Regulation of growth and development by light quantity- and quality-dependent redox changes at optimal and low temperature in wheat (ANN117949)

Final report

Summary

Both light quantity and quality affected the glutathione-dependent redox environment as it was shown by the changes in the size of the glutathione pool and the ratio of the oxidised and reduced forms in leaf extracts of wheat. This control took place at least partly at transcriptional level as shown by the influence of the light on the expression of the glutathione metabolism-related genes. The involvement of miRNAs in this regulatory process was also demonstrated. Light conditions affected the subcellular distribution of glutathione, too. The alterations in the activities of the antioxidant enzymes also contributed to the light condition-dependent redox changes which were accompanied by the reprogramming of the metabolism as indicated by the alterations in the free amino acid and polyamine levels. From comparison of the various parameters in roots, crowns and leaves turned out that the leaves were the most sensitive to the changes in the light conditions. Low temperature modified the physiological and biochemical effects of light conditions. Chemical modification of the redox state in wheat tissues or the use of mutant Arabidopsis lines with low glutathione or ascorbate levels indicated that the redox system is involved in the mediation of the effect of light on metabolism, growth and development.

Study of the effect of light intensity and spectrum on the redox system, physiological, biochemical and molecular biological parameters

The effects of various light intensities and spectral compositions on the redox system, physiological, biochemical and molecular biological parameters were compared in crown, leaf and root extracts of wheat seedlings. Although smaller effect of light conditions on the studied parameters could be also shown in crowns and roots, the greatest changes were observed in leaf extracts. Based on the results of time-course experiments, the 7-day cultivation under various light conditions was selected for further studies. Among the components of the redox system, the metabolites of the ascorbate – glutathione cycle (glutathione, ascorbate, NADPH) were investigated. In the first experiments the light conditions exhibited the greatest influence on the amount and redox state of glutathione, therefore it was investigated subsequently. The ascorbate – glutathione cycle degrades the H_2O_2, the amount of which was not or only slightly affected by the applied light intensities or spectral conditions in leaf extracts or in shoot and root apices (*in situ* detection by fluorescent dye). This result can be explained by the efficient activation of the glutathione synthesis and reduction ensuring the quick removal of the excess of H_2O_2 after the modification of the light conditions.

Elevation of light intensity (low – normal - high) was accompanied by a simultaneous increase in the shoot fresh weight, photosynthetic activity and glutathione content of wheat leaves (*Toldi et al., 2019, PLoS ONE 14(12): e0227271*). These parameters were also affected by the modification of the ratios of blue, red and far-red components (hereafter the three modified spectral conditions are referred to as blue, pink and far-red lights) compared to normal white light. The photosynthetic activity and the glutathione content decreased to 50% and the percentage of glutathione disulfide (characterising the redox state of the tissues) in the total glutathione pool doubled in far-red light. The alterations in the level and redox state of the

antioxidant glutathione resulted from the effect of light on its synthesis as it could be concluded from the changes in the transcription of the related genes and the level of its precursors. Modification of the light conditions also greatly affected both the amount and the ratio of free amino acids. The total free amino acid content was greatly induced by the increase of light intensity and was greatly reduced in pink light compared to the normal intensity white light. The concentrations of most amino acids were similarly affected by the light conditions as described for the total free amino acid content but Pro, Met, Thr, ornithine and cystathionine showed unique response to light. As observed for the amino acid levels, the expression of several genes involved in their metabolism also grew due to increased light intensity. Interestingly, the modification of the spectrum greatly inhibited the expression of most of these genes. Correlation analysis of the investigated parameters indicates that changes in the light conditions may affect growth through the adjustment of photosynthesis and the glutathione-dependent redox state of the tissues. This process modifies the metabolism of glutathione and amino acids at transcriptional level.

