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RESPONSE OF FIVE CHENOPODIUM QUINOA VARIETIES TO DIFFERENT SALINITY LEVELS, ELEVATED CO_2 AND UVB

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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

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Declaration of Original Work

I, Saif Ali Matar Al Blooshi, the undersigned, a graduate student at the United Arab Emirates University (UAEU), and the author of this thesis entitled "*Response of Five Chenopodium Quinoa Varieties to Different Salinity Levels, Elevated CO_2 and UVB*", 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, in the College of Agriculture and Veterinary Medicine at 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 Any materials borrowed from other sources (whether published or unpublished) and relied upon or included in my thesis have been properly cited and acknowledged in accordance with appropriate academic conventions.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.

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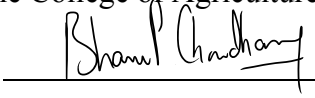
Signature  Date 21/03/2022

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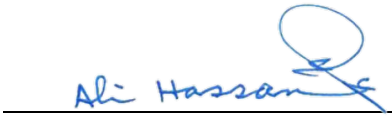
Signature  Date 21/03/2022

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Abstract

Ecosystems have been affected by climate changes. Both agriculture and environmental changes are correlated with various features since climate change is the main cause of abiotic and biotic stress which affects crop plants. The climate changes and its severe impact on plant productivity showed great intensities due to the effects of abiotic stress. In the present investigation, five quinoa varieties *viz* KAUST-05395/CHFN-68 (V1), KAUST-05398/PI-614889 (V2), KAUST-05397/PI-614885 (V3), KAUST-05403/ICBA-Q3 (V4), and KAUST-05399/PI-614888 (V5) were screened for their salinity stress response by measuring the morphological parameters such as total plant height, fresh and dry weight of shoot and roots. V4 and V5 varieties were identified as salt tolerant and selected to study the response to future climatic scenarios such as eCO_2, enhanced UVB radiation and UVB+eCO_2 combined effect in Open Top Chambers. The response of studied quinoa varieties were measured by analyzing the photosynthetic pigments, biochemical contents, proline metabolizing enzymes, non – enzymatic antioxidants and antioxidant enzymes activities. Based on the results obtained in the present investigation, further study is warranted for screening the more varieties with additions climate change factors such as temperature and humidity to find out more tolerant varieties of quinoa suitable for future climatic conditions.

Keywords: Quinoa, Climate change, UVB Radiation, Elevated Level CO_2, Morphology, Antioxidant.

Title and Abstract (in Arabic)

استجابة خمسة أصناف كينوا كينوبوديوم لمستويات مرتفعة من ثاني أكسيد الكربون والأشعة فوق البنفسجية باء ومستويات الملوحة المختلفة

الملخص

تأثرت النظم البيئية بالتغيرات المناخية المدمرة. حيث ترتبط كل من التغيرات الزراعية والبيئية بالأحداث المستقبلية المختلفة لأن تغير المناخ هو السبب الرئيسي للإجهاد الأحيائي والحيوي الذي يؤثر بدوره على المحاصيل والنباتات. أظهرت التغيرات المناخية الأثر الشديد على إنتاجية النبات بسبب تأثيرات الإجهاد الأحيائي. في هذه الدراسة، خمسة أصناف من الكينوا هي KAUST-05395/CHFN-68 (V1), KAUST-05398/PI-614889 (V2), KAUST-05397/PI-614885 (V3), KAUST-05403/ICBA-Q3 (V4), and KAUST-05399/PI-614888 (V5) تم فحصها لاستجابة إجهاد الملوحة عن طريق قياس العلامات المورفولوجية مثل الطول الكلي للنبات والوزن الرطب والجاف للساق والجذور. تم تحديد أصناف V4 و V5 على أنها تتحمل الملوحة وتم اختيارها لدراسة الإستجابة للسيناريوهات المناخية المستقبلية مثل eCO_2 وإشعاع UVB المعزز والتأثير المشترك لـ eCO_2 + UVB في الغرف المفتوحة العلوية. تم قياس استجابة أصناف الكينوا المدروسة من خلال تحليل أصباغ التمثيل الضوئي والمحتويات البيوكيميائية، إنزيمات استقلاب البرولين، ومضادات الأكسدة غير الأنزيمية وأنزيمات مضادات الأكسدة. يوصى بدراسة عدد أكبر من الأصناف وتأثير العوامل المختلفة عليها، مثل عوامل تغير المناخ، درجة الحرارة والرطوبة؛ وذلك لاكتشاف أصناف متنوعة أكثر تحملاً من الكينوا لتكون مناسبة للظروف المناخية المستقبلية.

مفاهيم البحث الرئيسية: الكينوا، تغير المناخ، الأشعة فوق البنفسجية، المستوى المرتفع من ثاني أكسيد الكربون، المورفولوجيا، مضادات الأكسدة.

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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

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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

Plants show wide variety of response to different environmental factors. They occur nearly everywhere and must therefore live under a wide range of dynamic environmental conditions. They also require appropriate conditions for their optimum growth. Physical main factors which affect the growth of plants are a) high temperature b) salinity c) exposure to UVB radiation d) recurrent drought e) rainfall (Piccini *et al.,* 2020). Choice of the physical factors is very important. Plants do not live for long in the environment due to their optimum growth conditions, change of environmental conditions of the climate due to accumulated industrial exhausts, vehicle emissions, ozone depletions, recurrent drought, global warming etc. The climate change affects the agricultural production in many other ways and the most important of which is that it gives rise to poor productivity of crops (Cline, 2007). Therefore, appropriate conditions should be provided for a long period without affecting their physical and chemical needs. Plants are always exposed to the hazards of climatic variations by several agents all around. While environmental changes, the plant body tries to defend by a number of defense mechanisms. One of the most effective mechanisms is the adaptability of the physiological tissues to resist continuous variation upon it by expressing tolerance (Hasanuzzaman *et al.,* 2013). Toleration or resistance is the reaction of plant tissues to changes in the atmosphere. Resistance might be natural. Secondary metabolites or phenolic compounds or pigment productions are natural defense mechanisms (Isah, 2019). These are formed by the plant tissues in response to the external climatic conditions. There are various types of agents that can induce physiological response. Some phenolic compounds produced by the plants induce the tolerance ability (Lin *et al.,* 2016).

Actually, it involves primary or secondary metabolites and their reactions to climatic variations. The manifestations of such reactions are of seen in shoot system or in leaves or stem or fruits or in flowers. Climate is inseparable from habitat and is an important abiotic factor in physiological investigations concerning phenology, physiology, biochemistry, genetics, etc. Some plants show the presence of hormones in tissues. These are invisible that can cause defending of plant tissues or enhance resistance by vigorous growth. The formation of morphological variances would be visible by the production of more sepals, more petals, more leaves, more fruits, plant height, plant width, biomass increase etc.

The impacts of soil salinity are well known issues in forestry and agriculture and also the subject of investigation by chemists because of their abiotic constraints which affect crop productions such as high temperature, sandy soils, water scarcity, and loss of top soil and also the wind erosion occur in the environment. Over 800 x 10^6 hectares of the global lands are affected by salt stress. Reports of affected people across the globe reveals that affected are as huge as 250 x10^6 people (Meredith *et al.,* 2019). Many regions of the world are revealing the saline soil and degrading soil presence particularly in arid and semi arid regions. Previous literature reported the presence of 12 hec of degraded land across the globe according to one assessment.

Because of great increase in food demand, large population the food production is found to be of shrinking as the fertile lands are also shrinking worldwide (Satterthwaite *et al.,* 2010). The strong winds are dangerous agents affecting arid and semi arid regions scrapping the top productive fertile soil layers. The known fact that the water potential is reduced where there is high salinity is soil. Therefore,

morphological and physiological traits of crops also affected by exhibiting their high osmotic balance and ROS, ion homeostasis, etc.

UV radiations do not ionize but it can intrude the exposed cells and is received specifically by various pigments or compounds and therefore is detrimental to plant health. The detrimental activity depends on various factors such as strength of radiation, duration, uniformity of exposure, air current, distance from the surface of the earth and etc. Ion toxicity might be evident in the high saline sandy soil and as a result stunted growth of plants which explain the fact that the salinity of the soil induces imbalance of nutrition in soil. When the salinity increases, the plant may show accumulation of K+ ions, nutrient non availability, physiological inactivation etc. Therefore, renewed effort is required to investigate the salinity of soil, development of saline tolerant crops of halophytes, drought resistant varieties. Increasing CO_2 gas is considered as the most common issue in the World. The global annual increase in CO_2 level to 1.8 u Mol can increase the risk of global warming, climate change, ocean atmospheric devastations, changes in the average rainfall, increase in greenhouse effect. Previous studies indicated that there this can increase the risk of changes in the cloud amount, distribution of cloud and changes in the atmospheric aerosols.

The greenhouse gases consisting of methane, chloroflurocarbon and nitrous oxide are considered to be hazardous yet they are also the interest of study by the environmentalists because of their climate change effects and their impacts with fisheries, animal husbandries and terrestrial vegetation and atmosphere in the ecosystem. Examination of CO_2 level reveals that available concentrations in the past 250 years may be as large as 100 ppm on earth (IPCC, 1994). Because of increasing atmospheric CO_2 gas to 400 ppm by the end of this 21^{st} century, doubling the amount

of atmospheric carbon dioxide is considered to be of very important so far as the climate change is concerned (Pachauri *et al.,* 2014). Examination of increasing global temperature predicts that air temperature would increase to 4.5˚C from 2.5˚C by the end of this 21st century. This reveals the fact that the drought and high temperature will be severe in near future (Pachauri *et al.,* 2014).

The environmental stress factors and their impacts are responsible for the climate change and therefore special protocols are needed to assess the impact of physical factors on plants and assess their physiological characteristics (Singh *et al.,* 2010; Vinebrooke *et al.,* 2004). Although the global warming is the most common cause that leads to decrease the crop yield, their negative impacts have also raised substantially in recent years. The temperature curve is also reaching high at the rate of 0.85°C during 2010 which might be attributed to the lack of proper conservation practices lack of CO_2 control protocols (Hartmann *et al.,* 2013).

1.1 Elevated Level eCO_2

Again, it should be remembered that the atmospheric Carbon dioxide concentration difference might show some impact on atmospheric temperature variation. A combination two more factors such as fossil fuel combustion and deforestation are involved for increasing the atmospheric concentration of CO_2. Previous report indicated that the CO_2 in the atmosphere was 280 ppm. But in 2021, the recent study demonstrated the presence of 415.13 ppm during April 2021. The report of IPCC too suggested that the global CO_2 concentration might increase to 1000 ppm by 2100.

The study results suggested the possession of both the ill and beneficial effects of CO_2 on crops. Presence of high levels of CO_2 can be considered as the factor that might probably increase the rate of photosynthesis. Their high level of CO_2 presence in the atmosphere indicates beneficial effects such as maximum yield and good plant growth (Long *et al.,* 2004; Ainsworth and Long, 2005; van der Kooi *et al.,* 2016). The photosynthesis is one of the most useful process used for this purpose of optimizing abiotic variables of plant growth (Wang *et al.,* 2012). Formation of activities of high ribulose 1,5 bisphosphate oxygenase/ carboxylase confirm the presence of elevated levels of carbon di oxide. According to Warren *et al.* (2014) and Ainsworth and Long (2005) the down regulation of photosynthesis might be due to the long term exposure of eCO_2 on plants. As it was evident from previous studies, eCO_2 is responsible for the development of tolerance of plants to high sugar concentrations, antioxidants (Huang and Xu, 2015) and the atmospheric temperature variable provides considerable information as the dependent variable involved. The highest vapour pressure which is generally followed by changes in temperature from warm to warmer air temperature conditions (Novick *et al.,* 2016). Stomatal closure is the phenomenon in which the rate of photosynthesis and transpiration reduced (Mott & Parkhurst, 1991) Such is the result of drying of soil to the faster rate by water absorption of root system (Will *et al.,* 2013).

The ill effects of eCO_2 which is also evident from the varying composition of proteins (Broberg *et al.,* 2017) vitamins, macro and micro elements in plants (Myers *et al.,* 2014). So, the eCO_2 concentrations indicate the various responses of crops. From the seed germination stage until the crop set to harvest, the plant is facing a number of threats by a variety of abiotic and biotic factors. During the earlier stages of vegetative growth open field plants are under constant threat by climatic variations. Often the

damage was caused by UVB radiation, uncertain weathering conditions, hottest temperature, recurrent wind and sudden rain etc. which decrease the yield. Minja *et al.* (2011) reported that low productivity is caused by a combination of factors including abiotic factors such as salinity, drought, excessive heat, soil fertility, pests and diseases, poor crop management and a lack of high yielding varieties.

