

RESEARCH

Open Access



Role of trehalose on antioxidant defense system and some osmolytes of quinoa plants under water deficit

Mervat Shamooun Sadak^{*} , Hala Mohammed Safwat El-Bassiouny and Mona Gergis Dawood

Abstract

Background: For scavenging reactive oxygen species, plant possess effective system that protect them from destructive oxidative reaction. Parts of this system as osmoprotectants and antioxidative enzymes are key elements in the defense mechanisms. A field experiment was conducted to evaluate the potential of foliar treatment of trehalose (Tre) with different concentrations (0, 0.1 mM, or 0.5 mM) in improving antioxidant defense system of quinoa plant under normal irrigation and drought stress conditions.

Results: Drought stress caused significant increases in some osmoprotectants as glucose, trehalose, TSS, free amino acids, and proline. Meanwhile, trehalose foliar treatment with different concentrations significantly decreases in free amino acids and proline contents. More accumulation of the tested organic solutes of leaves (glucose, sucrose, trehalose, TSS) of the trehalose-treated plant in both normal irrigated and drought-stressed quinoa plants as compared with the corresponding controls. Treating quinoa plants with trehalose resulted in significant decrease in lipid peroxidation, hydrogen peroxide contents, and LOX activity in normal irrigated and drought-stressed plants. These decreases correlated with significant increases in total phenolic contents as compared with untreated control. Different concentrations of trehalose resulted in significant increases in antioxidant enzymes. Maximum increase antioxidant enzymes were observed by treating plants Tre at 0.5 mM either under normal irrigation or drought conditions.

Conclusion: It could be concluded that foliar spray of trehalose was effective in improving quinoa performance by reducing hydrogen peroxide free radical and by enhancing antioxidant compounds (phenolics), compatible osmolytes, membrane stability, and antioxidant enzymes.

Keywords: Antioxidant enzymes, Drought, Phenolics, Proline, Quinoa, Trehalose

Introduction

Quinoa as a newly introduced food crop can replenish part of food gap. It is considered as a multipurpose crop, and seeds can be utilized for human food, in flour products, and in animal feedstock because of its high nutritive value (Bhargava et al. 2007). Quinoa can grow in sandy soil of arid and semiarid regions in addition to its tolerance to salinity, drought, and other abiotic stresses which reduce crop production (Fuentes et al. 2012). Quinoa seeds was recognized as high-quality protein seeds, especially rich in essential

amino acids, minerals, carbohydrates, antioxidant compounds as α -& γ -tocopherol, carotenoids, flavonoids, riboflavin, thiamine, vitamin C, folic acid, and dietary fiber, compared to that of cereals such as corn, oat, rice, and wheat (Repo-Carrasco-Valencia and Serna 2011). Quinoa crop was chosen by FAO as one of the important crops which play major role in food security assuring in the twenty-first century due to its high nutritional value and its good tolerance to adverse climatic conditions. Also, because this crop can grow in sandy soil of arid and semiarid regions, it can be used to replenish part of the food gap.

Water deficiency which is often linked with other major abiotic stress such as heat stress, salinity stress, etc. is considered as one of the primary factors responsible

* Correspondence: mervat_sh24@yahoo.co
Botany Department, Agriculture and Biology Division, National Research Centre, 33 El Bohouth st., Dokki, Giza 12622, Egypt

for crop productivity reduction (Dawood and Sadak 2014). Water shortage caused adverse effect on plants via reduced growth, nutrient acquisition reduction, and modulation, in water status of plants (Sadak 2016b). Protoplasma dehydration increases cellular electrolytes concentrations which cause substantial disturbance in a variety of metabolic reactions in the cell (Mahajan and Tuteja 2005). The reduction of photosynthetic efficiency and stomatal conductance, RuBisCo activity inhibition, and energy balance disruption and distribution during photosynthesis (Demirevska et al. 2010) cause increased accumulation of reactive oxygen species (ROS) under water deficient stress (Hasanuzzaman et al. 2014). Plants have evolved protective mechanisms to perceive and respond rapidly to the adverse environmental cues (Demirevska et al. 2010). These metabolic adaptations that improve plant tolerance to osmotic or water stress involve an increased synthesis of osmoprotectants as free amino acids, proline, and soluble sugars. Compatible osmolytes not only contribute to osmoregulation but they may also protect the structure of different biomolecules and membranes or act as free-radical scavengers that protect DNA from damaging effects of ROS (Ashraf and Foolad 2007). Phenolic compounds accumulation also plays an important role in scavenging free radicals and protects plants against the damaging effects of increased ROS levels due to water stress (Petridis et al. 2012). Moreover, to reduce oxidative damage resulting from water deficiency, plants have developed different adaptive mechanisms, via the biosynthesis of a cascade of antioxidants. Antioxidant defense system enhancement is an important strategy to scavenge ROS by antioxidant enzyme such as ascorbate peroxidase (APX), ascorbate reductase (AR), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPX), and superoxide dismutase (SOD) and with non-enzymatic antioxidants such as ascorbate (AsA), glutathione (GSH), carotenoids, flavanones, and anthocyanins (Hasanuzzaman et al. 2014).

Egypt presents a typical example of the drought problem faced in some arid regions. There is a critical need to balance water availability, water requirements, and water consumption. Thus, water conserving is becoming a crucial consideration for agriculture. To bridge the gap between different crop production and consumption, increasing and exploiting production of crops in these areas are necessary. So, application of osmoprotectants, as trehalose was found to extent positive effect of plant growth which overcomes the harmful effect of some environmental stress such as drought. Trehalose (Tre) is a non-reducing disaccharide of glucose that stabilizes biological structures and macromolecules such as membrane lipids and proteins during abiotic stresses (Luo et al. 2010). Exogenous treatment of trehalose is transported by leaf or root cells to

different cells and displays significant roles as osmoprotectant (Luo et al. 2010). Trehalose is distributed widely among different biological systems: bacteria, fungi, and lower and higher plants (Elbein et al. 2003). During a biotic stress, trehalose does not function directly in alleviation of stress; it may act as a signal molecule. Thus the aim of the present work was to improve drought tolerance of quinoa plants grown under newly reclaimed sandy soil by exogenous application of different concentrations of trehalose.

Materials and methods

Plant material and growth conditions

A field experiment was conducted at the Experimental Station of National Research Centre, Nubaria district Beheira Governorate, Egypt, during two successive seasons of 2013/2014 and 2014/2015. The soils of both experimental sites were reclaimed sandy soil where mechanical and chemical analysis is reported in Table 1 according to Chapman and Pratt (1978).

