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Hydroxamic acid levels in Chilean and British wheat seedlings

By S. V. COPAJA, H. M. NIEMEYER and S. D. WRATTEN*

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

* *Department of Biology, School of Biological Sciences, University of Southampton, Bassett Crescent East, Southampton SO9 3TU, UK*

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Summary

Fifty-two cultivars of Chilean wheats were screened, using high pressure liquid chromatography, for hydroxamic acid levels at the seedling stage when maximal levels occur. DIMBOA levels ranged from 1.4 to 10.9 mmol/kg fr. wt and DIBOA levels ranged from 0 to 1.1 mmol/kg fr. wt. The family of wheat cultivars leading through breeding to the British cultivar Maris Freeman was examined. While DIMBOA and DIBOA levels in the ancestors ranged from 1.6 to 3.8 mmol/kg fr. wt and 0.16 to 0.34 mmol/kg fr. wt, respectively, DIMBOA and DIBOA levels in Maris Freeman were 2.39 and 0.24 mmol/kg fr. wt, respectively. The results are discussed in terms of the possibility of breeding for aphid resistance by making use of hydroxamic acids.

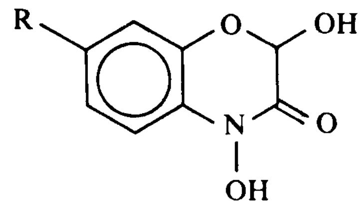
Key words: Hydroxamic acids, DIMBOA, DIBOA, wheat, aphid resistance, wheat breeding

Introduction

Hydroxamic acids (Hx) of the 4-hydroxy-1,4-benzoxazin-3-one type present in extracts of cereals such as wheat, maize and rye (Niemeyer, 1988) have been related to resistance of the plant to aphids (Long, Dunn, Bowman & Routley, 1977; Argandoña, Luza, Niemeyer & Corcuera, 1980; Argandoña, Niemeyer & Corcuera, 1981; Bohidar, Wratten & Niemeyer, 1986; Thackray, Wratten, Edwards & Niemeyer, 1990*a,b*; Wratten, Martin, Rhind & Niemeyer, 1990), components of resistance being both antibiosis (Corcuera, Argandoña & Niemeyer, 1982) and antixenosis (Argandoña, Corcuera, Niemeyer & Campbell, 1983; Givovich & Niemeyer, 1990; Leszczynski & Dixon, 1990; Leszczynski, Wright & Bakowski, 1990). The main Hx in wheat extracts is DIMBOA while in rye it is the demethoxylated analogue DIBOA (Fig. 1). Both compounds show comparable toxicity towards the greenbug *Schizaphis graminum* (Rond.) (Zúñiga, Argandoña, Niemeyer & Corcuera, 1983).

Potential limitations of hydroxamic acids in breeding programs for developing aphid resistant cereal cultivars have been pointed out: i) negative correlations between aphid performance and Hx levels are strong when *Triticum aestivum* (L.) and *T. durum* (L.) wheats are examined, but inclusion of primitive diploid and tetraploid wheats decreases considerably the correlation coefficient (Thackray *et al.*, 1990*a*); ii) after a steep increase of Hx levels soon after germination, levels drop in the plant as a whole, casting doubt on Hx as protective compounds against pests of mature plants (Argandoña *et al.*, 1981; Thackray *et al.*, 1990*a*); and iii) if correlations between resistance and Hx levels hold true only for modern wheats, germplasm available for breeders to increase Hx levels in wheat may be thought to be considerably limited.

This paper shows that within a limited set of modern wheats considerable variation of Hx levels may be found. The arguments raised above are also discussed.



DIBOA: R = H

DIMBOA: R = CH_3O

Fig. 1. Structures of hydroxamic acids from cereals.

Materials and Methods

Plant material

Seeds of Chilean cultivars of *T. durum* and *T. aestivum* were obtained from Instituto de Investigaciones Agropecuarias (INIA), Chile, and seeds from British cultivars were obtained from Plant Breeding International, Cambridge, UK. They were planted in 7-cm diameter, polystyrene pots containing vermiculite and were germinated in a growth chamber at 25 °C with a 6 °C range, with a 12 light: 12 dark photoperiod and 55% humidity with a 10% range. Light intensity was 75 $\mu E\ m^{-2}\ s^{-1}$. Hx levels were determined 4, 5, 7, 8, 10 and 15 days after germination.

