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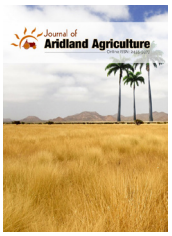
Research Article



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Response of two different *Phoenix dactylifera* cultivars to future climate conditions

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ABSTRACT

Plants are naturally exposed to various environmental stresses that affect their growth and development. As a desert plant, *Phoenix dactylifera* (date palm) has developed strategies to protect itself from most abiotic stresses. However, projected climate changes and the interaction between the various abiotic stressors will have profound effects on date palm adaptation and production In the present study, the two date palm cultivars, Sultana and Zamli cultivars were exposed to elevated levels of CO_2 and enhanced UVB radiation and non-enzymatic antioxidants (total phenols, α-tocopherol, reduced glutathione content) and antioxidant enzyme activities (polyphenol oxidase, peroxidase, superoxide dismutase, catalase, ascorbate peroxidase) activities were analysed. The results showed that the Sultana cultivar is tolerant to future climate conditions. However, more biotic stress and yield parameters are needed for the identification of biotic stress tolerant date palm cultivars.

KEYWORDS: *Phoenix dactylifera*, Future climate conditions, Antioxidant enzymes

INTRODUCTION

Climate change is one of the foremost vital threats facing the globe nowadays. It is largely driven by the magnified levels of greenhouse gases within the earth's atmosphere such as Methane (CH_4), Nitrous Oxide (N_2O), Chlorofluorocarbons (CFCs) and especially elevated levels of carbon dioxide resulting in changes in environmental factors like ozone depletion and high temperature, enhanced UV-B radiation, salinity, weather extremes, drought and variation in rainfall patterns and atmospheric humidity. The CO_2 concentration of the atmosphere is increasing day by day mainly due to deforestation and fossil fuel combustion. As per the IPCC (2014) report, it is predicted to reach 1000 ppm by the year 2100. Increasing CO_2 concentrations in the atmosphere have both positive as well as negative effects on plants. However, most of the effects are still unknown. Photosynthesis is a vital process for controlling variables of plant growth (Wang *et al.*, 2012). Elevated level of CO_2 (eCO_2) can stimulate the rate of net photosynthesis which subsequently leads the positive effects *viz.*, plant growth and yield (Long *et al.*, 2004; Ainsworth & Long, 2005; van der Kooi *et al.*, 2016).

The change in CO_2 and temperature accompanied by the emission of ozone depleting compounds such as chlorofluorocarbons (CFCs), methane and nitrous oxide causes a reduction in the thickness and affecting the distribution of stratospheric ozone columns, thus causing an increase in the amount of UVB radiation reaching on the earth's surface. Relative to the 1970s, the mid-latitudes O_3 column losses for the 2002-2005 periods were approximately 3% in the Northern and 6% in the Southern hemisphere (WMO, 2008). Plants are consistently exposed to solar UV radiation because they require sunlight to carry out photosynthesis. They are generally adapted to environmental UV-B radiation exposure since they have evolved mechanisms to avoid damage. Visual symptoms consisting of chlorotic or necrotic patches on leaves exposed to UV-B were not unique (Kakani *et al.*, 2003). Environmental factors have a direct effect on the global crop distribution and food production. Similar to other plants date palm is also adversely affected by climate change factors such as elevated levels of CO_2 and UVB radiation. Crop and admirably adapted to the arid and semi-arid environments of the Middle Eastern countries including the United Arab Emirates (UAE) (Kizhisseri *et al.*, 2021). However, variations in rainfall, global warming, gas pollution and decline of water resources are common concerns for date palm production. Most of the previous studies have largely focused on the quality aspects of the date palm fruits and seeds. However, the research on the

evaluation of the climate change impacts on date palm has received little attention. Thus, considering socio-economic importance of the date palms, the present study was focused on to evaluate the impacts of elevated CO_2 and enhanced UVB radiations in the antioxidant activity of two date palm cultivars growing in the UAE growing regions.

MATERIAL AND METHODS

Experimental Site

The study was conducted in Open Top Chambers facility in Al-Foah Experimental Farm [24°21′31.139″N 55°47′57.239″ E (Altitude 303 M)], College of Agriculture and Veterinary Medicine, United Arab Emirates University, Al Ain in natural conditions.

