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High blue light improves acclimation and photosynthetic recovery of pepper plants exposed to UV stress

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ABSTRACT

Intensity of photosynthetic active radiation (PAR) plays an important role in the acclimation of plants to UV radiation. Thereby, specific morphological and physiological characteristics influenced by high irradiance are also affected by blue light. With this background we conducted two experiments to evaluate the impact of light intensity and the relevance of blue light for the acclimation of pepper plants to UV. In this context we hypothesized that higher amount of blue light in the PAR spectrum significantly improves the plant acclimation and recovery to UV radiation. Our results demonstrate that UV stressed plants cultivated either under the higher light intensity (PAR 300 μmol m^{-2} s^{-1}) or under higher amount of blue light (62%) show better photosynthetic performance (i.e., higher photosynthetic rate (Pn), higher maximal photochemical efficiency of PSII (*Fv*/*Fm*) and lower non-photochemical quenching (NPQ)) than UV stressed plants grown under lower light intensity (PAR 100 μmol m^{-2} s^{-1}) or lower amount of blue light (30%). Contents of chlorophyll *a* and *b*, as well as carotenoids, had a stronger decrease due to UV in those plants cultivated either under the lower light intensity or under the lower amount of blue light. In contrast, plants grown either under 300 μmol m^{-2} s^{-1} or 62% blue light accumulated more epidermal flavonols. Analogous to the well described effects of high PAR intensity, we demonstrate here that high amount of blue light triggers specific biochemical and physiological processes resulting in better acclimation and recovery of plants to UV stress.

1. Introduction

The effects of UV on plant development, morphology and physiology have been intensively studied and summarized in several reviews (e.g., Teramura, 1983; Stapleton, 1992; Jordan, 1996; Mackerness, 2000; Frohnmeyer and Staiger, 2003; Kakani et al., 2003; Vass et al., 2005; Jenkins, 2009; Schreiner et al., 2012; Hideg et al., 2013). In general, energy-rich UV radiation leads to the generation of free radicals which damage DNA, proteins, membrane lipids and the photosynthetic machinery including chloroplasts and the degradation of photosynthetic pigments (a detailed review is presented by Hideg et al., 2013). In the sum, photosynthesis is impaired leading to a decrease in biomass accumulation (Smith et al., 2000; Kakani et al., 2003).

The damaging potential of UV irradiation forces plants to adapt to high energy fluxes. Typical morphological adaptations comprise the lower leaf area (Teramura, 1983) and higher leaf mass per area (LMA) resulting in lower penetration of UV light in the deeper layers of the tissue (see reviews of Teramura, 1983 and Kakani et al., 2003). Acclimation processes include also the accumulation of secondary metabolites in the tissues, particularly in the epidermal layer (Müller et al., 2013). Of particular note are flavonoids and hydroxycinnamic acids that screen UV radiation and shield the underlying tissues (Olsson et al., 1998; Cerovic et al., 2002; Falcone Ferreyra et al., 2012). Finally, the susceptibility of plants to UV strongly depends on their acclimation- and recovery-capacity which are also strongly influenced by the growth conditions (Ziska et al., 1992).

In general, plants cultivated under elevated intensities of PAR are better adapted to UV than plants cultivated under lower intensities of PAR (Walters, 2005). This phenomenon was elucidated very early, more than 30 years ago. For example Teramura (1980) found that soybeans cultivated under low PAR regimes were more affected by UV-B light, as shown by a stronger reduction in

Abbreviations: Abs., absorbance; Anth, anthocyanins; AOIs, areas of interest; BE, biologically effective; Car, carotenoids; CHS, chalcone synthase; Chl, chlorophyll; Chl-index, chlorophyll index; das, days after sowing; DW, dry weight; Flav, flavonoids; FLAV-index, flavonol-index; Fm, maximum chlorophyll fluorescence; Fo, ground chlorophyll fluorescence; *Fv*/*Fm*, maximum photochemical efficiency of the photosystem II; FW, fresh weight; LED, light emitting diodes; LMA, leaf mass per area; NPQ, non-photochemical quenching; n.s., non significant; PAR, photosynthetic active radiation; Pn, net photosynthetic rate; UV, ultraviolet.

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biomass. Also, classical works demonstrating the structural and functional adaptations of sun and shade leaves to UV-radiation (e.g., Lichtenthaler et al., 1980) contribute for a better understanding of the role of light intensity.

Apart from the impact of light intensity recent studies indicate that the light quality also regulates a variety of pathways as related to plant development, and might also influence certain acclimation processes. Photoreceptors such as the UV-A/blue light receptors cryptochrome and phototropin or the red/far red light receptor phytochrome perceive specific wavelengths and trigger morphological and functional adaptations at chloroplast and plant level (Banerjee and Batschauer, 2005; Taulavuori et al., 2005; Nagatani, 2010). As indicated, blue light (400–500 nm) can initiate plant responses and induce leaf characteristics that also develop under high irradiance (Hogewoning et al., 2010). Amongst others, chloroplast movement was induced by enhanced light intensities as well as by increased percentage of blue light, a process mediated by the phototropin (UV-A/blue light receptor) related NPL1 gene that controls the chloroplast relocation (Jarillo et al., 2001; Kagawa et al., 2001; Banás et al., 2012; Wada, 2013). In addition, experiments with barley and radish seedlings have shown that formation of sun type chloroplasts, which typically happens under high light conditions, can also be initiated at low intensities of blue light (Buschmann et al., 1978; Lichtenthaler and Buschmann, 1978). Similarly, the biosynthesis of phenolic compounds such as UV-screening flavonoids depends on both light intensity and light quality (Taulavuori et al., 2013). Cryptochrome and phytochrome photoreceptors are involved in the induction of CHS gene expression that leads to the formation of chalcone synthase (CHS) which is the first step in flavonoid biosynthesis (Feinbaum et al., 1991; Wade et al., 2001).

Up to now, the impact of light quality on the acclimation of plants to abiotic stress factors has hardly been taken into account. Nowadays LEDs providing 'consumer-tailored' light can be used as light sources for the commercial cultivation of horticultural crops in controlled and semi-controlled environments (Morrow, 2008). In parallel, basic and applied research evaluate the impact of light quality on plant development and physiology, more recently using LEDs as light sources. Thereby, most studies focus on the impact of light composition on biomass production and photomorphogenesis (e.g. germination, growth habit, flower production) (Brown et al., 1995; Carvalho et al., 2011; Abidi et al., 2013). Already about 20 years ago Adamse et al. (1994) demonstrated that supplemental blue light alleviates UV-B induced growth inhibition in cucumber. In a more recent study it is suggested that in diatoms the perception of blue light might be of central importance for high light acclimation. (Schellenberger Costa et al., 2013). However, besides the relevance of this topic, this research field remains widely underexplored.

In the present study we investigate the relevance of blue light for the UV acclimation of pepper plants (*Capsicum annuum* L.). Our studies base on the hypothesis that higher amount of blue light in the light spectrum induces similar structural and functional adaptations at chloroplast, leaf and plant level as observed for high light, resulting in lower susceptibility to UV. Non-destructive methods (fluorescence and gas exchange measurements) as well as biochemical indicators and biometric parameters were adopted to evaluate the effects of light intensity, light quality and UV.