Seeds of quinoa (*Chenopodium quinoa* Willd.) Quinoa 1 cultivar was obtained from Agricultural Research Centre Giza, Egypt. The experimental design was split-plot with four replications. The main plots were devoted to the irrigation treatments, while the trehalose concentrations treatments were randomly occupied the sub-plots. Quinoa seeds were sown on October in both seasons at the rate of 3 kg/faddan (one faddan = 0.42 ha) in rows 3.5 m long, and the distance between rows was 20 cm apart. Plot area was 10.5 m² (3.0 m in width and 3.5 m in length). The recommended agricultural practices of growing quinoa were applied. Pre-sowing 150 kg/feddans of calcium super-phosphate (15.5% P₂O₅) was applied to the soil. Nitrogen was applied after emergence in the form of ammonium nitrate 33.5% at a rate of 75 kg/feddans in five equal doses before the 1st, 2nd, 3rd, 4th, and 5th irrigation. Potassium sulfate (48.52% K₂O) was added in two equal doses of 50 kg/feddans, before the 1st and 3rd irrigations. Irrigation was carried out using the new sprinkler irrigation system where water was added every 5 days. Trehalose foliar treatment consisted of three levels of trehalose namely 0 mM (control), 0.1 mM, and 0.5 mM considered as Tre₀, Tre₁, and Tre₂ respectively. Drought stress including normal irrigation (D₀) and skipping two irrigation times (D₁). Different trehalose treatments were carried out twice, where plants were sprayed after 30 and 45 days from sowing. Skipping the irrigation at 50 and 60 days after sowing. Plant samples were taken after 75 days from sowing for estimation of some biochemical parameters in leaves of plant such as carbohydrate constituents (glucose, sucrose, trehalose, total soluble sugars, and starch), proline, total free amino acid, phenolic contents, as well as hydrogen

Table 1 Mechanical and chemical analysis of the experimental soil sites

A. Mechanical analysis:																							
Sand				Silt 20–0 $\mu\%$				Clay < 2 $\mu\%$		Soil texture													
Course 2000–200 $\mu\%$				Fine 200–20 $\mu\%$																			
47.46				36.19				12.86		4.28		Sandy											
B Chemical analysis:																							
pH 1:2.5		EC dSm ⁻¹		CaCO ₃		OM%		Soluble Cations meq/l				Soluble anions meq/l											
								Na ⁺		K ⁺		Mg ⁺		Ca ⁺⁺		CO ₃ ⁻⁻⁻		HCO ₃ ⁻		Cl ⁻		SO ₄ ⁻⁻⁻	
7.60		0.13		5.3		0.06		0.57		0.13		0.92		1.0		0.0		1.25		0.48		0.89	
Available nutrients																							
Macro element ppm						Micro element ppm																	
N		P		K		Zn		Fe		Mn		Cu											
52		12.0		75		0.14		1.4		0.3		0.00											

peroxide content (H₂O₂), lipid peroxidation lipoxigenase (LOX), and antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT), polyphenol oxidase (POX), and ascorbate peroxidase (APX)).

Biochemical analysis

Determination of glucose

Glucose estimations were performed using a modification of O-toluidine procedure (Feteris 1965). Sugars were extracted by overnight submersion of dry tissue in 80% (v/v) ethanol at 25 °C with periodic shaking. A 1.0 ml aliquot of the alcoholic extract was heated with 5.0 ml O-toluidine reagent (60 ml O-toluidine and 2.0 g thiourea made to 1 L with glacial acetic acid) for 15 min at 97 °C. Absorbance was measured at 630 nm.

Determination of sucrose

Sucrose contents were determined by first degrading reactive sugars present in 0.1 ml extracts with 0.1 ml 5.4 N KOH at 97 °C for 10 min (Handel 1968). Three milliliters of freshly prepared anthrone reagent were then added to the cooled reaction product, and the mixture was heated at 97 °C for 5 min, cooled, and read at 620 nm.

Determination of trehalose

Trehalose content in the quinoa leaves was determined following the method described by Li et al. (2014) with some modifications. The leaves (1.0 g) were homogenized in 5 ml of 80% (v/v) hot ethanol and centrifuged at 11,500×g for 20 min. The supernatants were dried at 80 °C followed by resuspension in 5 ml distilled water. The solution (100 μl) was mixed with 150 μl 0.2 N H₂SO₄ and boiled at 100 °C for 10 min to hydrolyze any sucrose or glucose-1-phosphate, etc., and then chilled on ice. NaOH (0.6 N, 150 μl) was added to the above mixture and boiled for 10 min to destroy reducing sugars,

and then chilled again. To the above mixture, 2.0 ml of anthrone reagent (0.2 g anthrone per 100 ml of 95% H₂SO₄) was added and boiled for 10 min to develop a color, and then chilled again. The absorbance was recorded at 630 nm, and trehalose concentration was calculated as micromoles per gram FW using a standard curve developed with commercial trehalose.

Determination of total soluble sugars

Total soluble sugars (TSS) were extracted by overnight submersion of dry tissue in 10 ml of 80% (v/v) ethanol at 25 °C with periodic shaking, and centrifuged at 600×g. The supernatant was evaporated till completely dried then dissolved in a known volume of distilled water to be ready for determination of soluble carbohydrates (Homme et al. 1992). TSS were analyzed by reacting of 0.1 ml of ethanolic extract with 3.0 ml freshly prepared anthrone (150 mg anthrone + 100 ml 72% H₂SO₄) in boiling water bath for 10 min and reading the cooled samples at 625 nm using Spekol Spectrocolorimeter-VEB Carl Zeiss (Yemm and Willis 1954).

Determination of starch

For starch measurement, the insoluble fraction remaining after ethanolic extraction of soluble sugars was resuspended in 2 ml of 2.5 M NaOH and boiled for 5 min. After cooling the solution, pH was adjusted to pH 4.5 with 2 M HCl, and the resulting gelatinized starch was hydrolyzed 10 min at 50 °C with buffered (0.1 M sodium acetate buffer, pH 4.5). After this process, starch was measured as reducing sugars by Nelson's method (Nelson, 1944) and expressed in maltose equivalents.

Determination of proline

Proline was assayed according to the method described by Bates et al. (1973). Then, 2.0 ml of proline extract,

2.0 ml of acid ninhydrin, and 2.0 ml of glacial acetic acid were added and incubated for 1 h in a boiling water bath followed by an ice bath. The absorbance was measured at 520 nm using Spekol Spectrocolorimeter VEB Carl Zeiss. A standard curve was obtained using a known concentration of authentic proline.

Determination of free amino acids

Free amino acid content was extracted according to the method described by Vartainan et al. (1992). Free amino acid was determined with the ninhydrin reagent method (Yemm and Cocking 1955). Further, 1.0 ml acetate buffer (pH 5.4) and 1.0 ml chromogenic agent were added to 1.0 ml free amino acid extraction. The mixture was heated in boiling water bath for 15 min. After cooled in tap water, 3 ml ethanol (60% v/v) was added. The absorbance at 570 nm was then monitored using Spekol Spectrocolorimeter VEB Carl Zeiss.

Determination of phenolic contents

A known weight of the fresh samples was taken and extracted with 85% cold methanol (v/v) for three times at 0 °C. The combined extracts were collected and made up to a known volume with cold methanol. And then 0.5 ml of the extraction was added to 0.5 ml Folin reagent, shaken, and allowed to stand for 3 min. Then 1 ml of saturated sodium carbonate was added to each tube followed by distilled water shaken and allowed to stand for 60 min. The optical density was determined at wave length of 725 nm using spectrophotometer as described by Danil and George (1972).

