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Certain Growth and Metabolic Indices of Stress induced by Visible Light and UV Radiation in Broad Bean Seedlings

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Summary

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The effects of either visible light or UV-radiation on growth and metabolism of broad bean (*Vicia faba*) seedlings were investigated. Exposure of seedlings to low and high visible light and UV-radiation, either alone or in combination, induced variable significant decreases in the levels of growth parameters throughout the experimental period, as compared with values of control seedlings grown in darkness or ambient visible light. In addition, induced pronounced significant changes in the total amount and in the relative composition of pigment fraction contents, associated with significant variable decreases in photosystem II (PSII) activity were observed. In relation to controls, direct exposure of broad bean seedlings to visible light and UV-radiation, induced significant variable changes in the total amount and in the relative composition of the carbohydrate pool. Concurrently with carbohydrate changes, significant variable increases in the activities of both invertase and α-amylase of broad bean seedlings were maintained throughout the entire period of the experiment.

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Zusammenfassung

YOUNIS M. E., HASANEEN M. N. A. & ABDEL-AZIZ H. M. M. 2012. Certain growth and metabolic indices of stress induced by visible light and UV radiation in broad bean seedlings. [Durch Lichtstress (UV und sichtbares Licht) hervorgerufene Änderungen der Wachstums- und Stoffwechselparameter bei Keimpflanzen der Saubohne]. – Phyton (Horn, Austria) 52 (2): 203–218.

Die Effekte, die sichtbares Licht bzw. UV-Licht auf das Wachstum und den Stoffwechsel von Keimpflanzen der Saubohne (*Vicia faba*) haben, wurden untersucht. Die Keimlinge, die starkem und schwachem sichtbaren Licht bzw. UV-Licht einzeln, oder in Kombination, ausgesetzt waren, zeigten, im Vergleich zu im Dunklen oder im Umgebungslicht aufgewachsenen Kontrollpflanzen, während des gesamten Untersuchungszeitraumes eine signifikante Abnahme der gemessenen Wachstumsparameter. Außerdem verringerte sich der Gesamtpigmentgehalt, genauso wie sich das Verhältnis der einzelnen Pigmente zueinander änderte. Dies war verbunden mit einer signifikant verringerten Aktivität des Photosystems II. Der Kohlenhydrat-Pool war sowohl in Menge, wie auch in der Zusammensetzung signifikant unterschiedlich zur Kontrolle. Während der gesamten Versuchsdauer ging mit diesen Veränderungen eine Abnahme der Aktivitäten von Invertase und α-Amylase einher.

Introduction

Light is a critical environmental signal that affects nearly every aspect of plant growth, development and metabolism. Plants require sunlight for photosynthesis and thus are constantly also exposed to potentially damaging ultraviolet radiation (UV) that is present in sunlight (KUCERA & al. 2003). Ultraviolet radiation is categorized into several components based on different wavelength ranges (UV-A 320–400 nm, UV-B 280–320 nm and UV-C shorter than 280 nm) in the biological sciences. Of these UV-B and UV-C have been known to affect most organisms harmfully.

The biological effects of UV radiation have received little attention and questions arise as to how plants combat the effects of UV. Thus, the negative effects of UV-A and UV-C radiation of various plant species result in deformed morphological parameters; decreased length of radicle, length of plumule, decreased leaf area per unit plant, biomass and dry mass accumulation (WEIH & al. 1998, KRIZEK & al. 1998, ZUK-GOLASZEWSKA & al. 2003). Species, cultivar and age are determining factors in the degree of susceptibility of plants to increased UV-radiation (TEVINI & TERAMURA 1989, CALDWELL & al. 1995). Because UV-A and photo-synthetically active radiation (PAR, 400–700 nm) can ameliorate the damaging effects caused by UV-B radiation by inducing photoreactivation processes in living cells (CALDWELL & al. 1995), the ratios of UV-B/UV-A and UV-B/PAR determine the susceptibility of organisms, as well as plant tissues, to UV exposure (BARNES & al. 1996).

Furthermore, MUSIL 1996 and SALEH & al. 2006 indicated that increasing supplemental doses of UV decreased significantly total carbohydrates; the reduction in carbohydrate contents in response to elevated UV

radiation was attributed to the destructive damage of photosystems induced by UV radiation, which led to the decrease in photosynthetic efficiency (SALEH & al. 2006). UV-A irradiation was also shown to damage the primary photochemistry of PSII to a larger extent than that of photosystem I (PSI) (NAYAK & al. 2003, IVANOVA & al. 2008).

The present study (ABDEL-AZIZ 2008) was carried out in an attempt to obtain better understanding towards the induced radiation stress effects and mechanisms involved either by UV-A and UV-C radiation or visible light either alone or in combination in *Vicia faba* seeds during germination. As a test plant, *Vicia faba* L. is one of the most important winter crops of high nutritive value in Egypt as well as in the world. Although faba beans are consumed less in western countries, it is one of the main sources of protein and energy for much people in Africa, Asia and Latin America. In this paper, the approach applied was to follow the effects of visible light and UV irradiations on growth and photosynthetic components as well as on the changes in carbohydrate constituents and certain related enzyme activities.

