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Protective Antioxidant Enzyme Activities are Affected by Drought in Quinoa (*Chenopodium Quinoa Willd*)

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Abstract
Changes in water availability are responsible for a variety of biochemical stress responses in plant organisms. Stress induced by this factor may be associated with enhanced reactive oxygen species (ROS) generations, which cause oxidative damage. In the present study we investigated the activities of antioxidant enzymes superoxide dismutase (SOD), polyphenoloxydase (PPO), peroxidase (POD) and catalase (CAT), measured at flowering in quinoa, subjected to varying levels of drought stress. Drought levels were 100, 50 and 33% of evapotranspiration (ETc), and rainfed. Compared to full water supply (100%ETc), the activities of SOD under dry conditions (33%ETc) increased significantly by 39 and 90%, in 2011 and 2012, respectively. Under rainfed conditions, the activities of SOD increased by 178.71 and 322.42 %. The CAT activity in rainfed treatment increased significantly by 103.15% (2011) and 87.4% (2012) compared to the full water supply treatment. Compared to the control, POD activity in both essays increased significantly by 50.2% in 33%ETc of 2012 assay and increased by 72.8 and 115.6% in the rainfed treatment of 2011 and 2012 assays respectively. In comparison to the full watered treatment, the PPO activity increased in all treatments. These results suggest that antioxidant enzymes play important roles in reducing oxidative stress in quinoa plant exposed to drought stress.
Keywords: Quinoa, water stress, deficit irrigation, biochemical responses, polyphenoloxydase (PPO), peroxidase (POD) and catalase (CAT)

1. Introduction

Changing climate is mainly characterized by increase in emission of greenhouse gases, global mean temperature, and changes in precipitation levels and patterns (IPCC 2007). Owing to changed patterns of precipitation, episodes of drought are increasing and are expected to increase in time to come (IPCC 2007). It is thus imperative to prepare our crops for drought and other changes. This requires better understanding of the mechanisms of drought tolerance (Farooq et al. 2009).

Among the stressful conditions of the environment, drought serves as a major constraint, limiting crop production in arid and semiarid areas of the world. Growth and the primary production of plants are severely reduced by water deficit (Fatima et al. 2005). The inhibition of carbon dioxide (C02) assimilation, coupled with the changes in photosystem activities and photosynthetic transport capacity under drought stress, result in the production AND THE accumulation of reactive oxygen species (ROS) (Asada 1999; Wang et al. 2012). ROS are the products of many degenerative reactions in crop plants, which affect the regu¬lar metabolism by damaging cellular components (Foyer and Noctor 2003). As a consequence, plants have evolved cellular adaptive responses like up-regulation of oxidative stress protectors and accumulation of pro¬tective solutes (Tahi et al. 2008). Antioxidant de¬fense enzymes such as superoxide dismutase (SOD), catalase (CAT), polyphenolperoxidase (PPO) and perox¬idase (POD) are the systems designed to minimize the concentrations of the ROS substances superox¬ide and hydrogen peroxide. These antioxidants operate co-ordinately to keep a low ROS level within cells. Overall, an increase in tolerance against

abiotic stress. SOD catalyzes the dismutation of superoxide into oxygen and hydrogen per¬oxide. H2O2 is then eliminated by catalase and peroxi¬dases, which include both enzymic and non-enzymic H2O2 degradation (Peltzer et al. 2002), Catalase dismutates H2O2 into water, whereas POD decomposes H2O2 by oxidation of co-substrates such as pheno¬lic compounds and antioxidants (Blokhina and Fagerstedt 2010). Higher level of these antioxidant enzyme activities is considered as one of tolerance mechanism in most plants (Ashraf, 2009). Previous studies showed that drought tolerant cultivars generally have an enhanced or higher constitutive antioxidant enzyme activity under drought stress when compared with the sensitive cultivars. This has been demonstrated in numerous plant species such as tomato (Tahi et al. 2008), olive (Aganchich et al. 2007), grapevines (Beis and Patakas 2012), Mentha pulegium (Candan and Tarhan 2012).