2. Material and methods

2.1. Plant material, growth conditions and experimental setup

Two experiments were conducted in order to evaluate the photosynthetic acclimation of pepper plants as affected by different light intensities and light qualities. The experiments were performed in a custom-built climate chamber that can be divided in up to six light compartments. During pre-cultivation, plants were illuminated with white fluorescence lamps (Master PL-L 4P, Philips, The Netherlands). As the different light treatments were initiated light emitting diodes (LEDs) from a prototype optimized for our research purposes (Ushio Lighting Inc., Japan) were used for illumination. The LED-modules are characterized by a 2:1 combination of red and blue LEDs with single peaks, respectively, at 665 nm and 445 nm. The LED settings (intensity and spectral composition) are controlled by the equipment-specific software. Interspacing the LED-modules we installed UV tubes (UV-XEFL 290BB, Ushio Lighting Inc., Japan). The tubes emit mostly (60%) in the UV-B region (280–320 nm) with a dominant peak at 290 nm; 30% is emitted in the UV-A spectrum (320–400 nm), 4% in the UV-C region (200–280 nm) and 6% in the visible spectrum (400–700 nm) (Fig. 1). Plants were grown under a photoperiod of 12 h, with day/night temperature of 21 °C/20 °C and relative humidity of 82%. During the experiments plants were irrigated with a standard Hoagland nutrient solution (pH 6.2).

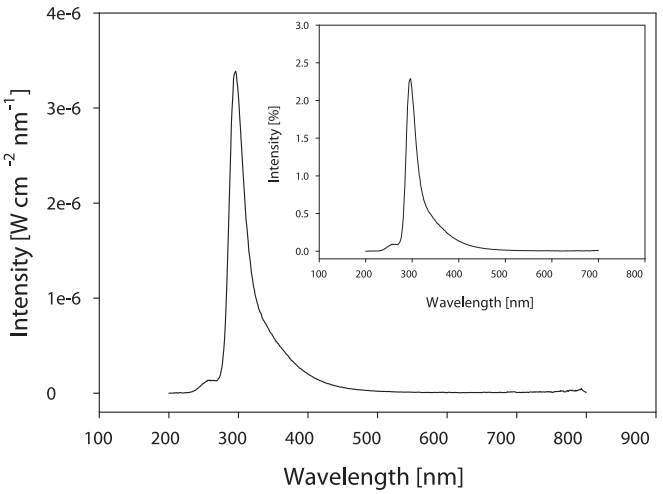

Fig. 1. Light spectrum of the UV-tube (UV-XEFL 290BB, Ushio Lighting Inc., Japan); inset indicates the normalized spectrum (200–700 nm) in percent. The measurement was done under standardized conditions (distance = 0.35 m; T = 22 °C) with the spectroradiometer OL 756 (Gooch and Housego's, Ilminster, UK).

Before running light treatments, seeds of the pepper (*C. annuum* L.) genotype Ziegenhorn Bello (Austrosaat AG, Austria) were sown in trays filled with a mixture of peat, sand and perlite and allocated under 100 μmol m^{-2} s^{-1} white fluorescence lamps (Philips, MASTER PL-L 4P). Four weeks after sowing in the 1st experiment and five weeks after sowing in the 2nd experiment, plantlets were transferred into standard pots (7 × 7 × 8 cm) and cultivated under same environmental conditions for four more weeks.

2.1.1. Impact of light intensity

In the first experiment we analysed the relevance of light intensity without any changes in the spectral quality. For this purpose, eight-week old plants were placed under LED lamps (45% blue light, 55% red light) either under 100 ± 5 μmol m^{-2} s^{-1} or 300 ± 5 μmol m^{-2} s^{-1}. Light intensities were measured and monitored with a radiometer (RM21, Dr. Gröbel UV Elektronik, Germany). One week after acclimation (58–64 das) to the new light intensities half of the plants of each light intensity were exposed to UV light of 4.98 kJ m^{-2} per hour per day which is equivalent to a biologically effective UV radiation of 5.53 kJ m^{-2} per hour per day ($UV\text{-}B_{BE}$ = 4.5, $UV\text{-}C_{BE}$ = 1.0, $UV\text{-}A_{BE}$ = 0.03 per hour per day). The biologically effective UV was calculated using the action spectrum of Flint and Caldwell (2003). UV radiation was supplied during

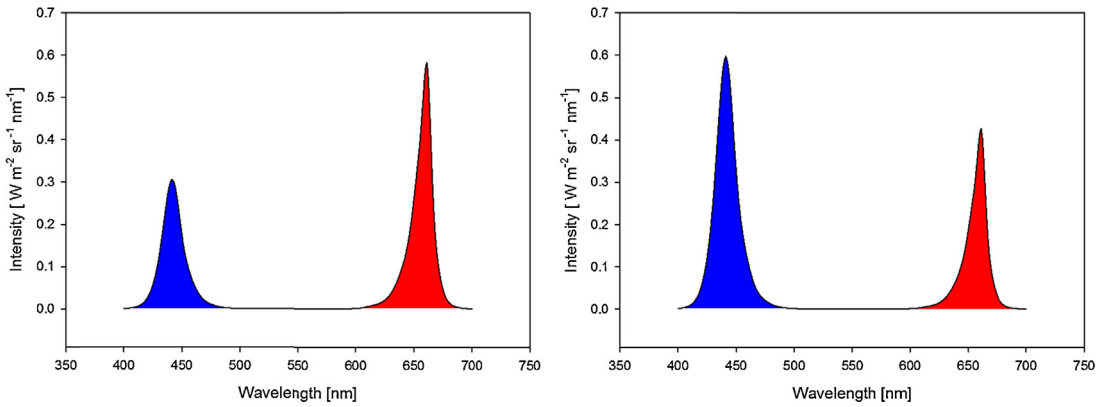

Fig. 2. Light spectra produced by the light-emitting diodes. Left side: Light spectrum with 30% blue light and 70% red light. Right side: Light spectrum with 62% blue light and 38% red light. Measurements were done with the spectroradiometer FieldSpec®3 (ASD Inc., USA).

one week (65–71 das) for one hour per day (6 a.m. to 7 a.m.). UV-intensity was measured in a testing chamber under standardised conditions at a distance of 0.35 m with the spectroradiometer OL 756 (Gooch and Housego's, Ilminster, UK) at 22 °C. Thereafter, plants were allowed to regenerate for eight days (72–79 das) either under $100 \pm 5\ \mu mol\ m^{-2}\ s^{-1}$ 1 or $300 \pm 5\ \mu mol\ m^{-2}\ s^{-1}$.

2.1.2. Impact of light quality

In the second experiment nine-week old plants were allocated under LED panels providing a similar light intensity ($100 \pm 5\ \mu mol\ m^{-2}\ s^{-1}$) but different spectral compositions (30% or 62% of blue light) (Fig. 2). One week after acclimation (65–71 das) half of the plants of each light treatment (30% or 62% blue light) were exposed to the UV light for one week (72–78 das), as described above. After eight days (79–86 das) of regeneration (either under 30% or 62% of blue light) the final evaluations were done. Each experiment consisted of four treatments with 10 plants per treatment.

2.2. Photosynthesis and chlorophyll fluorescence

Leaf gas exchange was measured weekly with a portable infrared gas analyzer (CIRAS-1, PP Systems, United Kingdom) equipped with a standard $2.5\ cm^{-2}$ leaf cuvette (PLC B, PP Systems, United Kingdom). Measurements were carried out under white fluorescence lamps in the climate chamber adopting standardized settings: CO_2 concentration 350 ± 5 ppm, light intensity $100 \pm 5\ \mu mol\ m^{-2}\ s^{-1}$ PAR, a boundary layer resistance (Rb) of $0.27\ m^2\ s\ mol^{-1}$ and a leaf chamber air flow rate of $200\ ml\ min^{-1}$. Net photosynthetic rate (Pn) was measured on the same leaf used for the chlorophyll fluorescence measurements (10 plants per treatment, one measurement per plant).

