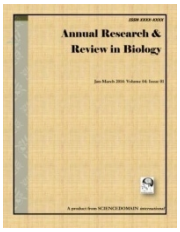

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Effects of Enhanced UV-B Radiation and Drought Stress on Photosynthetic Performance of Lettuce (*Lactuca sativa* L. Romaine) Plants

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Authors' contributions

This work was carried out in collaboration between all authors. They designed the Study, performed the statistical analysis and managed the literature searches. Author IAH managed the field work. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: The aim of the present study was to investigate some biochemical changes in field grown lettuce (*Lactuca sativa* L. cv Romaine) plants in terms of importance of the accumulation of anthocyanins, flavonoids and photosynthetic pigments as well as photosynthetic limitations which changed during exposure of plants to drought stress and UV-B radiation in order to circumvent the deleterious effects of these Stresses.
Place and Duration of Study: The experiment was conducted under filed conditions from November 2012 to January 2013, at the Agricultural Research Center, KAU.
Methodology: The experimental design was a factorial arrangement in randomized complete blocks with four replicates. The first factor was UV-B (300 nm). The second factor was irrigation regime (complete irrigation to field capacity and limited irrigation. Gas exchange measurements were carried out using a *LI-6200* portable IRGA. Chlorophyll fluorescence of Fv/Fm was measured by PAM 2000 fluorometer. Biochemical analyses and antioxidant enzymes assays were performed according to the appropriate methods.
Results: Exposure of lettuce plants to enhanced UV-B radiation and drought stresses (DS) negatively and significantly affected the process of photosynthesis including CO_2 assimilation (P_N), stomatal conductance to water vapour (g_s) and transpiration rate (E). However, the amplitude of the effects of both stressors was dependent on the interactions.

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This resulted in alleviation of the negative effect of drought on photosynthesis and transpiration by UV-B radiation as the water stress intensified. Intercellular CO_2 (C_i) concentration was only reduced due to water stress compared to control plants. The maximum efficiency of photosystem II (F_v/F_m) was not affected by UV-B radiation stress but reduced by drought. There was an increase in the activities of some antioxidant due to both stresses when applied singly and in combination UV-B irradiation increased the contents of the UV-B absorbing compounds (carotenoids, soluble phenols, anthocyanins), while drought stress caused a notable increase in free proline content. Increase in content of Proline may be the drought-induced factor which plays a protective role in response to UV-B.

Conclusion: UV-B radiation provoked in general more severe damage, evaluated as changes in the amounts of stress markers, than DS, 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. DS can induce accumulation of UV-B absorbing compounds (flavonoids, carotenoids and soluble phenols), which is likely to offer some increased protection from UV-B.

Keywords: UV-B − ultraviolet-B radiation; Ds – drought; PN – net photosynthetic rate; gs − stomatal conductance; F_v/F_m – The maximum efficiency of PSII photochemistry; PSII – photosystem II; PAR – Photosynthetically active radiation.

ABBREVIATIONS

UV-B: ultraviolet-B radiation; DS: drought; RWC: relative water content; Chl a(b): chlorophyll a(b); E: transpiration rate; F_v/F_m: The maximum efficiency of PSII photochemistry; PSII: photosystem II; C_i: internal CO_2 concentration; ROS: Reactive oxygen species; AR: Photosynthetically active radiation; FW: fresh wight.

1. INTRODUCTION

Environmental stresses, including UV-B irradiance and drought, trigger a wide variety of plant responses, ranging from altered gene expression and cellular metabolism (e.g. membrane injuries, photosynthetic disorders) to changes in growth rates and crop yields. They have been shown to inhibit photosynthetic electron transport, with PSII as the major site of damage [1,2]. A plethora of plant reactions exist to circumvent the potentially harmful effects of such stresses [3-12]. Since the mechanism of photosynthesis involves various components, including photosynthetic pigments and photosystems, the electron transport system and CO_2 reduction pathways, any damage at any level caused by a stress may reduce the overall photosynthetic capacity of a green plant [1,13,-16]. In natural conditions, there are various stresses that can alter the response of plants to the UV radiation effects [17-23]. Of these stresses, water stress is an important restricting factor that always affects agricultural productivity, particularly in arid and semi-arid regions [24-31. Supplementary UV radiation and drought could act synergically and one of them could alleviate the inhibitory effects of another under conditions of arid and semi-arid soils [32-34].

Negative effects of UV-B radiation was alleviated and masked in drought-stressed soybean due to anatomical (leaf thickening) and biochemical [flavonoids production] changes induced by drought stress [35]. However, deleterious effects of UV-B radiation in the presence of leaf thickening and elevated flavonoids concentrations were observed in earlier studies [36,37].

Both drought and UV-B radiation may independently affect photosynthesis, either directly through alteration photosystem II or indirectly through alteration in stomatal conductance [35,36,38]. Drought may reduce photosynthesis by both stomatal closure and biochemical effects [1,3,39]. Therefore the specific limitations on photosynthesis may vary in response to these specific stresses.

