



## Research article

# Effect of UV-B radiation on morphology, phenolic compound production, gene expression, and subsequent drought stress responses in chili pepper (*Capsicum annuum* L.)<sup>☆</sup>



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## ABSTRACT

It has been suggested that accumulation of flavonoids could be a key step in development of plant tolerance to different environmental stresses. Moreover, it has been recognized that abiotic stresses such as drought and UV-B radiation (280–315 nm) induce phenolic compound accumulation, suggesting a role for these compounds in drought tolerance. The aim of the present study was to evaluate the effect of UV-B exposure on chili pepper (*Capsicum annuum*, cv. ‘Coronel’) plant performance, phenolic compound production, and gene expression associated with response to subsequent drought stress. Additionally, the phenotypic response to drought stress of these plants was studied. UV-B induced a reduction both in stem length, stem dry weight and number of floral primordia. The largest reduction in these variables was observed when combining UV-B and drought. UV-B-treated well-watered plants displayed fructification approximately 1 week earlier than non-UV-B-treated controls. Flavonoids measured epidermally in leaves significantly increased during UV-B treatment. Specifically, UV-B radiation significantly increased chlorogenic acid and apigenin 8-C-hexoside levels in leaves and a synergistic increase of luteolin 6-C-pentoside-8-C-hexoside was obtained by UV-B and subsequent drought stress. Gene expression of phenylalanine ammonia lyase (*PAL*) and chalcone synthase (*CHS*) genes also increased during UV-B treatments. On the other hand, expression of genes related to an oxidative response, such as mitochondrial Mn-superoxide dismutase (*Mn-SOD*) and peroxidase (*POD*) was not induced by UV-B. Drought stress in UV-B-treated plants induced mitochondrial *Mn-SOD* gene expression. Taken together, the UV-B treatment did not induce significant tolerance in plants towards drought stress under the conditions used.

## 1. Introduction

Environmental stresses cause significant yield losses in agriculture and horticulture worldwide. Abiotic stress factors such as light, UV, drought, salinity, mechanical damage, among others, directly affect crop production (Nakabayashi et al., 2014). For instance, the severe US drought during 2012 caused serious losses of 45 and 83 million tonnes of maize and soybean production, respectively (Gilbert, 2012). Climate change developing over the coming decades is likely to bring more frequent episodes of severe drought, with potentially devastating

impact on the world's agricultural and horticultural capacity to feed a growing population. Therefore, in such a scenario, it will be critical to generate sustainable agricultural systems that also lead to more efficient crop production.

One strategy for this would be to improve the drought tolerance in crops using plant breeding and controlled elicitation of stress response-related phytochemicals (Varshney et al., 2012; Cardenas-Manríquez et al., 2016). Among phytochemicals related to drought tolerance are flavonoids, a group of phenolics (Nakabayashi et al., 2014). Flavonoids are a large group of ubiquitous plant-specific compounds that are vital

**Abbreviations:** CHS, chalcone synthase; PAL, phenylalanine ammonia lyase; PAR, photosynthetically active radiation; PCR, polymerase chain reaction; POD, peroxidase; PSII, photosystem II; ROS, reactive oxygen species; SOD, superoxide dismutase; UV-A, ultraviolet-A radiation (315–400 nm); UV-B, ultraviolet-B radiation (280–315 nm); UV-C, ultraviolet-C radiation (< 280 nm); VIS, visible radiation

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for plant growth, development, and stress amelioration (Julkunen-Tiitto et al., 2015). On the other hand, both biotic and abiotic stresses induce flavonoid production in plants. Specifically, there is a clear association between drought conditions and flavonoid accumulation in plants (Nakabayashi et al., 2014; Shojaie et al., 2016). For instance, drought and UV influence the accumulation of flavonoids in crop plants such as some cultivars of tomato (but not others) and lettuce (Klunklin and Savage, 2017; Zivanovic et al., 2017; Rajabbeigi et al., 2013). Flavonoid accumulation induced by UV-B radiation (280–315 nm) in plants has been shown to play a key role in cellular protection (Kusano et al., 2011). Flavonoids have great potential to scavenge reactive oxygen species (ROS) once they have formed during stress conditions, i.e. they act as antioxidants (Agati et al., 2012; Hideg and Strid, 2017). Also, detailed studies are needed to elucidate the precise combinations of environmental conditions that lead to the physiological states of either eustress or distress (Hideg et al., 2013). These insights will be valuable for designing drought tolerance strategies by manipulating the production of beneficial phytochemicals associated with drought tolerance (e.g. phenolic compounds, including flavonoids).

Chili pepper (*Capsicum* spp.) is an economically important crop exploited in horticulture, agriculture, and in pharmaceutical and medicinal industry. China, Mexico and Turkey are the main producers worldwide (Mejía-Teniente et al., 2013). This species is the most widely produced in America, for instance in Mexico more than 149,000 ha are used for its production (Secretariat of Agriculture, Livestock, Rural Development, Fisheries and Food of the Government of Mexico, 2016). *Capsicum* spp displays a flowering time between 55 and 60 days post-germination; the fruits are firm, aromatic and are 10–12 cm long and 3–4 cm wide at the base. Typically, the chili pepper produce 35–50 fruits per individual (Mejía-Teniente et al., 2013). Effects of UV radiation on chili pepper (especially UV-C; < 280 nm) have been documented, mainly in post-harvest studies (Mercier et al., 2001; Vicente et al., 2005) and in leaves of bell peppers (León-Chan et al., 2017). A study in *C. annuum* using special plastic films that cut off solar radiation between 280 and 400 nm (UV-B and UV-A; 280–315 nm and 315–400 nm, respectively), showed no significant changes on growth, morphology and leaf transmittance of UV in this species (Barnes et al., 2017).

In this work, we aimed at evaluating the effect of UV-B radiation on chili pepper plant morphology, phenolic compound production, and gene expression, also associated with drought as an additional subsequent stressor.