1.2 Enhanced UVB

High harvest of crops by controlled environmental conditions has become a field of interest in crop physiology. However, in recent years, extensive and intensive studies have been carried out on the climatic conditions influencing photosynthesis and respiration of cultivars. The green house gases that hinder the growth and harvest characteristics of plants are Carbon dioxides, Nitrogen oxides and Sulphur dioxide. Many of these gases might change the precipitation, daily temperatures, relative humidity of the atmosphere of soil. Among these, Carbon dioxide, receives the most crucial abiotic factor results in global warming. However, ozone depletion and Carbon dioxide influence are greatly referred as abiotic variables that has the ability to change the atmospheric weather conditions. Previous studies reported that the temperature increase and CO_2 are the agents that decrease or increase photosynthetic activity and also reflects its correlation with the relative increase in crop growth and yield. The ozone in the atmosphere plays a significant role in the physiology of crop and might act as resistance to external harsh environments. The depletion of ozone can react direct impact of UVB on earth. Previous studies have reported that the UVB has the intensity of < 315 nm is found to have disastrous influence on majority of crops.

The ozone concentration analysis in the atmosphere provides useful clue on the ill effect of UVB of sun. The presence of ozone in the stratosphere above 10 but below

50 km on the earth's surface always means that the biological species are protected but < 10 km from the Earths surface in the troposphere indicates excess pollution. There are several pollutants which influence the depletion of ozone in the atmosphere. These are halogenated compounds, methane, nitrous oxides and CFCs. The observed level of ozone depletion assessed during 2002 - 2005 which showed about 3% and 6% in the northern and southern hemisphere respectively (World Meteorological Organization, 2008). The UVB radiation of sun get into earth directly by many causes. The depletion of ozone in the stratosphere is one cause.

All plants on earth are susceptible to UV exposure. The ability of plants to do photosynthesis depend upon the exposure of plants to sunlight. When UV radiations expose on plant, they cause adaptability of the plant system and resolve the damages. The degree and time required for complete adaptations of plants to UV radiations depend upon the type of crop variety. Changes in the reproductive and vegetative structures, thickness of mesophyll, palisade and epidermal layers can be tested on the plants exposed under UVB radiation. The deleterious impacts of UV radiations against the crops can be observed in the anatomy of leaf grana and thylakoid membrane integrity, upregulation of flavonoid pathways, phenolic compound pathways, vegetative growth analysis, photomorphogenic systems and etc. It is also observed that the primary secondary and tertiary effects of radiations altered the growth and development of crops at a defined dose. Some varieties of crops showed yield reductions, biomass reductions, less fruit numbers, lowered photosynthesis canopy and less light interception (Kakani *et al.,* 2003).

1.3 Soil Salinity

The biogeochemical cycles, climatic variations and atmospheric changes occur over the surface of the earth on the soil. Especially, climate changes are much greater in the soil which influence soil properties and soil processes. Among many such changes, salinization is widely studied and their mobilization and fractionation of salts in soils has been established beyond doubt by Foster and Chilton in the year 2003. In Sumerian time, in Mesopotamian plains, the process of salinization was found to occur during 5000 years ago (Shahid *et al.,* 2018). This type of processes was also recorded during 3500 BC in the ancient Mesopotamia by Jacobsen and Adams in the year 1958. Although the salinization processes occur globally, arid and semi arid regions were most affected. This might be due to water scarcity in soil has been reported by Kurylyk and MacQuarrie (2013). Some of the factors such as growth of population, economic pressure for food production, impact of climate change and physical factors are other variables affecting a wide variety of soil and imparts salinization. Some other factors are also specific in their agricultural practice management. They are rate of fertilization, poor drainage conditions, quality of soil, micro topograph, water table depth, type of crops and quality of water irrigation (Meimei *et al.,* 2011; Nosetto *et al.,* 2013; Allbed *et al.,* 2014; Yahiaoui *et al.,* 2015). The climate changes are destructive because they cause soil salinization by catalysing the soil (Teh & Koh, 2016; Gorji *et al.,* 2019). The high salinization affects the yield of crops and the climate change might also act on to cause sea level rise.

1.4 Quinoa (*Chenopodium quinoa* Willd.)

Quinoa belongs to family Amaranthaceae of the kingdom plantae. They are widely present in European countries, South North western America, Kenya and India.

They are also present in Bolivia and Peru. They are herbaceous and dicotyledenous annual plant. They are 1 – 2 meters in height. The fruits are 2 mm in dia with white or black or red in colour (Vaughan & Geissler 2009).

They can be distinguished by their morphological appearances. They are also shown to be pseudocereal similar to spinach. The seeds are edible. The plant shows drought tolerant, saline tolerant specialization with regard to physiological features. This species may further be identified by their pistillate flowers, and panicles emerge from leaf axils. The flowers are hypogynous. They are highly nutritious. The proximal chemical composition of uncooked quinoa shows 14% gluten free protein, 64% carbohydrates, 13% water and 6% fat. The seeds that shows rich protein source of about 20% Daily value in addition with vitamin B, folate, dietery fiber, phosphorous, manganese and magnesium. They are high yielding type and experimental crop in NASA's controlled ecological life support system (James, 2009) for space flights (Schlick, 1993). The Quinoa plant is described to be facultative halophytes, highly nutritious and internationally recognized crop. The saline tolerance ability yield behaviour growth characteristics are little explored in this crop. The plant has wide variability in their genotype and phenotype and they are viable to adapt to any adverse hot arid to subtropical environments. Therefore it was attempted to cultivate them in controlled environmental conditions in our present study.

1.5 Cultivation of Quinoa in UAE

The average annual rainfall falls below 200mm, salinity of soil (2-16 dS/m in soil and 6 -18dS/m in ground water), low fertility nature of sandy soil, and alikaline pH of soil are well recognized in UAE. But the adaptability of halophilic drought tolerant Quinoa crop to grow as a saviour to be used purely for the welfare of UAE

native farmers to fulfil their food crisis has been appreciated only in recent years. Quinoa as staple crop in their nutritional and food self sufficiency, their role in boosting farmers income as well as their adaptability to wide harsh environment which facilitate its introduction as a staple crop in poor fertility nature of soils at various level of research in UAE.

There may not be one variable as to how the crop shall be tested in the field and what might be the dependent abiotic parameters. It is all the more difficult to select particular crop for the field. Learning to cultivate quinoa and to investigate their salt tolerance, UVB tolerance, high atmospheric CO_2, drought tolerance stresses are only a introduction to assuring the plant quinoa as opt cultivar containing greater biochemical contents, disease tolerance and enzymatic and non enzymatic antioxidants.

Quinoa as a staple crop in harsh environments and their abiotic stress tolerance, saline tolerances, their agro-physiological features such plant height, number of panicles, branches, panicle length, harvest index, dry bio mass of quinoa, biochemical features are well reported in the previous literatures (Hussain *et al.,* 2020; Rezzouk *et al.,* 2020) which facilitated the screening of different cultivars, investigation of their biochemical, physiological response to future dynamic climatic conditions. Investigating saline tolerance ability of Quinoa plant and their yield and stability features are of paramount important. Therefore much effort is made for assessment of Salinity tolerance, effect of UVB, adaptability of quinoa was conducted in the present study

1.6 Hypothesis

It is expected that the future generations will benefit from the present study on cultivation of Quinoa as they will suffer from many climatic change challenges such as increasing atmospheric CO_2 on one hand and enhanced UVB infiltration, salinity of the soil, environmental stresses, scarcity of ground water etc on the other hand.

1.7 Relevant Literature

Plants display wide variety of response to climatic variations. Some are sensitive to UV radiations of the environment in which they grow. Most of the plants have an ambient UV temperature range for growth. Some prefer extreme conditions and some are to adjust the climatic variations. Most plants however alter the physiological conditions as a result of the global changes in the climatic conditions. Saline halophytes prefer salty nature of the soil or sandy soils. Some plants might survive complete desiccation for prolonged duration, some might not withstand and die quickly. Spores of plants or vegetative structures of plants can however withstand drought for long durations.

The fact that the climate change are the integral part of our ecosystem has ever been reported in news magazine, journals, social media and daily news papers. But the impact of global warming on ecophysiology of all organisms and plant growth has been reported in recent years (Christopher *et al.,* 2015). A biotic stresses are of great impact on plants and of great influence as well on the yield and growth of plants (Suzuki, 2014; Benevenuto, 2017; Ashraf *et al.,* 2018). Considering the impact of climate change study on phenology, reproduction, distribution yield and growth of

plants has gained wide support from various agencies and has made several advances of high research value.

1.7.1 Effect of Elevated Level CO_2 on Plants

There are also many studies concerning the impact of elevated CO_2 on physiology of plants where studies have been made (Miglietta, 1998; Zhang *et al.,* 2012). It was observed that the results showed CO_2 level dependent changes in the secondary metabolite content, nitrogen and carbon contents in their plant tissues (Sun *et al.,* 2010). Some other studies have reported combinations of temperature and CO_2 levels are referred being interacted on development and growth of plants (Kirschbaum, 1994). The report showed the elevated CO_2 maximum light saturated photosynthetic activity (Long, 1991).

Previous literature with the plant *Raphanus sativus* and *Gossypium hirsutum* has demonstrated the presence of good growth in the high humidity ranges upto 90% and elevated levels of eCO_2 up to 350 ppm. Based on the dry matter yield assay, it was confirmed that the humidity and eCO_2 did not exhibit any response from *R. sativus.* So, the further studies were conducted with the seedlings of *Pinus koraiensis.* The work demonstrated the presence of high photosynthetic activity in 500 ppm CO_2 level and high soluble sugar and chlorophyll contents (Wong, 1993).

The photosynthetic rate study was conducted with the combination of heat stress (drought) and eCO_2 level with the *Larrea tridentata* seedlings. The seedlings showed decreased photochemical efficiency with photosystem, stomatal conductance and decreased photosynthetic rate when exposed to 53 C under 770, 550 and 360 ppm CO_2 concentrations in water and waterless regimes. The photosynthetic activity was

found to be effective in the plants exposed with ambient CO_2 doses (Hamerlynck 2000).

The eCO_2 was found to have increased water use efficiency, rate of photosynthesis, chlorophyll content, dry weight of leaves, stem, thickness, and height of the tomato plants. The growth chamber experiments confirmed the positive effect of eCO_2 and suggests the higher photosynthetic rate upto 55%, increased thickness of the stem to 24%, average height of the plant to 22%, greater biomass of the tomato plant with 67%. So, the study could act as supportive results for the positive effect of eCO_2 for physiological activity on tomato plants. The CO_2 concentrations such as 700 and 400 ppm were found to have Rubisco enzyme activity and influence photosynthesis. Lee *et al.* (2007) concluded their findings that the eCO_2 doses were found to be ambient and confirmed that these concentrations had possessed upregulation of photosynthesis in the start up phase while down regulation noticed during long term treatment.

Another study was aimed to carry out the water diffusion coefficient and root cell water permeability of *Zea mays* against varying concentrations of eCO_2 treatment such as 1200 and 800 ppm. In any study water is commonly employed for water use efficiency test but this study conducted by Suslov (2020) proved that the eCO_2 assisted in the regulatory decrease of H_2O conductivity and thus reduced water permeability of root cells. The study used two different concentrations of eCO_2 viz.,1200 and 800. The results were found to show that 800 ppm concentrations had decreased intensity of water transfer to roots while eCO_2 at 1200 ppm had increased intensity of water transfer of *Zea mays*.

According to Brito *et al.* (2020), the eCO_2 activity study shows the strong positive effect of eCO_2 on tomato plant. The results showed high assimilation rate and excellent growth of tomato variety with less levels of C_2H_2 and abcisic acid in roots and leaves. The high salinity of soil was found to be prevalent throughout the study which increased metabolites of photorespiratory mechanisms viz., glycine and serine while intermediates of Kreb's cycle decreased. The study suggested that eCO_2 promoted rate of photosynthesis, decreased absicic acid and precursor of ethylene 1 aminocyclopropane1 carboxulic acid concentration sin leaves and root systems. The study also suggested that this control plant restored their metabolites concentrations.

Another study carried out by Avila *et al.* (2020) and the study was aimed to show the effect of combinations of drought and eCO_2 on accumulation of biomass. The chosen eCO_2 concentrations of eCO_2 were 723 ppm and 386 ppm for a period of 210 days. The results have shown a strong interaction between eCO_2 and carbon assimilation of plants and stomatal conductance was unaltered. The carbon assimilation was found to be as high as 60%. The study also has shown that the eCO_2 had no role play to down regulate photosynthesis. The study also proved that eCO_2 was found to be effective under drought conditions and this suggested that the rate of photorespiration and oxidative pressure were decreased and biomass accumulation was increased but better water use efficiency was observed. The root and length of roots had attained maximum in measurement using eCO_2 treatments.

Based on the Alzate-Marin *et al.* (2021) study, it is found that the combinations of eCO_2 at 600 ppm dose and high temperature to 2C plus above optimum had exhibited high physiological activity. The results have shown that the Stylosanthes capitata had greater number of flowers increased to 62% per plot, flower beginning

rate was1 hour earlier. The control slots showed 9.59 while the test samples treated with eCO_2 showed 9.05 while the high temperature plots had shown 9.55. Based on the phenology of flowers it is concluded that the plant of Stylosanthes capitata exhibited positive response between phenological flower study and varying levels of eCO_2.

