Improving drought tolerance of quinoa plant by foliar treatment of trehalose

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Abstract: Two field experiments were conducted during two successive seasons (2014/2015 and 2015/2016) at the Experimental Station of National Research Centre, Nubaria district, Beheira Governorate, Egypt, to study the effect of foliar treatment of quinoa plants with trehalose (Tre) (100 µM and 500 µM) on the growth, photosynthetic pigments, seed yield quantity & quality, in fever of nutritional and antioxidant compounds in the yielded quinoa seeds which subjected to water deficiency (skipping two irrigation times at 50 & 60 days after sowing). Water deficiency caused marked decreases in quinoa plant growth parameters (shoot height, fresh and dry weights of shoot/plant) and photosynthetic pigments with marked increases in root growth parameters (root length, fresh and dry weight of root/plant). Drought stress decreased yield and yield attributes, carbohydrates, protein, nitrogen, phosphorous and potassium contents. Meanwhile, oil percentage, phenolic and flavonoids contents increased by drought stress. Antioxidant activity at 50 and 100 µg/L showed significant increases in response to drought stress. On the other hand, Tre treatments were proved to be effective in enhancing growth parameters and photosynthetic pigments of drought stressed plants. Trehalose treatments at different levels caused marked increases in yield and yield attributes, carbohydrate, protein, oil, nitrogen, phosphorous, potassium, total phenolic, flavonoids contents, and antioxidant activity of the yielded seeds either in non-stressed or drought stressed plants relative to corresponding controls. Generally, 500 µM Tre was the most pronounced and effective treatment in alleviating the deleterious effect of drought stress on quinoa plants.

Keywords: Antioxidant activity, drought, flavonoids, oil, phenolics, protein, Quinoa, trehalose

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1 Introduction

Quinoa (*Chenopodium quinoa*Willd), is the newly introduced food crop that can replenish a part of food gap all over the world. Quinoa is considered as a multipurpose crop, seeds can be utilized as human food or animal feedstock because of its high nutritive value (Repo-Carrasco *et al.*, 2003); high protein quality, different vitamins and essential minerals as mentioned by Jacobsen *et al*, (2003), Ogungbenle (2003) and Shams (2010). In addition, quinoa seeds have enormous potentials in the food industry as being gluten-free, highly nutritious an did not contain anti-nutritional factors (Doweidar & Kamel, 2011). Therefore, it is recommended as an useful staple food industry for formulations of baby food (Ogungbenle, 2003). Quinoa can grow in sandy soil of arid & semiarid regions in addition to its tolerance to salinity, drought and other abiotic stresses which reduce crop production (Jacobsen, 2003).

In many different regions of the world drought is one of the most important stress limiting plant production (Passioura, 2007). Drought stress happens when soil available water reduced and the atmospheric conditions caused continuous water loss by evaporation or transpiration (Khaje Hosseini *et al*., 2003). Drought stress severely reduced plant yield despite of the growth stage at which it occurs (Jensen & Mogensen, 1984). Plants under dry condition have the ability to change their metabolism

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to overcome the changed environmental condition. These changes include physiological and biochemical changes like reduced leaf size, stem extension, root proliferation, reduced water use efficiency (Farooq *et al.,* 2009), alteration in metabolic activities (Lawlor & Cornic, 2002), disturbances in accumulation of solute and ionic imbalance (Khan *et al.,* 1999) or a combination of all these factors.

To improve plant tolerance to drought stress over the last few decades, in parallel with traditional breeding and biotechnological strategies, several techniques have been proposed to improve plant performance in drought environments. These techniques include seed pre sowing treatment or foliar treatment with antioxidant, vitamins and osmoprotectant compounds as glycine betaine, proline, or trehalose (Dawood & Sadak, 2014). Trehalose (Tre) is a non-reducing disaccharide of glucose, plays an important role as a stress protectant in some plants (Duman*et al*., 2010 and Ali & Ashraf, 2011). Trehalose is an energy source in addition to its unique physicochemical properties in protecting cell components from damage during desiccation as well as stabilizes efficiently dehydrated enzymes, proteins, and lipid membranes (Fernandez *et al*. 2010). Tre acts as an antioxidant and signaling molecule as well as genes elicitor involved in detoxification and stress response (Bae*et al*., 2005). In most plants, interior Tre production is not sufficient to mitigate the adverse effects induced by stress. Thus, external application of Tre caused increases in its internal level (Chen and Murata 2002). Exogenous Tre alleviates the adverse effects of various abiotic stresses including drought in maize, heat and water deficit in wheat (Luo et al. 2010; Ma et al. 2013) and fenugreek (Sadak., 2016) .

