Hungarian University of Agriculture and Life Sciences
Institute of Food Science and Technology
Doctoral School of Food Sciences

The effect of different light conditions on glutathione and free amino acid metabolism on the expression of related genes in bread wheat (*Triticum aestivum* L.)

Dávid Zoltán Toldi

Budapest
2024

Name of Doctoral School	MATE, Hungarian University of Agriculture and Life Sciences, Institute of Food Science and Technology, Doctoral School of Food Sciences
Discipline:	Food Sciences
Head of Doctoral School:	Dr. Livia Simonné Sarkadi Doctor of Science MATE Faculty of Food Sciences
Supervisors:	Dr. Livia Simonné Sarkadi Professor, Doctor of Science MATE Institute for Food Science and Technology, Department of Nutrition Dr. Gábor Kocsy Scientivic advisor, Doctor of Science HUN-REN Center for Agricultural Research, Agricultural Institute Department of Biological Resources

.....

Dr. Livia Simonné Sarkadi

Approval of Head of

Doctoral School

.....	.....
Dr. Livia Simonné Sarkadi	Dr. Gábor Kocsy
Approval of Supervisor	Approval of Supervisor

1. PRECEDINGS, OBJECTIVES

The production of plants is basically determined by their genetic stock, but it is also greatly influenced by environmental conditions. One group of abiotic factors depends directly on the Sun and its position in the sky. Such factors are the temperature, including low and high temperature periods; the light, as well as the annual periodic changes of the length of the day, and the spectral composition and intensity of the light. Furthermore, abiotic factors include the composition of the atmosphere; the water supply, primarily the appropriate distribution of the amount of precipitation; the structure of the soil, its acidity or alkalinity and nutrient supply. Together with the biotic factors, these factors determine the specific conditions of habitat. (TOLNER, 1999).

One of the most important stages in the development of wheat, which also greatly affects the quality of the flour, is heading. This is a very complex process, which is determined by the set of environmental factors mentioned earlier. These environmental factors directly influence individual development and grain yield quality (VARGA *et al.*, 2003). In the case of wheat and barley (*Hordeum vulgare* L.), the basic relationships of the multifaceted regulatory processes given to different environmental factors have already been revealed (KOSOVÁ *et. al.*, 2014), but the molecular-genetic steps that take place during the adaptation processes have not yet been sufficiently clarified (LIPIEC *et al.*, 2013). A deeper understanding of the light-dependent physiological processes of plants, i.e. bread wheat (*Triticum aestivum* L.), which is the subject of this research, can help breeding of varieties that can best adapt to the specifics of the growing area and have reliable high breadmaking properties.

Objectives:

- tracking the changes of growth and development as well as tracking of the changes of photosynthesis, which fundamentally determines these processes, depending on the light conditions (i.e. different intensity and spectral composition of) in wheat in young and fully developed plants
- tracking the changes of growth and development in wheat as well as tracking of the changes of photosynthesis, which fundamentally determines these processes, according to the light conditions (i.e. different intensity and spectral composition of) in young and fully developed plants
- studying the effect of light intensity and spectrum on glutathione synthesis at the level of metabolic products
- investigation of the effect of light of different intensity and spectral composition on the concentration of free amino acids at the level of the metabolic product
- examining the effect of the different light environment used in the planned experiments on glutathione synthesis and free amino acid concentration at the level of gene expression

2. MATERIAL AND METHOD

2.1. Wheat varieties used in the research

My research was carried out on *Triticum aestivum* L. ssp. *aestivum* cv. Chinese Spring (CS) genotype (GALIBA *et al.*, 1989; GALIBA *et al.*, 1993; KEREPESI *et al.*, 1998). Another hexaploid wheat genotype (*Triticum aestivum* L. ssp. *aestivum* cv. 'Mv Kikelet') was used in experiments with plants grown until fruit maturity.

2.2. Growing conditions

Lighting in the plant breeding chamber was provided by several different types of LED light sources. These light sources are not monochromatic. The proportions of the blue, red or far-red spectral components was changed. White light had a similar composition to natural light, while in the case of blue, pink and far red, the spectrum was shifted in the direction of the given color. These light settings are intended to imitate changes in the light environment that also occur in nature (QUILES and LÓPEZ, 2004).

