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Mitigation of drought stress on Fenugreek plant by foliar application of trehalose

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Abstract: The present work aimed to study the alleviation effect of foliar treatment of different concentrations of trehalose at different water holding capacities (WHCs) on growth, photosynthetic pigments, seed yield quantity and quality in fever of nutritional value and antioxidant activities. In addition to raise the efficiency of Fenugreek plants to resist water stress to reduce the amount of irrigation water. So, this experiment was carried out at the green house of National Research Centre on Fenugreek plant. Three concentrations of trehalose were foliar sprayed (Tre0, Tre1 and Tre2). Plants were irrigated with different WHC 100% and 60%. Data showed that, irrigation of Fenugreek plants with lower WHC 60% resulted in decreases in all growth parameters, photosynthetic pigments, yield components, carbohydrate% and protein%. Meanwhile phenolic and flavonoids contents increased by drought stress. Antioxidant activity at 50 and 100µg/l showed significant increases in response to drought stress. On the other hand, treatment Fenugreek plant with different concentrations of trehalose led to increases in growth parameters, photosynthetic pigments, yield components, carbohydrate, protein, total phenolic, flavonoids contents, and antioxidant activity of the yielded seeds either in non stressed and 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 Fenugreek plants.
Key words: Antioxidant activity, Fenugreek, flavonoids, phenolics, protein, trehalose.

Introduction

Fenugreek (*Trigonella foenum graecum*) is an annual herb that belongs to the family *Leguminosae* widely grown in Egypt and Middle Eastern countries. Due to its strong flavor and its aroma, fenugreek is one of such plants whose leaves and seeds are widely consumed as a spice in food preparations, and as an ingredient in traditional medicine. It is rich source of calcium, iron, â-carotene and other vitamins[1]. Both leaves and seeds should be included in normal diet of family, especially diet of growing kids, pregnant ladies, puberty reaching girls and elder members of family because they have haematinic (i.e. blood formation) value[2]. The seeds of fenugreek contain lysine and L-tryptophan rich proteins, mucilaginous fiber and other rare chemical constituents such as saponins, coumarin, fenugreekine, nicotinic acid, sapogenins, phytic acid, scopoletin and trigonelline which are thought to account for many of its presumed therapeutic effects, may inhibit cholesterol absorption and to help lower sugar levels[3].

Drought is the most important limiting factor for crop production and it is becoming an increasingly severe problem in many regions of the world[4]. Despite, water is one of abundant compounds in earth and 2/3 of earth surface is covered by water, but water shortage is limitative to produce agriculture products in the world.

Drought is one of the major physical parameter of an environment, which determines the success or failure of plants establishment[5]. Generally drought stress occurs when the available water in the soil is reduced and atmospheric conditions cause continuous loss of water by transpiration or evaporation[6]. Plants under dry condition change their metabolism to overcome the changed environmental condition. Water stress reduces crop yield regardless of the growth stage at which it occurs[7]. Drought causes numerous physiological and biochemical changes in plants like reduced leaf size, stem extension, root proliferation, reduced water use efficiency[8], alteration in metabolic activities[9], ionic imbalance and disturbances in solute accumulation[10] or a combination of all these factors.

Over the last few decades, several techniques have been proposed to improve plant performance in drought environments. These include foliar treatment with osmoprotectants compounds as proline, glycinebetaine or trehalose[11]. Trehalose (Tre), is a non-reducing disaccharide of glucose, which plays an important role as a stress protectant in some plants[12,13&14]. In addition to being an energy source, the unique physicochemical properties of Tre efficiently stabilize dehydrated enzymes, proteins, and lipid membranes, as well as protect biological structures from damage during desiccation[15]. Tre has the added advantage of being a signaling and antioxidant molecule and acts as an elicitor of genes involved in detoxification and stress response[16]. However, Tre production in most plants is not sufficient to ameliorate stress-induced adverse effects. On the other hand, external Tre application increases the internal level of this osmolyte and has been suggested as an alternative approach to induce stress tolerance[11&17]. Exogenous Tre alleviates the adverse effects of various abiotic stresses including drought in maize[14], heat and water deficit in wheat[18&19].

