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Original Scientific Paper

UV-B induced changes in pea (*Pisum sativum*) pigments and antioxidative system: Effects of different UV dose distribution on immature and mature leaves

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ABSTRACT: Pea plants grown hydroponically under a 16/8 h photoperiod were treated with 16.2 kJ m^{-2} UV-B per day, provided as a single (1 x 30 min) or split (3 x 10 min) dose, to monitor the effects of dose splitting on protein content, photosynthetic pigments and total phenolics content, peroxidase and catalase activity. Apical immature leaves and youngest mature leaves were compared. UV-B treatment led to significant increases in chlorophyll and carotenoid concentrations in mature but not in immature leaves. A single dose was more effective than a split dose, inducing a greater increase in pigment contents. While immature leaves showed no significant differences in total phenolic contents upon treatment, mature leaves responded to the single dose with a slight decrease and the split dose resulted in an increase in total phenolics. A significant increase in catalase activity was observed in mature leaf samples in UV-B treated plants. Total protein content indicated that splitting the daily dose of UV-B induced less damage than the same total daily UV-B dose provided at once. These findings suggest that not only radiation amount, but time distribution of UV-B can also determine plant responses to high radiation doses.

Key words: pea, UV-B, dose distribution, photosynthetic pigments, antioxidative system

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INTRODUCTION

Depletion of the stratospheric ozone layer is leading to elevated UV radiation at the Earth surface. The emission spectrum of the sun includes 5-7% UV light (200-400 nm); however the largest part of this radiation is absorbed in the atmosphere. UV-C (100-280 nm) is completely absorbed, while UV-B (280-315 nm) is partially absorbed and less than 0.5% of total radiation that reaches the Earth surface falls in the UV-B region. This radiation, even in relatively low doses, can cause free radical formation and damage to biological macromolecules. Thus, living organisms should be able to react in some way specifically to UV-B to activate protection mechanisms.

UV-B radiation induces changes in gene expression, elevation of the contents of UV-absorbing compounds and altered phytochemical content (Liu *et al.* 2000; Lau *et al.* 2006, Brosché & Strid 2002). Ambient UV is generally recognized not to be an impediment to photosynthesis, while reductions in productivity are usually caused by a combination of ambient UV levels with another stress factor (Hofmann *et al.* 2003; Nogués & Baker 2000; Lau *et al.* 2006; Singh *et al.* 2011; Qaderi *et al.* 2010). Even elevated ambient UV caused by disturbance of the ozone layer can, in most cases, be regarded as a regulatory, and not a stress factor (Jansen *et al.* 2012).

Studying ROS metabolism related to UV treatment includes quantifying the activity of the enzyme components of the antioxidant system as proxies for oxidative pressure (Ravindran *et al.* 2010, Hideg *et al.* 2013). It has been established that UV induces elevation in ascorbate peroxidase, dehydroascorbate reductase, glutathione peroxidase, glutathione reductase, catalase and superoxide dismutase (reviewed in Hideg *et al.* 2013). However, there is still uncertainty whether these activities are upregulated in response to eustress, a stress not causing permanent

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damage (opposite of distress) but rather promoting health and growth (Hideg *et al.* 2013). Also, interpretation of results is complicated because different species, varieties of the same species as well as stages of development or even leaves on the same plant react differently (Hideg *et al.* 2013; Majer & Hideg 2012; He *et al.* 2006; Li *et al.* 2010; Frohnmeyer & Staiger 2003; Kakani *et al.* 2003).

In this work, we used a high UV-B dose supplied as a single 30 min treatment or as a split dose, divided into three treatments of 10 min during two days to establish whether splitting the dose and introducing "resting periods" between irradiations can reduce the damage caused by high UV-B treatment. We compared reactions of apical, immature leaves, and the youngest fully developed, mature, leaves to supplementary UV-B irradiation.

MATERIAL AND METHODS

Plant material and treatments. Pea seeds (*Pisum sativum* L., var. Mali provansalac, Superior DOO) were thoroughly rinsed and left to imbibe for 24 h, and then transferred to the dark, on moist filter paper to germinate at 22±2°C. After 4 d, germinated seeds were transferred to half-strength Höagland mineral nutrient solution (Höagland & Davis 1923) and grown at 22±2°C, with a photoperiod 16 h day/8 h night, under white light provided by four fluorescent tubes (Philips TLD 18 W) and two incandescent lamps (Philips Philinea 60 W).

