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United Arab Emirates University

College of Agriculture and Veterinary Medicine

Department of Integrative Agriculture

# ABIOTIC STRESS EFFECT ON GROWTH AND PHOTOSYNTHETIC CHARACTERISTICS IN EMIRATI DATE PALM VARIETIES

Nasser Abdullah Ghdayer Al Kaabi

This thesis is submitted in partial fulfilment of the requirements for the degree of Master of Science in Horticulture

Under the Supervision of Dr. Mohammed Abdul Muhsen Ali Salem Alyafei

November 2021

## Declaration of Original Work

I, Nasser Abdullah Ghdayer Al Kaabi, the undersigned, a graduate student at the United Arab Emirates University (UAEU), and the author of this thesis, entitled "*Abiotic Stress Effect on Growth and Photosynthetic Characteristics in Emirati Date Palm Varieties*", hereby, solemnly declare that this thesis is my own original research work that has been done and prepared by me under the supervision of Dr. Mohammed Abdul Muhsen Ali Salem Alyafei, of the Agriculture and Veterinary Medicine at the UAEU. This work has not previously formed the basis for the award of any academic degree, diploma or a similar title at this or any other university. Any materials borrowed from other sources (whether published or unpublished) and relied upon or included in my thesis have been properly cited and acknowledged in accordance with appropriate academic conventions. I further declare that there is no potential conflict of interest with respect to the research, data collection, authorship, presentation and/or publication of this thesis.

Student's Signature:  Date: 14/11/2021

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## Abstract

Plants are naturally exposed to various environmental stresses that affect their growth and development. As a desert plant, date palm has developed strategies to protect itself from most abiotic stresses. However, projected climate changes and the interaction between the various abiotic stressors will have profound effects on date palm adaptation and production In the present study, five date palm cultivars, namely Chichi, Kalas, Nabt Saif, Sultana and Zamli, were initially evaluated for salinity tolerance by measuring the growth parameters such as plant height, fresh weight and dry weight of shoots and roots. Based on the obtained results, Sultana and Zamli cultivars were identified as salinity tolerant and selected to study the response to future climate scenarios such as eCO2, enhanced UVB radiation and the combined effect of UVB and eCO2 in open chambers. The response of the date palm cultivars studied was determined by analysing photosynthetic pigments (chlorophyll 'a', 'b' and total chlorophyll, carotenoids), biochemical contents (proline, protein, amino acid), proline metabolising enzymes (γ-glutamyl kinase activity, proline oxidase activity, non-enzymatic antioxidants (total phenols, α-tocopherol, reduced glutathione content) and antioxidant enzyme activities (polyphenol oxidase, peroxidase, superoxide dismutase, catalase, ascorbate peroxidase) were analysed. The results also showed that the Sultana cultivar is tolerant to future climate scenarios. However, more biotic stress and yield parameters are needed for the identification of biotic stress tolerant date palm cultivars.

**Keywords**: Date Palm, UVB Radiation, Elevated Level $CO_2$, Morphology, Antioxidant Enzymes, Stress Tolerant.

## Title and Abstract (in Arabic)

# تأثير الإجهاد الغير حيوي على النمو والخصائص الضوئية في أصناف النخيل الإماراتية


***الملخص***

تتعرض النباتات بشكل طبيعي لضغوط بيئية مختلفة تؤثر على مراحل النمو. نظرًا لكونه نباتًا صحراويًا، فقد طور نخيل التمر استراتيجيات لحماية نفسه من معظم الضغوط الغير حيوية. ومع ذلك، فإن التغيرات المتوقعة في المناخ والتفاعل بين مختلف الضغوط الغير حيوية سيكون لها تأثير عميق على تكيف وإنتاج نخيل التمر. في هذا البحث، تم فحص خمسة أصناف من نخيل التمر وهي شيشي، خلاص، نبته سيف، سلطانة و أخيراً زاملي، في البداية لمعرفة مقدار تحمل الملوحة عن طريق قياس معاملات النمو مثل ارتفاع النبات، الوزن الطازج والجاف للساق والجذور. بناءً على النتائج التي تم الحصول عليها، تم تحديد صنفي سلطانة و زاملي على أنهما يتحملان الملوحة وتم اختيارهما لدراسة الاستجابة للسيناريوهات المناخية المستقبلية مثل ارتفاع نسبة ثاني أوكسيد الكربون، الأشعة فوق البنفسجية المعززة وتأثيرهما المشترك في البيوت المحمية المفتوحة من الأعلى . تم قياس استجابة صنفي سلطانة و زاملي من خلال تحليل أصباغ التمثيل الضوئي (الكلوروفيل أ، ب وإجمالي الكلوروفيل، الكارتونيات)، محتويات الكيمياء الحيوية (البرولين، البروتين، الأحماض الأمينية)، إنزيمات التمثيل الغذائي للبرولين (نشاط بيتا-جلوتاميل كيناز، نشاط أوكسيد البرولين، مضادات الأكسدة غير الأنزيمية، مجموع الفينولات، ألفا توكوفيرول، انخفاض محتوى الجلوتاثيون) والإنزيمات المضادة للأكسدة (أوكسيد بولي فينول، بيروكسيداز، ديسموتاز الفائق، الكاتلاز، أسكوربات بيروكسيداز). كما أظهرت النتائج أن صنف سلطانة يتحمل سيناريوهات المناخ المستقبلية، ومع ذلك، هناك حاجة لدراسة المزيد من عوامل الإجهاد الحيوي ومعايير الإنتاجية لتحديد أصناف نخيل التمر التي تتحمل الإجهاد الحيوي.



**مفاهيم البحث الرئيسية:** نخيل التمر، الأشعة فوق البنفسجية، ارتفاع نسبة ثاني أكسيد الكربون، إنزيمات مضادات الأكسدة، الإجهاد الحيوي.

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I wish to express my hearty, deep sense of gratitude and sincere thanks to Dr. Mohammed Abdul Muhsen Alyafei, Vice Dean, College of Food and Agriculture. I thank him for his periodical guidance in planning and execution of the work.

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Special thanks go to my parents and family members who helped me along the way. I am sure they suspected it was endless.

# Dedication

*To my beloved parents and family*

# Table of Contents

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# List of Abbreviations

| | |
|---|---|
| ATP | Adenosine Triphosphate |
| CRD | Completely Randomized Design |
| $FeCl_3$ | Iron(III) Chloride |
| FW | Fresh Weight |
| HCl | Hydrochloric Acid |
| M | Molar |
| mg | Milligram |
| $MgCl_2$ | Magnesium Chloride |
| mL | Milliliter |
| mM | Millimolar |
| N | Normality |
| NaCl | Sodium Chloride |
| NaOH | Sodium Hydroxide |
| OTCs | Open Top Chambers |
| PLC | Program Logic Control |
| SCADA | Supervisory Control and Data Acquisition |
| TCA | Trichloroacetic Acid |
| U | Unit |
| UVB | Ultraviolet B |
| v/v | Volume/Volume |

# Chapter 1: Introduction

## 1.1 Overview

### 1.1.1 Climate Change

Climate change is one in all the foremost vital threats facing the globe nowadays. It is largely driven by the magnified levels of greenhouse gases within the earth's atmosphere such as Methane ($CH_4$), Nitrous Oxide ($N_2O$), Chlorofluorocarbons (CFCs) and especially elevated level carbon dioxide resulting in changes into environmental factors like ozone depletion and high temperature, enhanced UV-B radiation, salinity, weather extremes, drought and variation in rainfall patterns and atmospheric humidity.

Climate change is already affecting the natural resources such as terrestrial vegetation, animal husbandry and fisheries that societies depend on to provide food, fibre, fuel, several industrial products and recreational services (World Bank, 2010). The atmospheric concentration of carbon dioxide ($CO_2$) has increased globally by more than 100 ppm (36%) over the last 250 years. The current $CO_2$ concentration in the atmosphere is approximately 400 ppm and it estimated to double the end of this 21$^{st}$ century (IPCC, 2014a). Changes projected in $CO_2$ and other greenhouse gases are expected to increase global air temperature by 2.5 to 4.5°C during the same period.

The intensity and frequency of extreme event such as high temperature and drought are expected to increase at global and regional scales due to the amplifying effect of the predicted changes in climate (Allison *et al*., 2009; IPCC, 2014a). Due to the increasing multiplicity and complexity of environmental stress factors linked with global climate change, there is urgent need to develop better understanding of the

interactive effect of multiple abiotic stresses on crops and trees system to predict their response to changing environment (Singh *et al*., 2010; Vinebrooke *et al*., 2004).

It is virtually certain that the average global surface temperature has successively increased since the mid-19$^{th}$ century (Stocker *et al*., 2013). IPCC's report states that the first decade of the 21$^{st}$ century was the warmest and the global temperature -increased by 0.85°C [0.65–1.06]. Edame et al. (2011) present startling evidence that crop yield has already changed in a manner associated with the warming.

### 1.1.2 Elevated Level of $CO_2$ ($eCO_2$)

The $CO_2$ concentration of the atmosphere is increasing day by day mainly due to deforestation and fossil fuel combustion. Before the industrial revolution it was 280 ppm but, now it rapidly reached to 415.13 ppm (April 2021). As per the IPCC (2014b) report, it is predicted to reach 1000 ppm by the year 2100. Increasing $CO_2$ concentrations in the atmosphere have both positive as well as negative effects on plants. However, most of the effects are still unknown. Photosynthesis is a vital process for controlling variables of plant growth (Wang *et al.,* 2012). Elevated level $CO_2$ ($eCO_2$) can stimulate the rate of net photosynthesis which subsequently lead the positive effects *viz.,* plant growth and yield (Long *et al.,* 2004; Ainsworth and Long, 2005; van der Kooi *et al.,* 2016).

Under $eCO_2$, the increased net photosynthetic rate is mainly due to the high carboxylase/oxygenase and ribulose-1,5-bisphosphate activity. Whereas, It is also found that $eCO_2$ has initially stimulated the net photosynthetic rate but under long term exposure a down-regulation of photosynthesis were noted in both chamber based (Warren *et al.,* 2014) and FACE experiments (Ainsworth & Long, 2005). Researchers found that the $eCO_2$ also improves the tolerance of plants to environmental stresses

such as antioxidants, increased soluble sugars and root exudates (Huang & Xu, 2015). But, it dependents on the air temperature of the environment since the greater vapor pressure deficit may occur due to warmer air (Novick *et al.,* 2016). By inducing stomatal closure, it can reduce the rate of photosynthesis in response to greater transpiration (Mott & Parkhurst, 1991) as well as faster soil drying through root uptake (Will *et al.,* 2013). Moreover, $eCO_2$ can also cause negative effects on plants which decline in a range of nutrients some macro- and micro-elements (Myers *et al.,* 2014) vitamins (Högy & Fangmeier, 2008) and concentrations of the protein in food crops (Broberg *et al.,* 2017). So, understanding of plant responses is important due to these effects.

### 1.1.3 Enhanced UVB

The atmospheric concentration of (Ozone) $O_3$ has also been altered. The stratospheric $O_3$ (10-50 km above the Earth's surface) protect the biological system from the harm full effect of Sun's UVB (280-320 nm) radiation whereas in the troposphere (< 10 km from Earth's surface) it acts as a pollutant. The depletion of stratospheric $O_3$ is strongly linked to the processes involving CFCs and other halogenated compounds. The change in $CO_2$ and temperature accompanied with emission of ozone depleting compounds such as chlorofluorocarbons (CFCs), methane and nitrous oxide cause reduction in the thickness and affect the distribution of stratospheric ozone columns, thus causing an increase in the amount of UVB radiation reaching on the earth's surface. Relative to the 1970s, the mid-latitudes $O_3$ column losses for the 2002-2005 periods were approximately 3% in the Northern and 6% in the Southern hemisphere (WMO, 2008). Current global distribution of mean erythemal

daily doses of UV-B radiation between the latitude 40°N and 40°S during summer ranges from 2 to 9 $kJ^{m-2}$ (McKenzie *et al.* 2007).

Plants are consistently exposed to solar UV radiation because they require sunlight to carry out photosynthesis. They are generally adapted to environmental UV-B radiation exposure since they have evolved mechanisms to avoid damaged. Visual symptoms consisting of chlorotic or necrotic patches on leaves exposed to UV-B were not unique. Both vegetative and reproductive morphology were altered by UV-B radiation. Leaf anatomy was altered due to changes in thickness of epidermal, palisade, and mesophyll layers. In most systems of exposure, enhanced UV-B radiation affected crop growth directly through several first order effects. These include leaf photosynthesis (photosystems, thylakoid and grana membrane integrity) and photomorphogenic systems (developmental rates), upregulation of pathways producing defence compounds (flavonoids and related phenolic compounds or waxes), decreased vegetative growth, and decreased developmental times. These primary effects have led to a myriad of secondary and tertiary effects resulting in altered crop growth and development, which in turn affected light interception that lowered canopy photosynthesis, reduced fruit numbers and retention, and finally, biomass and yield reductions (Kakani *et al.,* 2003).

### 1.1.4 Soil Salinity

Soils are well associated with the atmospheric and climatic systems through the nitrogen carbon, and hydrological cycles. Consequently, the climate change will affect soil processes and properties (Karmakar *et al.,* 2016). Salinization is a process in which the mobilization and/or fractionation of salts causes raised salt concentration in water as well as in soils (Foster & Chilton, 2003). Historically, 5000 years ago, the

soil salinity has been observed on the earth surfaces in earlier Sumerian time in the Mesopotamian plains (Santis, 1996; Shahid *et al.,* 2018). But the salinity in agricultural land were recorded ancient Mesopotamia 3500 BC (Jacobsen & Adams, 1958). Even though, the soil salinity exists globally with different geographical regions characterized by diverse climates, arid and semi-arid regions are much affected especially during the period of drought due to rainfall scarcity and low ground water quality (Kurylyk & MacQuarrie, 2013).

The soil salinity is the importance of a combination of four factors which includes physical factors, economic pressure for food production, growth of population and mainly impact of climate change (Dagar *et al.,* 2016). The first three factors are related to the related to the management of agricultural practices such as quality of water irrigation, type of crops, rate of fertilization, quality of soil quality of groundwater depth of water table, micro-topography and poor drainage conditions (Meimei *et al.,* 2011; Nosetto *et al.,* 2013; Allbed *et al.,* 2014; Yahiaoui *et al.,* 2015). But, the fourth factor, climate change is a severe and important phenomenon since it is affecting the soil salinization catalysing (Teh & Koh, 2016; Gorji *et al.,* 2019). Also, the high salinity influences the crop production where the rise of sea level due to climate changes.

### 1.1.5 Date Palm Varieties

The date palm is botanically known as *Phoenix dactilyfera* and it comes under the plant family *Aceraceae*. The date palm tree is the tallest of the *Phoenix* species growing to 30 m in some places. The leaf is large 4-5 m, alternate, sheathing in dense terminal rosette, the ends of leaf fronds are needle sharp protecting the growth tip from grazing animals. A fully productive palm gives 60-100 kg/date. The date palm grows in areas with

long dry summers and mild winters. It has a unique characteristic to thrive in desert and oasis where temperature could be high but with underground water close to the surface.

The fruit is used as food. It contains the sugar Sucrose (semidry and dry cultivars), fructose and glucose (soft cultivars), proteins, vitamins and minerals. The dates high energy food and very nutritious apart from that it plays as one of the important diets in the Arab countries and consumed fresh, dried or in various processed forms (Kader & Hussein, 2009). In addition to this the other parts of the tree are used in various purposes. The leaves are used in making fences, baskets, containers for domestic uses, sheath is used as fuel as well as in rope making, the drunk is used in roof, door, light foot bridge, pillar making and also as firewood (Barreveld, 1993). Date palm is thought to be originated from Iraq and the cultivation of this plant spread to the Arabian Peninsula, Middle East and North Africa (MENA) countries about 5000 years ago.

Dates are produced in hot arid regions of the world and marketed worldwide as a high value confectionery. It is considered as an important subsistence crop in most of the world's desert areas. The popular date palm producing countries are Egypt, Saudi Arabia, Iran, United Arab Emirates, Pakistan, Algeria, Sudan, Oman, Libya and Tunisia (El-Juhany, 2010). In these countries thousands of date palm cultivars are cultivated including soft, semi-dry and dry date fruits. Arab countries possess 70% of the 120 million world's date palms and are responsible for 67% of the global date production. During the past 50 years, date palm was extensively exploited due to increase in the human population and domestic animals. Date palm production faces serious problems such as low yields as well as marketing constraints. Technical and socio-economic factors contributed for date palm degradation (El-Juhany, 2010).

