Different Uv Radiation-Induced Changes in Antioxidant Defense System in Okra

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ABSTRACT

In this paper okra plants were treated by different UV radiation, UV-A, UV-B and UV-C, for 12 days and effects of different UV radiation on activities of antioxidant enzymes including superoxide dismutase (EC. 1.15.1.1, SOD), ascorbate peroxidase (EC 1.11.1.11, APX), guaiacol peroxidase (EC 1.11.1.7, GPX), catalase (EC 1.11.1.6, CAT), and glutathione reductase (EC 1.6.4.2, GR) in leaves, stems, and roots as well as antioxidant compounds including ascorbic acid (AA), anthocyanin, and flavonoid were examined under controlled conditions. The results show an increase in enzymatic and non-enzymatic antioxidants when plants are exposed to UV-B or UV-C radiation while antioxidant enzymes activities and antioxidant compounds did not significantly increase under the UV-A radiation treatments compared to control plants. Our results suggest that increased level of lipid peroxidation products and antioxidant system activity in the UV-B and UV-C treatments may be considered as biomarkers of intensity of UV radiation stress. However, more research is necessary to elucidate the precise role that the antioxidant system plays under UV radiation stress.

KEY WORDS:
Antioxidant compounds; Antioxidant enzymes; Okra; Oxidative stress; Ultraviolet radiation

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INTRODUCTION

In numerous studies, the effects of various radiations including magnetic radiation and UV radiation on different plants have been studied and different results have been reported. Indeed, the radiations based on intensity and radiation dose in some plants resulted in growth while they resulted in growth reduction in other plants [1, 2]. Increased UV radiation will influence the growth and metabolism of terrestrial plants due to their need for sunlight for photosynthesis [3]. Deleterious effects of natural UV radiation on plants is due to reduced photosynthesis, biomass reduction, decreased protein synthesis, impaired chloroplast function, and damage to DNA [4]. Previous studies have shown that high levels of antioxidant enzymes and secondary metabolites can lead to increased stress tolerance in some of plants [5-7]. However, some questions have still remained unanswered: Do plants enjoy a defense system to encounter the exerted stress in all UV radiations?; by which mechanism each family or one of its species over comes the stress produced by radiation?; which defense system or compound exhibit the most changes in response to stress?, and which plant compound or defense system could be used as marker in identifying the intensity of UV radiation effects?; Therefore, awareness of the mechanisms involved in response to the stresses exerted on different plants is still particularly important and could contribute greatly to managing the plantation and growing of the plants.

Since few have investigated the effect of different UV radiation on antioxidant enzymes and UV-absorbing pigments of Malvaceae family and okra (Hibiscus esculentus) is a member of the Malvaceae family, which is a good source of many nutrients including vitamins, protein, amino acids, minerals, and iodine, the aim of the present study was to investigate the effect of different artificially enhanced UV radiation on the MDA, H2O2, AA, UV-absorbing pigment contents, and antioxidant enzyme (SOD, CAT, APX, GR, GPX) changes of okra which may become a biomarker for assessing the biological impact on Malvaceae family in response to the UV radiation change.

MATERIAL AND METHODS

For culture conditions and treatments, okra seeds cultivar Clemson Spineless (obtained from Bakker brothers Co., Noord- Scharwoude, Holland) were sterilized with 10% sodium hypochlorite for 10 min and thoroughly washed with distilled water. The sterilized seeds were then germinated in an incubator at 25°C for 4 days. After germination seven homogeneous seedlings were visually selected and transferred to pot. The soil

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used in the pots was obtained from the same form and mixed with sand (1:5 v/v). The mixture was autoclaved for 4 h at 121°C before use [8]. Plants were grown in the greenhouse with diurnal regime of 16 h light at 27-29°C and 8 h dark at 18-20°C, light intensity 150 μmol.m⁻².s⁻¹ and relative humidity 30-40% and were fed with half-strength Hoagland solution and distilled water for 4 weeks. The plants were grown in 80 pots and after 28 days of growth they divided into 4 sets of 20 pots. One set served as the control, another set received UV radiation for 12 days. UV-B radiation was applied with two (15 w) UV-B lamps (LF-215 m, 312 nm), UV-A radiation was applied with two insecticide lamps (F2O/BL-Hitachi, Japan) and UV-C radiation was applied with a germicidal lamp (TUV30T8, Philips, Holland). The biologically effective level of UV-A, UV-B and UV-C radiation were 14.5 kJ m⁻²d⁻¹, 11.3 kJ m⁻²d⁻¹ and 6 kJ m⁻²d⁻¹ respectively.

