



Melatonin priming manipulates antioxidant regulation and secondary metabolites production in favour of drought tolerance in *Chenopodium quinoa* Willd.

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ABSTRACT

Melatonin can be considered a physiological protective agent against environmental stress because of its natural antioxidant ability. The main goal of this study was to investigate the possible melatonin-positive effects on the growth enhancement and drought tolerance of quinoa (*Chenopodium quinoa* Willd) cultivars. To accomplish this objective, we compared the effects drought stress (100 % field capacity as control and 40 % field capacity), priming (melatonin priming (MP), hydro priming (HP), non-priming (NP), and cultivars (Titicaca, Giza1). Thus, the obtained results indicated higher oxidative damage, lipid peroxidation, and reduced chlorophyll content, which decreased the seed yield more in Giza1 than in Titicaca in comparison with the control. MP remarkably enhanced endogenous melatonin content, superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) activity in Titicaca (1.57, 2.50, 1.40, 1.90, and 2 times, respectively) and Giza1 (1.68, 4.50, 4, 2 and 2.6 times, respectively), compared with NP, under drought stress. MP effectively protected Titicaca and Giza1 chlorophylls and carotenoids, while decreasing the malondialdehyde (MDA) content through antioxidant enzyme activation, facilitation of soluble protein, as well as sugar synthesis and osmolyte accumulation. Therefore, MP improved photosynthesis in Titicaca and Giza1 under drought stress compared with NP. Overall, Titicaca showed a relatively higher drought stress tolerance. Furthermore, MP ameliorated the adverse consequences of drought stress and facilitated the recovery of susceptible cultivars such as Giza1 through the enhancement of photosynthetic pigments, antioxidant systems, osmotic adjustment, and secondary metabolite production. The main novelty of the current study is the elucidation of the role of melatonin priming in the induction of drought tolerance in quinoa.

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1. Introduction

Plants are exposed to a range of stressful circumstances in their growth and development stages, which will have varying effects and reduce crop yield (Zhang et al., 2023). Drought stress can be regarded as the most destructive environmental stress affecting agricultural crops and products because it can severely reduce crop yields (Zarei et al., 2019; Bukhari et al., 2021; Siddiqui et al., 2021; Naservafaei et al., 2021), thus posing a threat to food security (Iqbal et al., 2018). Drought stress can affect physiological and biochemical processes that are of particular importance for plant development and growth

(Kosar et al., 2020; Kaya et al., 2020; Abrahimi et al., 2023; Ashrafi et al., 2022; Yousefvand et al., 2022). Drought can affect photosynthesis, assimilation partitioning, nutrient and water interactions, and growth, resulting in considerable declines in crop yields (Ashrafi et al., 2022; Khaleghnezhad et al., 2021; Mahdavi et al., 2020; Fahad et al., 2017). One primary effect is stomatal closure, which can restrict plant transpiration and CO₂ diffusion into leaves (Manaa et al., 2021). Consequently, the physical and biochemical processes that allow CO₂ assimilation from the air can be compromised by drought stress (Padilla et al., 2021). Owing to stomatal closure, leaf intercellular CO₂ concentration (C_i) may be reduced, thus restricting its diffusion and uptake through the mesophyll cells; this, in turn, leads to a decline in carbon assimilation (Manaa et al., 2021; Tankari et al., 2021).

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Owing to the reduction in the CO₂ assimilation rate, drought stress can lead to oxidative stress because the reducing power of light processes may overwhelm the rate of consumption in the Calvin cycle. In the electron transport chain of photosystems, reductant assimilation can cause overproduction of reactive oxygen species (ROS) and other destructive changes at the cell level, including a lack of membrane cohesion, compromising structural integrity, peroxidation of lipids, and cell destruction in plant tissues (Fahad et al., 2017; Iqbal et al., 2018; Khan et al., 2019; Ilyas et al., 2020; Patel and Mishra, 2021). Also by upsetting the equilibrium between the generation of ROS and the plant's antioxidant defense system, drought stress eventually leads to oxidative stress, which affects plant development stages, organ structure, and activity (Alharby and Fahad, 2020; Naghizadeh et al., 2019). Such damage could eventually limit the capacity of leaves for photosynthetic activity, particularly under high light and severe drought conditions, resulting in higher C_i, as compared to well-watered plants (Lawlor and Tezara, 2009).

Therefore, it seems that plants can detoxify the toxic effects of ROS, which could be regarded as the most significant factor influencing their tolerance to environmental stress conditions (Rajput et al., 2021). Toward this end, plants have established a set of intricate defensive systems like the ROS scavenging apparatus, which consists of antioxidant enzymes, secondary metabolites, etc. (Aziz et al., 2018; Saud et al., 2020). Therefore, the exploration and exploitation of drought stress tolerance in crops are crucial for the breeding and production of plants (Nguyen et al., 2021).

Chenopodium quinoa Willd. (Quinoa) can be regarded as a C₃ plant belonging to Chenopodiaceae, which is native to South America (Bazile et al., 2016), and has emerged as a promising food crop because of its remarkable nutritive characteristics, including high energy level as well as excellent protein quality, as well as its adaptability to unfavorable environmental conditions, such as drought stress (Aziz et al., 2018; Razzaghi et al., 2020), salinity (Hinojosa et al., 2018; Derbali et al., 2021; Manaa et al., 2021), frost (Jacobsen et al., 2005; Jacobsen et al., 2007; Yaqoob et al., 2019) and poor soils (Ruiz et al., 2015).

One of the most practical and cost-effective methods for minimizing the negative impacts of stress and increase plant tolerance to drought is priming (Alharby and Fahad, 2020; Saud et al., 2020). Seed priming can improve both germination and early stand establishment of crops under both normal and stressful circumstances (Fahad et al., 2017). In addition, it boosts the growth of plants and, consequently, their resistance to diverse types of stresses (Khan et al., 2020). Melatonin (N-acetyl-5-methoxytryptamine), a well-known indole compound, can be produced in the mitochondria and chloroplasts of plants and may be responsible for protecting tissues against oxidative damage that may arise under drought stress (Zheng et al., 2017). Among all plant growth regulators, melatonin has been identified as the most powerful antioxidant (Qi et al., 2018; Kaya et al., 2019; Fathi et al., 2023). Melatonin is a multifunctional agent effective on the growth and development process, as well as on the resistance to drought stress. Melatonin, through its antioxidant activity, could serve a noteworthy function in improving plant cell function by eliminating free radicals, promoting antioxidant activity, improving electron transfer system efficiency in mitochondria, regulates metabolite contents, photosynthesis and diminishing free radical production and electrolyte leakage (Zhang et al., 2014; Qi et al., 2018; Kaya et al., 2019; Alharby and Fahad, 2020). The outcomes of previous studies have provided an indication of the defensive function of melatonin against stresses, such as drought stress (Ahmad et al., 2019), salinity (Alinia et al., 2021), and low temperatures (Yang et al., 2018; Jahan et al., 2021). Melatonin priming (MP) reduced the destructive effects of water shortage in soybean (*Glycine max* L. Merr.) through enhancement of carbohydrate and ascorbate metabolism (Wei et al., 2015). In alfalfa (*Medicago sativa* L.), drought tolerance was improved through activity enhancement of superoxide

dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT) (Antonioni et al., 2017). In rapeseed (*Brassica napus* L.), antioxidant enzymes have been shown to be activated in parallel with the production of compatible organic solutes such as proline and glutathione (Khan et al., 2020). In response, tolerant plants employ various strategies to maintain and balance their physiological functions, including increasing antioxidant activities and osmotic regulation. Therefore, melatonin priming could alter some stress-responsive pathways in exerting its protective role against drought stress-induced damage, thus enhancing the tolerance of quinoa cultivars to drought. Considering the importance of quinoa for food security and the apparent role of melatonin in strengthening the antioxidant system, this research aimed to (i) study adaptive strategies to cope with drought stress in two quinoa cultivars and (ii) investigate the effects of melatonin in enhancing drought stress tolerance of quinoa through increased antioxidant capacity.

2. Materials and methods

2.1. Plant growth condition

A greenhouse pot experiment was conducted at Shiraz University, Shiraz, Iran (52°35' E, 29°43' N). The seeds were sown in May 2020, with the monthly average night and day temperatures of 33 °C/15 °C and an average solar radiation of 800 μmol m⁻² s⁻¹; pots of the 20 cm height and 18 cm diameter were allowed to be filled with 4.5 kg loam soil and used. Soil chemical assessment showed 1.68% N, 20.8 mg kg⁻¹ P, 212 mg kg⁻¹ K, 0.64 dS m⁻¹ EC_e, and a pH of 7.20. The pots were placed on tables and assigned to diverse treatments based on a three-factor (water levels, priming, cultivars) factorial experiment with a completely randomized design involving three replications. The three experimental factors and their levels are described in the following sections.

2.2. Cultivars and seed source

The two quinoa cultivars investigated in this study (Titicaca and Giza1, Fig. 8) were obtained from the Iranian National Salinity Research Center in Yazd, Iran (Table 3). In a separate experiment based on drought stress tolerance indices like STI (stress tolerance index), GMP (geometric mean productivity) and MP (mean productivity) among 7 cultivars, Titicaca was chosen as the tolerant cultivar and Giza1 as the susceptible cultivar (data not shown). Titicaca and Giza1 are the most popular cultivars among quinoa growers in Iran. The cultivars' seeds displayed a germination rate of ≥98 % at 25 °C.