2.3. The study of plant growth

During the research, I also monitored the growth of developing sprouts and shoots. The development stage of the fully grown plants was determined from Z13 to Z89 according to the Zadoks scale (ZADOKS *et al.*, 1974).

2.4. Examination of photosynthetic parameters

The pigment content (chlorophylls and carotenoids) of plant extracts was determined by a simple photometric method using a Cary-100 UV-VIS spectrophotometer (Varian, Middelburg, Netherlands) at four different wavelengths (750 nm, 664 nm, 646 nm and 470 nm) (LICHTENTHALER, 1987).

2.5. Determination of free amino acids

The free amino acid content was determined using the shredded and homogenized leaf samples with an AAA 400 Automatic Amino Acid Analyzer (Ingos Ltd., Czech Republic) equipped with an Ionex Ostion LCP5020 cation exchange column (22 cm x 0.37 cm). The chromatographic separation was carried out using a Li-citrate buffer system based on multi-step gradient elution. Detection was performed with a flow-through cuvette detector, after post-column ninhydrin derivatization, at 570 nm and 440 nm (Pro). To evaluate the results, I used the CHROMULAN v0.82 (PIKRON, Czech Republic) program (KOVÁCS *et al.*, 2012).

2.6. Determination of thiols by HPLC

To determine the amount of thiols i.e. GSH, cysteine, γ-glutamyl-cysteine (γ-EC), hmGSH and cysteinyl-glycine (Cys-Gly) in the plant leaf samples, a reversed-phase column HPLC apparatus (Waters, Milford, MA, USA) was used (KRANNER and GRILL 1996, KOCSY *et al.*, 2000) equipped with fluorescent detector (W474 scanning, fluorescent, Waters). (KRANNER és

GRILL 1996, KOCSY *et al.*, 2000). For the precise determination of the reduction potential, I used the formula of SCHAFER and BUETTNER (2001).

2.7. RNA isolation

For the isolation of the RNA required for gene expression studies (KHANDHAR *et al.*, 2022) the Direct-zol™ RNA Miniprep Kit (Zymo Research) was used. Solutions containing RNA were stored at -80 °C until further use.

2.8. cDNA writing, i.e. reverse transcription

The concentration of RNA in the solutions containing the extracted RNA was measured with a NanoDrop ND-1000 UV/VIS spectrophotometer, and then dilutions of uniform concentration were prepared from the extracts. After the reaction, the cDNA solution was diluted to finally obtain a cDNA solution of 10 ng/µl (VOGEL and WHEAT, 2011).

2.9. RT-qPCR based studies

For the gene expression test series, I used a CFX96 real-time PCR device (CFX96 Touch™ Real-Time PCR Detection System, Bio-Rad), and then analyzed it with the Bio-Rad CFX Manager software (Bio-Rad CFX Manager 3.1.1517.0823) belonging to the device. During the data analysis, the ΔCT method was used to determine the relative expression levels, for which in all cases the Ta30797 gene (PAOLACCI *et al.*, 2009) was used as a reference ("housekeeping" gene - HKG) gene (LIVAK and SCHMITTGEN, 2001).

2.10. Statistical analysis

The statistical analysis was performed using the IBM SPSS Statistics 22.0 (2013) program with a one-factor analysis of variance (ANOVA), and then the Tukey's post hoc test ($P<0.05$ %) (GUILFORD, 1950). Correlation analysis and data representation were performed using the Microsoft Office 365 (2019) Excel program, while hierarchical clustering was performed using the Multiexperiment Viewer V4.5 program.

3. RESULTS AND THEIR DISCUSSION

3.1. The effect of light conditions on the growth and metabolism of young wheat

3.1.1. Effect of light on growth

The change in the fresh weight of the wheat showed significant differences in correlation to the different light treatments. In all cases, I took white light of normal intensity as the basic/standard condition, which is routinely used for growing plants in the phytorotron chambers in Martonvásár. Compared to white light with normal intensity, I experienced significant differences due to changes in brightness and color spectrum. The average fresh weight of wheat increased almost linearly with increasing light intensity. The fresh weight of wheat grown at high light intensity was the highest.