The level of lipid peroxidation products was estimated following the method of Heath and Packer [9]. 0.2 g fresh weight was homogenized in 5 ml of 1% (w/v) TCA. The homogenate was centrifuged at 4000 g for 20 min and supernatant made up to 10 ml. To 1 ml of the aliquot of supernatant 1 ml of 0.17 mM nitroblue tetrazolium (NBT) salt in ethanol, 13 mM methionine, 0.007 mM riboflavin, 0.1 mM EDTA, and 1% PVP. The homogenate was centrifuged at 5000 g and supernatant made up to 10 ml, and was used for assay. To 1 ml of extract, 2 ml of DTC reagent was added and incubated for 3 h at 37°C. Then 0.75 ml of ice cold 65% H₂SO₄ was added, allowed to stand for 30 min at 30°C, and then resulting color was recorded at 520 nm. The ascorbic acid content was assayed using a standard curve prepared with AA and the results were expressed in µmol g⁻¹ FW.

Enzyme extraction was prepared for assay of CAT, APX, and GPX using modification method of Kang et al. [13]. 0.5 g of fresh tissues was homogenized under ice cold in 6 ml of extraction buffer containing, 50 mM Tris-HCl buffer (pH 7.5), 3 mM MgCl₂, 1 mM EDTA, and 1% PVP. The homogenate was centrifuged at 5000 g for 20 min. For APX extraction buffer containing 0.2 mM AA was also used. The supernatant was stored at -80°C and used for the assay of enzymes activities.

Ascorbate peroxidase assay (EC 1.11.1.11, APX) activity assay was performed according to Asada [14]. The reaction mixture contained 2.5 ml phosphate buffer (pH 7) containing 0.1 mM EDTA, 0.5 mM AA, 0.2 ml 1% H₂O₂ and 0.1 ml enzyme extract in final assay volume of 2.8 ml. The H₂O₂ dependent oxidation of AA was measured by decrease in the absorption at 290 nm (extinction coefficient 2.8 M/m/cm).

Guaiacol peroxidase (EC 1.11.1.7, GPX) activity was measured using a modification procedure of Upadhya [15]. The reaction mixture contained 2.5 ml phosphate buffer (pH 7), 1 ml 1% guaiacol, 1 ml 1% H₂O₂ and 0.3 ml enzyme extract in final assay volume of 4.8 ml. GPX activity was determined in the homogenates by measuring the increase in absorption at 470 nm (extinction coefficient 26.6 M/m/cm).

Catalase (EC 1.11.1.6, CAT) activity was measured according to Aebi [16] by measuring the decrease in absorption at 240 nm in a reaction medium containing 2.5 ml phosphate buffer (pH 7.4), 0.1 ml H₂O₂ and 0.3 ml enzyme extract in final assay volume of 2.9 ml (extinction coefficient 4.7 M/m/cm).

Glutathione reductase (EC 1.6.4.2, GR) activity was measured using modification of the procedure of Foyer and Halliwell [17] by following the decrease in absorption at 340 nm due to NADPH oxidation. The reaction mixture contained 50 mM phosphate buffer (pH 7) with 2.5 mM MgCl₂, 0.5 mM GSSG, 0.2 mM NADPH, and 0.3 ml enzyme extract in final assay volume of 2.8 ml (extinction coefficient 6.2 M/m/cm).

The contents of H₂O₂ production was measured colorimetrically using the method of Jana and Choudhuri [18]. The intensity of yellow color developed was recorded at 410 nm and the amount of H₂O₂ was calculated using a standard curve prepared with (H₂O₂) and the results were expressed in µmol (H₂O₂) g⁻¹ Fw⁻¹.

The anthocyanin content was determined according to the method described by Fullick and Francis [19]. The control and treated leaves (0.1 g) were homogenized in 10 ml of acidified methanol (1:99, HCl: methanol). The homogenate was centrifuged at 6000 g for 10 min. Extract was incubated at room temperature in dark for 24 h. Then, the absorption was read at 550 nm. The total flavonoid content was determined according to the aluminum chloride colorimetric method described by Chang et al. [20]. The control and treated leaves (0.1 g) were homogenized in 1 ml deionized water. This solution (0.5 ml) was mixed with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of deionized water. After incubation
at room temperature for 40 min, the reaction mixture absorption was measured at 415 nm. Then the absorbance was read at 415 nm.

For statistical analysis, each value was the mean of four replicate experiments (±SE). The data were statistically analyzed using (ANOVA), followed by Duncan's multiple range test. Standard error of mean was also calculated for presentation in figures.