2.3. Seed priming treatments

Melatonin (Merck, catalog number: M118674–1 g/ L1619020) was used as the priming treatment in this study. Several pre-experiments were carried out to choose the ideal priming concentrations of melatonin and priming period based on quinoa emergence as well as early seedling growth performance. Cultivars' seeds were placed in solutions that included melatonin (20, 50, 100, 250, and 500 μM) for a period of 4, 5, and 6 h. Melatonin (50 mg) was allowed to be dissolved in 100 % ethanol (one mL). The appropriate soaking time and melatonin concentration were 4 h and 20 μM, respectively (data not shown). Following incubation, the seeds were washed many times with distilled water to eliminate melatonin from the external surface. The treated seeds were allowed to be dried in the dark at the temperature of 25 °C they returned to their original weight. Then they were sterilized by using 3 % NaOCl solution for a period of 3 min and then washed three times with distilled water prior to seeding. In addition to melatonin priming (MP), two other seed pretreatments were examined: hydropriming, which was used with distilled water (HP) for 4 h, after which it was blotted dry at room temperature in the

dark until its initial weight was regained; and non-priming (NP), which was subjected only to surface sterilization, as a reference.

2.4. Drought stress treatments

There were two stress levels: 100 % of pot field capacity (FC, watered control) and 40 % FC as the drought treatment). All the pots were weighed every two days throughout the experiment, and the amount of irrigation water for 100 % FC treatment was specified according to the amount needed to raise the soil water content to 100 % of the considered pot FC. In addition, irrigation water amount required for 40 % FC was calculated based on the amount of irrigation for 100 % FC. Seeds were sowed (ten per pot) in plastic pots. After the completion of emergence, thinning was performed to maintain only five plants in each pot. Based on the BBCH (biologische bundesanstalt, bundessortenamt und chemische industrie) scale code for quinoa plants (Sosa-Zuniga et al., 2017), irrigation treatments were initiated at six pairs of visible leaves (BBCH 16). At the beginning of flowering (BBCH 60), young leaves (three plants from each pot) were sampled, immediately immersed, and kept in liquid nitrogen until they were utilized for biochemical investigation. Finally, to assess their performance in the physiological maturation stage (BBCH 95), the remaining two plants from each pot were harvested and seed yield was measured.

2.5. Determination of endogenous melatonin content

Samples from young middle leaves were taken at the beginning of flowering (BBCH 60) from 10:00 to 12:00 AM and frozen in liquid N₂ at –80 °C. Minor modifications were made to the methodology applied by Arnao and Hernandez-Ruiz (2009) (Arnao and Hernández-Ruiz, 2009) to measure the leaves' melatonin content. The fresh leaves (100 mg) were divided into small pieces and then put into vials which contained 1 mL chloroform. The mixture was then shaken at 4 °C overnight under N₂ gas, and the leftover material was allowed to be dissolved in 100 µL acetonitrile; then it was filtered through a 0.2-micron polyvinylidene fluoride (PVDF) membrane. The content of melatonin was measured by using the Knauer High-Performance Liquid Chromatography (Germany) and C-18 column (Phenomenex KINETEX 250 mm × 4.6 mm) with fluorescence detection. The isocratic mobile phase, which was made up of water as well as acetonitrile (50:50) at the 0.2 L/min flow rate, with the excitation wavelength of 280 nm and emission wavelength of 350 nm, were utilized for the purpose of detection. As a standard, the use was made of pure melatonin (Merck, Germany).

2.6. Gas exchange measurements

Gas exchange measurements related to stomatal conductance (g_s), transpiration rate (T_r), intercellular CO₂ concentration (C_i) as well as net photosynthetic rate (P_n) were conducted by applying a portable LCi photosynthesis system (ADC Bioscientific Ltd., UK) on fully developed leaves in each of the treatments. At BBCH 60, photosynthetic parameters were measured under ambient conditions for two successive days before noon (between 9:00 AM and 11:00 AM). To calculate the leaf-scale water use efficiency (WUE_l), the following equation was used:

$$WUE = P_n / T_r \quad (1)$$

2.7. Leaf pigment analyses

Leaf samples were taken at BBCH 60 for leaf pigment analysis. Measurement of chlorophyll a, b, and carotenoids in leaves was performed using Arnon's method (1949) (Arnon, 1949). Briefly, extraction of 0.1 g of leaf powder was extracted with 10 mL of 80 % acetone.

The extract was then subjected to centrifugation (300–500 × g) for 5 min at room temperature, and measurement was performed using a spectrophotometer (7315 UV/visible Spectrophotometer, Jenway, UK) at 646, 663, and 470 nm. Quantification followed Wellburn (1994):

$$Chl_a \text{ (mg g}^{-1} \text{ FW)} = 12 \cdot 21 A_{663} - 2 \cdot 81 A_{646} \quad (2)$$

$$Chl_b \text{ (mg g}^{-1} \text{ FW)} = 20 \cdot 13 A_{646} - 5 \cdot 03 A_{663} \quad (3)$$

$$\begin{aligned} \text{Carotenoids (mg g}^{-1} \text{ FW)} \\ = (1000 A_{470} - 3 \cdot 27 C_a - 104 C_b) / 198 \end{aligned} \quad (4)$$

2.8. ROS production (H₂O₂) and lipid peroxidation (MDA) measurement

Leaf tissue was sampled at BBCH 60 to determine ROS production. Assessment of hydrogen peroxide (H₂O₂) levels was done according to the technique developed by Alexieva et al. (2001) (Alexieva et al., 2001). Leaf tissues (500 mg) were allowed to be homogenized in liquid nitrogen with 5 mL 0.1 % (w/v) trichloroacetic acid (TCA). Following centrifugation at 12 000 × g for 15 min, 500 µL of supernatant, 500 µL of 10 mM potassium phosphate buffer solution (pH 7.0), and 1000 µL of potassium iodide were added, followed by mixing. The optical density (OD) related to the supernatant was read at 390 nm. The H₂O₂ concentration is represented by a standard curve. MDA (malondialdehyde) content was measured using the thiobarbituric acid test [(Heath and Packer, 1968). A leaf sample (0.2 g) was then ground with 5 mL of TCA (1 %). It was subjected to centrifugation at 8000 × g for 10 min. Addition of 1 mL of the supernatant was added to 4 mL of some solution that contained 20 % TCA and 0.5 % thiobarbituric acid (TBA). All samples were then kept in a water bath at the temperature of 95 °C for a 30 min period and allowed to be quickly cooled in ice; then they were subjected to centrifuging at 8000 g for 5 min. The absorbance of each sample was recorded at 532 and 600 nm. Determination of MDA was performed according to Eq. (5), where the extinction factor was 155 mM^{–1} cm^{–1}.

$$MDA \text{ (}\mu\text{mol g}^{-1} \text{ FW)} = [A_{532} - A_{600} / 155] \times 1000 \quad (5)$$

2.9. Determination of antioxidant enzyme activity

To determine the antioxidant enzyme activity, leaf sample was taken at BBCH 60. Then leaf material (0.5 g) was extracted in phosphate buffer (5 mL) ice cooled to a pH value of 7.6 and then centrifuged at 12,000 × g at the 4 °C temperature for a 15 min period. Subsequently, the supernatant was used for the measurement of the following enzymes and total soluble protein as U g^{–1} FW (Alinia et al., 2021). Measurement of superoxide dismutase (SOD) activity was done in accordance with the technique developed by Giannopolitis and Ries (1977). Phosphate buffer (50 mM, pH 7.6), methionine (13 mM), riboflavin (4 µM), nitro blue tetrazolium (NBT) (750 mM), and ethylenediaminetetraacetic acid (EDTA) (0.1 mM) were mixed with the solution (0.2 mL). The OD was recorded spectrophotometrically at 560 nm (7315 UV/visible Spectrophotometer, Jenway, UK) and calculated using the following equation:

$$\begin{aligned} \text{SOD activity (U mg}^{-1} \text{ protein)} \\ = (\Delta A_{560} \times Vt) / (W \times V_s \times 0 \cdot 05 \times t) \end{aligned} \quad (6)$$

where ΔA_{560} is the change in OD, Vt is the total volume of the reaction mixture, W is the sample weight, V_s = Crude enzyme extract volume, and t is reaction time (min).

Peroxidase (POD) activity was evaluated using the method which had been developed by Cakmak and Marschner (1992). POD activity

was measured by adding phosphate buffer (50 mM) at pH 7.0, guaiacol (16 mM), enzyme extract (0.2 mL), and 10 mM H₂O₂. The absorbance of the mixture was recorded at 470 nm using a spectrophotometer (7315 UV/visible Spectrophotometer, Jenway, UK) and calculated as follows:

POD activity (U mg⁻¹ protein)

$$= (\Delta A_{470} \times V_t) / (W \times V_s \times 0.01 \times t) \quad (7)$$

ΔA_{470} = change in OD, V_t = total volume of the reaction mixture, W = weight of the sample, V_s = crude enzyme volume, t = reaction time (min).

The protocol developed by Havir and Mchale (1987) was applied to estimate the catalase (CAT) activity (Havir and McHale, 1987). Then, phosphate buffer (50 mM) at pH 7.0 and H₂O₂ (12.5 mM) were mixed with the enzyme extract (0.2 mL). The mixture absorption was assessed spectrophotometrically at 240 nm (7315 UV/visible Spectrophotometer, Jenway, UK), as follows:

CAT activity (U mg⁻¹ protein)

$$= (\Delta A_{240} \times V_t) / (W \times V_s \times 0.01 \times t) \quad (8)$$

ΔA_{240} = change in absorbance, V_t = total enzyme solution volume extracted, W = sample weight, t = reaction time, V_s = crude enzyme volume.

