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Growth and glucosinolate profiles of *Eruca sativa* (Mill.) (rocket salad) and *Diplotaxis tenuifolia* (L.) DC. under different LED lighting regimes

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Abstract: In this study, the growth and glucosinolate (GSL) profiles of rocket salad *Eruca sativa* (Mill.) and *Diplotaxis tenuifolia* (L.) DC. were determined during 30 days growing under different lighting regimes; T5_ peak at 545 nm, LED1_ peak at 631 nm and LED2_ peak at 598 nm. The biggest increase of dry weight (DW) was measured in *E. sativa* under T5 (0.657 g DW/plant) and the lowest in *D. tenuifolia* under LED1 (0.080 g DW/plant). GSL content was found to vary significantly, regardless of the light treatment, but it is related with genotype (*E. sativa*, $r = 0.802^{**}$). On average, the highest amount of 4-methylsulfinylbutyl-GSL (glucosativin) (7.3248 mg/g DW) was quantified in *E. sativa* and *D. tenuifolia* (6.7428 mg/g DW) under the T5. The regression analysis between different light wavelengths and glucosinolates showed the strongest correlation between photosynthetic photon flux density (PPFD_B) and 4-methylthiobutyl-GSL (glucoerucin) in *E. sativa* ($r = 0.698^{*}$) and *D. tenuifolia* ($r = 0.693^{*}$), respectively, which indicates the effect of light on the response of plants to induced stress and changes in GSL biosynthesis.

Keywords: salad vegetables; antioxidant compounds; light; abiotic stress; phytohormone

Eruca sativa (Mill.), known as rocket (or arugula, rucola, roquette), and *Diplotaxis tenuifolia* (L.) DC. are vegetables considered to be increasingly important in the salad vegetable market in the areas surrounding the Mediterranean Sea (Pasini et al. 2012). Moreover, these plants contain biologically active antioxidant compounds such as glucosinolates (GSLs), which are induced upon pathogen recognition (Czerniawsky and Bednarek 2018).

GSLs are a group of sulphur- and nitrogen-containing secondary plant metabolites, which are classified on the origin of their side chain, and the main groups are aliphatic derived from alanine (Ala), leucine (Leu), isoleucine (Ile), valine (Val) and methionine (Met); benzenic derived from phenylalanine (Phe) or tyrosine (Tyr) and indolic derived from tryptophan (Trp), respectively (Figure 1) (Agerbirk and Olsen 2012).

GSLs biosynthesis is regulated by many different factors. Environmental regulation has been well-known (Grubb and Abel 2006) for decades, and it will be described later. In addition, great progress has been made in researching transcriptional regulation, e.g., the identification of subgroup 12 R2R3-MYB transcription factors and components acting upstream, including MYB28 and MYB29 (Hirai et al. 2007), basic nuclear-localised calmodulin-binding protein, IQ-domain1 (IQD1) (Levy et al. 2005), and ethylene-insensitive 3-like transcriptional

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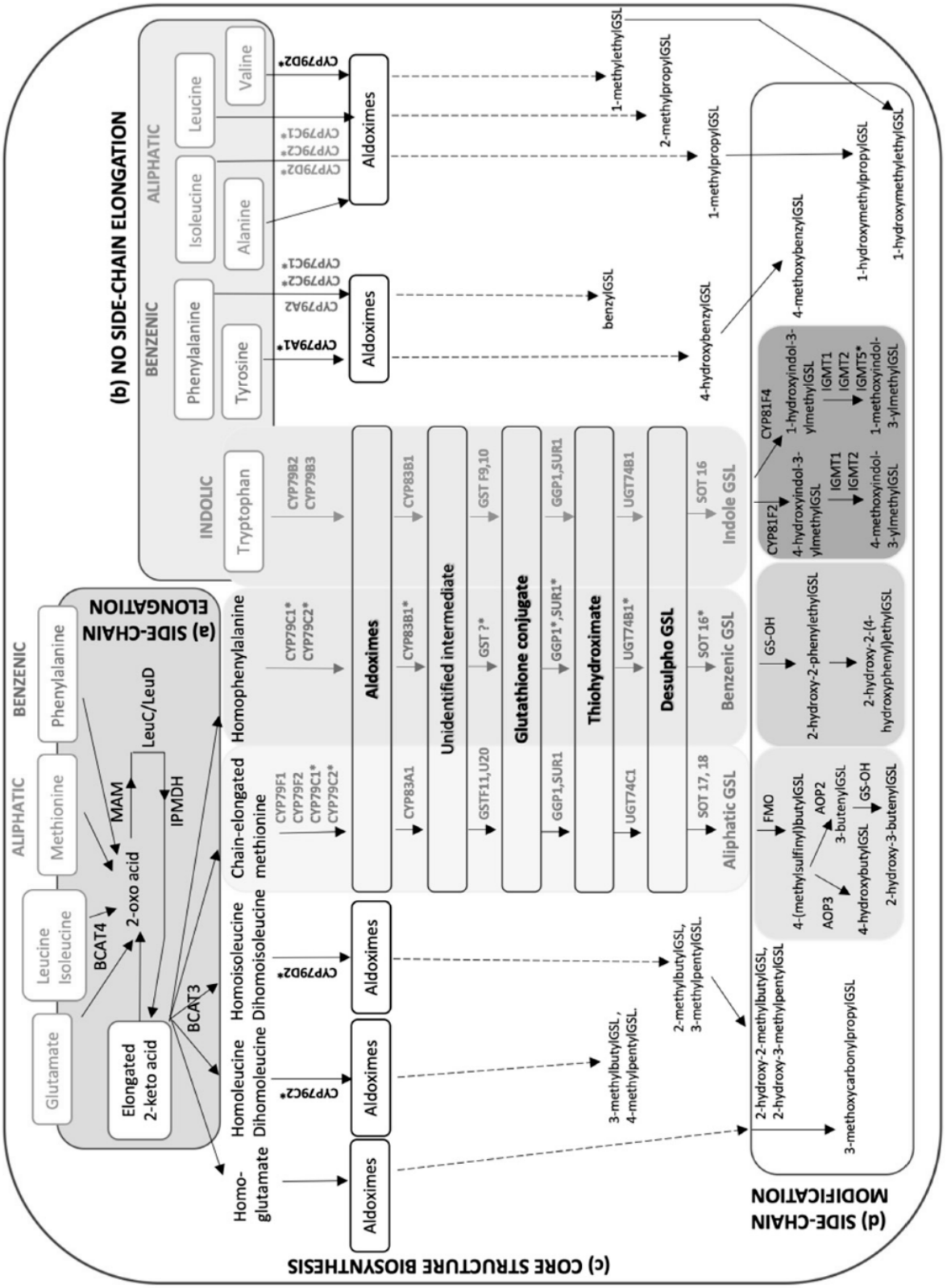

