Plant Archives Vol. 19, Supplement 2, 2019 pp. 215-225 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

PHYSIOLOGICAL ROLE OF TREHALOSE ON GROWTH, SOME BIOCHEMICAL ASPECTS AND YIELD OF TWO FLAX VARIETIES GROWN UNDER DROUGHT STRESS

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Abstract

Drought stress is an important adversely effective abiotic stresses reduce growth and yield of plant. Exogenous application of osmoprotectants is considered as a shotgun method for improving plant drought tolerance. Trehalose (Tre) is one of these effective osmoprotectants; it that has an effective role on enhancing plant tolerance to various stresses. Thus, the effect of foliar treatment of trehalose (2.5, 5 & 7.5 mM) on two varieties of flax plant (Azur and Sakha-1) growth, some biochemical aspects, antioxidant defense system and yield under drought stress (100%, 75% and 50% water holding capacity in sandy soil were investigated. Decreasing water irrigation requirement (WIR) from 100% to 75% to 50% (drought stress) decreased significantly different growth criteria, photosynthetic pigments, yield and yield components of the two studied flax varieties. Meanwhile, drought stress caused significant increases in phenolics contents, total soluble sugars, proline and free amino acids as well as some antioxidant enzymes (super oxide dismutase, (SOD), catalase, peroxidase and polyphenol oxidase). On the other hand, foliar treatment of trehalose with different concentrations could alleviate the adverse effects of drought stress and increased significantly growth parameters, yield quantity and quality of the two varieties of flax plant *via* improving photosynthetic pigments, indole acetic acid (IAA), phenolic, total soluble sugars (TSS), proline, free amino acid contents and antioxidant enzyme systems as compared with their corresponding untreated controls. Five mM foliar treatment of trehalose was the most effective treatment. Finally it could be concluded that, the promotive role of trehalose in improving tolerance of flax plant (two varieties) to drought stress. This promotive role may be ascribed to the enhancing photosynthetic pigments, osmoprotectants, and mitigation of oxidative damage caused by drought stress. The results suggest that trehalose could be considered as a potential plant growth regulator in improving crop drought tolerance.

Key words: Flax, Drought tolerance, free amino acid, Antioxidant enzymes, IAA, Melatonin, Phenolics and Yield.

Introduction

Flax plant (*Linum usitatissimum* L.) is one of the most important crops grown in Egypt as seed, fiber and dual purpose plant (fibers and seeds). Flax oil is edible as its seeds contain 30-40 percent of fatty acids with high amount of essential fatty acids as well as, proteins, mucilage and cyanogenic glycosides. Flax oil is also used in the production of paints, varnishes, printing ink, oil cloth and soap because it has a fast dyeing property. In Egypt, flax is considered second fiber crop after cotton. Recently, the importance of flax have passed all expectations, in addition to its uses in production of feeding poultry and animals, as well as, different types of compact wood (particle board). Flax is used also in many fine industries in electric insulations and non-textile medical materials. More essential uses are in producing bank note papers (Bakry *et al*., 2013). Flax varieties are greatly differed in yield and yield components quantity and quality in addition to oil percent (Darja and Trdane, 2008).

Drought is an adverse environmental stresses which affect on crop growth and productivity in various regions of the world (Passioura, 2007). However, water shortage is increasing and thus becomes the limitative to agriculture production all over the world. Plant suffers from drought stress because of increased transpiration rate or decreased supply of water to roots. Thus any level of water decrease at any growth stage adversely affects plant growth and development. Generally, the reason of drought is mainly the reduction of soil available water and bad atmospheric conditions which cause continuous loss of water by evaporation or transpiration (Khaje Hosseini *et al*., 2003). Drought stress adversely affects on cell enlargement and expansion, various metabolic activities and decrease various enzyme systems such as Calvin cycle enzymes (Ashraf *et al*., 2013) in addition to, respiration decreases, reduction in ion absorption and translocation and decrease growth regulators contents (Praba *et al.,* 2009). Plant response to drought stress by increasing the level of reactive oxygen species (ROS) which cause disturbance in various biochemical and metabolic processes such as photosynthesis, chlorophyll destruction and biological macromolecule deterioration, membrane dismantling, ion leakage, and DNA-strand cleavage. Moreover, damage to fatty acids of membrane could produce small hydrocarbon fragments including malondialdehyde (MDA) that is considered as one important sign of membrane system injury (Hossain *et al.,* 2013). Among enzymatic antioxidants superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), glutathione peroxidase (GPX) and the non-enzymatic antioxidants include ascorbate, glutathione and phenolic compounds (Hossain *et al*., 2013).