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

Materials and Methods

Experimental Conditions, Plant Materials, Growth and Treatment Conditions:

Seeds of Fenugreek (*Trigonella foenum graecum*) cv. Giza 30 were obtained from Agriculture Research Centre, Ministry of Agriculture and Land Reclamation, Egypt. Seeds were grown in Pots (diameter 30 cm) at two successive seasons (2012/2013 and 2013/2014), filled with clay and sand soil with the ratio of 2:1. Treatments of WHC were started after 45 days from sowing. Irrigation treatments were given to plants with different levels of water holding capacity (WHC) 100%, and 60%. Trehalose concentrations (0, 250 or 500 µM) were sprayed after 30 and 37 days of sowing. Fertilization with super phosphate (5 g / pot), potassium sulfate (2.5 g / pot) and urea (6 g / pot) were used.

Experimental Design, Growth and Yield & Yield components:

The pot experiment was conducted in the greenhouse of Botany Department, National Research Center. Experimental design was complete randomized blocks. Samples were taken after 60 days after sowing to analyze crop performed in terms of growth parameters, RWC% and photosynthetic pigment (chlorophyll a, chlorophyll b and carotenoids). Each treatment was replicated four times and each replicate had three plants. Three healthy plants were left in each pot to determine number of pods/ plant, number of seeds/pod weight of seeds/ plant, and seed index were determined. Air dried seeds were ground into a fine powder and kept in desiccators for chemical analysis.

Chemical Analysis:

Photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) of fresh leaves were determined[20]. For seed chemical analysis, seeds were powdered to determine carbohydrates, proteins, phenolic and flavonoids cntents. Protein contents were determined by microkjeldahl method[21]. Total carbohydrates were determined calorimetrically according to the method[22]. Total phenol content was measured[23]. Total flavonoid contents were measured by the aluminum chloride colorimetric assay[24]. The free radical scavenging activity was determined[25]using the 1.1-diphenyl-2-picrylhydrazil (DPPH) reagent.

Statistical Analysis:

The data were statistically analyzed on complete randomized design system according[26]. Combined analysis of the two growing seasons was carried out. Means were compared by using least significant difference (LSD) at 5% levels of probability.

Result

Changes in growth parameters:

Drought stress (60% WHC) caused significant decreases in Fenugreek plant growth parameters (shoot length, branches and leaves number/ plant, fresh & dry weight of shoot/plant, RWC of shoot) Meanwhile, increased significantly root length, fresh and dry weights of root/plant) relative to control plants (D0T0) (Table 1). On the other hand, Trehalose treatments 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 1). It was noted that Tre2 was more effective than Tre1 treatment at unstressed and drought stressed as caused significant increases in dry weight of shoot by 38.32% and 62.5% respectively, compared to 15.89% & 47.22% at Tre1. It was interesting to observe a considerable increment in root length of trehalose-treated plants more than the control and the drought-stressed plants. Fresh and dry mass of roots also increased in response to trehalose treatment under stress and un-stress conditions (Table 1).

Table 1. Effect of Trehalose at 250 µM (Tre1) and 500 µM (Tre2) on growth parameters of Fenugreek plants irrigated with 100%WHC (WHC0) or 60%WHC (WHC1). The presented results are means of the measurements taken at two successive seasons.

Drought	Trehalose conc (µM)	Shoot length (cm)	Leaves no/plant	Fresh wt/plant (g)	Dry wt (g)	RWC%	Root length (cm)	Root fresh wt (g)	Root dry wt (g)
WHC0	Tre0	24.00	14.00	6.3	1.07	83.02	15.00	1.59	0.37
	Tre1	27.00	16.00	8.26	1.24	84.99	19.00	2.30	0.38
	Tre2	32.00	18.33	9.92	1.48	85.08	21.30	2.50	0.39
WHC1	Tre0	16.00	10.67	4.05	0.72	82.22	18.00	2.27	0.47
	Tre1	21.33	12.00	6.39	1.06	83.41	21.33	3.40	0.58
	Tre2	24.33	13.00	7.36	1.17	84.10	22.33	3.40	0.59
LSD at 5%		1.25	0.64	0.79	0.07	0.86	1.25	0.45	0.03

