Effects of CO$_2$ concentration and UV-B radiation on date palm (*Phoenix dactylifera*) grown in open-top chambers

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Received: 11 July 2019; Accepted: 28 December 2019

INTRODUCTION

Plant primary production depends on many factors including the physiology and morphology of the plants, and environmental factors, such as irradiance, temperature, water and nutrient availability. Climate change is already affecting the natural resources such as terrestrial vegetation, animal husbandry and fisheries (World Bank, 2009). Climate change is mostly driven by the increased levels of greenhouse gases especially CO$_2$ in the earth's atmosphere leading to changes into environmental factors such as stratospheric ozone depletion and rise in solar UV-B radiation, high temperature, weather extremes and variation in humidity and rainfall patterns. The carbon dioxide concentration in the atmosphere is expected to double by the year 2050. Climate model studies projects that large areas of Middle Eastern countries such as Saudi Arabia might become climatically unsuitable for date palm growth due to the westward shift of the heat stress in these countries. The survival of the date palm trees and marketable fruit productivity are highly reduced under saline conditions (Yaish & Kumar, 2015).

Elevating atmospheric CO$_2$ concentration, rising global temperature and increasing amount of solar ultraviolet-B (UV-B) radiation reaching the earth are all considered to be the key climate change factors that affect the fitness of green plants. Despite the suppression of photorespiration,
CO$_2$ has typically been shown to have positive effects on the development of C$_3$ plants. A number of recent studies emphasize how the enhancement of atmospheric CO$_2$ directly impacts the physiology of plants and generally accelerates the photosynthetic rate and increases plant growth and yield (Zhang et al., 2018). Plants grown at elevated CO$_2$ compared to those grown at ambient CO$_2$ also exhibit increased growth and photosynthesis, lower transpiration, decreased respiration, better water quality, lower concentrations of mineral nutrients, increased content of plant hormones, reduced stomatal density and conductance. Not unexpectedly, several researchers focused on understanding CO$_2$-temperature interactions with plant growth and development (Singh et al., 2010).

Species grown in highlands appear to be better adapted to high doses of UV-B than those grown in coastal regions (Wang et al., 2016). Environments can affect plant exposure to UV-B radiation, including elevated CO$_2$ and temperature. Hypothetically, the assessment of UV-B engrossing mixes would increase and the negative effect of extended UV-B parts should be reduced due to an increased substance of auxiliary metabolites under elevated CO$_2$. In fact, previous studies have reported the damaging impacts of UV-B even under the elevated CO$_2$ conditions on row crops (Koti et al., 2007; Singh et al., 2010). In natural environments, the depth of UV-B radiation relies upon on the season, range, altitude and clouds, leading to wide temporal and spatial dynamics. Thus, investigation on date palm response to the combinations of elevated CO$_2$ and UV-B radiation under the UAE climate conditions will help to elucidate on the CO$_2$ compensatory effects, if any, on plant growth.

Date palm is an economically important crop and well adapted to the arid and semi-arid environments of the Middle Eastern countries including the UAE (Shabani et al., 2012). However, variation in rainfall, global warming, gas pollution and decline of water resources are common concern for date palm production. Climate model studies projects that large areas of Middle Eastern countries such as Saudi Arabia might become climatically unsuitable for date palm growth due to the westward shift of the heat stress in these countries (Shabani et al., 2012). Given the limited research and development activities, the Gulf Cooperation Council (GCC) countries ranked date palm as one of the high research priority as reflected in priorities settings for agricultural research (Erskine et al., 2004).

Atmospheric CO$_2$ production, temperature and UV-B radiation simultaneously increase and only a few studies have considered the combined effects of these three climate change influences on woody plant (Paajanen et al., 2010). It is one of the high research priorities as reflected in priority settings for agricultural research. Thus considering socio-economic importance of the UAE Date palm, the present study was on the evaluation of the impact of the climate change factors such as elevated CO$_2$, enhanced UV-B radiation and their interactions and climate change consequences on morphological and physiological changes. This study is part of a series of studies in which we attempted to elucidate the physiological and morphological responses of date palm to UV-B radiation and CO$_2$ concentration and to determine whether elevated CO$_2$ can reduce or eliminate the adverse effects of UV-B radiation, which can be well suited to the UAE climate conditions.