The influence of light conditions on the redox state of tissues and various physiological and biochemical parameters was also shown in flag leaves of fully developed wheat plants (*Monostori et al., 2018, Front. Plant Sci., 9:605*). The plants were grown under six regimens designed to compare the effects of LED and conventional fluorescent lights on growth and development, leaf photosynthesis, thiol and amino acid metabolism. Elevated light intensities made possible with LEDs increased photosynthetic activity, the number of tillers, biomass and yield. At lower light intensities, blue, green and far-red light operated antagonistically during the stem elongation period. High photosynthetic activity was achieved when at least 50% of red light was applied during cultivation. A high proportion of blue light prolonged the juvenile phase, while the shortest flowering time was achieved when the blue to red ratio was around one. Blue and far-red light affected the glutathione- and proline-dependent redox environment in leaves. The influence of light spectrum on the amount and redox state of glutathione derived from its altered synthesis as shown by the changes in the level of its precursors, cysteine and γ-glutamylcysteine. Besides proline, the amount of most amino acids was affected by spectrum as shown by their smaller levels in red and pink lights compared to the other spectral conditions. These results clearly show that comparison of the physiological and biochemical effects of various light intensities and spectral compositions make it possible to optimize the growth and metabolism of wheat.

The modification of spectrum (supplemental far-red and blue lights) also affected the diurnal oscillations in the size and redox state of glutathione pool and expression of the genes related to the redox-dependent metabolic processes as observed in leaves of barley seedlings. Supplemental far-red light induced the activation of several genes associated with the circadian clock, redox control, nitrate and sulfate reduction and various antioxidants, while supplemental blue light inhibited them during two days of light/dark cycles. However, in the subsequent 2-day constant light, it was observed that several genes were induced more remarkably by blue light than by white and far-red lights. Based on these results, we propose a coordinating role for far-red and blue lights in the transcriptional adjustment of the redox-dependent metabolic pathways to spatial and temporal changes in the light spectrum occurring at various latitudes and altitudes and during the day, season and year.

The light condition-dependent changes of glutathione content were also compared in leaf extracts and subcellular compartments of the monocotyledonous crop species, wheat (Chinese Spring variety, **Fig. 1**) and the dicotyledonous model plant, *Arabidopsis* [wild-type plants, ascorbate- and glutathione-deficient mutants]. The subcellular studies of the samples embedded in Martonvásár were done in Graz where our colleague, Ákos Boldizsár took also part in the work carried out in the laboratory of Maria Müller (Austrian project leader).

