**Identification of genes and metabolites involved in the redox control of the transitions between growth, development and quiescent period in cereals**
**Final report of grant K131638**

## Summary

The transitions between growth, development and quiescent period in cereals was induced either by direct (ascorbate, $H_2O_2$, NaHS – donor of $H_2S$) or indirect (low temperature, cadmium, changes in light conditions) modification of the redox environment in cereals. Depending on their level, the various redox changes either reduced or stopped growth, and modified the development of wheat and maize seedlings. Ascorbate and $H_2O_2$ could increase the shoot regeneration from wheat calli. $H_2S$ inhibited the formation of lateral roots in maize and Arabidopsis. These effects were due to the modification of the ascorbate- and glutathione-dependent redox environment (reactive oxygen species, antioxidants) and hormone (auxins, gibberellins, abscisic acid, salicylic acid, jasmonic acid) levels. In this process redox-responsive miRNAs (miR395), transcription factors (auxin response factor, oxidative stress 2 protein) and their target genes (encoding antioxidants, enzymes of primary and secondary metabolism) were involved. The observed transcriptional changes in turn modified the amount of many metabolites including carbohydrates, amino acids and polyamines. The results, obtained during the investigation of whole shoots and roots, were also confirmed in extracts of shoot and root tips and by *in situ* detection of reactive oxygen species, transcripts (encoded by vernalisation genes) and metabolites (glutathione) in this organ parts. We proved the role of the redox system in the control of growth and development by the study of mutant Arabidopsis genotypes deficient in ascorbate or glutathione. Our results indicate, that the induction and inhibition of the growth, development or break of these processes and their transitions are modulated by the environment through the redox and hormonal system which adjust the metabolism to the actual growth conditions.

## Modulation of the growth and development of wheat by ascorbate and $H_2O_2$ during germination

We could successfully modulate the growth and development of wheat (*Triticum aestivum* L. cv. Chinese Spring) shoot primordia by ascorbate (ASA) and $H_2O_2$ during germination (*Asghar et al., 2023, Physiol. Plant., 175(2): e13887*). Treatment with ASA resulted in a greater growth reduction than the addition of $H_2O_2$. ASA also had a larger effect on the redox state of the shoot tissues, as shown by the higher ASA and glutathione (GSH) levels, lower glutathione disulphide (GSSG) content, GSSG/GSH ratio and redox potential of the GSSG/GSH couple compared to the $H_2O_2$ treatment (**Fig. 1**). Apart from common responses (i.e., increase of *cis*-zeatin and its *O*-glucosides), the contents of several compounds related to cytokinin (CK) and abscisic acid (ABA) metabolism were greater after ASA application. These differences in the redox state and hormone metabolism following the two treatments may be responsible for their distinct influence on various metabolic pathways. Namely, the glycolysis and citrate cycle were inhibited by ASA but not by $H_2O_2$, while the amino acid metabolism was induced by ASA and repressed by $H_2O_2$ based on the changes in the level of the related carbohydrates, organic and amino acids. The first two pathways produce reducing power, while the last one needs it; therefore, ASA, as a reductant, may suppress and induce them, respectively. $H_2O_2$ as an oxidant had a different effect, namely, it did not alter glycolysis and citrate cycle but inhibited the formation of amino acids. The different effect on the metabolism resulted in a large and a small inhibition of growth by ascorbate and $H_2O_2$, respectively.

In this experimental system we also compared the effect of Asc and $H_2O_2$ on the redox environment and gene expression in whole shoots and roots and their tips. The accumulation of $H_2O_2$ was greater in tips (about 1 cm region) of both organs compared to their other parts based on the *in situ* study of $H_2O_2$ levels. Correspondingly, the total glutathione content was also greater in the root tips as shown by transmission electron microscope. The greater changes in the redox environment were accompanied by the greater expression of some redox-responsive genes (auxin response factor, oxidative stress2 protein and vernalisation genes) in the extracts of shoot and root tips. This change was also confirmed by the detection of mRNA levels by *in situ* hybridization in the case of vernalisation gene. These biochemical alterations following the treatments with certain concentrations of Asc and $H_2O_2$ were accompanied by developmental changes based on the number of lateral roots. The effect of these compounds on the growth and development was also confirmed in various *Brachypodium distachyon* (L.) P.Beauv. genotypes.

