The influence of organic and inorganic chemical compounds on the concentration of flavonoids, total phenols, antioxidants activity and leaf pigments in different wheat genotypes

[1,2]Ionuț Racz, [2]Rozalia Kadar, [3]Loredana M. Soran, [4]Florin D. Bora

[1] University of Agricultural Sciences and Veterinary Medicine Cluj Napoca, Romania; [2] Agricultural Research and Development Station Turda, Romania; [3] National Institute for Research and Development of Isotopic and Molecular Technologies Cluj Napoca, Romania; [4] Research Station for Viticulture and Enology Târgu Bujoru, Department of Physico-Chemistry and Biochemistry, Romania. Corresponding author: F. D. Bora, boraflorindumitru@gmail.com

Abstract. The present paper investigates the effect of two different treatments applied to wheat on its concentrations of flavonoids, total polyphenols, antioxidant and leaf pigments. The first treatment consists in the application of synthetic phytohormone -2,4-dichlorophenoxyacetic acid to winter wheat leaf at the stem elongation stage. The second treatment consists in a mixture of microelements as Fe, B, Mn, S and Mg applied to the same type of sample for testing their influence on the plant biochemical compounds. A higher content of flavonoids was recorded after both, the 2.4 D treatment and the mineral fertilization, compared to the controls. Total polyphenols, antioxidant and leaf pigments content were negatively influenced by the application of treatments.
Key Words: flavonoid, total polyphenols, antioxidant activity, leaf pigments, wheat.

Introduction. Plants represent a rich source of natural compounds which are responsible for many multifunctional biological effects (Molina-Garcia et al 2018). Wheat (*Triticum aestivum* L.) is one of the most important food ingredients worldwide. However, wheat is not only a source of basic nutrients, such as carbohydrates, proteins, and vitamins, but also a source of antioxidants, such as flavonoids and phenolic acids (Adom et al 2005). The scientific and medical communities consider that antioxidants have the ability to scavenge free radicals and reduce oxidative damage (Yu et al 2002).

Flavonoids are an important class of natural products. Particularly, they belong to a class of plant secondary metabolites having a polyphenolic structure, widely found in fruits, vegetables and certain beverages (Panche et al 2016). In plants, flavonoid compounds are products extracted from plants and they are found in several parts of the plant. Flavonoids are used by vegetables for their growth and defense against plaques (Havsteen 2002). Flavonoids protect plants from different biotic and abiotic stresses and act as unique UV filters (Takahashi & Ohnishi 2004) functioning as signaling molecules, allopathic compounds, phytoalexins, detoxifying agents and antimicrobial defensive compounds. Flavonoids have roles against frost hardiness, drought resistance and may play a functional role in plant heat acclimatization and freezing tolerance (Samanta et al 2011). Phenols are important constituents present in most plant materials (Johnson et al 2008). Phenolic compounds are important plant constituents with redox properties responsible for antioxidant activity (Soobrattee et al 2005). The accumulation of phenolics in plant tissues is considered as an adaptive response of plants to adverse environmental conditions (Naikko et al 2019). Plant phenolics confer various physiological functions for survival and adaptation to environmental disturbances (Landolt et al 1997; Andersen 2003).

Chlorophyll molecules are magnesium-tetrapyrrole pigments that give plants, algae and cyanobacteria their characteristic green color, being the primary pigments used

in plant photosynthesis (Kiang et al 2007). Chlorophyll pigments (chl) consist of two main types, a (chl a) and b (chl b) (Sonobe et al 2020). Their contents relate closely to primary production because they absorb sunlight and convert sunlight, water, and carbon dioxide into carbohydrates and oxygen (Gitelson et al 2006).

Nutrients supplied by fertilizers play a fundamental role in the structural and functional components of photosynthetic machinery (Schertz 1928; Pearman et al 1979; Young et al 2010), and an optimal nutrient supply is considered essential for the biosynthesis of plant photosynthetic pigments (Cai et al 2008; Hosseinzadeh et al 2016).

Antioxidants play a very important role in the body defense system against reactive oxygen species (ROS), which are harmful byproducts generated during normal cell aerobic respiration (Gutteridge & Halliwell 2000).