Chlorophyll fluorescence was recorded weekly under laboratory conditions (ambient CO_2 concentration) using an imaging pulse-amplitude-modulated fluorometer (Imaging PAM, Heinz-Walz GmbH, Effeltrich, Germany), equipped with 96 blue light-emitting diodes (peak at 470 nm) used for fluorescence excitation, actinic illumination and saturation pulses. Fluorescence images (640×480 pixel) were taken on the second fully expanded leaf of the third leaf level (eight plants per treatment, one measurement per plant). Plants were dark adapted (30 min) prior to the measurements. Ground fluorescence (Fo) was recorded after leaf illumination by the blue light-emitting diodes ($0.5\ \mu mol\ m^{-2}\ s^{-1}$); the maximum fluorescence (Fm) was determined after a blue light saturation pulse of $1000\ \mu mol\ m^{-2}\ s^{-1}$. The yield of variable chlorophyll fluorescence (Fv) was calculated as $Fm - Fo$ while the maximum photochemical efficiency of PSII was calculated as Fv/Fm. After the first saturation pulse actinic light ($110\ \mu mol\ m^{-2}\ s^{-1}$) was switched on and saturation pulses ($1000\ \mu mol\ m^{-2}\ s^{-1}$) were applied at 20 s interval within a time frame of 340 s. Non-photochemical quenching (NPQ) was calculated as $NPQ = (Fm - F'm)/F'm$. The recorded images were analyzed by ImagingWin v2.40b (Heinz-Walz GmbH, Effeltrich, Germany). From each image four areas of interest (AOIs) were selected. The mean of the four AOIs was then used for the analysis of variance.

2.3. Sensor-based determination of leaf pigments

The content of chlorophyll and epidermal flavonols were estimated non-destructively with the hand-held optical sensor Dualex® (FORCE-A, Orsay, France). At the start of the experiment (five weeks after sowing) one fully expanded leaf per plant was labeled at the third leaf level. Both the initial measurement as well as the records throughout the experiment (three times a week) were done on the same leaves. Four measurements were taken on the labeled leaf, on 10 plants per treatment. The difference in the transmission of the red and near infrared light is used as a basis for the calculation of the chlorophyll-index (Chl-index) which is directly related to the leaf chlorophyll content per unit area [$\mu g\ cm^{-2}$] (Cerovic et al., 2012). The flavonol-index (FLAV-index) is a fluorescence-based index using the UV-screening effect of epidermal flavonols. This index is directly linked to the epidermal polyphenol concentration of the leaf (Goulas et al., 2004; Cerovic et al., 2012).

2.4. Analytical determination of leaf pigments

The concentrations of chlorophylls, carotenoids, flavonoids and anthocyanins were determined photometrically according to established methods as described elsewhere (Solovchenko et al., 2001; Solovchenko and Schmitz-Eiberger, 2003). Briefly, for the extraction, 100 mg of MgO were added to 30 mg of the dried and grounded leaf material (leaf material was harvested separately per plant, 10 plants per treatment); then, after addition of 6 ml folch solution samples were shaken and centrifuged (10 min, 4000 rpm, 15 °C). The supernatant was transferred into graduate centrifuge tubes; after addition of 1.2 ml H_2O, the content was mixed and the tubes centrifuged until the chloroform and the water–methanol phases had separated (10 min, 3000 rpm, 15 °C). After determination of the volumes the water–methanol phase was transferred in another centrifuge tube. The chloroform was transferred into glass flasks and filled up to a defined volume (25 ml) with the folch solution. The centrifuge tube with the pellet was kept open to dry.

The absorbencies were measured with a UV/vis spectrophotometer (Lambda 35, Perkin-Elmer, USA) using dissolvent resistant quarz-glass cuvettes. The chloroform phase was measured at 750, 665.6, 647.6 and 480 nm. The water–methanol phase was measured at 750 and 360 nm; afterwards a drop of HCL (37%) was added to the water–methanol solution which was then measured at 750 and 530 nm. Four milliliter of a methanol–HCL solution (100 ml MeOH + 1 ml 37% HCL) was added to the dried pellet, well shaken and centrifuged (10 min, 4000 rpm, 4 °C). The supernatant was transferred to a graduate centrifuge tube. This process was done twice. The supernatant (8 ml) was measured at 750 and 360 nm, then acidified with one drop of HCL (37%) and measured once again at 750 and 530 nm.

Concentrations of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), carotenoids (Car), flavonoids (Flav) and anthocyanins (Anth) were calculated according to the following equations:

Chlorophyll:

$$C\,(\mathrm{Chl}\,a) = \frac{A \times \mathrm{Vol\,(chloroform)} \times 10^3}{\mathrm{M}(Chl\,a) \times \mathrm{DW}}$$

$$A = 11.47 \times (\mathrm{Abs.}\,665.6 - \mathrm{Abs.}\,750) - 2 \times (\mathrm{Abs.}\,647.6 - \mathrm{Abs.}\,750)$$

A: Chlorophyll *a* concentration in μg ml^{-1}; C(Chl *a*): Chlorophyll *a* in nmol g^{-1} DW; Vol (chloroform): Volume of the chloroform phase in ml; M (Chl *a*): Molar mass of chlorophyll *a* (893.49 g mol^{-1}).

$$C\ (\mathrm{Chl.}\,b) = \frac{B \times \mathrm{Vol\ (chloroform)} \times 10^3}{M\,(Chl\,b) \times \mathrm{DW}}$$

$$B = 21.85 \times (\mathrm{Abs.}\,647.6 - \mathrm{Abs.}\,750) - 4.53 \times (\mathrm{Abs.}\,665.6 - \mathrm{Abs.}\,750)$$

B: Chlorophyll *b* concentration in μg ml^{-1}; C(Chl *b*): Chlorophyll *b* in nmol g^{-1} DW; Vol (Chloroform): Volume of the chloroform phase in ml; M (Chl *b*): Molare mass of chlorophyll *b* (907.47 g mol^{-1}).

Carotenoids:

$$C\,(\mathrm{Car}) = \frac{[1000 \times (\mathrm{Abs.}\,480 - \mathrm{Abs.}\,750) - 1.33 \times A - 23.93 \times B] \times \mathrm{Vol\,(Chloroform)} \times 10^2}{202 \times M(\mathrm{Car}) \times \mathrm{DW}}$$

C (Car): Carotenoid concentration in nmol g^{-1} DW; M (Car): Average of the molar mass of carotenoids (536.87 g mol^{-1}).

Flavonoids:

$$C\,(Flav) = \frac{10^6 \times F_1}{23500 \times DW(g)}$$

$$F_1 = Vol_{(MeOH-H_2O-phase)} \times (Abs.\,360 - Abs.\,750) + Vol_{(pellet\,MeOH-HCl-phase)} \times (Abs.\,360 - Abs.\,750)$$

F_1: Combined absorption of the extract at 360 nm; C (Flav): Flavonoid concentration in nmol g^{-1} DW; Vol$_{(\mathrm{MeOH-H_2O\text{-}phase})}$: Volume of the water–methanol-phase in ml; $Vol_{(pellet\,MeOH\text{-}HCl\text{-}phase)}$: Volume of the methanol–HCl-phase of the pellet.

Anthocyanins:

$$C\,(Anth) = \frac{10^6 \times F_2}{30000 \times DW(g)}$$

$$F_2 = Vol_{(MeOH-H_2O-phase,\,acidified)} \times (Abs.\,530 - Abs.\,750) + Vol_{(pellet\,MeOH-HCl-phase,acidified)} \times (Abs.\,530 - Abs.\,750)$$

F_2: Combined absorption of the extracts at 530 nm; C(Anth): Anthocyanin concentration in nmol g^{-1} DW.