## 2. Materials and methods

### 2.1. Plant material

Chili pepper seeds (*Capsicum annuum* cv. ‘Coronel’, Harris Moran) were sown in peat moss using 52 cavity trays and maintained in a germination chamber (29 ± 1 °C, 80 ± 5% of relative humidity in darkness) during six days until radicle emergence. Seedlings were grown for 33 days under shade cloth in a greenhouse and with supplementary light were necessary. Seedlings were exposed to white light corresponding to 150–200 μmol m<sup>-2</sup> s<sup>-1</sup> for 13 h (from 06:00 h to 19:00 h) daily. Application of mineral nutrition started when seedlings developed their first pair of true leaves (See Supplementary Table S1). Seedlings were transplanted into individual pots at a phenological state of 6 true leaves. Cultivar ‘Coronel’ of *C. annuum* has a fast flowering time (55–60 days post germination), and the commercial desired fruit is medium to big sized (length from 10 to 12 cm).

### 2.2. UV-B irradiation

One week after transplanting, young plants (8–9 true leaves) were exposed to UV-B + white light. Control plants (VIS) were exposed to white light only in the same chamber as the UV-B plants by blocking UV

radiation using Perspex (Plastbearbetning AB, Norsborg, Sweden). Supplementary UV-B was given for 4 h per day (from 10:30 h to 14:30 h) and white light for 16 h per day (06:00 h to 22:00 h). UV-B irradiation was obtained from fluorescent lamps (Phillips TL40/12 UV, Eindhoven, The Netherlands) filtered through 0.13 mm cellulose acetate sheets (Nordbergs Tekniska AB, Vallentuna, Sweden) to remove any UV-C radiation, and normalized to 300 nm (Yu and Björn, 1997; Kalbina et al., 2008). 80 mW m<sup>-2</sup> plant weighted UV-B was applied for the four-hour exposure giving a total irradiation of 1.14 kJ m<sup>-2</sup> day<sup>-1</sup>. The UV was measured using a Gooch & Housego OL756 (Orlando, FL) double monochromator spectroradiometer. Photosynthetically active radiation was obtained from sodium-vapour lamps (Vialox NAV-T Super 4Y, Osram, Sweden).

### 2.3. Sample collection

Well-watered plants were exposed to supplementary UV-B or VIS for 14 days. Then, UV-B exposure was stopped and the plants were allowed to continue growing under well-watered conditions or moderate drought for a period of 14 days. Leaf samples were collected during UV-B exposure for gene expression studies 0, 4, 28, 52 and 76 h, and at day 7, 14 and 28. Samples were taken from both UV-B-exposed or VIS control plants as follows: leaves from the upper part of the canopy were collected manually and immediately frozen in liquid nitrogen, then stored at –80 °C until analysis. For flavonoid content, samples were collected on days 0, 7, 14, and 28, then frozen in liquid nitrogen, stored at –80 °C, and freeze-dried for 3 days (Lyolab 3000, ThermoFisher Scientific). Samples from roots were collected at day 0, 7, 14, and 28. The whole root was removed, thoroughly cleansed, gently dried with paper, and stored at –80 °C until freeze-drying. Three replicates per sampling point were collected for all the analyses.

### 2.4. Drought treatment

The soil moisture was set as follows: moderate drought (40–45% of the field capacity), well-watered (75% of the field capacity), resulting in four treatments: VIS + well-watered (general control), UV-B-treated plants + well-watered, VIS + drought, and UV-B-treated plants + drought. Recovery of plants from drought treatment was accomplished by again watering them such as the soil moisture was allowed to increase from 40–45% to 75% of field capacity. Soil humidity was monitored using a soil moisture sensor (type ML1) and a hand-held read out unit (type HH1; ThetaProbe England).

### 2.5. Morphology and photosystem II fluorescence measurements

Three plants per treatment were examined for the following dry weight parameters: a) dry weight of stem; b) dry weight of the root (washing the root gently to avoid loss of material); c) dry weight of the leaves (placing all the foliar material inside a plastic basket). These three plant parts were dried in an oven at 80 °C overnight.

In addition, d) stem length was measured from the soil level to apex of the newest and longest bifurcation; e) number of leaves where counted, including both mature and young leaves; f) number of floral primordia in all bifurcations were counted.

Finally, g) F<sub>v</sub>/F<sub>m</sub> chlorophyll fluorescence measurements (using a Plant Efficiency Analyzer (PEA), Hansatech Instruments, United Kingdom) on youngest mature leaves by placing the PEA analyzer clip between the second and third vein of the leaf where the clip could be position without harming the leaf.

### 2.6. General phenolic compound content

Upper surface flavonoids in young leaves were measured in triplicate using a DUALEX<sup>®</sup> SCIENTIFIC (ForceA, France) according to the instruction of the manufacturer. In this non-destructive real-time