Quinoa is an important crop because of its economic importance and nutritional value mainly due to the high protein content and wide range of minerals and vitamins (Stikic et al. 2012; Repo-Carrasco et al. 2003). The seed proteins are rich in amino acids like lysine, threonine and methionine that are deficient in cereals. Recently, attention has been given to quinoa for people with celiac disease (allergy to gluten), as an alternative to the cereals wheat, rye and barley (Jacobsen 2003). However, to the best of our knowledge, there is no available information on changes of antioxidant enzyme activities during developmental stages of quinoa. Therefore, Understanding the mechanism enabling quinoa plants to tolerate drought will help in the selection and development of drought tolerant cultivars. This study was therefore conducted to understand the possible responses and adaptations of quinoa to drought, and the function of physiological and biochemical processes, and the development of scientific strategies for alleviating negative effects of water deficiency.

2. Materiel and methods

2.1 Plant material and experimental location

The study was carried out on one quinoa variety (Chenopodium quinoa Willd) frequently cultivated in the South America and provided under the EU 7th Framework Program through the project "Sustainable water use securing food production in dry areas of the Mediterranean region (SWUP-MED)". Seeds were grown in a sandy loam soil (62% of sand, 36% of silt and 12% of clay) in farmer's fields at "Tnin Bouchan" Experimental Station of University Cadi Ayyad located in 70km south West Marrakech (32°14.6267'N, 8°19.8181'W, 280 m.a.s.1.). Field trials were conducted in February and harvested in June during two successive years (2011 and 2012).

2.2 Experimentation and measurements

The trial was conducted under four levels of irrigation (rainfed (0%ETc), full irrigated (100% ETc), and deficit irrigation DI (50 and 33% of ETc). There were four randomized blocks (30m²/plot). Quinoa seeds were sown directly with spacing of 0.2m between sowing pits of the same row and 0.8 m between rows. Buffer areas of 1 m between experimental units were sown to avoid border effects.

2.2.1 Climatic conditions

Meteorological data (minimum and maximum temperature, minimum and maximum relative humidity, wind speed, solar radiation) are permanently measured (figure 1) by iMETOS® ag weather stations installed in the field and automatically sent to internet climate data base.

2.2.1 Irrigation determination

Irrigation planning was based on a daily follow up of Reference evapotranspiration (ET0), calculated with the FAO-Penman-Monteith equation (Allen et al. 1998).

The crop coefficient of quinoa presented by Garcia et al. (2003) was corrected with validated procedures (Allen et al. 1998) for incomplete cover, incomplete wetting by irrigation and high convection due to the arid climate. Irrigation was efficiently applied by drip irrigation. Drip emitters were spaced 0.10 m along the lateral with a discharge of 1 LPH under an operation pressure of 1.5 kg cm-2. The rate of water flow in all drip laterals was equal and constant under all the treatments.

The net irrigation requirement is derived from the field balance equation :

$$IRn = (ET_0 \times K_C) - P_e$$

Where:

IRn : Net irrigation requirement (mm)

ET0: Reference evapotranspiration (mm)

Kc : Crop coefficient

Pe: Effective rainfall (mm)

The gross irrigation requirements account for losses of water incurred during conveyance and application to the field. This is expressed in terms of efficiencies when calculating project gross irrigation requirements from net irrigation requirements, as shown below (SAWA & FRENKEN 2002):

$$IR_g = \frac{IR_n}{E}$$

Where:
IRg : Gross irrigation requirements (mm)
IRn : Net irrigation requirements (mm)
E : Overall project efficiency which is equal to 0.85 for drip irrigation

2.3 Enzyme assays

Leaf segments (0.1 g) were crushed into fine powder in a mortar in an ice-bath. 1.0 mL of 0.05 mol l-l pH 6 phosphate buffer with 1% polyvinylpyrrolidone (PVP) was used as an extraction buffer. The homogenate was centrifuged at 15000xg for 15 min at 4°C, and the supernatant was used for soluble protein content and enzyme analysis of superoxide dismutase (SOD), polyphenoloxydase (PPO), peroxidase (POD) and catalase (CAT).

The activity of SOD was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetra-zolium (NBT) following the method of Beauchamp and Fridovich (1791). One unit of SOD activity was defined as the amount of enzyme which caused 50% inhibition of photochemical reduction of NBT. The enzyme activities were expressed as units mg-1 protein

PPO activity was determined by measuring the increase in absorbance at 420 nm with recording spectrophotometer. The reaction mixture contained 20mM catechol in 0.1 M phosphate buffer pH 6, 0.1 M phosphate buffer pH 7. The assay was initiated by the addition of enzyme extract. PPO activity was expressed in enzyme unit mg-1 protein. One unit of PPO activity was defined as the amount of enzyme, which caused an increase in absorbance of 0.001/ min.