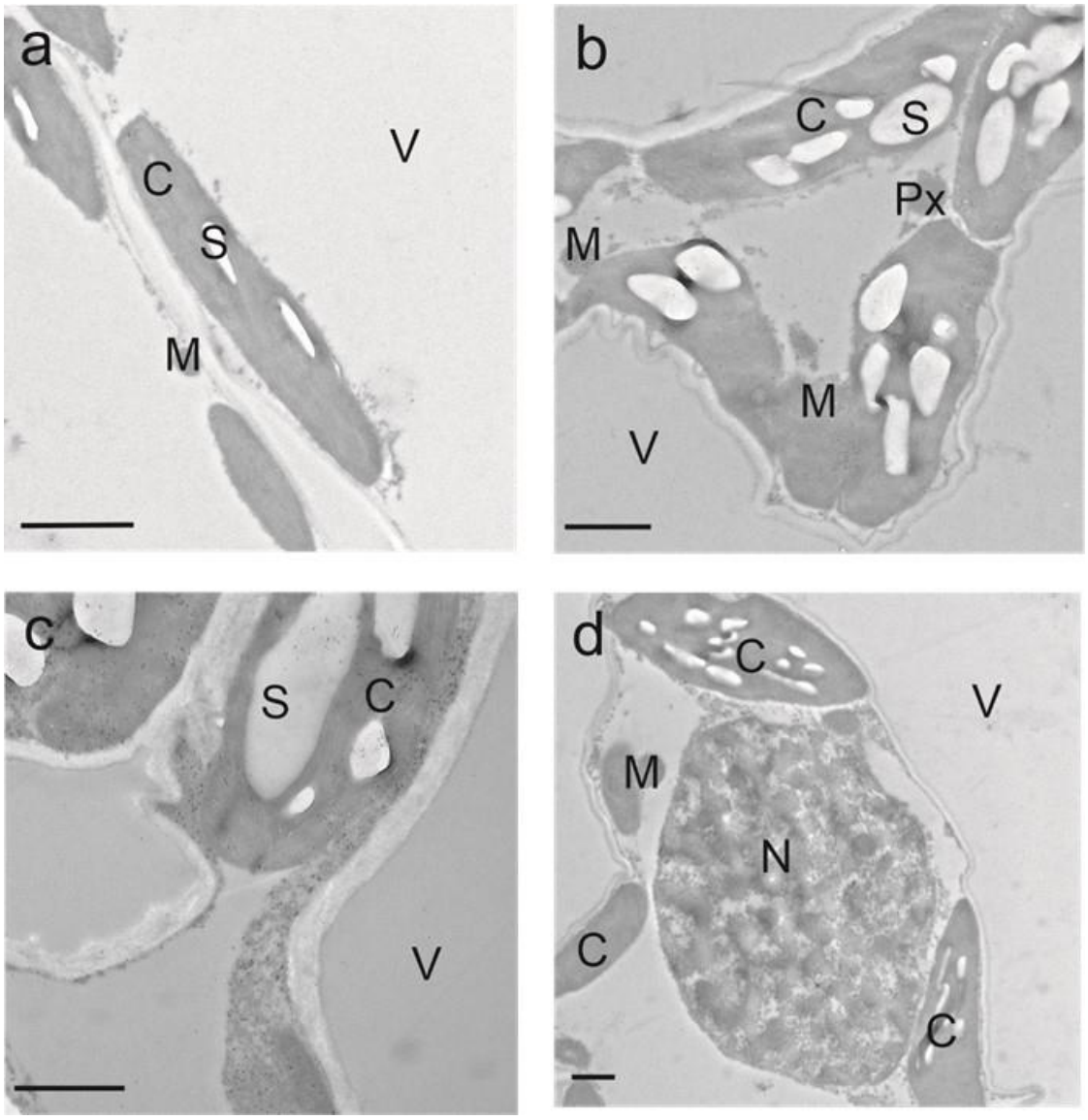

Figure 1. Subcellular distribution of glutathione in wheat grown under various light conditions. Representative transmission electron microscope images show compartment-specific glutathione distribution (dark dots) in parts of mesophyll cells from plants exposed to (a) low light, (b) normal light, (c) high light and (d) far-red light. C = chloroplasts with starch (S), M = mitochondria, N = nuclei, Px = peroxisomes, V = vacuoles. Bars =1µm.

The amount of its reduced (GSH) and oxidized (GSSG) form and their ratio increased with increasing light intensity in leaf extracts of both species including all genotypes, while far-red light induced the amount and ratio of GSH and GSSG only in wheat. Based on the expression changes of the glutathione metabolism-related genes, light intensity influenced the size and redox state of glutathione pool at transcriptional level in wheat, but not in *Arabidopsis*. In accordance with the results in leaf extracts, a similar inducing effect of both light intensity and far-red light was shown on total glutathione content at subcellular level in wheat. In contrast to the leaf extracts, the inducing influence of light intensity on glutathione level was only shown in the cell compartments of the GSH-deficient Arabidopsis line, and far-red light increased it in both mutants. The observed general and genotype-specific, light-dependent changes in the

accumulation and subcellular distribution of glutathione participate in the redox-dependent adjustment of metabolism to the actual environmental conditions.

Modification of the redox environment for the selection of redox-dependent genes and testing of their involvement in the response to changing light conditions

Changes in light conditions affected the redox environment and the redox-dependent biochemical processes as summarised in the previous section. The redox environment was modified by various chemicals in order to select redox-dependent genes. The various oxidants, antioxidants and osmotica (inducing oxidative stress) proved to be appropriate for the modification of the amount of GSH and GSSG under normal light conditions (250 umol/m^2/s) in wheat (**Fig. 2**). While 1-week treatment with 2 mM GSH reduced the levels of endogenous GSH, 1 mM GSH, 2 mM ascorbate, 2 mM H_2O_2, 100 mM NaCl and 15% PEG increased the amount of GSH and decreased that of GSSG.