Figure 1. Biosynthetic pathways of aliphatic, indole, and benzenic glucosinolates (GSLs) (Chhajed et al. 2020)

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factor (SLIM1) (Maruyama-Nakashita et al. 2006). In *Brassica* species, this subgroup 12 consists of 55 MYBs that have been reported to play roles in glucosinolate biosynthesis (Seo and Kim 2017).

Transcriptional regulators controlling glucosinolate biosynthesis with MYB28, MYB76, and MYB29 transcription factors were found to regulate aliphatic glucosinolate biosynthesis in three different ways. First, Hirai et al. (2007) found that MYB28 and MYB29 are co-regulated with known genes in glucosinolate biosynthesis. Second, a quantitative trait loci (QTL) an analysis identified that MYB28 is located within a genomic region that determines aliphatic glucosinolate levels (Sønderby et al. 2007). Third, MYB28, MYB29, and MYB76 were identified in a screen for their transactivation potential toward biosynthetic genes of aliphatic glucosinolates (Gigolashvili et al. 2008). Overexpression of MYB28, MYB29, and MYB76 resulted in increased accumulation of aliphatic glucosinolates (but not indole glucosinolates) in leaves and suspension cells, increased expression of aliphatic glucosinolate biosynthetic genes, and repression of the indole glucosinolate pathway (Hirai et al. 2007). However, the aliphatic and indolic MYB factors have been shown to be regulated differentially in *Arabidopsis* plants by light cycling (Huseby et al. 2013), and thus, the transcription factor HY5 acts as a repressor of the aliphatic MYB factors and as an activator of indolic MYB factors.

Biosynthesis of indole glucosinolates starts with the conversion of tryptophan to indole-3-acetaldoxime by CYP79B2 and CYP79B3. CYP83B1 then catalyses the aldoxime to produce an uncharacterised intermediate, which undergoes sulfur incorporation and thiohydroximate formation through the activities of GSTF9, GSTF10, GGP1, and SUR1. In a similar manner to aliphatic glucosinolate biosynthesis, UGT74B1 is required for thiohydroximate glucosylation, and SOT16 is responsible for the sulfation step to produce intact indole glucosinolates. In terms of modifications, CYP81Fs catalyse hydroxylation of indole GSLs, e.g., CYP81F2 is responsible for the production of 4-hydroxyindole glucosinolate (Grubb and Abel 2006). In addition, CYP86A7 and CYP71B26 may be responsible for the hydroxylation of indole GSLs, especially at 1-position (Mostafa et al. 2017).

The hydroxyindole GSLs can be further metabolised to methoxyindole derivatives through indole glucosinolate methyltransferases 1 and 2 (IGMT1 and IGMT2) (Pfalz et al. 2011). In addition, methylation of 4-hydroxyindol-3-ylmethyl glucosinolate (4MI3G) is controlled by cytoplasmic protein phosphatase 2A regulatory subunit B'(PP2A-B'), which physically interacts with IGMTs and regulates the IGMT activities in catalysing the O-methylation at the 4-position (Rahikainen et al. 2017). Recently, it was reported that methylation of 1-hydroxyindol-3ylmethylglucosinolate can take place *via* indole glucosinolate-O-methyl transferase 5 (IGMT5) (Pfalz et al. 2016). Furthermore, the PP2A-B' may affect the catabolism of indole GSLs through direct regulation of the phosphorylation of myrosinase TGG1 involved in glucosinolate hydrolysis (Durian et al. 2016). Posttranslational modification analysis of glucosinolate metabolic enzymes is an interesting research direction.

As the tryptophan is simultaneously a substrate of growth auxin phytohormones (indole-3-acetic acid – IAA) and melatonin (stress pleiotropic phytohormones), the biosynthesis of indole directly related to the hormonal control of the physiological processes of plants on biotic and abiotic stress stimuli through the molecular genetic mechanisms of accumulation secondary plant metabolites (Koprivova and Kopriva 2016). So-called crosstalk between indole glucosinolate biosynthesis and those of IAA and camalexin has been well-studied (Yan and Chen 2007). IAA and camalexin are connected to indole glucosinolates through indole-3-acetaldoxime (IAOx) and indole-3-acetonitrile (IAN). When indole glucosinolate biosynthesis was blocked, increased IAA production from IAOx and IAN was observed (Malka and Cheng 2017). This is a classic example of metabolic flux and pathway channelling. Many years ago, the connection between glucosinolate biosynthesis and the phenylpropanoid pathway was hinted at by a study of *A. thaliana* reduced epidermal fluorescence2 (*ref2*, also known as cyp83a1) mutant (Hemm et al. 2003).