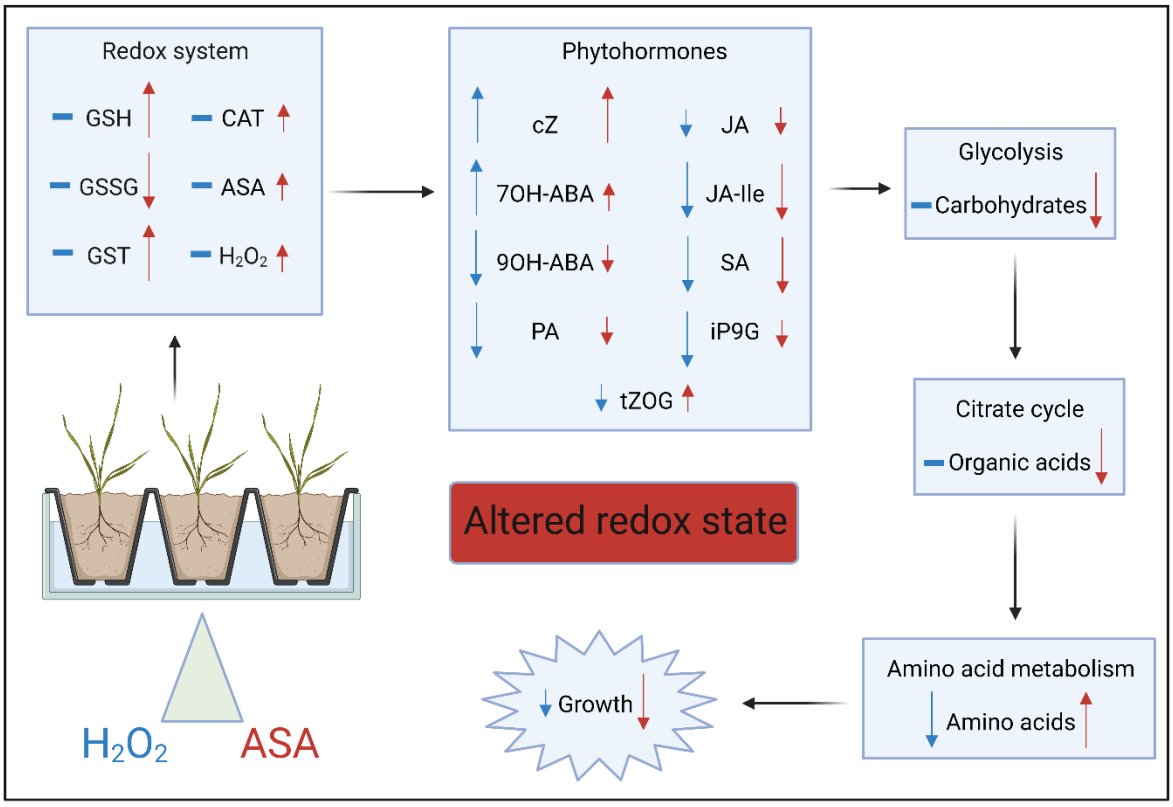


**Fig. 1.** An overview of metabolic changes involved in primary pathways of wheat seedlings exposed to an oxidant ($H_2O_2$) and a reductant (ASA). The blue and red arrows and horizontal lines refer to 7 d 5 mM $H_2O_2$ and 10 mM ASA treatments, respectively. Their relative changes compared to the control are indicated as follows: downregulation – small and large downwards arrows for 0.7- to 0.35-fold and <0.35-fold changes, respectively; no effect – horizontal lines for 0.7- to 1.4-fold changes; upregulation – small and large upwards arrows for 1.4- to 2.8-fold and >2.8-fold changes, respectively. The continuous lines show the direct metabolic connection between two metabolites, while the dashed lines show several intermediate products between them. Besides the three-letter abbreviations of the proteinogenic amino acids, the following ones were used: AAA: alfa-aminoadipic acid. GABA: gamma-aminobutyric acid, γGluCys: gamma-glutamylcysteine, γGluCysGly: gamma-glutamylcysteinylglycine, GSH: glutathione, Orn: ornithine, OPPP: oxidative pentose-phosphate pathway, TCA: tricarboxylic acid.

**Ascorbate and $H_2O_2$-dependent changes in the growth and development of 2-leaf old wheat seedlings**

ASA and $H_2O_2$ affected the growth and development of wheat also at the 2-leaf developmental stage (*Ashgar et al, 2023, J. Plant Growth Regul.*, ***42***, *6155–6170*). Interestingly, the redox environment became more oxidized after ASA treatment and more reduced after $H_2O_2$ addition based on the ratios of oxidised and reduced ascorbate and glutathione. The excess of ASA could inhibit, while $H_2O_2$ could induce the oxidative pentose phosphate pathway producing reducing power as shown by constant and decreased glucose-6-phosphate content, respectively (**Fig. 2**).

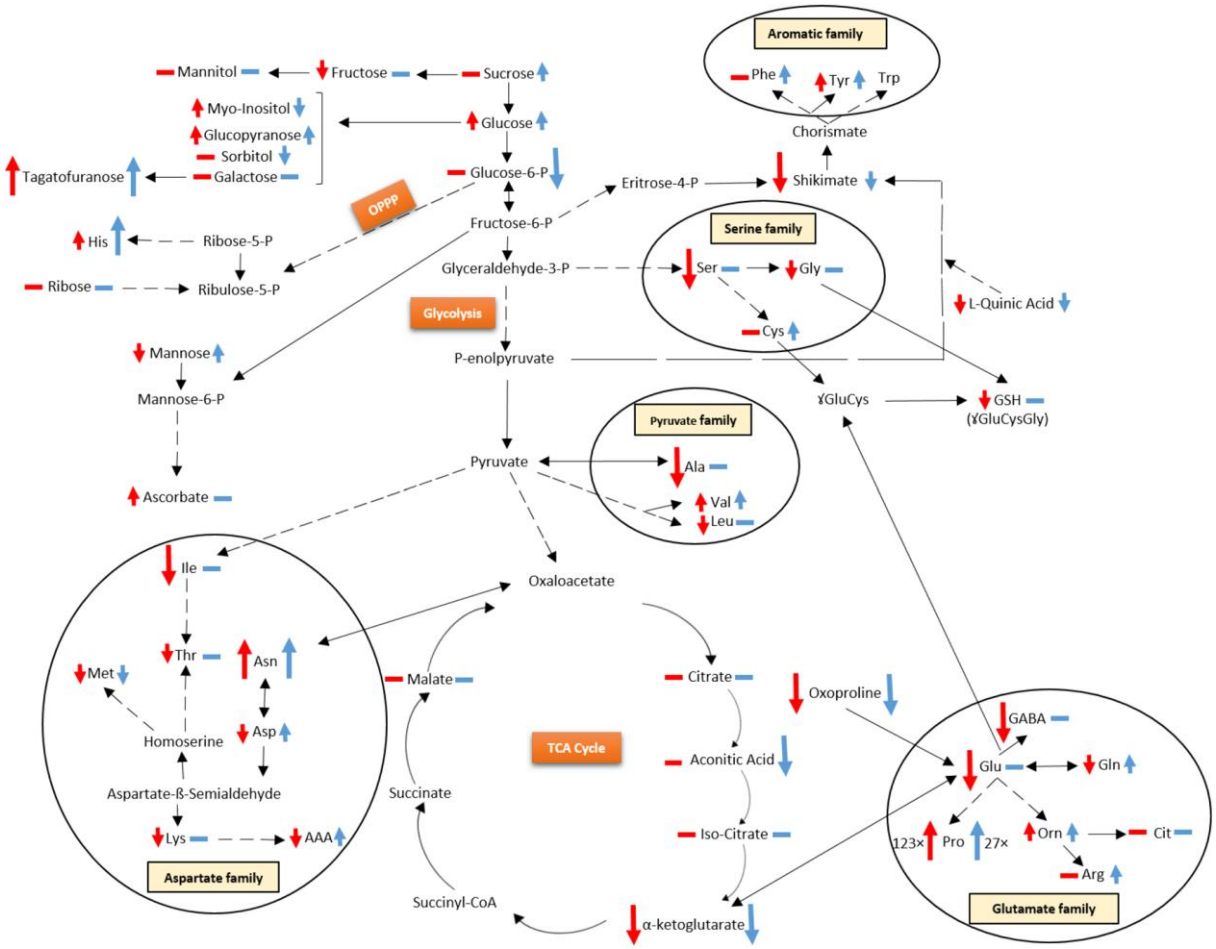


**Fig. 2.** An overview of metabolic changes involved in primary pathways of wheat seedlings exposed to a reductant (ASA) and oxidant ($H_2O_2$). The red and blue arrows and horizontal lines refer to 7 days 20 mM ASA and $H_2O_2$ treatments, respectively. Their relative changes compared to the control are indicated as follows: downregulation – small and large downwards arrows for 0.7- to 0.35-fold and < 0.35-fold changes, respectively; no effect – horizontal lines for 0.7- to 1.4-fold changes; upregulation – small and large upwards arrows for 1.4- to 2.8-fold and > 2.8-fold changes, respectively. The maximum ratio was around 7, but for proline the increase was much greater as indicated besides the arrows. The continuous lines show the direct metabolic connection between two metabolites, while the dashed lines show several intermediate products between them. Besides the three-letter abbreviations of the proteinogenic amino acids, the following ones were used: AAA alfa-aminoadipic acid. Cit citrulline, GABA gamma-aminobutyric acid, γGluCys gamma-glutamylcysteine, γGluCysGly gamma-glutamylcysteinylglycine, GSH glutathione, Orn ornithine, OPPP oxidative pentose phosphate pathway, TCA tricarboxylic acid.

