EFFECT OF SUPPLEMENTAL UV-B RADIATION ON GERMINATION, SEEDLING GROWTH, AND BIOCHEMICAL RESPONSES OF SUNFLOWER (*HELIANTHUS ANNUUS* L.)

S. SHAHID SHAUKAT[1], SAHAR ZAIDI[2] AND MOAZZAM A. KHAN[1]

[1]Institute of Environmental Studies, University of Karachi, Karachi-75270, Pakistan.
[2]Department of Botany, Federal Urdu University of Arts, Science and Technology, Gulshan-e-Iqbal Campus, Karachi-75300, Pakistan

Abstract

This investigation attempts to examine the effect of UV-B radiation on germination, seedling growth, chlorophyll a and b content, soluble phenol, anthocyanin and flavonoid contents of sunflower (*Helianthus annuus* L.). Final germination percentage was significantly reduced but germination velocity was enhanced. Chlorophylls a and b and total Chlophyll a+b content declined significantly in response to UV-B exposure. However, chlorophyll a/b ratio was substantially increased. Marked accumulation of soluble phenol was observed as a result of UV-stress. Anthocyanin and flavonoids were increased in response to UV-B radiation as they provide a protective mechanism to UV-radiations. These results are discussed in the light of the mode of action of UV-B radiation.

Introduction

Climate change and Ozone depletion are the greatest global threats to the life on earth. The depletion of stratospheric ozone has led to increased penetration of solar UV- B (280-320 nm) radiation through the atmosphere, reaching earth's surface. Unfortunately, the protective ozone layer is continuously being damaged by human activity, *i.e.,* by ozone depletion substances including chlorofluorocarbons, hydrochlorofluorocarbons, methybromide and other industrial products that contain halogens (Kerr, 1988). As a consequence, the level of UV radiation is being increased that can be harmful for all life forms, plants, animals and even microorganisms. Biologically effective UV radiation can be increased up to 2 %, due to 1% depletion of ozone layer (Madronich *et al*., 1998).

Studies have provided evidence that UV-B can induce some general stress responses and other photomorphogenic responses (Mackerness, 2000; Jansen, 2002). Several investigations have indicated the impact of supplemental UV-B radiation on growth, development, biomass accumulation, yield and metabolism of plants (Rozema *et al.,* 1997; Gao *et al*., 2003; Ravindran *et al*., 2008). Some studies have also demonstrated the inhibition of stem growth thereby altering the shoot morphology (Kim *et al.,* 1998; Kobzar *et al.,* 1998). Mechanisms such as increased leaf thickness alterations in cuticle and increased production of UV-B protective pigments have been studied in different plant species (Gwynn-Jones, 2001). In one such study 5% stimulated ozone depletion reduced biomass and leaf area under enhanced UV-B radiation (Barnes *et al.,* 1993). Physiological parameters such as UV- B absorbing compounds and chlorophylls have been found to be useful indicators of UV-B sensitivity and tolerance (Greenberg *et al*., 1997). If protective mechanism fails to protect the genome and photosynthetic machinery against UV-B, repair mechanisms are relied upon (Takeuchi *et al.,* 1998) One protective mechanism which seems to be common under stress conditions is the increase in the phenol content (Kozlowska *et al*., 2007; Ravindran *et al*., 2008). Exposure to near ambient UV-B results in increase in leaf phenolic content in soybean plants (Zavala *et. al*., 2001). UV-B can accelerate the biosynthesis of plant flavenoids and anthocyanins (Ravindran *et al*., 2008) and other phenolic compounds (Figueroa *et al*., 2009) which serve to protect the sensitive tissues from UV-B radiations (Beggs and Wellman, 1994).

The studies on the effects of UV-B on various plants are numerous, but little literature is available on the growth and physiological responses of sunflower plant *Helianthus annuus* L., an important oil-seed crop and a horticultural plant. This study focuses on germination, seedling growth and development and physiological and biochemical responses of sunflower to supplemental UV-B radiation.

