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Stigmasterol Relieves the Negative Impact of Drought on Flax through Modulation of Redox Homeostasis

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DROUGHT impacts on flax growth are aimed to be alleviated by soaking seeds in stigmasterol then sowing in pots. On the 24th day after sowing, water regime was applied and samples were harvested up to the 56th day after sowing for measuring growth parameters, free radicals, antioxidants and Ru-1,5-P carboxylase/oxygenase (Rubisco). At seed maturity, yield analysis measurements (capsules, seeds, oil yield and fatty acid composition) were performed. Drought provoked significant decreases in growth parameters, ascorbic acid and glutathione but elevated lipid peroxidation and H_2O_2 concurrently with significant inhibition in the activities of catalase, guiacol peroxidase, ascorbate peroxidase, glutathione reductase, glutathione peroxidase and Rubisco. Yield analysis demonstrated declines in capsule and seed numbers, the oil as well as both contents and composition of fatty acids. Nevertheless, stigmasterol diminished drought impacts on growth parameters, antioxidants and Rubisco and rendered the contents of lipid peroxides and H_2O_2 comparable to control. In the meantime, oil yield and fatty acid composition were improved in synchronization with the efficiency of antioxidants and reactive oxygen species (ROS) homeostasis. These findings conclude that stigmasterol alleviated these drastic impacts and improved oil yield and fatty acid composition via modulating efficient antioxidant capacity and ROS homeostasis.

Keywords: Fatty acid composition, Free radicals, Linseed, Tolerance, Water stress.

Introduction

In oil crops, the contents of oil and the compositions of fatty acids are important attributes. Flax (*Linum usitatissimum* L.) is a fibrous and oil important plant. Seeds are rich in essential fatty acids which are necessary for good health (Kajla et al., 2015). Johnson & Bradford (2014) indicated that the quality oil is worthy depending on essential fatty acids contents. These features are influenced by several factors; environmental, agronomic, genetic, etc. (Savoire et al., 2015).

Water stress limits plant growth and productivity. It inhibits growth and drops yield (Nayyar & Gupta, 2006). Moreover, drought over produces reactive oxygen species (ROS) and changes the antioxidative system (Nemat Alla et al., 2014). ROS include superoxide, peroxide or hydroxyl radicals (Mittler, 2002; Nemat Alla et al., 2008). ROS damage the cell membranes due to lipids peroxidation and the oxidation of both nucleic acids and proteins.

Plants have an endogenous system to tolerate stresses; nonetheless, they are usually disturbed under stress conditions (Perveen et al., 2018; Hassan et al., 2020). The antioxidative mechanisms of plants, either enzymatically or non-enzymatically, help plants to cope with the ROS drastic effects. The non-enzymatic antioxidants include ascorbic

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acid and reduced glutathione, both are among the ascorbate–glutathione cycle through which can eliminate H_2O_2 (Foyer et al., 2001). Ascorbate is major antioxidant that functions in protecting plants by degrading H_2O_2 (Nemat Alla et al., 2008). Glutathione is able to degrade epoxides and peroxides (Foyer et al., 2001). Moreover, several enzymes participate in the breakdown of ROS like catalase, guiacol peroxidase, glutathione reductase, ascorbate peroxidase and glutathione peroxidase (Blokhina et al., 2003; Hassan et al., 2020). Catalase decomposes H_2O_2 into water and oxygen. Guiacol peroxidase or ascorbate peroxidase oxidizes guiacol or ascorbate, respectively with the degradation of H_2O_2. Glutathione reductase participates in the reduction of oxidized glutathione for glutathione maintenance. On the other hand, Rubisco affects the photosynthetic activity as carboxylase and in photorespiration as oxygenase. Water stress inhibits activities of enzymes in photosynthesis causing a diminution in plant development, and yield (Nayyar & Gupta, 2006; Hassan et al., 2008). A net degradation of Rubisco has been detected under stress conditions (Thoenen et al., 2007). The environmental conditions affect the degradation velocity of Rubisco as well as its involved mechanisms (Feller et al., 2008).