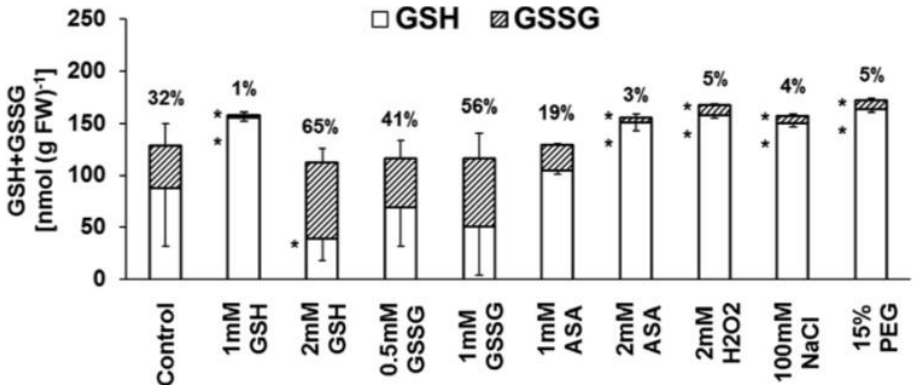

Figure 2. Effect of various oxidants, antioxidants and osmotica on the amount of reduced (GSH) and oxidised glutathione (GSSG) in leaves of wheat seedlings. ASA: ascorbate, PEG: polyethylene-glycole. Values indicated by asterisks are significantly different from the control at p<5% level.

The effect of these chemicals on the components of the ascorbate-glutathione cycle was also checked in high and far-red lights. Two-fold increase in the light intensity enhanced the influence of the various redox compounds on glutathione compared to their application in normal light as shown by the greater increase of the amount of both GSH and GSSG in high light. A similar influence of the light intensity was observed on the activity of glutathione reductase and ascorbate peroxidase which was the most pronounced in the case of GSSG and H_2O_2 treatments. In contrast, increase in the ratio of the far-red light did not change or only slightly modified the effect of the various used chemicals on the components of the ascorbate-glutathione cycle.

For the study of the redox-dependent genes, mRNAs and miRNAs were identified in wheat treated with 10 mM H_2O_2 for one day (*Cao et al., 2019, J. Exp. Bot., 70: 85–99; collaboration with the laboratory of Y.Y. Yao, Beijing, China*). This treatment modified the redox environment in the leaf tissues as shown by the decreased GSH content, increased half-cell reduction potential of the GSSG/GSH redox pair, and greater ascorbate peroxidase activity compared to the control plants. These changes were accompanied by alterations in the miRNA

transcript profile, with 70 miRNAs being identified with at least 1.5-fold difference in their expression between control and treated (0, 3, 6 h) seedlings. Degradome sequencing identified 86 target genes of these miRNAs, and 6722 possible additional target genes were identified using bioinformatics tools. The H_2O_2-responsiveness of 1647 target genes over 24 h of treatment was also confirmed by transcriptome analysis, and they were mainly found to be related to the control of redox processes, transcription, and protein phosphorylation and degradation. In a time-course experiment (0–24 h of treatment) a correlation was found between the levels of glutathione, other antioxidants, and the transcript levels of the H_2O_2-responsive miRNAs and their target mRNAs. This relationship together with bioinformatics modelling of the regulatory network indicated glutathione-related redox control of the selected miRNAs and their targets.

Another possibility for the selection of redox-responsive genes was the comparison of the miRNA and degradome (degraded target mRNAs of miRNAs) profiles using RNAseq in wild type and mutant Arabidopsis plants with altered antioxidant levels (*collaboration with Tamás Dalmay, Norwich, UK*). Eight redox-responsive miRNAs were selected and the effect of spectrum on the expression of sulfate starvation-inducible miR395 was investigated (**Fig. 3**).

