

# UV radiation, elevated CO<sub>2</sub> and water stress effect on growth and photosynthetic characteristics in durum wheat

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## ABSTRACT

Climate change studies are of considerable interest in agriculture and environmental science. The objective of this research was to investigate the changes in photosynthetic pigments and other physiological and biochemical traits of durum wheat exposed to ultraviolet A, B and C radiation, elevated CO<sub>2</sub> and water stress. The results showed that carotenoids, anthocyanins, flavonoids and proline content increased significantly by decreasing ultraviolet wavelength compared to control. Elevated CO<sub>2</sub> increased only height and specific leaf area. Water stress induced a significant increase in carotenoids, anthocyanins, flavonoids, proline and protein content. Interaction of UV-C and water stress in ambient CO<sub>2</sub> increased UV screen pigments and proline content, while under elevated CO<sub>2</sub> these increments were alleviated. Interaction among UV-C radiation, elevated CO<sub>2</sub> and water stress demonstrated a significant decrease in Fv/Fm, chlorophyll, protein, carbohydrates and specific leaf area compared to control. The results of this experiment illustrate that increased UV radiation and water stress induces an increase of screen pigments and elevated CO<sub>2</sub> prevents accumulation of these pigments.

**Keywords:** carbon dioxide; pigments; ultraviolet; water stress; wheat

Continued growth of the world population is resulting in an increased emission of greenhouse gases, especially CO<sub>2</sub>, from combustion of fossil fuels, industrial processes, and deforestation (Marland et al. 2002). Based on the reports by the IPCC (Intergovernmental Panel on Climate Change 2007), atmospheric CO<sub>2</sub> concentration is rising. Rising levels of atmospheric CO<sub>2</sub> can affect global changes. Several studies of responses of crops to CO<sub>2</sub> were conducted, and there is no advantage in repeating the detail of these here. At the leaf level, the two most well-known responses to elevated CO<sub>2</sub> are an increase in the rate of net photosynthesis and a decrease in stomatal conductance. Elevated CO<sub>2</sub> generally enhances leaf and canopy carbon assimilation rates. An increase in atmospheric CO<sub>2</sub> concentration mitigates drought stress directly by reduction of stomatal conductance in wheat which reduces transpiration (Kimball et al. 1999) and enables plants to avoid drought. Earlier studies documented that elevated CO<sub>2</sub> increases leaf net photosynthesis in C<sub>3</sub> plants (Zhao et al. 2003). Studies also showed that CO<sub>2</sub> did not alleviate the negative effects of high temperatures

in dry bean (Prasad et al. 2003) and in cotton (Reddy et al. 2005). Some reports even imply that the toxicity effect of CO<sub>2</sub> in high concentration reduces photosynthesis, dark respiration and yield in wheat (Reuveni and Bugbee 1997).

An increase in UV radiation at the earth surface due to ozone degradation will have potentially adverse effects on agricultural production and natural plant ecosystems (Rozema et al. 1997). A wide range of biological changes in plants were attributed to elevated UV radiation (Caldwell et al. 2007). There are three potential targets for UV radiation in plant cells, the genetic system, the photosynthetic system and membrane lipids (Jansen et al. 1998). These changes ultimately lead to decreased biomass production and grain yield. Enhanced UV radiation also affects plant development; in particular biomass distribution and the reproduction stage (Kakani et al. 2003). In natural conditions, effects of UV radiation on plants are related to other environmental factors such as environmental stress (Caldwell et al. 2003). Reports on influence of UV radiation on photosynthesis are inconsistent due to differences in crops, UV dosages, and other environmental conditions (Kakani et al.

2003). Coleman and Day (2004) reported that as the UV dose approached the ambient level, cotton and sorghum produced more branches or tillers, but with a smaller leaf area. Various stress factors competing with the supplemental UV radiation were shown to modify the UV radiation effects (Feng et al. 2000). Of these stresses, water stress is an important restricting factor that always affects agricultural productivity, particularly in arid and semi-arid regions. Feng et al. (2007) showed that co-stresses of supplementary UV radiation and drought functioned synergically and one of them could alleviate the inhibitory effects of another under conditions of arid and semi-arid soils. Plants developed different defense mechanisms against UV radiation, such as thicker and smaller leaves (Bornman and Vogelmann 1991), increased production of UV-absorbing compounds such as flavonoids, anthocyanins (Tevini et al. 1991), and higher amounts of reflective waxes (Rozema et al. 1997). The accumulation of flavonoids in the epidermis was shown to reduce epidermal transmittance of UV radiation and thus may provide some protection (Tevini et al. 1991). Flavonoids and soluble hydroxycinnamic acid derivatives appear to be the most important, as was shown for many plant species, including cereals (Ibdah et al. 2002). This study describes interaction of enhanced UV radiation, elevated CO<sub>2</sub> and water stress on growth, relative water content (RWC), Fv/Fm ratio, UV absorbing and photosynthetic pigments, proline, protein, carbohydrates and seed yield in durum wheat. Novelty of this experiment was a study of triple interaction of the mentioned factors. Its aim was to study the importance of the accumulation of anthocyanins, flavonoids and photosynthetic pigments which changed during exposure of wheat plants to UV-A, UV-B and UV-C radiation, CO<sub>2</sub> and water stress.

