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## Phenylpropanoid and Isopropanoid Enhance Tolerance to Increased Levels of UV<sub>A+B</sub> Radiation in Three Cultivars of Soybean (*Glycine max*) Seedlings

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**Abstract:** The present study was carried out to investigate the effect of different ultraviolet radiation doses on the growth and UV-screening compounds of three cultivars of soybean (*Glycine max* L.) (Giza-22, Giza-35 and Giza-111). The seeds were grown in plastic pots equally filled with a mixture of pre-sieved sandy loam soil, beat-moss and vermiculite (2:1:1) for two weeks. The planted pots were divided into four groups and exposed to white light (1100 Lux) (control), 3.2, 6.4 and 12.8 kJ m<sup>-2</sup> d<sup>-1</sup> UV<sub>A+B</sub> radiation, respectively for 30 days and then harvested. The results indicated that increasing ultraviolet radiation doses significantly induced decrease in shoot biomass and leaf area in Giza-22 and Giza-35 cultivars. On the other hand, ultraviolet radiations significantly induced accumulation in the root biomass of soybean cultivar Giza-111. While, by increasing ultraviolet radiation, the plant height was significantly increased in the three studied cultivars. Increasing UV<sub>A+B</sub> radiation doses induced a significant increasing in Phenylalanine Ammonia Lyase (PAL) activity, total phenolic compounds UV-absorbing compounds, anthocyanins content and carotenoids in the three soybean cultivars under investigation. The data also showed that Giza-111 cultivar was most tolerant to UV<sub>A+B</sub> radiation followed by Giza-22, while Giza-35 was the most sensitive cultivar to enhanced UV<sub>A+B</sub> radiation.

**Key words:** Anthocyanin, phenylalanine ammonia lyase, soybean plant, total phenolic compounds, ultraviolet radiation, UV-absorbing compounds,

### INTRODUCTION

The increase in solar ultraviolet radiation reaching the Earth's surface as a consequence of depletion of the ozone layer raises concerns since it may have deleterious effects on both animals and plants. Enhanced UV-B radiation can alter plant growth and development (Caldwell *et al.*, 1989). Since the evolutionary process is not perfect and plants are not necessarily best adapted for the current conditions they grow in, some plant species are less resistant to UV-radiation than other species. UV-radiation sensitivity can result in a decrease in leaf area and biomass and changes to flowering (Caldwell *et al.*, 1995). Tevini and Teramura (1989) noticed that UV-B radiation reduced plant height and leaf area in sensitive species and cultivars of cucumber. In addition, Teramura *et al.* (1991) found that, approximately one third of all cultivars tested of rice (*Oryza sativa* L.) showed a significant decrease in total biomass and leaf area with increased UV-B radiation. Also, the height, biomass and leaves area of the stems of silver birch saplings tended to be reduced after long-term exposure to UV-B (Tegelberg *et al.*, 2001; Tegelberg, 2002). Moreover,

Donald *et al.* (1997) investigated the inhibitory effects of UV-A and UV-B radiation on growth of lettuce. Their results indicated that plants grown in the absence of UV-B or UV-A radiation had greater fresh and dry weight than those grown under ambient UV-B and UV-A. Excluding ambient solar UV-B reduced the UV absorbance of flavonoids and related compounds and reduced the concentration of anthocyanin in the leaves which. There was a significant reduction in biomass and plant height of various plant species with increasing UV-B radiation, also measurements of stem length, branching, leaf thickness, flowering and total UV-B absorbing compounds of sub-arctic plants were affected significantly by ambient UV-B (Callaghan *et al.*, 2004; Donald, 2004).

There are many protective mechanisms that plants use to both tolerate and avoid UV-damage. The accumulation of UV-absorbing compounds can aid in avoidance of UV penetration to sensitive layers within the leaf. These compounds accumulate in the upper epidermal layers of the leaves of many higher plants following irradiance with UV-B (Schnitzler *et al.*, 1997). UV-screening compounds are thought to constitute phenyl-propanoid including flavones, flavonols,

cinnamoyl esters and anthocyanin that provide a UV-A and UV-B screens (Cockell and Knowland, 1999). In higher plants, flavonoids and other Phenylpropanoid derivatives that accumulate in large quantities in the vacuoles of epidermal cells effectively attenuate the UV component of sunlight with minimal effects on the visible region of the spectrum (Mazza *et al.*, 2000).

Phenylalanine ammonia-lyase (PAL) catalyses the first committed step of the core pathway of general phenylpropanoids metabolism. Branch pathways lead to the synthesis of compounds that have diverse functions in plants, notably in defense, such as cell wall strengthening and repair (e.g., lignin and suberin), antimicrobial activity (e.g., furanocoumarin, pterocarpan and isoflavonoid phytoalexins) and as signalling compounds such as salicylic acid (Hammerschmidt, 1999). The resulting phenolics are often converted into more reactive species by phenol oxidases and peroxidases (Mayer and Harel, 1979; Heath, 1980). Middleton and Teramura (1993) studied the role of flavonol glycosides and carotenoids in protecting soybean from ultraviolet-B damage. This study suggests that both Carotenoids and flavonoids may be involved in plant UV-B photo-protection but only carotenoids are directly linked to photo-protection of photosynthetic function.

Anthocyanin accumulates in young, expanding foliage of various plant species in response to ultraviolet (UV) radiation exposure (Close and Beadle, 2003). Exposure to ultraviolet light promotes the production of foliar anthocyanin (Lindoo and Caldwell, 1978) and it has been hypothesized that anthocyanin provides a UV sunscreen (Lee and Lowry, 1980). UV-B responsive anthocyanin production in a rice cultivar was associated with a specific phase of Phenylalanine Ammonia Lyase (PAL) biosynthesis (Reddy *et al.*, 1994). They found that the anthocyanin induction in rice seedlings is mediated exclusively by the ultraviolet (UV) component of sunlight.

Middleton and Teramura (1993) noticed that, Plants with higher levels of total flavonoids, not specific flavonol glycosides, were more UV-B tolerant as determined by growth, pigment and gas-exchange variables. UV-B radiation increased photosynthetic pigment content, along with UV-B-absorbing compounds, but only the former (especially carotenoids) was related to total biomass and to photosynthetic efficiency. They suggested that both carotenoids and flavonoids may be involved in plant UV-B photoprotection, but only carotenoids are directly linked to photoprotection of photosynthetic function. Their results additionally showed the importance of UV-A control in UV-B experiments conducted using artificial lamps and filters.

The main objective of this research was to study the effect of ultraviolet radiation on the production of UV-protective compounds of three cultivars of soybean (*Glycine max* L.) cultivated in Egypt (Giza-22, Giza-35 and Giza-111) to avoid UV-damage.

## MATERIALS AND METHODS

**Plant material and plantation:** Soybean (*Glycine max*) seeds of three cultivars were selected (Giza-22, Giza-35 and Giza-111). Seeds were sterilized with 2.5% sodium hypochlorite for 15 min and washed with distilled water. These seeds were then grown in plastic pots (25 cm in height and 20 cm in diameter) which equally filled with a mixture of pre-sieved sandy loam soil, beat-moss and vermiculite (2:1:1). All pots were gently watered up to saturation and kept in the open air and irrigated regularly every two days until UV-treatments.

