



Changes in Physiological and Biochemical Parameters of Gourd (*Cucurbita pepo* L.) Two Varieties under Drought and UV-B Radiation

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Abstract

It emerged recently that there is an inter-relationship between drought and Ultraviolet-B (UV-B) radiation in plant responses, in that both stresses provoke an oxidative burst. The objective of the present investigation was to study the effect of drought stress, UV-B radiation and the combined effects of UV-B and drought stresses on two cultivars of gourd seedling. The gourd plants were grown with diurnal regime of 16h light and 8h dark and temperature of 22/26°C (night/day). The relative humidity 60% and average of photosynthetically active radiation was 150 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Treatments was started 25 days after planting. Then, 25-days-old seedlings were divided in to four groups. The control and three groups subjected to stress conditions: UV-B radiation, drought stress conditions, UV-B radiation and drought stresses combined. The results of measurement on compounds which absorbs UV including flavonoids, anthocyanins, phenol and antioxidant enzymes activity including catalase, guaiacol peroxidase showed that these parameters increased when plant irradiated with UV-B, drought stress and their combination. Biochemical parameters indicate that with this experiment situation UV-B stress has stronger stress effectors than drought on the growth of seedling of both cultivars. The present results suggested that, under soil drought conditions, enhanced UV-B radiation had not let to greater decrease gourd biochemical markers. The results suggested that co-stresses of supplementary UV-B radiation and drought synergistically functioned and one of them could alleviate the inhibitory effects of another under the condition of arid and semiarid loessial soils.

Keywords: Gourd; Ultraviolet radiation; Drought stress; Antioxidant enzymes

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Introduction

The effects of extreme weather events on vegetation and ecosystem functioning are likely to be much stronger than the effects of changes in mean values of temperature and precipitation (Easterling et al., 2000). Ultraviolet radiation

plays a key role in several biological functions, sometimes detrimental (e.g. DNA damage, immune suppression, cataracts) and others beneficial (e.g. assimilation of vitamin D, diminishing of risk of some internal cancers). However, there is no general health benefit in exposing crops and medicinal plants to extra UV B and UV C radiations (Zhang and Bjorn, 2009). Drought is one of the most significant factors among abiotic stresses that limit plant

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performance, growth and productivity (Chaves and Oliveira, 2004). In addition, among the abiotic stressors, drought and increased levels of ultraviolet-B (UV-B) radiation have received much more attention, because of their potential to impair many plant species (Agrawal et al., 2009). The combination of stresses cause a variety of interaction responses that can be adaptive or cumulative. Because it is produced at protection mechanisms of plants against oxidative stress by both drought and UV-B (Cechin et al., 2008). Plants have two ways for thermolysis toxic of reactive oxygen species includes either non-enzymatic (flavonoids, carotenoids, tocopherols) or enzymatic constituents (Basu et al., 2010). Plants have developed a complex biochemical defence system that including carotenoids and flavonoids. Flavonoid compounds, as secondary metabolites are considered to play a major role in protecting plants from UV-B damage (Liang et al., 2006). Flavonoids are frequently found in or on epidermal layers where they can increase markedly following UV-B treatment. Studies with mutants further highlight the importance of flavonoids for UV-B protection (Ryan et al. 2001). Alexieva et al. (2001) concluded that both stresses acted synergistically to induce protective mechanisms (antioxidant compounds). Since drought and UV-B radiation induce similar protective mechanisms (e.g. Hofmann et al. 2003; Cechin et al. 2008). Therefore, the purpose of the present study is to further examine the interactive effects of UV-B radiation and mild water stress on some biochemical traits included proline, flavonoids, soluble phenols and some related enzyme activity in gourd seedlings.

Materials and Methods

Plant material and treatment conditions

The seeds were surface sterilized with 10% sodium hypochlorite for 10 min then thoroughly rinsed with distilled water before further experimentations. Seeds were germinated in petri dishes containing two sheets of filter paper and wetted with distilled water for two days. Two days later, germinated about 95% seeds. After germination, seedlings were transferred in plastic pots filled with soil/sand mixture (5:1) (Hosseini Sarghein et al., 2011). The

gourd plants were grown with diurnal regime of 16 h light and 8 h dark and temperature of 22/26 °C (night/day). The relative humidity was 60% and average photosynthetically active radiation 150 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}$. Treatments were started 25 days after planting. Then, 25-days-old seedlings were divided in four groups: the control and three groups subjected to stress conditions: UV-B radiation, drought stress conditions and UV-B radiation plus drought stress for 14 days. As a source of UV-B radiation was artificially supplied by two lamps 2(15w) (LF-215.312 nm) suspended above the plants, at 60 cm distance for 30 min daily. For drought treatment determining the field capacity of the soil, the plants were irrigated with 25% of soil field capacity (Amiry dehahmadi et al., 2012). All measurements were made after 14 d of the stress treatments. Biochemical analyses were carried out on fresh plant material, that was immediately extracted and assayed according to the appropriate methods listed here.