Another study conducted by Yang *et al.* (2021) carried out similar study with the intercropped *Echinochloa caudate* with the *Festuca arundinacea*. The efficacy of phytoremediation had shown that the CO_2 levels increased their efficacy by increasing and 550 ppm CO_2 levels from 400 and 280. The effect of eCO_2 has shown that there had no changes in the Cd content of *E. caudata* but substantial increase in their dry weight. The results obtained on comparison with the eCO_2 level and Cd phytoremediation, the study confirmed the presence of decreased efficacy under the eCO_2 level. The photosynthetic stimulation study shows the increased photosynthetic activity of *Panicum miliaceum*. The results showed the eCO_2 was effective which increased the water use efficiency and compensate physiological indices such as drought conditions on biomass and leaf area (Zhang *et al.,* 2021).

1.7.2 Effect of UVB on Plants

Many plants have been tested for their response to UVB exposure. There are several factors are important to prove their harmful effects. The sun light plays a crucial role in photosynthesis of plants is also widely known. The supportive results given by Barnes *et al.* (2016) showed that there was induction of phenolic and flavonoid compounds when plants were exposed to UV radiation. The results suggested that this compound had possessed defence mechanism such as increase in thickness of leaf, epicuticular wax production, antioxidant production, phtotsynthetic

mechanism changes, transpiration mechanism changes and canopy morphology changes (Barnes *et al.,* 2016).

UVB radiation treatment tests were done with the pollen germination stimulation tests under in vitro conditions. The test results showed germination of pollen was affected. Many species had shown insensitivity to UVB radiation. Similarly the growth of the pollen tube was also found to be affected (Feng *et al.,* 2002). Kakani *et al.* (2003), study showed that there were strong positive interaction between the high UVB levels and vegetative and reproductive parameters of *Gossypium hirsutum.*

The accumulation of wax content in the epicuticle and increased adaxial and abaxial leaf tissues were taken as a strong screening test for the effect of high UVB radiation against high rate of vegetative and reproductive growth characters. The UVB irradiation was found to be effective at 2-11 kJ m-2 d -1 and thus confirmed effective changes in number of anther in each flower, length of petals and bracts, number of main stem node, length of bracts and petals, number of node in the mains stem and length of internode and height of the plant and leaf area. Higher dose substantially had strong negative effect in their reproductive and vegetative growth characters. The UVB ambient exposure had positive influence in their thickness of mesophyll, epidermal and palisade tissues, stomatal index, Stomatal and epidermal densities.

The sensitivity tests were carried out by Singh *et al.* (2008) with the Vigna unguiculata exposed to UVB radiation. The plant showed less yield of seeds and reduction in length of flower and stem, The UVB was found to be effective which affected reproductive parameters and the study suggested the plant species were classified under three categories such as UVB tolerant, sensitive and intermediate. Kravets *et al.* (2012) reported the impact of UVB radiation. The UVB was found to

have decreased reproductive tissues by 33% and increase polymorphic in somatic tissue by 80% and increased leaf tissue structure, seedling viability, fertility of pollen, and statolistic starch, stability of embryos.

1.7.3 Combined Effects of eCO2 and UVB on Plants

The impact of climate change factors may be known from the historical times. Accordingly, a combination of eCO_2 and UVB factors and their effects were also studied by other researchers. The UVB radiations are referred to being affected plant growth. The UVB was found to decrease the biomass by 8% in comparison with the control plants. The *Elymus athericus* grass was treated with eCO_2, and UVB. The eCO_2 was found to increase the biomass under green house system. (van de Staaij *et al.,* 1993). The combined influence of eCO_2 and UV B radiation was studied with the faba bean seedlings under open top chambers system. The crop was CO2 treated at a dose of maximum of 700 ppm and minimum of 350 ppm. The increase in biomass, shoot length increase were confirmed in 14 days. The biomass reduction and growth stuntedness were observed from UVB exposed plants. The results showed maximum carbohydrate accumulation in the leaves.

It was observed that Faba bean (cv. Minica) showed time dependent decreased biomass activity of UVB radiation (Tosserams *et al.,* 2001). The study conducted by Zhao *et al.* (2004) reported that the eCO_2 and UVB did not have combined activity. It was also found that eCO2 showed higher net photosynthetic activity in leaf, and large leaf area. The rate of Rubisco activity, electron transport and CO_2 compensation were investigated and found that 360 and 720 ppm concentrations of eCO_2 had similar effect. Koti *et al.* (2005) conducted combinations of high temperature, eCO_2 and UVB and flower features, germination of pollen, petal features were studied. The results

were found to be positive and damaging effect of soyabean plants exposed to 10 kJ $m^{-}2\ d^{-1}$ radiation and 1720 ppm of CO_2. The results showed poor germination of pollen grain, minimum number of pollen, shorter staminal length and less number of flowers. The results were found to be effective and damages the morphology features of soyabean plants including pollen production, pollen germination, tube formation and length. Thus UVB activity was effective in combination with high temperature.

Six hybrids of maize were compared with physiological and morphological response by exposed to eCO_2 at 750 ppm and 400 ppm concentrations. Stunted growth was found in plants when treated with UV B radiations and it suggested that eCO_2 increased height of maize plant, increased photosynthetic pigment, increased leaf area (Wijewardana *et al.,* 2016).

The multiple factors affected the development and growth of crop plants as described by Singh *et al.* (2010) and the work of Breitburg *et al.* (1998) supported that the effect of drought and heat should be investigated separately. The need for drought tolerant, saline tolerant, UVB resistant cultivar has increased with the increase in CO_2 contaminated environments. With the scientists targeting drought resistant, UVB tolerant cultivar, there is huge demand for abiotic stress tolerant cultivar.

According to Reyes *et al.* (2018), the study was conducted based on the aim to study the effect of UVB radiation on quinoa. The photosynthetic activity test results have shown a negative correlation between UVB irradiation and photosynthetic pigments. The antioxidant capacity was found to be increased. The results have shown death of the quinoa crop and decreased stomatal conductance ROS production electron transport system inhibition and damage to the apparatus of photosynthesis occurred when quinoa was UV exposed for > 30 mts to 1.69 W m−2 UVB.

The response of lichen to ultraviolet radiation (300 - 400 nm) might induce ROS and RNS in skin. Skin cancer is a serious problem addressed by entire world characterized by onset of skin erythema, immune suppression, gene mutation and DNA damage. Skin protectants are substances in sun screen cream prevent skin against malfunction. Light screen compounds of certain lichen protect the light sensitive photobiont against high intensity of ultra violet exposture. The extract of the lichen *Usnea rocellina* M showed antioxidant and UVB and UVA absorbing property (Rojas *et al.,* 2015). They serve as UV filters for excessive UV B radiation. The compounds that regulate solar radiation are parietin, usnic acid, and vulpinic acid. The compounds of atranorin, calycin, pinastric acid, rhizocarpic acid filter UV radiation. The agents of lichen that serve promising photoprotection against UV A and UV B are calycin, bisxanthones and scytonemin (Nguyen *et al.,* 2013).

The response of Quinoa plant to UVB was studied by Mariotti *et al.* (2021). The plant was found to have increased abscisic acid (ABA) which is the major indicator for the UVB stress. The result has shown a negative correlation between UV doses and plant morphology and defence systems. The habitat UVB relation study shows that the UVB sensitivity did not correlate with the geographical distribution of quinoa.

Based on the response of palm plant to the eCO_2 and UVB radiation, it is confirmed that the date palm had exhibited increased growth, antioxidant enzymes, aminoacids, protein carotenoid contents, enzymes like glutamyl kinases, gama peroxidases, tocopherol and proline oxidases. The results also indicated that the UVB was found to be effective and thus suggested that it had damaging effect on palm growth Karthishwaran *et al.* (2020).

Chapter 2: Material and methods

2.1 Experimental Site and *Chenopodium quinoa* Cultivars

The present study was carried out in Al-Foah Experimental Farm [24°21′31.139″N 55°47′57.239″ E (Altitude 303 M)], College Agriculture and Vet. Medicine, UAEU, Al Ain. The salinity screening was carried out under shade house and a climate change study was carried out in Open Top Chambers facility. Five *Chenopodium quinoa* varieties i.e., KAUST-05395/CHFN-68 (V1), KAUST-05398/PI-614889 (V2), KAUST-05397/PI-614885 (V3), KAUST-05403/ICBA-Q3 (V4), and KAUST-05399/PI-614888 (V5) were used for the present study. The plants were grown in plastic pots and used for salinity screening and climate change study.

2.2 Salinity Stress

The selected Quinoa varieties were initially screened for salt salinity with two different concentrations *viz.,* 5000 (T1) and 10000 (T2) ppm. Laboratory grade NaCl (sodium chloride) was used to prepare different salinity levels and irrigated in alternate days for 45 days. A completely randomized design (CRD) was used to study the salinity tolerance experiment with three replicates.

2.3 Open Top Chambers Facility

The effect salt tolerant Quinoa varieties response to future climatic scenarios such as high atmospheric CO_2 and enhanced UVB radiation was studied in an Open Top Chambers facility (Figure 1). 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.

Figure 1: Open Top Chambers Facility in Al-Foah experimental farm, UAEU.

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 analyzer 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.

2.4 Morphological Parameters

After the salinity treatment, Quinoa verities were selected by measuring the growth parameters *viz.,* total plant height, fresh and dry weight of the control and salt treated plants. The selected varieties were used for the elevated atmospheric CO_2 and enhanced UVB radiation effect.

2.4.1 Total Plant Height

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

2.4.2 Fresh and Dry Weight of the Plant

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 at 50°C for three days and dry weight were measured and expressed in grams.

2.5 eCO_2 and UV-B Treatments

The effect of eCO_2 and enhanced UVB on selected Quinoa varieties 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}). 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.

2.6 Photosynthetic Pigments

2.6.1 Determination of Chlorophyll and Carotenoids Contents

Photosynthetic pigments such as Chlorophyll a, b and total chlorophyll and carotenoid contents of Quinoa varieties 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.

2.7 Biochemical Contents

2.7.1 Estimation of Proline Content

The estimation of proline content of Quinoa 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 percent 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. Immediately, the test tube was kept in an ice bath to terminate the reaction. 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.

2.7.2 Estimation of Protein

The soluble protein of Quinoa 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 minutes. 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. Reagent blank was prepared with 5 mL of NaOH (0.1 N) and 0.1 mL of distilled water. 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.

2.7.3 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 Quinoa leaves. 500 mg of fresh Quinoa 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.

2.8 Proline Metabolizing Enzymes

2.8.1 Estimation of γ – Glutamyl Kinase Activity

The γ – glutamyl kinase activity of Quinoa 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˚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 1mM 1, 4-dithiothreitol. The Final volume (2 mL) of the enzyme a mixture contain 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.

2.8.2 Estimation of Proline Oxidase Activity

Huang and Cavalieri (1979) method was adopted the determine the Proline oxidase activity of the Quinoa 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, the reduction rate of DCPIP was used and the results of the enzyme activity are presented in μg/min/mg.

2.9 Non – Enzymatic Antioxidants

2.9.1 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 Quinoa 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 was 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 phenol content is expressed as mg/g Fresh weight

2.9.2 Determination of α-Tocopherol Activity

α-Tocopherol activity was analyzed as described by Baker *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.

2.9.3 Reduced Glutathione Activity

A method described by Griffith (1980) was adopted to analyze 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 stabiliz it. Finally, Glutathione Reductase (10 µl) was added and the absorbance was read at 412 nm.

2.10 Antioxidant Enzymes

2.10.1 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.

2.10.2 Peroxidase Activity

Peroxidase activity of the Quinoa 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.

2.10.3 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.

2.10.4 Catalase Activity

The catalase activity of the leaves of Quinoa 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.

2.10.5 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.

2.11 Statistical Analysis

The obtained data related to both salinity tolerance and eCO_2 and UVB treatments were analyzed using SPSS (V. 21.0). The results were taken from three

replicates and data are expressed in Mean ± SE. Statistical significance was indicated at a probability level of $P > 0.05$.

Chapter 3: Results

3.1 Salinity Stress on Growth of *Chenopodium quinoa*

The effects of salinity on plant height, fresh and dry weights of five varieties of Quinoa were compared with untreated plants and the results are presented in Figure 2 to 5.

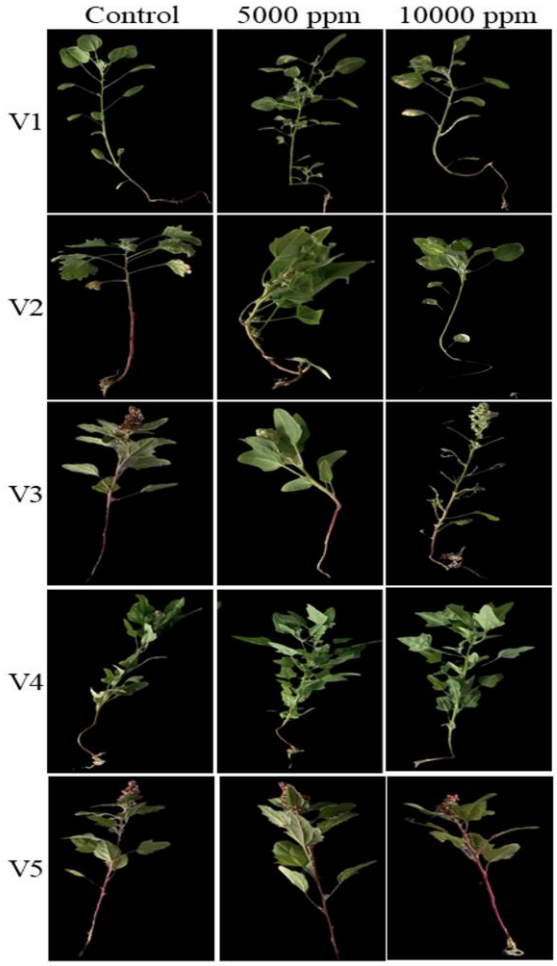

Figure 2: Morphology of different varieties of *Chenopodium quinoa* after salinity treatment

The results on the impact of two different level of salinity on plant height of Quinoa varieties are given in Figure 3. When compared to control, the total plant height of V1, V2 and V3 varieties were significantly reduced in T2. However, V4 and V5

varieties have less effect with the salinity treatment when compared to the control plant.