Plants under drought alternate their performance to alleviate the changed environmental conditions. These changes include physiological and biochemical changes such as decreased leaf size, stem elongation, root proliferation and water use efficiency (Farooq *et al.,* 2009), accumulation of solute and ionic imbalance or a combination of all these factors (Dawood and Sadak., 2014). To alleviate the adverse effect of abiotic stress, plants produce different organic solutes known as osmoprotectants or compatible solutes, which have the function of decreasing the osmotic potential caused by the stress. These osmoprotectant compounds have the ability of water molecule attraction into the plant cells and thus maintain cell turgor. Among these compounds soluble sugars, sugar alcohols, proline, trehalose and glycinebetaine. In general, these osmoprotectants protect plants from stress injury *via* many ways, including protection of cytoplasm and chloroplasts and lowering the increased levels of reactive oxygen species (Smirnoff and Cumbes, 1989), stabilizing protein molecules and protecting membrane structure (Bohnert and Jensen, 1996) and maintaining the osmotic balance (Farooq *et al.,* 2009). Moreover, the application of osmoprotectants has been considered as a shotgun method for improving plant drought tolerance. Trehalose (Tre) is one of these organic solutes, a non-reducing disaccharide of glucose, that has a very useful effect as an osmoprotectant in many crops (Garcia *et al.*, 1997; Duman *et al.*, 2010; Ali and Ashraf 2011; and Sadak 2016 & 2019). Moreover, it acts as an energy source, the important physiological effect of trehalose is in stabilizing dehydrated enzymes, proteins and lipid membranes, in addition to its role in protection of various biological cell structures from desiccation destruction (Fernandez *et al.,* 2010). Trehalose is also, considered as a signal and antioxidant molecule. Moreover, trehalose has an essential role as an elicitor of genes involved in detoxification and stress response (Bae *et al*., 2005). Exogenous trehalose treatment has the ability to increase the internal content of osmoprotectants and by this way plant can increase the low level of trehalose content and hence plant could ameliorate stress adverse effect induced by drought stress (Chen and Murata 2002). Thus, this study was done to investigate the physiological role of trehalose treatment on growth and yield via various physiological and antioxidant defense system of flax plant under drought stress conditions.

Materials and Methods

Two field experiments were carried out at the experimental station of National Research Centre, Al Nubaria district El-Behira Governorate-Egypt, in 2016/2017 and 2017/2018 winter seasons. Soil of the two experimental sites was sandy soil. Mechanical, chemical and nutritional analysis of the experimental soil is reported in Table-1 according to Chapman and Pratt (1978). The experimental design was split - split plot design with three replications, where water irrigation requirements (100%, 75% and 50%) were occupied the main plots, while, flax varieties (Azur (Romanian linseed variety) and Sakha-1 (local variety) were allocated in sub plots and the concentrations of trehalose (0.0, 2.5 mM, 5.0 mM and 7.5 mM) were allocated at random in sub-sub plots. A flax seed of Azur and Sakha-1 varieties were sown on the 17th November in both winter season 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 seeding rate was 2000 seeds/m^2. Pre-sowing, 150 kg/fed of calcium super-phosphate (15.5% P_2O_5) were used. Nitrogen was applied after emergence in the form of ammonium nitrate 33.5% at rate of (75 Kg/fed) in five equal doses. Potassium sulfate (48 % K_2O) was added at two equal doses of 50 kg/fed. Irrigation was carried out using sprinkler irrigation system.

Irrigation Water Requirements:

Three irrigation water requirements was calculated using Penman Monteith equation and crop coefficient according to Allen *et al.* (1989). The average amount of irrigation water applied with sprinkler irrigation system were 2500, 1875 and 1250 m^3 $fed.^{-1}$ $season^{-1}$ as (100%, 75% and 50%, respectively) for both seasons of 2016/2017 and 2017/2018.

The amounts of irrigation water were calculated according to the following equation:

$$IWR = \left(\frac{ET_0 \times Kc \times Kr \times I}{Ea} + LR \right) \times 4.2$$

Where:

IWR = Irrigation water requirement m^3/ fed/ irrigation
ET_0 = Reference Evapotranspiration (mm/day)
Kc = Crop coefficient.
Kr = Reduction factor (Keller and Karmeli, 1975)
I = Irrigation interval, day
Ea = Irrigation efficiency, 90%.
LR = Leaching requirement = 10% of the total water amount delivered to the treatment.

Foliar application of different concentrations of trehalose (0.0, 2.5, 5 and 7.5 mM) was carried out twice; where plants were sprayed after 30 and 45 days from sowing at rate of 200 L/faddan. Plant samples were taken after 60 days from sowing for measurements of growth characters and some biochemical parameters. Growth parameters in terms of, shoot length (cm), shoot fresh and dry weight (g), roots length (cm), root fresh and dry weight (g). Chemical parameters measured were photosynthetic pigments, total phenol contents and some antioxidant enzymes i.e. Polyphenol oxidase (PPO), Peroxidase (POX), Catalase (CAT) and Superoxide dismutase (SOD). Plant samples were dried in an electric oven with drift fan at 70°C for 48 hr. for determination of total soluble sugars (TSS), free amino acids and proline contents. Flax plants were pulled when signs of full maturity were appeared, then left on ground to suitable complete drying. Capsules were removed carefully. At harvest, plant height (cm), fruiting zone length (cm), number of fruiting branches/plant, number of capsules/plant, seed yield/plant (g), biological yield/plant (g) and 1000 seeds wt (g), were recorded on random samples of ten guarded plants in each plot. Also, seed yield/fed (Kg/Fed), straw yield Kg/fed, and biological yield Kg/fed were estimated. Water productivity as affected by foliar application of different concentrations of trehalose was measured as the ratio of output (straw, seed and oil yield) to the amount of water used in the production process. [WP $_{(kg\,/\,m3)}$ = Output $_{(kg\,/fed.)}$ / Water Applied $_{(m3\,/fed.)}$]

Table 1: Physical and chemical characteristics of the experimental soil

Soil characteristics			Mean of two seasons
Mechanical analysis			
Sand %			91.77
Silt %			3.33
Clay %			4.90
Texture			Sandy
Chemical analysis			
pH (1 : 2.5 water)			7.45
E.C. (mhos/cm)			0.40
CaCO3 %			1.53
O.M. %			0.27
Available	P	mg/100 g soil	0.23
	K		11.09
	Ca		91.0
	Mg		18.0
	Na		13.69
Available	Fe	mg/kg soil	4.46
	Mn		3.55
	Zn		0.09
	Cu		0.09

Chemical Analysis:

Photosynthetic pigments contents (chlorophyll a and b and carotenoids) in fresh leaves were estimated using the method of Lichtenthaler and Buschmann, (2001). Total phenol content was measured as described by Danil and George (1972). Total soluble sugars (TSS) were extracted by the method of Homme *et al.,* 1992) and analyzed using Spekol Spectrocolourimeter VEB Carl Zeiss (Yemm and Willis, 1954). Free amino acids were extracted according to Vartainan *et al.* (1992) and estimated according to (Yemm and Cocking, 1955). Proline was extracted as free amino acid and assayed according to Bates et al. (1973). The method used for extracting the enzyme was that of MuKherjee and Choudhuri (1983). Polyphenol oxidase (PPO, EC 1.10.3.1) activity was assayed using the method of Kar and Mishra (1976). Peroxidase (POX, EC 1.11.1.7) activity was assayed using to the method of Bergmeyer (1974). Catalase (CAT, EC 1.11.1.6) activity was assayed according to the method of Chen et al., (2000). Super oxide dismutase (SOD, EC 1.12.1.1) activity was measured according to the method of Dhindsa *et al*., (1981). The enzyme activities was calculated by Kong *et al*., (1999). Seed oil content was determined using Soxhlet apparatus and petroleum ether (40-60°C) according to A.O.A.C. (1990). Determination of total carbohydrates was carried out according to Herbert *et al.* (1971).

Statistical Analysis:

The data were subjected to statistical analysis of variance according to method described by (Snedecor and Cochran, 1980) since the trend was similar in both seasons the homogeneity test Bartlett's equation was applied and the combined analysis of the two seasons was done according to the method (Gomez and Gomez, 1984). Means were compared by using least significant difference (LSD) at 5%.

Results and Discussion

Changes in Growth Parameters

Growth parameters of two varieties (Azur and Sakha-1) of flax plants in response to treatment with different concentrations of trehalose foliar treatments (0.0, 2.5, 5.0 and 7.5 mM) grown under water deficit (by decreasing water irrigation requirements from 100% to 75% and 50%) are presented in Table (2). Results revealed that, drought stress (75% and 50%) decreased gradually and significantly growth parameters of the two flax varieties in the term of length, fresh and dry weight of shoot. But, increased root length, fresh and dry weight significantly as compared with control plant (100%). Meanwhile, trehalose foliar treatment with different concentrations not only, increased significantly shoot length, fresh and dry weight of shoot but also, could alleviate the negative effects of drought stress by increasing different studied growth parameters as compared to their corresponding control plants. Trehalose (5.0 mM) foliar treatment was the most effective treatment on increasing the studied growth parameters.

Changes in Photosynthetic Pigments:

Moderate and severe drought stress (75% and 50% of water irrigation requirements) caused significant decreases in photosynthetic pigments of the two flax tested varieties relative to control plant at 100% of water irrigation requirements (Table 3). On the other hand, trehalose treatments with different concentrations caused marked increases in photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids and total pigments) in fresh leaf tissues of unstressed plants at (100% of water irrigation requirements) as well as in fresh leaf tissues of plants that exposed to moderate (75%) and severe (50%) drought stress relative to their corresponding controls (Table 3).

Changes in Phenolic Contents:

Drought stress 75% and 50% from water irrigation requirements caused significant and gradual increases in phenolic contents of the two varieties of flax plant relative to control plant (100% of water irrigation requirements) (Table 4). Trehalose treatment with different concentrations (2.5, 5.0 and 7.5mM) caused also gradual increases in phenolic contents not only in plants grown under 100% water irrigation requirements, but also, for those irrigated by 75% or 50% from water irrigation requirements as compared with untreated controls.

Changes in Compatible Solutes (TSS, Proline and Free Amino Acids):

Data of compatible solutes of two varieties of flax plant exogenously applied by trehalose and subjected to drought stress (Table 4). Results stated the increased contents of total soluble sugars (TSS) in the two flax varieties subjected to drought stress (75% and 50% water irrigation requirements) as compared with those plants irrigated with 100% water irrigation requirements. Table 4 shows that, either moderate or severe drought stress increased significantly proline and free amino acids (FAA) contents of the two tested varieties of flax leaves (Azur and Sakha-1) relative to control plants (100%). On the other hand, trehalose treatment caused more increases in proline and free amino acids levels in the two flax varieties either under normal (100% water irrigation requirements) or drought stress conditions (75% and 50% water irrigation requirements) as compared with their corresponding untreated controls (Table 4).

Changes in Enzyme Activities

Moderate and severe drought stress by lowering water irrigation requirements (75% and 50% water irrigation requirements) caused significant increases in super oxide dismutase (SOD), catalase (CAT), peroxidase (POX) and polyphenol oxidase (PPO) compared with the control plants. Moreover, trehalose foliar treatment with different concentrations improved stress resistance by more significant increases in peroxidase and PPO activities as compared with corresponding drought stress levels. On the other hand, SOD and catalase activities decreased by increasing trehalose levels (Table5).

Yield and Yield Attributes:

Yield and yield components of the two varieties of flax plant were significantly affected by decreasing water irrigation requirements (Table 6, 7). 75% and 50% irrigation water caused significant decreases in all agronomic traits, as well as yield quality and quantity (plant height, fruiting zone length, technical shoot length, number of fruiting branches, capsules/plant, seeds weight/plant, 1000 seeds weight, biological & straw yield (ton/fed) and seed yield (kg/fed), in addition to seed oil %, seed yield/fed and seed carbohydrates % as compared with control treatment (100% of different water irrigation).