Material and Method. Eight winter wheat genotypes with different origin were analyzed for flavonoid, total phenols, antioxidant activity and leaf pigments. The plants were grown in field conditions at the Agricultural Research and Development Station of Turda, as a bifactorial experiment in tree replication. A basic mineral fertilization was applied in autumn (NPK 50:50:0) after plant emergence (T1 /Control). Two different foliar treatments (T2 and T3) were applied at the intensive growth stage (stem elongation) for stimulating growth, development and adaptability, and then compared with the control treatment (T1). The first foliar treatment (T2) contained a synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D) in dose of 20 ppm. 2.4 -D is one of the most common chemical substances used as herbicide, although this is a synthetic phytohormone with an important role in cell development. The second foliar treatment/fertilization (T3) consisted in a mixture of microelements as Fe (1000 mg L^{-1}), B, Mn, S and Mg (each of 500 mg L^{-1}). Teen leaf plant was randomly harvested after 10 days of foliar application for the determination of biochemical compounds.

Determination of total flavonoid. The method of the flavonoid content analysis was applied according to the Romanian Pharmacopeea (1993), based on the absorbance at 420 nm of the aluminium compounds resulted from the addition of the aluminum chloride ($AlCl_3$) solution (the aluminium complexation reaction) reading (Fernandes et al 2012).

Determination of total phenols. The determination of total phenolic content was carried out by the Folin-Ciocalteu colorimetric method, modified by Ivanova et al (2010). The spectrophotometric determination of total phenolic content is based on the absorbance at 765 nm.

Determination of antioxidant activity by DPPH assay. The measurement of the DPPH test was performed according to the methodology described by Brand-Williams et al (1995) at a 515 nm reading absorbance. The samples were reacted with stable DPPH radicals in an ethanol solution.

Leaf pigments. The study of the literature showed that an elegant and precise way of qualitative (but also quantitative) analysis of chlorophyll a, chlorophyll b and carotenoids in a plant tissue extract can be performed by UV-VIS spectroscopy. For that purpose, the absorbance at wavelengths of 662, 645 and 470 nm were recorded, and the concentrations for chlorophyll a (ca), chlorophyll b (cb) and carotenoids (c (x + c)) were then calculated following the formulas:

$$ca\ (\mu g\ mL^{-1}) = 11.24 * A662 - 2.04 * A645$$
$$cb\ (\mu g\ mL^{-1}) = 20.13 * A645 - 4.19 * A662$$
$$c\ (x+c)\ (\mu g\ mL^{-1}) = (1000 * A470 - 1.90 * ca - 63.14 * cb\ ) / 214$$

Results and Discussion. The flavonoid content of all the extracts is shown in Figure 1. The application of additional chemical compounds improved the flavonoid content especially for V2 and V4 variants. The application of a synthetic auxin treatment (2.4-D) increased the average of the flavonoid content (5.32 mg rutin g^{-1}) as a result of flavonols

accumulation. The application of the additional inorganic fertilizer increased also the mean of total flavonoid content compared with the control.

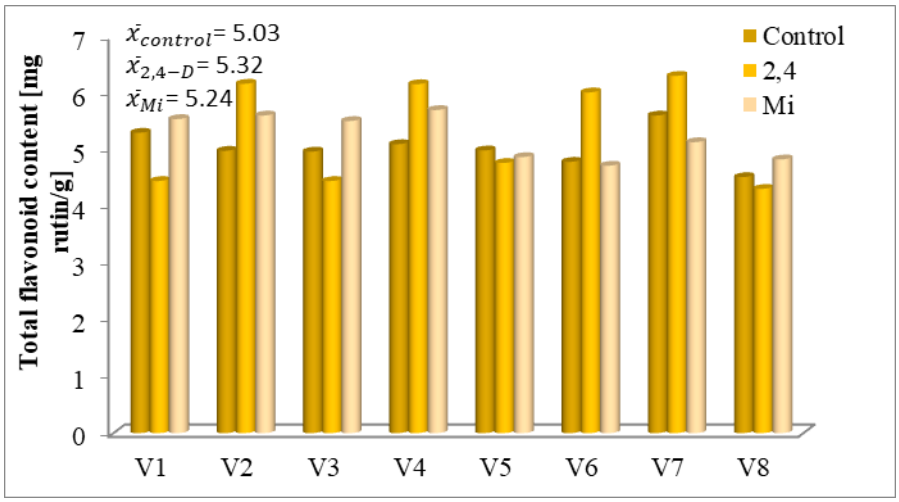

Figure 1. Total flavonoid content of studied genotypes with different treatments.

As shown in Figure 2, the application of different additional foliar fertilizers decreased the concentration of the phenolic compounds, compared with the control plants. A different behavior can be observed in the cases of V1, V4 and V8 where the application of microelements increases the total phenolic content. A specific reaction can be highlighted at V 6 in which case the application of 2.4-D increases the phenolic compounds concentrations, compared to the control and inorganic fertilizer treatments.