Measurement of Ascorbate peroxidase (APX) activity was measured according to the method proposed by Nakano and Asada (1981). According to this procedure, phosphate buffer (50 mM) with pH 7.0, EDTA (0.1 mM), AsA (ascorbate) (0.5 mM), and H₂O₂ (1.0 mM) was added to the crude enzyme extract (0.2 mL). The absorbance change was observed at 290 nm using a spectrophotometer (7315 UV/visible Spectrophotometer, Jenway, UK) and calculated as follows:

APX activity (U mg⁻¹ protein)

$$= (\Delta A_{290} \times V_t) / (2.8 \times M \times V \times t) \quad (9)$$

ΔA_{290} = change in absorbance during 30 s, V = crude enzyme volume, 2.8 mM = extinction coefficient, M = fresh material mass, and t = reaction time.

2.10. Measurement of total soluble protein (TSP), total soluble sugar (TSS), reducing & non-reducing sugar (RS & NRS) and total flavonoid contents

TSP quantification was performed according to the method developed by Bradford (1976). Briefly, 1 mL of Bradford reagent was allowed to be mixed with 100 mL of crude extract and then incubated for a 20 min period at 25 °C, recording to the absorbance was done by using a UV-vis spectrophotometer at 595 nm. TSS content was quantified according to Yemm and Willis (1954). Briefly, fresh leaves (0.25 g) was allowed to be immersed in 5 mL of ethanol (80 %). To 100 μ L of the considered sample extract, addition of 3 mL anthrone reagent (prepared in 72 % H₂SO₄) was done. The reaction mixture was allowed to be heated in some water bath at 95 °C for a 10 min period; then it was cooled and kept at the room temperature for a 20 min period. The OD was then read at 625 nm using a spectrophotometer (7315 UV/visible Spectrophotometer, Jenway, UK). Reducing sugar content was determined according to the method previously described (Karimmojeni et al., 2021). In short, 1 mL sample (the same as that used for TSS) was blended with 5 mL of 6 % O-toluidine; then, it was incubated at 95 °C for a period of 20 min and allowed to be cooled in an ice bath. The optical density was measured at 630 nm. The amount of reducing sugar was calculated using the following formula:

Non – reducing sugar

$$= \text{total soluble sugar} - \text{reducing sugar} \times 0.95 \quad (10)$$

Total flavonoid content was estimated according to Tsanova-Savova et al. (2016). Briefly, 1 mL of sample (the same as that applied for TSS) was homogenized with 300 μ L NaNO₂ and allowed to rest for 5 min. Then, 300 μ L of AlCl₃ was added and the mixture was allowed to rest again for 5 min. Next, 2 mL of 1 M NaOH was added to the mixture and kept for 10 min, and the volume was adjusted to 10 mL by adding distilled water. After incubation for 5–10 min, measurement of OD was measured at 510 nm.

2.11. Statistical analysis

Data analysis was conducted in three replicates based on an experimental design by applying SAS v. 9.1 software and Excel 2016 software. Significant differences between the treatment means were determined using the LSD test at 5 % probability level. If the three-way interaction effect was found to be non-significant, we only discussed and displayed two-way effects. The association between the physiological and biochemical parameters was examined using Pearson's correlation analysis. Correlation analysis was performed using XLSTAT ver. 2019 (Addinsoft, Paris, France) and Principal component analysis (PCA) was performed using R 4.1.0 software.

3. Results

3.1. Effects of water levels and priming on endogenous melatonin content of quinoa

A considerable raise of the endogenous melatonin content was observed in Titicaca (Fig. 1a) and Giza1 (Fig. 1b) due to drought stress. MP (melatonin priming) under 40 % FC (field capacity) enhanced the endogenous melatonin content by 1.6 and 1.7 times in Titicaca and Giza1, respectively, as compared with NP. In non-stressed conditions (100 % FC), MP enhanced the endogenous melatonin content by 2.6 and 2.3 times in Titicaca and Giza1, respectively.

3.2. Effect of water levels and priming on gas exchange parameters

As shown in Table 1, drought stress (40 % field capacity) resulted in the decrease of g_s (stomatal conductance), P_n (net photosynthetic rate) and Tr (transpiration rate), in comparison with the control (100 % FC) in both cultivars. The highest g_s (0.60 mol H₂O m⁻² s⁻¹), P_n (20.20 μ mol CO₂ m⁻² s⁻¹) and Tr (5.61 mmol H₂O m⁻² s⁻¹) were found under 100 % FC, MP and Titicaca cultivar. Under drought stress (40 % FC), MP could enhance g_s by 3.10 and 3.14 times; also P_n was raised by 1.56 and 1.66 times, and Tr 1.20 was increased by 1.18 times in Titicaca and Giza1, respectively, as compared with NP (non-priming). Drought stress could significantly increase C_i (intercellular CO₂ concentration) in both quinoa cultivars. This was partly counteracted by MP, which, in 40 % FC, decreased C_i by 9.7 and 15.6 % in Titicaca and Giza1, respectively, as compared with NP (Table 1). WUE (water use efficiency) was found to be significantly affected by the interactions between W, P and C. MP could increase WUE under drought stress conditions (40 % FC) by 2.45 and 2.20 times in Titicaca and Giza1, respectively, as compared with NP (Table 1). The same effect was seen, though to a lesser extent, under well-watered conditions (100 % FC).

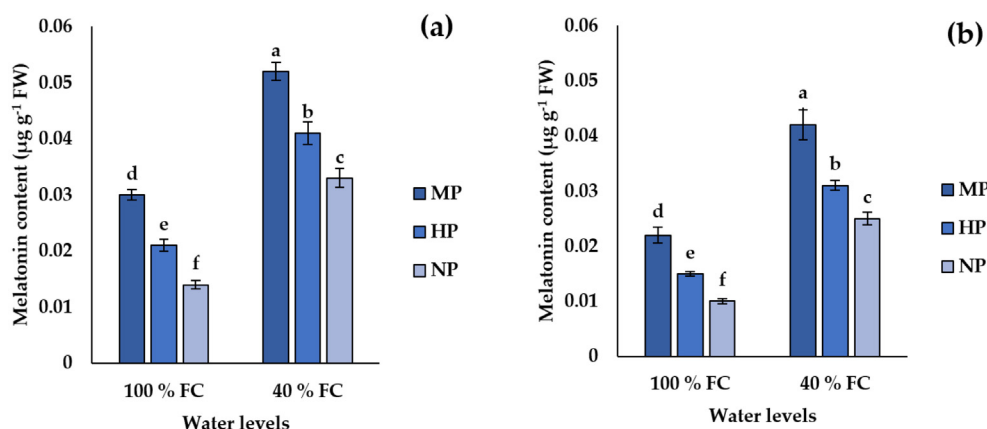


Fig. 1. Impacts of water level and priming treatments on melatonin content of Titicaca (a) and Giza1 (b) cultivars. MP: melatonin priming; HP: hydro priming; NP: non-priming. The data are presented as the means $\pm$ SEs ($n = 3$). Different letters indicate significant differences at $p < 0.05$ according to the LSD test.

3.3. Effect of water levels and priming on photosynthetic pigments of quinoa

The interaction of W (water levels) $\times$ P (priming) $\times$ C (cultivar) did not have a considerable effect on photosynthetic pigments. Watering levels and seed priming had a considerable effect on Chl a (chlorophyll a), Chl b (chlorophyll b), and carotenoid concentrations in both cultivars. The Chl a content was maximum at 100 % FC, but it was decreased at 40 % FC. Drought stress reduced Chl a by 24 % and 20 % in Titicaca and Giza1, respectively (Fig. 2a). The maximum and minimum chlorophyll a contents were observed in Titicaca with MP and Giza1 with NP (0.837 and 0.591 mg g⁻¹ FW, respectively). MP increased chlorophyll a content by 26 % and 51 % in Titicaca and Giza1, respectively, as compared with NP (Fig. 2b). The effect of priming on Chl b was observed as MP increased the chlorophyll b content in both cultivars. The Chl b content was affected by drought stress (Fig. 2c). The maximum chlorophyll b content was observed in Giza1 with MP, with no significant difference from Titicaca with MP (Fig. 2d). Drought stress dramatically decreased carotenoid content by 2.17 and times in Titicaca and Giza1, respectively (Fig. 2e). MP

could significantly enhanced the total carotenoid content as much as 39 and 64 % in Titicaca and Giza1, respectively, as compared to NP plants (Fig. 2f).

3.4. Effect of water levels and priming on production of H₂O₂ and MDA

H₂O₂ (hydrogen peroxide) and MDA (malondialdehyde) contents were significantly elevated by drought stress in both quinoa cultivars. In contrast, plants primed with melatonin showed lower hydrogen peroxide and MDA contents than those primed with HP and NP (Table 2). MP under 40 % FC led to 13.5 % and 41.5 % decreases in H₂O₂ and MDA content in Titicaca, respectively, compared with NP. In 40 % FC, H₂O₂ and MDA contents in Giza1 were shown to be higher than those found in Titicaca (Table 2).

3.5. Effect of water levels and priming on enzymatic antioxidant activity

The interaction of W $\times$ P $\times$ C had a significant impact on SOD (superoxide dismutase), POD (peroxidase), CAT (catalase), and APX (ascorbate peroxidase) activities (Table 2). MP and drought stress

Table 1
Impacts of water level and priming treatments on the gas exchange parameters of quinoa cultivars.