3.1.2. Photosynthetic pigments

By increasing the light intensity or increasing the proportion of blue light, the chlorophyll content of the leaves increased slightly, however, I could not demonstrate statistically significant significance. In general, I found that the amount of chlorophyll in the leaves changed depending on the energy content of the light. Similar to the chlorophyll content, when examining the results of the carotenoid content, I did not see any significant differences, but the trend is almost the same as the changes in the chlorophyll content

3.1.3. Photosynthetic activity of young wheat

To further investigate the photosynthetic activity, I examined the photosynthetic electron transport rate (ETR) of wheat. In the case of low-intensity white light, this parameter is drastically lower than the values measured in the case of other light treatments. As the intensity increased, the photosynthetic electron transport rate increased exponentially, and the highest value was measured in plants grown under high-intensity white light. Compared to normal white light, the ETR has increased by almost 50%.

3.1.4. The effect of light on the synthesis and redox state of glutathione

Their quantity changed under the influence of light with a different spectral composition, however, I experienced the most prominent effect in the case of plants grown under blue light. In this case, their quantity was the highest. The amount of GSH and GSSG was the lowest in the case of wheat grown under low-intensity white light, and did not reach half of that of plants grown under normal-intensity white light, which is related to the reduced functioning of photosynthesis, as in this case less ROF is produced. The GSH and GSSG contents were the highest in plants grown under normal and high-intensity white light.

3.1.5. Effect of light on free amino acids

Examining the total free amino acid content in wheat, I observed significant differences in all treatments compared to white light of normal intensity. By increasing the light intensity, the

free amino acid content increased almost linearly. In the case of wheat grown under high-intensity white light, the free amino acid content was almost twice as much as in the case of wheat grown under normal-intensity white light. Light with a different spectral composition also affected the free amino acid content of wheat. As a result of pink light, the content of all free amino acids significantly decreased compared to plants grown under normal, blue and red light. Hierarchical clustering showed the extent to which the concentrations of individual amino acids differ in each treatment. In the case of plants grown on low-intensity light, I experienced the most differences compared to the other light treatments. By comparing the treatments, two larger groups could be formed. These were blue and pink light, as well as normal, high and far-red light treatments. The highest free amino acid (FAA) concentrations were detected in wheat grown under high-intensity white light. The amount of amino acids increased parallel to increasing light intensity, except for Pro, Met and Orn. In pink light, the concentration of many amino acids was much lower, but the amount of Asp and Thr was greater than in normal intensity white light.

3.1.6. Effect of light on the expression of genes related to glutathione and amino acid metabolism

The products of the examined genes play a decisive role in the given metabolic pathway and were selected based on literature data. In the case of glutathione metabolism, the selection was made taking into account the publication of NOCTOR *et al.*, (2011) and the summary article of PRATELLI and PILOT (2014) regarding amino acid metabolism. The transcription level of genes related to glutathione and amino acid metabolism was also affected by the intensity and spectral composition of the light. In all cases, I compared the results to white light of normal intensity. In the light-dependent gene expression study of CysASTL, OrnATF and GluDH, I found that the expression increased with the increase in light intensity, i.e. the expression of the genes increased in parallel with the increase in light intensity-dependent assimilation. In the case of the P5CR and APX1 gene expression test, I found that the expression increased almost linearly with the increase in light intensity, i.e. the expression of the genes increased in parallel with the increase in light intensity-dependent assimilation. As a result of light with a different spectral composition, the expression of these genes decreased to a large extent compared to white light of normal intensity, and increasing the proportion of far red light slightly increased the expression of these genes, however, this is also lower than the values measured in the case of plants grown on white light of normal intensity. In the case of TaGR, AspTA and APSR gene expression studies, I found that similar to what has been described so far, the expression increased with increasing light intensity (almost linearly in the case of APSR). In the case of wheat grown under light with a different spectral composition, when examining the expression of these genes, I found that it was lower in plants grown under light with a pink spectral composition than in the case of light shifted towards

the blue or far red spectrum. Furthermore, in the case of APSR, I found that changing the spectral composition increased the expression and even exceeded the level measured in plants grown under high-intensity white light. In the case of the ADC and TaGSHS gene expression study, I found that the expression changes caused by the change in light intensity are related, but the relationship is not exactly linear.In the case of wheat grown under light with different spectral composition, the expression of the gene was lowest under the influence of far red light. Finally, for TaNR, TaGST and SerHMT, I found that the expression changes of NR as a result of changes in light intensity are inversely related, i.e. the expression of these genes decreased with increasing light intensity. The test results of the expression of the GST and SerHMT genes are different from the previous ones. The relationship between the change in white light intensity and gene expression is less apparent.In the case of wheat grown under white light of normal intensity, gene expression was the highest, i.e. both lack of light and high light intensity reduced the expression of these two genes. In the case of light treatments with different spectral compositions, the level of gene expression was lower than that observed in the case of plants grown under white light of normal intensity.