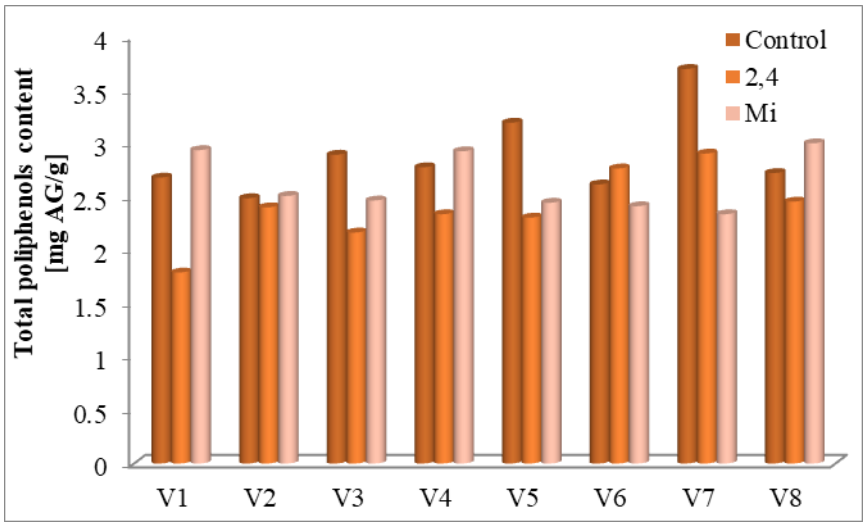

Figure 2. Phenolic compounds variation of studied winter wheat genotype under different treatments.

Generally, inorganic fertilizers reduce the antioxidant levels, which also happened in the case of the present experiment (Figure 3). Additional fertilization (T_2) had a negative impact on the plant antioxidant activity, compared to the check. Also, the application of synthetic phytohormone (2.4-D) caused a consistent reduction in the antioxidant activity of plants.

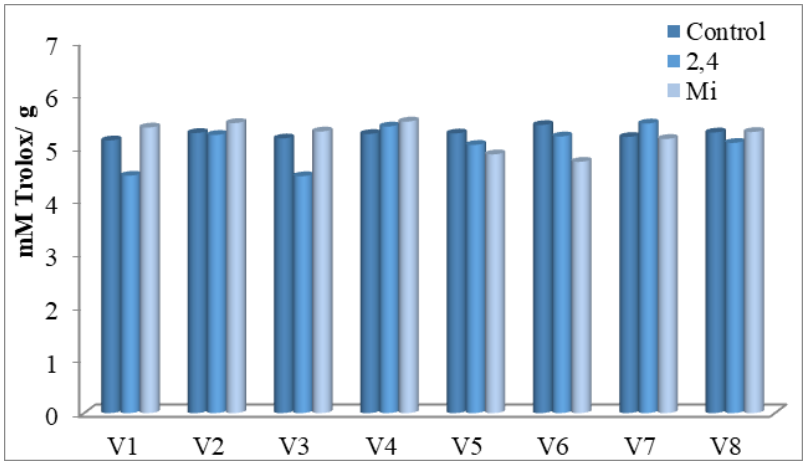

Figure 3. Antioxidant activity of studied winter wheat genotypes.

The application of different treatments (T_1 and T_2) on a wheat plant had a negative influence on chlorophyll a, chlorophyll b and carotenoids content (Figure 4). The average values obtained suggested that the application of synthetic phytohormone or any additional microelement on the wheat leafs has an unfavorable influence, even if certain genotypes react positively.

