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Comparison of responses of bean, pea and rape plants to UV-B radiation in darkness and in light

Elżbieta Skórska

Department of Physics, Agricultural University of Szczecin, ul. Pawła VI 3, 71-459 Szczecin,
e-mail: skorska@dedal.man.szczecin.pl

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Abstract

Effect of UV-B radiation on leaves of bean, pea and rape plants was studied. UV-B radiation (11.2 $kJ \cdot m^{-2}$) induced more distinct reduction of the primary photosynthesis activity when applied in darkness than the same UV-B dose, extended in time, and applied with photosynthetic active radiation (PAR). The pea plants were more susceptible to UV-B in darkness, but in the presence of PAR their tolerance was higher. The CO_2 fixation in the bean and rape plants, exposed to UV-B was decreased, but for the pea plants it remained unchanged. The UV-B irradiation caused an increase in the content of ultraviolet-absorbing pigments. Additionally, the bean plants grown at UV-B increased the thickness of leaves, described as SLW.

Introduction

The anticipated reduction in the stratospheric ozone column, resulting from the antropogenic release of chlorfluorinecarbonates and other air pollutants, increases the level of ultraviolet radiation in the range of UV-B (280-320 nm) reaching the Earth surface. A number of studies which have been carried out over the last 20 years shows that about 30 % to 50 % of all studied plant species are susceptible to the enhanced UV-B level; UV-B can finally reduce the yield of some crop species (Caldwell 1977, Teramura and Sullivan 1994). The susceptibility to UV-B radiation varies greatly among plant species. Even low UV-B level can adversely impact some sensitive species; other plants are tolerant to large doses of UV-B. We can explain the plant reactions to enhanced levels of UV-B on the anatomical, biochemical and physiological basis. Some anatomical changes in epidermis layer - increase in leaf mass and thickness - prevent the UV-B penetration to the susceptible internal structures or organs (Cen and Bornman 1990, Day *et al.* 1992). The penetration of UV-B through the epidermis into leaf mesophyll can induce physiological changes *i.e.* reduction of carbon dioxide fixation or the functioning stomatal apparatus. The UV-B damage can cause structural changes of tissues, as well as some quantitative changes in enzymes, proteins, lipids, chlorophyll and chromophores compounds (Stapleton 1992). It has been reported that the accumulation in the leaf epidermis layer of UV-B-absorbing compounds, *i.e.* flavonoids, constitute a protection against the damaging effect of UV-B radiation in some plant species (Caldwell *et al.* 1994; Deckmyn *et al.* 1994; Gonzalez *et al.* 1996).

This paper presents the results of the studies on the effect of increased UV-B irradiation on the photo-

synthesis of bean, pea and rape leaves, with particular focus on the processes of primary photochemical reactions. The aim of the studies was to compare the susceptibility of these species to UV-B applied in darkness and in light, as well as to examine the protective reactions during the growth at the low level of UV-B.

Material and methods

Plant material

The plant material used for experiment comprised of three species: of bean (*Phaseolus vulgaris* L.) cv. Aura, pea (*Pisum sativum* L.) cv. Sześciotygodniowy (Sixweeks), rape (*Brassica napus* var. *oleifera* L.) cv. Marita. The plants were grown in pots (250 cm^3) on sand, watered with Hoagland nutrient (KNO_3 - 304 $mg \cdot dm^{-3}$, $MgSO_4 \cdot 7\ H_2O$ - 124 $mg \cdot dm^{-3}$, $NH_4H_2PO_4$ - 12 $mg \cdot dm^{-3}$, $Ca(NO_3)_2 \cdot 4\ H_2O$ - 471 $mg \cdot dm^{-3}$, 0.001 % of iron citrate, microelements), in thermoluminostats under mercury lamp type LRF 250 W (140 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR), photoperiod 12 h/12 h, at a temperature 22 °C/18 °C day/ night respectively, with air humidity 50 %.

UV-B irradiation in darkness and in light

Leaf disks (diameter 15 mm), cut from the first leaves of three-week old plants, were placed on the surface of water with Hoagland nutrient (1:1) in open Petri dishes. The samples were divided into four groups. One group was exposed to UV-B for 60 minutes in darkness, and the second group was exposed to the UV-B in the presence of PAR 140 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The samples of two remaining groups, kept in darkness or in light (140 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR), were regarded as common controls, as there were no significant differences between the measured parameters of these groups. The source of UV-B was a lamp type VL-115 M (Vilber Lourmat, France), emitting in the range of 280 to 320 nm with the maximum emission at $\lambda_{max} = 311$ nm. The lamp was equipped with the special filter which did not transmit any radiation below 280 nm (Fig. 1). The intensity of irradiance was 8 $W \cdot m^{-2}$, which was measured by means of IL1400 radiometer with a calibrated photodetector SEL 240/UV-B1 (International Light Co., USA). The total biological effective dose, $UV\text{-}B_{BE}$ = 11.2 $kJ \cdot m^{-2}$, was calculated according to Caldwell (1977).

