Indian J. Plant Physiol., Vol. 16, No. 1, (N.S.) pp. 58-67 (Jan.-Mar., 2011)

AFTER EFFECTS OF TREATMENT WITH VISIBLE LIGHT AND UV RADIATION ON AMINO ACIDS, NUCLEIC ACIDS AND PROTEIN PATTERNS IN BROAD BEAN SEEDLINGS

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Received on 07th Oct., 2010, Revised and Accepted on 05th March, 2011

SUMMARY

The objective of the present study was to investigate the possible effects of direct exposure of broad bean seedlings to different light spectra (low visible light, high visible light, UV-A and UV-C), either alone or in combination, as stressful factors on amino acids, nucleic acids and protein patterns. High accumulation of both proline and glycine was operative under the above mentioned experimental conditions, in relation to controls. As for protein banding patterns, stressed broad bean seedlings showed the appearance of protein bands with molecular weights of 106, 102, 93, 91, 78, 72 and 28.5 kDa. On the other hand, disappearance of protein bands with molecular weights of 22.0, 18.4 and 16 kDa was operative. In addition, exposure of broad bean seedlings to the above mentioned stressful factors induced a significant decrease in the total amount and in the relative composition of the pool of nucleic acids (RNA and DNA), throughout the entire period of the experiment. The present results are discussed in relation to interference of the light radiations with the process of germination and with intermediary metabolism in broad bean seedlings.

Key words*: Glycine, proline, protein patterns, DNA, RNA, UV, *Vicia faba.

INTRODUCTION

The direct effects of ultraviolet radiations on plant cells are mostly damaging, because UV photons have enough energy to create lesions in important UV-absorbing biomolecules such as nucleic acids and proteins (Taylor *et al.* 1997). In the leaves of tropical trees, the ambient UV-B and UV-A radiation might contribute to the reversible decline in potential photosystem II (PSII) efficiency observed upon exposure to full, direct sunlight. Increased level of UV absorbing compounds, and protein damage were indicated by strong effect of photosynthetically active radiations PAR/UV light (Krause *et al*. 1999).

Furthermore, enhanced levels of UV_{A+B} (280 - 400 nm) radiation reaching the earth's surface due to stratospheric ozone depletion (Madronich *et al*. 1998) may damage DNA, proteins and lipids, impair chloroplast function and reduce photosynthesis, growth and development (Jansen *et al*. 1998). UV_{A+B} radiation also produces oxidative stress (Panagopoulos *et al*. 1990) although the mechanism of reactive oxygen species (ROS) generation in UV_{A+B} irradiated plants is not known (Rao *et al*. 1996). Inhibitions of DNA and RNA synthesis and enzymic activity have been also demonstrated in tobacco as a result of UV-A irradiation in the presence of psoralens, naturally occurring plant products (Heimer *et al*. 1977 and 1978).

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The present study (Abdel-Aziz 2008) was carried out to report further on the induced radiation stress effects and mechanisms involved either by UV-A and UV-C radiation or highlight intensity in *Vicia faba* seeds during germination. In our report (Younis *et al.* 2010), it is reported that exposure of dark- or ambient-visible light grown bean seedlings to low and high visible light intensities, UV-A or UV-C, either alone or in combination, induced significant increases in total phenolic compounds and anthocyanins content as well as significant increases in the contents of non-enzymatic antioxidants and enzymatic antioxidant activities. The work presented in this paper was designed to evaluate the interactive effects of visible light and UV radiations on the changes in proline and glycine, nucleic acids and protein patterns.

MATERIALS AND METHODS

Plant material and growth conditions

In the present investigation, a series of experiments, embodied in two separate sections were carried out. Homogeneous *Vicia faba* (cv. Egypt1) seeds were selected and surface sterilized by soaking in 10^{-3} M mercuric chloride solution for 3 min, thoroughly washed with sterile distilled water and then soaked for 24 h in sterile distilled water at 25 ± 1°C, with aeration to avoid anaerobiosis as a complicating factor. The seeds were divided into a number of sets of each 25 seeds. These sets were allowed to germinate in plastic boxes (22 × 14 × 10 cm) furnished with Whatman No.1 paper wetted with 20 ml of sterile distilled water. During the experimental period and when required each box was supplied with additional 20 ml of sterile distilled water.