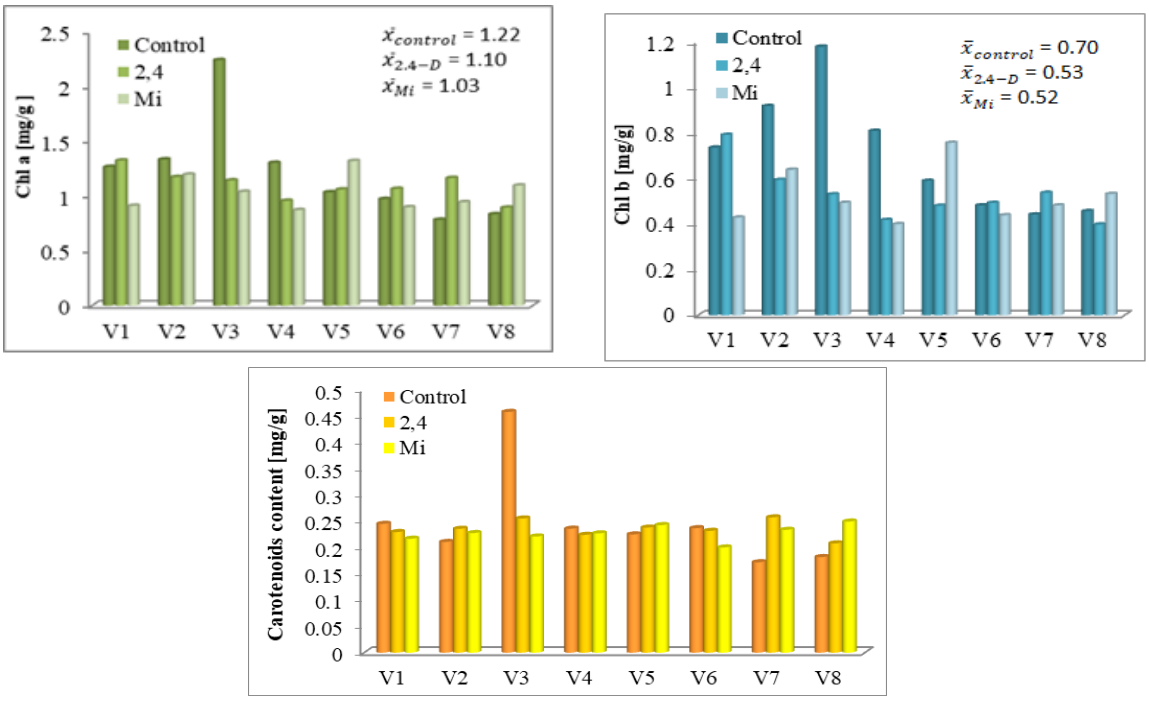

Figure 4. Chlorophyll a, chlorophyll b and carotenoids content.

Conclusions. The current study showed that the application of synthetic auxin (2.4-D in doses of 20 ppm) determines an increase of the flavonoid content, but decreasing the total polyphenols content and antioxidant activity in most of the winter wheat genotypes. Also, the foliar fertilization (T_3) had a positive influence on the flavonoid content and an inhibitory effect on the other biochemical compounds. The application of the two treatments had a negative influence on the leaf pigments.

References

Adom K. K., Sorrells M. E., Liu R. H., 2005 Phytochemicals and antioxidant activity of milled fractions of different wheat varieties. Journal of Agricultural and Food Chemistry 53:2297-2306.

Andersen C. P., 2003 Source–sink balance and carbon allocation below ground in plants exposed to ozone. New Phytologist 157:213-228.

Brand-Williams W., Cuvelier M. E., Berset C., 1995 Use of a free radical method to evaluate antioxidant activity. LWT—Food Science and Technology 28:25-30.

Cai R.-G., Zhang M., Yin Y.-P., Wang P., Zhang T.-B., Gu F., Dai Z.-M., Liang T.-B., Wu Y.-H., Wang Z.-L., 2008 Photosynthetic characteristics and antioxidative metabolism of flag leaves in responses to nitrogen application during grain filling of field-grown wheat. Agricultural Sciences in China 7:157–167.

Fernandes A. J. D., Ferreira M. R. A., Randau K. P., de Souza T. P., Soares L. A., 2012 Total flavonoid content in the raw material and aqueous extractives from Bauhinia monandra Kurz (Caesalpiniaceae). Scientific World Journal 923462, https://pubmed.ncbi.nlm.nih.gov/22701375/

Gitelson A. A., Vina A., Verma S. B., Rundquist D. C., Arkebauer T. J., Keydan G., Leavitt B., Ciganda V., Burba G. G., Suyker A. E., 2006 Relationship between gross primary production and chlorophyll content in crops: Implications for the synoptic monitoring of vegetation productivity. Journal of Geophysical Research Atmospheres 111, https://doi.org/10.1029/2005JD006017

Gutteridge J. M. C., Halliwell B., 2000 Free radicals and antioxidants in the year 2000: a historical look to the future. Annals of the New York Academy of Sciences 899:136–147.

Havsteen B., 2002 The biochemistry and medical significance of the flavonoids. Pharmacology & Therapeutics 96:67–202.

Hosseinzadeh S., Amiri H., Ismaili A., 2016 Effect of vermicompost fertilizer on photosynthetic characteristics of chickpea (*Cicer arietinum* L.) under drought stress. Photosynthetica 54:87–92.

Ivanova V., Stefova M., Chinnici F., 2010 Determination of the polyphenol contents in Macedonian grapes and wines by standardized spectrophotometric methods. Journal of the Serbian Chemical Society 75:45-59.