## MATERIALS AND METHODS

The seeds of wheat (*Triticum aestivum* L. c.v. Aria) were collected from the Seed and Plant Improvement Institute (SPII), Karaj, Iran. The experiment was conducted in the Faculty of

Agriculture, Tarbiat Modares University, Tehran, Iran, (35°41'N latitude, 51°19'E longitude and altitude of 1215 m) at the 2007 growing season. The yearly average precipitation (30-year long term period) which is mostly concentrated during the autumn and winter months was 298 mm. The mean annual temperature was 18.8°C. In brief, the experimental design was a factorial arrangement in randomized complete blocks with three replicates. The first factor included three levels of UV radiation (UV-A: wavelength > 320 nm or solar radiation, UV-B: 280–320 nm and UV-C: wavelength < 280 nm). The second factor included two CO<sub>2</sub> levels (ambient concentration, i.e. 400 µl/l, and elevated concentration, i.e. 900 µl/l). The third factor was irrigation regime [complete irrigation and limited irrigation (60% field capacity)]. Before the beginning of experiment, soil samples were taken in order to determine their physical and chemical properties. A composite soil sample was collected at a depth of 0–20 and 20–40 cm. Soil characteristics are shown in Table 1.

Each plot consisted of four rows, 1 m width and 2 m length. Between all plots, a 1 m alley was kept to eliminate all influence of lateral water movement. Before seeding, 100 kg/ha N as urea was broadcasted and incorporated into the soil. The seeds were hand-planted in early October, with plant density of approximately 350 plant/m<sup>2</sup>. Wheat seeds were disinfected with fungicide before planting. Weed control was realized manually without any chemical input. The plots were top-dressed with 50 kg/ha N at jointing stage.

In each experimental plot, a sheltered frame was erected (1.5 m × 2.5 m × 2 m) made of light alloyed-metal. The frames were covered with 0.03 mm thick polyethylene plastic film to prevent CO<sub>2</sub> escaping. The top of frames were opened. All plots were irrigated same at field capacity until flag leaf appeared. Soil volumetric water contents were monitored daily in surface to a depth of 70 cm using the time-domain reflectometry (TDR, FM-Trime -IMKO- GmbH, D-76275-Germany) and probes of 70 cm length, previously calibrated, were inserted in the middle plot of each treatment and. From appearance of

Table 1. Physical and chemical properties of the soil

Depth of sampling (cm)	Soil texture (%)			Field capacity	Wilting point	N (%)	P (ppm)	K (ppm)	pH
	Clay	Silt	Sand						
0–20				12.6	7.0				
20–40	13	19	68	14.8	8.2	0.231	5.5	340	7.6

the flag leaf until the end of flowering, the soil-moisture stresses were maintained at 60 percent moisture of field capacity. During water stress, UV-B and C radiation were delivered to plants by fluorescent lamps (UV-B Philips 40W/12; UV-C Philips TUV 30W/G30T8). Radiation intensity of UV-A (Solar radiation or control treatment), UV-B and UV-C were measured (18, 25 and 40  $\mu\text{W}/\text{cm}^2$ , respectively) by a spectroradiometer.

The lamps were suspended above the canopy of plants. The distance from lamps to the top of plants was always maintained at 0.5 m throughout the experiment. Concurrent with water stress and UV radiation,  $\text{CO}_2$  concentration was elevated to 900  $\mu\text{l}/\text{l}$ . Carbon dioxide was adjusted by electronic sensor manufactured by Testo (Germany).

**Maximum photochemical efficiency.** Maximum photochemical efficiency was determined by a portable fluorometer (PAM-2000, H WalsGmbH, Effeltrich, Germany) connected with a leaf-clip holder (2030-B, Walz) and with a trifurcated fibre-optic (2010-E,

Walz). Before measurement, the leaves were dark-adapted for 30 min. The maximum photochemical efficiency of PSII was determined from the ratio of variable (Fv) to maximum (Fm) fluorescence.

**Relative water content.** In order to RWC assay and other biochemical traits, flag leaves were sampled. Relative water content of fully expanded last leaves was estimated. Leaf material was weighed (0.2 g) to determine fresh weight and placed in double-distilled water for 4 h and then turgid weight was recorded. Finally, the samples were dried in an oven at 65°C for 48 h and the dry weights were recorded. Relative water content was calculated as:

$$[(\text{fresh weight} - \text{dry weight}) / (\text{saturated weight} - \text{dry weight})] \times 100.$$

**Sampling.** At the end of treatments, the flag leaves were sampled and frozen in liquid  $\text{N}_2$  and stored at -80°C until biochemical analysis.

**Chlorophyll assay.** Chlorophyll was extracted in 80% acetone from the leaf samples, according

Table 2. Analysis of variance on physiological and biochemical traits of wheat exposure to elevated  $\text{CO}_2$ , ultra-violet and water stress

Source of variation	df	Fv/Fm	RWC	Chlorophyll	Carotenoid	Anthocyanin	Flavonoid 270	Flavonoid 300
Replication	2	ns	ns	ns	ns	ns	ns	ns
UV	2	**	**	**	**	**	**	**
$\text{CO}_2$	1	**	**	ns	ns	**	**	**
Drought	1	**	**	**	**	**	**	**
$\text{UV} \times \text{CO}_2$	2	**	ns	**	**	*	**	**
$\text{UV} \times \text{Drought}$	2	**	ns	**	**	**	**	**
$\text{CO}_2 \times \text{Drought}$	1	*	ns	**	**	ns	**	ns
$\text{UV} \times \text{CO}_2 \times \text{Drought}$	2	*	*	ns	ns	**	**	**
<i>C.V</i>		6.52	8.35	6.53	7.91	9.79	7.31	9.48
Source of variation	df	Flavonoid 330	Proline	Protein	Carbohydrate	Height	SLA	Yield
Replication	2	ns	ns	ns	ns	ns	ns	ns
UV	2	**	**	**	**	**	**	**
$\text{CO}_2$	1	**	*	**	**	*	**	ns
Drought	1	**	**	**	**	**	**	**
$\text{UV} \times \text{CO}_2$	2	**	*	**	**	ns	**	ns
$\text{UV} \times \text{Drought}$	2	**	**	**	ns	ns	**	**
$\text{CO}_2 \times \text{Drought}$	1	**	**	**	*	ns	**	**
$\text{UV} \times \text{CO}_2 \times \text{Drought}$	2	**	**	**	*	ns	**	ns
<i>C.V</i>		9.00	7.80	4.35	7.12	7.41	7.88	11.82

ns – not significant; \*and \*\*significant at  $P < 0.05$  and  $P < 0.01$ , respectively

to the method of Arnon (1949). Extracts were filtrated and then absorbances of chlorophyll a, b were determined by spectrophotometer (UV-S, Sinco 2100) at 645 and 663 nm.