The experimental set-up, treatment and sampling were the same as previously described by Younis *et al.* (2010). Thus, the plastic boxes containing the seeds were divided into two separate sections. In the first section (A) the allotted germination boxes were incubated in the dark whereas those boxes allotted for the second section (B) were incubated in ambient light (12 h day and 12 h night, photosynthetically active radiation, PAR = 280 µmol m^{-2} s^{-1}). After 14 days from the start of incubation at 25 ± 0.1°C, the allotted boxes of each section were subdivided into a number of subgroups, one being left without treatment and the other subgroups were irradiated, 1 h daily for 6 days, then quickly returned back to the original germination conditions. For clarity the following treatments were used:

Dark growth conditions (section A):

1- Control (C).

2- Exposure of seedlings for 1 h to low visible light level (LL) (180 µmol m^{-2} s^{-1}).

3- Exposure of seedlings for 1 h to UV-C radiation (UV-C) (254 nm, 50 kJ m^{-2}).

4- Exposure of seedlings for 1 h to low visible light level (180 µmol m^{-2} s^{-1}) in combination with UV-C radiation (254 nm, 50 kJ m^{-2}) (LL + UV-C).

5- Exposure of seedlings for 1 h to UV-A radiation (UV-A) (365 nm, 80 kJ m^{-2}).

6- Exposure of seedlings for 1 h to low visible light level (180 µmol m^{-2} s^{-1}) in combination with UV-A radiation (365 nm, 80 kJ m^{-2}) (LL + UV-A).

Ambient light growth conditions (section B):

1- Control (C).

2- Exposure of seedlings for 1 h to high visible light level (HL) (735 µmol m^{-2} s^{-1}).

3- Exposure of seedlings for 1 h to UV-C radiation (UV-C) (254 nm, 50 kJ m^{-2}).

4- Exposure of seedlings for 1 h to high visible light level (735 µmol m^{-2} s^{-1}) in combination with UV-C radiation (254 nm, 50 kJ m^{-2}) (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 (80 kJ m^{-2}) and 254 nm (50 kJ 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 (180 µmol m^{-2} s^{-1}) (Express factory, China) and high visible light level was supplied from blended-light mercury lamps (735 µmol m^{-2} s^{-1}) (Simlux factory, Egypt) fixed at the same distance.

Spectral irradiances at the plant level were measured with a Bentham spectroradiometer (Bentham, UK). Photon flux densities (PFD) of PAR (400- 700) were measured with a LI-185 A quantameter (Lambda, USA) and irradiances were calculated according to conversion factors given by McCree (1981).

Triplicate samples of seedlings were taken for determination of free amino acids, electrophoretic determination of proteins and for estimation of nucleic acids. All measurements were carried out on the 2nd, 4th and 6th days from the date of exposure to different wavelengths of light. 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 $*P < 0.05$ and all the analyses were made using the SPSS 13.0 for Windows software package (SPSS Inc., Chicago, IL, USA).

Determination of free amino acids

The method of extraction of amino acids was essentially that adopted by Yemm and Willis (1956). Glycine was estimated by the method of Muting and Kaiser (1963). The extract was deproteinized with ethanol/acetone mixture and then glycine was determined colorimetrically at 580 nm with ninhydrin. The method adopted for estimation of proline was essentially that described by Snell and Snell (1954).

Electrophoretic determination of proteins

Broad bean samples were subjected to protein analysis according to their molecular weights by denatured sodium dodecyl sulphate (SDS)-PAGE as described by Laemmli (1970). Protein bands were visualized by naked eye and the data were recorded on photographs.

Estimation of nucleic acids

Method of extraction

As adopted by Mohammed and El-sayed (1982), a known fresh weight of seedlings was crushed with acid-washed sand in a glass mortar using 4 ml of Tris-EDTA buffer (0.05 M Tris-EDTA + 0.1 M NaCl + 0.1 M EDTA, pH 8.0), 2 ml SDS and 1 ml of 1× standard saline citrate solution (0.015 M NaCl + 0.0015 M trisodium citrate, pH 7.0). The mixture was transferred into a measuring flask with 7 ml of chloroform: isoamyl alcohol (24:1). The contents were shaken for 10 min, and then centrifuged at 3000 g for 10 min at 4°C. The clear supernatant contained the nucleic acids.