Materials and Methods

Treatments and Germination conditions: For the current study a series of experiments with seeds and seedlings of sunflower (*Helianthus annuus* L.) var. Sun (obtained from Agricultural Research Centre, Tandojam) were performed. Sunflower is an important oil-yielding crop that provides oil and is cultivated throughout Asia and Africa. Clean seeds of sunflower were first surface sterilized with 0.5 percent sodium hypochlorite for 2 min., rinsed and soaked in distilled water for 2 h and then 20 seeds were placed in 9 cm diameter sterile Petri plates fitted with two discs of Whatman No.1 filter paper, subsequently transferred to

radiation chamber and exposed to fluorescent UV-B tube. The chamber was covered by wooden lid for safety reasons. Within the chamber a UV-B fluorescent tube (TL40W/12, Philips, Eindhoven, The Netherlands) was installed, which exhibited its emission >280nm to a maximum at 312 nm (the actual UV-B range is 280-320nm). UV was filtered through cellulose acetate filter paper to avoid transmission of wavelength below 290 nm.

The Petri plates containing 20 sunflower seeds were exposed for 10, 20, 30, and 40 minutes to UV-B radiation. Five replicates were kept for each treatment and control. Initially, 5 ml sterile distilled water was added to each Petri plate. For germination study Petri plates were kept at 28° C and 50 % humidity on a laboratory bench. Day light was supplemented by light from two fluorescent tubes. Observations on germination were recorded daily. Small amounts of distilled water were added periodically when Petri plates were beginning to dry out. Germination was recorded daily up to 10 days. At the end root and shoot lengths, of the seedlings and their fresh weights were recorded. Germination velocity (GV) was measured using the index proposed by Khandakar and Bradbeer (1983), as follows:

$$GV= [N_1/1 + N_2/2 + N_3/3 + ….+ N_n/n] X 100/1$$

Where N_1, N_2, N_3,…N_n are the proportion of seeds that germinated on day 1,2 3, … n respectively.

Estimation of chlorophyll: Seeds were exposed to UV-B radiation for 0 (control) ,10,20, 30 and 40 min and the seedlings grown as described above, subsequently at eighth day chlorophyll in the leaf and the cotyledons was determined. Chloropyll a and b were extracted from the irradiated shoots and estimated by the method of Maclachlan and Zalik (1963). For extraction 0.5g of shoots were grounded in 10ml of 80% (v/v) acetone and centrifuged at 2000rpm for 15 minutes to clear the suspension. Supernatant, which contained soluble pigments was used for determination of chlorophylls. Absorbance of the extract was recorded at 663, 645 and 630nm on Shimadzu UV-1201 spectrophotometer against 80% (v/v) acetone blank. The chlorophyll content was calculated using the formula given below and expressed in mg/g fresh weight.

Chl a =11.64 D 663-2.16 D 645 + 0.1 D 630
Chl b =20.97 d 645- 3.94 D 663- 3.363 D 630

Soluble Phenols: Treatments and cultural conditions of sunflower seedlings are described above. Soluble phenol contents were ascertained in the seedlings of sunflower. Total soluble phenols were determined following the method of Gonzalez *et al*., (2003) with minor modifications. Plant tissues (500 mg) were taken from seedlings in each Petri dish and homogenized in an ice bath with 2 ml cold 80% ethanol (v/v). The homogenate was centrifuged at 6000 g for 3 min. One hundred µl of the supernatant was added to 0.5 ml Folin-Ciocaleau reagent and 1 ml of 20 percent sodium carbonate. Finally, distilled water was added to make a final volume of 10 ml. The mixture was incubated at 40º C for 30 min and the absorbance of the developed blue color was read at 750 nm using a Shimadzu UV-1201 spectrophotometer. Catechol was used as standard. The amount of soluble phenols was expressed as µg mg^{-1} fresh weight.