Determination of Malondialdehyde (MDA) content

The level of lipid peroxidation was measured by the amount of malondialdehyde (MDA), a product of unsaturated fatty acid peroxidation. Rate of membrane lipid peroxidation was determined by estimating malondialdehyde (MDA) content with thiobarbituric acid (TBA) and trichloroacetic acid (TCA) (Ghanati et al., 2013).

Antioxidant enzymes assay

Antioxidant enzymes extracts were prepared according to the Kang and Kang and Saltveit (2002) methods. The GPX activity was determined using the method of Plewa et al, (1991) following the formation of tetraguaiacol by measuring the absorbance at 470nm and using an extinction coefficient $25.5\text{ mM}^{-1}\text{ cm}^{-1}$. Catalase activity was assayed by measuring the initial rate of H_2O_2 disappearance at 240nm using the extinction coefficient $40\text{ mM}^{-1}\text{ cm}^{-1}$ for H_2O_2 (Velikova et al., 2000).

Table 1

MDA content ($\mu\text{M/g}$ FW) in shoot and root of two Gourd cultivars (Mashhadi and Hamadani) subjected to drought and UV-B stresses applied separately or in combination. Data are means of three replicates with standard errors (Mean \pm SE, n=3).

Cultivar	Treatment	Root MDA ($\mu\text{M/g}$ FW)	Shoot MDA ($\mu\text{M/g}$ FW)
Mashhadi	Control	0.300 \pm 0.005 ^c	0.133 \pm 0.003 ^c
	UV-B radiation	0.390 \pm 0.005 ^a	0.243 \pm 0.003 ^a
	Drought	0.320 \pm 0.010 ^{bc}	0.166 \pm 0.003 ^b
	UV-B +Drought	0.353 \pm 0.008 ^b	0.243 \pm 0.003 ^a
Hamadani	Control	0.376 \pm 0.008 ^c	0.140 \pm 0.005 ^c
	UV-B radiation	0.623 \pm 0.003 ^a	0.246 \pm 0.003 ^a
	Drought	0.503 \pm 0.008 ^b	0.186 \pm 0.003 ^b
	UV-B +Drought	0.483 \pm 0.088 ^b	0.240 \pm 0.005 ^a

*Different letters in each column show significant differences at $P \leq 0.05$

Determination of total phenolics content (TPC)

The TPC was determined using the Folin-ciocalteu reagent (FCR) and gallic acid as standard based on the method described by Marinova et al. (2005). The sample (25 μl) and 1.25 ml of sodium carbonate (75g/L) were added to 2.5 ml of 10% (v/v) FCR. After 1.5 h of reaction at room temperature, the absorbance was measured at 765 nm in a spectrophotometer. The results were given as mg gallic acid equivalent/100 g extract (mg GAE/100 g extract).

Determination of total flavonoid content (TFC)

The TFC was determined using aluminium chloride (AlCl_3) and quercetin (standard) as described by Chang et al., (2002). 0.1 ml plant extract was added to 0.3 ml distilled water followed by 0.03 ml NaNO_2 (5%). Then, AlCl_3 (0.03 ml, 10%) was added after 5 min and at 25 °C. After further 5 min, the reaction mixture was treated with 0.2 ml of 1 mM NaOH. Finally, the reaction mixture was diluted to 1 ml with water and the absorbance was measured at 510 nm. The results were expressed as mg quercetin equivalent/ 100 g extract (mg QE/100 g extract).

