CONCENTRATION OF HYDROXAMIC ACIDS IN POLISH WHEAT VARIETIES

Oleszek W.[1], Stochmal A.[1], Kuś J.[2]

[1]Department of Biochemistry and [2]Department of Systems and Economics of Crop Production, Institute of Soil Science and Plant Cultivation, ul. Czartoryskich 8, 24-100 Pulawy, Poland

INTRODUCTION

Secondary metabolites present in plant tissue may play an important function as allelochemicals, plant insect, plant pathogen or plant herbivores mediators. Wheat may accumulate several groups of secondary metabolites the most important of which seem to be hydroxamic acids (benzoxazinones). Available literature indicate that hydroxamic acids are not present in wheat seed but are synthesized early at seed germination with highest concentration occurring at seedling stage (1-3). There are however discrepancies on the concentration of these chemicals over growing season. Present work was aimed to shed more light on this question.

MATERIALS AND METHODS

Plant material

For this purpose two field experiments were performed. First one included six varieties being under cultivation: Kobra, Roma, Korweta, Sukces, Zyta and Mewa and one primitive wheat variety - Orkisz (*Triticum spelta*). These varieties were grown in two cultivation systems – organic and conventional. Each system included about 1 ha field (about 0.15ha for each variety). Additionally ten old varieties, Wysokolitewka, Ostka kazimierska, Banatka kresowa, Magnatka rogalinska, Tiroler Roter (Orkisz), Spelt Droogendijk (Orkisz), Rottweiler (Orkisz), Dankowska zachodnia, Blondynka and Kujawianka wieclawicka were grown in 1 x 1m plots in conventional systems. These ten varieties are presently not under cultivation and seeds were obtained from a seed bank. Samples were collected from each variety at three growing stages: 2 leaf, 4 leaf and tillering. Samples were separated into roots and aerial parts and freeze dried and powdered.

Analytical procedure

Powdered material was extracted by overnight cold extraction with acidified methanol followed by purification on C18 cartridges prior to HPLC analysis. Quantification of hydroxamic acids was based on HPLC equipped with Photo Diode Array detection. Separations were performed on Synergi MAX-RP 80A column using linear gradient of 1% H_3PO_4 and 40% AcN in 1% H_3PO_4. The solvent flow rate was 1 ml/min and total time of analysis was 80 min per one sample. These conditions were different from the those used by the other FATEALLCHEM partners who performed LC-MS analysis, but the correctness of the method was evaluated at inter-laboratory tests performed between partners. Hydroxamic acids (DIBOA, DIMBOA-glc, HMBOA, DIMBOA, BOA and MBOA) were determined in Polish wheat varieties.

RESULTS AND DISCUSSION

The analytical protocol developed under this study had some limitations. The whole plant extract besides of hydroxamic acids contained number of impurities, predominantly flavonoids. They were overlapping some hydroxamic acids and in consequence only HMBOA and MBOA were detected with Photodiode Array Detector. Root extract contained less impurities in the matrix and six hydroxamic

acids, including DIBOA, DIMBOA-glc, HMBOA, DIMBOA, BOA and MBOA, could be determined when HPLC profiles were integrated at 255 or 229 nm (Figure 1). Using this method hydroxamic acids could be only found in leaf tissue at the first sampling stage (2 leaf stage). At second sampling stage (4 leaf stage) no hydroxamic acids could be detected. In roots hydroxamic acids were found in all sampling stages (2 and 4 leaf, tillering). The most abundant compound in all sampling stages was DIMBOA, while its glucoside could be found only in a trace amounts (Figure 2).