Anthocyanin and flavonoids: UV-absorbing pigments including anthocyanins and flavonoids were determined spectrophotometrically. Fresh leaves from seedlings were ground and extracted in acidified methanol (1: 99 HCl: methanol , v/v) using 100mg of leaf tissue. The extract was kept at 0º C for 24h., the content was made up to 10 ml and the absorbance was noted at 530nm as described by Mancinelli *et al.,* (1975). Flavonoids were extracted and measured as described by Mirecki and Teramura (1984). Leaf tissue 100mg were placed in 80 percent acidified methanol (methanol:water:HCl – 80:20:1 v/v/v) for 12 min in dark at 4° C to extract flavonoids and the absorbance recorded at 315 nm.

Statistical analysis: The data were subjected to appropriate statistical analysis which included the one-way analysis of variance (ANOVA) and a post-hoc test namely, Duncan's multiple range test (DMRT) following Zar (1999). Computer programs for all the statistical analyses were developed by the first author (S.S.S.) in C++ and are available on request at a nominal cost.

Results

***Seed germination*:** The final germination percentage was significantly (p at the most 0.05) reduced at 20, 30 and 40 min exposures to UV-B radiation compared to controls (Table 1). However, germination velocity (GV) increased substantially at 10 and 20 min exposure though it was markedly retarded at 30 and 40 min exposure, relative to controls (Table 1).

***Seedling growth*:** Root growth was significantly retarded in the treatments (20, 30, and 40 min UV-B exposure periods) (p at the most 0.05) compared to unexposed controls (Table 2). Likewise shoot growth was also

suppressed at 20 min UV-B exposure and onwards. Similarly, root and shoot fresh weights also declined significantly at 20 min or more exposure to UV-B radiation compared to controls ($p<0.05$).

***Chlorophylls*:** Following exposure to UV-B radiation both chlorophyll a and b as were significantly (p at the most 0.05) decreased compared to controls (Table 3). The total chlorophyll (a+b) also declined remarkably (P at the most 0.05) relative to controls. The chlorophyll a/b ratio, however, decreased owing to the fact that reduction in chlorophyll b content was not as high as in chlorophyll a (Table 3).

***Anthocyanins, flavonoid and soluble phenol*:** As a result of exposure to UV-B radiation the anthocyanin content of the seedlings was elevated significantly (p at the most 0.05) at all the exposure periods (Table 4). Similarly the flavonoid content of the seedling was also increased significantly ($P<0.01$) and interestingly the increase in flavonoid content over the controls was more pronounced than that of anthocyanins. About four-fold increase in flavonoid content was observed in response to UV-B radiation exposure for 40 min. when compared with the controls. A significant accumulation of total soluble phenol in the seedlings over the controls was recorded at all the UV-B exposure periods (p at the most 0.05). The phenol content increased rapidly with the increase in the UV-exposure time.

Table 1. Final germination percentage and germination velocity (GV) of sunflower wet seeds exposed to various durations (min) of UV-B radiation. Mean ± standard error. Means in columns not sharing a letter are significantly different (P <0.05).

Treatments UV-B exposure	Final germination (%)	Germination velocity (GV)
0 (control)	92.0a ± 2.0	24.61
10 min	90.0a ± 2.5	27.65
20 min	86.5b± 3.8	28.09
30 min	73.7c ± 4.6	17.54
40 min	75.2c ± 2.7	16.34

Table 2. Effect of UV-B radiation exposure for different duration (min) on root and shoot growth. Means ± standard error. Means in the same column not sharing a letter are significantly different (P<0.05).

Treatments UV-B exposure	Root length (cm)	Shoot length (cm)	Root wt. (gm)	Shoot wt. (gm)
0 (controls)	2.24a± .13	8.1a± 0.82	0.65a±0.033	1.65a ± 0.18
10 min	2.04a± .16	6.5b± 0.75	0.47b± .024	1.16a±0..17
20 min	1.76b±0.11	6.1b±0.56	0.41b±0.017	0.95b± 0.08
30 min	1.80b±0.12	4.7c ± 0.61	0.24c±0.015	1.04b± 0.13
40 min	1.72b± .08	4.4c ± 0.72	0.24c±0.016	0.85b± 0.16

Table 3. Effect of UV-B radiation exposure for different duration (min) to sunflower on chlorophyll a and b content, total chlorophyll (a+b) and the ratio a/b. Means ± standard error. Chl.= chlorophyll. Means in the same column not sharing a letter are significantly different (P<0.05).