This different effect on glucose-6-phosphate content can also explain the reduced formation of several amino acids from the intermediate products of glycolysis after ASA treatment and their constant or greater levels after $H_2O_2$ addition. In contrast to most amino acids, the accumulation of Pro was greatly induced by ASA, and this change was fivefold greater than after $H_2O_2$ addition. This difference could also contribute to the distinct redox shifts after the two treatments, since NADPH is oxidised during Pro synthesis. The more oxidising environment after ASA treatment activated several transcripts related to the ascorbate–glutathione cycle and the pentose phosphate pathway. Our results indicate the overcompensating effect of ASA and $H_2O_2$ on the redox environment in leaf tissues and the subsequent different adjustment of metabolite profile, the related transcript levels, growth and development.

**Induction of shoot regeneration or growth inhibition of wheat calli by ascorbate and $H_2O_2$**

We assumed that ASA and $H_2O_2$, depending on their concentrations, can induce shoot regeneration or modify the growth of wheat calli through their effects on the metabolism. To test this hypothesis, calli were treated with 0, 10, 20 and 40 mM ASA and $H_2O_2$ for 1 week (*Kulman et al., the manuscript for J. Plant Growth Regulation is being prepared*). Lower concentrations of these compounds increased the shoot regeneration, but their greater amounts resulted in reduced growth or even death of calli without shoot formation (**Fig. 3**). Both com-

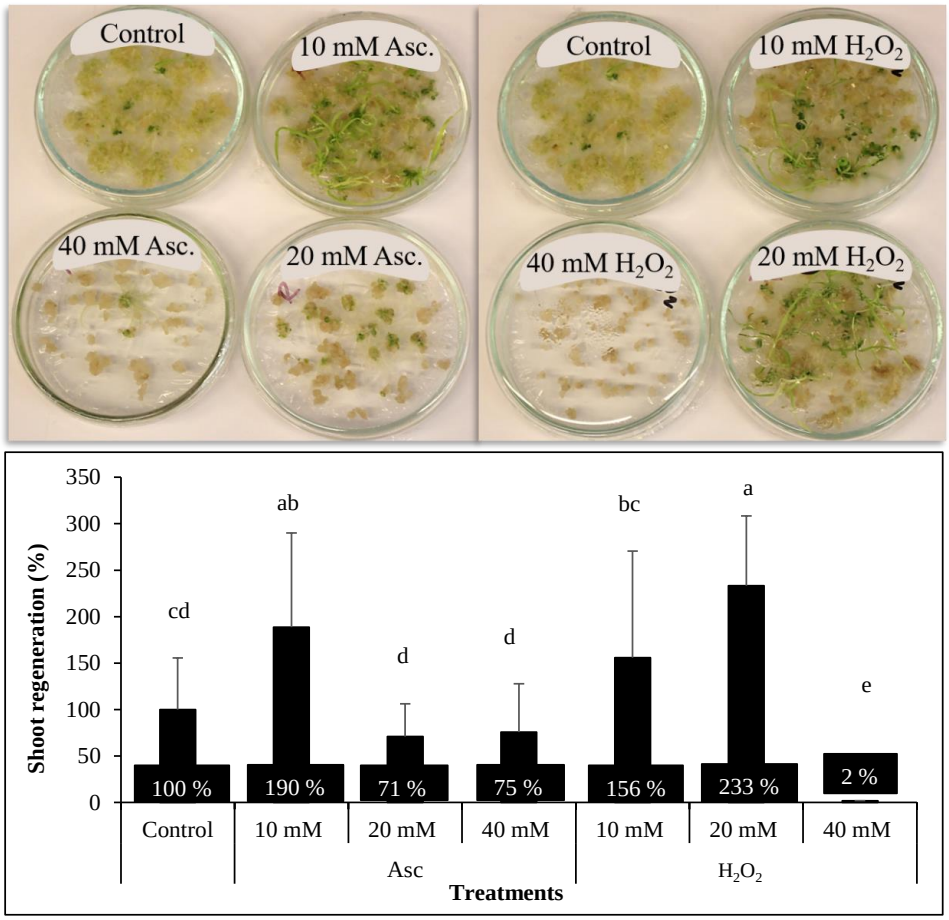


**Fig 3.** The effect of 10, 20 and 40 mM ascorbate (Asc) and $H_2O_2$ treatments on shoot regeneration and damage of calli. The bar chart shows the percentage of shoot regeneration compared to the control. The control is taken as 100 per cent. Columns marked with different letters indicate significantly different values ($P \leq 0.05$).

pounds decreased glutathione level in a concentration-dependent manner, and at higher concentrations, they also changed its redox state as shown by the half-cell redox potential values. Furthermore, the activity of the antioxidant enzymes was significantly lower after the addition of ASA and $H_2O_2$ in higher concentrations. The induced redox changes were accompanied by an increase in the amount of jasmonic acid-isoleucine, auxin precursor indol-3-aldehyde and auxin degradation product oxo- indole-3-acetic acid, and abscisic acid-glucose ester, and a decrease in the amount of cytokinin precursors: trans-zeatin-O-glucoside and trans-zeatin riboside-O-glucoside. These alterations of redox and the hormonal system also affected the metabolism. Thus, 10 mM ASA and $H_2O_2$ had different effects compared to their higher concentrations, since the Pro (osmoprotection, antioxidant), myoinositol (ascorbate synthesis), and amphetamine (may play a role in increasing antioxidant activity) levels declined in the latter case. The concentration-dependent differences in the effects of ASA and $H_2O_2$ on the redox and hormonal system resulted subsequently in specific metabolic changes leading either to improved shoot regeneration or growth inhibition.

**Modulation of the development of 2-leaf stage maize seedling by ascorbate, $H_2O_2$ and $H_2S$**

The effect of ASA and $H_2O_2$ on development was also tested in maize seedlings. In addition, $H_2S$ was included into these experiments, too. According to our hypothesis, these compounds may have specific effects on redox and hormonal systems and subsequently on physiological and biochemical processes (**Fig. 4**, *Singh et al., the manuscript was submitted to Phys. Plant.*).