UV-B treatment was provided by a Estus 2 UV-B lamp, with maximum emission at 312 nm, and energy 9 J m^{-2} s^{-1}, daily dose being 16.2 kJ m^{-2}. Treatment began when plants were 8 d old, and lasted for two days with different daily distributions of the same dose of UV-B radiation. One experimental group was irradiated with supplemental UV-B once a day, at noon, for 30 min (single dose, 1*30 min). The second experimental group was irradiated three times a day, at 9, 12 and 15 h, for 10 minutes per treatment (split dose, 3*10 min) for 2 d. UV-B was supplied in addition to background white light.

To establish whether a low UV-B dose would increase resistance to UV-B induced stress, a daily 5 min UV-B pretreatment (2.7 kJ m^{-2} per day for two days) was included in the morning, 2 d prior to the beginning of treatment.

Sample preparation. After treatment, plants were harvested and height and total weight were measured. For biochemical analyses, stem apex (5th and 6th node with undeveloped leaves), and 4th node with fully developed leaves were collected, frozen in liquid nitrogen and stored at -80°C until extraction.

Samples were homogenized in liquid nitrogen using a mortar and pestle. Total proteins were extracted from powdered tissue in isolation medium consisting of 50 mM potassium phosphate buffer pH 6.3, 2 mM EDTA, 2 mM dithiothreitol, 1% polyvinylpyrrolidone, and 0.05% Triton X-100. After centrifugation (10 min, 13000 x *g*) the supernatant was used for protein and enzyme determination, while the pellet was used for photosynthetic pigment determination.

Photosynthetic pigments were extracted in 80% acetone for 24 h at 4°C. Samples were then centrifuged for 10 min at 13000 x *g* and the supernatant was used for pigment determination.

For total phenolic contents, powdered tissue was extracted in 80% methanol at 60°C for 3 h, centrifuged (10 min at 13 000 x *g*) and the supernatant was used for subsequent analyses.

Biochemical analyses. Photosynthetic pigment contents were determined spectrophotometrically in 80% acetone, according to Lichtenthaler (1987).

Total phenolic content was estimated essentially by the method of Singleton & Rossi (1965) with gallic acid as a standard. Samples (50 ml) were incubated with 0.475 ml 5% Na_2CO_3 for 3 min, then 0.475 ml 1N Folin-Ciocalteu reagent was added. The mixture was placed in the dark at room temperature and absorbance at 724 nm was measured after 1 h incubation.

Protein content was estimated according to Bradford (1976) modified for a microtiter plate, with BSA as a protein standard.

Peroxidase activity was detected spectrophotometrically by monitoring the production of purpurogallin at 420 nm (Chance & Maehly 1955) and calculated using an extinction coefficient of 0.264 $mM^{-1}cm^{-1}$. The assay mixture contained 50 mM potassium-phosphate buffer pH 6.3, 42 mM pyrogallol and 8 mM hydrogen peroxide in 1ml volume.

Catalase activity was measured in a mixture containing 50 mM potassium-phosphate buffer pH 7 and 7.4 mM hydrogen peroxide (Claiborne 1984). The reaction was started by adding 10 µl sample, and decrease of H_2O_2 absorbance was monitored for 3 min at 240 nm, with an extinction coefficient of 43.6 $\mu M^{-1}cm^{-1}$.

All measurements were made with four separately extracted biological replicates, and all parameters were measured in triplicates for each sample. Data were tested by one-way ANOVA. Statistical analyses were performed using Microcal Origin 6.1

RESULTS

Pea leaf responses concerning photosynthetic pigments differed in apical immature and subapical mature leaves. In the control group, pigment concentrations were 10-20% lower in apical immature leaves than in mature leaves. Upon UV-B treatment these concentrations were almost 40% lower than control concentrations in immature leaves treated with both split and a single daily dose. Mature leaves showed pronounced elevation of pigment contents (Figs 1, 2). Chlorophyll concentration (Fig. 1) was almost two times higher (90%) than the control when plants were

