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The reactivation of detoxification enzymes by red light at night contributes to a more successful defence against *Fusarium graminearum* in wheat ears

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ABSTRACT

Nocturnal red light application affects defence-related enzymes in wheat, in particular antioxidants and glutathione transferases (GSTs), which are crucial for successful plant defence. In our study, we sought to answer the question of whether night-time illumination during anthesis induces detectable changes in the infection responses of wheat cultivars with different levels of *Fusarium resistance*. To address this question, we investigated a range of factors from yield parameters and membrane status to changes in enzyme activities and gene expression and its regulation. Nocturnal red light treatment increased the activities of superoxide dismutase, catalase, guaiacol-dependent peroxidase and GSTs. In parallel with increased activity of detoxification enzymes, a higher grain yield was observed in the more resistant wheat line during infection by *Fusarium graminearum*. Notably, *GSTs*, which are typically down-regulated in darkness, were strongly up-regulated by red light at night in the more susceptible cultivar, especially *TaGSTU1B* and *TaGSTF5*. In addition, an exceptionally high and light-dependent transcriptional activity of *TaGSTF5* was detected in the sensitive line. Sequence analysis of the regulatory region of *TaGSTF5* identified an additional GATA box, a circadian regulatory element, which may be responsible for the increased transcript levels. These results suggest that nocturnal red light can effectively enhance antioxidant and GST activity as part of the defence of wheat against Fusarium head blight, but this depends on the Fusarium resistance of the selected wheat genotypes.

1. Introduction

Fusarium head blight (FHB) of wheat (also known as wheat scab) is one of the most severe fungal diseases affecting kernel development, resulting in widespread problems and serious yield losses worldwide (Dweba et al., 2017). During infection, Fusarium species are known to produce epoxy-sesquiterpenoid compounds known as trichothecenes, such as deoxynivalenol (DON), nivalenol and T-2 toxin (McCormick et al., 2011; Wang et al., 2020). This trichothecene contamination of cereal grains leads to immunotoxicity and cytotoxicity in consumers and thus has raised concerns about food and feed safety (Hathout and Aly, 2014; Arumugam et al., 2021). Therefore, methods to control fungal infection in wheat can contribute to crop protection and food safety.

Several studies have reported quantitative trait loci (QTLs), differentially expressed genes (DEGs) and proteins that can be associated with Fusarium responses and successful defence in wheat (Zhou et al., 2006; Walter and Doohan, 2011; Erayman et al., 2015; Dweba et al., 2017; Kosovaá et al., 2021). These studies reported total thousands of DEGs, proteins and metabolites in more than 60 publications (Ma et al., 2020). According to Pan et al. (2018), as a result of *F. graminearum* infection, 220 DEGs were associated with resistance in three resistant wheat lines. Based on this study, upregulation of glutathione transferase (*GST*) genes associated with FHB resistance was common in the three resistant wheat genotypes. Recently, Kosová et al. (2021) provided proteomic data on plant acclimation to Fusarium infection, which may underlie the superior resistance of the Sumai3 cultivar. In addition to GSTs, members of another phase II enzyme family, UDP-glycosyltransferases (UGTs), were also associated with the superior resistance of Sumai3 to Fusarium infection (Ma et al., 2020). Several UGTs have been reported to be associated with FHB resistance in wheat (Sharma et al., 2018). At the same time, *TaUGT4* was reported to show the highest expression induction in the FHB-resistant cultivar Sumai 3 in contrast to the susceptible wheat lines after treatment with DON or methyl jasmonate (MeJA) (Ma et al., 2020). Despite efforts screening a large number of

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wheat accessions, a limited number of QTLs have been verified to confer a stable effect on FHB resistance (Hao et al., 2020).

Plant GSTs (EC 2.5.1.18; GSTs) are a diverse group of multifunctional enzymes that catalyse a wide range of reactions involving the conjugation of glutathione (GSH; γ-Glu-Cys-Gly) to several electrophilic and/or harmful compounds to form more soluble conjugates, which can be further metabolized (Labrou et al., 2015; Gallé et al., 2019). Plant GSTs are divided into the following groups: tau (U), phi (F), theta (T), zeta (Z), lambda (L), γ-subunit of the eukaryotic translation elongation factor 1B (EF1Bγ), dehydroascorbate reductase (DHAR), metaxin, tetrachlorohydroquinone dehalogenase (TCHQD), Ure2p, microsomal prostaglandin E synthase type 2 (mPGES-2), hemerythrin (GSTH), iota (GSTI), and glutathionyl-hydroquinone reductases (GHRs) (Csiszár et al., 2014; Labrou et al., 2015; Gallé et al., 2019).

The presence of the fungus *Fusarium graminearum*, like all stresses, leads to a rapid accumulation of reactive oxygen species (ROS) both locally and systemically in plants, collectively termed as oxidative burst, which results in the activation of defence responses in all parts of the plant (Mittler et al., 2022). At the same time, GST activity and GSH levels are highly dependent on light and show lower levels at dawn in the dark when various fungal pathogens such as *Fusarium* species show more activity (Beyer et al., 2004; Zechmann, 2017; Gallé et al., 2019). However, not only the light quantity but also the light quality can control GST and antioxidant levels and activity, such as red light as a molecular switch e.g. of the rising sun (Ahn et al., 2015; Pelsőczi et al., 2022). It has been reported that the transcriptional and post-transcriptional regulation of GSTs by red light is related to GSH homeostasis and GSH/GSSG ratio and regulates phytohormone-mediated defence under biotic stress (Yang et al., 2015). Thus, light, especially red light, may determine detoxification and stress resistance of plants, for example under pathogen attack (Gallé et al., 2021).

As the role of GSTs and other proteins has been associated with a more successful defence against the cellular and oxidative damage caused by *Fusarium sp*., the control of gene expression in flowering wheat systems is crucial for the analysis of phase II defence. Inducible gene expression can be used for different purposes, e.g. light with a suitable wavelength can be easily applied with high temporal resolution to influence the transcriptome and cellular redox state (Müller et al., 2014). Among the various possibilities for the application of a specific light wavelength as a molecular gene expression controller, a short exposure to light in the middle of the night period (often referred to as night break; NB; Cao et al., 2016) has previously been found to be effective in enhancing resistance to, for example, *Pseudomonas syringae* (Yang et al., 2015) and *Botrytis cinerea* in tomato (Hui et al., 2017). The use of NB as a resistance-regulating signal may be particularly important for plant defence against pathogenic fungal attacks, as night darkness is favourable for fungal metabolism, hyphal development, sexual reproduction, sporulation and fungal virulence (Yu and Fischer, 2019; Pelsőczi et al., 2022), while it can cause a weakening of plant defences (Bhardwaj et al., 2011; Butt et al., 2020). In addition, the application of NB light exposure can be well planned in the case of wheat, as the early period of wheat ear development and the short flowering period provides the greatest exposure to Fusarium infection (Mesterházy et al., 2011). This timed illumination, which uses special wavelengths of light, can be combined with night-time spraying to protect bees (Karbassioon and Stanley, 2023) or drone-mounted LEDs (Rejeb et al., 2022; Ünler, 2024), but scale can be limited.

The aim of our work was to investigate whether the defence of flowering wheat against *Fusarium graminearum* can be enhanced by the reactivation of detoxifying enzymes such as GSTs, using nocturnal red light illumination. In addition, changes in the activity of key antioxidant enzymes were also monitored following night-time red light at dawn, which may also contribute to a more successful defence against oxidative stress induced by *F. graminearum* infection. Moreover, the promoter region of the most highly expressed *TaGSTs* is being analysed and sequenced to better understand their light regulation.

2. Materials and methods

2.1. Plant material and growth conditions

Two wheat cultivars were used to study the effect of night breaking red illumination on *F. graminearum* infection based on our previous work (Pelsőczi et al., 2022): *Triticum aestivum* cv. GK Ígéret (resistant to *Fusarium*) and cv. GK Arató (more sensitive to *Fusarium*) (Cereal Research Non-Profit Ltd., Szeged, Hungary). The plants were grown under normal field conditions in the field of Cereal Research Non-Profit Ltd., Szeged, Hungary (GPS coordinates: 46.24137, 20.10214).

2.2. Experimental design

The experiments were carried out and repeated in three different years (2019, 2020 and 2022) on parallel plots. In our experimental design we have a control, a red light illuminated, an infected and an illuminated and infected group (Fig. 1), so there were 4 groups of each cultivar according to treatments.

