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Chlorophyll content and photosystem II efficiency in soybean exposed to supplemental ultraviolet-B radiation

Z. HU[*,**,+], H. LI[*], S. CHEN[*], and Y. YANG[*]

Yale-NUIST Center on Atmospheric Environment & School of Environmental Science and Engineering, Nanjing University of Information Science & Technology, Nanjing – 210044,China[*]
Key Laboratory of Agrometeorological Safeguard and Applied Technique, China Meteorological Administration, Zhengzhou – 450003, China[**]

Abstract

Chlorophyll (Chl) *a* fluorescence parameters and rapid light curves of soybean [*Glycine max* (L.) Merrill] were measured by pulse amplitude modulation fluorometry. Measurements were taken during different stages of soybean growth under field conditions with 20% enhancement in ultraviolet-B (UV-B) radiation. Results showed that supplemental UV-B radiation decreased Chl contents by 5.5% (P=0.048), 8.7% (P=0.046), and 10.5% (P=0.005) in seedling, in branching-flowering, and in pod-setting stages, respectively. In the branching-flowering and pod-setting stages, maximum quantum yield of photosystem (PS) II photochemistry (F_v/F_m) decreased by 6.1% (P=0.001) and 3.0% (P=0.009), respectively. Supplemental UV-B radiation significantly decreased the effective quantum yield (Y). The photosynthetic capacity at light saturation (P_m) also decreased in both the seedling and branching-flowering stages by 28.9% (P=0.007) and 15.5% (P=0.041), respectively. However, Y and P_m showed no significant difference in the trefoil and pod-setting stages with and without the UV treatment. The light saturation parameter (E_k) decreased by 21.1% (P=0.000) and 23.2% (P=0.029) in the trefoil and seedling stages, respectively. Moreover, the initial slope (α) decreased by 21.1% (P=0.001) in the branching-flowering stage. Nonphotochemical quenching (NPQ) in the seedling stage and photochemical quenching coefficient (q_p) in the branching-flowering stage decreased significantly under UV-B treatments. The results of the present study suggest that supplemental UV-B radiation adversely affected Chl content and electron transport activity in PSII and consequently decreased the photosynthetic efficiency of soybean plants.

Additional key words: chlorophyll content; fluorescence quenching; *Glycine max*; rapid light curves; ultraviolet-B radiation.

Introduction

Atmospheric chlorofluorocarbons and N_2O continue to rise significantly and it leads to stratospheric ozone depletion. As a result, the amount of biologically harmful UV-B radiation (280–320 nm) reaching the Earth's surface is rising (Erickson *et al*. 2000). The effects of UV-B radiation have been intensively studied on crop plants (Yannarelli *et al*. 2006, Feng *et al*. 2007, Pradhan *et al*. 2008). Enhanced UV-B radiation damaged the chloroplast structure, reduced Chl and Rubisco contents, decreased the Hill reaction rate (He *et al*. 2004, Yao and Liu 2006), inhibited photosynthesis and transpiration rates (Pandey *et al*. 2007, Yang *et al*. 2007), and reduced a biomass production (Agrawal *et al*. 2006, Yao *et al*. 2006).

Rapid light curve (RLC) is a powerful tool for assessing photosynthetic activity (White and Critchley 1999, Ralph and Gademann 2005) that has been widely used to quantify the photosynthetic activity of photoautotrophic organisms (Serôdio *et al*. 2005, Belshe *et al*. 2007, Cruz

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[+]Corresponding author; fax: +86 025 58731090, e-mail: zhhu@nuist.edu.cn

Abbreviations: Chl – chlorophyll; DAS – dark-adapted state; E_k – light saturation parameter; ETR – electron transport rate; F – fluorescence yield; F_o – minimum fluorescence yield of a dark-adapted leaf; F_m – maximum fluorescence yield of a dark-adapted leaf; F_m' – maximum fluorescence yield of a light-adapted leaf; F_v/F_m – maximum quantum yield of PSII photochemistry; FPs – chlorophyll fluorescence parameters; NPQ – nonphotochemical quenching; NR – nitrate rdeuctase; PAR – photosynthetically active radiation; P_m – photosynthetic capacity at light saturation; PS – photosystem; q_p – photochemical quenching coefficient; RLCs – rapid light curves; *SPAD-502* – chlorophyll meter; Y – effective quantum yield; UV-B – ultraviolet-B radiation; α – initial slope.

