# Project: Genotype specificity of wheatgrass (Triticum aestivum L.) highly nutritional natural food supplement

**Rebekić, Andrijana; Lisjak, Miroslav; Mišković Špoljarić, K.; Guberac, Sunčica; Orkić, Vedran**

*Source / Izvornik:* **53. hrvatski i 13. međunarodni simpozij agronoma: zbornik radova, 2018, 211 - 215**

**Conference paper / Rad u zborniku**

*Publication status / Verzija rada:* **Published version / Objavljena verzija rada (izdavačev PDF)**

*Permanent link / Trajna poveznica:* https://urn.nsk.hr/urn:nbn:hr:151:850216

PRELIMINARY COMMUNICATION

# Project: Genotype specificity of wheatgrass (*Triticum aestivum* L.) highly nutritional natural food supplement

Andrijana Rebekić[1], Miroslav Lisjak[1], Katarina Mišković Špoljarić[2], Sunčica Guberac[1], Vedran Orkić[1]

[1]*Faculty of Agriculture, University of J.J. Strossmayer in Osijek, Vladimira Preloga 1, Osijek, Croatia (arebekic@pfos.hr)*
[2]*Faculty of Medicine, University of J.J. Strossmayer in Osijek, Cara Hadrijana 10/E, Osijek, Croatia*

## Abstract

The aim of this paper is to present Installation research project „Genotype specificity of wheatgrass (*Triticum aestivum* L.) highly nutritional natural food supplement" financed by Croatian Science Foundation. With an aim to examine nutraceutical composition of wheatgrass the proposed study will investigate (1) genotype specificity of wheatgrass regarding the *in vitro* bioavailability of Zn, Fe, Se, Mn, Mg, Ca, K and Na, antioxidant activity and the content of chloroplast pigments, (2) the influence of Zn and Se biofortification on mineral composition, antioxidant activity and content of chloroplast pigments and (3) antiproliferative effect of wheatgrass extracts on human tumor cell lines.

**Key words**: Croatian Science Foundation, wheatgrass, micronutrients, bioavailability, antioxidative activity

## Introduction

Due to growing demand for food products that have an increased beneficial effect on human health part of agricultural production is directed towards production of food with improved characteristics and to production of functional foods. From the aspect of agricultural crop production, biofortification of wheat is accepted as an agronomic measure of grain enrichment with minerals and vitamins (Saltzman et al., 2017, Finkelstein et al., 2017). Such enriched products are functional foods, that belongs to group of enriched and improved agricultural products.

Wheatgrass represents young shoots of the species of Genus *Triticum*, which are cultivated and used in a form of dietary supplements as fresh juice, powder or tablets. Wheatgrass contains high concentrations of chlorophylls, bioflavonoids, essential amino acids, vitamins and minerals and has high antioxidant activity, which is why it is recommended in the treatment of chronic diseases (Sharma et al., 2013). One of the most important properties of wheatgrass is high chlorophyll content. In fresh juice of wheatgrass grown in open air, chlorophyll content is higher than in broccoli and kale samples (Wakeham, 2013) and it was found that it can be up to 70% (Swati et al., 2010) and it is dependable on the growing conditions (Wakeham, 2013).

Due to the high content of bioflavonoids (Singh et al., 2012) and the high antioxidant activity of wheatgrass, its effect on cell lines of various human cancers was studied (Altinok et al., 2008, Avci et al., 2008, Karadag et al., 2007), where the antiproliferative effect of wheatgrass has been determined.

Few research studies were engaged in investigation of optimal agrotechnic conditions for wheatgrass cultivation. The effect of different light spectrum illumination on the

antioxidative properties of wheatgrass of different wheat genotypes was tested (Urbonavičiūtė et al., 2009), method of drying wheatgrass for powder production (Das et al., 2011) and different germination and breeding conditions (Kulkarni et al., 2006) were studied. In addition, only one publication was published so far in which *in vitro* bioavailability of elements was investigated only one wheat genotype.