Plants developed different defense mechanisms against UV radiation, such as thicker and smaller leaves [15], increased production of UV-absorbing compounds such as flavonoids, anthocyanins [7,40] and higher amounts of reflective waxes [41-44]. The accumulation of flavonoids in the epidermis was shown to reduce epidermal transmittance of UV radiation and thus may provide some protection [19,45,46]. In fact, measurements of several physiological parameters, such as the gas exchange parameters, levels of UV-B absorbing compounds, and Chl contents, have been proved as useful indicators of UV-B tolerance or sensitivity [3]. The different sensitivities of plants are partially explained by their abilities to respond to UV-B through the induction of defensive pathways [5]. Ultraviolet-B radiation has been shown to reduce photosynthesis by direct alterations of photosystem II [46], while its effect on stomatal conductance is minimal [47]. Drought may reduce photosynthesis by both stomatal closure and biochemical effects [11]. Therefore the specific limitations on photosynthesis may vary in response to these specific stresses. Photosynthetic limitations may be evaluated by gas exchange analyses including the functional responses of photosynthesis to light, internal CO_2 concentrations and O_2 sensitivity [7,24,48].

Evidence of interaction between UV-B exposure and drought stress in plants has emerged in recent years, but the mechanisms involved have received little attention [2,3,7]. Elucidation of the interaction between drought and UV-B stresses would help in understanding the potential impact of partial stratospheric ozone depletion on plant adaptation to changing environmental condition [49]. However, the mechanisms of sensitivity or tolerance of crop plants, either in growth and yield, to combined stresses remain unknown.

Both stresses cause physiological and morphological alterations. Therefore, it is worth to further our understanding of the physiological bases of individual stresses in order to lead to a better explanation of their interactions.

It seems that under multiple stresses, each of the stress factors may bring out some adaptive effects to reduce the damage experienced by plants and caused by the other stress [50]. UV-B irradiation could alleviate the negative effects of water stress on plants or exert an additional inhibitory effect on the functional processes in plants. For example, exposure to both UV-B and water stress led to decreased growth in cucumber and radish, but protein content was increased by the combined stresses [51]. Similar additional injurious effects of UV-B on net photosynthesis of soybean under drought stress were recorded also [51,52]. The interaction between soil water deficit and UV-B stresses in cowpeas resulted in benefits from the combined stresses in terms of greater growth and development as compared with exposure to single stresses [16,53,54].

A common drought stress, UVB radiation, and other environmental stresses could cause the accumulation of reactive oxygen species (ROS) and thus result in oxidative damage [5]. ROS are highly reactive and, in the absence of effective protective mechanism, they can compromise normal metabolism through oxidative damage to pigments, lipids and protein [42,55].

In wheat seedlings, drought stress and UV-B irradiation resulted in the high H_2O_2 accumulation, which caused lipid peroxidation along with the reduction of growth. Moreover, UV-B treatment was found to cause a more severe damage than drought stress on wheat seedlings measured as more obvious reduction in growth and much more strong accumulation of H_2O_2 and increased lipid peroxidation [56,57]. This data corresponded well to [3,4] who also obtained similar results for pea and wheat seedlings. However, the combination of drought stress and UV-B irradiation was additive, in contrast to the other researcher data suggesting an antagonistic effect [3,58]. The growth of wheat seedlings under combined stress was much more retarded than when stresses were applied separately [3].

Many contradictory results about antioxidant enzyme response to different stresses have emerged due to the fact that the levels of enzyme responses depend on the plant species, the developmental stage, the organs, as well as on the duration and severity of the stress [59,60]. In many plants, free proline accumulates in response to biotic and abiotic stresses, including UV-B irradiation [17,23,25]. In wheat seedlings, proline contents were up to 1.71 times higher under drought, UV-B, and combined stresses as compared with the control, respectively.

The aim of the present study was to investigate some biochemical changes in field grown lettuce plants in terms of importance of the accumulation of anthocyanins, flavonoids and photosynthetic pigments as well as photosynthetic rates which could change during exposure of plants to DS and UV-B radiation in order to circumvent the deleterious effects of these stresses.

2. MATERIAL AND METHODS

2.1 Plant Materials and Growth Conditions

The experiment was conducted under filed conditions from November 2012 to January 2013, at the Agricultural Research Center, on a silt loam soil (pH 6.5, CEC 4.6 meq 100 g^{-1} and organic matter 1.0%). Seeds of lettuce (*Lactuca sativa* L. cv. Romaine) were sown directly in late Nov. 2012 in rows spaced 0.5 m apart at a seeding density of 25 seeds m^{-1} in four plots of 5 m x 3 m each. After emergence of first foliage leaf, seedlings were thinned to one seedling/1 cm^2.

2.2 Water Stress and UV-B Irradiation Treatments

Ten days after germination, when first foliage appeared, plants were thinned for uniformity in growth to 15 seedlings m^{-1}. Soil water potential was maintained at approximately -0.5 MPa in well-watered plots and at -2.0 MPa in water-stressed plots by supplemental irrigation.