3.2. The effect of light conditions on the growth, development and metabolism of plants grown until seed ripening

3.2.1. The effect of light conditions on plant morphology and flowering time

The average height of wheat was the highest under the far red light treatment, while the lowest under the blue light treatment. The differences due to height were not accompanied by a change in the number of leafs. Developmental differences during ripening (Z71-89) under the influence of different light sources were not significant, except in the case of the blue treatment, where ripening was completed four days later than in the case of the other treatments.

3.2.2. Effect of light conditions on thiol levels in wheat flag leaves

Different light treatments affected the thiol level of wheat flag leaves. Total cysteine was greater in plants grown under far red light than in wheat grown under fluorescent white, pink, blue and red, and normal intensity red light. Among the three investigated thiol molecules, the most light-dependent change was detected in the case of γ-glutamylcysteine (γEC). The examination of the flag leaves showed that the highest amount of γEC was found in plants grown under high-intensity red and the lowest under pink light treatment, and the ratio of reduced and oxidized forms of γEC was influenced by light conditions. Under fluorescent white, blue, high and normal intensity red light treatment, this ratio was low, while for pink and far red it was high in the flag leaves of wheat. The lowest value of the total amount of glutathione was detected in the flag leaves of the plants grown under normal intensity red light treatment.

3.2.3. The effect of light conditions on the amino acid content of flag leaves

The intensity and spectral composition of the light influenced the quantity and ratio of free amino acids in the flag leaves of wheat. Lower amounts of free amino acids were detected in blue and normal-intensity red light treatments, while the highest amount of free amino acids was observed in the flag leaves of wheat grown under high-intensity red light treatment. Based on the analysis carried out on the flag leaf, it can be concluded that the relative amount of amino acids in it changed to a lesser extent due to different light treatments than in the case of young wheat, where the GABA, Asn, Glu, Ser, Asp, Ala, Thr, Gln and Gly groups can be clearly distinguished. The amount of GABA was crucial in the case of high-intensity light treatment of both rearing systems, thereby indicating its central role in metabolism.

4. CONCLUSIONS AND RECOMMENDATIONS

Changes in light intensity and spectrum affect photosynthesis and the closely related redox system in wheat. Changes in the quantity and redox state of glutathione may be related to the transcriptional regulation of light-dependent glutathione and amino acid metabolism. The existence of this relationship is supported by the positive correlation of the GSH concentration and the negative correlation of the GSSG ratio with ETR. This means that with an increase in GSH concentration, these parameters will also be higher, while with an increase in the GSSG ratio, which results in a more oxidizing environment in the cells, they will be lower.

During the complete cultivation of wheat, changes in light conditions had an impact on development and metabolism. During the research, the light intensity was primarily the most effective for the photosynthetic activity and thus the biomass production of the plants. In addition to all this, it was also of outstanding importance in the tillering phase of the plants. The quality of light affected the stem elongation of wheat, which was most affected by blue and far-red light as antagonists. A prolongation of the heading time was observed under blue light treatment, while the earliest earing time was observed when the ratio of blue and red spectrum was approximately the same. Light quality and quantity also changed the metabolism of flag leaves, which serve as an important source for the accumulation of reserve nutrients in wheat grains. Even though the blue treatment stimulated the opening of stomates, the CO_2 assimilation capacity of the flag leaves remained low. The redox state of thiols was primarily influenced by far-red light treatment. The ratio of reduced and oxidized forms of these metabolites, such as γ-EC, cysteine, and glutathione, was altered. Blue light treatment stimulated proline metabolism. All in all, these results allow us to conclude that different light conditions and their modification during development enable the optimization of wheat growth conditions, contributing to the change of certain properties.