Material and Methods

Plant Material and Growth Conditions

In this investigation, a series of experiments, embodied in two separate sections were carried out. A homogeneously-sized lot of *Vicia faba* (cv. Egypt1) seeds was selected and surface sterilized by soaking in 10^{-3} M mercuric chloride solution for 3 min, washed thoroughly with sterile distilled water and then soaked for 24 h in sterile distilled water at $25 \pm 1^{\circ}$C, with aeration to avoid anaerobiosis as a complicating factor. The seeds were divided into a number of sets; each of 25 seeds. These sets were allowed to germinate in plastic boxes ($22 \times 14 \times 10$ cm) furnished with Whatman No.1 paper moistened by adding 20 cm^3 of sterile distilled water. During the experimental period, each box was supplied with 20 cm^3 of sterile distilled water every other day.

In the first section (A), the germination boxes were incubated in the dark at $25 \pm 0.1^{\circ}$C. After 14 days from the start, the boxes containing seedlings were subdivided into six subgroups, each of 4 boxes. One of the subgroups was left without treatment to serve as control and the other five subgroups were irradiated, one h daily, for six days, then quickly returned back to the original germination conditions in darkness. The treatment scheme adopted in this first section (A) can be summarized as follows:

1) Control.
2) Exposure of seedlings for 1 h to low visible light level (LL) (40 W m^{-2}).
3) Exposure of seedlings for 1 h to UV-C radiation (UV-C) (254 nm).
4) Exposure of seedlings for 1 h to low visible light level (40 W m^{-2}) in combination with UV-C radiation (254 nm) (LL + UV-C).
5) Exposure of seedlings for 1 h to UV-A radiation (UV-A) (365 nm).
6) Exposure of seedlings for 1 h to low visible light level (40 W m^{-2}) in combination with UV-A radiation (365 nm) (LL + UV-A).

In the second section (B), the germination boxes were incubated in ambient light (12 h day and 12 h night, photosynthetically active radiation, PAR = 280 μmol m^{-2} s^{-1}) at 25 ± 0.1°C. After 14 days from the start of germination, the appropriate boxes containing seedlings were sub-divided into four subgroups each of 4 boxes; one of them was left without treatment to serve as control and the other three subgroups were irradiated, one h daily, for six consecutive days, and then quickly returned back to the original ambient light germination conditions. The treatment scheme adopted in the second section (B) can be summarized as follows:

1) Control.
2) Exposure of seedlings for 1 h to high visible light level (HL) (160 W m^{-2}).
3) Exposure of seedlings for 1 h to UV-C radiation (UV-C) (254 nm).
4) Exposure of seedlings for 1 h to high visible light level (160 W m^{-2}) in combination with UV-C radiation (254 nm) (HL + UV-C).

All the exposure treatments were performed in UV-light boxes (70 × 50 × 70 cm) in which UV radiation was supplied from a compact UV lamp, 365 nm and 254 nm (8 W m^{-2}) (UVP factory, USA) which was suspended above and perpendicular to the germination boxes at a distance of 50 cm. Low visible light level was supplied from filament lamps with soft white bulbs (40 W m^{-2}, 400–700 nm) (Express factory, China) and high visible light level was supplied from blended-light mercury lamps (160 W m^{-2}, 400–700 nm) (Simlux factory, Egypt) fixed at the same distance.

It should be mentioned that the broad bean seedlings appeared unable to tolerate the specified UV-radiation doses for a long 6-h period; most seedlings failed to survive. Thus, as an alternative, consecutive 6 h treatment period; one h daily for 6 days was used throughout the experimental period as the sustainable treatment.

The differently treated seedlings were examined every other day for morphological appearance and for determination of length of radicle and plumule. Other growth parameters, as well, were also determined over a period of six days from the date of exposure to different wavelengths of light, either alone or in combination.

Since seed coat, in general, is considered as not being utilized in germination, the decoated seed represents the living portion, i.e. embryo and cotyledons, in which resides the potential for growth. Thus, decoated triplicate samples of broad bean seedlings were taken for determination of their water contents, fresh and dry matter accumulation, photosynthetic pigments, PSII activity, carbohydrates and carbohydrate-related enzyme activities. All measurements were carried out on the 2nd, 4th and 6th days from the date of exposure immediately after treatment.

It should be mentioned that the results obtained from the analyses of duplicate determinations and triplicate samples were remarkably close, thus the data presented in the corresponding tables are the means of triplicate samples. The full data of the different stressed groups of seedlings were statistically analyzed using one-way analysis of variance (ANOVA) and comparison among means was carried out by calculating the Post Hoc L.S.D. with a significant level at $*P < 0.05$. The Pearson's correlation coefficients between the growth parameters and the various metabolic changes were also carried out in order to substantiate our conclusions. All the analyses were made using the SPSS 13.0 for Windows software package (SPSS Inc., Chicago, IL, USA).

For better quantitative comparison among the different treatments, the percentage of change (increase or decrease) in response to each treatment, in relation to control level, was calculated throughout this investigation as follows:

- Percentage change (increase or decrease) immediately after each specific treatment: [(level after treatment – control level) / control level] × 100.