For measurement of POD activity, assay solution (3 mL) contained 50 mM phosphate buffer (pH 7.0), 20 mM guaiacol, 40 mM H2O2 and 0.1 mL enzyme extract. The reaction was initiated by adding the enzyme extract. Increase in absorbance of the reaction solution at 470 nm was recorded after every 30 s. One unit PO activity was defined as an absorbance change of 0.01 units min-1.

For measurement of CAT activity, assay solution (3 mL) contained 50 mM phosphate buffer (pH 7.0), 5.9 mM H2O2 and 0.1 mL enzyme extract. Decrease in absorbance of the reaction solution at 240 nm was recorded after every 30 s. An absorbance change of 0.01 units min-1 was defined as one unit CAT activity.

Total soluble protein concentration was measured by dye binding assay as described by Bradford (1976)

2.4 Lipid peroxidation analysis

The level of lipid peroxidation was estimated in terms of MDA level, which was determined by thiobarbituric acid reaction according to Heath and Packer (1968). A 0.1 g sample (fresh weight) was homogenized in 1.5 ml 0.1% TCA. The homogenate was centrifuged at 10 000g for 10 min. to a 1 ml of the supernatant, 4 ml 20% TCA containing 0.5% thiobarbituric acid was added. The mixture was heated at 95% for 30 min and then quickly cooled in ice bath. After the tubes were centrifuged at 10 000*g for 10 min. MDA content was calculated by multiplying the difference between absorbance at 532 nm and 600 nm by the extinction coefficient of 155 nM-1 cm-1 (Heath & Packer 1968).

2.5 Statistical analyses

The experimental layout was a completely randomized design. The means were compared with least significant difference test using CoStat version 6.3.

3. Results

3.1 Proteins

The soluble protein content of the control treatment in 2011 and 2012 was 4.47 and 3.52 mg g-1 FW. In rainfed treatment they were 62.83% and 47.73 of control in 2011 and 2012, respectively, and in 50ETc and 33ETc treatments they were 71.13 and 61.12% of those in 2011 control. Under drought stress, the amount of reduction was related to drought intensity (Figure 2) the leaves content of soluble protein was reduced in both assays (2011 & 2012) in comparison to the full irrigated for all irrigation treatments, however we not that the same effect between 2011 and 2012 results except for the 50ETc and 33ETc treatments of 2012 assay which had respectively an increasing of 32.4 and 6.56% respectively in comparison to the same treatments in 2011. There was no significant difference between 100ETc of the 2011 assay and 50 ETc of the 2011 assay as first group, between 100ETc and 33ETc of the 2012 assay

as a second group, and between 33ETc and RFETc of the 2011 assay.

3.2 Antioxidant enzymes

Adaptation to drought stress may depend on different mechanisms, including the capacity to maintain high levels of antioxidants and through the induction of antioxidant enzymes. The effects of drought stress on the protective enzymes PPO, CAT, PO and SOD in leaves are shown in Figure 3. The activities of PPO, CAT, POD and SOD in leaves were increased with increasing drought. The protective enzyme activities in 2011 were lower than those in 2012 assay due to the climate conditions.

Compared to full water supply (100%ETc), the activities of SOD in 2012 and 2011 assays in treatment 33%ETc increased significantly by 90.1 and 38.72%, respectively, compared to 2012 and 2011 full water supply treatment. In rainfed treatment, the activities of SOD in 2012 and 2011 assays increased drastically by 322.42 and 178.71% respectively.

The catalase activity in rainfed increased significantly ($P<0.05$) by 87.4% (2012) and 103.15% (2011) compared to the full water supply treatment.

Compared to the controls, POD activity increased significantly by 50% in 33E2 treatment and increased by 116 and 73% in the rain fed treatment of 2012 and 2011 assays respectively.

PPO activity increased in 2011 and 2012 by 190 and 253% in 50%ETc treatment, 463 and 229% in 33%ETc treatment, and by 520 and 106, % in rainfed treatment.