**Carotenoids assay.** Total carotenoids were determined according to the method of Lichtenthaler and Wellburn (1983). Leaves were extracted in 80% acetone. The extract was centrifuged twice at 5300 g for 10 min, then supernatant was filtrated and absorbance of carotenoids was determined at 470 nm. Carotenoid content was expressed as  $\mu\text{mol/g FW}$  and concentrations of carotenoids were calculated using an extinction coefficient  $\text{og } \epsilon = 33\,000\mu\text{M/cm}$ .

**Anthocyanin assay.** Anthocyanin content was estimated according to the method of Krizek et al. (1993). Leaf samples were homogenized in a mortar and pestle with 3 ml 1% HCl-methanol solvent (1: 99, v: v). The homogenate was centrifuged at 18 000 g for 30 min at 4°C, and then the supernatant was filtered through Whatman #1 to remove particulate matter and was stored in darkness at 5°C for 24 h. The amount of anthocyanin was determined from the absorbance at 550 nm. Anthocyanin content was expressed as  $\mu\text{mol/g FW}$  and the concentration of anthocyanin was calculated using the extinction coefficient of anthocyanin  $\epsilon = 33\,000/\text{mol}^2 \text{ cm}$ .

**Flavonoids assay.** Flavonoids were estimated according to the method of Krizek et al. (1993). Leaf samples were homogenized in a mortar and pestle with 3 ml 1% acetic acid-ethanol solvent (1:99, v:v). The homogenate was centrifuged at 18 000 g for 30 min, and then the supernatant was incubated in a water bath for 10 min at 80°C and then allowed to cool to room temperature. The amount of flavonoids was determined from the absorbance at 270, 300 and 330 nm. Flavonoid content was expressed as  $\mu\text{mol/g FW}$  and the concentration of flavonoids was calculated using an extinction coefficient of flavonoids  $\epsilon = 33\,000/\text{mol}^2 \text{ cm}$ .

**Proline assay.** Proline content was determined according to the method of Bates et al. (1973). Samples of leaves (0.2 g) were homogenized in a mortar and pestle with 3 ml sulphosalicylic acid (3% w/v), and then the homogenate was centrifuged at 18 000 g for 15 min. Supernatant was then put into a test tube into which 2 ml glacial acetic acid and 2 ml freshly prepared acid ninhydrin solution were added. Tubes were incubated in a water bath for 1 h at 100°C, and then allowed to cool to room temperature. Four ml of toluene were added and mixed on a vortex mixer for 20 s. After, the toluene phase was carefully pipetted out into a glass test

tube, and its absorbance was measured at 520 nm in a spectrophotometer. The content of proline was calculated from a proline standard curve and was expressed as  $\mu\text{g/g FW}$ .

**Protein assay.** Total protein content was determined using bovine serum albumin (BSA) as a standard, according to the method of Bradford (1976), using 1 ml Bradford solution and 100  $\mu\text{l}$  crude extract. The protein concentration was calculated from a BSA standard curve. Protein content was expressed as  $\mu\text{g/g FW}$ .

**Total soluble carbohydrate assay.** Total carbohydrate determination in leaves was carried out using the phenol-sulphuric acid method (Dubois et al. 1956). The values of total soluble carbohydrate were calculated as  $\text{mg/g FW}$ .

**Growth parameters.** At the end of vegetative stage, plant height and area of flag leaf were measured; then, leaves were oven-dried for 72 h at 70°C in order to measure specific leaf area (SLA). At the end of growing season the final yield was also determined.

**Statistical analysis.** Main and interaction effects of treatments were determined from analysis of variance (ANOVA) using the GLM procedure in SAS (SAS Institute, 2002). The assumptions of variance analysis were tested by insuring that the residuals were random, homogeneous, with a normal distribution about a mean of zero. Duncan's Multiple Range Tests was used to measure statistical differences between treatment methods and controls.

## RESULTS AND DISCUSSION

Results of analysis of variance are shown in Table 2. Comparison of the main effects demonstrated that UV-B and UV-C irradiance decreased significantly Fv/Fm ratio, RWC, chlorophyll content, protein, carbohydrate, plant height, SLA and final yield (Table 3). A significant increase was observed in total carotenoid, total anthocyanin, flavonoids contents and proline accumulation in plants treated with UV-B and C (Table 3).

The height of plants and SLA of flag leaf were significantly increased in  $\text{CO}_2$ -treated plants – by 5.7 and 20.04%, respectively; in contrast, elevated  $\text{CO}_2$  had a reducing effect on other traits (Table 3). In our study, the content of UV-absorbing compounds was significantly decreased under high level of  $\text{CO}_2$ . This may imply that the secondary metabolisms were negatively affected by high level of  $\text{CO}_2$  radiation. Elevated  $\text{CO}_2$  did not

Table 3. Comparison main effects elevated CO<sub>2</sub>, ultraviolet and water stress on physiological, biochemical traits and yield of wheat