Determination of Photosynthetic Components

The photosynthetic pigments (Chl a, Chl b, and Car) were determined in fresh tissues after extraction with 85% acetone using the spectrophotometeric method as described and recommended by METZNER & al. 1965.

PSII activity, as indicated by the rate of 2,6-dichlorophenol indophenol (DCPIP) was monitored at 600 nm using a spectrophotometer. According to ARNON 1949, 4 g of fresh tissues were used for preparation of chloroplast pellets that were suspended in 1 mM Na-Tricine (pH 7.8), 10 mM NaCl and 10 mM $MgCl_2$ and then kept at 0–4 °C until required. The assay reaction mixture for determination of PSII activity contained 200 mM Na-phosphate (pH 7.2), 2 mM $MgCl_2$ and 0.5 mM 2,6-DCPIP. A calibration curve in terms of micromoles of dye reduced (DEAN & MISKIEWICZ 2003) was made using 2,6-DCPIP range between 10–50 μM in the reaction mixture (4 cm^3).

Estimation of Carbohydrates

The method of extraction of different carbohydrate fractions (glucose, fructose, sucrose and starch), used was patterned after those adopted by YEMM & WILLIS 1954 and VAN HANDEL 1968. In the ethanolic extract of the broad bean seedlings, glucose was estimated using the o-toluidine procedure of FETERIS 1965. Fructose was estimated in the ethanolic extract using the resorcinol method of ROE 1934 as described by DEVI 2002. Sucrose was determined by first degrading reactive sucrose present in 0.1 cm^3 extract with 0.1 cm^3 5.4 N KOH at 97 °C for 10 min. Three cm^3 of freshly prepared anthrone reagent [150 mg anthrone + 100 cm^3 72% (w/w) H_2SO_4] were then added to the cooled reaction products and the mixture was heated at 97 °C for 5 min, cooled and the developed colour was read at 620 nm, using spectrophotometer (VAN HANDEL 1968). Polysaccharides were determined by the method of THAYUMANAVAN & SADASIVAM 1984.

Invertase Activity

Extraction of crude invertase was performed using the method of PRESSEY & AVANTS 1980. The reaction mixture for the invertase assay consisted of 0.1 cm^3 enzyme preparation, 0.2 cm^3 of 0.1 M sodium acetate (pH 5), 0.1 cm^3 of 0.15 M NaCl, and 0.1 cm^3 of 0.73 M sucrose. The enzyme preparation was diluted with 0.15 M NaCl to produce approximately l-μmol reducing groups. The blank prepared for each sample was the same as the assay mixture but was heated before the addition of sucrose (NELSON 1944).

α-Amylase Activity

α-amylase was extracted and assayed according to the methods adopted by GIBBS 1955 and STREET & CLOSE 1956.

Results and Discussion

Changes in Growth Parameters

The length of both radicle and plumule as well as fresh and dry mass accumulation and water content of the control as well as of the differently radiated broad beans appeared to increase progressively and significantly throughout the duration of the experiment reaching a maximum on the sixth day. Exposure of broad beans to LL, UV-C and UV-A either alone or in combination led, in general, to significant decreases in all the above mentioned growth parameters throughout the germination period as compared with control values (Table 1). The apparent decreased levels maintained for all the various growth parameters, in response to treatment with UV radiations, seem, in general, to be slightly augmented when LL was used in combination with UV-C and UV-A. The following sequence of treatments: LL+UV-C > LL+UV-A > LL > UV-C > UV-A, and LL+UV-A > LL > LL+UV-C > UV-A > UV-C, were displayed with respect to the percentage decrease in the length of both radicle and plumule and the percentage decrease in the water content and fresh and dry matter accumulation respectively (Table 1).

Again in both control and irradiated seedlings grown in ambient light, there were progressive significant increases in length of radicle and plumule, fresh and dry mass accumulation, throughout the entire period of germination. But, as compared with control, all the detected growth parameters were variably and significantly decreased in response to irradiation of the seedlings with HL, UV-C and HL+UV-C (Table 1). Thus, the following sequence of treatments (UV-C > HL+UV-C > HL) was displayed with respect to the percentage decrease maintained in all the growth parameters during the experimental period (Table 1).

These induced negative alterations in all growth parameters may be attributed to photomorphogenetic UV-radiation effects associated with changes in cell division and/or cell elongation (Gehrke 1999). In hypocotyls of sunflower seedlings, a clear interaction with the growth regulator indole-3-acetic acid (IAA) was detected (Tevini & al. 1991). IAA absorbs in the UV waveband and can be converted in vitro and in vivo to various photo-oxidative products. One of these products is 3-methylene-oxindole which inhibits hypocotyl growth when applied exogenously. The primary effect of enhanced UV-radiation appears to be subtle photomorphogenic responses that induce altered carbon partitioning and allocation rather than significant reductions in growth or biomass accumulation (Caldwell & al. 2003, Saleh & al. 2006).

In support of the present results, Musil & al. 1998 found that germination of *Leucadendron laureolum* seeds was depressed following short (7-day) exposures to UV radiation. This depression was intensified with increased UV exposure dose, and most pronounced at shorter UV-B wave-

Table 1. The effects of visible light or UV radiations, either alone or in combination, on growth parameters; length of radicle (cm seedling^{-1}), length of plumule (cm seedling^{-1}), fresh mass (g seedling^{-1}), dry mass (g seedling^{-1}) and water content (g seedling^{-1}) of *Vicia faba* seedlings germinated in darkness and in ambient light. Mean values are significantly different from control at *P ≤ 0.05.