2.3. Treatments

Fifteen-minutes of red light treatments were applied at midnight with LEDs (V-TAC; Plovdiv; Bulgaria; 50 mW m^{-2}; 590–660 nm interval with a maximum at 630 nm) for 7 days, 4 days before anthesis and 3 days after anthesis (Fig. 2). The LED strips were mounted on posts driven into the ground at a distance of 10–15 cm from the wheat ears. After 3 days of pre-treatment with red light for 15 min, the spikelets were infected with *F. graminearum* sensu stricto isolate suspension (conidium concentrations of the inocula was 10^6 mL^{-1}) by the staff of Cereal Research Non-Profit Ltd. in the morning (9:00) based on the method of Mesterházy et al., (1999), (2005). Infected groups/batches contained 15–20 ears of wheat and were sprayed (approximately 20 mL per group) using a hand sprayer (1 L). Plastic bags were used to maintain the required humidity for 48 h after infection. At least 6 plots (1 ×2 m) with 300–400 wheat ears m^{-2} were used for the experiments (Mesterházy et al., 2011). For the control treatment, distilled water was used and similarly covered with plastic bags for the same period of time. After the treatment, the plants were illuminated 3 more times for 15 min at midnight. Samples were collected 4 days after infection at 3:00 am. The experiments were repeated in parallel plots and in three different years.

2.4. Determination of yield parameters

Before harvest, the number of grains and grain weight per wheat ear for each treatment were determined manually and using a laboratory balance (Radwag, Radom, Poland).

2.5. Determination of malondialdehyde content

For the determination of malondialdehyde (MDA), wheat ear samples (100 mg) were ground in liquid nitrogen to a homogeneous powder. To prevent lipid peroxidation, 100 μL of 4 % butylhydroxytoluene (BHT) was added, and the subsequent steps were identical to those described in the article by Ederli et al., (1997).

2.6. Determination of cell viability

Electrolyte leakage (EL) was measured according to Sun et al. (2010). 100 mg of wheat ear was placed in 20 mL of double-distilled water and incubated in the dark at room temperature for 2 h. The electrical conductivity (C1) was then measured, and the samples were boiled at 100 °C for 40 min. After cooling back to room temperature, the electrical conductivity (C2) was measured again using a conductivity

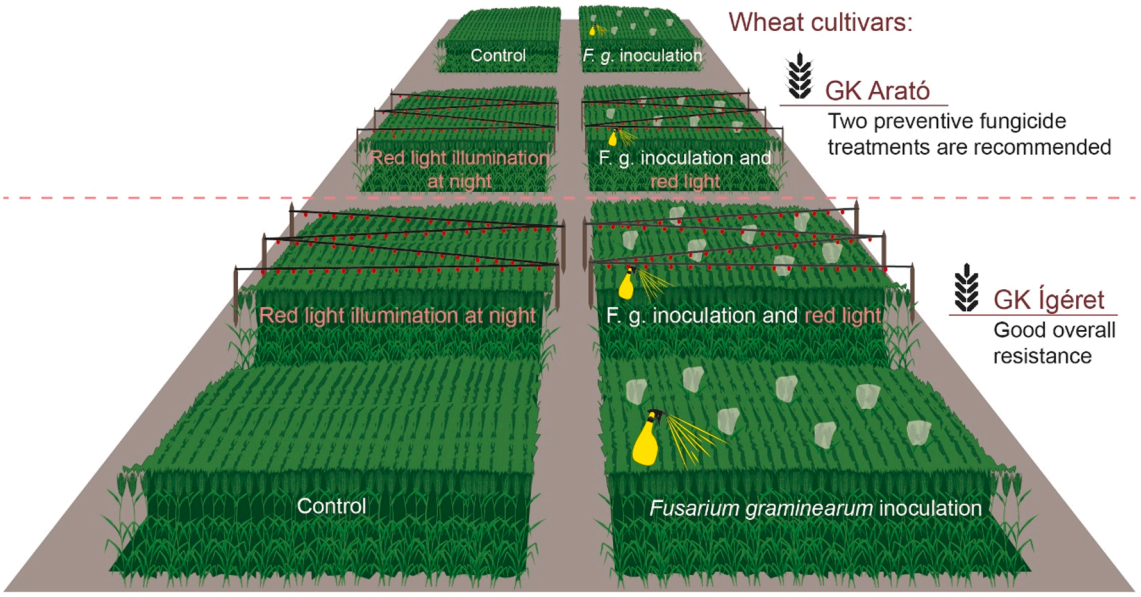

Fig. 1. The experimental design and treatments: In addition to the control group, there were *F. graminearum* inoculated, night red light pre-treated, and both *F. graminearum* inoculated and red light pre-treated groups. The wheat cultivars were GK Arató and GK Ígéret with different susceptibility to FHB and other pathogens: GK Ígéret is moderately resistant to *Fusarium* and GK Arató is rather sensitive to *Fusarium* (Cereal Research Non-Profit Ltd., Szeged, Hungary).

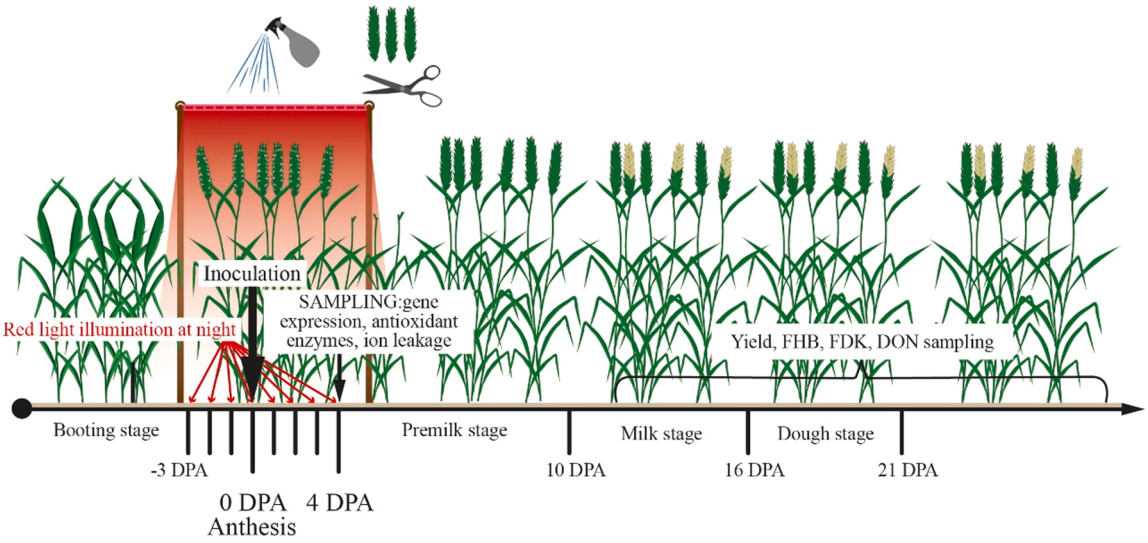

Fig. 2. Time of treatment, illumination and sampling of wheat lines at the end of the booting stage, before anthesis, 15 min of red light illumination (pre-treatment) was started. After 3 days, spikelets were infected with *F. graminearum* sensu stricto isolate suspension (provided by Cereal Research Non-Profit Ltd). Samples were taken at dawn (3:00 a.m.) four days after infection (at the beginning of the necrotrophic phase of the fungus). Evaluation of FHB disease severity began on 17 DPA and was repeated on 21 DPA (at the end of the dough stage).

meter (Mettler Toledo, Columbus, USA). The following formula was used to calculate the percentage of EL: EL % = (C1/C2) x 100.

2.7. Determination of the activity of key antioxidant and GST enzymes

For enzyme activity measurements, tissue homogenisation and extraction steps were performed at 4 °C immediately after sampling at dawn. Crude protein extracts were prepared by homogenising (250 mg) wheat ears with 1 mL ice-cold extraction buffer (0.1 M phosphate buffer (pH 7); 1 mM phenylmethylsulphonyl fluoride) and 10 mg polyvinylpolypyrrolidone. The homogenised samples were centrifuged (20 min, 4 °C, 12000 rpm), and then used to determine the activity levels of the key antioxidant and GST enzymes using a spectrophotometer (KONTRON, Milan, Italy) (Gallé et al., 2009; Tari et al., 2015; Pelsőczi et al., 2022).

SOD activity was defined by the inhibition of the photochemical reduction of nitro blue tetrazolium (0.05 M NBT) in the presence of riboflavin (0.2 mM) under light irradiation. Formazan absorbance was determined at 560 nm after 1 min. One unit (U) is the amount of SOD enzyme that causes 50 % inhibition of NBT reduction under light irradiation.