**MATERIALS AND METHODS**

The experiment was conducted under natural light conditions at Al-Foah Experimental farm, College of Food and Agriculture, UAEU, in an open top chamber covered with the transparent plastic on the top to allow the passage of maximum sunlight. The OTCs were 3 m in diameter and 5 m in height with a 45° sloping frustum and the minimum distance between any two chambers was 3.5 m. The 24 month old of commercially grown Date palm (LaLd), obtained from Date palm Research unit, UAE University, were planted (pots with sand. Plant was supplied well with the nutrient and water as recommended for the plant cultivation.

The experimental design was based on completely randomized plots including four treatments belonging to 6 OTCs: (1) control (2) elevated CO$_2$ concentration (400-600 ppm); (3) enhanced UV-B radiation (9.50 kJ d$^{-1}$ m$^{-2}$); (4) CO$_2$ + UV-B (elevated CO$_2$ concentration + enhanced UV-B radiation, 9.50 kJ d$^{-1}$ m$^{-2}$). There were no air filtered chambers in all OTCs.

**UVB and CO$_2$ treatments**

The UVB radiation treatments of the ambient level (control) and elevated (U- VB) level were imposed using eight fluorescent UV-313 (Q-Panel Company, Cleveland, OH, USA) with the UVB radiation (280 and 320 nm) emission. The UVB lamps were directly mounted above each set of the plants receiving 8 hours of the UVB radiation. The elevated CO$_2$ treatments of the current level (control) and elevated CO$_2$ levels as 8 hrs per day were imposed using CO$_2$ sensors were used for the CO$_2$ enrichment to the plants with the increased CO$_2$ level (500 ppm) concentration. The actual concentration of carbon dioxide within the OTC was measured by a CO$_2$ analyzer and controlled by computer supported regulation of inlet valves (Upreti et al., 2006; Vanaja et al., 2006).

**Estimation of chlorophylls, carotenoids**

Chlorophyll contents were extracted from the matured leaves and estimated according to the method of Arnon, et al. (2004).
(1949) with slight modification on the plant material added. 500 mg of fresh leaf material was ground with 10 ml of 80 per cent acetone at 4°C in a pestle and mortar and centrifuged at 2,500 g for 10 minutes. The absorbance was read at 645, 663 and 480 nm in a Spectrophotometer (U-2001–Hitachi) against 80 per cent acetone as a blank. The carotenoids content was calculated using the formula of Kirk and Allen (1965) method and expressed in milligrams per gram dry weight. Carotenoid (mg/g) = A.480 + (0.114 × A.663 − 0.638 × A.645).

Estimation of protein content
Soluble protein determination was made according to the Bradford (1976). One gram of plant material was ground in mortar and pestle with 20 ml of 20 per cent TCA. The homogenate was then centrifuged at 800 rpm 15 minutes and the supernatant was discarded. To the pellet, 5 ml of 0.1 N NaOH was added to solubilize the protein and the solution was centrifuged again at 800 rpm (Pro-Analytical, Centurion Scientific, UK) for 15 minutes. The absorbance at 595 nm was measured after 2 minutes against a reagent blank. The protein weight was plotted against the corresponding absorbance resulting in a standard curve and used in unknown samples to assess the protein and the findings were expressed in milligrams per gram of dry weight.

Estimation of amino acid content
Extraction and estimation of the total amino acid content was measured by the method of Moore & Stein (1948). 1 ml of the sample was mixed with 1 ml of Ninhydrin and kept in a boiling bath for 20 minutes, and then 5 ml of diluting reagent (equal volume of water and n-propanol) was added and incubated for 15 minutes and diluted to 25 ml with distilled water. At 570 nm, the absorbance was read against a blank reagent. Using glycine, the standard graph was prepared. The content of amino acid was determined using the standard chart. The calculation was made in triplicates and the findings are presented as a measurement of mg/g.