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and Serôdio 2008). Compared with traditional measurement methods, RLCs can significantly decrease measurement time and interferences in natural photosynthesis (White *et al.* 1999). In fact, the RLC method takes only about 10 s to retrieve stable information on photochemical electron transport under different PAR levels (Rascher *et al.* 2000). In addition, RLCs provide detailed information on PSII and on the photosynthetic performance of plants and can describe the light adaptation state of a plant and its capacity to tolerate short-term light changes (Ralph and Gademann 2005). Hence, RLCs have been extensively used in plant physiological and ecological studies (Belshe *et al.* 2007, Cruz and Serôdio 2008).

Several studies have shown that UV-B radiation can decrease Chl content and a net photosynthetic rate of high plants (Brandle *et al.* 1977, Teramura 1983, Teramura and Sullivan 1994, Liang *et al.* 2011). However, these studies were performed under controlled laboratory conditions. In this study, we hypothesized that enhanced UV-B radiation might change the characteristics of the Chl fluorescence of soybean under field conditions. To test this hypothesis, a soybean experimental field was exposed to supplemental UV-B radiation, which was generated by an automatic control system under field conditions. The primary aim of the present study was to investigate the response of the Chl content and fluorescence characteristics of soybean to supplemental UV-B radiation during different growth stages.

Materials and methods

Experimental design: The field experiment was performed during the soybean-growing season. The experiment was arranged in a randomized plot design, with 4 plots of the UV-B treatment and 4 plots of the ambient control. Each plot had an area of 3 m × 4 m and featured 11 rows arranged at interval distances of 30 cm each.

Seeds of soybean were sown on 6 July 2008. The main growth stages of soybean were: seedling, 9 July; trefoil, 14 July; branching, 14 August; flowering, 23 August; pod, 7 September; grain-filling, 19 September; harvest, 13 October. Neither basal fertilizer nor additional organic manure was applied.

UV-B treatments: Supplemental UV-B radiation was artificially provided by 40 W fluorescent lamps (*UV-B*, *Huade Instrument Factory*, Shanghai, China). The lamps were hung above and parallely to the planted rows, which were arranged east-westward to minimize shading. The experiment consisted of the UV-B treatment group (UV-B, with 20% enhancement in UV-B radiation), and the control group (control, with UV-B lamp exposure filtered by a 125 µm thick polyester plastic film, *Mylar-D*, purchased from *DuPont Co*., Wilmington, DE, USA). The film covering the control group, which filtered off UV-B radiation (Fig. 1), was replaced weekly to ensure uniformity in UV-B absorption. Plants under the polyester-filtered lamps received only ambient levels of UV-B radiation, whereas plants beneath the lamps received ambient and supplemental levels of UV-B radiation.

At the canopy level, the PAR photon flux density for the two treatments was consistent and varied with weather conditions. Irradiance of the biologically effective UV-B of the two treatments varied with the solar radiation. The irradiance of UV-B in the two treatments was recorded automatically with the UV-B radiance measurement instrument, which consists of UV-B radiation sensors (spectral range = 280–315 nm; *SKU430*, *Skye Co*., Powys, UK) and a data logger (*Skye-Datahog*, *Skye Co*., Powys, UK). The sensors were installed at the vegetation level at the center of the plots. The maximum UV-B radiation intensity of the treatment was 518.49 µW cm^{-2} and the average UV-B radiation intensity of the treatment and the control were 288.45 µW cm^{-2} and 239.73 µW cm^{-2}, respectively. The plants were irradiated daily for 8 h (08:00–16:00) from the seedling to the harvest stage.

