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EFFECT OF SUPPLEMENTAL UV-B RADIATION ON GROWTH AND STRESS RESPONSE OF *VIGNA RADIATA* (L.) WILCZEK

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ABSTRACT

This investigation attempts to examine the effects of UV-B radiation on seedling growth, chlorophyll content and total soluble phenol accumulation in the roots of mungbean. Imbibed seeds of mungbean (*Vigna radiata* (L.) Wilczek) were exposed to UV-B radiation in a radiation chamber for 10, 20, 30 and 40 minutes. At each radiation dose both root and shoot growth of seedlings were markedly suppressed while shoot weights were significantly reduced. The inhibitory effect increased with increasing exposure period. In response to UV-B irradiation, chlorophyll a and b as well as total chlorophyll (a and b) contents were greatly reduced. UV-B irradiation resulted in substantial accumulation of total soluble phenols in the roots of the seedlings. These results are discussed with respect to the mode of action of UV-B radiation.

Key-words: UV-B radiation, growth, stress, mungbean, *Vigna radiata*

INTRODUCTION

A high proportion (70%) of the electro-magnetic radiation emitted from the sun belongs to the UV range of the spectrum (200-400nm). In the past sixty years or so , the concentration of ozone has declined to about 5%, primarily due to release of anthropogenic pollutants such as chlorofluorocarbons (Pyle *et al*., 1997). As a result, a large proportion of UV-B radiation reaches Earth's surface which is deleterious to all living organisms (Caldwell *et al*., 2003). Because of the continuous increase in ambient UV-B radiation levels owing to climatic change (Taalas *et al*, 2000) it is necessary to evaluate the degree of sensitivity of various crop plants to UV-B radiation. Different species and even varieties of the same crop species are known to have different responses to the levels of UV-B radiation (Mathew *et al.*, 1996; Skorska, 1996a,b; Tivini and Teramura, 2008). The potential impact of increased UV-B radiation on plants has been investigated for the last three decades by several workers (Teramura & Sullivan, 1994; Frohmeyer & Staiger, 2003; Tivini and Teramura, 2008). All types of UV radiation cause damage to plants in basically two ways, i.e., damage to DNA (which can cause mutations that are heritable) and damage or alteration of physiological processes. UV-B radiation has many direct and indirect effects on plants. UV-B radiation has pleiotropic effect on plant development, morphology, and many physiological processes (Frohmeyer and Staiger (2003). Sisson and Caldwell (1977) showed that enhanced UV-B radiation (280-320nm) inhibits photosynthesis and leaf expansion. The effect on photosynthesis was cumulative but the effect on leaf growth varied with leaf age. It was shown that the suppression of leaf growth is not entirely due to inhibition of photosynthesis but the low irradiance phytochrome system is also involved. The damage by UV-B radiation to photosystem II and Rubisco has also been reported which may lead to reduction in photosynthetic capacity, RuBP regeneration and quantum yield (Teramura and Sullivan, 1994). Changes in plants seen after providing supplemental UV-B radiation include biomass reduction (Tivini *et al*,1989), decrease in the percentage of pollen germination (Flint and Caldwell, 1984; Torabinejad *et al*., 1998), decrease in the competitive ability of crops (Barnes *et al*., 1988, 1990; Conner and Neumeir, 2002) damage and deformation of epidermal tissue, increase in flavonoids (Jansen *et al*., 1998; Liu *et al*, 2006) and elevation of anthocyanin pigments (Strid and Porra, 1992; Ambasht and Agarwal, 1998) and accumulation of soluble phenols (Ambasht and Agarwal, 1998; Jansen *et al.*, 2001; Ravindran *et al*., 2008).

The objectives of the present study were: (1) to examine the effect of supplemental UV-radiation on germination and seedling growth of *Vigna radiata* (L.) Wilczek (mung-bean, (2) to investigate the effect on chlorophylls, and (3) to examine the accumulation of soluble phenol content in response to UV-B radiation.