Determination of lipid peroxidation

The level of lipid peroxidation was measured by determining the levels of malondialdehyde (MDA) content using the method of Hodges et al. (1999). A sample (200 mg) was homogenized in 10 ml of 5% trichloroacetic acid (TCA). The homogenate was centrifuged at 15,000×g for 10 min to 2.0 ml aliquot of the supernatant 4.0 ml of 0.5% thiobarbituric acid (TBA) in 20% TCA was added. The mixture was heated at 95 °C for 30 min and then quickly cooled in an ice bath and centrifuged at 10,000×g for 10 min, then the absorbance of supernatant was recorded at 532 nm by spectrophotometer (Shimadzu). The value for non-specific absorption at 600 nm was subtracted. The MDA content was calculated using its absorption coefficient of 155 nmol⁻¹ cm⁻¹ and expressed as nmol (MDA) g⁻¹ fresh weight.

Assay of enzymes activities

Enzyme extracts were collected following the method described by Chen and Wang (2006). Leaf tissues were homogenized in ice-cold phosphate buffer (50 mM, pH 7.8), followed by centrifugation at 8000 rpm and 4-C for

15 min. The supernatant was used immediately to determine the activities of enzymes.

Lipoxygenase activity Lipoxygenase (LOX) (EC 1.13.11.12) activity was estimated according to the method of Doderer et al. (1992) by monitoring the increase in absorbance at 234 nm using linoleic acid as a substrate. The activity was calculated using the extinction coefficient (25 mM⁻¹ cm⁻¹) and express as units (1 nmol of substrate oxidized per minute) per mg protein.

Peroxidase activity Peroxidase (POX) (EC 1.11.1.7) activity was assayed by the method of Kumar and Khan (1982). The reaction mixture used for estimating the peroxidase enzyme (POX) contained 2 ml of 0.1 M phosphate buffer (pH 6.8), 1 ml of 0.01 M pyrogallol, 1 ml of 0.005 M H₂O₂, and 0.5 ml of the enzyme extract. The solution was incubated for 5 min at 25 °C after which the reaction was terminated by adding 1 ml of 2.5 N H₂SO₄. The amount of purpurogallin formed was determined by measuring the absorbance at 420 nm against a reagent blank prepared by adding the extract after the addition of 2.5 N H₂SO₄ at the zero time.

Superoxide dismutase activity Superoxide dismutase (SOD) (EC 1.12.1.1) activity was spectrophotometrically assayed at 560 nm by nitro-blue-tetrazolium (NBT) reduction method (Chen and Wang 2006). The reaction mixture (3 ml) contained 150 μg riboflavin (13 μM), 2.5 ml methionine (13 μM), 250 μg NBT (63 μM), 50 μg phosphate buffer (50 mM, pH 7.8), and 50 μg enzyme extract. One unit of SOD activity was defined as the amount of enzyme protein required for inhibition of the 50% reduction of NBT.

Catalase activity Catalase (CAT) (EC 1.11.1.6) activity was determined spectrophotometrically by following the decrease in absorbance at 240 nm (Chen and Wang 2006). The mixture (3 ml) contained 1.9 ml phosphate buffer (50 mM, pH 7.0), 100 μl enzyme extract, and 1 ml 0.3% H₂O₂. The reaction was initiated by adding enzyme extract. One unit of CAT activity was defined as the 0.01 deduction in absorbance at 240 nm per minute. The enzyme activities were calculated by Kong et al. (1999).

Ascorbate peroxidase activity Ascorbate peroxidase (EC 1.11.1.11) activity was determined as described by Nakano and Asada (1987). The reaction mixture (1 ml) contained 50 mM potassium phosphate (pH 7.0), 1.2% sucrose, 1 mM hydrogen peroxide, 0.5 mM ascorbate, and 10 mM 3-aminotriazole (an inhibitor of catalase). The hydrogen peroxide-dependent oxidation of ascorbate was

followed by monitoring the decrease in absorbance at 290 nm, using the extinction coefficient of 2.8 mM cm⁻¹.

Statistical analysis

The analysis of variance procedure of split-plot design according to Snedecor and Cochran (1990), treatments means were compared using Duncan (1955) test at 5% of probability. Combined analysis of the two growing seasons was carried out.

Results

Carbohydrate constituents

Glucose, sucrose, trehalose, TSS, and starch contents of quinoa leaves in response to treatment of quinoa plants with different concentrations of trehalose under drought stress are presented in Fig. 1. Data clearly show that skipping irrigation at 50 and 60 days after sowing increased significantly glucose, trehalose, total soluble sugars, and starch contents of quinoa leaves as compared with those irrigated normally (control plants); meanwhile, sucrose contents decreased. More accumulation of the tested organic solutes of

leaves of the trehalose-treated plant in both normal irrigated and drought stressed quinoa plants.

Changes in proline and free amino acid contents

Data clearly show that treatments with different concentrations of trehalose on quinoa plant under normal and drought stress are presented in Fig. 2. Skipping two irrigation times at 50 and 60 days after sowing caused significant increases in proline and free amino acids of quinoa leaves as compared with normal irrigated plants (control). Meanwhile, foliar treatment of different concentrations of trehalose 0.1 mM and 0.5 mM (Tre₁ or Tre₂) decreased significantly and gradually proline and free amino acid contents.

Changes in phenolic contents

Data presented in Fig. 2 showed the effect of different concentrations of trehalose treatment on quinoa plant under drought stress. Drought stress (D1) caused significant increases in phenolic contents relative to control plant (D0 Tre0). Trehalose foliar treatment with