Therefore, this study was conducted to investigate the effects of exogenous applications of trehalose on various growth parameters, photosynthetic pigments, yield, yield attributes and some nutritional constituents of the yielded seeds of quinoa plants grown under drought stress.

2 Materials and Methods

2.1 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 2014/2015 and 2015/2016. The soils of both experimental sites were newly reclaimed sandy soil where mechanical and chemical analysis is reported in Table 1 according toto Chapman and Pratt (1978).

Table 1 Mechanical and chemical analysis of the experimental soil sites

A- Mechanical analysis

Sand		Silt 20-0 µ%	Clay<2 µ%	Soil texture
Course 2000-200 µ%	Fine 200-20 µ%			
47.46	36.19	12.86	4.28	Sandy

B- Chemical analysis

pH 1:2.5	EC, dSm^{-1}	$CaCO_3$, %	OM, %	Macroelement ppm			Microelement ppm		
				N	P	K	Zn	Fe	Mn
7.60	0.13	1.5	0.06	52	12.0	75	0.14	1.4	0.3

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 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 meters long, and the distance between rows was 20 cm apart. Plot area was 10.5 m^2 (3.0 m in width and 3.5 m in length). The recommended agricultural practices of growing quinoa were applied. Pre-sowing, 150 kg/feddan of calcium super-phosphate (15.5% P_2O_5) was applied to the soil. Nitrogen was applied after emergence in the form of ammonium nitrate 33.5% at a rate of 75 kg/feddan in five equal doses before the 1st, 2nd, 3rd, 4th and 5th irrigation. Potassium sulfate (48.52% K_2O) was added in two equal doses of 50 kg/feddan, 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 (D_0) and skipping two irrigation times (D_1). Different trehalose treatments were carried out twice; where plants were sprayed after 30 and 45 days from sowing. Normal water irrigation was skipped twice at 50 and 60 days after sowing. Plant samples were taken after 75 days from sowing for estimation of some growth parameters as plant height (cm), branches & leaves number/plant, fresh & dry weight of shoot/plant (g), relative water content% (RWC), root length (cm), fresh & dry weight of root/plant (g), photosynthetic pigments of leaves. Yield and its components as plant height (cm), number of fruiting branches/plant, dry weight of plant (g), seed weight/plant and 1000 seeds wt (g). Nutritive value of the yielded seeds as total carbohydrates%, protein%, oil%, phenolic contents, flavonoids content,nitrogen, phosphorus and potassium contents, in addition to antioxidant activities % at 50% and 100% µg/mL of quinoa extract.

2.2 Biochemical analysis

Chlorophyll a, chlorophyll b and carotenoids concentrations were estimated using the method of Moran (1982). Total carbohydrateswere determined according to Dubois *et al.* (1956). The protein content was determined by microkjeldahl method according to AOAC (1990). Seed oil content was determined using soxhlet apparatus and petroleum ether (40ºC-60ºC) according to AOAC (1990). Total phenolic compounds were determined according to the method described by Danil and George (1972). Total flavonoid contents were measured by the aluminum chloride colorimetric assay as described by Ordoñez*et al*. (2006). The free radical scavenging activity was determined according to Brand- Williams *et al*. (1995) using the 1.1-diphenyl-2-picrylhydrazil (DPPH) reagent. nitrogen, phosphorus and potassium contentswere determined according to the method described by Chapmen & Pratt (1978). N and P were determined using SpekolSpectrocolourimeter VEB Carl Zeiss. While, estimation of K content wasdone by the use of flame photometer.