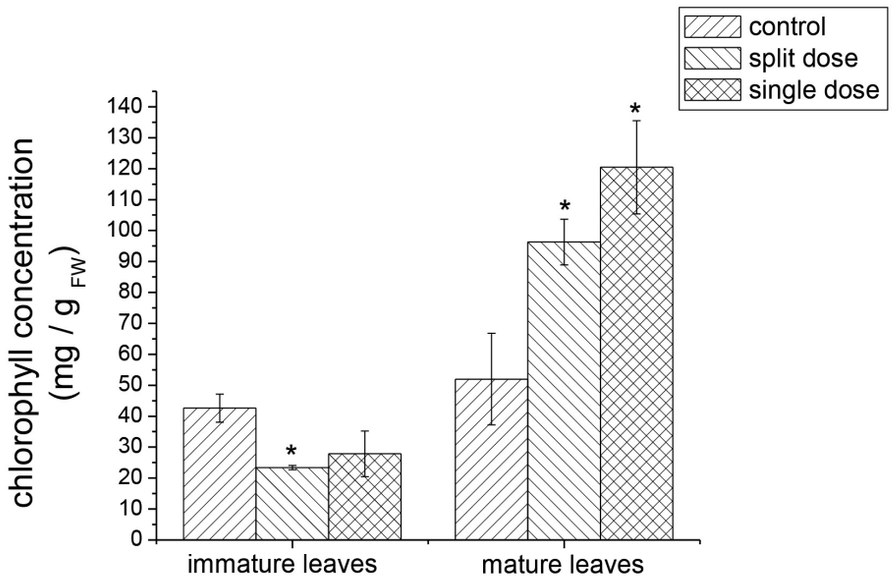

Fig 1. Total chlorophyll (*a+b*) concentrations in apical immature and subapical mature leaves in the control group, split dose group (3*10 min supplemental UV-B daily) and single dose group (1*30 min supplemental UV-B daily). Each value represents the mean of 4 replicates ± SE. Asterisk denotes statistically significant differencies compared to the corresponding control at 0,05 level.

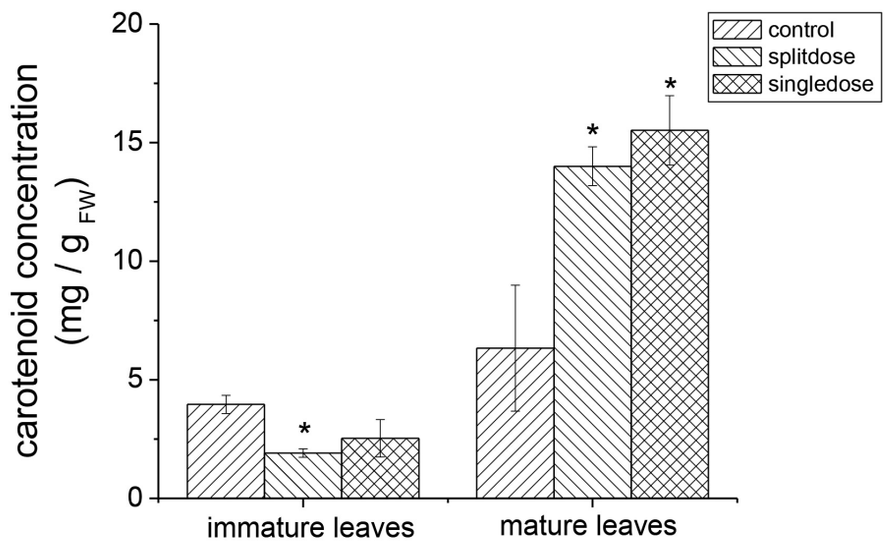

Fig 2. Total carotenoid concentrations in apical immature and subapical mature leaves in the control group, split dose group (3*10 min supplemental UV-B daily) and single dose group (1*30 min supplemental UV-B daily). Each value represents the mean of 4 replicates ± SE. Asterisk denotes statistically significant differencies compared to the corresponding control at 0,05 level.

treated with the split UV-B dose (3*10 min) and even higher (120%) when plants received the full UV-B dose at once (single dose, 1*30 min). The chlorophyll *a:b* ratio of 1.95±0.01 in immature leaves was independent of UV-B treatment. In mature leaves, the chlorophyll *a:b* ratio was more variable amongst the replicates, but not in response to UV treatment; control, single and split-dose groups all exhibited chlorophyll *a:b* ratios around 1.6. Total leaf carotenoid content showed the same trend as chlorophyll (Fig. 2) with more pronounced treatment effects: split dose induced a *ca*. 130% and the single dose induced a 160% rise in carotenoid content in mature leaves, while in immature leaves the total carotenoid content was decreased by *ca*. 40%.

