# Chromosomal location of genes for hydroxamic acid accumulation in *Triticum aestivum* L. (wheat) using wheat aneuploids and wheat substitution lines

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Hydroxamic acids are defensive secondary metabolites in wheat, the most abundant aglucones being DIMBOA and DIBOA. The location of genes coding for the accumulation of hydroxamic acids by wheat (*Triticum aestivum* L.) seedlings was explored comparing the euploid of cultivar Chinese Spring with aneuploids of the same cultivar, and substitution lines of cultivar Chinese Spring with cultivar Cheyenne. Significant differences in DIBOA and DIMBOA concentrations in young seedlings with respect to the euploid (0.08 and 5.9 mmol kg$^{-1}$ fresh weight, respectively) were found in the following lines: monosomic lines 4A (3.9 and 2.8) and 5B (0.29 and 2.7), substitution line 4A (1.7 and 3.2) and nulli/tetra lines N4AT4B (0.39 and 7.0), N4AT4D (0.20 and 0.48), N5BT5A (0.12 and 0.34) and N5BT5D (0.06 and 0.63). These results suggest the involvement of chromosomes of group 4 and also chromosome 5B in the accumulation of hydroxamic acids in wheat seedlings.

**Keywords:** DIMBOA, hydroxamic acids, wheat.

## Introduction

Hydroxamic acids (Hx) are a family of secondary metabolites of cereals discovered over three decades ago in relation to the resistance of rye to fungal diseases (Niemeyer & Pérez, 1995). They are present in wheat and maize, as well as in a wide range of wild Gramineae (Niemeyer, 1988a,b; Niemeyer *et al.*, 1992). Hx exist in the intact plant as glucosides that, upon injury to the plant tissue, are transformed to the more toxic aglucones by an endo-$\beta$-glucosidase (Hofman & Hofmanova, 1969; Cuevas *et al.*, 1992). In wheat extracts, the most abundant aglucone is 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), whereas its demethoxylated analogue, DIBOA, is a minor constituent (Niemeyer, 1988a).

Two different ways of producing DIMBOA aglucone have been reported: synthesis via the methoxylated lactam, supported by time-course studies of the incorporation of radiolabel from putative precursors into Hx in maize seedlings (Tipton *et al.*, 1973), and synthesis from DIBOA, supported by time-course studies of the accumulation of Hx in wheat callus cultures (Zúñiga *et al.* 1990) (Fig. 1). The hydroxamic acid aglucones are further transformed into glucosides for storage (Leighton *et al.* 1994).

Several lines of argument point to the potential usefulness of Hx in developing wheat cultivars resistant to aphids, a major cereals pest worldwide: (i) Hx are capable of reducing cereal aphid populations through antibiosis (Argandoña *et al.* 1980) and antixenosis (Nicol *et al.* 1992; Givovich & Niemeyer 1995), and of decreasing infection of wheat by barley yellow dwarf virus through deterrence of aphid feeding (Givovich & Niemeyer 1991); (ii) the developmental time of the aphid-predatory ladybird *Eriopis connexa* (Germar) is shorter, and the number of aphids ingested higher, when the beetle feeds on aphids from a high-Hx wheat cultivar compared with an intermediate-Hx cultivar (Martos *et al.* 1992), suggesting that higher Hx levels in wheat could promote the beneficial effects of the predator; (iii) sublethal doses of insecticide are more effective on aphids feeding on a high-Hx wheat cultivar than on a low one (Nicol *et al.* 1993); and (iv) increased levels of Hx do not involve a yield penalty in field-grown wheat (Gianoli *et al.* 1996).