2.3 Statistical analysis

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

3 Results

3.1 Changes in growth parameters

Table 2 represents the effect of Tre foliar treatment on the growth parameters quinoa plant under drought stress. Drought stress (skipping irrigation at 50 and 60 days after sowing) caused decreases in most of quinoa plant growth parameters and markedly increased root length, fresh and dry weights of root/plant relative to control plants (D0T0) (Table 2). On the other hand, Trehalose treatments was proved to be effective in enhancing shoot height, root length, fresh and dry weights of shoot and root under unstressed and drought stressed plants (Table 2). It was noted that Tre2 was more effective than Tre1 treatment under all conditions.

Table 2 Effect of trehalose on morphological criteria of quinoa plants subjected to drought stress

Drought	Trehaloseconc, µM	Shoot height, cm	Branches number/plant	Leaves number/plant	Shoot fresh weight/plant, g	Shoot dry weight/plant, g	RWC %	Root length, cm	Root fresh weight/plant, g	Root dry weight/plant, g
D0	Tre0	34.0 ± 0.41^{b}	1.97 ± 0.24^{d}	24.0 ± 1.89^{d}	23.97 ± 2.12^{e}	4.49 ± 0.16^{d}	81.26 ± 0.49^{a}	14.67 ± 0.41^{c}	1.59 ± 0.11^{c}	0.37 ± 0.01^{b}
	Tre1	47.0 ± 1.55^{a}	2.97 ± 0.24^{c}	30.7 ± 0.71^{b}	54.90 ± 1.45^{b}	9.51 ± 1.48^{ab}	82.67 ± 0.53^{a}	18.33 ± 0.23^{b}	2.30 ± 0.14^{b}	0.45 ± 0.02^{b}
	Tre2	52.0 ± 1.18^{a}	4.90 ± 0.24^{a}	35.3 ± 1.08^{a}	59.90 ± 5.81^{a}	10.18 ± 0.26^{a}	83.01 ± 1.42^{a}	20.30 ± 0.23^{ab}	2.50 ± 0.04^{b}	0.48 ± 0.02^{b}
D1	Tre0	26.0 ± 1.47^{b}	1.10 ± 0.23^{e}	17.7 ± 0.41^{e}	19.50 ± 2.62^{f}	3.82 ± 0.19^{d}	80.41 ± 4.70^{a}	18.00 ± 0.71^{b}	2.09 ± 0.07^{c}	0.44 ± 0.02^{b}
	Tre1	31.3 ± 1.22^{b}	2.93 ± 0.25^{c}	$26.70\pm.49^{c}$	45.07 ± 1.03^{d}	8.26 ± 0.04^{c}	81.67 ± 0.34^{a}	19.67 ± 0.41^{ab}	3.16 ± 0.12^{a}	0.58 ± 0.03^{a}
	Tre2	32.3 ± 1.25^{b}	3.67 ± 0.41^{b}	35.30 ± 0.24^{a}	51.64 ± 0.16^{c}	9.36 ± 0.09^{b}	81.87 ± 0.16^{a}	21.67 ± 0.33^{a}	3.40 ± 0.07^{a}	0.59 ± 0.01^{a}

Note: Each value represents the mean ± standard error (*n*=3). Means with different letters were significantly different at the 0.05 level according to Duncan's multiple range test.

3.2 Changes in photosynthetic pigments

Chlorophyll (Chl. a, b), total chlorophyll, carotenoids and total photosynthetic pigments content of quinoa leaves were significantly decreased by drought stress (skipping irrigation time at 50 and 60 days after sowing) as compared with the control plants (Figure 1). The percentages of decreases were 18.32%, 18.67%, 18.42%, 27.20% and 19.78% in chlorophyll a, chlorophyll b. carotenoids and total pigments, respectively. Meanwhile, different concentrations of Trehalose significantly increased different components of photosynthetic pigments in quinoa leaf under normal irrigated and drought stressed plants as compared to corresponding controls. Data clearly showed gradual increases of photosynthetic pigments with increasing trehalose concentrations.