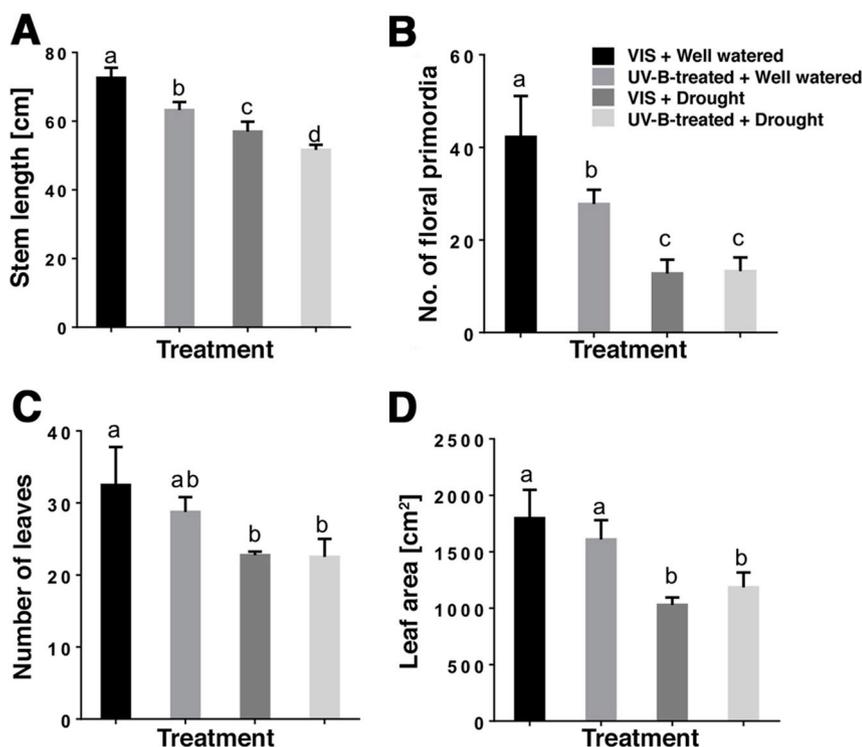


Fig. 1. Effects of UV-B treatments and drought on stem length (A), number of floral primordia (B), and number (C) and area (D) of leaves of chili pepper plants after 28 days. All plants were kept well-watered for 14 days (with or without UV-B exposure), whereas for the next 14 days, half of them were still kept well-watered and the other half under drought conditions. Between Day 14 and 28, no UV-B exposure was given. The number of samples per point,  $n = 3$ . Different letters in each histogram indicate significant difference between the treatments using Tukey's test ( $P = 0.05$ ).

method, the fact that leaf epidermal pigments (in particular flavonols) are screening out fractions of the UV light (375 nm in this case) that can be absorbed by chlorophylls in the mesophyll and then be emitted as fluorescence, is used for estimation of the leaf epidermal flavonol content (Bilger et al., 1997; Cerovic et al., 2002). As a reference for the chlorophyll fluorescence, red light (655 nm), for which the epidermis hardly has any absorption, is used. At a frequency of 1 kHz, the studied leaf is illuminated with UV and red light in an alternating fashion.

To analyse the phenolic compounds using HPLC, 20 mg lyophilized powdered leaf samples were extracted according to Neugart et al. (2015). For the quantitative analysis of flavonoid glycosides and hydroxycinnamic acid derivatives, an HPLC series 1100 (Agilent Technologies, Waldbronn, Germany) was used as described in Neugart et al. (2017). Standards (chlorogenic acid, apigenin 3-glucoside, and luteolin 7-glucoside; Roth, Karlsruhe, Germany) were used for external calibration curves in a semi-quantitative approach. Results are presented in  $\text{mg g}^{-1}$  dry weight. Three biological replicates per sampling point were collected for all the analysis. HPLC measurements were performed with technical duplicates.

### 2.7. Gene expression analysis of molecular markers *Mn-SOD*, *POD*, *PAL*, *CHS*

Two molecular markers for flavonoid biosynthesis (phenylalanine ammonia lyase, *PAL*, GenBank accession number AF081215, and chalcone synthase, *CHS*, GenBank accession number FJ705842.1) and two molecular markers for oxidative pressure (mitochondrial manganese superoxide dismutase, *Mn-SOD*, GenBank accession number AF036936.2, and peroxidase, *POD*, GenBank accession number FJ596178.1) were used in this study as an indication of effects of the treatments on these pathways. The choice of genes was based on previous results where inductions were shown by UV-B, drought and abiotic stress. Triplicate leaf samples were ground in liquid nitrogen, and RNA extracted (RNAeasy Plant Mini Kit, Qiagen). RNA of high purity (260/280 nm absorbance ratio above 2.0 and 260/230 nm absorbance ratio 1.8–2.0) was used to synthesize cDNA (Mastercycler gradient, Eppendorf) using the Maxima First Strand cDNA Synthesis

#K1612 (ThermoFisher Scientific) for RT-qPCR according to the instructions of the provider (10 min at 25 °C followed by 15 min at 50 °C). Primers for *Mn-SOD* (forward 5'-CTC TGC CAT AGA CAC CAA CTT-3'; reverse 5'-CCA AGT TCG GTC CTT TAA TAA-3'), that we have previously shown to be induced by drought in chili pepper (Rodríguez-Calzada and Guevara-González, unpublished results), *POD*, reported to be induced by abiotic stress in *Capsicum annum* (forward 5'-GCA GCA TTC CTC CTC CTA CT-3'; reverse 5'-ATT TCT TTG CCT TGT TGT TG-3'), *PAL* (forward 5'-ATT CGC GCT GCA ACT AAG AT-3'; reverse 5'-CAC CGT GTA AGG CCT TGT TT-3'), *CHS* (forward 5'-TCG ACC CTC AGT CAA ACG AC-3'; reverse 5'-TGG GCC ACG GAA AGT AAC TG -3'); and beta-tubulin (*β-TUB* GenBank accession number EF495259.1, forward 5'-GAG GGT GAG TGA GCA GTT C-3'; reverse 5'-CTT CAT CGT CAT CTG CTG TC), all provided by Eurofins, Germany, were utilized to amplify the genes using Applied Biosystems™ PowerUp™ SYBR™ Green Master Mix to perform qPCR analysis (Step One Plus Real-Time PCR System, ThermoFisher Scientific). Reaction conditions for all the genes were: 2 s, 95 °C, and 40 cycles of 3 s, 95 °C and 30 s, 60 °C.

