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Comparative physiological and biochemical mechanisms of drought tolerance in three contrasting cultivars of quinoa (*Chenopodium quinoa*)

Yemeng ZHANG[1], Qian YANG[2], Lili ZHU[3], Zhiguo CHEN[4*]

[1,2,3,4]Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining 810001, P.R. China

[1,2,3,4]Institute of Three-River-Source National Park, Chinese Academy of Sciences, Xining 810001, P.R. China

[1,3,4]University of the Chinese Academy of Sciences, Beijing 100081, P.R. China

[*]Correspondence: zgchen@nwipb.cas.cn
[1]https://orcid.org/0000-0003-0676-5904, [2]https://orcid.org/0000-0003-2540-6456
[3]https://orcid.org/0000-0002-9988-4306, [4]https://orcid.org/0000-0001-5474-2606

Abstract. Quinoa (*Chenopodium quinoa Willd.*) is a halophytic, pseudocereal crop, which has a richer nutritional value than other major cereals and is highly resistant to multiple abiotic stresses. In this study, the germination characteristics, morphological, physiological and biochemical changes of three contrasting quinoa cultivars under drought stress were compared. The results indicated that 'Chaidamuhong' and 'Gongzha No.3' showed stronger drought tolerance than 'Qingli No.1'. This was mainly manifest in seed germination index, activity of antioxidant enzymes, cell membrane damage and morphological changes. We speculate that the increase in the activity of many antioxidant enzymes and the lower stomatal density make 'Chaidamuhong' and 'Gongzha No.3' superior in release of reactive oxygen species and water retention than 'Qingli No.1', thus reducing the degree of cell damage, and improving drought resistance.

Keywords. Quinoa, drought, reactive oxygen species, antioxidants, germination.

Resumen. La quinua (*Chenopodium quinoa Willd.*) es un cultivo de pseudocereal halófilo, que tiene un valor nutricional más rico que el de otros cereales importantes y es altamente resistente a múltiples estreses abióticos. En este estudio, se compararon características de germinación, cambios morfológicos, fisiológicos y bioquímicos de tres cultivares de contrastantes quinua bajo estrés por sequía. Los resultados indicaron que 'Chaidamuhong' y 'Gongzha No.3' mostraron una mayor tolerancia a la sequía que 'Qingli No.1'. Esto se manifestó principalmente en el índice de germinación de las semillas, la actividad de las enzimas antioxidantes, el daño de la membrana celular y los cambios morfológicos. Especulamos que el aumento en la actividad de muchas enzimas antioxidantes y la menor densidad estomática hacen que 'Chaidamuhong' y 'Gongzha No.3' sean superiores en la liberación de especies reactivas de oxígeno y la retención de agua que 'Qingli No.1', reduciendo así el grado de daño celular y mejorando la resistencia a la sequía.

Palabras clave. Quinoa, sequía, especies de oxígeno reactivo, antioxidantes, germinación.

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INTRODUCTION

Drought is an important factor that negatively affects seed germination, plant growth, and crop yield. Rapid climate change has further increased the risk of drought in arid and semi-arid areas, which may threaten food security and production. In such alarming circumstances, it is important to ensure a sustained increase in the food supply (Mensbrugghe & al. 2009). Therefore, in a deteriorating environment, the development of some halophytic crops such as quinoa is the best way to cope with the growing demand for food (López-Marqués & al. 2020). Quinoa originated in the Andean region of South America and has a history of approximately 7,000 years under human cultivation (Dillehay & al. 2007; Zurita-Silva & al. 2014). Quinoa has rich nutritional value, containing protein, starch, dietary fiber, oil, and many minerals (Vega-Gálvez & al. 2010), and has the ability to grow under adverse environments, such as soil salinity (Parvez & al. 2020), drought (Hinojosa & al. 2019), and heat (Ivanov & al. 2017). The Food and Agriculture Organization of the United Nations (FAO) defined 2013 as the "Year of quinoa" and identified that quinoa plays an important role in ensuring food security in the future (Choukr-Allah & al. 2016). The cultivation and investigation of quinoa are increasing rapidly all over the world. It is cultivated in more than 95 countries (Jacobsen

2003). As a new crop introduced into China, it is necessary to evaluate its adaptability and stress resistance. Different quinoa resources were systematically screened, and their agronomic characteristics were identified in order to evaluate the adaptability of quinoa in the Qinghai-Tibet Plateau to provide a theoretical basis for its cultivation and breeding.