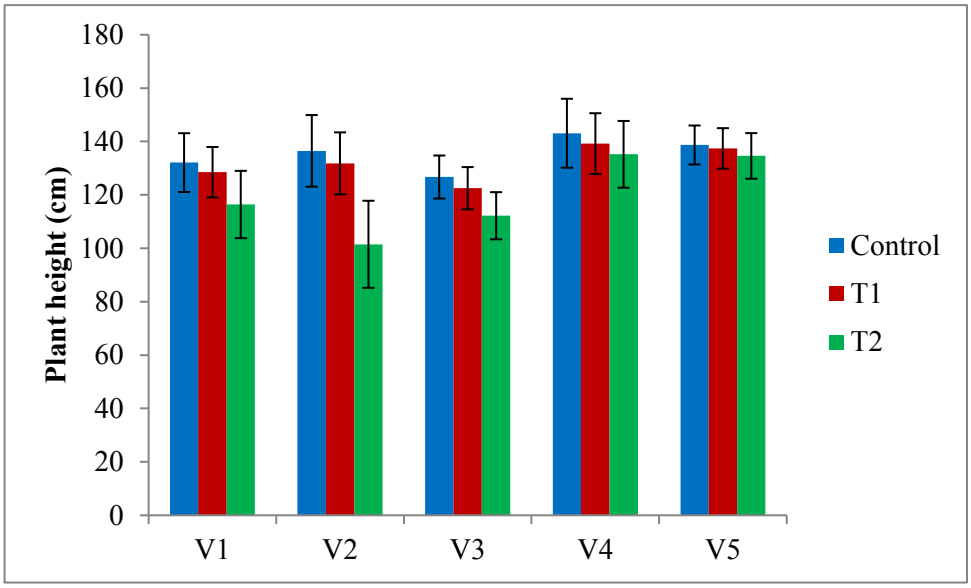

Figure 3: Effect of different levels of salinity on plant height of Quinoa varieties

Figure 4 represents the fresh weight of five varieties of Quinoa plants after treated with two different levels of salinity. After the salinity treatment, the fresh weights were reduced in all the varieties. However, in T1 and T2 treatments, there was no significant variations were observed in V3. But, compared to control plants, the fresh weight was reduced in saline treated plants. In other varieties, the fresh weight were reduced gradually when increase the salinity levels.

The total plant dry weight of salinity treated Quinoa varieties along with control plants are given in Figure 5. The plants dry weight was reduced in T1 and T1 treatments in all the Quinoa varieties studied when compared to untreated plants. Based on the morphological variations, V4 and V5 varieties were identified as a salinity resistant and selected to study the effect of elevated level CO_2 and UVB under Open Top Chambers facility.

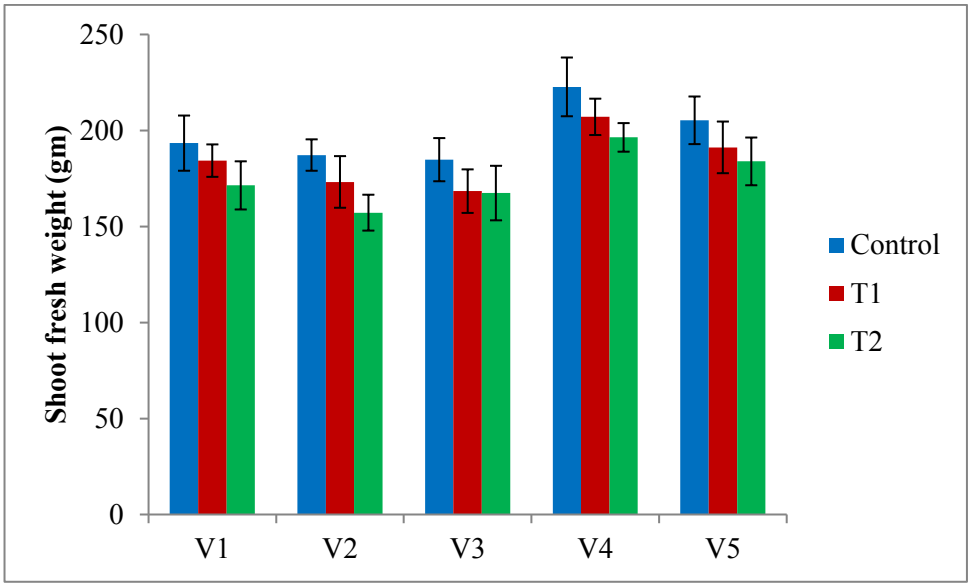

Figure 4: Effect of different levels of salinity on fresh weight of Quinoa varieties

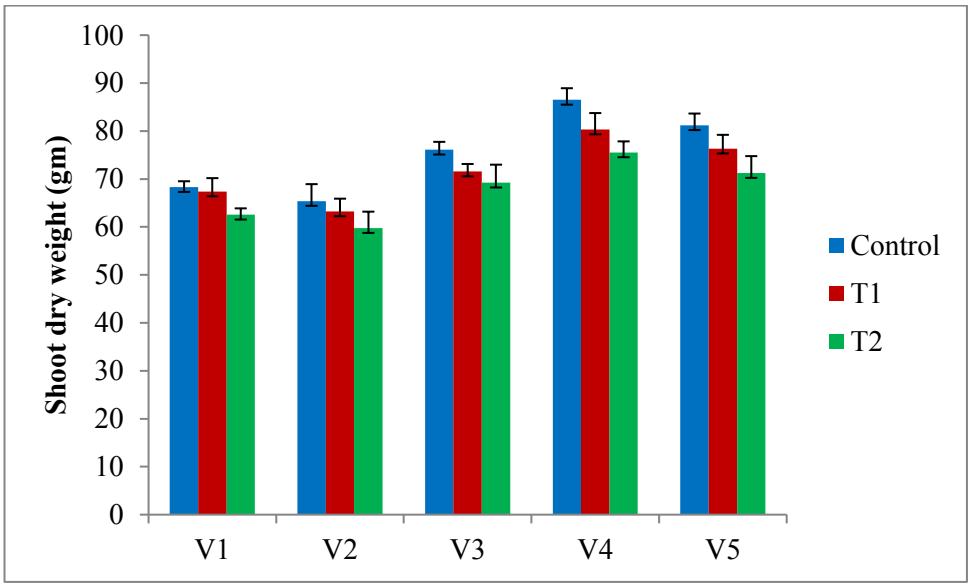

Figure 5: Effect of different levels of salinity on dry weight of Quinoa varieties

3.2 eCO_2, UV-B and Combined Effect on Selected Varieties of Quinoa

3.2.1 Photosynthetic Pigments

In the present study, the effect of eCO_2, UVB and eCO_2+UVB on photo synthetic pigments of V4 and V5 varieties are presented in Figures 6-9. The climate

change scenarios such as eCO_2, UVB and their combined effect showed a significant effect of photosynthetic pigments of selected Quinoa varieties. The effect of eCO_2, UVB and eCO_2+UVB on chlorophyll a content of both V4 and V5 varieties are given in Figure 6. In both the varieties, chlorophyll a content was reduced in UVB treatment. Whereas, eCO_2 showed an increased level of chlorophyll a in both the varieties studied.

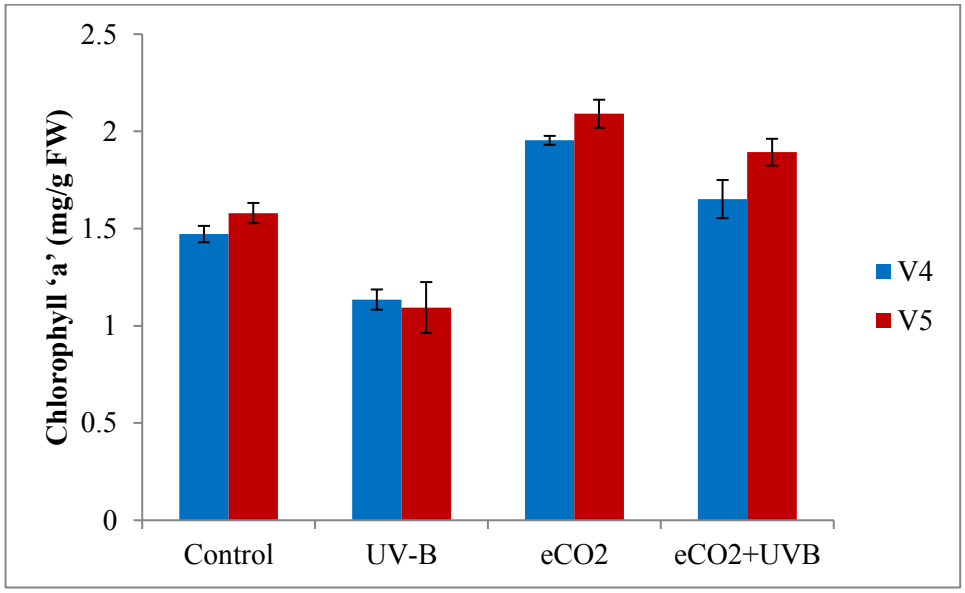

Figure 6: Effect of eCO_2, UVB and combined treatment on chlorophyll 'a' content of selected Quinoa varieties.

The chlorophyll a content values Quinoa varieties were V4=1.472 ± 0.042 (control), 1.135 ± 0.052 (UVB), 1.954 ± 0.023 (eCO_2) and 1.652 ± 0.098 (mg/g FW) and V5=1.58 ± 0.052 (control), 1.094 ± 0.131 (UVB), 2.091 ± 0.072 (eCO_2) and 1.893 ± 0.069 (mg/g FW).

Figure 7 represents the effect of eCO_2, UVB and eCO_2+UVB on chlorophyll b content in two salt tolerant varieties of Quinoa. A reduced level of chlorophyll b content was observed in UVB treatment when compared to control. Chlorophyll b content of C4 variety was 0.728 ± 0.012 (control), 0.525 ± 0.016 (UVB), 0.797 ± 0.018

(eCO_2) and 0.641 ± 0.013 (UVB+eCO_2) mg/g FW. Whereas in V5 variety 0.684 ± 0.032 (control), 0.512 ± 0.041 (UVB), 0.765 ± 0.018 (eCO_2) and 0.598 ± 0.023 (UVB+eCO_2) mg/g FW of chlorophyll b were recorded.

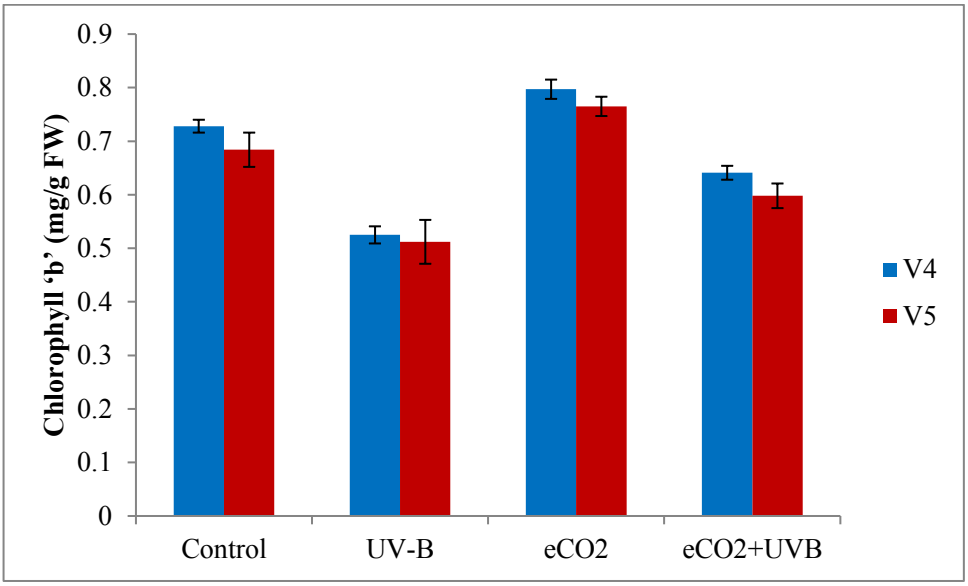

Figure 7: Effect of eCO_2, UVB and combined treatment on chlorophyll 'b' content of selected Quinoa varieties.