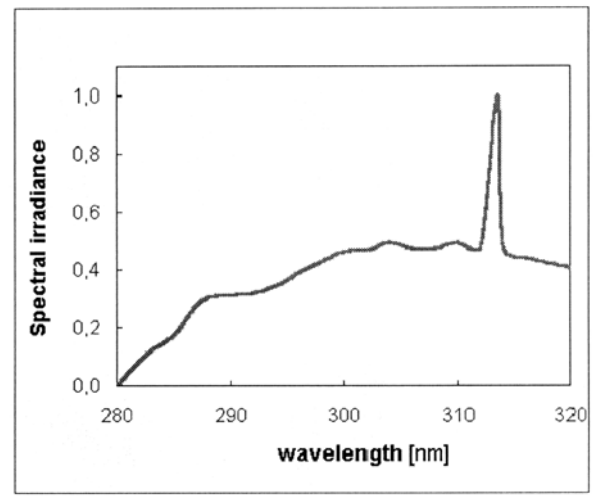


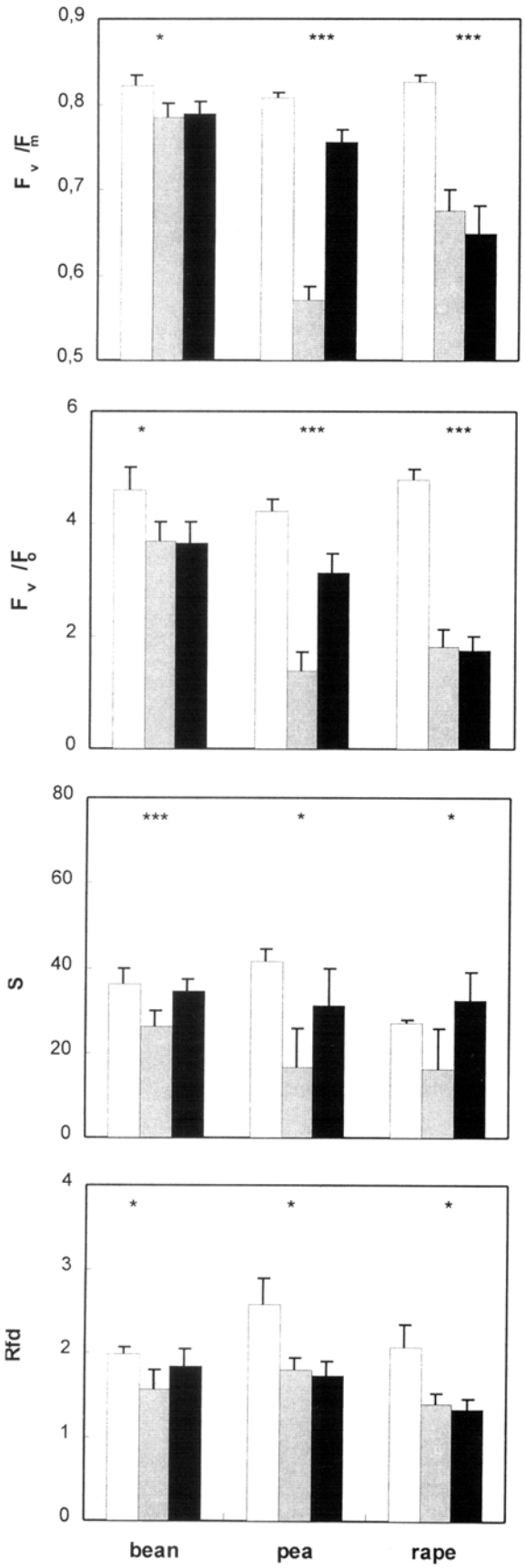
Fig. 1. Spectral characteristics of the lamp VL-115 used in the experiments. Energetic emission spectrum is normalised to the maximum (311 nm).