2.4 Lipid peroxidation analysis

The product of membrane peroxidation (MDA) has been used as a direct indicator of lipid peroxidation and membrane damage. Under environmental stresses, ROS attack the most sensitive biological macro-molecules in cells to impair their function. Therefore, we determined the MDA levels in order to investigate the extent of the damage to membranes caused by drought stress (figure 4).

The highest lipid peroxidation products were detected in 2012 rainfed treatment, while the lowest was the fully irrigated treatment in 2011. The MDA was increased significantly ($P<0.05$) by 63% in 50ETc (2012 assay), 81,6% in 33ETc(2012 assay), 125.8% in RFETc (2012 assay) in comparison to 100ETc (2012 assay) and by 141,9% in 33ETc (2011 assay) and 172,3% in RFETc (2011 assay) in comparison to 100ETc (control). There was no significant difference between stressed treatments except for the 50ETc (2011 assay) and RFETc (2012 assay).

4. Discussion

Active oxygen is accumulated in plants under drought stress. Fortunately, plants have an internal protective enzyme-catalyzed cleanup system, to avoid injury from active oxygen, thus guaranteeing normal cellular function (Wang et al., 2002). When the plants suffer from drought stress, the whole defensive system needs to be activated in order to tolerate the active oxygen. It was found that the activities of protective enzymes of plants were affected by drought (Bian & Jiang 2009).

Many studies have demonstrated up-regulation of the antioxidant defense system in plants subjected to different degrees of drought stress (Adolf, Jacobsen & Shabala 2012; Aganchich et al. 2009; Tahi et al. 2008)). This provide protection against irreversible damage to the photosynthetic machinery, thereby allowing the photosynthetic apparatus to function. In our study there was a marked stimulation in the activity of SOD, CAT, PPO and POD in response to the irrigation treatments (Figure 3). The activities of soluble POD and PPO were significantly higher in 2012 assay especially under drought. Furthermore, the degree of leaf injury caused by drought could be lessened through morphological adaptation and regulation of stem and sheath water content, stem diameter or plant height. Leaves reduced water transpiration by curling and stomata regulation In order to minimize oxidative damage, similar effect was mentioned by many authors for olives (Aganchich et al. 2007; Anjum et al. 2012; Liu et al. 2009) and tomato (Tahi et al. 2008). Wang et al., (2012) noted that, both the activities of antioxidant enzymes and antioxidant concentrations are increased in the leaves of apple in response to drought stress.

ROS is produced by the photosynthetic electron transport chain in chloroplasts (Asada 1999). Increased SOD activity in response to water and nutrient deficiency indicates that the chloroplast antioxidant apparatus is immediately activated in response to a stress signal, and that this activation may occur long before the stress is detectable by physiological measurements. Drought caused a significant decrease in APX and SOD activities (de Campos et al. 2011) and induced decreases of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) activities (Fu et al. 2010).

The oxidative damage can usually be seen by monitoring changes in MDA concentrations. Here, MDA in stressed plants increased With increasing drought, indicating that the MDA concentration was directly related to drought, as

was also previously reported for many cultivars like, maize (Ge et al. 2006), rice (Fu et al. 2010) and olive (Aganchich et al. 2007; Aganchich et al. 2009). In leaves, less MDA accumulated in 2011 assay than 2012assay, suggesting that less lipid peroxidation developed because higher plant water status was maintained in first assay.

Conclusion

Plants under drought stress are highly regulated by components of osmoregulative and antioxidative systems and secondary metabolites. Our results suggest that in quinoa, under drought stress, the activities of PPO, SOD, POD, and CAT increase. Therefore antioxidant activity responses are contributing to defense of quinoa to drought stress. The total antioxidant capacities were dependent on the stress levels. Further studies regarding other detoxifying enzymes and antioxidants are being performed in order to clarify and provide additional information for the complex response of quinoa to drought stress.