Date Palm Cultivars

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

Open Top Chambers Facility

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

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 of *P. dactylifera* leaves was homogenized with 80% of 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 volume of each test tube was made to 3 mL. The test tubes were placed in a water bath after adding 0.5 mL of Folin-Ciocalteau reagent and absorbance was read at 660 nm. Different concentrations of catechol solutions were prepared as above and standard curve was prepared. The results of the phenolic content are expressed as mg/g Fresh weight.

Determination of α-tocopherol activity

α-Tocopherol activity was analysed as described by Backer *et al*. (1980). 10 mL of petroleum ether and ethanol (2:1.6 v/v) was used to homogenize 500 mg of fresh tissue and centrifuged for 20 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 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.

Reduced glutathione activity

A method described by Griffith (1980) was adopted to analyse the reduced glutathione activity. 200 mg of plant material was ground with 2% metaphosphoric acid (5 mL). After grinding, it was centrifuged for 10 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 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 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 turn in to orange-red colour. The obtained results are expressed in U mg^{-1} protein.

Peroxidase activity

Peroxidase activity of the date palm leaves was determined by the method of Kumar and Khan (1982). The assay mixture [0.1 M phosphate buffer (2 mL), 0.01 M pyrogallol (1 mL), 0.005 M of H_2O_2 and enzyme extract (0.5 mL)] was incubated at 25 °C (5 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 *P. dactylifera* 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 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 analysed using SPSS (V. 21.0). The results were taken from three replicates and date data are expressed in Mean±SE.

RESULTS AND DISCUSSION

P. dactylifera is an economically important crop and adapted to the arid and semi-arid environments of the Middle Eastern countries including the UAE (Shabani *et al.*, 2012). However, variations in rainfall, global warming, gas pollution, drought, salinity and decline of water resources are common concerns for date palm production. In the present study, two selected date palm varieties were exposed to elevated level CO_2 concentrations and enhanced UVB and the non-enzymatic and enzymatic antioxidant activities were studied. The results on the effect of high atmospheric CO_2 and enhanced UVB radiation on non-enzymatic enzymes of Sultana and Zamli date palm cultivars are graphically presented in Figures 1-3. An increased level of total phenol content was recorded in both the cultivars at UVB and UVB+CO_2 treatments (Figure 1). The highest content of total phenol was recorded in the leaves of the Zamli variety treated with UVB radiation (0.079±0.004 mg/g). The same variety had the lowest content (0.041±0.002 mg/g) of phenol when the plant was treated with eCO_2. Also, the highest α-tocopherol activity was recorded in the Sultana variety (Figure 2) treated with UVB+eCO_2 (9.562±0.992 mg/g FW). Whereas, the UVB treated plants of the Zamli variety showed the lowest α-tocopherol activity (8.628±0.672 mg/g FW). In both *P. dactylifera* cultivars, reduced glutathione activity was increased in UVB treatment then decreased in eCO_2 treatment and again increased in the combined treatment when compared to control (Figure 3). The results on enzymatic antioxidants such as polyphenol oxidase, peroxidase, superoxide dismutase, catalase and ascorbate peroxidase activities of UVB and eCO_2 treated *P. dactylifera* cultivars, Sultana and Zamli are graphically

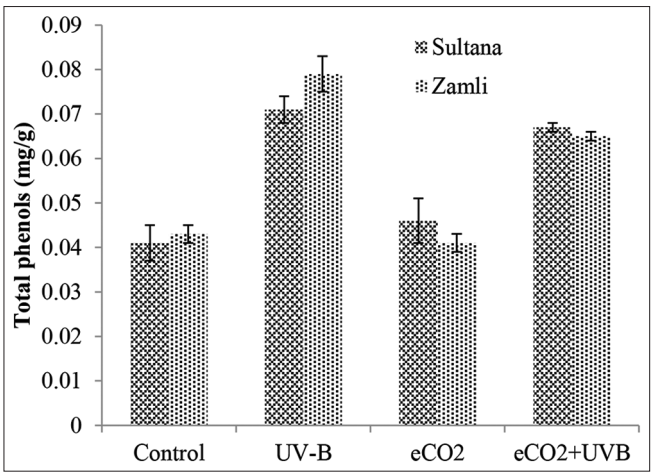

Figure 1: Effect of eCO_2, UVB and combined treatment on total phenol content of Sultana and Zamli *P. dactylifera* cultivars