The environmental factors, such as light (Engelen-Eigles et al. 2006), temperature (Velasco et al. 2007) and drought (Radovich et al. 2005), may modify glucosinolate composition and effect their physiological role in response to abiotic stress.

Water stress increased the glucosinolate accumulation in *Brassica oleracea* L. var. *capitate* (Radovich et al. 2005); *Brassica oleracea* L. var. *italic* (Champolivier and Merrien 1996); *Brassica napus* L. (Jensen et al. 1996); *Brassica rapa* ssp. *rapifera* L. (Zhang et al. 2008); and *Brassica carinata* L. (Schreiner et al. 2009) in agreement with the prediction of the "protein competition model", where

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drought is expected to reduce some vegetative growth parameters with the subsequent increase of secondary metabolites at the expense of primary metabolism (Jones and Hartley 1999).

Although the induction of GSLs accumulation by drought conditions has been reported as part of the plant response to stress through the process of osmotic adjustment (Schreiner et al. 2009), contradictory results have been observed in the literature when high drought (30% of the amount of water received by well-watered plants) had no effect on the concentration of total GSLs in *Brassica oleracea* L. var. *gemmifera* (Brussels sprouts) (Gutbrodt et al. 2012) or in *Brassica napus* L. under mild drought stress (Jensen et al. 1996).

GSLs content also varies in response to temperature and light quality (Engelen-Eigles et al. 2006). The seasonal variation for the glucosinolate content in different *Brassica* sp., such as radish (Schreiner et al. 2002), oilseed rape (Petersen et al. 2019), turnip (Zhang et al. 2008) and cabbage (Charron and Sams 2004), has been reported. In these studies, it was observed that spring season conditions, such as moderate temperatures, low humidity, high light intensity and longer photoperiods, induced higher glucosinolate accumulation than autumn/winter season conditions. Thus, elevated temperatures have been shown to increase glucosinolate levels in *Brassica rapa* (Seo and Kim 2017), and a positive relationship between soil temperature and GSLs has also been documented in *Brassica oleracea* (Charron and Sams 2004). Interaction between temperature, solar radiation or plastic mulch properties may condition glucosinolate content in greenhouse or field experiments. In general, indole GSLs are more sensitive to elevated temperatures than aliphatic or aromatic GSLs (Bones and Rossiter 2006). Therefore, the contribution of each individual and specific glucosinolate to the variation of total glucosinolate levels by the temperature regime results in decisive importance.

Katsarou et al. (2016) showed nitrogen (N) and sulphur (S) supply had a significant and interactive effect on the GSL content of leaves, which were significantly correlated with the relative expression of the genes involved in their biosynthesis. Moreover, under the same conditions, genes associated with aliphatic GSLs biosynthesis (EsMAM-1, EsCYP79F1, EsCYP83A1, EsSUR1 and EsFMOGSox-5) in plant leaves were strongly suppressed, leading to a reduced aliphatic GSLs abundance. The positive correlation calculated between the relative expression of these genes and aliphatic GSLs content further supports the hypothesis of the direct regulation of biosynthesis and transcription of GSL by N and S availability. S supply has a stronger effect on the relative expression of genes associated with aliphatic GSLs content since under these conditions (-N/-S), their relative expression was significantly lower compared to all other treatments. This was not the case for indole GSLs biosynthesis, so no significant correlation between the metabolite content and the relative expression of the associated genes (EsCYP79B2, EsCYP79B3 and EsCYP83B1) was found.

Despite the link that seems to exist between light and the biosynthesis of GSL, their total content often fluctuates more than the gene expression, and elevated levels can be seen during the dark period when the genes have low expression levels (Schuster et al. 2006). In addition, the closely related sulphate assimilation is also regulated by light. In *Brassicaceae* microgreens, PPFD-B increased xanthophyll, β-carotene contents and lutein synthesis (Brazaitytė et al. 2015). Enhanced blue light (400–500 nm) might remarkably increase the biosynthesis of phenolic compounds, as well as epidermal flavonoids (Hoffmann et al. 2015). In their recent study, Zhuang et al. (2022) showed the correlation between the expression levels of genes related to aliphatic glucosinolates biosynthesis (CYP79F1, CYP83A1, UGT74B1 and FMOGS-OX1) and yellow, blue and purple LED lights treatments.

The effect of light on the response of plants from the *Brassicaceae* family in relation to induced stress and changes in GSL biosynthesis was researched in several studies; however, contradictory results were often revealed. For instance, Kopsell et al. (2015) reported that different RB LED light treatments of 5% blue (470 nm)/95% red (630 nm), 5% blue/85% red/10% green (530 nm), and 20% blue/80% red at an intensity of 250 ± 10 mmol/m^2/s cause significantly higher individual and total aliphatic and total indole GSLs in broccoli (*Brassica oleracea* var. *italica*) grown under a fluorescent light treatment. Tan et al. (2020) showed that the growth of choy sum (*Brassica rapa* subsp. *chinensis* var. *parachinensis*) under 160 µmol/m^2/s red-blue (160RB) LED light produced the highest shoot fresh weight (FW) and dry weight (DW) for all three growth stages tested (i.e., one-leafed seedlings, three-leafed seedlings and adult plants). Furthermore, plants exposed to 160 µmol/m^2/s red-blue LED lights contain significantly higher glucoerucin compared to those having undergone

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a white light treatment with equal photosynthetically active radiation (PAR).