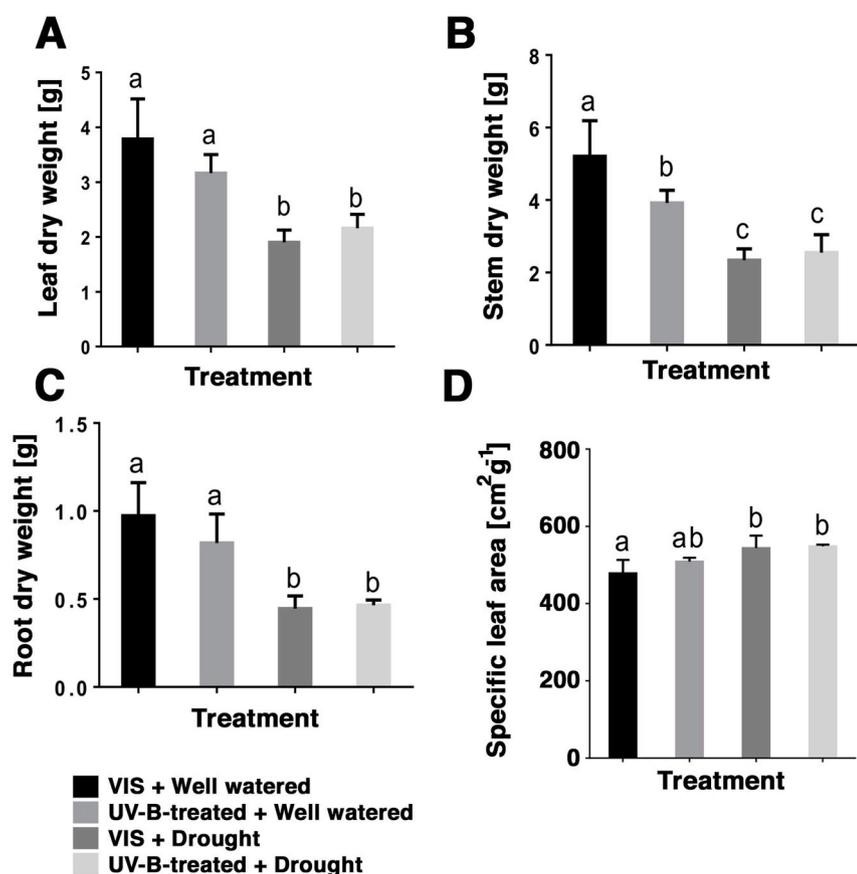
### 2.8. Statistical analysis

For all experiments, two-way ANOVA was performed, and for multiple comparison tests, Tukey's test ( $P = 0.05$ ) was applied. In the case of phenolic compounds, for each compound the cell averages were compared, regardless of row and columns. The highest value is encoded with "a" etc. In all cases the factors evaluated were the number of days of treatment and the different treatments themselves (i.e. the combination of the type of light and water regimen).

## 3. Results and discussion

### 3.1. Effects of UV-B treatment on morphological features of chili pepper

The treatment of chili pepper plants with UV-B radiation led to significant decreases in stem length (from 72.6 to 63.2 cm) and number of floral primordia (from 42 to 28) under well-watered conditions (Fig. 1A and B; Supplementary Table S2). VIS + drought plants showed



**Fig. 2.** Effects of UV-B treatments and drought on dry weight of leaves (A), stems (B), roots (C), and specific leaf area (SLA, in  $\text{cm}^2$  of leaf area per g of leaf dry weight) of chili pepper plants after 28 days. All plants were kept well-watered for 14 days (with or without UV-B exposure), whereas for the next 14 days, half of them were still kept well-watered and the other half under drought conditions. The number of samples per point,  $n = 3$ . Between Day 14 and 28, no UV-B exposure was given. Different letters in each histogram indicate significant difference between the treatments using Tukey's test ( $P = 0.05$ ).

even larger decreases for these two parameters (stem length 57.0 cm and 13 floral primordia only) than those observed for plants treated with UV-B under well-watered conditions, although for floral primordia the effect was the same independently of whether the plants had been exposed to UV-B before the drought treatment or not. Moreover, UV-B + drought treatment caused the highest significant decrease observed in stem length (51.6 cm under these conditions). No statistically significant effects of UV-B on leaf number and total leaf area were observed in plants kept well-watered (Fig. 1C and D; Supplementary Table S2). However, significant differences in total leaf area were seen when comparing plants grown under the two different light conditions for 14 days and then kept under well-watered conditions for another 14 days on the one hand, and similar plants kept under drought conditions (Fig. 1D; Supplementary Table S2). For the number of leaves present on the plants, a significant difference was found only between well-watered plants grown under VIS control light (32 leaves), and plants subjected to drought (23 leaves), independently of what light conditions the latter plants had experienced between days 0 and 14 (Fig. 1C; Supplementary Table S2).

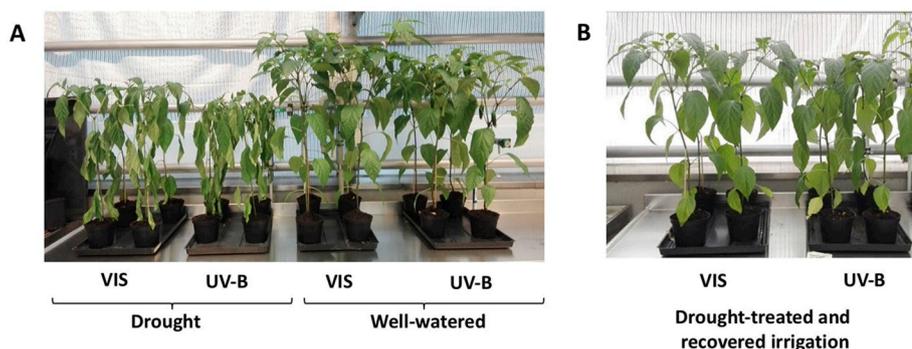
Exposure of plants to supplementary UV-B radiation have in a number of previous studies led to significantly lower biomass production compared to the situation in non-exposed plants. This has in turn led to losses in yield (Kataria and Guruprasad, 2012; Paul et al., 2012; Choudhary and Agrawal, 2014; Lee et al., 2014). Thus, similarly to studies in other species (Bandurska et al., 2012; Kataria and Guruprasad, 2012; Zhang et al., 2014), we here show that also chili pepper plants displayed a clear reduction in plant height. It is obvious from the data that the drought regime applied is a more severe stress than the UV-B and only for the stem length parameter there seems to be an additive response of the two environmental factors under the conditions used. However, the drought stress applied (shown in Fig. 3A) were not more severe than that plants that were capable of recovering and re-establishing a normal phenotype (Fig. 3B) proving that the

drought conditions as such were not lethal to the plants.