Lamps for supplemental UV-B radiation were suspended above and perpendicular to the planted rows [rows oriented in an east-west direction to minimize shading] and filtered with either presolarized 0.13 mm thick cellulose diacetate [transmission down to 290 nm] for supplemental UV-B radiation in order to give a biologically effective dose of UV-B equivalent to 10 kJ m^{-2} d^{-1} or 0.13 mm polyester plastic films [absorbs all radiation below 320 nm] as a control to give a biologically effective dose of UV-B equivalent to 5 kJ m^{-2} d^{-1} . We did not use a control plots with 0 kJ $m^{-2}d^{-1}$ UV-B as this is a normal procedure in this type of experiments Moreover, a polyester film curtains were hung between treatments to prevent

the UV-B radiation reaching the treatment 5 kJ $m^{-2}d^{-1}$UV-B. The spectral irradiance from the lamps was determined with an Optronic 742 Spectroradiometer model (Laboratories Inc.), equipped with a dual holographic grating modified to maintain constant temperature. The lamp height above the plants was adjusted weekly to maintain a distance of 60 cm between the lamps and the top of the plants. Daily UVB radiation was for 10h, from 8:00 to 18:00h for the entire period of the experiment. To minimize positional effects, plants were rotated within the sections every 2 days.

One half of the plants, under each UVB condition, were watered to field capacity (well-watered) every day and the other half were watered after the leaves showed visible signs of wilting (water-stressed). These watering regimes were continued until the conclusion of the experiment. Soil water potential at a depth of 0.25 m was monitored continuously using psychrometers to prevent drought stress beyond -0.5 MPa. These watering regimes were continued until the conclusion of the experiment.

The experimental design was a factorial arrangement in randomized complete blocks with four replicates. The first factor was UV-B and the second factor was irrigation regime complete irrigation to field capacity and limited irrigation.

2.3 Net photosynthetic rate [P_N] and stomatal conductance [g_s]

They were measured on the youngest fully expanded leaf. Gas exchange measurements were carried out seven times at 5 d intervals using a *LI-6200* portable IRGA (*LI-COR*, Lincoln, USA) between 10:00 and 14:00 h (Local time). All plants were measured on the same day. Apparent quantum efficiency (AQE) was calculated from initial slope of P_N - PAR response curve [2].

2.4 Chlorophyll fluorescence

Chl fluorescence parameters were measured with a portable fluorometer (*PAM-2000, Walz GmbH*, Effeltrich, Germany) on the same leaves as used for gas-exchange determination. Prior to the measurements, leaves were kept in dark-dapted state for 30 min. Different Chl fluorescence yields were measured and maximum quantum yield of photosynthesis (Fv/Fm), was calculated [61,62]

2.5 Biochemical Analyses and Antioxidant Enzymes Assays

Fresh leaves collected from the four treatments (control, DS, UV-B and DS+ UV-B) were cut and immersed in liquid nitrogen and kept in a deep freezer at -80ºC until the analyses were performed. Extractions of antioxidant enzymes, pigments, proline from the leaves were performed according to the appropriate methods.

Samples were weighed and ground at about ºC in 25 m Tris–HCl buffer containing 3 mM $MgCl_2$, then the homogenates were centrifuged at 20 000 *g* for 15 min (Centrifuge 17 S/RS, Heraeus Sepatech). The supernatants were used for the enzyme assays and the results were expressed on protein basis [15]. All assays were performed using a final volume of 1 mL, with at least duplicate assays undertaken on each sample. Moreover, the assays were end-point determinations [30].

2.5.1 Superoxide dismutase (EC 1.15.1.1, SOD)

The extraction mixture contained 50 mM phosphate buffer solution (pH 7.8), 13 mM L-methionine, 63 µM nitro blue tetrazolium and 2 µM riboflavin. The ability of the extract to inhibit the photochemical reduction of nitro blue tetrazolium was determined at 560 nm (Schimadzu UV-1201 spectrophotometer). The amount of the extract resulting in 50% inhibition of nitro blue tetrazolium reaction is defined as one unit of SOD activity [47].

2.5.2 Catalase [EC, 1.11.1.6, CAT]

The reaction was started by adding 10 mM H_2O_2, and the reduction in absorbance was determined at 240 nm [30,49].

2.5.3 Ascorbate peroxidase [EC, 1.11.1.11, APX]

The reaction mixture contained 50 mM potassium phosphate, 0.5 mM ascorbate, 0.1 mM ethylenedimethyl tartaric acid (EDTA) and 0.1 mM H_2O_2 and the absorbance was determined at 290 nm [30,49]. Protein concentrations of leaf extracts were determined as described earlier [30].

2.6 Photosynthetic Pigments and UV-B Absorbing Compounds

2.6.1 Anthocyanins

They were extracted with methanol: water: concentrated HCl (80: 20: 1, v/v); samples were shaken in the dark at 4ºC per 48 h; anthocyanins was measured spectrophotometrically [22].

2.6.2 Flavonoids

Fresh leaves (1g) were immersed in 20 mL of acidified methanol (MeOH: H_2O: HCl = 79:20:1, v/v). The relative UV-B absorbing compounds of flavonoids were determined using a spectrophotometer [42,63]

.