CAT activity was measured with hydrogen peroxide (H_2O_2) substrate using a quartz cuvette with a final volume of 1500 µL (1350 µL 0.05 M pH 7 mixed phosphate buffer, 50 µL sample and 100 µL 1 % H_2O_2). The reaction was initiated with H_2O_2 and measured after 0.5 min against phosphate buffer at 240 nm. One U is the amount of CAT that catalyses the dissolution of 1 µmol H_2O_2 in 1 min (ε_{240} = 39.4 mM^{-1} cm^{-1}).

POD activity was determined with guaiacol (1 %) and the reaction was initiated with H_2O_2 (1 %). The absorbance was measured at 470 nm. One U is the amount of enzyme that catalyses the formation of 1 μmol of tetraguaiacol in one minute (ε_{470}=26.6 mM^{-1} cm^{-1}).

GST activity was determined using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate. The final volume was 1000 μL (850 μL 0.1 M phosphate and sodium buffer (pH 6.5), 50 μL sample, 50 μL reduced glutathione (GSH) and 50μl CDNB). The reaction was started with CDNB and read at 340 nm after 1.5 min.

2.8. RNA extraction, cDNA synthesis, and expression analysis by quantitative real-time PCR

Wheat ear samples were crushed with liquid nitrogen. Then 1 mL of TRI reagent was applied according to Chomczynski and Sacchi (1987), with the following differences: after the application of TRI reagent (without 2-mercaptoethanol), 200 μL of chloroform was added and, after vortexing, the samples were incubated for 3 min at room temperature. The samples were then centrifuged at 10,000 rpm for 15 min in a centrifuge at 4 °C. The supernatant was pipetted into a mixture of 375 μL chloroform and isoamyl alcohol (24:1), the subsequent steps were following the description published by Chomczynski and Sacchi (1987). The supernatant was discarded, the pellet dried and suspended in 30 μL molecular RNase-free sterile water.

Genomic DNA was removed using DNase I (Thermo Scientific, Waltham, MA, USA). After DNase treatment, RNA was purified by phenol-chloroform extraction and ethanol precipitation. RNA concentration and purity (OD260/OD280, OD260/OD230) were assessed using a Nanodrop spectrophotometer (Thermo Scientific, USA).

cDNA was synthesised from a total of 1 μg RNA using reverse transcriptase (Thermo Scientific, Waltham, USA).

Quantitative real-time reverse transcription PCR (qRT-PCR; qTOWER Real-Time qPCR System, Analytik Jena, Jena, Germany) was performed using diluted cDNA as template, SYBR Green qPCR Master Mix and primers in a final volume of 10 μL according to the manufacturer's instructions. The selected genes were detected using the qTOWER 2.0 real-time qPCR system (Analytik Jena, Jena, Germany). In our experiments, we used the 18S and EF genes for normalisation, as the expression of these genes is constant even under stress. The data obtained from the RT-qPCR measurement were then further calculated using the $2^{-(\Delta\Delta Ct)}$ method (Livak and Schmittgen, 2001).

The primers used in the assays were designed to target genes considered to be the most important in stress tolerance and protection against *Fusarium* wilt in wheat, based on previous publications (Table 1). According to Thom et al. (2002) and Cummins et al. (2003), the selected *GSTs* showed activity against CDNB and crotonaldehyde (stress metabolite analogue) and exhibited glutathione peroxidase (GPOX) activity. Among the selected enzymes, some showed outstanding activity: The TaGSTU1–1 protein shows outstanding conjugation activity against crotonaldehyde (stress metabolite analogue), and among the four tau group GSTs they examined, the CDNB conjugation activity was the second highest, while TaGSTU2 showed the highest. All phi group GSTs showed conjugation activity against stress metabolites, with TaGSTF1 showing higher activity against CDNB (Thom et al., 2002; Cummins et al., 2003; Gallé et al., 2009; 2022).

2.9. DNA sequencing and analysis

DNA fragments (approximately 400 bp long) containing sequences of *TaGSTU1B* and *TaGSTF5* were amplified using DreamTaq DNA Polymerase (Thermo Scientifi, Waltham, USA), according to the manufacturer's instructions. The primers used in the amplification are listed as supplement (Supplemental Table 1). Sequencing was conducted on both strands and reactions were performed at the sequencing center at the Sequomics Company and was done using the NextSeq500 Sequencer (Illumina, San Diego, CA). DNA sequence assemblies were performed manually using a word processor (Supplemental Tables 2–3). Due to the allelic variability of allohexaploid wheat, one section out of ten could not be sequenced independently (the PCR products originated from two different genomes), and it is marked 'N' in the supplement. Alignments of the −1000 bp long 5' flanking regions of the reference genome, *TaGSTU1B* and *TaGSTF5* were performed using Clustal Omega (EMBL-EBI, Sievers et al., 2011). The cis-acting regulatory DNA elements in the promoter regions of the genes were analysed using New Place (https://www.dna.affrc.go.jp/PLACE/?action=newplace; Supplemental Tables 4-7).

Table 1
Primer pairs used for qRT-PCR.

Genome locus identifier	Wheat genes	Primer sequence (5'-3')
KX533924 F	*TaEF*	F:CCAAGACGAAGCAGAACAGA
		R: ACACATCCAACGCAAGAGAA
AY049040 F	*Ta18S*	F:GTGACGGGTGACGGAGAATT
		R:GACACTAATGCGCCCGGTAT
AJ414697 F	*TaGSTU1A*	F:GTGGTTGGTGGTTGTTGGTT
		R:ATGGAAATGGAATGGCAGGT
AJ414698 F	*TaGSTU1B*	F:CGGAGGGAAGGAACAAATAA
		R:CACTGACTGACCCAACCAAC
AJ414700 F	*TaGSTU2*	F:CCGTGCTCGCTTGGAT
		R:CGGACTCAGACACACACAAACA
AJ414701 F	*TaGSTU3*	F:CCGCCTATGTGAACGACAA
		R: GGGTCTCCTCCATCTTACCG
AJ440796	*TaGSTF1*	F: GTGCCTTATCCATCCAATGC
		R: AGGAAGTCTCCAGCCAGGTA
AJ440792 F	*TaGSTF3*	F:CAAGAAGGTGCTGGAGGTGT
		R: AAGGGGAAGTGGCTGAGGT
AJ440793	*TaGSTF4*	F: TATTCTCCGCAAACACAAGC
		R: CGACCCATACATCCACCATC
AJ440794	*TaGSTF5*	F: GCGAGAACGGAATAAAATCG
		R: AACAGCAGCAACACCAAGAA
AJ441055 F	*TaGSTF6*	F: CAAGAAGCCGTGATTTGCTA
		R: GCGACACCAACAAGAAAAGA
MK166043	*TaUGT4*	F: AAGGGGGAAGTTCGGACAAG
		R: AGCATCGTTCCAAATGGTGAG
AY641449	*TaCytP450*	F: TGGCATCAAAGTGACCGAAGG
		R: GGCCCACTGGAGAAAGACAAT

2.10. Statistical analysis

Data were processed and analysed using Office Excel 2016 and SigmaPlot 11.0 (Systat Software Inc., Erkrath, Germany). Significant differences from the control groups were determined using one-way ANOVA with Duncan's multiple range test. The difference was considered significant if $P \leq 0.05$.

3. Results

In this work, the effects of 15 min nocturnal red light treatments were investigated in different wheat cultivars (Fig. 1), GK Ígéret and GK Arató, at the anthesis stage to reduce the extent of *F. graminearum* infection (Fig. 2). However, nocturnal red light treatments significantly reduced grain number and grain weight in the Fusarium resistant wheat cultivar GK Ígéret (Fig. 3), but the effects of fungal infection were more significant in the reduction of yield parameters in this genotype (Fig. 3). In addition, nocturnal red light application was beneficial in alleviating the decrease in yield parameters in GK Ígéret (Fig. 3). In contrast, nocturnal red light treatments had no significant effect on yield parameters in the Fusarium sensitive wheat genotype GK Arató (Fig. 3), but effectively alleviated the decrease in grain number per ear under Fusarium infection (Fig. 3A).