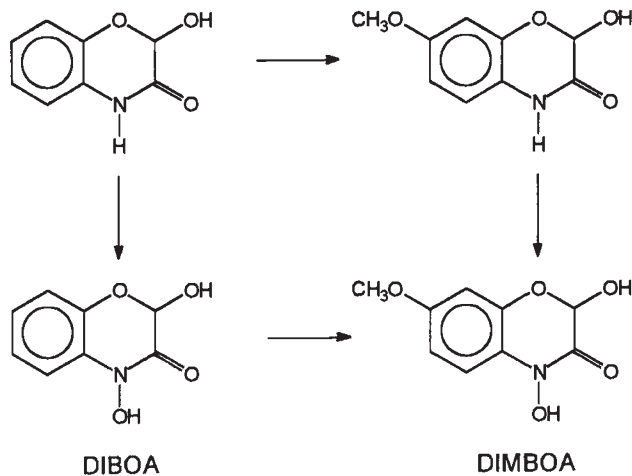*Correspondence. E-mail: niemeyer@abulafia.ciencias.uchile.cl

**Fig. 1** Possible biosynthetic pathways leading to the production of DIMBOA in cereals.

Efficient breeding of wheat for higher levels of hydroxamic acids may benefit from knowledge of the chromosomal location of genes coding for their accumulation. In this paper, we explore this location using wheat aneuploids and substitution lines.

## Materials and methods

Seeds were obtained from the University of Missouri (monosomic lines of hexaploid wheat *Triticum aestivum* L. cv. Chinese Spring) or from the AFRC Institute of Plant Science Research (substitution lines of *T. aestivum* cv. Cheyenne into cv. Chinese Spring, and nullisomic/tetrasomic lines of *T. aestivum* cv. Chinese Spring). Seeds were sown individually in 5-cm diameter pots containing vermiculite and kept in a plant growth chamber at 20°C, 60 per cent relative humidity, with a 12-h photophase. Five days after planting, seedlings in decimal growth stage 10 (Zadoks *et al.* 1974) were harvested. Fresh material was weighed and the aqueous extracts produced were subjected to high-performance liquid chromatography on a reversed-phase column, as described previously (Niemeyer *et al.* 1989). The aerial part of one seedling constituted one replicate; a variable number of replicates, up to 10, was analysed from each line. Significant differences between wheat lines analysed were determined by ANOVA ($P<0.05$) and the multiple comparisons between mean values of Hx concentrations were made using the Tukey test.

Karyotypes were not determined for each of the seedlings analysed. Segregation of monosomic lines is expected to lead to about 75 per cent monosomic and 25 per cent disomic seedlings.

## Results and discussion

The basis for these studies was the hexaploid wheat cultivar Chinese Spring. This cultivar has been the subject of extensive genetic studies and also showed a high concentration of DIMBOA at the seedling stage (5.9 mmol kg$^{-1}$ fresh weight). Analysis of Chinese Spring monosomic lines is shown in Fig. 2. Significant differences in Hx concentrations were obtained when either chromosome 4A or 5B was absent, suggesting a dosage effect and the presence in these chromosomes of genes involved in Hx accumulation.

The significantly lower concentration of DIMBOA and higher concentration of DIBOA in monosomic line 4A compared with the euploid suggests that this chromosome contains a gene(s) involved in the transformation of DIBOA into DIMBOA. The fact that *T. urartu*, the putative donor of genome A of hexaploid wheat, accumulates DIBOA but not DIMBOA further suggests this gene(s) is not present in the diploid genome. One possibility is that the gene(s) may have been introduced into genome A of the hexaploid through translocations from a homoeologous chromosome, most probably chromosome 4B, as *T. tauschii*, the putative donor of genome D of hexaploid wheat, does not accumulate DIMBOA to any substantial extent, whereas *T. speltoides*, the putative donor of genome B of hexaploid wheat, accumulates DIMBOA in substantial amounts (Niemeyer *et al.* 1992). However, numerous restriction fragment length polymorphism (RFLP) studies have failed to show the occurrence of translocations between chromosomes 4A and 4B. Alternatively, the differential accumulation of DIBOA in the 4A monosomic line may result from compensation by gene(s) of other chromosomes, probably chromosome 4B, for the reasons just given.