The unique contribution of the proposed research is in determination of the genotypic specificity of wheat with respect to the nutraceutical properties of wheatgrass. Furthermore, proposed research will contribute to the development of a methodology for determination of the *in vitro* bioavailability of minerals in wheatgrass. These results will contribute to a better understanding of the beneficial characteristics of wheatgrass, and obtained results will serve as a basis for future research in the same area.

## Material and methods

### Plant material and cultivation of wheatgrass

A 100 wheat genotypes that will be used in this research are part of the collection of the Agricultural Faculty in Osijek and are grown in the field experiment conducted within the Installation project PHENOWHEAT No. 2000 funded by the Croatian Science Foundation.

Wheatgrass will be cultivated from conventionally cultivated grain and biofortified wheat grain under controlled conditions. The grain will be sown in peat pellets that will be placed in the growing chamber (20 °C, at 12h / 12h night), and a growth will be monitored on a daily basis. Cultivation of wheatgrass in growth chamber will be carried out according to the completely randomized design with three replicates.

### Determination of chloroplast pigments

Determination of the content of chloroplast pigments (chlorophyll a, chlorophyll b and carotenoids) will be performed by spectrophotometric determination by Lichtenthaler and Welburn (1983). The pigment concentrations will be expressed in milligrams per gram of fresh weight of plant material. Chloroplast pigments will be determined in a total of 320 samples.

### Preparation of samples for *in vitro* digestion and extraction

Simulation of *in vitro* digestion will be carried out on: (1) fresh wheatgrass juice and (2) wheatgrass powder.The extraction of wheatgrass samples will be carried out as extraction by maceration with ultra pure water as a solvent (Kulkarni et al., 2006) and by using an ultrasonic water bath according to Akbas et al. (2017).

### Simulation of *in vitro* digestion and determination of *in vitro* bioavailability of Zn, Fe, Se, Mn, Mg, Ca, K and Na

Concentration of elements in the samples before *in vitro* digestion will be determine by ICP-OES (induced coupled plasma-optical emission spectrometry, Perkin Elmer 2100 DW technique after digestion of samples by wet digestion according to (Kingston and Lassie, 1986). Simulation of *in vitro* digestion will be carried out according to Kiers, Nout and Rombouts (2000) and according to Minekus et al. (2015). Bioavailability will be calculated as B(%) = (concentration in samples after in vitro digestion * 100) / concentration in samples before in vitro digestion. The simulation of *in vitro* digestion and the determination of the bioavailability of Zn, Fe, Se, Mn, Mg, Ca, K and Na will be carried out on 1920 samples, and 15360 single concentration measurements will be made.

**Determination of antioxidant activity of wheat grass extract**

The antioxidant capacity will be tested using the DPPH method (2,2-diphenyl-1-picryl hydrazil) according to Brand-Wiliams et al. (1995) with necessary modifications due to sample characteristics. In addition, the antioxidant capacity will be determined based on the ORAC method (Oxygen Radical Absorbance Capacity) according to Prior et al. (2003).The total content of phenol and flavonoid will be determined using the Folin–Ciocalteu method (Ainsworth and Gillespie, 2007) and the results will be calculated on the basis of a standard curve of gallic acid. Determination of the antioxidant activity will be carried out on a 2360 samples and in total 7080 measurements will be made.

***In vitro* methods used for estimation of antiproliferative effect.**

Antiproliferative effect of wheatgrass extracts will be tested on five human tumour cell lines. There are: acute promyelocytic leukaemia (HL-60: ATCC®CCl-240TM ), acute lymphoblastic leukaemia (MOLT-4: ATCC®CRL-1582TM ), cutaneous T lymphocyte (HuT-78: ATCC®TIB-161TM ), gastric adenocarcinoma (AGS: ATCC®CRL-1739TM ), and duodenum adenocarcinoma (HuTu-80: ATCC®HTB-40TM ). Antiproliferative effect will be estimated by spectrophotometric MTT assay (Mickisch et al., 1991).