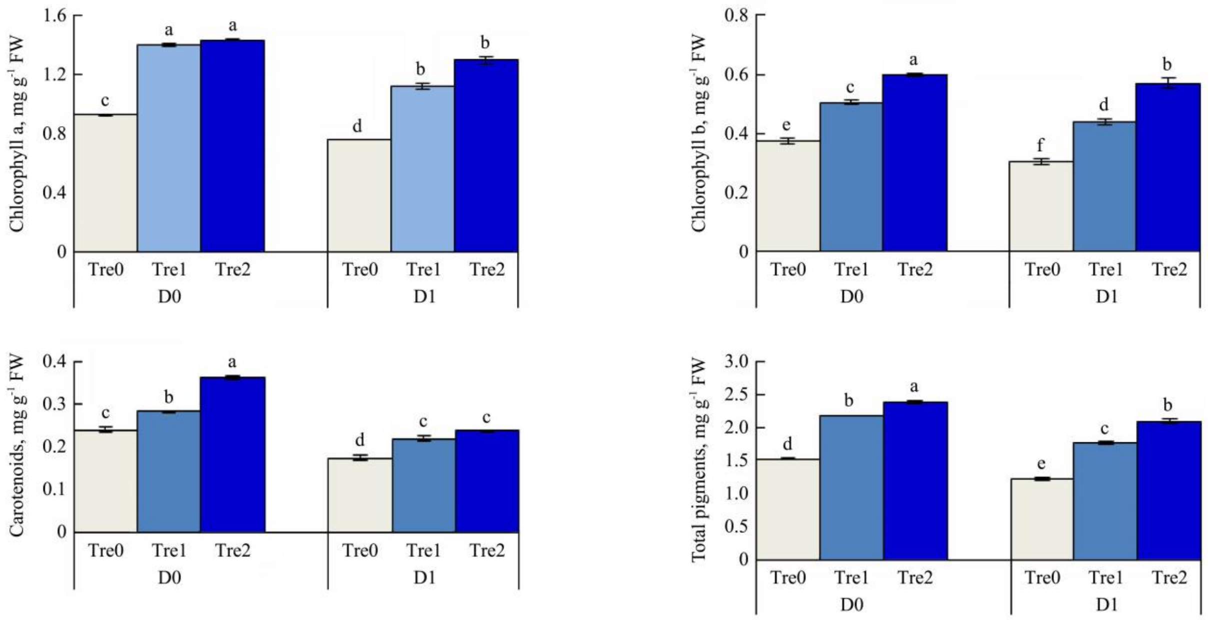

Figure 1 Effect of trehalose on photosynthetic pigments (mg/g fresh weight) of quinoa leaves grown under drought stress. Each value represents the mean ± standard error (*n* =3)

Means with different letters above bars were significantly different at the 0.05 level according to Duncan's multiple range test

3.3 Yield and yield Components

Shoot height, number of fruiting branches/plant, shoot weight, seed weight/plant as well as 1000 seed weight of the quinoa plant significantly decreased by skipping irrigation times (Table 3). On the other hand, foliar treatment of quinoa plant with different concentrations of trehalose (100 and 500 µg/l) under normal conditions and drought stressed conditions caused significant increases in all parameters of yield components as compared to thecorresponding control plants; the most prominence concentration was 500 µg/L.

Table 3 Effect of trehalose on yield components of quinoa plants subjected to drought stress

Drought	Trehaloseconc, µM	Shoot height, cm	Number of fruiting branches	Shoot dry weight/plant, g	Seeds weight/plant, g	1000 seed weight, g
D0	Tre0	39.00±0.85^c	20.67±0.41^c	4.82±0.11^c	3.35±0.07^d	0.380.±0.00^c
	Tre1	51.00±0.62^b	23.67±1.03^b	7.18±0.18^{ab}	4.63±0.08^b	0.43±0.01^b
	Tre2	75.00±0.41^a	25.00±0.24^a	8.22±0.06^a	5.82±0.06^a	0.49±0.01^a
D1	Tre0	28.33±0.00^d	17.00±0.62^f	3.44±0.21^d	1.16±0.21^f	0.35±0.01^d
	Tre1	38.67±0.82^c	18.67±0.08^e	7.05±0.08^b	2.68±0.33^e	0.41±0.01^b
	Tre2	40.00±1.22^c	20.33±0.11^d	7.31±0.11^{ab}	4.02±0.09^c	0.48±0.01^a

Note: Each value represents the mean ± standard error (n =3). Means with different letters were significantly different at the 0.05 level according to Duncan's multiple range test.