| | 3 d | | | | 7 d | | | |
|---|---|---|---|---|---|---|---|---|
| ng g$^{-1}$ FW$^{-1}$ | C | Asc | $H_2O_2$ | NaHS | C | Asc | $H_2O_2$ | NaHS |
| Bound *o*HCA | b 9488.00 | b 10775.00 | a 12362.33 | b 9575.00 | b 9519.00 | c 6054.00 | a 12497.00 | c 5718.00 |
| Free SA | c 22.00 | b 38.00 | bc 31.33 | bc 25.33 | c 23.67 | a 62.67 | bc 30.00 | c 24.33 |
| Bound SA | de 284.33 | b 460.67 | bc 383.67 | cd 359.00 | e 251.00 | a 567.33 | bc 403.67 | bc 373.67 |
| Auxin | a 2.15 | ab 1.86 | ab 2.04 | ab 1.90 | ab 2.02 | b 1.34 | ab 1.65 | ab 1.82 |
| ABA | d 4.38 | b 65.90 | a 117.50 | d 9.57 | d 5.47 | c 32.23 | b 53.93 | d 3.74 |
| PA | d 2.42 | bc 8.71 | a 20.73 | d 3.82 | d 3.49 | cd 5.94 | b 10.75 | d 2.20 |
| DPA | d 7.37 | cd 25.57 | a 155.50 | cd 21.50 | cd 13.16 | c 33.23 | b 92.77 | d 12.00 |
| JA | ab 160.33 | ab 202.00 | a 236.00 | ab 182.33 | b 90.10 | ab 199.67 | ab 160.00 | b 103.35 |
| JA-LE/ILE | a 6.41 | a 7.58 | a 8.29 | a 5.58 | a 3.01 | a 6.43 | a 6.03 | a 5.16 |

**Fig. 4.** Hormonal profile after the addition of 5 mM ascorbate (Asc), 5 mM hydrogen peroxide ($H_2O_2$), and 1 mM sodium hydrosulfide (NaHS) after 3 and 7 days. The colouring is done individually for each row with blue to red as the lowest to highest. Values with different letters are significantly different from each other at $p \leq 0.05$ levels (one-way ANOVA followed by Tukey's HSD test for the data obtained by 3 independent experiments with 3 biological parallels each). *o*HCA: *ortho*-hydroxycinnamic acid, SA: salicylic acid, ABA: abscisic acid, PA: phaseic acid, DPA: dihydrophaseic acid, JA: Jasmonic acid, JA-LE/ILE: JA-isoleucine/ leucine.

Interestingly, the $H_2S$-donor NaHS affected the root development of maize, since it reduced the number of lateral roots. The other two compounds did not have such influence. In contrast to NaHS, Asc and $H_2O_2$ significantly reduced shoot growth after 7 d, which can be the result of membrane damage indicated by the greater increase in electrolyte leakage and lipid peroxidation after 3 d compared to control plants. These changes led to the induction of enzymes of the ascorbate-glutathione cycle after 3 days at gene expression (measured in shoot tips, too) and after 7 d at activity level following Asc and $H_2O_2$ treatments in shoots. In addition, these two treatments also resulted in greater increase in salicylic acid, abscisic acid and jasmonate levels compared to NaHS. The activation of redox and hormonal system was accompanied by greater accumulation of vanillin, vanillic acid, ferulic acid 2 in Asc- and $H_2O_2$ -treated plants after 7 d which compounds have antioxidant function. Besides these similar effects, the GSH synthesis was only induced by $H_2O_2$, and the decreased accumulation of most amino acids and citrate was only observed after Asc treatment. Interestingly, a many-fold increase in the reduced to oxidised glutathione ratio occurred only after NaHS addition. We could show both specific and general effects of the three studied compounds on the studied physiological and biochemical parameters, which results can be useful in the plant breeding to increase adaptation ability.

**Glutathione-dependent regulation of amino acid metabolism in *Arabidopsis***

We could show that modification of the redox environment in plant tissues results in altered amino acid metabolism and growth in wheat and maize. The assumed glutathione-dependent redox regulation of the α-aminoadipic acid (AAA, non-proteinogenic amino acid, an intermediate product in the Lys catabolism on the saccharopine pathway) metabolism was not tested earlier. The effect of the modified size and redox state of glutathione pool (reduced + oxidised, GSH + GSSG) on the AAA content and related amino acid and transcript levels was compared in the wild-type Col-0 plants and the GSH-deficient *pad2-1 Arabidopsis* mutant with and without (control) glutathione (GSH) treatment (*Gulyás et al., 2023, J. Plant Biochem. Biotechnol., 32, 204–210*). Modification of the size and redox state of glutathione pool resulted in the alteration of Lys, Glu, Pro and AAA contents and the transcription of the three investigated genes of the saccharopine pathway. Both the AAA content and the expression of the two genes encoding enzymes of its synthesis (ketoglutarate reductase/saccharopine dehydrogenase, aminoadipic semialdehyde dehydrogenase) was lower in the *pad2-1* mutant, and these parameters, together with the GSSG content, were increased by GSH treatment both in Col-0 and mutant plants. The GSSG content and its ratio in the glutathione pool exhibited a positive correlation with the AAA content and the transcription of the two genes of its synthesis and the AAA catabolic gene (encoding dihydrolipoamide-succinyltransferase), which indicates a GSH-dependent control of AAA metabolism. This latter process in turn can affect the redox environment, because of the NADPH use/formation in its several steps.

**Effect of low temperature on polyamine metabolism**

Besides certain amino acids (α-aminoadipic acid, proline), polyamines can also modify the redox environment of plant tissues, since $H_2O_2$ is formed during their degradation. The appropriate timing of the accumulation of polyamines is very important during cold acclimation due to their direct protective role and their involvement in the signalling processes. The time course of changes in the amount of six of them was compared during a 3-week acclimation period in a freezing tolerant and a sensitive genotype of rye, barley and wheat (*Asghar et al., 2021, Braz. J. Bot. 44:11-15*). In general, a greater and faster cold-induced increase in biogenic

amine content was observed in the tolerant genotypes of the three species compared to the sensitive ones (**Fig. 5**). This change was very quick in the case of putrescine, spermidine and cadaverine reaching a maximum after three days in the freezing-tolerant rye genotype. There was a continuous increase in the spermine and tyramine contents during the whole acclimation period in the tolerant wheat genotype while nearly constant levels were detected in the sensitive one. The amount of these two amines exhibited a positive correlation with the level of freezing tolerance in each of the five sampling points. Based on the correlations, a coordinated adjustment of the level of the six studied biogenic amines occurred during the acclimation period which could contribute to the efficient adaptation to cold. In addition, the earlier induction of the biogenic amine accumulation in the freezing tolerant genotypes may contribute to their better cold acclimation and adjustment their growth to the stress conditions.