### 1.1.6 Date Palm in the UAE

The date palm has been an important part of survival in the UAE for close to 7000 years, the oldest seeds found on Delma Island dating back to 5110 BC. The UAE has the largest number of date palms for any single country in the world. It was reported to have 40 million date palm trees and a minimum of 200 cultivars, 68 of which are commercially the most important commercially (Jaradat & Zaid, 2004). In the UAE, date palm is one of the most important crops due to their economic values, high nutritional value, aesthetic and religious significance. The date palm production and consumptions both have greatly increased in last decades. Date palm tree also have positive impact on environments by providing shades and comforts, and sequestering more carbon form the atmosphere. Moreover, date palm can grow in different types of soil, including dry, clay and sandy soils. It is highly salt tolerant (Sharifa *et al.,* 2010).

Environmental factors have direct effect on the global crop distribution and food production. Similar to other plants date palm is also adversely affected by the climate change factors such as elevated level $CO_2$, UVB radiation and salinity. Climate model studies projects those 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. Crop and admirably adapted to the arid and semi-arid environments of the Middle Eastern countries including United Arab Emirates (UAE) (Kizhisseri *et al.,* 2021). However, variation in rainfall, global warming, gas pollution, and decline of water resources are common concern for date palm production. The UAE Government emphasizes the establishment and development of date palm production industries as one of its priorities in the framework of its development plans.

Most of the previous studies have largely focused on the quality aspects of the date palm fruits and seeds. However, the research on the evaluation of the climate change impacts on the date palm have received little attention. Given the limited research and development activities, the Gulf Cooperation Council (GCC) countries ranked date palm as one of the high research priorities as reflected in priority settings for agricultural research. The overall objective will be to understand the response mechanisms and to identify cultivars and plant traits tolerant to various environmental stresses, which can be well suited to the UAE growing conditions.

Thus, considering socio-economic importance of the date palm, the present study was focused on to evaluate the impacts of salinity and climate change factors such as elevated $CO_2$ and enhanced UVB their interactions on some date palm cultivars growing in the UAE growing regions with the following objectives:

i) To screen some UAE date palm cultivars *viz.,* chichi, kalas, Nabt saif, sultana, zamli for salinity stress based on grown parameters.
ii) To investigate the salt tolerant date palm cultivars response to future climatic scenarios such as high atmospheric $CO_2$ and enhanced UVB radiation for various traits and responses of date palm plants such as photosynthetic pigments, biochemical, non-enzymatic and enzymatic antioxidants.
    a. Photosynthetic pigments (chlorophyll ‘a’, ‘b’ and total chlorophyll, carotenoids).
    b. Biochemical contents (proline, protein, amino acid).
    c. Proline metabolizing enzymes (γ - glutamylkinase activity, proline oxidase activity.

d. Non – enzymatic antioxidants (total phenols, α-tocopherol, reduced glutathione contents).
e. Antioxidant enzymes (polyphenol oxidase, peroxidase, superoxide dismutase, catalase, ascorbate peroxidase activities).

iii) To identify date palm cultivars tolerant to environments stresses and best suited for the current and future growing conditions of UAE.

iv) To identify agronomic and physiological traits that warrants greater date palm growth and improved tolerance to environmental stress.

**1.2 Hypothesis**

i) Varietal difference exists to allow selection of date palm cultivars tolerant to environmental stresses such as salinity, elevated level $CO_2$ and enhanced UVB.

ii) The increased atmospheric $CO_2$ of the future will benefit, at least partially, data palm from the adverse effect of environmental stresses.

iii) The climate change factors will alter the morphology, physiology and biochemical processes of the date palm cultivars.

# Chapter 2: Literature Review

Currently, changes in climate denote greatest challenges in research faced by the scientists. Since the climate change factors have great impact on plant growth, ecophysiology of the plants and interactions with other organisms (IPCC, 2014b). Especially, abiotic stresses such as heat, cold, salinity, drought, UV-B, flooding, physical and chemical factors, light intensities, and gas emissions have much influence on the plant growth and yield (Suzuki *et al*., 2014; Benevenuto *et al*., 2017; Ashraf *et al*., 2018). Consequently, the effects of climate change factors on the growth, yield, reproduction, phenology and distribution of plants have studied and reported in many articles. Parmesan and Hanley (2015) published long-term observational records of 4000 eukaryote species. Among that about 42% were plants (terrestrial plants) spread across the world.

### 1.2.1 Effect of Elevated Level $CO_2$ on Plants

A number of recent papers and reviews 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 (Miglietta *et al.,* 1998; Morison & Lawlor, 1999; Sun *et al.,* 2010; Vannette & Hunter, 2011; de la Mata *et al.,* 2012; Zhang *et al.,* 2012). Moreover, elevated level $CO_2$ can lead to reallocation of carbon and nitrogen resources among plant organs and change the secondary metabolites content of plant tissues (Sun *et al.,* 2010). Not surprisingly, some researchers have focused on understanding of the interactions between the $CO_2$ and temperature on plant growth and development (Kirschbaum, 1994; van Bel, 1996; Morison & Lawlor, 1999; Singh *et al*., 2010). In theory, a progressive increase in $CO_2$

could decrease the ratio of photosynthesis to photorespiration and the ratio of gross photosynthesis to dark respiration at higher temperature.

Long (1991) has demonstrated that an increase in $CO_2$ from 350 to 650 ppm could raise the optimum temperature of light-saturated leaf photosynthesis by 5°C and the relative stimulation of light compensation point by elevated $CO_2$ was reduced at high temperature. In 1993, Wong studied the effect of two different levels of $eCO_2$ (350 and 700 ppm) and humidifies (35 and 90%) on *Gossypium hirsutum* and *Raphanus sativus* grown under full sunlight. *G. hirsutum* showed good response to $eCO_2$ and increased humidity. The dry matter yield of the *G. hirsutum* was higher at low humidity compared to high humidity. In contrast, *R. sativus* did not respond to the $eCO_2$ and increased humidity apart from early stage of development. Plant height, taproot length and diameter, above ground and below ground part dry weight of the plant, stomatal conductance, activity of ribulose 1,5-biphosphate carboxylase and the net $CO_2$ assimilation rate of *Pinus koraiensis* seedlings were studied after exposure to the $eCO_2$ for six weeks under open top chamber facility.

Two different $CO_2$ concentrations (500 and 700 ppm) were used. The results showed the stomatal conductance and biomass of the plants were greatly increased at $eCO_2$. Also, soluble sugar and chlorophyll contents, activity of RuBP case and acclimation of photosynthesis were higher in 500 ppm $CO_2$ level than 700 ppm (Shi-Jie *et al*., 2000). Photosynthesis rate of one-year old seedlings of *Larrea tridentata* were studied for the interaction of drought and eCO2 under heat stress (Hamerlynck *et al*., 2000). The seedlings were exposed to high temperature (53°C) under three different $CO_2$ concentrations *viz.,* 360, 550 and 700 ppm with 2 water regimes such as drought and well-watered.

Chlorophyll fluorescence, Photosynthetic gas exchange and response of water potential were measured before, during and after the experiment. The results of the showed heat stress decreased photosystem photochemical efficiency, net photosynthetic rate and stomatal conductance in all the plants except plants grown in well-watered and high $CO_2$ 700 ppm level. Down-regulation of photosynthesis was recorded by the extreme heat under $eCO_2$ and well-watered conditions. Also, the similar effect was observed in drought condition. Seedlings grown in ambient $CO_2$ conditions did not show the recovery of photosynthetic capacity significantly.

Li *et al*. (2007) studied growth-chamber experiments to know the effect of $eCO_2$ on growth the water usage of tomato seedlings under different ratio of ammonium nitrate. The results showed the $eCO_2$ increased plant height, total dry weight, thickness of the stem, dry weights of roots, stem and leaves, chlorophyll content, rate of photosynthesis and water use efficiency with the increasing proportion of ammonium nitrate. The authors also noted that the $eCO_2$ treatment has higher biomass of the plant (67%), height of the plant (22%), thickness of the stem (24%) and photosynthetic rate (55%) when compared to ambient $CO_2$ concentration. The study concludes both $eCO_2$ and ambient $CO_2$ concentration levels, ammonium nitrate ratios were influencing all parameters.

Photosynthetic responses of European beech to UV-B and $eCO_2$ were evaluated. The experiment was done with two different concentrations (400 and 700 ppm) of $CO_2$. Activity of Rubisco enzyme and biochemical were analysed throughout the experiment. The authors found a positive response of $eCO_2$ on plant photosynthesis at beginning of the vegetation season. Whereas the down regulation of photosynthesis noted in long-term cultivation (Urban *et al*., 2019).

Suslov (2020) studied the intercellular water transfer of *Zea mays* roots under $eCO_2$ concentration of 800 and 1200 ppm and the results showed the decreased root water transfer intensity. Also, the $eCO_2$ concentration decreased the water diffusion coefficient as well as the root cell water permeability by 30-35% approximately. Whereas 1200 ppm $eCO_2$ increased water permeability of *Zea mays* roots. The reduction of root cells water permeability under $eCO_2$ may be due to the regulatory decrease of water conductivity.

By decreasing the levels of ethylene and abscisic acid in both leaves and roots, $eCO_2$ improves the growth and assimilation rate of tomato under high soil salinity. The study was done with fluctuating temperature and irradiance under $eCO_2$ by analysing the biomass of the plant, primary metabolism and biosynthesis of hormone. The $eCO_2$ enhanced the plant growth by stimulating the photosynthetic rate and reduced concentrations of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid and abscisic acid in both roots and leaves. The high salinity increased the photorespiratory metabolites such as serine and glycine and reduced intermediates of Krebs Cycle concentration. But it was restored to the control level by the $eCO_2$ (Brito *et al.,* 2020).

An open top chamber experiment was done to study how the carbon use and assimilation of coffee plants affected by the $eCO_2$ with a drought combination (Avila *et al.,* 2020). Also, the impact of combination of drought and $eCO_2$ on biomass accumulation also studied. For that the plants were treated with ambient (386 ppm) as well as $eCO_2$ (723 ppm) for seven months. Results indicated that $eCO_2$ improves the rate of carbon assimilation (> 60 %) without altering the stomatal conductance and also, down regulation of photosynthesis also not deducted. Moreover, under drought condition, degreased oxidative pressure and rates of photorespiration and increased

biomass accumulation water-use efficiency and respiration rates were observed. Also, the $eCO_2$ promoted the tolerance of drought by biomass partitioning towards roots with better root length.

The combined effect of high temperature (+ 2°C above ambient) and $eCO_2$ concentration (600 ppm) were studied on pattern of phenological, interactions of plant-insect and rates of outcrossing in *Stylosanthes capitata* were studied (Alzate-Marin *et al*., 2021). The results indicated greater number of flowers (+ 62%)/per plot were observed significantly. The flowers began opening was earlier (1 hr approximately, ~09:05) in high temperature plots than the $eCO_2$ (~09:55) and control (~09:59) treatments. Also, in high temperature the lower closure occurred about 3 h later. These flower phenology variations increased the availability of floral resources as well as the pollinator's attractiveness.

It was found that phytoremediation efficacy of *Festuca arundinacea* intercropped with *Echinochloa caudate* was increased when increasing the difference (280, 400, and 550 ppm) $CO_2$ levels (Yang *et al.,* 2021). The authors found that $eCO_2$ significantly increased the *E. caudata* dry weight but there was no alteration in in the Cd content of the plant parts. Nevertheless, the blow ground and aerial parts biomass of *F. arundinacea* were decreased in all the treatments. Overall, under the $eCO_2$ level, the efficacy decreased the Cd phytoremediation *F. arundinacea* intercropped with *E. caudate*.

Recently, Zhang *et al*. (2021) studied the effect of $eCO_2$ and drought on water-use efficiency, gas exchange, physiological indices in response to tolerance of drought, leaf area, above ground biomass of *Panicum miliaceum* under open-top chamber facility. The authors found that the $eCO_2$ compensate the effect of drought on leaf area

as well as biomass of the plant. It may be due to the stimulation of photosynthesis by uptake of carbon under $eCO_2$. Under drought condition, $eCO_2$ also enhanced the efficiency of water-use. While, efficiency of PSII, peroxidase and malondialdehyde defence capacity, osmotic adjustment such as proline and the content of soluble sugar were not affect significantly under $eCO_2$.

### 1.2.2 Effect of UVB on Plants

Exposure of plants to the natural sunlight is unavoidable since the plants require light for photosynthetic process. However, the UV radiation especially UV-B in the natural sun light has important role in the development of plants (Bornman *et al*., 2015). But it is also causing harmful effects by acting as a plant stressor. Plants had multiple defence mechanisms to the UV-B radiation viz. thickness of leaf, changes transpiration as well as photosynthetic system, pigments of leaf, epicuticular waxes, canopy morphology, and antioxidant defence system. A key defence mechanism of plants against UV- radiation is production of UV observing secondary metabolites such as flavonoids and phenolic compounds (Barnes *et al*., 2016; León-Chan *et al*., 2017). This highly flexible phenomenon can happen in minutes to hours (Barnes *et al.,* 2016). Previously, there are numerous researchers have been done to find out the response of plants to the UV-B radiation.

19 species of plants were screened to determine the pollen response to two levels of UV-B radiation in *in vitro* condition. It was found that germination of pollen and growth of tube were significantly affected by the UV-B in most of the plant species tested. Also, most of the species showed insensitivity, even pollen germination stimulation and growth of tube to the UV-B radiation (Feng *et al.,* 2002).

*Gossypium hirsutum* grown in controlled environment chambers was exposed to 2-11 kJ $m^{-2}$ $d^{-1}$ of UV-B radiation in a to find out the changes of height of the plant, leaf area, length of branch and internode, number of mainstem node, area and length of petals and bracts, number of anthers per flower. Also, leaf thickness, Epidermal cell and stomatal density and index of the stomata, thickness of palisade, epidermal mesophyll tissue were measured. High UV-B radiation reduced the vegetative and reproductive parameters which results the smaller canopy. Moreover, epicuticular wax content and index of the stomata were increased in both abaxial and adaxial leaf tissues. The high-level UV-B radiation affects the vegetative parameters, but the reproductive parameters were reduced in ambient and high UV-B levels (Kakani *et al*., 2003).

Sensitivity of *Vigna unguiculata* genotypes to different UV-B radiation levels was studied. The results revealed a significant genotypic variability to the UV-B treatment. All genotypes of *V. unguiculata* were reduced stem, length of flowers and lower yield of seeds compared to control plants. Also, most of parameters related to vegetative showed optimistic response to the UV-B radiation. While the negative response was observed in reproductive parameters. Based on the response to the UV-B, the genotypes were classified as sensitive, intermediate and tolerant to the UV-B radiation (Singh *et al*., 2008). Two barley cultivars were exposed to elevated level UV-B and leaf tissue structure, fertility of the pollen, seedling viability stability of embryo roots statolitic starch were analysed. Also, ISSR marker based genetic analysis showed that the polymorphic level was increased (80%) in somatic tissue and decreased (33%) in reproductive tissue under the UV-B radiation (Kravets *et al*., 2012).

### 1.2.3 Combined Effects of $eCO_2$ and UV-B on Plants

Many studies were reported the effect of climate change factors on plants independently. Similarly, the combined effect of $eCO_2$ and UV-B were also reported. It is known that the enhanced UV-B radiation reduced the plant growth. But the $CO_2$ enrichment has ability to ameliorate effect of UV-B. A greenhouse experiment was conducted to study the effect of $eCO_2$ and UV- radiation on a $C_3$ grass *Elymus athericus*. The plant was treated with $eCO_2$, UV-B and both $eCO_2$ +UV-B. As observed in many other studies, the $eCO_2$ increased the growth of the plant and the production of biomass. While UV-B treated plants showed less growth and the production of biomass around 31% than the control. In $eCO_2$ and UV-B combination, the biomass depression was 8% than the control plants (van de Staaij *et al*., 1993).

Effect of $eCO_2$ and UV-B irradiance on the seedlings of faba bean were studied by Visser *et al.* (1997) under open top chambers facility. Faba bean seedlings were treated with 350 or 700 ppm $CO_2$ levels and the growth and physiological changes were analyzed. In all harvests, enrichment of $CO_2$ resulted the increased biomass production but after 14 days treatment biomass production decreased significantly. Throughout the experiment, shoot length was increased at both $CO_2$ levels when reduce the level of UV-B radiation. Alteration in biomass and reduction of growth were noted in UV-B treatment. Net photosynthetic rate was stimulated by 26 and 38 days of $CO_2$ enrichment treatment. The result of accumulation of carbohydrates in the leaves, $CO_2$ enrichment for 26 days showed photosynthetic acclimation. Whereas it was not present after 38 days of treatment.