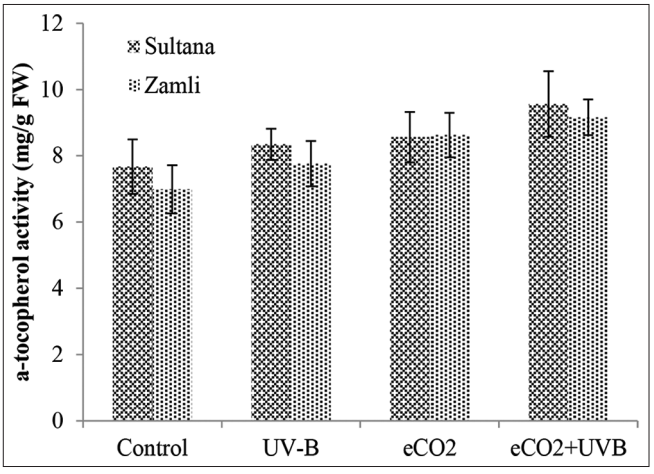

Figure 2: Effect of eCO_2, UVB and combined treatment on α-tocopherol activity of Sultana and Zamli *P. dactylifera* cultivars

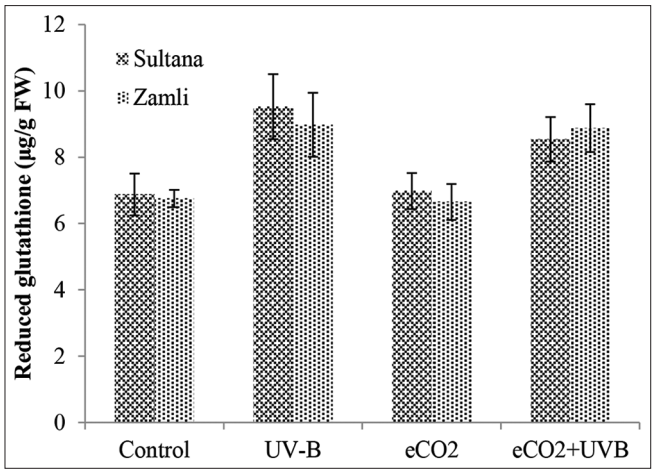

Figure 3: Effect of eCO_2, UVB and combined treatment on reduced glutathione activity of Sultana and Zamli *P. dactylifera* cultivars

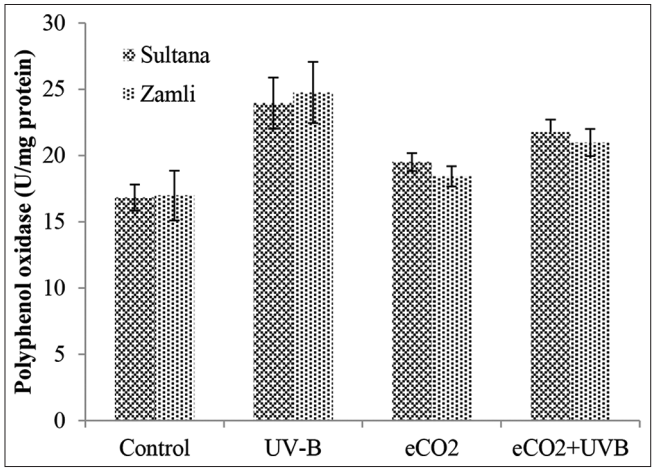

Figure 4: Effect of eCO_2, UVB and combined treatment on polyphenol oxidase activity of Sultana and Zamli *P. dactylifera* cultivars

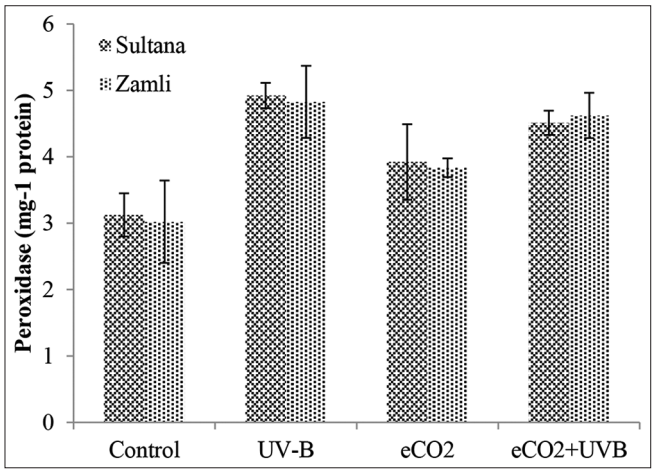

Figure 5: Effect of eCO_2, UVB and combined treatment on peroxidase activity of Sultana and Zamli *P. dactylifera* cultivars

presented in Figures 4-8. The polyphenol oxidase activity was increased in all the treatments when compared to control