Factors	Levels	Fv/Fm	RWC (%)	ChlorophyllII (%)	Carotenoid (μmol/g FW)	Anthocyanin (μmol/g FW)	Flavonoid 270 (μmol/g FW)	Flavonoid 300 (μmol/g FW)
UV-Radiation	UV-A	0.454333 ± 0.02 <sup>a</sup>	51.13 ± 2.36 <sup>a</sup>	51.18 ± 1.87 <sup>a</sup>	0.10 ± 0.01 <sup>c</sup>	0.36 ± 0.03 <sup>c</sup>	4.37 ± 0.10 <sup>c</sup>	1.17 ± 0.19 <sup>c</sup>
	UV-B	0.348583 ± 0.01 <sup>b</sup>	46.06 ± 1.97 <sup>b</sup>	48.18 ± 1.65 <sup>b</sup>	0.18 ± 0.02 <sup>b</sup>	0.61 ± 0.01 <sup>b</sup>	6.64 ± 0.45 <sup>b</sup>	3.96 ± 0.22 <sup>b</sup>
	UV-C	0.000000 ± 0 <sup>c</sup>	39.45 ± 3.19 <sup>c</sup>	22.38 ± 1.58 <sup>c</sup>	0.25 ± 0.01 <sup>a</sup>	0.91 ± 0.06 <sup>a</sup>	34.43 ± 11.52 <sup>a</sup>	8.56 ± 0.96 <sup>a</sup>
CO <sub>2</sub> Concentration	400 μl/l	0.28 ± 0.05 <sup>a</sup>	52.23 ± 1.67 <sup>a</sup>	42.03 ± 3.37 <sup>a</sup>	0.17 ± 0.02 <sup>a</sup>	0.69 ± 0.06 <sup>a</sup>	23.09 ± 8.44 <sup>a</sup>	5.33 ± 0.99 <sup>a</sup>
	900 μl/l	0.25 ± 0.04 <sup>b</sup>	38.87 ± 1.77 <sup>b</sup>	39.12 ± 3.43 <sup>b</sup>	0.17 ± 0.01 <sup>a</sup>	0.55 ± 0.05 <sup>b</sup>	7.21 ± 0.80 <sup>b</sup>	3.80 ± 0.68 <sup>b</sup>
Irrigation	complete	0.31 ± 0.05 <sup>a</sup>	49.13 ± 2.19 <sup>a</sup>	45.33 ± 3.32 <sup>a</sup>	0.14 ± 0.01 <sup>b</sup>	0.53 ± 0.05 <sup>b</sup>	7.75 ± 1.00 <sup>b</sup>	3.34 ± 0.57 <sup>b</sup>
	limit	0.22 ± 0.03 <sup>b</sup>	41.97 ± 2.20 <sup>b</sup>	35.83 ± 3.11 <sup>b</sup>	0.21 ± 0.01 <sup>a</sup>	0.71 ± 0.06 <sup>a</sup>	22.54 ± 8.48 <sup>a</sup>	5.78 ± 1.01 <sup>a</sup>
Factors	Levels	Flavonoid 330 (μmol/g FW)	Proline (μg/g FW)	Protein (μg/g FW)	Carbohydrate (mg/g FW)	Height (cm)	SLA (g/cm <sup>2</sup> )	Yield (g/m <sup>2</sup> )
UV-Radiation	UV-A	5.12 ± 0.23 <sup>c</sup>	6.49 ± 1.14 <sup>c</sup>	734.93 ± 58.47 <sup>a</sup>	357.10 ± 28.95 <sup>a</sup>	26.58 ± 0.52 <sup>a</sup>	292.41 ± 32.53 <sup>a</sup>	159.42 ± 25.75 <sup>a</sup>
	UV-B	7.63 ± 0.54 <sup>b</sup>	9.82 ± 1.35 <sup>b</sup>	487.33 ± 20.83 <sup>b</sup>	282.97 ± 19.12 <sup>b</sup>	22.25 ± 0.62 <sup>b</sup>	183.35 ± 3.18 <sup>b</sup>	133.46 ± 21.75 <sup>b</sup>
	UV-C	37.07 ± 11.74 <sup>a</sup>	14.87 ± 1.93 <sup>a</sup>	426.23 ± 24.44 <sup>c</sup>	166.28 ± 12.56 <sup>c</sup>	19.17 ± 0.69 <sup>c</sup>	150.74 ± 3.05 <sup>c</sup>	111.82 ± 18.74 <sup>c</sup>
CO <sub>2</sub> Concentration	400 μl/l	24.29 ± 8.72 <sup>a</sup>	10.76 ± 1.83 <sup>a</sup>	603.82 ± 54.81 <sup>a</sup>	327.25 ± 26.05 <sup>a</sup>	22.00 ± 0.93 <sup>b</sup>	185.58 ± 9.47 <sup>b</sup>	134.66 ± 15.10 <sup>a</sup>
	900 μl/l	8.92 ± 1.18 <sup>b</sup>	10.03 ± 0.99 <sup>b</sup>	495.17 ± 25.76 <sup>b</sup>	210.31 ± 15.23 <sup>b</sup>	23.33 ± 0.81 <sup>a</sup>	232.09 ± 27.26 <sup>a</sup>	135.14 ± 21.40 <sup>a</sup>
Irrigation	complete	8.10 ± 0.84 <sup>b</sup>	6.06 ± 0.78 <sup>b</sup>	516.51 ± 22.88 <sup>b</sup>	290.47 ± 24.49 <sup>a</sup>	23.83 ± 0.82 <sup>a</sup>	235.94 ± 27.29 <sup>a</sup>	205.40 ± 8.93 <sup>a</sup>
	limit	25.11 ± 8.67 <sup>a</sup>	14.72 ± 1.24 <sup>a</sup>	582.47 ± 58.00 <sup>a</sup>	247.09 ± 25.65 <sup>b</sup>	21.50 ± 0.86 <sup>b</sup>	181.72 ± 8.07 <sup>b</sup>	64.40 ± 4.66 <sup>b</sup>

Each value is mean ± SE (*n* = 3). The means within each column followed by the same letter are not significantly different (*P* < 0.05)

significantly affect carotenoid (Table 3); moreover, it can partially mitigate some of the adverse effects of UV and decreases UV screening pigment production.