In order to tolerate drought conditions, halophytic plants have evolved a variety of physiological mechanisms, such as osmotic regulation (Gámez & al. 2019), enhanced antioxidant response (Hinojosa & al. 2019), ion accumulation or excretion, ion dynamic balance (Cai & Gao 2020) in order to maintain plant growth. Chenopodium quinoa responds to water deficits in seed germination characteristics and stomatal density (Gámez & al. 2019), phenotypic and physiological changes (Bascuñán-Godoy & al. 2016; Aziz & al. 2018), and biochemical adaptation (Bohnert & Jenson 1998). The evaluation of seed germination resistance enables us to identify tolerance in the early growth stage, which can save time for detecting tolerance under drought conditions. In addition, the ability of quinoa to tolerate drought stress differs. Some studies have shown that drought-tolerant cultivars have lower water loss rate and cell damage than sensitive cultivars (Amjad & al. 2020). Sufficient evidence has shown that drought stress can lead to excessive production of reactive oxygen species (ROS) in quinoa (Iqbal & al. 2018; Yang & al. 2020;), such as hydrogen peroxide (H_2O_2), and superoxide radical (O^{2-}), and hydroxyl (HO^-) ions. These ROS can cause serious damage to proteins, DNA, lipids, and chlorophyll in plant cells (Raja & al. 2017). In the process of plant evolution, a variety of antioxidant enzymes have been developed to eliminate ROS, including the antioxidant enzymes superoxide dismutase (SOD), peroxidase (POD), peroxidase, catalase (CAT), glutathione peroxidases (GPX), and glutathione reductase (GR) (Hasanuzzaman & al. 2020). However, the activities of these antioxidant enzymes are also different among different cultivars. Tolerant cultivars have higher antioxidant enzyme activities than sensitive cultivars.

In this experiment, three kinds of quinoa cultivars were selected to compare seed germination and physiological and biochemical responses to drought stress. These three quinoa cultivars are suitable for planting in arid and semi-arid regions of Northwest China, but there are differences in their tolerance to drought. Therefore, The purpose of this study was to: (i) detect the germination characteristics of three, contrasting, plateau quinoa cultivars under PEG-6000 stress; (ii) evaluate the degree of cell damage in three contrasting plateau quinoa cultivars under water deprivation; (iii) compare the activities of antioxidant enzymes in three contrasting plateau quinoa cultivars under water deprivation; (iv) explore the differences in tolerance of drought stress due to the different activities of antioxidant enzymes in different quinoa cultivars; and (v) screened the drought-resistant quinoa by seed germination index, activity of antioxidant enzymes, cell membrane damage and morphological changes.

MATERIAL AND METHODS

Plant material

Seeds of three quinoa cultivars (Table 1) were provided by Qinghai Seed Management Station in Ulan County, Qinghai Province, China. The quinoa seeds were screened by a 1.5 mm mesh and the seeds of good maturity, fullness, and of the same size were selected. The selected seeds were soaked in 70% ethanol for 5 min to sterilize them, after which they were washed three times in distilled water. After that, the seeds were stored at 4 °C in a refrigerator for later use. The content of protein, fat and starch was determined by the Analysis and Testing Center of Northwest Plateau Institute of Biology, Chinese Academy of Sciences. Qingli No.1, Chaidamuhong, and Gongzha No.3 have protein contents of 14.5%, 14.7%, and 14.0%, fat contents of 5.7%, 4.4.0%, and 6.0%, and starch contents of 21.8%, 19.0%, and 17.0%, respectively.

Table 1. Information of three quinoa cultivars used in this study.

Plant name	Kilo-grain Weight (g)	Planting	Origin	Ecotype
Qingli No.1	3.44	Northeast edge of Chaidamu basin	Bolivia	Alpine
Chaidamuhong	3.31	Northeast of Chaidamu basin	Bolivia	Alpine
Gongzha No.3	3.46	Hinterland of qing-hai-tibet plateau	Bolivia	Valley

Seed germination test

The 15% and 25% PEG-6000 (bought from Sangon Biotech in Shanghai, China) solutions were prepared, and distilled water was used as a control. Then, 12 mL of each gradient solution was poured into a germination box (12 cm × 12 cm × 6 cm) covered with two layers of filter paper. Thirty seeds were placed in the germination box and incubated in a growth chamber at 20 **°C**, a 16-h photoperiod, an irradiance of 150 μmol $m^2 \cdot s^{-1}$, and a relative humidity of 65 % to induce germination. Taking a radicle length of more than 2 mm as the standard, the seed germination number was counted every day for 7 d, and the seed germination rate (GR), germination potential (GP), germination index (GI), mean germination time (MGT), and seed germination index of drought resistance (PIS / PIC) were calculated.

Seedling growth physiological test.

Three, 2-week-old quinoa seedlings of similar size were selected and transplanted into vermiculite pots (every pot contained 0.13 kg vermiculite), and placed in the cultivation cabinet. The cabinets containing the quinoa seedlings are randomly placed, and the positions are changed randomly each week. Dehydration treatment was carried out by withholding water in the vermiculite pot for about a month. The quinoa samples were harvested at 1, 7, 14, 21, 28, and 35 d after treatment to determine various physiological and biochemical indices.

Measurement of germination index

The seeds germination situation and the number of germinated seeds were recorded every day. The seeds were germinated for eight days to calculate the final germination.

$GR = \left(\frac{\text{Germinated seed number}}{\text{Test seed number}}\right) \times 100\%$

$GP = \frac{\sum Gi1\sim4}{\text{Test seed number}}$, where Gi is the number germination at the ith day.

$GI = \Sigma\left(\frac{Gi}{Ti}\right)$, where Gi is the number germination at the ith day (Wang & al. 2004).

$MGT = \frac{\Sigma(f \times i)}{\Sigma f}$, where f is the number of newly germinated seeds at the ith day (Ellis & Roberts 1980).

$\frac{PIS}{PIC} =$; seed promptness index under water stress (PIS)/ controlled seed promptness index (PIC);

$\text{Promptness index}\ (PI) = Gi2 \times (1.00) + Gi4 \times (0.75) + Gi6 \times (0.50)$; where Gi is the number germination at the ith day (Ranal & Santana 2006).

