



Effect of UV-B radiation on photosynthetic pigments and UV-absorbing compounds of three different soybean cultivars (*Glycine max* L.)

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Abstract

The effects of UV-B radiation on the amount of chlorophyll a, b, carotenoids, flavonoids, anthocyanins, and polyphenols of three soybean cultivars was studied. After germination in the incubator, plants were put in pots of soil and during 3 weeks and irrigated with Hoagland solution every day. After 3 weeks, plants were exposed to UV-B for 20 min every day for a week. In each cultivar, UV-B reduced chlorophyll a, b, and carotenoids and increased anthocyanins, flavonoids, and polyphenols. The reduction of chlorophyll a and b content was significant between two cultivars *Williames*, *Linforced* and *L17*, *Linforced*. Carotenoids content showed significant decrease between cultivars *Linforced*, *Williames* and *Linforced*, *L17*. The anthocyanin and flavonoid contents were affected by UV-B and there was a significant difference in the increase between *Williames* and *L17* and also between *L17* and *Linforced* cultivars. Also increase in phenol content was significantly different between *Williames* and *L17* and also between *Williames* and *Linforced* cultivars.

Keywords: UV-B; soybean; photosynthetic pigment; phenolic compounds

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Introduction

UV radiation is generally classified into three wavelength ranges: UV-A (320-400 nm), UV-B (320-280 nm) and UV-C (less than 280 nm) (Stapleton and Walbot, 1994). The stratospheric ozone layer efficiently filters most of the detrimental, shortwave UV radiation, shorter than 200 nm. A small decrease in ozone levels may cause a large relative increase in biologically effective UV radiation. The decrease in ozone level originates from human activities such as the release of chlorofluorocarbons and nitrogen oxides,

which act as ozone antagonists (Hollosoy, 2002). Like all living organisms, plants sense and respond to UV radiation. All types of UV radiation are known to damage plant growth (Stapleton, 1992). As stratospheric ozone layer becomes thin, this leads to an increase in solar UV-B radiation (320-280 nm) on the surface of earth. Consequently, major research efforts have been conducted to study the effects of UV-B on photosynthetic organisms (Heinrich et al., 2003).

Deleterious effects of UV radiation on the growth, productivity, and photosynthesis of higher plants have been extensively studied. UV radiation also produces oxidative stress which arises from the deleterious effects of reactive oxygen species (ROS), which react with lipids,

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pigments, proteins, and nucleic acids (Nasibi and Kalantari, 2005). However, many plants are quite resistant to UV radiation and possess a number of UV protection mechanisms. One of the important mechanisms is a variety of secondary metabolites including flavonoids and anthocyanins. These compounds often accumulate in the upper epidermis cells of leaves. Other mechanisms that have received less attention than epidermal screening mechanisms are enzymatic and non-enzymatic antioxidative defense systems that may mitigate UV-induced damage that occurs due to the production of reactive oxygen species (ROS) (Xu et al., 2007).

Soybean is an annual plant with branched stems and oval leaves. Its fruit is bean-like. Three to five seeds are in each pod. Soybeans provide a great source of protein and oil for human and animal consumption. The purpose of our study was to assess the effect of UV-B radiation on some physiological traits of three different soybean cultivars (Williams, Linford, and L17).

Materials and Methods

Plant growth and treatment

Seeds of three soybean cultivars (Williams, Linford and L17) were obtained from Seed and Plant Improvement Institute, Karaj, Iran in October 2012. Seeds were sterilized with sodium hypochlorite 1% for 20 min and then soaked in distilled water. The soil used in pots was obtained from a field and mixed with sand (1:5 v/v). The mixture was autoclaved at 20 °C for 4h. The germinated seeds were grown in 33 pots of 35 cm in diameter in a greenhouse. After 21 days of growing in a uniform condition, selected plants were exposed to ultraviolet radiation with UV-B=2 (15W) (LF-25m.312nm) lamps (Philips). Each pot was treated with UV-B for 20 min per day during their light period for a week (Hosseini Sargheini et al., 2008).

Pigment assay

For analysis of chlorophylls a, b, and carotenoid content, 0.1 g leaf material was put in 2 ml acetone 80%. Then the extract was centrifuged at 2700 g for 10 min. The absorbance of the supernatant was recorded at three

wavelengths 663, 647, and 470 nm by spectrophotometer (Lichtenthaler and Welburn, 1983).

Total phenolic assay

For extracting total phenols 5 g of fresh leaf tissues was ground with 10 ml of 80% methanol. The resulting mixture was centrifuged at 10000 g for 15 min at 4 °C (Horii and Shetty, 2007). Nine ml of distilled water was added to 1 ml of the extract. Then 1 ml Folin ciocalteu was added to the mixture and stirred. After 5 minutes, 10 ml of 7% sodium bicarbonate was added and after 90 min the absorbance was recorded at 750 nm (Marinova et al., 2005).

