Journal of Phytology 2023, 15: 172-178
doi: 10.25081/jp.2023.v15.8738
https://updatepublishing.com/journal/index.php/jp

Research Article

ISSN: 2075-6240

The influence of elevated CO_2 concentrations and UVB radiation in antioxidant activity of selected *Chenopodium quinoa* varieties

Saif Ali Matar Al Blooshi, Nasser Abdullah Ghdayer Al Kaabi, Karthishwaran Kandhan, Abdul Jaleel, Mohammed Abdul Mohsen Alyafei*

Department of Integrative Agriculture, College of Agriculture and Veterinary Medicine, United Arab Emirates University, PO Box No. 15551, Al Ain, UAE

ABSTRACT

Ecosystems have been affected by climate change. 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. Climate change and its severe impact on plant productivity showed great intensity due to the effects of abiotic stress. In the present investigation, we selected two quinoa varieties to study the response to future climatic factors such as eCO_2, enhanced UVB radiation, and UVB+eCO_2 combined effects in open-top chambers in the hot climate of the UAE. The treatments were administered for 90 days in the hot UAE weather conditions and the experiment was carried out in a transparent OTC facility. The response of the studied quinoa varieties was measured by analyzing their non-enzymatic antioxidant and antioxidant enzyme activities. Our findings showed that quinoa varieties are suitable as industrial crops for their levels of antioxidants under stimulating climatic conditions because the quantity and quality of their yield have not been affected. Based on the results obtained in the present investigation, further study is warranted for screening more varieties with the addition of climate change factors such as temperature and humidity to find more tolerant varieties of quinoa suitable for future climatic conditions.

Received: June 05, 2023
Revised: December 10, 2023
Accepted: December 15, 2023
Published: December 20, 2023

***Corresponding Author:**
Mohammed Abdul Mohsen Alyafei
E-mail: mohammed.s@uaeu.ac.ae

KEYWORDS: *Chenopodium quinoa*, Climate change, UVB radiation, Elevated level CO_2, Antioxidant

INTRODUCTION

Plants show a wide variety of responses to different environmental factors (Ncube *et al.*, 2012). They occur nearly everywhere and must therefore live under a wide range of dynamic environmental conditions. Climate change affects 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. During environmental changes, the plant body tries to defend itself with several defense mechanisms. One of the most effective mechanisms is the adaptability of the physiological tissues to resist continuous variation it by expressing tolerance (Hasanuzzaman *et al.*, 2013). Toleration or resistance is the reaction of plant tissues to changes in the atmosphere. 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 responses. Some phenolic compounds produced by the plants induce the tolerance ability (Lin *et al.*, 2016).

It should be remembered that the atmospheric CO_2 concentration difference might show some impact on atmospheric temperature variation. A combination of two more factors such as fossil fuel combustion and deforestation are involved in increasing the atmospheric concentration of CO_2. Previous reports indicated that the CO_2 in the atmosphere was 280 ppm. But, a recent study demonstrated the presence of 415.13 ppm during April 2021. According to Ainsworth and Long (2005) and Warren *et al.* (2014), the down regulation of photosynthesis might be due to long-term exposure to 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 and antioxidants (Huang & Xu, 2015) and

the atmospheric temperature variable provides considerable information as the dependent variable involved. The highest vapour which is generally followed by changes in temperature from warm to warmer air temperature conditions (Novick *et al.*, 2016). 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).

The depletion of ozone has a direct impact of UVB on earth. Previous studies have reported that UVB has an intensity of <315 nm is found to have a disastrous influence on the majority of crops. The observed level of ozone depletion assessed during 2002-2005 showed about 3% and 6% in the northern and southern hemispheres respectively (WMO, 2008). The UVB radiation of the sun gets into earth directly by many causes. The depletion of ozone in the stratosphere is one cause. The ability of plants to do photosynthesis depends upon the exposure of plants to sunlight. When UV radiations are exposed to plants, 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.

