



# Light spectrum modifies the drought-induced changes of glutathione and free amino acid levels in wheat

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## Abstract

The proposed spectral control of the adaptive metabolic responses to drought was tested by the comparison of two wheat genotypes with different stress tolerance. White light was used as the reference to see the effects of the three altered ratios of blue, red, and far-red spectral components, which conditions are referred to as blue, pink, and far-red lights. Under all spectral conditions, drought greatly reduced the growth of both genotypes. However, the glutathione content decreased and the proline level increased, independently of the spectrum, only in the sensitive genotype. Far-red light greatly decreased the amount of cystine and glutathione disulphide during the stress which resulted in their lower ratios compared to the reduced forms only in the tolerant genotype. The maintained more reducing redox environment contributes to its better stress tolerance. In far-red light, drought induced a greater accumulation of several free amino acids (mHis, Val, Ile, Leu, Asn, His, Tyr, Lys, Arg) in the tolerant genotype, while a smaller one in the sensitive genotype compared to the other spectral conditions. The transcript level of the genes related to amino acid and glutathione metabolism was also different between the two genotypes under this condition. The present results indicate the adaptive adjustment of glutathione and amino acid levels by far-red light during drought which observation can serve as a basis for the spectrum-dependent modification of the protective metabolites (glutathione, proline) of crops to reduce the stress-induced damages.

**Keywords** Drought · Free amino acids · Light spectrum · Glutathione · Wheat

## Abbreviations

APX	Ascorbate peroxidase	CySS/Cys	Cystine/cysteine ratio
AspTA	Aspartate transaminase	CysGly	Cysteinylglycine
B	Blue light	CySSGly	Cystinylglycine
CD	Cappelle Desprez	CySSGly/CysGly	Cystinylglycine/cysteinylglycine ratio
Cys	Cysteine	ETR	Electron transport rate
CySS	Cystine	FR	Far-red light
		GR	Glutathione reductase
		GS2	Glutamine synthase2
		GSH	Glutathione
		GSMC	Gravimetric soil moisture content
		GSSG	Glutathione disulphide
		GSSG/GSH	Glutathione disulphide/glutathione ratio.
		GST	Glutathione S-transferase
		hmGSH	Hydroxymethylglutathione
		hmGSSG	Hydroxymethylglutathione disulphide
		hmGSSG/hmGSH	Hydroxymethylglutathione disulphide/hydroxymethylglutathione ratio
		NR	Nitrate reductase
		P	Plainsman

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P	Pink light
PAR	Photosynthetically active radiation
P5CR	Pyrroline-5-carboxylate reductase
R/FR	Red/far-red ratio
ROS	Reactive oxygen species
W	White light
Y(II)	Effective quantum yield
$\gamma$ ECS	$\gamma$ -Glutamylcysteine synthetase
$\gamma$ EC	$\gamma$ -Glutamylcysteine
$\gamma$ ESSE	$\gamma$ -Glutamylcystine
$\gamma$ ESSE/ $\gamma$ EC	$\gamma$ -Glutamylcystine/ $\gamma$ -glutamylcysteine ratio

## Introduction

In nature, light spectrum is subjected to temporal and spatial changes as indicated by the greater ratio of blue light with increasing latitude and altitude and the lower red/far-red (R/FR) ratio at higher latitude and during sunset (Kotilainen et al. 2020). The modifications of the spectrum affect directly the photosynthesis which was reduced by the low R/FR ratio in *Oenothera biennis* (Qaderi et al. 2015) and was increased by greater percentage of blue light in cucumber (Hogewoning et al. 2010). These alterations in photosynthesis influence the formation of reactive oxygen species (ROS) during the photosynthetic electron transport and consequently the antioxidant activity (Kocsy et al. 2013, Conside and Foyer 2014, Roach et al. 2018). Accordingly, the greater ratio of blue light at higher altitudes resulted in a larger ascorbate content (Wildi and Lutz 1996) and activated the antioxidants under controlled conditions in *Rehmannia glutinosa* (Manivannan et al. 2015). A fivefold decrease in the R/FR ratio greatly reduced the levels of antioxidants in the shade leaves of bean (Bartoli et al. 2009). The spectrum-dependent changes in the amount of antioxidants modify the cellular redox environment which also affects the metabolism of amino acids (Geigenberger and Fernie 2014). Thus, blue light positively influenced the free amino acid levels in young barley plants (Koga et al. 2013), while they were not affected by the modification of the R/FR ratio in the internodes of sunflower (Mazzella et al. 2008). However, in flag leaves of wheat, decrease in R/FR and increase in red/blue ratios enhanced the amount of several free amino acids (Monostori et al. 2018). These biochemical changes affect the growth and development of plants (Lakshmanan et al. 2015) as shown by the negative effect of blue light on growth in Scots pine (Taulavuori et al. 2005) and by the positive influence of low R/FR ratio on the elongation process in *Chrysanthemum morifolium* and *Phaseolus vulgaris* (Lund et al. 2007; Bartoli et al. 2009). Both antioxidants and certain free amino acids have important role in the stress

response; therefore, the light spectrum-dependent control of their levels is probably involved in the reduction of drought-induced damages.

The drought periods have negative influence on physiological and biochemical processes and yield of wheat (Sallam et al. 2019), and therefore, the mitigation of yield loss is very important for the food safety based on the effect of ‘Millennium Drought’ (2002–2009) in Australia (Van Dijk et al. 2013). This loss is a result of the decline in leaf water relations and many related biochemical processes leading to greater formation of ROS, lipid peroxidation, and membrane injury as observed in wheat leaves after withholding of water (Abid et al. 2018). The negative processes were smaller and the induction of protective mechanisms was greater in the tolerant wheat genotype compared to the sensitive one based on the greater level of antioxidants, soluble sugars, and free amino acids in the former one. Similarly, the increase in proline and carbohydrate contents during drought-induced osmotic stress was also associated with the level of drought tolerance by comparison of other two wheat genotypes (Marcinińska et al. 2013). The very close relationship between the drought-induced changes in the free amino acid contents and the level of drought tolerance was shown by the comparison of eight wheat genotypes, since 98% of the genetic yield gap-based drought tolerance variance could be explained by the altered amounts of serine, asparagine, methionine, and lysine (Yadav et al. 2019). In addition, the tolerant wheat genotype could maintain a higher photosynthetic rate during drought and exhibited a faster recovery due to its more efficient protective mechanisms, which could contribute to its better productivity (Abid et al. 2018).

Growth of plants under modified spectral conditions can function as a hardening through physiological and biochemical adjustments resulting in improved tolerance during the subsequent stress period. The drought-induced water loss can be reduced by the changes in R/FR ratio, which has a regulatory effect on stomatal conductance and transpiration by the involvement of phytochrome B (for review, see: Demotes-Mainard et al. 2016). Its smaller expression delayed the drop of stomatal conductance in *Arabidopsis* during the decrease in the soil water availability which reduced drought tolerance (González et al. 2012). Additional far-red light resulted in greater stomatal conductance and in lower water use in Scots pine seedlings (Riikonen 2017). However, its negative effect on this parameter was shown in *Arabidopsis*, since the blue light-stimulated opening of stomata was reversed by far-red light (Talbot et al. 2003). During drought, the greater induction of the stomatal opening was observed in blue light than in red one in *Vicia faba* (Sun et al. 2014). A mixture of blue and red lights could improve drought tolerance of *Melissa officinalis* L. compared to monochromatic light as shown by the better growth and reduced lipid peroxidation due to the activation of various antioxidants (Ahmadi et al.