Stem dry weight of UV-B-treated plants also significantly decreased (from 5.2 to 3.9 g), while leaf and root dry weights were significantly affected by the water status only (Fig. 2; Supplementary Table S2), where root biomass decreased from approximately 0.89 g on average to approximately 0.45 g, and leaf dry weight from approximately 3.5 g to approximately 2.0 g on average. Inhibition of root growth during drought is a characteristic of the chili pepper cv. Coronel (Arrowsmith et al., 2012).

Just as the plant dry weight of leaves and total leaf area per plant was not significantly changed when comparing VIS control and UV-B-treated plants grown under 28 days of well-watered conditions, the dry weight per leaf area did not differ between the two treatments either ( $2.0\text{--}2.1 \text{ mg cm}^{-2}$ ; not shown). Thus, under our conditions, UV-B treatment did not lead to morphological changes in the form of thicker leaves of UV-B-treated plants, an effect that has been seen in previous studies (Wargent and Jordan, 2013). However, both drought and the combination of UV-B and drought significantly increased leaf thickness (Fig. 2D), expressed as a decrease in specific leaf area (SLA, in  $\text{cm}^2$  of leaf area per gram of leaf dry weight). Our specific conditions for instance included a pre-treatment of UV-B before the onset of drought. It is still possible that applying both stresses simultaneously, or at other severities or levels of photosynthetically active radiation, could have resulted in other outcomes, e.g. UV-B-induction of drought tolerance. Therefore, the interplay of UV-B and drought deserves to be explored in more detail using a number of combinations of UV regions and doses, PAR levels, and temporal patterns of UV and drought applications.

Although, as was mentioned above, there was no statistically significant effect of UV-B on leaf number or leaf area, the surface of a number of leaves of UV-B-treated plants showed a curled or mis-formed morphology (Supplementary Fig. S1A), indicating deleterious effects on morphogenesis, possibly during cell division or leaf expansion. In line with this, it has recently been shown in maize that UV-B affects cell



**Fig. 3.** Morphological aspects of chili pepper plants treated with UV-B or VIS radiation under well-watered or drought conditions. Panel A, general appearance of plants after 28 days of cultivation in different regimes of radiation and irrigation; panel B, plants with 24 h of recovery irrigation after the drought period. Both VIS control plants and UV-B-treated plants were used.

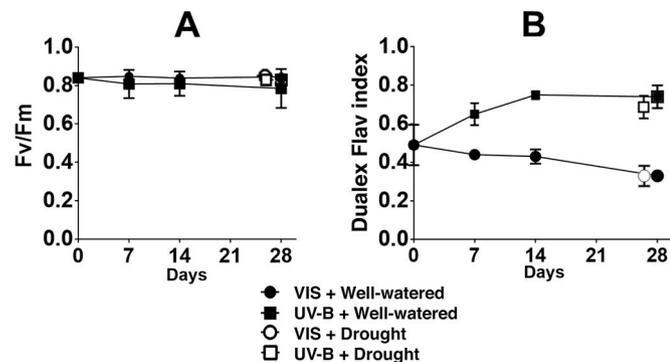
growth and leaf concentrations of auxins, cytokinins, jasmonic acid and a precursor of gibberellin that correlate with changes in leaf morphology (Fina et al., 2017). It is worth mentioning that these morphological effects were induced by UV-B levels lower than those under natural solar UV-B (see Materials and Methods, section 2.2).

Fruit development in UV-B-treated chili pepper was not affected. UV-B exposed plants grew fruits of normal appearance (Supplementary Fig. S1B). Interestingly, UV-B treatment induced earlier fructification by 1 week in comparison with control (VIS) plants (data not shown), which may indicate an effect of UV-B on hormonal action (i.e. gibberellin production). Thus, in total, UV-B radiation induces selective regulation of development, morphology and growth in chili pepper, similarly to previous results with other plant species (e.g. Robson et al., 2015; Suchar and Robberecht, 2015).

### 3.2. Function of photosystem II and phenolic compound levels in leaves and roots of UV-B-treated chili pepper plants

UV-B treatment of chili pepper plants did not affect the maximum quantum yield of photosystem II ( $F_v/F_m$ ) in any statistically significant fashion, neither under well-watered nor drought conditions, during the 28 days of cultivation (Fig. 4A). This indicates that neither the UV-B nor the drought treatments affected the primary photochemical efficiency of photosystem II (PSII) in these plants negatively. Again, this means that the plants were not stressed to the point where energy supply to the plant through photosynthesis was severely curtailed.

Flavonol levels (expressed as Dualex Flav index) in the superior



**Fig. 4.** (A) Maximum quantum yield of photosystem II ( $F_v/F_m$ ) and (B) upper surface flavonoids levels (expressed as “Dualex absolute value”) of chili pepper cultivated under well-watered or drought conditions during 28 days of cultivation. All plants were kept well-watered for 14 days (with or without UV-B exposure), whereas for the next 14 days, half of them were still kept well-watered and the other half under drought conditions. Between Day 14 and 28, no UV-B exposure was given. Due to overlap of results and standard deviations, for the four day 28 points we have separated the points for the sake of clarity. The number of samples per point,  $n = 3$ . The standard deviation of the measurements is shown.

epidermis of UV-B-treated chili pepper leaves were significantly increased under both well-watered and drought conditions compared with the VIS control (Fig. 4B). Approximately, a doubling was obtained during the experimental period. Increased abundance of flavonoids as a UV-B response in crop plants is a well-studied phenomenon (Neugart and Schreiner, 2018). Drought has previously been shown to give rise to contrasting effects on flavonoid content depending on e.g. the species, the cultivar, and the developmental stage of the tissue (Alonso et al., 2015; Klunklin and Savage, 2017; Ngwene et al., 2017; Zandalinas et al., 2017).