Quinoa is widely present in European countries, United States, Kenya and India. They are also present in Bolivia and Peru. The plant has wide variability in their genotype and phenotype and they are viable to adapt to any adverse hot arid to subtropical environments. Due to its high nutritive values it has increased interest throughout the world, since it can be consumed directly or after processing. This crop can be considered an alternative to rice due rich protein content. Studies showed that Quinoa has a protein content which is two times higher than wheat (Ceccato *et al.*, 2011). Quinoa is an adaptive plant and resistant to various stresses (Jacobsen *et al.*, 2009). However, the present climate change factors such as elevated level of CO_2 and UVB radiation has a potential impact on the growth of Quinoa plants. In the present study, selected varieties were studied for the effect of climate change factors such as eCO_2 and UVB radiation.

MATERIAL AND METHODS

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. Selected *Chenopodium quinoa* varieties i.e., KAUST-05403/ICBA-Q3 (V1), and KAUST-05399/PI-614888 (V2) were used for the present study.

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. The chambers are fabricated with a Galvanized steel squire tube with a size of 3×3×3 m dimension. The OTCs are covered with 80 to 85% transparent polycarbonate sheets with open top to maintain the near-natural conditions of temperature and relative humidity. Also, the 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 radiation between 280 and 320 nm.

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 h/day.

Proline Metabolizing Enzymes

Estimation of γ – glutamψl 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). The 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 min at 10000 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 min at 20000 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 min 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.

Estimation of proline oxidase activity

Huang and Cavalieri (1979) method was adopted to 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 min at 10000 rpm and supernatant was collected and it was centrifuged for 25 min 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.

Non–enzymatic Antioxidants

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 were homogenized with 80% ethanol (10X) and it was centrifuged for 20 min 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 the 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 the standard curve was prepared. The results of the phenol content is expressed as mg/g Fresh weight

Determination of α-tocopherol activity

α-Tocopherol activity was analyzed 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 min 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 min. After getting a 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 a known quantity of α-tocopherol.

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 min 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 μL of neutralized extract and 100 μL of distilled water. The mixture was kept for 4 min at 25 °C to stabilize it. Finally, Glutathione Reductase (10 μL) was added and the absorbance was read at 412 nm.

Antioxidant Enzymes

Polyphenol oxidase activity

The activity of polyphenol oxidase was determined as per the method described by Kumar and Khan (1982). Briefly, the 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 min 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 turned into an orange-red colour. The obtained results are expressed in U mg^{-1} protein.

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 min) 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.

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 min at 12500 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, the 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.

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 contains PMSF (1 mM). The obtained extract was centrifuged at 12500 rpm for 20 min 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.

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 50 mM potassium phosphate buffer (10 mL) and liquid nitrogen. The homogenate was filtered and centrifuged for 20 min 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.

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 $P > 0.05$.

RESULTS AND DISCUSSION

The effect of UVB, eCO_2 and UVB+ eCO_2 on the proline metabolizing enzymes (γ– glutamyl kinase, proline oxidase and total phenol content) activity on the selected Quinoa varieties are presented in Figures 1 and 2. The results on proline metabolizing enzyme, γ–glutamyl kinase were decreased in eCO_2 treatment and increased with UVB treatment (Figure 1). The activity of proline oxidase was reduced in UVB as well as UVB+CO_2 treated plants. But, the elevated level of CO_2 has not reduced the activity of proline oxidase significantly (Figure 2). This result is in accordance with the previous report on date palms (Karthishwaran *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 an 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 maintaining NADPH/NADP balance (Miller *et al.*, 2009).

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 3 to 5. The results on enzymatic and non-enzymatic antioxidants were increased in the UVB treatment and decreased