Estimation of DNA

The method followed was based on the quantitative reaction of deoxysugar with diphenylamine reagent (Sadasivam and Manickam 1996). In a test tube, an aliquot of extract was followed by the addition of 6 ml of diphenylamine reagent (mixture of 5 g crystallized diphenylamine, 500 ml glacial acetic acid and 13.75 ml concentrated H_2SO_4). The contents were then mixed, heated in a boiling water bath for 10 min, then cooled and the absorbance of the developed blue solution read at 600 nm against a blank (H_2O instead of plant extract). Recovery of pure DNA added to broad bean extract was 97 %.

Estimation of RNA

The widely used reaction of ribose in RNA with orcinol is used here (Sadasivam and Manickam 1996). An aliquot of extract was pipetted into a tube, followed by the sequential addition of 6 ml of orcinol acid reagent (2 ml 10 % solution (w/v) $FeCl_3 \cdot 6H_2O$ + 400 ml of concentrated HCl) and 0.4 ml 6.0 % alcoholic orcinol (6 g orcinol in 100 ml 95 % ethanol). The tubes were first shaken, heated in a boiling water bath for 20 min and then allowed to cool. Absorbance was read at 600 nm against a blank prepared by adding all reactants plus water instead of the plant extract. Recovery of pure RNA added to the broad bean extract was 98 %.

RESULTS AND DISCUSSION

Changes in amino acid content

The contents of proline and glycine of the control as well as of the differently treated broad bean seedlings are illustrated in Figure 1A and B; a progressive significant increase above the control levels, throughout the duration of the experiment, being apparent in response to the various light radiations used. The following sequence of treatments (LL+UV-A > UV-A > LL+UV-C > LL > UV-C) and (HL > HL+UV-C > UV-C) for the dark- and light-germinated seedlings were respectively displayed with respect to percentage increase in both proline and glycine contents, calculated as percentage of control (Table 1).

In accordance with the above mentioned changes in amino acid contents of *Vicia faba* seedlings, as influenced by exposure to LL, HL, UV-A, UV-C, either alone or in combination, Broeckling *et al.* (2005) reported that exposure of *Medicago truncatula* cells to UV resulted in simultaneous accumulation of several amino acids including proline and glycine. Furthermore, Saleh *et al.* (2006) and Demir (2000) showed that increasing UV radiation doses induced a highly significant increase in the levels of proline in soybean and wheat. When cultivars Giza-35 and Giza-111 were irradiated with 3.2 kJ m^{-2} d^{-1}, the percentages of increase in proline content were 202 and 307 respectively (Saleh *et al.* 2006).

Proline metabolism is a typical mechanism of the biochemical adaptation in living organisms subjected to stress conditions (Yao and Liu 2007). In addition to its function as (1) an osmoprotectant for intracellular osmotic adjustments, (2) as a storage sink for carbon and nitrogen and (3) as a stabilizer for subcellular structures (plasma membranes and proteins), proline could also function as a free-radical scavenger (Chinnusamy *et al*. 2005). Though mainly involved in the water-stress syndrome, proline is reported to accumulate in the shoots of rice, mustard and mung bean seedlings exposed to UV radiation (Saradhi *et al*. 1995); this being supposed to protect plant cells against peroxidative processes.

In the present study on broad bean seedlings, enhanced UV-A, UV-C, LL and HL, either alone or in combination, significantly increased proline and glycine contents, throughout the experimental period, suggesting the important role of both amino acids in enhanced UV- and visible light-tolerance. The magnitude of increase appeared to depend on the set of experimental conditions under investigation. Thus, careful examination of Table 1 indicates greater accumulation of glycine than that of proline in broad bean seedlings grown in darkness under single irradiation stress (LL, UV-C or UV-A) and double

Table 1. Changes in the content as well as in the percentage change for the amino acids and nucleic acids, in response to treatment of broad bean seedlings with visible light and UV on the 6th day after treatment. Mean values are significantly different from control at *P ≤ 0.05.