Estimation of phenolic compounds
Malik & Singh (1980) method was used to estimate the total phenolic compounds. In brief, the 500 mg of fresh plant tissue with 10 ml of 80% ethanol and the homogenate was centrifuged at 10000 rpm for 20 minutes. The residue was dissolved in 5 ml distilled water and 0.5 ml Folin–Ciocalteau reagent was added to the extract. 2 ml of 20 % Na₂CO₃ solution was subsequently added and completely mixed. The mixture was kept in boiling water for 1 minute and after cooling the absorbance was read at 650 nm. The total phenol content in the test samples was calculated from the standard curve and expressed as milligrams of gallic acid equivalent of wet mass (mg of GAE/100 g).

γ-glutamyl kinase
The activity of γ-glutamyl kinase was assessed using Hayzer and Leisinger (1980) method. One gram of plant tissue was extracted for 3 minutes in a vortex homogenizer with 10 ml of 50 mM Tris-HCl buffer (pH 7.2) and 10,000 g centrifugation at 4°C for 20 minutes. In a final volume of 0.25 ml, the enzyme assay mixture contained; L-glutamate, 50 mM; ATP, 10 mM; MgCl₂, 20 mM; Hydroxylamine HCl, 100 mM; Tris base 50 mM, balanced (pH 7.0) and 100 ml of desalted extract containing approximately 3.0 mg of enzyme protein at a final 2 ml volume. The reaction began with the addition of extract of the enzyme. 2 ml of stop buffer (FeCl₃,3H₂O (2.5% w/v) and TCA (6% w/v) in 2.5 M HCl) was added to incubate at 37°C for 30 minutes. Activity of γ-glutamyl kinase measured at 535 nm by a spectrophotometer and expressed in units (U). One unit of the activity of the γ-glutamyl kinase may be defined as any γ-glutamyl hydroxamate formed per minute min⁻¹ mg⁻¹ protein.

Proline oxidase
Based on the method outlined by Huang & Cavalieri (1979), proline oxidase activity was determined. 1 g of plant tissue was homogenized with 5 ml of homogenizing medium and filtered homogenously at 10,000 g for 10 minutes at 4°C. This pellet was mixed with 1 ml 5 mM Tricine – KOH buffer (pH 7.5) with 6 M sucrose. 3 ml enzyme assay mixture contained 0.1 ml enzyme extract, 1.2 ml 50 mM Tris-HCl buffer at pH 8.5, 1.2 ml 5 mM MgCl₂, 0.1 ml 0.5 mM NADP, 0.1 ml 1 mM potassium cyanide (KCN), 0.1 ml 1 mM phenazine methosulfate (PMS), 0.1 ml 0.06 mM 2, 6-dichlorophenol indophenol (DCPIP) and 0.1 ml 0.1 ml 0.1 ml proline. The reaction was monitored at 600 nm in at 25°C using proline to initiate the reaction. The activity of the enzyme is expressed as a reduced mM of DCPIP per minute per mg protein.

α-Tocopherol
α-Tocopherol activity was measured as described by Backer et al. (1980). 500 mg of fresh tissue was homogenized with 10 ml of a petroleum ether and ethanol mixture (2:1.6 v/v). The extract was centrifuged for 20 minutes at 10,000 rpm, and the supernatant was used to estimate α-tocopherol. 1 ml of extract, 0.2 ml of 2% 2, 2-dipyridyl was applied and thoroughly dissolved in ethanol and kept in the dark for 5 minutes. The resulting red color was diluted and mixed well with 4 ml of distilled water. The resulting color was assessed at 520 nm in the aqueous layer and the α-tocopherol content was calculated using a standard graph made with a known volume of α-tocopherol.

Peroxidase activity
Peroxidase activity was evaluated using the Kumar & Khan method (1982). The experimental peroxidase mixture
contained 2 ml 0.1 M phosphate buffer (pH 6.8), 1 ml 0.01 M pyrogallol, 1 ml 0.005 M H₂O₂ and 0.5 ml enzyme extract. The solution was incubated for 5 minutes by adding 1 ml of 2.5 N H₂SO₄ to complete the reaction. The quantity of purpurogallin formed was estimated by measuring the absorption at 420 nm against a blank prepared by adding the extract at zero time after adding 2.5 N H₂SO₄. The POX activity is expressed in unit mg⁻¹ protein.