Treatments UV-B exposure	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Chl. a+b (mg/g)	Chl. a/b (mg/g)
0 (Control)	3.7a± 0.28	1.3a± 0.16	5.0a±.32	2.84a±0.3
10 min	3.2a± 0.22	1.2a± 0.17	4.2b± 27	2.60b±0.1
20 min	3.1a± 0.18	1.4a± 0.12	4.5b± 23	2.21c±0.2
30 min	2.8b± 0.20	1.2b± 0.14	3.7c± 25	2.33c±0.1
40 min	2.9b± 0.16	1.1b± 0.11	3.6c± 21	2.62b±0.3

Table 4. Effect of UV-B radiation exposure for different duration (min) to sunflower wet seeds on the anthocyanin and flavonoids content of the seedlings. Means in the same column not sharing a letter are significantly different (P<0.05).

Treatments UV-B exposure	Anthocyanins A_{53} g^{-1} fr. Wt.	Favonoids A_{315} g^{-1} fr. Wt.	Total soluble Phenols µg/g
0 (Control)	0.04a ± 0.02	0.19a ± 0.08	52.7a ± 5.9
10 min	0.08b± 0.02	0.32b ± 0.16	71.31b ± 3.2
20 min	0.09b± 0.03	0.51c ± 0.22	77.74 c± 4.6
30 min	0. 13c± 0.05	0.45c ± 0.17	84.17 cd± 8.8
40 min	0.14c 0.06	0.68d ± 0.26	92.25d ± 5.7

Discussion

Several investigations have demonstrated that increasing exposure to UV-B or increasing intensities of UV-B radiations induce several morphological (Day and Demchik, 1996; Furness *et al*., 1999; Bilger *et al*., 2001; Frohmeyer and Staiger, 2003) and a number of physiological/biochemical changes in higher plants (Bilger *et al.*, 2001; Jansen, 2002; Warren *et al.*, 2003; Ranjbarfordoei *et al.*, 2007; Kozlowska *et al*., 2007). In the present study, UV-exposure of hydrated seeds of sunflower for 20, 30, 40 min significantly reduced the final germination percentage but the speed of germination (GV) was substantially increased at 20 and 30 min exposure to UV-B radiation. Our result pertaining to final germination percentage contradicts the findings of many other workers (Tosserams *et al*., 1997; Dai and Upadhaya, 2002). On the other hand, Wagne (1966) investigated the effect of UV light on lettuce seeds and noted improvement in germination (at 254-405 nm). Noble (2002) investigated the effect of UV-B irradiation on seeds of four species and found that germination was not affected. However, he showed that the speed of germination was increased which is consistent with the result of the present study.

The results of the experiments clearly demonstrated the deleterious effects of UV-B radiation on sunflower (*Helianthus annuus*) seedlings in terms of resulting physical and biochemical changes. UV-B radiation exposure not only caused decrease and in root and shoot growth and their corresponding weights but also resulting in curling of roots and shoots. These results corroborate the findings of earlier workers (Barness *et al*., 1990; Greenberg *et al*., 1997; Furness *et al*., 1999; Bilger *et al*., 2001; Warren, 2003; Kozlowska *et al*., 2007), who reported marked changes in the morphological traits such as reduction in plant height, decreased leaf expansion, curling of leaves, etc. However, plant response to UV-B radiation varies among species (Barnes *et al*., 1990; Musil, 1995; Cybulski and Peterjohn, 1999) and even different species of the same genus (Johanson *et al*., 1995). The differences among species, though not examined here, can be attributed to the mechanism whereby the plants reduce or tolerate the damage inflicted by UV-B radiation. In a comparative study, Furness *et al.* (1999) examined the effect of UV-B radiation on three weeds (*Cyanoglossum officinale, Centauria diffusa* and *Tragopogon pratensis*), the UV-B radiation decreased the growth and leaf area in all three weeds while most susceptible was *Cyanoglossum officinale*. The results of the current experiment show that the level of UV-B radiation used has measurable suppressive impact on root and shoot growth of sunflower seedlings. The fresh weights of the seedling were significantly reduced by the UV-B radiation which was presumably due to inhibition of photosynthesis (Fegueroa *et al.*, 2000). The suppression of seedling growth could also result due to the accumulation of phenolic compounds synthesized in the plant in response to UV-B stress (Warren *et al*., 2003).