**Irradiation system and growth conditions:** After 15 days from seed soaking, the planted pots (each pot contains 4 plants) from each cultivar were randomly divided into equal groups (four groups). Plants of first groups exposed to white light (1100 Lux) (control). The second group exposed to UV<sub>A+B</sub> radiation for 2.5 h/day (3.2 kJ m<sup>-2</sup> d<sup>-1</sup>). The third group exposed to UV<sub>A+B</sub> radiation for 5 h/day (6.4 kJ m<sup>-2</sup> d<sup>-1</sup>). The fourth group exposed to UV<sub>A+B</sub> radiation for 10 h/day (12.8 kJ m<sup>-2</sup> d<sup>-1</sup>). Seedlings were exposed to (14:10) light: dark periods allow the experimental period. Irradiation treatments were applied using OSRAM SYLVANIA ULTRAVIOLET 350 BL F40 lamp. Timers were set to automatically turn irradiation lamps on at midday time as possible. Radiation doses and radiation power emitted from were calculated according distance between lamp axis and soybean as presented in the lamp instruction manual (Gilbert, 1996). Distance between lamp and upper trifoliolate leaves of soybean were set to 45 cm and were periodically monitored and reset as soybean grows. Plants were exposed to UV-radiation for 30 days and were harvested after 45 days of germination.

**Growth parameters:** Shoot and root biomass, biomass allocation, leaf area and plant height were estimated.

### **Biomass and biomass allocation**

**Shoot and root biomass, shoot/root ratio:** The fresh weight of shoot and root systems were weighed and recorded as root biomass (Chapman, 1976).

**Estimation of Phenylalanine Ammonia Lyase activity (PAL):** Phenylalanine ammonia lyase activity was determined spectrophotometrically by following the

formation of *trans*-cinnamic acid which exhibit an increase in absorbance at 290 nm (Sadasivam and Manickam, 1992). PAL activity was expressed as micromoles *trans*-cinnamic acid formed per g fresh weight.

**Determination of total phenolic compounds:** Phenolic compounds were estimated by Malik and Singh (1980) in which phenols was allowed to react with phosphomolybdic acid in Folin-Ciocalteu reagent in alkaline medium and produce blue colored complex (molybdenum blue). Standard curve were prepared to calculate the concentration of phenolic compounds per 100 g fresh weight.

**Determination of UV-absorbing compounds:** Accumulation of UV-absorbing compounds was estimated on the basis of absorbance of methanol: HCl: H<sub>2</sub>O (90:1:1 volume) extracts at 300 nm (Flower-Ellis and Olsson 1993). Absorbance was expressed on leaf area basis.

**Estimation of leaf flavonoids:** Fresh soybean leaves (0.5 g) were extracted in a solution of methanol, water and 2 M HCl (79:20:1 volume) as described by Mirecki and Teramura (1984). Absorbance of the extract was scanned from 200 to 700 nm using scanning UV/Visible Spectrophotometer. Absorbance readings were normalized to leaf sample fresh weight. Leaf flavonoids conc. (A/g) = Absorbance/sample fresh weight.

**Estimation of anthocyanin content:** Fresh soybean leaves were homogenized with a mixture of 1% HCl (v/v) in methanol and kept at 4°C overnight (Lange *et al.*, 1970). After centrifugation, the amount of anthocyanin in the resulting extract was quantified spectrophotometrically. The values are reported as  $A_{535} \cdot 0.25 (A_{650})/g$  fresh weights.

**Estimation of carotenoids content:** The carotenoids were determined by the spectrophotometrically method recommended by Metzner *et al.* (1965). A known fresh weight of leaves was homogenized in 85% aqueous acetone. The homogenate was centrifuged and measured the absorbance against a blank at 3 wavelengths of  $A_{452.5}$ ,  $A_{644}$  and  $A_{663}$  (nm) using Spekol spectrophotometer VEB Carl Zeiss.

**Statistical analysis:** Differences with each treatment were statistically using one way ANOVA. On the other hand, difference between ultraviolet radiations treatments and control treatment were performed using two-way-

ANOVA. Differences in the mean values were regarded as significant at the levels of  $p < 0.05^*$  and  $p < 0.01^{**}$ . Pearson Rank Correlation test and simple linear regression test were used between variables measured and ultraviolet radiation doses. Statistical analyses were performed by using SPSS (Version 9.00),

## RESULTS

**Shoot biomass:** The reduction of shoot biomass was negatively correlated with elevated UV radiation doses in Giza-22 and Giza-35 cultivars. On the other hand, the lowest UV-dose ( $3.2 \text{ kJ m}^{-2} \text{ d}^{-1}$ ) induced a significant increase in shoot biomass of Giza-111 cultivar and a highly significant decrease (-65.5% reduction) at  $12.8 \text{ kJ m}^{-2} \text{ d}^{-1}$  UV dose as compared with control as shown in Fig. 1A.

**Root biomass:** Ultraviolet radiations significantly induced accumulation in the root biomass of soybean cultivar Giza-111, the plant showed an increase by 12.8, 214.2 and 89.8% of the root biomass as compared to control when subjected to 3.2, 6.4 and  $12.8 \text{ kJ m}^{-2} \text{ d}^{-1}$ , respectively as shown in Fig. 1B. Low doses of UV radiation significantly induced a reduction in root biomass (g) in Giza-22 and Giza-35 cultivars. On the other hand, high ultraviolet radiation dose ( $12.8 \text{ kJ m}^{-2} \text{ d}^{-1}$ ) induced accumulation of root biomass by 15 and 316% in Giza-22 and Giza-111 cultivars, respectively as shown in Fig. 1B.

**Biomass allocation (Shoot/Root ratio):** Biomass allocation was estimated based on the shoot/root ratio. By increasing enhanced ultraviolet radiations, the shoot/root ratio was first increased at low ultraviolet dose and then decreased significantly at the three tested soybean cultivars. The highest reduction rate of 81.1% of shoot/root ratio was recorded at soybean cultivar Giza-111 at UV-dose of  $12.8 \text{ kJ m}^{-2} \text{ d}^{-1}$ . In the meantime, at the highest enhanced UV-radiation dose of  $12.8 \text{ kJ m}^{-2} \text{ d}^{-1}$ , reduction values of 41.3 and 31.4% were recorded at soybean cultivars Giza-22 and Giza-35, respectively Fig. 1C.

**Leaf area (cm<sup>2</sup>):** Increasing ultraviolet radiation doses on different soybean cultivars, the upper leaf area (foliage leaves) was significantly decreased ( $F = 17.69$ ,  $p = 0.000^{***}$ ). Figure 1D showed that, Giza-111 was more sensitive to UV-radiations than the other two cultivars. The present data showed that, leaf area was significantly decreased with increasing UV<sub>A+B</sub> radiations at  $12.8 \text{ kJ m}^{-2} \text{ d}^{-1}$ . The leaf area reduced by 55.2, 47.9 and 47.9% in Giza-111, Giza-35, Giza-22, respectively.