Amino acids					
Treatments / Parameters		Glycine	% change	Proline	% change
Darkness	Control	160.4	-	194.4	-
	LL	218.6*	+36.28	219.7*	+13.01
	UV-C	176.6*	+10.10	209.1*	+7.56
	LL +UV-C	235.2*	+46.63	226.9*	+16.72
	UV-A	297.4*	+85.41	297.1*	+52.83
	LL + UV-A	315.3*	+96.57	308.6*	+58.74
Light	Control	113.30	-	14.80	-
	HL	166.00*	+46.51	36.10*	+143.92
	UV-C	121.00*	+6.80	23.00*	+55.41
	HL +UV-C	147.00*	+29.74	29.00*	+95.95

Nucleic acids					
Treatments / Parameters		DNA	% change	RNA	% change
Darkness	Control	51.9	-	27.70	-
	LL	47.9*	-7.71	21.3*	-23.10
	UV-C	50.1*	-3.47	22.1*	-20.22
	LL +UV-C	45.1*	-13.10	20.0*	-27.80
	UV-A	41.2*	-20.62	19.0*	-31.41
	LL + UV-A	39.2*	-24.47	19.7*	-28.88
Light	Control	51.20	-	24.90	-
	HL	43.60*	-14.84	17.20*	-30.92
	UV-C	49.80*	-2.73	22.10*	-11.25
	HL +UV-C	45.60*	-10.94	19.30*	-22.49

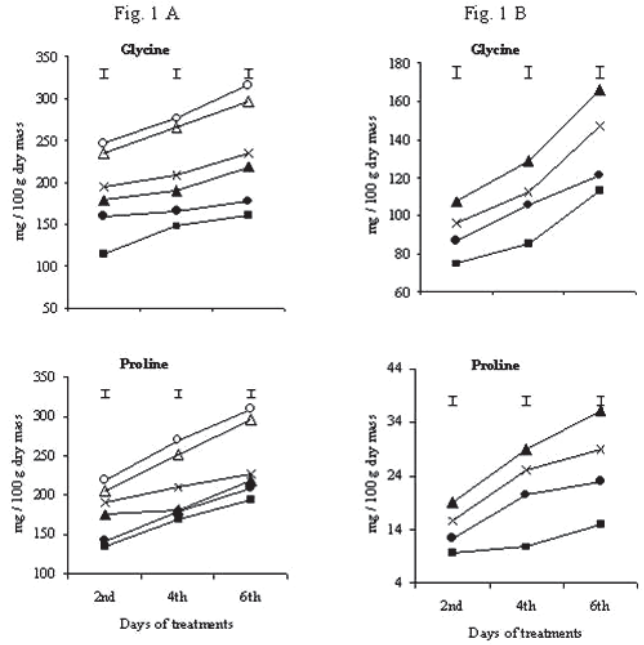


Fig. 1A. The effects of low visible light (LL) or UV radiations (UV-A and UV-C) on glycine and proline contents of *Vicia faba* seedlings germinated in darkness. (—■— Control; —▲— LL; —●— UV-C; —×— LL+UV-C; —Δ— UV-A; —o— LL+UV-A).Vertical bars represent the standard error (± S.E.).

Fig. 1B. The effects of high visible light (HL) or UV-C radiation on glycine and proline contents of *Vicia faba* seedlings germinated in ambient light (—■— Control; —▲— HL; —●— UV-C; —×— HL+UV-C). Vertical bars represent the standard error (± S.E.).

irradiation stress (LL+UV-C) and (LL+UV-A) conditions. An opposite situation was, however, observed under ambient light growth conditions; proline accumulation being more operative. Under this last condition, UV-C used in combination with HL appeared to nullify the accumulation effect, induced by high visible light treatment, on glycine and proline contents.