Treatments			Gs (mol H ₂ O m ⁻² s ⁻¹)	Pn (μmol CO ₂ m ⁻² s ⁻¹)	Tr (mmol H ₂ O m ⁻² s ⁻¹)	Ci (μmol CO ₂ mol ⁻¹)	WUE (μmol CO ₂ mmol H ₂ O ⁻¹)
Water levels (W)	Priming (P)	Cultivars (C)					
100 % FC	MP	Titicaca	0.60 ^a	20.20 ^a	5.61 ^a	249.62 ⁱ	3.63 ^c
		Giza1	0.48 ^b	15.41 ^b	4.20 ^b	272.66 ^h	3.67 ^c
	HP	Titicaca	0.36 ^c	13.69 ^c	3.50 ^c	271.00 ^h	3.94 ^{bc}
		Giza1	0.27 ^{de}	9.28 ^d	3.23 ^c	297.65 ^g	2.87 ^d
	NP	Titicaca	0.24 ^{de}	7.33 ^f	3.93 ^b	301.33 ^g	1.86 ^f
		Giza1	0.15 ^{fg} _h	5.84 ^g	2.77 ^d	347.00 ^{de}	2.11 ^{ef}
40 % FC	MP	Titicaca	0.31 ^{cd}	9.13 ^{de}	1.46 ^f	320.20 ^f	6.26 ^a
		Giza1	0.22 ^{ef}	8.28 ^e	1.30 ^g	362.12 ^{bc}	6.25 ^a
	HP	Titicaca	0.19 ^{efg}	6.12 ^g	2.40 ^e	344.00 ^e	4.44 ^b
		Giza1	0.15 ^{fg} _h	5.94 ^g	2.11 ^e	372.33 ^b	4.29 ^b
	NP	Titicaca	0.10 ^{gh}	5.82 ^{gh}	1.21 ^h	354.67 ^{cd}	2.55 ^{de}
		Giza1	0.07 ^h	4.97 ^h	1.01 ⁱ	429.23 ^a	2.83 ^d
Analysis of variance							
	W		**	**	**	**	**
	P		**	**	**	**	**
	C		**	**	**	**	NS
	W × P		**	**	**	*	**
	W × C		NS	**	**	**	NS
	P × C		NS	**	**	*	NS
	W × P × C		NS	**	**	*	*

W, P, and C are the water levels, priming, and cultivars, respectively. MP, HP, and NP represent melatonin priming, hydropriming, and non-priming, respectively. gs: stomatal conductance; Pn: net photosynthetic rate; Tr: transpiration rate; Ci: intercellular CO₂ concentration and WUE: water use efficiency.

Values in the same column followed by different letters indicate statistical significance at $p \leq 0.05$ according to LSD test. NS, *, **, and *** indicate nonsignificant and significant at 5 %, 1 %, and 0.1 %, respectively. Data shown are means of three replicates per treatment.

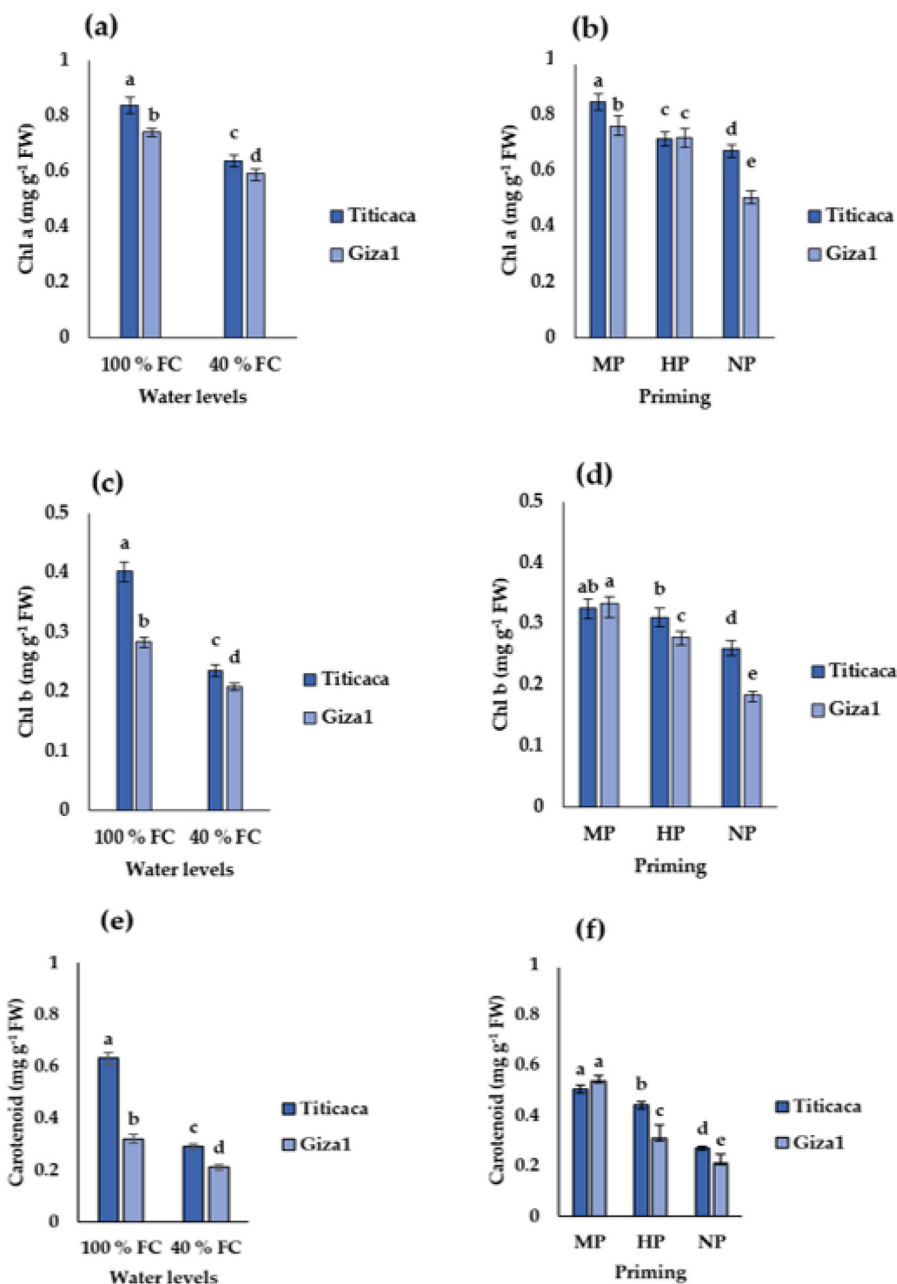


Fig. 2. Impacts of water level and priming treatments on Chl a: chlorophyll a (a and b); Chl b: chlorophyll b (c and d); and carotenoid contents (e and f) of quinoa cultivars. MP: melatonin priming; HP: hydro priming; NP: non-priming. The data are presented as the means $\pm$ SEs ($n = 3$). Different letters indicate significant differences at $p < 0.05$ according to the LSD test.

(40 % FC) in Titicaca resulted in the highest activity of SOD (7.52 U mg⁻¹ protein), POD (0.80 U mg⁻¹ protein), and APX (1.78 U mg⁻¹ protein), which were 1.18, 1.37 and 1.89 times higher than that found in Giza1, respectively; however, the CAT activity in MP under drought stress was not found to be significantly different between cultivars (Table 2). Under non-stressed conditions, Titicaca had a higher antioxidant enzyme content than Giza 1 (Fig. 3a). In both cultivars, a marked increase in SOD, POD, CAT was observed under drought stress as compared with the control, but Titicaca showed a significantly higher of SOD, POD, and APX content in 40 % FC (Fig. 3b).

3.6. Effect of water levels and priming on leaf total soluble protein (TSP), total soluble sugar (TSS) and reducing sugar (RS) contents

Watering level, priming, and cultivar interaction significantly influenced TSP (total soluble protein), TSS (total soluble sugar), and RS (reducing sugar) content. A significant increase in TSP content was observed in both cultivars under drought stress. MP, under 40 % FC, could enhance TSP by 8.3 and 24.3 % in Titicaca and Giza1, respectively, as compared with NP (Table 2). Imposition of drought stress increased TSS and RS content in both cultivars, whereas TSS and RS content in Titicaca was found to be higher than that in Giza1 (Table 2).

Table 2
Impacts of water level and priming treatments on hydrogen peroxide and malondialdehyde contents, antioxidant enzymes, total soluble protein, total soluble sugar and reducing sugar contents of quinoa cultivars.

Treatments			H ₂ O ₂ (μmol g ⁻¹ FW)	MDA (μmol g ⁻¹ FW)	SOD (U mg ⁻¹ protein)	POD (U mg ⁻¹ protein)	CAT (U mg ⁻¹ protein)	APX (U mg ⁻¹ protein)	TSP (mg g ⁻¹ FW)	TSS (mg g ⁻¹ FW)	RS (mg g ⁻¹ FW)
Water levels (W)	Priming (P)	Cultivars (C)									
100 % FC	MP	Titicaca	0.25 ^g	0.32 ^{fg}	5.07 ^d	0.57 ^c	0.47 ^e	0.72 ^d	5.32 ^{bc}	21.18 ^a	9.00 ^{cd}
		Gizal	0.26 ^g	0.23 ^h	3.61 ^f	0.26 ^f	0.45 ^e	0.30 ^e	5.18 ^c	20.22 ^d	8.05 ^d
	HP	Titicaca	0.28 ^{ef}	0.33 ^{ef}	4.43 ^e	0.55 ^d	0.44 ^e	0.30 ^e	4.4 ^{d-f}	23.66 ^a	8.11 ^d
		Gizal	0.26 ^{fg}	0.28 ^{gh}	3.57 ^f	0.14 ^s	0.42 ^e	0.35 ^e	4.27 ^{ef}	18.90 ^d	6.07 ^e
40 % FC	NP	Titicaca	0.29 ^e	0.35 ^{ef}	2.84 ^g	0.50 ^d	0.26 ^f	0.35 ^e	4.22 ^{ef}	18.39 ^d	5.92 ^e
		Gizal	0.28 ^e	0.32 ^{ef}	2.31 ^h	0.13 ^g	0.20 ^f	0.33 ^e	4.08 ^f	13.36 ^e	4.05 ^f
	MP	Titicaca	0.32 ^d	0.38 ^{de}	7.52 ^a	0.80 ^a	1.37 ^a	1.78 ^a	5.72 ^a	34.08 ^a	15.07 ^a
		Gizal	0.35 ^c	0.44 ^c	6.33 ^b	0.58 ^c	1.28 ^a	0.94 ^{bc}	5.68 ^{ab}	29.51 ^a	11.97 ^b
	HP	Titicaca	0.35 ^c	0.42 ^{cd}	5.68 ^c	0.74 ^b	0.80 ^b	1.02 ^b	5.82 ^a	32.28 ^a	10.02 ^c
		Gizal	0.38 ^b	0.54 ^b	5.18 ^d	0.32 ^e	0.68 ^c	0.71 ^d	5.39 ^{bc}	24.24 ^c	9.85 ^c
	NP	Titicaca	0.37 ^b	0.65 ^a	3.87 ^f	0.59 ^c	0.54 ^d	0.78 ^{cd}	5.28 ^c	28.88 ^b	12.01 ^b
		Gizal	0.43 ^a	0.69 ^a	1.75 ^h	0.15 ^g	0.49 ^{de}	0.45 ^e	4.57 ^{de}	15.20 ^e	9.85 ^c
Analysis of variance			**	**	**	**	**	**	**	**	**
	W		**	**	**	**	**	**	**	**	**
	P		**	**	**	**	**	**	**	**	**
	C		**	NS	NS	**	**	**	NS	**	**
	W × P		*	**	**	**	**	**	*	*	*
	W × C		**	**	**	NS	NS	**	**	**	**
	P × C		NS	NS	**	**	**	**	**	**	**
	W × P × C		*	*	**	**	*	*	**	**	**