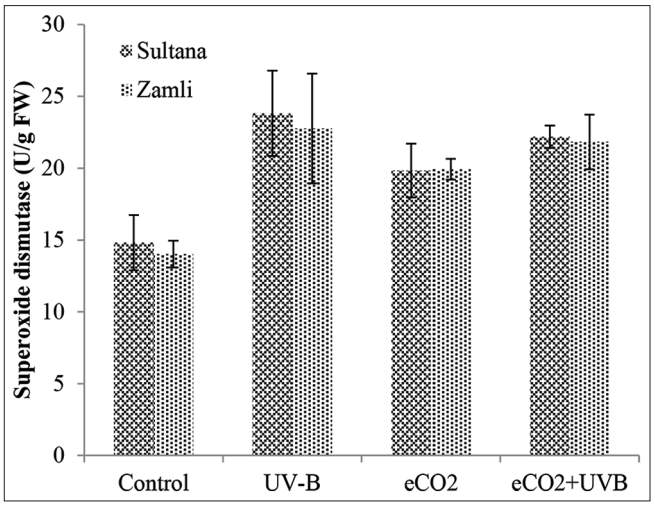

Figure 6: Effect of eCO_2, UVB and combined treatment on superoxide dismutase activity of Sultana and Zamli *P. dactylifera* cultivars

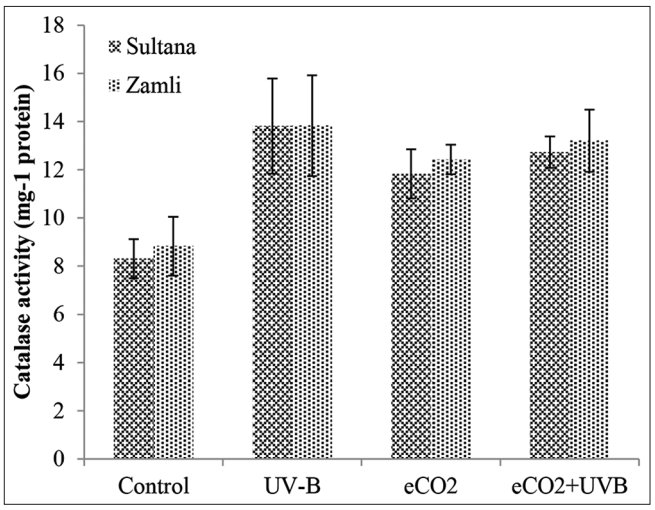

Figure 7: Effect of eCO_2, UVB and combined treatment on catalase activity of Sultana and Zamli *P. dactylifera* cultivars

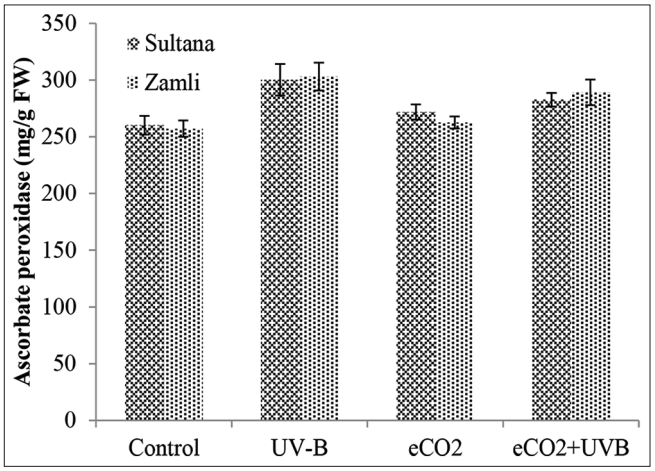

Figure 8: Effect of eCO_2, UVB and combined treatment on ascorbate peroxidase activity of Sultana and Zamli *P. dactylifera* cultivars