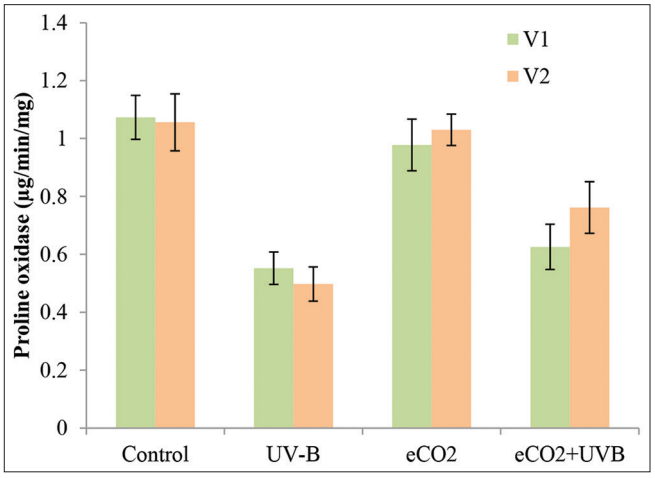

Figure 2: Effect of eCO_2, UVB and combined treatment on Proline oxidase activity of selected Quinoa varieties

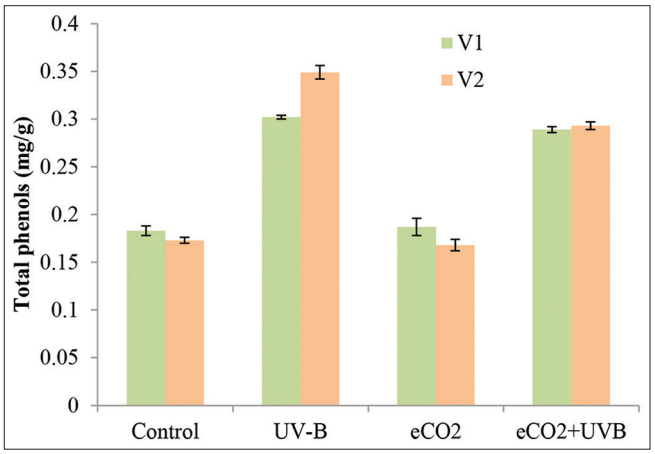

Figure 3: Effect of eCO_2, UVB and combined treatment on total phenol content of selected Quinoa varieties

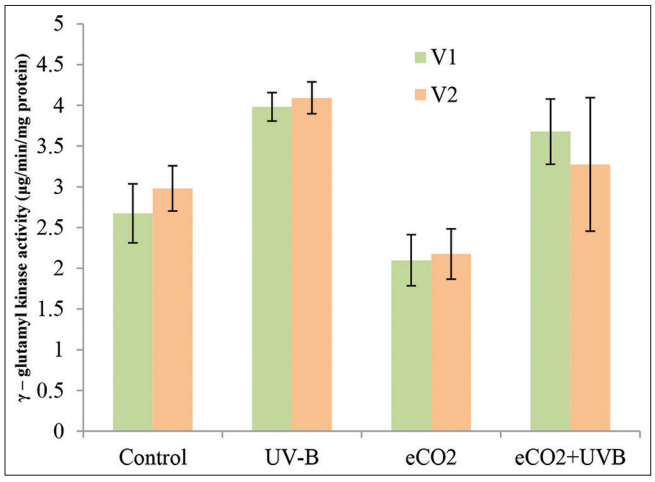

Figure 1: Effect of eCO_2, UVB and combined treatment on γ – glutamyl kinase activity of selected Quinoa varieties

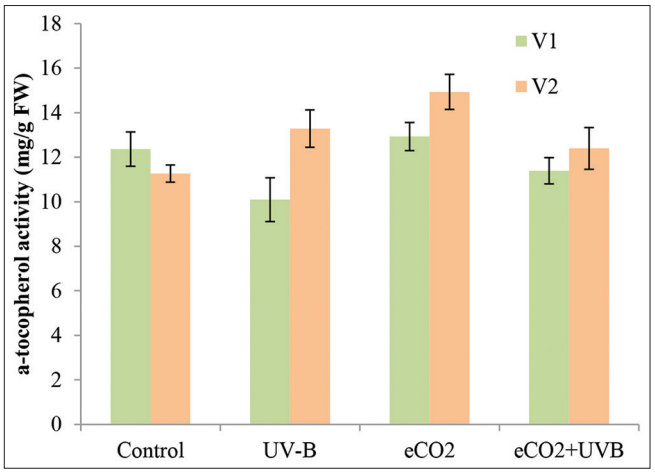

Figure 4: Effect of eCO_2, UVB and combined treatment on α-tocopherol activity of selected Quinoa varieties

