Triticum* species and two modern wheat cultivars. *Ann Appl Biol* 101: 99-107.
- Thompson, J.P. & J. Nath, 1986. Elucidation of the B-genome donor to *Triticum turgidum* by unique- and repeated-sequence DNA hybridizations. *Biochem Gen* 24: 39-50.
- Toth Toldi, E., 1984. Relationship between DIMBOA content and *Helminthosporium turcicum* resistance in maize. *Novenytermeles* 33: 213-218.
- Tyler, J.M., J.A. Webster & E.L. Smith, 1985. Biotype E greenbug resistance in wheat streak mosaic virus-resistant wheat germplasm lines. *Crop Sci* 25: 686-688.
- Woodward, M.D., L.J. Corcuera, J.P. Helgeson, A. Kelman & C.D. Upper, 1979. Quantitation of 1,4-benzoxazin-3-ones in maize by gas-liquid chromatography. *Plant Physiol* 63: 14-19.
- Zuñiga, G.E., V.H. Argandoña, H.M. Niemeyer & L.J. Corcuera, 1983. Hydroxamic acid content in wild and cultivated Gramineae. *Phytochemistry* 22: 2665-2668.