Chl content: Total Chl content of soybean leaves was measured using a *SPAD-502* meter (*Minolta Camera Co*., Osaka, Japan), which has a weigh of 225 g, 0.06 cm^2 measurement area, and an index is calculated in 'SPAD units'. The claimed accuracy of the *SPAD-502* is ± 1.0 SPAD units. The principle of measurement is based

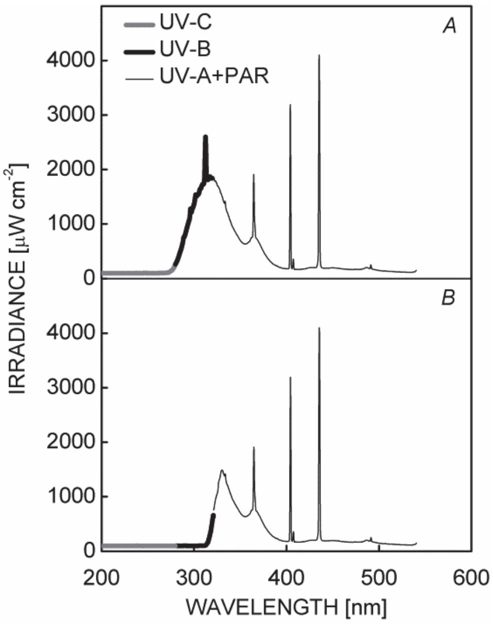

Fig. 1. Emission spectra of *A*: UV-B lamps, and *B*: *Mylar* film-filtered UV-B lamps.

on the difference in a light attenuation at 650 and 940 nm. From the difference in a light attenuation, a dimensionless SPAD unit, ranging from 0 to 80, is calculated by the microprocessor in the *SPAD-502* meter (Ruiz-Espinoza *et al.* 2010). The relationship between the output of the *SPAD-502* and absolute Chl content can be described by the equation: Chl [μmol m^{-2}] = $10^{(M\wedge 0.265)}$, $r^2 = 0.94$ (M, SPAD values) (Markwell *et al.* 1995). In general, SPAD values may increase with leaf thickness, and specific leaf mass (Marenco *et al.* 2009). There are highly positive exponential relationships between SPAD values and Chl (*a*+*b*), Chl *a*, and Chl *b* contents (Mielke *et al.* 2010).

Leaves at different developmental stages, from very young to fully expanded functional leaves, were selected to obtain a wide range of total Chl contents. The *SPAD-502* measurements were conducted in the field between 08:00 and 10:00 h. Ten readings were taken from the widest portion of the leaf blade at about 1.5 cm from the leaf margin. The adaxial leaf surface was positioned toward the emitting window of the *SPAD-502* avoiding major veins.

Chl fluorescence and RLCs: Chl fluorescence parameters (FPs) were measured with a *Diving-PAM* fluorometer (*Walz Co*., Effeltrich, Germany), and the type of radiation measured was set to red. RLCs were constructed on four randomly chosen soybean shoots from each plot. Prior to the measurements, leaves were kept in a dark-adapted state (DAS) for about 2 h after sunset. During DAS, all reaction centers and electron carriers of PSII were re-oxidized, which was essential for recording FPs (Genty *et al.* 1989, van Kooten and Snel 1990, Baker 2008). The leaves were exposed to different irradiation intensity (112, 234, 366, 557, 771; 1,146; 1,561; and 2,351 μmol m^{-2} s^{-1}), with the exposure to each irradiation intensity occurring for approximately 10 s.

The following Chl FPs were measured: fluorescence yield (F), maximum fluorescence yield of a light-adapted leaf (F_m'), and minimum and maximum Chl fluorescence yields in DAS (F_o and F_m). Other Chl FPs were subsequently calculated using the following equations (Ranjbarfordoei et al. 2011):

$$F_v/F_m = (F_m - F_o)/F_m \quad (1)$$

$$Y = (F_m' - F)/F_m' \quad (2)$$

$$q_p = (F_m' - F)/(F_m' - F_o) \quad (3)$$

$$NPQ = (F_m - F_m')/F_m' \quad (4)$$

$$\text{Electron transport rate (ETR)} = Y \times PAR/2 \times 0.84 \quad (5)$$

In the last equation above, $Y = (F_m' - F)/F_m'$; PAR/2, the absorbed photon energy, is assumed to be equally distributed between PSI and PSII; 0.84 is the assumed light absorptance of the leaf (Belshe *et al.* 2007).