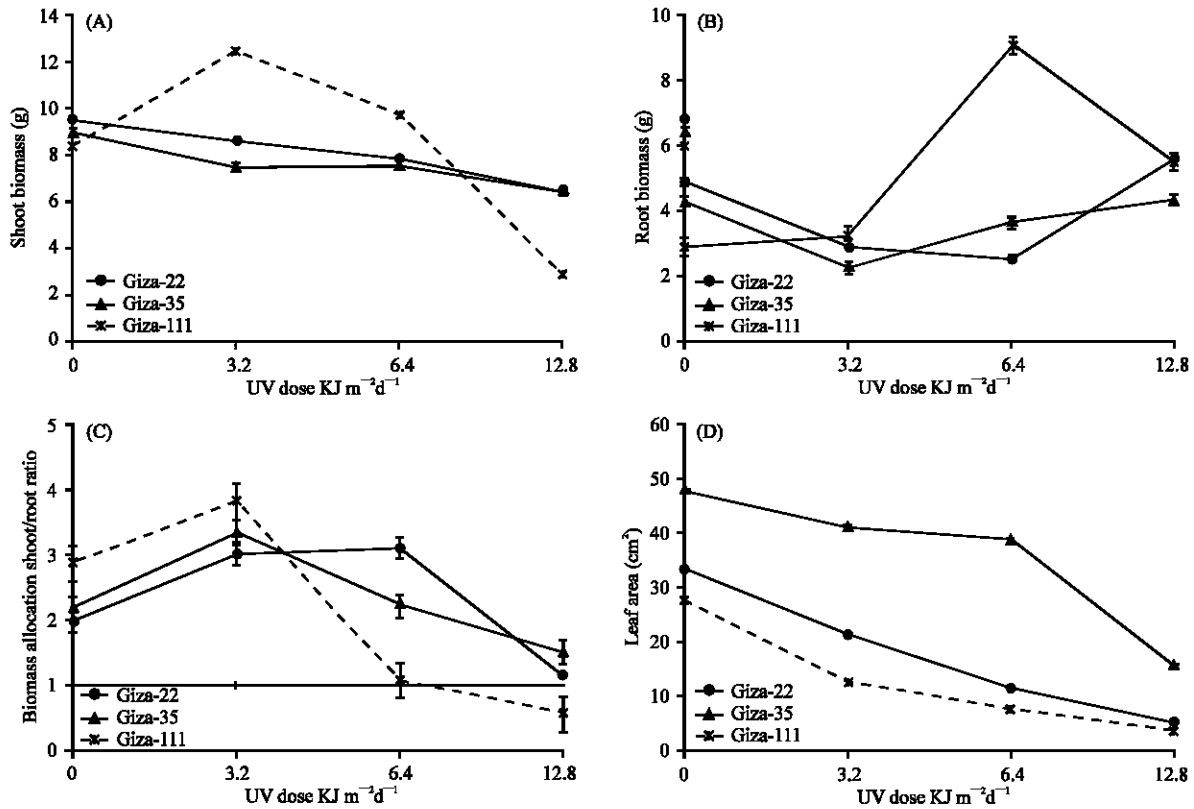


Fig. 1: Effect of different doses of UV<sub>A+B</sub> radiation (kJ m<sup>-2</sup> d<sup>-1</sup>) on shoot biomass (A), root biomass (B), biomass allocation (C) and leaf area (D) of three *Glycine max* L. cultivars

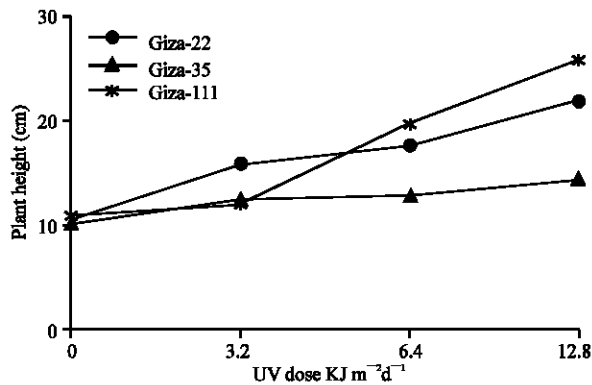


Fig. 2: Effect of different doses of UV<sub>A+B</sub> radiation (kJ m<sup>-2</sup> d<sup>-1</sup>) on plant height (cm) of three *Glycine max* L. cultivars

**Plant height:** The data recorded in Fig. 2 showed that, generally, by increasing enhanced ultraviolet radiation the plant height (cm) was statistically significantly increased in the three studied cultivars. Two-Way-ANOVA assessed that soybean cultivar Giza-22, Giza-35 and

Giza-111 showed significant variation in increasing plant height (cm<sup>2</sup>) (ANOVA;  $F = 58.37, p = 0.000^{***}$ ). At high dose of UV radiation (12.8 kJ m<sup>-2</sup> d<sup>-1</sup>) the plant height significantly increased by 138, 106, 40.9% for Giza-111, Giza-22 and Giza-35, respectively as compared to the control. It was found that plant height (cm) significantly increased by ultraviolet radiations even at low supplemented dose of UV<sub>A+B</sub> radiations, especially for Giza-22 and Giza-111. However, Giza-35 showed a limited increase in the plant height in response to enhanced ultraviolet radiations. Significances of each treatment against control were tested using ANOVA ( $F = 130.07, p = 0.000^{***}$ ) and least significance statistic test Fig. 2.

**Phenylalanine ammonia-lyase activity (PAL):** The data of Table 1 showed that increasing UV radiation doses induced a significant increasing in phenylalanine ammonia lyase (PAL) activity in the three soybean cultivars; Giza-22, Giza-35 and Giza-111 ( $r = 0.94, 0.57, 0.97; p = 0.000^{***}, 0.052^*, 0.000^{***}$ , respectively). Soybean cultivar, Giza-111 showed the highest increase of 152% in PAL activity at

Table 1: Effect of different doses of UV<sub>A+B</sub> radiation (KJ m<sup>-2</sup> d<sup>-1</sup>) on Phenylalanine-Ammonia Lyase (PAL) activity (nmole/sec/g-FW) of three *Glycine max* L. cultivars

UV dose (KJ m <sup>-2</sup> d <sup>-1</sup> )	Giza-22		Giza-35		Giza-111	
	Mean±SE (nmol/s/g)	LSD (Sig)	Mean±SE (nmol/s/g)	LSD (Sig)	Mean±SE (nmol/s/g)	LSD (Sig)
0	8.4±0.37	-	9.9123±0.00	-	9.0826±0.00	-
3.2	11.137±0.28	0.015*	13.207±0.00	0.000***	12.812±0.00	0.000***
6.4	12.125±0.10	0.003**	12.444±0.00	0.000***	13.709±0.17	0.000***
12.8	16.0±1.15	0.000***	12.633±0.05	0.000***	22.902±0.30	0.000***
F-ratio	22.19		3484.00		1147.37	
p-value	0.0002***		0.000***		0.000***	
Two-way ANOVA						
Among cultivars	F-ratio	67.36	Among ultraviolet treatment	F-ratio	235.13	
	p-value	0.000***		p-value	0.000***	

\* Significant at p<0.05, \*\* highly significant at p<0.01, \*\*\* Very high significant at p<0.001

Table 2: Effect of different doses of UV<sub>A+B</sub> radiation (kJ m<sup>-2</sup> d<sup>-1</sup>) on total phenolic compounds content (mg/100 g) of three soybean cultivars

UV dose (KJ m <sup>-2</sup> d <sup>-1</sup> )	Giza-22		Giza-35		Giza-111	
	Mean±SE (mg/100 g)	LSD (Sig)	Mean±SE (mg/100 g)	LSD (Sig)	Mean±SE (mg/100 g)	LSD (Sig)
0	6.8122±0.11	-	6.7686±0	-	6.2021±0.00	-
3.2	7.5212±0.25	0.000***	9.0185±0	0.000***	8.7486±0.0	0.000***
6.4	8.2792±0.07	0.000***	8.4974±0	0.000***	9.3614±0.11	0.000***
12.8	8.8047±0.12	0.000***	8.6262±0.03	0.000***	10.485±0.19	0.000***
F-ratio	33.54		3520.16		276.55	
p-value	0.000***		0.000***		0.000***	
Two-way ANOVA						
Among cultivars	F-ratio	61.77	Among ultraviolet treatment	F-ratio	353.33	
	p-value	0.000***		p-value	0.000***	

\* Significant at p<0.05, \*\* highly significant at p<0.01, \*\*\* Very high significant at p<0.001

enhanced ultraviolet radiation dose of 12.8 kJ m<sup>-2</sup> d<sup>-1</sup> from control samples (Table 1). Two-Way-Analysis of Variance proved that there was highly significant variation between cultivars (F = 67.36, p = 0.000\*\*\*) and among ultraviolet radiation treatments (F = 235.1, p = 0.000\*\*\*).