The content of specific phenolic compounds in whole leaves was also assayed. Generally, apigenin (4',5,7-trihydroxyflavone) was a more common flavonoid aglycon than luteolin (3',4',5,7-tetrahydroxyflavone) in pepper leaves and chlorogenic acid had an intermediate abundance compared with the first two. This is in line with previous findings (Marin et al., 2004; Materska and Perucka, 2005; Mikulic-Petkovsek et al., 2013). Up to  $3.8 \text{ mg g}^{-1}$  dry weight of apigenin compounds were present, whereas the maximal levels of chlorogenic acid and luteolin compounds were  $0.6$  and  $0.045 \text{ mg g}^{-1}$  dry weight, respectively. The levels of chlorogenic acid in leaves showed considerable variation between samples. However, the increases of this compound in UV-B-treated plants were significant after 7 and 14 days of exposure (3.2- and 2.2-fold, respectively; Table 1 and heatmap in Supplementary Fig. S2). Accumulation of chlorogenic acid in chili pepper leaves have also previously been found in a UV-B study with bell pepper carried out by León-Chan et al. (2017). Furthermore, a subsequent drought treatment of both UV-B-treated and VIS control chili pepper plants, led to a significant decrease in chlorogenic acid content after 28 days as previously shown for indigenous African species (Ngwene et al., 2017).

Significant increases from low levels were also found in luteolin-8-C-hexoside after UV-B exposure, (2.5- and 2.0-fold, after 7 and 14 days of treatment, respectively). The aglycone lutein has a catechol structure in the B ring similarly to quercetin, which is a well-known antioxidant (Agati and Tattini, 2010; Agati et al., 2011, 2013; Fiol et al., 2012). Luteolin 6-C-pentoside-8-C-hexoside levels were unaltered after 14 days of UV exposure as well as in VIS control plants subjected to drought. However, a subsequent drought stress in UV-B-treated plants led to a synergistic effect in the form of a more than 50% increase of this compound, which is in line with literature (Alonso et al., 2015; Ngwene et al., 2017; Zandalinas et al., 2017). Traces of luteolin 7-O-(2-apiosyl-6-acetyl)glucoside were found primarily under control conditions (Supplementary Fig. S1).

For apigenin 8-C-hexoside many of the changes were significant with a general decreasing trend under VIS conditions (down by 85% over 14 days) and a general increasing trend during UV-B exposure (by approximately 50% over the 14 days) in accordance with literature (Agati et al., 2011; Tattini et al., 2005). After the end of UV exposure, the levels of this phenolic compound decreased again by about 70% under well-watered conditions. Under drought the apigenin 8-C-hexoside leaf concentrations decreased sharply both in VIS controls and in

**Table 1**

Phenolic compound content at days 0, 7, 14, and 28 in leaves of chili pepper either treated with supplementary UV-B radiation or kept in PAR only (VIS control) for 14 days and then grown for 14 days (until day 28 of the experiment) under either drought or well-watered conditions. Three biological replicates per sampling point were collected for all the analyses. HPLC measurements were performed with technical duplicates. Results are presented in mg g<sup>-1</sup> dry weight.

Phenolic compound	Water regimen	Irradiation type	Time of exposure to supplementary radiation			Watering or drought
			Day 0	Day 7	Day 14	Day 28
Chlorogenic acid	Well-watered	VIS	0.185 ± 0.120 b	0.014 ± 0.017 c	0.054 ± 0.051 c	0.590 ± 0.244 a
		UV-B		0.593 ± 0.207 a	0.406 ± 0.056 a	0.498 ± 0.096 a
	Drought	VIS				0.053 ± 0.001 c
		UV-B				0.153 ± 0.135 b
Luteolin 6-C-pentoside-8-C-hexoside	Well-watered	VIS	0.029 ± 0.007 b	0.043 ± 0.003 a	0.029 ± 0.006 b	0.023 ± 0.006 b
		UV-B		0.026 ± 0.007 b	0.023 ± 0.003 b	0.025 ± 0.009 b
	Drought	VIS				0.028 ± 0.007 b
		UV-B				0.044 ± 0.006 a
Luteolin 8-C-hexoside	Well-watered	VIS	0.004 ± 0.001 b	0.001 ± 0.001 b	0.001 ± 0.001 b	0.001 ± 0.001 b
		UV-B		0.010 ± 0.002 a	0.008 ± 0.002 a	0.004 ± 0.006 ab
	Drought	VIS				0.001 ± 0.001 b
		UV-B				0.001 ± 0.001 b
Apigenin 6-C-pentoside-8-C-hexoside	Well-watered	VIS	2.522 ± 0.523 a	1.751 ± 0.232 b	1.443 ± 0.416 b	1.499 ± 0.749 ab
		UV-B		2.453 ± 0.662 ab	2.712 ± 0.278 a	2.175 ± 0.224 a
	Drought	VIS				1.749 ± 0.153 b
		UV-B				1.355 ± 0.747 ab
Apigenin 8-C-hexoside	Well-watered	VIS	0.733 ± 0.137 b	0.179 ± 0.036 d	0.109 ± 0.062 d	0.249 ± 0.035 c
		UV-B		1.141 ± 0.225 a	1.051 ± 0.260 a	0.326 ± 0.068 c
	Drought	VIS				0.060 ± 0.021 d
		UV-B				0.115 ± 0.007 d

UV-B-exposed plants until day 28. The apigenin 6-C-pentoside-8-C-hexoside leaf content decreased over time during the experimental period in VIS control plants (most significantly after 14 days) but not in UV-B-treated plants, in line with the above discussion.