2020). A similar result was obtained in rocket, but not in lettuce (Ginzburg and Klein 2020). Besides antioxidants, proline as an osmoticum has an important role during osmotic stress induced by drought or high salt concentrations. During salt stress, its accumulation was induced by monochromatic red and blue lights, but not by the far-red one in *Arabidopsis* (Kovács et al. 2019). However, decrease in the red/far-red ratio of white light increased proline content and growth of salt-stressed tomato (Cao et al. 2018).

Besides the previously observed involvement of phenolic compounds, ascorbate peroxidase and proline in the light spectrum-controlled adaptation to drought, probably other antioxidants and amino acids, participate in this process. We assumed an important role of the altered ratios of blue, red, and far-red spectral components in this regulation based on our previous experiments in which these spectral modifications greatly affected the antioxidant and glutathione levels under optimal growth conditions (Monostori et al. 2018; Toldi et al. 2019). In addition, drought-induced changes in the level of these parameters were affected by light intensity in wheat (Gyugos et al. 2019). Thus, we also wanted to check whether a similar influence exists for the various spectral components. To test this hypothesis, the effect of three different spectral conditions (with modified ratios of blue, red and far-red components) on the amount and redox state of glutathione, the level of the individual amino acids, and the transcription of the genes related to their metabolism was compared in two wheat genotypes with different drought tolerance using white light as a reference. Growth and photosynthetic parameters were also studied to check the possible stress-induced damages.

## Materials and methods

### Plant material and growth conditions

The effect of light spectrum on the drought tolerance of wheat (*Triticum aestivum* L.) was compared in the drought-tolerant Plainsman and in the drought-sensitive Cappelle Desprez varieties (Varga et al. 2013) obtained from the Martonvásár Cereal Gene Bank. After germination between wet filter papers (1 days 25 °C, 3 days 4 °C, 3 days 25 °C), 12 seeds were sown in 11-cm-diameter pots containing a 2:1:1 (v/v/v) mixture of garden soil, humus, and sand. The pots were mixed each day to avoid the edge effect of the growth conditions in the chamber. The seedlings were cultivated at 250  $\mu\text{mol}/\text{m}^2/\text{s}$  (Kocsy et al. 2000) LED light for 2 weeks, with a photoperiod of 16 h, at 20/17 °C and 75% RH in growth chambers (Convion PGR15; Controlled Env. Ltd., Winnipeg, Canada). Four different light spectra were used: white light regimen as a reference and three specific spectral conditions with modified ratios of the blue, red, and

far-red spectral components as summarized in Table 1 and referred to as blue, pink, and far-red lights thereafter (Monostori et al. 2018). In the case of all four spectral conditions, four LEDs of the light modules (covering the whole ceiling of the chamber to ensure homogenous illumination) were used including a continuous wide-spectrum LED (Philips Lumileds, LXZ2-5790-y, San Jose, CA, USA) and three narrow spectrum LED armatures with the dominant wavelengths of 448 nm (Philips Lumileds, LXZ1-PR01, San Jose, CA, USA), 655 nm (Philips Lumileds, LXZ1-PA01, San Jose, CA, USA) and 750 nm (Edison Edixeon, 2ER101FX00000001, New Taipei, Taiwan). The whole spectrum of these four LEDs is shown in Fig. S1.

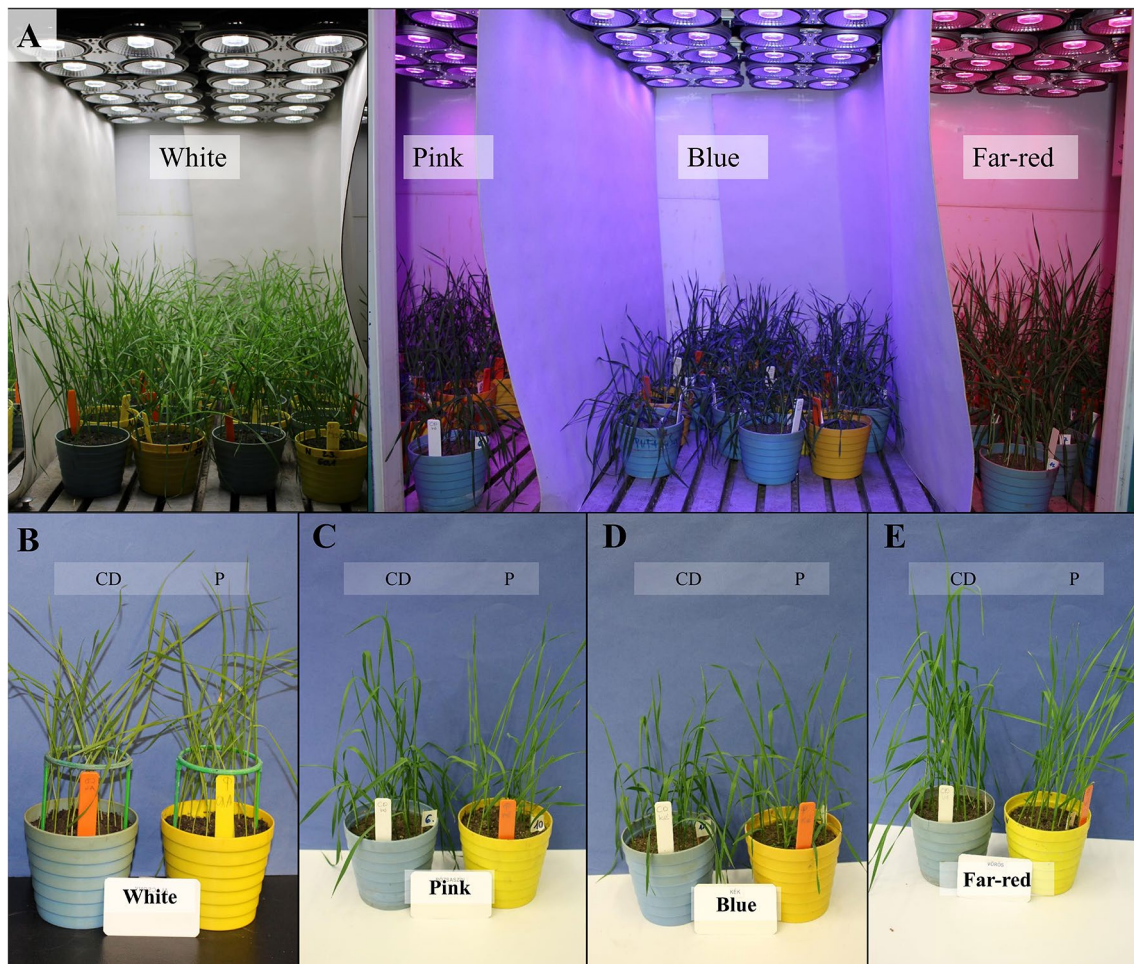
The gravimetric soil moisture content (GSMC) was adjusted to 24% by irrigation. Determination of the GSMC was achieved by measurement (Moisture Meter HH2, Delta-T Devices Ltd, Cambridge, UK) and also by daily weighing of the pots. From the weight data, the GSMC was calculated by the following equation:  $[(W_w - W_d)/W_d] \times 100$ , where  $W_w$  is the wet weight of the soil and  $W_d$  is the dry (drying at 105 °C for 24 h) weight of the soil. The seedlings had three fully developed leaves before drought treatment. During drought stress, the water was withheld until GSMC decreased to 14% (6 days without irrigation) (Fig. 1). During recovery (6 days with irrigation), it was adjusted again to the initial value (24%) for a week. The fresh weight of the shoots of 12 plants was determined before and after the drought stress and at the end of the recovery period. The measurements and samplings (mixture of the youngest fully developed leaves from 12 plants in each sample) were done in three independent experiments, each with three parallels.