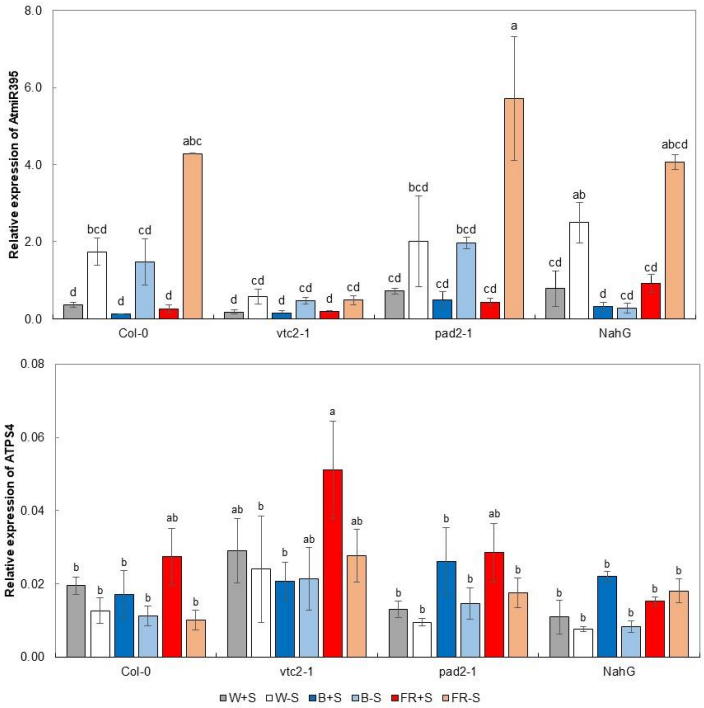

Figure 3. Comparison of the expression of miR395 and its target, ATP-sulfurylase 4 (ATPS4) in Arabidopsis genotypes with altered antioxidant levels under three spectral conditions. The plants were cultivated under optimal sulfate supply (+S) or under sulfate starvation (-S). Col-0: wild type plant, vtc2-1: ascorbate-deficient mutant, pad2-1: glutathione-deficient mutant, NahG: overexpressor of the gene encoding an enzyme of salicylate degradation (decreased salicylate content, release of catalase inhibition by salicylate). Walues indicated by different letters are significantly different at p<5% level. W: white, B: blue, FR: far-red light.

As expected, sulfate starvation increased the expression of miR395, however a corresponding reduction in the level of the target ATP-sulfurylase 4 mRNA was only observed under some light conditions in some genotypes. The expression of miR395 was lower in *vtc2-1* compared to the other genotypes and sulphur starvation in far-red light had a greater activating effect on its transcription than white or blue lights except for *vtc2-1*.

The effect of high and far-red lights on 5 redox-responsive miRNAs was tested in wheat. Among them the expression of miR167 was greatly increased by both the modification of the intensity and spectrum. High and far-red lights reduced the transcription of two target genes of miR167, which are encoding cystathionine gamma-synthetase 1 (catalyzes the formation of cystathionine from cysteine and an activated derivative of homoserine) and 4-aminobutyrate transaminase (catalyses the reaction of 4-aminobutanoate and 2-oxoglutarate forming succinate semialdehyde and L-glutamate). Since cysteine and glutamate are precursors of GSH, these enzymes are associated with the GSH metabolism which was also affected by the light conditions in this experiment.

Effect of light conditions on the response to abiotic stresses

We tested whether modification of the light conditions influences the response to abiotic stresses. Both high and far-red lights increased the cold-induced accumulation of GSH by 40-60% in all investigated four wheat genotypes independently of the level of their freezing tolerance. In contrast, they did not affect usually the cold-induced increase in the expression of genes encoding antioxidant enzymes **(Fig. 4**). The ascorbate peroxidase and glutathione reductase genes were only influenced in Cheyenne (freezing-tolerant) variety by far-red light compared to normal light during cold. A similar effect of high light on glutathione reductase was observed in Cappelle Desperz (freezing-sensitive) and Cheyenne varieties. Thus, alterations in the light conditions did not affect all studied components of the ascorbate-glutathione cycle during cold treatment and the detected changes were not associated with the level of freezing tolerance.