In roots (Table 2), the chlorogenic acid content was generally much lower than in leaves (maximally 0.19 mg g<sup>-1</sup> dry weight under any of our experimental conditions), which has also been shown in faba bean (Li et al., 2012), but increased over time, primarily under well-watered conditions and more so in plants not treated with UV-B. This might be due to interaction with microbiota (Witzel et al., 2017). The drought treatment between days 14 and 28 resulted in an at least 85% decrease in root chlorogenic acid content and is similar to the results obtained in shoots. Two other unknown phenolic compounds were also found in roots at low levels but with unclear trends of abundance with regards to the two environmental factors.

### 3.3. Gene expression changes of phenolic compounds and oxidative stress response genes by UV-B treatments in chili pepper

Gene expression associated with phenolic compound biosynthesis (*PAL* and *CHS*) displayed a clear and significant early induction by UV-

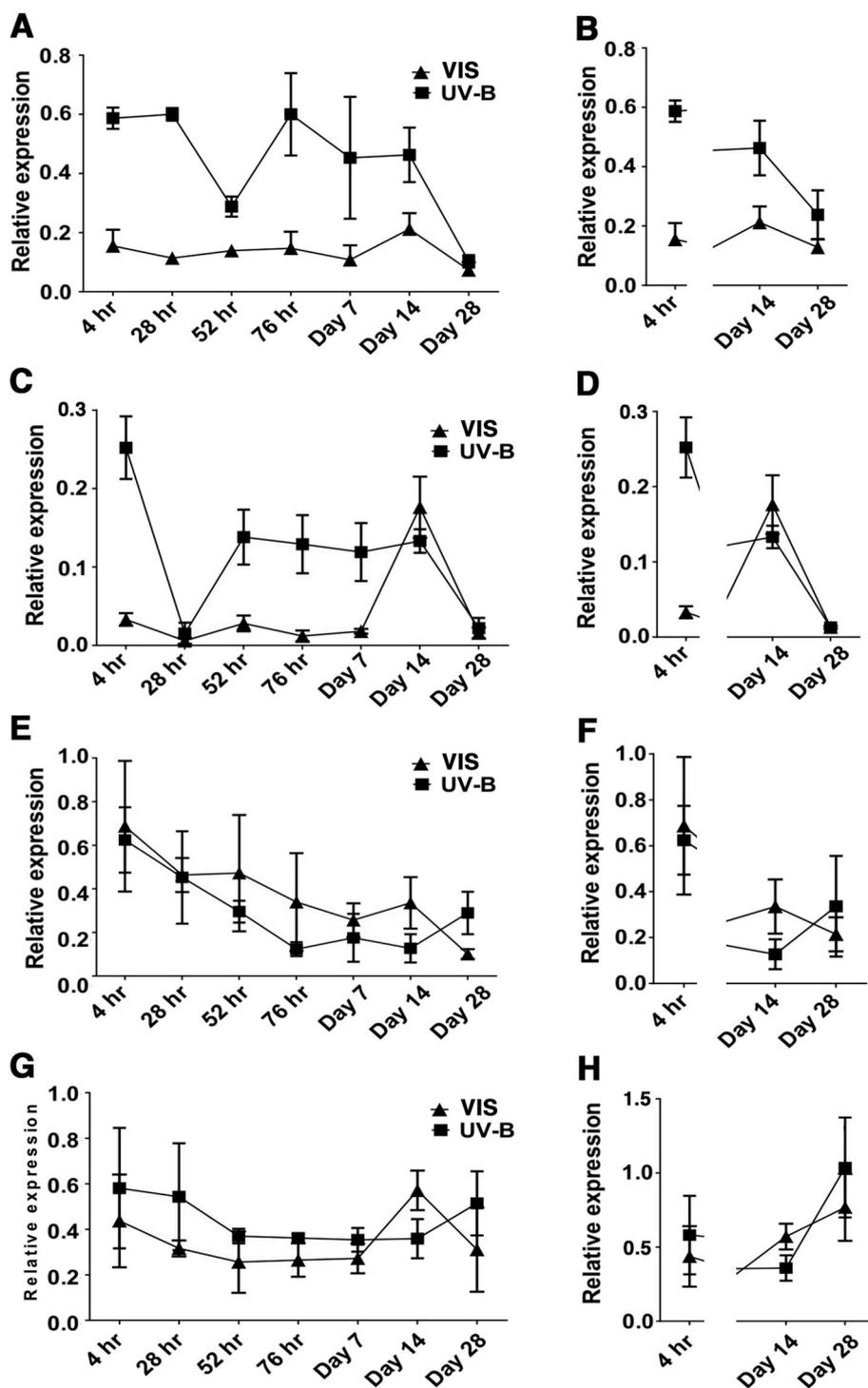
B treatment compared with VIS controls, from 4 h after the commencement of the treatment until 14 days (Fig. 5A and C). Under the treatment that followed (i.e. from day 15–28), either under well-watered or drought conditions in the absence of UV-B, a clear decrease in expression of these genes was found (samples collected on day 28; Fig. 5A–D). In addition, an approximate doubling of transcript levels for *PAL* on day 28 in plants subjected to drought (Fig. 5B) was obtained compared to plants kept well-watered (Fig. 5A), both in VIS controls and plants previously exposed to UV-B. For *CHS*, no such drought effect was seen on day 28 (compare Fig. 5C and D). These results suggest a molecular effect of UV-B on the control of gene expression of the phenylpropanoid biosynthetic pathway in chili pepper, as has been shown in many other plant species (Chappell and Hahlbrock, 1984; Strid, 1993; Sävenstrand et al., 2004; Morales et al., 2010). Also, drought seems to a certain extent be a determinant of expression of the *PAL* gene but not the *CHS* gene.