Decreases in biological yield, straw yield and seeds yield in Azur variety, were 39.08%, 42.18% and 26.70%, respectively at 75% water irrigation requirement and reached to 61.34%, 59.33% and 70.55% at 50% water irrigation requirement, while the reductions in Sakha-1 variety reached to 38.52%, 37.05% and 44.56%, respectively in plants irrigated with 75% water irrigation requirement, versus 55.93%, 51.96% and 71.87% at 50% water irrigation requirement. On the other hand, foliar treatment of the two flax varieties (Azur and Sakha-1) with different concentrations of trehalose (2.5, 5 and 7.5 mM) significantly increased yield and yield attributes as compared to the corresponding untreated control plants under at 100% 75% and 50% irrigation water (Table 6,7). Trehalose foliar treatment at 5.0 mM was the most effective treatment on increasing different yield and yield component parameters in the two studied flax varieties (Azur and Sakha-1) as compared with their corresponding controls at different irrigation water levels (100%, 75% and 50%). Data clearly show that, Sakha-1 variety gave higher yield and yield components than Azur variety.

Effect of interaction between drought stress and trehalose treatments on two varieties of flax water productivity:

Data presented in Fig (1) show that, decreased water irrigation requirement (75% and 50%) caused marked decreases in water productivities of seed and oil yield (kg/m^3) of the two tested flax varieties (Azur and Sakha-1) as compared with 100% water irrigation requirement. Meanwhile, different concentrations of trehalose caused significant increases in the above mentioned parameters.

LSD at 5%: WP straw yield (kg / m^3.) 0.11, WP seed yield (kg / m3) 0.01 and WP oil yield (kg / m3) 0.05.

The obtained data in Fig (1) indicated that water productivity of flax under sprinkler irrigation system increased significantly with increasing the foliar treatments of trehalose up to (5 mM) and decreasing with the foliar treatment of trehalose (7.5 mM) with all water irrigation requirements, in details; the highest values of water productivity for straw yield (kg/m^3) was achieved using 50 % of water regime with Azur linseed variety and the foliar treatments of trehalose (5 mM), while, water productivity of seed and oil yields were achieved using 100% of water regime with Sakha-1 variety and the foliar treatments of trehalose (5 mM). Generally, the most effective concentration was 5.0 mM of trehalose as it gave the highest increases over the two concentrations (2.5 and 7.5 mM).

Table 2 : Effect of trehalose on growth parameters of two flax varieties under different water irrigation requirements (Combined data across two seasons)

Varieties	Trehalose (mM)	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)
		100 % Water irrigation requirement					
Azur	0.0	63.00	3.47	1.17	7.33	0.53	0.31
	2.5	78.00	5.13	1.77	9.00	0.96	0.37
	5.0	85.33	7.30	2.98	8.67	1.27	0.61
	7.5	69.33	5.80	2.46	8.67	0.95	0.51
Sakha-1	0.0	54.33	2.60	0.51	8.33	0.43	0.18
	2.5	83.00	5.67	1.60	11.00	0.70	0.19
	5.0	83.67	6.30	1.73	14.00	0.95	0.21
	7.5	74.33	4.20	1.27	13.00	1.10	0.27
		75 % Water irrigation requirement					
Azur	0.0	57.67	3.27	0.99	11.00	0.79	0.41
	2.5	66.67	4.77	1.22	11.33	0.74	0.43
	5.0	73.33	6.93	2.06	12.00	0.95	0.61
	7.5	66.33	5.30	1.79	12.00	0.73	0.42
Sakha-1	0.0	50.33	2.17	0.46	12.67	0.62	0.28
	2.5	73.00	3.00	1.13	13.33	1.30	0.32
	5.0	82.00	5.10	1.52	15.67	1.51	0.35
	7.5	78.00	3.33	1.23	12.33	1.22	0.35
		50 % Water irrigation requirement					
Azur	0.0	52.67	3.17	0.85	12.33	1.29	0.44
	2.5	54.00	3.97	1.26	15.33	1.40	0.44
	5.0	59.33	6.17	1.73	15.33	2.07	0.53
	7.5	58.67	3.47	1.41	12.00	1.27	0.55
Sakha-1	0.0	44.67	2.17	0.39	15.00	1.01	0.33
	2.5	57.67	2.37	0.53	15.33	1.47	0.35
	5.0	62.00	3.90	0.61	15.67	1.73	0.32
	7.5	62.00	3.27	0.55	14.33	1.27	0.35
LSD (5 %)		**2.15**	**0.23**	**0.11**	**0.50**	**0.28**	**0.05**

Table 3 : Effect of trehalose on photosynthetic pigments of two flax varieties under different water irrigation requirements (Combined data across two seasons).

		Photosynthetic Pigments			
Varieties	**Trehalose (mM)**	**Chlorophyll a**	**Chlorophyll b**	**Carotenoids**	**Total pigments**
		mg/g fresh wt			
		100 % Water Irrigation Requirement			
Azur	0.0	1.03	0.51	0.26	1.8
	2.5	1.23	0.54	0.28	2.05
	5	1.37	0.56	0.32	2.25
	7.5	1.37	0.54	0.31	2.22
Sakha-1	0.0	0.9	0.55	0.29	1.74
	2.5	0.99	0.61	0.3	1.9
	5	1.25	0.66	0.42	2.33
	7.5	1.02	0.63	0.32	1.97
		75 % Water Irrigation Requirement			
Azur	0.0	0.94	0.45	0.22	1.61
	2.5	0.99	0.47	0.23	1.69
	5	1.05	0.48	0.26	1.79
	7.5	1.13	0.49	0.26	1.88
Sakha-1	0.0	0.63	0.41	0.13	1.17
	2.5	0.78	0.43	0.21	1.42
	5	0.87	0.57	0.24	1.68
	7.5	0.83	0.47	0.25	1.55
		50 % Water Irrigation Requirement			
Azur	0.0	0.87	0.39	0.17	1.43
	2.5	1.03	0.42	0.19	1.64
	5	1.08	0.47	0.21	1.76
	7.5	0.96	0.45	0.24	1.65
Sakha-1	0.0	0.46	0.34	0.08	0.88
	2.5	0.66	0.37	0.12	1.15
	5	0.86	0.41	0.12	1.39
	7.5	0.68	0.41	0.13	1.22
LSD (5 %)		**0.16**	**0.03**	**0.01**	**0.12**