### Determination of photosynthetic activity, chlorophyll, and carotenoid contents

For the determination of the chlorophylls and carotenoids, the leaf samples (50 mg) were extracted with 1 mL of 80% acetone and the amount of the photosynthetic pigments was determined by a spectrophotometer based on the absorbance of the extracts at 470, 647, and 663 nm (Varian 100-Scan, Palo Alto, CA, USA) according to Lichtenthaler (1987). The photosynthetic electron transport rate (ETR) was analyzed by a PAM-2100 fluorometer (Heinz Walz GmbH, Effeltrich, Germany) on intact, attached leaves adapted to the actual light conditions. At least 15 leaves were measured

**Table 1** Detailed data about light intensity and spectral conditions

	White	Blue	Pink	Far-red
PAR ( $\mu\text{mol}/\text{m}^2/\text{s}$ )	250	250	245	250
Blue/red	1:2	5:1	1:1	1:5
Red/far-red	15:1	17:1	37:1	10:1



**Fig. 1** The plants grown under different light conditions at the end of the drought stress. **a** The plants in the growth chamber; **b–e** comparison of the drought-sensitive Cappelle Desprez (CD) and drought-

tolerant Plainsman (P) wheat varieties subjected to drought in white, pink, blue and far-red lights

before the drought stress, at the end of the drought (6 days after withholding water), and recovery (6 days after recovery) periods. ETR represents the relative apparent photosynthetic electron transport rate in  $\mu\text{mol electron m}^{-2} \text{s}^{-1}$ . It was calculated from the measured values of the effective quantum yield [Y(II)] parameter and photosynthetically active radiation (PAR) according to the equation of  $\text{ETR} = Y(\text{II}) \times 0.5 \times \text{PAR} \times A$ , where  $A$  is the absorptivity of leaves.

### Measurement of thiols by HPLC

Leaf sample (200 mg) was extracted in 1 ml 0.1 M HCl. After reduction, the thiols were derivatized with monobromobimane as described earlier (Gulyás et al. 2014). Cysteine (GSH precursor),  $\gamma$ -glutamylcysteine ( $\gamma\text{EC}$ , intermediate product of GSH synthesis),

hydroxymethylglutathione (hmGSH, a homologue of GSH in *Gramineae*), GSH, and cysteinylglycine (CysGly, degradation product of GSH) were separated by reverse-phase HPLC (Waters, Milford, MA, USA) and determined by a W2474 scanning fluorescence detector (Waters, Milford, MA, USA). A mixture of the Cys,  $\gamma\text{EC}$ , GSH, and CysGly in a dilution series (0, 1, 2, 3, 4, 5 mM) was used for their qualitative and quantitative determination in the present experiment (Fig. S2). hmGSH is not available commercially, and therefore, we let it synthesize earlier (Kocsy et al. 2000) and observed its appearance between the peaks of  $\gamma\text{EC}$  and GSH. The areas of the hmGSH and GSH were equal at the same concentration, so the GSH standard is appropriate for the quantification of hmGSH; therefore, we did not include hmGSH standard into later experiments because of its instability (degradation after 6 months) and lack of commercial availability.

## Analysis of free amino acid content

Leaf samples of 300 mg were extracted with 2 mL of cold, 10% trichloroacetic acid, and free amino acid level was determined according to (Gulyás et al. 2017) using an automatic amino acid analyser (Ingos Ltd., Praha, Czech Republic) equipped with an Ionex Ostion LCP5020 cation-exchange column (22 cm × 0.37 cm).

## Study of gene expression

Total RNA was extracted from leaf samples using Direct-zol™ RNA Miniprep Kit (Zymo Research, Irvine, CA, USA) according to the instructions of the manufacturer. Reverse transcription and qPCR were carried out using the earlier described qPCR BIOSyGreen Blue Mix Separate-Roxas, using a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA) with primers as detailed in Table S1 to determine the expression of the genes related to the redox system and amino acid metabolism. Fold change ( $2^{\Delta\Delta Cq}$ , where  $\Delta Cq = Cq(\text{ref}) - Cq(\text{target})$ ) values of the expression were calculated (Livak and Schmittgen 2001) using the *Ta30797* gene as reference, the product of which is similar to the phosphogluconate dehydrogenase protein (Paolacci et al. 2009).

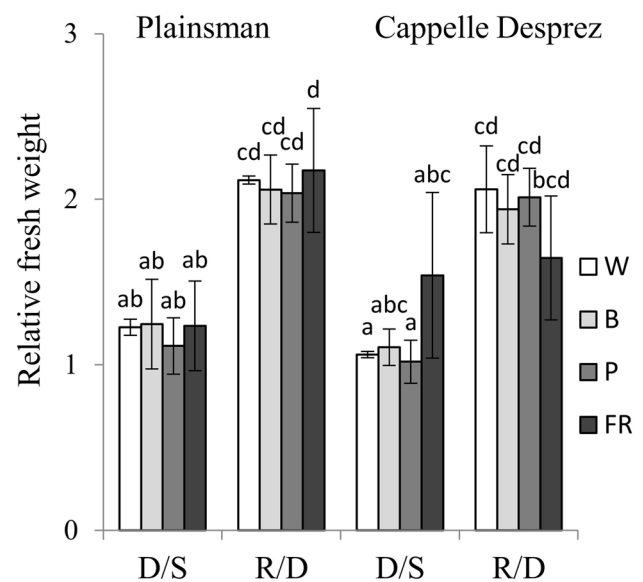
## Statistics

Significant differences between the control and the treated groups were determined by ANOVA using the SPSS program. Normality of the data distribution was checked by Kolmogorov–Smirnov test ( $p > 0.05$ ). Homogeneity of variance was checked by Levene test. If the variances were equal ( $p > 0.05$ ), Tukey's *b* test was used. If they were not equal ( $p < 0.05$ ), Dunnett's T3 post hoc test was applied.

## Results

### Effect of light spectrum on drought-induced changes in growth and photosynthetic parameters

The seedlings of both genotypes did not grow during drought except for Cappelle Desprez in far-red light, while their fresh weight significantly increased during the recovery (Table S2). The modification of spectral composition did not effect this parameter compared to white light in Plainsman, while it was greater in far-red light both during drought and recovery and smaller in blue light in Cappelle Desprez. Regarding the relative fresh weight data, they were significantly greater in both genotypes at the end of the recovery compared to drought except for Cappelle Desprez in blue and far-red lights (Fig. 2).