Changes in photosynthetic pigments:

Chlorophyll (Chl. a, b), total chlorophylls, carotenoids and total pigments contents of Fenugreek leaves were significantly decreased by drought stress (60%WHC) as compared with control plants (Table 2). The percentages of decreases were 14.33%, 6.29%, 12.03%, 14.06% and 12.29% in chlorophyll a, chlorophyll b, chloro a+b, carotenoids and total pigments, respectively. Different concentrations of trehalose foliar treatment to Fenugreek plant significantly increased photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophylls, carotenoids and total pigments) as compared to untreated plants in normal irrigated and drought stressed plants. Data clearly show the gradual increases of photosynthetic pigments with increasing trehalose concentrations.

Table2. Effect of Trehalose at 250 µM (Tre1) and 500 µM (Tre2) on photosynthetic pigments (mg/g FW) of Fenugreek plants irrigated with 100%WHC (WHC0) or 60%WHC (WHC1). The presented results are means of the measurements taken at two successive seasons.

Drought	Trehalose conc (µM)	Chlo a	Chlo b	Chl a+b	Car	Total pig
WHC0	Tre0	1.186	0.477	1.663	0.249	1.912
	Tre1	1.66	0.608	2.268	0.295	2.563
	Tre2	1.693	0.701	2.394	0.312	2.706
WHC1	Tre0	1.016	0.447	1.463	0.214	1.677
	Tre1	1.383	0.54	1.923	0.358	2.281
	Tre2	1.557	0.672	2.229	0.436	2.665
LSD at 5%		1.245	0.067	0.112	0.052	0.342

Yield and Yield Components:

Data in Table 3 show that, at harvest, pods number/plant seeds number/pod, seeds weight/plant and seed index of Fenugreek plant significantly affected by drought stress. Drought stress induced significant decreases in all the previous parameters of yield and its components as compared with control treatment. For instance, the reduction in seed weight/plant and seed index reached to 37.79%, 14.90%, respectively as compared with normal irrigated plants. On the other hand, foliar treatment of Fenugreek plant with different concentration of trehalose (250 and 500 µM) under normal conditions and drought stressed conditions caused significant increases in all parameters of yield components as compared to the corresponding control plants, the most prominence concentration was 500µM.

Table 3. Effect of Trehalose at 250 µM (Tre1) and 500 µM (Tre2) on yield parameters of Fenugreek plants irrigated with 100%WHC (WHC0) or 60%WHC (WHC1). The presented results are means of the measurements taken at two successive seasons.

Treatment		Pods			
WHC	Trehalose conc (µM)	no/plant	Seeds no/pod	Seeds wt/plant	Seed index
100%	Tre0	7.67	6.33	2.985	5.154
	Tre1	8.33	7.00	3.735	5.827
	Tre2	9.00	8.33	4.542	6.431
60%	Tre0	5.33	4.67	1.857	4.386
	Tre1	6.33	5.33	2.687	5.021
	Tre2	8.67	6.67	3.383	5.674
LSD at 5%		0.225	0.245	0.145	0.177

Biochemical constituents of the yielded Fenugreek seeds:

Carbohydrate Contents:

Data in Table 4 demonstrate that, WHC at 60% led to marked decrease in total carbohydrates of the yielded seeds compared to plants grown under 100% of field capacity. Foliar trehalose treatment on Fenugreek plant under different WHC (100% and 60% WHC) led to marked increases in total carbohydrates when compared with the corresponding controls especially at 100% WHC.

Protein Contents:

Table (4) show that, the decrease of WHC (60%) of soil led to marked decreases in protein content of yielded seeds compared to plants grown under 100% WHC. The results showed the stimulatory effects of trehalose treatment especially at 500 µM under normal or under different levels of water stress on protein contents compared to untreated plants.