Reference compounds

DIMBOA was isolated from extracts of *Zea mays* (L.) cv. T129, as described by Queirolo, Andreo, Niemeyer & Corcuera (1984). DIBOA was synthesised as described by Jernow & Rosen (1975).

Hydroxamic acid quantification

Aerial parts of seedlings (20 – 70 mg fr. wt, usually equivalent to 3 to 4 seedlings) were macerated successively with three batches of 0.33 ml H_2O each, using a mortar and pestle. The aqueous extract was left at room temperature for 15 min and was then taken to pH 3 with 0.1 NH_3PO_4. The extract was then centrifuged at 10 000 g for 10 min and the supernatant filtered (0.45 mμ). Fifty μl of the filtrate were injected into a high performance liquid chromatograph (Shimadzu LC-6A). A Lichrosfer RP-18 column (100 × 4 mm) was used with a constant solvent flow of 1.5 ml/min and the following linear gradients between solvents A (MeOH) and B (0.5 ml H_3PO_4 in 1 litre H_2O): 0 to 9.5 min, 30 to 50% A; 9.5 to 10 min, 50 to 30% A; 10 to 13 min, constant at 30% A. Detection was carried out at 263 nm. Retention times were 3.5 $\pm$ 0.3 min for DIMBOA and 4.5 $\pm$ 0.3 min for DIBOA.

Three replicated analyses were carried out for each Chilean cultivar and eight for each British cultivar.

Results and Discussion

Hydroxamic acid levels of a broad spectrum of currently used Chilean cultivars are shown in Fig. 2. Levels reported correspond to the age at which seedlings contained maximal levels, usually around the fourth or fifth day after germination. While at this stage of development some cultivars showed measurable levels of DIBOA, these levels decreased and became unmeasurable in all cases after the tenth day, probably reflecting the transformation of DIBOA into DIMBOA; this has also been observed in wheat callus culture (Zúñiga, Copaja, Bravo & Argandoña, 1990).

