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*Original Research*

# Effect of UV Radiation and Elevated $CO_2$ on Physiological Attributes of Canola (*Brassica napus* L.) Grown under Water Deficit Stress

**Hamid Reza Tohidi-Moghadam[1]*, Farshad Ghooshchi[1], Farshid Jamshidpour[1], Hossein Zahedi[2]**

[1]Department of Agronomy, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran
[2]Department of Agronomy and Plant Breeding, Eslamshahr Branch, Islamic Azad University, Eslamshahr, Iran

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**Abstract**

An experiment was conducted to study the effects of solar UV radiation, UV-B, UV-C radiation, and elevating $CO_2$ on some physiological attributes of two canola cultivars (*Brassica napus* L.) under complete irrigation or limited irrigation in two continuous years. Generally, elevated $CO_2$ increased leaf-soluble carbohydrates, reducing sugars, glucosinolate, and Fv/Fm ratio while carotenoids and soluble protein were decreased due to elevated $CO_2$. In addition, UV radiation decreased leaf-soluble carbohydrates, reducing sugars, chlorophyll, proline, and Fv/Fm ratio and increased UV absorbing pigments, soluble proteins, and glucosinolate.

**Keywords:** canola, elevated $CO_2$, UV radiation, water stress

## Introduction

The recent depletion of stratospheric ozone can significantly increase the quantity of ultraviolet radiation reaching the earth's surface [1]. Elevated UV radiation causes a wide range of morphological, physiological, and metabolic responses in plants, for example increases in UV absorbing compounds such as flavonoids, anthocyanin, and carotenoids, and a decrease in the efficiency of photosystem II due to chlorophyll degradation have been reported [2, 3]. Some of the mechanisms that could lead to these alterations are damages to DNA [4]. Zahedi and Tohidi reported that antioxidant activities increased in many physiological cycles such as the response to water deficit stress [5]. However, many plants are quite resistant to UV radiation. In contrast, sensitive plants are able to develop several repair and adaptive mechanisms. The first and foremost adaptations are structural modifications such as thickening of cell walls, epicuticular wax formation, and the synthesis of anthocyanin and flavonoid [6, 7]. One of the most important mechanisms is the screening out UV radiation by accumulation of flavonoids, anthocyanins, or other UV absorbing compounds in the leaf epidermis [8]. The influence of other environmental factors such as water stress and increasing $CO_2$ also can interact and alter the balance or consequences of these defence mechanisms. Current atmospheric levels of $CO_2$ may double from 340 $\mu L \cdot L^{-1}$ to 680 $\mu L \cdot L^{-1}$ by the middle of the 21$^{th}$ century [9]. Simultaneous with elevation of $CO_2$, an increase in photosynthesis and biomass can be expected in $C_3$ plants. In UV sensitive plants, photosynthetic capacity may be reduced directly by the effect of UV radiation on photosynthetic enzymes or disruption of PSII

*e-mail: hamid_tohidi2008@yahoo.com

reaction centers, or indirectly by affecting photosynthetic pigments and stomata function [7]. Both $CO_2$ and UV radiation are expected to increase simultaneously with future changes in global climate in the world. Thus, an experiment was performed in order to study the effects of these three environmental factors and their interactions on two canola cultivars. In this study, we investigated the effect of water stress, different UV radiation, and elevated $CO_2$ on two canola cultivars: Okapi and Talaye. The attributes analyzed were UV-absorbing compounds, leaf-soluble carbohydrates, reducing sugars, chlorophyll content, soluble proteins, glucosinolate, Fv/Fm, and endogenous content of proline accumulated in the tissues as a result of water stress, UV radiation, and $CO_2$ treatments.

## Materials and Methods

### Plant Material and Growth Conditions

The experiment was conducted at Karaj province, Iran in the 2008 and 2009 growing seasons. The study site was located at 35º59′ N latitude, 50º75′ E longitude. The experimental design was a randomized complete blocks arrangement in factorial with three replicates. The first factor included two varieties of canola, the second factor was irrigation regimes (complete irrigation and limited irrigation of 60% field capacity). The third factor included two $CO_2$ levels (atmospheric concentration and 400 $\mu L \cdot L^{-1}$ and 900 $\mu L \cdot L^{-1}$) and the fourth factor was different levels of UV radiation (UV-A: wavelength > 320 nm or solar radiation, UV-B: 280-320 nm and UV-C: wavelength < 280 nm). UV-B and C radiation were delivered to plants by fluorescent lamps (UV-B Philips 40W/12 and 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 µw/cm d, respectively) by a spectroradiometer. Sunlight was considered as UV-A.

Each experimental unit included an erected sheltered frame (1.5 m×2.5 m×2 m) covered with polyethylene plastic film to prevent $CO_2$ escaping. Disinfected canola seeds (Okapi and Talaye cultivars) were sown at a depth of 2-3 cm and irrigation was done immediately. All experimental units were irrigated at field capacity until seedling establishment. After that, in water stress units soil moisture was maintained at 60 percent of field capacity using Time-Domain Reflectometry (T.D.R, soil moisture, model 4593).

During water stress, UV-B and C radiation were delivered to plants by UV lamps from 10:00 to 13:00. Simultaneous with water stress and UV radiation, $CO_2$ concentration was increased to 900 $\mu L \cdot L^{-1}$ for treated units. One $CO_2$ capsule was used and $CO_2$ concentration was elevated into covered frames. Carbon dioxide was adjusted to 900 $\mu L \cdot L^{-1}$ by an electronic sensor (Testo Co. Germany). Nitrogen fertilizer (Urea) was applied in three stages: seed sowing, stem elongation, and flowering. A systemic insecticide (Metasystox) was used at the flowering stage of canola to protect them against aphids.