The nulli/tetra line, CS-N4AT4B, accumulates high concentrations of DIMBOA with hardly any accumulation of DIBOA (Table 1), suggesting that chromosome 4B is able to compensate for the absence of 4A and, hence, that 4B may also contain a locus for the transformation of DIBOA into DIMBOA, providing support for the compensation hypothesized earlier. It is not clear, however, why monosomic line 4B accumulates hydroxamic acids in concentrations similar to the cultivar.

A comparison of the Hx levels in tetraploid wheats with genome AABB and hexaploid wheat with genome AABBDD shows that the incorpora-
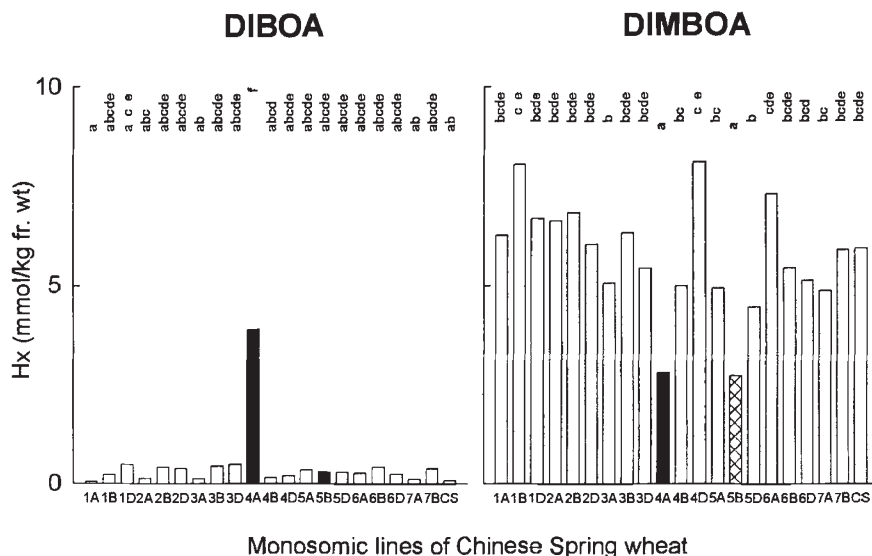
**DIBOA** **DIMBOA**



Fig. 2 Hydroxamic acid (Hx) concentrations in monosomic lines of Chinese Spring wheat (*Triticum aestivum* L.). Bars represent mean Hx values. Means sharing a letter are not significantly different (*P* > 0.05, Tukey test).

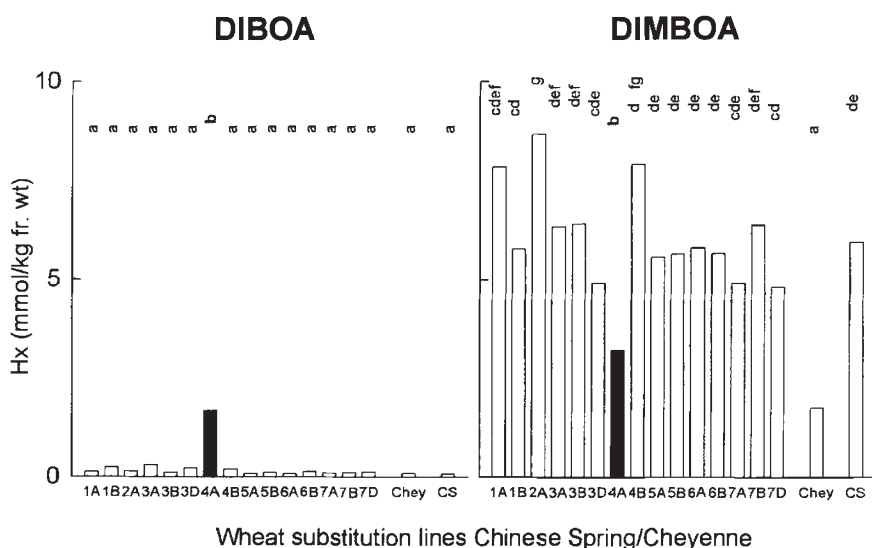Monosomic lines of Chinese Spring wheat

**DIBOA** **DIMBOA**



Fig. 3 Hydroxamic acid (Hx) concentrations in substitution lines of wheat (*Triticum aestivum* L.) cultivar Chinese Spring with wheat cultivar Cheyenne. Bars represent mean Hx values. Means sharing a letter are not significantly different (*P* > 0.05, Tukey test).