in the control as well as in the elevated level CO_2 conditions. The α-tocopherol activity was slightly increased in all the treatments (Figure 4). However, in UVB treatment, a decreased level of α-tocopherol activity was recorded in the V1 variety. The CO_2 enrichment showed the highest α-tocopherol activity in the V2 quinoa variety (14.930 ± 0.792 mg/g FW). An increased activity level of reduced glutathione (Figure 5) was recorded in both V1 and V2 varieties when the plants were treated with UVB (V1=18.273 ± 0.918; V2=19.289 ± 2.357 µg/g FW) and UVB+eCO_2 (V1= 17.938 ± 1.293; V2=18.291 ± 2.392 µg/g FW). The increasing level of antioxidants might be associated with the production of plant cells from UVB radiation. Mainly, the total phenol content was dramatically increased when the plants were treated with UVB radiation. Usually, the UV absorbing compounds act as a shield that protects the plant cells from UVB radiation (Köhler *et al.*, 2017).

In the present study, the activity of enzymatic antioxidants was increased when compared to control (Figures 6-10). The total phenol content was increased in UVB (V1=0.302 ± 0.002; V2=0.349 ± 0.007 mg/g) and eCO_2+UVB (V1=0.289 ± 0.003; V2= 0.293 ± 0.004 mg/g) treatments. However, the CO_2 enrichment has not affected the phenol content of the studied Quinoa varieties when compared to the control plant. It was also reported that the phenols synthesized through the

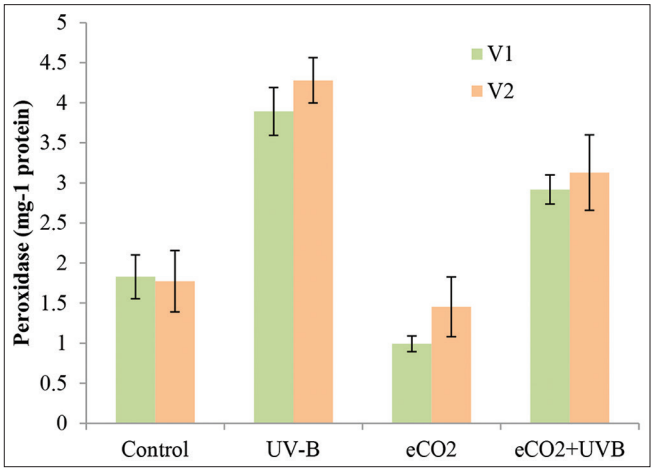

Figure 7: Effect of eCO_2, UVB and combined treatment on peroxidase activity of selected Quinoa varieties

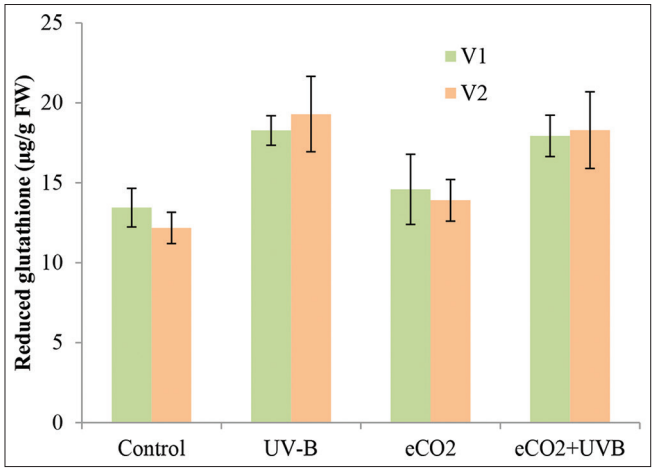

Figure 5: Effect of eCO2, UVB and combined treatment on reduced glutathione activity of selected Quinoa varieties