2.6.3 Chlorophylls and carotenoids

Fresh leaves from different treatments were extracted with 80% acetone and absorbance of supernatants was measured spectrophotometrically. Chlorophyll a was determined at wavelength 663 nm and b at 645 nm and total chlorophyll at 652 nm [6] carotenoids were determined by extraction of 200 mg of fresh weight of leaves with 4 ml of dimethyl sulfoxide for 12 h in the dark at 45ºC and the absorbance was calculated at 450nm [12,64].

2.6.4 Phenols

Leaves were placed in vials with distilled water, in liquid nitrogen at −80ºC for 12 h; after that they were washed and shaken for 24 h. Absorbance of soluble phenols was measured at 260 nm [24].

2.7 Free Proline

0.2g of fresh leaves was homogenized in 5mL of 3% sulphosalicyclic acid. After centrifugation, 2mL supernatant, 2mL glacial acetic acid and 2 mL 2.5% acid ninhydrin solution were added in a test tube covered with Teflon cap. The absorbance of the free proline concentration was measured at 520 nm. The proline content was expressed as mg g^{-1} fresh mass [8].

2.8 Lipid Peroxidation

The amount of malondialdehyde (MDA), a product of unsaturated fatty acid peroxidation, MDA concentration [32].

2.9 Hydrogen Peroxide

Fifteen leaf discs (10-mm diameter) were submerged in 750 µL reagent mixture containing 0.05% guaiacol and horseradish peroxidase (350 µL L^{-1}, 250 U mL^{-1}) in 25 mM sodium phosphate buffer (pH 7.0) and incubated for 2h at 20ºC in the dark [76]. Then, a volume of 250 µL was transferred into 96-well microlitre plates and the absorbance was immediately measured at 450 nm in a plate reader photometer (SLT, Spectra, Dixons Ltd, Pure Chemicals for Laboratories, Switzerland). Commercial H_2O_2, which was used for standard curves was calibrated by titration with $KMnO_4$ [30].

2.10 Leaf relative Water Content (RWC)

Four (outer and inner) leaves was weighed to determine fresh weight and placed in distilled water at +4º*C* for 19h and turgid weight was recorded [42]. Finally the samples were dried in an oven at 65ºC for 48 h and dry weights were recorded. RWC was calculated as: RWC= ((fresh weight- dry weight)/(turgid weight- dry weight)) x100

2.11 Statistical Analysis

Data of gas-exchange parameters, photosynthetic pigments, free radicals and MDA, activities of antioxidant enzymes and UV-B absorbing compounds were expressed as means ± standard error. All data were subjected to a two-way analysis of variance (*ANOVA)* which tested main effects of UV-B radiation and drought and their interaction. Significantly different means were separated using *Tukey'*s multiple comparison test (p = 0.05) using the *SPSS 11.5 (SPSS Inc*., USA) statistical package.

3. RESULTS AND DISCUSSION

Fig. 1 shows the changes in plant water status. Relative water content (RWC) of well-watered plants [control] was maintained around 98% throughout the experimental period. However, drought stress greatly lowered the RWC of lettuce leaves, by as much as 29% at the end of the stress period (Fig. 1). UV-B radiation had no significant effect (P>0 .05), while both stresses together reduced it to 19% on the 5th week of stress (Fig. 1a). After rewatering or/and withholding excess UV-B radiation, the water content of leaves generally returned to the control level (data not shown).

The daily evaporation rate (E) increased with plant growth in all treatments throughout the experiment with control plants having the highest rates and plants exposed to drought have lowest rates (Fig. 1). Daily evaporation rates were 43.2, 23.5, 32.6 and 28.1 g H_2O d^{-1} for well-watered (control0 plants, plants exposed to drought stress alone, UV-B stress alone and plants exposed to both stresses, respectively.

Water potential (ψ_w) of well-watered leaves was maintained at about –1.2 MPa throughout the entire course of the experiment. It had dropped below -1.2 MPa in plants exposed to either UV-B and/ or DS (Fig.1).

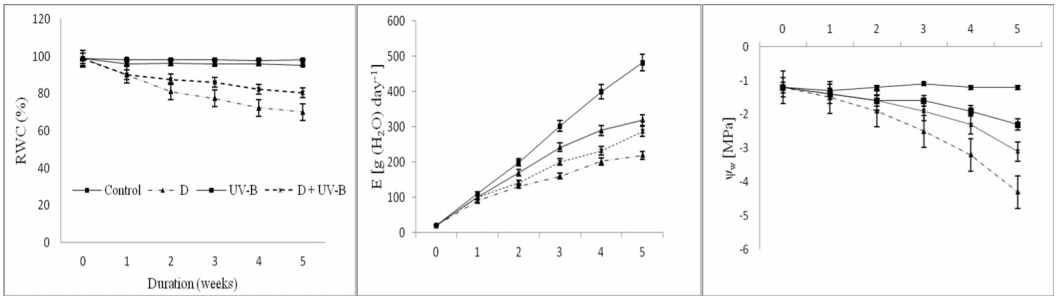

Fig. 1. Changes in Relative water content [RWC], transpiration (*E)* and water potential (ψ_w) in Lettuce (*Lactuca sativa* L.) leaves exposed to drought stress and/or UV-B radiation. Data are mean values ± 1 SE [*n* = 10].