W, P, and C are the water levels, priming, and cultivars, respectively. MP, HP, and NP represent melatonin priming, hydropriming, and non-priming, respectively. H₂O₂: hydrogen peroxide; MDA: malondialdehyde; SOD: superoxide dismutase; POD: peroxidase; CAT: catalase; APX: ascorbate peroxidase; TSP: total soluble protein, TSS: total soluble sugar and RS: reducing sugar. Values in the same column followed by different letters indicate statistical significance at $p \leq 0.05$ according to LSD test. NS, *, **, and *** indicate nonsignificant and significant at 5 %, 1 %, and 0.1 %, respectively. Data shown are means of three replicates per treatment.

Table 3
Detail of two quinoa cultivars used in this study.

Number	Name	Origin	Characters
1	Titicaca	Denmark	Salinity tolerant, early mature, day length neutral
2	Giza 1	Egypt	Suitable for spring cultivation season, early mature, day length neutral

Under drought stress conditions, MP could raise the levels of TSS and RS by 18 and 25 % in Titicaca and 94 and 22 % in Giza1, respectively, as compared with NP (Table 2). No changes could be observed in TSP, TSS, and RS of the two cultivars under 100 % FC (Fig. 3a). TSP content of Giza1 was found to be higher than that of Titicaca at 40 % FC (Fig. 3b).

3.7. Effect of water levels and priming on non-reducing sugar (NRS) and total flavonoid contents

NRS (non-reducing sugar) and total flavonoid contents were not significantly affected by the interaction of W, P, and C. Seed priming significantly enhanced the NRS content. Results thus revealed that drought stress could increase the NRS content in both cultivars and the maximum NRS (17.4 mg g⁻¹ FW) was found in the Titicaca cultivar under drought stress, whereas the lowest (10.3 mg g⁻¹ FW) was recorded in Giza1 under well water conditions. However, no consistent differences between cultivars were recorded under well-watered conditions (Fig. 4a). Hydropriming in the Titicaca cultivar was effective in enhancing NRS content (Fig. 4b). Drought stress (40 % FC) significantly reduced the accumulation of total flavonoids in both cultivars in a significant manner; in Giza1, as compared to Titicaca, the deleterious impact of drought stress on the total flavonoid content was greater (Fig. 4c). Seed priming significantly increased total flavonoid content, and the highest levels of total flavonoids were found in the MP treatment of both cultivars (Fig. 4d).

3.8. Effect of water levels and priming on seed yield

The interaction between W, P, and C had no appreciable impact on seed yield. Drought stress drastically reduced seed yield, but it significantly increased with seed priming treatments. Seed priming with melatonin had a significant promoting effect on seed yield; accordingly, the highest seed yield was observed in MP under well-watered conditions. Under drought stress, the MP treatment led to a 2.2-fold increase in the seed yield in comparison with NP (Fig. 5a), against a 1.5-fold increase in the 100 % FC treatment. Seed yield was significantly reduced by 2.74 and 2.83 times in the Titicaca and Giza1 cultivars, respectively, under drought stress conditions (Fig. 5b).

3.9. Relationship between seed yield (SY), net photosynthetic rate (Pn) and some biochemical attributes of quinoa

SY (seed yield) and Pn were shown to have a significant association; SY increased as Pn increased, based on the correlation graph (Fig. 6). Similarly, positive associations were discovered between SY and chlorophyll a, b, carotenoid, and flavonoid contents. MEL (melatonin content) had a highly significant positive association with antioxidant enzymes, TSS, RS and NRS. However, there was also a negative but not strong correlation when MEL content was compared with Chl a and b (Fig. 6). Unsurprisingly, the negative correlations of MDA and H₂O₂ with all chlorophyll pigments, flavonoid content, Pn, and SY were found to be significant. This indicates that photosynthetic pigments serve as important limiting growth factors (Fig. 6). A positive and significant interaction was also observed between TSS content with SOD, CAT, APX, TSP and NRS (Fig. 6). Consequently, the findings of the current study showed that higher seed yield and Pn

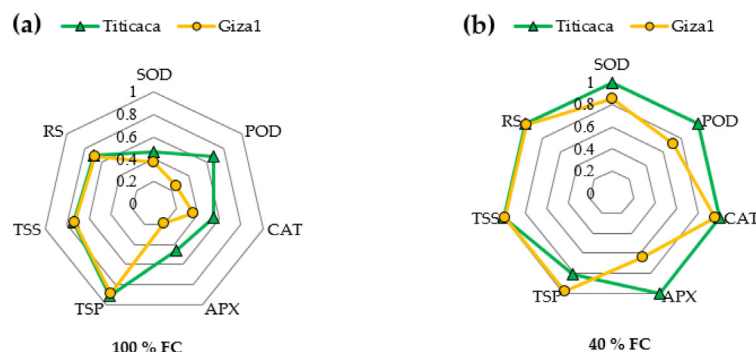


Fig. 3. Radar analysis for changes in relative contents of some biochemical traits in two quinoa cultivars under 100 % FC (a) and 40 % FC (b). SOD: Superoxide dismutase; POD: peroxidase; CAT: catalase; APX: ascorbate peroxidase; TSP: total soluble protein; TSS: total soluble sugar and RS: reducing sugar. Relative contents were calculated as the ratio of the relative content for each parameter to their control sample. Values are mean $\pm$ SE ($n = 3$).

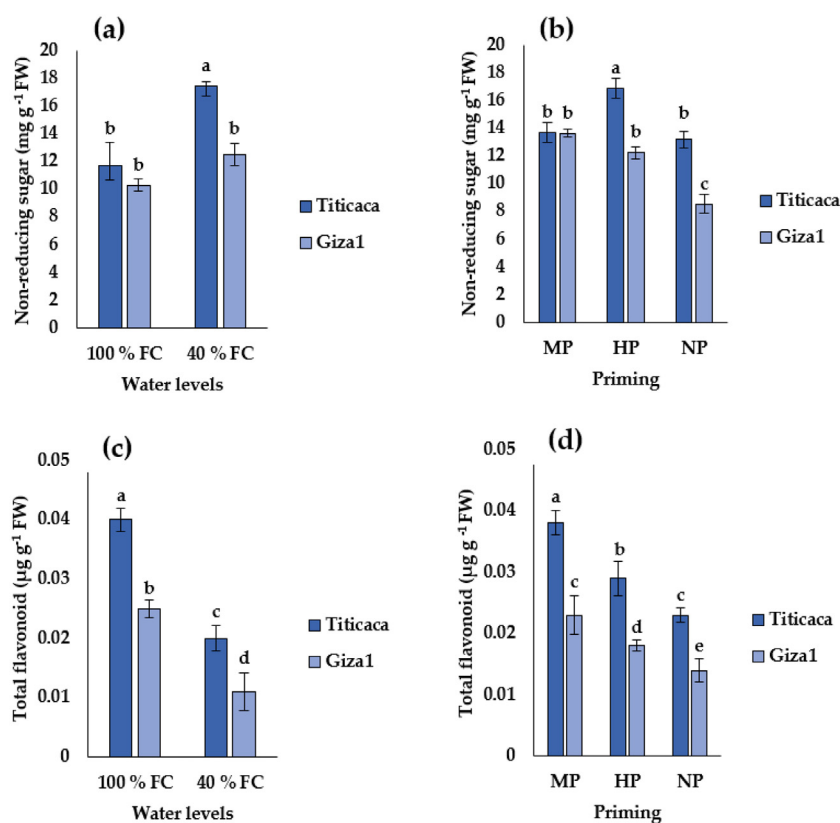


Fig. 4. Impacts of water level and priming treatments on non-reducing sugar (a and b) and total flavonoid contents (c and d) of quinoa cultivars. MP: melatonin priming; HP: hydro priming; NP: non-priming. The data are presented as the means $\pm$ SEs ($n = 3$). Different letters indicate significant differences at $p < 0.05$ according to the LSD test.