**Statistical analysis**

The data analysis of variance (ANOVA) was performed using IBM SPSS Statistics for Windows (IBM Corp., Armonk, NY). Two-way Analysis of Variance (ANOVA) procedure was used to analyze the data with CO₂ and UV-B as two independent variables. The significant difference between mean was determined by Duncan’s multiple range test at the P ≤ 0.05 levels. Values are given as mean SD of three experiments in each group.

**RESULTS**

It was found that CO₂ and UV-B radiation caused significant morphological and biochemical changes in the date palm plant being examined. Evidence on the chlorophyll content of the studied date palm plants showed that the content of chlorophyll ‘a’ and ‘b’ in UV-B reduced gradually and increased in the date palm variety of LuLu after 8 hours of CO₂ and UV-B treatment. Plants grown under OTC conditions maintained a higher content of chlorophyll than plants grown under environment conditions (Fig. 1). The date palm crop analyzed after UVB treatment the overall chlorophyll content was decreased compared to untreated crops. Carotenoid content (0.575 mg/g) was decreased in the treatment plant compared to untreated control. After 8 hours of UV-B + CO₂ treatment, the carotenoid content of the date palm variety of LuLu did not show much difference. However, after 8 hours of CO₂ treatment, the carotenoid content of LuLu was significantly increased (0.712 mg/g).

In the UV-B treated crop, the protein concentration of date palm variety content decreased compared to control CO₂ treatments showed higher protein content relative to UV-B treated with CO₂. In date palm 8 hours of CO₂ and UV-B treatment, the protein content gradually decreased in UV-B and increased in CO₂ treatment. There was also a slight decrease in more reliable data at 500 ppm CO₂, where the combined treatments showed little difference in protein content compared to untreated control (Fig. 2).

In the date crop variety of LuLu, the free amino acid content increased significantly as compared to control (Fig. 3). CO₂ treatment at the date plants reduced the amount of free amino acids in the test of leaves relative to plants treated with UV-B. Date palm plants in 500 ppm CO₂ had the highest average free amino acid content (5.522 mg/g) compared to CO₂ treatment control.

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**Fig 1.** Effect of CO₂, UV-B and their combination induced changes in leaf (A) chlorophyll ‘a’, (B) chlorophyll ‘b’, (C) total chlorophyll and (D) carotenoid content of date palm native plants. (Values are expressed as mean ± SEM, n=5).
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The total phenolic content of gallic acid equivalent was expressed in μg. Phenolic compounds can directly contribute to the action of antioxidants. UV-B treated date palm has the highest phenol content recorded in a 280-300 nm UV-B environment with a value of 0.062 mg/g compared to other treated plants in the concentration of total phenol content. Fig. 4 shows the standard curve generated with gallic acid for the determination of total phenolic content. The UV-B stimulated the total phenolics throughout the date palm plants, while under the elevated CO₂ it decreased.

In date palm variety LuLu, the activity of γ-glutamyl kinase has been significantly increased in UV-B treatment compared to control (Fig. 5). We observed that in high and moderate water stress treatments under CO₂ enriched environments (500 ppm) γ-glutamyl kinase of date palm plants decreased compared to the OTC environment. In contrast to control (1.163 μg/min/mg protein) and other treated plants, the combined effect of CO₂ and UV-B treatments increased slightly (2.206 μg/min/mg protein) in the activity of γ-glutamyl kinase. CO₂ treatments at the date palm decreased the activity of γ-glutamyl kinase compared to other treatments, but the maximum similarity of control was apparent.