MATERIAL AND METHODS

Irradiation of seedlings

For the current study a series of experiments with seeds and seedlings of mungbean (*Vigna radiata* (L.) Wilczek) were performed. Mungbean is an important legume that provides dietary protein and is cultivated throughout Asia and Africa. Clean seeds of mungbean were first surface sterilized with) 0.5 percent sodium hypochlorite (2 min.), rinsed and soaked in distilled water for 2 h and then placed in 9 cm diameter sterile Petri

plates. The Petri plates were cushioned with two discs of Whatman No.1 filter paper. The Petri plates were marked as: control, 10, 20, 30, and 40 minutes for exposure to UV-B radiation. For each treatment and control three replicates of Petri plates with seedlings were taken and 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), which exhibited its emission >280nm to a maximum at 312 nm (the actual UV-B range is 280-320nm), was installed, suspend above the seedlings. For each experiment, intended doses of UV-B were administered to different soaked seeds. . After each radiation, seedlings were kept in laboratory bench in day light supplanted by light from a fluorescent tube, at room temperature 20±2°C (day) and 18±2°C (night). The Petri plates were kept wet throughout the experiment and small amount of distilled water was added periodically when Petri plates were going to dry out. At the end of four days radiation treatment, the physical parameters which included: shoot length, hypocotyls length, radical length, seedling weight and chemical parameters, including chlorophylls a and b concentrations and total phenol contents were measured.

Estimation of chlorophyll:

Chlorophyll 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 µg/g fresh weight.

Chl a =11.64 D 663- 2.16 D 645 + 0.10 D 630

Chl b = 20.97 D 645- 3.94 D663 - 3.363 D630

Soluble phenols:

Soluble phenol contents were ascertained in the roots of plants. Total soluble phenols were determined in accordance with Gonzalez *et al*., (2003) with minor modifications. Root tissues (500 mg) were taken from each Petri-plate (replicate) and homogenized in an ice bath with 2 ml 80% methanol v/v. The homogenate was centrifuged three times at 6000 g for 3 min. One hundred µl of the supernatant was added to 0.5 ml Folin-Ciocalteau reagent and 1 ml of 20 percent sodium carbonate. Finally, distilled water was added until a final volume of 10 ml was attained. The mixture was incubated at 40°C for 30 min. and the absorbance of the developed blue colour 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.

Statistical analysis

The data were subjected to appropriate statistical analysis which involved the analysis of variance (ANOVA) and post-hoc test namely, Duncan's multiple range test (DMRT) following Zar (1999).

RESULTS

Both root length and shoot length were decreased significantly (P at the most 0.05) following exposure to UV-B radiation compared to controls (Table 1). In addition, curling of the shoot and root was also observed. The suppressive effect in both cases increased with the increasing time of exposure and at the highest exposure period (40 min.) shoot and root growth were found most retarded. Seedling dry weights were drastically reduced at each period of UV radiation treatment (P<0.001) (Table 1). Both chlorophyll a and b contents as well as the total chlorophylls were reduced significantly by UV-B radiation exposures compared to the controls (P at the most 0.05) (Table 2).. On the other hand, the total phenol content was elevated significantly (P at the most 0.01) in the UV-irradiated treatments . The total phenol content increased with the increase in UV- radiation exposure time (Table 2).

Table 1. Effect of UV-B radiation on Shoot and root length and shoot dry weight of mungbean *Vigna radiata* seedlings. Mean ± standard error. Means followed by a different letter in a column are significantly different (p<0.05).

Time of exposure	Shoot length (cm)	Root length (cm)	Shoot dry wt. (mg)
Control (0 min)	6.66 a ±1.18	6.47 a± 0.55	37 a± 0.22
10 min	3.23 b ± 0.84	5.24 b± 0.42	16.6b ± 0.35
20 min	2.23 b± 0.72	5.39 b± 0.54	8 .4± c 0.28
30 min	2.10 c± 0.81	3.12 c± 0.46	6.7 ± c 0.24
40 min	1.76 d± 0.65	2.41d ± 0.39	4 .2± d 0.15

Table 2. Effect of UV-B radiation on chlorophyll a and b content of shoot and total soluble phenol content of mungbean *Vigna radiata* seedlings Mean ± standard error. Means followed by a different letter in a column are significantly different ($p<0.05$).