**Total phenolic compounds content:** The data in Table 2 showed that increasing UV radiation doses induced a significant increasing in total phenolic compounds in the three soybean cultivars; Giza-22, Giza-35 and Giza-111 (r = 0.93, 0.57 and 0.91; p = 0.000\*\*\*, 0.052\* and 0.000\*\*\*, respectively). Soybean cultivar Giza-111 showed the highest increase in total phenolic compounds content ultraviolet radiation dose of 12.8 kJ m<sup>-2</sup> d<sup>-1</sup> by 69.1% from control samples (Table 2). Least significant difference statistics test revealed that there was a significant difference in the phenolic compounds accumulation between different UV-treatments and the control for cultivars Giza-22, Giza-35 and Giza-111 (Table 2). Two-Way-ANOVA which revealed that there was a highly significant variation among cultivars in total phenolic compounds content (F = 61.7, p = 0.000\*\*\*) and between ultraviolet radiation treatments (F = 353.3, p = 0.000\*\*\*).

**UV-absorbing compounds:** The enhanced supplemental doses of ultraviolet radiations induced a significant accumulation in UV-absorbing compounds in soybean cultivars Giza-111 and non-significant accumulation on Giza-22 and Giza-35 (r = 0.57, 0.15, 0.03; p = 0.000\*\*\*, 0.399, 0.861, respectively). The variation in mean UV-absorbing compounds among the three cultivars following the exposure to enhanced levels of ultraviolet radiations were tested using Two-way-ANOVA which revealed that there was a highly significant variation in foliage leaves UV-absorbing compounds among cultivars and among ultraviolet radiation treatments (F = 10.80, p = 0.000\*\*\*, F = 3.76, p = 0.014\*). The foliage leaf UV-absorbing compounds of the three studied cultivar Giza-22 exposed to enhanced doses of ultraviolet radiations were totally decreased by about-55% from the control one (Table 3). However, soybean cultivars, Giza-22 and Giza-111 were totally increased by about 47.9 and 171%, respectively. In the meantime, soybean cultivar Giza-111 showed the highest increase in UV-absorbing compounds enhanced ultraviolet radiation dose of 12.8 kJ m<sup>-2</sup> d<sup>-1</sup> by 73.4% from controlled samples (Table 3).

Table 3: UV-absorbing compounds of the three soybean cultivars Giza-22, Giza-35 and Giza-111 under different ultraviolet radiation dose

Cultivars	UV-dose (KJ m <sup>-2</sup> d <sup>-1</sup> )	UV-Absorbing compounds (Absorbance/g)									
		A <sub>280</sub>	A <sub>290</sub>	A <sub>300</sub>	A <sub>310</sub>	A <sub>320</sub>	A <sub>330</sub>	A <sub>340</sub>	A <sub>350</sub>	A <sub>total</sub>	A <sub>mean</sub>
Giza-22	0.0	5.35	4.72	4.30	3.98	3.99	3.14	3.29	3.03	31.80	3.98
	3.2	5.12	4.58	4.06	3.70	3.59	2.92	2.71	2.47	29.17	3.65
	6.4	3.36	2.89	2.60	2.37	2.36	2.13	1.96	1.78	19.46	2.43
Giza-35	12.8	4.84	4.32	3.99	3.76	3.81	2.92	2.97	2.68	29.28	3.66
	0.0	5.06	4.48	4.03	3.67	3.64	2.89	2.90	2.58	29.26	3.66
	3.2	6.19	5.93	5.73	5.42	5.44	3.26	3.48	3.46	38.90	4.86
Giza-111	6.5	5.09	4.57	4.14	3.82	3.83	2.87	2.81	2.63	29.76	3.72
	12.8	5.53	5.06	4.66	4.35	4.33	3.03	3.13	3.05	33.13	4.14
	0.0	2.36	3.11	2.10	2.25	2.20	1.22	2.17	1.98	17.39	2.17
Giza-111	3.2	4.77	4.13	2.60	3.65	2.80	1.12	2.10	2.85	23.72	2.97
	6.6	3.88	3.38	3.00	2.66	3.98	3.76	3.60	3.91	28.17	3.52
	12.8	4.09	3.62	3.27	2.99	5.20	2.47	3.55	4.95	30.15	3.77

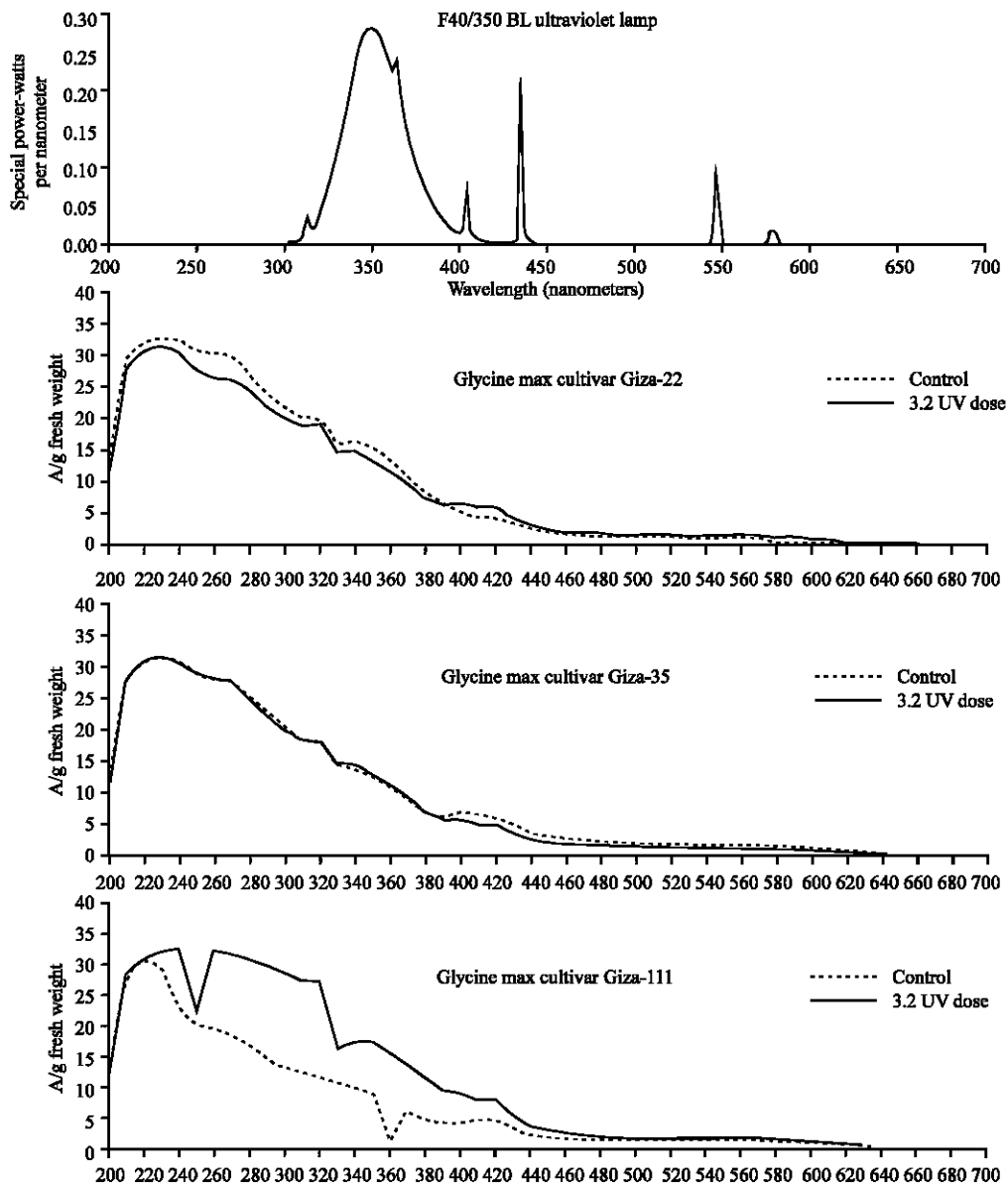


Fig. 3: Showing total foliage leaf flavonoids from 200 to 700 nm wavelengths, of plants irradiated with 3.2 kJ m<sup>-2</sup> d<sup>-1</sup>