2.5. Biomass and leaf mass per area (LMA)

At the end of the experiments leaves and stalks of each plant (10 plants per treatment) were harvested separately into previously weighed plastic bags. Fresh weight (FW) was determined directly using a precision scale (BP210S, Sartorius, Chicago, USA) while leaf area was measured with a leaf area meter (LI-COR, Lincoln, Nebraska, USA). Stems and leaves were then frozen at −25 °C, lyophilized (Gamma 1-16 LSC; Christ, Osterode am Harz, Germany) and weighed again in order to quantify their dry weight (DW). Leaf mass per area (LMA) was calculated according to the equation

$$\mathrm{LMA}\ \left[\mathrm{g\ \ cm^{-2}}\right] = \frac{\text{leaf-DW [g]}}{\text{leaf area}\ \left[\mathrm{cm^2}\right]}.$$

2.6. Statistics

Data were checked for normal distribution (Kolmogorov–Smirnov-test) and homogeneity of variance (Levene-test). If both conditions were fulfilled statistical analyses were performed by one-way analysis of variance (ANOVA, $p \leq 0.05$) using SPSS statistic software (PASW statistics version 20.0, SPSS Inc., Chicago, USA). Where applicable, the Duncan's multiple range test ($p \leq 0.05$) was used to determine the differences among the four treatments. The impact of light intensity and light quality as well as the interaction between light intensity or light quality and the UV-B treatment was determined by a two-factor analysis of variance. In those cases where homogeneity of variance was not given non-parametric tests were performed (Kruskal–Wallis-test ($p \leq 0.05$) and Mann–Whitney-*U*-test). The use of non-parametric tests is indicated in the subtitles of the respective figures. Graphs were drawn with SigmaPlot 11.0 (Systat Software Inc., Richmond, CA, USA).

3. Results

3.1. Impact of light intensity

3.1.1. Photosynthetic performance

The dynamics of photosynthetic rate (Pn) is shown in Fig. 3. After five days of LED illumination (62 das) under 100 or 300 μmol m^{-2} s^{-1} (PAR 100 and PAR 300, respectively), Pn did not differ significantly among the four treatments under the comparatively low light intensity (100 μmol m^{-2} s^{-1}) used for measurements. Exposure to UV induced significant lower Pn in those plants grown under 100 μmol m^{-2} s^{-1} whereas no significant differences were noted in plants grown under 300 μmol m^{-2} s^{-1} (69 das). In the period after UV exposure (76 das) Pn was higher in plants grown under 300 μmol m^{-2} s^{-1}. Under both light intensities, 100 and 300 μmol m^{-2} s^{-1}, plants exposed to UV had a lower photosynthetic rate. A two-factor analysis of variance revealed a high significant impact of light intensity ($p \leq 0.01$) and the UV treatment ($p \leq 0.01$) during and after UV radiation, but no interaction of both experimental factors was detected.

As demonstrated by *Fv*/*Fm* (Fig. 4), plants without UV exposure (PAR100, PAR300) had a higher maximum photochemical efficiency of the photosystem II during the stress phase as compared to plants that were subjected to UV. A two-factor analysis of variance indicates a highly significant impact ($p \leq 0.01$) of the UV irradiation but no influence of light intensity. The regeneration period allowed

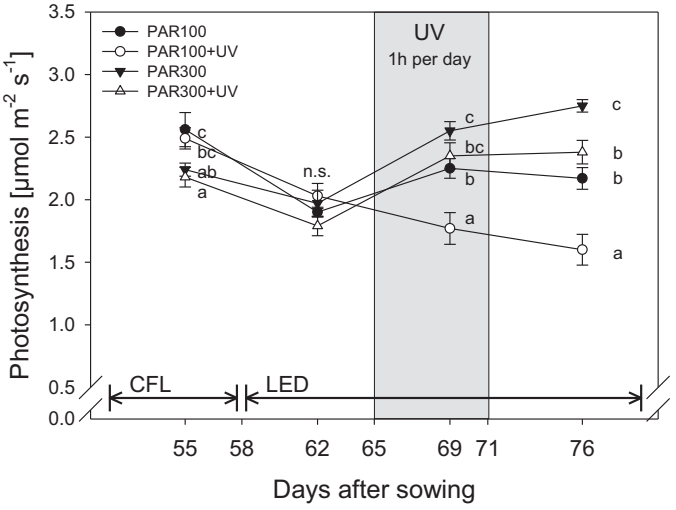

Fig. 3. Influence of light intensity and UV irradiation on the development of net photosynthesis. Pepper plants were cultivated under 100 µmol m^{-2} s^{-1} or 300 µmol m^{-2} s^{-1}, with or without supplemental UV (UV_{BE} = 5.53 kJ m^{-2} per hour per day, 65–71 das). Means ± SE (n = 10) followed by the same letters (within the evaluation day) do not differ significantly according to the Duncan test ($p \leq 0.05$); n.s., non significant.

PAR300+UV to approach the same level as control, non-stressed plants, whereas *Fv*/*Fm* decreased further in PAR100+UV.

Evaluations of the non-photochemical quenching (NPQ) indicate the impact of both the light intensity and UV exposure on the heat dissipation by the leaves (Fig. 5A–C). Here, we observe a general increase of NPQ in those plants grown under 100 µmol m^{-2} s^{-1}, irrespective of the UV irradiation. However, a more detailed view indicates higher NPQ values at 70 das for the plants of the treatment PAR100+UV (Fig. 5B). In contrast, no differences between the PAR300 and PAR300+UV groups could be observed during the experiment. During the UV period (70 das) the two-factor analysis of variance indicated a high significant impact of light intensity ($p \leq 0.01$) from 120 s until 180 s, a high significant impact of UV light ($p \leq 0.01$) from 80 s until 100 s and a significant interaction of both factors ($p \leq 0.05$) from 80 s until 100 s. At 77 das a high significant impact of light intensity ($p \leq 0.01$) could be proven from 100 s until

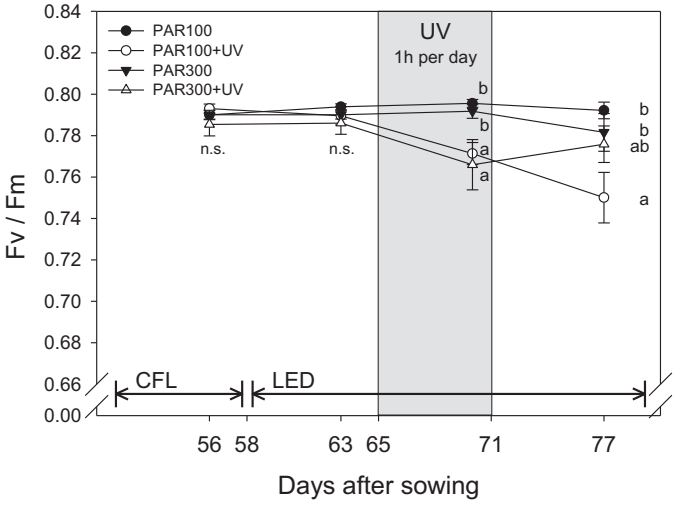

Fig. 4. Influence of light intensity and UV irradiation on the development of the maximum photochemical efficiency of PSII (*Fv*/*Fm*). Pepper plants were cultivated under 100 µmol m^{-2} s^{-1} or 300 µmol m^{-2} s^{-1}, with or without supplemental UV (UV_{BE} = 5.53 kJ m^{-2} per hour per day, 65–71 das). Means ± SE (n = 8) followed by the same letters (within the evaluation day) do not differ significantly according to the Duncan test ($p \leq 0.05$); n.s., non significant.