Net photosynthetic rate (P_N) was decreased by drought (23%) and UV-B (although it was insignificant, $P \geq 0.05$) when applied singly. However, when both stresses applied simultaneously, their effects were less than additive as their combination caused a reduction by 13% (Fig. 2a). Total stomatal conductance (g_s) showed a contrasting response to drought and UV-B, where the first caused a reduction by 34% while the latter caused insignificant effect Interaction between both stresses decreased g_s by about 24% (Fig. 2b).

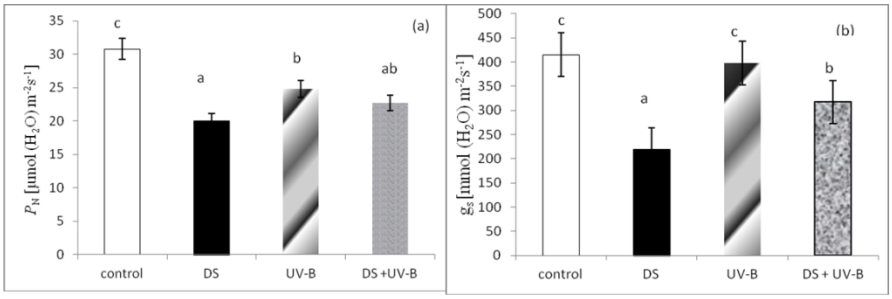

Fig. 2. Changes in the rates photosynthesis (P_N) (a) and stomatal conductance (g_s) (b) in lettuce leaves exposed to drought stress and/or UV-B radiation. PAR and CO_2 concentrations were 500 µmol m^{-2} s^{-1} and 365 µl L^{-1}, respectively during the experiment. Data are mean values ± 1 SE [*n* = 10].

The photosynthetic response over a range of PAR was generally altered by UV-B and drought (Fig. 3). The irradiance required to saturate P_N was reduced compared to the well-watered controls by both UV-B radiation and drought but no additive effects were observed. P_N was reduced at PAR 200 µmol $m^{-2}s^{-1}$ in plants exposed to both stresses singly and in combination. By contrast, above PAR of 600 µmol $m^{-2}s^{-1}$ light saturation of P_N required a

higher PPFD in the plants receiving the combination of stresses compared to drought alone (Fig. 3). However, the photosynthetic response over a range of C_i was generally not altered by UV-B (Fig. 4), while drought stress reduced it (averaged throughout the entire course of experiment) at a C_i of about 400 µl L^{-1} and above.

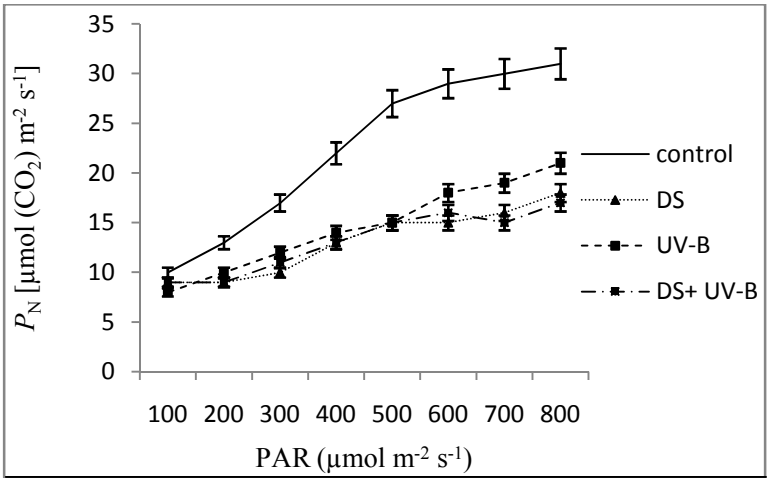

Fig. 3. Photosynthesis light-response curve (P_N/PAR) of Lettuce leaves exposed to drought stress (D) and/or UV-B radiation. (n=10±1 SE). Legends and symbols as Fig. 1

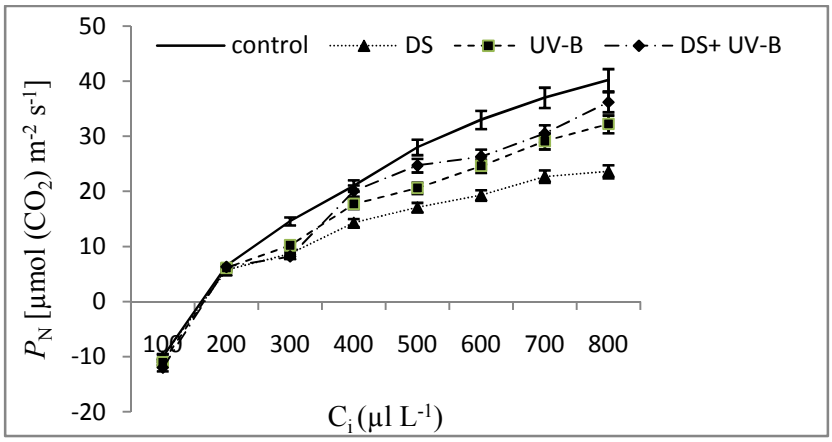

Fig. 4. Photosynthetic response to internal CO_2 partial pressures (C*i*) for lettuce subjected to drought stress and UV-B singly and in combination. (n = 10 ± 1 SE).