Based on the results, instead of the general plant spacing used in wheat cultivation, choosing a species-specific one can help to develop better light adaptation of the plants, as well as to develop a better protection against oxidative stress. By fine-tuning the antioxidant system, it is possible to breed new varieties that can more easily adapt to different light conditions. Since the different light environments affected the amount and ratio of free amino acids in addition to the redox system, based on the results, it would be worthwhile to influence the metabolism by strengthening certain metabolic pathways, so that the plant would be able to make better use of the available resources as efficiently as possible, thus reducing, for example, nitrogen fertilizer demand. Given that, in addition to the redox system, the different light environments also affected the quantity and ratio of free amino acids, based on the results, it would be worthwhile to influence the metabolism by strengthening certain metabolic pathways, so that the plant is able to make better use of the available resources as efficiently as possible, thereby reducing for example, the need for nitrogen fertilizer. Since light affects the amount and ratio of free amino acids, it may be worth considering, for example, the application of top dressing of wheat plants depending on the time of day in order to create the most suitable physiological state of the plants and the highest possible quality of the grain yield.

5. NEW SCIENTIFIC RESULTS

1. The key role in wheat shoot growth is played by light intensity, which increases shoot length. Among the tested spectral components, blue and far-red light had the greatest effect on plant height.
2. Examining the development of the plants, it was proven that the degree of tillering depends to a small extent on the light intensity, but not on the spectral composition. Blue light slightly delays the ear ripening processes of wheat, but does not cause any morphological difference.
3. The amount of chlorophyll a+b, which is the basis of photosynthesis, and carotenoids were not significantly affected by the investigated light conditions. The expression of genes coding the enzymes involved in pigment biosynthesis did not change either.
4. The total amount and redox state of antioxidants (GSH/GSSG and the precursor CysS/Cys) also showed light-dependent changes both at the metabolic product and gene expression levels. Parallel to the light intensity decrease the ratio of GSH/GSSG decreased, and far-red light made decrease the amount of glutathione.
5. As the light intensity increased, the amount of free amino acids increased due to the gene expression level regulation of enzymes that are key in their metabolism. In addition, the amount of blue, red and far-red spectral components also affects the amount of amino acids.

6. PUBLICATIONS RELATED TO THE TOPIC OF THE DISSERTATION

MTMT identifier: 10056968

DÁVID TOLDI, MÓNIKA GYUGOS, ÉVA DARKÓ, GABRIELLA SZALAI, ZSOLT GULYÁS, KRISZTIÁN GIERCZIK, ANDRÁS SZÉKELY, ÁKOS BOLDIZSÁR, GÁBOR GALIBA, MARIA MÜLLER, LIVIA SIMON-SARKADI, GÁBOR KOCSY (2019): Light intensity and spectrum affect metabolism of glutathione and amino acids at transcriptional level, PLOS Published: December 31, 2019 https://doi.org/10.1371/journal.pone.0227271, IF.: 3.24 D1, független hivatkozások száma: 24

MONOSTORI I, HEILMANN M, KOCSY G, RAKSZEGI M, AHRES M, ALTENBACH SB, SZALAI G, PÁL M, **TOLDI D**, SIMON-SARKADI L, HARNOS N, GALIBA G AND DARKO É. (2018): LED Lighting – Modification of Growth, Metabolism, Yield and Flour Composition in Wheat by Spectral Quality and Intensity, Front. Plant Sci. (2018) 9:605. doi: 10.3389/fpls.2018.00605, IF.: 5.75 D1, független hivatkozások száma: 66

KOCSY, GÁBOR ; GALIBA, GÁBOR ; MEDNYÁNSZKY, ZSUZSANNA ; KOVÁCS, ZITA ; **TOLDI, DÁVID** ; SALGÓ, ANDRÁS ; SIMONNÉ, SARKADI LIVIA (2019): A szabad aminosavak és a poliaminok szerepe a gabonafélék sztresszválaszában, In: Simonné, Sarkadi L. (szerk.) 374. Tudományos kollokvium előadásainak rövid kivonata (2019) p. 6

TOLDI, DÁVID ; KOCSY, GÁBOR ; SIMONNÉ, SARKADI LIVIA (2019): A búza szabad aminosav anyagcseréjének fényfüggő változásai, In: MKE Vegyészkonferencia 2019 (2019) p.