Days		Parameters Treatments	Length of radicle	% change	Length of plumule	% change	Fresh mass	% change	Dry mass	% change	Water content	% change
2nd	Darkness	Control	4.50	–	5.20	–	2.55	–	0.60	–	1.95	–
		LL	4.40*	– 2.22	5.12*	– 1.54	2.37*	–7.06	0.50*	–16.67	1.87*	–4.10
		UV-C	4.43*	– 1.56	5.13*	– 1.35	2.50*	–1.96	0.60	0.00	1.90*	–2.5
		LL +UV-C	4.26*	– 5.33	5.00*	– 3.85	2.38*	–6.67	0.50*	–16.67	1.88*	–3.59
		UV-A	4.45*	– 1.11	5.16*	– 0.77	2.43*	–4.71	0.54*	–10.0	1.89*	–3.08
		LL+UV-A	4.33*	– 3.78	5.10*	– 1.92	2.36*	–7.45	0.50*	–16.67	1.86*	–4.62
	Light	Control	4.56	–	5.28	–	2.62	–	0.66	–	1.96	–
		HL	4.53	– 0.66	5.26	– 0.38	2.48*	–5.34	0.64	– 3.03	1.84*	–6.12
		UV-C	4.40	– 3.51	5.20*	– 1.52	2.42*	–7.63	0.60*	– 9.09	1.82*	–7.14
		HL +UV-C	4.50	– 1.32	5.25	– 0.57	2.46*	–6.11	0.62*	– 6.06	1.84*	–6.12
4th	Darkness	Control	4.70	–	5.73	–	2.66	–	0.62	–	2.04	–
		LL	4.45*	– 5.32	5.25*	– 8.38	2.44*	–8.27	0.55*	–11.29	1.89*	–7.35
		UV-C	4.50*	– 4.26	5.30*	– 7.50	2.60*	–2.26	0.61	– 1.61	1.99*	–2.45
		LL +UV-C	4.30*	– 8.51	5.18*	– 9.60	2.55*	–4.14	0.60*	– 3.23	1.94*	–4.90
		UV-A	4.60*	– 2.13	5.40*	– 5.76	2.55*	–4.14	0.60*	– 3.23	1.95*	–4.41
		LL+UV-A	4.40*	– 6.38	5.19*	– 9.42	2.41*	–9.40	0.54*	–12.90	1.87*	–8.33
	Light	Control	5.28	–	6.50	–	2.74	–	0.69	–	2.05	–
		HL	4.70*	–10.98	6.30*	– 3.08	2.67*	–2.55	0.67	– 2.90	2.00*	–2.44
		UV-C	4.46*	–15.53	5.80*	–10.77	2.60*	–5.11	0.63*	– 8.70	1.96*	–4.39
		HL +UV-C	4.55*	–13.83	5.85*	–10.00	2.61*	–4.74	0.65*	– 5.80	1.97*	–3.90
6th	Darkness	Control	4.90	–	6.80	–	2.74	–	0.64	–	2.10	–
		LL	4.55*	– 7.14	5.50*	–19.12	2.57*	–6.20	0.58*	– 9.37	2.00*	–4.76
		UV-C	4.65*	– 5.10	5.50*	–19.12	2.67*	–2.55	0.63	– 1.56	2.04*	–2.86
		LL +UV-C	4.35*	–11.22	5.26*	–22.65	2.64*	–3.64	0.63	– 1.56	2.01*	–4.29
		UV-A	4.84*	– 1.22	5.65*	–16.91	2.65*	–3.28	0.63	– 1.56	2.01*	–4.29
		LL+UV-A	4.53*	– 7.55	5.36*	–21.18	2.57*	–6.20	0.57*	–10.94	2.00*	–4.76
	Light	Control	5.40	–	7.20	–	2.89	–	0.71	–	2.18	–
		HL	4.75*	–12.04	7.10*	– 1.39	2.77*	–4.15	0.68	– 4.23	2.09*	–4.13
		UV-C	4.63*	–14.26	6.80*	– 5.56	2.72*	–5.88	0.65*	– 8.45	2.07*	–5.05
		HL +UV-C	4.70*	–12.96	7.00*	– 2.78	2.74*	–5.19	0.66*	– 7.04	2.08*	–4.59

lengths. One explanation for this discrepancy in UV dose effectiveness may be the greater penetration into tissues of longer UV-A wavelengths (LOWE & SHAATH 1990, ÅLENIUS & al. 1995). This suggests a much greater impact of solar UV radiation on seed deterioration processes under natural conditions, since the UV-A component of the solar UV flux constitutes a much greater fraction (39%: 3.45 kJ m^{-2} d^{-1}) of the total daily biologically effective flux, according to the QUAITE & al. 1992 action spectrum, than that supplied by the fluorescent sun lamps (0.45 kJ m^{-2} d^{-1}).