Total flavonoids determination

For extraction of flavonoids, 0.1 g leaf material was ground in 10 ml methanol and then 1 ml of extract was added to 1 ml of Aluminum trichloride (AlCl₃) 2% in ethanol. The volume of extract was increased to 25 ml by adding ethanol. After centrifugation at 3000 g for 10 min, the absorbance of the supernatant was recorded at three wavelengths, namely, 270, 300, and 330 nm (Markantonuts and Qrskov, 1993).

Anthocyanin assay

For analysis of anthocyanin, 0.1 g leaf material was put in 10 ml acidified methanol (99% methanol and 1% HCl in volume). Samples were centrifuged at 6000 g for 10 min. The supernatant of each sample was put in darkness and at room temperature for 24 h. Sample absorbance was recorded at 550 nm (Fulcki and Francis, 1968).

Statistical analysis

Quantitative changes of different parameters were analyzed through analysis of variance (Anova) and Tukey's multiple range test was used to determine if there are significant differences among treatments.

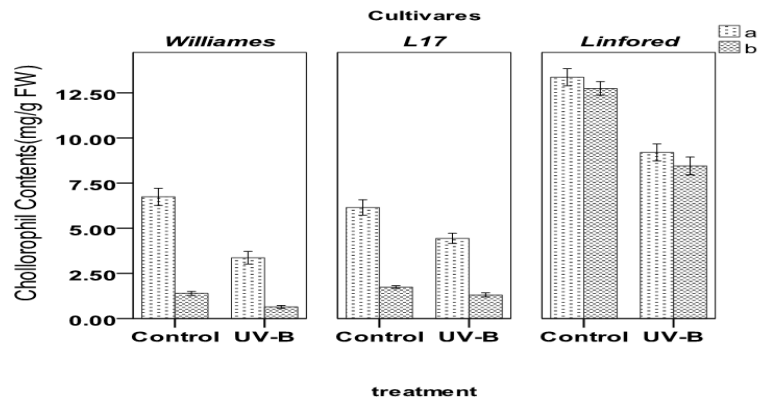


Fig. I. Changes in chlorophyll a and b content in leaves of soybean cultivars in control samples and UV-B treatments (Mean of 3 replicates \pm SE, $P < 0.05$)

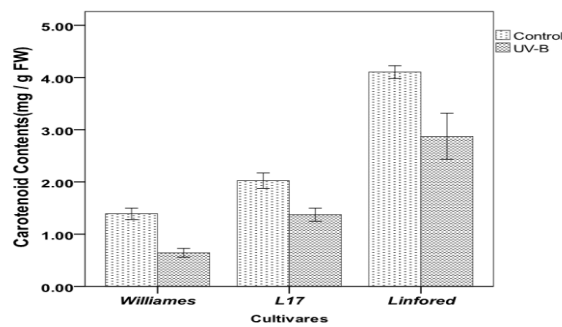


Fig. II. Changes in carotenoid content in the leaves of soybean cultivars in control samples and UV-B treatments (Mean of 3 replicates \pm SE, $P < 0.05$)

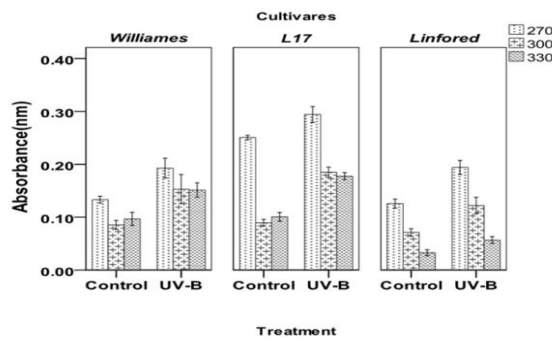


Fig. III. Changes in flavonoids content in leaves of soybean cultivars in control samples and UV-B treatments (Mean of 3 replicates \pm SE, $P < 0.05$)

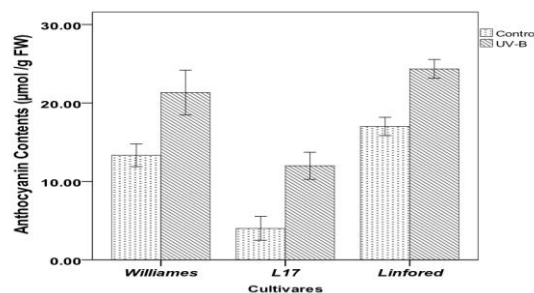


Fig. IV. Changes in anthocyanin content in leaves of soybean cultivars in control samples and UV-B treatments (Mean of 3 replicates \pm SE, $P < 0.05$)

Results

Pigment concentration

The amounts of chlorophyll-a and chlorophyll-b decreased when the plants were exposed to UV-B. Reduction of chlorophyll-a was significant in Linford and Williams cultivars as compared with the control, but there was no significant difference in L17. Reduction in chlorophyll-b was significant in Linford cultivar in comparison with control; however, it was not significant in Williams and L17. Decreased chlorophyll-a and b were significant between Linford, Williams and Linford, and L17 cultivates (Fig. I).