Table 4 : Effect of trehalose on phenolic, total soluble sugar (TSS), proline and free amino acids (FAA), of two flax varieties under different water irrigation requirements (Combined data across two seasons)

		Phenol	**TSS**	**Proline**	**FAA**
Varieties	**Trehalose**	**mg/g dry wt**			
		75 % Water irrigation requirement			
Azur	**0.0**	28.11	4.17	56.17	211.53
	2.5	84.86	4.28	57.32	226.78
	5.0	89.51	4.96	60.37	247.53
	7.5	79.66	4.63	57.17	251.53
Sakha-1	**0.0**	20.70	5.48	36.37	147.53
	2.5	35.62	5.91	40.89	151.11
	5.0	56.65	6.00	44.66	153.98
	7.5	69.60	6.61	44.44	157.88
		75 % Water irrigation requirement			
Azur	**0.0**	59.46	5.00	61.82	258.78
	2.5	62.56	5.21	71.97	265.53
	5.0	69.51	5.30	78.62	288.78
	7.5	62.46	5.79	66.67	299.03
Sakha-1	**0.0**	42.60	6.10	39.81	154.73
	2.5	56.65	6.34	42.35	167.41
	5.0	65.81	6.94	45.35	172.41
	7.5	73.64	6.97	48.21	176.73
		50 % Water irrigation requirement			
Azur	**0.0**	62.56	5.82	74.07	286.03
	2.5	70.11	5.96	76.42	297.78
	5.0	65.16	5.96	79.92	315.28
	7.5	62.26	6.15	76.22	326.78
Sakha-1	**0.0**	72.90	6.40	45.35	183.41
	2.5	78.78	6.99	52.88	190.08
	5.0	79.60	7.19	52.22	199.78
	7.5	85.60	7.43	52.23	211.83
LSD (5 %)		**3.22**	**0.37**	**1.45**	**16.55**

Table 5 : Effect of trehalose on antioxidant activities SOD, CAT, POX and polyphenol oxidase (PPO) of two flax varieties under different water irrigation requirements (Combined data across two seasons)

Varieties	**Trehalose**	**Antioxidant Activities**			
		SOD	**CAT**	**POX**	**PPO**
		u activity/g fresh wt/hr			
		100 % Water Irrigation Requirements			
Azur	0.0	32.72	32.58	84.66	6.09
	2.5	30.57	19.38	92.97	6.51
	5.0	26.72	18.38	106.16	7.01
	7.5	23.52	12.03	114.56	7.87
Sakha-1	0.0	12.72	12.93	109.58	10.72
	2.5	12.24	9.29	121.22	11.27
	5.0	11.01	7.99	122.99	12.57
	7.5	10.79	7.10	130.24	12.65
	75 % Water Irrigation Requirements				
Azur	0.0	45.17	38.58	116.70	9.04
	2.5	38.32	34.93	123.03	9.43
	5.0	34.97	28.98	131.53	10.12
	7.5	33.02	27.43	144.08	11.27
Sakha-1	0.0	16.16	28.18	140.49	14.59
	2.5	12.70	19.91	145.56	15.36
	5.0	11.70	15.67	148.02	16.12
	7.5	11.56	12.69	148.49	16.62
	50 % Water Irrigation Requirements				
Azur	0.0	50.42	43.48	150.04	14.05
	2.5	42.77	38.28	151.00	14.69
	5.0	36.27	34.03	160.48	15.88
	7.5	32.57	28.78	170.18	16.49
Sakha-1	0.0	21.70	48.24	180.78	19.44
	2.5	19.23	43.66	183.49	20.53
	5.0	17.57	38.69	187.78	21.26
	7.5	17.58	27.64	192.68	22.28
LSD (5 %)		**2.07**	**3.13**	**5.35**	**1.14**

Table 6 : Effect of trehalose on agronomic traits of the two flax varieties under different water irrigation requirements. (Combined data across two seasons)

Varieties	**Trehalose (mM)**	**Plant height (cm)**	**Fruiting zone length (cm)**	**Technical stem length (cm)**	**No. of fruiting branches/ plant**	**No. of capsules/ plant**
		100 % Water irrigation requirements				
Azur	0.0	63.33	16.67	46.66	6.67	16.00
	2.5	76.00	19.67	56.33	8.00	17.67
	5.0	82.00	22.67	59.33	10.00	19.00
	7.5	79.33	20.00	59.33	9.33	21.33
Sakha-1	0.0	81.33	21.33	60.00	14.67	14.67
	2.5	84.33	22.33	62.00	15.00	17.33
	5.0	88.67	25.67	63.00	16.00	24.00
	7.5	86.33	23.67	62.66	15.67	21.33
		75 % Water irrigation requirements				
Azur	0.0	62.33	14.33	48.00	5.33	12.67
	2.5	66.33	18.33	48.00	7.33	15.67
	5.0	82.00	20.33	51.67	9.00	18.67
	7.5	82.33	19.33	63.00	8.00	20.00
Sakha-1	0.0	76.33	18.67	57.66	11.00	12.67
	2.5	79.67	21.33	58.34	13.33	14.67
	5.0	83.00	23.33	59.67	15.33	16.67
	7.5	82.67	22.67	60.00	12.67	20.67
		50 % Water irrigation requirements				
Azur	0.0	61.67	12.67	49.00	4.67	8.33
	2.5	62.67	14.67	48.00	8.33	10.33
	5	67.33	18.33	49.00	6.67	12.33
	7.5	63.67	16.00	47.67	8.00	11.00
Sakha-1	0.0	72.00	16.67	55.33	9.83	4.00
	2.5	75.33	17.33	58.00	11.00	9.33
	5	81.00	19.33	61.67	13.00	13
	7.5	80.33	18.67	61.66	11.33	15.33
LSD 5 %		**2.08**	**1.57**	**2.17**	**1.07**	**1.05**