Determination of total anthocyanin content (TAC)

The total anthocyanin was extracted with MeOH: HCl (99:1), and were measured at 550 nm (Masukasu et al., 2003)

Determination of free proline

The free Proline content was determined according to Bates et al. (1973). Frozen leaf tissue (0.5 g) was homogenized with 10 ml of 3% sulfosalicylic acid at 4 °C. The extract was filtered with Whatman No. 2 filter paper. In a test tube, 2 ml of filtrate, 2 ml of acid-ninhydrin, and 2 ml of glacial acetic acid were mixed and incubated at 100 °C for 1 h. The reaction was terminated on ice, and the reaction mixture was then extracted with 4 ml of toluene. The chromophore-containing toluene was separated from the hydrated phase. The absorbance at 520 nm was spectrophotometrically determined with toluene as the blank. The Proline concentration was calculated based on a standard curve.

Statistical analysis

The data were statistically analysed from a one-way ANOVA using the SPSS (version 21). The mean value of three replicates and standard errors were calculated. Tukeys multiple range testes ($p < 0.05$) were performed to determine the significance of the results.

Table 2

Changes in the content of anthocyanin, phenol and flavonoid in leaves of two Gourd cultivars (Mashhadi and Hamadani) subjected to drought and UV-B stresses applied separately or in combination. Data are means of three replicates with standard errors (Mean± SE, n=3)

Cultivar	Treatment	Phenol ($\mu\text{M/g FW}$)	Flavonoid ($\mu\text{g/g FW}$)	Anthocyanin ($\mu\text{M/g FW}$)
Mashhadi	Control	1.95±0.028 ^b	4.46±0.035 ^d	18.00±0.173 ^d
	UV-B radiation	2.60±0.028 ^a	6.33±0.033 ^a	25.03±0.145 ^a
	Drought	2.43±0.044 ^a	5.18±0.006 ^c	23.96±0.072 ^b
	UV-B +Drought	2.58±0.043 ^a	5.76±0.120 ^b	19.66±0.240 ^c
Hamadani	Control	3.02±0.037 ^c	6.13±0.120 ^d	24.20±0.115 ^c
	UV-B radiation	3.93±0.033 ^a	8.45±0.028 ^a	28.00±0.288 ^a
	Drought	3.33±0.044 ^b	6.52±0.014 ^c	26.00±0.115 ^b
	UV-B +Drought	3.88±0.008 ^a	8.10±0.057 ^b	24.23±0.185 ^c

*Different letters in each column show significant differences at $P \leq 0.05$

Results

The data indicated that plant responses to simultaneously imposed stress conditions were distinctly different from the responses displayed by plants when the stresses were applied individually. In both cultivars UV irradiation applied alone generally enhanced the biochemical parameters such as root and shoot MDA, anthocyanins, flavonoids, phenol and antioxidants enzymes (CAT and GPX) but did not significantly effects root and leaf Proline. Drought stress enhanced the root and leaf Proline. There was no marked effect of drought on other parameters. It could be expected drought significantly influenced the Proline, and UV-B affected MDA, anthocyanins, flavonoids, phenol

and antioxidants enzymes (CAT and GPX). When UV-B radiation and drought stress were applied together the effects were reduced less comparison with the injurious effects of a single stress factor. The effects of the stresses on the MDA content measured in two cultivars seedlings are documented in Table 1. MDA content were significantly enhanced in roots and shoots of two cultivars after application of drought and UV-B radiation treatment and their combination. But this increase in shoots of Mashhadi was higher than Hamadani cultivar and in roots of Hamadani cultivar was higher than Mashhadi cultivar. In shoots of both cultivars increase was higher than roots. MDA content was higher by UV-B treatment than drought treatment. In both cultivars UV irradiation and drought stress

Table 3

CAT activity in roots and leaves of two Gourd cultivars (Mashhadi and Hamadani) under UV-B radiation, drought and combination of two treatments. Data are means of three replicates with standard errors (Mean± SE, n=3)

Cultivar	Treatment	Root CAT ($\mu\text{M min}^{-1}/\text{g FW}$)	Leaf CAT ($\mu\text{M min}^{-1}/\text{g FW}$)
Mashhadi	Control	5.00±0.115 ^c	3.28±0.029 ^c
	UV-B radiation	12.23±0.145 ^a	5.93±0.044 ^a
	Drought	9.16±0.088 ^b	5.26±0.018 ^b
	UV-B +Drought	9.00±0.057 ^b	5.35±0.076 ^b
Hamadani	Control	8.33±0.120 ^c	6.50±0.115 ^c
	UV-B radiation	13.00±0.057 ^a	8.50±0.057 ^a
	Drought	10.43±0.120 ^b	7.78±0.109 ^b
	UV-B +Drought	10.65±0.076 ^b	7.83±0.088 ^b

*Different letters in each column show significant differences at $P \leq 0.05$

Table 4

GPX activity in roots and leaves of two Gourd cultivars (Mashhadi and Hamadani) under UV-B radiation, drought and combination of two treatments. Data are means of three replicates with standard errors (Mean±SE, n=3).