On the other hand, Signore et al. (2020) showed that fresh yield and dry matter of *E. sativa* and *D. tenuifolia* are influenced primarily by nitrogen level, although red light increased yields with respect to blue and red + blue. Contrary, in *D. tenuifolia*, the red component of the light alone or mixed with blue (in *E. sativa*) increased the GSLs content.

However, while the influence of light is well established and the data have been obtained in different *Brassica* species in various growth conditions, very little is known about the regulation of GSL biosynthesis with different light spectrum.

The main purpose of our study was, therefore, to investigate the influence of photosynthetic photon flux density (abiotic stress) on the yield and profile of GSLs after 30 days of growing of rocket salad *E. sativa* (Mill.) and *D. tenuifolia* (L.) DC. under different LED lighting since those two species are most commonly grown indoors and accepted most among consumers in the Mediterranean countries.

MATERIAL AND METHODS

Plant materials and growing conditions. Seeds of two species of rocket *E. sativa* (Mill.) and *D. tenuifolia* (L.) DC. were evenly sown by hand in germination trays (54 cm × 27 cm × 6 cm) with BIO potgrond mix (Klasmann-Deilmann GmbH, Geeste, Germany). Approximately 1.4 g of *E. sativa* seeds were sown in each germination tray before 3 L of tap water was added. Subsequently, each tray was watered every two days with 1 L of tap water through sub-irrigation. The plants were not thinned out after sowing in trays.

Germination took place in a dark chamber (temperature: 20 °C) for 3 days. Then, the plastic film was removed, and plants were grown indoors at 25 ± 2 °C/22 ± 2 °C (light/dark) and 65 ± 5% relative humidity under three different light regimes; T5 – as a standard light (LUMii, 60 cm, 87 W supplied by EnviroGro, LED1 (TXVSO 600W LED Grow Light, unknown supplier) and LED2 (Samsung LM301B, 80W supplied by EasyGrow S600). Each light was applied in a separate controlled growing chamber 20 cm above the plants in a photoperiod of 14/10 h (day/night) in three repetitions, as represented in Table 1. The plants were sampled randomised within the trays after 15 and 30 days.

Table 1. Different combinations of *Eruca sativa* and *Diplotaxis tenuifolia* and light were investigated in the experiment

Treatment	
T5	
LED1	*E. sativa*
LED2	
T5	
LED1	*D. tenuifolia*
LED2	

Light characterisation. The spectral characteristics expressed as photosynthetic photon flux density (PPFD); PPFD-UV, PPFD-B, PPFD-G, PPFD-R and PPFD-FR per (μmol/m^2/s) were determined using a light spectrometer (UPRtek AI-MK350D, Hunan, Taiwan).

The spectral output of all lights is visualised in Figure 2, where it can be seen that most energy packets arrive in different wavelength bands with peaks at

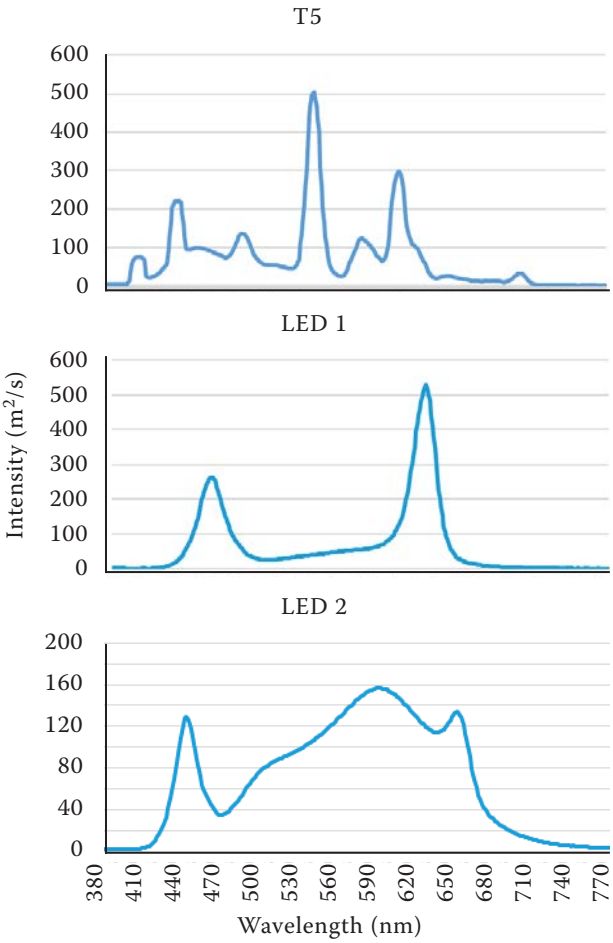

Figure 2. The spectral output of T5, LED1 and LED2 lights installed in the grow-cell

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545 nm (T5), 631 nm (LED1) and 599 nm (LED2). Secondly, a broad wavelength PPFD expressed as PPFD_B, PPFD_G and PPFD_R (Table 2) showed differences in distribution between the lights in the area 0.2 m directly below and adjacent to the light source. The T5 has the biggest PPFD output in the PPFD_G band (54.85 μmol/m^2/s), while LED1 and LED2 have it in the PPFD_R band (73.13 μmol/m^2/s, 54.69 μmol/m^2/s, respectively). In contrast, LED2 shows practically the same PPFD_G as well as the PPFD_R band (52.53 μmol/m^2/s and 54.69 μmol/m^2/s, respectively).