### Biochemical Determinations

#### Soluble Carbohydrate

At flowering stage, five plants were harvested randomly and bulk fresh tissues were collected and frozen in liquid nitrogen until biochemical analysis. Soluble carbohydrate, including glucose xylose and mannose, were estimated according to the method of Dubois et al. [10]. Leaf samples were homogenized in a mortar and pestle with 3 ml distilled water and the homogenate was filtered by filter paper. Half ml phenol 5% and 2.5 ml sulphuric acid 98% were added to homogenate. After reaction, the test tubes were allowed to cool at room temperature. The amount of glucose, xylose, and mannose was determined from the absorbance at 480, 485 and 490 nm, respectively. The sugar concentration was calculated from a glucose, xylose, and mannose standard curve.

#### Reducing Sugars

Reducing sugars were measured by dinitrosalicylic acid [11]. Sucrose was determined after incubation of 0.5 ml of the extract with acetate buffer (pH 4.5) containing 0.05% invertase. The sucrose level was related to the difference in optical density values between the reactions with and without invertase. The supernatant that remained after ethanol extractions were analyzed for starch [12].

#### Chlorophyll and Carotenoid Assay

Chlorophyll was extracted in 80% acetone from the leaf samples, according to the method of Arnon [13]. Extracts were filtrated and then absorbance of chlorophyll a, b, and carotenoids were determined by spectrophotometer (UV-S, Sinco 2100) at 645, 663, and 470 nm. The content of chlorophyll was expressed as $mg \cdot g^{-1}$ FW. Total carotenoids were determined according to the method of Lichtenthaler and Wellburn [14]. Leaves were extracted in 80% acetone. The extract was centrifuged twice at 5,300 g for 10 min, then supernatant was filtrated and absorbance of carotenoids was determined at 470 nm. Carotenoid content was expressed as $\mu mol \cdot g^{-1}$ FW and concentrations of carotenoids were calculated using an extinction coefficient of $\varepsilon = 33{,}000\ \mu M \cdot cm^{-1}$.

#### Flavonoids Assay

Flavonoids were estimated according to the method of Krizek et al. [15]. 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 80ºC and allowed to cool to room temperature. The amount of flavonoids was determined from the absorbance at 270, 300, and 330 nm. The content of flavonoids were determined using the extinction coefficient of flavonoids ($\varepsilon$=33,000 $mol^{-2} \cdot cm^{-1}$). Flavonoid content was expressed as $\mu mol \cdot cm^{-1}$.

### Anthocyanin Assay

Anthocyanin content was estimated according to the method of Krizek et al. [15]. 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 4ºC, and then the supernatant was filtered through Whatman #1 to remove particulate matter and stored in darkness at 5ºC for 24 h. The amount of anthocyanin was determined from the absorbance at 550 nm. The content of anthocyanin was determined using the extinction coefficient of anthocyanin ($\varepsilon$=33,000 $mol^{-2} \cdot cm^{-1}$). Anthocyanin content was expressed as $\mu mol \cdot cm^{-1}$.

### Proline Assay

Proline content of leaves was determined according to a modification of the method of Bates et al. [16]. Samples of leaves (0.5 g) were homogenized in a mortar and pestle with 10 ml sulphosalicylic acid (3% w/v), and then centrifuged at 18,000 g for 15 min. Two millilitres of the supernatant was then added to a test tube, to which 2 ml glacial acetic acid and 2 ml freshly prepared acid ninhydrin solution (1.25 g ninhydrin dissolved in 30 ml glacial acetic acid and 20 ml 6 M orthophosphoric acid) were added. The test tubes were incubated in a water bath for 1 h at 100ºC and then allowed to cool to room temperature. Four ml of toluene were added to the tubes and then mixed in a vortex mixer for 20 s. The test tubes were allowed to stand for at least 10 min to allow separation of the toluene and aqueous phases. The toluene phase was carefully pipetted into a glass test tube and its absorbance was measured at 520 nm in a spectrophotometer. The content of proline was calculated from a standard curve.

### Soluble Proteins

The protein content of the crude extract was determined using bovine serum albumin (BSA) as a standard, according to the method of Bradford [17]. One ml of Bradford solution was added to 100 µl crude extract and the absorbance recorded at 595 nm for estimation of total protein content. The protein concentration was calculated from a BSA standard curve.

### Glucosinolate Assay

Glucosinolate content was measured according to Embaby et al. [18]. Two hundred mg of canola meal were transferred to a test tube and heated in a water-bath at 75ºC for 1 min. Two ml of boiling methanol solution (70% v:v) were added and 200 µl of 20 mmol/internal standard solution of sinigrin were added immediately. The heating at 75ºC was continued for a further 10 min, shaking the tube at regular intervals. The tube was centrifuged at 3,000 g for 3 min and the supernatant was transferred to another tube. Two millilitres of boiling methanol solution were added to the tube containing the solid residue and the tube was reheated for 10 min, and then centrifuged for 3 min, as described above. The supernatant was added to the tube containing the first supernatant and the volume of the combined extracts was adjusted to 5 ml with water. Pasteur pipettes were placed vertically on a stand and a glass wool plug placed in the neck of each pipette. Half 1 ml of suspension of ion exchange resin was transferred to each pipette. The pipettes were rinsed with 2 ml of the imidazole format solution (6 mol) followed with 1 ml of water. One ml of the glucosinolate extract was transferred to a prepared column and two ml portions of sodium acetate buffer were added. The buffer was drained after each addition. Diluted purified sulfatase solution was added to the column (75 µl) and left to act overnight at ambient temperature. The second day, the desulfoglucosinolate was eluted with two 1 ml portions of water and collected in a tube placed under the column. Then the sample was ready for HPLC analysis. The different glucosinolates in canola meal were determined using high performance liquid chromatography (HPLC) (1100 Hewlett Packard, Palo Alto, CA). The desulfoglucosinolates were separated using a type C18 column with a flow rate of 0.5 ml/min at 30ºC. Elution of desulfoglucosinolates from HPLC was performed by a gradient system of water (A) and acetonitrile/water (25:75, v/v, B). The total running time was 45 min with a gradient as follows: 100% A and 0% B for 5 min, then in 35 min to 0% A and 100% B and in 5 min back to 100% A and 0% B. A UV detector was used at a wavelength of 229 nm. Individual glucosinolates were identified in comparison with the retention time of sinigrin standard.