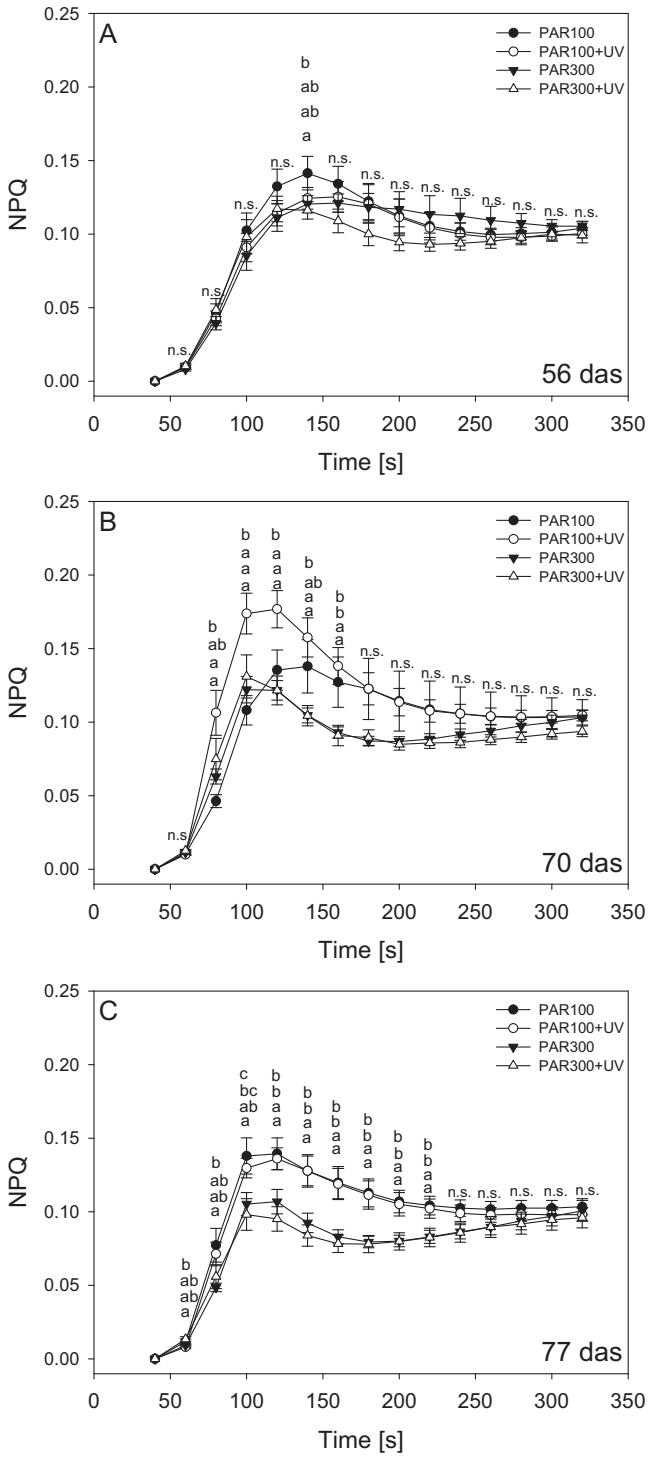

Fig. 5. Influence of light intensity and UV irradiation on the development of the non-photochemical quenching (NPQ). Pepper plants were cultivated under 100 µmol m^{-2} s^{-1} or 300 µmol m^{-2} s^{-1}, with or without supplemental UV (UV_{BE} = 5.53 kJ m^{-2} per hour per day, 65–71 das). Evaluation was done before LED-treatment initiation (56 das, A), during the UV period (70 das, B) and after the UV period (77 das, C). Means ± SE (n = 8) followed by the same letters do not differ significantly according to the Duncan test ($p \leq 0.05$); n.s., non significant.

220 s. There was no longer a significant UV effect, although an interaction of light intensity and UV irradiation could be seen at 60 s.

3.1.2. Leaf pigments

Alterations in the content of leaf pigments were determined non-destructively with an optical sensor in the time-course of the

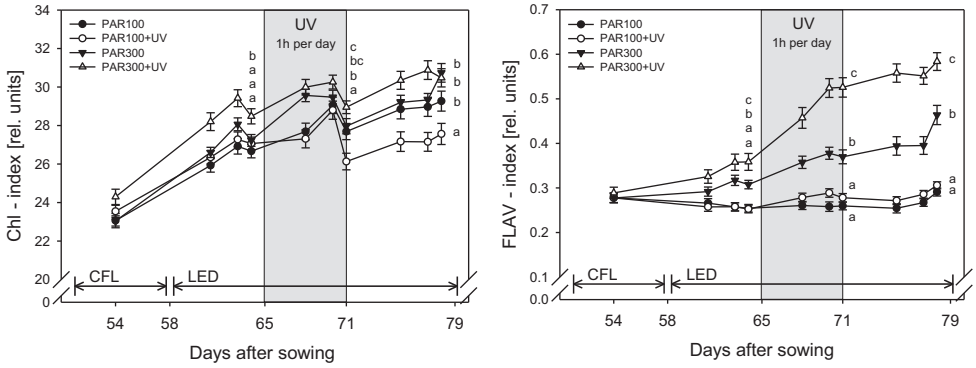

Fig. 6. Influence of light intensity and UV irradiation on the development of the Chl—index (left) and the FLAV—index (right). Pepper plants were cultivated under 100 μmol m^{-2} s^{-1} or 300 μmol m^{-2} s^{-1}, with or without supplemental UV (UV_{BE} = 5.53 kJ m^{-2} per hour per day, 65–71 das). Chl-index was statistically analysed by one-way ANOVA and means were separated by a Duncan test ($p \leq 0.05$). FLAV-index was analysed by Kruskal–Wallis-test and means were separated by the Mann–Whitney-*U*-test ($p \leq 0.05$). Irrespective of the statistical procedure, means ± SE (n = 40) followed by the same letters (within selected days) do not differ significantly.

experiment (Chl-index and FLAV-index) and compared to a wet-chemical analysis at the end of the experiment.

Irrespective of light intensity and UV irradiation we observed an increase of the Chl-index in the time-course of the experiment (Fig. 6). A negative impact of UV was observed particularly in the plants grown under 100 μmol m^{-2} s^{-1}, which presented a lower Chl-index after the period of UV exposure (71 das). Differently, the destructive analysis of the chlorophyll content at the end of the experiment reveal a significantly lower chlorophyll concentration in those plants cultivated under 300 μmol m^{-2} s^{-1} (Table 1). Since the concentration of Chl *a* and Chl *b* was affected in a similar way by the treatments (*data not shown*), we pooled the data to calculate the total chlorophyll content. The UV irradiation led to a significant decrease in the content of chlorophyll and carotenoids in plants cultivated under 100 μmol m^{-2} s^{-1}, whereas this was not the case for plants grown under 300 μmol m^{-2} s^{-1} (Table 1). Moreover, the leaf mass per unit area (LMA) indicates a significant impact of light intensity, and supports the results of the Chl-index recorded with the optical sensor.

Also the other leaf pigments were affected by the experimental treatments, although in a different way. Plants grown under 300 μmol m^{-2} s^{-1} revealed a strong increase in the FLAV-index, which was more accentuated after irradiation with UV (PAR300+UV). In contrast, plants grown under 100 μmol m^{-2} s^{-1} (PAR100, PAR100+UV) showed no significant changes in the FLAV-index, which remained at the same level throughout the experiment. The stronger impact of light intensity was also confirmed with the determination of the flavonoid content in the leaves. However, here we also observed a positive effect of UV radiation on the accumulation of flavonoids (Table 1), but no effect on the concentration of anthocyanins.