Chl *b* and total chlorophyll showed no significant ($P \geq 0.05$) response to UV-B and/or drought stress (Table 1). However, Chl *a* was decreased by 11% due to water stress while UV-b and its interaction with drought caused no significant effect (Table1).

The changes in the maximum efficiency of PSII photochemistry (F_v/F_m) showed no significant response ($P>0.05$) to UV-B, while drought stress decreased it slightly by about 9% (Table 1). Moreover, DS reduced number, length, fresh and dry weight of leaves, 10, 19, 18 and 22%, respectively, while UV-B had no significant effect on growth parameters

(P>0.05). Interaction between both stresses caused reductions by 13, 23, 23 and 24%,in these parameters, respectively (Table 1).

There was a strong increase of anthocyanins after the application of UV-B (1.3-fold), while drought did not cause a significant effect (Fig. 5a). Interaction between both stresses was less than additive (there was an increase in amount of anthocyanin by 18%). Nevertheless, UV-B had no significant effect on flavonoids, while drought stress increased it by ca 4-fold (Fig 5b). Exposure to both stresses was less than additive, as it caused an increase by ca 1-fold. The tendency of the action of UV irradiation to increase the UV absorbing compounds was supported by the increase in the content of carotenoids and soluble phenols (14 and 70%, respectively) (Figs. 5c and 5d). Interaction between DS and UV-B was less than additive as it caused increases in these pigments by 14% each.

Table 1. Chl *a* and *b* content (mg g^{-1}), Chl fluorescence (F_v/F_m), AQE and growth parameters in *response to* UV-B and/ or drought stress. *Different letters* express significant difference between treatments ($P \leq 0.05$). (n=10±1 SE)

Parameter	TREATMENT				
	Control	DS	UV-B	DS + UV-B	LSD at *P* = 0.05
Chl *a*	10.19±1.13^{c}	6.45+1.71^{a}	12.45+1.32^{c}	8.23±1.12^{b}	1.49
Chl *b*	9.83±1.14^{a}	9.14+0.89^{a}	8.46+0.98^{a}	9.07±1.08^{a}	2.14.
Total chl	20.02±2.10^{ab}	15.59+1.41^{b}	20.91+1.64^{bc}	17.30±2.21^{a}	1.82
F_v/F_m	0.841±0.02^{bc}	0.765+0.03^{a}	0.880+0.04^{c}	0.816+0.03^{ab}	0.051
AQE	0.072±0.001^{c}	0.063+0.003^{b}	0.061+0.001^{b}	0.054+0.001^{a}	0.006
No. of leaves	19.72±2.21^{c}	15.24±1.72^{a}	16.50±5.11^{ab}	17.23±1.94^{b}	1.42
Leaf length (cm)	22.61±3.03^{c}	17.22±1.97^{a}	20.01±2.19^{b}	17.45±2.01^{a}	1.002
FW of leaves (g)	25.68±30.09^{c}	17.84±19.18^{a}	20.24±20.61^{ab}	19.87+19.56^{b}	26.76
DW of leaves (g)	7.92±1.12^{c}	4.75±0.98^{b}	5.96+1. 13^{b}	6.03+0.76^{b}	0.69

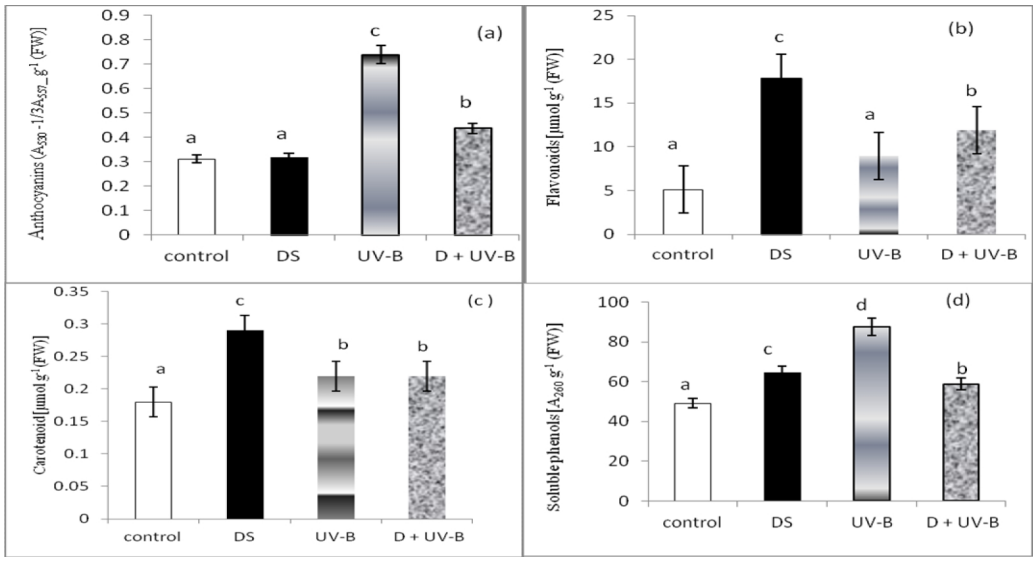

Fig.5. Interactive effects of drought stress and UV-B on Photosynthetic pigments and UV-B absorbing compounds. (a) Anthocyanin, (b) flavonoids, (c) carotenoids and (d) soluble phenols. Data are mean values+1 SE (n = 5)