**Fig. 2** Effect of light spectrum and drought on relative fresh weight of the shoots of the drought-tolerant Plainsman and the drought-sensitive Cappelle Desprez wheat varieties. The values are calculated as the ratio of the fresh weights measured before and after the drought and recovery, respectively (absolute values are given in Table S2). After 17 days of growth, the water was withheld for 6 days resulting in a decrease of soil moisture from 24 to 14%. The drought was followed by 6 days recovery with normal irrigation (24% soil moisture content). Three independent experiments, each with 12 parallels, were done. Significant differences between the control and the treated groups were determined by one-way ANOVA. *S* start of the drought, *D* end of the drought, *R* end of the recovery, *B* blue light, *P* pink light, *FR* far-red light. Information about light intensity and ratios of blue, red, and far-red spectral components is given in Table 1. Values indicated with different letters are significantly different from each other at  $p \leq 0.05$  levels

They were not influenced by the spectrum at the individual sampling points. The dry weight of the two species was not affected by drought, but it increased during the subsequent recovery period except for Cappelle Desprez in far-red light where the increase was detected during drought (Table S3). The spectrum did not influence the dry weight during the whole experiment in Plainsman; however, it was greater in far-red light after drought and lower in blue light after recovery in Cappelle Desprez compared to the other spectral conditions. The water content of the leaves was not influenced significantly by the applied moderate drought stress and the spectrum (Table S4). Comparing all data, among the investigated growth parameters, the relative fresh weight was significantly affected by treatment and the genotype × drought × spectrum interaction (Table S5). The fresh and dry weights and the water content were influenced by all three factors individually and the two former ones also by the genotype × drought, drought × spectrum and genotype × spectrum interactions.

The amount of chlorophylls and carotenoids was not affected significantly by drought nor by light spectrum compared to the starting and white light values, respectively (Fig. S3). The electron transport rate was significantly lower in far-red light compared to white light at all sampling points in both genotypes (Fig. 3). In addition, it was significantly lower in Cappelle Desprez than in Plainsman after drought in far-red light. Based on the comparison of all data, treatment and spectrum affected ETR values and pigment contents (Table S5). In addition, an effect of genotype on chlorophyll levels, and that of treatment  $\times$  spectrum interaction on ETR was shown.

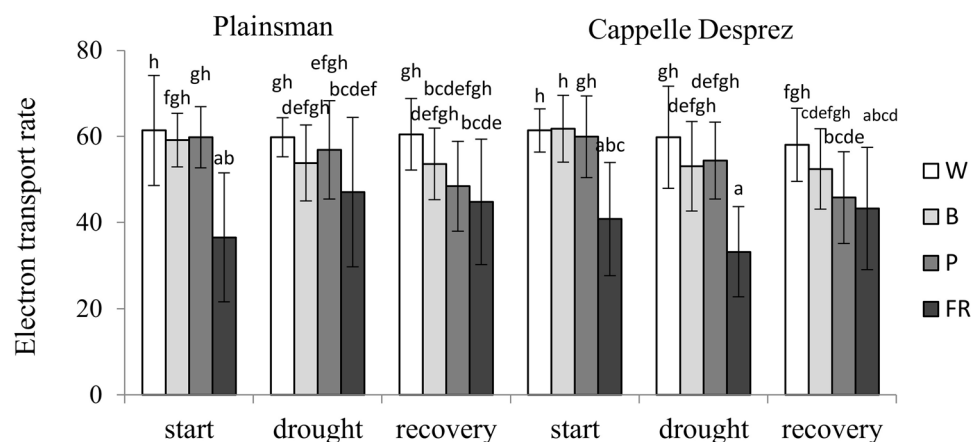
### Differences in glutathione metabolism during drought under various spectral conditions

The concentration of the cysteine (precursor of glutathione, reduced form) was influenced by far-red light during the recovery; namely, it was twofold greater compared to white light in Plainsman (Fig. 4a). The cystine (CySS, disulphide formed from 2 cysteine molecules) content was significantly lower (0.25-fold) after drought and recovery compared to the starting value in far-red light in Plainsman and in blue light in Cappelle Desprez (0.4-fold) (Fig. 4b). Comparing the four light conditions, its amount was smaller in far-red light than in white one at these sampling points only in Plainsman. The CySS/Cys ratio was greatly reduced by drought in far-red light in Plainsman and in white and far-red lights in Cappelle Desprez (Fig. 4c). During the recovery this ratio was significantly lower nearly in all light conditions compared to the starting values in both genotypes except for white light in Plainsman and blue and far-red lights in Cappelle

Desprez. It was affected by the spectral composition during drought and recovery in Plainsman, namely it was lower in far-red light than in the other three ones.

The  $\gamma$ -glutamylcysteine ( $\gamma$ EC, intermediary product in GSH synthesis) content was not affected by drought and light spectrum in the two genotypes (Fig. S4A). The amount of  $\gamma$ -glutamylcysteine ( $\gamma$ ESSE, its oxidized form) was greatly increased by drought in far-red light in Plainsman and in all spectral conditions in Cappelle Desprez (Fig. S3B) which high levels were reached and kept, respectively, in all four lights except for pink light in Plainsman. The effect of spectrum on  $\gamma$ ESSE was observed only in Plainsman during drought, since its amount was threefold greater in far-red light compared to the other three ones. The  $\gamma$ ESSE/ $\gamma$ EC ratio was not significantly changed by drought or spectral changes in the two genotypes (Fig. S4C).