The results of eCO_2, UVB, combined treatment of eCO_2 and UVB on total chlorophyll content of V4 and V5 Quinoa varieties are given in Figure 8. The total chlorophyll content was reduced in UVB and eCO_2+UVB treatments when compared to control. Whereas, enriched CO_2 has increased total chlorophyll level in both the varieties. The total chlorophyll content of control, eCO_2, UVB and eCO_2+UVB treated Quinoa varieties were V4=2.309 ± 0.073, 1.660 ± 0.081, 2.751 ± 0.065, 2.293 ± 0.059 mg/g FW and V5=2.264 ± 0.068, 1.606 ± 0.102, 2.847 ± 0.074 and 2.491 ± 0.076 mg/g FW respectively. The results on the carotenoid content of studied Quinoa varieties after the eCO_2, UVB, combined treatments of eCO_2 and UVB are given in Figure 9. As observed in chlorophyll content, the carotenoid content of the Quinoa varieties also decreased in UVB treatment as well as in the combined treatments of eCO_2+UVB.

Whereas, when compared to control, the carotenoid content of the plants was increased during CO_2 enrichment.

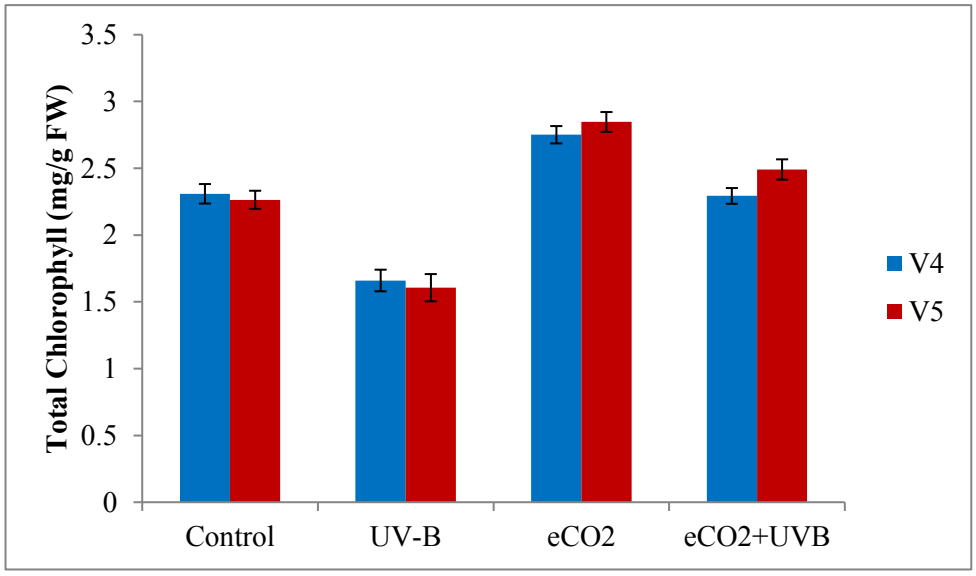

Figure 8: Effect of eCO_2, UVB and combined treatment on total chlorophyll content of selected Quinoa varieties.

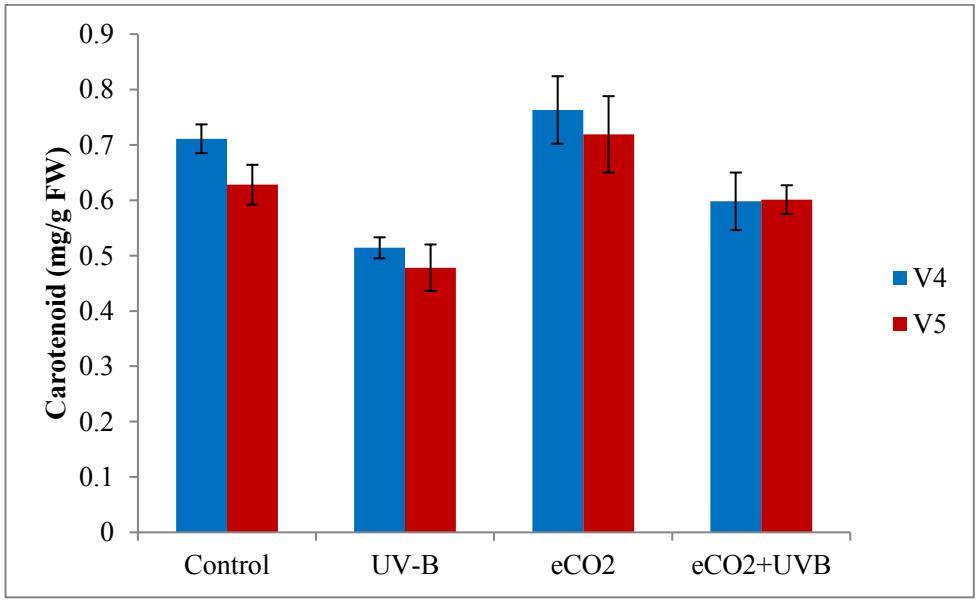

Figure 9: Effect of eCO_2, UVB and combined treatment on carotenoid content of selected Quinoa varieties.

Carotenoid content of V4 and V5 varieties were 0.711 ± 0.026 (control), 0.514 ± 0.019 (UVB), 0.763 ± 0.061 (eCO_2), 0.598 ± 0.052 (UVB+eCO_2) and 0.628 ± 0.036

(control), 0.478 ± 0.042 (UVB), 0.719 ± 0.069 (eCO_2) and 0.601 ± 0.026 (UVB+eCO_2) mg/g FW respectively.

3.2.2 Biochemical Contents

The biochemical such as proline, protein and amino acid contents of the V4 and V5 Quinoa varieties were analysed after treated with eCO_2, UVB, eCO_2+UVB and the results are given in Figures 10-12.

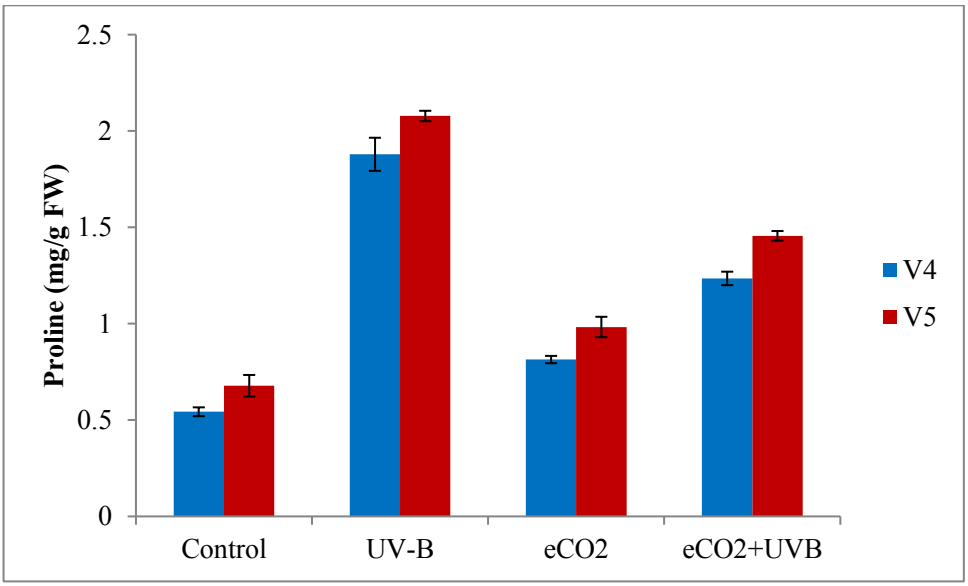

Figure 10: Effect of eCO_2, UVB and combined treatment on proline content of selected Quinoa varieties.

The proline content of the studied Quinoa varieties was dramatically increased in UVB treated plants (Figure 10). When compared to control, an increased level of proline content was recorded in eCO_2 as well as eCO_2+UVB treatments. In all the treatments the proline content of V5 variety was higher than V4 variety. The proline content of control, eCO_2, UVB and eCO_2+UVB treated Quinoa varieties were 0.543 ± 0.023 (control), 1.879 ± 0.086 (UVB), 0.814 ± 0.019 (eCO_2) 1.235 ± 0.035

(UVB+eCO_2) and 0.678 ± 0.056 (control), 2.078 ± 0.027 (UVB), 0.983 ± 0.053 (eCO_2), 1.456 ± 0.025 (UVB+eCO_2) mg/g FW respectively.

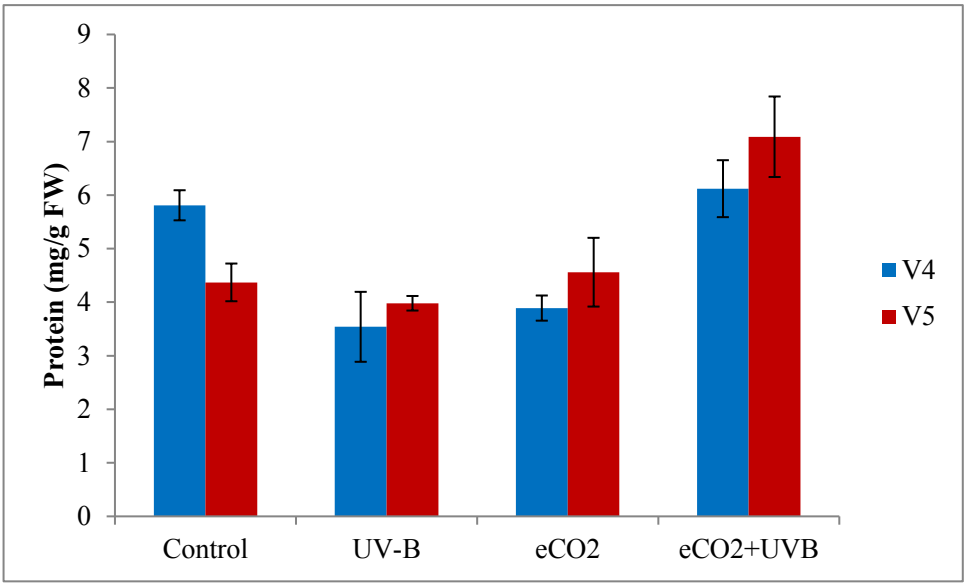

Figure 11: Effect of eCO_2, UVB and combined treatment on protein content of selected Quinoa varieties.

The effects of eCO_2, UVB and eCO_2+UVB on protein content of Quinoa varieties are graphically represented in Figure 11. An increased level of protein content was observed in combined treatments of eCO_2 and UVB. But in UVB treatment the content of the protein was decreased. The protein content of the control, eCO_2, UVB and eCO_2+UVB treated Quinoa varieties were V4=5.81 ± 0.281, 3.54 ± 0.653, 3.89 ± 0.235, 6.12 ± 0.532 mg/g FW and V5= 4.37 ± 0.352, 3.98 ± 0.136 4.56 ± 0.642, 7.09 ± 0.725 mg/g FW respectively. Among the varieties, in control, the protein content was high in V4. Whereas, in eCO_2, UVB and eCO_2+UVB treated plants V5 has high content of the protein.

The amino acid content of two salt tolerant Quinoa varieties treated with eCO_2, UVB and eCO_2+UVB are given in Figure 12. The amino acid content was increased in UVB treated plant. But, decreased level of amino acid content was obtained in both

eCO_2 and eCO_2+UVB treated plants compared to control. The amino acid content of untreated, UVB, eCO_2 and UVB+ eCO_2 combined treated plants of V4 variety was 6.26 ± 0.424, 8.37 ± 0.751, 6.21 ± 0.639, 5.71 ± 0.781 mg/g FW and in V5 variety it was 5.72 ± 0.501, 7.76 ± 0.303, 6.01 ± 0.791 and 4.56 ± 0.890 mg/g FW respectively.

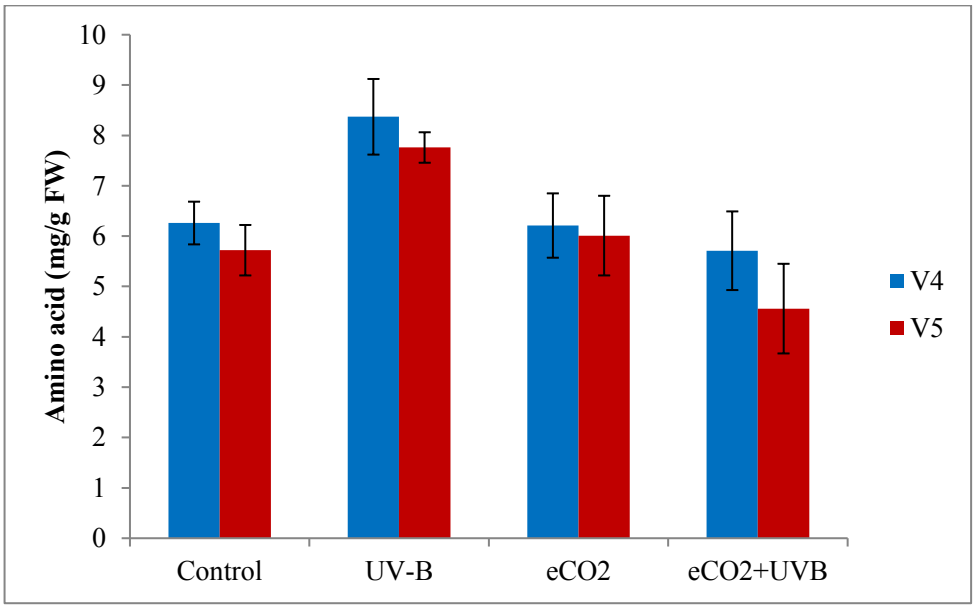

Figure 12: Effect of eCO_2, UVB and combined treatment on amino acid content of selected Quinoa varieties.