**Statistical data analysis**

The data collected in the first two years of the study will be used to examine the specificity of wheat genotypes with respect to nine variables (concentration of essential elements in the samples before and after in vitro digestion, in vitro bioavailability, total phenolic content and flavonoid content, DPPH test and ORAC test, content of chlorophyll a, chlorophyll b and carotenoids). For each of the examined variables, the corresponding measurements of the descriptive statistics will be calculated. Examined genotypes will be grouped according to the similarity of examined traits by hierarchical cluster analysis. The distances between individual groups will be calculated by Squared Euclidean Distance, and the dendrogram will be based on the average linkage within the group.

Differences between investigated treatments (genotype, biofortification, days after germination, sample type and extraction method) and their interactions will be tested by factorial analysis of variance. Differences between levels of individual treatments will be tested using Tukey's HSD test at significance level of $p<0.01$. The interdependence of the examined traits will be investigated by correlation and regression analysis ($p<0.05$). The statistical analysis will be carried out using the SAS 9.4 for Windows, SAS Enterprise Guide 7.1 and JMP 9.0.

**Results and discussion**

In general, project activities can be divided into five groups: (1) activities leading to determination of genotypic specificity of the examined genotypes regarding examined traits, (2) activities carried out to determine the effects of Zn and Se biofortification, the age of the tillers, type of sample, and extraction method on the examine traits of the wheatgrass, (3) to investigate the biological effect of wheatgrass extract on human carcinoma cell lines, (4) dissemination of results and (5) application to proposals for different sources of finance in order to continue the work of the research group. The research results will be significant on an international level, because the determination of wheat genotype specificity will result in a recommendation on which genotypes can be used to produce wheatgrass as a mineral food supplement and which genotypes are better to use for their antioxidant properties. Results of this research will be used for PhD thesis of young PhD student who will be employeed during

the project implementation. In addition, members of research group will actively apply on call's for other sources of financing in order to continue this research.

## Conclusions

Considering that research of this type and this scope on wheatgrass has not been yet carried out, this project will result in a new knowledge of importance to the Croatian and international scientific community. According to our knowledge, this project is a first project in the Republic of Croatia that will research nutraceutical properties of wheatgrass. In addition, within the project, a method for simulation of *in vitro* digestion which is not used by any other research group in Croatia, will be indirectly developed. By developing research in the new field and developing a new methodology, the project and research group will become recognizable in the scientific community.

## Acknowledgement

This work has been supported in a part by Croatian Science Foundation under the project 4292.

## References

Ainsworth E. and Gillespie K. (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nature Protocols, 2(4), pp.875-877.

Akbas E., Kilercioglu M., Onder O., Koker A., Soyler B. and Oztop M. (2017). Wheatgrass juice to wheat grass powder: Encapsulation, physical and chemical characterization. Journal of Functional Foods, 28, pp.19-27.

Altinok B., Ozkan T., Karadag A., Aydos S., Biyikli Z. and Sunguroglu A. (2008). Apoptotic effect of wheatgrass (*Triticum aestivum* L.) extract on Baf3p210 and Baf3p210-T315I leukemia cell lines. Planta Medica, 74(09).

Avci A., Gurleyik E., Ozkan, T., Altinok B., Karadag A., Aydos S. and Sunguroglu A. (2008). Effect of wheatgrass (*Triticum aestivum* L.) on oxidant/antioxidant status in Baf 3p210 and T315i cell lines. Planta Medica, 74(09).

Brand-Williams W., Cuvelier M.E. and Berset C. (1995). Use of free radical method to evaluate antioxidant activity. Lebensmittel-Wissenschaft & Technologie, 28, pp.25-35.

Das A., Raychaudhuri U. and Chakraborty R. (2011). Effect of freeze drying and oven drying on antioxidant properties of fresh wheatgrass. International Journal of Food Sciences and Nutrition, 63(6), pp.718-721.