Measurement of leaf water content

The same three plants were harvested and the fresh weight (FW) was determined immediately, and the dry weight (DW) was determined after 24 h of incubation at 80 °C. $\text{Leaf water content}\ (LWC) = \left(\frac{FW - DW}{FW}\right) \times 100\%$.

Measurement of proline content

Proline contents of the quinoa experimental group and control group were estimated with standard L-proline. Briefly, the sample (0.5 g) was extracted in 3% (w/v) sulfosalicylic acid before 2 mL of ninhydrin reagent and 2 mL of glacial acetic acid were added. Well mixed solutions were boiled at 100 °C for 40 min. After cooling to room temperature, the absorbance at 520 nm was measured (Bates & al. 1973).

Measurement of electrolyte leakage (EL)

For the EL assay, approximately 0.1 g of leaves were placed in 10 mL of double distilled water and shaken at room temperature for 6 h. The initial conductivity (Ci) was measured, and the mixture was boiled for 20 min to completely induce all electrolytes. After cooling to room temperature, the ultimate conductivity (Cmax) was determined (Luo & al., 2011). $EL = \left(\frac{Ci}{Cmax}\right) \times 100\%$

Measurement of malondialdehyde

Malondialdehyde (MDA) contents of the quinoa experimental group and control group was measured with thiobarbituric acid (TBA), as previously reported (Shi & al. 2012). Samples (0.5 g) were ground in 2.5 mL of reagent (0.25% (w/v) TBA in 10% (w/v) trichloroacetic acid), and then boiled at 100 °C for 20 min. The MDA content was determined by subtracting the non-specific absorption at 600 nm from the absorbance of the sample supernatant at 532 nm.

Measurement of H_2O_2 level and antioxidant enzyme activities

The extraction procedures for antioxidant enzymes and H_2O_2 were carried out at 4 °C. Samples of quinoa leaves (0.5 g) were crushed and mixed in 2 mL extraction buffer (0.1 M potassium phosphate buffer with 0.1 mM EDTA, pH 7.0), and centrifuged in 15000 × g refrigerated centrifuge for 20 min, then the supernatant was collected and set aside at -20 °C. The level content of H_2O_2 and the activity of antioxidant enzymes were determined using Assay Kit (bought from Comin, Suzhou, China), and the procedures were as described by the Suzhou Comin Biotechnology Research Institute.

Measurement of stomatal density

Fully spread leaves were harvested from ~4-week-old plants under withholding water conditions for stomatal density measurement. The upper epidermis and lower epidermis of the leaf from the same part were peeled off, and photographed by a microscope (BX43, OLYMPUS, Guangzhou, China). The stomata numbers were counted and the density was calculated. Five leaves from three cultivars were used for each replicate, with three replicates for each cultivar (Paul & al. 2017).

Statistical analysis

Statistical analysis at a significance level $P < 0.05$ was performed using the Statistical Product and Service Solutions (version 22.0). The data are presented as means ± standard error (SE). Asterisk symbols indicate significant differences at $P < 0.05$ (Tukey's test). Statistical analysis values shown in the figure are the means of three independent replicates.

RESULTS

Germination characteristics of three quinoa cultivars under PEG-6000 stress

Quinoa seeds were treated with different concentrations of PEG-6000, and GP (Fig. 2a), GR (Fig. 2b), GI (Fig. 2c), MGT (Fig. 2d), and PIS/PIC (Fig. 2e) were recorded. Different concentrations of PEG-6000 had an inhibitory effect on the three kinds of quinoa seeds, and the inhibitory effect was the most obvious in the 25% PEG-6000 treatment. Under 25% PEG-6000 treatment, the GP, GR, GI, and PIS/PIC of 'Chaidamuhong' and 'Gongzha No.3' was not as obviously decreased as that of 'Qingli No.1'; and the MGT of 'Qingli No.1' was significantly longer than that of'Chaidamuhong' and 'Gongzha No.3'. In addition, the radicle length of the three quinoa cultivars also decreased under 25% PEG-6000 treatment, especially 'Qingli No.1' (Fig. 2f). Therefore, we speculate that the drought resistance of 'Chaidamuhong' and 'Gongzha No.3' under 25% PEG-6000 stress is stronger than that of 'Qingli No.1'.

LWC and proline contents of three quinoa cultivars under water deprivation

To observe the physiological and biochemical changes of the three quinoa cultivars under water deprivation treatment, we detected the LWC of quinoa leaves (Fig. 2a). The LWC of 'Qingli No.1' decreased sharply after 21 days of treatment, while that of 'Chaidamuhong' and 'Gongzha No.3' decreased sharply after 35 days of treatment. The dipolarity of proline can maintain the morphology of membrane proteins, thus reducing plant water loss (Per & al. 2017). The increase in proline content can reduce the cell osmotic potential and improve the drought resistance of the plant (Meena & al. 2019). In this experiment, the proline content of three quinoas cultivars was very low at 0 days of water deprivation, but the proline content increased significantly after 28 days of water deprivation (Figs. 2b, 2c). Interestingly, the proline content of 'Qingli No.1' increased significantly after 28 days of water deprivation, which may be due to the significant decrease in leaf water content of 'Qingli No.1' at 28 days of water deprivation. After 35 days of water deprivation, there were no significant differences in leaf water content among the three quinoa cultivars, and proline content was similar.