Antioxidant Substances of the Yielded Seed and its Activity:

Data in Table (4) show the variation in antioxidant substances of the yielded seeds of Fenugreek plant in response to spraying with different concentrations of trehalose and subjected to different levels of WHC. WHC decreases at 60% caused marked significant increases in both total phenolic contents and flavenoids content as compared with those of the corresponding controls. Spraying Fenugreek plants with different concentrations of trehalose increased significantly total phenolic contents and flavenoids content as compared with the corresponding control plants. Regarding to antioxidant activity as WHC decreased from 100% to 60% caused significant increases at all treatments. Foliar treatment with trehalose at different concentrations caused significant increases in antioxidant activities at 50 & 100 µg/l of the yielded seeds of Fenugreek plant grown under 100% and 60% WHC as compared with the corresponding untreated controls.

Table 2. Effect of Trehalose at 250 µM (Tre1) and 500 µM (Tre2) on carbohydrate%, protein%, Flavonoids and phenolic contents and antioxidant activities% at 50&100 µg/l of Fenugreek plants irrigated with 100%WHC (WHC0) or 60%WHC (WHC1). The presented results are means of the measurements taken at two successive seasons.

Drought	Trehalose conc	Carbohydrate%	Protein%	Flavonoids mg/g DW	DPPH% (µg/l)		Phenolics
					50	100	
WHC0	Tre0	41.64	23.35	1.504	24.80	40.80	47.60
	Tre1	42.75	25.77	2.517	36.80	49.20	65.00
	Tre2	43.25	26.34	3.080	39.35	51.82	76.56
WHC1	Tre0	40.18	20.92	2.239	27.67	44.89	79.32
	Tre1	41.14	22.76	3.154	38.77	52.00	92.62
	Tre2	42.32	23.24	4.430	41.39	57.45	124.34
LSD at 5%		1.021	0.574	0.245	2.411	3.489	2.985

Discussion

Water is one of the major factors limiting crop production, affecting not only agricultural output but also food security. Consequently, developing drought tolerant plants/crops is a major research goal for both plant scientists and farmers. Trehalose as an osmoprotectant maintains cellular osmotic balance It protects biological structures from damage at desiccation[28]. Previous studies proved Tre as efficient protectant against drought stress or under water deficit conditions[14&29]. Our results of drought stress are in harmony with[11]which stated that both fresh and dry weights of shoots of canola decreased with increasing drought stress and these reductions may be due to the metabolic disorders induced by stress and generation of ROS. Also, the decrease in shoot height in response to drought might be due to decrease in cell elongation, cell turgor, cell volume and eventually cell growth[30]. In addition, drought affects plant–water relations, reduces water contents of shoot, causes osmotic stress, inhibits cell expansion and cell division as well as growth of plants as a whole[31]. Also, the effect of drought in reduced plant height (Table 1) might be due to the adaptation of Fenugreek plants to cope with drought stress. With the initial effects of drought stress, Fenugreek plants started to divert the assimilates from stem and utilized them for increased root growth in order to increase the water absorption. Regarding to trehalose effect, Tre application alleviated the adverse effects of drought stress of Fenugreek plant by improving their growth and physiological attributes. However, water content or growth reduction was restored by exogenous Tre supplementation under drought stress as evidenced by improved shoot RWC and fresh weight and dry weight of plant. Similar findings were documented previously by Tre addition with abiotic stress [13,14&32]. Trehalose reduced the inhibitory effects of drought on growth may be through improving the water status of the plant tissues, since the relative water content of the shoot increased (Table 2). Also, trehalose treatment on maize plants improves water retention and plant tolerance through osmoregulation and stomatal closing at stress[33].