**Total flavonoids content:** Total foliage leaf flavonoids content were estimated by scanning UV/visible spectrophotometer. Graphs obtained from leaf flavonoids analysis of the three cultivars were presented in Fig. 3-5 for 3.2, 6.4 and 12.8 kJ m<sup>-2</sup> d<sup>-1</sup>, respectively. Active UV lamp spectra were presented to allow comparisons with

applied wavelengths. Levels of foliage leaf flavonoids content in control sample were presented with treated one to allow comparisons. From the three figures of foliage leaf flavonoid contents it was clearly that lamp poses several spectral peaks (in term of spectral power of each wavelength) and these peaks were,  $\lambda_{320}$ ,  $\lambda_{350}$ ,  $\lambda_{370}$ ,  $\lambda_{420}$ ,  $\lambda_{440}$ ,

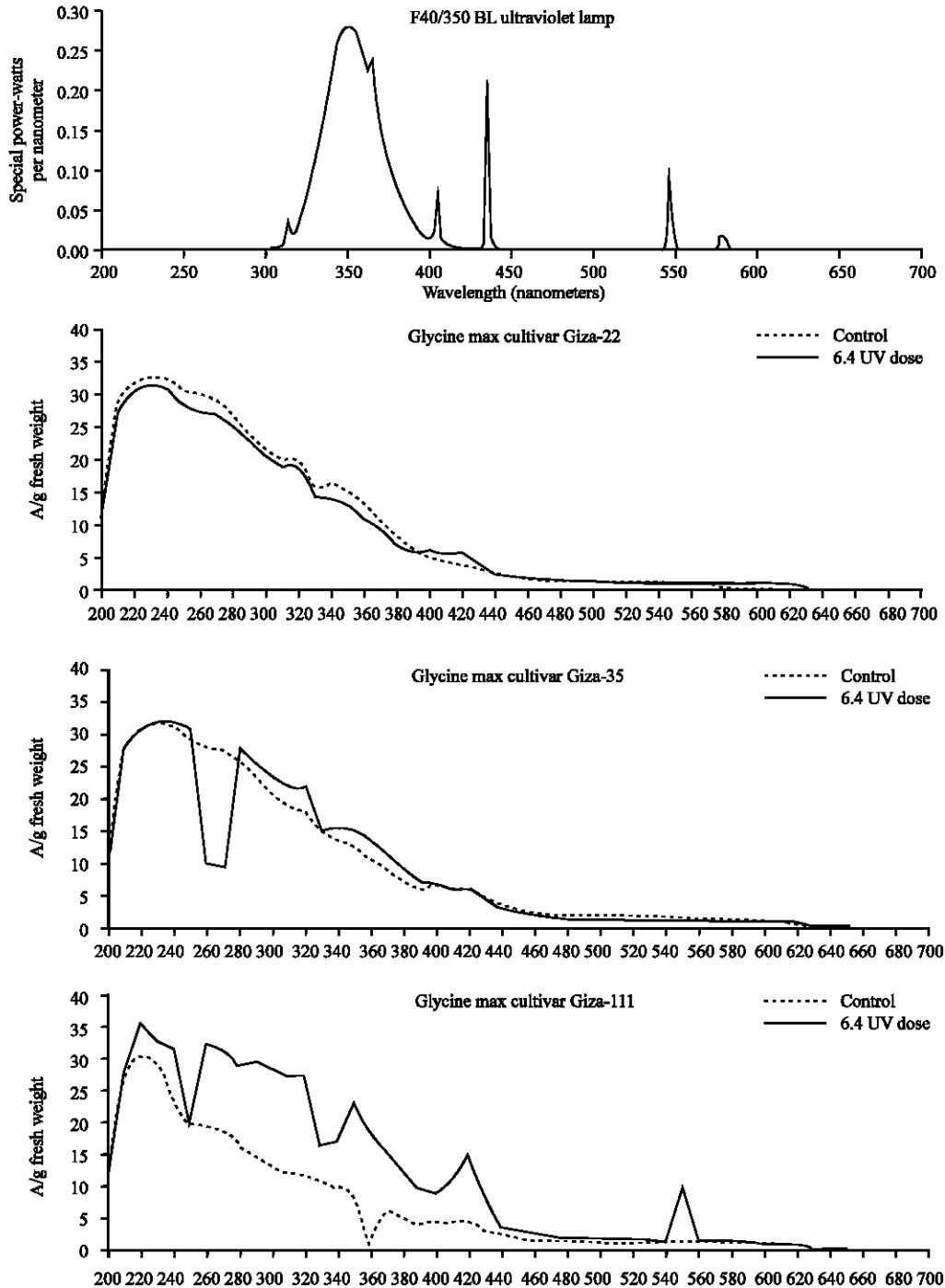


Fig. 4: Showing total foliage leaf flavonoids from 200 to 700 nm wavelengths, of plants irradiated with 6.4 kJ m<sup>-2</sup> d<sup>-1</sup>



$\lambda_{350}$  and  $\lambda_{580}$ . The maximum spectral power was at 350 nm (i.e.,  $\lambda_{max} = 350$  nm)

For both cultivars Giza-22 and Giza-35; an increase in flavonoids peaks were lying between  $\lambda_{240}$  to  $\lambda_{400}$ , compared to the control flavonoids concentration. However, the change in total leaf flavonoids content in

3.2, or 6.4, or 12.8  $\text{kJ m}^{-2} \text{d}^{-1}$  showed a very limited increase after exposure to enhanced ultraviolet radiations.

On the other hand, soybean cultivar Giza-111, showed an absolutely different response to enhanced ultraviolet radiations as follow: at enhanced UV radiation dose of 3.2  $\text{kJ m}^{-2} \text{d}^{-1}$ , remarked increase in total leaf