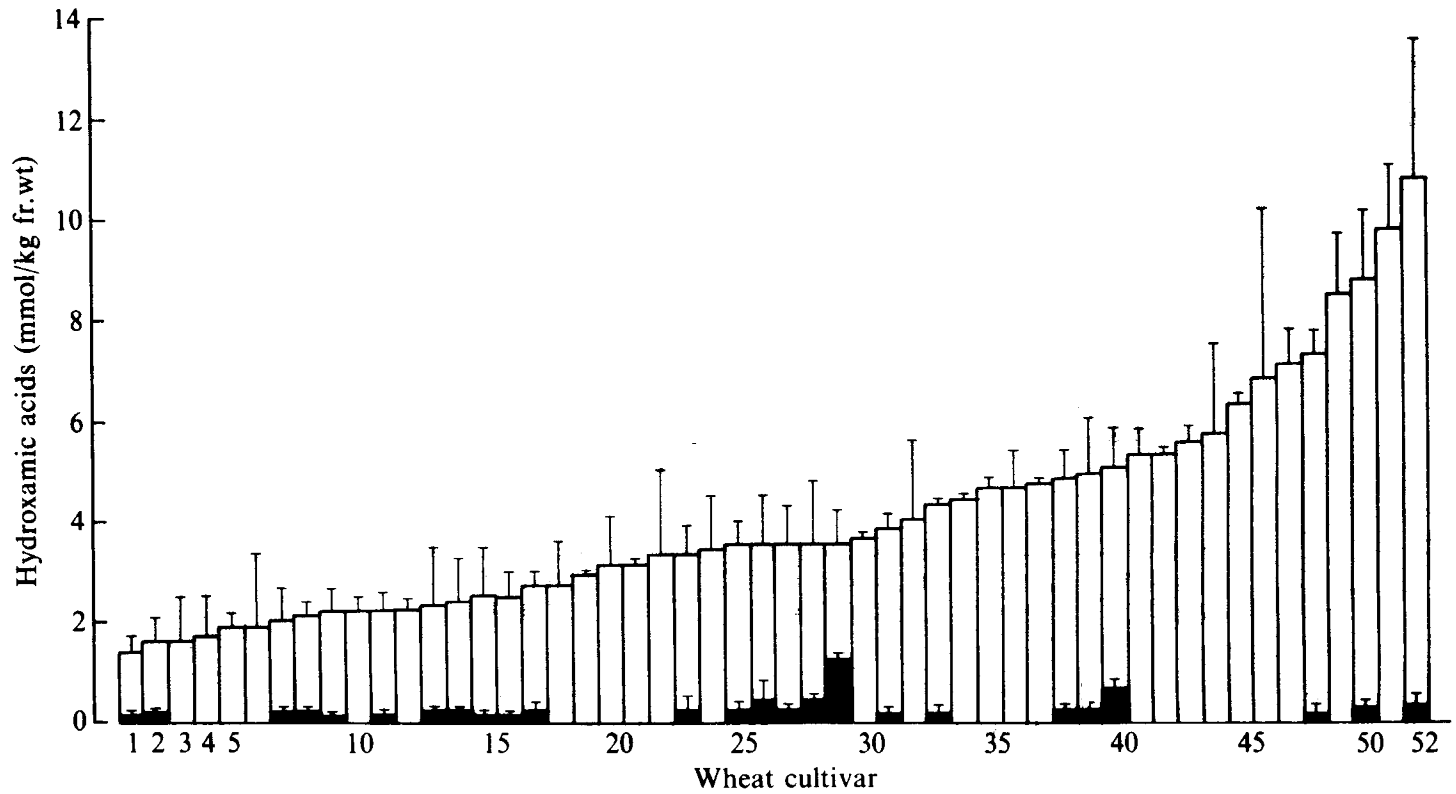


Fig. 2. Maximum DIBOA (■) and DIMBOA (□) levels in seedlings of Chilean wheat cultivars: 1 (Perquenco), 2 (Vilufén), 3 (Antufén, 4 (Andalién), 5 (Talhuén), 6 (Lilén), 7 (Nobo), 8 (Huenufén), 9 (Canelo), 10 (Reihue), 11 (Lancero), 12 (Ancoa), 13 (Pumafén), 14 (Maitén), 15 (Chifén), 16 (Talafén), 17 (Rancofén), 18 (Laurel), 19 (T-1500), 20 (Aurifén), 21 (Malifén), 22 (Yafén), 23 (Budifén), 24 (Mexifén), 25 (Lautaro), 26 (Collafén), 27 (Cunco), 28 (Patagua), 29 (Labriego), 30 (Etoile de Choisy), 31 (Millaleu), 32 (Linaza), 33 (Manella), 34 (Likafén), 35 (Manquefén), 36 (Vilmorin), 37 (Cisne), 38 (Toquifén), 39 (Chasqui), 40 (Lanco), 41, (Panguifén), 42 (Lilifén), 43 (Lancofén), 44 (Sonka-INIA), 45 (Andifén), 46 (Trisa), 47 (SNA-3), 48 (Naofén), 49 (Likay), 50 (Sipa), 51 (Alifén) and 52 (Quilafén). All cultivars were *T. aestivum*, except for 47 and 52, which were *T. durum*. Bars represent the upper 95% confidence limit.

The levels of DIMBOA ranged from 1.4 to 10.8 mmol/kg fr. wt. In artificial diets, DIMBOA has been shown to significantly decrease aphid reproduction rates at concentrations as low as 0.05 mM (Corcuera *et al.*, 1982), corresponding approximately to a bulk tissue concentration of 0.05 mmol/kg fr. wt. Hence, even in those cultivars containing the lowest levels of DIMBOA, deleterious effects may be expected on aphid pests attacking the plant at this stage of development, provided the overall DIMBOA concentrations reflect that encountered by the feeding aphid. Although the precise distribution of Hx within a plant remains unknown, they are mainly concentrated in the vascular bundles (Argandoña & Corcuera, 1985; Argandoña, Zúñiga & Corcuera, 1987), the site of aphid feeding (Pollard, 1973).

Wild diploid and tetraploid wheats do not follow the pattern of aphid performance in relation to Hx levels followed by modern tetraploid and hexaploid wheats (Thackray *et al.*, 1990*a*). This is not unexpected since different taxa, especially relatively distant ones, may depend on different combinations of mechanisms for their defence against a given pest. On the other hand, large variations may be expected between individual plants when measuring quantitative traits in wild populations. Hence, the different patterns found when these plants are analysed may also be caused partly by aphid performance having, of necessity, been measured on individual plants different from those used in assessing Hx levels, coupled with the fact that the number of replications performed may have been too low.