As stated by Stewart and Lee (1974) and Younis *et al.* (2009 a) the role of amino acids (e.g. phenylalanine, taurine, glycine, alanine and glutamic) cannot be ignored in stress phenomena. Waditee *et al.* (2005) working on *Synechococcus* and *Arabidopsis* and Younis *et al.* (2009 a and b) working on broad bean seedlings and lettuce plants stated that glycine seems to meet the requirements of a compound that can have a role in stress tolerance. In this context, Waditee *et al.* (2005) obtained results indicating that glycine is limiting for maximal salt-stress tolerance in plants. This may provide an ecological adaptation for broad bean seedlings by enhanced defence substance content under stress conditions (Yao and Liu 2007).

Changes in protein patterns

Protein banding patterns of the control as well as of the differently treated broad bean seedlings throughout the entire period of the experiment are shown up in Fig. 2 and 3 and the protein banding patterns of the marker and of the query proteins are recorded in Tables 2 and 3. Each protein band was treated as a unit character and scored as + (present) or – (absent). The total number of polypeptide molecules and the number of polymorphic bands observed in each treatment are given in Tables 2 and 3. Careful examination of Table 2 and Fig. 2 revealed the following main points:

a) Seven monomorphic bands (106, 91, 78, 34.7, 28.5, 16 and 14 kDa) were expressed in the control and the different treatments.

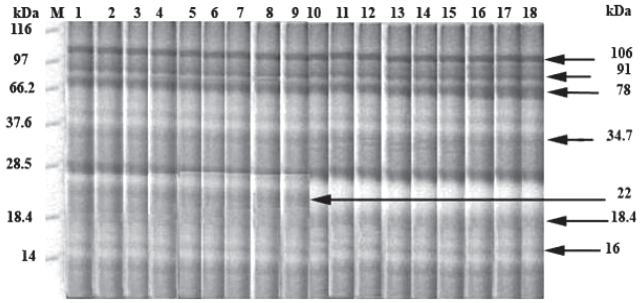


Fig. 2. Gel of the dark germinated seeds (A)

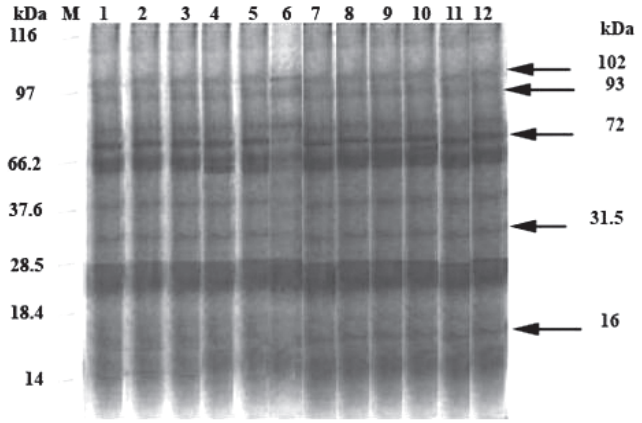


Fig. 3. Gel of the light germinated seeds (B)

Table 2. Analysis of protein profiles induced by LL, UV-A or UV-C radiations, either alone or in combination, in *Vicia faba* seedlings germinated in darkness (+; present; –; absent).

Band no.	Mol. wt. of marker	Day	2nd						4th						6th						Mol. wt. of bands
		Treatments	C	LL	UV-C	LL+ UV-C	UV-A	LL+ UV-A	C	LL	UV-C	LL+ UV-C	UV-A	LL+ UV-A	C	LL	UV-C	LL+ UV-C	UV-A	LL+ UV-A	
		Lane no. / Rf	1	4	7	10	13	16	2	5	8	11	14	17	3	6	9	12	15	18	
1	116	0.07																			
2		0.13	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	106
3	97	0.18																			
4		0.20	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	91
5		0.24	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	78
6	66.2	0.28																			
7	37.6	0.40																			
8		0.43	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	34.7
9	28.5	0.56	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	28.5
10		0.63	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	22
11	18.4	0.73	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	18.4
12		0.81	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16
13	14	0.87	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Total no. of bands			8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	
Monomorphic bands			7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	
Polymorphic bands			2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	
% of Polymorphism			25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	

b) 22 kDa protein was expressed only in control, LL and UV-C treatments and was absent in all other treatments.

c) 18.4 kDa polypeptide was expressed only in LL+UV-C, UV-A and LL+UV-A treatments and was not detected in all other treatments including the control seedlings.