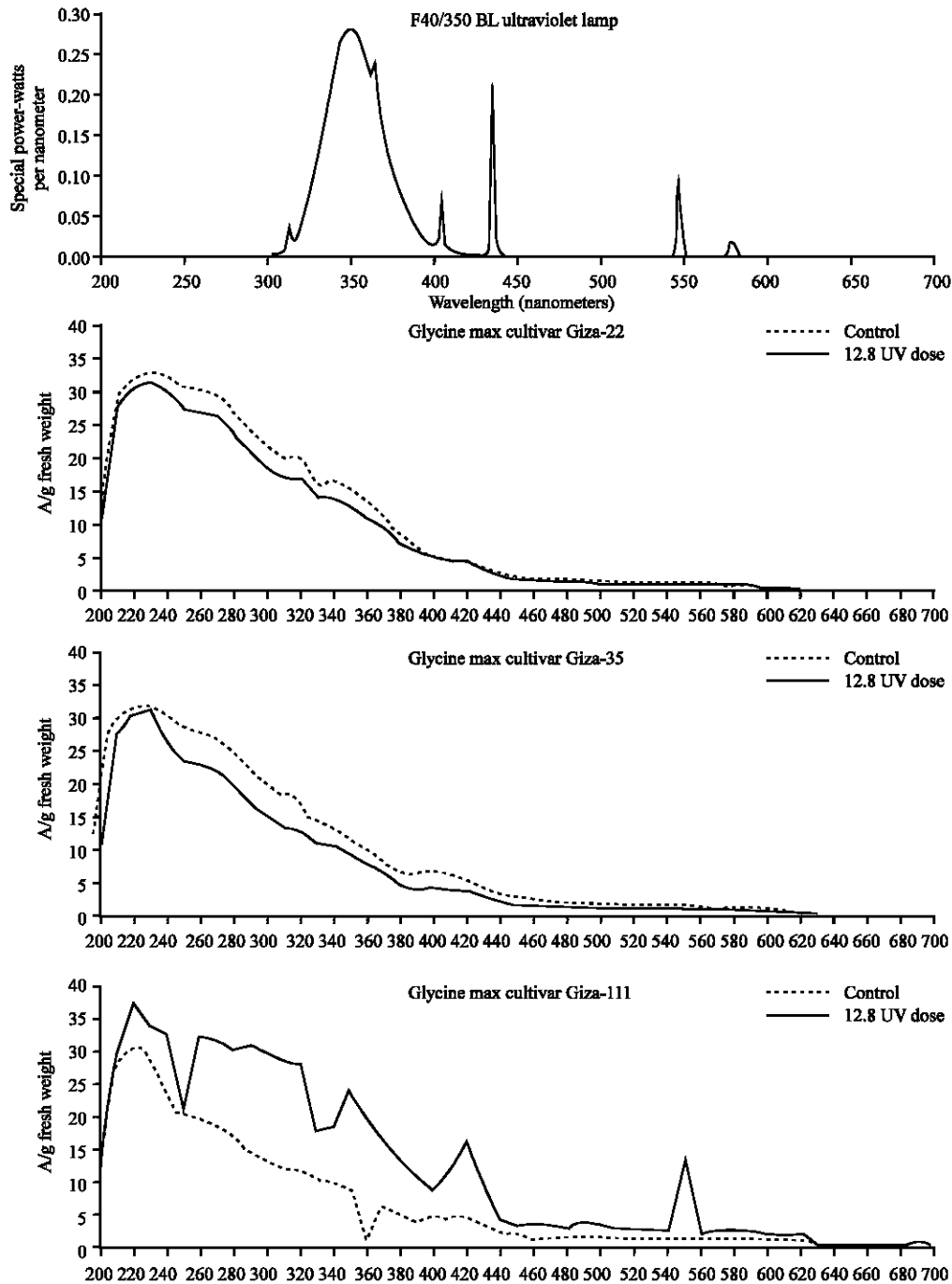


Fig. 5: Showing total foliage leaf flavonoids from 200 to 700 nm wavelengths, of plants irradiated with 12.8  $\text{kJ m}^{-2} \text{d}^{-1}$

flavonoids content was observed. Also, appearance of new peaks of leaf flavonoids following exposure to  $3.2 \text{ kJ m}^{-2} \text{ d}^{-1}$  at  $\lambda_{240}$ ,  $\lambda_{280}$ ,  $\lambda_{320}$ ,  $\lambda_{350}$  and small peak at  $\lambda_{420}$  were detected. In addition, increased flavonoids concentration in the range from  $\lambda_{230}$  to nearly  $\lambda_{500}$  and newly appeared flavonoids peaks can be easily correlated to peaks in the UV-lamp at the same wavelengths.

With increasing UV radiation dose, there was an increase in total leaf flavonoids content. Also, appearance of new peaks of leaf flavonoids at  $\lambda_{220}$ ,  $\lambda_{240}$ ,  $\lambda_{280}$ ,  $\lambda_{300}$ ,  $\lambda_{320}$ ,  $\lambda_{350}$ ,  $\lambda_{420}$  and new peak at  $\lambda_{550}$  and at  $\lambda_{220}$ ,  $\lambda_{240}$ ,  $\lambda_{280}$ ,  $\lambda_{300}$ ,  $\lambda_{320}$ ,  $\lambda_{350}$ ,  $\lambda_{420}$ ,  $\lambda_{550}$  and  $\lambda_{620}$  were observed after exposure to 6.4 and  $12.8 \text{ kJ m}^{-2} \text{ d}^{-1}$ , respectively.

**Anthocyanin content:** The data in Table 4 showed that as UV doses increased, anthocyanin content increased in the three soybean cultivars Giza-22, Giza-35 and Giza-111 ( $r = 0.87, 0.73, 0.99$ ;  $p = 0.000^{***}, 0.006^{**}, 0.000^{***}$ , respectively). Two-Way-ANOVA which revealed that there is highly significant variation between cultivars ( $F = 77.03, p = 0.000^{***}$ ) and among ultraviolet radiation treatments ( $F = 525.13, p = 0.000^{***}$ ). Soybean cultivar Giza-22 showed the highest accumulation of anthocyanin in leaves after exposure UV-dose of  $12.8 \text{ kJ m}^{-2} \text{ d}^{-1}$  by

about 392% as compared to control (Table 4). Least significant difference statistics test revealed that there was a statistically significant variation in the anthocyanin accumulation level among different UV-treatments and control in the foliage leaves of soybean cultivars Giza-22, Giza-35 and Giza-111 (Table 4).

**Carotenoids levels:** The enhanced supplemental doses of ultraviolet radiations significantly induced accumulation of carotenoids in foliage leaves of soybean cultivar Giza-111 and non-significantly in cultivars Giza-22 and Giza-35 ( $r = 0.42, 0.075$  and  $0.94$ ;  $p = 0.163, 0.815$  and  $0.000^{***}$ , respectively). Both Giza-22 and Giza-111 accumulated highest level of leaf carotenoids after exposure to enhanced ultraviolet radiation dose of  $12.8 \text{ kJ m}^{-2} \text{ d}^{-1}$  by about 262 and 265% from control samples (Table 5). Least significant difference test statistic revealed that there was a significant difference in the leaf carotenoids content among UV-treated cultivars Giza-22, Giza-35 and Giza-111 when compared to the control (Table 5). Two-way-ANOVA proved that there was a highly significant variation among the leaf carotenoids content of different enhanced ultraviolet radiation treatments ( $F = 7.22, p = 0.001^{***}$ ).

Table 4: Effect of different doses of UV<sub>A+B</sub> radiation ( $\text{kJ m}^{-2} \text{ d}^{-1}$ ) on anthocyanin content (anthocyanin/g) of three *Glycine max* L. cultivars

UV dose ( $\text{kJ m}^{-2} \text{ d}^{-1}$ )	Giza-22		Giza-35		Giza-111	
	Mean±SE (Anth/g)	LSD (Sig)	Mean±SE (Anth/g)	LSD (Sig)	Mean±SE (Anth/g)	LSD (Sig)
0	0.50±0.04	-	1.03±0.05	-	0.72±0.01	-
3.2	1.52±0.05	0.000***	1.15±0.0	0.091	1.39±0.04	0.000***
6.4	2.38±0.03	0.000***	1.76±0.06	0.000***	2.14±0.09	0.000***
12.8	2.46±0.04	0.000***	1.58±0.04	0.000***	3.20±0.12	0.000***
F-ratio	506.43		62.12		189.36	
p-value	0.000***		0.000***		0.000***	
Two-Way ANOVA						
Among cultivars	F-ratio	77.03	Among ultraviolet treatment	F-ratio	525.13	
	p-value	0.000***		p-value	0.000***	

\* Significant at  $p < 0.05$ , \*\* highly significant at  $p < 0.01$ , \*\*\* Very high significant at  $p < 0.001$