Growth of plants under UV-B radiation

The other set of the three-week old plants was exposed to PAR intensity 140 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at the conditions similar to the previously described (Skórska *et al.* 1997). The plants were then irradiated for six days with the lamp type VL-115 M (Fig. 1). The total $UV\text{-}B_{BE}$ dose during six days was 11.2 $kJ \cdot m^{-2}$ (daily dose 1.86 $kJ \cdot m^{-2} \cdot d^{-1}$). The control plants were grown without the UV-B lamps.

Measurement methods

The measurements of chlorophyll fluorescence induction were done using a Plant Efficiency Analyser PEA (Hansatech Ltd., England) on the intact first leaves. Before measurements the leaves were dark-adapted for 30 minutes in special leaf clips. The adaxial leaf surface was excited with red light of photon flux density 1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (40 % on the PEA screen) and the fluorescence signal was recorded for two minutes. The parameters $\mathbf{F_v/F_m}$ and **S** (the area between the curve of fluorescence induction and the line F_m) were measured automatically, and $\mathbf{F_v/F_o}$ and **Rfd** were calculated as a ratio $F_m\text{-}F_s/F_s$ (F_s – an intensity of stationary fluorescence), according to Lichtenthaler *et al.* (1986).



The intensity of net photosynthesis ($\mathbf{P_N}$) and transpiration rate (**E**) were measured in an open circuit using a portable LCA-4 analyser with a PLC-4 camera (ADC Ltd., England). The comparative measurements of total chlorophyll (a+b) content were done using a non-destructive method, by means of a portable chlorophyll meter SPAD 502 (Minolta, Japan), previously calibrated (Castelli *et al.* 1996), determining chlorophyll concentration according to Lichtenthaler (1987). As a result of the calibration, 10 SPAD units corresponded to 0.8 mg chl·dm^{-2} leaf area for pea and rape, and 1.0 mg chl·dm^{-2} leaf area for bean leaves. The content of UV-B absorbing compounds (mainly flavonoids) was measured spectrophotometrically, according to Caldwell *et al.* (1994), and presented as the absorbance values at 305 nm per 1 dm^2 of leaf area (A_{305}·dm^{-2}). As the coefficient of leaf thickness, the specific leaf weight (**SLW**) was assumed, *i.e.* the leaf dry weight per leaf area unit (g·m^{-2}).

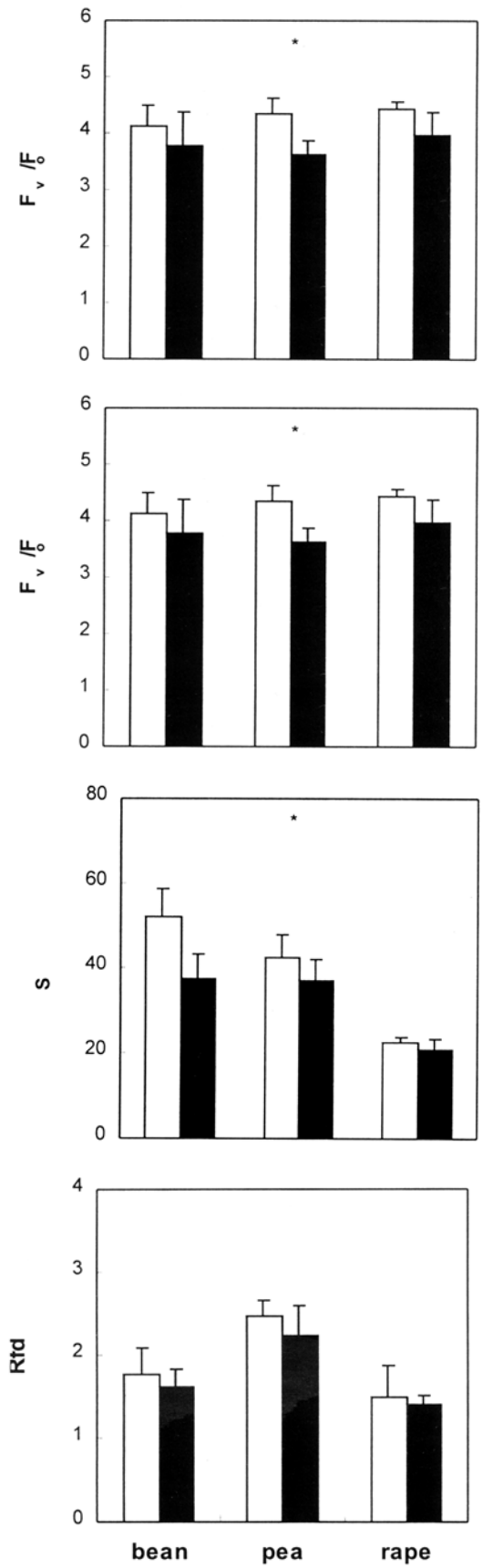
Statistical analysis

All the results were presented as the means from 5-7 replications (plants). The data were analysed by a one-way analysis of variance and the significantly different means were separated using the Newman-Keuls multiple range test (Statistica 5.0, Statsoft). The asterisks in the tables and Fig. 3 indicate significant differences as compared with the control, and in the Fig. 2— significant differences between the control and the two UV-B irradiated samples (*p<0.05, **p<0.01, ***p<0.001).