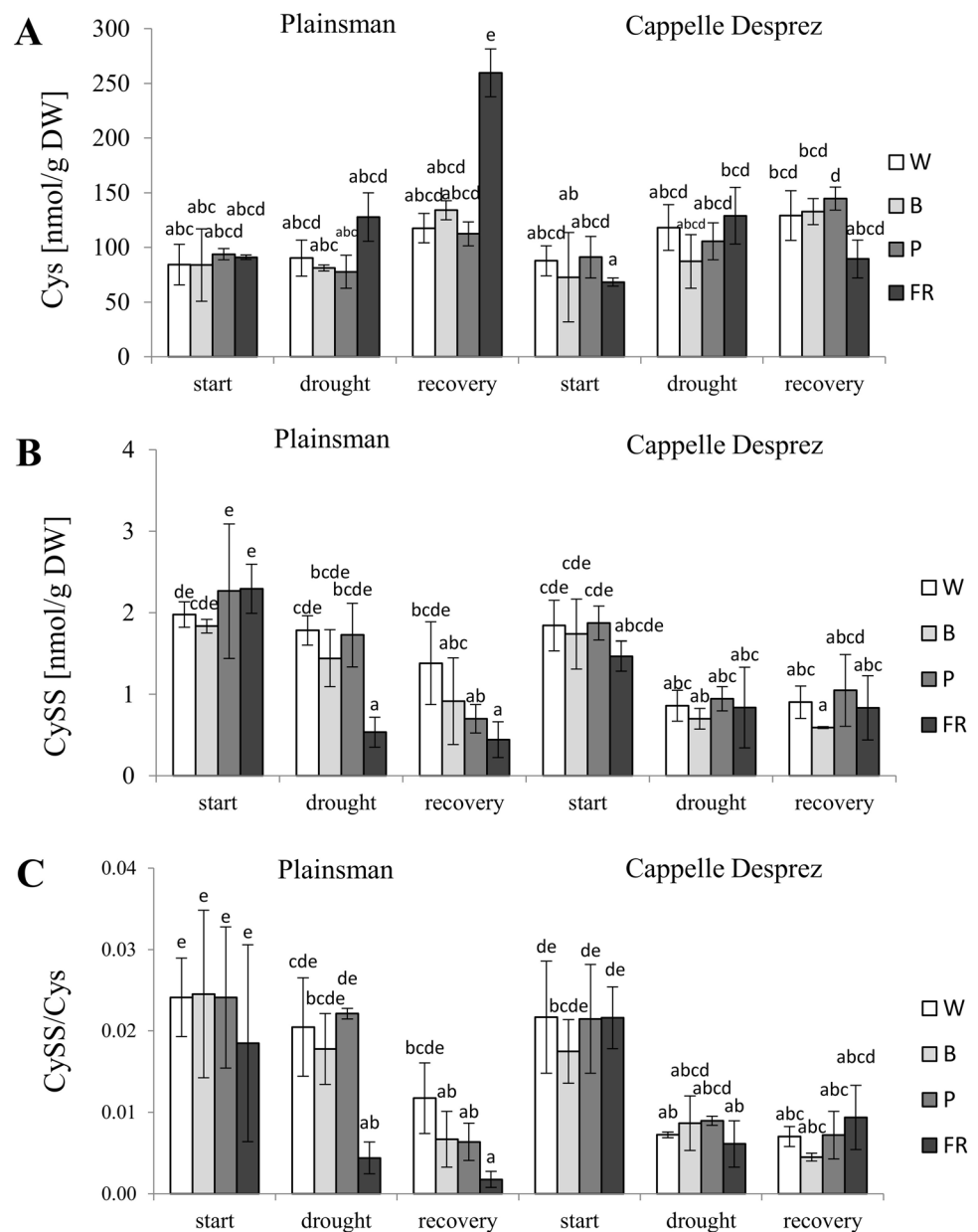
The amount of hydroxymethylglutathione (hmGSH, a homologue of GSH in which the Gly is replaced by a Ser) was significantly greater compared to the starting values in far-red light after drought and recovery in Plainsman and Cappelle Desprez, respectively (Fig. S5A). In these sampling points, its level was also significantly greater in far-red light than in white one. The hydroxymethylglutathione disulphide (hmGSSG) content was not influenced by drought or spectrum in Plainsman (Fig. S5B). However, it was greater in pink and far-red lights in Cappelle Desprez following drought, in which sampling point its level was higher under these light conditions compared to white light. The hmGSSG/hmGSH ratio greatly decreased during recovery in far-red light in Plainsman, whereas it large increase was detected during drought in pink light in Cappelle Desprez (Fig. S5C). It was significantly affected by the spectrum during drought and recovery in Cappelle Desprez as shown by



**Fig. 3** Effect of light spectrum and drought on the electron transport rate in the leaves of the drought-tolerant Plainsman and the drought-sensitive Cappelle Desprez wheat varieties. It was analyzed on intact, attached leaves adapted to the actual light conditions. At least fifteen leaves were measured before the drought stress, at the end of

the drought and recovery periods. The experimental system and the abbreviations are explained in the legends of Fig. 2. Values indicated with different letters are significantly different from each other at  $p \leq 0.05$  levels

**Fig. 4** Effect of light spectrum and drought on the amount and redox state of cysteine in the leaves of the drought-tolerant Plainsman and the drought-sensitive Cappelle Desprez wheat varieties. **a** Cysteine (Cys), **b** cystine (CySS), and **c** CySS/Cys ratio. Each sample contained the mixture of the youngest fully developed leaves from 12 plants. Three independent experiments, each with three parallels, were done. *W* white light, *B* blue light, *P* pink light, *FR* far-red light. The experimental system, samplings, and the other abbreviations are explained in the legends of Fig. 2. Values indicated with different letters are significantly different from each other at  $p \leq 0.05$  levels



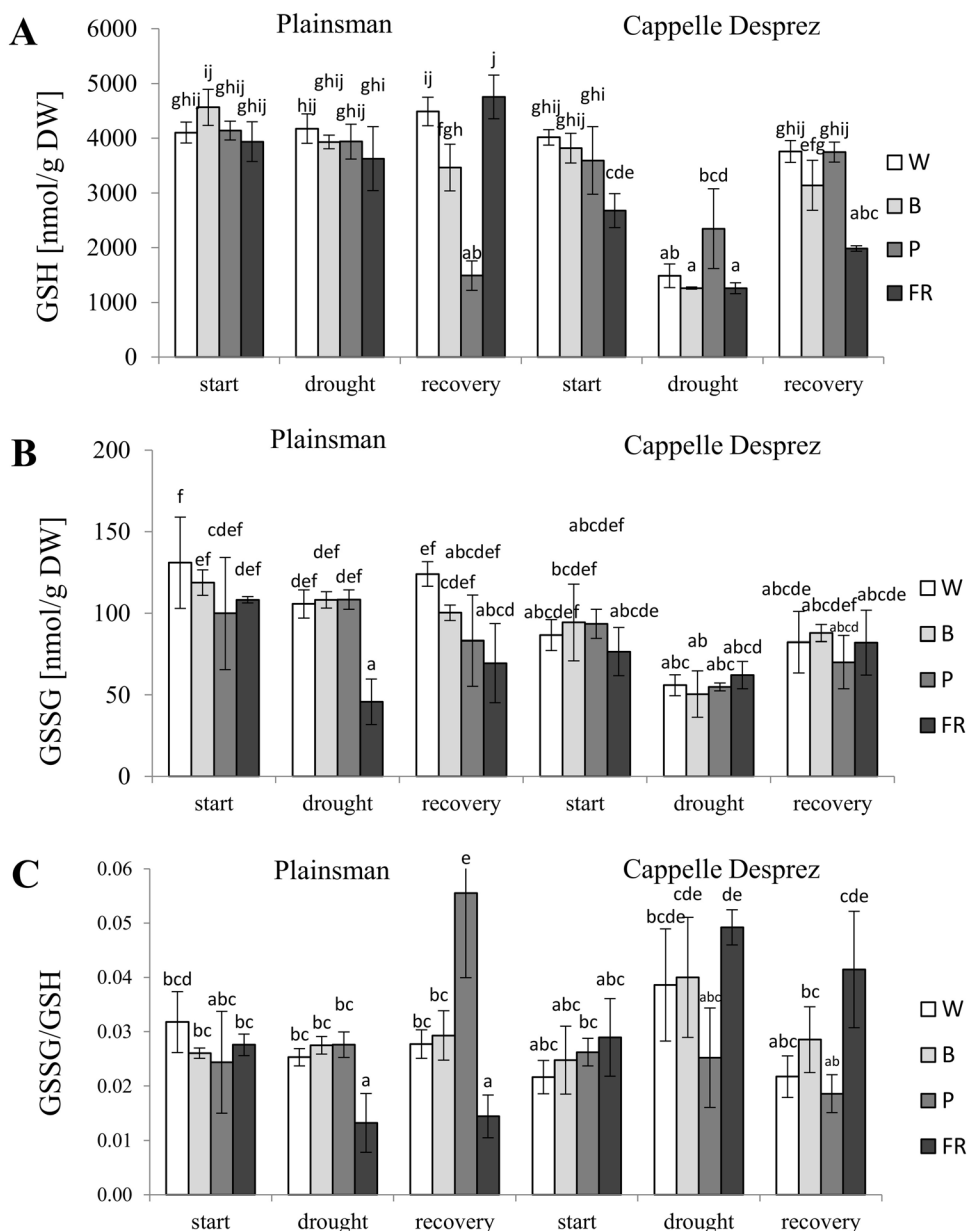
the threefold greater level in pink light compared to the other spectral conditions.