Growth and photosynthetic response of faba bean (cv. Minica) under $eCO_2$ and four level of UV-B radiation were reported by Tosserams *et al*. (2001). By decreased

biomass production, faba bean showed its sensitivity to the UV-B treatment. In the highest UV-B level, $CO_2$ enrichment growth stimulation was reduced greatly. Even though, biomass partitioning was affected by both the treatments, UV-B showed most pronounced. It is also found that the effect of studied climate change factors on the growth faba bean were time dependent and developmental stages.

Both $eCO_2$ and UV-B radiation on cotton's Leaf and canopy photosynthetic characteristics were studied (Zhao *et al.* 2004). Two $CO_2$ levels *viz.* 360 ppm (ambient) and 720 ppm (elevated), three levels of biologically effective UV-B *viz.* 0 (control), 8 and 16 kJ $m^{(-2)}$ $d^{(-1)}$) were given for 66 days from the emergence of crop. Plants with $eCO_2$ had large leaf area, higher net photosynthesis of leaf and canopy, lesser light compensation point and lesser dark respiration rate compared to the control. Also, rate of Rubisco activity, $CO_2$ compensation point and rate of electron transport were same in both the $Co_2$ treatments.

Besides, photosynthetic parameters plants grown with the radiation of 8 kJ $m^{(-2)}$ $d^{(-1)}$ UV-B did not show any difference compared to the control plants. Whereas 16 kJ $m^{(-2)}$ $d^{(-1)}$ UV-B showed much impact on the pants by reducing the leaf area (47-50%), lower Rubisco activity (72-74%), less net photosynthetic rates (38-44%) and lower rate of electron transport (61-66%) when compared to control. The authors deducted that there was not interactive effect of measured photosynthetic parameters of both $eCO_2$ and UV-B treatment.

Koti *et al*. (2005) studied the long-term exposure of $eCO_2$ and UV-B along with high temperature on morphology and production of pollen, germination and tube lengths of soybean genotypes under controlled environmental chamber. The plants were treated with 720 ppm of $CO_2$ and 10 kJ $m^{-2}$ $d^{-1}$ of UV-B. The results showed that

flowers of the plants grown either at temperature/UV-B treatment alone or in combination were smaller, shorter petal and staminal column lengths. Also, those flowers had less pollen with shorter tube lengths and germination of the pollen was poor. The study also finds that the damaging effect of UV-B and temperature were not ameliorated by $eCo_2$.

Morphological and physiological response of maize hybrids to $eCO_2$ (400 and 750 ppm), UV-B (10 kJ $m^{-2)}$ and evapotranspiration-based irrigation treatments (50 and 100%) were studied (Wijewardana *et al.,* 2016). Six hybrids *viz.,* N77P-3111, DKC 65–81, P1319, N75H-GTA, P1498 and DKC 66–97 was used for the study. Plants treated with UV-B alone or 50% evapotranspiration irrigation produced shorter plants with lesser leaf area. But $eCO_2$ treatments ameliorated the effect of UV-B and drought. Also, the $CO_2$ enrichment increased the height of the plants, dry matter, leaf area, photosynthetic pigments and net photosynthesis.

Some UAE date palm cultivars were screened for susceptibility to the elevated level $CO_2$, UVB radiation and their combined effects. The experiment was conducted in an open top chambers facility and the plants were treated for 120 days. After the treatment, photosynthetic pigment analysis such as chlorophyll and carotenoid content, amino acids, protein, phenol and enzymes like proline oxidase, a-tocopherol, γ-peroxidases activity, glutamyl kinase were analysed. The results of the study revealed that $CO_2$ treated plants increased the growth of the plants. Whereas UVB treated plants significantly affect the growth of the date palm plants. Whereas the level antioxidant enzymes were significantly increased (Karthishwaran *et al*., 2020).

In nature, plants are routinely exposed to multiple abiotic stresses and recent studies demonstrate that plant responses to a single factor are much different than those

responses under multiple stress conditions (Rizhsky *et al*., 2004; Singh *et al*., 2010). Understanding the impact of multiple stressors is particularly important when their combined effect cannot be predicted based on evidence from single-stressor studies (Breitburg *et al*., 1998). Most of our knowledge of the effect of the abiotic stresses on crop productivity is entirely based on the experiments with single abiotic stress. Which is understandable because multiple abiotic stress research programs are complex in nature, requires state-of-the-art facilities and financially prohibitive.

Singh *et al*. (2010) reported that, the multiple stresses affected the most to the growth and development of crop plants as compared to single stressor. Therefore, the combined effect of two or more stress factors such as heat and drought should be addressed as if it is a new state of abiotic stress and not simply the sum of two different stresses (Breitburg *et al*., 1998; Singh *et al*., 2010).

The experiments designed to evaluate the interaction among the abiotic stressors are critical to understand whether interaction between heat and drought will be negatively additive or greater-than-additive effect might occur, and whether $CO_2$ enriched environments will counteract the negative effect or enhance the sensitivity of crops to abiotic stresses (Breitburg *et al*., 1998; Singh *et al*., 2010; Vinebrooke *et al*., 2004). Therefore, an understanding of plant response to multiple environmental factors that mimic the weather variability under current and future climate change scenario would be one of the primaries focuses of the research program.

# Chapter 3: Material and Methods

## 3.1 Experimental Site

The study was conducted in Al-Foah Experimental Farm [24°21′31.139″N 55°47′57.239″ E (Altitude 303 M)], College of Food and Agriculture, UAEU, Al Ain in natural conditions. Shade house and open top chambers facility were used for salt tolerance experiment and climate change studies, respectively.

## 3.2 Date Palm Cultivars

Two years old seedlings of five UAE date palm cultivars *viz.,* Chichi, Kalas, Nabt saif, Sultana, and Zamli were received from Date Palm Research Laboratory, UAEU and transferred to the in PVC cylinder pots (60 cm (H), 25 cm (D) filed with sand and manure (1:1) and used for the present study.

## 3.3 Open Top Chambers Facility

The effect salt tolerant date palm cultivars response to future climatic scenarios such as high atmospheric $CO_2$ and enhanced UVB radiation was studied in an Open Top Chambers facility, as shown in Figure 1.

Figure 1: Open top chambers facility located in UAEU Al Foah experimental farm

The chambers are fabricated with Galvanized steel squire tube with the size of 3×3×3 m dimension. The OTCs is covered with 80 to 85% transparent poly carbonate sheets with open top to maintain the near-natural conditions of temperature and relative humidity. Also, plenum at the base chambers provides $CO_2$ circulation in the chambers. Commercial grade $CO_2$ gas (95.5%) was used for the $CO_2$ enrichment through a manifold fitted with copper tubing. $CO_2$ was maintained at set levels using manifold gas regulators, solenoid valves, $CO_2$ analyser PC linked Program Logic Control (PLC) and Supervisory Control and Data Acquisition (SCADA). For UV-B treatment, fluorescent (UV-313) lamps (Q-Panel, OH, USA) were used to emit the radiation between 280 and 320 nm.

### 3.4 Salinity Stress

Initially, the selected date palm cultivars were screened for salt salinity with two different concentrations *viz.,* 5000 (T1) and 10000 (T2) ppm (Figure 2). Laboratory grade NaCl (sodium chloride) was used to prepare different salinity level and irrigated in alternate days for 30 days. A Completely Randomized Design (CRD) was used to study the salinity tolerance experiment with three replicates.

Figure 2: Date palm plants under salinity treatment

### 3.5 Growth Parameters

After the salinity treatment, salt tolerant date palm cultivars were selected by growth parameters *viz.,* shoot length, root length, fresh and dry weight of root and shoot measurements to study the response to future climatic scenarios such as high atmospheric $CO_2$ and enhanced UVB radiation.

#### 3.5.1 Plant Height

The total plant height from the longest root to first cotyledonary of the date palm plants and the values were expressed in cm.

#### 3.5.2 Fresh and Dry Weight of Root and Shoot

After the salinity treatment, the plants were harvested and washed with tap water and the fresh weight and dry weight of the samples were measured by an electronic balance and the values were expressed in grams. The same samples were dried in a hot air oven and dry weight were measured and expressed in in grams.

### 3.6 $eCO_2$ and UV-B Treatments

The effect $eCO_2$ and enhanced UVB on selected date palm cultivars was studied by the completely randomized design method which includes four treatments as follows Chamber 1: Control (Ambient), Chamber 2: Elevated level $CO_2$ (550 ppm), Chamber 3: Enhanced UV-B radiation (9.50 kJ $d^{-1}$ $m^{-2}$) and Chamber 4: $eCO_2$ (550 ppm) + UV-B radiation (9.50 kJ $d^{-1}$ $m^{-2}$) (Figure 3). Three replicates were maintained in each treatment. Samples were taken for photosynthetic pigments, biochemical contents, proline metabolizing enzymes, non–enzymatic and enzymatic antioxidants analysis after 45 days of treatment with $eCO_2$, UV-B and $eCO_2$+UV-B for 8 hrs/day.


Figure 3: Selected Date palm cultivars under eCO2 and UV-B treatment

### 3.7 Photosynthetic Pigments

#### 3.7.1 Determination of Chlorophyll and Carotenoids Contents

Photosynthetic pigments such as Chlorophyll a, b and total chlorophyll and carotenoid contents of date palm plant cultivars were estimated using the method described by Arnon (1949). Fresh leaf material (500 mg) was ground using pestle and mortar with 10 ml of 80 % acetone and the extract was centrifuged at 2500 rpm for 10 minutes. Until the residue became colourless, same procedure was repeated. Then the extract was transferred to a graduated tube and 10 ml of 80 % was used to make up to 10 ml. For analysis, 3 mL of extract aliquots were transferred to a cuvette to read the absorbance at 645, 663 and 480 nm using a spectrophotometer (U-2001-Hitachi). 80 % acetone was used as a blank.

The content of the chlorophyll was calculated using the following formula. Chlorophyll ‘a’ (mg/ml) = (0.0127) × (A.663) – (0.00269) × (A.645); Chlorophyll ‘b’ (mg/ml) = (0.0229) × (A.645) – (0.00468) × (A.663) and Total chlorophyll (mg/ml) =

(0.0202) × (A.645) + (0.00802) × (A.663). The values of the chlorophyll contents are expressed in mg/g Fresh Weight. The content of the carotenoid was calculated according to Kirk and Allen (1965) using the following formula Carotenoid (mg/g) = A.480 + (0.114 × A.663 – 0.638 × A.645) and values are expressed in mg/g Fresh Weight.

### 3.8 Biochemical Contents

#### 3.8.1 Estimation of Proline Content

The estimation of proline content of date palm plants was performed by the method of Bates *et al*. (1973). 500 mg of leaf samples was homogenized with 10 ml of 3% aqueous sulfosalicylic acid using a pestle and mortar. Then it was filtered through Whatman No. 2 filter paper and re-extraction was done with 3% sulfosalicylic acid with the same residue, all the filtrates were pooled, and 3% sulfosalicylic acid was used to make up to 20 ml and used for the proline estimation. For estimation, in a test tube, 2 mL of extract, 2 ml of glacial acetic acid and 2 ml of acid ninhydrin reagent were taken, and it was incubated in a water bath at 100ºC for one hour. Then 4 ml of toluene was added mixed vigorously for 20 seconds and the aqueous phase was separated using a separating funnel. Finally, absorbance was measured at 520 nm in a spectrophotometer, standard curve was plotted to determine the proline content and results are expressed in mg/g Fresh Weight.

#### 3.8.2 Estimation of Protein

The soluble protein of date palm plants was determined according to the method of Bradford (1976). Briefly, 20 ml of 20% Trichloro Acetic Acid (TCA) was added with 1 gm of plant sample and ground using mortar and pestle. Then the homogenate was centrifuged at 800 rpm for 15 mts. The pellet was taken and 0.1 N NaOH (5 mL) was added and again centrifuged for 15 mts at 800 rpm. Finally, 0.1 N NaOH (10 mL) was

added to the supernatant and used for the soluble protein estimation. 5 mL of protein reagent was added to the 0.1 ml protein solution (containing 10-50 μg soluble protein) and mixed well and the absorbance was measured at 595 nm. A standard curve was plotted with obtained absorbance values to determine the soluble protein content of the samples and the values are expressed in mg/g Fresh Weight.

### 3.8.3 Estimation of Amino Acid

A method described by Moore and Stein (1948) was adopted for the extraction and estimation of total free amino acid content of the date palm leaves. 500 mg of fresh date palm leaves was homogenized with 80% boiled ethanol (10 mL) and centrifuged for 15 mts at 800 rpm. The supernatant was taken and made up to 10 mL with 80% ethanol and used for the estimation of total free amino acid content. 1 mL of extract was neutralized with 0.1 N NaOH and methyl red indicator. Then ninhydrin reagent (1 mL) was added and kept in a water bath for 20 mts. Diluting solution (5 mL) was added, cooled and distilled water was added to make up to 25 mL. Finally, absorbance was read at 570 nm, standard graph was prepared for the estimation of total free amino acid content and values are expressed in mg/g Fresh Weight.

## 3.9 Proline Metabolizing Enzymes

### 3.9.1 Estimation of γ-Glutamyl Kinase Activity

The γ-glutamyl kinase activity of date palm leaves after $eCO_2$ and UV-B radiation treatment was assessed by the method of Hayzer and Leisinger (1980). Plant sample (1 gm) was extracted with 50 mM Tris-HCl buffer (10 ml; pH 7.2) using a vortex homogenizer and centrifuged for 20 mts at 10,000 rpm. Again, it was washed with the same buffer and stored at – $20^0$C. The sample was suspended in 50 mM Tris–HCl buffer (7 mL) with 7.2 pH which contains 1 mM 1, 4-dithiothreitol. A French press at

38.5 MPa was used to affect the Cellular disruption and the sample was centrifuged for 30 minutes at 20,000 rpm to remove the cell debris. Finally, γ – glutamyl kinase activity was measured by the crude extract. For enzyme assay, 2.5 ml of enzyme extract was desalted with a SephadaxG-25 column equilibrated with Tri-HCl buffer (50 mM) which contains 1 mM 1, 4-dithiothreitol.

The Final volume (2 mL) of the enzyme a mixture contains ATP (50 mM), L-glutamate (0.25 mL), $MgCl_2$ (10 mM), Tris base 50 mM (pH 7.0), Hydroxylamine HCl (20 mM) and 100 µl of desalted extract. The reaction was initiated by adding the enzyme extract and it was stopped after 30 mts by a solution contains trichloroacetic acid (6% w/v) and $FeCl_3.3H_2O$ (2.5% w/v). The sample was centrifuged at 10000 rpm to remove the precipitated protein and absorbance was read at 535 nm. The activity of one unit of γ-glutamyl kinase can be defined as µg of γ-glutamyl hydroxamate formed per minute per mg protein.

**3.9.2 Estimation of Proline Oxidase Activity**

Huang and Cavalieri (1979) method were adopted the determine the Proline oxidase activity of the date palm leaves after the treatment. 1 gm of plant sample was homogenized in a pre-chilled pestle and mortar using 5 ml of homogenizing medium and it was filtered using two layers of muslin cloth. The filtrate was centrifuged for 10 mts at 10000 rpm and supernatant was collected and it was centrifuged for 25 mts at 20000 rpm. The obtained pellet was mixed with 5 mM Tricine – KOH buffer (1 mL) and used for the estimation of proline oxidase activity. The enzyme reaction was monitored by reading the absorbance at 600 nm. For the enzyme activity determination, rhe reduction rate of DCPIP was used and the results of the enzyme activity are presented in µg/min/mg.

### 3.10 Non–Enzymatic Antioxidants

#### 3.10.1 Estimation of Total Phenols

A method described by Malik and Singh (1980) was adopted to determine the total phenol content of the samples. 0.5 g of date palm leaves was homogenized with 80% of ethanol (10X) and it was centrifuged for 20 mts at 10000 rpm. This extraction process was repeated with ethanol. The obtained supernatants were pooled together and evaporated. Then the residue was dissolved with distilled water. Different aliquots were taken, and volume of each test tube was made to 3 mL. The test tubes were placed in a water bath after adding 0.5 mL of Folin-Ciocalteau reagent and absorbance was read at 660 nm. Different concentrations of catechol solutions were prepared as above and standard curve was prepared. The results of the phenolic content are expressed as mg/g Fresh weight

#### 3.10.2 Determination of α-Toczopherol Activity

α-Tocopherol activity was analysed as described by Backer *et al.* (1980). 10 ml of petroleum ether and ethanol (2:1.6 v/v) was used to homogenize 500 mg of fresh tissue and centrifuged for 20 mts at 10000 rpm. After centrifugation, the supernatant was taken for the α-tocopherol estimation. 0.2 mL of 2, 2-dipyridyl (2%) in ethanol was added in 1 mL of extract and kept in a dark room for 5 mts. After getting red colour, the mixture was diluted with distilled water (4 mL) and absorbance was read at 520 nm. A standard graph was used to calculate the content of α-tocopherol with known quantity of α-tocopherol.