Cell membrane damage and H_2O_2 of three quinoa cultivars under water deprivation

Generally, drought stress leads to an imbalance in ROS content in plant cells, causing damage to the cell membrane. Therefore, we measured EL (Fig. 3a), and MDA (Fig. 3b), and H_2O_2 (Fig. 3c) content of plant leaves. In three quinoa cultivars, the content of MDA, H_2O_2, and EL increased with the time of water deprivation. Moreover, the EL, MDA, and H_2O_2 content of 'Qingli No.1' increased the earliest, followed by 'Chaidamuhong' and 'Gongzha No.3'.

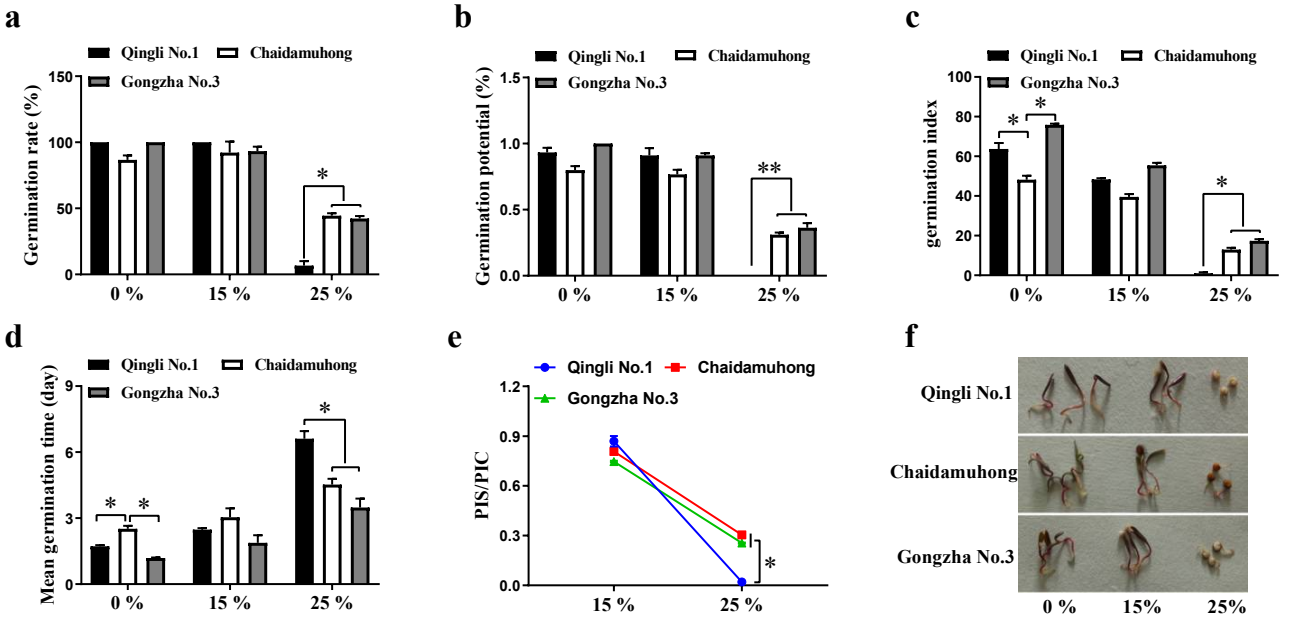

Fig. 1. Germination related index of three quinoa materials under different concentrations of PEG-6000: **a,** germination percentage; **b,** germination potential; **c,** germination index; **d,** mean germination time; **e,** seed germination index of drought resistance; **f,** photo of quinoa on the 7th day of germination [0%, 15%, and 25% represent the concentration of PEG-6000; each value is the mean ± standard deviation of three replicates; *P < 0.05].

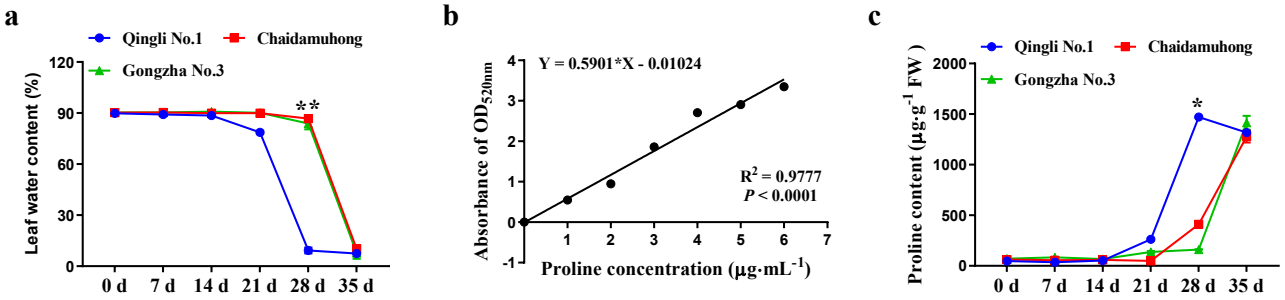

Fig. 2. LWC and proline contents of three quinoa cultivars under water deprivation: **a,** LWC content; **b,** proline standard curve; **c,** proline content [each value is the mean ± standard deviation of three replicates; *P < 0.05].