On the contrary, the oxidative stress response genes evaluated (*POD* and mitochondrial Mn-*SOD*) did not show significant changes in their expression under the same levels of UV-B exposure (Fig. 5E and G). However, although not significant in most cases, an interesting trend is seen in the Mn-*SOD* and *POD* samples where gene expression increased

**Table 2**

Phenolic compounds content at days 0, 7, 14, and 28 in roots of chili pepper either treated with supplementary UV-B radiation or kept in PAR only (VIS control) for 14 days and then grown for 14 days (until day 28 of the experiment) under either drought or well-watered conditions. Three biological replicates per sampling point were collected for all the analyses. HPLC measurements were performed with technical duplicates. Results are presented in mg g<sup>-1</sup> dry weight.

Phenolic compound	Water regimen	Irradiation type	Time of supplementary irradiation			Watering or Drought
			Day 0	Day 7	Day 14	Day 28
Chlorogenic acid	Well-watered	VIS	0.010 ± 0.007 b*	0.004 ± 0.002 bc	0.011 ± 0.005 b	0.187 ± 0.091 a
		UV-B		0.009 ± 0.006 b	0.003 ± 0.001 c	0.100 ± 0.041 a
	Drought	VIS				0.029 ± 0.013 b
		UV-B				0.015 ± 0.003 b
Unknown 1	Well-watered	VIS	0.004 ± 0.002 a	0.006 ± 0.002 a	0.008 ± 0.001 a	0.015 ± 0.010 a
		UV-B		0.008 ± 0.008 a	0.005 ± 0.003 a	0.006 ± 0.003 a
	Drought	VIS				0.008 ± 0.001 a
		UV-B				0.012 ± 0.002 a
Unknown 2	Well-watered	VIS	0.039 ± 0.002 ab	0.045 ± 0.003 a	0.045 ± 0.007 a	0.031 ± 0.006 b
		UV-B		0.049 ± 0.011 a	0.041 ± 0.008 ab	0.024 ± 0.003 b
	Drought	VIS				0.027 ± 0.005 b
		UV-B				0.034 ± 0.004 b



**Fig. 5.** Changes in expression of genes associated with phenolic compound synthesis and oxidative stress responses in leaves of chili pepper plants. The genes under study were those for the phenylpropanoid biosynthesis genes phenylalanine ammonia lyase (*PAL*; panels A and B) and chalcone synthase (*CHS*; panels C and D), and reactive oxygen species-scavenging enzymes peroxidase (*POD*; panels E and F) and Mn-superoxide dismutase (*Mn-SOD*; panels G and H). All plants were kept well-watered for 14 days (with or without UV-B exposure), whereas for the next 14 days, half of them were still kept well-watered (panels A, C, E, and G) and the other half under drought conditions (panels B, D, F, and H). Between Day 14 and 28, no UV-B exposure was given. The number of samples per point,  $n = 3$ . The standard deviation of the measurements is shown.

after cessation of UV-B treatment between days 14 and 28 compared with the VIS controls. This might indicate a remaining ROS pressure in UV-B treated plants after the end of the UV-B exposure (Czégény et al., 2014). Just as for *PAL* (Fig. 5A and B), *Mn-SOD* expression doubled at 28 days in plants exposed to drought compared with well-watered plants, again independent of whether the plants had been treated with UV-B for 14 days or were VIS controls (Fig. 5G and H). Such an effect was not seen for *POD*. Thus, drought appears to regulate mitochondrial *Mn-SOD* expression but not *POD* expression under the conditions used here.

Regarding the gene expression studies carried out in this work, it is noteworthy that the qRT-PCR analysis was oriented only to the

individual genes corresponding to the GenBank accession numbers indicated in the Materials and methods section. This may be of importance since *PAL*, *CHS*, *Mn-SOD* and *POD* comprises gene families in plants (The Tomato Genome Consortium, 2012; Albert and Chang, 2014). Thus, other genes in each gene family might display different gene expression patterns to the environmental factors that are evaluated in this work.

#### 4. Conclusions

Both dicot and monocot plants exposed to UV-B radiation in addition to white light have previously been shown to grow less well when

compared with non-exposed plants. We here show that chili pepper plants display a clear reduction in plant height under UV-B. UV-B also negatively affected floral primordial development, but with no significant effects on leaf photosynthetic performance ( $F_v/F_m$ ) and fruit development. Additionally, UV-B treatment caused earlier fructification by 1 week. Although no drought tolerance was displayed in the chili pepper plants after using UV-B pre-treatment, increased levels of some of the leaf phenolic compounds (of interest for food and pharmacological industries due to their nutraceutical properties) were found. It is likely that the types and levels of phenolic compounds in chili pepper tissue induced by UV-B in this work were not significant enough to increase drought tolerance. Such a drought tolerance (Nakabayashi et al., 2014) or co-variance (Shojaie et al., 2016) have previously been shown to occur upon flavonoid accumulation in *Arabidopsis thaliana*. A more detailed study using several UV-B dose and exposure times, and probably during different phenological stages of chili pepper development, need to be conducted in order to exhaustively evaluate increased UV-induced drought tolerance in this crop. This also includes a larger transcriptome study.

### Author contributions

Tania Rodríguez-Calzada and Minjie Qian carried out the cultivation and experimentation associated with morphological changes and gene expression. Irineo Torres-Pacheco contributed plant seeds of chili pepper and the design of some experiments regarding cultivation (nutrient solution used). Susanne Neugart and Monika Schreiner carried out the analysis of phenolic compounds. Åke Strid and Ramon G. Guevara-González conceived the whole study and wrote the draft of the manuscript. All authors have read the final version of the manuscript and declare no conflicting interest.

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### Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.plaphy.2018.06.025>.

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