#### 3.10.3 Reduced Glutathione Activity

A method described by Griffith (1980) was adopted to analyse the reduced glutathione activity. 200 mg of plant material was ground with 2 % metaphosphoric

acid (5 mL). After grinding, it was centrifuged for 10 mts at 17000 rpm and supernatant was used for the estimation of reduced glutathione. To neutralize the extract for estimation, 0.6 ml (10%) sodium citrate buffer was added to 0.9 ml of the extract. 1 ml of the extract contains 100 µL Dithionitrobenzoic acid, 700 µL NADH, 100 µ1 of neutralized extract and 100 µ1 of distilled water. The mixture was kept for 4 mts at 25°C to stabilize it. Finally, Glutathione Reductase (10 µl) was added, and the absorbance was read at 412 nm.

### 3.11 Antioxidant Enzymes

#### 3.11.1 Polyphenol Oxidase Activity

The activity polyphenol oxidase was determined as per the method described by Kumar and Khan (1982). Briefly, assay mixture contained 0.1 M phosphate buffer (2 mL), 0.1 M catechol (1 mL) and enzyme extract (0.5 mL). This mixture was incubated at 25°C for 5 mts then the reaction was stopped by the addition of 1 mL of $H_2SO_4$ (2.5 N). The absorbance was read at 495 nm after the mixture turn in to orange-red colour. The obtained results are expressed in U $mg^{-1}$ protein.

#### 3.11.2 Peroxidase Activity

Peroxidase activity of the date palm leaves was determined by the method of Kumar and Khan (1982). The assay mixture [0.1 M phosphate buffer (2 mL), 0.01 M pyrogallol (1 mL), 0.005 M of $H_2O_2$ and enzyme extract (0.5 mL)] was incubated at 25°C (5 mts) and the reaction was stopped by the addition of 1 ml of 2.5 N $H_2SO_4$. The amount of orange-red colour formation was determined by reading the absorbance at 420 nm. The results on the activity of peroxidase are expressed as $mg^{-1}$ protein.

### 3.11.3 Superoxide Dismutase Activity

Based on Hwang *et al.* (1999) method, the Superoxide dismutase activity was determined. For extraction, 1 gm of fresh plant sample was homogenized by adding 50 mM sodium phosphate buffer which contains 1 mM PMSF. The extract was filtered and centrifuged for 20 mts at 12,500 rpm. By adding extraction buffer, the supernatant was made up to 10 ml and used for the estimation of superoxide dismutase activity by the method of Beauchamp and Fridovich (1971). In 1 ml of enzyme extract, 3 ml reaction medium was added, and the reaction mixture was illuminated in clear glass test tubes with the help of Philips 40 W fluorescent tubes. For blank, reaction mixture was without illumination and kept in a dark place. Finally, the absorbance was read at 560 nm and the results are expressed in U/g FW.

### 3.11.4 Catalase Activity

The catalase activity of the leaves of date palm cultivars was analyzed by the method of Chandlee and Scandalios (1984). 500 mg of frozen plant material was homogenized with 50 mM sodium phosphate buffer which contain PMSF (1 mM). The obtained extract was centrifuged at 12500 rpm for 20 mts and the supernatant was saved and used for estimation. The method of Chandlee and Scandalios (1984) was adopted to determine the catalase activity with slight modification. Briefly, the assay mixture contains 50 ml of 50 mM potassium phosphate buffer + 0.4 ml of 15 mM $H_2O_2$ + 0.04 mL of enzyme extract. The $H_2O_2$ decomposition was followed by reading the absorbance at 240 nm and the results are expressed in $mg^{-1}$ protein.

### 3.11.5 Ascorbate Peroxidase Activity

The method of Asada and Takahashi (1987) was used to determine the activity of ascorbate peroxidase. 500 mg of fresh samples was ground using 50mM potassium

phosphate buffer (10 mL) and liquid nitrogen. The homogenate was filtered and centrifuged for 20 mts at 15000 rpm and supernatant was used for the estimation. 1 mL reaction mixture was taken and read the absorbance at 290 nm. The results are presented in μg/g FW.

### 3.12 Statistical Analysis

The obtained data related to both salinity tolerance and $eCO_2$ and UVB treatments were analysed using SPSS (V. 21.0). The results were taken from three replicates and date data are expressed in Mean±SE.

# Chapter 4: Results

## 4.1 Effect of Salinity on Grown Parameters

The results of salinity tolerance experiment on morphological parameters of five UAE date palm cultivars viz., Chichi, Kalas, Nabt saif, Sultana, Zamli are presented in Figures 4 to 9. The salinity in the water can cause significant alterations in the morphology of plants. In the present study, the high salinity irrigation showed different morphological responses in the studied date palm cultivars. The leaf appearance of five date palm cultivars after the saline treatment is given in Figure 4. The variation in seedling height of studied date palm cultivars is given in Figure 5. The two different concentrations of salinity affect the growth of Chichi, Kalas and Nabt Saif cultivars significantly when compared to the control.

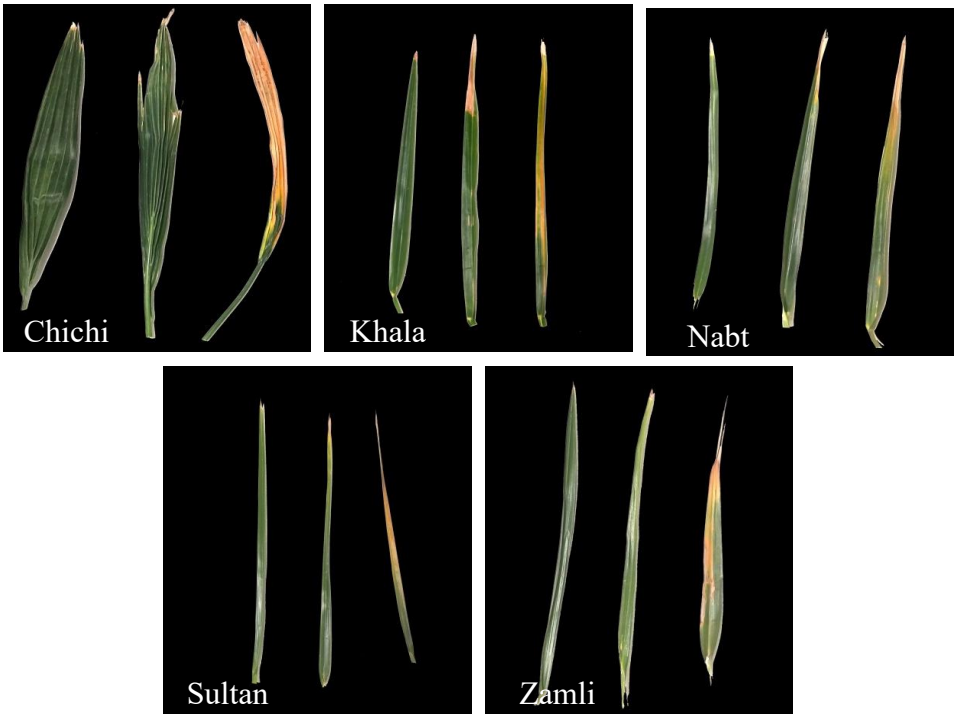


Figure 4: Leaf appearance of the date palm cultivars under saline and non-saline conditions

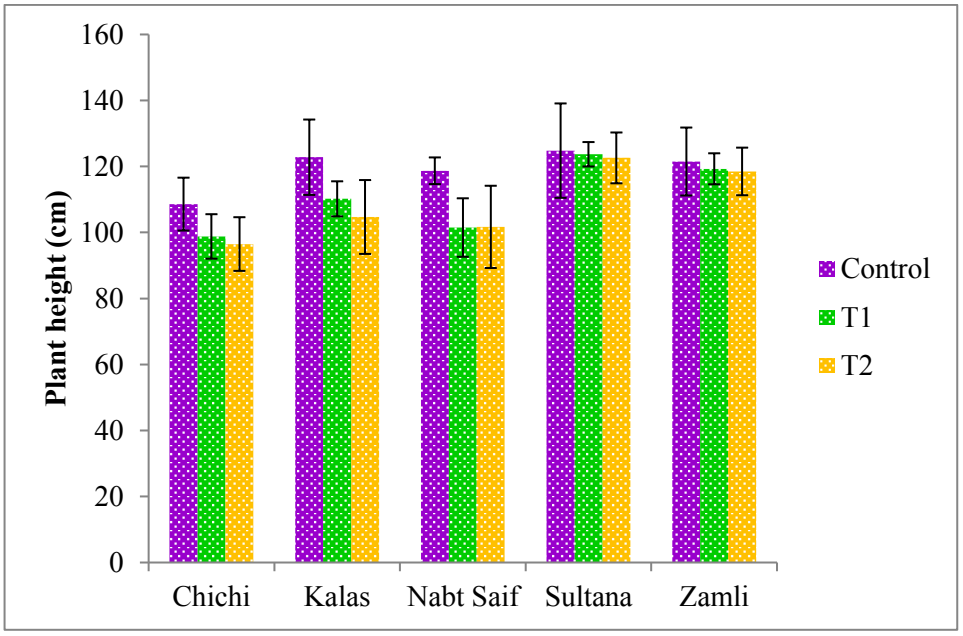


Figure 5: Variations in plant height of date palm cultivars after salinity treatment (T1- 5000 ppm; T2-10000 ppm)

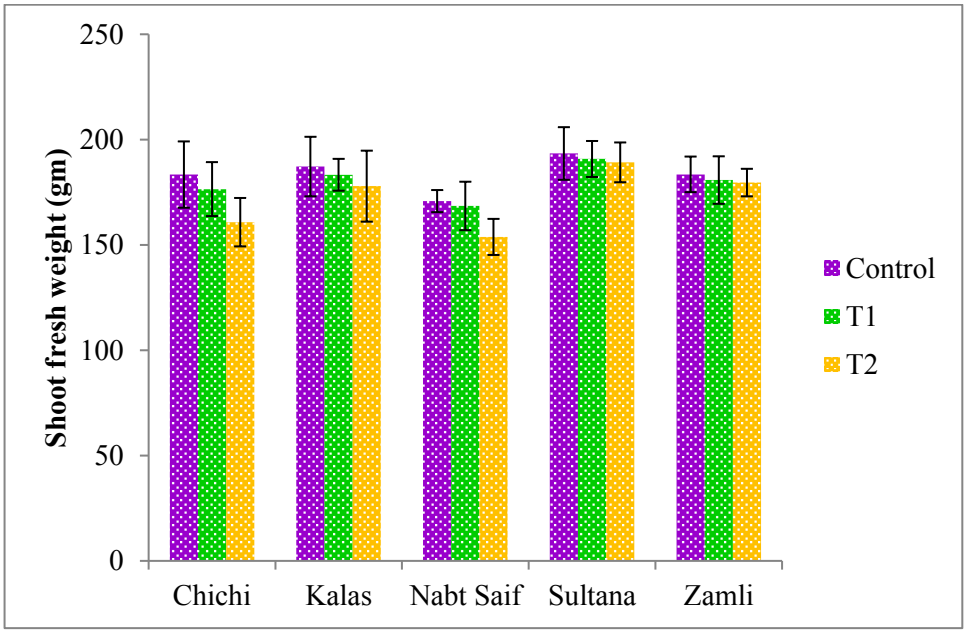


Figure 6: Effect of salinity treatment on shoot fresh weight of date palm cultivars (T1- 5000 ppm; T2-10000 ppm)

Whereas, plant height of Sultana and Zamli was not affected by the salinity treatment. The results on the shoot fresh weight of all the studied date palm cultivars are

given in Figure 6. Similar to the plant height, the salinity affected the shoot fresh weight of Chichi, Kalas and Nabt Saif date palm cultivars. The shoot fresh weight of Chichi, Kalas, Nabt Saif, Sultana and Zamli cultivars after 10000 ppm salinity treatment were 160.8 ± 11.69, 177.9 ± 16.85, 153.8 ± 8.56, 189.2 ± 9.45 and 179 ± 6.54 gm respectively. Whereas the shoot fresh weight of control plants was 183.4 ± 15.74, 187.2 ± 14.13, 170.8 ± 5.25, 193.4 ± 12.50 and 183.5 ± 8.45 gm. The results on the shoot dry weight of date palm cultivars after the salinity treatment are given in Figure 7. The shoot dry weight of control plants was 25.8 ± 2.67 (Chichi), 26.7 ± 3.69 (Kalas), 21.6 ± 1.82 (Nabt Saif), 28.4 ± 4.21 (Sultana) and 26.2 ± 1.52 (Zamli) gm. While the T1 showed the shoot dry weight of 22.7 ± 1.49 (Chichi), 21.9 ± 3.56 (Kalas), 18.5 ± 2.58 (Nabt saif), 26.8 ± 1.13 (Sultana) and 24.5 ± 1.0 (Zamli) gm. The shoot dry weight of T2 were 21.4 ± 2.63 (Chichi), 18.5 ± 3.67 (Kalas), 16.8 ± 1.34 (Nabt saif), 2.6 ± 2.94 (Sultana) and 23.8 ± 1.74 (Zamli) cm.

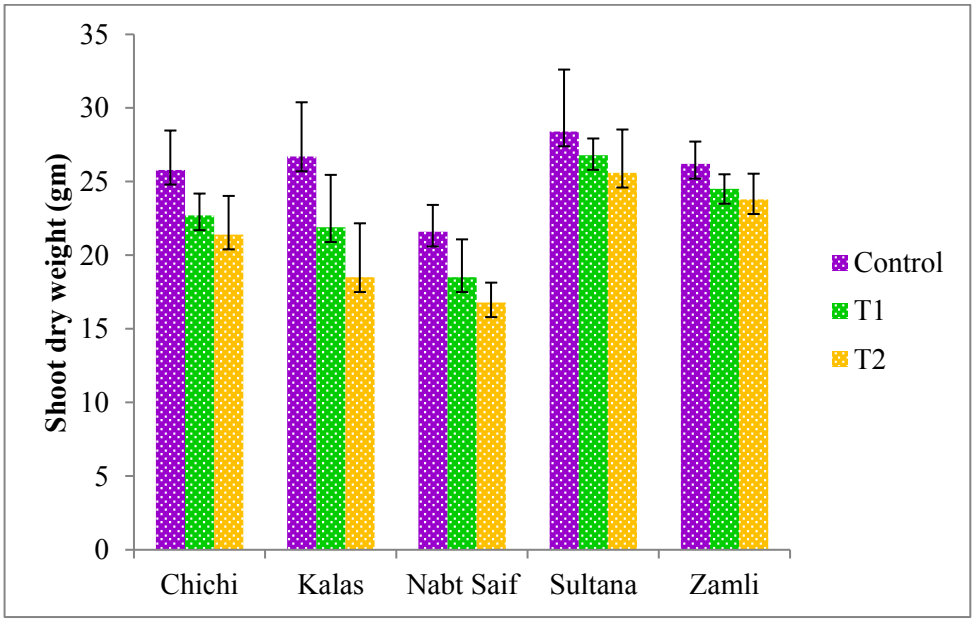


Figure 7: Effect of salinity treatment on shoot dry weight of date palm cultivars (T1-5000 ppm; T2-10000 ppm)

Figure 8 represented the root fresh weight of control and salinity treated date palm cultivars. The fresh weight of roots was reduced when increase the salinity level. In T1 and T2 treatments, the highest root fresh weight was observed in Sultana variety. The fresh weight of roots of control plants were 74.6 ± 2.12 (Chichi), 86.7 ± 0.90 (Kalas), 54.8 ± 4.2 (Nabt saif), 97.3 ± 2.3 (Sultana) and 83.7 ± 1.8 (Zamli) gm. The root fresh weight of salinity treated plants were T1=67.3 ± 1.8 (Chichi), 80.2 ± 4.6 (Kalas), 52.5 ± 3.8 (Nabt saif), 94.4 ± 1.4 (Sultana) and 82.3 ± 2.5 (Zamli) gm; T2=62.5 ± 2.1 (Chichi), 76.7 ± 3.2 (Kalas), 43.4 ± 1.8 (Nabt saif), 93.2 ± 2.8 (Sultana) and 78.2 ± 1.2 (Zamli) gm.