Water stress caused reduction of the Fv/Fm ratio, RWC, chlorophyll content, carbohydrates, height, SLA and final yield, while pigments, proline and protein were significantly increased (Table 3). Triple interaction was significant in all of traits except RWC, carotenoid content and plant height (Table 2). There were significant interactions between UV and CO<sub>2</sub> (Figure 1), UV and water stress

(Figure 2) and CO<sub>2</sub> and water stress (Figure 3) on carotenoid content. Changes in the fluorescence parameters indicate the sensitivity of PSII to UV-B and C radiation (Djanaguiraman et al. 2005). The decline in the Fv/Fm ratio is a good indicator of photoinhibitory damage caused by light or other environmental stresses. The highest and the lowest Fv/Fm ratio were observed in solar radiation + ambient CO<sub>2</sub> + complete irrigation treatment and in plants treated with UV-C radiation, respectively (Table 4). In general, wavelength reduction caused the decline of Fv/Fm ratio and water stress had

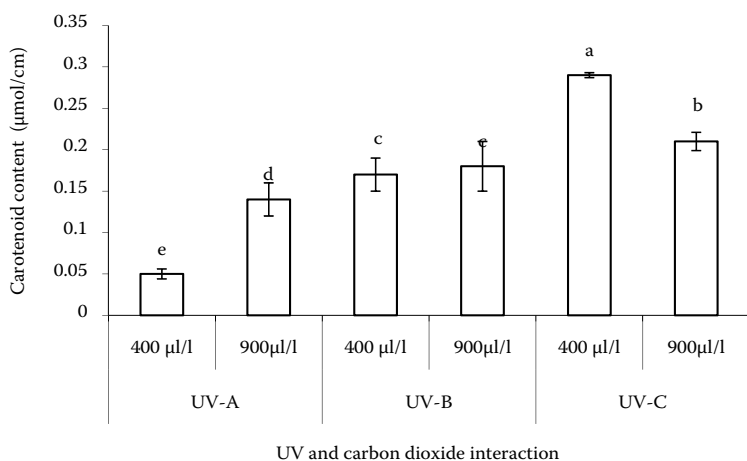


Figure 1. Changes in carotenoid content due to ultraviolet radiation and elevated carbon dioxide. Means followed by the same letter do not differ among themselves in the Duncan's Multiple Range Tests at 5% probability. Each value is mean  $\pm$  S.E. ( $n = 3$ )

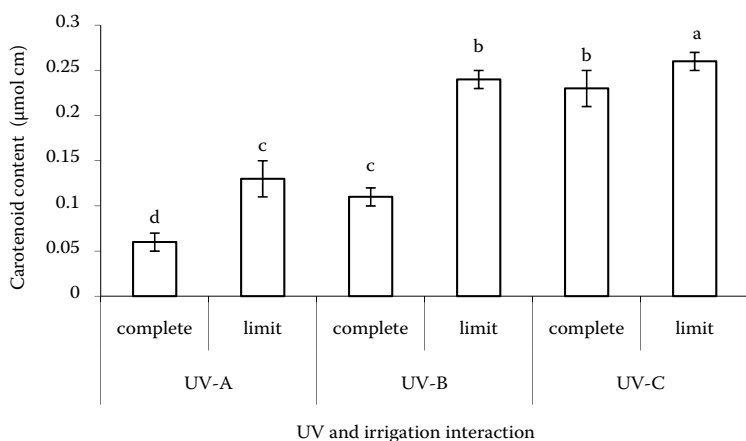


Figure 2. Changes in carotenoid content due to ultraviolet radiation and water stress. Means followed by the same letter do not differ among themselves in the Duncan's Multiple Range Tests at 5% probability. Each value is mean  $\pm$  S.E. ( $n = 3$ )

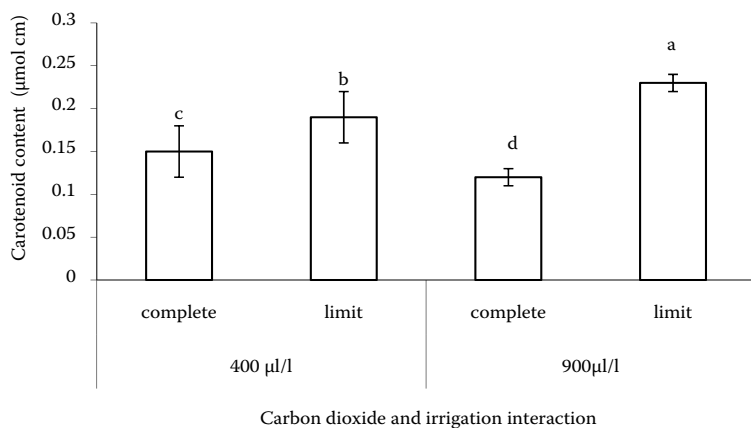


Figure 3. Changes in carotenoid content due to elevated carbon dioxide and water stress. Means followed by the same letter do not differ among themselves in the Duncan's Multiple Range Tests at 5% probability. Each value is mean  $\pm$  S.E. ( $n = 3$ )