3.2. Impact of light quality

3.2.1. Photosynthetic performance

Although Pn showed unexpected discrepancies in the initial measurement, after seven days of illumination with the LEDs the four treatments (30%_B, 30%_B+UV, 62%_B, 62%_B+UV) did not differ significantly under the comparatively low light intensity (100 μmol m^{-2} s^{-1}) used for measurements. Four days after starting UV radiation significant effects could be observed but the most pronounced influence of the applied treatments became apparent at the end of the experiment (Fig. 7). Here, plants cultivated under 62% of blue light showed a higher Pn than those cultivated under 30% of blue light. Furthermore, the UV irradiated plants had significant lower Pn values than the respective control groups.

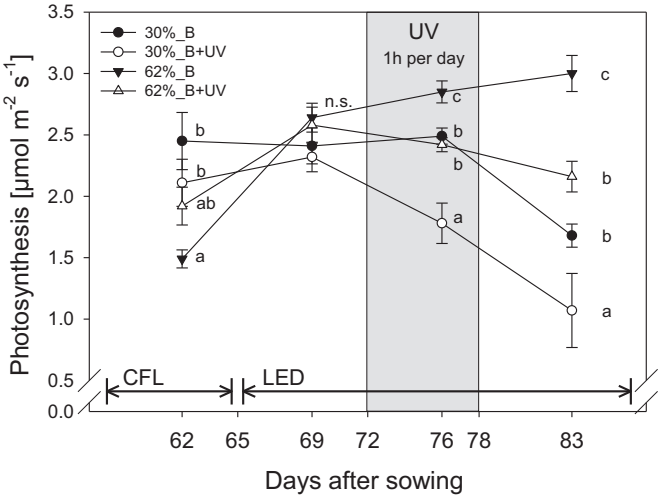

Fig. 7. Influence of light quality and UV irradiation on the development of net photosynthesis. Pepper plants were cultivated under 30% or 62% blue light, with or without supplemental UV (UV_{BE} = 5.53 kJ m^{-2} per hour per day, 72–78 das). Means ± SE (n = 10) followed by the same letters (within the evaluation day) do not differ significantly according to the Mann–Whitney-*U*-test ($p \leq 0.05$); n.s., non significant.

The UV induced stress was also confirmed by chlorophyll fluorescence measurements. Here, plants grown under 30% blue light and exposed to UV had significantly lower *Fv*/*Fm* during (77 das) and after (84 das) the UV phase (Fig. 8). In contrast, plants cultivated under 62% blue light were not significantly affected by UV radiation. This effect was more pronounced in the plants cultivated under 30% blue light in the PAR composition. The stress situation also influenced the heat dissipation, as expressed by the non-photochemical quenching. In general, the effects of the light quality were stronger at the end of the experiment, the plants cultivated under 30% blue light showing a stronger increase of NPQ (Fig. 9A–C). However, the major alterations in the intensity of the values and the shape of the NPQ time-resolved curve were induced by the UV radiation (Fig. 9B and C). Plants that were cultivated under 30% blue light showed the highest NPQ values during (Fig. 9B) and after (Fig. 9C) the UV irradiation. In contrast, plants grown under 62% blue light approached the values of the respective control plants which were not irradiated with UV.

3.2.2. Leaf pigments

Contrasting the results of photosynthesis and chlorophyll fluorescence, the experimental treatments had only a minor influence

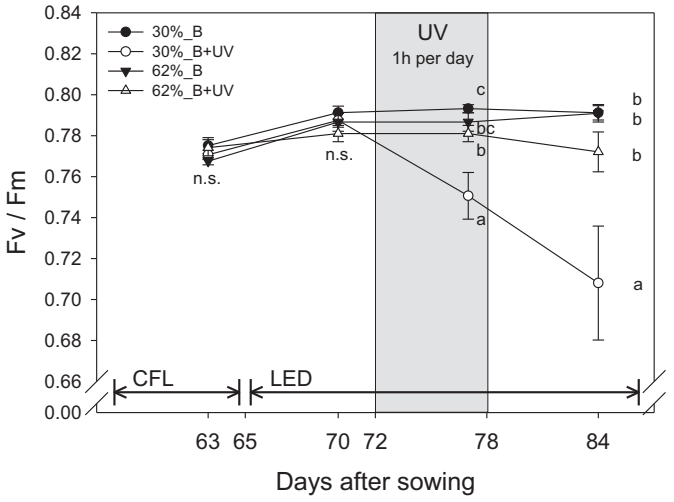

Fig. 8. Influence of light quality and UV irradiation on the development of the maximum photochemical efficiency of PSII (*Fv*/*Fm*). Pepper plants were cultivated under 30% or 62% blue light, with or without supplemental UV (UV_{BE} = 5.53 kJ m^{-2} per hour per day, 72–78 das). Means ± SE (n = 8) followed by the same letters (within the evaluation day) do not differ significantly according to Mann–Whitney-*U*-test ($p \leq 0.05$); n.s., non significant.

on the Chl-index (Fig. 10). On the last day of the UV irradiation 30%_B showed a significantly higher Chl-index as compared to the other treatments. However, the analysis of the chlorophyll concentration indicates that UV treated plants (30%_B+UV, 62%_B+UV) had significantly less Chl *a* and Chl *b* (*data not shown*), resulting in lower concentration of total chlorophylls (Table 2). Thereby, we observed a more pronounced reduction in Chl and carotenoids in those plants grown under a lower portion of blue light.

On the other hand, the FLAV-index was slightly higher in plants cultivated under 62% of blue light. In addition UV radiation slightly increased the FLAV-index, particularly in the 30%_B treatment group. The only minor difference between the treatments was confirmed by the analysis of total flavonoids and anthocyanins at the end of the experiment. Here, UV treated plants (30%_B+UV, 62%_B+UV) had a higher concentration of flavonoids and a lower concentration of anthocyanins as compared to the respective control plants.

4. Discussion

In our work we exploited the causal relationship between the biochemical and physiological mechanisms of light acclimation and the susceptibility of pepper plants to UV radiation. We hypothesized that higher amount of blue light in the PAR spectrum contributes for the acclimation to UV light as already described for high light intensities. Here, we focused on light induced modifications in the composition and accumulation of leaf pigments such as chlorophylls, carotenoids and flavonoids and the impact on the photosynthetic performance. In addition, morphological adaptations at leaf and whole plant level were determined.

4.1. Relevance of light intensity and light quality for the photosynthetic acclimation of plants to UV stress

When exposed to UV radiation, non-adapted plants might show distinct responses including damages to the photosynthetic machinery and the degradation of photosynthetic pigments (Smith et al., 2000; Kakani et al., 2003). In our study plants were generally less affected by the UV stress (1 h per day) when grown under higher light intensity (300 μmol m^{-2} s^{-1}) or higher amount of blue light (62%); this is clearly demonstrated by the CO_2 fixation