Cultivar	Treatment	Root GPX ($\mu\text{M min}^{-1}/\text{g FW}$)	Leaf GPX ($\mu\text{M min}^{-1}/\text{g FW}$)
Mashhadi	Control	130.21±1.340 ^c	105.66±1.20 ^c
	UV-B radiation	150.33±0.881 ^a	171.33±3.28 ^a
	Drought	138.35±1.207 ^b	140.00±2.88 ^b
	UV-B +Drought	136.33±0.881 ^{bc}	149.66±1.45 ^b
Hamadani	Control	151.39±1.19 ^d	84.33±1.45 ^c
	UV-B radiation	198.00±1.52 ^a	141.33±0.881 ^a
	Drought	176.66±1.20 ^b	113.00±2.51 ^b
	UV-B +Drought	164.66±2.33 ^c	135.00±1.54 ^a

*Different letters in each column show significant differences at $P \leq 0.05$

applied along generally increased the MDA content.

The amounts of anthocyanins increased in both cultivars after application of UV-B irradiation and this increase in mashhadi was higher than hamadani cultivar and in UV-B radiation was higher than drought treatment and in two stresses combination was lower than alone stress (Table 2). The amounts of anthocyanins increased in both cultivars after application of UV-B irradiation and drought stress and it was more pronounced in Mashhadi cultivar and in Hamadani cultivar. The combination of stresses resulted in anthocyanins that were no higher than those induced by

drought alone. The UV absorbing compounds (flavonoids and phenolics) were also increased after the application of UV-B irradiation and drought stresses (Table 2). CAT activity increased in roots and leaves of both cultivars after drought and UV-B radiation treatment and their combination. But this increase in Mashhadi was higher than Hamadani and in roots of both cultivars was higher than leaves (Table3). The combined application of both stresses did not lead to any significant changes in the enzyme activities in comparison with the UV-B application alone. This class of substances was also affected significantly by UV-B and in both cultivars the

Table 5

Effect of drought and UV-B irradiation applied alone or in combination on free proline content in two Gourd cultivars (Mashhadi and Hamadani) in roots and leaves. Data are means of three replicates with standard errors (Mean± SE, n=3).

Cultivar	Treatment	Root Proline ($\mu\text{g}/\text{g DW}$)	Leaf Proline ($\mu\text{g}/\text{g DW}$)
Mashhadi	Control	0.850±0.011c	0.923±0.014b
	UV-B radiation	0.993±0.014b	1.013±0.008b
	Drought	1.073±0.008a	1.49±0.023a
	UV-B +Drought	0.950±0.011b	1.45±0.028a
Hamadani	Control	0.900±0.005b	1.35±0.028b
	UV-B radiation	0.950±0.011b	1.41±0.016b
	Drought	1.056±0.008a	1.88±0.008a
	UV-B +Drought	1.050±0.011a	1.89±0.014a

*Different letters in each column show significant differences at $P \leq 0.05$

combination of stresses resulted in flavonoids that were no higher than those induced by UV-B alone. Guaiacol peroxidase activity were enhanced in roots and leaves of both cultivar after application of drought and UV-B radiation treatment and their combination. But this increase in hamadani cultivar higher than mashhadi cultivar and in leaves of both cultivar higher than roots. GPX activity was higher in UV-B treatment than drought treatment (Table4).

Proline content was significantly enhanced in roots and leaves of two cultivars after application of drought and UV-B treatment and their combination. But this increase in mashhadi cultivar was higher than hamadani cultivar and in leaves of both cultivars was higher than roots. Proline content was higher by drought treatment than UV-B treatment (Table5). An increase in the amount free Proline was observed in roots and leaves both cultivars due to the action drought stress. A significant increase was not observed after UV-B treatment in both cultivars. These data support the hypothesis that what may be the protective interaction between UV-B and drought stresses a UV-B positive effect on drought-stressed gourd plant.