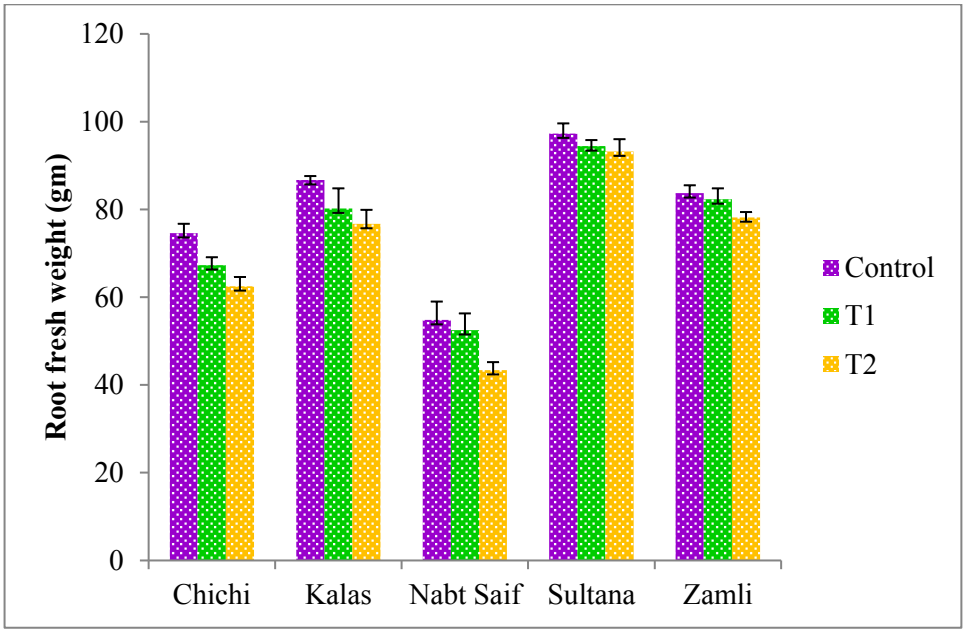


Figure 8: Effect of salinity treatment on root fresh weight of date palm cultivars (T1-5000 ppm; T2-10000 ppm)

The results on the dry weight of control and salinity treated date palm cultivars are given in Figure 9. In Chichi, Kalas and Nabt saif cultivars, the salinity treatment the root dry weight much reduced when compared to the control plant. The root dry

weight of control plants was 19.1 ± 1.56 (Chichi), 22.4 ± 0.84 (Kalas), 13.2 ± 2.13 (Nabt saif), 25.7 ± 1.35 (Sultana) and 20.8 ± 2.5 (Zamli). In T1 treatment, the dry weight of the date palm plants was 16.4 ± 2.81 (Chichi), 12.2 ± 0.68 (Kalas), 10.5 ± 1.85 (Nabt saif), 22.5 ± 1.59 (Sultana) and 19.5 ± 1.81 (Zamli) gm. While root dry weight of 10000 ppm salinity treated date palm cultivars such as Chichi, Kalas, Nabt Saif, Sultana and Zamli were 15.7 ± 1.23, 11.4 ± 0.6, 9.4 ± 1.4, 22.3 ± 1.7 and 19.0 ± 1.0 gm respectively.

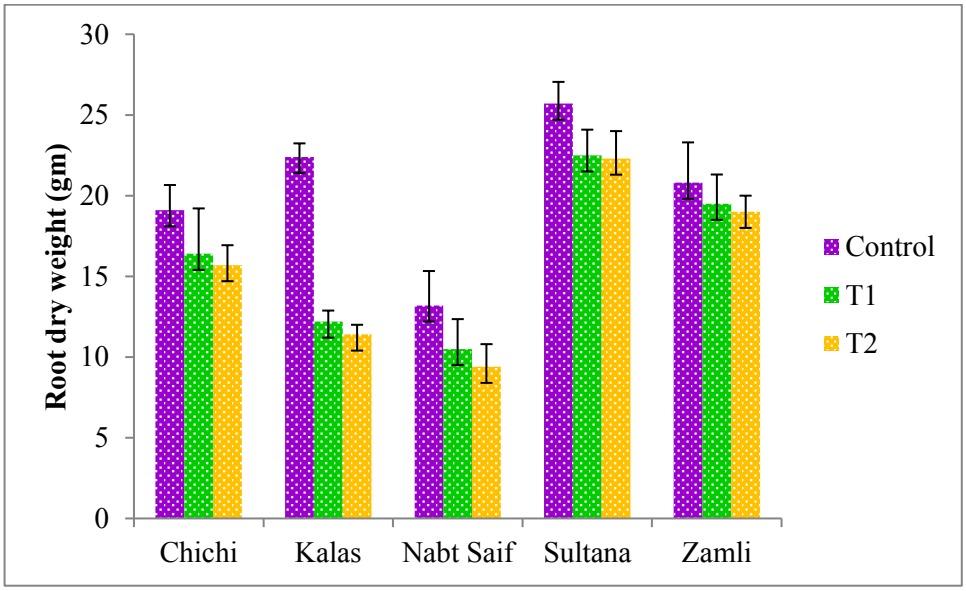


Figure 9: Effect of salinity treatment on root dry weight of date palm cultivars (T1-5000 ppm; T2-10000 ppm)

Sultana and Zamli cultivars of date palm seedlings had less effect against the salinity treatment when compared to the control. Based on the morphological responses such as plant height, shoot fresh and dry weight, root fresh and dry weight, Sultana and Zamli cultivars were characterized as salinity tolerant cultivars selected to find out the response to future climatic scenarios such as high atmospheric $CO_2$ and

enhanced UVB radiation for various traits and responses of date palm plants such as photosynthetic pigments, biochemical, non-enzymatic and enzymatic antioxidants.

### 4.2 $eCO_2$ and UV-B and Combined Effect on Selected Cultivars of Date Palm

#### 4.2.1 Photosynthetic Pigments

The effect of $eCO_2$, UVB and combined treatments on photosynthetic pigments of salt tolerant date palm cultivars *viz.* Sultana and Zamli are presented in Figures 10-13. Chlorophyll 'a' content of Sultana and Zamli date palm cultivars treated with $eCO_2$, UVB and $eCO_2$+UVB are presented in Figure 10.

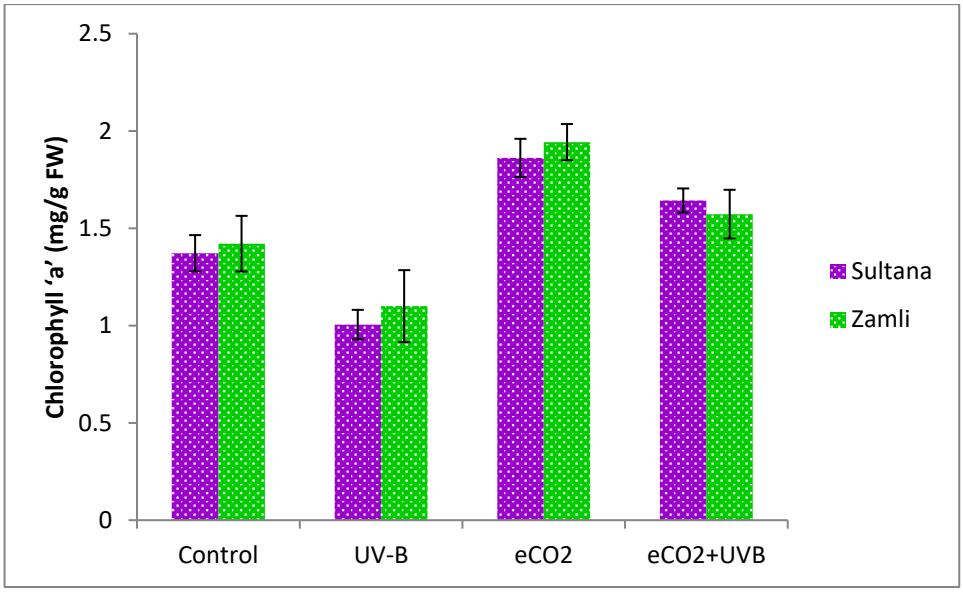


Figure 10: Effect of $eCO_2$, UVB and combined treatment on chlorophyll 'a' content of Sultana and Zamli date palm cultivars

In both the cultivars, the chlorophyll a content was dramatically reduced in UVB treatment when compared to control. Whereas the enrichment of $CO_2$ has increased the content of chlorophyll a. The chlorophyll a content of control, UV-B, $eCO_2$ and UVB+ $eCO_2$ treated plants were 1.372 ± 0.093, 1.005 ± 0.076, 1.862 ± 0.098

and 1.643 ± 0.062 mg/g FW for Sultana and 1.421 ± 0.143, 1.100 ± 0.185, 1.943 ± 0.093 and 1.573 ± 0.125 mg/g FW for Zamli respectively.

Chlorophyll b content of control and UV-B, $eCO_2$ and UVB+ $eCO_2$ treated plants of two cultivars of date palm is graphically represented in Figure 11. The UV-B treated plants have less content of chlorophyll b. But the $eCO_2$ has increased the photosynthetic pigment in both the cultivars. However, Sultana had more pigments when compared to Zamli. The values of chlorophyll b content in Sultana variety were 0.467 ± 0.009 (Control), 0.342 ± 0.065 (UV-B), 0.572 ± 0.002 ($eCO_2$) and 0.532 ± 0.012 (UV-B+$eCO_2$) mg/g FW. While in Zamli variety, 0.472 ± 0.023, 0.282 ± 0.056, 0.501 ± 0.029 and 0.481 ± 0.056 mg/g FW of chlorophyll b content were recorded in control, UV-B, $eCO_2$, UV-B+$eCO_2$ treatments, respectively.

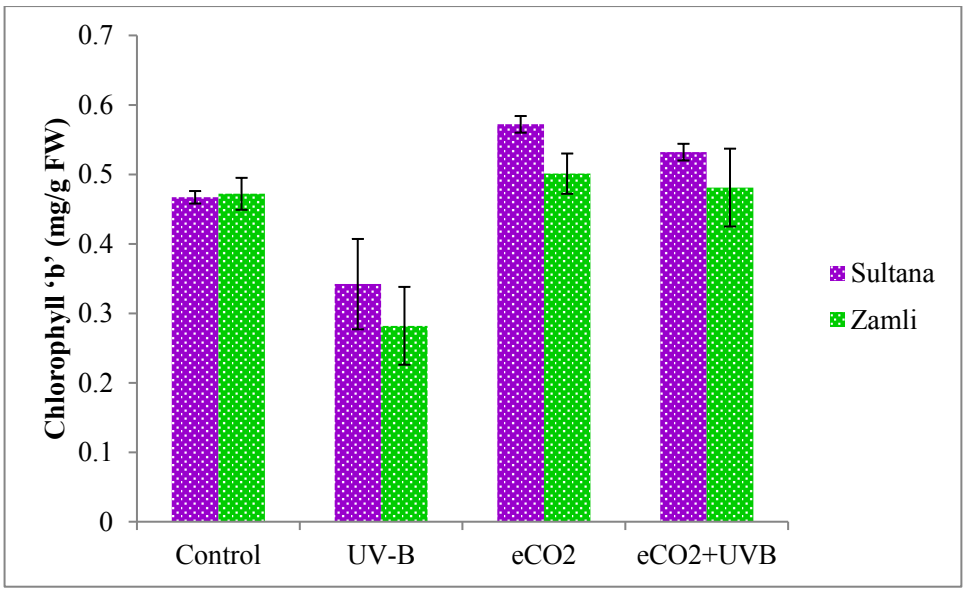


Figure 11: Effect of $eCO_2$, UVB and combined treatment on chlorophyll 'b' content of Sultana and Zamli date palm cultivars

The total chlorophyll content of Sultana and Zamli date palm cultivars after treatment of $eCO_2$, UVB is given in Figure 12. The total chlorophyll content has

reduced in UVB treatment. However, it has increased in $eCO_2$ and combined treatment of UV-B+ $eCO_2$ when compared to control. The total chlorophyll content in Sultana variety was 1.843 ± 0.092 (Control), 1.378 ± 0.059 (UVB), 2.428 ± 0.041 ($eCO_2$) and 2.156 ± 0.072 (UVB+$eCO_2$) mg/g FW. Whereas the total chlorophyll in the leaves of Zamli variety was 1.892±0.173 (Control), 1.390 ± 0.087 (UVB), 2.440 ± 0.06 ($eCO_2$) and 2.054 ± 0.122 (UVB+$eCO_2$) mg/g FW.

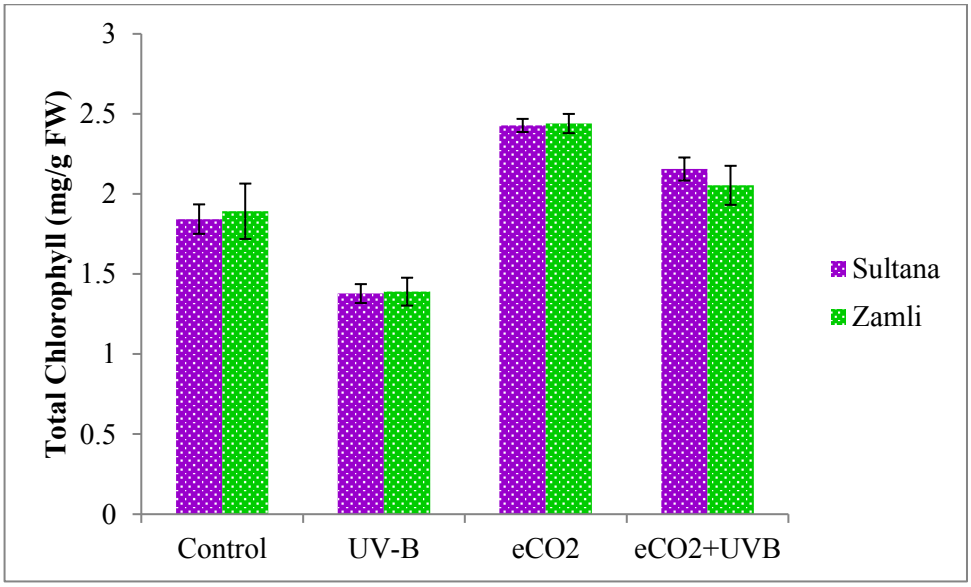


Figure 12: Effect of $eCO_2$, UVB and combined treatment on total chlorophyll content of Sultana and Zamli date palm cultivars

A similar trend was observed in carotenoid content as observed in the chlorophyll content in the leaves of Sultana and Zamli date palm cultivars, after the $eCO_2$ and UVB treatments (Figure 13). The carotenoid content was decreased in UVB treatment and increased in both $eCO_2$ and the combined treatment of $eCO_2$+UVB. The values of carotenoid content in control, UV-B, $eCO_2$ and UVB+ $eCO_2$ treated plants were 0.615 ± 0.034, 0.498 ± 0.017, 0.749 ± 0.072 and 0.682 ± 0.041 mg/g FW for Sultana and 0.557 ± 0.029, 0.472 ± 0.027, 0.669 ± 0.067 and 0.621 ± 0.011 for Zamli respectively.

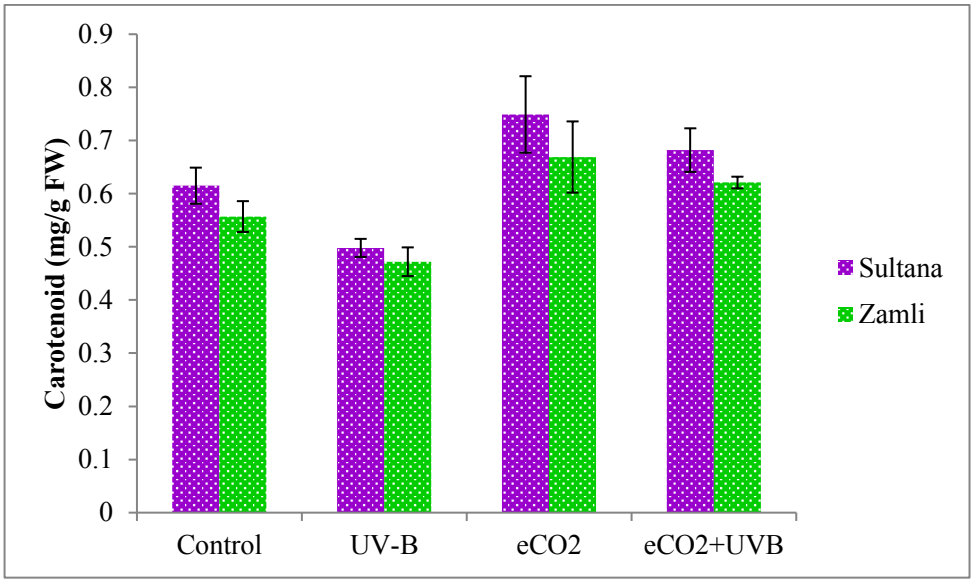


Figure 13: Effect of $eCO_2$, UVB and combined treatment on carotenoid content of Sultana and Zamli date palm cultivars

### 4.2.2 Biochemical Contents

The effect of UVB, $eCO_2$ and combined treatment on biochemical contents in the leaves of Sultana and Zamli date palm cultivars are presented in Figures 14-16. The proline content of the date palm cultivars was increased tremendously in UVB treatment, as shown in Figure 14. But, the $eCO_2$ treatment has slightly increased the proline content. Among the two cultivars of date palm, Zamli had high content of proline in UVB and $eCO_2$+UVB combined treatments. The values of proline content in Sultana were 0.634 ± 0.056 (Control), 1.542 ± 0.072 (UVB), 0.783 ± 0.042 ($eCO_2$) and 1.023 ± 0.021 (UVB+$eCO_2$) mg/g FW. In Zamli variety, 0.573 ± 0.049 (Control), 1.743 ± 0.062 (UVB), 0.657 ± 0.032 ($eCO_2$) and 1.234 ± 0.034 (UVB+$eCO_2$) mg/g FW proline were observed.