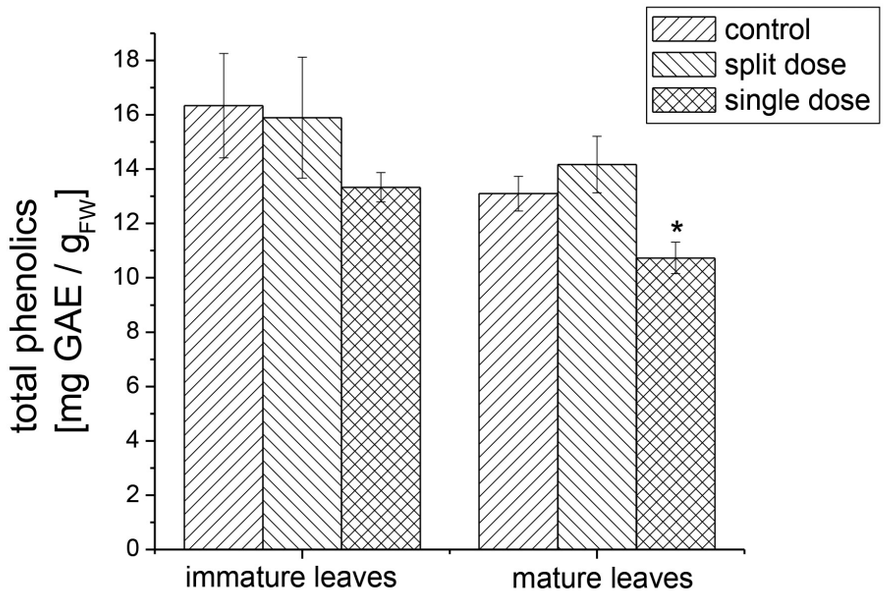

Fig 3. Total phenolic compounds concentrations expressed as mg gallic acid equivalents (GAE) in apical immature and subapical mature leaves in the control group, split dose group (3*10 min supplemental UV-B daily) and single dose group (1*30 min supplemental UV-B daily). Each value represents the mean of 4 replicates ± SE. Asterisk denotes statistically significant differencies compared to the corresponding control at 0,05 level.

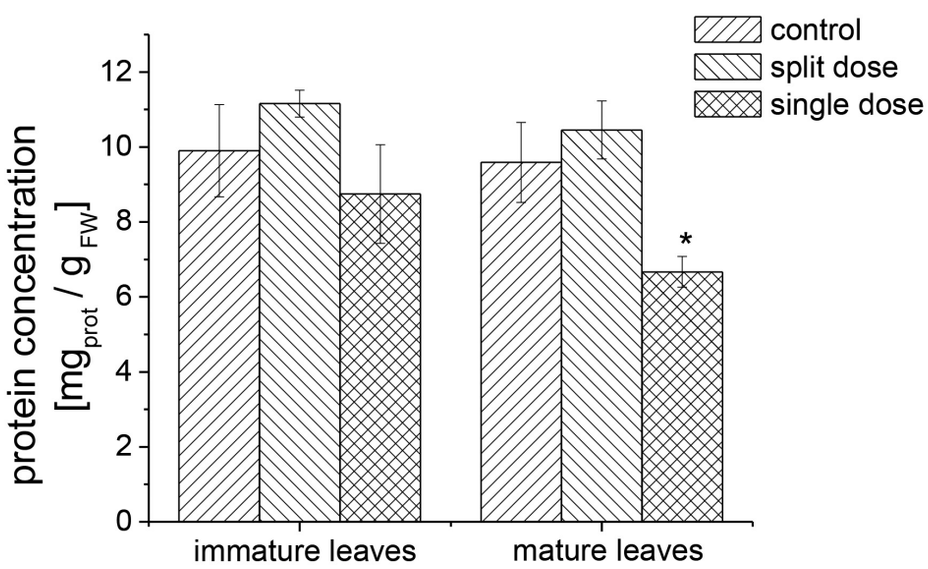

Fig 4. Total protein concentrations in apical immature and subapical mature leaves in the control group, split dose group (3*10 min supplemental UV-B daily) and single dose group (1*30 min supplemental UV-B daily). Each value represents the mean of 4 replicates ± SE. Asterisk denotes statistically significant differencies compared to the corresponding control at 0,05 level.

Effects of UV-B treatment on phenolic compound contents (Fig. 3) were not pronounced in apical leaves. In fully-developed leaves, the split dose caused a slight increase (9%) in total phenolic compounds while the single dose treatment caused an 18% decrease in phenolic content that was statistically significant. Total protein amount (Fig. 4) showed no significant differences in young immature leaves, while in fully developed leaves, the single UV-B dose decreased total proteins to 70% of the control level.