WEIH & al. 1998 stated that enhanced UV-A decreased leaf area per unit plant biomass (leaf area ratio). Qualitative effects of solar UV-A radiation on higher plants have been reported (TEZUKA & al. 1994), but quantitative data are minimal. Recent UV-exclusion studies conducted on cucumber (KRIZEK & al. 1997) and a red-pigmented lettuce (KRIZEK & al. 1998) indicate that ambient UV-A greatly inhibits leaf enlargement, stem elongation and biomass production over and above that under ambient UV-B. The above mentioned reports and conclusions lend a strong support to our results.

Changes in Photosynthetic Parameters

It is apparent from Table 2 that the irradiation with LL, UV-A or UV-C, either alone or in combination, induced variable significant decreases in all the detected pigment contents below the control levels. The interrelationships between Chl a and Chl b fractions can be better evaluated when the values of Chl a/b ratios are taken into consideration. In broad bean seedlings, treatment with LL, UV-A or UV-C, either alone or in combination, lowered these ratios below those of controls maintained throughout the entire period of the experiment (Table 2). In response to irradiation, the calculated percentage negative change in all pigment contents of broad bean seedlings are also shown in Table 2.

In broad bean seedlings treated with HL, UV-C and HL+UV-C, the pigment contents as well as Chl a/b ratios appeared to be lowered below those of control throughout the experimental period except on the 6th day when the ratio of HL irradiated seedlings appeared comparable with that of control seedlings (Table 2).

The calculated percentages of decrease in all pigment fraction contents of the variously treated seedlings (Table 2) are in accord with the following sequence of treatments: (LL > UV-C > LL+UV-A > UV-A > LL+UV-C) and (HL > HL+ UV-C > UV-C) in the case of the dark and ambient-light germinated seeds, respectively.

As compared with dark- or ambient light-grown broad bean seedlings, exposure of the seedlings to LL, UV-A, UV-C and HL, either alone or in combination, induced a significant decrease in PSII activity (Table 2). The sequence of the maintained percent change (decrease) in the activity of PSII of the variously treated broad bean seedlings appeared as follows: (LL+UV-

Table 2. The effects of visible light or UV radiation, either alone or in combination, on photosynthetic pigments (μg/100 g fresh mass) and on PSII activity (μM DCPIP reduced/100 mg Chl/ h) of *Vicia faba* seedlings germinated in darkness and in ambient light. Mean values are significantly different from control at *P ≤ 0.05.

Days		Parameters Treatments	Chl a	% change	Chl b	% change	Chl a+b	Chl a/b	Car	% change	Total pigments	% change	PS II	% Change
2nd	Darkness	Control	120	–	330	–	450	0.36	270	–	720.0	–	4.1	–
		LL	66.6*	–44.50	91.1*	–72.39	157.7*	0.73*	263.1*	– 2.56	420.8*	–41.56	3.7*	– 9.76
		UV-C	84.4*	–29.67	242.4*	–26.55	326.8*	0.35	172.2*	–36.22	499.0*	–30.69	3.1*	–24.34
		LL +UV-C	110.5*	– 7.92	292.8*	–11.27	403.3*	0.37	195.2*	–27.70	598.5*	–16.88	2.2*	–46.34
		UV-A	107.3*	–10.58	285.4*	–13.42	392.7*	0.37	196.7*	–27.15	589.4*	–18.14	2.1*	–48.78
		LL+UV-A	102.8*	–14.33	249.1*	–24.52	351.9*	0.40*	214.5*	–20.56	566.4*	–21.33	1.2*	–70.73
	Light	Control	543.60	–	427.90	–	971.50	1.27	339.30	–	1310.80	–	8.40	–
		HL	321.90*	–40.78	305.80*	–28.53	627.70*	1.05*	261.60*	–22.90	889.30*	–32.16	4.70*	–44.05
		UV-C	481.20*	–11.48	374.50*	–12.48	855.70*	1.28	296.30*	–12.67	1152.00*	–12.11	6.40*	–23.81
		HL +UV-C	351.20*	–35.39	333.70*	–22.01	684.90*	1.05*	282.20*	–16.83	967.10*	–26.22	5.10*	–39.29
4th	Darkness	Control	238.0	–	519.9	–	757.9	0.45	352.3	–	1110.2	–	5.7	–
		LL	72.3*	–69.62	165.8*	–68.11	238.1*	0.43	160.2*	–54.53	398.3*	–64.12	4.1*	–28.07
		UV-C	84.2*	–83.80	143.3*	–39.79	227.5*	0.59*	175.0*	–50.33	490.2*	–55.85	3.9*	–31.58
		LL +UV-C	91.6*	–61.51	237.2*	–54.38	328.8*	0.39	209.1*	–40.65	537.9*	–51.54	3.1*	–45.61
		UV-A	86.2*	–63.78	270.0*	–48.07	356.2*	0.32*	167.6*	–52.43	523.8*	–52.82	2.7*	–52.63
		LL+UV-A	101.4*	–57.39	276.0*	–46.91	377.4*	0.37*	187.2*	–46.86	514.6*	–53.65	2.1*	–63.16
	Light	Control	607.30	–	485.50	–	1092.80	1.25	496.70	–	1589.50	–	15.90	–
		HL	251.50*	–58.59	300.80*	–38.04	552.30*	0.84*	239.20*	–51.84	791.50*	–50.20	10.80*	–32.08
		UV-C	457.30*	–24.70	360.40*	–25.77	817.70*	1.27	266.10*	–46.43	1083.80*	–31.82	13.80*	–13.21
		HL +UV-C	311.20*	–48.76	303.60*	–37.47	614.80*	1.03*	248.60*	–49.95	863.40*	–45.68	11.70*	–26.42
6th	Darkness	Control	331.9	–	661.3	–	993.2	0.50	402.9	–	1396.1	–	7.3	–
		LL	61.2*	–81.56	141.2*	–78.65	202.4*	0.43*	137.8*	–65.80	340.2*	–75.63	4.8*	–34.25
		UV-C	72.1*	–78.28	241.4*	–63.50	313.5*	0.30*	134.2*	–66.69	447.7*	–67.93	4.2*	–42.47
		LL +UV-C	161.2*	–51.43	70.1*	–89.4	231.3*	2.30*	251.7*	–37.53	483.0*	–65.40	3.9*	–46.58
		UV-A	82.7*	–75.08	243.1*	–63.24	325.8*	0.34*	174.0*	–56.81	472.8*	–66.13	3.6*	–50.68
		LL+UV-A	66.2*	–80.05	216.2*	–67.31	282.8*	0.30*	176.3*	–56.24	459.1*	–67.12	2.8*	–61.64
	Light	Control	675.70	–	566.40	–	1242.10	1.19	520.60	–	1762.70	–	21.20	–
		HL	202.00*	–64.34	241.00*	–64.33	443.00*	0.84	231.60*	–55.51	674.60*	–61.73	14.60*	–31.13
		UV-C	382.30*	–43.42	336.20*	–40.64	718.50*	1.14*	246.30*	–52.69	964.80*	–45.27	17.50*	–17.45
		HL +UV-C	281.90*	–58.28	292.10*	–48.43	574.00*	0.97*	238.90*	–54.11	812.90*	–53.88	15.30*	–27.83