Wheat substitution lines Chinese Spring/Cheyenne

Table 1 Hydroxamic acids in nullisomic/tetrasomic lines of wheat cultivar Chinese Spring

| Genome | n | Hydroxamic acids (mmol kg$^{-1}$ fresh weight) | |
| --- | --- | --- | --- |
| | | DIBOA | DIMBOA |
| Chinese Spring | 10 | $0.08 \pm 0.023$ | $5.9 \pm 2.0$ |
| CS-N4AT4B | 5 | $0.39 \pm 0.20$ | $7.0 \pm 3.4$ |
| CS-N4AT4D | 1 | 0.20 | 0.48 |
| CS-N5BT5A | 4 | $0.12 \pm 0.12$ | $0.34 \pm 0.60$ |
| CS-N5BT5D | 4 | $0.06 \pm 0.04$ | $0.63 \pm 0.69$ |

tion of genome D leads to a considerable decrease in Hx accumulation (Niemeyer 1988b). The low concentrations of both DIBOA and DIMBOA in the nulli/tetra line, CS-N4AT4D (Table 1), is consistent with this and suggests the presence in chromosome 4D of a gene inhibiting the accumulation of Hx.

In the case of monosomic line 5B, the significant decrease in DIMBOA with no significant change in DIBOA with respect to the cultivar suggests the presence in chromosome 5B of genes coding for a pathway producing DIMBOA without the involvement of DIBOA, presumably through the methoxylated lactam (Fig. 1). Thus, when this chromosome is present in reduced proportion, as in the monosomic line 5B, less DIMBOA is produced without a concomitant increase in DIBOA concentration. The suggestion is supported by results from the analysis of nulli/tetra lines, CS-N5BT5A and CS-N5BT5D, in which replacement of 5B chromosomes by either 5A or 5D led to lines that accumulate very small concentrations of DIMBOA and DIBOA.

A screening was performed of different wheat cultivars for which substitution lines with Chinese Spring were available, to search for one showing particularly low levels of Hx. The cultivar selected was Cheyenne, which showed only 1.2 mmol/kg fresh weight of DIMBOA at the seedling stage. Results from the analysis of substitution lines of Chinese Spring with Cheyenne, shown in Fig. 3, are consistent with those of Fig. 2, in that substitution of chromosome 4A leads to a decrease in DIMBOA accumulation and an increase in DIBOA accumulation with respect to other substitution lines. Surprisingly, no significant effect was shown by chromosome 5B.

In summary, the results of this study suggest a multigenic control of the accumulation of hydroxamic acids in wheat involving chromosomes 4A, 4B, 4D and 5B.

## Acknowledgements

## References

ARGANDOÑA, V. H., LUZA, J. G., NIEMEYER, H. M. AND CORCUERA, L. J. 1980. Role of hydroxamic acids in the resistance of cereals to aphids. *Phytochemistry*, **19**, 1665–1668.

CUEVAS, L., NIEMEYER, H. M. AND JONSSON, L. M. V. 1992. Partial purification and characterization of a hydroxamic acid glucoside β-D-glucosidase from maize. *Phytochemistry*, **31**, 2609–2612.

GIANOLI, E., PAPP, M. AND NIEMEYER, H. M. 1996. Costs and benefits of hydroxamic acids-related resistance of winter wheat against the bird cherry-oat aphid of hydroxamic acids-related resistance of winter wheat against the bird cherry-oat aphid, *Rhopalosiphum padi*, L. *Ann. appl. Biol.*, **129**, 83–90.