3.2.3 Proline Metabolizing Enzymes

Figures 13 and 14 represent the proline metabolizing enzymes activity (γ – glutamyl kinase and proline oxidase) of untreated, UVB, eCO_2 and UVB+ eCO_2 combined treated plants Quinoa varieties. The results on γ – glutamyl kinase activity of V4 and V5 Quinoa varieties are given in Figure 13. A decreased level of enzyme activity was observed in both the varieties of eCO_2 treated plants. Whereas, γ – glutamyl kinase activity was high in the leaves of UVB treated plants when compared to control. The value of the γ – glutamyl kinase activity was 2.673 ± 0.362, 3.982 ± 0.174, 2.098 ± 0.314, 3.679 ± 0.401 µg/min/mg protein in V4 variety and 2.981 ±

0.278, 4.092 ± 0.194, 2.174 ± 0.309 and 3.274 ± 0.819 µg/min/mg protein in V5 variety for untreated, UVB, eCO_2 and UVB+ eCO_2 treatments respectively.

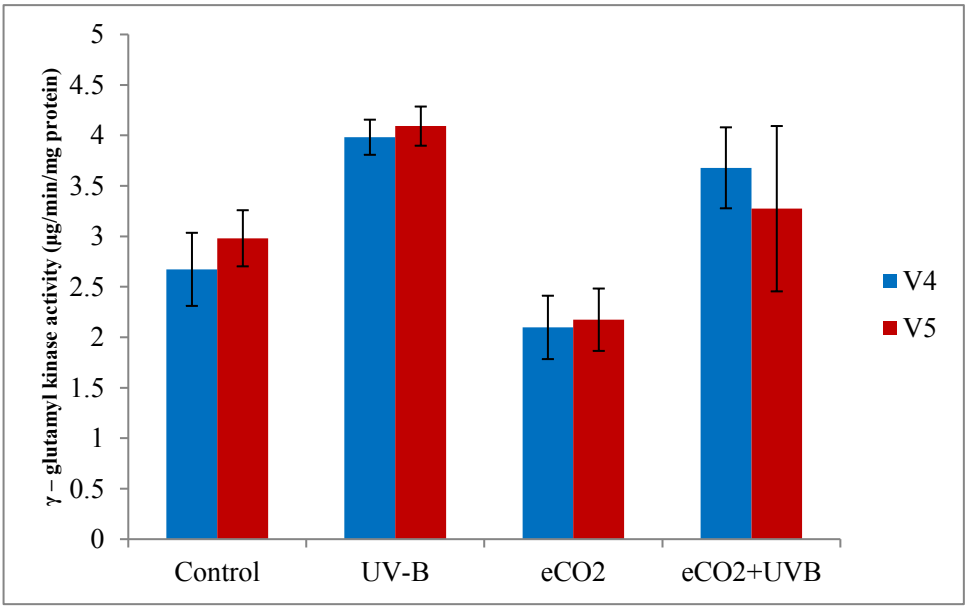

Figure 13: Effect of eCO_2, UVB and combined treatment on γ – glutamyl kinase activity of selected Quinoa varieties.

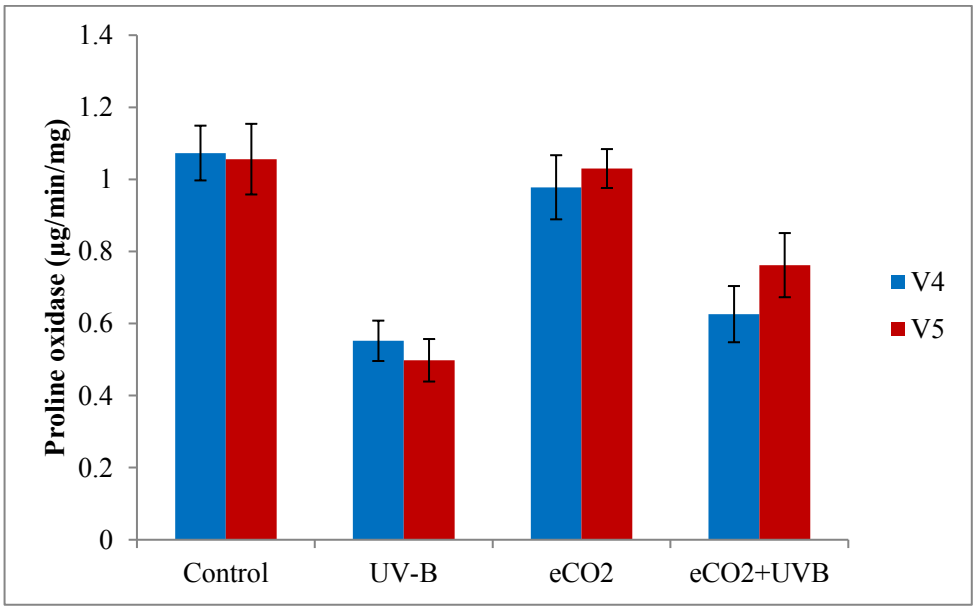

Figure 14: Effect of eCO_2, UVB and combined treatment on Proline oxidase activity of selected Quinoa varieties.

Proline oxidase activity of control, UVB, eCO_2 and UVB+CO_2 treated leaves of V4 and V5 varieties is graphically represented in Figure 14. The activity of proline oxidase was reduced in UVB as well as UVB+CO_2 treated plants. But, the elevated level CO_2 has not reduced the activity of proline oxidase significantly. The proline oxidase activity of untreated, UVB, eCO_2 and UVB+ eCO_2 combined treated plants of V4 variety was 1.073 ± 0.076, 0.552 ± 0.056, 0.978 ± 0.089, 0.626 ± 0.078 µg/min/mg and in V5 variety 1.056 ± 0.098, 0.498 ± 0.059, 1.030 ± 0.054 and 0.762 ± 0.089 µg/min/mg respectively.

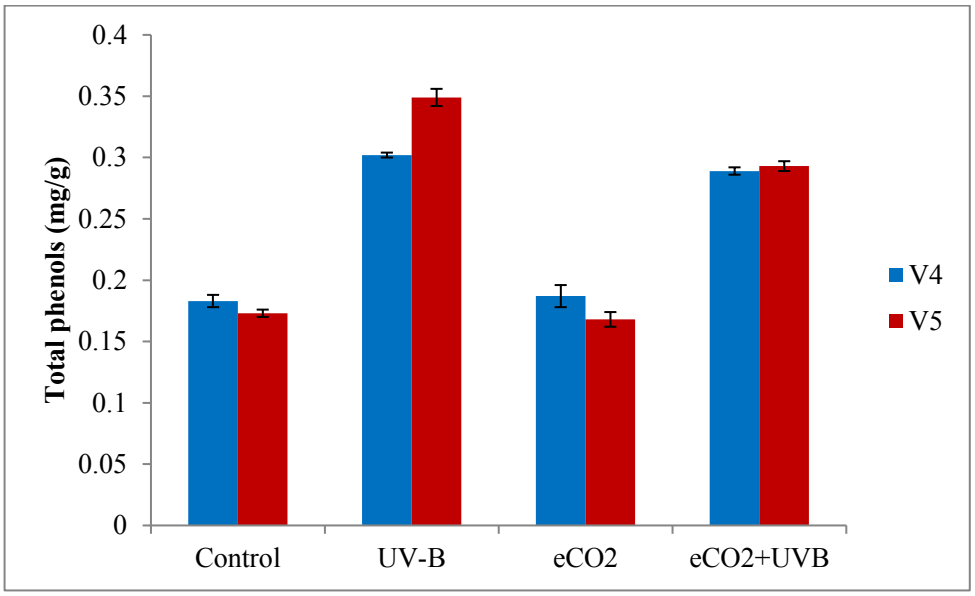

Figure 15: Effect of eCO_2, UVB and combined treatment on total phenol content of selected Quinoa varieties.

3.2.4 Non – Enzymatic Antioxidants

The effect of climate change factors results on non-enzymatic antioxidants such as phenol content, α-tocopherol and reduced glutathione activities of studied Quinoa varieties are presented in Figures 15-17. The total phenol content of V4 and V5 Quinoa varieties treated with eCO_2, UVB and eCO_2+UVB are given in Figure 15.

The phonol content was increased in UVB and eCO_2+UVB treatments. But, the CO_2 enrichment has not affect phenol content of the studied Quinoa varieties when compared to the control plant. The phenol content of untreated, UVB, eCO_2 and UVB+ eCO_2 combined treated plants of V4 variety was 0.183 ± 0.005, 0.302 ± 0.002, 0.187 ± 0.009 and 0.289 ± 0.003 mg/g and in V5 variety it was 0.173 ± 0.003, 0.349 ± 0.007, 0.168 ± 0.006 and 0.293 ± 0.004 mg/g respectively.

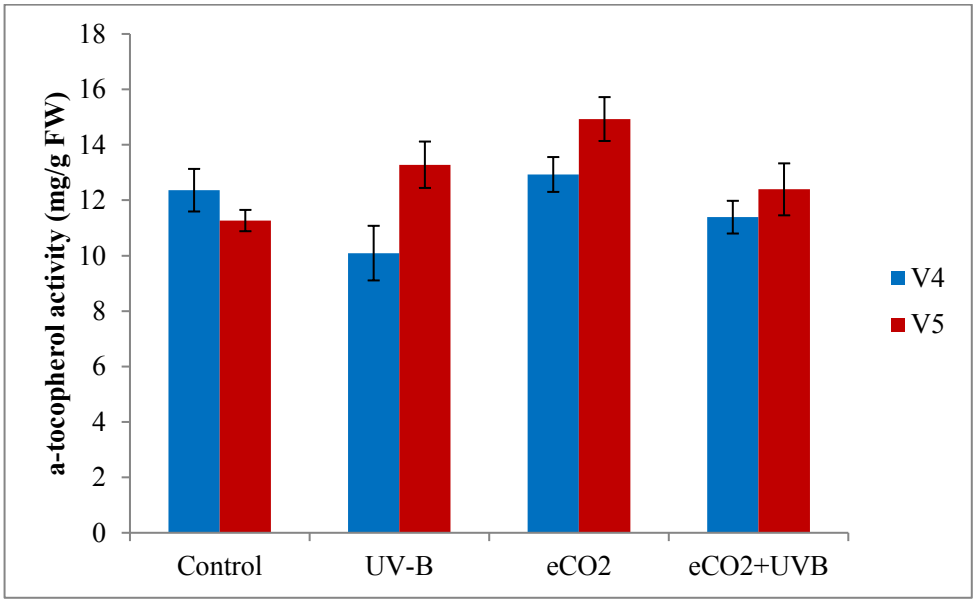

Figure 16: Effect of eCO_2, UVB and combined treatment on α-tocopherol activity of selected Quinoa varieties.

A graphical representation of Figure 16 showed the α-tocopherol activity of untreated, eCO_2, UVB and eCO_2+UVB Quinoa varieties. The α-tocopherol activity was slightly increased in all the treatments. However, in UVB treatment, a degreased level of α-tocopherol activity was recorded in V4 variety. The CO_2 enrichment showed a highest α-tocopherol activity in V5 quinoa variety. The α-tocopherol activity of V4 variety was 12.362 ± 0.768 (control), 10.092 ± 0.985 (UVB), 12.930 ± 0.629 (eCO_2), 11.389 ± 0.590 (UVB+eCO_2) mg/g FW. Whereas, in V5 variety it was 11.267 ± 0.384

(control), 13.281 ± 0.837 (UVB), 14.930 ± 0.792 (eCO_2), 12.393 ± 0.938 (UVB+eCO_2) mg/g FW.

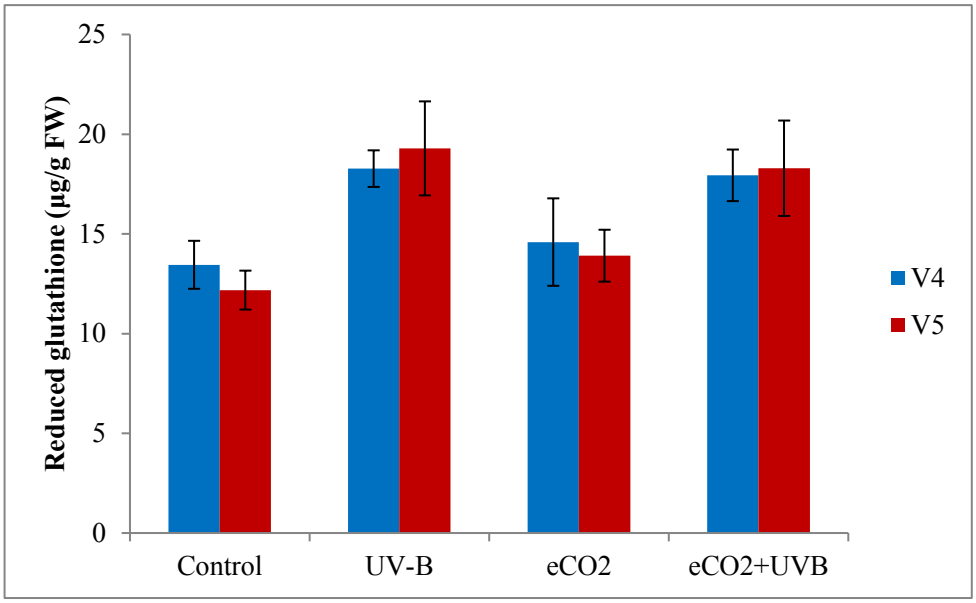

Figure 17: Effect of eCO_2, UVB and combined treatment on reduced glutathione activity of selected Quinoa varieties.