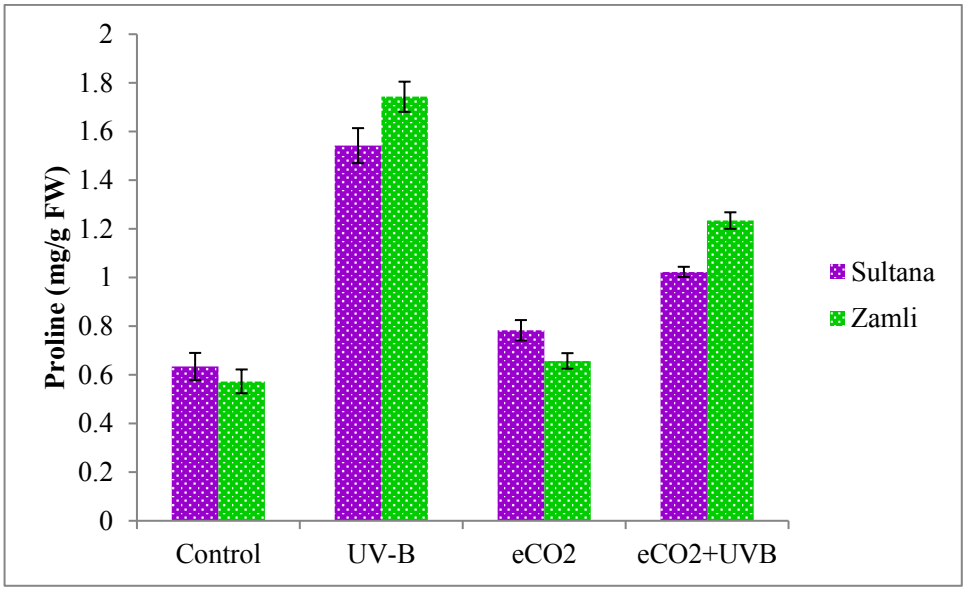


Figure 14: Effect of $eCO_2$, UVB and combined treatment on proline content of Sultana and Zamli date palm cultivars

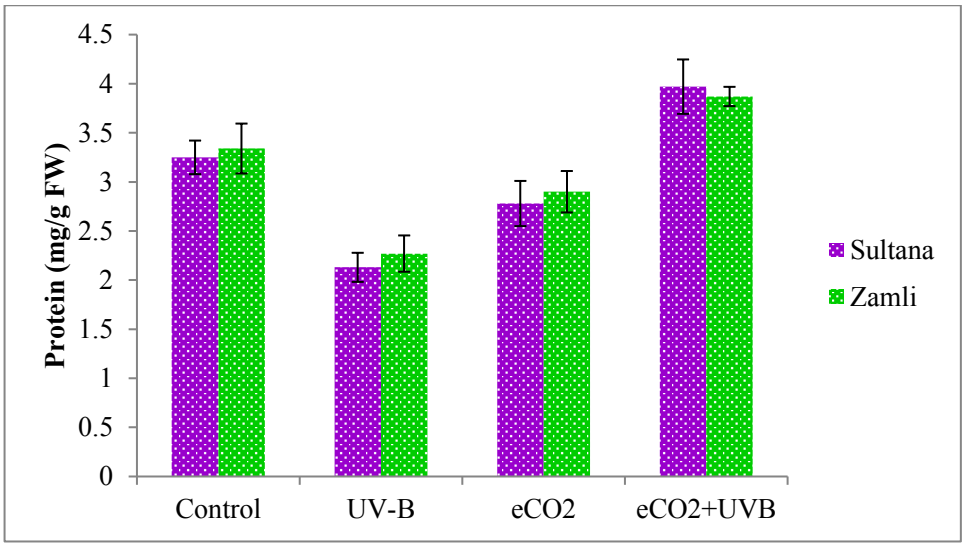


Figure 15: Effect of $eCO_2$, UVB and combined treatment on protein content of Sultana and Zamli date palm cultivars

The protein content in both the cultivars was decreased in UVB treatment and then gradually increased in the $eCO_2$ and UVB+$eCO_2$ treatments (Figure 15). A higher content of protein was observed in the leaves of Sultana variety with $eCO_2$+UVB combined treatment. Whereas the lowest protein content also noted in the same variety

in the UVB treatment. Protein values in the leaves of Sultana variety was 3.25 ± 0.171 (Control), 2.13 ± 0.148 (UVB), 2.78 ± 0.230 ($eCO_2$) and 3.97 ± 0.277 (UVB+$eCO_2$) mg/g FW. While Zamli variety contains 3.34 ± 0.254 (Control), 2.27 ± 0.185 (UVB), 2.90 ± 0.211 ($eCO_2$) and 3.87 ± 0.098 (UVB+$eCO_2$) mg/g FW protein in the leaves.

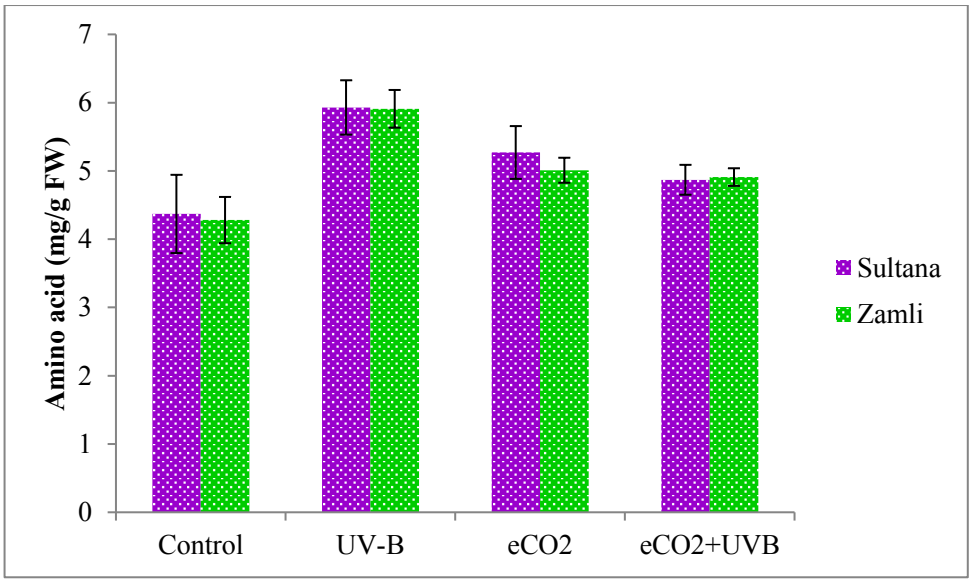


Figure 16: Effect of $eCO_2$, UVB and combined treatment on amino acid content of Sultana and Zamli date palm cultivars

Figure 16 represents the amino acid content of two date palm cultivars after treated with $eCO_2$, UVB and $eCO_2$+UVB. When compared to control, the amino acid content was increased in UVB treatment then decreased in $eCO_2$ as well as in $eCO_2$+UVB combined treatments. A highest content of amino acid was recorded in Sultana variety after treated with UVB. Amino acid values of control, UVB, $eCO_2$ and UVB+ $eCO_2$ treatments in Sultana verity were 4.37 ± 0.573, 5.93 ± 0.398, 5.27 ± 0.387 and 4.87 ± 0.219 mg/g FW. The Zamli variety contains 4.28 ± 0.339 (control), 5.91 ± 0.276 (UVB), 5.01 ± 0.183 ($eCO_2$) and 4.91 ± 0.129 (UVB+ $eCO_2$) mg/g FW amino acids in the leaves.

### 4.2.3 Proline Metabolizing Enzymes

The proline metabolizing enzymes such as γ-glutamyl kinase and Proline oxidase were analysed in the leaves of Sultana and Zamli cultivars and the results are presented in Figures 17 and 18. A maximum γ-glutamyl kinase activity was observed in Sultana variety after the treatment of UVB (Figure 17). But it was degreased in the $eCO_2$ treatment and again increased in the UVB+ $eCO_2$ combined treatment. Among the treatments, a highest γ – glutamyl kinase activity was observed in Sultana variety. The values of γ – glutamyl kinase activity of control, UVB, $eCO_2$ and UVB+ $eCO_2$ combined treatments in Sultana verity were 1.172±0.118, 2.789±0.176, 1.276±0.098 and 2.389 ± 0.110 µg/min/mg protein, respectively. In Zamli variety, γ-glutamyl kinase activity were 1.275 ± 0.182 (control), 2.583 ± 0.090 (UVB), 1.324 ± 0.106 ($eCO_2$) and 2.498 ± 0.176 (UVB+$eCO_2$) µg/min/mg protein.

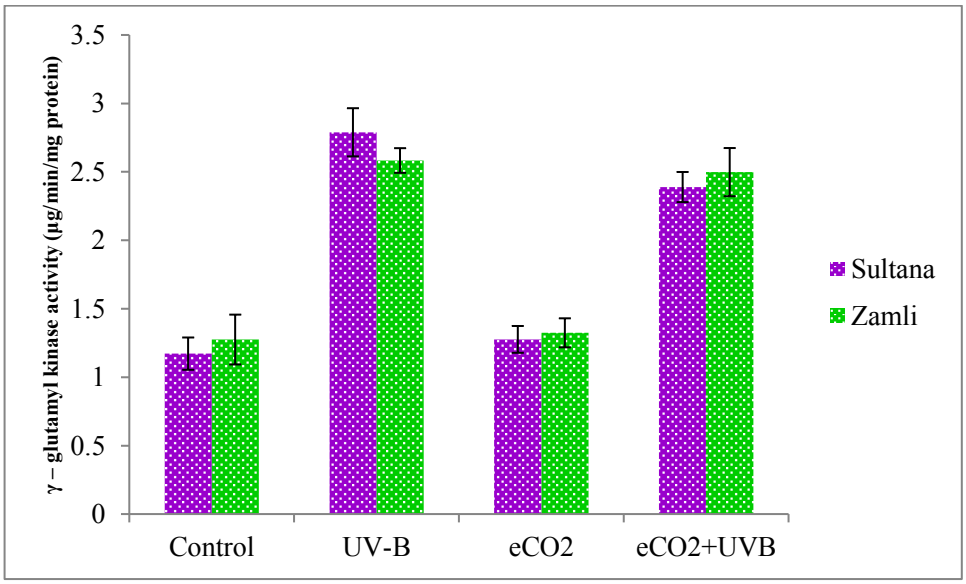


Figure 17: Effect of $eCO_2$, UVB and combined treatment on γ-glutamyl kinase activity of Sultana and Zamli date palm cultivars

The results on proline oxidase activity recorded in the leaves of Sultana and Zamli date palm cultivars are graphically represented in Figure 18. The proline oxidase

activity was reduced in all the three treatments when compared to control. Specially, in control treatment, a highest enzyme activity was observed in the leaves of Zamli. But, in the UVB, $eCO_2$ and combined treatments it was reduced when compared to Sultana variety. The proline oxidase activity in Sultana variety was 0.627±0.021 (Control), 0.438±0.030 (UVB), 0.590±0.027 ($eCO_2$) and 0.428±0.070 (UVB+$eCO_2$) µg/min/mg. While control, UVB, $eCO_2$ and UVB+ $eCO_2$ combined treated plants of Zamli verity contains 0.698 ± 0.054, 0.401 ± 0.010, 0.556 ± 0.042 and 0.419 ± 0.019 µg/min/mg proline oxidase activity respectively.

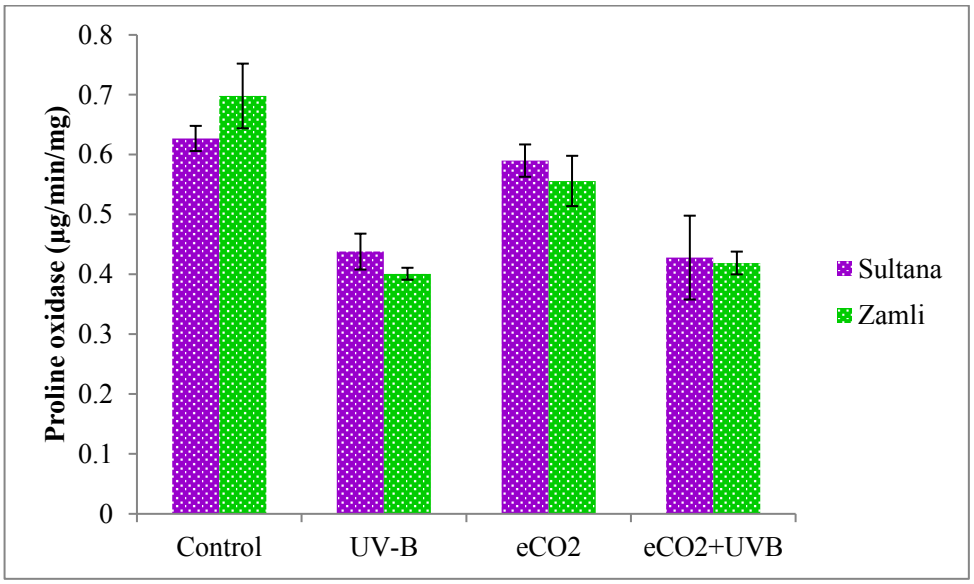


Figure 18: Effect of $eCO_2$, UVB and combined treatment on proline oxidase of Sultana and Zamli date palm cultivars

### 4.2.4 Non – Enzymatic Antioxidants

The results on the effect of future climatic scenarios such as high atmospheric $CO_2$ and enhanced UVB radiation on non-enzymatic enzymes of Sultana and Zamli date palm cultivars are graphically presented in Figures 19-21. The total phenol contents of studied date palm cultivars after UVB and $eCO_2$ treatments are given in Figure 19. An increased level of total phenol content was recorded in both the cultivars at UVB and

UVB+$CO_2$ treatments. The highest content of total phenol was recorded in the leaves of Zamli variety treated with UVB radiation. The same variety has the lowest content of phenol when the plant was treated with $eCO_2$. Total phenol content of untreated, UVB, $eCO_2$ and UVB+ $eCO_2$ combined treatments in Sultana verity were 0.041 ± 0.004, 0.071 ± 0.003, 0.046 ± 0.005 and 0.067 ± 0.001 mg/g and in Zamli variety it was 0.043 ± 0.002, 0.079 ± 0.004, 0.041 ± 0.002 and 0.065 ± 0.001 mg/g respectively.

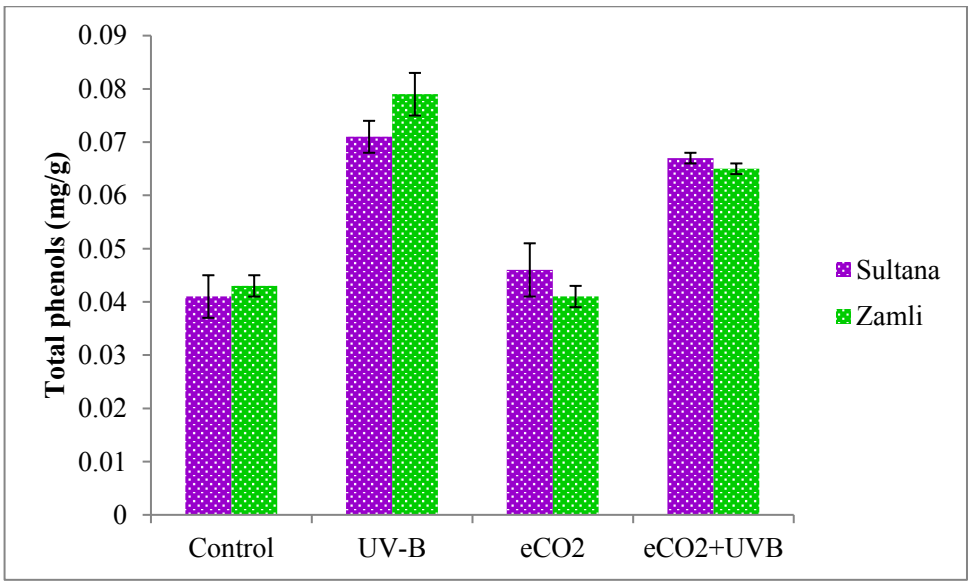


Figure 19: Effect of $eCO_2$, UVB and combined treatment on total phenol content of Sultana and Zamli date palm cultivars

Figure 20 represents the α-tocopherol activity of Sultana and Zamli date palm cultivars treated with UVB, $eCO_2$ and UVB+ $eCO_2$. The α-tocopherol activity was gradually increased in all the treatments when compared to control plants. Among the treatments, the highest α-tocopherol activity was recorded in Sultana variety treated with UVB+$eCO_2$. Whereas the UVB treated plants of Zamli variety showed a lowest α-tocopherol activity. The α-tocopherol activity of Sultana cultivars was 7.671 ± 0.822 (Control), 8.345 ± 0.467 (UVB), 8.563 ± 0.762 ($eCO_2$) and 9.562 ± 0.992

(UVB+$eCO_2$) mg/g FW. In Zamli variety, 6.987 ± 0.726 (Control), 7.765 ± 0.682 (UVB), 8.628 ± 0.672 ($eCO_2$) and 9.162 ± 0.536 (UVB+$eCO_2$) mg/g FW α-tocopherol activity was observed.