A > UV-A > LL+ UV-C > UV-C > LL) and (HL > HL+ UV-C > UV-C) in case of the dark- and ambient-light germinated seeds, respectively.

Thus, the present results, concerning the negative effects of UV irradiation upon the photosynthetic machinery (pigment content and PSII activity) appeared to coincide with the negative effects on growth components. In accord with the above mentioned results, YAO & LIU 2007 demonstrated that enhanced UV-radiation significantly decreased Chl a, Chl b, Chl a+b and Car contents of *Picea asperata* seedlings. A parallel changing trend in Chl a and Chl b resulted in no significant change in Chl a/b ratio under enhanced UV-radiation. Furthermore, the decreasing tendency of chl content and chl fluorescence appeared parallel to the biomass reduction in plants. The decrease in Chl a+b content was mainly attributed to the distribution of Chl b, which is more sensitive to radiation than Chl a (YAO & LIU 2007). In addition the decreases of total chlorophyll content may be due to the decreases of Car, since Car protect chlorophyll from photooxidative destruction (SINGH 1996).

The decrease in content of Car due to enhanced UV- or high light intensity radiations was attributed to considerable oxidative stress by accumulation of reactive oxygen species (ROS) (YAO & LIU 2007). Carotenoids, being considered as a quenching agent of short wave with high energy, could exert their protective function as antioxidants to inactivate UV- induced radicals in the photosynthetic membranes (GÖTZ & al. 1999).

Moreover, PSII inactivation can be induced by both visible (LONG & al. 1994) and UV irradiation (KRAUSE & al. 1999). That PSII inactivation can be increased by abiotic stresses, such as nutrient deficit (GROSSMAN & TAKAHASHI 2001). UV-A also causes PSII inactivation and contributes to oxidative pressure (WHITE & JAHNKE 2002).

Recently, VASS & al. 2002 and IVANOVA & al. 2008 studied the effect of UV-A radiation on the function of the photosynthetic apparatus in thylakoid membranes and they suggested that the primary target of UV-A radiation in thylakoid membranes is the PSII complex. Several UV sensitive sites are supposed to exist in this complex, including the redox-active tyrosine, the Mn cluster on the donor side and the plastosemiquinones on the acceptor side (IVANOVA & al. 2008). The primary site of UV-A radiation damage is thought to be the catalytic Mn cluster of the oxygen evolving system (VASS & al. 2002).The inactivation of the electron transport between the Mn cluster and the tyrosine electron donors was proposed to be the immediate cause for the loss of O_2 evolution by UV radiation (IVANOVA & al. 2008).

Changes in Carbohydrate Content and Carbohydrate-Related Enzyme Activities

The pattern of changes herein obtained (Table 3) indicates that glucose, fructose, sucrose, starch and total saccharide contents of the control

Table 3. The effects of visible light or UV radiation, either alone or in combination, on carbohydrate content given as mg glucose equivalent/100 g dry mass and on activities of invertase and α-amylase (units 100^{-1} cm^3 enzyme preparation) of *Vicia faba* seedlings germinated in darkness and in ambient light. Mean values are significantly different from control at *P ≤ 0.05.