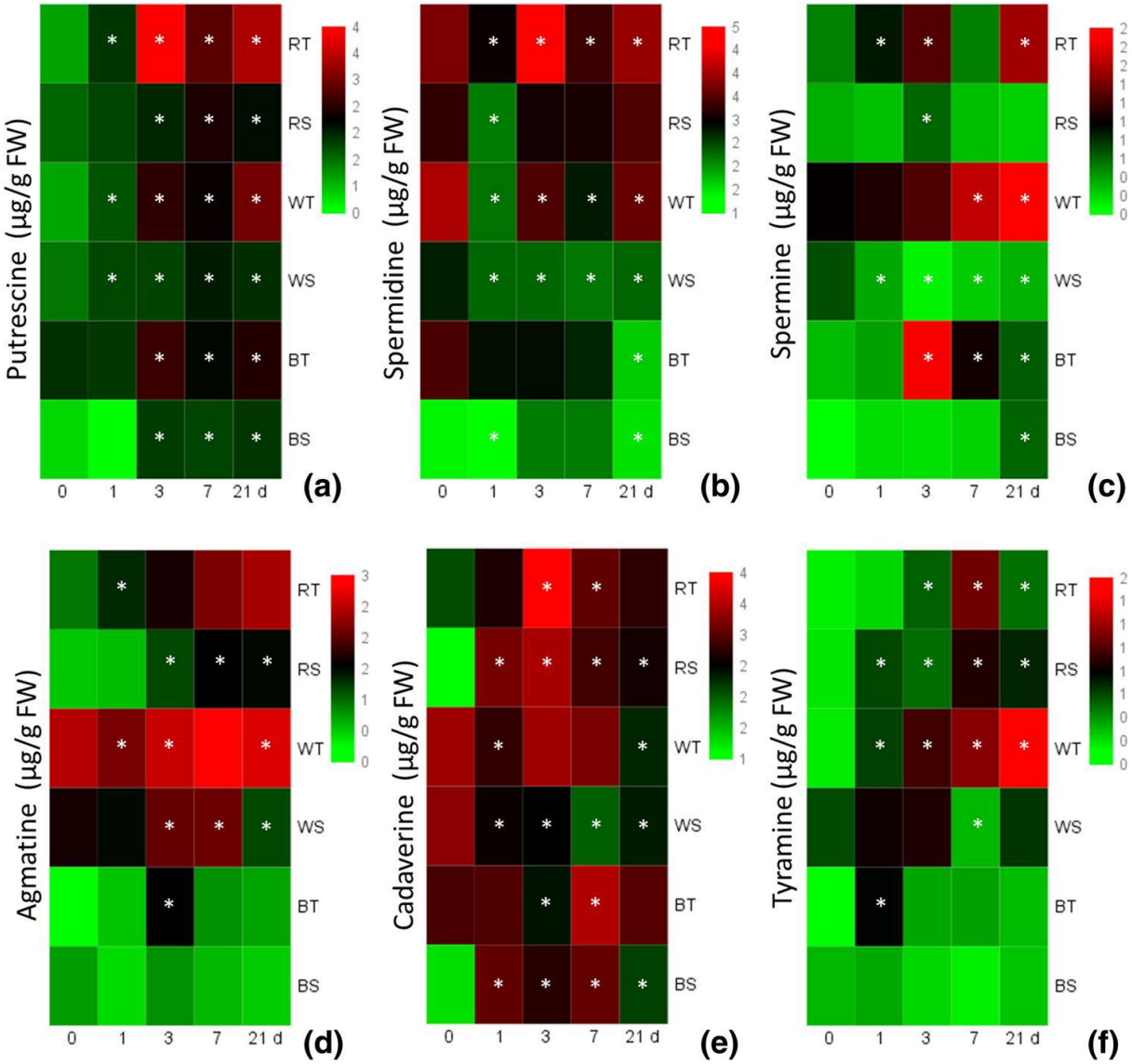


**Fig. 5.** The cold-induced accumulation of biogenic amines in Triticeae. Log2-values of the amine contents are shown on the heatmaps. The values indicated by asterisks are significantly different from those ones detected at the beginning of the cold acclimation at $p < 0.05$ level (ANOVA, t-test, 3 independent experiments, each with 3 parallels). RT: tolerant rye, RS: sensitive rye, WT: tolerant wheat, WS: sensitive wheat, BT: tolerant barley, BS: sensitive barley variety.

**Modification of the metabolism and growth of plants by light conditions at optimal and low temperature and by cadmium**

Besides the originally planned low temperature and cadmium stresses in cereals, we also included various light treatments (modification of intensity and spectrum) into our experiments for the indirect modulation of the redox environment under optimal or stress conditions based on our previous preliminary results using model plants (*Arabidopsis thaliana, Lemna minor*), too. Changes in the light conditions affect this environment through the induction of the formation of reactive oxygen species and modification of the activity of antioxidants in the plant tissues (Gulyás et al., 2023, Int. J. Mol. Sci., 2023, 24, 8323). Many metabolic processes, thus the biogenesis and function of miRNAs, are redox-responsive. The miRNAs, in turn, can modulate various components of the redox system, and this process is also associated with the alteration in the intensity and spectrum of the light. Daily and seasonal fluctuations in the intensity and spectral composition of the light can affect the expression of miRNAs, which can fine-tune the various physiological and biochemical processes due to the effect of miRNAs on their target genes. There is a light-controlled interaction between the redox system and miRNAs which ensures the efficient control of the various biochemical, physiological and molecular biological processes in plants and optimization of growth and development under various environmental conditions.

miRNAs are important in the control of the various metabolic processes being sensitive to the changes in intensity and spectrum of light under both optimal and stress conditions (*Borbély et al. 2022, Antioxidants, 11, 1311*). The blue and red spectral regimens are decisive in the regulation of metabolism because of the absorption maximums of chlorophylls and the sensitivity of photoreceptors. Photoreceptor-controlled transcription factors such as ELONGATED HYPOCOTYL5 (HY5) and changes in the cellular redox environment may have a major role in the coordinated fine-tuning of basic (carbon, nitrogen, amino acid, sulphur, lipid, and nucleic acid metabolism), and secondary (related to terpenoids, flavonoids, and alkaloids) metabolic processes during changes in light conditions. This reprogramming of metabolism is the basis for proper growth and development of plants; therefore, its better understanding can contribute to more efficient crop production.

To identify redox-responsive miRNAs, the transcriptome profile was compared in wild-type Arabidopsis plants and lines with decreased ascorbate (ASA), glutathione (GSH), or salicylate (Sal) levels (*Székely et al., 2023, Physiol. Plantar., 175(6), e14070*). GSH deficiency did not influence the miRNA expression, whereas lower levels of ASA and Sal reduced the accumulation of 9 and 44 miRNAs, respectively, but only four miRNAs were only upregulated. Bioinformatics analysis revealed that their over-represented target genes are associated with the synthesis of nitrogen-containing and aromatic compounds, nucleic acids, and sulphate assimilation. Among them, the sulphate reduction-related miR395 – ATP-sulphurylase couple was selected to check the assumed modulating role of light spectrum (**Fig. 6**). A greater induction of the ASA- and Sal-responsive miR395 was observed by sulphur starvation in far-red light compared to white and blue light in wild-type and GSH-deficient Arabidopsis lines. Sal-deficiency inhibited the induction of miR395 by sulphur starvation in blue light, whereas ASA-deficiency greatly reduced it independently of the spectrum. Interestingly, sulphur starvation decreased only the level of *ATP sulfurylase 4* among the target genes of miR395 in far-red light. The expression level of *ATP sulfurylase 3* was higher in far-red light than in blue light in wild-type and ASA-deficient lines. This results indicate that the expression of ASA- and Sal-responsive miRNAs can be modulated by light spectrum as shown for miR395. miR395 in turn regulates sulfate assimilation, and the end product of this pathway is cysteine, the precursor of GSH. Thus, this miRNA can affect the redox system, and subsequently the growth and development of plants.