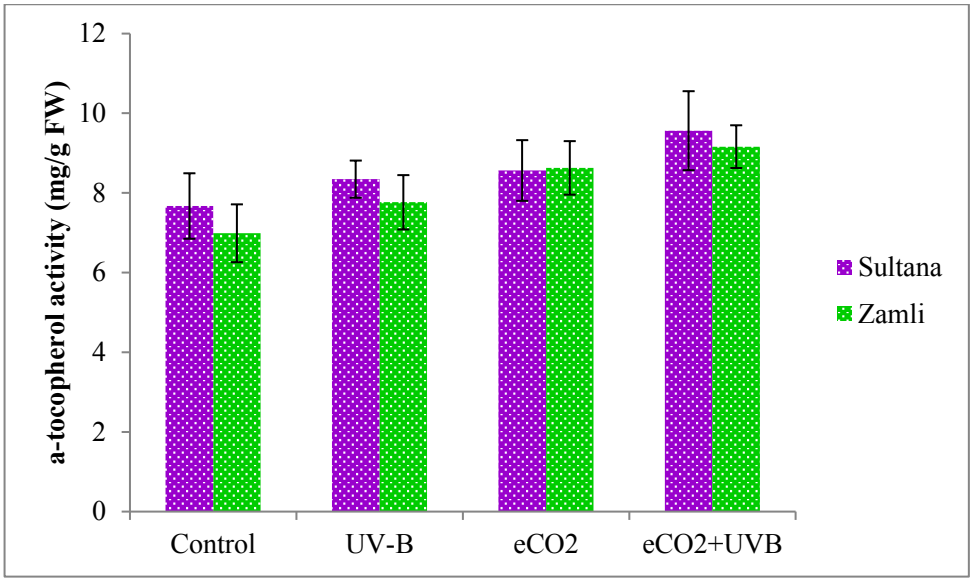


Figure 20: Effect of $eCO_2$, UVB and combined treatment on α-tocopherol activity of Sultana and Zamli date palm cultivars

In both date palm cultivars, reduced glutathione activity was increased in UVB treatment then decreased in $eCO_2$ treatment and again increased in the combined treatment when compared to control (Figure 21). A highest reduced glutathione activity (9.521 ± 0.981 µg/g FW) was recorded in UVB treated Sultana variety. In other treatments, Sultana variety showed 6.982 ± 0.541 ($eCO_2$) and 8.542 ± 0.671 (UVB+$eCO_2$) µg/g FW reduced glutathione activity. Zamli variety had 6.756 ± 0.264, 8.976 ± 0.962, 6.651 ± 0.541 and 8.876 ± 0.716 µg/g FW reduced glutathione activity in control, UVB, $eCO_2$ and UVB+$CO_2$ treatments, respectively.

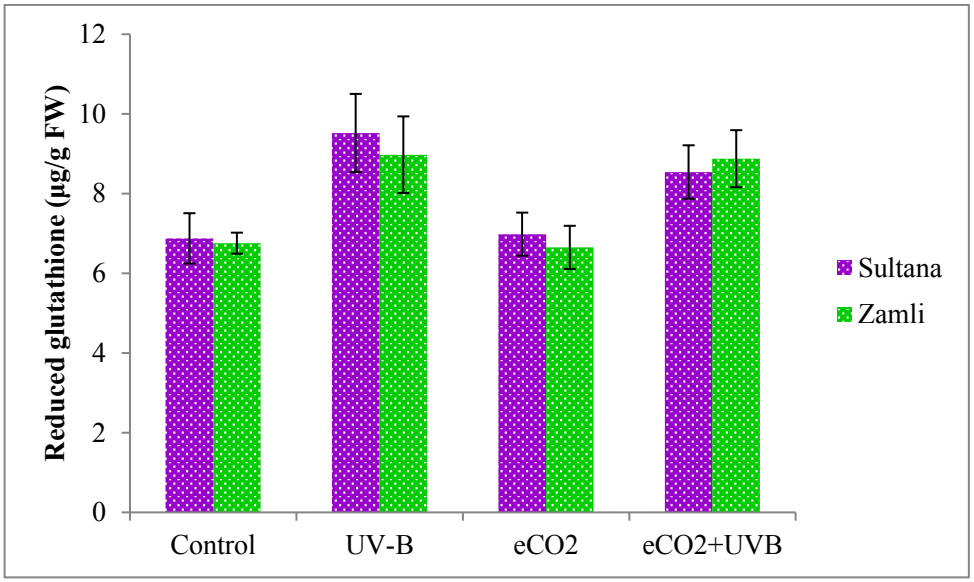


Figure 21: Effect of $eCO_2$, UVB and combined treatment on reduced glutathione activity of Sultana and Zamli date palm cultivars

### 4.2.5 Enzymatic Antioxidants

The results on enzymatic antioxidants such as polyphenol oxidase, peroxidase, superoxide dismutase, catalase and ascorbate peroxidase activities of UVB and $eCO_2$ treated date palm cultivars, Sultana and Zamli are graphically presented in Figures 22-26. The polyphenol oxidase activity was increased in UVB treated plants (Figure 22). Also, polyphenol oxidase activity was increased in the $eCO_2$ and UVB+$CO_2$ treatments when compared to control. Among the treatments, a highest polyphenol oxidase activity was observed in UVB treated Zamli variety.

When compared to Sultana the polyphenol oxidase activity was less in $eCO_2$ and UVB+$CO_2$ treatments. The values of polyphenol oxidase activity of control, UVB, $eCO_2$ and UVB+ $eCO_2$ combined treatments in Sultana verity were 16.83 ± 0.987, 23.95 ± 1.920, 19.51 ± 0.671 and 21.76 ± 0.956 U/mg protein, respectively. Whereas the polyphenol oxidase activity in Zamli variety was 16.98±1.872 (control), 24.76 ± 2.309 (UVB), 18.43 ± 0.762 ($eCO_2$) and 20.98 ± 1.023 (UVB+$eCO_2$) U/mg protein.

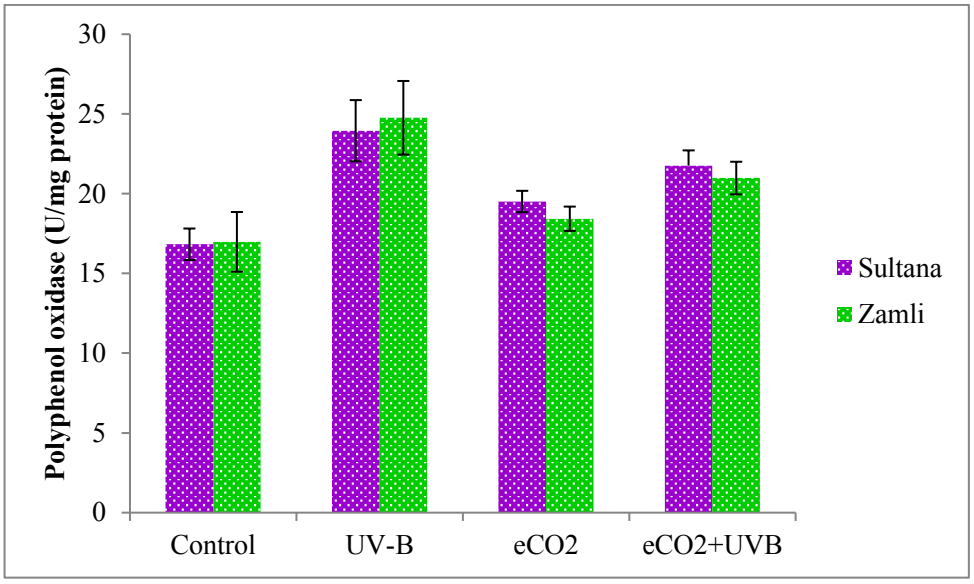


Figure 22: Effect of $eCO_2$, UVB and combined treatment on polyphenol oxidase activity of Sultana and Zamli date palm cultivars

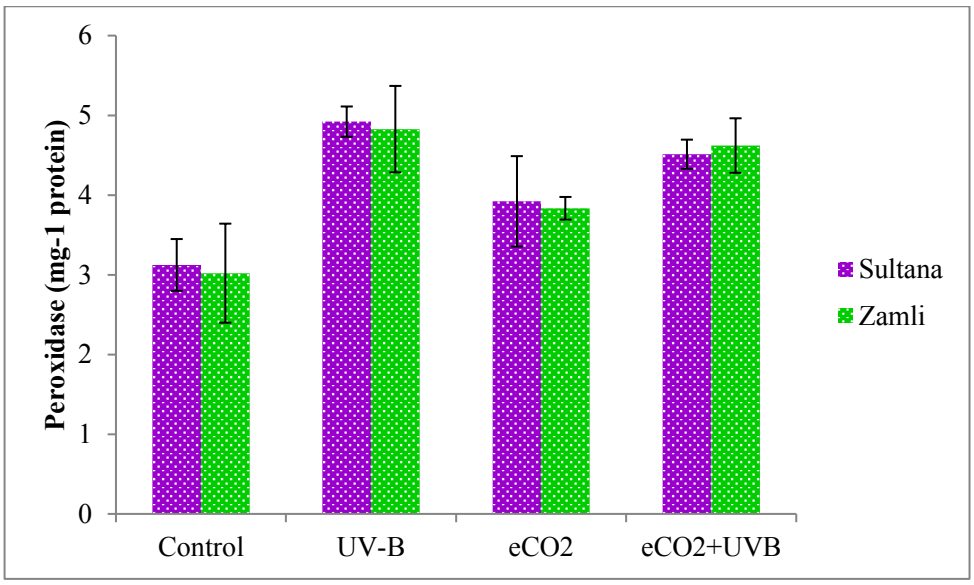


Figure 23: Effect of $eCO_2$, UVB and combined treatment on peroxidase activity of Sultana and Zamli date palm cultivars

The results on peroxidase activity of UVB and $eCO_2$ treated date palm plants are given in Figure 23. The peroxidase was increased in both the cultivars in all the treatments compared to control. A highest peroxidase activity was observed Sultana

variety with UVB treatment. The values of peroxidase activity in Sultana and Zamli cultivars were 3.123 ± 0.325, 4.921 ± 0.190, 3.921 ± 0.567, 4.512 ± 0.183 and 3.02 ± 0.621, 4.827 ± 0.542, 3.834 ± 0.142, 4.621 ± 0.342 $mg^{-1}$ protein in untreated, UVB, $eCO_2$ and UVB+$CO_2$ treatments, respectively. Figure 24 shows the superoxide dismutase activity of Sultana and Zamli date palm cultivars treated with UVB and $eCO_2$. Superoxide dismutase activity was higher in all the treated plants compared to untreated plants.

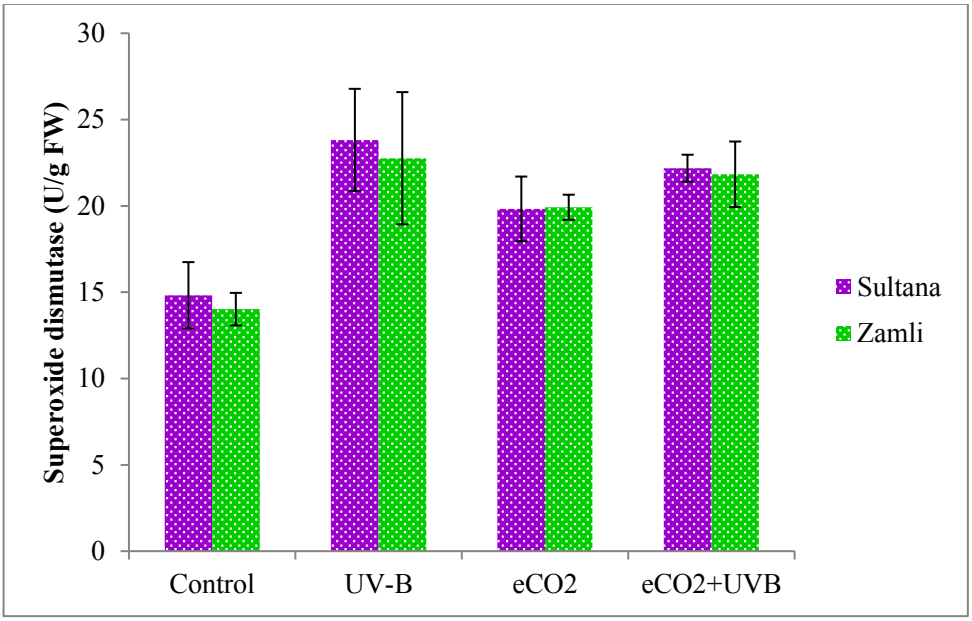


Figure 24: Effect of $eCO_2$, UVB and combined treatment on superoxide dismutase activity of Sultana and Zamli date palm cultivars

The highest content of dismutase activity also recorded in the leaves of Sultana date palm cultivars treated with UVB. The values of superoxide dismutase activity in Sultana variety were 14.823 ± 1.920 (control), 23.82 ± 2.961 (UVB), 19.827 ± 1.872 ($eCO_2$) and 22.182v0.782 (UVB+ $eCO_2$) U/g FW. While the leaves of Zamli variety have 14.021 ± 0.941 (control), 22.762 ± 3.829 (UVB), 19.922 ± 0.726 ($eCO_2$) and 21.829 ± 1.900 (UVB+ $eCO_2$) U/g FW of superoxide dismutase in their leaves. The

results of catalase activity of studied date palm cultivars treated with UVB and $eCO_2$ are presented in Figure 25.

In both the cultivars, the catalase activity higher when the plants expose to UVB radiation. But degreased catalase activity values were recorded in other two treatments. In UVB treatment, both the cultivars have similar amount of catalase. The values of Catalase activity in Sultana and Zamli cultivars were 38.312 ± 0.812, 13.812 ± 1.980, 11.829 ± 1.019, 12.732 ± 0.652 and 8.827 ± 1.219, 13.829 ± 2.092, 12.426 ± 0.617, 13.201 ± 1.292 $mg^{-1}$ protein in untreated, UVB, $eCO_2$ and UVB+$CO_2$ treatments, respectively.