Free proline is a good indicator of water stress, it has been increased by 46% due to drought while UV-B had no significant effect (Fig 6 a). The combination of stresses was antagonistic (i.e. less than additive) as it caused undetectable change in the concentration of free proline. Ultraviolet-B irradiation acted in a more destructive manner than the drought stress and caused considerable membrane damage as assessed by lipid peroxidation (measured as malondialdehyde "MDA") (21%) (Fig 6b) and by H_2O_2 level which increased by 25% (Fig 6c). On the other hand, drought had no significant effect on MDA while it caused an increase in H_2O_2 by ca 40% (Fig. 6c). Intraction between both stresses was less than additive and no change was noticed in the concentrations of any of these biomarkers.

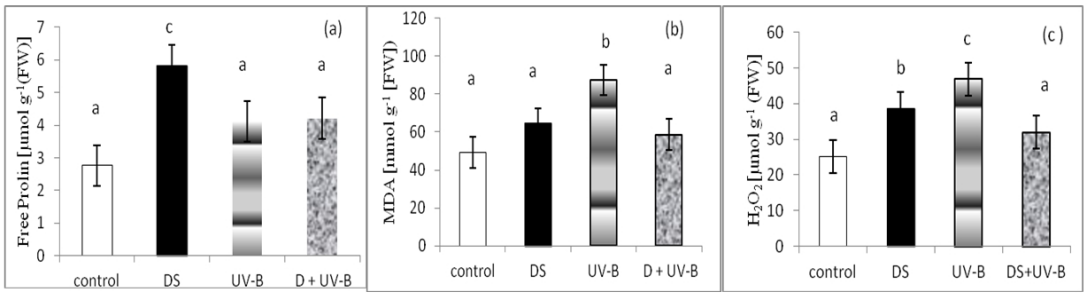

Fig. 6. Interactive effects of drought stress and UV-B on free proline [a], MDA [b]. H_2O_2 [c]. Means bot followed by the same letter(s) are significantly different from each other at P < 0.05.

Drought caused a reduction in activity of SOD and CAT by 32 and 36%, respectively, while UV-B increased their activities by 35 and 60%, respectively (Fig 7). The combination of stresses suppressed the drought effect and SOD activity content was maintained at control level while a detectable increase in CAT activity by ca 34% was observed. On contrary, APX activity was increased by 12 and 44% after exposure to drought and UV-B, respectively (Fig 7). The application of drought together with UV irradiation seemed to have a protective effect by lowering the activity (19%) caused by UV-B alone *i.e.* less than additive.

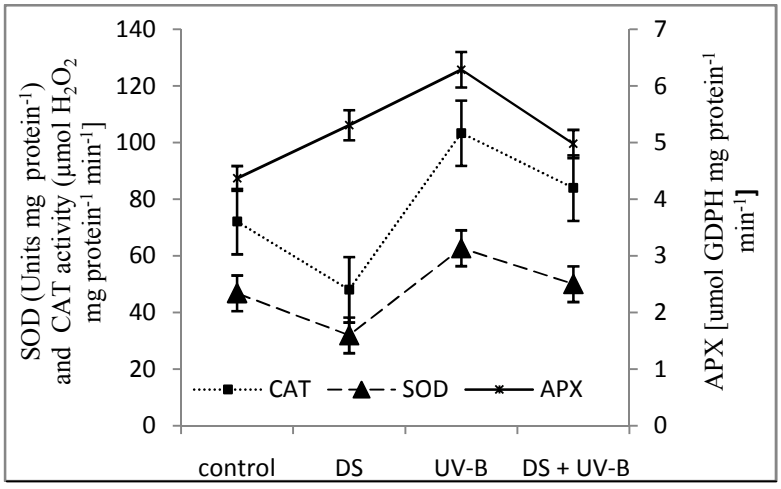

Fig. 7. Interactive effects of drought stress and UV-B on antioxidant enzymes. (n=8)

Plant productivity and agricultural ecosystems have changed greatly due to global change. However, the effects on plants will be different for each region depending on the preexisting climatic conditions and the adaptation potential of local cultivated species [73-76].