GIVOVICH, A. AND NIEMEYER, H. M. 1991. Hydroxamic acids affecting barley yellow dwarf virus transmission by the aphid *Rhopalosiphum padi*. *Entomologia exp. appl.*, **59**, 79–85.

GIVOVICH, A. AND NIEMEYER, H. M. 1995. Comparison of the effect of hydroxamic acids from wheat on five species of cereal aphids. *Entomol. exp. appl.*, **74**, 115–119.

HOFMAN, J. AND HOFMANOVA, O. 1969. 1,4-Benzoxazine derivatives in plants. Sephadex fractionation and identification of a new glucoside. *Eur. J. Biochem.*, **8**, 109–112.

LEIGHTON, V., NIEMEYER, H. M. AND JONSSON, L. M. V. 1994. Substrate specificity of a glucosyltransferase and an N-hydroxylase involved in the biosynthesis of cyclic hydroxamic acids in Gramineae. *Phytochemistry*, **36**, 887–892.

MARTOS, A., GIVOVICH, A. AND NIEMEYER, H. M. 1992. Effect of DIMBOA, an aphid resistance factor in wheat, on the aphid predator *Eriopis connexa* Germar (Coleoptera: Coccinellidae). *J. Chem. Ecol.*, **18**, 469–479.

NICOL, D., COPAJA, S. V., WRATTEN, S. D. AND NIEMEYER, H. M. 1992. A screen of worldwide wheat cultivars for hydroxamic acid levels and aphid antixenosis. *Ann. appl. Biol.*, **121**, 11–18.

NICOL, D., EATON, N., WRATTEN, S. D. AND COPAJA, S. V. 1993. Effects of DIMBOA levels in wheat on the susceptibility of the grain aphid (*Sitobion avenae*) to deltamethrin. *Ann. appl. Biol.*, **122**, 427–433.

NIEMEYER, H. M. 1988a. Hydroxamic acids (4-hydroxy-1, 4-benzoxazin-3-ones), defence chemicals in the Gramineae. *Phytochemistry*, **27**, 3349–3358.

NIEMEYER, H. M. 1988b. Hydroxamic acid content of *Triticum* species. *Euphytica*, **37**, 289–293.

NIEMEYER, H. M. AND PÉREZ, F. J. 1995. Potential of hydroxamic acids in the control of cereal pests, diseases, and weeds In Inderjit, Dakshini, K. M. N. and Einhellig, F. A. (eds) *Allelopathy. Organisms, Processes, and Applications.* ACS Symposium Series 582, pp. 260–270. American Chemical Society, Washington, DC.

NIEMEYER, H. M., PESEL, E., COPAJA, S. V., BRAVO, H. R., FRANKE, S. AND FRANCKE, W. 1989. Changes in hydroxa-

mic acid levels of wheat plants induced by aphid feeding. *Phytochemistry*, **26**, 447–449.

NIEMEYER, H. M., COPAJA, S. V. AND BARRÍA, B. N. 1992. The Triticeae as sources of hydroxamic acids, secondary metabolites in wheat conferring resistance against aphids. *Hereditas*, **116**, 295–299.

TIPTON, C. I., WANG, M.-C., TSAO, F. H.-C. AND LIN TU, C.-C. AND HUSTED, R. R. 1973. Biosynthesis of 1, 4-benzox-azin-3-ones in *Zea mays*. *Phytochemistry*, **12**, 347–352.

ZADOKS, J. C., CHANG, T. T. AND KONSAK, C. F. 1974. A decimal code for the growth stage of cereals. *Weed Res.*, **14**, 415–421.

ZÚÑIGA, G. E., COPAJA, S. V., BRAVO, H. R. AND ARGAN-DOÑA, V. H. 1990. Hydroxamic acids accumulation by wheat callus. *Phytochemistry*, **29**, 2139–2141.