Peroxidase (POD) and catalase (CAT) activities (Fig. 5) were affected by UV-B irradiation, though POD

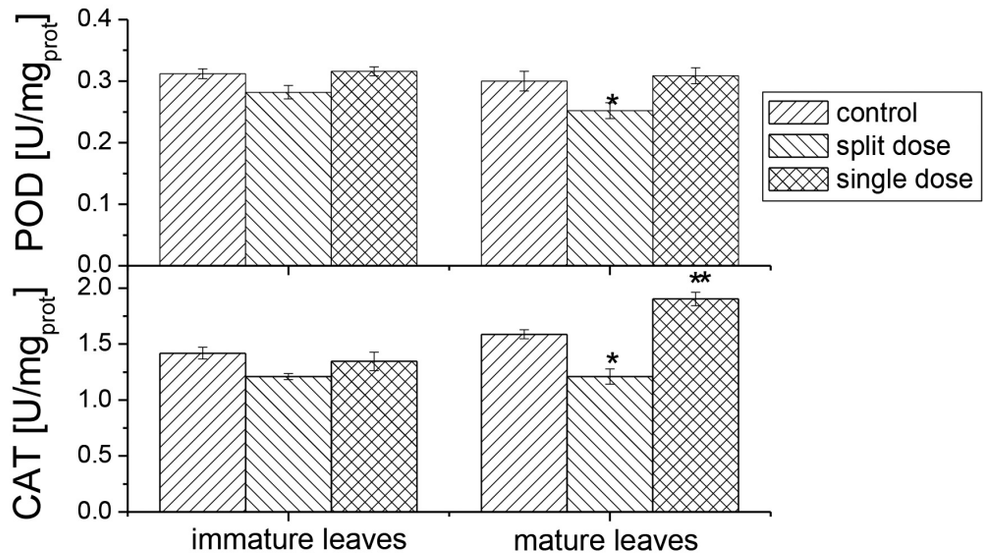

Fig 5. Catalase and pyrogallol peroxidase-specific activities in apical immature and subapical mature leaves in the control group, split dose group (3*10 min supplemental UV-B daily) and single dose group (1*30 min supplemental UV-B daily). Each value represents the mean of 4 replicates ± SE. Asterisk denotes statistically significant differencies compared to the corresponding control at 0,05 level.

effects were quite small. POD was practically unaffected after the single dose treatment, and the split dose caused decreases of about 10% in immature and 17% (significant at $p<0.05$) in mature leaves. Catalase activity in mature leaves changed significantly upon treatment, with the split dose inducing a decrease and the single dose an increase in CAT activity. In immature leaves, CAT activity changes were not significant.

Pretreatment that was introduced two days before the UV treatment started, aiming to harden plants, had little effect. Changes were detected in mature leaves when control plants were pretreated, with no subsequent UV-B treatment, where POD and CAT activities were significantly lower ($p<0.05$) than in the control (data not presented). Also, total phenolics were slightly elevated in mature leaves when the pretreatment was included, but changes were not statistically significant.

DISCUSSION

Changes in both chlorophylls and total carotenoids exhibited the same trend irrespective of the distribution of UV-B irradiation in our experiments. In apical immature leaves, applied levels of UV-B caused a slight decline in photosynthetic pigment contents. These leaves would not have been photosynthetically active, and were more susceptible to UV-B induced damage. This may be because young leaves had not yet developed protection mechanisms and direct photooxidation of pigments could occur. Immature leaves do not have developed cuticle wax layers that were shown to act as protectors and in preventing a decrease in photosynthesis under UV-B treatment (Skórska 2000). Casati & Walbot (2004) showed that immature leaves also have a specific gene expression pattern related to active developmental processes and differ from adult leaves in their transcriptome changes upon UV-B treatment. This is probably the reason for immature leaves not being able to react as efficiently as mature leaves to elevated UV–B levels. In contrast, subapical mature leaves promptly reacted to UV-B treatment by elevation of photosynthetic pigment contents. Elevated UV-B has led mainly to decreases in photosynthetic pigments content (He *et al.* 2006), due to reduced carbon allocation to chlorophyll synthesis and increased chlorophyll degradation (Lau *et al.* 2006). In only a few cases have these pigments been up-regulated by UV-B exposure (reviewed by Kakani *et al.* 2003). It was shown that responses are different in primary and trifoliate leaves of cowpea seedlings (Premkumar & Kulandaivelu 2001), and in plants subjected to different ambient white light conditions (Cen & Bornman 1990). Ravindran *et al.* (2008) showed that during the first few days of UV-B treatment chlorophyll content was elevated, while prolonged treatment led to a decrease in photosynthetic pigments. Our investigation was short-term and elevation of total photosynthetic pigments might have been due to up-regulation of chlorophyll-degrading enzymes and carbon allocation not yet reaching their maxima under conditions of elevated UV-B level, while extensive direct photooxidation is unlikely because of the protection mechanisms present in mature leaves. In our study, UV-B supplemental radiation had the same effect on chlorophyll *a* and chlorophyll *b* contents, thus not changing the chlorophyll *a:b* ratio as reported previously (He *et al.*

2006). Dose responses were shown to differ significantly between species and even lines or cultivars (González *et al.* 1998, He *et al.* 2006, Baroniya *et al.* 2011). The elevation of pigment contents under short-term high-dose UV-B that we have applied, could lead to increased photosynthetic activity as well as more efficient photoprotection, as reported during the first days of UV treatment in *Indigofera tinctoria* L. by Ravindran *et al.* (2008).