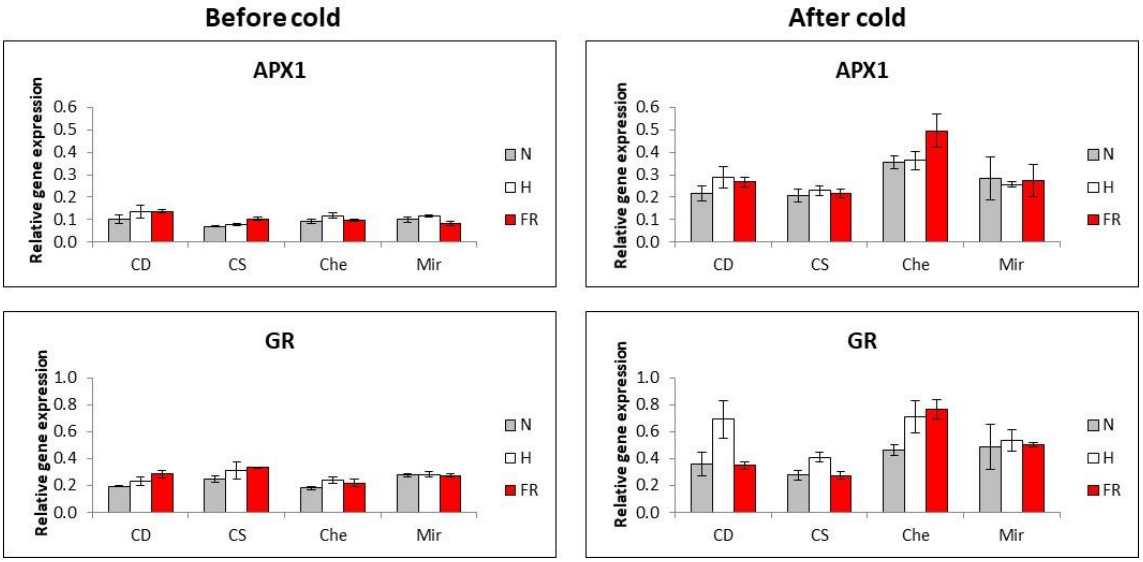

Figure 4. Effect of light spectrum on the cold-induced activation of genes encoding ascorbate peroxidase 1 (APX1) and glutathione reductase (GR) in wheat leaves. Cappelle Desprez (CD) and Chinese Spring (CS) are freezing-sensitive genotypes and Cheyenne (Che) and Miranoskaya (Mir) are freezing-tolerant ones. N: normal, H: high, FR: far-red light.

The effect of light-intensity (low, normal and high) on the response to drought was also investigated in wheat (*Gyugos et al., J Agro Crop Sci. 2019;205:562–570*). The drought-tolerant genotype (Plainsman) recovered better than the sensitive one (Cappelle Desprez) after the stress as shown by growth and photosynthetic parameters, the levels of which were lower and greater in low and high lights, respectively. Glutathione as an antioxidant contributed to this difference, since its level was twofold greater in Plainsman throughout the experiment. In addition, the accumulation of most amino acids even increased in normal light during drought in Plainsman, while such change occurred in Cappelle Desprez only in high light. The higher contents of proline, glutamate and γ-aminobutyrate are especially important because of their involvement in the protection against drought. The transcription of certain genes related to amino acid and glutathione metabolism and various antioxidants was even induced by higher light intensities before drought, which can contribute to the subsequent increase in the amount of the corresponding metabolites during stress. Increase in light intensity activated various protective mechanisms including greater accumulation of glutathione, proline and other amino acids during drought, which contributed to the efficient recovery of wheat after stress.