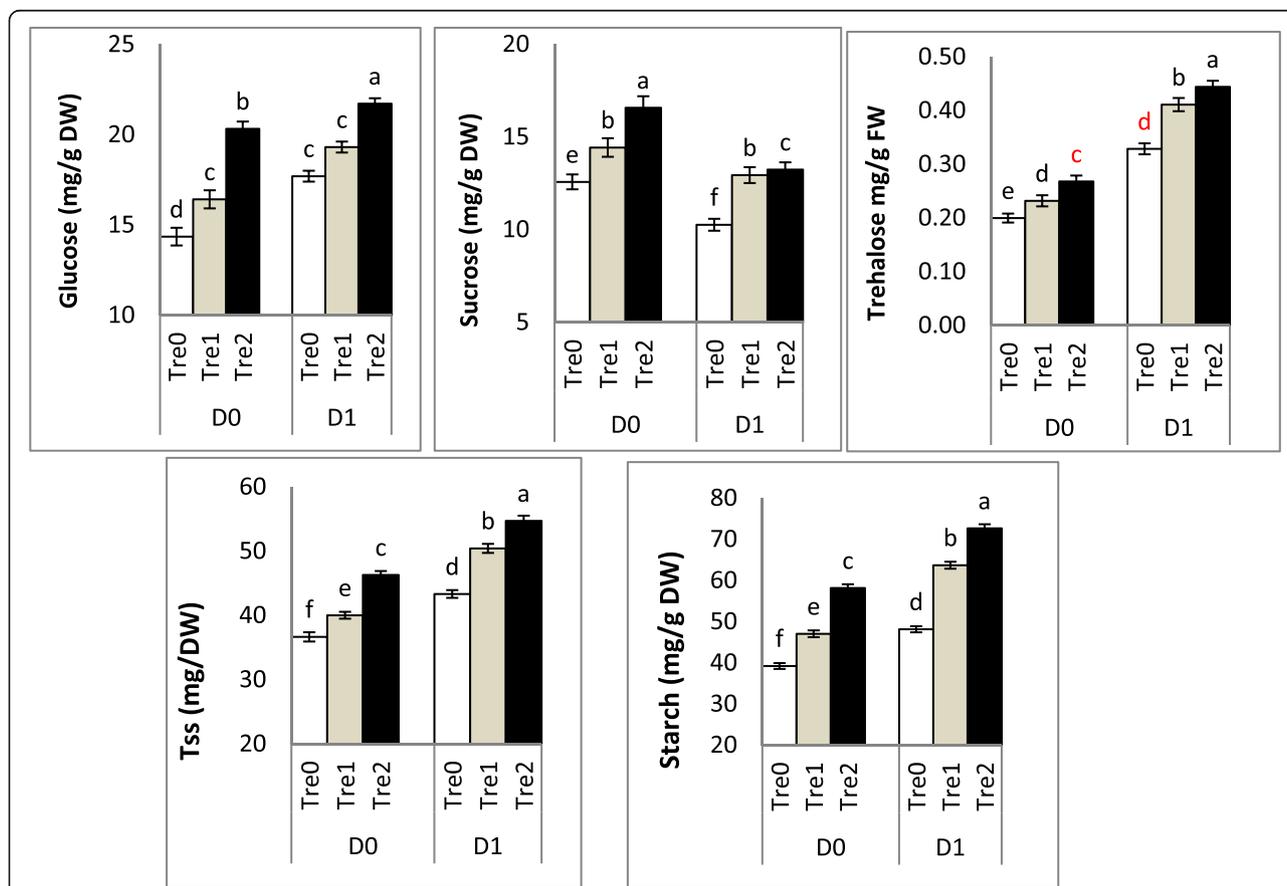


Fig. 1 Effect of trehalose (Tre1, 0.1 mM and Tre2, 0.5 mM) on carbohydrates constituents (TSS, glucose, sucrose, starch mg/g DW and trehalose μg/g FW) of quinoa plant under drought stress (at 75 days from sowing). Each value represents the mean ± standard error (n = 3). Means with different letters above bars were significantly different at the 0.05 level according to Duncan's multiple range test

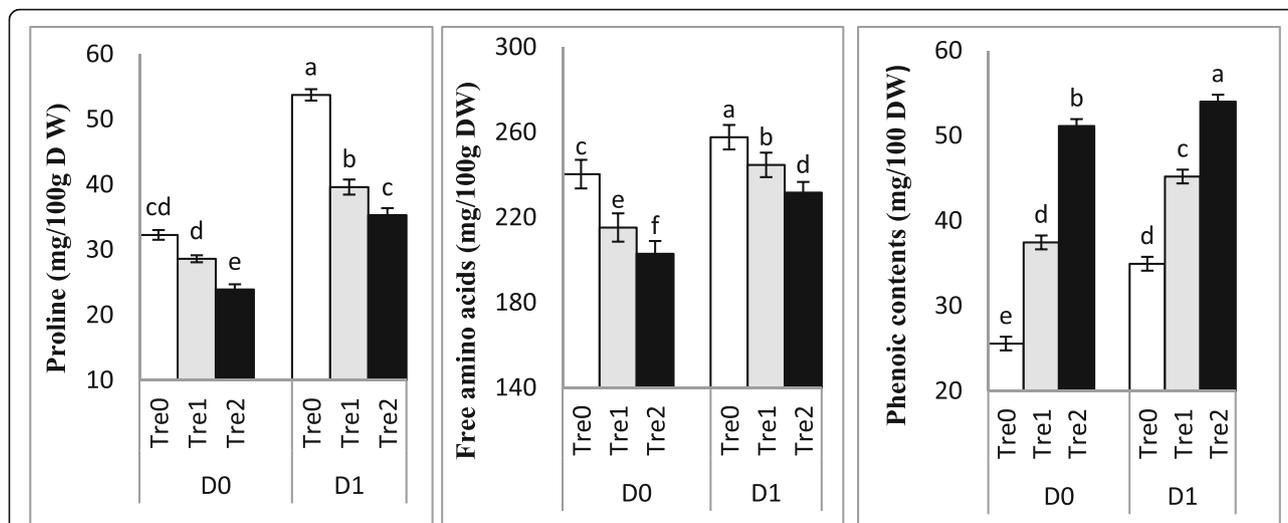


Fig. 2 Effect of trehalose (Tre1, 0.1 mM and Tre2, 0.5 mM) on proline, free amino acids and phenolic contents (mg/100 g DW) of quinoa plant under drought stress (at 75 days from sowing). Each value represents the mean \pm standard error ($n = 4$). Means with different letters above bars were significantly different at the 0.05 level according to Duncan's multiple range test

different concentrations (Tre1 and Tre2) in normal irrigation caused more significant increases in phenolic content as compared with control plant (D0 Tre0). Then, 0.5 mM trehalose was more effective than 0.1 mM trehalose in improving phenolic contents.

Hydrogen peroxide

The level of hydrogen peroxide (H_2O_2) upon drought stress is presented in (Fig. 3), H_2O_2 level sharply increased in quinoa leaves. The H_2O_2 level increased by 43% in the D1 drought stress plants compared with the

control plants (D0). Trehalose foliar treatment decreased significantly the level of H_2O_2 in the normal irrigated plants and drought compared with the corresponding control.

Lipid peroxidation

The effect of trehalose foliar treatment on quinoa plants grown under drought stress are presented in (Fig. 3). Drought stress induced oxidative damage, assessed by increasing in lipid peroxidation in the tested quinoa plant (Fig. 3) as compared with unstressed control

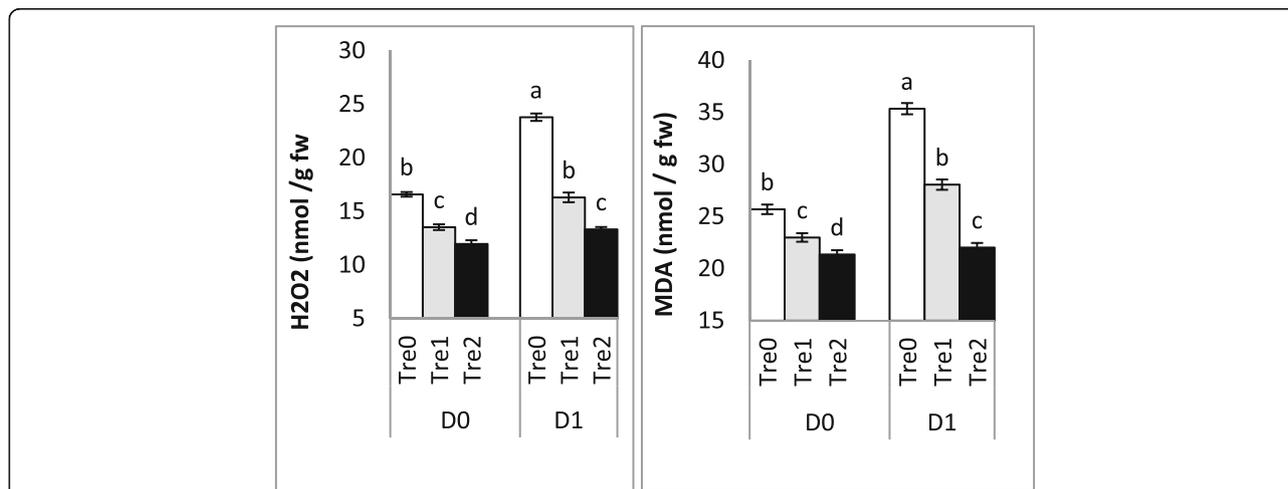


Fig. 3 Effect of trehalose (Tre1, 0.1 mM and Tre2, 0.5 mM) on H_2O_2 , MDA contents (nmol/g fw) of quinoa plant under drought stress (at 75 days from sowing) Each value represents the mean \pm standard error ($n = 3$). Means with different letters above bars were significantly different at the 0.05 level according to Duncan's multiple range test