Drought did not affect the GSH levels in Plainsman but induced pronounced decrease in white (0.3-fold), blue (0.3-fold) and far-red (0.5-fold) lights in Cappelle Desprez (Fig. 5a). During recovery, the original GSH level was restored in blue light in Cappelle Desprez, while its concentration decreased 0.4-fold in pink light in Plainsman. Its level was significantly lower during recovery in pink and far-red light compared to the white one in Plainsman and Cappelle Desprez, respectively. The concentration of glutathione disulfide (GSSG) and the GSSG/GSH ratio decreased 0.5-fold in Plainsman, while they did not change significantly in Cappelle Desprez in far-red light during

drought (Fig. 5b, c). Both parameters were lower in far-red light than in white one during drought and recovery in Plainsman. They were not influenced by the modification of the spectrum compared to white light in Cappelle Desprez.

The GSH degradation, as indicated by cysteinylglycine (CysGly) and cystinylglycine (CySSGly, oxidized form of CysGly) contents, and the CySSGly/CysGly ratio were not affected significantly by drought or spectral composition except for the increase in CySSGly content during drought and recovery in white light, as well as in pink light during recovery in Cappelle Desprez (Fig. S6). The effect of spectrum on these parameters was not shown at any sampling point.

**Fig. 5** Effect of light spectrum and drought on the amount and redox state of glutathione in the leaves of the drought-tolerant Plainsman and the drought-sensitive Cappelle Desprez wheat varieties. *A* glutathione (GSH), *B* glutathione disulphide (GSSG), *C* GSSG/GSH ratio. Each sample contained the mixture of the youngest fully developed leaves from 12 plants. Three independent experiments, each with three parallels, were done. *W* white light, *B* blue light, *P* pink light, *FR* far-red light. The experimental system, samplings, and the other abbreviations are explained in the legends of Fig. 2. Values indicated with different letters are significantly different from each other at  $p \leq 0.05$  levels



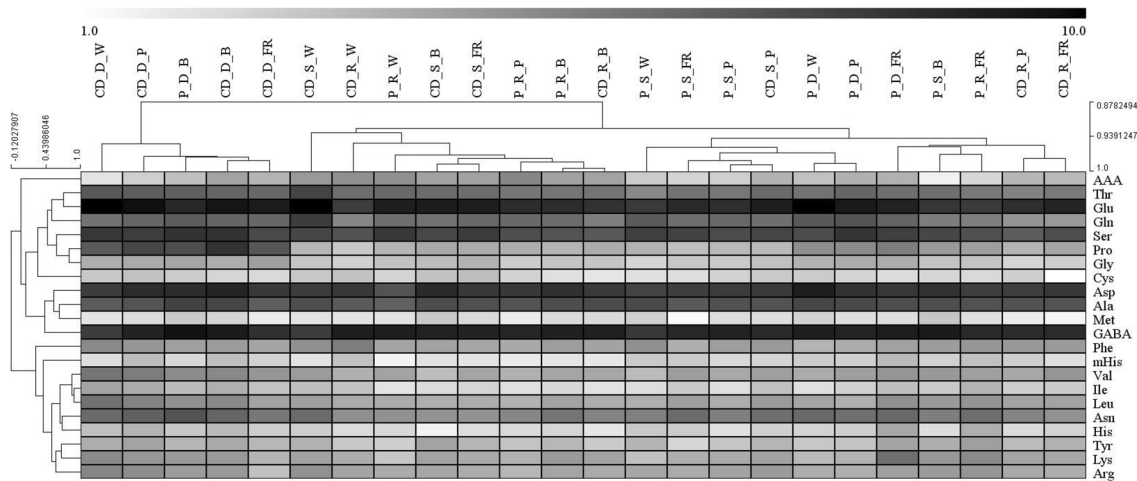
Comparing all thiol data by analysis of variance, the GSH and hmGSH contents were significantly affected by genotype, drought, spectrum, and all their possible interactions (Table S5). The Cys and  $\gamma$ ESSE levels and the GSSG/GSH ratio were influenced by all possible interactions of these three factors. CySS, GSSG, and hmGSSG were individually affected by them. The spectrum alone or in an interaction with the other two factors had an

influence on all thiols related parameters except for the CysGly content or the  $\gamma$ ESSE/ $\gamma$ EC ratio.

**Effect of light spectrum on free amino acid levels during drought**

The total free amino acid content was not affected by drought and light spectrum in the two examined wheat varieties (data not shown). The individual amino acids





**Fig. 6** Effect of light spectrum and drought on the free amino acid contents in the leaves of the drought-tolerant Plainsman and the drought-sensitive Cappelle Desprez wheat varieties. The grayscale indicates the  $\log_2$  concentration values (white: the lowest value,

black: the highest value). Three independent experiments, each with three parallels, were done. The experimental system, samplings, and the other abbreviations are explained in the legends of Fig. 2. *P* Plainsman, *CD* Cappelle Desprez

were grouped into two major clusters based on the similar pattern of their levels after the various treatments in the two genotypes (Fig. 6). In the first cluster, their levels were in general greater compared to the second one except for, alpha-aminoadipate, Gly, Cys, and Met. Interestingly, the Pro accumulation was greatly induced by drought in all light condition in Cappelle Desprez, and in blue light in Plainsman resulting in its very high levels compared to the other samples. Such large difference between the treatments was not observed for other amino acids. In the first large cluster belonged mainly the amino acids of the aspartate, glutamate and serine family except for Ala and alpha-aminoadipate. However, Lys, Ile, and Asn from aspartate family and Arg from glutamate family were grouped into the other large cluster containing amino acids present in lower level. In this latter cluster, a greater effect of drought was observed on mHis, Val, Ile, Leu, Asn, His, Tyr, Lys, and Arg concentrations under certain spectral conditions. Thus, their levels were increased by drought in Plainsman in far-red light but not in pink and blue ones. In contrast, greater concentrations of these amino acids were observed only in pink and blue light during the stress period in Cappelle Desprez. This difference was also maintained during the recovery in Plainsman but not in Cappelle Desprez.

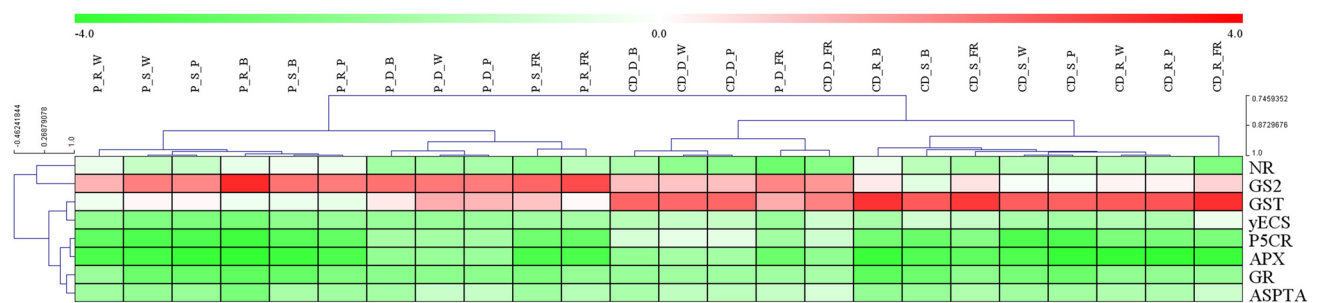
The hierarchical clustering revealed that among the three examined factors, drought had the greatest effect on the free amino acid levels, since one of the two main clusters contained only values observed after drought (Fig. 6). While data from all four light conditions in Cappelle Desprez were grouped here, in the case of Plainsman

only those one is in this group which was obtained in blue light. The effect of genotype and spectrum is weaker than that of drought, since only smaller subclusters were created based on them following the final branches.