KOCSY, G ; **TOLDI, D** ; GYUGOS, M ; DARKÓ, É ; PÁL, M ; GIERCZIK, K ; GALIBA, G ; MÜLLER, M ; SIMON - SARKADI, L (2018): Control of photosynthesis, glutathione and amino acid metabolism by light quantity and quality in wheat In: 22nd Meeting Austrian Society of Plant Biology (ATSPB). Conference book. (2018) p. 31

TOLDI, D ; KOCSY, G ; SIMON, SARKADI L (2018): Influence of different spectra on metabolism of nitrogen-containing components of wheat, In: István, Dalmadi; László, Baranyai; Quang, Duc Nguyen Third International Conference on Food Science and Technology Budapest, Magyarország : Szent István Egyetem, Élelmiszertudományi Kar (2018) 182 p. pp. 167-168. , 2 p.

TOLDI, D ; KOCSY, G ; SIMON, SARKADI L (2018): Influence of Light Conditions on the Composition of free Amino Acids in Wheat Seedling In: Hungarian, Chemical Society (szerk.) II. Young Researchers' International Conference on Chemistry and Chemical Engineering : Program and Book of Abstracts Budapest, Magyarország : Hungarian Chemical Society (2018) p. 42

TOLDI, DÁVID ; KOCSY, GÁBOR ; SIMONNÉ, SARKADI LIVIA (2018): Fényfüggő változások a búza szabad aminosav anyagcseréjében, In: Majdik, Kornélia (szerk.) XXIV.

Nemzetközi Vegyészkonferencia : 24th International Conference on Chemistry Erdélyi Magyar Műszaki Tudományos Társaság (EMT) (2018) p. 49

KOCSY, G ; **TOLDI, D** ; GULYÁS, ZS ; BOLDIZSÁR, Á ; DARKÓ, É ; SZALAI, G ; SZÉKELY, A ; JÄGER, K ; MEDNYÁNSZKY, ZS ; SIMON-SARKADI, L (2017): Light-quality and quantity-dependent redox control of metatbolism in wheat, In: Buerstmayr, H; Lang-Mladek, C; Steiner, B; Michel, S; Buerstmayr, M; Lemmens, M; Vollmann, J; Grausgruber, H (szerk.) Proceedings of the 13th International Wheat Genetics Symposium Vienna, Ausztria : Universität für Bodenkultur Wien (2017) p. 301

TOLDI, D ; PÁL, M ; DARKÓ, É ; KOCSY, G ; SIMON-SARKADI, L (2017): Influence of different light conditions on the metabolism of nitrogen-containing components in wheat, In: Györgyey, János (szerk.) A Magyar Növénybiológiai Társaság XII. Kongresszusa Szeged, Magyarország : Magyar Növénybiológiai Társaság (2017) 72 p. pp. 39-39. , 1 p.

TOLDI, D. ; SIMONNÉ, SARKADI L. ; KOCSY, G. (2017): A különböző fényviszonyok hatása a búza szabad aminosav tartalmának változására Influence of Different Light Conditions on the Content of Free Amino Acids in Wheat In: MAJDIK, Kornélia (szerk.) XXIII. Nemzetközi Vegyészkonferencia : 23rd International Conference on Chemistry Kolozsvár, Románia : Erdélyi Magyar Műszaki Tudományos Társaság (EMT) (2017) 148 p. p. –

TOLDI, DÁVID ; KOCSY, GÁBOR ; SIMONNÉ, SARKADI LIVIA (2016): Influence of light on the metabolism of nitrogen-containing components of wheat, In: MAJDIK, Kornélia (szerk.) XXII. Nemzetközi Vegyészkonferencia Hungarian Technical Scientific Society of Transylvania (HTSST) (2016) 140 p. p.

Bibliography

GALIBA, G., KOCSY, G., KAUR-SAWHNEY, R., SUTKA, J., GALSTON, A.W. (1993): Chromosomal localization of osmotic and salt stress-induced differential alterations in polyamine content in wheat. Plant Sci., 92, 203-211.

GALIBA, G., SIMON-SARKADI, L., SALGO, A., KOCSY, G. (1989): Genotype dependent adaptation of wheat varieties to water stress in vitro. J. Plant Physiol., 134, 730-735.