Days		Parameters Treatments	Glucose	% change	Fructose	% change	Sucrose	% change	Starch	% change	Total saccharides	% change	Invertase	% change	α-amylase	% change
2nd	Darkness	Control	19.8	–	2.85	–	285.7	–	4160	–	4468.35	–	80.3	–	11.2	–
		LL	18.0*	–9.09	2.51*	–11.93	307.1*	+ 7.49	3060*	–26.44	3387.61*	–24.19	90.3*	+12.45	16.6*	+48.21
		UV-C	19.0*	–4.04	2.60*	– 8.77	302.6*	+ 5.92	3120*	–25.0	3444.2*	–22.92	84.2*	+ 4.86	16.3*	+45.54
		LL +UV-C	17.1*	–13.63	2.37*	–16.84	309.4*	+ 8.30	3000*	–27.88	3328.87*	–25.50	94.3*	+17.43	16.9*	+50.89
		UV-A	16.2*	–18.18	2.22*	–22.11	312.8*	+ 9.49	2680*	–35.57	3011.22*	–32.61	96.5*	+20.17	18.6*	+66.07
		LL+UV-A	15.4*	–22.22	2.13*	–25.26	329.6*	+15.37	2480*	–40.38	2827.13*	–36.73	101.2*	+26.03	18.7*	+66.96
	Light	Control	19.60	–	2.95	–	305.10	–	4560.00	–	4887.65	–	89.10	–	15.70	–
		HL	14.10*	–28.06	2.00*	–32.20	309.10*	+ 1.31	3640.00*	–20.18	3965.20*	–18.87	96.70*	+ 8.53	21.20*	+35.03
		UV-C	17.20*	–12.24	2.62*	–11.19	318.10*	+ 4.26	3880.00*	–14.91	4217.92*	–13.70	90.40*	+ 1.46	19.60*	+24.84
		HL +UV-C	16.30*	–16.84	2.52*	–14.58	311.20*	+ 1.99	3700.00*	–18.86	4030.02*	–17.55	92.70*	+ 4.04	20.30*	+29.30
4th	Darkness	Control	25.52	–	3.42	–	293.2	–	6440	–	6762.14	–	83.6	–	13.5	–
		LL	24.32*	–4.70	3.38	– 1.16	322.1*	+ 9.86	4160*	–35.40	4509.8*	–33.31	99.4*	+18.90	22.4*	+65.93
		UV-C	24.9*	–2.43	3.40	– 0.58	314.1	+ 7.13	4360*	–32.30	4702.4*	–30.46	97.3*	+16.39	20.8*	+54.07
		LL +UV-C	20.1*	–21.24	3.19*	– 6.73	338.1*	+15.31	3400*	–47.20	3761.39*	–44.38	104.1*	+24.52	25.3*	+87.41
		UV-A	18.2*	–28.68	3.15*	– 7.89	352.1*	+20.09	3320*	–48.45	3693.45*	–45.38	107.0*	+27.99	26.0*	+92.59
		LL+UV-A	16.1*	–36.91	3.04*	–11.11	361.2*	+23.19	3160*	–50.93	3540.34*	–47.64	109.6*	+31.10	26.6*	+97.04
	Light	Control	24.90	–	3.01	–	319.60	–	6260.00	–	6607.51	–	96.00	–	21.00	–
		HL	16.10*	–35.34	2.49*	–17.28	321.10*	+ 0.47	3820.00*	–38.98	4159.69*	–37.05	114.00*	+18.75	29.40*	+40.00
		UV-C	18.10*	–27.31	3.00	– 0.33	329.10*	+ 2.97	4460.00*	–28.75	4810.20*	–27.20	105.20*	+ 9.58	25.40*	+20.95
		HL +UV-C	17.20*	–30.92	2.80*	– 6.98	328.10*	+ 2.66	4140.00*	–33.87	4488.10*	–32.08	107.30*	+11.77	28.00*	+33.33
6th	Darkness	Control	33.1	–	5.09	–	297.7	–	8560	–	8895.89	–	88.7	–	16.4	–
		LL	21.9*	–33.84	4.63*	– 9.04	343.3*	+15.32	5060*	–40.89	5431.79*	–38.94	105.6*	+19.05	26.3*	+60.37
		UV-C	22.3*	–32.63	4.86*	– 4.52	340.2*	+14.28	5160*	–39.72	5527.36*	–37.87	104.6*	+17.92	24.0*	+46.34
		LL +UV-C	21.1*	–36.25	3.43*	–32.61	346.1*	+16.26	4340*	–49.30	4710.63*	–47.05	109.8*	+23.79	27.1*	+65.24
		UV-A	19.6*	–40.79	4.11*	–19.25	361.1*	+21.30	4260*	–50.23	4644.81*	–47.79	110.6*	+24.69	31.7*	+93.29
		LL+UV-A	17.2*	–48.04	4.08*	–19.84	374.3*	+25.73	4160*	–51.40	4555.58*	–48.79	118.2*	+33.26	33.4*	+103.66
	Light	Control	34.90	–	4.82	–	329.30	–	8520.00	–	8889.02	–	102.10	–	26.10	–
		HL	18.10*	–48.14	2.80*	–41.91	334.20*	+ 1.49	4560.00*	–46.48	4915.10*	–44.71	120.30*	+17.83	36.20*	+38.70
		UV-C	20.10*	–42.41	3.70*	–23.24	341.10*	+ 3.58	5520.00*	–35.21	5884.90*	–33.80	112.70*	+10.38	33.70*	+29.12
		HL +UV-C	19.20*	–44.99	3.00*	–37.76	336.10*	+ 2.06	4900.00*	–42.49	5258.30*	–40.85	118.00*	+15.57	35.00*	+34.10