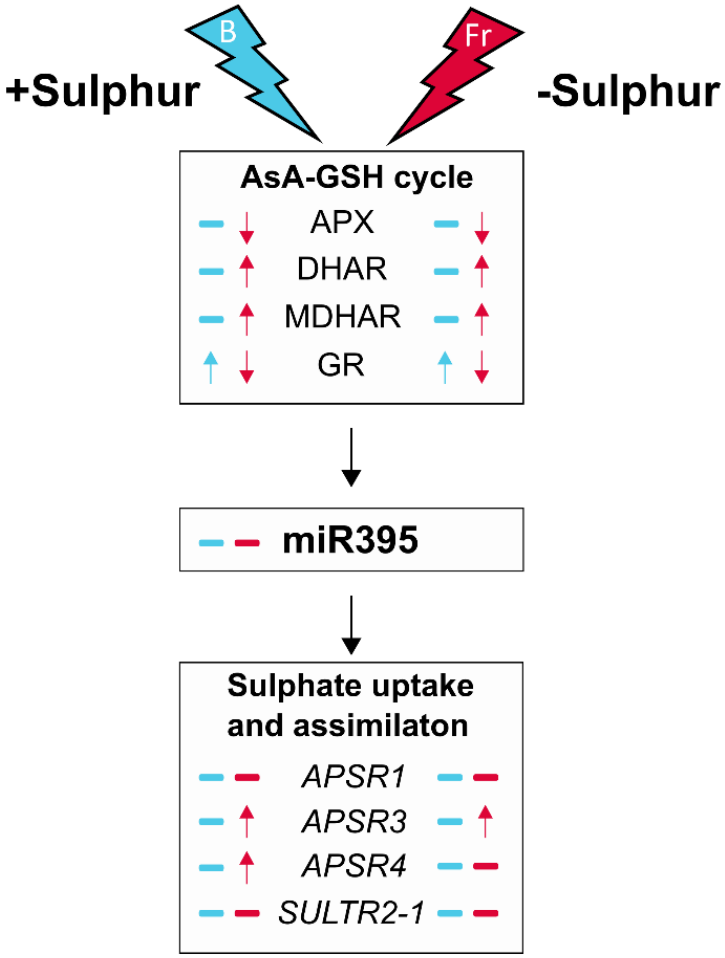


**Fig. 6.** Involvement of the ascorbate-glutathione cycle in the spectral control of miR395 and its target genes. Supplemental far-red light modifies the activities of ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR) and glutathione reductase (GR) compared to white light. This change is followed by a greater induction of miR395 and a smaller expression of *ATP sulfurylase4* (*APS4*) during sulphur starvation than under optimal sulphur supply in far-red light. *SULTR2-1: SULPHATE TRANSPORTER2-1*. ▬ : No effect, ↑: Positive effect, ↓: Negative effect, +Sulphur: sulphur supplementation, -Sulphur: sulphur starvation, Far-red light (red colour), Blue light (blue colour).

Light intensity and spectrum play in important role in the synchronization of nitrate and sulfate assimilations with photosynthesis, which ensures energy and reductants for these pathways. However, photosynthesis is also a source of reactive oxygen species, whose levels are controlled by glutathione and other antioxidants. We investigated the effect of supplemental far-red (735 nm) and blue (450 nm) lights on the diurnal expression of the genes related to photoreceptors, the circadian clock, nitrate reduction, glutathione metabolism (**Fig. 7**) and various antioxidants in barley (*Balogh et al. 2022, Int. J. Mol. Sci., 23, 7479*). The maximum expression of the investigated four photoreceptor and three clock-associated genes during the light period was followed by the peaking of the transcripts of the three redox-responsive trans-