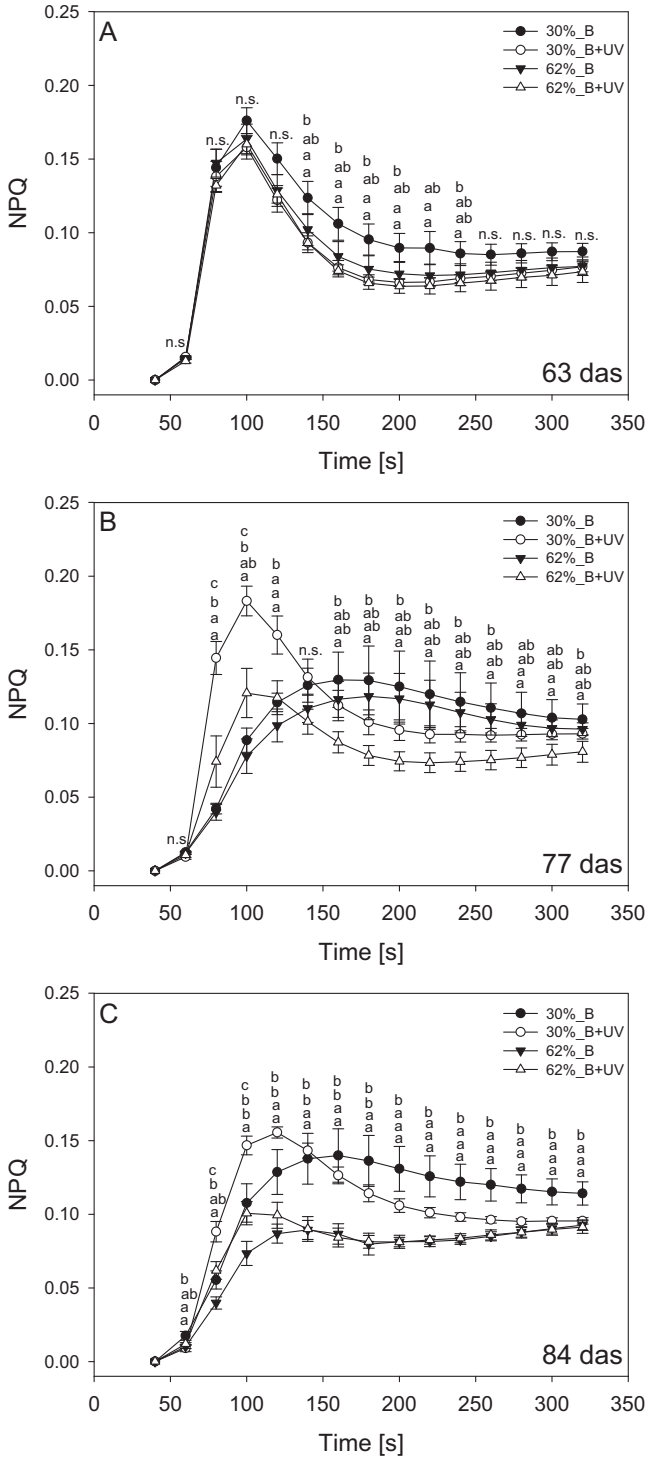

Fig. 9. Influence of light quality and UV irradiation on the development of the non-photochemical quenching (NPQ). Pepper plants were cultivated under 30% or 62% blue light, with or without supplemental UV (UV_{BE} = 5.53 kJ m^{-2} per hour per day, 72–78 das). Evaluation was done before treatment initiation (63 das, A), during the UV period (77 das, B) and after the UV period (84 das, C). Means ± SE (n = 8) followed by the same letters do not differ significantly according to the Duncan test ($p \leq 0.05$); n.s., non significant.

rates (Pn) (Figs. 3 and 7) and the maximum quantum yield of PSII photochemistry (Figs. 4 and 8) during and after UV application. Particularly *Fv*/*Fm* has been proposed as a reliable indicator to evaluate light acclimation of leaves (e.g. sun and shade leaves) (Lichtenthaler et al., 2013) as well as the impact of UV and other abiotic and

Table 1
Influence of light intensity and UV irradiation on the leaf mass per area, chlorophyll *a* + *b*, carotenoid, flavonoid and anthocyanin concentrations of pepper leaves. Plants were cultivated under 100 μmol m^{-2} s^{-1} or 300 μmol m^{-2} s^{-1}, with or without supplemental UV (UV_{BE} = 5.53 kJ m^{-2} per hour per day, 65–71 das). Evaluation was done at the end of the experiment. Means ± SE (*n* = 10) within the columns followed by the same letters do not differ significantly according to the Duncan test ($p \leq 0.05$); n.s., non significant.

Treatment	leaf mass per area [g cm^{-2}]	Chlorophyll *a* + *b* [μmol g^{-1} DW]	Carotenoids [μmol g^{-1} DW]	Flavonoids [μmol g^{-1} DW]	Anthocyanins [μmol g^{-1} DW]
PAR100	0.20 ± 0.004a	17.96 ± 0.37c	6.92 ± 0.15c	18.24 ± 0.48a	0.50 ± 0.02n.s.
PAR100+UV	0.21 ± 0.005a	16.69 ± 0.32b	6.48 ± 0.11b	22.27 ± 0.01b	0.51 ± 0.02
PAR300	0.24 ± 0.007b	14.95 ± 0.33a	5.90 ± 0.12a	25.71 ± 1.35c	0.52 ± 0.02
PAR300+UV	0.25 ± 0.006b	14.35 ± 0.62a	5.68 ± 0.22a	29.02 ± 0.58d	0.54 ± 0.02

Table 2
Influence of light quality and UV irradiation on leaf mass per area, chlorophyll *a* + *b*, carotenoid, flavonoid and anthocyanin concentrations of pepper leaves. Plants were cultivated under 30% or 62% blue light, with or without supplemental UV (UV_{BE} = 5.53 kJ m^{-2} per hour per day, 72–78 das). Evaluation was done at the end of the experiment. Means ± SE (*n* = 10) within the columns followed by the same letters do not differ significantly according to the Duncan test ($p \leq 0.05$).

Treatment	leaf mass per area [g cm^{-2}]	Chlorophyll *a* + *b* [μmol g^{-1} DW]	Carotenoids [μmol g^{-1} DW]	Flavonoids [μmol g^{-1} DW]	Anthocyanins [μmol g^{-1} DW]
30%_B	0.20 ± 0.026b	17.39 ± 0.30c	6.72 ± 0.10c	21.46 ± 1.05ab	0.485 ± 0.01c
30%_B+UV	0.16 ± 0.004ab	15.25 ± 0.34a	5.99 ± 0.14a	22.67 ± 0.86b	0.457 ± 0.03bc
62%_B	0.15 ± 0.005a	17.55 ± 0.31c	6.73 ± 0.11c	19.02 ± 1.37a	0.423 ± 0.02ab
62%_B+UV	0.17 ± 0.005ab	16.42 ± 0.30b	6.33 ± 0.10b	19.72 ± 1.33ab	0.390 ± 0.02a

biotic stress factors (Murchie and Lawson, 2013). The suitability of both, Pn and *Fv*/*Fm*, to evaluate physiological alterations induced by UV was confirmed in our studies, even if the measuring conditions (100 μmol m^{-2} s^{-1}) were not ideal to demonstrate the full photosynthetic performance of the plants and their acclimation to different light conditions. On the other hand, additional measurements indicate higher CO_2 assimilation rates in control plants (no UV) when cultivated under 300 μmol m^{-2} s^{-1} or 62% blue light, irrespective of the light intensity (100–500 μmol m^{-2} s^{-1}) selected for photosynthesis measurement (*data not shown*). It is interesting to note that while the effect of UV radiation on the stomatal conductance is well described (Nogués et al., 1999), in the present study we observed no significant impairment of the stomatal conductance (*data not shown*).