Biomass and growth parameter analyses. For growth assessment, the total fresh and dry weight measurements of individual plants were recorded 30 days after seeding. Twenty-five plants (n = 25) per treatment were randomly selected from each growing tray at both intervals and destructively sampled for biomass analysis.

All solvents and chemicals used were of LC-MS grade and obtained from Sigma-Aldrich (Poole, UK) unless otherwise stated.

Glucosinolate extraction. The described method was reported by Jin et al. (2009) and Pasini et al. (2012). The content of GSL was determined using ~50 mg of frozen plant material with three experimental replicates. Each sample was prepared as a precautionary measure to inactivate as much myrosinase enzyme as possible before liquid extraction. After the samples were put into a drier (70 °C), they were centrifuged (10 000 rcf, 18 °C) to collect loose material into a pellet. Two mL of supernatant was then taken and again poured with preheated 70% (v/v) methanol (70 °C) for the second centrifugation, followed for 10 min. The second supernatant was adjusted to 1 mL with 70% (v/v) methanol and frozen at 80 °C until analysis by LC-MS.

Determination of GSLs. Individual GSLs were determined on HPLC (high-performance liquid chromatography) in coupled by detectors PDF-EIS-MS (photodiode array-electrospray ionisation-mass spectrometer) controlled by CromQuest 4.0 chromatography workstation software (Thermo Scientific, Waltham, USA).

Table 2. The difference in photosynthetic photon flux density (PPFD) spectrum between different lights (μmol/m^2/s)

PPFD	T5 (87 W)	LED1 (78 W)	LED2 (80 W)
PPFD-UV	0.48	0.05	0.17
PPFD-B	36.07	30.98	17.36
PPFD-G	54.85	21.07	52.53
PPFD-R	34.15	73.13	54.69
PPFD-FR	3.02	0.75	0.85
Total	125.86	125.98	125.60

The concentration of individual GSLs was based on UV absorption at 229 nm and calculated according to response factors, so the contents were expressed in μmol equivalents of sinigrin per g DW. Sinigrin standard was acquired from Sigma-Aldrich. Identification was done by liquid chromatography-mass spectrometry (LC-MS), using atmospheric pressure chemical ionisation and the +H+ molecular ion. The following GSL were identified: 4-methylthiobutyl-GSL (glucoerucin), 4-methylsulfinylbutyl-GSL (glucoraphanin), 3-indolylmethyl-GSL (glucobrassicin), 4-mercaptobutyl-GSL (glucosativin) and 4-(β-D-glucopyranosyldisulfanyl) butyl-GSL (diglucothiobeinin).

Statistical analyses. All statistical analyses were performed using IBM SPSS Statistics 25 (IBM 2020), using a two-way analysis of variance (ANOVA) for the interaction effects of factors on the dependent parameter. When an interaction effect was not confirmed, one-way ANOVA was used for interpreting the effects of each individual factor. The differences in the content levels were estimated by Duncan's test. P-values of less than 0.05 were considered statistically significant. Effects of the light treatments on plant characteristics and GSL content were analysed using generalised linear mixed models (GLMM) followed by Tukey post hoc tests.

RESULTS AND DISCUSSION

Glucosinolate identification and concentration. Table 3 lists the concentrations of different GSLs in the shoots of *E. sativa* and *D. tenuifolia* after 30 days of growing. On average, 93.2% of the total GSL concentration represents 4-mercaptobutyl-GSL (glucosativin) (Figure 3). However, significant differences were identified between the biggest amount (7.3248 mg/g DW) quantified in *E. sativa* under T5 and LED2 (6.9820 mg/g DW), respectively, and the lowest identified in *D. tenuifolia* under LED1 (4.9325 mg/g DW), which is contrary to results from Tan et al. (2020), who reported concentrations 4 times higher in young choy sum plants compared to adult ones.

Contrary, our data is very close to the SR5 accession of *E. sativa* described by Bell et al. (2015), which amounts

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Table 3. Effect of different LED lights on the concentration of specific glucosinolates (GSLs) detected in the shoots

Treatment	Glucoraphanin	Glucothiobeinin	Glucosativin	Glucoerucin	Glucobrassicin
	(mg/g DW)				
T5 *Eruca sativa*	0.0096^{e}	0.0957^{a}	7.3248^{a}	0.2744^{d}	0.0079^{d}
LED2 *E. sativa*	0.0169^{b}	0.0676^{b}	6.9820^{b}	0.4769^{a}	0.0051^{e}
T5 *Diplotaxis tenuifolia*	0.0298^{a}	0.0629^{b}	6.7428^{bc}	0.3168^{c}	0.0755^{a}
LED1 *E. sativa*	0.0163^{b}	0.0646^{b}	6.6002^{bc}	0.3838^{b}	0.0085^{d}
LED2 *D. tenuifolia*	0.0152^{c}	0.0652^{b}	5.2496^{c}	0.4733^{a}	0.0479^{b}
LED1 *D. tenuifolia*	0.0135^{d}	0.0465^{c}	4.9325^{d}	0.2142^{e}	0.0225^{c}

^{a,b,c}significant at $P < 0.05$ (Duncan's test); DW – dry weight

on average to 7.7 ± 0.8 mg/g DW when grown under a light intensity of 200 µmol/m^2/s for 16 h. This is approximately 50 µmol/m^2/s more than in our experiment; unfortunately, no detailed information about different PPFDs was provided. On the other hand, our results are much higher than the proportions presented in previous studies reported by Pasini et al. (2012).