A significant reduction in chlorophyll a and b content was recorded in the present study. Similarly, Day and Vogelmann (1995), Ambasht and Agarwall (1998) , Skorska (2000) and Ravindran *et al*. (2008) reported a marked reduction in total chlorophyll (about 50% of the controls), Strid *et al*. (1990) and Hoffman (1999) also demonstrated significant reduction in chlorophyll content following UV-B radiation. The decrease in chlorophyll a/b ratio could be explained on the grounds that the degradation pathway of chlorophyll a is different from that of chlorophyll b. Chlorophyll a may undergo degradation under stress condition first prior to degradation of chlorophyll b. This may account for decreased a/b ratios with increasing UV-B stress.

Our results showed an increase in the content of anthocyanins, a pigment that plays an important role in radiation absorption, following exposure of plants to UV-B radiation. Both anathocyanins and flavonoids are protective pigments that depict marked changes in response to UV-B radiation. The increase in anathocyanin pigment in response to UV-B radiation corroborates the findings of Tevini *et al*. (1991), Pinter *et al*. (2007) and Ravindran *et al*. (2008). Likewise, flavonoid production was promoted in response to UV-B exposure. This is in accordance with the observations of several workers (Warren, 2003; Ravindran *et al*., 2008; Ranjbarfordoei *et*

al., 2009). Accumulation of UV-B absorbing pigments such as flavonoids provide one of the mechanism whereby plants alleviate the harmful effects of UV-B radiations.

Exposure of sunflower hydrated seeds to UV-B radiation resulted in accumulation of soluble phenols in the seedlings. Accumulation of phenols as a result of exposure of plants to UV-B radiation has also been recorded by Ambasht and Agarwal (1998), Kozlowska *et al*. (2007) and Ravindran *et al*. (2008) which provides a protection against UV radiation. It has been established that phenol metabolism is activated in plants as a reaction to abiotic stress (Abreu and Mazzafera, 2005; Olenchenko and Zagoskina, 2005; Ganeva and Zozikova, 2007). Shaukat *et al*. (1999, 2010) demonstrated that the exposure of plants to heavy metals such as Cd, Cr, Pb and Zn results in the accumulation of soluble phenols. Plant phenolics have been regarded as defenses against pathogens and herbivores (Dixon and Paiya, 1995; Shaukat and Khan, 2009) and provide protective mechanism against a variety of abiotic stresses including stress due to heavy metals, pathogens and UV radiations. Our results provide additional support to this conjecture. Secondary metabolic pathway is physiologically important as it provides the means of channeling and storing carbon compounds, accumulated from photosynthesis during periods when nitrogen is limiting and whenever leaf growth or cotyledons are suppressed, e.g., by UV-B radiations. In this connection it is noteworthy that the cotyledons and first leaf growth was suppressed by the UV-B radiation. The protective role of phenolics may be due to structural stabilization of cell wall through condensation-polymerization of phenols and quinines. Secondly, they can provide photoprotective mechanism *i.e*., by altering the absorbance of visible and UV-radiation. Thirdly, they act as powerful antioxidant and antiradical agents (Harborne, 1999, 2007; Edreva *et al*., 2008).

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