Synthesis of phenolic compounds is known to be the first line of defense when plants are exposed to UV-B radiation (Li *et al.* 1993; Landry *et al.* 1995; Day & Vogelmann 1995). Synthesis of UV-B absorbing compounds lowers the amount of radiation that penetrates cells, thus lowering damage to macromolecules (González *et al.* 1996). In our study, effects were not pronounced on immature leaf phenolic contents, as differences between experimental groups were not significant (Fig. 4), although a 19% decrease in total phenolics was evident in plants treated with a single dose. In the case of mature leaves, results were rather unexpected. It has been shown (Skórska 2000; Bieza & Lois 2001) that exposure to UV-B radiation increases the content of total phenolic compounds, while our results were the opposite. Down-regulation of UV-B absorbing compounds was recorded in only a few studies (Deckmyn & Impens 1997; Bornman & Vogelmann 1991). Partial inhibition of the synthesis of UV-B screening pigments was detected when plants were exposed to high UV-B doses, and in some cases it was shown that very high UV-B doses could inhibit flavonoid biosynthesis (Deckmyn & Impens 1997; Tevini *et al.* 1983). UV-B irradiation provided as a single dose was high enough to inhibit UV-absorbing pigment synthesis, but the same dose split into three sub-doses promoted the biosynthesis of UV screening compounds.

The reduction in total protein content that was observed in our work upon single-dose treatment has also been recorded by others, but in a highly species-dependent manner, as the same dose could lead to a drop in protein content in one species and a rise in other species (Baumbusch *et al.* 1998). It seems that splitting the dose gives cells time to remove ROS between treatments, and can even be stimulating, increasing total protein content, and as such can be regarded as 'eustress' (Hideg *et al.* 2013).

González *et al.* (1998), and Deckmyn & Impens (1997) showed that UV-responses might be non-linear, and that effects could be greater at low UV-B doses, with further increase in dose reversing changes that occurred at low doses. If we consider the split dose as if it was a functionally lower dose, this non-linearity was obvious in enzymes in our experiments. Both catalase and peroxidase activities were decreased when plants were treated with the split UV-B dose. The single UV-B dose, leading to more elevated ROS and causing more damage, led to reversal of the POD level to control levels and elevation of CAT activity when compared with control plants. This implies high H_2O_2 production under these conditions. Reduction in CAT was observed by Ravindran *et al.* (2010) and Baumbusch *et al.* (1998). However, Yang *et al.* (2007) found both POD and CAT to be elevated. This was again species-specific, as in Baumbusch *et al.* (1998) pine showed decrease in both enzyme activities, while in spruce, under the same conditions, activities were not affected by a low UV-B dose. In our study, the single daily dose was high enough to cause severe stress that lowered the phenolics content and enhanced antioxidative enzymes, and these effects could be overcome by splitting the dose.

CONCLUSION

Treatment of pea plants with a high UV-B dose provided in a single dose led to increases in photosynthetic pigments and signs of oxidative stress in mature leaves. In immature leaves, pigments were down-regulated while POD and CAT did not change significantly. Splitting the daily dose into sub-doses lowered the effects even though the total UV-B dose that plants received during the day remained the same. When UV-B is not supplied as a single dose during the day but divided into smaller doses, the time between irradiations gave plants a chance to lower the oxidative pressure and repair the damage, showing effects as though the total dose was lower than actual. This implies that the effects of high daily doses can be significantly reduced by periodic shading of incident UV radiation. Daily doses of UV-B applied in our experiments exceeded average ambient levels of UV-B. Even though one might expect higher damage resulting from applying UV-B as high intensity even for a short period, it seems that daily distribution, or more likely daily distribution of "resting periods" determines the extent of damage by UV-B.

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REFERENCES

Baroniya SS, Kataria S, Pandey GP & Guruprasad KN. 2011. Intraspecific variation in sensitivity to ambient ultraviolet-B radiation in growth and yield characteristics of eight soybean cultivars grown under field conditions. *Braz. J. Plant Physiol.* **23**: 197-202.

Baumbusch LO, Eiblmeier M, Schnitzler JP, Heller W, Sandermann H & Polle A. 1998. Interactive effects of ozone and low UV-B radiation on antioxidants in spruce (*Picea abies*) and pine (*Pinus sylvestris*) needles. *Physiologia plantarum* **104**: 248-254.