Time of Exposure min.	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Chlorophyll a+b (mg/g)	Total soluble Phenols (µg/g)
Control (0 min.)	0.54 a± 0.046	0.32 a±0.033	0.86a ± 0.67	48.5a ± 5.9
10 min	0.43 b± 0.044	0.29ab±0.035	0.72b ± 0.65	64.75b ± 3.2
20 min	0.38 b± 0.048	0.35 b± 0.025	0.73 b± 0.061	73.75 c± 4.6
30 min	0.31c± 0..29	0.23 c± 0.022	0.54c ± 0.050	79.5 cd± 5.8
40 min	0.34c ± 0.0.32	0.27 c ± 0.026	0. 61ca ± 0.052	90.76e ± 7.7

DISCUSSION

The attenuation of solar UV-B radiation by filters or enhancement of UV-B radiation using the UV-B emitting lamps or tubes are the two major approaches to investigate the influence of such radiations in both field and laboratory investigations. Thus, supplemental UV-B radiation was given by a UV-tube. The results of the experiment clearly demonstrated the deleterious effects of UV-B radiation on the mungbean (*Vigna radiata*) seedlings in terms of the resulting physical and chemical damage. UV-B radiation not only caused decrease in shoot and root growth but also resulted in the curling of roots and the shoots. These results corroborate the findings of earlier studies of Barness *et al*., (1988, 1990) Greenberg *et al*., (1997) and Furness *et al*., (1999) who reported marked changes in the morphological traits such as reduction in plant height, decreased leaf area , curling of leaves, etc. However, the response to UV-B radiation varies among species (Barness *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. The presence of leaf hairs (Karabourniotis *et al*., 1992), a high content of UV-B screening compounds (Day , 1993; Lois and Buchanan, 1994; Day *et al*., 1999) or the production of thick leaves that reduce the penetration of UV-B radiation (Teramura and Sullivan, 1994; Sullivan *et al*., 1996). In addition, the effect on perennial plants is cumulative and in long term studies the magnitude of effect varies from year to year (Sullivan and Teramura, 1990; Johanson *et al*., 1995). In a comparative study (Furness *et al*., 1999) of the effect of UV-B radiation on three weeds (*Cynoglossum officinale, Centauria diffusa* and *Tragopogon pratensis*), the UV-B radiation decreased the growth and leaf area in all three weeds while most susceptible was *Cynoglosum officinale*. The results of the current experiment show that the level of UV-B radiation used has measurable suppressive effects on root and shoot growth of mungbean seedlings. The dry weights of shoots were reduced significantly by the UV-B radiation which was presumably due to inhibition of photosynthesis and disruption of photosynthetic pigments. A similar response to these radiations has been reported previously for other species (Rozema *et al*., 1997; Deckmyn and Impens, 1999; Gonzalez, 1996; Gonzalez *et al*., 1998).

A significant reduction in chlorophyll a and b as well as total chlorophyll content was recorded in the present study. Similarly, Day and Vogelmann (1995), Ambasht and Agarwal (1998), Skorska (2000) and Ravindran *et al*., (2008) reported a marked reduction in total chlorophyll (about 50 % of controls). Strid *et al.,* (1990) and Hoffman (1999) also demonstrated significant reduction in chlorophyll content following UV-B radiation.

Exposure of bungbean seedlings to UV-B radiation resulted in accumulation of soluble phenols. 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 & Mazzafera, 2005; Olenchenko & Zagoskina, 2005; Ganeva & 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 defences against pathogens and herbivores (Dixon & Paiva, 1995; Shaukat and Khan, 2009) and provide protective mechanism against a variety of abiotic stresses including stress due to heavy metals. Our results provide additional support to this conjecture. Secondary metabolic pathway is physiologically important as it provides the means of channelling and storing carbon compounds, accumulated from photosynthesis during periods when nitrogen is limiting and whenever leaf growth or cotyledons are suppressed. In this connexion 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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