Table 7 : Effect of trehalose on yield and quality traits of two flax varieties under different water irrigation requirements (Combined data across two seasons)

Varieties	Trehalose (mM)	Seed wt/plant (g)	1000 Seeds wt (g)	Carbohy drates %	Oil %	Straw yield (ton/fed)	Seed yield (kg/fed)	Biological yield (ton/fed)	Oil yield (Kg/fed)
		100 % Water irrigation requirements							
Azur	0.0	0.67	6.20	30.75	32.53	1.98	395	2.38	128.5
	2.5	1.17	6.37	31.75	33.05	2.31	450	2.76	148.7
	5.0	1.47	7.61	31.98	33.99	3.62	517	3.82	175.7
	7.5	1.15	6.07	32.56	33.00	3.10	500	3.51	165.0
Sakha-1	0.0	1.48	7.30	33.56	29.82	2.15	558	2.70	166.4
	2.5	1.49	7.30	34.55	30.77	2.74	686	3.42	211.1
	5.0	1.79	8.92	35.57	31.67	3.69	842	4.53	266.7
	7.5	1.53	8.01	34.33	30.66	2.37	711	3.08	218.0
		75 % Water irrigation requirements							
Azur	0.0	0.44	5.35	30.60	32.09	1.15	289	1.45	92.7
	2.5	0.59	5.58	31.77	32.72	1.79	340	2.13	111.2
	5.0	1.06	6.58	32.50	32.55	3.20	498	3.60	162.1
	7.5	0.64	6.17	32.00	32.50	2.87	380	3.15	123.5
Sakha-1	0.0	1.14	6.04	31.67	28.56	1.35	309	1.66	88.3
	2.5	1.23	6.49	32.80	29.70	1.88	354	2.23	105.1
	5.0	1.49	7.57	32.70	30.50	2.61	577	3.13	176.0
	7.5	1.26	7.09	33.53	30.95	1.93	513	2.50	158.8
		50 % Water irrigation requirements							
Azur	0.0	0.26	4.98	29.05	31.60	0.81	116	0.92	36.7
	2.5	0.36	5.00	30.69	31.98	0.93	176	1.11	56.3
	5	0.47	5.96	31.67	31.70	2.49	355	2.74	112.5
	7.5	0.38	5.39	31.53	31.44	1.86	229	1.39	72.0
Sakha-1	0.0	0.81	5.19	30.35	28.53	1.03	157	1.19	44.8
	2.5	0.91	5.39	31.66	29.72	1.43	263	1.70	78.2
	5	1.22	5.46	32.15	30.29	1.59	292	2.29	88.4
	7.5	1.08	5.64	32.00	30.30	1.70	324	2.02	98.2
LSD 5 %		0.10	0.25	0.45	**0.21**	**0.16**	**13.05**	**0.36**	**5.62**