Bieza K & Lois R. 2001. An *Arabidopsis* mutant tolerant to lethal ultraviolet-B levels shows constitutively elevated accumulation of flavonoids and other phenolics. *Plant Physiol.* **126**: 1105-1115.

Bornman JF & Vogelmann TC. 1991. Effect of UV-B radiation on leaf optical properties measured with fiber optics. *J. Exp. Bot.* **42**: 547-554.

Bradford M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72:** 248-254.

Brosché M & Strid Å. 2002. Molecular events following perception of ultraviolet-B radiation by plants. *Physiologia Plantarum* **117**: 1–10.

Casati P & Walbot V. 2004. Rapid transcriptome responses of maize (*Zea mays*) to UV-B in irradiated and shielded tissues. *Genome Biology* **5**: R16.

Cen YP & Bornman JF. 1990. The response of bean plants to UV-B radiation under different irradiances of background visible light. *J. Exp. Bot.* **41**: 1489-1495.

Chance B & Maehly AC. 1955. Assay of catalases and peroxidases. *Methods In Enzymology* **2**: 764-775.

Claiborne A. 1984. Catalase activity. In: Greenwald RA (ed.). Handbook of Methods for Oxygen Radical Research, pp 283-284, CRC Press, Boca Raton.

Day TA & Vogelmann TC. 1995. Alterations in photosynthesis and pigment distributions in pea leaves following UV-B exposure. *Physiologia Plantarum* **94**: 433–440.

Deckmyn G & Impens I. 1997. The ratio UV-B/photosynthetically active radiation (PAR) determines the sensitivity of rye to increased UV-B radiation. *Environ Exp Bot* **37**: 3-12.

Frohnmeyer H & Staiger D. 2003. Ultraviolet-B radiation-mediated responses in plants. balancing damage and protection. *Plant Physiol.* **133**: 1420–1428.

González R, Paul ND, Percy K, Ambrose M, McLaughlin CK, Barnes JD, Areses M & Wellburn AR. 1996. Responses to ultraviolet-B radiation (280-315 nm) of pea (*Pisum sativum*) lines differing in leaf surface wax. *Physiol. Plant.* **98**: 852-860.

González R, Wellburn AR & Paul ND. 1998. Dose responses of two pea lines to ultraviolet-B radiation (280-315 nm). *Physiologia plantarum* **104**: 373-378.

He LL, Zu YQ, Li Y & Wu YS. 2006. Intraspecific differences in physiological responses of different wheat cultivars to enhanced UV-B radiation. *Chinese Journal of Applied Ecology* **17:** 153–165.

Hideg É, Jansen MAK & Strid Å. 2013. UV-B exposure, ROS, and stress: inseparable companions or loosely linked associates? *Trends in Plant Science* **18**: 107-115.

Höagland DR & Davis AR. 1923. The uptake and accumulation of electrolytes in plant cells. *Protoplasma* **6**: 610-626.

Hofmann RW, Campbell BD & Fountain DW. 2003. Sensitivity of white clover to UV-B radiation depends on water availability, plant productivity and duration of stress. *Global Change Biol.* **9**: 473–477.

Jansen MAK, Hideg É & Lidon FJC. 2012. UV-B radiation: "When does the stressor cause stress?". *Emir. J. Food Agric.* **24**: 1-5.

Kakani VG, Reddy KR, Zhao D & Sailaja K. 2003. Field crop responses to ultraviolet-B radiation: a review. *Agricultural and Forest Meteorology* **120**: 191–218.

Landry LG, Chapple CCS & Last RL. 1995. Arabidopsis mutants lacking phenolic sunscreens exhibit enhanced ultraviolet-B injury and oxidative damage. *Plant Physiol.* **109**: 1159–1166.

Lau TSL, Eno E, Goldstein G, Smith C & Christopher DA. 2006. Ambient levels of UV-B in Hawaii combined with nutrient deficiency decrease photosynthesis in near-isogenic maize lines varying in leaf flavonoids: flavonoids decrease photoinhibition in plants exposed to UV-B. *Photosynthetica* **44**: 394–403.

Li J, Ou-Lee T-M, Raba R, Amundson RG & Last RL. 1993. Arabidopsis flavonoid mutants are hypersensitive to UV-B irradiation. *Plant Cell* **5**: 171–179.

Li Y, He LL & Zu YQ. 2010. Intraspecific variation in sensitivity to ultraviolet-B radiation in endogenous hormones and photosynthetic characteristics of 10 wheat cultivars grown under field conditions. *South African Journal of Botany* **76**: 493–498.

Lichtenthaler HK. 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Meth. Enzymol.* **148**: 350-382.