Table 4 represents the correlations between GSLs and the DW in shoots of *E. sativa* and *D. tenuifolia*, respectively. As seen in both species, the LED1 has the biggest effect on the amount of GSL. In *E. sativa*, the strongest correlation (0.888**) was estimated for 3-indolylmethyl-GSL (glucobrassicin), while in *D. tenuifolia*, it was (4-methylthiobutyl-GSL) glucoerucin (r = 0.693*). The regulation of the content of individual GSL in both compared species indicates differences in the selection pressure of both species, which manifested itself in different phenotypes, which are determined by the regulation of GSLs gene expression, and which are a response to environmental conditions – to light radiation.

Contrary to total light emissions, the influence of specific PPFD can only be determined for a particular GSL (Table 5). In *E. sativa* the strongest correlation (r = 0.836**) was estimated between PPFD_R and 4-methylsulfinylbutyl-GLS (glucoraphanin) and in *D. tenuifolia* between PPFD_B and 4-methylthiobutyl-GSL (glucoerucin) (r = 0.693*), respectively.

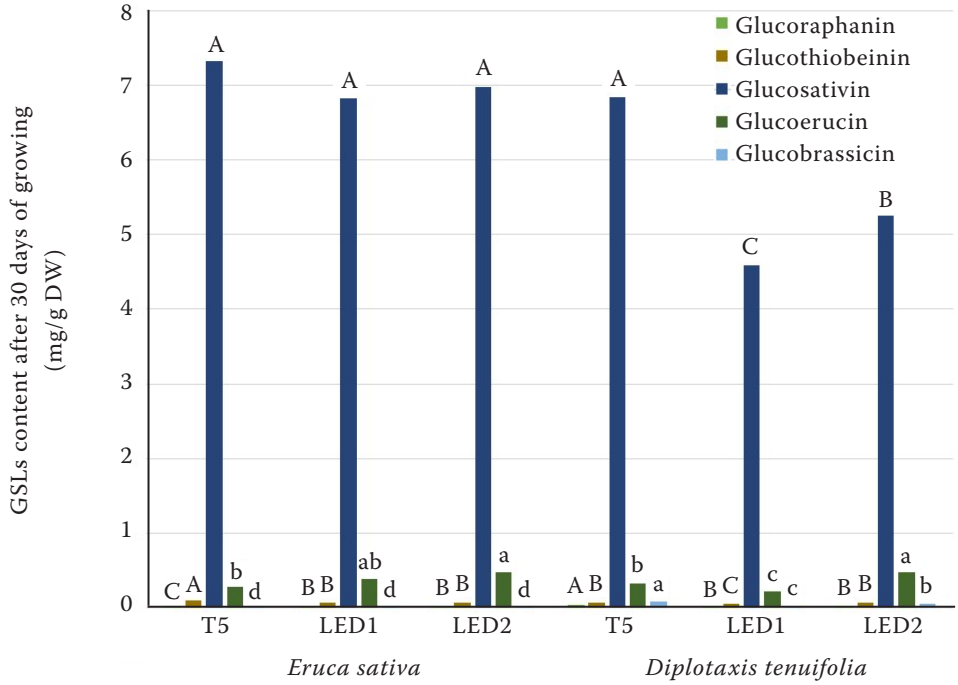

Figure 3. Glucosinolates (GSLs) content after 30 days of growing under different light regimes. ^{a,b,c}significant at $P < 0.05$ (Duncan's test); DW – dry weight

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Table 4. Correlation coefficients between total dry weight (DW) and glucosinolates (GSLs) in shoots of *Eruca sativa* and *Diplotaxis tenuifolia*

Treatment	Glucoraphanin	Glucothiobeinin	Glucosativin	Glucoerucin	Glucobrassicin
	(mg/g DW)				
LED1 *E. sativa*	0.678*	0.378	0.488*	0.688**	0.888**
LED2 *E. sativa*	0.678*	0.378	0.483*	0.583*	0.703**
T5 *D. tenuifolia*	0.678*	0.478*	0.385	0.485*	0.585*
LED1 *D. tenuifolia*	0.467	0.367	0.870**	0.670**	0.370
LED2 *D. tenuifolia*	0.469*	0.366*	0.869*	0.769*	0.769**
T5 *E. sativa*	0.501*	0.469*	0.766*	0.769**	0.469*

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

These findings are very close to those from Tan et al. (2020), who showed the correlation between RB light and growth stage on one hand, and 4-methylthiobutyl-GSL (glucoerucin) content on the other, but they summarised that the overall level of GSL is affected by the interaction between environmental and genetic factors.

As seen from both Tables 4 and 5, the correlation between specific PPDF and GSLs in the DW differs from species to species meaning that the overall changes in the regulation vary for aliphatic and indolic GSLs. In *E. sativa* and *D. tenuifolia*, the PPFD_B might be involved in the biosynthesis of aliphatic GSLs but not in indolic GSL (3-indolylmethyl-GSL, glucobrassicin). GSL biosynthesis was regulated by light and HY5, a transcriptional factor functioning downstream of phytochromes, cryptochromes and PPFD_B, which regulated other transcriptional factors involved in aliphatic and indolic GSLs biosynthesis (Huseby et al. 2013). Therefore, the fact that PPFD_B accelerated GSLs accumulation in *E. sativa* and *D. tenuifolia* would be the result of up-regulated gene expression in aliphatic GSL synthesis through the association of the PPFD_B photoreceptors and function of HY5. Moreover, as suggested by Zhuang et al. (2022), the expression levels of genes related to aliphatic glucosinolates biosynthesis, especially CYP79F1, CYP83A1, UGT74B1 and FMOGS-OX1, were dramatically upregulated by PPFD_B in broccoli sprouts. Thus, 4-methylthiobutyl-GSL (glucoerucin) contents in *E. sativa* and *D. tenuifolia* PPFD_B could also be significantly higher than those in other treatments.