Drought reduced photosynthesis in lettuce, but no reductions in plants received enhancing UV-B radiation. Masking of drought effects in the presence of UV-B radiation may be due in part to anatomical [leaf thickening] or biochemical [pigment accumulation] adjustments to drought which ostensibly also protect plants from UV-B radiation through screening mechanisms [17,23,47,66]. Both drought and UV-B radiation reduced the photosynthetic capacity of in the present study and altered the apparent limitations to assimilation. Stomatal limitations on photosynthesis may be reduced under drought or other stress conditions [66,67]; although, in this study we did not investigate these limitations and it is worth to investigate in the future.This may imply that some synergistic effects increased the biochemical limitations present. The use of path-dependent methodology of could partition diffusional and non-diffusional limitations [68-71]. It seems likely that the mesophyll-first path would be more appropriate, although stomatal conductance g_s did not affected by UV-B in the present study. However, in drought, where significant reductions in stomatal conductance were observed, the stomata-first path might, however, be more appropriate.

The changes in AQE observe in the present study suggest that DS and UV-B radiation may damage electron transport in PSII [15,22,36]. Reductions in P_N at saturating C_i indicated that drought increased the substrate regeneration limitations on P_N [72,73]. These limitations, which generally arise due to a lack of light reaction products and/or enzyme limitations, may be more sensitive to the mild drought conditions used in this study or may have been more readily detected than direct effects on AQE by the methods employed.

Both drought and UV-B radiation altered the fundamental biochemical and photochemical processes of photosynthesis. These results suggest that UV-B radiation may significantly affect and photosynthesis primarily when water availability is high and that these effects may be obscured by drought. These findings supported earlier when they stated that the effectiveness of UV-B radiation is strongly influenced by the concurrent temperature, precipitation patterns and visible irradiance in their field studies on soybean [51], [52]. Therefore, the magnitude of the effect of increased solar UVB radiation on plant productivity may be modified by concurrent changes in global temperature and precipitation patterns.

Drought stress and UV-B radiation could cause oxidative damage through the accumulation of reactive oxygen species “ROS” [3,68]. ROS are highly reactive and in the absence of effective protective mechanism, they can compromise normal metabolism through oxidative damage to pigments, lipids, proteins and nucleic acids. Drought stress and UV-B irradiation, in the present study resulted in the high H_2O_2 accumulation, which caused lipid peroxidation measured as MDA content along with the reduction of growth (15%, presented as length of leaves). This is in agreement with the results on wheat seedlings [73]. Moreover, UV-B treatment was found to cause a more severe damage than drought stress [high accumulation of H_2O_2 and MDA]. Our results indicated that interaction between both stresses was additive on some traits [76], while others [3,4,74] showed antagonistic response of both stresses. Such variations may be due to experimental conditions and plants used.

Nevertheless, environmental stresses cause disturbance in plant metabolism which lead to oxidative injuries by enhancing ROS, plant cells develop endogenous protective mechanisms to tolerate ROS, including the antioxidant enzymes, to prevent stress damage.

The increased activity of SOD, the first antioxidant enzyme for scavenging, increased under UV-B, and combined stresses is in agreement with the results of other researchers on different plants including lettuce [3,4,74,75]. Moreover, APX using ascorbic acid as a substrate to detoxify H_2O_2, also increased under drought, UV-B and combined stresses. Its activity under combined stress was lower than when they were implied separately but also higher than in the unstressed plants [66]. Moreover, the increase in CAT activity under UV-B stress, compared to the control is consistent with the changes in the H_2O_2 in our study. Many contradictory results about antioxidant enzyme response to different stresses have emerged due to the fact that the levels of enzyme responses depend on the plant species, the developmental stage, the organs, as well as on the duration and severity of the stress [61,76].

Accumulation of free proline in response water stress in the present study is in agreement with other results [3,4,67,73,74,76]. Proline is generally assumed to serve as a physiologically compatible solute that increases as needed to maintain a favourable osmotic potential between the cell and its surroundings [75] and it is known to be involved in alleviating cytosolic acidosis associated with several stresses [35,56,76]. The control of water loss constitutes a UV-B positive effect on drought-stressed plants could be the protective interaction between UV-B and drought stresses [3,4]. 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.

Accumulation of anthocyanins and other UV-absorbing compounds, anthocyanins, carotenoids and total phenols, after UV irradiation may act in the leaf as solar screens by absorbing UV before it reaches UV-sensitive targets such as chloroplasts and other organelles [3,4,57]. The greater membrane damage, measured as lipid peroxidation after UV-B treatment in comparison with the drought stress may be explained by the fact that the increase of anthocyanins and phenols was not enough to absorb the UV radiation that can reach the cell organelles and cause damage. Moreover, the reduced chlorophyll content can be used to monitor the stress- induced damage in leaves [48]. The reduced chlorophyll accumulation in leaves was explained by the inhibition of different stages of chlorophyll biosynthesis [5,59].

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 plants.

4. CONCLUSION

In conclusion, UV-B radiation provoked in general more severe damage, evaluated as changes in the amounts of stress markers, than drought stress, when applied separately. Accumulation of UV-B absorbing compounds (flavonoids, carotenoids and phenols) in response to DS is likely to offer some increased protection from UV-B. Moreover, there was an interaction between DS and UV-B; where UV-B reduced and delayed the severity of DS through reduction in g_s (although it was not significant) and leaf area (indicated by lowering in number and fresh weight of leaves).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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