Curve-fitting: To quantitatively compare RLCs, a curve fittind is done using an equation according to Smith (1936):

$$P = P_m \times \alpha \times PAR/\text{sqrt}\,[P_m^2 + (\alpha \times PAR)^2] \quad (6)$$

where P is the relative electron transport rate, P_m is the photosynthetic capacity at saturation, and α is the initial slope of the RLC. The RLC parameter is a measurement of the ETR value under different PAR. The least squares method was used to fit the equation and the corresponding values of P_m, and α was obtained by using the fitting equations.

$$E_k = P_m/\alpha \text{ (Sakshaug et al. 1997)} \quad (7)$$

Plant sample analysis: The biomass of soybean was determined by oven-drying to a constant mass at about 70°C. Extraction and assay for nitrate reductase (NR) activity were conducted by the method outlined by Natali *et al.* (2009). NR activity was reported in units of NO_2^- produced per hour per gram leaf fresh mass. Soluble protein contents were determined at 595 nm by the protein-dye binding method, using bovine albumin as a standard (Bradford 1976, Azevedo Neto *et al.* 2009).

Statistical analyses: *ANOVA* was performed to test the effects of supplemental UV-B radiation on the Chl fluorescence characteristics of soybean with the *SPSS* statistical package, *version 13* (*SPSS lnc*., Chicago, IL, USA). Differences among treatments were considered highly significant at $P \leq 0.01$, as well as significant at $P \leq 0.05$.

Results

Chl content: The supplemental UV-B radiation had no significant effect on total Chl content in the trefoil stage (*P*=0.549) (Table 1). In contrast, supplemental UV-B radiation significantly decreased Chl contents by 5.5% (*P*=0.048), 8.7% (*P*=0.046), and 10.5% (*P*=0.005) in the seedling, branching-flowering, and pod-setting stages, respectively.

F_v/F_m: The supplemental UV-B radiation had no significant effect on F_v/F_m in the trefoil (*P*=0.089) and seedling (*P*=0.116) stages (Table 1). However, F_v/F_m values significantly decreased by 6.1% (*P*=0.001) and 3.0% (*P*=0.009) in the branching-flowering and pod-setting stages, respectively.

Y values of the control and UV-B treatments showed no significant difference during the trefoil and pod-setting stages (Fig. 2). In contrast, *Y* values significantly decreased during the UV-B treatment in the seedling and branching-flowering stages.

RLCs: P_m values significantly decreased by 28.9% (*P*=0.007) and 15.5% (*P*=0.041) during the UV-B treatment in the seedling and branching-flowering stages,

Table 1. Effects of supplemental UV-B on the chlorophyll (Chl) content and maximum quantum yield of PSII photochemistry (F_v/F_m) of soybean leaves. Data are mean values ± SD (Chl content, $n = 6$; F_v/F_m, $n = 6$). *, and ** – the significant difference between control and supplemental UV-B treatments in *ANOVA* at $P \leq 0.05$, and 0.01, respectively.

Stage	Chl content [SPAD]		F_v/F_m	
	Control	UV-B	Control	UV-B
Trefoil	36.92 ± 1.92	35.88 ± 3.60	0.84 ± 0.00	0.83 ± 0.02
Seedling	33.93 ± 0.74	32.06 ± 1.40*	0.83 ± 0.01	0.80 ± 0.03
Branching-flowering	36.40 ± 1.46	33.23 ± 3.08*	0.82 ± 0.01	0.77 ± 0.01**
Pod-setting	34.62 ± 1.63	31.00 ± 1.54**	0.83 ± 0.01	0.80 ± 0.01**