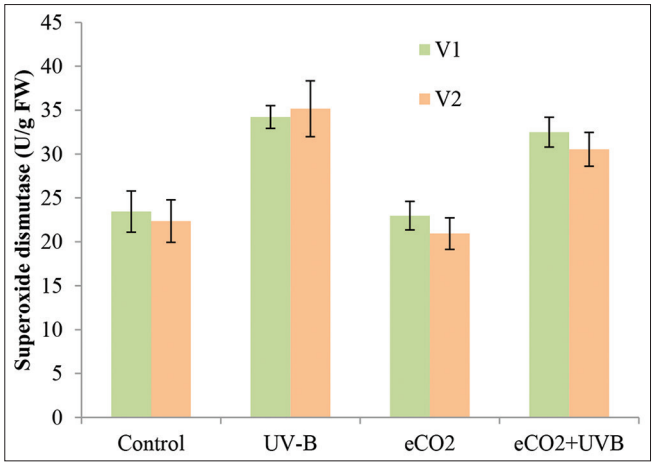

Figure 8: Effect of eCO_2, UVB and combined treatment on superoxide dismutase activity of selected Quinoa varieties

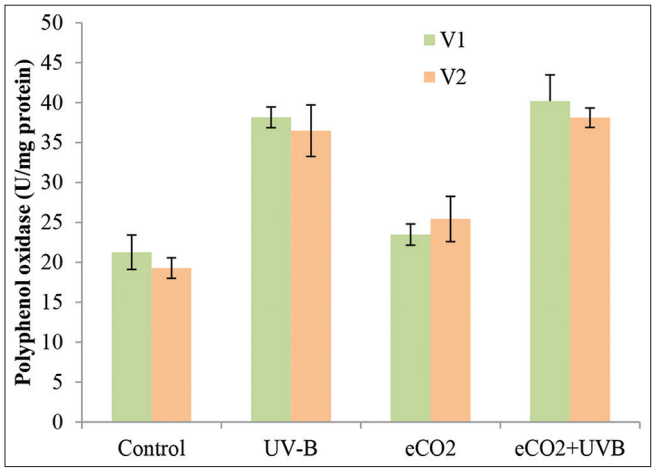

Figure 6: Effect of eCO_2, UVB and combined treatment on polyphenol oxidase activity of selected Quinoa varieties

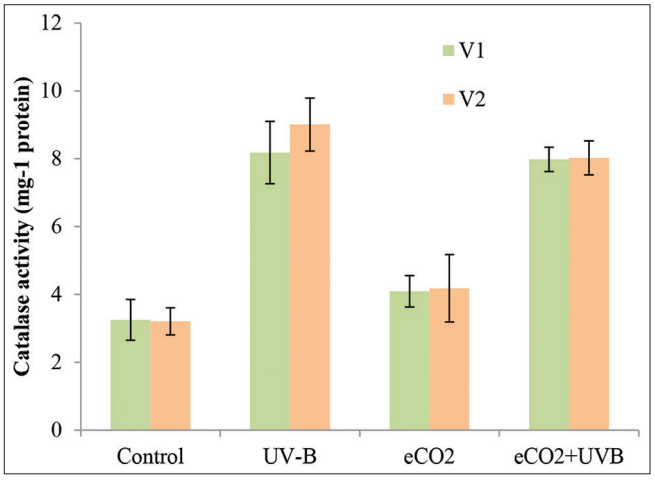

Figure 9: Effect of eCO_2, UVB and combined treatment on catalase activity of selected Quinoa varieties

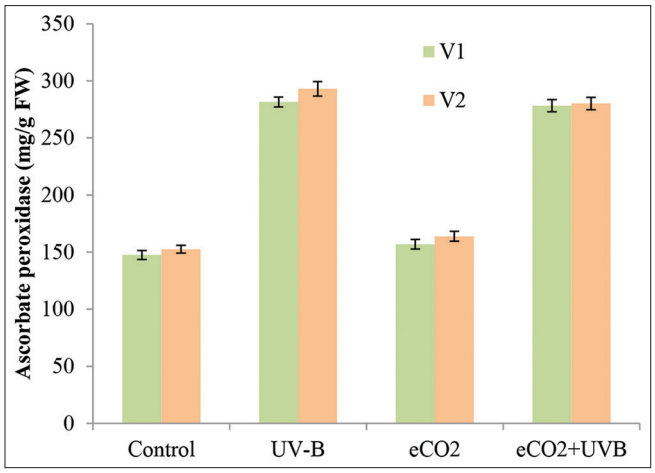

Figure 10: Effect of eCO_2, UVB and combined treatment on ascorbate peroxidase activity of selected Quinoa varieties