Results:

UV had a significant effect on AA content in okra plants. In the leaves, stems, and roots of plants treated with UV-B, the AA content significantly increased to respectively 41.31, 44.77, and 32.94% and with UV-C treatment significantly increased to respectively 38.9, 58.42, and 42.4% of the controls. But in the different parts of plants treated with UV-A, AA content increased by 9.59, 9.75, and 12.30%, respectively, not significantly compared to the control plants (Figure 1.a). MDA content in both leaves and stems of the plants treated with UV-B significantly increased by 42.30 and 41.53%, respectively, but in the root of plants treated with UV-B, the MDA content increased by 15.78%, not significantly compared to the control plants. UV-C treatments also increased MDA content in the three parts of the treated plants significantly (46.43, 46.2, and 30% respectively in leaves, stems, and roots), but in UV-A treated plants there was no significant increase (7.4, 3.4 and 7.35% respectively in leaves, stems, and roots in comparison with the controls) (Figure 1.b).

Okra plants grown under the UV-A, UV-B, and UV-C treatment showed different enzymatic activities. SOD activity significantly increased in UV-B and UV-C treated leaves by 44.3 and 50.63% respectively. Also, in the stems of SOD activity significantly increased by 37.45 and 47.66%, respectively. But in leaves and stems UV-A treated plants there was no significant increase (13.87 and 7.16% respectively in comparison with the control). In the root of plants treated with UV-C the SOD activity significantly increased by 41.9%, but in the leaf, stem and root of plants treated with UV-A (13.87, 7.17, and 10.38%, respectively) and in the root of UV-B treatment (17.04%) SOD activity did not significantly increase when compared to the control plants (Figure 2.a). CAT activity increased due to UV-B in the leaves, stems, and roots when compared to control (Figure 2.b). Significant increase (55.1, 63 and 50%) in CAT activity was observed in leaves, stems and root respectively of UV-B treated plant as compared to leaves, stems and roots of control. CAT activity in leaves, stems and roots significantly increased by about 64.38, 67.5, and 62% at UV-C respectively, but no significant changes in CAT activity were observed in the leaves, stems and roots of okra exposed to UV-A treatment while the activities showed increase by about 16.11, 11.5, and 4.9% in respectively leaves, stems and roots when compared to the control plants (Figure 2.b). Figure 3.a shows changes of APX activity in leaves, stems, and roots of okra plant under different UV treatment. APX activity significantly increased in UV-B and UV-C treated leaves by 44.7 and 62.79%, respectively. APX activity in stems significantly increased by about 43.66 and 50.25% at UV radiation of UV-B and UV-C, respectively. APX activity in roots significantly increased by about 40.8 and 44.22% at UV-B and UV-C, respectively. But no significant changes in APX activity were observed in the leaves, stems and roots of okra exposed to UV-A treatment while the activities showed increase by about 6.7, 13.19, and 9.37%, respectively. Changes in GPX activity of leaves, stems, and roots of okra plants at different UV treatments are shown in figure 3.b. GPX activity increased due to UV-B in the leaves, stems, and roots compared to the control. GPX activity of both leaves and stems of plants treated with UV-B significantly increased by 52.43 and 48%, respectively, but, in the root of plants treated with UV-B, the GPX activity increased by 26.76%, not significant compared to the control plants. UV-C treatments also increased GPX activity in the leaves, stems and roots of the treated plants. Significant increase in UV-C treated okra leaves, stems, and roots (by 76.30, 57.36, and 42.21%, respectively) was observed, but in UV-A treated plants there was no significant increase (by 11.29, 13.26, and 4.48%, respectively) in comparison with the control (Figure 3.b). GR activity increased during UV radiation treatment in the leaves, stems, and roots of okra plants (Figure 4.a). In the leaves and stems of plants treated with UV-B, the GR activity significantly increased by 52.44 and 49.2%, respectively, but in the roots of plants treated with UV-B, GR activity increased by 29.74%, not significantly compared to the control plants. In the leaves, stems and roots of the plants treated with UV-C, GR activity significantly increased by 72.84, 63, and 55% compared to the control plants (Figure 1.a). But in the leaves, stems, and roots of plants treated with UV-A, GR activity increased by 35.35, 15.81, and 3.64%, respectively, not significant compared to the control plants (Figure 4.a).