Johnson C. E., Oladeinde F. O., Kinyua A. M., Michelin R., Makinde J. M., Jaiyesimi A. A., Mbiti W. N., Kamau G. N., Kofi-Tsekpo W. M., Pramanik S., Williams A., Kennedy A., Bronner Y., Clarke K., Fofonoff P., Nemerson D., 2008 Comparative assessment of total phenolic content in selected medicinal plants. Nigerian Journal of Natural Products and Medicine 12:40-42.

Kiang N. Y., Siefert J., Govindjee R. E., Blankenship V. S., Meadows, 2007 Spectral signatures of photosynthesis I: Review of Earth organisms. Astrobiology 7:222-251.

Landolt W., Gunthardt-Goerg M. S., Pfenninger I., Eining W., Hampp R., Maurer S., Matyssek R., 1997 Effect of fertilization on ozone-induced changes in the metabolism of birch (*Betula pendula*) leaves. New Phytologist 137:389-397.

Molina-García L., Martínez-Expósito R., Fernández-de Córdova M. L., Llorent-Martínez E. J., 2018 Determination of the phenolic profile and antioxidant activity of leaves and fruits of spanish *Quercus coccifera*. Hindawi Journal of Chemistry 573270, https://doi.org/10.1155/2018/2573270

Naikoo M. I., Dar M. I., Raghib F., Jaleel H., Ahmad B., Raina A., Khan F. A., Naushin F., 2019 Role and regulation of plants phenolics in abiotic stress tolerance: An overview. In: Plant signaling molecules: Role and regulation under stressful environments. Khan M. I. R., Reddy P. S., Ferrante A., Khan N. A. (eds), pp. 157–168, Woodhead Publishing, Sawston, Cambridge, UK.

Panche A. N., Diwan A. D., Chandra S. R., 2016, Flavonoids: an overview. Journal of Nutritional Science 5:e47, doi: 10.1017/jns.2016.41

Pearman I., Thomas S. M., Thorne G. N., 1979 Effect of nitrogen fertilizer on photosynthesis of several varieties of winter wheat. Annals of Botany 43:613–621.

Samanta A., Das G., Das S., 2011 Roles of flavonoids in plants. International Journal of Pharmaceutical Science and Technology 6:12–35.
Schertz F. M., 1928 The quantitative determination of chlorophyll. Plant Physiology 3:323–334.
Sonobe R., Yamashita H., Mihara H., Morita A., Ikka T., 2020 Estimation of leaf chlorophyll a, b and carotenoid contents and their ratios using hyperspectral reflectance. Remote Sensing 12:3265, https://doi.org/10.3390/rs12193265
Soobrattee M. A., Neergheen V. S., Luximon-Ramma A., Aruoma O. I., Bahorun T., 2005 Phenolics as potential antioxidant therapeutic agents: Mechanism and actions. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 579:200–213.
Takahashi A., Ohnishi T., 2004 The significance of the study about the biological effects of solar ultraviolet radiation using the exposed facility on the international space station. Biological Sciences in Space 18:255–260.
Yong J. W. H., Ng Y. F., Tan S. N., Chew A. Y. L., 2010 Effect of fertilizer application on photosynthesis and oil yield of *Jatropha curcas* L. Photosynthetica 48:208–218.
Yu L., Haley S., Perret J., Harris M., Wilson J., Qian M., 2002 Free radical scavenging properties of wheat extracts. Journal of Agricultural and Food Chemistry 50:1619-1624.
*** Farmacopeea Romana, 1993, edition X, Editura Medicala, 1319 p.

Received: 12 October 2020. Accepted: 23 November 2020. Published online: 08 December 2020.
Authors:
Ionuț Racz, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Agriculture, 3-5 Calea Mănăştur St., 400488 Cluj-Napoca, Romania, e-mail: ionut.racz@usamvcluj.ro
Rozalia Kadar, Agricultural Research and Development Station Turda, 27 Agriculturii St., Turda, Cluj-Napoca, Romania, e-mail: rodica.kadar@scdaturda.ro
Loredana Maria Soran, National Institute for Research and Development of Isotopic and Molecular Technologies, 67-103 Donath St., 400293 Cluj Napoca, Romania, e-mail: loredana.soran@itim-cj.ro
Florin Dumitru Bora, Research Station for Viticulture and Enology Târgu Bujoru, Department of Physico-Chemistry and Biochemistry, 65 Eremia Grigorescu St., 805200 Targu Bujor, Romania, e-mail: boraflorindumitru@gmail.com
This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.
How to cite this article:
Racz I., Kadar R., Soran L. M., Bora F. D., 2020 The influence of organic and inorganic chemical compounds on the concentration of flavonoids, total phenols, antioxidants activity and leaf pigments in different wheat genotypes. AAB Bioflux 12(3):89-94.