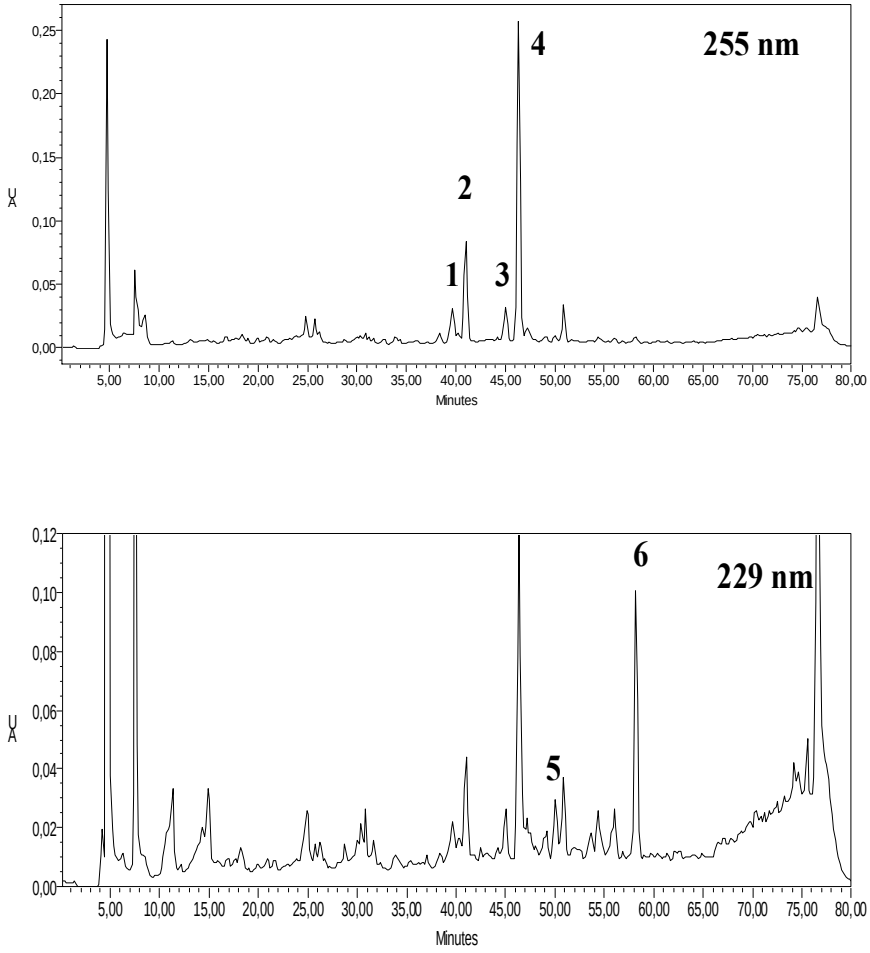

Figure 1.HPLC profile of hydroxamic acids in wheat root extracts: **1** – DIBOA, **2** – DIMBOA-glc, **3** – HMBOA, **4** – DIMBOA, **5** – BOA, **6** – MBOA

There was small differences found between hydroxamic acid concentration between Polish wheat varieties. Only Kobra showed slightly elevated level in relation to average for all varieties. There was also no distinct differences in hydroxamic acids concentration found between samples collected from organic versus conventional cultivation system (Figure 3).

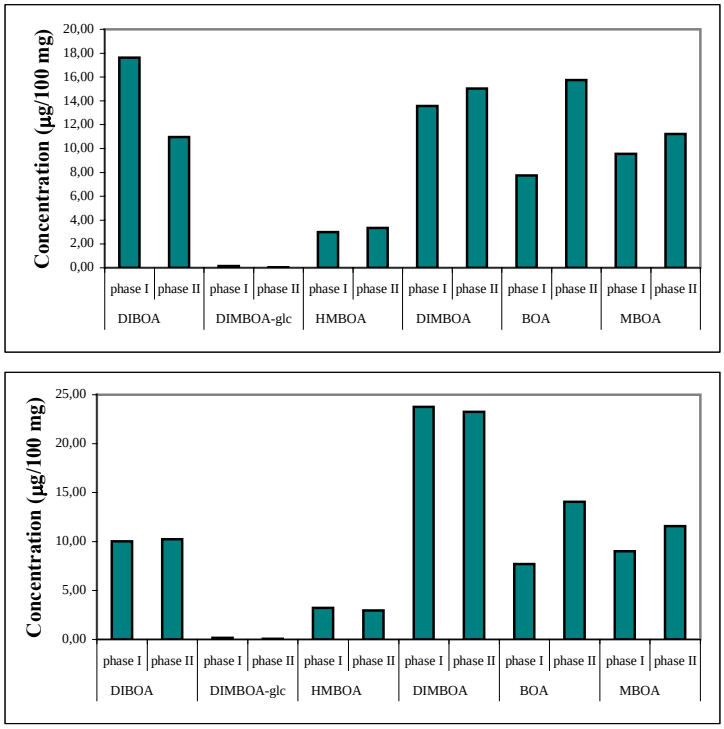

Figure 2. Hydroxamic acids concentration in roots of Polish wheat varieties; two growing phases (2 and 4 leaf) collected in autumn 2003 in organic and conventional systems