3.4 Nutritional values of the yielded seeds

Data presented in Figure 2 clearly showed that the skipping irrigation two times significantly decreased the nutritional values of the yielded seeds (carbohydrates%, protein%, phosphorous, potassium and nitrogen contents) and significantly increased oil% of the yielded seeds as compared with unstressed plants. Trehalose treatments significantly enhanced the nutritional values of the yielded seeds as compared with the corresponding untreated controls. Increasing trehalose concentrations increased gradually the studied parameters.

Figure 3 clearly showed that skipping irrigation two times increased markedly phenolic contents and flavonoids% of the yielded seeds. Moreover, Tre foliar treatment to quinoa plant caused significant increases of phenolic and flavonoids contents of the yielded seeds as compared with untreated controls either at normal irrigated or drought stressed plants. Increasing trehalose concentrations caused gradual increases of flavonoids and phenolics content.

3.5 Antioxidant activity

The 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity, a measure of seed total antioxidant activity, expressed the percentage reduction of the initial DPPH absorption by the tested antioxidants. Drought stress significantly decreased (DPPH) radical scavenging activities at 50 and 100 μg/mL in quinoa seed methanolic extract. Exogenous application of trehalose increased significantly the seed antioxidant activities under stress and non-stress conditions, respectively (Figure 4).