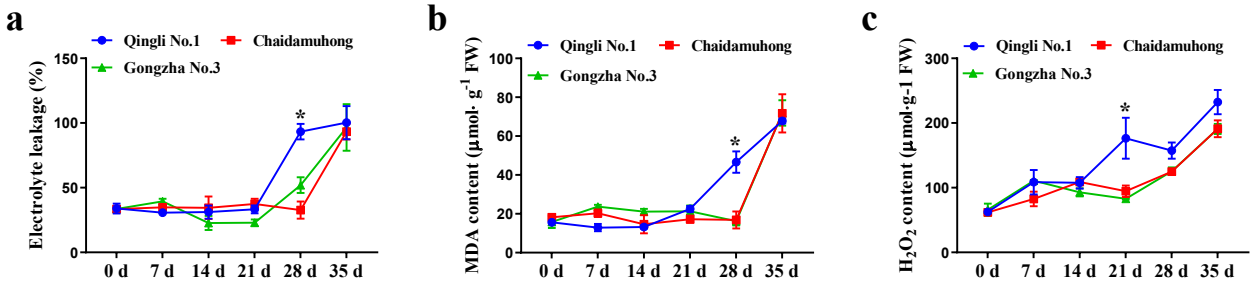

Fig. 3. Cell membrane damage and H_2O_2 of three quinoa cultivars under water deprivation: **a,** electrolyte leakage; **b,** malondialdehyde (MDA) content; **c,** H_2O_2 content [each value is the mean ± standard deviation of three replicates; *P < 0.05]

Activities of antioxidant enzymes in three quinoa cultivars under water deprivation

Generally, when plants are subjected to abiotic stress, the ROS in their cells are out of balance, and the activities of antioxidant enzymes will change accordingly. In this experiment, the measurement of antioxidant enzyme activities showed that there was no significant difference in the activities of four antioxidant enzymes among the three quinoa cultivars at 0 d of water deprivation. However, with the increase in water deprivation time, the activities of the four antioxidant enzymes in the three quinoa cultivars also changed. The SOD activity of 'Chaidamuhong' and 'Gongzha No.3' increased significantly at 21 days, and then decreased in 'Gongzha No.3', while 'Chaidamuhong' maintained high activity until 35 days. The SOD enzyme activity of 'Qingli No.1' increased at 28 days, and then decreased (Fig. 4a). The POD enzyme activity of ''Chaidamuhong' increased rapidly after 7 days of water deprivation, and maintained a high enzyme activity, while the POD enzyme activity of 'Gongzha No.3' and 'Qingli No.1' did not increase significantly under water deprivation (Fig. 4b). The GR enzyme activity of 'Chaidamuhong' and 'Gongzha No.3' increased after 28 days of water deprivation, but the GR enzyme activity of 'Qingli No.1' did not increase significantly under water deprivation (Fig. 4c). Under water deprivation, GPX enzyme activity of 'Chaidamuhong' increased with the increase in treatment time, but GPX enzyme activity in 'Gongzha No.3' did not increase significantly, while GPX enzyme activity in 'Qingli No.1' decreased after 28 days of treatment (Fig. 4d).

Phenotype and stomatal density of three quinoa cultivars under water deprivation

To observe the phenotypic changes in the three cultivars under water deprivation, we took photos at every sampling site. There were obvious phenotypic differences after 28 days of water deprivation. The quinoa cultivar of 'Qingli No.1' was drying up, and 'Chaidamuhong' and 'Gongzha No.3' remained relatively complete. However, after 35 days, the three quinoa cultivars all dried up, and the rehydration reaction was carried out, but the three quinoa cultivars could not recover (Fig. 5a). Furthermore, the stomatal density of three quinoa cultivars was observed after 28 days of water starvation treatment. The results showed that the stomatal density of 'Qingli No.1', 'Chaidamuhong', and 'Gongzha No.3' were 110 mm^{-2}, 100 mm^{-2} and 148 mm^{-2} on the upper epidermis and 145 mm^{-2}, 116 mm^{-2}, and 163 mm^{-2} on the lower epidermis, respectively (Fig. 5b). Compared with 'Qingli No.1' and 'Gongzha No.3', 'Chaidamuhong' showed lower stomatal density in both epidermises, especially in the upper