The flowering stage of wheat is very sensitive to Fusarium infection, which is associated with oxidative stress in plants. Therefore, the effects of red light treatment and Fusarium infection on lipid peroxidation, based on changes in MDA content, and on cell viability, based on electrolyte leakage from the ear cells, were investigated. None of the

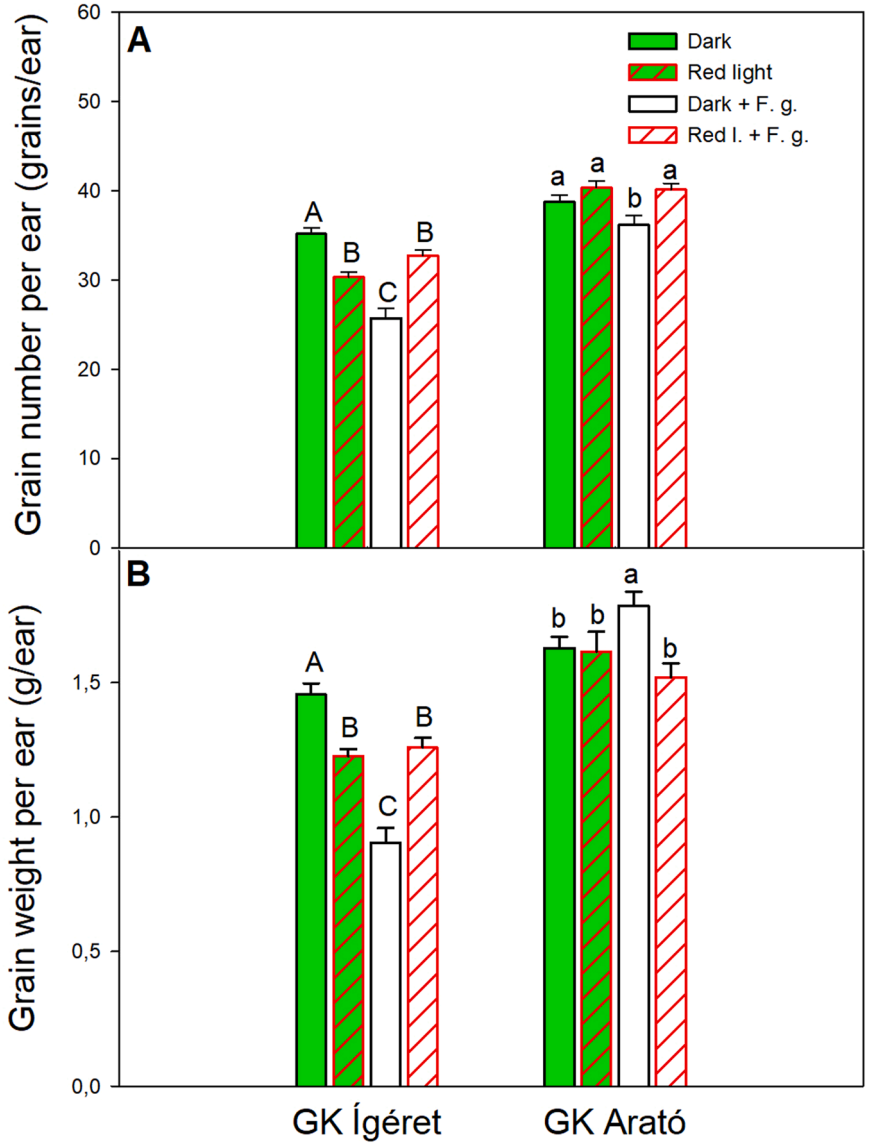

Fig. 3. Changes in the number of grain number (A) and grain weight (B) per ear in the wheat varieties GK Ígéret and GK Arató after the red light treatment at midnight during the anthesis and after the infection with *Fusarium graminearum* (F. g.). The samples were taken before harvest (mean+SE, n = 30). Columns marked with different letters are significantly different from each other at $P \leq 0.05$ as determined by Duncan's multiple comparisons in the case of each wheat cultivar.

treatments caused a significant change in the MDA content of wheat ears at dawn, but GK Ígéret generally had a much higher MDA level than GK Arató (Fig. 4A). At this time point, EL also did not change significantly in GK Iígéret, but red light application decreased EL from ear cells under *F. graminearum* infection in the GK Arató wheat genotype (Fig. 4B).

Antioxidant enzymes play a crucial role in the regulation of Fusarium-induced oxidative stress in plants thus changes in the activities of SOD, CAT, POD were also determined at dawn in the ears of the selected wheat genotypes.

Based on the results, SOD activity at dawn was significantly increased by 15 min of red light treatment at midnight in the ears of both wheat cultivars (Fig. 5A). *F. graminearum* infection also increased SOD activity in wheats (Fig. 5A). Interestingly, nocturnal red light treatment reduced SOD enzyme activity under Fusarium infection at dawn in the ears of both wheat cultivars (Fig. 5A).

In the case of CAT enzyme activity, nocturnal red light increased it in GK Ígéret (Fig. 5B). At the same time, no significant changes were observed in the selected wheat cultivars under Fusarium infection and the combined treatment with red light at dawn (Fig. 5B).

POD activity was significantly increased in the ears of both wheat genotypes by the nocturnal red light application at dawn (Fig. 5C). In addition, *F. graminearum* infection more significantly increased POD enzyme activity in the ears of GK Ígéret (Fig. 5C), which was alleviated by nocturnal red light application (Fig. 5C). At the same time, significantly higher POD activities were also detected in the ears of GK Arató after nocturnal red light treatment and Fusarium infection.

In addition to the key antioxidant enzymes, changes in GST activities were also monitored in the ears of the selected wheat genotypes at dawn following red light and Fusarium treatments. Nocturnal red light application increased GST activity at dawn in the ears of both wheat cultivars (Fig. 6). Moreover, *F. graminearum* infection induced a significant increase in GST activity in the ears of both wheat genotypes (Fig. 6). GST activity was also significantly higher in wheat ears after red light and Fusarium exposure at dawn compared to the dark control (Fig. 6).

Nocturnal red light treatments significantly increased GSH levels in the ears of both selected wheat genotypes at dawn (Fig. 7A). *F. graminearum* infection also resulted in higher GSH levels in GK Ígéret (Fig. 7A). Similarly, GSH levels were increased by both treatments in ears of GK Ígéret compared to the untreated control at dawn (GK Ígéret).

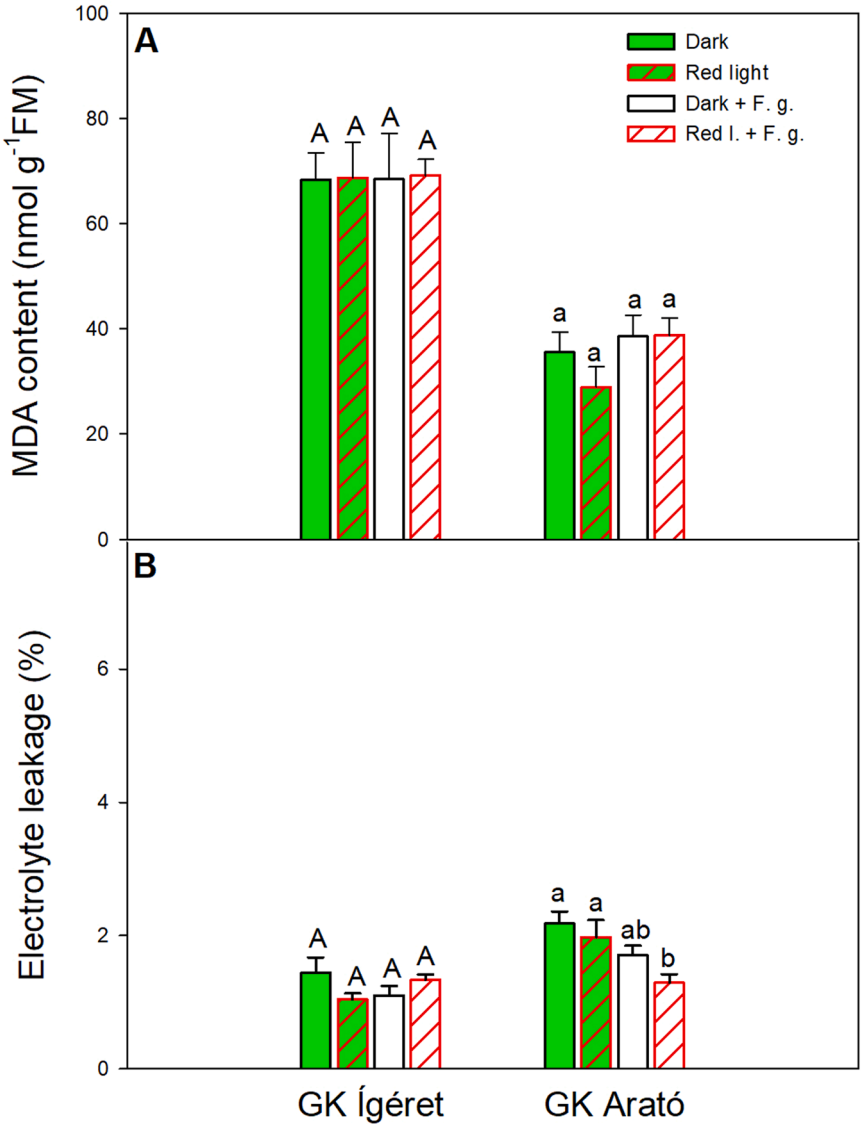

Fig. 4. Changes in MDA content (A) and electrolyte leakage (B) in the wheat varieties GK Ígéret and GK Arató after the red light treatment at midnight during the anthesis and after the infection with *F. graminearum* (F. g.). The samples were taken at dawn (mean+SE, n = 6). Columns marked with different letters are significantly different from each other at $P \leq 0.05$ as determined by Duncan's multiple comparisons in the case of each wheat cultivar.