### Changes in the expression of the genes related to thiol and amino acid metabolism

The two studied genes related to the incorporation of nitrogen into amino acids (encoding nitrate reductase and Glu synthase) formed a main cluster based on their expression changes under the various experimental conditions. While the expression of nitrate reductase gene in general decreased during drought, that of Glu synthase increased (Fig. 7). The other main cluster consisted of genes related to the glutathione metabolism, except for those ones associated with Pro and Asp metabolism. Their expression was increased by drought with the exception of glutathione S-transferase gene in Cappelle Desprez.

The hierarchical clustering demonstrated the greatest effect of genotype on the gene expression among the three studied factors, since the two genotypes were grouped separately in the two main clusters except for Plainsman after drought in far-red light. Drought was the second most effective factor based on the common subclusters of the related transcript levels. The spectrum had the smallest influence on the gene expression as indicated by the clustering.



**Fig. 7** Effect of light spectrum and drought on the relative expression of the genes related to the glutathione and amino acid metabolism in the leaves of the drought-tolerant Plainsman and the drought-sensitive Cappelle Desprez wheat varieties. Three independent experiments, each with 3 parallels, were done. The experimental system, samplings, and the other abbreviations are explained in the legends of Fig. 2. *P* Plainsman, *CD* Cappelle Desprez, *NR* nitrate reductase,

*GS2* glutamine synthase2, *GST* glutathione S-transferase,  $\gamma$ *ECS*  $\gamma$ -glutamylcysteine synthetase, *P5CR* pyrroline-5-carboxylate reductase, *APX* ascorbate peroxidase, *GR* glutathione reductase, *AspTA* aspartate transaminase. Log<sub>2</sub> expression values are shown on a color scale where green and red colors indicate a decrease and increase, respectively, and the intensity of colors shows the level of changes

## Discussion

### Growth and photosynthesis during drought under various spectral conditions

Based on the relative fresh weight data, both genotypes were released from the drought-induced inhibition of growth under all three spectral conditions. According to the analysis of the general effect of genotype, drought, and spectrum, the relative fresh weight (showing the change during cold and recovery) was not affected by the spectrum, only by the drought treatment. Thus, in general, we could observe probably the effect of compensatory growth which was independent of light conditions. However, pairwise comparison of fresh weight data indicated, that in far-red light, the recovery of plants was better than in white light. This better recovery can be explained the inducing effect of far-red light on stomatal closure reducing vaporization as observed in *Arabidopsis* (Talbot et al. 2003). The positive effect of far-red light on the response to drought was also shown by the greater dry weight of the sensitive wheat genotype following the treatment. In certain species, the spectral composition of the light affected the growth even under optimal conditions, since supplementation of the basic white illumination with blue and far-red light increased the growth of lettuce and tomato, respectively (Johkan et al. 2010; Zhang et al. 2019). Similarly to the drought-induced osmotic stress in the case of the sensitive wheat variety in the present experiments, low red/far-red ratio improved the growth of tomato under salt-induced osmotic stress (Cao et al. 2018). These observations indicate the control of growth by the spectral conditions both under optimal and stress conditions.

The electron transport rate was lower in far-red light compared to white, blue, and pink lights before drought in both genotypes and during the drought period in Cappelle

Desprez. In addition, such difference between white and far-red lights existed during the whole experiment in both genotypes. The reduction of electron transport rate decreases the formation of ROS in excess and subsequently the oxidative stress-induced damages. The regulatory effect of far-red light on the electron transport rate and the association between this control and stress response was also shown in the leaves of tomato (Cao et al. 2018). As observed for the supplementary blue light in the case of the electron transport rate in wheat subjected to drought, monochromatic blue light did not influence most of the photosynthetic parameters in rose plants compared to white light during a subsequent cultivation in high light inducing oxidative stress (Bayat et al. 2018). The lack of the photosynthetic adjustment decreased the ability to tolerate the stress.

### Light spectrum-dependent control of glutathione metabolism during drought

At seedling stage, the GSH level was affected by the spectrum even under optimal growth conditions, since it was lower before drought in far-red light than in white, blue, or pink lights in Cappelle Desprez. Far-red light also influenced the GSH content in bean (Bartoli et al. 2009). However, in leaves of Plainsman wheat variety at 3-leaf stage, as observed in flag leaves of the MvKikelet wheat variety, the amount of GSH was not significantly influenced by light regimens under optimal conditions (Monostori et al., 2018). The concentration of GSSG was in turn significantly lower in the far-red regimen compared to blue regimen in the flag leaves (Monostori et al., 2018). A similar difference was only observed after drought at seedling stage in Plainsman in the present experiments. Thus, the effect of light on glutathione depends both on the developmental stage and the environmental conditions.

The great difference in the effect of far-red light on glutathione metabolism compared to white, blue, and pink light during drought should be emphasized in the drought-tolerant wheat variety based on the results of the present study. Many-fold lower CySS and GSSG contents, as well as CySS/Cys and GSSG/GSH ratios, were detected during drought in far-red light compared to white, blue, and pink lights in Plainsman but not in Cappelle Desprez. These parameters indicate the maintenance of a more reducing redox environment in Plainsman which may contribute to its better drought tolerance due to the efficient removal of the excess of ROS (Considine and Foyer 2014a). Besides the reduction state of glutathione, its contribution to the better drought tolerance is also demonstrated by the twofold greater GSH content during drought in white, blue, and far-red light in Plainsman compared to Cappelle Desprez. However, during the rewatering, an increase of GSH levels in Cappelle Desprez was observed resulting in their similar amounts in the two genotypes except for far-red light. Interestingly, the difference between the two genotypes in GSH content was not observed in the amount of the precursors (Cys and  $\gamma$ EC) which can be explained by their immediate use for the GSH synthesis. It is also not a result of the differential degradation of GSH in the two genotypes as shown by the similar levels of CysGly under the various spectral conditions. The observed differences in the amount and redox state of GSH in the two genotypes under various spectral conditions did not derive from a transcriptional regulation based on the expression of the related genes, since corresponding differences in the related transcript levels were not detected. The effect of far-red light was also shown on hmGSH (a GSH homologue in *Gramineae*) which is less important in the maintenance of the cellular redox state because of its much lower concentration (15% that of the GSH). Its amount was far greater in far-red light than in blue and pink light after drought only in Cappelle Desprez and after recovery in both genotypes. hmGSH may have some special functions in the control of protein structure and activity through its interaction with the sulphhydryl groups because of its larger size compared to GSH (Gly is replaced by Ser in hmGSH).