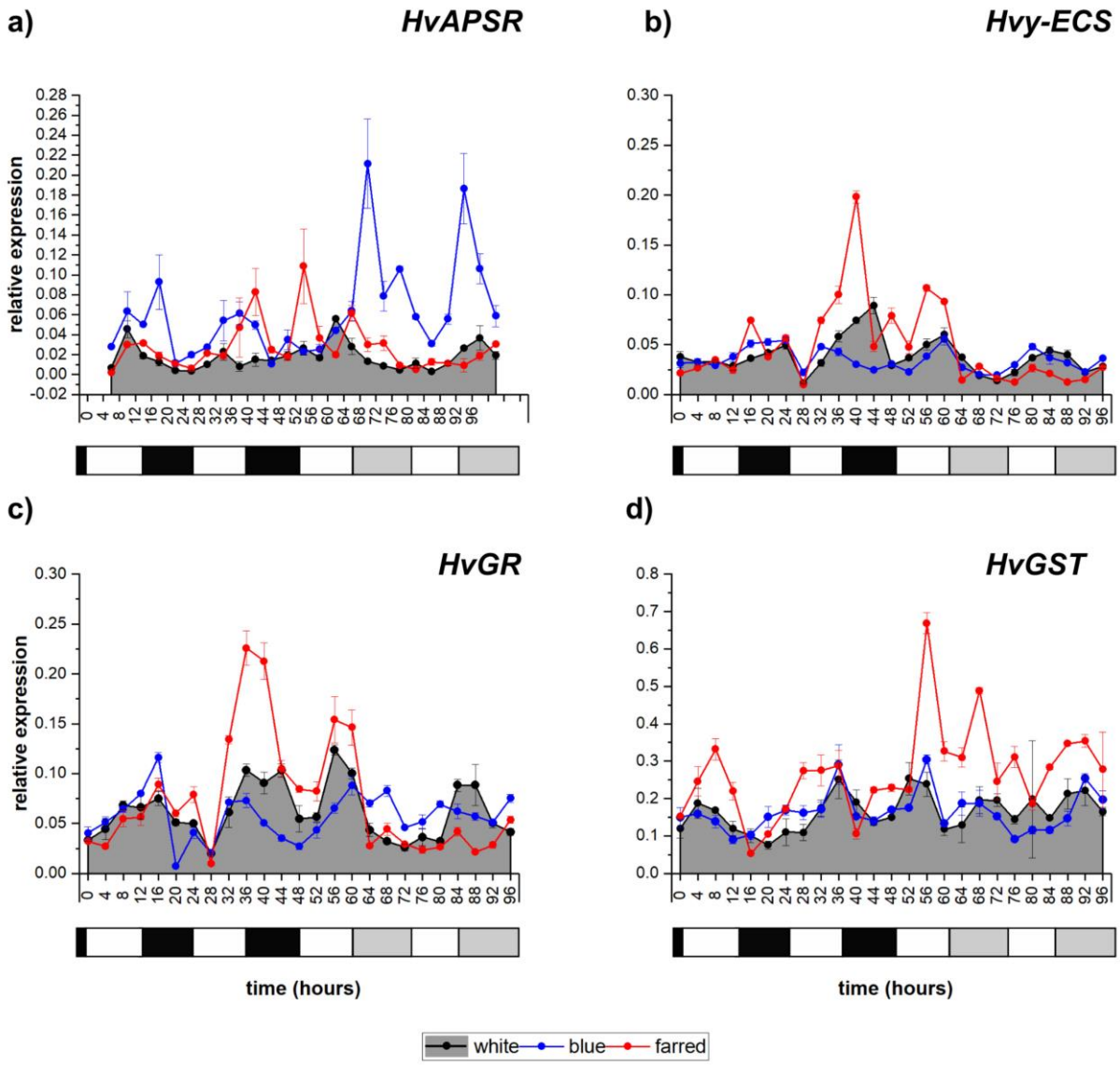


**Fig. 7**. Expression patterns of barley genes related to glutathione metabolism: APS reductase (HvAPSR), (**a**) γ-glutamylcysteine synthetase1 (Hv γ-ECS), (**b**) glutathione reductase (HvGR) (**c**) and glutathione S-transferase (HvGST) (**d**), under white, blue and far-red light illumination, respectively. Calculating methods and the legends of graphs are the same as described in Figure 1. The following significant differences were calculated at p 5% level: HvAPSR - 0.019; Hv-ECS - 0.023; HvGR - 0.016; HvGST -0.019.

cription factors during the dark phase, while most of the nitrate and sulfate reduction, glutathione metabolism and antioxidant enzyme-related genes exhibited high expression during light exposure in plants grown in light/dark cycles for two days. These oscillations changed or disappeared in constant white light during the subsequent two days. Supplemental far-red light induced the activation of most of the studied genes, while supplemental blue light did not affect or inhibited them during light/dark cycles. However, in constant light, several genes exhibited greater expression in blue light than in white and far-red lights. Based on a correlation analysis of the gene expression data, we propose a major role of far-red light in the coordinated transcriptional adjustment of nitrate reduction, glutathione metabolism and antioxidant enzymes to changes of the light spectrum.

Besides the light spectrum-dependent adjustment of the redox environment at organ level, the light intensity and spectrum-dependent modulation of the amount of various antioxidants at cellular and subcellular level is also important in the regulation of growth and development under optimal and stress conditions. In a review we provided an overview on how different light spectra and light intensities affect ascorbate and glutathione distribution at the cellular and subcellular levels in plants based on our joint research with our Austrian collaborators and results of other research groups (**Fig. 8**, *Gasperl et al. Histochem. Cell Biol. 158, 213–227*). Findings obtained in our most recent study demonstrated that both light intensity and spectrum significantly affected glutathione metabolism and its amount in different organelles of wheat and *Arabidopsis* cells.

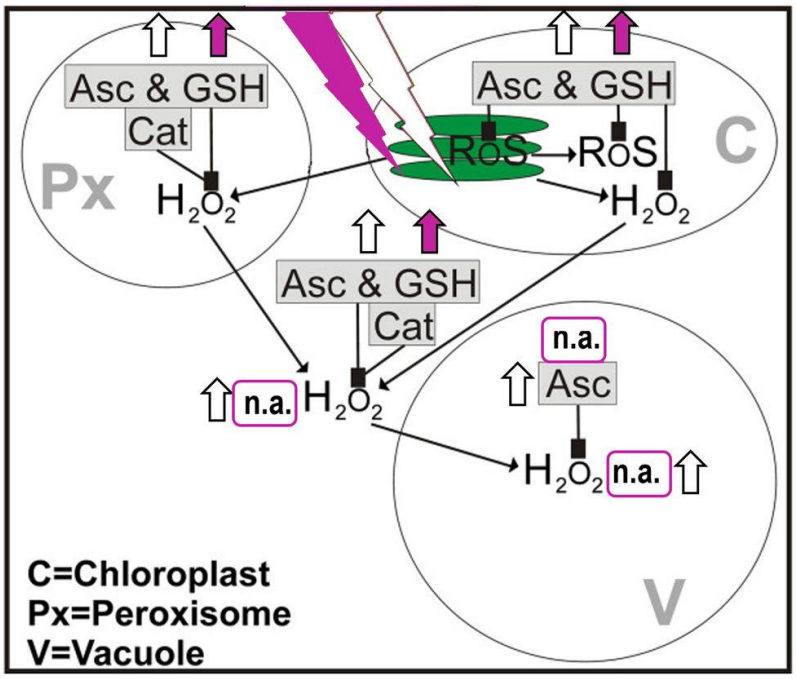


**Fig. 8.** Adapted model of subcellular reactive oxygen species (ROS) accumulation and detoxification by antioxidants and catalase in plants under conditions of excess light or white light with lower red/farred ratio

The regulation of metabolism by light conditions is also important in the adaptation to various environmental stresses. Light-dependent (250 or 500 μmol/$m^2$/s, red/far-red: 15/1; 250 μmol/$m^2$/s and red/far-red: 10/1) adjustment of glutathione metabolism during cold acclimation (5°C, 7 d) was compared in four wheat genotypes (14-day-old) differing in freezing tolerance (*Asghar et al., 2022, J. Agron. Crop Sci., 208: 65–75*). Only the shoot fresh weight of the two tolerant genotypes increased during cold, regardless of light conditions. Their electrolyte leakage was decreased in high light intensity. Cold greatly increased both the amount of γ-glutamylcysteine (glutathione precursor) and cysteinylglycine (degradation product) in all genotypes grown in high intensity and far-red lights, and consequently, the ratio of their oxidised forms exhibited a great decrease. However, cold induced a fivefold increase in the amount of glutathione and hydroxymethylglutathione disulphides only in the two sensitive genotypes grown in far-red light (**Fig. 9**). In general, the activities of the four enzymes of ascorbate–glutathione cycle were decreased by cold, except for the two tolerant genotypes cultivated in high light intensity. The gene expression studies did not reveal any transcriptional control of the changes observed at metabolite and activity levels. Our results show that both high intensity and far-red lights are involved in the control of the cold-induced changes in the