phenylpropanoid biosynthesis pathway can protect the plants by the absorption of UV-B radiation (de Vries *et al.*, 2017; Escobar *et al.*, 2017). Fu *et al.* (2021) discovered that the phenolic substances of *Porphyra haitanensis* was unregulated significantly under UV-B radiation. In plants, the non-enzymatic and enzymatic antioxidants provide sufficient production against the free radicals under UVB radiation. Previously, there are many studies were made concerning the 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 the 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 has less activity when compared to the control. Agrawal *et al.* (2009) found that an increased superoxide dismutase activity in 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 studied quinoa varieties showed a good response to the climate change factors. The antioxidant enzymes showed good defense against UVB radiation. The authors found that the non-antioxidant enzyme activities were increased when the plants were treated with supplementary. The previous studies were mainly focused on the effect of salinity on quinoa varieties. But, the effect of climate change scenarios on quinoa plants is limited. So, the results of the present study may help to find out a more tolerant variety of quinoa for future climatic conditions.

REFERENCES

Agrawal, S. B., Singh, S., & Agrawal, M. (2009). Ultraviolet-B induced changes in gene expression and antioxidants in plants. *Advances in Botanical Research, 52*, 47-86. https://doi.org/10.1016/S0065-2296(10)52003-2

Ainsworth, E. A., & Long, S. P. (2005). What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. *New Phytologist, 165*(2), 351-371. https://doi.org/10.1111/j.1469-8137.2004.01224.x

Asada, K., & Takahashi, M. (1987). Production and scavenging of active oxygen in photosynthesis. In D. J. Kyle, C. B. Osmond & C. J. Arntzen (Eds.), *Photoinhibition* (pp. 227-287) Amsterdam, Netherlands: Elsevier.

Backer, H., Frank, O., Angelis, B. D., & Feingold, S. (1980). Plasma tocopherol in man at various times after ingesting free or acetylated tocopherol. *Nutrition Reports International, 21*(4), 531-536.

Beauchamp, C., & Fridovich, I. (1971). Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry, 44*(1), 276-287. https://doi.org/10.1016/0003-2697(71)90370-8

Broberg, M. C., Högy, P., & Pleijel, H. (2017). CO_2-induced changes in wheat grain composition: meta-analysis and response functions. *Agronomy, 7*(2), 32. https://doi.org/10.3390/agronomy7020032

Ceccato, D. V., Bertero, H. D., & Batlla, D. (2011). Environmental control of dormancy in quinoa (*Chenopodium quinoa*) seeds: two potential genetic resources for pre-harvest sprouting tolerance. *Seed Science Research, 21*(2), 133-141. https://doi.org/10.1017/S096025851100002X

Chandlee, J. M., & Scandalios, J. G. (1984). Analysis of variants affecting the catalase developmental program in maize scutellum. *Theoretical and Applied Genetics, 69*, 71-77. https://doi.org/10.1007/BF00262543

Cline, W. R. (2007). *Global Warming and Agriculture: Impact Estimates by Country* Washington: Center for Global Development and Peterson Institute for International Economics.

De Vries, J., de Vries, S., Slamovits, C. H., Rose, L. E., & Archibald, J. M. (2017). How embryophytic is the biosynthesis of phenylpropanoids and their derivatives in streptophyte algae?. *Plant & Cell Physiology, 58*(5), 934-945. https://doi.org/10.1093/pcp/pcx037

Escobar, A. L., de Oliveira Silva, F. M., Acevedo, P., Nunes-Nesi, A., Alberdi, M., & Reyes-Díaz, M. (2017). Different levels of UV-B resistance in *Vaccinium corymbosum* cultivars reveal distinct backgrounds of phenylpropanoid metabolites. *Plant Physiology and Biochemistry, 118*, 541-550. https://doi.org/10.1016/j.plaphy.2017.07.021