as well as of the differently treated broad bean seedlings appeared to show a significant progressive increase throughout the experimental period. Exposure of dark- or ambient light-grown seedlings to LL, HL, UV-C, UV-A radiations, either alone or in combination, throughout the experimental period, led to significant variable decreases in all saccharide contents in relation to levels of control seedlings. The following sequence of treatments; (LL+UV-A > UV-A > LL+UV-C > LL > UV-C) for the dark germinated seedlings and (HL > HL+UV-C > UV-C) for the light germinated ones, were displayed with respect to the percentages of decrease in the various saccharide contents, calculated as percentage of control (Table 3). On the other hand, sucrose content showed significant variable increases above the control levels (Table 3). The following sequence of treatments: (LL+UV-A > UV-A > LL+UV-C > LL > UV-C) for the dark germinated seedlings and (UV-C > HL+UV-C > HL) for the light germinated ones were displayed with respect to percentages of increase in the sucrose content.

Furthermore, broad bean seedlings grown under dark and ambient light conditions, when exposed to LL, HL, UV-A and UV-C, either alone or in combination, showed significant variable increases in the activities of invertase and α-amylase throughout the experimental period, as compared with controls levels (Table 3). The order of the calculated percentages of increase in the activities of both enzymes goes along the following sequence of treatments: LL+UV-A > UV-A > LL+UV-C > LL > UV-C for dark-germinated seeds and HL > HL+UV-C > UV-C for ambient-light germinated seeds (Table 3).

In support of the present results, MUSIL 1996 and SALEH & al. 2006 indicated that increasing supplemental doses of UV radiations significantly decreased the concentration of total carbohydrates of certain plant species. When soybean cultivars were irradiated with UV_{A+B} 12.8 kJ m^{-2} d^{-1}, the percentage of inhibition in total carbohydrates were 25.9, 28.1 and 36.5 for Giza-22, Giza-35 and Giza-111, respectively in comparison with control (SALEH & al. 2006). Also, in a long term study, enhanced UV-radiations significantly decreased the ratio of storage starch to chloroplast area in field-grown silver birch leaves (KOSTINA & al. 2001).

The reduction in glucose, fructose, starch and total saccharide contents of broad bean seedlings grown under dark or ambient light conditions, in response to exposure to LL, HL, UV-A, UV-C, either alone or in combination, throughout the experimental period, could be attributed to the destructive damage of photosynthetic machinery induced by UV radiation. As already stated by ALLEN & al. 1998 and SALEH & al. 2006, it is evident that UV-radiation can potentially impair the performance of the main component processes of photosynthesis; the photophosphorylation reactions of the thylakoid membrane, the CO_2-fixation reactions of the Calvin cycle and stomatal control of CO_2 supply.

Moreover, in accord with our results, BROECKLING & al. 2005 reported increases in the activities of some carbohydrate-related enzymes due to exposure of seedlings of certain plant species to UV-radiation. Also, of interest in this connection, DARBELLEY & al. 1997 investigated the changes in both invertase and α-amylase activities and in starch and free sugar contents in correlation with lipid mobilization in *Helianthus annuus* during the first 15 days of seedling growth in discontinuous light and in darkness. Throughout the seedlings development, invertase and α-amylase activities increased more significantly in light than in darkness. The study of induced changes in several soluble sugars indicated that: 1) sucrose stored in cotyledons of mature seeds was used at the onset of seedling growth, more rapidly in light than in darkness, 2) glucose, fructose and maltose accumulated in old etiolated cotyledons in contrast to what occurred in the light.

The data herein obtained clearly demonstrate that optimum levels of starch and reducing soluble sugars reached in light-grown broad bean seedlings do not coincide with the maintained maximal amount of chlorophyll. These results might be the reflection of much more stronger mobilization of carbohydrates in light than in darkness, in correlation with a more rapid development of embryo axis in light, as was observed in lupin cotyledons (CRAWSHAW & REID 1984). These data were consistent with the fact that the 12-day-old cotyledons of sunflower kept less glucose and fructose in light than in darkness (DARBELLEY & al. 1997).

In view of the above mentioned changes in growth and metabolism induced by visible light and UV-radiation, we can finally conclude that a close parallelism appeared to exist between the growth pattern and dry matter accumulation throughout the experimental period. This appears to be a consequence of the maintained disruption of the functional intensity of the photosynthetic apparatus which also led to decreased levels in the various carbohydrate fractions as well as in the total carbohydrate content. However, the increased levels of sucrose content can be explained on the basis of contribution to the maintenance of osmotic pressure in the rapidly expanding cells of broad bean seedlings, as was proposed by PFEIFFER & KUTSCHERA 1996 during light induced expansion of sunflower cotyledons.

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