Complementary to Pn and *Fv*/*Fm* the non-photochemical quenching provided meaningful data to better understand the acclimation of pepper plants to the different light treatments. Non photochemical quenching competes with the photochemical quenching and the fluorescence emission (Murchie and Lawson, 2013). In physiological experiments the steady-state values of NPQ are commonly used to conclude about the efficiency of energy use in the chloroplasts, although the speed of induction and relaxation provides insight into this topic (D'Haese et al., 2004). In the current study plants grown under 100 μmol m^{-2} s^{-1} had faster induction of NPQ with higher peaks as compared to 300 μmol m^{-2} s^{-1}, however reaching similar steady-state values (Fig. 5B and C). This result is not surprising since the actinic light (about 110 μmol m^{-2} s^{-1}) was in best case similar to the light intensity used for plant cultivation. Another relevant aspect is the higher NPQ during UV exposure (Fig. 5B) and its matching to the values of control plants in the sequential phase (Fig. 5C), indicating a reversible slow-down of the energy flux during the stress phase in plants cultivated under 100 μmol m^{-2} s^{-1}. Stronger increase of NPQ in dependence to UV was also observed in plants grown under 30% blue as compared to 62% blue light (Fig. 9B), indicating a higher demand for energy dissipation particularly shortly after illumination of dark-adapted tissues. However, as relaxation time was very short (few minutes) and the steady state levels were similar, it is assumed that photoprotective processes such as high energy quenching by light-absorbing pigments and state transition processes significantly contributed to these results (D'Haese et al., 2004; Horton et al., 1996).

Finally, the described responses in the photosynthetic performance were strongly affected by the degradation of photosynthetic pigments, a process which was more evident in plants grown either under 100 μmol m^{-2} s^{-1} or 30% of blue light (Tables 1 and 2). UV radiation generates free radicals that cause damage to the photosynthetic machinery and lead to degradations of Chl *a* + *b* and carotenoids (Hideg et al., 2013). The stronger the degradation of those pigments, the higher will be the thermal dissipation as this

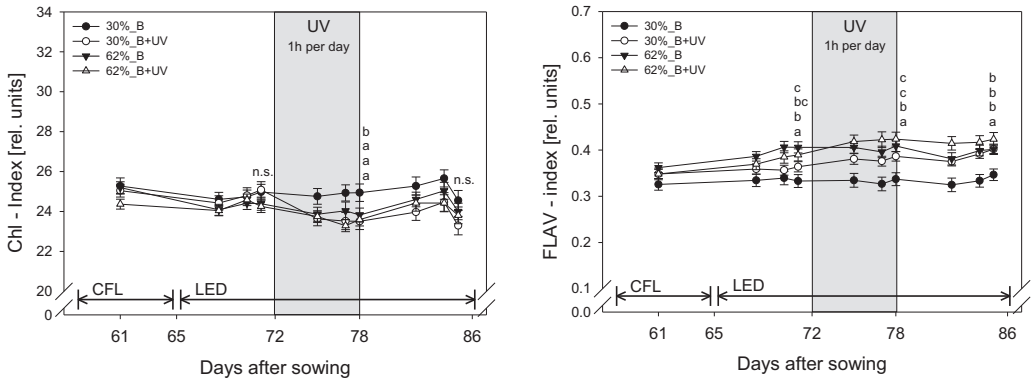

Fig. 10. Influence of light quality and UV irradiation on the Chl—index (left) and the FLAV—index (right). Pepper plants were cultivated under 30% or 62% blue light, with or without supplemental UV (UV_{BE} = 5.53 kJ m^{-2} per hour per day, 72–78 das). Chl-index was statistically analysed by one-way ANOVA and means were separated by a Duncan test ($p \leq 0.05$). FLAV-index was analysed by Kruskal–Wallis-test and means were separated by the Mann–Whitney-*U*-test ($p \leq 0.05$). Irrespective of the statistical procedure, means ± SE (*n* = 40) followed by the same letters (within selected days) do not differ significantly; n.s., non significant.

protects PSII reaction centers against photoinhibitory damages (Moon et al., 2011).

4.2. Light intensity and high blue light influence pigment composition in distinct ways

The lower UV susceptibility induced by both higher light intensity and higher amount of blue light, and characterized by the photosynthetic performance, relies on specific acclimation processes that include pigments which contribute the photochemical adaptation (Chl *a*, Chl *b*, Car) as well as pigments that serve as shielding compounds (e.g. epidermal flavonols). In the present study light intensity and light quality had different effects on leaf pigment compositions. While the photochemical acclimation was particularly influenced by light quality, the accumulation of flavonoids was affected by both light intensity and light quality. Control plants grown under 100 μmol m^{-2} s^{-1} had a 17% higher concentration of Chl *a* + *b* and a 15% higher concentration of Car than control plants grown under 300 μmol m^{-2} s^{-1} (Table 1). Both, Chl *a* + *b* and Car improve light harvesting (Matsubara et al., 2009). Under low light conditions (PAR100) plants invest more energy in light harvesting than in biomass production (Matsubara et al., 2009) which was also proven by us in terms of biometric data (Tables S1 and S2). Thinner leaves with larger chloroplasts and higher amounts of chlorophyll improve light harvesting in a light-limited environment (Boardman, 1977). In our study the morphological adaptations induced changes in the LMA which was 17% lower in plants grown under 100 μmol m^{-2} s^{-1} (Table 1). The modifications in LMA explain why the area based Chl-index was less suitable to detect modifications in the chlorophyll concentration, contrasting the dry weight based analytics. It must also be taken into consideration that alterations in LMA leads to changes in the frequency of photosynthetic reaction centers per leaf area unit that consequently influences net photosynthesis and *Fv*/*Fm* as already described for sun and shade leaves (Lichtenthaler and Babani, 2004; Lichtenthaler et al., 2013). In contrast to light intensity, light quality did not affect the amount of photosynthetic related pigments as demonstrated by wet-chemical analysis (Table 2) and the non-invasive measurements (Fig. 10). The ratio of Chl *a*/Chl *b* (*data not shown*) which is known to be sensitive to light intensity and light quality was not affected in both experiments. The results suggest that adaptations in the pigment composition are strongly limited by the amount of blue light. According to Buschmann et al. (1978) and Lichtenthaler and Buschmann (1978), the formation of sun type chloroplasts is already induced at low percentage of blue light. Both light qualities used in our study (30% and 62% blue light) contained comparatively high amounts of blue light. It must therefore be assumed that sun type chloroplasts were formed also under 30% blue light since the composition of photosynthetic pigments did not differ between plants grown under 30% and 62% of blue light.

Irrespective of that, flavonoids working as UV screeners and reactive oxygen species (ROS) scavengers, are of central importance in the context of UV acclimation (see review of Falcone Ferreyra et al., 2012; Gould, 2004). Flavonoid synthesis depends on light intensity as well as on light quality (Feinbaum et al., 1991). Moreover, we point out that light intensity and light quality influence the ability to accumulate epidermal flavonols during and after UV exposure. It is conspicuous that the FLAV-index (Fig. 6) and the total flavonoid concentration followed a similar trend as affected by different light intensities whereas this was not the case when analyzing the effect of light quality. In contrast to light intensity high blue light increased the amount of epidermal flavonols (Fig. 10), whereas the amount of anthocyanins and the total amount of flavonoids were not affected (Table 2). Hereby, the results confirm that the light dependent accumulation of flavonoids is specific for each single component of the flavonoid family. Furthermore, we observed that light intensity (1st experiment) had no significant effect while light quality (2nd experiment) significantly affected the anthocyanin accumulation. This suggests that particularly under low fluence rates light quality is an important factor that determines anthocyanin synthesis and accumulation. This hypothesis is also supported by Sarala et al. (2011) who found that the removal of blue light from the normal light spectrum significantly reduced anthocyanin concentration in different plant species.

5. Conclusion

In our study we demonstrate that high amount of blue light triggers specific biochemical and physiological processes resulting in better acclimation and recovery of plants to UV stress. Amongst others this was clearly proven by gas exchange and fluorescence measurements. UV induced degradation of photosynthetic related pigments (chlorophyll *a* and *b*, as well as carotenoids) was stronger in those plants cultivated either under lower light intensity or lower amount of blue light, whereas plants grown either under 300 μmol m^{-2} s or 62% blue light accumulated more epidermal flavonols.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.envexpbot.2014.06.017.

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