### Maximum Photochemical Efficiency

Maximum photochemical efficiency was determined by a portable fluorometer (PAM-2000, H Wals GmbH, Effeltrich, Germany). 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 [2].

### Statistical Analysis

All data were subjected to SAS software, and Duncan's Multiple Range Tests was used to identify statistical differences between treatments.72 samples were biochemical analyzed.

## Results and Discussion

### Leaf-Soluble Carbohydrates

Analysis of variance demonstrates that water stress, carbon dioxide, and UV radiation had significant effects on soluble carbohydrates in canola leaves, and these results were similar in both years of experiment (Table 1). Also, we observed that canola cultivars were unlike each other in terms of leaf-soluble carbohydrates with leaf-soluble carbohydrate in Talaye higher than Okapi. In addition, water deficit stress and UV radiation significantly decreased leaf-

Table 1. Analysis of variance on some physiological attributes of two canola cultivars affected by water stress, carbon dioxide, and UV radiation.

| S.O.V | df | Leaf-soluble carbohydrates | Reducing sugars | Chlorophyll | Carotenoids | Flavonoids | Anthocyanin | Proline | Soluble proteins | Glucosinolate | Fv/Fm |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Year | 1 | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| R (Year) | 4 | * | ns | * | ns | ns | ns | ns | ns | ns | ns |
| V | 1 | ** | ** | ns | ** | ns | ns | ** | ** | ns | ns |
| W | 1 | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** |
| C | 1 | ** | ** | ns | ** | ns | ns | ns | ** | ** | ** |
| U | 2 | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** |
| V*W | 1 | ns | * | ns | ns | ns | * | ns | ** | ns | ** |
| V*C | 1 | ns | ns | ** | ns | ns | * | ns | ns | ** | ns |
| V*U | 2 | ** | ns | ns | ** | ** | ns | ns | ns | ns | ** |
| W*C | 1 | ** | ** | ** | ** | * | ns | ns | ns | ** | ns |
| W*U | 2 | ns | ** | ** | ** | ** | ** | ns | ** | ** | ** |
| C*U | 2 | ** | ns | ns | ** | ** | ** | ns | ** | ** | ** |
| VWC | 1 | ns | ns | ** | ** | * | ns | * | ns | ** | * |
| VWU | 2 | ** | ** | ns | ** | ** | ** | ns | ns | * | ** |
| WCU | 2 | ** | ** | ns | ** | ns | ns | ** | ** | ** | ** |
| VCU | 2 | ** | ** | ns | ** | ns | ns | ns | ns | ns | ns |
| VWCU | 2 | ** | ns | ** | ** | ** | ** | ns | ns | ns | ** |
| Year (V) | 1 | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| Year (W) | 1 | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| Year (C) | 1 | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| Year (U) | 2 | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| C.V | | 5.97 | 8.09 | 8.05 | 4.94 | 10.19 | 23.54 | 17.92 | 10.80 | 6.41 | 4.66 |

R – replication; V – variety; W – water stress; C – carbon dioxide; U – UV radiation
*, ** significant at the 0.05 and 0.01 probability levels, respectively and ns no significant.

soluble carbohydrates. In contrast, elevated $CO_2$ increased leaf-soluble carbohydrates (Table 2). Interaction between cultivar and the other treatments showed that Talaye cultivar had the highest soluble carbohydrate in comparison to Okapi cultivar (Table 3). The results showed that under conditions of complete irrigation or limited irrigation, increasing $CO_2$ can increase soluble carbohydrate in leaves. Furthermore, regardless of the presence of water stress or elevated $CO_2$, UV radiation decreased leaf-soluble carbohydrates (Table 3). Three-way interactions on leaf-soluble carbohydrates are shown in Table 4. According to Table 5, the highest leaf-soluble carbohydrates were observed in Talaye cultivars when these plants were grown under condition of complete irrigation and elevated $CO_2$ under natural sunlight. UV-C radiation and water stress significantly decreased leaf-soluble carbohydrates in Okapi cultivars under condition of ambient $CO_2$, as this cultivar had the lowest leaf-soluble carbohydrates affected by these treatments. It is reported that thylakoid membranes are destroyed due to oxygen-free radicals induced by UV stress and then thylakoid membrane integrity would be decreased and thus photosynthetic process and energy production would be decreased [19]. Additionally, several studies on the effects of UV radiation on plant carbohydrates have been carried out, some indicating increases in response to UV-B and others indicating decreases [20, 21]. This may be due to plant diversity or experimental conditions. In the present work, significant effects of UV radiation on total soluble carbohydrates was observed at both UV-B and UV-C radiation. Such increases have been reported in UV-B irradiated leaves of pea and corn [22, 23].