(Figure 4). Among the treatments, the highest polyphenol oxidase activity was observed in UVB treated Zamli variety (24.76±2.309 U/mg protein). Also, the same trend was observed for highest peroxidase activity (4.921±0.190 mg^{-1} protein) in the Sultana variety with UVB treatment (Figure 5). Whereas, the increased level of peroxidase activity was Sultana variety with UVB treatment (Figure 6). In both cultivars, the catalase activity is higher when the plants are expose to UVB radiation. But, degreased catalase activity values were recorded in other two treatments. In UVB treatment, both the cultivars have similar amounts of catalase. The values of catalase activity in Sultana and Zamli cultivars were 38.312±0.812, 13.812±1.980, 11.829±1.019, 12.732±0.652 and 8.827±1.219, 13.829±2.092, 12.426±0.617, 13.201±1.292 mg^{-1} protein in untreated, UVB, eCO_2 and UVB+CO_2 treatments, respectively (Figure 7). As observed in the other antioxidant enzymes, ascorbate peroxidase activity also increased in both the *P. dactylifera* cultivars treated with UVB radiation (Figure 8). The highest ascorbate peroxidase activity was observed in the leaves of the Zamli variety with UVB treatment (302.98±12.298 μg/g FW).

Generally, all the plant species had antioxidative defence mechanisms which provided enough production to alleviate the adverse impact of stress. Non-antioxidant enzymes such as total phenols, α-tocopherol and reduced glutathione contents also played a vital role in alleviating the effects of ROS. The synthesis of UV absorbing constituents is the potential non-enzymatic antioxidant mechanism which quenchers of ROS or reduces the penetration of radiation (Del Valle *et al.*, 2020).

Zagoskina *et al.* (2003) studied the tissue localization and accumulation of phenolic compounds in callus cultures of *Camellia sinensis* after UVB radiation. The results showed that UVB promotes the accumulation of phenol content by increased deposition of phenolic compounds in cell walls. On the other hand, the antioxidant enzyme activities are also induced by abiotic stress. In particular, UVB radiation enhanced the activities of polyphenol oxidase, peroxidase, superoxide dismutase, catalase and ascorbate peroxidase activities in the present study. This activity is supported by the previous studies (Kondo & Kawashima, 2000; Mishra *et al.*, 2009). Moghimifam *et al.* (2020) reported that the antioxidant enzymes such as catalase, ascorbate peroxidase, peroxidase, glutathione reductase, superoxide dismutase and polyphenol oxidase were increased in *Withania somnifera* after the treatment of UVB. Dwivedi *et al.* (2015) reported that varied level of antioxidant enzymes with the plant species and UV-B exposure.

Agarwal (2007) studied the UVB oxidative damage of *Cassia auriculata* seedlings. The study showed that the level of antioxidant enzymes such as catalase, superoxide dismutase, polyphenol oxidase and peroxidase were increased significantly. Similarly, the increased antioxidant enzyme level was noted in wheat and maize (Mackerness *et al.*, 1999), cucumber (Kondo & Kawashima, 2000), *Plectonema boryanum* (Prasad & Zeeshan, 2005) and potato (Santos *et al.*, 2004). Various reports showed that the increased enzyme activities are linked with better tolerance to environmental stress (Zaefyzadeh *et al.*, 2009; Chen *et al.*, 2011). For example, the increased superoxide dismutase activity is directly correlated with stress tolerance and all nuclear encoded superoxide dismutase forms target the subcellular compartments through a targeting sequence of amino terminals (Bowler *et al.*, 1992). Also, the immediate expression of multiple enzymes had more potential for stress tolerance than the single/double expression (Lee *et al.*, 2007). It is also reported UVB inhibits the rate of net photosynthesis by decreasing the rate of CO_2 assimilation which reduces the RUBISCO content as well as carboxylation velocity (Allen *et al.*, 1997). For example, UVB increased the antioxidant defence efficacy of *Picea asperata* seedlings through the antioxidant enzymes (Han *et al.*, 2009). Both the varieties had good responses to photosynthetic pigments, biochemical contents, proline metabolizing enzymes, non-enzymatic and enzymatic defence to UVB, eCO_2 and the combined treatments when compared to the control. The results also showed that the sultana cultivar had stress tolerance ability and it could be suited for the UAE growing conditions. The results also advance our understanding by elucidating the various physiological and biochemical mechanisms responsible for the abiotic stress tolerant characteristics among the *P. dactylifera* varieties. Moreover, other a biotic stress and yield parameters are warranted for the identification of biotic stress tolerant *P. dactylifera* cultivars.

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