Results and discussion

The short-duration UV-B irradiation in darkness strongly reduced the measured parameters of chlorophyll fluorescence (grey bars in Fig. 2), in comparison with the control samples (white bars in Fig. 2), indicating an inhibition of primary photosynthesis reactions (Krause and Weis 1984, Murkowski and Skórska 1997). The decrease of the parameter

Fig. 2. Parameters of chlorophyll fluorescence ($\mathbf{F_v/F_m}$, $\mathbf{F_v/F_o}$, **S**, **Rfd**) of the control plants (white bars) and the plants treated with UV-B in 60 minutes (UV-B_{BE} = 11.2 kJ·m^{-2}) in darkness (grey bars) or in the presence of PAR 140 µmol·m^{-2}·s^{-1} (black bars). Asterisks indicate significant differences between the UV-B irradiated and the control plants at the p level: 0.05 (*), 0.01 (**) or 0.001 (***).



F_v/F_m, was stronger in pea (from 0.807 to 0.570) than in rape leaves (from 0.826 to 0.676). Bean leaves were more tolerant, as the value of this parameter decreased only by about 4 %. The decrease of F_v/F_m, due to UV-B treatment, is mainly the result of a decrease in F_m, but some increase in F_o could also be discerned. Other researchers, who found that F_v/F_m declined following UV-B treatment in pea and other species, concluded that reduction in photosynthesis of pea leaves under enhanced UV-B levels was primarily related to PSII. Tevini *et al.* (1988) suggested that PSII reaction centres are transformed into dissipative sinks for excitation energy following UV-B treatment, and Greenburg *et al.* (1989) showed that turnover of the D1 protein on the reducing side of PSII is altered by UV-B. Greater changes were found for the parameter F_v/F_o, which decreased by 67 %, 62 % and 19 % for pea, rape, and bean leaves respectively. Decrease of the value of F_v/F_o is associated with a disruption of photosynthesis process in the donor part of the photosystem II (Schreiber *et al.* 1994). The decrease of S parameter indicates a disturbance of plastoquinone pool on the reduction side of the photosystem II (Schreiber *et al.* 1994). Its decrease was considerable, especially for the pea leaves. The Rfd is referred to a vitality index and indicates the interaction between primary photosynthetic reactions and dark enzymatic reactions, leading to CO_2 assimilation (Lichtenthaler *et al.* 1986). Earlier studies showed a similar decrease of the above parameters in cucumber, rape and pea plant leaves exposed to UV-B in darkness (Skórska 1996abc). However, when the pea leaves were exposed to the same dose of UV-B irradiation — this time in the presence of PAR (black bars in Fig. 2) — these parameters were less changed. Unlike for bean and rape, in pea leaves the light in the PAR range alleviated the damages — induced by UV-B — of the light photosynthesis reactions.

Also the distribution of the UV-B dose over several days diminished its adverse effect. The UV-B irradiation of the plants growing at PAR caused a less

Fig. 3. Parameters of chlorophyll fluorescence (**F_v/F_m**, **F_v/F_o**, **S**, **Rfd**) of the control plants (white bars) and the plants grown six days in presence of UV-B radiation (UV-B_{BE} = 11.2 kJ·m^{-2}) and PAR 140 µmol·m^{-2}·s^{-1} (black bars). Asterisk denotes significant difference compared to the control ($p<0.05$)

Table 1. Net photosynthesis (P_N), transpiration (E) rate and specific leaf weight (SLW) of the control plants and the plants grown six days in the presence of UV-B and PAR as in the Figure 3.