Interestingly, GSSG levels did not change significantly after treatments in the ears of both wheat plants (Fig. 7B).

Analysis of key wheat GST genes was also carried out in the ears of the selected wheat cultivars after the treatments to test the reactivation effect of nocturnal red light application on gene expression levels in the dark.

For our further investigations, we selected several genes that may play a role in the general stress response or specifically in the biotic stress response. These genes were selected primarily on the basis of our previous findings and two publications. The genes have two known nomenclatures: Cummins and Thom (Cummins et al., 2003; Thom et al., 2002) named the proteins they studied, while Wang et al. assigned gene names based on their genomic positions. For simplicity, we use the former protein nomenclature in this article, which includes a smaller number of names that are easier to remember. According to our previous results, the mRNA levels of the *TaGSTU1B* (*TaGSTU120*), *TaGSTU2* (*TaGSTU145*), and *TaGSTF6* (*TaGSTF58*) proteins increased upon infection in lines more resistant to *Fusarium*. Among the tau class GSTs, the TaGSTU3 (TaGSTF59) protein, as well as the phi class TaGSTF1,3,4, 5 proteins (TaGSTF73, ~188, ~50, ~40) exhibit glutathione peroxidase activity in addition to their conjugation activity (Cummins et al., 2003; Thom et al., 2002). Interestingly, all the selected *GSTs* were up-regulated by the nocturnal red light treatments in the ears of GK Arató at dawn, especially *TaGSTU1B* and *TaGSTF5* (Fig. 8). At the same time, the expression of *TaGSTU1B* and *TaGSTF5* was generally higher in GK Arató than in GK Ígéret at dawn in the dark (Fig. 8). In addition, the expression of *TaUGT4* and *TaCytP450* was also increased by nighttime red light exposure in this genotype at dawn (Fig. 8). In contrast, only the expression of *TaGSTU1B* and *TaUGT4* was significantly induced by nocturnal red light in the ears of GK Ígéret (Fig. 8). However, the same two genes were induced by *F. graminearum* infection in this wheat genotype at dawn in the dark (Fig. 8). In contrast, the expression of *TaGSTU1B* and *TaGSTF5* was highest in the ears of GK Arató under Fusarium infection at dawn, but all *GSTs* were increased under FHB and after red light application at night in this genotype (Fig. 8).

Based on the results of the gene expression analysis, the *TaGSTF5* and *TaGSTU1B* genes were selected for further studies, because the up-regulation of these two genes (among the GSTs) was outstanding in GK Arató under the influence of the fungal pathogen. First, we searched for a 5′ regulatory region of −1500 base pairs using the reference

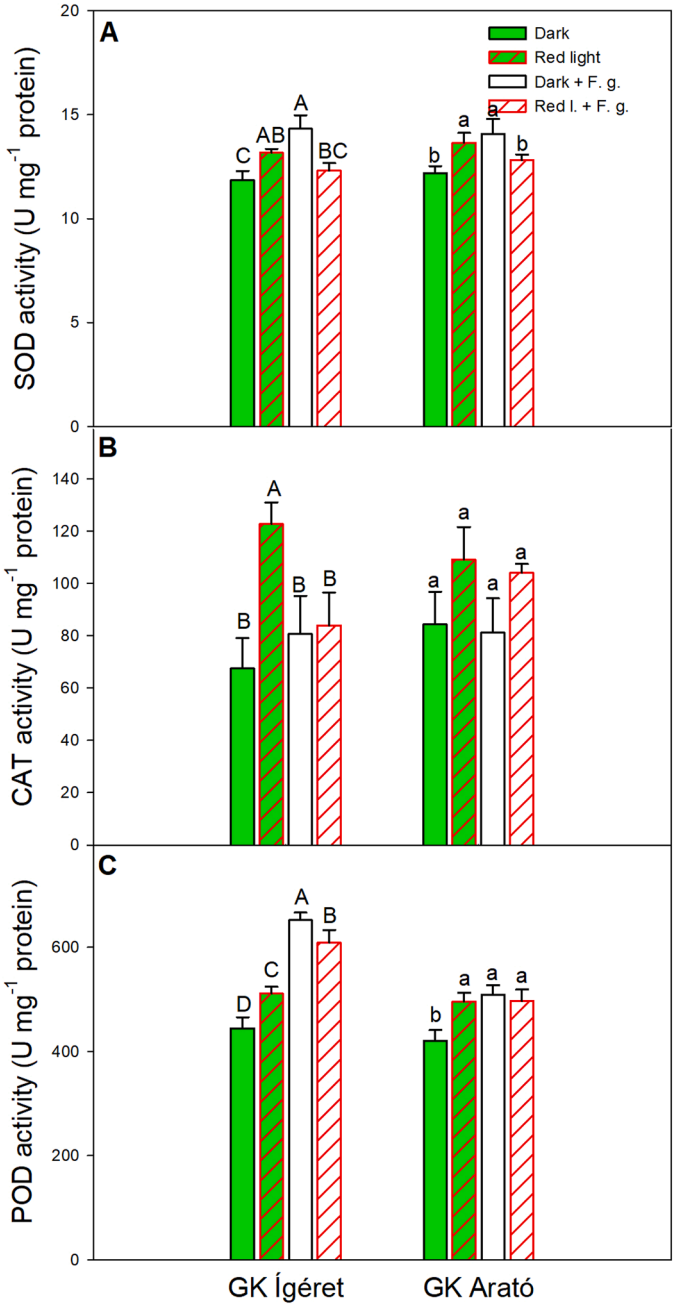

Fig. 5. Changes in the activites of superoxide dismutase (SOD; A), catalase (CAT; B) and guaiacol peroxidase (POD; C) in the wheat varieties GK Ígéret and GK Arató after the red light treatment at midnight during the anthesis and after the infection with *F. graminearum* (F. g.). The samples were taken at dawn (mean+SE, n = 6). Columns marked with different letters are significantly different from each other at $P \leq 0.05$ as determined by Duncan's multiple comparisons in the case of each wheat cultivar.

genome (EnsemblPlants, https://plants.ensembl.org/Triticum_aestivum/Info/Index, IWGSC RefSeq v2.1.; 2023). The 5' *cis* regulatory elemets of *TaGSTU1B* according to the genome assembly of the reference *Triticum aestivum* cv. Chinese Spring were listed (Supplemental Tables 4 and 5). In the promoter of *TaGSTU1B,* 31 elements were found that could be related to light responsiveness (7 GT-1, 6 GATA boxes, 2 IBOXes and 1 Inr initiator elements), 3 elements are connected to circadian regulation and 12 elements are related to phytochrome signalling (Supplemental Table 4). While 34 elements were found in the promoter of *TaGSTUF5* that could be associated with light