Finkelstein J., Haas J. and Mehta S. (2017). Iron-biofortified staple food crops for improving iron status: a review of the current evidence. Current Opinion in Biotechnology, 44, pp.138-145.

Karadag A., Ozkan T., Altınok B., Aydos S. and Sunguroglu A. (2007). Antiproliferative and apoptotic effects of wheatgrass (*Triticum aestivum* L.) extracts on chronic myeloid leukemia (CML) cell line. Planta Medica, 73(09).

Kiers J., Nout R. and Rombouts F. (2000). In vitro digestibility of processed and fermented soya bean, cowpea and maize. Journal of the Science of Food and Agriculture, 80(9), pp.1325-1331.

Kingstone H.M. and Lassie L.B. (1986). Microwave energy for acid decomposition at elevated temperatures and pressures using biological and botanical samples. Analytical Chemistry, 58, pp. 2534-2541.

Kulkarni S., Tilak J., Acharya R., Rajurkar N., Devasagayam T. and Reddy A. (2006). Evaluation of the antioxidant activity of wheatgrass (Triticum aestivum L.) as a function of growth under different conditions. Phytotherapy Research, 20(3), pp.218-227.

Lichtenthaker H. and Welburn A. (1983). Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochemical Society Transactions, 11(5), pp.591-592.

Mickisch G., Fajta S., Bier H., Tschada R. and Alken P. (1991). Cross-resistance patterns related to glutathione metabolism in primary human renal cell carcinoma. Urological Research, 19(2), pp.99-103.

Minekus M., Alminger M., Alvito P., Ballance S., Bohn T., Bourlieu C., Carrière F., Boutrou, R., Corredig M., Dupont D., Dufour C., Egger L., Golding M., Karakaya S., Kirkhus B., Le Feunteun S., Lesmes U., Macierzanka A., Mackie A., Marze S., McClements D., Ménard O., Recio I., Santos C., Singh R., Vegarud G., Wickham M., Weitschies W. and Brodkorb A. (2014). A standardised static in vitro digestion method suitable for food – an international consensus. Food & Function, 5(6), pp.1113-1124.

Prior R.L., Hoang H. and Gu L. (2003). Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORAC (FL)) of plasma and other biological and food samples, Journal of Agricultural and Food Chemistry, 51, pp.3273–3279.

Saltzman A., Birol, E., Oparinde, A., Andersson M., Asare-Marfo D., Diressie M., Gonzalez C., Lividini K., Moursi M. and Zeller M. (2017). Availability, production, and consumption of crops biofortified by plant breeding: current evidence and future potential. Annals of the New York Academy of Sciences, 1390(1), pp.104-114.

Sharma S., Shrivastav V.K., Shrivastav A. and Shrivasta B.R. (2013). Therapeutic potential of wheatgrass (Triticum aestivum L.) for the treatment of chronic diseases. South Asian Journa of Experimental Biology,3(6), pp. 3018-313.

Singh N., Verma P. and Pandey B.R. (2012). Therapeutic Potential of Organic *Triticum aestivum* Linn. (Wheat Grass) in Prevention and Treatment of Chronic Diseases: An Overview. International Journal of Pharmaceutical Sciences and Drug Research, 4(1), pp.10-14.

Swati P., Sushma D., Indira R., Alka G. and Mamta D. (2010). Multitude potential of wheatgrass juice (Green Blood): An overview. Chronicles of young scientists, 1(2), pp.23-28.

Urbonavičiūtė A., Samuolienė G., Brazaitytė A., Duchovskis P., Ruzgas V. and Žukauskas A. (2009). The effect of variety and lighting qualityon wheatgrass antioxidant properties. Zemdirbyste-Agriculture, vol. 96(3), pp. 119–128.

Wakeham, P. (2013). The medicinal and pharmacological screening of wheatgrass juice (Triticum aestivum L.): an investigation into chlorophyll content and antimicrobial activity. The Plymouth Student Scientist, 6(2), pp. 20-30.