Table 5: Effect of different doses of UV<sub>A+B</sub> radiation ( $\text{kJ m}^{-2} \text{ d}^{-1}$ ) on carotenoids level ( $\mu\text{g g}^{-1}$ ) of three *Glycine max* L. cultivars

UV dose ( $\text{kJ m}^{-2} \text{ d}^{-1}$ )	Giza-22		Giza-35		Giza-111	
	Mean±SE ( $\mu\text{g/g}$ )	LSD (Sig)	Mean±SE ( $\mu\text{g g}^{-1}$ )	LSD (Sig)	Mean±SE ( $\mu\text{g/g}$ )	LSD (Sig)
0	0.487±0.05	-	1.59±0.36	-	1.05±0.241	-
3.2	1.6314±0.86	0.140	1.84±0.39	0.607	1.79±0.10	0.027**
6.4	1.1659±0.42	0.358	1.55±0.09	0.941	2.01±0.075	0.007**
12.8	1.7616±0.21	0.105	1.58±0.41	0.984	3.88±0.273	0.000***
F-ratio	1.36		0.16		39.43	
p-value	0.321		0.918		0.001***	
Two-way ANOVA						
Among cultivars	F-ratio	6.52	Among ultraviolet treatment	F-ratio	7.22	
	p-value	0.005**		p-value	0.001***	

\* Significant at  $p < 0.05$ , \*\* highly significant at  $p < 0.01$ , \*\*\* Very high significant at  $p < 0.001$

## DISCUSSION

Because plants must be exposed to sunlight to power photosynthesis, they are exposed to high level of UV-B radiation in the biosphere might damage the performance of many crop species. In the present study, UV-radiations reduced shoot and root biomass of the three soybean cultivars (Giza-22, Giza-35 and Giza-111) with increasing levels of ultraviolet radiations (Fig. 1). The UV-B induced alterations in shoot growth parameters may be attributed to photomorphogenetic mechanisms. Photomorphogenetic UV-B effects may be 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 demonstrated (Tevini *et al.*, 1991). IAA absorbs in the UV-B waveband and can be converted *in vitro* and *in vivo* to various photooxidation products. One of these products is 3-methylene-oxindole which inhibits hypocotyl growth when applied exogenously. The primary effect of enhanced UV-B radiation appears to be subtle photomorphogenic responses that induce altered carbon partitioning and allocation rather than significant reductions in growth or biomass accumulation (Barnes *et al.*, 1996; Caldwell *et al.*, 2003).

Lowered above and below ground, total biomass and plant height in response to UV-B radiation is commonly observed in both laboratory and field grown conifers (Sullivan *et al.*, 1996; Poulson *et al.*, 2002). Decreased biomass is likely the result of lower rates of CO<sub>2</sub> assimilation in plants grown with UV-B radiation. Changes in biomass induced by ultraviolet radiations which observed in the three cultivars under investigation (Giza-22, Giza-35 and Giza-111) may increase their environmental stress tolerance. Change in plant height often occurs in conjunction with change in stem diameter and self-shading by foliage, which reduces heat load at the base of the seedling and minimizes cellular damage that occurs at high surface soil temperatures (Helgerson, 1990).

A highly significant positive correlation was observed between increasing UV radiation and phenylalanine ammonia-lyase (PAL) activity in the three soybean cultivars under investigation (Table 1). Phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) is a key enzyme of phenylpropanoid metabolism involved in the production of numerous, potentially protective compounds, such as flavonoids, furanocoumarin phytoalexins and cell wall components. The photoinduced accumulation of flavonoids is preceded by the induction of several enzymes involved in phenylpropanoid metabolism (Hahlbrock and Scheel, 1989). Photoregulation

of enzymes involved in anthocyanin and other flavonoids biosynthesis including PAL, chalcone synthase and dihydroflavonol reductase have been studied in many systems (Beggs *et al.*, 1986; Kubashek *et al.*, 1992). In maize, sunlight triggered the photoinduction of PAL with two distinct peaks in all organs of seedlings (Singh *et al.*, 1999). It is likely that the first PAL peak is induced by phytochrome, the second peak was seemed to be induced specifically by UV-B light. It is possible that two peaks of PAL activity arise by the stimulation of two different genes of PAL by phytochrome and UV-B photoreceptor on different temporal scales. The individual members of the PAL gene family respond differentially to photoinduction leading to the biphasic appearance of PAL. For example, in *Arabidopsis*, transcripts encoding PAL and other enzymes of the flavonoid biosynthetic pathway are induced independently by three photoreceptor namely, phytochrome, blue/UV-A photoreceptor and UV-B photoreceptor in a temporally determined fashion (Kubashek *et al.*, 1992).

Campos *et al.* (1991) observed an enhancement of Phenylalanine ammonia lyase mRNA levels, a group I enzyme in flavonoid synthesis. DNA itself may act as a UV-B photoreceptor, stimulating synthesis of both pigments, a mechanism already proposed for flavonoids (Beggs *et al.*, 1986) and stilbenes (Fritzemeier and Kindl, 1981). By whatever means these two pigments are biochemically associated, increased flavonoid levels may serve as an indicator or a correlate to the actual, physiologically relevant photoprotector of the photosynthetic apparatus, the carotenoids. Under UV-B irradiation, increases in flavonoid content have been far easier to recognize than have increases in photosynthetic pigments, probably due to lack of control for UV-A radiation. Consequently, flavonoids have been assumed to protect the photosystems, based largely on circumstantial evidence. The UV-B induced PAL accumulation was similar to cold stress-treated maize seedlings, where anthocyanin induction was preceded by the modulation of *pal* transcript levels. It is therefore plausible that U-B induced PAL enzymes may play a role in anthocyanin formation in maize seedlings (Singh *et al.*, 1999).

Accumulation of the UV-B-absorbing pigments is one of the ways by which plants alleviate the harmful effect of UV-B light (Beggs *et al.*, 1986; Bieza and Lois, 2001). The UV-B light-absorbing flavonoids are implicated as protective pigments in shoots and leaves exposed to UV-B light and their specific location in the epidermal layer protects internal cell layers by attenuating the impinging UV-B radiation at the epidermis (Braun and Tevini, 1993). It has been shown that the photoinduced

accumulation of these flavonoids is preceded by an induction of several enzymes of phenylpropanoid biosynthetic pathway such as PAL and chalcone synthase of the flavonoid biosynthetic pathway (Hahlbrock and Scheel, 1989; Schmelzer *et al.*, 1989).

A highly significant positive correlation was observed between increasing UV radiation and phenolic compounds, flavonoids, anthocyanins and UV-absorbing compounds in the three soybean cultivars under investigation (Table 2-4 and Fig. 3-5). Phenolics are believed to protect plants against UV-B radiation damage (Caldwell *et al.*, 1993). In some species, biosynthesis of phenolics is accelerated by UV-B radiation (Bornman and Teramura, 1993). Phenolics are located mainly in the epidermis (Caldwell *et al.*, 1983), on the cuticle (Wollenweber, 1985) and in the cell walls of the trichomes (Karabourniotis *et al.*, 1992).

Most evidence for the role of phenolics as UV screens has been correlative. Generally, when plants are allowed to develop under enhanced UV-B regimes or allowed to acclimate to doses of supplemental UV-B radiation, soluble phenolics and photosynthetic tolerance to UV increase simultaneously. In addition to increased phenolics, other presumably adaptive responses have been observed. These include increases in leaf thickness, free radical scavenging capacity, PSII protein turnover rates and altered chlorophyll and carotenoid contents (Jansen *et al.*, 1998; Jansen, 2002). Secondary phenols have been implicated in a wide range of plant environmental and developmental interactions not limited to UV stress responses. For example, phenols have been implicated as required oxidase co-factors in auxin catabolism (Stafford, 1991), regulators of auxin transport (Brown *et al.*, 2001), in vacuolar metal sequestering through chelation (Brouillard, 1988; Hale *et al.*, 2002) and as a structural component of the primary cell wall matrix (Hatfield *et al.*, 1999).