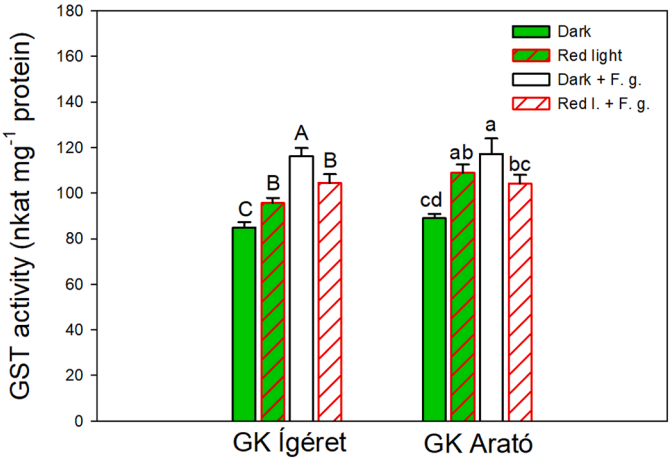

Fig. 6. Changes in the activity of glutathione transferase (GST) in the wheat varieties GK Ígéret and GK Arató after the red light treatment at midnight during the anthesis and after the infection with *Fusarium graminearum* (F. g.). The samples were taken at dawn (mean+SE, n = 6). Columns marked with different letters are significantly different from each other at $P \leq 0.05$ as determined by Duncan's multiple comparisons in the case of each wheat cultivar.

responsiveness (9 GT-1, 13 GATA boxes, 6 IBOXes, 3 Inr initiator elements, 1 psbD and 1 BOX II), but only one element is connected to circadian regulation and 10 to phytochrome signalling (Supplemental Table 5). In order to determine the possible differences in the corresponding regions in the genomes of GK Ígéret and GK Arató wheat lines, five DNA sections were amplified and sequenced using five primer pairs in both wheat lines. The alignment of the reference genome and the two sequenced 5′ regions can be found as supplementary files (Supplemental Tables 6 and 7).

Based on the sequencing, there were no differences between the three wheat cultivars (GK Arató, GK Ígéret and the reference Chinese Spring) in the 5′ regulatory regions for the *TaGSTU1B* gene, while there were several changes between the sequences of the reference genome and the GK Arató and GK Ígéret lines upstream of the *TaGSTF5* coding region: GK Arató contains a longer insert (69 bp), while a shorter region is missing, which present in the reference genome and in the GK Ígéret (26 bp). Two *cis*-acting elements, which may be responsible for the higher expression and light regulation of *TaGSTF5,* were found on the longer insert: GATA box and a Circadian (CAANNNNATC) expression regulator element.

4. Discussion

Plants can be induced to develop enhanced resistance to pathogen infection by treatment with a variety of abiotic and biotic inducers, such as cell wall components, plant extracts, compounds of biological origin and synthetic chemicals (Walters and Fountaine, 2009; Romanazzi et al., 2016). Among the various possibilities, light as a potential regulator of gene expression and resistance, the usage of light treatments may be a few of the most environmentally friendly and sustainable options. There are several examples of the use of light for this purpose: red light completely suppressed broad bean leaf spot disease caused by *Alternaria tenuissima* (Romanazzi et al., 2016) or *Botrytis cinerea* in tomato (Hui et al., 2017). At the same time, light can also inhibit fungal growth and metabolism, such as hyphal development and sporulation, as reported for *F. graminearum* (Beyer et al., 2004) and *Fusarium verticillioides* (Velmurugan et al., 2010). Thus, the application of a specific wavelength of light can be an effective and environmentally friendly solution to influence plant resistance mechanisms and reduce fungal diseases. Furthermore, it is known that the anthesis stage is the most sensitive to Fusarium infection (Mesterházy et al., 2011; Ma et al., 2020), so it can be

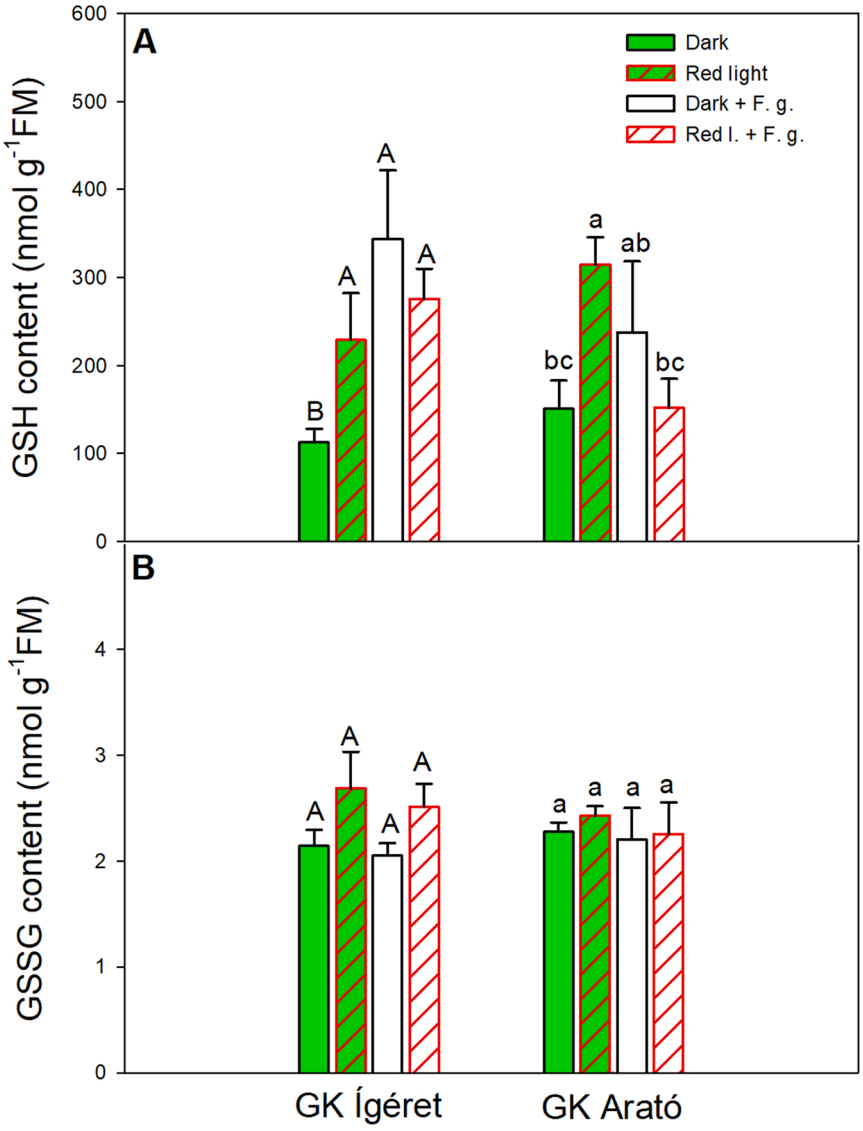

Fig. 7. Changes in the contents of reduced glutathione (GSH; A) and oxidised glutathione (GSSG; B) in the wheat varieties GK Ígéret and GK Arató after the red light treatment at midnight during the anthesis and after the infection with *Fusarium graminearum* (F. g.). The samples were taken at dawn (mean+SE, n = 6). Columns marked with different letters are significantly different from each other at $P \leq 0.05$ as determined by Duncan's multiple comparisons in the case of each wheat cultivar.

a well-planned period for light induction of plant defence responses, a few days before flowering.

Here, the effects of 15 min nocturnal red light treatments were investigated in ears of wheat genotypes with different *Fusarium* susceptibility to test the reactivation of plant defence responses at dawn in the dark. *F. graminerarum* infection significantly reduced grain number at harvest in both wheat genotypes. At the same time, the beneficial effects of red light exposure on yield parameters were observed in the moderately Fusarium resistant wheat cultivar GK Ígéret under fungal infection. However, no significant effects on yield parameters were observed in GK Arató. At the same time, the nocturnal red light treatments themselves significantly reduced grain number and weight in GK Ígéret. In the background of these changes can be environmental factors, as well as anatomical and morphological differences between the two selected wheat genotypes. The red light itself can activate different hormonal pathways (Yang et al., 2015; Gallé et al., 2019), resulting in different effects depending on the genotype. Furthermore, GK Arató has greater abiotic resistance can lead to important but not investigated physiological and biochemical changes. The yield and development of wheat grains are regulated by various environmental factors, such as light intensity, quality, and duration (photoperiod) (Dreccer et al., 2022; Li et al., 2022). However, the enhanced resistance and yield observed in this case could be due to the reactivation of plant defence responses by red light under the fungal infection. At the same time, the *GSTF5* gene, also known as *TraesCS3D02G445400*, was identified earlier as a novel quantitative trait locus (QTL) controlling spikelet number and grain yield in wheat (Li et al., 2021). Moroever, it is known that plant GSTs play a crucial role in protecting plants against oxidative stress (Dixon et al., 2002; Hasanuzzaman et al., 2017). Higher antioxidant and GST activity caused by red light treatment can enable more efficient scavenging of ROS, reducing cell damage during the critical periods of ear formation and grain filling. Consequently, the plant can allocate more energy to grain filling and experience less assimilate loss due to stress responses. GSTs are also involved in the detoxification of xenobiotics and lipid peroxidation products (Nianiou-Obeidat et al., 2017; Estévez and Hernández, 2020). This contributes to the stability of the photosynthetic apparatus, which indirectly can increase wheat yield.