plants. Moreover, gradual decreases in lipid peroxidation by trehalose foliar treatment with different concentrations (Tre1 and Tre2) as compared with the corresponding control in normal and stressed plants.

Enzyme activities

Lipoxygenase activity

Lipoxygenase activities in quinoa leaves were increased by 141% under drought stress (D1) compared to non-stress control (D0) Fig. 4. In non-stressed plants, trehalose treatment had no significant effect on LOX activity. Regarding to trehalose treatment combined with

drought treatment (Fig. 4), LOX enzyme activity decreased gradually and significantly as compared with their corresponding control.

Antioxidant enzymes

Activities of various antioxidant enzymes in drought-stressed and trehalose foliar-treated plants are presented in (Fig. 4). Drought stress by skipping irrigation caused significant increases in SOD, POX, and APX, but CAT activity significant decreased. Trehalose foliar treatment with different concentrations Tre1 and Tre2 increased plant tolerance to stress via significant increases in SOD,

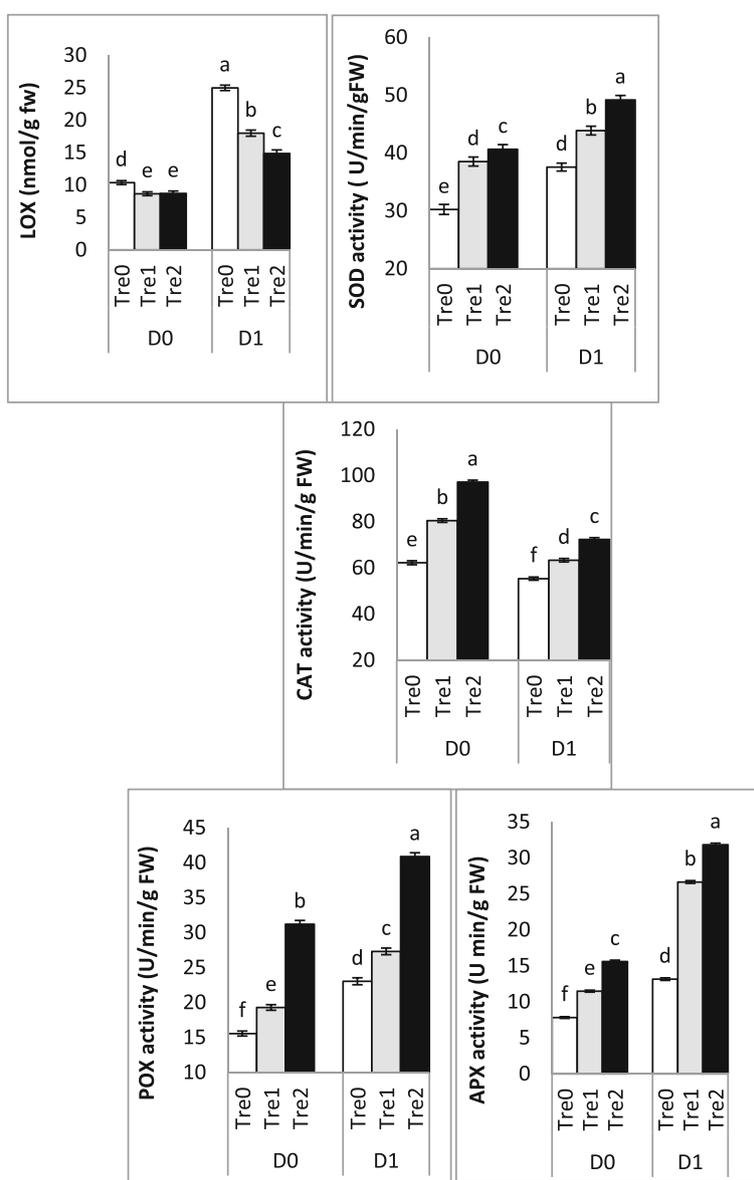


Fig. 4 Effect of trehalose (Tre1, 0.1 mM and Tre2, 0.5 mM) on LOX activity (nmol/g FW) and antioxidant enzyme activities (SOD, CAT, POX, and APX) (U/min/gFW) of quinoa plant under drought stress (at 75 days from sowing); each value represents the mean \pm standard error ($n = 3$). Means with different letters above bars were significantly different at the 0.05 level according to Duncan's multiple range test

CAT, POX, and APX activities as compared with D0 and D1. Further, 0.5 mM trehalose was more effective than 0.1 mM in increasing enzyme activities of quinoa plants.

Discussion

Carbohydrates constituents

Regarding carbohydrates constituents of quinoa leaves treated with trehalose different concentrations and grown under drought stress (Fig. 1). Data show accumulation of different carbohydrates constituents as glucose sucrose, trehalose, TSS, and starch contents. Although accumulation of high soluble sugar levels have also been demonstrated in shoots of different plant species under drought stress conditions (Bakry et al. 2012 and Dawood and Sadak 2014). Increased levels of soluble sugars in drought-stressed plants may help in turgor upkeeping and cellular membrane stabilization (Hosseini et al., 2014). This increases of soluble sugars is a response of plants to drought stress that has been widely reported despite the decrease in net CO₂ assimilation rate (Mura-keozy et al. 2003). In addition, soluble sugars may act as ROS scavengers to improve membrane stabilization (Hosseini et al. 2014).

During plant development, trehalose could play an important role in regulating carbohydrate allocation, as Tre acting as soluble sugars in quinoa leaves and its function is an osmoprotectant under water deficit-induced stresses. The present study show that in quinoa leaves, trehalose accumulation is concomitant with increasing sucrose level under normal and skipped irrigation (Fig. 1). Garg et al. (2007) confirmed these obtained results. The indirect effect of Tre on carbohydrate metabolism may be via conversion and utilization of other sugars and interfering with photosynthetic capacity (Ranwala and Miller 2009). Application of trehalose induced an additive stimulatory effect on the accumulation of trehalose contents in the drought plants. These results are in good agreements with those obtained by Ma et al. (2013). The improving effect of trehalose may be due to its physicochemical properties that stabilize dehydrated enzymes, proteins, and lipid membranes, as well as protect structures from damage during desiccation (Fernandez et al. 2010). Also, Tre involved in gene detoxification and response to stress as it acts as gene elicitor (Bae et al. 2005).