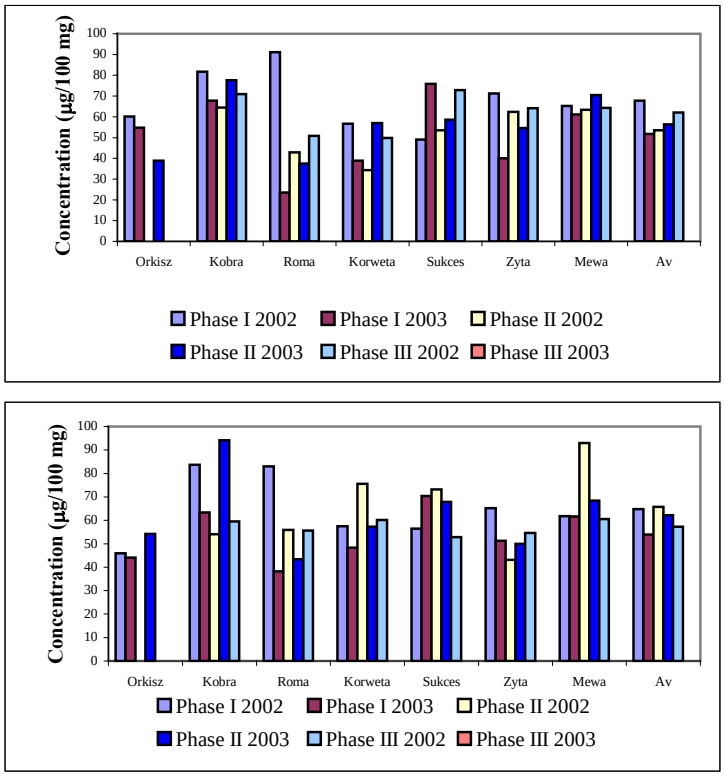

Figure 3. Total hydroxamic acid concentration in modern Polish wheat varieties grown in organic and conventional systems

For the old varieties and primitive forms (Okrisz, *Triticum spelta*), which are not under cultivation anymore hydroxamic acid concentration was very similar to that found in modern varieties (figure 3). This indicates that breeding for qualitative traits in wheat did not change substantially synthetic pathway for benzoxazinone synthesis.

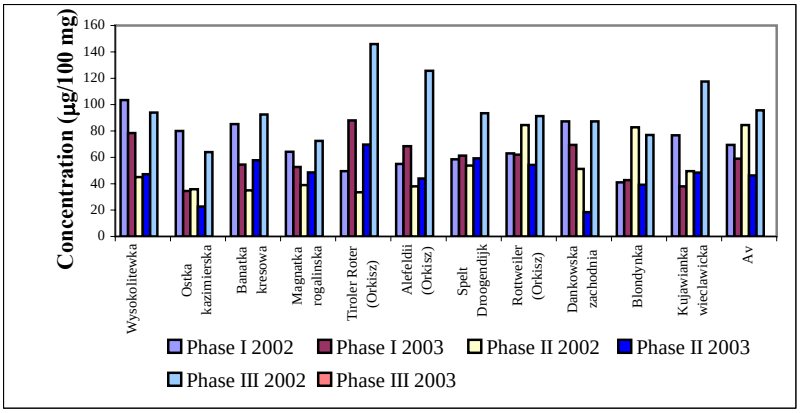

Figure 4. Total hydroxamic acid concentration in old varieties and primitive forms of wheat

ACKNOWLEDGMENTS

The research described in this abstract was performed as part of the project "FATEALLCHEM", "Fate and Toxicity of Allelochemicals (natural plant toxins) in Relation to Environment and Consumer". The project was carried out with financial support from the Commission of the European Communities under the Work programme Quality of Life, contract no. QLK5-CT-2001-01967, from Polish Ministry for Science and from Institute of Soil Science and Plant Cultivation.

REFERENCES

1. Argandona V. H., Luza J. G., Niemeyer H. M., Corcuera L. J.: Role of hydroxamic acid in the resistance of cereals to aphids. Phytochemistry, 1980, 19: 1665-1668.
2. Copaja S. V., Nicol D., Wratten S. D.: Accumulation of hydroxamic acids during wheat germination. Phytochemistry Oxford, 1999, 50: 17-24.
3. Corcuera J. J., Argandona V. H., Zuniga G. E.: Allelochemicals in wheat and barley: role in plant-insect interactions. In: Rizvi S. J. H., Rizvi V. (Eds.), Allelopathy: Basic and applied aspects. Chapman and Hall, London, 1992, 119-127.