The first days after Fusarium inoculation are crucial for disease

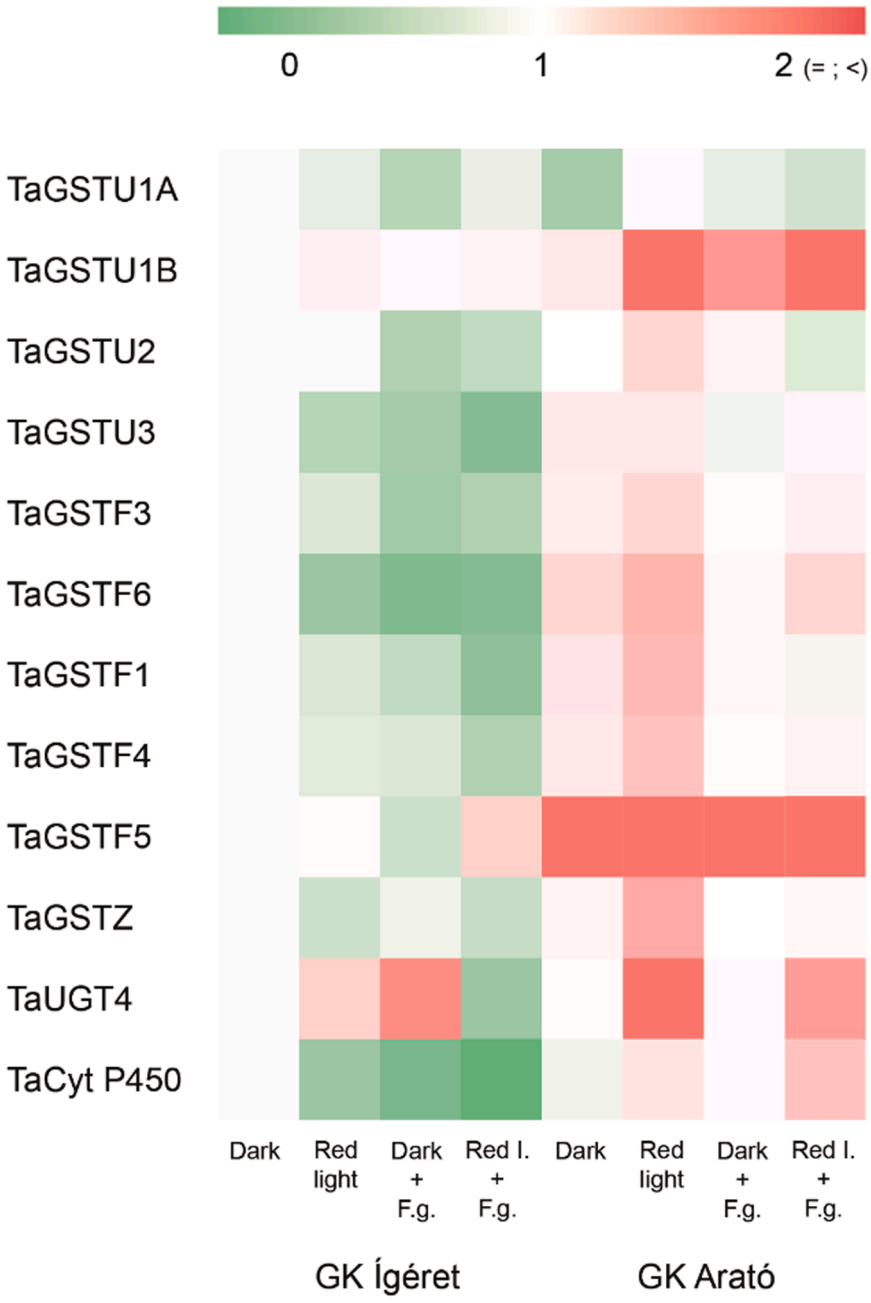

Fig. 8. Changes in the relative expression levels of selected *glutathione transferases* (*GSTs*) genes (phi, tau and zeta group), one UDP-glycosyltransferase gene (*TaUGT4*), and one cytochrome P450 gene (*TaCytP450*) in the wheat varieties GK Ígéret and GK Arató after the red light treatment at midnight during the anthesis and after the infection with *Fusarium graminearum* (F. g.). The samples were taken at dawn. The relative transcript levels of the genes were determined by qPCR. $2^{(-\Delta\Delta Ct)}$ data were presented as a heat map. Green colours show repression, while red colours show activation, as it is indicated on the upper colour scale bar.

development (Ma et al., 2020). The day chosen in this study is based on relevant studies (Alderete et al., 2018; Ahn et al., 2015; Gallé et al., 2018) to test the relative early defence responses of plants in the dark and the potential reactivation effects caused by nocturnal red light pre-treatment and defence against fusariosis at dawn. It is well known that most of the key antioxidant enzymes are basically downregulated at dawn, such as SOD (Kerdnaimongkol et al., 1997; Dutilleul et al., 2003), CAT (Michael and McClung, 2002; Mhamdi et al., 2010) and peroxidases (Pignocchi et al., 2003; Chen et al., 2015). Recently, ROS scavenging by Cu/Zn-SOD, Fe-SOD, Mn-SOD and CAT during the day/night cycle was studied by Jiménez et al. (2021), and the SOD enzymes were found to have a peak in scavenging at the end of the light period, while CAT showed higher activity from the beginning to three quarters of the photoperiod. Thus, the activities of these enzymes are regulated by light, especially the red light component (Mhamdi et al., 2012; Hui et al., 2017; Yang et al., 2018). Therefore, artificial lighting at night could be a method to reactivate these antioxidant enzymes for the most critical period of infection when the fungi are more active. However, plant defence responses under Fusarium infection are highly mediated by ROS, the levels of which may be significantly lower in the dark (Poór et al., 2018).

In this study, no significant ROS-induced lipid peroxidation was detected, based on changes in MDA content of wheat ears, after day 4 of *F. graminearum* infection at dawn. Similarly, there were no changes in EL at this relatively early stage of Fusarium infection. At the same time, a significant increase in SOD and POD activity was observed in wheat ears

under fusariosis at this time point at dawn. This is in agreement with some previous data that found similar higher antioxidant enzyme activities under Fusarium infection (Mohammadi and Kazemi, 2002; Spanic et al., 2017; Gallé et al., 2022). At the same time, red light pretreatment significantly affected the antioxidant enzymes in wheat ears. Application of red light at night increased the activity of SOD, an enzyme that dismutates the harmful superoxide radical anion, and the activity of CAT and POD in wheat ears, enzymes that neutralises large amounts of H_2O_2. This may contribute to the control of *F. graminearum* and FHB disease by reducing oxidative stress in wheat plants. It is known that fungi and fungal toxins can induce lethal oxidative stress and inhibit antioxidant enzyme activities such as CAT (Zhao et al., 2015; Iqbal et al., 2021). It has also been shown that red light treatment of strawberry leaves reduces H_2O_2 levels and makes them more resistant to infection by the plant pathogenic fungus *Botrytis cinerea* (Meng et al., 2019). Moreover, these results are in line with other observations where reduced H_2O_2 levels and higher antioxidant enzyme activities were found during cucumber mosaic virus infection in tobacco (Chen et al., 2015), or in the case of *B. cinerea* infection in tomato leaves (Hui et al., 2017) when exposed to red light. At the same time, significant differences can be found in the different wheat genotypes depending on the degree of Fusarium resistance. In addition, there were no significant differences between the two species in the response to the combined treatment compared to the red illuminated wheat ears.

Besides the key antioxidant enzymes as a crucial part of plant defence system, the focus of this study is on GSTs and how they can be reactivated by red light at dawn in the dark, where they basically showed lower activities (Gallé et al., 2018, 2019; Pelsőczi et al., 2022). Based on these results, red light application at night was effective in increasing GST activity both by itself and under Fusarium infection in ears of wheat plants. This effect of red light can influence Fusarium tolerance; GST activity was induced more by red light in the more Fusarium resistant cultivar compared to the more sensitive GK Ígéret wheat genotype. In addition, the levels of GSH, a substrate of GSTs, increased in the same way in both wheat genotypes under red light and Fusarium infection. Similar increases in GSH levels were measured in other plant species upon combined treatments with red light and nematodes (Yang et al., 2018) or cucumber mosaic virus (Chen et al., 2015).