Fu, S., Xue, S., Chen, J., Shang, S., Xiao, H., Zang, Y., & Tang, X. (2021). Effects of different short-term UV-B radiation intensities on metabolic characteristics of *Porphyra haitanensis*. *International Journal of Molecular Sciences, 22*(4), 2180. https://doi.org/10.3390/ijms22042180

Gao, Q., & Zhang, L. (2008). Ultraviolet-B-induced oxidative stress and antioxidant defense system responses in ascorbate-deficient vtc1 mutants of Arabidopsis thaliana. *Journal of Plant Physiology, 165*(2), 138-148. https://doi.org/10.1016/j.jplph.2007.04.002

Griffith, O. W. (1980). Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. *Analytical Biochemistry, 106*(1), 207-212. https://doi.org/10.1016/0003-2697(80)90139-6

Hasanuzzaman, M., Nahar, K., Alam, M. M., Roychowdhury, R., & Fujita, M. (2013). Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. *International Journal of Molecular Sciences, 14*(5), 9643-9684. https://doi.org/10.3390/ijms14059643

Hayzer, D. J., & Leisinger, T. H. (1980). The gene-enzyme relationships of proline biosynthesis in *Escherichia coli*. *Journal of General Microbiology, 118*(2), 287-293. https://doi.org/10.1099/00221287-118-2-287

Huang, A. H. C., & Cavalieri A. J. (1979). Proline oxidase and water stress induced proline accumulation in spinach leaves. *Plant Physiology, 63*(3), 531-535. https://doi.org/10.1104/pp.63.3.531

Huang, B., & Xu, Y. (2015). Cellular and molecular mechanisms for elevated CO_2–regulation of plant growth and stress adaptation. *Crop Science, 55*(4), 1405-1424. https://doi.org/10.2135/cropsci2014.07.0508

Hwang, C.-S., Rhie, G., Kim, S.-T., Kim, Y.-R., Huh, W.-K., Baek, Y.-U., & Kang, S.-O. (1999). Copper- and zinc-containing superoxide dismutase and its gene from *Candida albicans*. *Biochimica et Biophysica Acta (BBA) - General Subjects, 1427*(2), 245-255. https://doi.org/10.1016/s0304-4165(99)00020-3

Isah, T. (2019). Stress and defense responses in plant secondary metabolites production. *Biological Research, 52*, 39. https://doi.org/10.1186/s40659-019-0246-3

Jacobsen, S. E., Liu, F., & Jensen, C. R. (2009). Does root-sourced ABA play a role for regulation of stomata under drought in quinoa (*Chenopodium quinoa* Willd). *Scientia Horticulturae, 122*(2), 281-287. https://doi.org/10.1016/j.scienta.2009.05.019

Karthishwaran, K., Senthilkumar, A., Alzayadneh, W. A., & Salem, M. A. A. (2020). Effects of CO2 concentration and UV-B radiation on date palm (*Phoenix dactylifera*) grown in open-top chambers. *Emirates Journal of Food and Agriculture, 32*(1), 73-81. https://doi.org/10.9755/ejfa.2020.v32.i1.2062

Köhler, H., Contreras, R. A., Pizarro, M., Cortés-Antíquera, R., & Zúñiga, G. E. (2017). Antioxidant responses induced by UVB radiation in *Deschampsia antarctica* Desv. *Frontiers in Plant Science, 8*, 921. https://doi.org/10.3389/fpls.2017.00921

Koubouris, G. C., Kavroulakis, N., Metzidakis, I. T., Vasilakakis, M. D., & Sofo, A. (2015). Ultraviolet-B radiation or heat cause changes in photosynthesis, antioxidant enzyme activities and pollen performance in olive tree. *Photosynthetica, 53*(2), 279-287. https://doi.org/10.1007/s11099-015-0102-9

Kumar, K. B., & Khan, P. A. (1982). Peroxidase and polyphenol oxidase in excised ragi (*Eleusine coracana* cv. PR 202) leaves during senescence. *Indian Journal of Experimental Botany, 20*, 412-416.