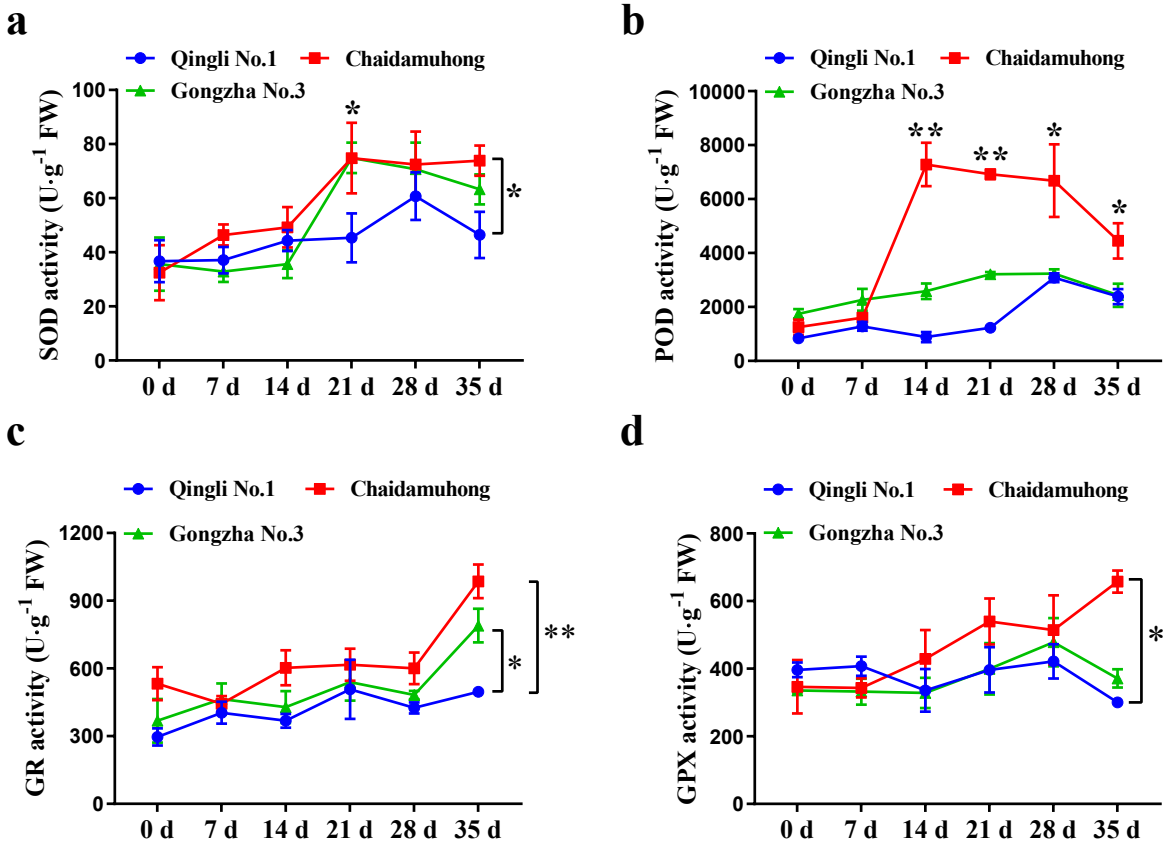

Fig. 4. Activities of antioxidant enzymes in three quinoa cultivars: **a,** activities of SOD; **b,** activities of POD; **c,** activities of GR; **d,** activities of GPX [each value is the mean ± standard deviation of three replicates; *P < 0.05, **P < 0.01]

epidermis. These results show that 'Chaidamuhong' may have a lower transpiration rate than 'Qingli No.1' and 'Gongzha No.3', thus reducing leaf water evaporation and ensuring higher leaf water content.

DISCUSSION

Drought is an important limiting factor in plant growth, which will lead to a decrease in respiration and photosynthetic rate, an imbalance in osmotic pressure, damage to the membrane system, seriously affecting the metabolic activities in all stages of plant growth, and leading to the failure in quality and yield of crops (Cohen & al. 2021). Therefore, breeding and screening of drought-resistant grains is particularly important for ensuring food security. Quinoa grain has high protein content, coordinated proportions of amino acids, is rich in vitamins (A, B2, E) and minerals (Ca, Fe, Cu, Mg, Zn), and has the titles of "mother of grain", "golden grain", and "sacred food" (Filho & al. 2017). In addition, quinoa has the characteristics of cold tolerance, drought tolerance, saline-alkali tolerance, and barren tolerance (Jacobsen & al.2003). Therefore, it is of great significance to screen and cultivate better stress-resistant quinoa to develop future agro-ecosystems.

It is well known that the period from seed germination to seedling growth is the most sensitive period in the plant life cycle, which is very easily affected by various factors in the external environment (Weitbrecht & al. 2011). Drought stress can delay seed germination or reduce seed germination power, so it is a major limiting factor in the process of seed germination (Ishibashi & al. 2018). The response of seed germination to drought stress reflects the ecological mechanism of its adaptation to the environment. In this experiment, drought stress was simulated by PEG-6000 to treat three contrasting plateau quinoa seeds, and their seed germination characteristics were observed. The results showed that there was no significant decrease or difference in the GP, GR, GI, MGT, and PIS/PIC among the three cultivars treated with 15% PEG-6000. However, under the 25% PEG-6000 treatment, the quinoas germination was obviously inhibited, but there were differences in drought tolerance among three cultivars. This was mainly manifest in the decline of GR, GP,GI, PIS / PIC and the in-