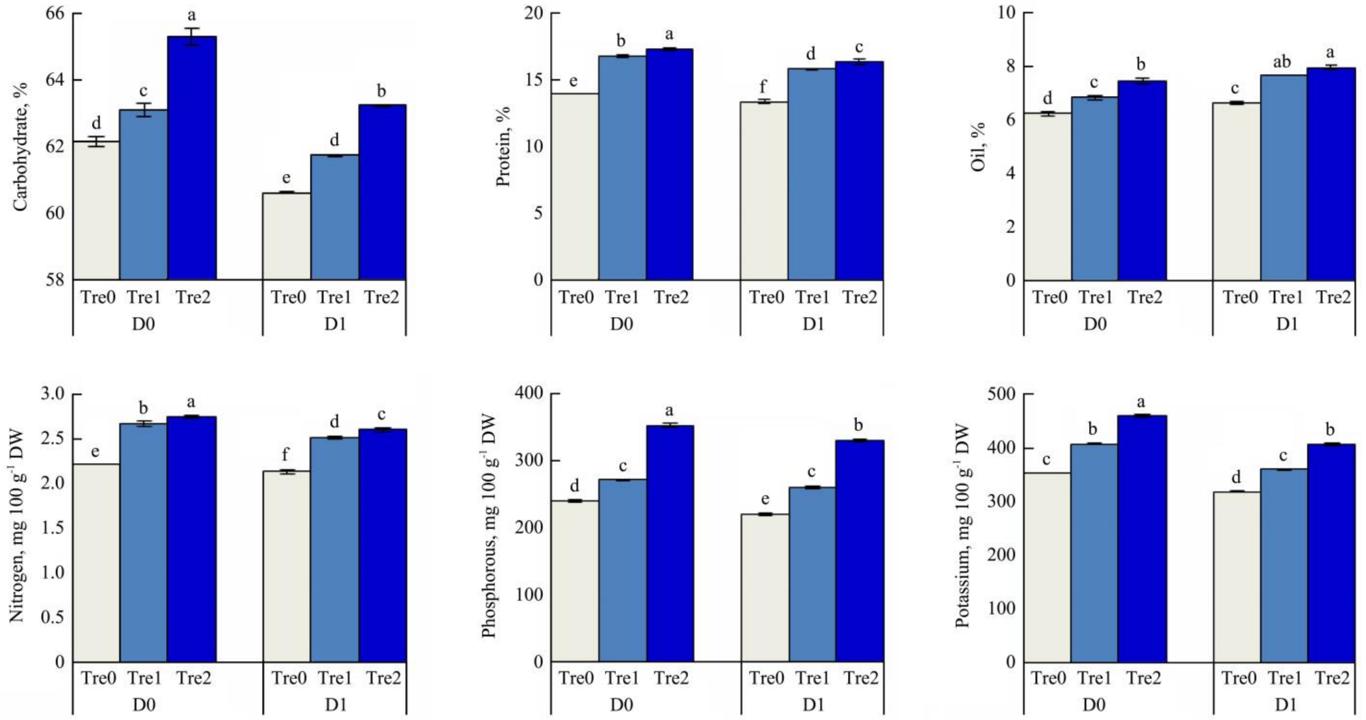

Figure 2 Effect of trehalose on nutritional values of the yielded quinoa seeds. Each value represents the mean ± standard error (n =3). Means with different letters above bars were significantly different at the 0.05 level according to Duncan's multiple range test

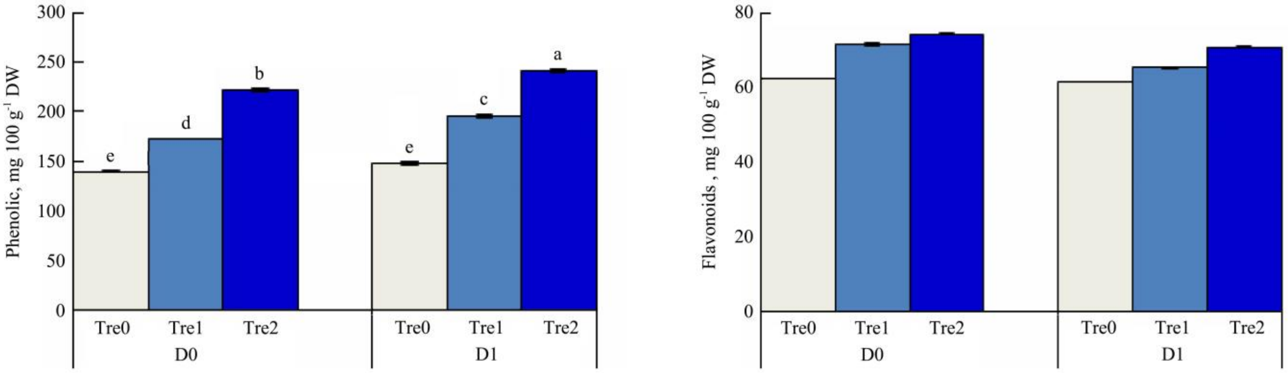

Figure 3 Effect of trehalose on phenolic and flavonoids contents of the yielded quinoa seeds

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

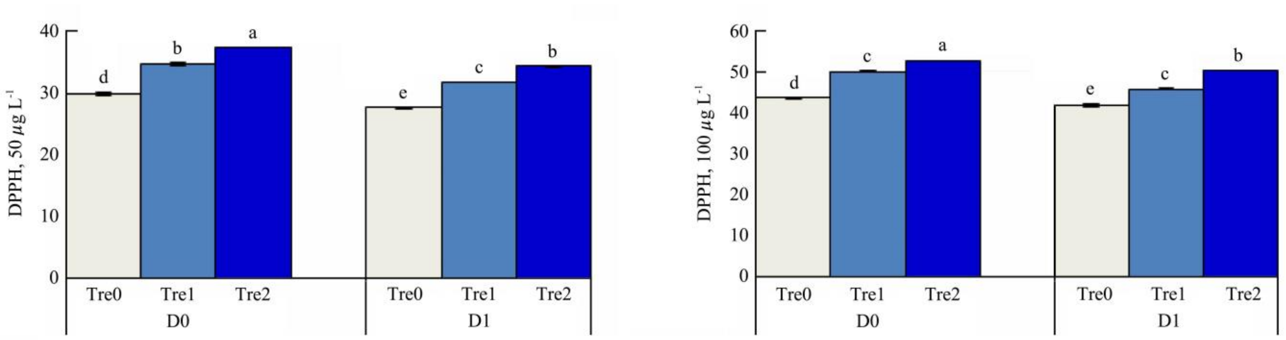

Figure 4 Effect of trehalose on antioxidant activity (at 50 and 100 µg/L) of the yielded quinoa seeds