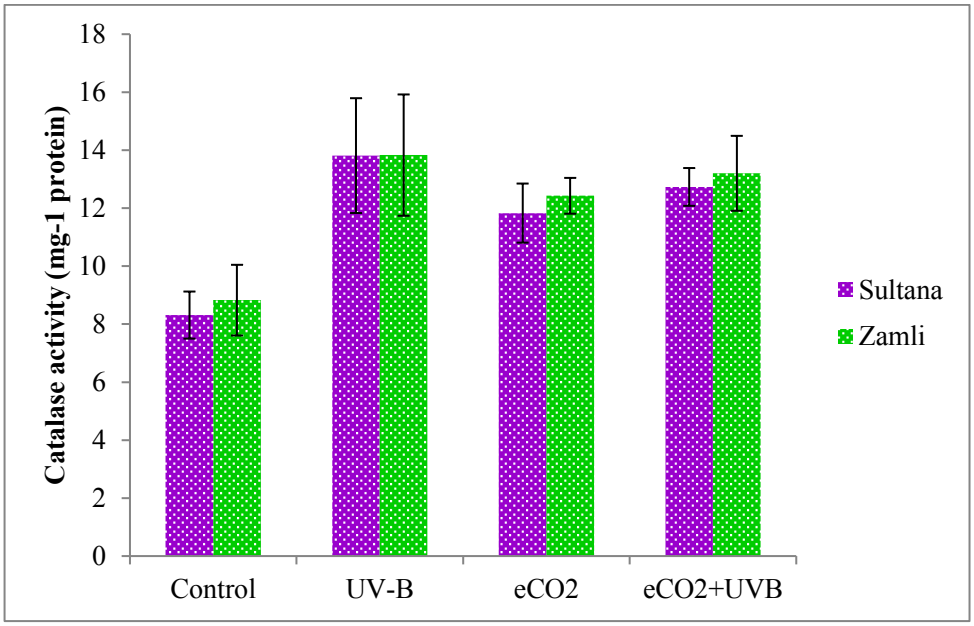


Figure 25: Effect of $eCO_2$, UVB and combined treatment on catalase activity of Sultana and Zamli date palm cultivars

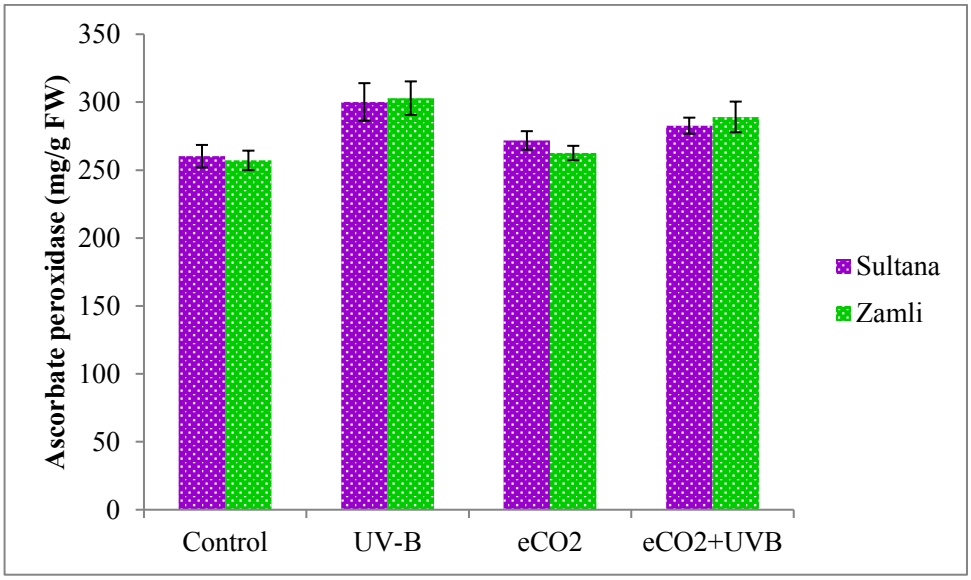


Figure 26: Effect of $eCO_2$, UVB and combined treatment on ascorbate peroxidase activity of Sultana and Zamli date palm cultivars

As observed in other antioxidant enzymes, ascorbate peroxidase activity also increased in both the date palm cultivars treated with UVB radiation (Figure 26). A highest ascorbate peroxidase activity was observed in the leaves of Zamli variety. Ascorbate peroxidase activity of untreated, UVB, $eCO_2$ and UVB+ $eCO_2$ combined treatments in Sultana verity were 260.18 ± 8.318, 300.17 ± 13.820, 271.82 ± 6.810 and 282.67 ± 5.928 µg/g FW, respectively. Whereas the values of ascorbate peroxidase activity in the leaves of Zamli variety were 257.10 ± 7.192 (control), 302.98 ± 12.298 (UVB), 262.56 ± 5.292 ($eCO_2$) and 289.12 ± 11.292 (UVB+$eCO_2$) µg/g FW.

# Chapter 5: Discussion

Date palm is an economically important crop and 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, drought, salinity and decline of water resources are common concern for date palm production. In the present study, five date palm cultivars *viz.,* Chichi, Kalas, Nabt saif, Sultana, Zamli were initially screened for salinity tolerance by measuring the morphological parameters. Since the excess salinity can alter the plant morphology significantly. Also, the vegetative phase of date palm varieties showed different response to the high salinity (Alhammadi & Kurup, 2012).

The results of the present study showed the salinity treatments affect the morphological parameters (plant height, fresh and dry weight of shoots and roots) of Chichi, Kalas and Nabt Saif cultivars significantly. However, there were no significant changes in the morphology of Sultana and Zamli cultivars after the treatment. Basically, date palms are salinity tolerant plants the can withstand salinities up to 24 dS/m (Yaish, 2015). The salinity tolerance results of the present study are agreed with previous reports. Al Kharusi *et al.,* (2017) previously screened ten date palm cultivars (Zabad, Umsila, Nagal, Abunarenja, Fard, HilaliOmani, Nashukharma, Barni, Manoma, and Khalas) for salinity tolerance.

The studied date palm cultivars have no significant difference at 160 mM (lower) and 320 (higher) salinity levels. Whereas the authors found that 240 mM salt salinity showed a difference among the date palm cultivars studied. The growth as well as salt update capacity of *deglet noor* and *medjool* date palm cultivars were studied after treated with salinity range of 520 to 24,000 ppm (Furr & Ream, 1968). The study

showed that the growth rate date palm cultivars plants was decreased with increased salinity levels. Seventeen years old *halawy* and *medjool* cultivars were treated with the salinity levels between 2,500 and15,300 ppm and the cultivars showed less effect in terms of leaves growth rate and fruit yield (Furr & Armstrong, 1962).

The reduction of growth parameters in salt sensitive species (Chichi, Kalas, Nabt saif) may be due to the Na and Cl toxicity or the deficiency of vital nutritional elements which is essential for photosynthesis (Darwesh, 2013). Hormonal imbalance and inhibition also responsible for the reduction of apical growth of date palm cultivars caused by the salinity stress (Younis *et al*., 2003). Nonetheless, the growth reduction could have been caused by the toxicity of $Na^+$ and $Cl^-$ irons on adverse water relations or metabolism (Le Rudulier, 2005). The growth reductions as well as stunted shoots are the general phenomenon when salinity level increased. This effect was also observed in other plants (Mittal *et al*., 2012; Rahneshan *et al*., 2018; Bhattarai *et al*., 2020).

In the present study, two cultivars (sultana and zamli) were identified as salt tolerant and further used to find out the response to future climatic scenarios such as high atmospheric $CO_2$ and enhanced UVB radiation. After the treatments, the plant were studied for the content of photosynthetic pigments, biochemical, non-enzymatic and enzymatic antioxidants. The photosynthetic pigment *viz.,* chlorophyll ‘a’, ‘b’ and total chlorophyll, carotenoids were decreased in UVB treatment when compared to control. This reduction may be due to the Rubisco and Photosystem II caused by the radiation. It leads to the secondary effects such as regeneration of RuBP, photosynthetic capacity and yield of quantum (Teramura & Sullivan, 1994).

Also, UVB reduces the photosynthetic pigments either affecting the enzymes of pigments biosynthetic pathway or the synthesis of photosynthetic pigments (Ranjbarfordoei *et al.,* 2011; Kataria *et al.,* 2014). The results of the present study are comparable with previous reports. The content of the photosynthetic pigments date palm plants was reduced in UVB radiation then gradually increased in $eCO_2$+UVB combined treatments under a controlled environment (Karthishwaran *et al.,* 2020).

UVB also showed selective destruction of biosynthesis or its precursors degradation of chlorophyll a since UV radiation reduces the chlorophyll a content than chlorophyll b (Marwood & Greenberg, 1996). Carotenoids could protect the plants from different environmental stresses. It has the ability to photo protect and stabilize the thylakoid membranes lipid phase, particularly xanthophyll and other terpenoids (Velikova *et al*., 2005; Camejo *et al*., 2006). Furthermore, it was reported that the carotenoids were significantly reduced in barley (Cicek *et al*., 2012) when the plant treated with UVB radiation. So, the reduction in carotenoids could have severe impacts on chlorophyll content during UVB radiation since it defends chlorophyll from the destruction of photooxidative (Agrawal & Rathore 2007; Mishra *et al*., 2008).

Nevertheless, $eCO_2$ stimulates the net photosynthetic rate and increase the biomass production (Cure & Acock, 1986; Visser *et al.,* 1997) either by giving protection to the photosynthetic apparatus or compensate the damage of UV-B radiation (Sullivan, 1997). For example, the UVB radiation directly affects the Rubisco activity (Jordan *et al*., 1996), but it can be compensated through $eCO_2$ by increased net carboxylation, and it was confirmed in previous reports in other plants. Wijewardana *et al*. (2016) studied the multiple environmental factors including $eCO_2$ and UVB radiation to find the multiple stress tolerant hybrids of maize. The authors found that

the growth of UV-B treated plants had smaller leaf area and shorter maize plants. But UVB damaging effects was ameliorated by $eCO_2$ treatments.

$eCO_2$ also showed increased leaf area, height of the plant, total dry weight, photosynthetic pigments and net photosynthetic rate. Lavola *et al*. (2013) studied the interactive effect of $eCO_2$ and UVB radiation on the growth and phytochemistry of *Betula pendula* seedlings. Around six genotypes were used to study the effect in a closed chamber for seven weeks. The results indicated that the UV-B radiation had no effect on the *Betula pendula* seedlings on growth. Whereas increased the growth of the plants as well as phytochemical were observed in $eCO_2$.

In the present study, the proline content was increased in UV-B treatment and decreased in $eCO_2$. This was similar to the results obtained by Balouchi *et al.* (2009) in durum wheat. In 1995, Saradhi *et al.* reported that the plants accumulate the proline when treated with UV radiation and it could protect the cells from peroxidative processes induced by the UV irradiation. Demir (2000) and Amal *et al.* (2006) observed that UV increased proline content in wheat. Likewise, Alia *et al.* (1997) reported the increased level of proline in the shoots of Mung bean and rice when planted espoused to UV radiation.

It was also observed that the UV radiation increased the proline content whereas, the combined effect of UV-B and $eCO_2$ decreased the content of proline. Salama *et al*. (2011) studied the effect of UV radiation on *Malva parviflora*, *Plantago major*, *Rumex vesicarius*, *Sisymbrium erysimoides*. The increased accumulation of proline content was observed in both root and shoots of the studied plant species compared to control. During UVB stress, there are three causes of proline accumulation. Proline synthesis stimulation from glutamic acids, proline oxidation

inhibition and protein synthesis inhibition (Salama *et al.,* 2011). So, the increased level of proline is an important factor that provides tolerance to the plants during UVB radiation. Furthermore, proline referred as a protective mechanism against the reactive oxygen species generated by the UVB irradiation.

The protein content of studied date palm cultivars was decreased in UVB treatment and gradually increased in $eCO_2$ and UVB+$eCO_2$ treatments, respectively. Generally, plants are susceptible to UVB radiation since most of the cellular components i.e. proteins, quinones, nucleic acids, lipids can directly absorb UV-B radiation (Jordan, 1996). Moreover, UV exposure can reduce the growth vigour, photosynthetic pigments, proteins and total sugars (Musil, 1996). The results obtained in the present study on protein content are similar to the previous report published by (Salama *et al.,* 2011). The results indicated that the decreased protein content was observed in both root and shoot of the tested plants. Whereas the increased levels of UV radiation showed higher content of protein.

Bassman (2004) stated that, UV radiation reduce the macro molecules which causing adaptation changes in their structure. Plants may decrease changes in synthesis of protein under different stress conditions which enable the plants to manage such a stress (Santos *et al*., 1998). Also, it was reported that the decreased protein content might be associated with underdeveloped rate of growth caused by decreased photosynthetic rate. Furthermore, in higher plants, UV radiation damages the proteins, lipids and nucleic acids (Jordan, 1996; Vass, 1996). In the present study, an increased level of amino acid content was observed UVB treatment when compared to control. In contrast, Bandurska *et al*. (2012) observed a decreased level of amino acid content in UVB+water deficit treated barley seedlings shoot and root. It may be due to the

water deficit which contributed to increased level of amino acids (Bandurska *et al*., 2005). Also, it decreased at $eCO_2$ as a significance of lower photorespiration rate (Stitt & Krapp, 1999). But Rogers *et al*. (2006) reported that the $eCO_2$ has no effect on amino acid content in *Glycine max*.

Proline metabolizing enzyme, γ-glutamyl kinase activity was increased in UVB treatment but in $eCO_2$ treatment the enzyme activity was equal to control plants. This enzyme showed a primary role in biosynthetic pathway of proline (Smith *et al.,* 1984). Under different environmental stress, the proline concentration increased up to 80% of the amino acid pool but in normal conditions it comprises less than 5% (Shahbaz *et al*., 2013). Karthishwaran *et al*. (2020) reported the increased proline metabolizing enzyme in date palm cultivars treated with UVB and $eCO_2$. So, proline metabolism is correlated positively with the exposure of plants to various environmental stresses (Saeedipour, 2013).

In the present study, increased level of non-antioxidant and antioxidant enzymes were observed in UVB treated date palm cultivars. Whereas those enzyme activities were decreased in elevated level $CO_2$ treatments. Generally, all the plant species had antioxidative defence mechanism which provides enough production to alleviate adverse impact of stress. Non-antioxidant enzymes such as total phenols, α-tocopherol and reduced glutathione contents also played a vital role in alleviating the effects of ROS. The synthesis of UV absorbing constituents is the potential non-enzymatic antioxidant mechanism which quenchers of ROS or reduce the penetration of radiation (Del Valle *et al*., 2020).

Zagoskina *et al*. (2003) studied the tissue localization and accumulation of phenolic compounds in callus cultures of *Camellia sinensis* after UVB radiation. The

results showed that UVB promotes the accumulation phenol content by increased deposition of phenolic compound in cell walls. On the other hand, the antioxidant enzymes activities are also induced by the abiotic stress. Especially, UVB radiation enhanced the activities of polyphenol oxidase, peroxidase, superoxide dismutase, catalase, ascorbate peroxidase activities in the present study. This activity is supported by the previous studies (Mishra *et al*., 2009; Kondo & Kawashima, 2000). Moghimifam *et al*. (2020) reported that the antioxidant enzymes such as catalase, ascorbate peroxidase, peroxidase, glutathione reductase, superoxide dismutase and polyphenol oxidase were increased in *Withania somnifera* after the treatment of UVB. Dwivedi *et al*. (2015) reported that varied level of antioxidant enzymes with the plant species and UV-B exposure.

Agarwal (2007) studied the UVB oxidative damage of *Cassia auriculata* seedlings. The study showed that the level of antioxidant enzymes such as catalase, superoxide dismutase, polyphenol oxidase and peroxidase were increased significantly. Similarly, the increased antioxidant enzyme level was noted in wheat and maize (Mackerness *et al.,* 1999), cucumber (Kondo & Kawashima, 2000), *Plectonema boryanum* (Prasad & Zeeshan, 2005) and in potato (Santos *et al.,* 2004). Various reports showed that the increased enzyme activities linked with the better tolerance to the environmental stress (Zaefyzadeh *et al.,* 2009; Chen *et al.,* 2011). For example the increased superoxide dismutase activity directly correlated with the stress tolerance and all are nuclear encoded superoxide dismutase forms target the subcellular compartments through a targeting sequence of amino terminal (Bowler *et al.,* 1992).

Also, immediate expression multiple enzymes had more potential on stress tolerance than the single/double expression (Lee *et al*., 2007). It is also reported UVB inhibits the rate of net photosynthesis by decreased the rate of $CO_2$ assimilation which reduce the RUBISCO content as well as carboxylation velocity (Allen *et al*., 1997). For example, UVB increased the antioxidant defence efficacy of *Picea asperata* seedlings through the antioxidant enzymes (Han *et al.*, 2009).

# Chapter 6: Conclusion

Since the date palm is considered as an important subsistence crop in most of the world's desert areas, the study on the effect of day-to-day increasing stress factors on the much important plant. Abiotic stresses such as elevated level $CO_2$, UVB, and salinity cause substantial damage to date palm resulting in annual losses estimated in billions of dollars worldwide. The crop, being a desert plant, has evolved strategies to protect itself against most of these stresses. However, the projected changes in climate, weather extremes and interaction among the various abiotic stresses will have profound impact on the date palm adaptation and production. Thus, it is imperative to evaluate the responses of various growth and biochemical parameters of data palm to the combinations of environmental factors. Five cultivars of date palm *viz.,* Chichi, Kalas, Nabt saif, Sultana, Zamli were initially screened for salinity tolerance with two concentrations of salinity.

Based on the morphological parameters Sultana, and Zamli cultivars were identified as a salinity tolerance and used for effect of climate change factors such as elevated level $CO_2$ and UVB irradiation under open top chambers facility. Both the varieties had good response to photosynthetic pigments, biochemical contents, proline metabolizing enzymes, non-enzymatic and enzymatic defence to UVB, $eCO_2$ and the combined treatments when compared to control. The results also showed that sultana cultivar had stress tolerance ability and it can be suited for the UAE growing conditions. The results also advance our understanding by elucidating the various physiological and biochemical mechanisms responsible for the abiotic stress tolerant characteristics among the date palm varieties. Moreover, other a biotic stress and yield parameter are warranted for the identification of biotic stress tolerant date palm cultivars.

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