Light-induced molecular changes may underline the increased GST activity following nocturnal red light treatment (Nagatani, 2004; Cheng et al., 2021). Since proteins encoding GSTs, UGTs, and Cytp450 are involved in the second phase of detoxification, the main activity of these enzymes is to catalyse conjugations that result in a less toxic compound ready to be transported to the vacuole (Nianiou-Obeidat et al., 2017; Gallé et al., 2019). The substrate preferences of certain wheat GST enzymes belonging to the phi and tau groups have been determined, allowing their role in stress responses to be inferred (Thom et al., 2002, Cummins et al., 2003). GSTs belonging to the phi and tau groups have significant GST enzyme activities. According to these data, the TaGSTU1B protein has a high conjugation activity towards 1-chloro-2, 4-dinitrobenzene (CDNB), benzyl isothiocyanate (BITC) and the stress metabolite analogue crotonaldehyde (Thom et al., 2002). *TaGSTU1B* mRNA levels were induced by drought in both tolerant and sensitive wheat cultivars, and transcript levels of this gene increased with senescence of flag leaf (Gallé et al., 2009). In seedlings, osmotic stress increased transcript levels mainly in roots of sensitive varieties (Galleé et al., 2009). Gene expression levels of *TaGSTU1B* were increased in flag leaves upon infection with *F. graminearum* and *F. culmorum*, which may be part of a systemic response in wheat (Galleé et al., 2009). In this study, *TaGSTU1B* expression showed notable response to both light and *F. graminearum* infection, which underlines the importance of this gene and may raise questions about the elements of its transcriptional regulation. The induction of *TaUGT4* by *F. graminearum* was consistent with other data: *TaUGT4* was strongly induced by *F. graminearum* or DON in both the FHB-resistant and susceptible cultivars (Ma et al., 2020). In terms of light activation, the transcript levels of several *GSTs* were increased by light in GK Arató, but these differences were only observed for one gene and to a lesser extent in the resistant cultivar. *TaUGT4* seems to be activated by both red light and *F. graminearum*. The transcriptional regulation of this gene has been linked to the salicylic acid and MeJA pathways as well (Ma et al., 2020), which phytohormones are highly light regulated (Czékus et al., 2021). Interestingly, the expression of *TaCytP450* is also induced by red light, but only in the more *Fusarium* sensitive GK Arató.

The light response of *TaGSTU1B* and *TaGSTF5* may come from variations in the flanking DNA sequence elements located upstream of the gene. Many light-responsive elements (LREs), such as GATA (GATA), sequences are overrepresented in light-induced promoters (SORLIP) (GCCAC), I-box (GATAA), and GT-1 (GRWAAW), and these have been proposed to confer light-mediated gene regulation (Khan et al., 2023); also these elements are present in the upstream sequence of *TaGSTU1B* and *TaGSTF5* in both cultivars (Supplemental Tables 2 and 3). Of the total twelve (*TaGSTU1B*) and ten (*TaGSTF5*) light-responsive elements, thirteen are linked to phytochrome and two to PHY A. Therefore, it seems likely that the expression of the *TaGSTU1B* gene is regulated by red light, which is mediated by phytochrome A (among others) and the transcription factors and *cis*-acting elements such as SORLIP, GT and GATABOX.

At the same time, certain elements are present only in the regulatory region of GK Arató *TaGSTF5*, and there aren't any functional regulatory elements at the same positions in GK Ígeret: GATA box and a Circadian (CAANNNNATC) expression regulator element. These elements may be responsible for the increased transcription of *TaGSTF5* in GK Arató compared to GK Ígéret.

It can be concluded that the decrease in key antioxidant enzymes and GSTs activity at night was partially compensated by a short-term application of red light at midnight in wheat ears, and the results are more visible in the expression levels of *GST* genes. Red light and Fusarium infection significantly increased the expression levels of various *GSTs* at dawn, depending on the Fusarium tolerance of the wheat genotypes. Among others, *TaGSTU1B*, *TaGSTF5* and *TaUGT4* are the most important genes that can be reactivated by red light at night and increase their expression in the dark at dawn. The promoter of the genes (based on consensus genome data) contains many light-dependent elements, (GATA- and -CAANNNNATC) and these may be responsible for the higher red light dependence of *TaGSTF5* in GK Arató. Based on the data, these *GSTs* is a promising candidate gene for regulating FHB resistance in wheat. Other studies using transgenic plants have confirmed that various *GSTs* play a crucial role in the defence reaction of plants against various fungi (Gullner et al., 2018). *Nicotiana benthamiana* plants silenced for *NbGSTU1* showed significantly higher susceptibility to *Colletotrichum destructivum* and greater colonization by the fungus compared with control plants (Dean et al., 2005). Among the GSTs, the Arabidopsis *gstf9* mutant was more susceptible to *Verticillium* wilt than wild-type plants (Gong et al., 2018). Moroever, the overexpression of *LrGSTU5*, which was isolated from Lilium regale Wilson, in *Nicotiana tabacum* resulted in increased resistance to *Fusarium oxysporum* infection (Han et al., 2016). In addition, the overexpression of *OsGSTU5* in rice also resulted in tolerance to sheath blight disease, which is caused by the necrotrophic fungus *Rhizoctonia solani* (Tiwari et al., 2020). This confirms the significant role of plant *GSTs* in the outcome of plant-fungus interactions.

The activation of GSTs by night illumination can be effective if GST detoxification is a relevant participant in the plant defence system (type III and possibly type II). The shift in GSH levels and oxidation balance caused by red light at night may influence several other defence processes. The protective role of GSTs in the elimination of fungal toxins such as DON or plant metabolites can influence the severity of the infection and their concentrations. Thus, the increased activity of antioxidant and detoxifying enzymes improves stress tolerance and grain filling efficiency, resulting in an increase in grain yield. Among the GSTs studied, *TaGSTF5* and *TaGSTU1B* were transcriptionally induced both

by *F. graminearum* infection and red light in a genotype-dependent manner. According to our results, night lighting can be effective in reactivating both antioxidant enzymes and GSTs as part of the plant defence system under FHB.

5. Conclusion

NB by light is a possible alternative and environmentally friendly method of reactivating plant defence mechanisms in the dark, when fungal pathogens can be more active. The use of a specific wavelength of light, such as red light, can act as a molecular regulator of gene expression. In the case of wheat ear fusariosis, the anthesis stage is one of the most critical for ear infection by *Fusarium sp*. At the same time, this developmental process and the potential application methods can be well planned. In this study, the effects of 15 min red light treatments on the ears of two selected wheat genotypes were investigated. Red light application was effective in alleviating *F. graminearum*-induced yield losses in the Fusarium resistant GK Ígéret. Red light significantly increased the activities of key antioxidant enzymes in the ears of this wheat genotype at the beginning of the dark period but the red light treatment only reactivated SOD and POD in the sensitive GK Arató. In addition, GST activity is also increased by red light, contributing to a more successful defence of the wheat plants. The results indicate that red light at night can reactivate and increase the expression of several *TaGSTs* in a genotype-dependent manner. The most significant changes were measured in GK Arató, where not only red light but also *F. graminearum* up-regulated of GSTs, especially *TaGSTF5* and *TaGSTU1B* contributing to the successful defence and reduced yield loss in this genotype. The extra GATA box and the circadian regulatory element (CAANNNNATC), which was found in the 5' flanking regulatory region of *TaGSTF5*, may play a major role in the measured expression pattern and the exceptionally high transcriptional activity of the *TaGSTF5* gene in the GK Arató variety. It can be concluded that nocturnal red light application can be an effective and well-planned method to reactivate plant defence and detoxification systems, such as antioxidant and GST enzymes, at dawn during the dark period, without significant energy cost and yield inhibition. However, the effectiveness of the red light exposure depends on the plant genotypes selected.

CRediT authorship contribution statement

Alina Pelsőczi: Writing – original draft, Investigation. **Edit Horváth:** Investigation. **Péter Körmőczi:** Investigation. **Beáta Tóth:** Investigation. **Jolán Csiszár:** Investigation. **Ádám Hajnal:** Investigation. **Péter Poór:** Writing – review & editing, Investigation, Conceptualization. **Ágnes Gallé:** Writing – review & editing, Investigation, Conceptualization.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eja.2026.128027.

Data availability

Data will be made available on request.

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