Species	P_N [μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$]		E [mmol $H_2O \cdot m^{-2} \cdot s^{-1}$]		SLW [$g \cdot m^{-2}$]	
	Control	UV-B	Control	UV-B	Control	UV-B
Bean	9.9	6.6**	0.28	0.16	19.4	22.0***
Pea	11.6	12.8	0.58	0.79	14.4	13.6
Rape	13.0	7.0**	1.16	0.95	13.0	12.3

* Significant difference between the means of the control and the UV-B irradiated plants (** $p<0.01$, *** $p<0.001$)

change in the process of primary photosynthesis reactions (Fig. 3). The value of F_V/F_O in the pea leaves decreased by about 10 %, and S in the bean leaves - by almost 30 %. The other parameters, including Rfd, were not modified. The alleviating influence of PAR against injuries induced by UV-B was described by Teramura *et al.* (1980) for soybean.

The intensity of net photosynthesis in rape and bean leaves was reduced nearly about 50 % and 35 % respectively (Table 1), but was not changed for pea. The wax layer, covering pea leaves, was presumably the protective agent, as described Gonzalez *et al.* (1996) in an experiment with mutant pea plants, entirely or partially stripped of the wax layer.

Under the relatively low dose of UV-B, a considerable increase in ultraviolet absorbing pigments in the leaves of all studied species was observed (Table 2). In the literature there is however no agreement, whether UV-B accelerates the synthesis of flavonoids in particular plant species. The UV-B dose applied, as well as the conditions during the growth, has the strongest impact on the synthesis of flavonoids. For example, Gonzalez *et al.* (1996) found that the content of UV-absorbing compounds in the expanded leaves of pea plants, grown at UV-B_{BE} = 6.5 $kJ \cdot m^{-2} \cdot d^{-1}$ and PAR 950 $\mu mol \cdot m^{-2} \cdot s^{-1}$, increased by only 10 % in comparison with the control plants. Reductions in growth occurred without any changes in chlorophyll fluorescence or photosynthetic rate. However, a line of wax-devoid pea had less of these pigments and UV-B did not induced their synthesis. In the field experiment with modulated field lamp banks, which simulated 0 %, 16 % or 24 % ozone depletion Day *et al.* (1996) found no significant UV-B effects on the pea height, aboveground biomass, total leaf area or average leaf area. Total leaf concentrations of UV-B-absorbing compounds were also unaffected by UV-B level. Nogues *et al.* (1998) did not observe any loss of mesophyll light-saturated photosynthetic activity of pea plants grown under high UV-B radiation compared with those grown without UV-B radiation. The growth in UV-B radiation resulted in large reductions of leaf area and plant biomass, which were associated with a decline in leaf cell number and cell division. The UV-B radiation also inhibited epidermal cell expansion on the exposed surface of leaves.

The bean plants, grown under different total irradiance levels but constant UV-B/PAR ratios, show large differences in growth rate, morphology, photosynthesis and pigmentation (Deckmyn *et al.* 1994). These plants show similar reductions of photosynthesis and growth when exposed to the UV-B irradiance, which was increased by 15 %. Low PAR levels only increase the sensitivity of plants when the UV-B irradiation is kept constant.

Table 2. Content of total chlorophyll and UV-B absorbing pigments in leaves of the control plants and the plants treated with UV-B/PAR as in the Figure 3.

Species	Chl [SPAD]		A_{305} [dm^{-2}]	
	Control	UV-B	Control	UV-B
Bean	32.4	32.2	21.9	25.4*
Pea	32.2	33.6	9.6	13.7*
Rape	25.6	25.2	16.7	19.2***

* Significant difference between the means of the control and the UV-B irradiated plants (* $p<0.05$, *** $p<0.001$)

Plants grown under higher total irradiance develop leaves that are more resistant to UV-B damage: higher SLW and higher content of UV-B-absorbing pigments.

The parameter SLW, proportional to leaf thickness, increased for the bean plants grown under the UV-B/PAR radiation, which represents an additional protective reaction of this species against harmful UV-B. A similar effect of UV-B radiation on rape leaf thickness was observed by Cen and Bornman (1993), who measured it by means of scanning electron micrographs.

No changes of chlorophyll content were recorded in the studied plant species growing at the UV-B, which corresponds with the results of Sullivan and Teramura (1989) for pine, however, the high level of UV-B usually caused some decrease of this pigment content (Cen and Bornman 1990, Strid *et al.* 1990, He *et al.* 1993).

To summarise, one could conclude that bean plants showed relatively higher tolerance – on the stage of the light reactions of photosynthesis – to the applied UV-B dose, in comparison with the pea and rape plants. It can be probably related to the increased thickness of leaves. The pea plants did not show changes in CO_2 fixation rate, which can be attributed to the presence of protective wax layer on the leaves. However, in darkness this protection was insufficient as a defence against the damage caused by the UV-B irradiation.

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