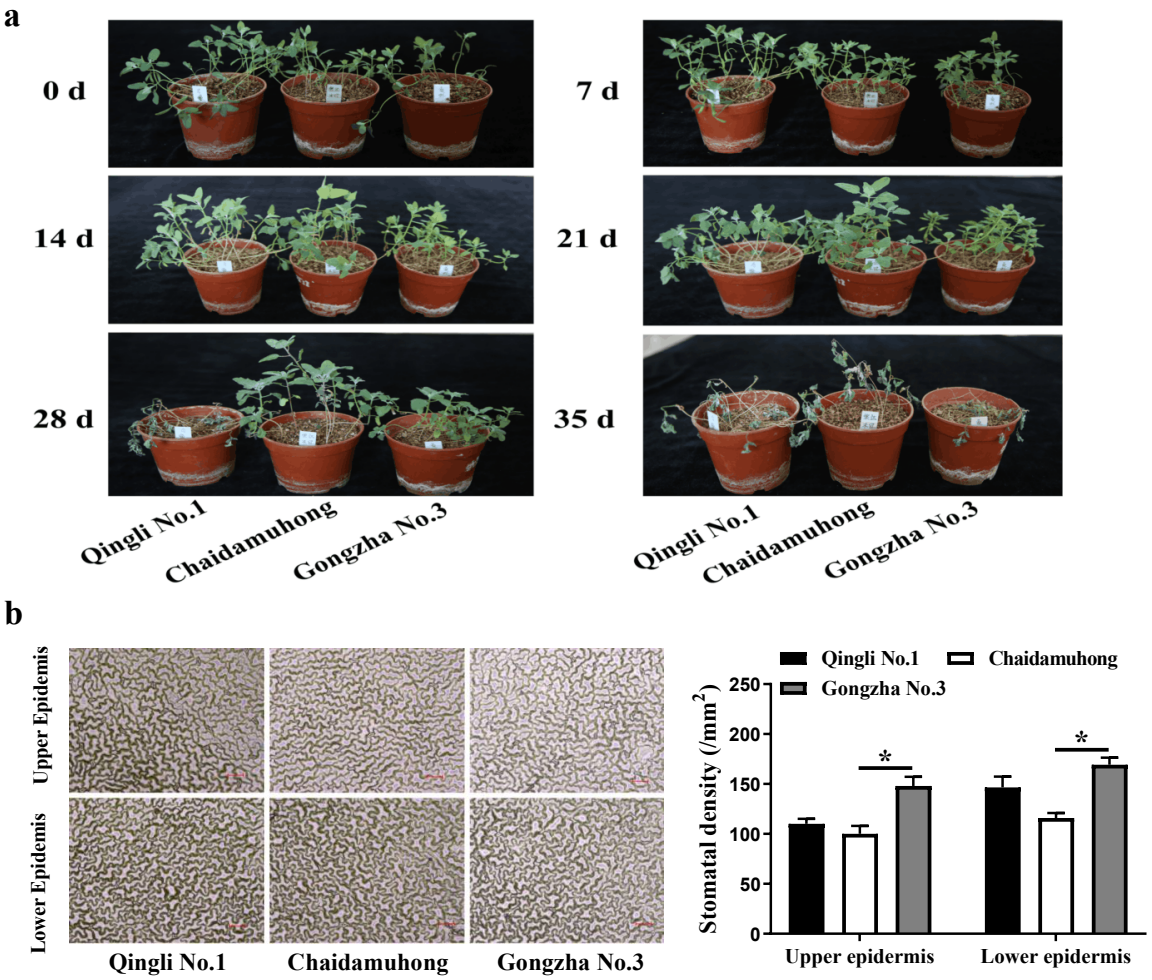

Fig. 5. Phenotype and stomatal density of three quinoa cultivars under water deprivation: **a,** photos of quinoa cultivars under water deprivation over 35 days; **b,** photo of epidermis and graphic showing stomatal density in three quinoas [d = day; bar = 50 μm; each value is the mean ± standard deviation of three replicates; *P < 0.05].

crease of MGT. Among that, 'Chaidamuhong' and 'Gongzha No.3' showed higher GR, GP,GI, PIS/PIC and shorter MGT compared with 'Qingli No.1'. Therefore, we speculate that 'Chaidamuhong' and 'Gongzha No.3' are the most survivability to PEG-6000 compared to 'Qingli No.1'.

Water is an essential component of living cells and an important substance in metabolic activities. The leaf structure of plants with strong drought resistance is more conducive to reducing water loss, so the water retention of leaves directly reflects the drought resistance of plants (Liu & al. 2006). Li & al. (1990) showed that the water content of wheat leaves was proportional to drought resistance. In this experiment, the LWC of three kinds of quinoa seedlings was measured under water deprivation stress. After 28 days of water deprivation, the LWC of 'Qingli No.1' was significantly lower than 'Chaidamuhong' and 'Gongzha No.3'. Therefore, compared with 'Qingli No.1', the leaves of 'Chaidamuhong' and 'Gongzha No.3' had higher water retention capacity and stronger drought resistance. In addition, some studies have shown that proline accumulates in plant cells under drought stress, while an increase in proline content helps to maintain cell osmotic potential, prevents cell dehydration, and protects the stability of the cell membrane system (Kumar & al. 2021). The proline content of the three quinoa cultivars increased with time under stress. However, on the 28th day, the proline content of 'Qingli No.1' was significantly higher than that of 'Chaidamuhong' and 'Gongzha No.3', which may be due to the significant decrease of LWC of 'Qingli No.1' on the 28th day, resulting in a decrease in intracellular water content, thus increasing the intracellular proline content.