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

4 Discussion

Water is one of the most important factors affecting not only agricultural output but also food security (Garg *et al.*, 2002). Therefore, improvement of plant tolerance towards drought stress is a major goal for both farmers and scientists. Trehalose as an osmoprotectant could alleviate the reduced effect of drought stress via its effect on cellular osmotic potential. In harmony with our results of drought stress, Dawood and Sadak (2014) stated that both fresh and dry weights of canola shoots decreased with decreasing water holing capacity and referred these decreases to disorders induced by stress and generation of reactive oxygen species (ROS). Sadak (2016b) reported that drought stress reduced growth characters of fenugreek plant. Drought affects plant–water relations, decreases shoot water contents, causes osmotic stress, inhibits cell expansion and cell division as well as growth of plants as a whole (Bakry *et al*., 2012; Alam *et al*., 2013).

The decrease in shoot height in response to drought stress (Table 2) might be due to the decrease in cell elongation, cell turgor, cell volume and eventually cell growth (Banon *et al.*, 2006). Meanwhile, the increases in root length, fresh and dry weight of root/plant and decreases in fresh & dry weight of quinoa shoot under drought stress may be due to the starting quinoa plants to divert the assimilates from stem and utilized them for increased root growth in order to increase water absorption. On the other hand, Tre application could alleviate the adverse effects of drought stress on quinoa plant via improving their growth and physiological attributes.

Water content or growth reduction was restored by exogenous Tre supplementation under drought stress as evidenced by improving water status of plant tissues, RWC percentage, fresh weight and dry weight of plant. Similar findings were observed previously on different plants (Duman*et al*., 2010, Ali and Ashraf, 2011, Theerakulpisut & Gunnula, 2012, Alam *et al.*, 2014 and Sadak, 2016b).

Chlorophyll (Chl. *a*, *b* and *a*+*b*), carotenoids and total pigments content of quinoa leaves was significantly decreased by drought stress (Figure 1). The loss of chlorophyll is one visible symptom of water stress in leaves (Dawood & Sadak, 2014; Sadak, 2016b) indicating some form of disruption of the chloroplasts. The reduced effect of drought on photosynthesis were attributed to oxidation of photosynthetic pigments and impaired pigment biosynthesis (Gonzàlez *et al*., 2009; Anjum *et al*., 2011; Pandey *et al*., 2012), damaging photosynthetic apparatus that leads to decrease in photosynthetic carbon assimilation (Din *et al.*, 2011). On the other hand, trehalose treatment exhibited a positive effect on the photosynthetic pigments (Chlorophyll (Chl. *a*, *b*, *a*+*b*), carotenoids and total pigments content in drought stressed and unstressed leaves. This stimulatory effect of Tre treatment might be due to the role of Tre in preserving chlorophyll envelope stability and maintenance of chloroplast osmotic potential. This effect of Tre is corroborated with the results of previous studies with *Lemmnagibba* L. where exogenous Tre improved photosynthetic pigment contents under cadmium stress (Duman *et al*., 2010). In addition, Theerakulpisut and

Phongngarm (2013) stated that trehalose treatment enhanced photosynthetic pigments of rice plant under salinity stress. Sadak (2016b) found that trehalose treatment enhanced photosynthetic pigments of fenugreek plant under drought stressed conditions by up-regulating photosynthetic and water relation attributes as well as antioxidant defense mechanism.