Figure 4.b shows that the exposure to UV radiation increased the H$_2$O$_2$ content in the leaves, stems and roots compared to the control plants. H$_2$O$_2$ content significantly increased by 40.52, 51, and 36.05% in respectively leaves, stems, and roots of the UV-B treated plants, and significantly increased by 50.85, 41 and 59.1% of okra leaves, stems, and roots respectively, compared to the control plants. However in the leaves, stems and roots of plants treated with UV-A, H$_2$O$_2$ content increased by 3.88%, 6.2%, and 16.07%, respectively, not significant compared to the control plants (Figure 4.b).

Significant changes in total flavonoid contents were observed in the leaves under different UV treatments when compared to the controls. In the leaves of plants treated with UV-B and UV-C, total flavonoid content significantly increased by 16.66 and 30.55%, respectively. However in the leaves of plants treated with UV-A
total flavonoid content increased by 3.83%, not significant in comparison with the controls (Figure 5.a). Results of anthocyanin content are given in figure 5.b. In the leaves of the plants treated with UV-B, the anthocyanin content significantly increased by 21.78% and with UV-C treatment significantly increased by 29.16% compared to the control plants, but in the leaves of the plants treated with UV-A, anthocyanin content increased by 2.82%, not significant when compared to the control plants (Figure 5.b).

Discussion:
Production of ROS, can be induced by interaction between UV-B radiation, oxygen, and certain organic compounds, such as dissolved organic matter or humic substances [21]. SOD converts O$_2$ to H$_2$O$_2$ that is then scavenged in chloroplast by a series of oxidation/reduction reactions known as the Halliwell-Asada pathway, using AA, GSH, and NADPH as electron donors [22]. In the present study, the treatment with UV-B and UV-C increased the H$_2$O$_2$ amount in okra plants significantly. Similar increase in concentration of H$_2$O$_2$ was also in Cassia seedlings [23], in Crotalaria juncea [24], in Pea [25] and in peanut seedling [6], probably as a result of induced superoxide dismutase activity during the treatment [26]. The available report indicate that H$_2$O$_2$ could be important as a secondary messenger that induces different defense processes in plants and could play a role in lignin synthesis [27, 28]. We can consider that one of the causes of the increase in antioxidant defense system in okra plant is the increase of H$_2$O$_2$ in UV-B and UV-C treated plants. Also, H$_2$O$_2$ might act as a signal molecule to induce different defense systems. Significantly increased concentrations of MDA as an index of lipid oxidation in biological system in the okra plants treated by UV-B and UV-C was in agreement with earlier observations of Agarwal (2007) in Cassia seedlings [23], Dai et al. (1997) in rice (Oryza sativa) leaves [29], Hernan et al. (2002) in sunflower cotyledons [30], and in peanut seedlings [6]. MDA is the product of peroxidation polyunsaturated fatty acid, in particular linolenic acid and is used as indicator of degree of membrane injury and indicates lipid peroxidation level [31]. Our results suggest that increase in ROSs in the UV treatment increases MDA content in okra. In this study, the SOD, CAT, APX, GPC and GR activity significantly increased in UV-B and UV-C treated okra plants after a 12 day treatment. Increased SOD activity have also been reported in other plant species in response to UV radiation, Agarwal (2007) in Cassia seedlings [23], in Crotalaria juncea [24], in pea [25], in peanut seedlings [6], and in cucumber [32]. In this study, the total SOD activity in okra treated with different UV illuminations. Moreover, previous studies suggested that increased activity of plant enzymes, such as SOD after the UV radiation was assessed as adaptive response of the plant towards oxidative stress caused by harmful factors [33]. Induction of CAT activity after treatment of the plants with different UV radiation has previously been reported in Cassia seedlings [23], in peanut seedlings [6] and in Capsicum annuum [7]. Decreased CAT activity in the vtc1 mutants of A. thaliana during UV-B treatment has been observed and it might be due to destruction of the peroxisome via increase in lipid peroxidation [34]. The APX is one of the important peroxidases in H$_2$O$_2$ detoxification, which operate both in cytosol and chloroplasts. In the first step of the ascorbate-glutathione cycle, APX consumed two AA as electron donor to reduce H$_2$O$_2$ to H$_2$O. GR is the key enzyme of the ascorbat-glutation cycle for the removal of H$_2$O$_2$ in different cellular compartments [35] and a member of flavoenzyme family which catalyzes the regenerates GSH from GSSG, with NADPH as a source of reducing power. This reaction maintains a proper GSH/GSSG concentration ratio in cells [36, 37]. Induction of APX enzymes activity after treatment of plants with UV radiation has previously been reported [6, 7]. In the present study, UV stress caused an increase in activity of GPX. This enzyme is an important member from peroxidase and is used as a substrate for the measurement of POX activity [38], which is one of the most widely distributed antioxidant enzymes in the plant cells [39]. In agreement with our results, Hernan et al. (2002) have reported that UV-B stress caused an increase in activity of GPX and CAT which have an important role in the control of endogenous H$_2$O$_2$ content. Increased POX activity has also been reported in other plant species in response to UV radiation [30].