Careful examination of Table 3 and Fig. 3 revealed the following main points:

a) Seven monomorphic bands (102, 93, 72, 66.2, 37.6, 31.5 and 28.5 kDa) were expressed in the control and the variously treated seedlings.

b) 16 kDa protein was expressed only in UV-C and HL+UV-C treatments and was not detected in control and HL treatment.

These polypeptides may be normal or Late Embryogenesis Abundant (LEA)-stress proteins which appeared in response to exposure of dark- and ambient light-germinated broad bean seedlings to LL, HL, UV-A and UV-C, either alone or in combination, for 6 consecutive daily-hours throughout the entire period of the experiment. In support of these results, Santos *et al*. (1993) stated that under various stress conditions, plants may induce specific changes in protein synthesis that enhance them to cope with such stress. Bassman (2004) reported that enhanced UV-B radiation is strongly

Table 3. Analysis of protein profiles induced by HL or UV-C radiation, either alone or in combination, in *Vicia faba* seedlings germinated in ambient light (+ present; – absent).

Band no.	Mol. wt. of marker	Day	2nd				4th				6th				Mol. wt. of bands
		Treatments	C	HL	UV-C	HL+ UV-C	C	HL	UV-C	HLL+ UV-C	C	HL	UV-C	HL+ UV-C	
		Lane no.	1	4	7	10	2	5	8	11	3	6	9	12	
		Rf													
1	116	0.07													
2		0.13	+	+	+	+	+	+	+	+	+	+	+	+	102
3	97	0.18													
4		0.20	+	+	+	+	+	+	+	+	+	+	+	+	93
5		0.24	+	+	+	+	+	+	+	+	+	+	+	+	72
6	66.2	0.28	+	+	+	+	+	+	+	+	+	+	+	+	66.2
7	37.6	0.40	+	+	+	+	+	+	+	+	+	+	+	+	37.6
8		0.50	+	+	+	+	+	+	+	+	+	+	+	+	31.5
9	28.5	0.56	+	+	+	+	+	+	+	+	+	+	+	+	28.5
10		0.63													
11	18.4	0.73													
12		0.81	-	-	+	+	-	-	+	+	-	-	+	+	16
13	14	0.87													
Total no. of bands			7	7	8	8	7	7	8	8	7	7	8	8	
Monomorphic bands			6	6	7	7	6	6	7	7	6	6	7	7	
Polymorphic bands			1	1	1	1	1	1	1	1	1	1	1	1	
% of Polymorphism			143	14.3	12.5	12.5	14.3	14.3	12.5	12.5	14.3	14.3	12.5	12.5	

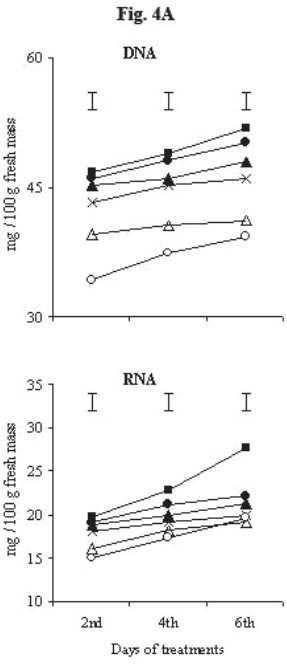
absorbed by proteins, nucleic acids and other macromolecules, which causes conformational changes in their structure. Furthermore, he stated that proteins that have strong absorption spectrum at about 260 nm as well as at higher wavelengths of the UV-region due to absorption by aromatic amino acids can be targets of UV- radiation.

Furthermore, Temprio *et al.* (2008) noted that acclimation of photosynthetic light reactions to high light requires both structural and functional dynamics, which occur in the time scale from seconds to several days. In high light (HL), feedback de-excitation (qE) is a well known photoprotective mechanism that dissipates excess excitation energy in the light-harvesting protein of photosystem II (LHCP-PSII). The xanthophylls zeaxanthin and lutein function in qE, but also have roles as antioxidants. Temprio *et al.* (2008) by using a reversed genetic approach, identified a chloroplast targeted protease FtsH6 being responsible for the degradation of LHCP-PSII, which is a regulator under various environmental conditions, e.g. light stress, to prevent photochemical damage to the reaction centre.