Chlorophyll (Chl. *a*, *b*), chlorophyll a+b, carotenoids and total pigments content of Fenugreek leaves were significantly decreased by drought stress (Table 2). Numerous studies reported that inhibition of photosynthesis were attributed by damages to photosynthetic pigments due to oxidation of pigments, impaired pigment biosynthesis[34,35&36]. One visible symptom of water stress in leaves is a concomitant loss of chlorophyll[11] which indicating some form of disruption of the chloroplasts. The reduction in growth criteria as a result of reducing the anabolic processes by the influence of drought could be attributed to the limiting effect of drought-induced stress on the chlorophyll content and photosynthetic activity. Drought inhibits photosynthesis process of plants may be due to oxidative stress by causing pigment photo-oxidation, damaging to photosynthetic apparatus and leading to decrease in photosynthetic carbon assimilation[37]. Trehalose treatment exhibited a positive effect on the photosynthetic pigments (Chlorophyll (Chl. *a*, *b*), chlorophyll a+b, carotenoids and total pigments content) in drought stressed and unstressed leaves. Trehalose may be preserved the stability of the chloroplast envelope and maintained the osmotic potential of the chloroplast. Interaction of drought and trehalose improved the photosynthetic pigment levels in studied plant (Table.2) which is corroborated with the results of previous studies with *Lemmna gibba* L. where exogenous Tre improved photosynthetic pigment contents under cadmium stress[13]. Moreover, trehalose treatment enhanced photosynthetic pigments of rice plant under salinity stress[38] drought stressed conditions[39].

It is worthy to mention that water availability to plant in different growth stages affect on plant yield and biochemical constituents of the plant and the yielded seeds. Table 3&4 indicates that drought stress significantly decreased yield and yield attributes of Fenugreek plant accompanied with significant decreases in biochemical constituents and nutritional values of the yielded seeds (Carbohydrates, proteins) relative to control plants. The reduction in yield of quinoa plant is mainly due to the reduction in growth parameters (Table 1) and photosynthetic pigments (Table 2). Carbohydrate changes are of particular importance because of their direct relationship with such physiological processes as photosynthesis, translocation, and respiration. Water stress decreased the pigments concentration in leaves which results in inhibition of photosynthetic activity, in turn 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[40]. The decreases in seed chemical composition might be due to the reason that low water supply during the plant life affects many enzymes whose activity is reduced under water stress conditions and leading to changes in metabolic activities that result in altered translocation of assimilates to seeds. Moreover, it was mentioned that the main cellular components susceptible to damage by free radicals are lipids (peroxidation of unsaturated fatty acids in membranes), proteins (denaturation), carbohydrates and nucleic acids[42].

Regarding to the promotive effect of trehalose foliar treatments on yield and yield components and chemical composition of the yielded seeds. In recent decades exogenous protectant such as osmoprotectant (proline, glycinebetaine, trehalose, etc) have been found effective in mitigating the stress induced damage in plant[43&44]. Trehalose can serve as a carbohydrate storage molecule as well as a transport sugar, similar to the function of sucrose[45]. It can also stabilize proteins and membranes of plants when exposed to stress by replacing hydrogen bonding through polar residues, preventing protein denaturation and fusion of membranes[46]. Moreover, trehalose acts as a source of carbon and energy and a protector against stresses.

In the present study, data revealed that drought stress increased the amount of flavonoids and total phenols. Flavonoids are one of the largest classes of plant phenolics performing different functions in plant system, including pigmentation and defense[47]. Intriguingly, the conditions leading to inactivation of antioxidant enzymes can also upregulate flavonoids biosynthesis, suggesting that flavonoids constitute a secondary ROS-scavenging system in plants exposed to prolonged stress[47]. Coinciding with the results obtained in the present study, it was recorded[48] that water stress enhanced the accumulation of flavonoids in *Plantago ovata* plants. Similarly, drought increased the concentration of total phenols in the leaves of five tomato cultivars[49]. 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[50]. Application of Tre markedly increased total phenols and flavonoids contents. Generally, Tre treatment appeared to be effective treatment in counteracting the negative effects of water stress on total phenols and flavonoids contents. Hence, Tre has been proposed to be a signaling molecule which induce 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[51].

In the present study, seed antioxidant activities in methanolic extract of Fenugreek seeds was positively related to seed phenolics and flavonoids contents Table 4. The strong positive correlation between total phenolics and antioxidant activity as observed in the present study had already been observed in cereals[52] and soybean[53] 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[54,14&55].

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