On the other hand, the biosynthesis and quantity of 3-indolylmethyl-GSL (glucobrassicin) is more likely to be connected with the N and S applied into the growing substrate as the genes associated with indolic GSL biosynthesis followed a different expression pattern in plants. According to Katsarou et al. (2016), the expression of genes encoding cytochromes CYP79B2 and CYP79B3 were both significantly affected by the implemented N and S treatments. The second explanation for the differences in the content of 3-indolylmethyl-GSL might be the presence of the specific pathogen, which according to Frerigmann et al. (2016), can activate MYB51 and WRKY transcription factors and consequently increase indole glucosinolates, which are important in plant resistance.

Table 5. Correlation coefficients between different photosynthetic photon flux density (PPFD) and glucosinolates (GSLs) concentrations in *Eruca sativa* and *Diplotaxis tenuifolia* shoots

Treatment	Glucoraphanin	Glucothiobeinin	Glucosativin	Glucoerucin	Glucobrassicin
	(mg/g DW)				
PPFD_B *E. sativa*	0.230	0.006	0.092	0.698*	0.739*
PPFD_G *E. sativa*	0.473	0.013	0.126	0.032	0.435*
PPFD_R *E. sativa*	0.836**	0.023	0.250	0.356	0.045
PPFD_B *D. tenuifolia*	0.162	0.049	0.112	0.693*	0.017
PPFD_G *D. tenuifolia*	0.024	0.026	0.005	0.072	0.023
PPFD_R *D. tenuifolia*	0.054	0.017	0.002	0.025	0.046

*$P < 0.05$; **$P < 0.01$; DW – dry weight

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Table 6. Effects of different LED lighting on shoot fresh weight (FW)

Treatment	Total FW	Root FW	Leaf FW	Stem FW
		(g)		
T5 *Eruca sativa*	10.809^{a}	0.478^{ab}	10.155^{a}	0.176^{c}
LED2 *Diplotaxis tenuifolia*	7.898^{b}	0.577^{ab}	6.528^{b}	0.793^{a}
LED1 *E. sativa*	7.695^{b}	0.259^{b}	7.270^{b}	0.166^{c}
LED2 *E. sativa*	6.968^{b}	0.254^{b}	6.486^{b}	0.228^{c}
LED1 *D. tenuifolia*	5.842^{bc}	0.672^{a}	4.643^{bc}	0.527^{b}
T5 *D. tenuifolia*	2.413^{c}	0.046^{c}	2.320^{c}	0.047^{d}

^{a,b,c}significant at $P < 0.05$ (Duncan's test)

Growth assessment of *E. sativa*/*D. tenuifolia* in response to different light regimes. The biggest total content of FW was measured in *E. sativa* and *D. tenuifolia* under LED1 light (Table 6), whereby the average FW of *E. saliva* was significantly greater than those for *D. tenuifolia*. When comparing the plant parts that are of commercial interest (leaves and stems), the biggest fresh yield (10.331 g) was again measured in *E. sativa* under T5 light (Figure 4). Unlike in *D. tenuifolia*, these findings do not

Figure 4. *Eruca sativa* (upper images) and *Diplotaxis tenuifolia* (bottom images) plants after 30 days of growing under (A) T5, (B) LED1 and (C) LED2

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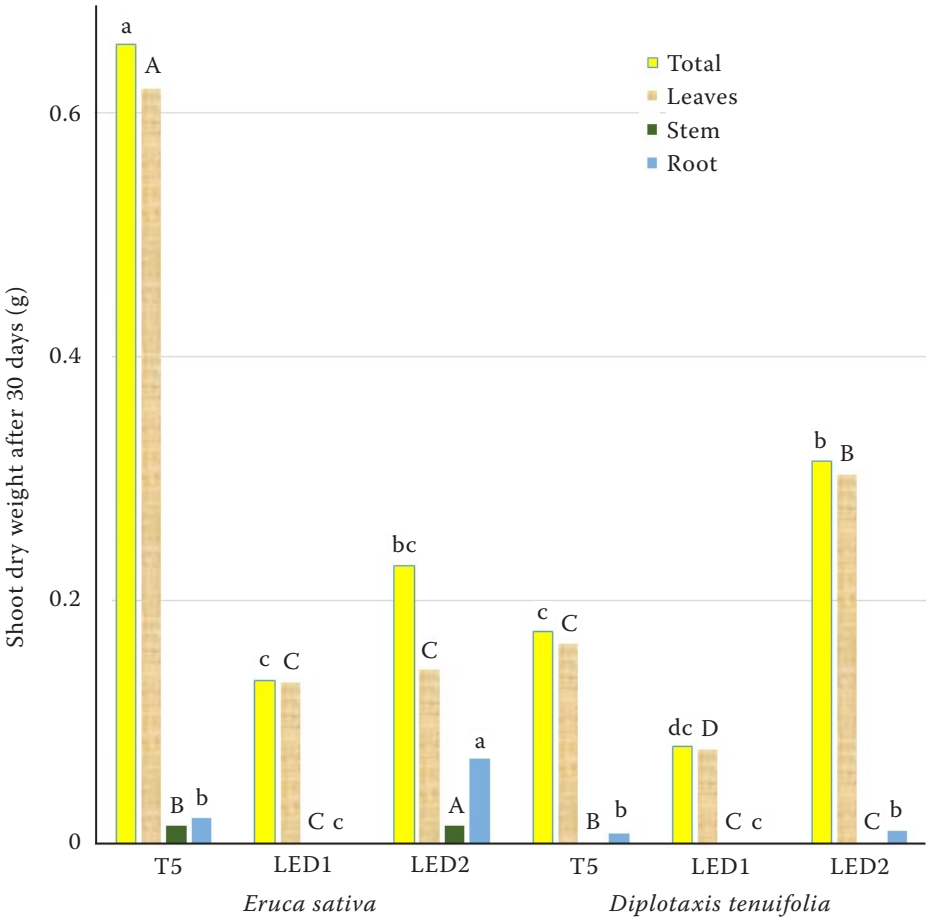