a synergistic effect on a decrease of this ratio, while elevated CO<sub>2</sub> had not effect on alleviation of these stresses. Chlorophyll fluorescence that descended under UV radiation at both ambient and elevated CO<sub>2</sub> indicates that UV radiation might have damaged the D<sub>1</sub> and D<sub>2</sub> proteins of PS II (Olsson et al. 2000) and degraded chlorophyll, which might have resulted in reduced quantum efficiency or lower photosynthetic capacity. In the case of photosynthesis, chlorophyll has a crucial role in the production of assimilates. The highest chlorophyll content was found in control plants, plants treated with UV-B radiation in normal conditions of CO<sub>2</sub> and irrigation and plants treated with CO<sub>2</sub> in normal condition (Table 4). The lowest chlorophyll content was achieved in plants exposed to UV-C radiation + ambient CO<sub>2</sub> and water stress (Table 4). Water stress resulted in an accelerated chlorophyll breakdown starting in the leaves. Elevated CO<sub>2</sub> did not modify this effect, while under high CO<sub>2</sub> concentrations also accelerated leaf senescence. Elevated CO<sub>2</sub> decreased the leaf chlorophyll content under water stress conditions, which corresponds to previous findings (Ommen et al. 1999). A decrease in photosynthetic pigments was evident during exposure to enhanced UV radiation in most crop species (Kakani et al. 2003). It is noteworthy that a decrease of total chlorophyll occurred under reduced UV radiation, and this implied that the UV-B and C radiation has a negative impact on the parameters related to photosynthesis. Decrease in chlorophyll concentration due to salinity and UV-B radiation was reported previously (Agarwal 2007). Reduced chlorophyll concentration may be due to increased chlorophyllase activity.

On the other hand, carotenoids, anthocyanins and flavonoids are affected differently by UV radiation. These pigments play an important role against UV damage in higher plants (Middleton and Teramura 1993). The highest levels of anthocyanin and flavonoids were obtained in UV-C radiation + ambient CO<sub>2</sub> + limited irrigation treatment while the lowest anthocyanin content was observed in plants exposed to solar radiation + elevated CO<sub>2</sub> + complete irrigation treatment (Table 4). Nogues and Baker (2000) reported that anthocyanin content was increased in plants exposed to UV radiation. An increase of UV absorbing compounds caused by UV was well documented in previous studies (Rozema et al. 2002). These results suggest that the UV-B absorbing compounds are mainly synthesized in leaves and they are used to protect leaf tissue under exposure to UV. However, it seems

to be produced through similar mechanisms as in the case of UV induction. Flavonoids and related compounds absorb strongly in the UV-region but not in the photosynthetically active regions of the spectrum (Cen and Bornman 1993), allowing photosynthesis to continue while UV wavelengths are attenuated at the epidermis.

It is reported that UV radiation stimulates synthesis of flavonoids and polyphenolic compounds such as tannin and lignin (Smrkolj et al. 2005). The flavonoid assay showed that elevated CO<sub>2</sub> decreased these compounds under UV and water stress conditions (Table 4). In this experiment plants grown under ambient CO<sub>2</sub> + water stress and submitted to UV-C radiation had a significant accumulation of the proline content (Table 4). RWC of the UV-irradiated plants decreased, and thus there is no doubt that some wilting-induced proline accumulation occurred. Saradhi et al. (1995) were the first to show that plants exposed to UV radiation accumulate proline that could protect plant cells against UV radiation-induced peroxidative processes. Water stress and UV radiation lead to the increase of the contents of proline but it was observed that under water stress conditions and UV radiation, elevated CO<sub>2</sub> decreased proline accumulation (Table 4). In the present study, a marked increase in proline accumulation under UV-B and C treatment (Table 3) represents adaptive responses to oxidative damage induced by UV radiation. Proline is known to be involved in alleviating cytosolic acidic associated with several stresses (Kurkdjian and Guern 1989). The removal of excess H<sup>+</sup> occurring as a result of proline synthesis may have a positive effect on reduction of the UV-B and UV-C induced damage. It suggests that UV radiation-induced proline accumulation protects plants against UV radiation-promoted peroxidation processes.

The highest and the lowest soluble protein content were found in plants grown under solar radiation + ambient CO<sub>2</sub> + limited irrigation and UV-C radiation + elevated CO<sub>2</sub> + limited irrigation, respectively (Table 4). Increase of leaf soluble protein content by UV radiation and water stress was decreased when compared with plants grown at elevated CO<sub>2</sub> (Table 4). Total soluble carbohydrates of flag leaf were significantly decreased, in plants exposed to UV-C radiation + elevated CO<sub>2</sub> + water stress than in plants grown under normal condition (Table 4). In general, wavelength reduction, elevated CO<sub>2</sub> and water stress have diminishing effect on total carbohydrates. Carbohydrate is another molecule that accumulates in chloroplasts and is used in plants as a reserve of carbon. In

Table 4. Interaction among elevated CO<sub>2</sub>, ultraviolet and water stress on physiological, biochemical traits and yield of wheat