It is worthy to mention that water availability to plant in different growth stages affects plant yield and biochemical constituents of the yielded seeds. Drought stress significantly decreased yield and yield attributes (Table 3) as well as nutritional values of the yielded seeds (Figure 2) and decreased oil% relative to control plants. This reduction in yield of quinoa plant is mainly due to the reduction in growth parameters (Table 2) and photosynthetic pigments (Figure 1). Carbohydrate changes of the yielded seeds are of particular importance because of their direct relationship with such physiological processes as photosynthesis, translocation, and respiration (Sadak *et al.*, 2010). Drought stress decreased chlorophyll contents in leaves that caused inhibition of photosynthetic activity. Thus, it leads to less accumulation of carbohydrates in mature leaves and consequently may decrease the rate of transport of carbohydrates from leaves to the developing seeds (Anjum *et al*., 2003). These decreases in seed chemical composition might be due to the low water supply during the life of plant that reduced many enzyme activities and this leading to metabolic activities changes that result in altered in translocation of assimilates to seeds (Ali *et al.* 2010). Moreover, Ali and Alqurainy (2006) mentioned that the main cellular components susceptible to damage by free radicals werelipids (peroxidation of unsaturated fatty acids in membranes), proteins (denaturation), carbohydrates and nucleic acids. Regarding promotive effect of trehalose treatments on yield and yield components and chemical composition of the yielded seeds. In recent decade's exogenous protectant such as osmoprotectant (proline, glycinebetaine, trehalose, etc) have been found effective in mitigating the stress induced damage in plant (Hasanuzzaman *et al*., 2013; Sadak 2016b). Tre serves as a carbohydrate storage molecule as well as a transport sugar, similar to the function of sucrose (Muller *et al*., 1999). In addition, it can also stabilize proteins and membranes of plants when exposed to stress via replacing hydrogen bonding through polar residues, preventing protein denaturation and fusion of membranes (Iturriaga*et al*., 2009).

Phenolic and flavonoid contents increased in response to drought stress of quinoa plant (Figure 3). Flavonoids are one of the largest classes of plant phenolics performing different functions in plant system, including pigmentation and defense (Harborne& Williams 2000). Flavonoids constitute a secondary ROS-scavenging system in plants exposed to stress (Fini*et al.*, 2011). Coinciding with the results obtained in the present study, Haghighi*et al*. (2012) recorded that water stress enhanced the accumulation of flavonoids in *Plantagoovata* plants. Moreover, they postulated that this increment in flavonoid content might be due to the induction in enzymatic activity occurring under stress, thereby favoring the production of different flavonoid compounds. These nonstructural carbohydrates then tend to accumulate and thus trigger the synthesis of carbon-based defensive substances. It is well known that many phenolics are stress-induced metabolites that accumulate in plant tissues after different abiotic and biotic stress stimuli. These metabolites may participate in reactive oxygen species (ROS) scavenging mainly through the antioxidative enzymes utilizing polyphenols as co-substrates (Sgherri *et al*., 2003). In accordance with these results, water stress brought about marked increase in the total amount of phenolic compounds in pea, wheat and tomato plants as indicated by Alexieva *et al*. (2001), Sadak (2016a) and Ianovici (2011). Application of Tre markedly increased total phenolic and flavonoids contents. Generally, Tre treatment appeared to be effective treatment in counteracting the negative effects of water stress on total phenolic and flavonoids contents. Hence, Tre has been proposed to be a signaling molecule which induces plants to speed up their rate of ROS production that sends signal to activate non-enzymatic antioxidants for ROS scavenging in order to counteract stress-associated oxidative stress. Beside, phenolic the non-photosynthetic pigments investigated in the present study may contribute to the antioxidant activity of wheat plants (Aldesuquy and Ghanem, 2015; Sadak, 2016).

Drought stress decreased significantly DPPH radical scavenging activities at 50 & 100 μg/mL in methanolic extract of quinoa seed. Exogenous application of trehalose increased significantly the seed antioxidant activities under stress and non-stress conditions, respectively (Table 5). In the present study, seed antioxidant activities in methanolic extract of quinoa seeds was positively related to seed phenolics, flavonoids, and oil contents (Table 5). The strong positive correlation between total phenolics and antioxidant activity as observed in the present study had already been observed in cereals (Dykes, *et al.*, 2007) and soybean (Kumar *et al.*, 2009), which suggests that this increase in seed antioxidant activity is contributed by the presence of high amount of phenolic compounds. Similar positive correlation in seed antioxidant activity and different antioxidant compounds under water deficit condition and due to exogenous application of organic osmolytes had already been reported in some earlier studies in maize (Ali & Ashraf, 2011, Dawood & Sadak 2014; Sadak 2016).

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