The results on the reduced glutathione activity of studied Quinoa varieties after the eCO_2, UVB, combined treatments of eCO_2 and UVB are given in Figure 17. An increased activity level of reduced glutathione was recorded in both V4 and V5 varieties when the plants were treated with UVB and UVB+eCO_2. When compared to control, CO_2 enrichment did not increase the reduced glutathione activity in both the varieties. The recoded reduced glutathione activity in the present study were V4=13.450 ± 1.203 (control), 18.273 ± 0.918 (UVB), 14.590 ± 2.193 (eCO_2), 17.938 ± 1.293 (UVB+eCO_2) µg/g FW and V5= 12.182 ± 0.976 (control), 19.289 ± 2.357 (UVB), 13.908 ± 1.301 (eCO_2) and18.291 ± 2.392 (UVB+eCO_2) µg/g FW respectively.

3.2.5 Enzymatic Antioxidants

In the present study, the salt tolerant varieties V4 and V5 were treated with eCO_2, UVB, combined treatments of eCO_2 and UVB and enzymatic antioxidants were analysed and the results are presented in Figures 18-22. The obtained results indicated that UVB and eCO_2 treatments increased the enzymatic antioxidants.

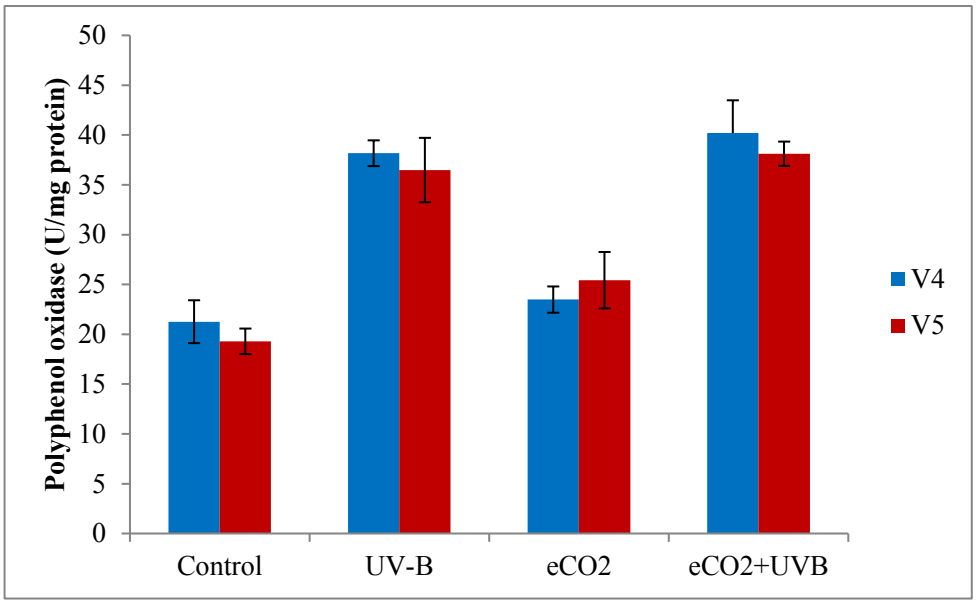

Figure 18: Effect of eCO_2, UVB and combined treatment on polyphenol oxidase activity of selected Quinoa varieties.

Figure 18 represents the polyphenol oxidase activity of two varieties of Quinoa treated with eCO_2, UVB and combined treatment of UVB and eCO_2. The results showed UVB and UVB+eCO_2 combined treatment increased the polyphenol oxidase activity in V4 and V5 varieties. The polyphenol oxidase activity values in the leaves of studied Quinoa varieties were 21.26 ± 2.150, 38.17 ± 1.293, 23.48 ± 1.319, 40.19 ± 3.291 U/mg protein and 19.29 ± 1.283, 36.48 ± 3.231, 25.43 ± 2.827 and 38.12 ± 1.218 U/mg protein in untreated, eCO_2, UVB and eCO_2+UVB treatments respectively.

The results of peroxidase activity of untreated, UVB, eCO_2 and UVB+ eCO_2 combined treated V4 and V5 Quinoa varieties are given in Figure 19. The peroxidase activity was dramatically increased in the UVB treated plants. But, the CO_2 enrichment has decreased the combined treatment of UVB and eCO_2 also increased the activity of peroxidase. The values of peroxidase activity of untreated, eCO_2, UVB and eCO_2+UVB treatments were 1.829 ± 0.273, 3.892 ± 0.298, 0.991 ± 0.098, 2.918 ± 0.182 mg^{-1} protein in V4 variety and 1.773 ± 0.382, 4.280 ± 0.282, 1.454 ± 0.372 and 3.129 ± 0.471 mg^{-1} protein in V5 variety respectively.

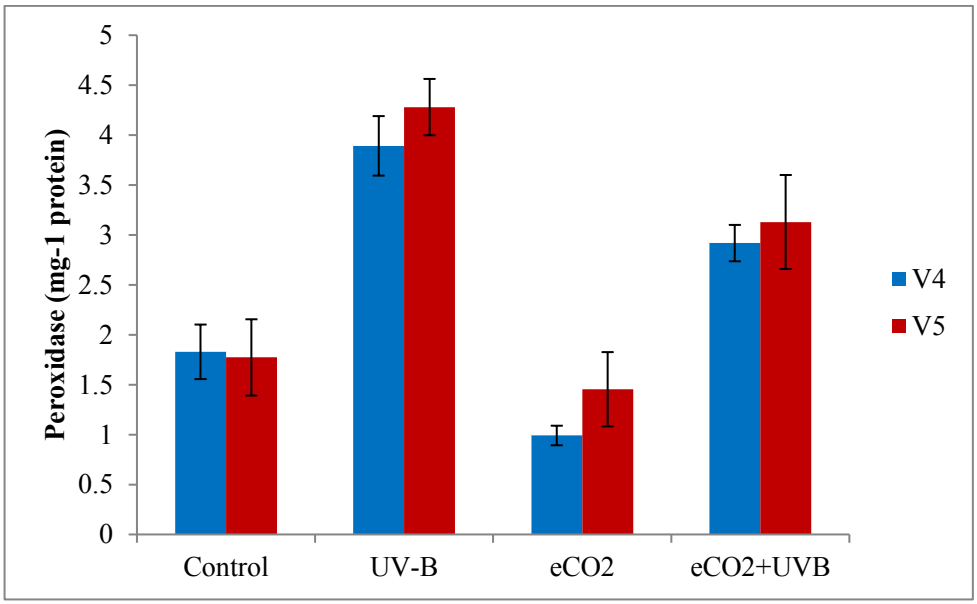

Figure 19: Effect of eCO_2, UVB and combined treatment on peroxidase activity of selected Quinoa varieties.

Figure 20 shows the superoxide dismutase activity of selected Quinoa varieties treated with UVB, eCO_2 and UVB+eCO_2. The enzyme activity was high in UVB and UVB+eCO_2 combined treatments. Moreover, the values of the enzyme activity were similar to the control plants. The superoxide dismutase activity in the leaves of C4 variety was 23.45 ± 2.345 (control), 34.23 ± 1.289 (UVB), 22.98 ± 1.637 (eCO_2), 32.50 ± 1.698 (UVB+eCO_2) U/g FW. While, the enzyme activity in V5 variety were 22.36

± 2.418 (control), 35.16 ± 3.182 (UVB), 20.94 ± 1.790 (eCO_2), 30.54 ± 1.928 (UVB+eCO_2) U/g FW.

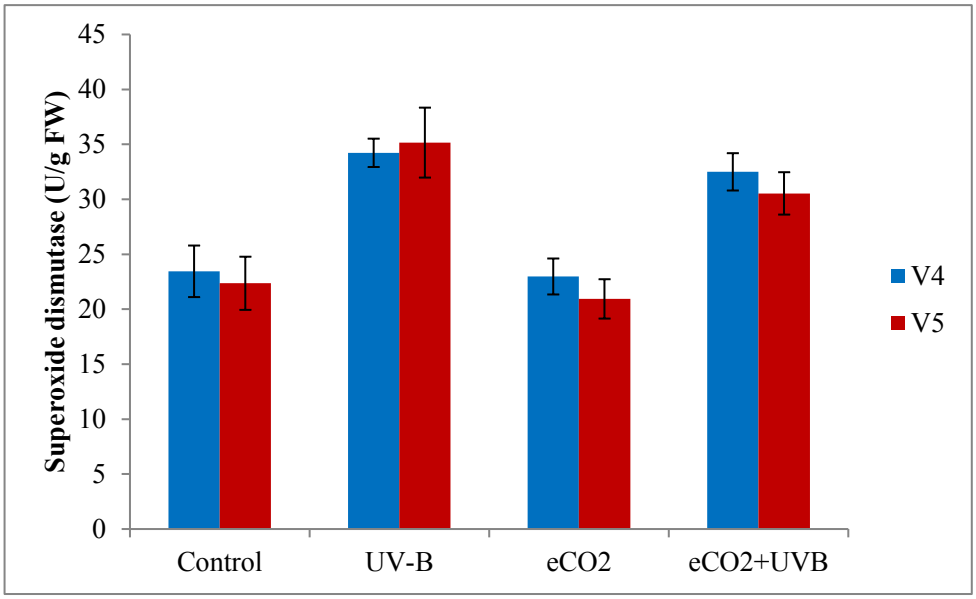

Figure 20: Effect of eCO_2, UVB and combined treatment on superoxide dismutase activity of selected Quinoa varieties.

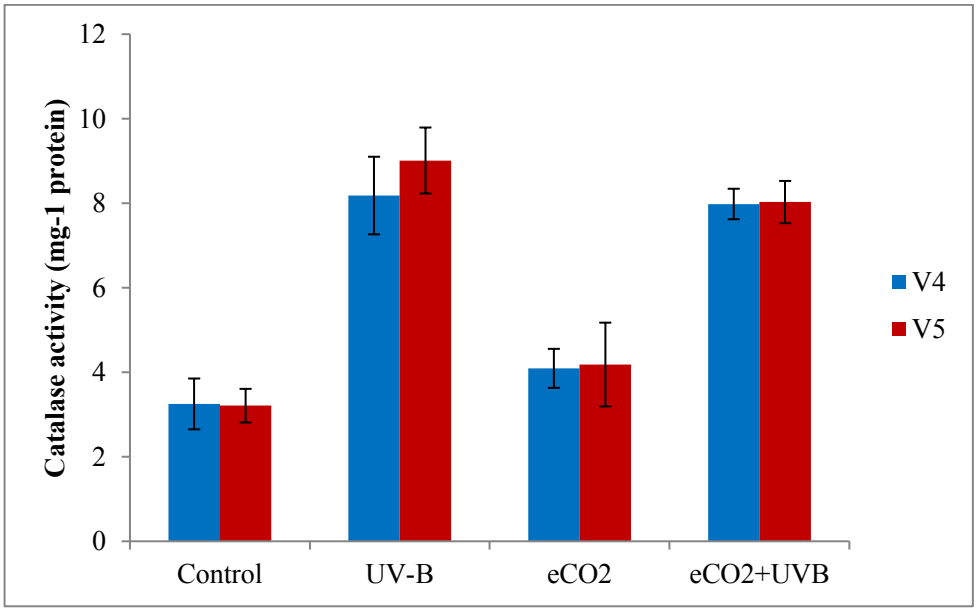

Figure 21: Effect of eCO_2, UVB and combined treatment on catalase activity of selected Quinoa varieties.

The catalase activity was increased when the Quinoa varieties exposed to UVB and UVB+CO_2 combined treatments (Figure 21). A highest catalase enzyme activity was recorded in V5 variety with UVB treatment. But, in UVB and CO_2 combined treatment there was no variation were recorded in the enzyme activity even though it was increased. The catalase activity of the control, eCO_2, UVB and eCO_2+UVB treated Quinoa varieties were V4=3.251 ± 0.601, 8.182 ± 0.918, 4.092 ± 0.462 7.981 ± 0.361 mg^{-1} protein and V5= 3.209 ± 0.398, 9.010 ± 0.781, 4.182 ± 0.992 and 8.027 ± 0.500 mg^{-1} protein respectively.