Table 2. Main effects of year, variety, water stress, carbon dioxide, and UV radiation on some physiological attributes.

| Treatments | Levels | Leaf-soluble carbohydrates (mg·g$^{-1}$ FW) | Reducing sugars (mg·g$^{-1}$ FW) | Chlorophyll (mg·g$^{-1}$ FW) | Carotenoids (mM·cm$^{-1}$) | Flavonoids (mM·cm$^{-1}$) | Anthocyanin (mM·cm$^{-1}$) | Proline (mg·g$^{-1}$ FW) | Soluble proteins (mg·g$^{-1}$ FW) | Glucosinolate (as µmol·g$^{-1}$) | Fv/Fm |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Year | First | 18.80a | 22.50a | 2.38a | 0.80a | 0.81a | 0.64a | 0.06a | 0.63a | 20.22a | 0.41a |
| | Second | 18.43a | 22.13a | 2.42a | 0.76a | 0.83a | 0.67a | 0.07a | 0.61a | 20.19a | 0.39a |
| Variety | Okapi | 17.51b | 20.76b | 2.39a | 0.74b | 0.82a | 0.64a | 0.06a | 0.65a | 20.39a | 0.41a |
| | Talaye | 20.10a | 24.25a | 2.38a | 0.78a | 0.80a | 0.64a | 0.05b | 0.61b | 19.99a | 0.41a |
| Water stress | Complete | 24.64a | 28.50a | 3.34a | 0.67b | 0.74b | 0.53b | 0.04b | 0.46b | 18.77b | 0.45a |
| | Limited | 12.97b | 16.51b | 1.43b | 0.85a | 0.88a | 0.75a | 0.07a | 0.80a | 21.61a | 0.36b |
| Carbon dioxide | 400 ppm | 17.62b | 21.30b | 2.36a | 0.77a | 0.80a | 0.62a | 0.05a | 0.69a | 18.93b | 0.39b |
| | 900 ppm | 19.98a | 23.70a | 2.41a | 0.75b | 0.82a | 0.66a | 0.06a | 0.57b | 21.45a | 0.42a |
| UV radiation | A | 21.15a | 25.01a | 2.55a | 0.63c | 0.57c | 0.24c | 0.04c | 0.44c | 14.52c | 0.49a |
| | B | 18.28b | 22.37b | 2.40b | 0.80b | 0.89b | 0.72b | 0.06b | 0.67b | 21.39b | 0.43b |
| | C | 16.98c | 20.12c | 2.20c | 0.85a | 0.97a | 0.96a | 0.07a | 0.77a | 24.66a | 0.30c |

Means with similar letter are not significant at the 5% probability level

## Reducing Sugars

Reducing sugar content was significantly affected by water stress, elevated $CO_2$, and UV radiation. Although water stress and UV radiation led to a reduction in reducing sugars, the elevating $CO_2$ obviously increased reducing sugars (Table 2). The results showed that Talaye cultivar had more sugar content than Okapi cultivar, and these results were similar in both years of study. Alternatively, reducing sugars might increase during water stress, if sugar formation is a response to either osmotic regulation or respiration needs. Reducing sugars might increase after water stress due to failures in starch deposition or the conversion of starch to sugars [24, 25]. Total reducing sugar content generally decreased by UV radiation. A decline in reducing sugar content due to UV radiation could be due to the damaged caused to chloroplasts and photosynthetic systems. High levels of UV-B radiation have been reported to cause down-regulation of photosynthetic genes, leading to reduced levels of glucose in common bean leaves [26].

## Chlorophyll

Significant effects of treatments and the changes in total chlorophyll content due to different treatments are shown in Table 1-5.

Significant negative effects of water stress and UV radiation on total chlorophyll content (compared to the control treatments) states the adverse effects of these abiotic stress on this plant. It is notable that no significant difference in total chlorophyll content was observed between ambient $CO_2$ concentration and elevated $CO_2$ concentration. Similar results were found when two canola cultivars were compared in term of chlorophyll content. Exposure of canola plants to increasing UV-B and UV-C intensity reduced the content of chlorophyll. The lowest chlorophyll content was found in Okapi plants grown under ambient $CO_2$ concentration and subjected to water stress and UV-C radiation (Table 5). Chlorophyll is the central part of the energy capturing system in plants, and so any significant alteration in their concentrations is likely to cause a marked effect on plant performance [27]. Water stress causes damage to the pigments, and plastids also decreased chlorophyll content because of water stress [28]. The authors also found that water stress also increases the speed of chlorophyll severance [29]. Reduction in chlorophyll contents by excess UV-B radiation has been reported in oak (*Quercus petraea* L.) [30]. A diminished chlorophyll concentration is the most common symptom due to UV radiation stress. This can be attributed to inhibition of biosynthesis of pigments under UV exposure [31]. Mackerness et al. [32] suggested that under UV-B stress plants sacrifice their chloroplasts in order to protect the rest of the cell.

## UV Absorbing Pigments

Carotenoids, flavonoids, and anthocyanin concentration showed an increasing trend with decreasing UV wavelength and water deficit stress. Elevated $CO_2$ had no significant effect on UV absorbing pigments except for a little decline in carotenoid content. Also, there was no significant difference between canola cultivars and the results were similar in both years of the experiment (Tables 2-5).

The increase in UV-absorbing pigments due to UV radiation points to the photo-protection role of these pigments

Table 3. Two-way interaction between treatments on some physiological attributes.