Liu Z, Hossain GS, Islas-Osuna MA, Mitchell DL & Mount DW. 2000. Repair of UV damage in plants by nucleotide excision repair: *Arabidopsis UVH1* DNA repair gene is a homolog of *Saccharomyces cerevisiae* Rad1. *The Plant Journal* **21**: 519-528.

Majer P & Hideg E. 2012. Developmental stage is an important factor that determines the antioxidant responses of young and old grapevine leaves under UV irradiation in a green-house. *Plant Physiology and Biochemistry* **50**: 15-23.

Nogués S & Baker NR. 2000. Effects of drought on photosynthesis in Mediterranean plants grown under enhanced UV-B radiation. *J. Exp. Bot.* **51**: 1309–1317.

Premkumar A & Kulandaivelu G. 2001. Influence of increased solar UV-B radiation on magnesium deficient cowpea seedlings: Changes in growth and foliar constituents. *Plant Science* **161**: 1–8.

Qaderi MM, Basraon NK, Chinnappa CC & Reid DM. 2010. Combined effects of temperature, ultraviolet-B radiation, and watering regime on growth and physiological processes in canola (*Brassica napus*) seedlings. *Int. J. Plant Sci.* **171**: 466–481.

Ravindran KC, Indrajith A, Balakrishnan V, Venkatesan K & Kulandaivelu G. 2008. Determination of defense mechanism in *Phaseolus trilobus* Ait. seedlings treated under UV-B radiation. *African Crop Science Journal* **16**: 111 – 118.

Ravindran KC, Indrajith A, Pratheesh PV, Sanjiviraja K & Balakrishnan V. 2010. Effect of ultraviolet-B radiation on biochemical and antioxidant defence system in *Indigofera tinctoria* L. seedlings. *International Journal of Engineering, Science and Technology* **2**: 226-232.

Singh S, Kumari R, Agrawal M & Agrawal SB. 2011. Modification in growth, biomass and yield of radish under supplemental UV-B at different NPK levels. *Ecotoxicol. Environ. Saf.* **74**: 897–903.

Singleton VL & Rossi JA. 1965. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid "reagents". *Am. J. Enol. Vitic.* **16**: 144-158.

Skórska E. 2000. Comparison of responses of bean, pea and rape plants to UV-B radiation in darkness and in light. A*cta Physiologiae Plantarum* **22**: 163-169.

Tevini M, Iwanzik W & Teramura AH. 1983. Effects of UV-B radiation on plants during mild water stress II. Effects on growth, protein and flavonoid content. *Z Pflanzenphysiol.* **110**: 459–467.

Yang SH, Wang LJ & Li SH. 2007. Ultraviolet-B irradiation-induced freezing tolerance in relation to antioxidant system in winter wheat (*Triticum aestivum* L.) leaves. *Environmental and Experimental Botany* **60**: 300–307.

Botanica SERBICA

REZIME

UV-B utiče na promene pigmenata i antioksidativnog sistema kod graška (*Pisum sativum*): efekat različite dozne distribucije UV zračenja kod mladih i starih listova

Dragoljub Dimitrijević, Tijana Cvetić Antić

Biljke graška tretirane su UV-B zračenjem, pri čemu su ukupnu dnevnu dozu zračenja od 16.2 kJ m^{-2} UV-B dobijale odjednom (cela doza, 1 x 30 min) ili iz tri puta (podeljena doza, 3 x 10 min) u toku dana, i praćen je efekat tretmana na sadržaj proteina, fotosintetskih pigmenata, fenola, i na aktivnosti peroksidaza i katalaze. Poređeni su apikalni nepotpuno razvijeni i subapikalni razvijeni listovi. Tretman UV-B zračenjem je doveo do povećanja količine fotosintetskih pigmenata u razvijenim i smanjenja količine pigmenata u nerazvijenim listovima. U zrelim listovima pri tretmanu podeljenom dozom došlo je do povećanja količine ukupnih fenola, dok je cela doza dovela do smanjenja količine fenola. Kod zrelih listova je došlo i do povećanja aktivnosti katalaze pri tretmanu celom dozom, dok je tretman podeljenom dozom doveo do smanjenja aktivnosti katalaze. Promene u količini ukupnih proteina ukazuju da je podela dnevne doze na tri dela dovela do manjih oštećenja nego kada se biljke ozrače celom dnevnom dozom odjednom. Ovo ukazuje da ne samo količina već i vremenska distribucija UV-B zračenja u toku dana utiče na odgovor biljke na visoke doze UV-B zračenja.

Ključne reči: grašak, UV-B, fotosintetski pigmenti, antioksidativni sistem