Data analysis showed that the reduction in the amount of carotenoids was significant in Williams, L17, and Linford compared with the control and this reduction between Linford and Williams and also between Linford and L17 was significant and (Fig. II).

UV absorbing compounds

Exposure to UV-B radiation was found to cause an increase in the UV absorbing compounds. Increase in the concentration of flavonoid and anthocyanin was significant in the three treated soybean cultivars compared with the control samples. Moreover, increase in the concentration of flavonoids and anthocyanin was significant between Williams and L17 cultivars and also between Linford and L17 cultivars (Fig. III, IV). Also, increase in the amount of phenol under UV-B radiation was significant in Linford and Williams in comparison with controls while this increase was not significant in L17 cultivar. The results showed that increased phenolic content was significant between Williams and L17 and also between Linford and Williams cultivars (Fig. V).

Discussion

The chloroplast was the first organelle to show response to injury when exposed to UV-B radiation (Ravindran et al., 2010). The reduction of chlorophyll content has a negative effect on plant photosynthetic efficiency. It has been reported that photosynthesis is dependent on the light harvesting properties of chlorophylls (Mahdavian

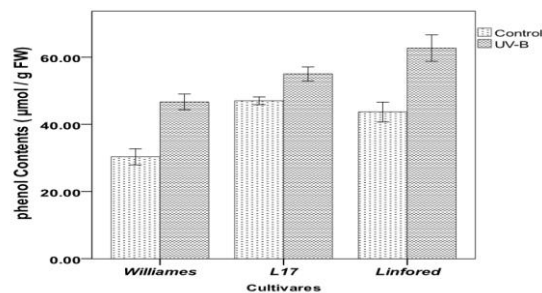


Fig. V. Changes in phenol content in the leaves of soybean cultivars in control samples and UV-B treatments (Mean of 3 replicates \pm SE, $P < 0.05$)

et al., 2008). Also it has been reported that UV-B radiation resulted in greater reduction in the amount of chl-b as opposed to chl-a biosynthesis or degradation of precursors. However the effect of UV-B radiation on chl a/b ratios varies among growth conditions and species (Hollosoy, 2002). It was reported that carotenoid content decreased under UV-B radiation. The decrease in chlorophyll a/b ratio was also reported by Gitz et al., (2004) and this 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 prior to degradation of chlorophyll b. This may account for decreased a/b ratios with increasing UV-B stress. Carotenoid content reduction may result either from breakdown of the pigments or from inhibition of synthesis. Since the carotenoids are involved in the light harvesting and protection of chlorophyll from photo oxidative destruction, any reduction in carotenoid could have serious consequences of chlorophyll pigments (Ravindran et al., 2010). Increased levels of UV-B absorbing compounds lessen the symptoms of UV-B damage, such as growth inhibition and photosynthetic damage (Murali and Teramura, 1986). Flavonoids play many defensive roles in plants and inhibition of UV-B by epidermal flavonoids is often recommended as an adaptive mechanism preventing UV-B from reaching the mesophyll and affecting photosynthesis (Liu et al., 1995). Flavonoids and related compounds are absorbed strongly in the UV-region but is not absorbed in the photosynthetically active regions of the spectrum (Cen and Bornman, 1993). This allows photosynthesis to continue while the wavelengths of UV at the epidermis are attended. Cultivars with higher levels of these compounds prior to the

onset of UV-B treatment have been reported before the start of UV-B treatment to be better protected against UV-B damage (Gonzalez et al., 1996). Similarly, cultivars of a species that can rapidly accumulate these compounds during UV-B exposure are better protected (Murali and Teramura, 1986). It is reported that flavonoids and anthocyanin protect leaf cells from photo oxidative damage from UV radiation (Chalker-Scott, 1999). In potato plants it has been shown that UV exposure caused accumulation of constitutive flavonoids and the induction of two new flavonoid types (Hosseini Sarghein et al., 2008). In *Satureja hortensis* L. it has been observed that exposure to UV radiation stimulated the production of both anthocyanins and flavonoids (Rahimzadeh et al., 2011).

Conclusion

By considering the results in this study, it is concluded that all three cultivars of soybean are sensitive to UV-B radiation. But their sensitivity to UV is different. Differences in UV-B sensitivity were observed in a range of vegetable crop species. Greater levels of biomass accumulation, which indicate higher growth rates were associated with greater UV-B sensitivity. Parameters such as chlorophyll content and the accumulation of UV-B absorbing compounds were useful indicators of the plants' response to UV-B, but could not be used to predict the sensitivity of plant growth to UV-B.

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