UV-B-induced formation of phenolics had some protective function (Lavola, 1998). In particular, the increase in concentrations of specific flavonoids (myricetin and quercetin derivatives), cinnamic acid derivatives and chlorogenic acid may play a protective role against photo-oxidation damage by UV-B radiation (Liu *et al.*, 1995; Reuber *et al.*, 1996; Sheahan, 1996). In a leaf, flavonoids absorb UV-radiation at 200-380 nm and cinnamic acid derivatives mainly absorb at 270 to 350 nm, attenuating UV-B even more effectively than flavonoids (Sheahan, 1996; Lavola *et al.*, 1997). Because several flavonoids and phenolic acids are antioxidants, they may scavenge the free oxygen radicals generated by UV-light (Foyer *et al.*, 1994). It has been suggested that a specific flavonoid is responsible for UV-protection (Liu *et al.*,

1995; Reuber *et al.*, 1996; Lavola *et al.*, 1997). The defense mechanisms do not provide complete protection against increased UV-B, because their effectiveness depends on environmental factors and the developmental stage of the plant (Caldwell *et al.*, 1983; Wand, 1995).

The differences in UV-B sensitivity between soybean cultivars under investigation may be partly explained by differences in the capacity of screening UV-B radiation, which depends on the concentration and spatial location and quality of UV-B-absorbing compounds (Sullivan *et al.*, 1996). The increase in both the amount of UV-B absorbing compounds and electron dense inclusions represents a response in maize pollen to UV-B stress. It is also possible that these storage compounds could be used as an energy source for pollen tube growth or a reserve of precursors utilized for membrane and wall synthesis during the growth of pollen tubes (Santos *et al.*, 1998).

Protective responses are stimulated mainly by UV-B radiation, including increased production of UV-B-absorbing compounds (e.g., flavonoids), secondary compounds ubiquitous in higher plants (Tevini *et al.*, 1991). Flavonoids are thought to protect photosynthetic tissues by acting as screening pigments, absorbing UV-B radiation. Flavonoids protect photosynthetic cells by absorbing UV-B radiation, the photosynthetic pigments should be maintained at normal levels as flavonoids increase in response to UV-B irradiation; if flavonoids fail to increase, as often observed in UV-sensitive plants, losses in photosynthetic pigments would be expected. However, an increase in photosynthetic pigments would imply that their synthesis is also induced, directly or indirectly, by UV-B radiation (Middleton and Teramura, 1993). Flavonoids accumulation also proved to be a useful indicator of leaf UV-B screening ability and potential plant sensitivity to increased UV-B radiation (Wand, 1995). Soybean *Glycine max* genotypes exhibit a wide range in sensitivity to UV-B radiation could be attributed to differences in flavonoid content (Sullivan and Teramura, 1990; Reed *et al.*, 1992).

The main changes in the carotenoids content are resulted from the increase of the amount of the xanthophylls cycle components. High light intensity increased the activity of xanthophylls cycle, the important protective mechanism against photoinhibition and photodamage of PSII (Long *et al.*, 1994). Flavonoids can effectively screen the UV-B radiation thereby protect the primary metabolism and furthermore they have antioxidant properties. Thus flavonoids accumulation can improve plant tolerance to abiotic stresses such as UV-B radiation by reducing damage to the photosynthetic apparatus (Estiarte *et al.*, 1999).

The UV-B component of sunlight plays a major role in anthocyanin induction. Moreover, the irradiation of seedlings with artificial UV-B light also elicits strong induction of anthocyanin similar to the sunlight (Singh *et al.*, 1999). A highly significant positive correlation was observed between increasing UV radiation and anthocyanin in the three soybean cultivars under investigation (Table 4). Since anthocyanin level induced by UV-B decreased with infra red (FR) pulse, which could be nullified by Red Light (RL) pulse (Reddy *et al.*, 1994), it is evident that phytochrome plays a secondary role in modulating the UV-B response in purple puttu seedlings, as in parsley and sorghum seedlings. In comparison to the cyanic purple puttu cultivar, the cyanic black puttu cultivar only a marginal amount of anthocyanin is induced under sunlight (SL). By contrast, both cultivars induce UV-B-absorbing compounds to the same extent under SL. This is further evidence that the UV-B component of SL is responsible for the specific induction of anthocyanin. The accumulation of anthocyanin may be of advantage to plants as anthocyanin can protect the growing meristematic zones from likely genetic damage caused by UV-radiation (Stapleton and Walbot, 1994). The studies comparing the role of anthocyanin and the DNA repair system as a protection against UV-B radiation have shown anthocyanin production to be the predominant mechanism of protection in the young plant (Hays and Pang, 1994). Moreover, anthocyanin could reduced DNA damage by reducing the level of dimmer formation (Stapleton and Walbot, 1994).

The present study showed a significant accumulation of carotenoids in response to elevated UV radiation doses (Table 5). This accumulation could be a protection against the harmful effect of UV radiation in soybean cultivars. The efficacy of carotenoids in protecting the photosystems is likely due to their function as efficient quenchers of high energy shortwave radiation. The mechanism by which this is accomplished was first proposed to involve photochemical state change of singlet oxygen to triplet form by interaction with carotenoids, removing the potentially dangerous oxygen radicals produced in photo-oxidative processes (Krinsky, 1979). Functionally, the carotenoids, especially xanthophylls, absorb the shortest wavelength radiation within the light harvesting complexes. A radiation-less dissipation process (i.e., heat) involving the xanthophyll cycle in the presence of excessive light has been proposed (Demmig-Adams and Adams, 1990). Zeaxanthin, produced by conversion from violaxanthin (or  $\beta$ -carotene) in excessively high radiation, lowers the excited singlet state of Chl's in the photosynthetic pigment antennae complexes, diverting excitation energy away from the

reaction centers. Larger pool sizes of the photosynthetic carotenoids, especially the xanthophylls, result under long-term exposure to high radiation (Demmig-Adams, 1990).

The photosynthetic pigments (especially carotenoids) and not the UV-B absorbing compound levels, could be related to photosynthesis and overall productivity. This relationship was best seen in the normal isolines; the increase in carotenoids was weaker in the flavonoid-deficient lines and unrelated to increases in UV-B-absorbing compounds in the Chl-deficient isolines, perhaps revealing an uncoupling of separate processes. This suggests that both pigments perform a photo-protective function, but only the carotenoids are implicated in photoprotection of the photosystems. Many studies have shown that carotenoids serve a protective function against UV-B (Rau *et al.*, 1991) and UV-C radiation (Campos *et al.*, 1991). The increase in both classes of pigments (photosynthetic and UV-B-absorbing compounds) with UV-B irradiation might indicate a similar UV-B induction stimulus for their biosynthesis. Campos *et al.* (1991) suggested that UV-B and UV-C irradiation increases the levels of 3-hydroxy-3-methylglutaryl COA reductase mRNA, inducing additional synthesis of carotenoids for protection of Chl against UV damage, which could be used as a marker of UV-induced stress.

It could be concluded that three studied soybean cultivars respond to elevated UV<sub>A+B</sub> radiation by increasing PAL activity, phenolic compounds, UV-absorbing compounds, flavonoids, anthocyanins and carotenoids. It was also found that Giza-111 cultivar was most tolerant to UV<sub>A+B</sub> radiation followed by Giza-22, while Giza-35 was the most sensitive cultivar to enhanced UV<sub>A+B</sub> radiation.

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