In the present study, the AA and UV absorb compounds (anthocyanin and flavonoid) content significantly increased in UV-B and UV-C treated okra plants after 12 days of treatment. Nasibi et al. (2005) found that DHAS and AA contents of Brassica napus increased after exposure to UV-B and UV-C [40]. Observed that the AA deficient sozI A. thaliana mutants were sensitive to oxidative damage caused by exposure to UV-B and sulphur dioxide [41]. AA is a substrate for APX enzyme in detoxification of H$_2$O$_2$ and has the capacity to directly eliminate several different ROSs including singlet oxygen, superoxide, and hydroxyl radicals. Also AA has a major role in photo protection as a cofactor utilized in xanthophyll cycle [42]. As a result, the increase in AA content observed in UV-B and UV-C treated plants might act as an antioxidant compound to reduce oxidative damage in different parts of okra plants. Some plants are more tolerant to UV-B than others because they produce a variety of secondary metabolites that effectively absorb UV radiation and prevent it from penetrating into the leaf mesophyll cells [5]. Flavonoids are the most common group of polyphenolic compounds and useful for screening out damaging UV-B radiation [43]. For conclusion increase in UV absorbing compounds prevents from penetration of UV radiation to sensitive tissues. UV-B radiation can stimulate key transcripts of PAL in the phenylpropanoid pathway [44, 45]. Increase in PAL activity stimulates the synthesis of flavonoid and anthocyanin. The increase in PAL activity in Phyllanthus amarus L. and
increased synthesis of flavonoid, and anthocyanin have been shown in UV-B treated <i>Arabidopsis thaliana</i> L. seedlings [46], and treated with UV was observed [5]. Ravindran (2008) reported that the flavonoids and anthocyanin had an important role in the solar screens by absorbing UV and preventing penetration of this radiation into sensitive mesophyll tissue [37].

Fig. 1: Effects of different UV treatments on okra leaves, stems and roots. (a) Ascorbic acid (AA) content and (b) Lipid peroxidation (MDA) content. (Mean±SE, n = 4), P < 0.05.

Fig 2: Effects of different UV treatments on okra leaves, stems and roots. (a) Superoxide dismutase (SOD) activity, and (b) catalase (CAT) activity. (Mean±SE, n = 4), P < 0.05.

Fig. 3: Effects of different UV treatments on okra leaves, stems and roots. (a) Ascorbate peroxidase (APX), and (b) guaiacol peroxidase (GPX) activity; (Mean±SE, n = 4), P < 0.05.
Fig. 4: Effects of different UV treatments on okra leaves, stems and roots (a) glutathione reductase (GR) activity, and (b) hydrogen peroxide (H$_2$O$_2$) content; (Mean±SE, n = 4), P < 0.05.

Fig. 5: Effects of different UV treatments on okra leaves, stems and roots (a) flavonoid content (b) anthocyanin content. (Mean±SE, n = 4), P < 0.05.

Conclusion:
The results show an increase in enzymatic and non-enzymatic antioxidant when plants are exposed to UV-B or UV-C radiation while antioxidant enzymes activities and antioxidant compounds do not significantly increase under the UV-A radiation treatments compared to the control plants. Exposure of the okra plants to UV radiation led to the accumulation of H$_2$O$_2$ in different tissues, which increased antioxidant enzymes activity (SOD, APX, CAT, GR, and GPX) and AA. Flavonoids and anthocyanins might be involved as part of the defenses against UV stress. Therefore, increased level of lipid peroxidation products and anyioxidant system activity may be considered as biomarkers of intensity of UV radiation stress in this species and family.

Abbreviations:
AA (Ascorbic acid), GSH (Reduced glutathione), GSSG (Glutathione disulfide), H$_2$O$_2$ (Hydrogen peroxide), MDA (Malondialdehyde), ROS (Reactive oxygen species), TBA (Thiobarbituric acid), TCA (Trichloroacetic acid), NBT (Nitroblue etrazolium), FW (Fresh weight), PAL (Phenylalanine ammonia lyase), DHAS (Dehydroascorbate), POX (Peroxidase), UV (Ultraviolet).

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