Similarly to the spectrum-dependent differences in the amount of the non-enzymatic antioxidant, GSH under various spectral conditions in wheat seedlings, the activity of antioxidant enzymes was also affected by light spectrum indicating their coordinated regulation. Thus, the activities of superoxide dismutase and peroxidase, which are also involved in the control of  $H_2O_2$  levels as the GSH, decreased in red light and increased in blue and orange lights in ramie (Rehman et al. 2020). Blue light also increased the antioxidant capacity in *Rehmannia glutinosa* (Manivannan et al. 2015) and the total antioxidant content in lettuce (Johkan et al. 2010) which can be explained by its inducing effect on the intracellular ROS as observed in human epithelial

cells (Lockwood et al. 2005). In addition, the activating effect of blue light on the enzymes of the ascorbate–glutathione cycle was shown in tomato (Thomashow 1994). Like during the drought-induced osmotic stress in wheat, the  $H_2O_2$ -decomposing system was also influenced by the light spectrum during the salt-induced osmotic stress in tomato as shown by the induction of catalase activity by low red/far-red ratio (Cao et al. 2018). The level of oxidative stress after cadmium treatment also depended on the light spectrum, since more superoxide radical and less  $H_2O_2$  was detected in rice seedlings grown in blue or red lights compared to those ones cultivated in white light (Sebastian and Prasad 2014). These results can be explained by the efficient removal of  $H_2O_2$  by the components of the ascorbate–glutathione pathways, catalase, and guaiacol peroxidase reaching greater levels in blue and red light than in white one. The experimental data indicate that the light spectrum participates in the aligned adjustment of the levels of thiols and other antioxidants both under optimal and stress conditions which contributes to the adequate growth and development of the plants under various environmental conditions. It also contributes to the efficient recovery after the stress as shown by the spectrum-dependent differences in the amount and redox state of glutathione in the present experiment.

### Combined influence of light spectrum and drought on free amino acids

The level of nearly all minor amino acids (2–20  $\mu$ g/g FW) was affected by drought and light spectrum, while the concentrations of the major ones (Asp, Asn, GABA, Glu, Gln, Ser, Thr, Ala) being present in high concentration (50–600  $\mu$ g/g FW) remained unchanged during the whole experiment in all three light conditions. In accordance with this observation, the reduction of R/FR ratio did not influence the Ala, Asp, Asn and Glu, concentrations in the internodes of sunflower (Mazzella et al. 2008). The major amino acids are available in sufficient amount for the various metabolic pathways; therefore, the adjustment of their concentration to the changing environmental conditions is not necessary. They may have a buffering effect due to the large size of their pools which provide these amino acids in sufficient amount for the various metabolic pathways. In contrast, the level of the minor amino acids should be modified if the metabolism will be re-programmed because of the changing environmental factors. Thus, compared to the other two light regimens, the lowest Ile and Leu levels were detected in pink light in Plainsman throughout the experiment, while the highest levels were observed in Cappelle Desprez before drought. The special role of far-red light in the control of the accumulation of minor amino acids (Tyr, Lys, His, mHis, Arg, Asn, Val, Ile, Leu) should be emphasized, since their levels were higher in Plainsman than in Cappelle Desprez

after stress in far-red light. These observations demonstrate the genotype-specific, light-dependent control of the level of certain amino acids during drought stress. In addition, the spectrum-dependent differences in the level of the amino acids listed above was also kept during the subsequent rewatering, which may influence many physiological and biochemical processes including the compensatory growth of wheat during recovery.

Many-fold drought-induced changes occurred only in the Pro content which can be a result of the activation of the gene encoding pyrroline-5-carboxylate reductase involved in Pro synthesis as shown by the increased transcript levels. The amount of Pro reached the level of major amino acids during the stress which finding corresponds to the observations obtained in other plant species (see for review: Ashraf and Foolad 2007; Per et al. 2017). Interestingly, these changes were greater in the drought-sensitive Cappelle Desprez compared to Plainsman, showing that this change is rather an indicator of drought stress than a sign of adaptation. Blue light was the most effective in the induction of Pro accumulation, since the Pro concentrations were 2–3-fold greater in blue light in the two genotypes compared to pink and far-red light. The Pro content was also greater in blue light than in red one in ramie and rapeseed cultivated under optimal growth conditions (Mh et al. 2019; Rehman et al. 2020). Blue light (but not red or green) also increased the Pro level in tomato which change was accompanied by its greater tolerance to biotic stress (gray mold disease) (Hee-Sun Kook 2013). In Arabidopsis, it was shown, that besides blue light, red light but not far-red light is important for the accumulation of Pro by the upregulation of its synthesis and downregulation its degradation at transcriptional level during salt-induced osmotic stress (Kovács et al. 2019). In contrast, the Pro content increased with decreasing red/far-red ratio during salt stress in tomato (Cao et al. 2018). These observation shows the importance of light spectrum in the control of Pro levels, the alterations of which can contribute to the better adaptation to the changing environmental conditions. Interestingly, in the amount of the Pro precursor Glu, no light spectrum-dependent differences were observed which can be explained by the large size of Glu pool compared to the Pro content throughout the experiment.

The light spectrum-dependent modification of the metabolite-profile including free amino acid composition and levels could be interesting for the increase of stress tolerance of plants cultivated at high latitudes or altitudes where special spectral conditions occur or in glass houses. The protective role of amino acids against drought was shown by a foliar application of a mixture of amino acids (Delfan, containing all proteinogen amino acids except for Gln, Asn, and Trp) which decreased the reduction of growth and yield loss due to the increasing the level of antioxidants (peroxidases, phenols) and osmoprotectants (soluble carbohydrate and

amino acids) in wheat leaves (Hammad and Ali 2014). The drought-induced changes in the level of proline, methionine, arginine, lysine, and aromatic and branched chain amino acids in wheat leaves were also associated with the level of drought tolerance (Michaletti et al. 2018). In addition, the important involvement of four amino acids (Ser, Asn, Met, Lys) in the response to drought was demonstrated by the comparison of eight wheat genotypes with different drought tolerance under control conditions and in the field (Yadav et al. 2019). Among them, the drought-induced changes in the level of Asn and Lys were greatly affected by far-red light in the present experimental system which result can be a basis for the reduction of the drought-induced damages of crops.

## Conclusions

Different effect of far-red light compared to white, pink, and blue light regimens was observed on redox state of glutathione in Plainsman and on the levels of certain minor amino acids in both genotypes during drought. Our results indicate that light spectrum plays an important role in the adjustment of metabolism to the stress conditions and this process is different in the studied drought-tolerant and sensitive wheat genotype. In general, a suitable spectral composition can mitigate the damaging effects of different environmental stresses including drought in various plant species through the coordinated fine tuning of various metabolic processes leading to adaptive changes in growth and development. This study may serve as a starting point for the future application of light irradiation (selection of lands with special spectral conditions or using special spectrum in glass houses) as a new, low cost, and safe method for protecting plants against deleterious effects of the adverse environmental conditions including water deficit.

**Author contribution statement** MG: design of experiments, preparation of figures and tables; MA: analysis of gene expression, statistical analysis, preparation of figures and tables; ZG: analysis of gene expression; GS: measurement of thiols; ÉD: determination of electron transport rate; ZM: analysis of free amino acids; ND: determination and evaluation of growth parameters; RKK: participation in writing of draft; L.S–S: supervision, data analysis; GK: funding acquisition, conceptualization, and writing of manuscript.

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