Kumari, R., Singh, S., & Agrawal, S. B. (2010). Response of ultraviolet-B induced antioxidant defense system in a medicinal plant, *Acorus calamus*. *Journal of Environmental Biology, 31*(6), 907-911.

Lin, D., Xiao, M., Zhao, J., Li, Z., Xing, B., Li, X., Kong, M., Li, L., Zhang, Q., Liu, Y., Chen, H., Qin, W., Wu, H., & Chen, S. (2016). An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes. *Molecules, 21*(10), 1374. https://doi.org/10.3390/molecules21101374

Malik, C. P., & Singh M. B. (1980). *Plant Enzymology and Hittoenzymology*. (1st ed.). New Delhi, India: Kalyani Publishers.

Miller, G., Honig, A., Stein, H., Suzuki, N., Mittler, R., & Zilberstein, A. (2009). Unraveling Δ^1-pyrroline-5-carboxylate-proline cycle in plants by uncoupled expression of proline oxidation enzymes. *Journal of Biological Chemistry, 284*(39), 26482-26492. https://doi.org/10.1074/jbc.M109.009340

Myers, S. S., Zanobetti, A., Kloog, I., Huybers, P., Leakey, A. D. B., Bloom, A. J., Carlisle, E., Dietterich, L. H., Fitzgerald, G., Hasegawa, T., Holbrook, N. M., Nelson, R. L., Ottman, M. J., Raboy, V., Sakai, H., Sartor, K. A., Schwartz, J., Seneweera, S., Tausz, M., & Usui, Y. (2014). Increasing CO_2 threatens human nutrition. *Nature, 510*, 139-142. https://doi.org/10.1038/nature13179

Ncube, B., Finnie, J. F., & Staden, J. V. (2012). Quality from the field: The impact of environmental factors as quality determinants in medicinal plants. *South African Journal of Botany, 82*, 11-20. https://doi.org/10.1016/j.sajb.2012.05.009

Novick, K. A., Miniat, C. F., & Vose, J. M. (2016). Drought limitations to leaf-level gas exchange: results from a model linking stomatal optimization and cohesion–tension theory. *Plant, Cell & Environment, 39*(3), 583-596. https://doi.org/10.1111/pce.12657

Rácz, A., Czégény, G., Csepregi, K., & Hideg, E. (2020). Ultraviolet-B acclimation is supported by functionally heterogeneous phenolic peroxidases. *Scientific Reports, 10*, 16303. https://doi.org/10.1038/s41598-020-73548-5

Rao, M. V., Paliyath, G., & Ormrod, D. P. (1996). Ultraviolet-B and ozone-induced biochemical changes in antioxidant enzymes of *Arabidopsis thaliana*. *Plant Physiology, 110*(1), 125-136. https://doi.org/10.1104/pp.110.1.125

Roychoudhury, A., & Basu, S. (2012). Ascorbate-Glutathione and plant tolerance to various abiotic stresses. In N. A. Anjum, S. Umar & A. Ahmad (Eds.), *Oxidative Stress in Plants: Causes, Consequences and Tolerance* (pp. 177-258) New Delhi, India: IK International Publishers.

Sharma, S., Kataria, S., Joshi, J., & Guruprasad, K. N. (2019) Antioxidant defense response of fenugreek to solar UV. *International Journal of Vegetable Science, 25*(1), 40-57. https://doi.org/10.1080/19315260.2018.1466844

Warren, J. M., Jensen, A. M., Medlyn, B. E., Norby, R. J., & Tissue, D. T. (2014). Carbon dioxide stimulation of photosynthesis in *Liquidambar styraciflua* is not sustained during a 12-year field experiment. *AoB Plants,* 7, plu074. https://doi.org/10.1093/aobpla/plu074

WMO. (2008). *WMO Statement on the Status of the Global Climate in 2007* (WMO, 1031). Geneva: World Meteorological Organization.

Zhu, L., Huang, R., Zhou, L., & Chen, Y. (2021). Responses of the ecological characteristics and antioxidant enzyme activities in *Rotaria rotatoria* to UV-B radiation. *Hydrobiologia, 848*, 4749-4761. https://doi.org/10.1007/s10750-021-04671-1