In the date palm plants, an activity of proline oxidase was largely inhibited by elevated CO₂ compared to control (Fig. 6). In contrast to UV-B treated plants, the addition of UV-B + CO₂ treated date palm increased the activity of proline oxidase. All three different plant treatment show increased activity in control groups compared to other

(4.056 mg/g). The CO₂ was compared with the other known CO₂ + UV-B sequences and showed the maximum similarity of LuLu’s date palm variety compared to other two different groups such as UV-B and control. It would however give an insight into what actually contributed more to the content of amino acids.
groups followed by CO₂ treatment. A proline oxidase was found in the mitochondrial fraction when the leaf organelles were isolated on a sucrose gradient. Compared to UV-B and UVB + CO₂ plants, CO₂ treatments at the date palm plant increased proline oxidase activity.

In the date palm variety of LuLu tested after 8 hours of treatment, the α-tocopherol content of CO₂ and UV-B treated plants was not significantly different compared to control (Fig. 7). A closer look would show that CO₂ enrichment significantly increased the content of α-tocopherol relative to the OTC environment. The α-tocopherol content was slightly increased (9.14 mg/g) after UV-B treatment (8.588 mg/g) in the combined effect of CO₂ and UV-B treated crop. The treatment with UV-B + CO₂ significantly increased the content of α-tocopherol in all growth stages and increased the influence of increasing α-tocopherol compared to control.

Peroxidase activity in the ambient environment under OTC was lowest (3.253 u/mg protein) and highest (4.33 u/mg protein) at 500 ppm CO₂ with UV-B (Fig. 8). The most important findings were that treatments with CO₂ (500 ppm) + UVB (300 nm) have the highest date palm plant activity relative to other treatments. Compared to the other identified UV-B sequences, the CO₂ was observed to have the highest date palm variety similarity compared to other two different groups. It would however give an insight into what actually contributed more to the content of amino acids. The combined effect showed a higher free peroxidase activity followed by UV-B, CO₂ and the lowest controlled activity among the 8 hour treatment of date palm plants.

**DISCUSSION**

Although most of the calculated growth parameters increased as a result of increased concentration of CO₂ and enhanced UV-B, the accumulation pattern of phytochemicals did not change dramatically as a result of the combination of treatments tested, nor were there any significant interactive effects due to treatments. Our findings suggest that different response mechanisms depending on the high dosage of CO₂ and UV-B irradiation, indicating the ability to maintain its photosynthetic activity of the date palm. Adverse environmental factors can affect metabolic and pathological changes in plants, including UV-B radiation and CO₂.

In this study, a decreased concentration of chlorophyll b is a more common symptom of UV-B radiation stress. This can be attributed to inhibition of UV-B pigment biosynthesis (Charles et al., 2002). Chlorophylls and carotenoids that are affected by various high UV radiations, where carotenoids are usually less affected than chlorophylls. Buckeridge et al., (2002) explains that increased CO₂ availability should increase the photosynthetic rate and allow greater carbon accumulation, thus increasing the size of plants.

Total phenolics increased, but protein decreased in plants treated with UV-B in Open top chambers Langerbartels et al. (1998) also reported increases in total phenolic compounds at high CO₂ rates. The decrease in the total protein content of all plant species could be a concomitant of the delayed growth rate of the treated plants due to reduced photosynthetic output, resulting in a projected decrease in the nitrogen pool. In contrast, Ultraviolet radiation in the leaves of higher plants destroys lipids, nucleic acids and proteins (Vass, 1997). The decrease in protein content in unsettling stressed plants was associated with increased accumulation of proline. This may be due to protein hydrolysis or protein synthesis inhibition due to oxidative stress that leads to proline accumulation (Feng et al., 2003).

Amino acids are regarded as precursors and protein constituents, as well as a well-known biostimulant that has
positive effects on plant growth, yields and significantly mitigates abiotic stress injuries (Kowalczyk & Zielony, 2008). The increased levels of free amino acids in plants throughout the life cycle also revealed the potential for these compounds to participate in osmoregulation. They also suggested that increased amino acid content could be due to increased protein degradation under pressure conditions. In addition, amino acids serve as precursors for many secondary plant metabolites that perform critical functions such as signaling, defense, interactions with other organisms, and photoprotection. (Pratelli & Pilot, 2014).