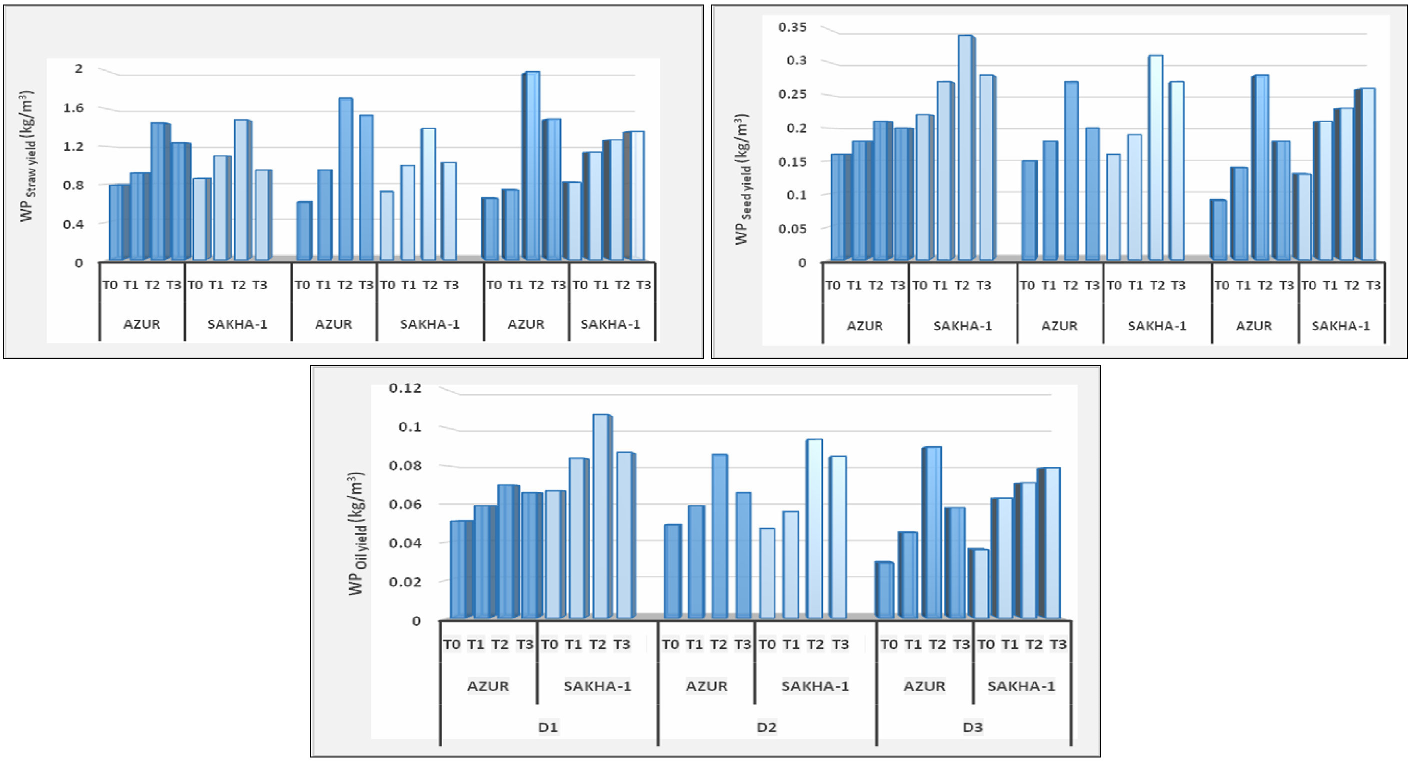

Fig. 1 : Effect of interaction between water irrigation requirement and trehalose treatments on flax water productivity. (Combined data across two seasons)

Discussion

The negative effects of drought on growth of the two varieties of flax plant are presented in Table (2). The obtained data are in accordance with those obtained by Elewa *et al.* (2017 a &b) who reported that, shoot length and both fresh and dry weights of shoots and roots of quinoa plant reduced under drought and they referred these decreases to the metabolic disorders induced by drought and increased levels of ROS by various metabolic processes such as photosynthesis and respiration. Also, Dawood *et al.* (2019) found that, drought stress reduced growth criteria of sunflower plant. These decreases resulted by drought stress could be due to reduce in cell elongation, cell turgor, cell volume and consequently cell growth (Banon et al., 2006). Meanwhile, the increases in root length, fresh & dry weight of the two varieties of flax plant resulted from the first effects of drought; flax cells began to divert assimilates from stem and utilized them for increased root growth in order to increase water absorption. On the other hand, foliar treatment of trehalose increased growth of the two varieties of flax plant (Table 2). Similar findings were observed previously by trehalose treatment on different plants (Duman *et al.,* 2010, Ali and Ashraf, 2011, Theerakulpisut and Gunnula, 2012, Sadak, 2016 and Sadak *et al.* 2019). This promotive effect might be due to enhancing water status of plant cell and osmoregulation and closing of stomata to reduce loss of water (Sadak, 2019).

Drought stress decreased photosynthetic pigments of flax plant (Table 3). Dawood & Sadak (2014), Elewa *et al.* (2017a and b) and Dawood *et al.* (2019) confirmed these obtained reduced effect of drought stress on quinoa plant. These reduced effect might be due to the oxidation of chloroplast lipids and the alteration occurred in the structure of pigments and proteins (Marcinska, *et al.* 2013), and damaging to photosynthetic apparatus thus leading to decreases in photosynthetic carbon assimilation (Din *et al.* 2011). On the other hand, trehalose foliar treatment enhanced different photosynthetic pigments constituents (Table 3). This effect of trehalose is corroborated with the results of previous studies with rice plant, where exogenous trehalose improved photosynthetic pigment contents under stress (Theerakulpisut and Gunnula, 2013) In addition, Sadak (2019) confirmed the promotive role of trehalose on photosynthetic pigments of wheat plant under drought stress. This stimulatory effect might be due to the role of trehalose in maintaining stability of chlorophyll envelope and maintaining chloroplast osmotic potential (Elewa *et al.*, 2017a).

Drought stress 75% and 50% from water irrigation requirements caused significant and gradual increases in phenolic contents of the two varieties of flax plant relative to control plant (100%) (Table 4). Plants evolved different ways of response to different abiotic stress especially drought stress; one of these ways is enhancing phenolic contents in plant cells. Total phenolic contents in oilseed crops are very important for the oxidative stability of the polyunsaturated fatty acids of oils and indicative of antioxidant activity (Ali *et al.,* 2010 and Ali *et al*., 2013). Trehalose treatment caused more gradual increases in phenolic contents. Drought stress caused disorders in various physiological process causing increases in the synthesis of phenolic compounds (Elewa *et al.*, 2017a). Trehalose promotive effect might result from its role as signal molecule thus inducted different metabolic pathways and stimulating the production of various substances, preferably operating under stress (Alam *et al.*, 2014).

When subjecting to environmental adverse conditions, plants increased contents of compatible organic solutes, which shield them from stress through stabilizing of membranes, tertiary structures of enzymes and proteins (Ashraf and Foolad, 2007). The accumulation of TSS caused by drought stress in the two varieties of flax plants (Table 4) are in harmony with those obtained in shoots of various plant species (Bakry *et al.*, 2012, Dawood and Sadak 2014; Sadak, 2016 and Dawood *et al*., 2019). However increased levels of soluble sugars is particularly significant in plants undergoing drought stress (Sadak *et al.*, 2019), it seems that increasing total soluble sugar to reduce osmotic potential is a resistance mechanism of plants against moderate stress. Moreover, trehalose foliar treatment on the two varieties of flax plants induced more significant increases in TSS under normal and drought stress conditions (Table 4). Trehalose, might play an effective role in regulating carbohydrate allocation in plants during development, has often been proposed as acting as soluble sugars in flax leaves may function as an osmotic osmoprotectant during periods of drought or water deficit-induced stresses (Hosseini *et al*., 2014).