The effect of light spectrum on the response to drought was also compared in Plainsman and Cappelle Desprez wheat varieties cultivated under high ratio of either blue or far-red spectral components using the pink light regimen with their low ratios as a reference (*Gyugos et al., Acta Physiol. Plant., submitted*). Drought greatly influenced the growth, antioxidant and free amino acid levels in both genotypes. However, the light spectrum did not affect the relative fresh weight data after drought and recovery, it modified only the level of the studied photosynthetic and biochemical parameters. The stronger harmful effect of drought in far-red light on Cappelle Desprez genotype compared to the other light conditions is indicated by the lower electron transport rate compared to the other two light regimens. The amounts of the antioxidant glutathione and of several free amino acids also were the lowest in Cappelle Desprez in far-red light. At the transcript level, the pronounced negative effect of far-red light was shown only for the gene encoding nitrate reductase but not for the genes of the various antioxidant enzymes during recovery. Such negative effect of far-red light on the investigated parameters was not observed in Plainsman which difference can contribute to its better tolerance to drought also under special spectral conditions during dusk and greater latitudes and altitudes.

The effect of light conditions on the response to osmotic stress, which is an important consequence of drought was also investigated in the Cappelle Desprez and Plainsman varieties. The osmotic stress was induced by a 3-day treatment with 18% polyethylene-glycol. It was a moderate stress as indicated by the unchanged electrolyte leakage values (no membrane damage), and this parameter was also not influenced by high or far-red lights. The expression of the redox system-related genes was not or only slightly affected by osmotic stress in normal light in both genotypes. Far-red light reduced their transcription during the stress in Plainsman. Most of the studied polyamines was not influenced by the osmotic stress and the light conditions. The amount of spermidine decreased in general in the two genotypes during the stress (**Fig. 5**). High light increased the spermidine content except for Plainsman under control conditions while far-red light did not influence it. Since the spermidine levels and their drought-induced changes were similar in the two genotypes with different drought tolerance, this polyamine is not associated with the level of stress tolerance in the present experimental system.

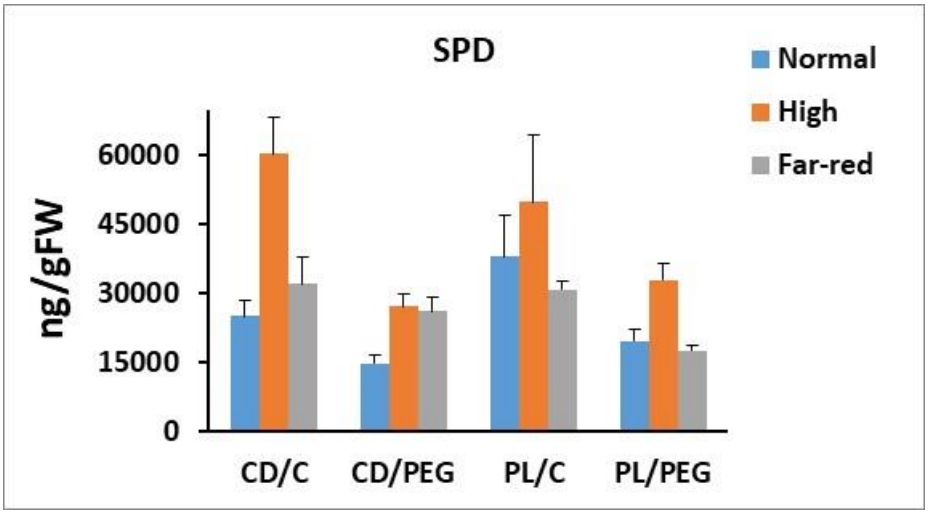

Figure 5. Effect of light conditions on the spermidine (SPD) accumulation under control (C) conditions and during osmotic stress (PEG, polyethylene--glycol) in the drought-sensitive Cappelle Desprez (CD) and the drought-tolerant Plainsman (P) varieties.

Conclusions

Changes in the light intensity and spectrum affected the glutathione-dependent redox environment in leaf tissues of wheat which resulted in the light condition-dependent, redox-mediated adjustment of metabolism (antioxidants, free amino acids, polyamines) and consequently growth and development. The involvement of the redox system in this process was also shown by its modification (redox treatment of wheat, Arabidopsis mutants). Changes in light intensities and spectrum can be involved in the more efficient adaptation to low temperature and drought through their effect on the redox environment.