The decrease in Hx levels with age of the plant has been taken as an argument against Hx being a character desirable to be bred into agronomically useful wheat. It should be

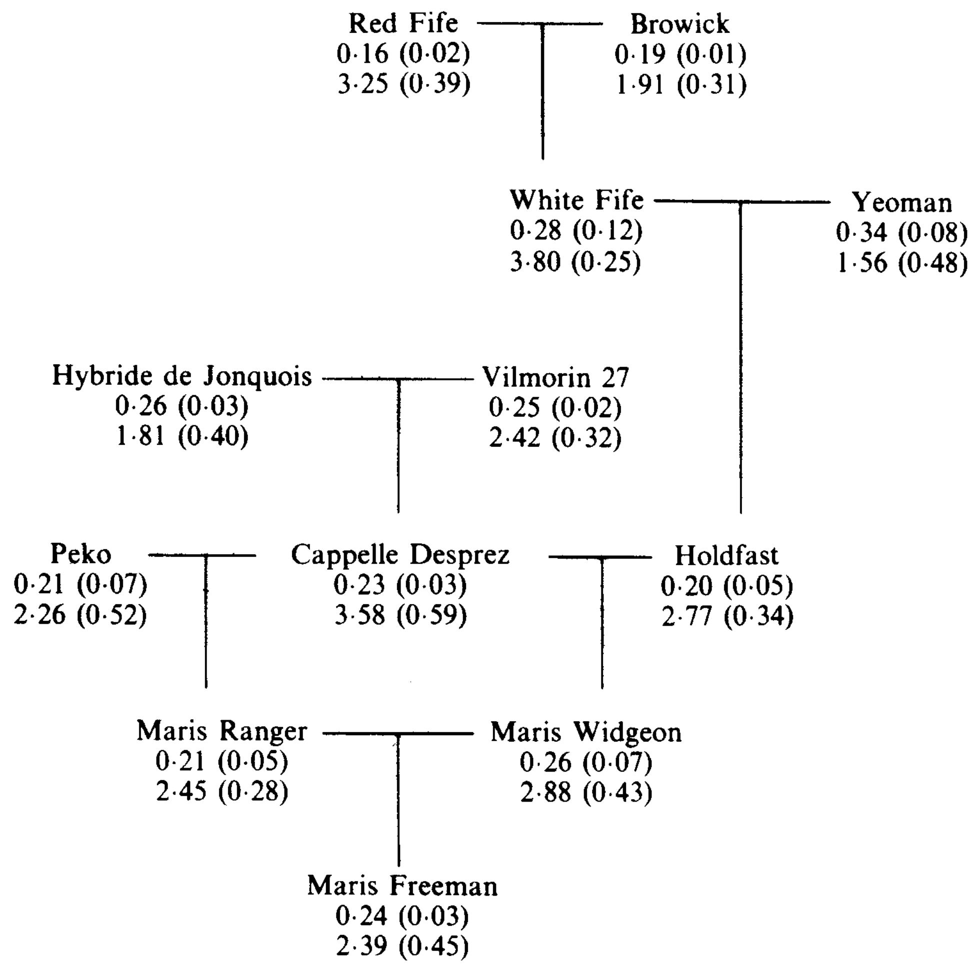


Fig. 3. Pedigree of English winter wheats. Values given are mean DIBOA (above) and DIMBOA (below) levels in seedlings, with 1.96 × S.E.

emphasised, however, that although this decrease does occur when the plant is considered as a unit, the youngest tissue in the plant still retains a high Hx level. Thus, in a 6-leaf wheat plant, while the oldest leaf may contain 0.6 mmol/kg fr. wt, the youngest may contain as much as 2.2 mmol/kg fr. wt (Thackray *et al.*, 1990*a*). The Hx concentration determined for the plant as a whole would lie closer to the value determined for the oldest leaf, since it is this one which contributes most to plant weight. Moreover, the flag leaf has been shown in a small selection of *Triticum* material to contain high Hx levels (Thackray *et al.*, 1990*a*; Leszczynski *et al.*, 1990). This pattern of distribution of Hx within the plant may be of importance in the defence of the plant against organisms colonising younger tissue, and is therefore relevant to the aphids *Sitobion avenae* (F.), which feeds on flag leaves and ears (Wratten, 1975) and *Rhopalosiphum padi* (L.), which is a seedling pest in western Europe (Vickerman & Wratten, 1979).

Fig. 3 shows Hx levels in a group of related wheats consisting of cultivars bred in the UK during the course of the present century and their immediate foreign ancestors (Lupton, 1987). The values show that the search for agronomically useful traits such as yield and disease resistance has not been paralleled by significant changes in Hx levels. Additionally, the average values fall well below the highest values determined in Chilean wheats, showing considerable potential for breeding for higher Hx levels by exploiting a wide, internationally-available range of *Triticum* material.

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