Table (4) shows that either moderate or severe drought stress (75% and 50% water irrigation requirements) caused significant increases in proline and free amino acid, contents of the two tested varieties of flax leaves relative to control plants (100% water irrigation requirements). These findings are in consistency with those of Dawood & Sadak (2014), Elewa *et al.* (2017 a & b) and Khater *et al.* (2018) who found that under water stress, proline is accumulated in canola, quinoa and cowpea plants respectively, and this accumulation is positively correlated with stress tolerance. These increases may be attributed to reduced proline oxidase, proline catabolising enzymes as mentioned by Farooq *et al.* (2016). Proline has vital roles in osmotic adjustment, stabilization and protection of membranes, proteins and enzymes (Ashraf and Foolad, 2007) from damaging effects of drought-osmotic stresses.

Drought stress by lowering water irrigation requirements (75% and 50%) caused significant increases in super oxide dismutase (SOD), catalase (CAT), peroxidase (POX) and polyphenol oxidase (PPO) compared with the control plants (Table 5). Trehalose foliar treatment with different concentrations improved stress resistance by more significant increases in peroxidase and PPO activities as compared with corresponding drought stress level. However, SOD and catalase activities decreased. Plant possess' efficient system for scavenging ROS which protect them from destructive oxidative reaction. As part of this system antioxidative enzymes are key elements in the defense mechanisms. Many changes have been detected in the activities of antioxidant enzymes in plants under stress (Mahatma *et al.*, 2009). Superoxide dismutase, catalase, peroxidase and polyphenol oxidase are of enzymes that are responsible for ROS-scavenging. These results are in agreement with those reported by Abdelgawad *et al*., (2014).

Antioxidant defense system is an effective technique in improving plant tolerance to abiotic stress *via* overcoming the adverse effect of ROS (Singh et *al*., 2010). Thus, a lot of variations were noticed in antioxidant enzymes activities in different crops under stress (Mahatma *et al.,* 2009). Superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and peroxidase (POD) are among antioxidant enzymes are responsible for ROS-scavenging. SOD is first line of defence responsible for detoxification of superoxide radicals to hydrogen peroxide (H_2O_2) that can be scavenged by catalase (CAT) and different classes of peroxidases and ascorbate peroxidase, thereby preventing oxidative damage (Noctor *et al.,* 2000). Moreover, POX is one of the major systems for the enzymatic removal of H_2O_2 efficiency in plants (Srivastava *et al*., 2010). Studies on oxidative stress showed that these antioxidants may be higher during the recovery than during the stress period, as observed in pea (Mittler and Zilinskas, 1994). Improving peroxidase and catalase activities increased resistance against harmful free radicals under stress conditions (Jin *et al.,* 2006). In agreements with these results Khater *et al*., (2018) stated that peroxidase activity was increased in response to drought stress. Higher increments in the activities of SOD, CAT and POX were also recorded in resistant varieties of horse gram and common bean under drought stress (Sadak *et al*., 2019). Trehalose application may be the most effective treatment in alleviating the adverse effects of water stress on various plant species, since; trehalose plays an important direct and indirect role as scavenger of ROS (Stolker, 2010). Trehalose was considered as a signaling molecule under abiotic stresses (Fernandez *et al.,* 2010) which stimulate cells to increase ROS production which sends signal to activate enzymatic antioxidants for ROS scavenging in order to counteract stress-associated oxidative stress. Previous studies prove the roles of exogenous trehalose in modulating SOD and CAT activities under abiotic stress conditions (Duman *et al.,* 2010; Ali and Ashraf, 2011 and Khater *et al*., 2018). CAT is an important enzymes having the highest turnover rates among all enzymes (Garg and Manchanda, 2009).

Water availability to plant in different growth stages affect on plant yield and biochemical constituents of the plant and the yielded seeds. These reductions in yield of flax plant are mainly due to the reduction in growth parameters (Table 2) and photosynthetic pigments (Table 3). Drought stress reduced the crop yield due to reduction in photosynthetic pigments and diminished activities of calvin cycle enzymes (Ashraf *et al*., 2013). On the other hand, foliar treatment of two flax varieties with different concentration of trehalose under normal and drought stress conditions caused significant increases in all parameters of yield components as compared to the corresponding control plants. This promotive effect of trehalose foliar treatments on yield and yield components of the yielded seeds are reported earlier on Quinoa (Elewa *et al*., 2017 a & b) and wheat (Sadak., 2019). In recent decade's exogenous protectants such as osmoprotectant (proline, glycinebetaine, trehalose, etc) have been found effective in alleviating stress induced damage in plant (Hasanuzzaman *et al*., 2013). The promotive effect of Trehalose might be because trehalose serve 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 exposed to stress *via* replacing hydrogen bonding through polar residues, preventing protein denaturation and fusion of membranes (Iturriaga *et al*., 2009). Moreover, trehalose acts as a source of carbon and energy and a protector against stresses.

Conclusion

From these results it could be concluded that, subjecting flax plant to decrease in irrigation water (drought stress) decreased growth and yield of the two tested varieties of flax plant (Azur and Sakha-1). Meanwhile, trehalose foliar treatment could alleviate these negative effects in growth and yield of the two flax varieties.

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