UV-radiation	CO <sub>2</sub> Conc. (μl/l)	Irrigation	Fv/Fm	Chlorophyll (%)	Anthocyanin (μmol/g FW)	Flavonoid 270 (μmol/g FW)	Flavonoid 300 (μmol/g FW)
UV-A	400	complete	0.55 ± 0.009 <sup>a</sup>	57.56 ± 2.18 <sup>a</sup>	0.38 ± 0.02 <sup>g</sup>	4.11 ± 0.06 <sup>de</sup>	0.96 ± 0.02 <sup>g</sup>
		limit	0.38 ± 0.006 <sup>bc</sup>	47.13 ± 1.55 <sup>bc</sup>	0.47f ± 0.04 <sup>g</sup>	4.74 ± 0.12 <sup>de</sup>	1.86 ± 0.00 <sup>f</sup>
UV-B	400	complete	0.40 ± 0.009 <sup>b</sup>	56.06 ± 0.52 <sup>a</sup>	0.57 ± 0.01 <sup>ef</sup>	6.19 ± 0.13 <sup>d</sup>	4.36 ± 0.11 <sup>d</sup>
		limit	0.35 ± 0.006 <sup>c</sup>	43.60 ± 1.45 <sup>c</sup>	0.69 ± 0.01 <sup>cd</sup>	9.07 ± 0.44 <sup>c</sup>	4.40 ± 0.10 <sup>d</sup>
UV-C	400	complete	0.00 ± 0 <sup>e</sup>	26.50 ± 2.30 <sup>d</sup>	0.80 ± 0.06 <sup>bc</sup>	13.82 ± 0.47 <sup>b</sup>	7.18 ± 0.03 <sup>c</sup>
		limit	0.00 ± 0 <sup>e</sup>	21.36 ± 0.08 <sup>e</sup>	1.23 ± 0.03 <sup>a</sup>	100.53 ± 2.17 <sup>a</sup>	13.23 ± 0.81 <sup>a</sup>
UV-A	900	complete	0.53 ± 0.026 <sup>a</sup>	56.06 ± 1.32 <sup>a</sup>	0.18 ± 0.00 <sup>h</sup>	4.03 ± 0.06 <sup>e</sup>	0.21 ± 0.00 <sup>h</sup>
		limit	0.35 ± 0.004 <sup>c</sup>	43.96 ± 1.41 <sup>c</sup>	0.39 ± 0.03 <sup>g</sup>	4.57 ± 0.18 <sup>de</sup>	1.66 ± 0.05 <sup>fg</sup>
UV-B	900	complete	0.37 ± 0.013 <sup>bc</sup>	49.03 ± 1.77 <sup>b</sup>	0.55 ± 0.02 <sup>ef</sup>	5.17 ± 0.07 <sup>de</sup>	2.66 ± 0.09 <sup>e</sup>
		limit	0.25 ± 0.002 <sup>d</sup>	44.03 ± 2.00 <sup>c</sup>	0.60 ± 0.00 <sup>de</sup>	6.10 ± 0.04 <sup>de</sup>	4.40 ± 0.08 <sup>d</sup>
UV-C	900	complete	0.00 ± 0 <sup>e</sup>	26.76 ± 1.51 <sup>d</sup>	0.71 ± 0.06 <sup>c</sup>	13.15 ± 0.06 <sup>b</sup>	4.66 ± 0.11 <sup>d</sup>
		limit	0.00 ± 0 <sup>e</sup>	14.86 ± 0.86 <sup>f</sup>	0.88 ± 0.03 <sup>b</sup>	10.21 ± 0.08 <sup>c</sup>	9.14 ± 0.12 <sup>b</sup>
UV-radiation	CO <sub>2</sub> Conc. (μl/l)	Irrigation	Flavonoid 330 (μmol/g FW)	Proline (μg/g FW)	Protein (μg/g FW)	Carbohydrate (mg/g FW)	SLA (g/cm <sup>2</sup> )
UV-A	400	complete	5.14 ± 0.30 <sup>e</sup>	2.60 ± 0.15 <sup>e</sup>	653.00 ± 21.82 <sup>b</sup>	468.51 ± 6.03 <sup>a</sup>	247.60 ± 18.42 <sup>b</sup>
		limit	5.43 ± 0.33 <sup>e</sup>	10.14 ± 0.35 <sup>c</sup>	1067.33 ± 14.53 <sup>a</sup>	427.30 ± 16.14 <sup>b</sup>	217.57 ± 4.63 <sup>cd</sup>
UV-B	400	complete	6.73 ± 0.18 <sup>e</sup>	3.94 ± 0.10 <sup>e</sup>	437.00 ± 11.40 <sup>e</sup>	357.74 ± 25.17 <sup>c</sup>	183.10 ± 1.37 <sup>ef</sup>
		limit	10.62 ± 0.61 <sup>d</sup>	16.28 ± 0.99 <sup>b</sup>	574.07 ± 11.11 <sup>c</sup>	325.11 ± 3.66 <sup>d</sup>	174.33 ± 5.84 <sup>efg</sup>
UV-C	400	complete	13.42 ± 0.09 <sup>c</sup>	7.28 ± 0.60 <sup>d</sup>	382.97 ± 14.81 <sup>f</sup>	196.93 ± 2.47 <sup>f</sup>	150.63 ± 4.53 <sup>gh</sup>
		limit	104.37 ± 2.47 <sup>a</sup>	24.28 ± 0.66 <sup>a</sup>	508.57 ± 13.41 <sup>d</sup>	187.90 ± 5.32 <sup>f</sup>	140.23 ± 4.69 <sup>h</sup>
UV-A	900	complete	4.71 ± 0.21 <sup>e</sup>	2.83 ± 0.10 <sup>e</sup>	603.43 ± 2.03 <sup>c</sup>	300.62 ± 0.26 <sup>d</sup>	474.87 ± 21.04 <sup>a</sup>
		limit	5.18 ± 0.88 <sup>e</sup>	10.37 ± 0.05 <sup>c</sup>	615.93 ± 14.67 <sup>bc</sup>	231.95 ± 7.11 <sup>e</sup>	229.60 ± 7.63 <sup>bc</sup>
UV-B	900	complete	6.34 ± 0.25 <sup>e</sup>	8.35 ± 0.06 <sup>d</sup>	527.10 ± 20.86 <sup>d</sup>	235.17 ± 6.90 <sup>e</sup>	197.43 ± 5.50 <sup>de</sup>
		limit	6.80 ± 0.33 <sup>e</sup>	10.68 ± 0.12 <sup>c</sup>	411.17 ± 11.89 <sup>ef</sup>	213.85 ± 11.75 <sup>ef</sup>	178.53 ± 2.42 <sup>efg</sup>
UV-C	900	complete	12.23 ± 0.75 <sup>cd</sup>	11.34 ± 0.32 <sup>c</sup>	495.60 ± 10.02 <sup>d</sup>	183.85 ± 11.16 <sup>f</sup>	162.03 ± 6.26 <sup>fgh</sup>
		limit	18.23 ± 0.35 <sup>b</sup>	16.57 ± 0.65 <sup>b</sup>	317.77 ± 11.60 <sup>g</sup>	96.46 ± 4.29 <sup>g</sup>	150.07 ± 1.99 <sup>gh</sup>