| Treatments | | Leaf-soluble carbohydrates (mg·g$^{-1}$ FW) | Reducing sugars (mg·g$^{-1}$ FW) | Chlorophyll (mg·g$^{-1}$ FW) | Carotenoids (mM·cm$^{-1}$) | Flavonoids (mM·cm$^{-1}$) | Anthocyanin (mM·cm$^{-1}$) | Proline (mg·g$^{-1}$ FW) | Soluble proteins (mg·g$^{-1}$ FW) | Glucosinolate (as µmol·g$^{-1}$) | Fv/Fm |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Okapi | Complete | 23.33b | 26.41b | 3.36a | 0.64d | 0.75b | 0.50b | 0.05c | 0.46c | 18.98a | 0.44b |
| | Limited | 11.69d | 15.10d | 1.42b | 0.84b | 0.89a | 0.78a | 0.07a | 0.84a | 21.80a | 0.37c |
| Talaye | Complete | 25.94a | 30.58a | 3.33a | 0.69c | 0.73b | 0.55b | 0.04d | 0.46c | 18.56b | 0.46a |
| | limited | 14.26c | 17.91c | 1.44b | 0.87a | 0.87a | 0.72a | 0.06b | 0.76b | 21.42a | 0.36d |
| Okapi | 400 ppm | 16.16c | 19.54d | 2.31b | 0.75b | 0.82a | 0.65ab | 0.06a | 0.72a | 19.65c | 0.39b |
| | 900 ppm | 18.86b | 21.97c | 2.46a | 0.72c | 0.82a | 0.64ab | 0.06a | 0.58c | 21.14b | 0.42a |
| Talaye | 400 ppm | 19.09b | 23.06b | 2.40ab | 0.79a | 0.78b | 0.59b | 0.05b | 0.66b | 18.21d | 0.39b |
| | 900 ppm | 21.11a | 25.43a | 2.36b | 0.78a | 0.83a | 0.68a | 0.05b | 0.56c | 21.77a | 0.42a |
| Okapi | A | 19.22b | 22.88c | 2.52ab | 0.59e | 0.62c | 0.28c | 0.05c | 0.48c | 14.88c | 0.50a |
| | B | 17.37d | 20.59d | 2.44bc | 0.79c | 0.89b | 0.73b | 0.06b | 0.69b | 21.30b | 0.42c |
| | C | 15.93e | 18.80e | 2.20d | 0.84b | 0.96a | 0.93a | 0.07a | 0.78a | 25.00a | 0.31d |
| Talaye | A | 23.09a | 27.13a | 2.59a | 0.67d | 0.53d | 0.21c | 0.04d | 0.41d | 14.16c | 0.49a |
| | B | 19.18b | 24.15b | 2.35c | 0.81c | 0.89b | 0.71b | 0.05c | 0.65b | 21.48b | 0.44b |
| | C | 18.03c | 21.45d | 2.21d | 0.86a | 0.98a | 0.98a | 0.06b | 0.76a | 24.33a | 0.30d |
| Complete | 400 ppm | 23.19b | 26.84b | 3.39a | 0.65d | 0.71c | 0.48c | 0.04b | 0.51c | 16.14b | 0.44b |
| | 900 ppm | 26.09a | 30.15a | 3.30a | 0.68c | 0.77b | 0.57b | 0.04b | 0.41d | 21.41a | 0.46a |
| Limited | 400 ppm | 12.06d | 15.76d | 1.33c | 0.88a | 0.89a | 0.76a | 0.07a | 0.86a | 21.72a | 0.35d |
| | 900 ppm | 13.88c | 17.25c | 1.53b | 0.82b | 0.87a | 0.75a | 0.07a | 0.73b | 21.50a | 0.38c |
| Complete | A | 26.97a | 30.63a | 3.41a | 0.50d | 0.45d | 0.11d | 0.03e | 0.28d | 12.97d | 0.56a |
| | B | 24.16b | 29.37b | 3.43a | 0.74c | 0.87b | 0.69b | 0.04d | 0.47c | 18.65b | 0.46b |
| | C | 22.78c | 25.50c | 3.19b | 0.75c | 0.91b | 0.78b | 0.06c | 0.63b | 24.70a | 0.33e |
| Limited | A | 15.33d | 19.39d | 1.70c | 0.75c | 0.70c | 0.38c | 0.06c | 0.61b | 16.07c | 0.43c |
| | B | 12.40e | 15.38e | 1.37d | 0.86b | 0.90b | 0.75b | 0.07b | 0.87a | 24.13a | 0.39d |
| | C | 11.18f | 14.75e | 1.21e | 0.95a | 1.04a | 1.13a | 0.08a | 0.91a | 24.63a | 0.27f |
| 400 ppm | A | 20.31b | 23.85b | 2.51ab | 0.59e | 0.57d | 0.25d | 0.04c | 0.48e | 13.91e | 0.49b |
| | B | 17.57d | 21.57c | 2.37c | 0.82b | 0.90bc | 0.76bc | 0.06b | 0.73b | 19.08c | 0.40d |
| | C | 14.99e | 18.48d | 2.19d | 0.89a | 0.93b | 0.85b | 0.07a | 0.85a | 23.81b | 0.29f |
| 900 ppm | A | 22.00a | 26.17a | 2.60a | 0.66d | 0.58d | 0.24d | 0.04c | 0.41f | 15.14d | 0.50a |
| | B | 18.98c | 23.18b | 2.43bc | 0.78c | 0.87c | 0.68c | 0.06b | 0.60d | 23.70b | 0.45c |
| | C | 18.97c | 21.77c | 2.21d | 0.81b | 1.01a | 1.07a | 0.07a | 0.69c | 25.52a | 0.31e |

Means with similar letter are not significant at the 5% probability level

in photosynthetic systems by dissipating excess excitation energy through the xanthophylls cycle [33]. Accumulation of UV-absorbing pigments such as carotenoids, flavonoid, and anthocyanin is one of the ways by which plants alleviate the harmful effects of UV stress. An increase in flavonoid content is in support of the results obtained by Shweta and Agrawal [27] in spinach (*Spinacia oleracea* L.), by Mirna et al. [20] in quinoa (*Chenopodium quinoa* Willd.), and by Rathore et al. [34] in wheat (*Triticum aestivum* L.). In this study, UV-absorbing pigment concentrations were significantly increased in leaves of canola plants exposed to UV-C radiation. Although water stress had an additive effect on these pigments, the effect of UV radiation, especially UV-C radiation, was more noticeable.