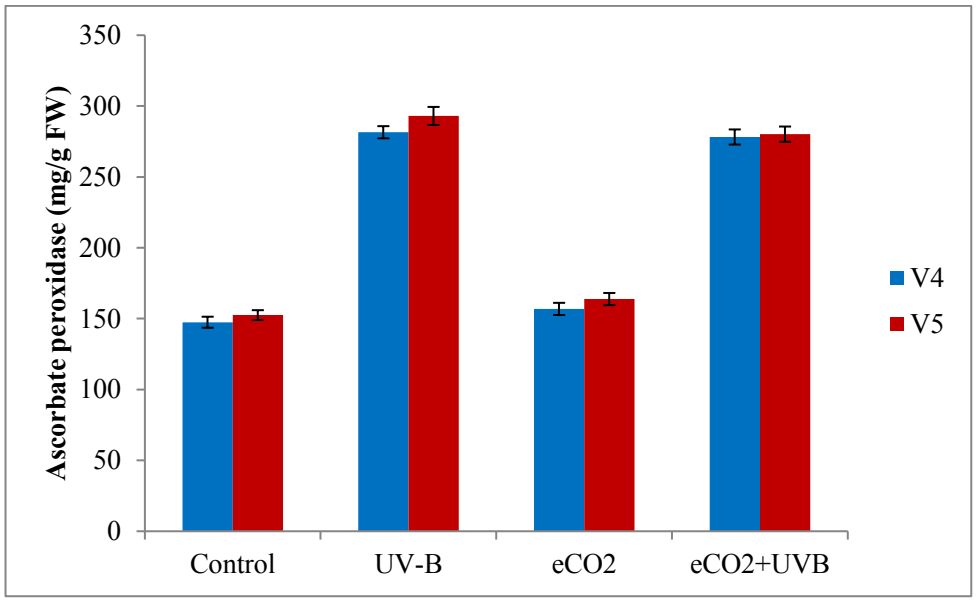

Figure 22: Effect of eCO_2, UVB and combined treatment on ascorbate peroxidase activity of selected Quinoa varieties.

The graphical representation of Figure 22 shows the ascorbate peroxidase activity of V4 and V5 varieties of Quinoa treated with UVB, eCO_2 and it combination. As observed in other antioxidant enzymes, ascorbate peroxidase activity also increased in the leaves of UVB and eCO_2+UVB treated plants. The values of ascorbate peroxidase activity in V4 variety were 147.49 ± 3.892 (control), 281.54 ± 4.281 (UVB), 156.87 ± 4.293 (eCO_2) and 278.19 ± 5.328 (UVB+eCO_2) µg/g FW. While the enzyme activity in

V5 variety were 152.50 ± 3.481 (control), 293.03 ± 6.371 (UVB),163.84 ± 4.291 (eCO_2) and 280.18 ± 5.381(UVB+eCO_2) μg/g FW.

Chapter 4: Discussion

High nutritive value of Quinoa has increase the interest throughout the world. Since, it can be consumed directly or after processing. This crop can be considered as alternative for rice due rich protein content. Studies showed that Quinoa has protein content which is two times higher than wheat (Ceccato *et al.,* 2011). Quinoa is adaptive plant and resistance to various stresses (Jacobsen *et al.,* 2009). However, the present climate change factors such as elevated level CO_2, UVB radiation and salinity has potential impact on the growth of Quinoa plants. In the present study, five Quinoa varieties were initially screened for salinity stress and two salt resistance varieties were studied for the effect of climate change factors such as eCO_2 and UVB radiation.

In the present study, the selected Quinoa varieties (V1, V2, V3, V4 and V5) were screened for salinity stress based on the morphological parameters such as total plant height, fresh and dry weight of the plants. The salinity stress has significant effects on the morphological characters of V1, V2 and V3 varieties. Whereas, V4 and V5 varieties had no morphological variations after salinity treatment. It is well known that the Quinoa plants have potential to grow in saline soils (Roman *et al.,* 2020). Also, the results of the present study are similar to the previous studies on Quinoa. Buedo and Gonzáleza (2020) reported the effect of salinity on germination of Quinoa. The seeds were treated with different concentrations (100 to 400 mM) of NaCl and KCl. The authors found that the increasing salinity influences the germination of the Quinoa. Moreover, the ionic factor of both NaCl and KCl salts influenced much on the germination of the seeds. Toderich *et al.* (2020) studied the response of high yielding Quinoa genotype to salt stress. The plant was exposed to sodium chlorid, sodium sulfate and their combination then the growth performance, quality and yield of seed

were studied. The results of the study showed that the mixed salinity (Na_2SO_4 + NaCl) reduces the plant height, shoot and root weights. Also, the high salinity reduced the weight of the panicle as well as the yield of the seed when compared to control. Abdallah *et al.* (2020a) reported the enhancing salinity tolerance of quinoa using proline, trehalose and compost. It was found that the salinity decreased the height of the plants, shoot fresh and dry weight when compared to control. The reduction of plant height and during salinity may be due to the effect of salinity inhibitory through metabolic activities or toxicity of of Na and Cl and ion interference caused deficiency of nutrient.

The effect of salinity on plant growth parameters were also studied on other crops. Hussein *et al.* (2019) found that the 4000 ppm of salinity decreased the height of the plant, leaf area, leaf number, shoot, leaves and spike dry weight of barley. Abdallah *et al.* (2020b) studied the growth parameters of wheat cultivars. The results showed that the growth of the plant was affected by the salinity due to the effect of salt stress on functions of cells with different functions of metabolism. Qados (2011) reported that the effect of salinity on growth, chlorophyll, protein content and osmatic potential of seedlings of *Vicia faba*. The highest concentration of salinity decreased the plant height. However, there was no significant effect on leaf area as well as the number of leaves.

Based on the results of the present study V4 and V5 varieties were selected for the effect of climate change factors such as eCO_2, UVB and their combined effect on photosynthetic pigments, biochemical contents, proline metabolizing enzymes, non-enzymatic and enzymatic antioxidants. After treatment, the photosynthetic pigments

such as chlorophyll a, b, total and carotenoid contents were decreased in the UVB treatment and increased in the CO_2 enrichment when compared to control.

Previous studies were made in respect to the effect on abiotic stress on the fluorescence of photosynthetic pigments (Zribi *et al.,* 2009; Ogweno *et al.,* 2009). Mainly, the photosynthetic fluorescence emitted by photosystem II and it can serve as intrinsic probe of transformation of energy (Strasser *et al.,* 2004). Moreover, it was reported that the reduction of photosynthetic pigments is mainly due to the degradation of proteins of PSII, reduced activity of Rubisco, destruction of carotenoids and chlorophyll and effects on the functions of stomata (Kataria *et al.,* 2014). Prado *et al.* (2016) reported the effect of UVB on photosynthetic pigments such as chlorophyll a, b, total and carotenoid contents, soluble sugars as well as UV productive compounds of five quinoa varieties collected from different geographic regions. Reyes *et al.* (2018) studied the effect of UVB on photosynthetic activity of quinoa. The study finds that different levels of UVB affects the photosynthetic pigments, chlorophyll fluorescence, accumulation of ROS and photosynthetic rate.

The increased level of photosynthetic pigments under elevated level CO_2 was reported in many studies. Singh and Agrawal (2015) reported that the eCO_2 was significantly increased the photosynthetic pigments as well as the efficiency of photosynthesis in the leaves of *Catharanthus roseus*. The photosynthetic pigment concentration changes in the leaves are mainly associated with the plant productivity (Blaceburn, 1998). It was confirmed by the previous studies. During leaf development, photosynthetic pigments biosynthesis of *Glycine max* leaves under eCO_2 in open top chambers facility were reported previously (Zhao *et al.,* 2003; Jiang *et al.,* 2006). The results revealed that the photosynthetic pigments were increased under elevated level

CO_2. On the contrary, a FACE study may by Hao *et al.* (2012) on soybean indicated, the photosynthetic pigments such as chlorophyll a, b, total chlorophyll and carotenoid contents were not affected under elevated level CO_2.

The results on the biochemical content of the present study showed that the proline and amino acid contents were increased in the UVB treated plants and decreased in eCO_2 and combined treatments. But, the protein was decreased in the UVB treated plants. The decreased protein content may due to the delayed growth rate of UVB treated plants. Amino acids and proline are increased in plants to resist the stress (Yue *et al.,* 1998; Martínez-Lüscher *et al.,* 2014). Through the osmatic cellular regulation, the proline can neutralize the stress damage by removal of protective membrane integrity and ROS (Ashraf *et al.,* 2007). The increased level of amino acid and proline contents under UVB stress were reported previously. The increased level of amino acid UVB treated plants of *Porphyra haitanensis* was reported by Fu *et al.* (2021). A short term UVB radiation affect the glutathione metabolism, carbohydrate metabolism, amino acid metabolism and biosynthesis of phenylpropane. Also, the study showed that the increased level of tyrosine, threonine and phenylalanine suggesting the UVB radiation increase the amino acid metabolism. The decreased level of amino acid content in the eCO_2 conditions may be due to the N assimilation inhibition (Serret *et al.,* 2018).

The results on proline metabolizing enzyme γ – glutamyl kinase and were decreased in eCO_2 treatment and increased with UVB treatment. However, proline oxidase was decreased in UVB and combined treatments and increased in the eCO_2 treatment. This result is in accordance with the previous report on date palm (Karthiswaran *et al.,* 2020). The date palm plants were grown in open top chambers

and treated with UVB and elevated level CO_2. The UVB treated date palm cultivar showed increased level of γ-glutamyl kinase. Whereas, proline oxidase activity was decreased in the UVB treatment. Moreover, in plants the metabolism of proline gives production against stress by maintain NADPH/NADP balance (Miller *et al.,* 2009).

The results on enzymatic and non-enzymatic antioxidants were increased in the UVB treatment and decreased in the control as well as in the elevated level CO_2 conditions. The increasing level of antioxidants might be associated with the production of plants cells from UVB radiation. Mainly, the total phenol content was dramatically increased when the plants treated with the UVB radiation. Usually, the UV absorbing compounds are acting as a shield that product the plant cells from UVB radiation (Köhler *et al.,* 2017). In plants, the non-enzymatic and enzymatic antioxidants provide a sufficient production against the free radicals under UVB radiation. Previously, there are many studies were made in relation to effect of UVB on antioxidant enzymes (Kumari *et al.,* 2010; Koubouris *et al.,* 2015; Zhu *et al.,* 2021). Rao *et al.* (1996) found that ascorbate peroxidase activity was increased *Arabidopsis thaliana* under UVB. Gao and Zhang (2008) reported the response of antioxidant defense system of *Arabidopsis thaliana* induced by UVB. The authors found that short term UVB radiation showed oxidative damage in the plant. Also, the reduced ratio of total glutathione and increased level of total ascorbate found in the plants. Moreover, ROS shaving enzymes like catalase, superoxide dismutase and ascorbate peroxidase hasless activity when compared to control. Agrawal *et al.* (2009) found that an increased superoxide dismutase activity wheat, rice and *Arabidopsis*. Also, a field based study increased superoxide dismutase activity when wheat and mungbean were exposed to UVB. Roychoudhury and Basu (2012) reported that ascorbic acid and reduced glutathione increased in plants when it exposed to UVB. Sharma *et al.* (2019)

studied the response of UV induced antioxidant defense of fenugreek. The results showed that the activity of antioxidant enzymes such as ascorbic acid, malondialdehyde, ASA peroxidase, malondialdehyde and guaiacol peroxidase were decreased during UV treatment. Rácz *et al.* (2020) reported the effect of UVB and CO_2 Tobacco plants. The authors found that the non-antioxidant enzyme activities were increased when the plants treated with supplementary UV-B radiation.

Chapter 5: Conclusion

Recently, quinoa is gaining attention due to its good source of quality protein with balanced profile of amino acids. The protein content of quinoa is 12 to 17% which is higher than rice, wheat and barley. Quinoa is adaptive to harsh environments and highly tolerant to the salinity stress. The previous studies were mainly focused on the effect of salinity on quinoa varieties. But, the effect of climate change scenarios on quinoa plant is limited. So, the present study was aimed to screen five varieties (V1, V2, V3, V4 and V5) of quinoa for salinity stress. Based on the morphological characters (total plant height, fresh and dry weight of the plants), V4 and V5 varieties were selected for the effect of climate change scenarios such as elevated CO_2 and UVB radiation. After treatment, the plants were analyzed for photosynthetic pigments, biochemical contents, proline metabolizing enzymes, enzymatic and non-enzymatic antioxidants. An increased level of protein content was observed in combined treatments of eCO_2 and UVB. Also, amino acid content was increased in UVB treated quinoa varieties. The effect of climate change factors results on non-enzymatic antioxidants such as phenol content, α-tocopherol and reduced glutathione activities and enzymatic antioxidant *viz.* polyphenol oxidase, peroxidase activity, superoxide dismutase, catalase and ascorbate peroxidase were analysed. The studied quinoa varieties showed good response to the climate change factors. The antioxidant enzymes showed good defense against the UVB radiation. Quinoa is getting good attention as an alternative for rice and wheat. Based on the present results, further study is warranted for screening the more varieties with additions climate change factors such as temperature and humidity to find out more tolerant variety of quinoa suitable for future climatic conditions.

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