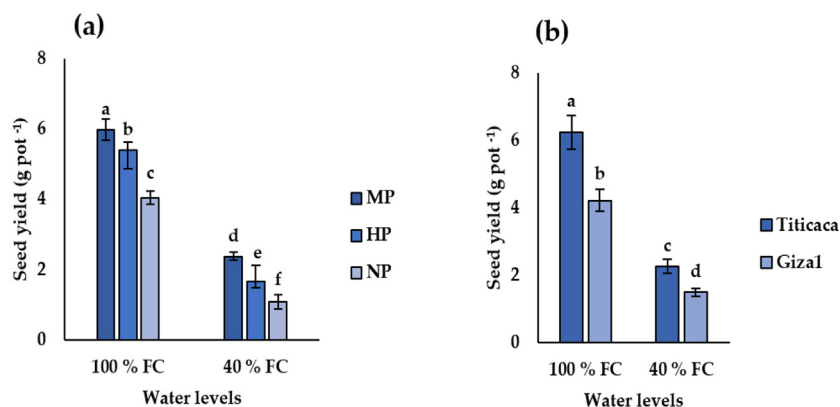


Fig. 5. Response of seed yield to different water levels and priming treatments (a), and to different water levels and quinoa cultivars (b). MP: melatonin priming; HP: hydro priming; NP: non-priming. The data are presented as the means $\pm$ SEs ($n = 3$). Different letters indicate significant differences at $p < 0.05$ according to the LSD test.

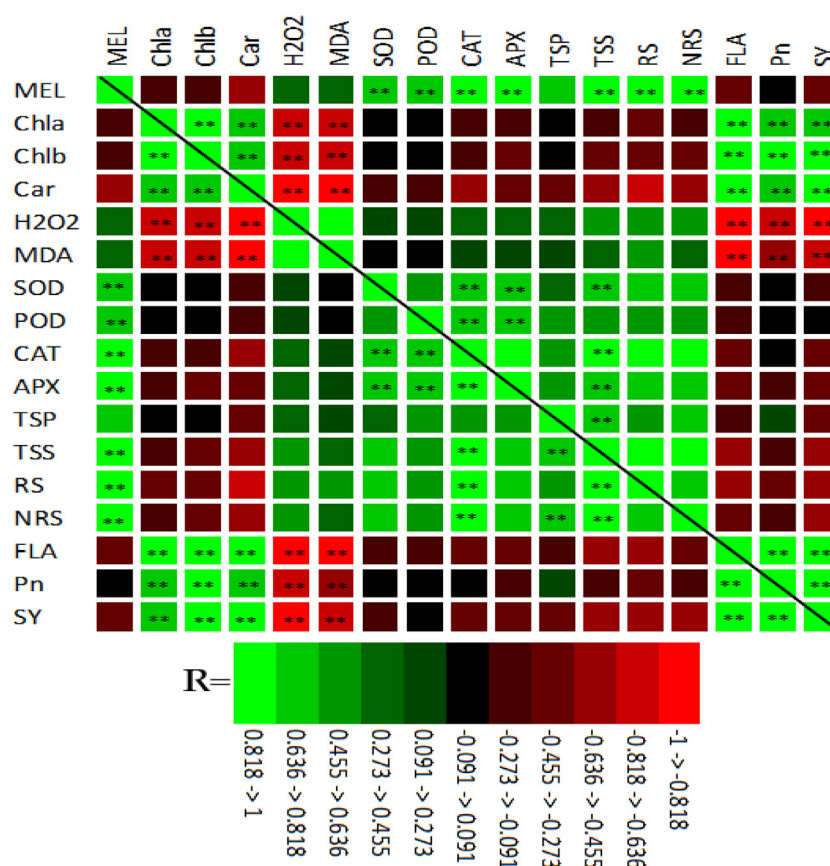


Fig. 6. Correlation analysis of the seed yield and some biochemical traits of quinoa cultivars. MP: melatonin priming; HP: hydro priming; NP: non-priming; MEL: melatonin content; Chl a: chlorophyll a content; Chl b: chlorophyll b content; Car: carotenoid content; H₂O₂: hydrogen peroxide content; MDA: malondialdehyde content; SOD: superoxide dismutase; POD: peroxidase; CAT: catalase; APX: ascorbate peroxidase; TSP: total soluble protein content; TSS: total soluble sugar content; RS: reducing sugar content; NRS: non reducing sugar content; FLA: flavonoid content; Pn: net photosynthetic rate and SY: seed yield. ** Significant at $p \leq 0.01$. * Significant at $p \leq 0.05$.

were associated with photosynthetic pigments and flavonoid content and higher melatonin content was associated with antioxidant enzymes and sugars.

3.10. Principal component analysis

The principal component analysis (PCA) for biochemical traits under 100 %FC could explain 85.2 % of overall variance, with PC1 could account for 70.2 % and PC2 15 % of the variance, respectively (Fig. 7a). The angles between the vectors in the PC biplot show the approximate relationship of the variables. In addition, the distance between the biochemical traits indicates the relationship between them. APX, SOD, Chl a and MEL are closer to the vector of Pn. Melatonin priming in Titicaca under 100 % FC has nearest association with POD and Chl b. This result shows that Titicaca has been more predominant for these traits (Fig. 7a).

Under 40 % FC the PC plot indicated that the first two principal components account for 87.5 % of the total variance, with PC1 could account for 75.2 % and PC2 12 % of the variance, respectively (Fig. 7b). The most important antioxidant contributors are APX, SOD and MEL that closer to the vector of Pn, play an important role to increase Pn and SY under 40 % FC. Additionally angles between vectors indicated that SOD, CAT, TSS and NRS showing association with Pn. Antioxidant enzymes (APX, POD, SOD and CAT), osmolytes (FLA, RS) and MEL were grouped together with acute angle in Titicaca and MP. These parameters positively contributed for Titicaca in drought tolerance. Additionally, melatonin priming in Giza1 located near to Chl a and Chl b. NP in Titicaca and Giza1 were located near the MDA and H₂O₂, which indicate that non priming in both cultivars had the most ROS production (Fig. 7b).

4. Discussion

In this research study, an increase in melatonin content could be observed under stressed conditions (Fig. 1). Under abiotic stresses melatonin might act as a signal molecule, as it upregulates anti-stress genes and endogenous melatonin levels under stress conditions (Zhao et al., 2015). Previous studies have also suggested that upregulated expression of melatonin biosynthesis genes, may be considered responsible for the increased endogenous melatonin content which was observed after exposing plants to extreme environmental conditions (Zhang et al., 2014; Wei et al., 2015). The significant increase of melatonin contents in the MP plants of both cultivars supported its potential role in stress tolerance (Fig. 1). Additionally, MP in Titicaca (Fig. 1a) and Giza1 (Fig. 1b) was found to have greater endogenous melatonin content compared to HP and NP. This may due to the absorption of exogenous melatonin or exogenous application of melatonin induced the expression of melatonin biosynthesis genes (Shreya et al., 2022). Based on many investigations, plants which were treated with melatonin under stress conditions displayed an increase of endogenous melatonin content stress (Shi et al., 2015; Arnao and Hernández-Ruiz, 2009; Arnao and Hernández-Ruiz, 2013).

Photosynthesis is a primary physiological process affected by heat stress and drought in plants. The reduction in leaf expansion, incorrect operation of the photosynthetic machinery, and leaf aging are regarded as the key factors affecting leaf expansion (Fahad et al., 2017). In our study, melatonin priming significantly increased g_s and Pn in comparison with the control in both cultivars (Table 1), which, together with the concomitant increase in WUE, might explain why MP accumulated more photosynthetic assimilates under drought stress, thus corroborating previous studies (Ye et al., 2016).