Table 4. Three-way interaction between treatments on some physiological attributes.

| Treatments | | | Leaf-soluble carbohydrates (mg·g$^{-1}$ FW) | Reducing sugars (mg·g$^{-1}$ FW) | Chlorophyll (mg·g$^{-1}$ FW) | Carotenoids (mM·cm$^{-1}$) | Flavonoids (mM·cm$^{-1}$) | Anthocyanin (mM·cm$^{-1}$) | Proline (mg·g$^{-1}$ FW) | Soluble proteins (mg·g$^{-1}$ FW) | Glucosinolate (as µmol·g$^{-1}$) | Fv/Fm |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Okapi | Complete | 400 ppm | 21.59c | 24.74c | 3.42a | 0.64ef | 0.75b | 0.51c | 0.05d | 0.51d | 16.38e | 0.43c |
| | | 900 ppm | 25.07b | 28.08b | 3.29a | 0.63f | 0.75b | 0.50c | 0.05d | 0.41e | 21.59bc | 0.46b |
| | Limited | 400 ppm | 10.73g | 14.34g | 1.20d | 0.86b | 0.90a | 0.79a | 0.07b | 0.92a | 22.92a | 0.36e |
| | | 900 ppm | 12.64f | 15.86f | 1.63b | 0.81c | 0.89a | 0.78a | 0.08a | 0.75c | 20.69d | 0.38d |
| Talaye | Complete | 400 ppm | 24.78b | 28.94b | 3.35a | 0.66e | 0.68c | 0.46c | 0.03e | 0.51d | 15.91e | 0.45b |
| | | 900 ppm | 27.10a | 32.23a | 3.30a | 0.73d | 0.79b | 0.64b | 0.04e | 0.40e | 21.22cd | 0.47a |
| | Limited | 400 ppm | 13.40e | 17.18e | 1.45c | 0.91a | 0.88a | 0.72ab | 0.06c | 0.80b | 20.52d | 0.34f |
| | | 900 ppm | 15.12d | 18.64d | 1.42c | 0.82c | 0.86a | 0.73ab | 0.06c | 0.71c | 22.31ab | 0.38d |
| Okapi | Complete | A | 25.00b | 28.78b | 3.37ab | 0.41g | 0.52e | 0.16e | 0.03de | 0.29f | 13.80d | 0.56a |
| | | B | 22.72cd | 26.71c | 3.48a | 0.74e | 0.85c | 0.63c | 0.05c | 0.47e | 18.37b | 0.44c |
| | | C | 22.26d | 23.74d | 3.22bc | 0.77e | 0.89bc | 0.72bc | 0.07b | 0.63c | 24.79a | 0.33e |
| | Limited | A | 13.44f | 16.98f | 1.67d | 0.76e | 0.71d | 0.40d | 0.06b | 0.68c | 15.97c | 0.44c |
| | | B | 12.03g | 14.47gh | 1.40e | 0.84d | 0.93b | 0.83b | 0.08a | 0.90a | 24.22a | 0.39d |
| | | C | 9.59h | 13.85h | 1.18f | 0.91b | 1.04a | 1.13a | 0.08a | 0.93a | 25.22a | 0.28f |
| Talaye | Complete | A | 28.94a | 32.47a | 3.45a | 0.59f | 0.38f | 0.06e | 0.02e | 0.27f | 12.15e | 0.56a |
| | | B | 25.59b | 32.02a | 3.37ab | 0.74e | 0.90bc | 0.75bc | 0.04d | 0.46e | 18.93b | 0.49b |
| | | C | 23.29c | 27.26c | 3.16c | 0.74e | 0.93b | 0.84b | 0.05c | 0.64c | 24.62a | 0.33e |
| | Limited | A | 17.23e | 21.79e | 1.73d | 0.74e | 0.69d | 0.36d | 0.05c | 0.54d | 16.18c | 0.43c |
| | | B | 12.77fg | 16.29f | 1.34ef | 0.87c | 0.88bc | 0.68c | 0.06b | 0.84b | 24.04a | 0.38d |
| | | C | 12.77fg | 15.65fg | 1.25ef | 0.99a | 1.04a | 1.13a | 0.08a | 0.89ab | 24.03a | 0.27g |
| Complete | 400 ppm | A | 25.05b | 28.30c | 3.48a | 0.42g | 0.41g | 0.10g | 0.04e | 0.29g | 11.61g | 0.55b |
| | | B | 23.44c | 27.72cd | 3.45a | 0.75de | 0.89d | 0.72de | 0.04de | 0.57e | 13.12f | 0.44d |
| | | C | 21.06d | 24.50e | 3.23bc | 0.79d | 0.85d | 0.62e | 0.05d | 0.68d | 23.69c | 0.33g |
| | 900 ppm | A | 28.89a | 32.95a | 3.34ab | 0.59f | 0.48f | 0.12g | 0.02f | 0.27g | 14.33e | 0.57a |
| | | B | 24.87b | 31.01b | 3.40a | 0.73e | 0.86d | 0.66e | 0.04de | 0.36f | 24.17bc | 0.49c |
| | | C | 24.49b | 26.50d | 3.15c | 0.72e | 0.97bc | 0.94c | 0.06c | 0.59e | 25.71a | 0.33g |
| Limited | 400 ppm | A | 15.56e | 19.40f | 1.54e | 0.77d | 0.73e | 0.40f | 0.05d | 0.67d | 16.21d | 0.43d |
| | | B | 11.71g | 15.42h | 1.29fg | 0.89b | 0.92cd | 0.80d | 0.07bc | 0.90b | 25.04ab | 0.36f |
| | | C | 8.92h | 12.47i | 1.15g | 1.00a | 1.02ab | 1.07b | 0.08a | 1.01a | 23.92c | 0.26i |
| | 900 ppm | A | 15.11e | 19.38f | 1.86d | 0.73e | 0.68e | 0.35f | 0.06c | 0.55e | 15.94d | 0.44d |
| | | B | 13.09f | 15.34h | 1.45ef | 0.83c | 0.89d | 0.70de | 0.07bc | 0.84c | 23.22c | 0.41e |
| | | C | 13.44f | 17.03g | 1.28g | 0.89b | 1.06a | 1.20a | 0.08ab | 0.80c | 25.33a | 0.29h |
| Okapi | 400 ppm | A | 18.65de | 22.38de | 2.45abc | 0.57i | 0.61e | 0.27f | 0.05de | 0.52e | 14.73e | 0.49b |
| | | B | 16.08f | 19.74g | 2.39bc | 0.81cde | 0.92bcd | 0.81cd | 0.06c | 0.76b | 19.55d | 0.40e |
| | | C | 13.75g | 16.50h | 2.09d | 0.88b | 0.94bc | 0.87bc | 0.07ab | 0.86a | 24.67ab | 0.30gh |
| | 900 ppm | A | 19.79c | 23.39cd | 2.59a | 0.60h | 0.62e | 0.28f | 0.05ef | 0.44f | 15.04e | 0.51a |
| | | B | 18.67de | 21.44ef | 2.49ab | 0.77f | 0.86d | 0.65e | 0.07bc | 0.61d | 23.04c | 0.43d |
| | | C | 18.11e | 21.09efg | 2.31c | 0.80def | 0.98ab | 0.99b | 0.08a | 0.69c | 25.33ab | 0.32f |