In comparison, Tripathi et al. (2011) documented increases in plant leaf phenols under UV-B radiation treatment and/or O3 treatment. In addition, the present study showed a significant difference in the concentration of flavonoids in response to increased O3 and or UV-B radiation. UV exposure significantly increased total phenol, similar findings in durum wheat (Balouchi et al., 2009) and Phaseolus trilobus (Ravindran et al., 2008) were found to increase UV absorbing compounds. The increased oxidation of phenolics due to the application of fungicides can thus contribute to the acceleration of oxidative damage. The perception of it as a healthier food is due to the red pigmentation in the plant tissues in response to the different types of phenolic and flavonoid compounds (Llorach et al., 2008).

Variations in γ-glutamyl kinase activities in tomatoes in various physiological regions have been identified (Gruenberg, 2001). In addition, Misra and Misra (2012) proposed increase in proline metabolism enzymes (pyrroline-5-carboxylate and γ-glutamyl kinase) and decreased proline oxidase activity in retaliation for abiotic stress. The decrease in proline oxidase activity with concomitant increase in γ-glutamyl kinase activity may be the reason for increased proline accumulation in date palm plants treated with UVB.

Prolines role in plants is related to survival rather than growth preservation (Jaleel et al., 2007). Other crops such as corn (zea mays), (Serraj & Sinclair, 2002) and peanut (Arabidopsis hypogaea) have also reported accumulation of proline under environmental stress (Smith et al., 2002). Proline oxidase, oxidize the proline and give it glutamic acid again. This enzyme also influences the free proline level (Jaleel et al., 2007). Generous plant work has shown that proline accumulation is positively correlated with oxidative stress resistance in plants under various abiotic stresses. (Saeedipour, 2013).

α-Tocopherol, found in green plant areas, scavenges lipid peroxide radicals by the concerted action of other antioxidants (Kiffin et al., 2006). In addition, α-tocopherols have also been known to protect lipids and other membrane components by physically quenching and chemical reaction with O$_3$ in chloroplasts, thus preserving the structure and function of PSII (Igamberdiev et al 2004). Conserving the synthesis of α-tocopherol during the growth of oxygenic photosynthetic organisms indicates that this molecule has one or more essential functions. With increased levels of α-tocopherol and β-carotene, active oxygen species produced at the membrane of wheat leaves under environmental stress are efficiently extracted after rehydration (Bartoli et al. 1999).

In the combined effect of CO$_2$ and UV-B under OTC conditions, peroxidase production in the date palm was increased. In many crops, such as rice, similar results have been observed (Shao et al., 2005). Peroxidases are monomeric hemoproteins that catalyze the oxidation by hydrogen peroxide of a range of substrates. Such enzymes are involved in physiological processes such as phenoloxidation. A common response to oxidative and abiotic stress is increased peroxidase activity. Peroxidase could therefore be part of the enzymatic process associated with increased ethylene production in plants such as spinach (Ozturk & Demir, 2003). Overall, according to OTC, the smallest changes in leaf phytochemicals and antioxidant accumulation were observed in combination with studied environmental factors such as UV-B and CO$_2$.

**CONCLUSION**

Based on the results, it can be inferred that plants make improvements in some of their physiological and biochemical characteristics in environmental stress. Our main objective was to assess the effects of elevated CO$_2$ and increased UV-B radiation both individually and in combination with plant growth and date palm activity, and we found that elevated CO$_2$ and increased UV-B radiation individually inhibited plant growth and date palm activity. Increases in photosynthetic pigments are of paramount importance for environmental stress sensitivity in addition to other factors. These changes highlight the importance of physiological and biochemical studies in revealing the mechanisms that underlie stress responses. Therefore, this study includes a combined effect of UV-B radiation with other stress parameters as well as a field level establishment and yield characteristics for improvement in the identification of new stress resistant cultivars of date palm plants.

**ACKNOWLEDGEMENTS**

We wish to express our gratitude to the United Arab Emirates University for providing the facilities for the study.
Authors’ contributions
KK and SK conducted the research work, interpreted the data, and prepared the manuscript; MAS and WAA provided the guidance for experimental design and help interpreted the data; MAS and KK critically reviewed the manuscript.

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