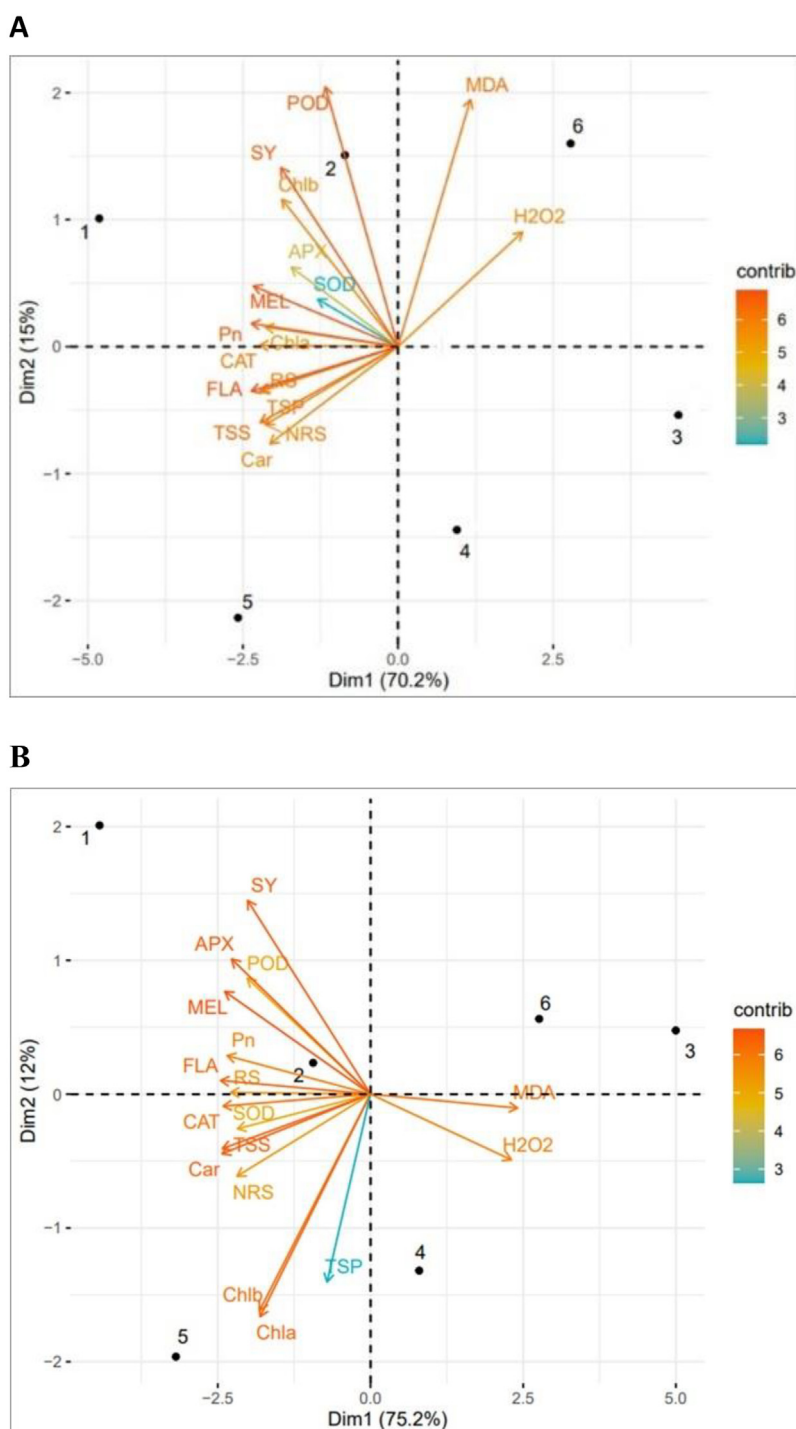


Fig. 7. Principal component analysis (PCA) plot of biochemical traits in two quinoa cultivars (Titicaca and Giza1) and three priming (MP, HP and NP) in (a) 100 % FC and (b) 40 % FC. MEL: melatonin content; Chl a: chlorophyll a content; Chl b: chlorophyll b content; Car: carotenoid content; H₂O₂: hydrogen peroxide content; MDA: malondialdehyde content; SOD: superoxide dismutase; POD: peroxidase; CAT: catalase; APX: ascorbate peroxidase; TSP: total soluble protein content; TSS: total soluble sugar content, RS: reducing sugar content, NRS: non reducing sugar content, FLA: flavonoid content; Pn: net photosynthetic rate and SY: seed yield. 1–6 stand for code of the cultivars- priming interaction. 1: Titicaca-HP; 2: Titicaca-MP; 3: Titicaca-NP; 4: Giza1-HP; 5: Giza1-MP and 6: Giza1-NP.

Melatonin enhances photosynthetic ability by improving stomatal conductance under stress conditions (Alinia et al., 2021). In addition, it was found to increase photosynthetic capacity by raising the leaf content of Rubisco, consequently improving shoot dry weight (Zhao et al., 2015). The latter is consistent with our results, as both well-watered and drought-stressed plants displayed improved Pn and yield when the seeds were treated with melatonin (Table 1; Fig. 5b).

Thus, our study indicated that the highest reduction in pigment content (Chl a, Chl b) and increased intercellular CO₂ concentration (Ci) were observed in Giza1 under drought stress (Fig. 2a, c; Table 1). These results imply that drought stress was severe (Lawlor and Tezara, 2009) and that the induced oxidative damage to the photosynthetic apparatus was more severe in Giza1 than in Titicaca, while MP mitigated the decrease in the concentration of Chl and carotenoids (as an essential part of photo-protection) in both cultivars



Fig. 8. Two quinoa cultivars investigated in this study: Titicaca (A and B) and Giza1 (C and D).

(Fig. 2). The reduction in photosynthetic pigments induced by drought stress has been previously documented in quinoa (Aziz et al., 2018), mung bean (*Phaseolus vulgaris* L.) (Uddin et al., 2021) and spinach (*Spinacia oleracea* L.) (Bukhari et al., 2021). A probable cause for the pigments degradation in stressful situations is the oxidation and breakdown of chlorophyll precursors, blockage of the stimulus of chlorophyll formation, induction of chlorophyll damaging enzymes such as chlorophyllase, leading to the destruction of chloroplasts (Naghizadeh et al., 2019). The decreased amounts of carotenoids under drought stress might be attributed to β -carotene disintegration and interference with the xanthophyll cycle, which produces zeaxanthin (Sultana et al., 1999). Melatonin priming can increase chlorophyll content and photosynthetic rates by either directly or indirectly delaying protein breakdown, lowering ROS, and boosting antioxidant capacity (Wang et al., 2013; Gibas et al., 2014). Similarly, MP elevated chlorophyll content in rapeseed (Khan et al., 2019) and maize (*Zea mays* L.) (Ye et al., 2016) when exposed to drought stress. In this study, melatonin priming had significant effects on carotenoid content in both cultivars (Fig. 2f). The induction of carotenoid biosynthesis through MP could be related to its protective function in the photosynthetic machinery because this pigment becomes chargeable for scavenging ROS, stopping lipid peroxidation, and lowering oxidative stress (Zhang et al., 2013).

Thus, our study confirmed that H_2O_2 content increased with drought stress in both quinoa cultivars, but MP decreased ROS production (Table 2). When plants are exposed to environmental stress conditions, they commonly produce ROS, such as H_2O_2 (Alharby and Fahad, 2020). ROS production and accumulation may cause

mitochondrial dysfunction, impairment of the antioxidant system, protein lipid oxidation, and cell's death (Mittler, 2002; Alharby and Fahad, 2020). As a very common ROS, H_2O_2 participates in several processes such as cell growth, development, and stress responses (Alinia et al., 2021). Our results also indicated that the MDA content was lower in MP plants under drought stress in both cultivars (Table 2). As melatonin is thought to be a lipophilic and hydrophilic molecule, it may be found in cytoplasmic and lipid membranes (Alharby and Fahad, 2020). Under such stressful circumstances, lipid peroxidation could be regarded as a sign of cell membrane deterioration, and the increase in MDA content could be generally considered as a reliable measure of oxidative damage, thus indicating perturbation of the cell membrane's stability (Zhang et al., 2021). Although the production of oxygen free radicals and MDA was lower in the tolerant cultivar (Titicaca) than in the sensitive cultivar (Giza1), it was further decreased by melatonin priming (Table 2). Overall, these results highlight that melatonin could serve a key function in ROS scavenging and the relief of oxidative stress by inducing antioxidant enzyme activity. This could facilitate the preservation of cell balance and integrity, decrease harm to cells, and improve recovery from drought stress (Arnao and Hernández-Ruiz, 2014). Research has also revealed the role of melatonin as a brake on lipid peroxidation, which may be associated with its capacity to react with lipid alkoxy ($LO^\bullet$) and lipid peroxy ($LOO^\bullet$) radicals, thus retarding the process of peroxidation (Li et al., 2012; Zhang et al., 2013). Melatonin maintains the redox state of membrane proteins by suppressing the oxygen radicals generated by drought stress. In addition, MP could mitigate MDA storage under normal and stressed conditions, as also confirmed by Khan et al. (2020) in rapeseed plants (Khan et al., 2020).

Reactive oxygen species are actively scavenged by a range of antioxidant enzymes, lipids, and water-soluble compounds in plants. Antioxidant enzymes are thought to be most effective in minimizing oxidative damage (Saad et al., 2020). We found that SOD, POD, CAT, and APX activities were notably higher in the Titicaca cultivar than in Giza1, thus indicating the high protection ability of Titicaca against oxidative stress under drought (Table 2). This is also supported by previous studies (Aziz et al., 2018; Lin and Chao, 2021). Alharby and Fahad (2020) claimed that the increased production of ROS and concurrent increases in membrane lipid peroxidation, as opposed to the decrease in antioxidant ability to quench ROS, can be responsible for the decrease in antioxidant activities in a vulnerable cultivar (Alharby and Fahad, 2020). In our study, melatonin-pretreated plants under drought stress showed increased antioxidant enzyme activity (Table 2; Fig. 1) because of their higher endogenous melatonin content. Melatonin upregulates the transcript levels and stimulates the activities of antioxidant enzymes (Arnao and Hernández-Ruiz, 2019). It also regenerates some endogenous antioxidants to enhance cellular antioxidant system (Arnao and Hernández-Ruiz, 2009). These results are supported by the strong and positive association between melatonin content and antioxidant enzyme activity (Fig. 6). These findings imply that MP could play a noteworthy role in increasing the activities of enzymes and lowering ROS levels in drought stress tolerance in Titicaca. Previous studies have also indicated this mechanism of action (Khan et al., 2019; Alharby and Fahad, 2020; Bidabadi et al., 2020; Hossain et al., 2020).

Cellular osmotic adjustment is of particular importance to endure drought-induced stress resulting from a drought. Plants maintain cell turgor pressure at a certain level and modify several physiological functions to delay and survive the prevailing dehydration conditions (Razzaghi et al., 2015; Alharby and Fahad, 2020). Drought stress denatures protein and enzyme structures. Osmo protectants are substances that plants use to prevent them (Bukhari et al., 2021). Our study, therefore, indicated that the TSP content of melatonin-treated seeds was greater than that of dry seeds under drought stress conditions (Table 2; Fig. 3). TSPs can be regarded as necessary materials that play a considerable role in drought stress tolerance of plants

(Arnao and Hernández-Ruiz, 2019). Therefore, the increase in TSP could be due to the accumulation of LEA proteins. By attaching water molecules to their hydrophilic surfaces, LEA proteins can play a crucial role in membrane protein stability, osmotic adjustment, and macromolecular stabilization, which could lead to reversing or stopping further denaturation of cellular proteins during drought stress (Smith and Graether, 2022). Melatonin can increase soluble protein content by reducing excessive intracellular ROS and, at the same time, suppresses protein degradation or facilitates the synthesis of new proteins under drought stress (Gao et al., 2019). These results agree with those of other studies, where soluble protein content was found to be increased in plants treated with melatonin under conditions of drought stress (Cao et al., 2019; Alharby and Fahad, 2020).