Discussion

UV-B treatment caused a stronger effect than drought on the main growth parameters of Gourd seedlings. There was no marked effect of drought on shoots and roots MDA content. In results when UV irradiation and drought stress applied together the effects were reduced less in comparison with the effects of a single stress factor. The negative UV irradiation effects in gourd seedlings were alleviated when UV stress was applied to moderately water-stressed seedlings. Leaf MDA content, the product of lipid peroxidation, is a prominent indicator of membrane impairment and free radical production (Katsuhara et al., 2005).

Ghanati et al. (2013) concluded that Increase of flavonoids, anthocyanins, tannins and wall-bound phenolics in UV-treated *Malva* cells might be a part of their defensive response.

Mutants of *Arabidopsis* lacking flavonoids are hypersensitive to UV radiation whereas an *Arabidopsis* mutant possessing constitutive elevated accumulation of flavonoids and other phenolics is tolerant to lethal UV level (Bieza and Lois, 2001). They may act in the leaf as solar screens by absorbing UV before it reaches UV-sensitive targets such as chloroplasts and other organelles. An increase of anthocyanins and soluble phenols after UV-B irradiation was observed either in wheat or pea leaves (Alexieva et al., 2001). Feng et al. (2007) concluded that flavonoid concentration can reduce the UV-B penetration and protect the photosynthetic apparatus to some extent depending on a threshold level, which may vary indifferent species. Flavonoid accumulation is also considered a defense mechanism against UV-B radiation and protects the mesophyll tissue through epidermal screening. UV-B screening by epidermal flavonoids is often proposed as an adaptive mechanism to prevent this radiation from reaching the mesophyll. The interactive role of UV-B and soil drought may improve the tolerance to one of two stresses, accordingly, alleviate the adverse effect on spring wheat biomass and yield (Feng et al., 2007).

The present results suggested that, under soil drought conditions enhanced UV-B radiation had not let to greater decrease gourd biochemical markers these were attributed to synergistically effects between UV-B and water stress. The changes of MDA content, anthocyanins, phenols and flavonoid content under the combined conditions could explain our results. Between the markers and enzymes assayed, Proline can be put forward as the main drought-induced factor that can exert a protective action on UV-B radiation stress (Alexieva et al, 2001). Proline acts as an osmolite beside enzymes and other macromolecules, and therefore, protects the plant against low water potential and causes osmotic regulation in plant organs. Also proline can act as an electron receptor preventing photosystems injuries in dealing with ROS function. Proline accumulation facilitates the permanent synthesis of soluble substances in closing stomata (Ghorbanli et al, 2013). The removal of excess H⁺ occurring as result of proline

synthesis may have a positive effect on the reduction of the UV-B-induced damage (Alexieva et al., 2001). Proline accumulation is a very common responses in plants subjected to drought stress (Alexieva et al., 2001; Yang et al., 2005). The combination of stresses suppressed the drought effect and proline content detectable increases both cultivars. In previous studies, free proline accumulation increases after enhanced UV-B radiation in *Trifolium repens* (Hofmann et al., 2003) and *Vicia faba* (Shetty et al., 2002).

In a research effect of drought stress on sunflower (Nazarli et al., 2011) and effect of UV-B radiation and their combination in cucumber was enhanced GPX activity (Kubis and Rybus, 2008). CAT is the most efficient antioxidant enzyme which protects plant by scavenging free radicals and H₂O₂ (Gao et al., 2008). Results of CAT activity showed that UV-B lead increase of this enzyme in treatment plants. Determine of CAT activity alfalfa (*Medicago sativa* L.) plant in drought stress conditions showed significantly was enhanced with increase of treatment (Safernejad, 2004). Veselá et al, 2013 concluded that UV-B radiation moderates the negative effects of drought on photosynthesis due to increased content of flavonols and improved water use efficiency. The initial hypothesis was confirmed in both mono- and dicotyledon species.

The data presented showed that UV-B radiation provoked in general more severe damage, evaluated as changes in the amounts of stress markers, than drought stress, when applied separately. Under multiple stress conditions, each of the stress factors seems to bring out some adaptive effects to reduce the damage experienced by plants caused by the other one in gourd plants. In general, the cultivar difference in response to the stresses was found in this experiment. The Mashhadi cultivar was resistible than Hamadani cultivar because higher proline was induced to resistance of drought stress and higher anthocyanins, UV absorbing compounds (flavonoids and phenolics) was produced to UV-B protection of plants.

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