Figure 5. Effects of different LED lighting on dry weight (DW) after 30 days of growing. [a,b,c]significant at $P < 0.05$ (Duncan's test)

correspond with those of Jamal et al. (2021), who reported a significant effect of RB LED light on shoot growth in *E. sativa*. On the other hand, Kopsell et al. (2015) found no positive effect for the blue light on mustard (*Brassica juncea* L.) microgreens as well.

Figure 5 represents the content of dry matter (DM) in particular plant parts. As seen, the biggest total yield of DM was measured in *E. sativa* (0.657 g) under T5, whereby the majority of the DW was found in the leaves. On the contrary, the smallest DW was measured in *D. tenuifolia* under LED1 (0.080 g). Contrary to the results of fresh weight, the percentage of DM depends on the part of the plant, and species (Table 7), meaning that on average, *E. sativa* (10.28%) contains significantly more DM as compared with *D. tenuifolia* (8.45%). However, different light affects the content of DW differently, i.e. in *E. sativa* (13.40% in leaves and 13.55% in stem) was estimated under the LED2 light and in *D. tenuifolia* (6.29% leaves, 6.78% stem) under the LED1 light, respectively.

Table 7. Effects of different LED lighting on the percentage of dry weight (DW)

Treatment	Total after	Root after	Leaves after	Stem after
	(%)			
T5 *Eruca sativa*	13.98[a]	17.07[a]	13.40[a]	13.55[a]
T5 *Diplotaxis tenuifolia*	10.76[b]	17.85[a]	10.48[b]	12.82[a]
LED2 *D. tenuifolia*	8.50[c]	8.66[b]	8.54[c]	8.69[b]
LED2 *E. sativa*	8.49[c]	8.11[b]	9.04[b]	8.08[b]
LED1 *E. sativa*	8.38[c]	8.49[b]	8.84[bc]	8.08[b]
LED1 *D. tenuifolia*	6.11[d]	6.88[c]	6.29[d]	6.78[c]

[a,b,c]significant at $P < 0.05$ (Duncan's test)

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Table 8. Correlation coefficients between different photosynthetic photon flux density (PPFD) wavelengths and dry weight (DW)

Treatment	Leaves	Stem	Roots	Total
PPFD_R *Eruca sativa*	0.394*	0.038^{ns}	0.350*	0.380*
PPFD_G *E. sativa*	0.451*	0.245^{ns}	0.552**	0.434*
PPFD_B *E. sativa*	0.686***	0.188^{ns}	0.742***	0.661***
PPFD_R *Diplotaxis tenuifolia*	0.526**	0.005^{ns}	0.506**	0.521**
PPFD_G *D. tenuifolia*	0.478*	0.189^{ns}	0.487*	0.488*
PPFD_B *D. tenuifolia*	0.792***	0.158^{ns}	0.785**	0.796**

*P < 0.05; **P < 0.01; ***P < 0.001; ns – not significant

These results are in contrast to findings from Signore et al. (2020), who reported no difference between the DM content of *E. sativa* and *D. tenuifolia*. This was probably affected by different genotypes (verities) and growing conditions.

The correlation between the PPFD wavelengths and DW of plant parts is represented in Table 8. The strongest correlation with total DW was found with the PPFD_B wavelength in both species, *E. sativa* (r = 0.661***) and *D. tenuifolia* (r = 0.796**), respectively. The same was found out for the leaves and roots DW, while there was no correlation with the stem.

In *D. tenuifolia*, the PPFD_R wavelength correlated considerably (r = 0.521**) and more strongly with total DW than in *E. sativa* (r = 0.380*). On the other hand, PPFD_G showed the weakest correlation for total DW in *D. tenuifolia* (r = 0.488).

Our results are very difficult to compare with other studies due to different species and higher lighting intensity as well as quality, which varies considerably among particular experiments. Generally, the effect of PPFD_B and PPFD_R on *D. tenuifolia* corresponds with those from Tan et al. (2020), who reported positive LED light intensity and spectrum effect on the growth of choy sum under 160 μmol/m^2/s as compared to our light intensity (~30 μmol/m^2/s).

We believe that crosstalk between indole glucosinolate biosynthesis and those of IAA (Yan and Chen 2007) in case of increased IAA production from IAOx and IAN, when indole glucosinolate biosynthesis was blocked, is the main reason for the low concentrations of 3-indolylmethyl-GSL (glucobrassicin). Thus, higher IAA expression and, subsequently highest content of FW and DW was measured in *E. sativa* under T5 (0.657 g DW/plant) and the lowest in *D. tenuifolia* under LED1 (0.080 g DW/plant).

As the content and regulation of individual GSLs in both compared plants is different, at the moment, this feature might be used for the taxonomic evaluation of differences between species in connection with their exposure to stress conditions.

Since there is still the unclear role of specific PPFD wavelengths (abiotic stress) in the biosynthesis of particular aliphatic GSLs, future research should involve the application of monochromatic LED light, with which one could investigate the expression of genes necessary for the biosynthesis of both phytohormones and glucosinolates more precisely.

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