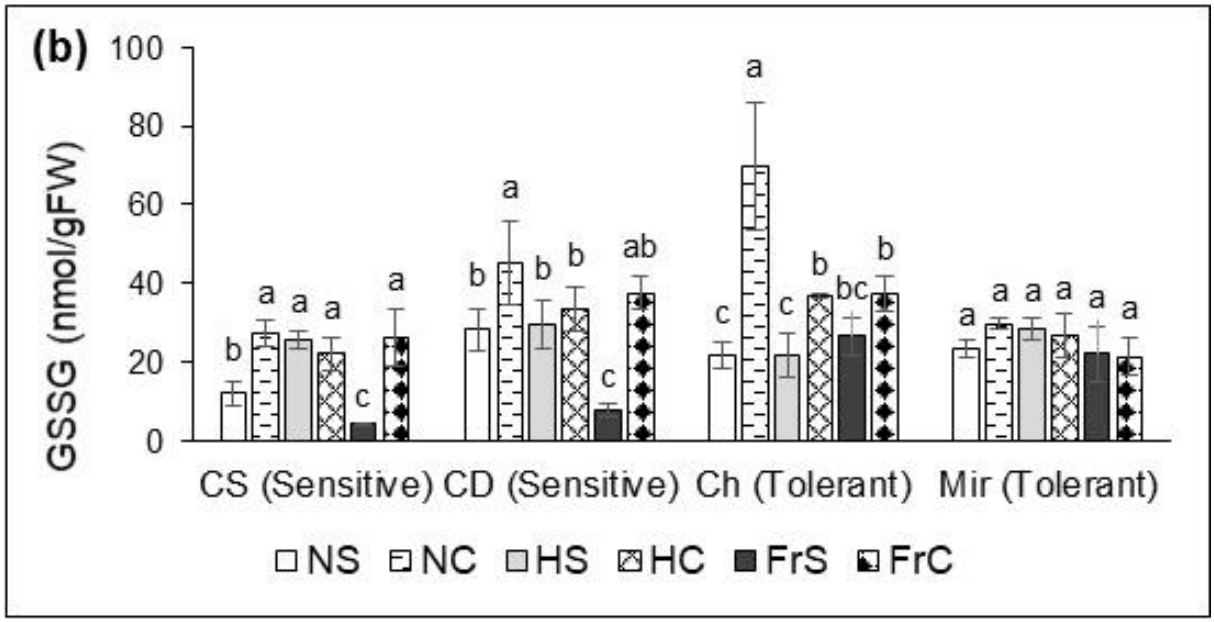


**Fig. 9.** Effects of high light intensity and far-red light on the amount of glutathione disulphide during cold acclimation. CS (Chinese Spring) and CD (Cappelle Desprez) are freezing-sensitive, and Ch (Cheyenne) and Mir (Miranovskaya 808) are freezing-tolerant genotypes. C, End of cold; FR, Far-red light; H, High light intensity; N, Normal light intensity; S, Start of cold. Values marked with different letters are significantly different from each other at $p \leq 0.05$ levels in the case of each genotype (ANOVA followed by least significant difference test, three independent experiments with 3 parallels each).

amount/activity of the studied antioxidants, and some of these alterations have positive correlation with the level of freezing tolerance and they are important in the modification of growth and development during the adaptation to low temperature..

Light spectrum-dependent changes in the amount of the glutathione, the precursor of phytochelatins, also affected the response to cadmium exposure in the model plant *Lemna minor* L. The assumed modulating effect of supplementary blue (B) and far-red (Fr) light on the Cd-induced changes of the various biochemical processes was clarified in our experiments (*Török et al., manuscript was submitted to Plant Stress*). While the tolerance of plants against Cd is mainly based on its chelation by phytochelatins in white (W) light, the defence process was also enhanced by its lower uptake in B and Fr light (**Fig. 10**). Interestingly, the reduced synthesis of phytochelatins from glutathione (GSH) in B and Fr lights was only associated with greater GSH levels in Fr light. This increased GSH content could contribute to the maintenance of the proper redox environment in the plant tissues, which reduced the Cd-induced changes in the level of most chemical elements and free amino acids in Fr light compared to W and B. However, Cd-treatment greatly increased the Zn, Cu and alpha-aminoadipic acid content in W light and the Na, K, glycine, cystathionine, proline, serine and glutamine content in B light. The present results demonstrate that the greatest Cd-extraction capacity of *L. minor* can be reached in W light. However, lower Cd uptake occurred in B and Fr light, which was accompanied by increased and decreased changes in the element and amino acid contents, respectively. These metabolic changes in turn modified the growth of the plants based on their fresh weight data.

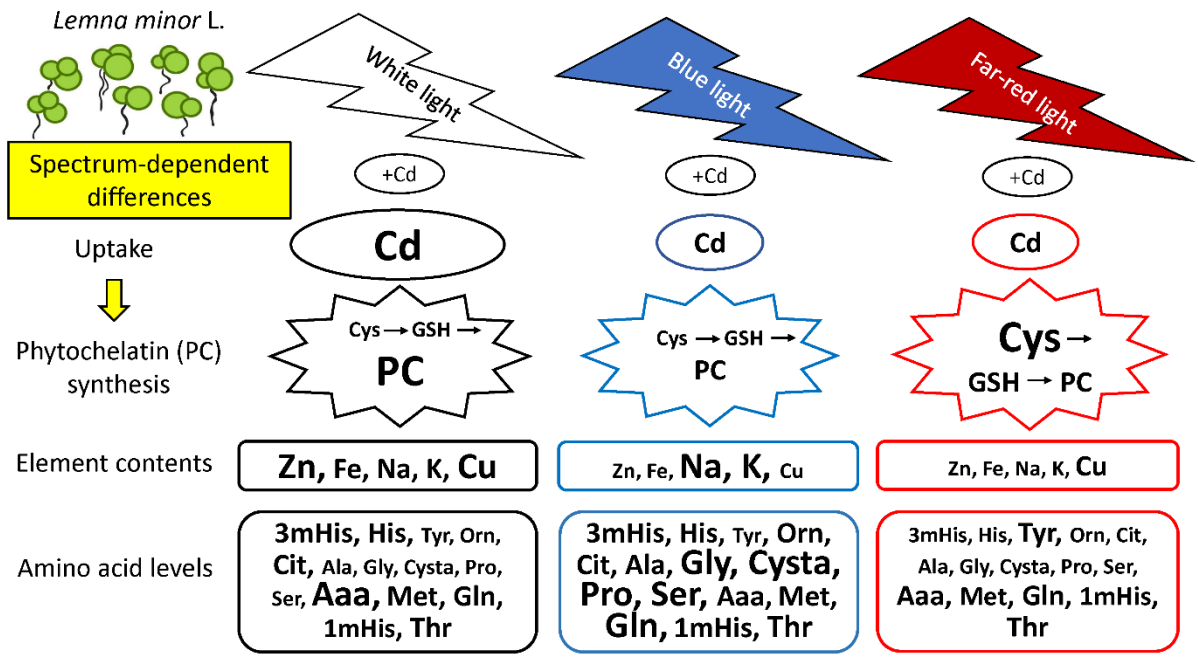


**Fig. 10.** The effect of light spectrum on cadmium uptake, phytochelatin synthesis, chemical element and amino acid levels in *Lemna minor* L.

## Conclusions

Our experimental system seemed to be appropriate for the pharmacological simulation of the physiological and biochemical processes induced by the environmental changes in plants. The used compounds (ASA, $H_2O_2$, $H_2S$) or experimental conditions (cadmium, low temperature, changes in light conditions) affected the redox environment of the plant tissues and subsequently the hormone levels, gene expression and the metabolite profile which modified the growth, development and their breaks (quiescent period) as well as their transitions. The miR395, auxin response factor, compounds of the ascorbate-glutathione cycle, auxins, cytokinins, abscisic acid, salicylic acid, certain amino acids, polyamines and flavonoid have important role in the control of these physiological processes based on our results in cereals and model species. Their redox-dependent alterations are the indicators of the adaptation ability to the environmental changes, therefore they could be used for the selection of stress tolerant crop genotypes in the future.