Each value is mean ± S.E. (*n* = 3). The means within each column followed by the same letter are not significantly different (*P* < 0.05)

this experiment, UV radiation, elevated CO<sub>2</sub> and water stress limited drastically the carbohydrate assimilation. This effect was exacerbated when UV-C radiation, elevated CO<sub>2</sub> and water stress were superimposed on that condition. Present investigation showed that at elevated CO<sub>2</sub> photosynthetic rate and carbohydrate synthesis were decreased due to a rise of temperature. This was probably due to the change in kinetic parameters of rubisco and decreased solubility of CO<sub>2</sub> compared to O<sub>2</sub> that increased the photorespiration. It

is plausible that a decrease of chlorophyll content and photosynthesis efficiency due to UV radiation reduced total carbohydrates. Elevated CO<sub>2</sub> increased SLA under conditions of solar radiation and complete irrigation only. The lowest SLA was found in plants submitted to UV-C radiation and water stress (Table 4). It is well known that the development of leaf area is the result of two phenomena: cell division and cell expansion (Tsukaya 2003). Literature indicates that both phenomena are affected by UV-B irradiation (Tosserams et al.



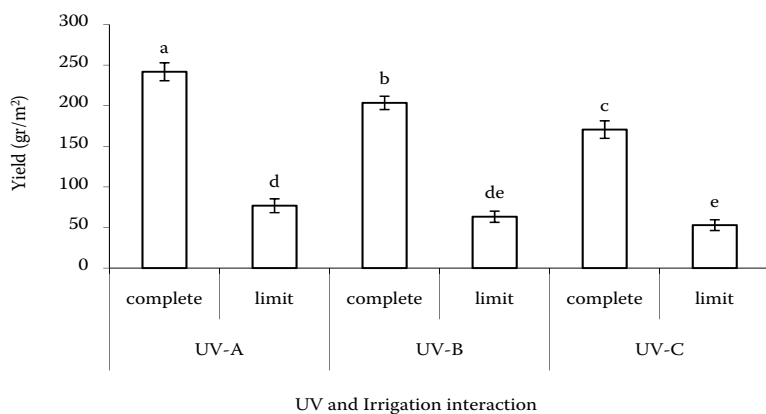


Figure 4. Changes in seed yield due to ultraviolet radiation and water stress. Means followed by the same letter do not differ among themselves in the Duncan's Multiple Range Tests at 5% probability. Each value is mean  $\pm$  S.E. ( $n = 3$ )

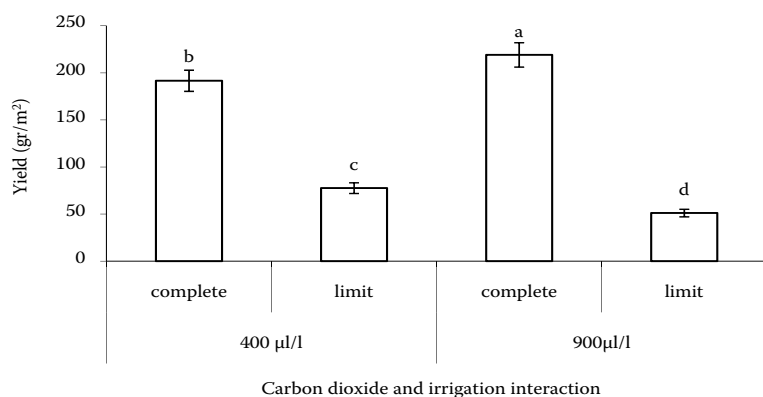


Figure 5. Changes in seed yield due to elevated carbon dioxide and water stress. Means followed by the same letter do not differ among themselves in the Duncan's Multiple Range Tests at 5% probability. Each value is mean  $\pm$  S.E. ( $n = 3$ )

2001). It is possible that the reduction in leaf area (data not shown), caused by the UV-B irradiation in this case, was mainly due to a reduction in cell expansion. Previously, we reported that plant height and SLA of wheat were affected negatively by UV radiation, particularly at the ambient level of CO<sub>2</sub>. We also demonstrated that level of CO<sub>2</sub> elevated to 900 µl/l can partially ameliorate some of the adverse effects of UVB on growth. Seed yield was affected by UV-B and C radiation and water stress (Table 3). Decrease of wavelength and water stress significantly decreased seed yield. Reduction of seed yield due to UV radiation and water stress was previously reported (Caldwell et al. 2007, Feng et al. 2007). Interaction between UV radiation and water stress showed that the highest seed yield was obtained from UV-A and complete irrigation, while the lowest yield was observed from UV-C and limited irrigation (Figure 4). It was observed that an increase of CO<sub>2</sub> concentration increased seed yield in complete irrigation conditions but in stress conditions, an increase of CO<sub>2</sub> concentration had a negative effect on seed yield (Figure 5). It seems that an increase of air temperature due to elevated CO<sub>2</sub> decreased seed yield through decreased seed weight (data not shown).

In conclusion, we found that UV-B and C radiation and water stress increased UV screen pigments

although elevated CO<sub>2</sub> decreased seed yield and pigment production. Our understanding of the relationships between crop growth and the atmospheric environment was developed substantially in the past few decades. Still, the factor of climate change and its impact on crops and food production will be further explored in future studies because global change climate might be critical event in future centuries; available data may not adequately characterize the potential effect of future, such as simultaneous changes in CO<sub>2</sub> concentration and UV-B radiation.

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