In the present study, the starch content increased in quinoa plant normal irrigated and drought stressed in response to trehalose corresponding control and consequence of trehalose accumulation (Fig. 1). Bae et al. (2005) reported that in *Arabidopsis thaliana* seedlings, starch was threefold greater in trehalose-treated plants than in the control. The increase in the level of sucrose, trehalose, and total soluble sugar (Fig. 1) may be linked

to the changes in starch content. Accumulation of starch in quinoa leaves may be a protective mechanism as it is an important component of plant cells and its increases in leaves as a reserve form of carbon (Mishra and Prakash 2010). The increases in starch by trehalose treatment might be due to increased activity of ADP-glucose pyrophosphorylase (AGPase), a major enzyme controlling starch synthesis in *Arabidopsis* (Bae et al. 2005).

Changes in proline and free amino acids

In the present work, water-deficient stress caused significant increases of proline and free amino acids, whereas decreased by trehalose application (Fig. 2). Accumulation of compatible osmolytes high concentrations causing the osmotic adjustment in plants under drought stress. Proline has vital roles in osmotic adjustment, stabilization, and protection of enzymes, proteins, and membranes (Ashraf and Foolad 2007) from damaging effects of drought-osmotic stresses. Also, reducing oxidation of lipid membranes (Demiral and Türkan 2004). Trehalose addition with drought stress reduced proline and free amino acids levels in quinoa plant (Fig. 2). Prevention of extra proline biosynthesis due to exogenous trehalose addition under drought stress suggests that trehalose prevented quinoa seedlings from adverse effects of drought stress by other means so that the studied quinoa seedlings did not need to increase the proline levels further. These results are corroborated to previous studies (Alam et al. 2014 and Abdallah et al. 2016).

Phenolic contents

Figure 2 shows that drought stress and/or trehalose foliar treatments enhanced the phenolic content of quinoa leaves. Increase in phenol contents in different tissues under osmotic stress have been reported in many plants (Rady et al. 2011, Dawood and Sadak 2014 and Sadak 2016a, b). These increases may be due to total phenols role that play a significant mechanism in regulation of plant metabolic processes and consequently overall plant growth (Abdallah et al. 2015). Moreover, phenols act as a substrate for many antioxidant enzymes; so, it mitigates the drought stress injuries (Bakry et al. 2012). Another mechanism underlying the antioxidative properties of phenolic compounds is the ability of phenols to decrease membrane fluidity (Sadak 2016b). In addition, the antioxidant role of phenolic compounds as free radical scavenger through their reactivity as electron or hydrogen donor, to stabilize and delocalize the unpaired electron, and from their role as transition metal ions chelater (Huang et al. 2005). The improvement in the role of trehalose might be due to its signaling function, via different metabolic pathways induction and induce the production of various substances, preferably operating under stress (Alam et al. 2014).

H₂O₂ content and lipid peroxidation

As shown in Fig. 3, quinoa plants responded to drought stress by inducing marked increases in H₂O₂ and MDA contents. Under stress, accumulation of ROS including H₂O₂ caused oxidative damage in plants and produced MDA, the potential biomarker of membrane lipid peroxidation in the cellular environment (Abdelhamid et al. 2013; Orabi and Sadak 2015; El Bassiouny et al. 2015). Drought stress caused changes in physical membrane organization and modification in the lipid matrix of the plasma membrane. In addition, it caused marked increases in MDA and H₂O₂ levels in different plant species including rapeseed and mustard may be due to inadequate induction of antioxidant system as mentioned by Hossain et al. (2013). Similar results of increased H₂O₂ and MDA contents under stress were obtained by El Bassiouny et al. (2015) on wheat, Dawood and Sadak (2014) on canola, and Mostafa et al. (2015) on rice plant. However, trehalose foliar treatment prior to drought stress resulted in lower H₂O₂ production, and reduced MDA content, which may be achieved via trehalose-mediated direct ROS scavenging, antioxidative mechanism involved in eliminating ROS, or stabilizing of membrane. Exogenous trehalose also reduced the levels of H₂O₂ and MDA in rice seedlings under salt stress, respectively (Mostafa et al. 2015).

Lipoxygenase activity

Lipoxygenase (LOX) activities increased in quinoa plants subjected to water deficiency (Fig. 4), which concomitant with higher MDA levels (Fig. 3). In addition, increased LOX activity might have contributed to the lipid peroxidation of membrane lipids and thereby significantly participate to the oxidative damage in water-deficient plant. Increased LOX activity is responsible for oxidation of polyunsaturated fatty acids and thus enhances lipid peroxidation under stress conditions (Sánchez-Rodríguez et al. 2012). The beneficial roles of Tre in drought-affected quinoa plants were observed as those treatments reduced oxidative stress (decreased H₂O₂ reduced LOX and lowered MDA levels; Figs. 3 and 4), compared to Tre-untreated drought-affected plants. These results are in agreement with Nounjan and Theerakulpisut (2012), Ma et al. (2013), and Liu et al. (2013); they confirmed that exogenous application of Tre was effective in reducing oxidative stress of different plants in different abiotic stresses.

Antioxidant enzymes activities

Plant possess' efficient system for scavenging ROS. Antioxidative enzymes are not part of this system but are key elements in the defense mechanisms. Activities of antioxidant enzymes of plants under stress show many changes (Abdelhamid et al. 2013). Superoxide dismutase,

catalase, ascorbate peroxidase, and peroxidase are of enzymes that are responsible for ROS-scavenging. Drought exhibited increased SOD or POX and APX activities in quinoa leaves as compared to normal irrigated plants (Fig. 4). These results are in agreement with (Abdelgawad et al. 2014). Higher levels of enzyme activities in the quinoa plant under water deficiency may be due to its resistance. NAD⁺ recovering and CO₂ fixation at the Calvin cycle decrease under drought stress cause damage to cell membrane due to the increases of free radicals. Adverse environmental stresses increase catalase and ascorbate peroxidase activities in several cycles of physiological processes. At stress conditions, higher content of hydrogen peroxide is detoxified by catalase and glutathione peroxidase (Dat et al. 2000). Plant tolerance to harmful free radicals increase by ascorbate peroxidase and catalase activities increases (Jin et al. 2006). Superoxide dismutase (SOD) is the first defense enzyme that converts superoxide to H₂O₂, which can be scavenged by catalase (CAT) and different classes of peroxidases (POX) and ascorbate peroxidase. These results are in agreement with the results observed by El-Bassiouny and Sadak (2015), Sadak and Orabi (2015), and Sadak and Abdelhamid (2015).

Foliar treatment of trehalose increased tolerance to stress by the increase in CAT, SOD, POX, and APX activities. Abdallah et al. (2016) confirmed these results. Under drought stress, Tre treatment improve SOD activity (Nounjan and Theerakulpisut 2012). However, Nounjan et al. (2012) also confirmed Tre major role in O₂⁻ scavenging directly than modulating SOD activity. Although, CAT is one of the most important enzyme of antioxidant system having the highest turnover rates among all enzymes (Garg and Manchanda 2009). However, enhanced CAT activity in Tre-pretreated plants represents an efficient H₂O₂ scavenging mechanism contributing to tolerance to water stress-induced oxidative stress (Mostafa et al. 2015). CAT, a peroxisomal enzyme, has a lower affinity for H₂O₂ and, therefore, is regarded as a bulk remover of excess H₂O₂ produced under stress conditions.