Table 4. Continued.

| Treatments | | | Leaf-soluble carbohydrates (mg·g$^{-1}$ FW) | Reducing sugars (mg·g$^{-1}$ FW) | Chlorophyll (mg·g$^{-1}$ FW) | Carotenoids (mM·cm$^{-1}$) | Flavonoids (mM·cm$^{-1}$) | Anthocyanin (mM·cm$^{-1}$) | Proline (mg·g$^{-1}$ FW) | Soluble proteins (mg·g$^{-1}$ FW) | Glucosinolate (as µmol·g$^{-1}$) | Fv/Fm |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Talaye | 400 ppm | A | 21.96b | 25.32b | 2.56a | 0.61h | 0.53f | 0.23f | 0.03g | 0.43fg | 13.09f | 0.49b |
| | | B | 19.07cde | 23.39cd | 2.35bc | 0.83c | 0.89cd | 0.71de | 0.05de | 0.71c | 18.61d | 0.40e |
| | | C | 16.24f | 20.46fg | 2.29c | 0.91a | 0.92bcd | 0.82cd | 0.06cd | 0.83a | 22.94c | 0.29h |
| | 900 ppm | A | 24.21a | 28.95a | 2.61a | 0.72g | 0.54f | 0.19f | 0.04gf | 0.38g | 15.23e | 0.50ab |
| | | B | 19.29cd | 24.92bc | 2.36bc | 0.78ef | 0.89cd | 0.71de | 0.05ef | 0.60d | 24.36b | 0.47c |
| | | C | 19.83c | 22.44de | 2.12d | 0.82cd | 1.04a | 1.15a | 0.06bc | 0.70c | 25.71a | 0.31fg |

Means with similar letter are not significant at the 5% probability level

Table 5. Four-way interaction between treatments on some physiological attributes.

| Treatments | | | | Leaf-soluble carbohydrates (mg·g$^{-1}$ FW) | Chlorophyll (mg·g$^{-1}$ FW) | Carotenoids (mM·cm$^{-1}$) | Flavonoids (mM·cm$^{-1}$) | Anthocyanin (mM·cm$^{-1}$) | Fv/Fm |
|---|---|---|---|---|---|---|---|---|---|
| Okapi | Complete | 400 ppm | A | 23.43e | 3.51a | 0.37i | 0.53i | 0.17gh | 0.54cd |
| | | | B | 20.72f | 3.49a | 0.75g | 0.86de | 0.67cd | 0.42g |
| | | | C | 20.63f | 3.27abcd | 0.81ef | 0.87cd | 0.69cd | 0.32j |
| | | 900 ppm | A | 26.58b | 3.23bcd | 0.46h | 0.51i | 0.14gh | 0.58a |
| | | | B | 24.73de | 3.47ab | 0.73g | 0.84de | 0.60cde | 0.45e |
| | | | C | 23.90de | 3.17cd | 0.72g | 0.90cd | 0.76cd | 0.34ij |
| | Limited | 400 ppm | A | 13.88hi | 1.39h | 0.77fg | 0.70gh | 0.37f | 0.45ef |
| | | | B | 11.44lm | 1.30hi | 0.87cd | 0.98abc | 0.96b | 0.37h |
| | | | C | 6.87n | 0.91j | 0.94b | 1.02ab | 1.06ab | 0.27k |
| | | 900 ppm | A | 13.00ijk | 1.94e | 0.75g | 0.73fgh | 0.43ef | 0.44efg |
| | | | B | 12.61ijkl | 1.51gh | 0.82ef | 0.88cd | 0.70cd | 0.42g |
| | | | C | 12.32jklm | 1.45h | 0.87cd | 1.06a | 1.21a | 0.29k |
| Talaye | Complete | 400 ppm | A | 26.68b | 3.45ab | 0.46h | 0.30j | 0.03h | 0.56bc |
| | | | B | 26.17bc | 3.41abc | 0.76g | 0.92bcd | 0.78c | 0.45e |
| | | | C | 21.50f | 3.20cd | 0.76g | 0.82def | 0.56de | 0.34ij |
| | | 900 ppm | A | 31.21a | 3.45ab | 0.73g | 0.46i | 0.10gh | 0.56ab |
| | | | B | 25.01cd | 3.34abcd | 0.73g | 0.89cd | 0.72cd | 0.52d |
| | | | C | 25.09cd | 3.13d | 0.73g | 1.03a | 1.11ab | 0.33j |
| | Limited | 400 ppm | A | 17.24g | 1.68fg | 0.77fg | 0.76efg | 0.44ef | 0.42g |
| | | | B | 11.97klm | 1.29hi | 0.91bc | 0.86d | 0.650cd | 0.36hi |
| | | | C | 10.97m | 1.39h | 1.06a | 1.02a | 1.08ab | 0.24l |
| | | 900 ppm | A | 17.22g | 1.78f | 0.72g | 0.63h | 0.28fg | 0.44efg |
| | | | B | 13.57hij | 1.39h | 0.84de | 0.89cd | 0.71cd | 0.41g |
| | | | C | 14.57h | 1.11ij | 0.91bc | 1.06a | 1.19a | 0.29k |