In addition, under drought stress, the production and elimination of ROS in plant cells will be out of balance (Janků & al. 2019; Winterbourn & al. 2016). H_2O_2 is an

important ROS (Quan & al. 2008). Recent studies have found that the massive increase in ROS (mainly H_2O_2) is a common characteristic of plants in response to external biotic and abiotic stresses (Luna & al. 2005). Sufficient evidence shows that high accumulation of H_2O_2 in plant cells leads to the destruction of cell membrane structure and DNA denaturation, eventually leading to cell death (Zhang & al. 2014). MDA is a lipid membrane peroxide with high activity, which can affect the balance of the active oxygen metabolism system by affecting membrane proteins (Campos & al. 2003), and the content of MDA is an important sign of membrane structure damage (Toscano & al. 2016). The increase in EL in plant tissue under drought stress is the result of the increase in cell membrane permeability caused by drought stress (Demidchik & al. 2014), so that electrical conductivity reflects the degree of damage to the plant leaf membrane structure. In order to study the degree of damage to the cell membrane caused by the changes in H_2O_2 in different quinoa cultivars under drought stress, we detected the EL and contents of H_2O_2 and MDA in plant cells under water deprivation. 'Qingli No.1' had higher EL, H_2O_2, and MDA content than 'Chaidamuhong' and 'Gongzha No.3' under water deprivation. Compared with 'Qingli No.1', the leaves of 'Chaidamuhong' and 'Gongzha No.3' had more complete membrane structure under water deprivation stress. This phenomenon also corresponds to the assumption that 'Chaidamuhong' and 'Gongzha No.3' are more drought-resistant than 'Qingli No.1'.

In general, under drought stress, excess ROS produced in plant cells can be eliminated by antioxidant enzymes to protect plants from oxidation (Sheoran & al. 2015). SOD in antioxidant enzymes is the first line of defense for the scavenging of reactive oxygen species in plant cells. SOD converts O^{2-} to O_2 and H_2O_2 by disproportionation, followed by the decomposition of H_2O_2 into H_2O and O_2 by the catalysis of POD (Bowler & al. 2003). In addition, GPX catalyzes the reaction of glutathione (GSH) with H_2O_2 to form oxidized glutathione (GSSG) and H_2O (Foyer & Noctor 2005), while glutathione reductase (GR) catalyzes the reduction of GSSG to GSH to maintain the content of GSH, thus preventing the production of OH^-, avoiding plasma membrane peroxidation, and protecting the structural and functional integrity of cell membranes. Therefore, the detection of the activities of these antioxidant enzymes can effectively evaluate the drought resistance of plants. The response of POD was the fastest under drought stress. Under drought treatment, the activity of POD in 'Chaidamuhong' and 'Gongzha No.3' increased significantly at 14 days, while that of 'Qingli No.1' increased significantly at 28 days. The POD activity of 'Chaidamuhong' was significantly higher than that of 'Gongzha No.3' and 'Qingli No.1' under drought stress. Under the same drought treatment, the SOD activity of 'Chaidamuhong' reached a maximum at 21 days, and then remained high, but the SOD activities of 'Gongzha No.3' and 'Qingli No.1' reached their maximum at 21 and 28 days, respectively, and then decreased. The trend of SOD activity is similar to that of POD activity of 'Chaidamuhong', which has higher SOD activity than 'Gongzha No.3' and 'Qingli No.1' under drought conditions. Under drought stress, the activities of GR and GPX of 'Chaidamuhong' and 'Gongzha No.3' increased significantly at 35 days, while the GR of 'Qingli No.1' did not increase significantly, and GPX even decreased at 35 days. Under drought stress, the activity of antioxidant enzymes in 'Chaidamuhong' was the highest, followed by 'Gongzha No.3', and 'Qingli No.1' was the lowest. Therefore, we speculate that under drought stress, 'Chaidamuhong' has higher antioxidant enzyme activity, which can quickly reverse the ROS imbalance caused by drought stress, and alleviate the oxidative damage of cell membranes caused by ROS.

Water deprivation not only induces physiological changes, but also triggers morphological and stomatal density changes in plant (Punchkhon & al. 2020). Therefore, to observe the morphological and stomatal density changes of three, contrasting, plateau quinoa cultivars under water deprivation. These results proved that quinoa plants can adapt to short-term water deprivation through their own physiological, biochemical, and morphological changes, but long-term water deprivation will cause irreversible damage to quinoa plants.

Overall, 'Chaidamuhong' has lower stomatal density physiologically, which can reduce the water loss rate and increase water use efficiency of 'Chaidamuhong' under water deprivation. In addition, the accumulation of intracellular proline under water deprivation also regulates cell osmotic pressure and cell water loss to protect the structure of the cell membrane. Higher antioxidant enzyme activity gives 'Chaidamuhong' stronger ability to reverse ROS imbalance under drought stress, which can reduce the irreversible damage of cells caused by excessive ROS. These results clearly show that 'Chaidamuhong' has higher drought resistance than 'Gongzha No.3' and 'Qingli No.1'. Similarly, the germination characteristics under PEG-6000 stress and the morphological observation under water deprivation also showed that 'Chaidamuhong' had higher drought resistance. Although the study does not include the explanation of genomics and genetics; however, the drought tolerant quinoa could be screened by comparing seed germination index, activity of antioxidant enzymes, cell membrane damage and morphological changes, which provides a theoretical basis for the breeding and cultivation of quinoa.

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