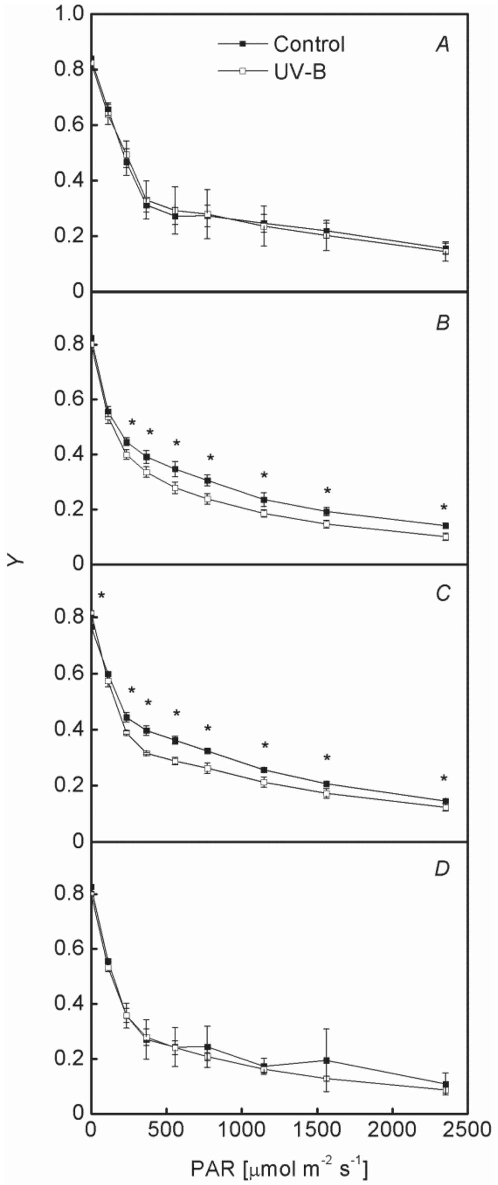

Fig. 2. Changes in the effective quantum yield (*Y*) with photosynthetically active radiation (PAR). *A*, *B*, *C*, and *D* indicate the trefoil, seedling, branching-flowering, and pod-setting stages, respectively. Data are mean values, and error bars are SDs ($n = 6$). * – significant differences between means ($P \leq 0.05$).

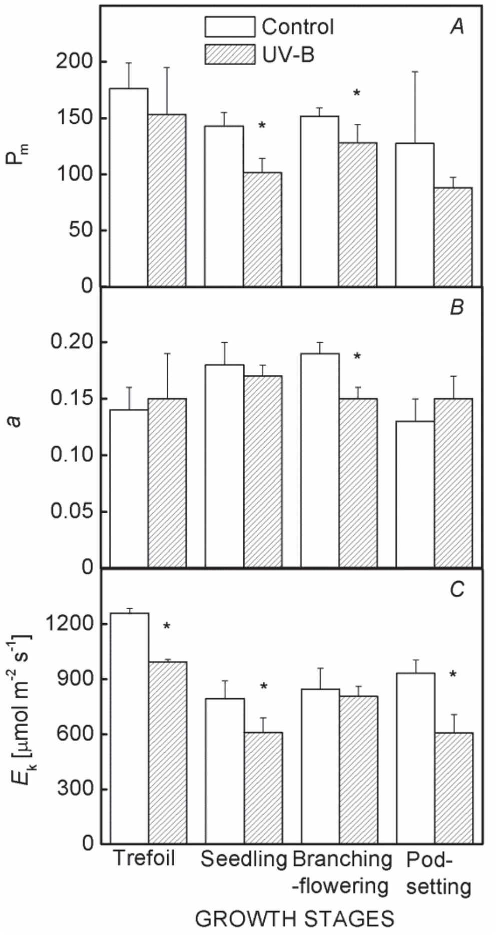

Fig. 3. Changes in the photosynthetic parameters of soybean at different growth stages under supplemental UV-B radiation. *A*: maximum fluorescence yield of a dark-adapted leaf (P_m), *B*: initial slope (*α*), and *C*: light saturation parameter (E_k). Data are mean values ± SD ($n = 6$). * – significant difference between treatments in the same growth stage ($P \leq 0.05$).

respectively (Fig. 3). Supplemental UV-B radiation decreased *α* by 21.1% (P=0.001) in the branching-flowering stage. Moreover, E_k decreased by 21.1% (P=0.000), 23.2% (P=0.029), and 34.9% (P=0.017) in the trefoil, seedling, and pod-setting stages, respectively.

q_p and NPQ: In the trefoil stage, supplemental UV-B radiation had no significant effect on q_p at different PAR intensities, but NPQ decreased at PAR > 1,561 μmol m^{-2} s^{-1} (Fig. 4). In the seedling stage, UV-B radiation significantly decreased q_p at PAR > 557 μmol m^{-2} s^{-1} and NPQ decreased at PAR > 366 μmol m^{-2} s^{-1}. In the branching-flowering stage, UV-B radiation decreased q_p at PAR > 112 μmol m^{-2} s^{-1}, and NPQ decreased at PAR > 771 μmol m^{-2} s^{-1}. In the pod-setting stage, UV-B radiation decreased NPQ at PAR > 771 μmol m^{-2} s^{-1}, but had no significant effect on q_p.