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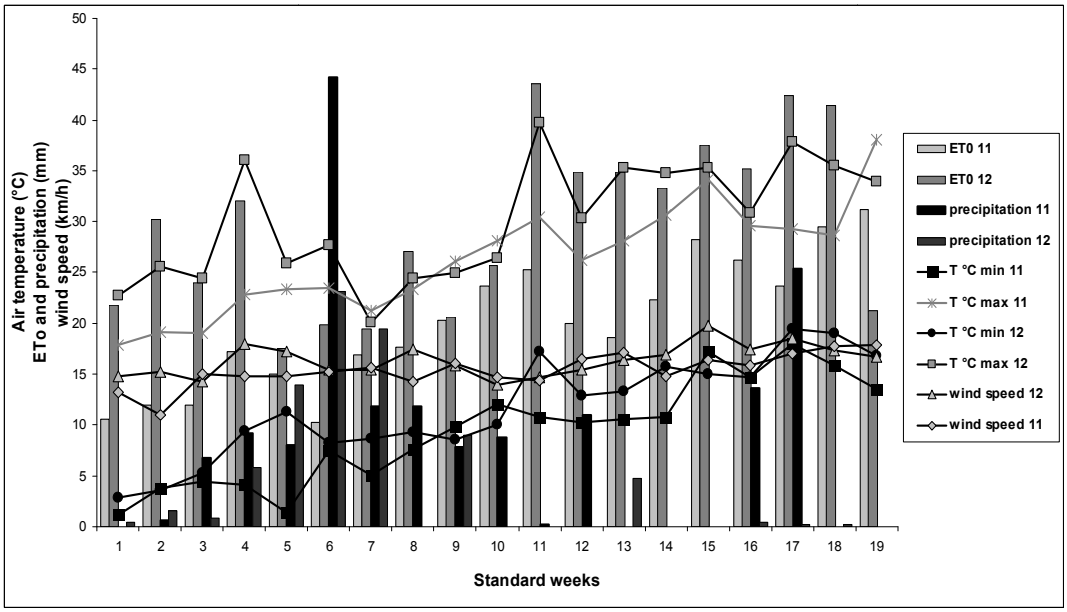

Figure 1: Climate weather data during the culture seasons (2011 and 2012)

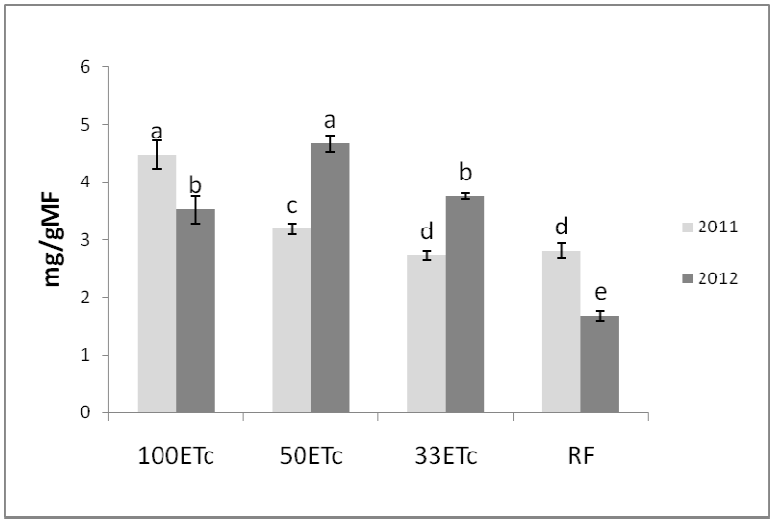

Figure 2: Soluble protein in leaves of quinoa grown under different levels of drought stress. 100ETc: 100% of the crop Evapotranspiration; 50 ETc: 50% of the crop Evapotranspiration; 33 ETc: 33% of the crop Evapotranspiration; RF: rainfed;