Conclusion

Osmolytes as Tre improved drought tolerance of quinoa plant via increasing formation of osmoprotectant compounds as glucose, trehalose, total soluble sugars, proline, and free amino acids. In addition to enhancing antioxidant enzyme activities. On the other hand, MDA and H₂O₂ contents decrease.

Acknowledgements

Not applicable.

Funding

Not applicable.

Availability of data and materials

Not applicable.

Authors' contributions

All authors share in every step of this work and all of them contribute in writing the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 27 June 2018 Accepted: 7 December 2018

Published online: 17 January 2019

References

- Abdallah MMS, Abdelgawad ZA, El-Bassiouny HMS (2016) Alleviation of the adverse effects of salinity stress using trehalose in two rice varieties. *S Afr J Bot* 103:275–282
- Abdallah MMS, El-Bassiouny HMS, Bakry AB, Sadak MS (2015) Effect of arbuscular mycorrhiza and glutamic acid on growth, yield, some chemical composition and nutritional quality of wheat plant grown in newly reclaimed Sandy soil. *RJPBCS* 6(3):1038–1054
- Abdelgawad ZA, Khalafa Allah AA, Abdallah MM (2014) Impact of methyl jasmonate on antioxidant activity and some biochemical aspects of maize plant grown under water stress condition. *Agric Sci* 5:1077–1088
- Abdelhamid MT, Sadak MS, Schmidhalter U, El-Saad A (2013) Interactive effects of salinity stress and nicotinamide on physiological and biochemical parameters of faba bean plant. *Acta Biol Colomb* 18(3):499–510
- Alam M, Nahar K, Hasanuzzaman M, Fujita M (2014) Trehalose-induced drought stress tolerance: A comparative study among different Brassica species. *Plant Omics J* 7(4):271–283
- Ashraf M, Foolad MR (2007) Roles of glycine betaine and proline in improving lant abiotic stress tolerance. *Environ Exp Bot* 59:206–216
- Bae H, Herman E, Bailey B, Bae HJ, Sicher R (2005) Exogenous trehalose alters Arabidopsis transcripts involved in cell wall modification, abiotic stress, nitrogen metabolism and plant defense. *Physiol Plant* 125:114–126
- Bakry BA, El-Hariri DM, Sadak MS, El-Bassiouny HMS (2012) Drought stress mitigation by foliar application of salicylic acid in two linseed varieties grown under newly reclaimed Sandy soil. *J Appl Sci Res* 8(7):3503–3514
- Bates LS, Waldan RP, Teare LD (1973) Rapid determination of free proline under water stress studies. *Plant Soil* 39:205–207
- Bhargava A, Shukla S, Ohri D (2007) Genetic variability and interrelationship among various morphological and quality traits in quinoa (*Chenopodium quinoa* Willd.). *Field Crop Res* 101:104–116
- Chapman HO, Pratt PE (1978) Methods of analysis for soils, plants and water. Division of Agriculture Sciences University California, Berkley, pp 5–6
- Chen JX, Wang XF (2006) Plant physiology experimental guide. Higher Education Press, Beijing, pp 24–25:55–56
- Danil AD, George CM (1972) Peach seed dormancy in relation to endogenous inhibitors and applied growth substances. *J Amer Soc Hort Sci* 17:621–624
- Dat J, Vandenabeele S, Vranova E, Van Montagu M, Inzé D, Van Breusegem F (2000) Dual action of the active oxygen species during plant stress responses. *Cell Mol Life Sci* 57:779–795
- Dawood MG, Sadak MS (2014) Physiological role of glycinebetaine in alleviating the deleterious effects of drought stress on canola plants (*Brassica napus* L.). *Middle East J Agric Res* 3(3):638–644
- Demiral T, Türkan I (2004) Does exogenous glycine betaine affect antioxidative system of rice seedlings under NaCl treatment? *J Plant Physiol* 161:108
- Demirevska K, Simova-Stoilova L, Fedina I, Georgieva K, Kunert K (2010) Response of oryzacystatin I transformed tobacco plants to drought, heat and light stress. *J Agron Crop Sci* 196:90–99
- Doderer A, Kokkelink I, van der Veen S, Valk B, Schram A, Douma A (1992) Purification and characterization of two lipoxygenase isoenzymes from germinating barley. *Biochim Biophys Acta* 112:97–104
- Duncan DB (1955) Multiple range and multiple F-tests. *Biometrics* 11:1–42
- El Bassiouny HMS, Abd Allah MMS, Rady MM, Gaballah MS, El-Sebai TN (2015) Role of blue-green algae, glutathione and salicylic acid on the oxidative defense systems of wheat plant grown in saline soil. *Int J PharmTech Res* 8(10):18–31
- El-Bassiouny HMS, Sadak MS (2015) Impact of foliar application of ascorbic acid and α -tocopherol on antioxidant activity and some biochemical aspects of flax cultivars under salinity stress. *Acta Biol Colomb* 20(2):209–222
- Elbein AD, Pan YT, Pastuszak I, Carroll D (2003) New insights on trehalose: a multifunctional molecule. *Glycobiology* 13(4):17r–27r ISSN 0959–6658
- Fernandez O, Béthencourt L, Quero A, Sangwan RS, Clément C (2010) Trehalose and plant stress responses: friend or foe? *Trends Plant Sci* 15:409–417
- Feteris AW (1965) A serum glucose method without protein precipitation. *Am J Medical Technol* 31:17–21
- Fuentes FF, Bazile D, Bhargava A, Martínez EA (2012) Implications of farmers' seed exchanges for on-farm conservation of quinoa, as revealed by its genetic diversity in Chile. *J Agric Sci* 150:702–716
- Garg AK, Ranwala AP, Miller WB, Wu RJ (2007) HPLC analysis of trehalose and other soluble carbohydrates from the leaf tissue of Indica and Japonica rice varieties. *Rice Genetics Newsletter* 4:44–47 U.S.A
- Garg N, Manchanda G (2009) ROS generation in plants: boon or bane? *Plant Biosys* 143:8–96
- Handel EV (1968) Direct microdeterminations of sucrose. *Anal Biochem* 22:280–283
- Hasanuzzaman M, Nahar K, Gil SS, Fujita M (2014) Drought stress responses in plants, oxidative stress and antioxidant defense. In: Gill SS, Tuteja N (eds) *Climate change and plant abiotic stress tolerance*. Wiley, Weinheim, pp 209–249
- Hodges DM, De Long JM, Forney C, Prange PK (1999) Improving the thiobarbituric acid reactive substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta* 207:604–611
- Homme PM, Gonzalez B, Billard J (1992) Carbohydrate content, frutane and sucrose enzyme activities in roots, stubble and leaves of rye grass (*Lolium perenne* L.) as affected by sources / link modification after cutting. *J Plant Physiol* 140:282–291
- Hossain MA, Mostofa MG, Fujita M (2013) Cross protection by cold-shock to salinity and drought stress-induced oxidative stress in mustard (*Brassica campestris* L.) seedlings. *Molecular Plant Breeding* 4:50–70
- Hosseini SM, Hasanloo T, Mohammadi S (2014) Physiological characteristics, antioxidant enzyme activities, and gene expression in 2 spring canola (*Brassica napus* L.) cultivars under drought stress conditions. *Turkish journal agricultural. Forestry* 38:1–8
- Huang C, He W, Guo J, Chang X, Su P, Zhang L (2005) Increased sensitivity to salt stress in ascorbate-deficient Arabidopsis mutant. *J Exp Bot* 56:3041–49
- Jin J, Shan N, Ma N, Bai J, Gao J (2006) Regulation of ascorbate peroxidase at the transcript level is involved in tolerance to postharvest drought stress in the cut rose (*Rosa hybrida* L.) cv. Samantha. *Postharvest Biol Tec* 40:236–243
- Kong FX, Hu W, Chao WL, Sang WL, Wang LS (1999) Physiological responses of Mexicana to oxidative stress of SO₂. *Environ Exp Bot* 42:201–209
- Kumar KB, Khan PA (1982) Peroxidase and polyphenol oxidase in excised ragi (Eleusine coracana cv. PR 202) leaves during senescence. *Indian J Exp Bot* 20:412–416
- Li ZG, Luo LJ, Zhu LP (2014) Involvement of trehalose in hydrogen sulfide donor sodium hydrosulfide-induced the acquisition of heat tolerance in maize (*Zea mays* L.) seedlings. *Bot Stud* 55:20
- Liu X, Hung C, Chen Q, Wu X, Zhang J (2013) Alleviates effects of exogenous trehalose on oxidative damage metabolism in *Pleurotus pulmonaris* under heat stress. *Acta Hort Sin* 40:1501–1150
- Luo Y, Li F, Wang GP, Yang XH, Wang W (2010) Exogenously-supplied trehalose protects thylakoid membranes of winter wheat from heat-induced damage. *Biol Plant* 54:495–501
- Ma C, Wang Z, Kong B, Lin T (2013) Exogenous trehalose differentially modulate antioxidant defense system in wheat callus during water deficit and subsequent recovery. *Plant Growth Regul* 70:275–285
- Mahajan S, Tuteja N (2005) Cold, salinity and drought stresses: an overview. *Arch Biochem Biophys* 444:139–158
- Mishra MK, Prakash V (2010) Response of non- enzymatic antioxidants to zinc induced stress at different pH in *Glycine max* L. cv. Merrill. *Acad J Plant Sci* 1:1–10