Discussion

The majority of Chl *a* and Chl *b* are engaged exclusively in light harvesting process, they are not directly involved in the excitation energy dissipation. Only 8 Chls *a* participate in the light reactions, four in the reaction center of PSII and four in the reaction center of PSI (Papageorgiou and Govindjee 2011). It is very sensitive to environmental stress, such as enhanced UV-B radiation. In the present study, supplemental UV-B radiation significantly decreased the Chl content of soybean leaves from the seedling to the pod-setting stage. This result was in agreement with a previous report by Kakani *et al.* (2004), who found that UV-B radiation can lower total Chl contents and damage the photosynthetic capacity of chloroplasts. Gao *et al.* (2004) suggested that under UV-B stress, the contents of Chl *a*, *b*, and (*a*+*b*) of maize leaves were reduced. Another study by Yao *et al.* (2006) found that enhanced UV-B radiation can inhibit the production of photosynthetic pigments and destroy the structure of chloroplasts and their membrane systems.

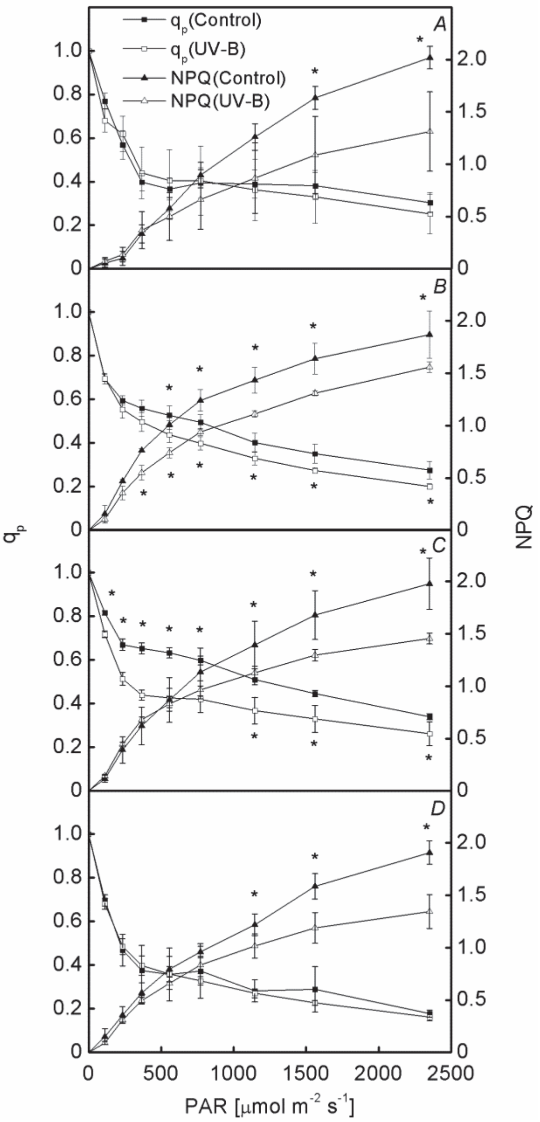

Fig. 4. Changes in photochemical quenching coefficient (q_p) and nonphotochemical quenching (NPQ) with photosynthetically active radiation (PAR). *A*, *B*, *C*, and *D* indicate the trefoil, seedling, branching-flowering, and pod-setting stages, respectively. Data are mean values, and error bars are SDs (*n* = 6). * – significant differences between means ($P \leq 0.05$).