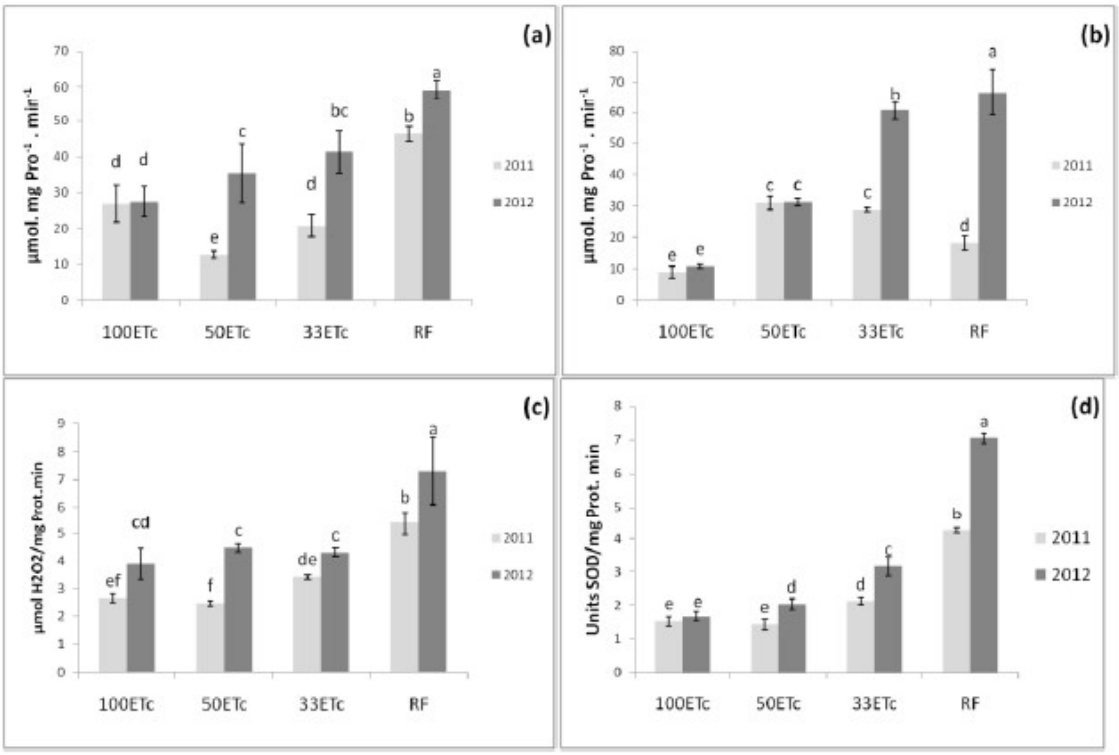

Figure 3: antioxidant enzymes (POD(a), PPO(b), CAT(c), SOD(d)) activity protein in leaves of quinoa grown under different levels of drought stress. 100ETc: 100% of the crop Evapotranspiration; 50 ETc: 50% of the crop Evapotranspiration; 33 ETc: 33% of the crop Evapotranspiration; RF: rainfed;

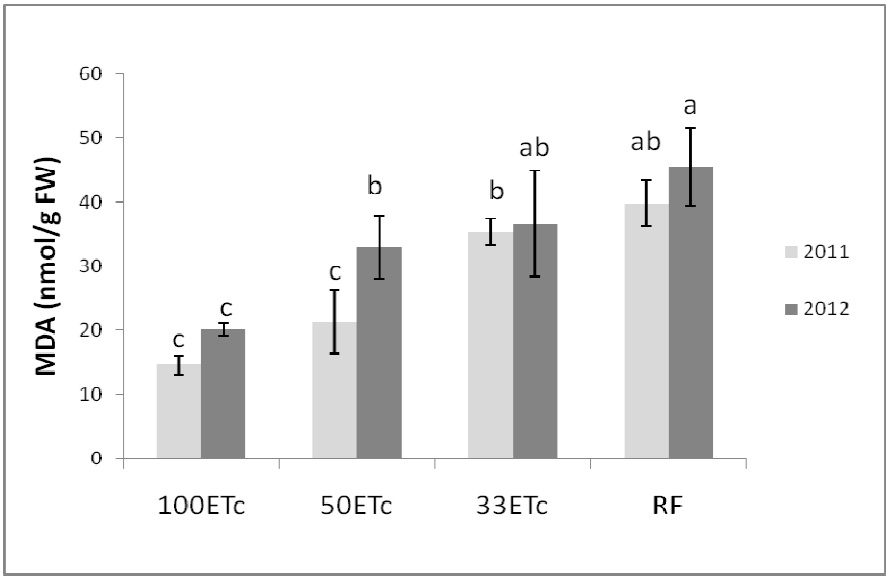

Figure 4: MDA protein in leaves of quinoa grown under different levels of drought stress. 100ETc: 100% of the crop Evapotranspiration; 50 ETc: 50% of the crop Evapotranspiration; 33 ETc: 33% of the crop Evapotranspiration; RF: rainfed;

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