Means with similar letter are not significant at the 5% probability level

### Proline

Water stress and UV radiation stress significantly increased proline content in leaves of both canola cultivars, while elevated $CO_2$ had not significant effect (Table 2). Accumulation of proline due to water stress as a water status regulator amino acid has been known previously. According to Saradhi et al. [35], free proline might have the capacity to scavenge and/or reduce the production of free radicals and could be an essential tool in UV protection as well as the relative contribution of other mechanisms to the overall tolerance of plants to UV radiation. Thus, we concluded that proline accumulation in subjected plants to UV radiation may be attributed to the regulator effect of proline in cell water status.

### Soluble Proteins

The results showed that soluble proteins were increased due to water stress and UV radiation while the increase of $CO_2$ decreased soluble proteins in canola leaf tissues. Also, Okapi cultivar had higher levels of proteins in comparison to Talaye cultivar (Table 2).

It seems that water stress or UV radiation leads to protein breaking down and soluble protein content would be increased in plant tissues [18].

### Glucosinolate

Glucosinolate content increased under conditions of water stress, elevated $CO_2$, and UV radiation. There was no significant difference between cultivars on glucosinolate content. Enhancement of glucosinolate content was paralleled with a decrease in UV wavelength so that in those plants subjected to UV-C radiation, glucosinolate content was at maximum amount (Table 2). Interaction among different treatments showed that the highest glucosinolate content was observed in those plants that received UV radiation and high $CO_2$ concentration with water stress. There are a few studies about glucosinolate accumulation in response to water stress, although the previous studies indicate that environmental factors such as light, temperature, and heavy metals alter glucosinolate content [36-38]. Increase of glycerinate in response to water stress may be a strategy to increase plant resistance to water stress. In addition, it has been suggested that high concentrations of organic solutes in the cytoplasm, including proline, sucrose, and glycinebetaine, and secondary metabolites such as glucosinolates contribute to the osmotic balance [39]. Other studies have reported that mechanical impacts also increase glucosinolate concentration in *Brassica* vegetables [40]. Some abiotic stress factors, such as UV-B and water stress, lead to increased glucosinolate concentrations in nasturtium and turnip [41, 42].

### Fv/Fm

In this study, maximum photochemical efficiency decreased due to water stress and UV radiation. In contrast, elevating $CO_2$ increased the Fv-to-Fm ratio (Table 2). The decline in the Fv/Fm ratio is a good indicator of photo inhibitory damage caused by light or other environmental stresses. The reduction in chlorophyll fluorescence under UV radiation at both ambient and elevated $CO_2$ indicates that UV radiation might have damaged the D1 and D2 proteins of PS II and degraded chlorophyll, which might have resulted in reduced quantum efficiency or lower photosynthetic capacity [41]. In the case of photosynthesis, chlorophyll has a crucial role in the production of assimilates. Also, we observed that increasing $CO_2$ concentration improved maximum photochemical efficiency; it seems that elevated $CO_2$ can improve photosynthesis efficiency via increase of $CO_2$ accessibility.

## Conclusions

Water stress, carbon dioxide, and UV radiation had significant effects on soluble carbohydrates in canola leaves. Also, we observed that canola cultivars were unlike each other in terms of leaf-soluble carbohydrates with leaf-soluble carbohydrate in Talaye higher than Okapi. In addition, water deficit stress and UV radiation significantly decreased leaf-soluble carbohydrates. In contrast, elevated $CO_2$ increased leaf-soluble carbohydrates. The results showed that under conditions of complete irrigation or limited irrigation, increasing $CO_2$ can increase soluble carbohydrates in leaves. Furthermore, regardless of the presence of water stress or elevated $CO_2$, UV radiation decreased leaf-soluble carbohydrates. UV-C radiation and water stress significantly decreased leaf-soluble carbohydrates in Okapi cultivars under condition of ambient $CO_2$ as this cultivar had the lowest leaf-soluble carbohydrates affected by these treatments. Reducing sugar content was significantly affected by water stress, elevated $CO_2$, and UV radiation. Talaye cultivar had more sugar content than Okapi cultivar. Significant negative effects of water stress and UV radiation on total chlorophyll content, compared to the control treatments, states adverse effect of these abiotic stress on this plant. It is mentionable that no significant difference in total chlorophyll content was observed between ambient $CO_2$ concentration and elevated $CO_2$ concentration. Exposure of canola plants to increasing UV-B and UV-C intensity reduced the content of chlorophyll. Carotenoids, flavonoids, and anthocyanin concentration showed an increasing trend with decreasing UV wavelength and water deficit stress. Water stress and UV radiation stress significantly increased proline content in leaves of both canola cultivars, while elevated $CO_2$ had no significant effect. Soluble proteins were increased due to water stress and UV radiation, while an increase of $CO_2$ decreased soluble proteins in canola leaf tissues. Also, Okapi cultivar had higher levels of proteins in comparison to Talaye cultivar. Glucosinolate content increased under conditions of water stress, elevated $CO_2$, and UV radiation. There was no significant difference between cultivars on glucosinolate content. Enhancement of glucosinolate content was paralleled

with decreases in UV wavelength so that in those plants subjected to UV-C radiation glucosinolate content was at maximum amount. Maximum photochemical efficiency decreased due to water stress and UV radiation. In contrast, elevating $CO_2$ increased the Fv-to-Fm ratio.

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