The efficiency and stability of PSII, a major component of the photosynthetic apparatus, was monitored by means of F_v/F_m during the experiment. The alterations in F_v/F_m implied changes in the photochemical conversion efficiency of PSII and a possible photoinhibition of photosynthesis (Ranjbarfordoei *et al.* 2011). F_v/F_m is relatively steady under nonstress conditions and may significantly decrease in the stress conditions (Björkman and Demming 1987). The decrease in F_v/F_m shows that environmental stress damages the PSII photochemical reaction center. In the present study, UV-B significantly decreased F_v/F_m in soybean leaves during the branching-flowering and pod-setting stages, demonstrating that the supplemental UV-B radiation lowered PSII efficiency. In addition, the supplemental UV-B radiation decreased the biomass of soybean plants in the branching-flowering and pod-setting stages (Table 2). In a similar study, UV-B exposure was found to decrease grain yield in wheat (Paknejad *et al.* 2007). Therefore, UV-B radiation has detrimental effects on photosynthetic processes and crop growth. Correia *et al.* (2005), Cechin *et al.* (2007), and Ranjbarfordoei *et al.* (2011) also found similar results for other plants.

The *Y* index is an effective measure of the operating photochemical efficiency of PSII in plant leaves (Rascher *et al.* 2000). In the seedling and branching-flowering stages, the supplemental UV-B radiation significantly decreased the *Y* of soybean. The cause of the reduction might be associated with enhancement of excitation energy quenching in PSII antennae. The decline in *Y* is generally considered to indicate physiological regulation of electron transport (Skórska 2011). Day and Vogelmann (1995) also suggested the *Y* is lower in UV-B

Table 2. Effects of supplemental UV-B on biomass of soybean. Data are mean values ± SD ($n = 3$). *, and ** – the significant difference between control and supplemental UV-B treatments in *ANOVA* at $P \le 0.05$, and 0.01, respectively.

Stage	Shoot biomass [g m^{-2}]		Root biomass [g m^{-2}]		Total biomass [g m^{-2}]	
	Control	UV-B	Control	UV-B	Control	UV-B
Branching-flowering	215.82 ± 10.06	148.52 ± 7.49**	29.13 ± 1.50	21.93 ± 1.52**	244.95 ± 9.21	170.45 ± 6.34**
Pod-setting	776.06 ± 23.14	666.75 ± 29.30**	55.14 ± 4.63	64.11 ± 1.91*	831.20 ± 18.73	730.86 ± 28.58**

Table 3. Effects of supplemental UV-B on nitrate reductase (NR) activity and soluble protein content in soybean leaves. Data are mean values ± SD (NR activity, $n = 3$; soluble protein content, $n = 3$). *, and ** – the significant difference between control and supplemental UV-B treatments in *ANOVA* at $P \le 0.05$, and 0.01, respectively.

Stage	Nitrate reductase activity [μg(NO_2^-) g^{-1} h^{-1}]		Soluble protein content [mg g^{-1}]	
	Control	UV-B	Control	UV-B
Branching-flowering	293.19 ± 20.22	131.95 ± 12.46**	7.81 ± 0.01	7.47 ± 0.06*
Pod-setting	468.53 ± 15.42	279.22 ± 9.90**	13.86 ± 0.64	8.59 ± 0.07**

exposed pea leaves due to a lower F_v/F_m in these leaves, which implies a lower efficiency of electron transport in open PSII reaction centers. Decreases in *Y* are associated with increases in the excitation energy quenching in the PSII antennae, and it can be understood as a physiological regulation of electron transport by increasing the excitation energy quenching in the PSII antennae (Skórska 2011).

RLCs can provide information on the saturation characteristics of electron transport, as well as the overall photosynthetic performance of a plant (Cruz and Serôdio 2008). FPs in RLC also provide photosynthetic information on injured plants, such as α, P_m, E_k, and electron transfer capability (van Kooten and Snel 1990, Ralph and Gademann 2005). In the present study, RLCs provided photosynthetic information on soybean leaves exposed to UV-B radiation, such as lower P_m and E_k in the UV-B treated leaves. Rubisco, enzyme, which is related to photosynthetic carbon assimilation, might play a role in the reduction of P_m. Karpinski *et al.* (1997) detected a damage and degradation of the D1 protein in rice leaves after an exposure to UV-B. In our study, the supplemental UV-B radiation significantly decreased NR activity and soluble protein content in the leaves during the branching-flowering and pod-setting stages (Table 3). Previous studies also suggested that UV-B radiation decreases enzyme activities (Ranjbarfordoei *et al.* 2011), and sharply decreases the Rubisco protein content in leaves (Fedina *et al.* 2010).

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