GUILFORD, J. P. (1950): Creativity. american psychologist, 5(9), 444–454. https://doi.org/10.1037/h0063487

KEREPESI, I., GALIBA, G., BÁNYAI, É. (1998): Osmotic and salt stresses induced differential alteration in water-soluble carbohydrate content in wheat seedlings. J. Agric. Food Chem., 46(12), 5347-5354.

KHANDHAR, D.,BHATT, P., & THAKER, V. (2022): Improved protocol of Rna isolation for transcriptome analysis of poaceae plants. International journal of agriculture, environment and biotechnology citation: IJAEB: 15(01): 25-31, March 2022 doi: 10.30954/0974-1712.01.2022.4

KOCSY, G., SZALAI, G., VÁGÚJFALVI, A., STÉHLI, L., OROSZ, G., & GALIBA, G. (2000): Genetic study of glutathione accumulation during cold hardening in wheat. Planta, 210(2), 295–301. doi:10.1007/pl00008137

KOSOVÁ, K., VÍTÁMÁS, P., & PRÁSIL, I. T. (2014): Proteomics of stress responses in wheat and barley-search for potential protein markers of stress tolerance. Frontiers in Plant Science, 5. doi:10.3389/fpls.2014.00711

KOVÁCS, Z., SIMON-SARKADI, L., VASHEGYI, I., & KOCSY, G. (2012): Different accumulation of free amino acids during short- and long-term osmotic stress in wheat. The Scientific World Journal, 2012, 1–10. doi:10.1100/2012/216521

KRANNER, I., & GRILL, D. (1996): Determination of glutathione and glutathione disulphide in lichens: a comparison of frequently used methods. Phytochemical Analysis, 7(1), 24–28. doi:10.1002/(sici)1099-1565(199601)7:1<24::aid-pca277>3.0.co;2-2

LICHTENTHALER, H. K. (1987): Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. Plant Cell Membranes, 350–382. doi:10.1016/0076-6879(87)48036-1

LIPIEC, J., DOUSSAN, C., NOSALEWICZ, A., & KONDRACKA, K. (2013): Effect of drought and heat stresses on plant growth and yield: a review, Int. Agrophys. 27, 463-477 doi: 10.2478/intag-2013-0017

LIVAK, K. J., & SCHMITTGEN, T. D. (2001): Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. Methods, 25(4), 402–408. doi:10.1006/meth.2001.1262

NOCTOR, G., MHAMDI, A., CHAOUCH, S., HAN, Y., NEUKERMANS, J., MARQUEZ-GARCIA, B., QUEVAL G., FOYER, C. H. (2011): Glutathione in plants: an integrated overview. Plant, Cell & Environment, 35(2), 454–484. doi:10.1111/j.1365-3040.2011.02400.x

PRATELLI, R., & PILOT, G. (2014): Regulation of amino acid metabolic enzymes and transporters in plants. Journal of Experimental Botany, 65(19), 5535–5556. doi:10.1093/jxb/eru320

QUILES, M. J., & LÓPEZ, N. I. (2004): Photoinhibition of photosystems I and II induced by exposure to high light intensity during oat plant growth. Plant Science, 166(3), 815–823. doi:10.1016/j.plantsci.2003.11.025

SCHAFER, F. Q., & BUETTNER, G. R. (2001): Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. Free Radical Biology and Medicine, 30(11), 1191–1212. doi:10.1016/s0891-5849(01)00480-4

TOLNER, L. (1999): Termel&i tényezők (1.2. fejezet). In.: Füleky, Gy. (szerk.): Tápanyag-gazdálkodás. Mezőgazda Kiadó, Budapest. 18-21.

VARGA, B., SVECNJAK, Z., JURKOVIC, Z., KOVACEVIC, J. & JUKIC, Z. (2003): Wheat grain and flour quality as affected by cropping intensity UDC 633.11:632.534 original scientific paper ISSN 1330-9862 Food Technol. Biotechnol. 41 (4) 321–329 (2003) https://hrcak.srce.hr/file/180933

VOGEL, H., & WHEAT, C. W. (2011): Accessing the transcriptome: how to normalize mRNA pools. Molecular methods for evolutionary genetics, 105–128. doi:10.1007/978-1-61779-228-1_6

ZADOKS, J., CHANG, T., & KONZAK, C. (1974): A decimal growth code for the growth stages of cereals. Weed Res. 14, 415–421. doi: 10.1111/j.1365-3180.1974.tb01084.x