- Mostafa MG, Hossain MA, Fujita M (2015) Trehalose pretreatment induces salt tolerance in rice (*Oryza sativa* L.) seedlings: oxidative damage and co-induction of antioxidant defense and glyoxalase systems. *Protoplasma* 252:461–475
- Murakeozy EP, Nagy Z, Duhaze C, Bouchereau A, Tuba Z (2003) Seasonal changes in the levels of compatible osmolytes in three halophytic species of inland saline vegetation in Hungary. *J Plant Physiol* 160:395–401
- Nakano Y, Asada K (1987) Purification of ascorbate peroxidase in spinach chloroplast; its inactivation in ascorbate-depleted medium and reactivation by monodehydroascorbate radical. *Plant Cell Physiol* 28:131–140
- Nelson NA (1944) (1944) photometric adaptation of the Somogyi method for the determination of glucose. *J Biol Chem* 153:375–380
- Nounjan N, Nghia PT, Theerakulpisut P (2012) Exogenous proline and trehalose promote recovery of rice seedlings from salt-stress and differentially modulate antioxidant enzymes and expression of related genes. *J Plant Physiol* 169:596–604
- Nounjan N, Theerakulpisut P (2012) Effects of exogenous proline and trehalose on physiological responses in rice seedlings during salt-stress and after recovery. *Plant Soil Environ* 58:309–315
- Orabi SA, Sadak MS (2015) Alleviation of adverse effects of salinity stress on wheat (*Triticum aestivum* L.) by exogenous application of hydrogen peroxide. *J Basic Appl Res Inte* 8(4):287–303
- Petridis A, Therios I, Samouris G, Tananaki C (2012) Salinity induced changes in phenolic compounds in leaves and roots of four olive cultivars (*Olea europaea* L.) and their relationship to antioxidant activity. *Environ Exp Bot* 79:37–43
- Rady MM, Sadak MS, El-Bassiouny HMS, Abd El-Monem AA (2011) Alleviation of the adverse effects of salinity stress in sunflower cultivars using nicotinamide and α -tocopherol. *Aust. J Basic Appl Sci* 5(10):342–355
- Ranwala AP, Miller WB (2009) Comparison of the dynamics of non-structural carbohydrate pools in cut tulip stems supplied with sucrose or trehalose. *Postharvest Biol Technol* 52:91–96
- Repo-Carrasco-Valencia RAM, Serna LA (2011) Quinoa (*Chenopodium quinoa* Willd.) as a source of dietary fiber and other functional components. *Cienc Tecnol Aliment* 31:225–230
- Sadak MS (2016a) Mitigation of salinity adverse effects on wheat by grain priming with melatonin. *Int J Chem Tech Res* 9(2):85–97
- Sadak MS (2016b) Mitigation of drought stress on fenugreek plant by foliar application of trehalose. *Int J Chem. Tech. Res.* 9(2):147–155
- Sadak MS, Abdelhamid MT (2015) Influence of amino acids mixture application on some biochemical aspects, antioxidant enzymes and endogenous polyamines of *Vicia faba* plant grown under seawater salinity stress. *Gesunde Pflanzen* 67:119–129
- Sadak MS, Orabi SA (2015) Improving thermo tolerance of wheat plant by foliar application of citric acid or oxalic acid. *Int J Chem Tech Res* 8(1):111–123
- Sánchez-Rodríguez E, Rubio-Wilhelmi MDM, Blasco B, Leyva R, Romero L, Ruiz JM (2012) Antioxidant response resides in the shoot in reciprocal grafts of drought-tolerant and drought-sensitive cultivars in tomato under water stress. *Plant Sci* 188–189:89–96
- Snedecor GW, Cochran WG (1990) *Statistical methods*, 8th edn. Iowa State University Press, Ames
- Theerakulpisut P, Gunnula W (2012) Alleviation of adverse effects of salt stress on rice seedlings by exogenous trehalose. *Asian J Crop Sci* 4(4):405–415.
- Vartanian N, Hervochon P, Marcotte L, Larher F (1992) Proline accumulation during drought rhizogenesis in *Brassica napus* var. *Oleifera*. *Plant Physiol* 140:623–628
- Yemm EW, Cocking EC (1955) The determination of amino acids with ninhydrin. *Analyst* 80:209–213
- Yemm EW, Willis AJ (1954) The respiration of barley plants. IX The metabolism of roots during assimilation of nitrogen. *New Phytol* 55:229–234

Submit your manuscript to a SpringerOpen[®] journal and benefit from:

- Convenient online submission
- Rigorous peer review
- Open access: articles freely available online
- High visibility within the field
- Retaining the copyright to your article

Submit your next manuscript at ► [springeropen.com](https://www.springeropen.com)