Thus, SDS-PAGE protein patterns, in the present study, showed a disturbed pattern of protein bands in response to HL, LL, UV-A and UV-C, either alone or in combination; the appearance of some protein bands and the disappearance of other ones being operative. The up-regulation of some proteins in association with down-regulation of others suggest a protective role under the present set of light conditions. Nevertheless, the identification of these types of proteins requires amino acid sequencing awaits further investigation.

Changes in nucleic acid contents

Figs. 4 A and B show the contents of DNA and RNA in the control as well as in the variously treated broad bean seedlings during the experimental period in darkness and in ambient light; the nucleotide levels detected appeared to increase with an increase in the duration of germination period. As compared with control, RNA and DNA contents in the variously treated seedlings showed a significant decrease throughout the duration of the experiment. Thus, 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 light germinated seeds were displayed with respect to decrease in RNA and DNA contents of broad bean seedlings; calculated as percentage of control (Table 1). Thus, it is evident that treatment with UV-A was more effective than the treatment with UV-C in reducing the DNA and RNA contents, in complete darkness. When UV-A or UV-C was used in combination with LL additive diminution was operative. Under ambient light conditions, treatment with HL induced the highest reduction in nucleotide contents, but combination of UV-C with HL appeared to counteract the high diminution induced by HL.



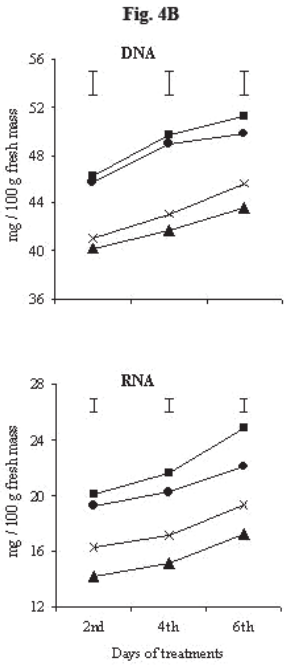


Fig. 4A. The effects of low visible light (LL) or UV radiations (UV-A and UV-C) on nucleic acid contents of *Vicia faba* seedlings germinated in darkness. (—■— Control; —▲— LL; —●— UV-C; —×— LL+UV-C; —Δ— UV-A; —o— LL+UV-A). Vertical bars represent the standard error (± S.E.).

Fig. 4B. The effects of high visible light (HL) or UV-C radiation on nucleic acid contents of *Vicia faba* seedlings germinated in ambient visible light. (—■— Control; —▲— HL; —●— UV-C; —×— HL+UV-C). Vertical bars represent the standard error (± S.E.).

In support of the present results, Hidema and Kumagai (2006) and Saleh *et al*. (2006) demonstrated a significant decrease in both DNA and RNA of *Oryza sativa* seedlings and soybean cultivars exposed to UV-A and UV-B radiations. Furthermore, in certain tissues, UV radiation has been shown to interfere with processes such as transcription and replication, resulting in reduction of RNA synthesis, arrest of cell cycle progression, and apoptosis (Sancar *et al*. 2004; Hidema and Kumagai 2006).

It is possible that some of the morphological responses are mediated, at least in part, by UV-induced damage to DNA (Jordan 2002). Absorption of DNA by UV can result in genome alterations and these changes can impact cellular metabolism (Barnes *et al*. 2005). Action spectra for DNA damage typically peak at wavelengths below 300 nm, but appreciable damage can be induced by wavelengths well into the UV-A (Quaite *et al*. 1992).

From the above mentioned expression of results and discussions, it could be concluded that broad bean seedlings grown in the presence of visible light and UV radiation were able to produce significant amounts of free amino acids which act, in part, as an osmoticum in the cell against stress conditions for the maintenance of normal metabolic processes and, in part, as storage compounds for nitrogen. Furthermore, the prevailing light conditions reduced DNA and RNA contents which appeared in association with marked changes in protein patterns that provide information concerning the structural genes and their regulatory systems that control varied biosynthetic pathways.

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