In our study, soluble sugar, reducing, and non-reducing sugar contents increased considerably in the Titicaca cultivar under drought stress conditions (Table 2; Fig. 4a), which is in accordance with other studies on quinoa (Aziz et al., 2018) and rice (*Oryza sativa* L.) (Wang et al., 2019). This indicated that this cultivar was more tolerant than the other one. Higher rates of soluble sugar accumulation in plants appear to be associated with drought tolerance (Iqbal et al., 2018). This is demonstrated by the ability of sugars to reduce water loss, support osmotic adjustment and thus maintain high turgor, reduce cellular damage, and maintain photosynthetic mechanisms for plant growth and development under drought stress conditions. High levels of soluble sugars have been shown to act as ROS scavengers, and sugar availability may lead to the feeding of oxidative pentose phosphate, which can initiate the ROS scavenging process (Kobylnska et al., 2018). In addition, we found that seeds primed with melatonin increased the amount of TSS, RS, and NRS (Table 2, Fig. 4b), which is in line with many previous studies (Sarropoulou et al., 2012; Naghi-zadeh et al., 2019). Melatonin participates in readjusting the osmotic potential and osmolyte accumulation of cells in response to drought stress, such as soluble sugars (Ahmed et al., 2020).

Results of our study, thus, provided the confirmation that total flavonoids were reduced in quinoa plants kept under stress conditions, whilst MP counteracted this decrease (Fig. 4c and d). Secondary metabolic compounds, including flavonoids, are considered essential for scavenging ROS and decreasing oxidative stress in plants (Ma et al., 2014; Nichols et al., 2015). Total flavonoid content was significantly increased in buckwheat (*Fagopyrum esculentum* L.) treated with melatonin (Tiwari et al., 2021). Aziz et al. (2018) also found that flavonoid content was reduced in quinoa under severe drought stress (Aziz et al., 2018). Such contrasting effects could be due to species-specific effects or the intensity of hydric stress in plant cells and tissues. The results obtained in this study revealed that the enhanced flavonoid content of quinoa cultivars primed with melatonin was not fully capable of relieving the harmful consequences of drought stress, thus leading to a decrease in seed yield. Based on these results, the protective effect of melatonin on quinoa plants is related to the activation of the antioxidant protection system.

This study also revealed that drought stress could be associated with a significantly stronger decrease in seed yield in Giza1 plants than in Titicaca plants (Fig. 5b). The stronger yield decrease in the former cultivar could be ascribed to higher oxidative damage, which conformed to previous research conducted on quinoa (Iqbal et al., 2018; Aziz et al., 2018; Nadali et al., 2020). The drought-induced reduction in yield might be due to a decrease in chlorophyll content and photosynthetic rate (Fahad et al., 2017). As shown in Fig. 6, there was a strong and negative correlation between seed yield and H_2O_2 , MDA levels. Hence, the decline in quinoa growth under stress conditions could be explained by the increase in ROS production. However, MP led to improved seed yield in both cultivars under drought stress and under well-watered conditions (Fig. 5). Melatonin, which could be regarded as a growth promoter, has been shown to be involved in many aspects of plant stress tolerance (Arnao and Hernández-Ruiz, 2014). We found that MP significantly increased antioxidant enzyme

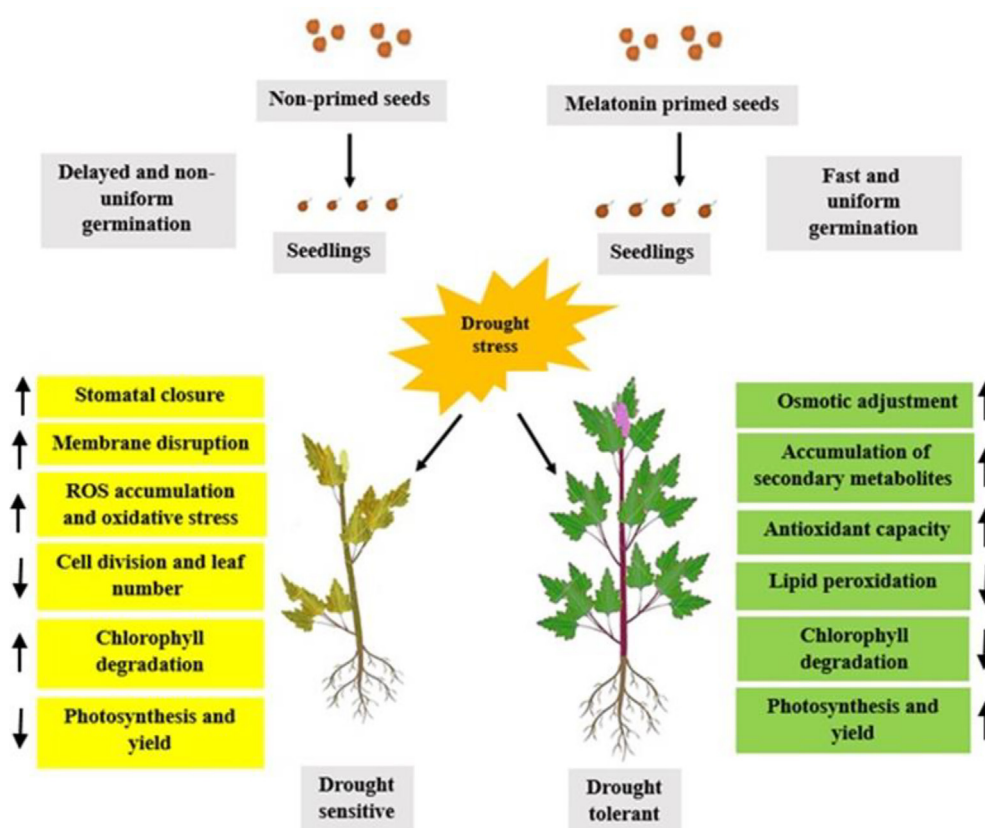


Fig. 9. A schematic model figure showing how melatonin priming confers drought stress in the quinoa cultivars.

activity and reduced H₂O₂ and MDA contents under both irrigation treatments. The priming effects found in 100 % FC seem to support the notion that plants are frequently stressed, even under well-watered conditions, as simple fluctuations in light levels may boost ROS production. It is likely that the higher activity observed in antioxidant enzymes contributed to enhancing the photosynthetic efficiency and improved the production of secondary metabolites, soluble protein, and sugar content in plants grown under stressful conditions (Mohamed et al., 2020). Under drought stress conditions, the yield increase due to MP, as compared with NP, has been previously reported in rapeseed (Khan et al., 2020). The induced tolerance to drought stress could be due to melatonin-induced changes in the expression of genes involved in the oxidative defence system and osmoprotection (Khan et al., 2020). These results clearly indicated that Titicaca displayed higher endogenous melatonin content and antioxidant activity than Giza1 under drought stress.

The results of PCA plot indicated that in drought stress conditions melatonin priming leads to increase in melatonin content and antioxidant enzyme activities in Titicaca more than Giza1. Melatonin priming reduced contents of H₂O₂ and MDA under drought stress. Also oxidative stress was lower in the tolerant cultivar (Titicaca) than in the sensitive cultivar (Giza1). Melatonin enhanced the antioxidant machinery by reduction of over-accumulation of ROS, which is primarily due to enhanced resilience to drought stress (Khan et al., 2020; Khan et al., 2019). The PCA biplot showed several significant biochemical parameters as potent explanatory factors, which could account for quinoa drought tolerance, thus suggesting their usefulness for cultivar screening. In particular, the higher accumulation of melatonin content and osmolytes, as well as the improved content of antioxidants, are promising traits that could be applied as potential selection markers for screening drought-tolerant quinoa cultivars. Therefore, it is plausible and logical to infer that Titicaca has better potential for drought tolerance than Giza1, which is susceptible to drought stress.

5. Conclusions

In summary, the above results showed that MP improved the drought stress tolerance of quinoa cultivars. MP had a significant protective effect on quinoa seed yield during drought stress, which led to an increase in endogenous melatonin content, the effective alleviation of membrane lipid peroxidation and the formation of H₂O₂ through osmolyte production and antioxidant system activation. The higher antioxidant enzyme activity observed in Titicaca was associated with enhanced Pn and compatible organic material accumulation in comparison with Giza1, which produced higher MDA and H₂O₂ contents. The current study, thus, highlighted the important role of melatonin in quinoa in stimulating the number of pigments, antioxidant defense systems, osmolyte production, and sugar and protein content for increasing or maintaining the yield (Fig. 9). These findings could open a new avenue for research on the molecular mechanisms of melatonin's contribution to drought stress tolerance in quinoa and other crops.

CRedit authorship contribution statement

Maryam Samadi: Conceptualization, Formal analysis, Writing – original draft. **Seyed Abdolreza Kazemeini:** Conceptualization, Methodology, Formal analysis, Data curation, Writing – original draft, Writing – review & editing. **Fatemeh Razzaghi:** Methodology, Formal analysis. **Mohsen Edalat:** Methodology, Formal analysis. **Mathias Neumann Andersen:** Data curation, Writing – review & editing. **Sven-Erik Jacobsen:** . **Andrea Mastinu:** Data curation, Writing – review & editing, Funding acquisition.

Data availability

The availability of data will be provided upon appropriate request to the corresponding authors.

Declaration of competing interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

CRedit authorship contribution statement

Maryam Samadi: Conceptualization, Formal analysis, Writing – original draft. **Seyed Abdolreza Kazemeini:** Conceptualization, Methodology, Formal analysis, Data curation, Writing – original draft, Writing – review & editing. **Fatemeh Razzaghi:** Methodology, Formal analysis. **Mohsen Edalat:** Methodology, Formal analysis. **Mathias Neumann Andersen:** Data curation, Writing – review & editing. **Andrea Mastinu:** Data curation, Writing – review & editing, Funding acquisition.

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