Stigmasterol is among the plant sterols. It is a structural component of the lipid core of cell membranes and is the precursor of numerous secondary metabolites, including plant steroid hormones, sugar and protein transport and plays an important role in plant development influencing vascular differentiation, affecting gene expression, as well as many processes involved in development (Rao et al., 2002). Sterols participate in plant cell elongation and morphogenesis of organs, cell division, modulation of hormonal responses, and responses to stresses (Nakashita et al., 2003). Stigmasterol regulate plant growth and development participating in transmembrane signal transduction by forming lipid microdomains (Valitova et al., 2016). It has essential functions in plant growth in response to stresses (Arnqvist et al., 2008). Valitova et al. (2016) indicated the utilization of most of the stigmasterol in the stressed cells, and so they suggested that stigmasterol affects the distribution of other membrane lipids, the metabolic processes in membranes, and signaling pathways influencing the expression of stress genes reporting that stigmasterol is a stress sterol. Moreover, they evidenced that sterols play a key role in cell response under oxidative stress. Stigmasterol application acts as effective stimulator for tolerance of flax and faba bean plants to salinity stress (Hashem et al., 2011; Hassanein et al., 2012) and so, this work aims to assess the role of stigmasterol in the alleviation of drought impacts on flax too. Therefore, seeds were soaked in stigmasterol and allowed to germinate under drought stress to check plant tolerance to drought as well as quality and quantity of oil yield and if whether this alleviation could result through modulation of efficient antioxidants and ROS homeostasis.

Materials and Methods

Growth conditions

Flax (*L. usitatissimum* L., cultivar Giza 10) seeds were surface sterilized by soaking in 3% of a Clorox disinfectant solution that contains 5.25% of sodium hypochlorite for 10min, then thoroughly washed with water. The seeds were divided into three groups; one was soaked in water to act as control, another group was soaked in water to be subjected to water stress and the third group was soaked in stigmasterol solution (100ppm) to be subjected to water stress. Stigmasterol solution was prepared in a minimum amount of dimethyl sulfoxide (the nontoxic polar solvent that dissolves both polar and nonpolar compounds (Kangsamaksin et al., 2017) and is miscible in water), then completed to the appropriate volume by water, an equivalent amount of dimethyl sulfoxide was added for soaking the other groups. The seeds were spread on a filter paper overnight for air-drying and then germinated (about 50-60 seeds per pot) in clay/sand soil (about 15kg; 2:1, v/v) in plastic pots (40cm diameter x 25cm height). The pots were kept at 12hrs photoperiod with 450-500µmol m^{-2} s^{-1} photosynthetic photon flux density obtained from 6 LED light source (sum of 150W m^{-2} at 1m below fixtures with the factor 0.327-0.342), 75-80% relative humidity, and 22/12±2ºC day/night regime. The pots were irrigated every 4 days (2L per pot) until emergence of seedlings (7-8cm height), then the seedlings were thinned on the 18th day after sowing to 20 seedlings per pot and full strength nutrient Ashton solution was applied once (600mL per pot). After 6 days, on the 24th day after sowing, water regime was applied; the control pots (water-soaked seeds) were irrigated every week while the two other groups (water- or stigmasterol-soaked seeds) were irrigated every two weeks throughout the

experimental period up to seed maturity (about 5 months). Seedling samples generated from water-soaked seeds and grown normally (control), water-soaked seeds and grown under water stress (drought) and stigmasterol-soaked seeds and grown under water stress (drought + stigmasterol) were harvested just before the application of regime (24 days after sowing) and on the 34th, 45th and 56th day after sowing. At harvest, shoots and roots were separated and used for growth parameters measurements in replicates (n= 6) Fresh weights were determined in these replicates, then dried at 80 °C for 2 days for determination of dry weight and water content. Liquid N was used to freeze young leaves for subsequent determinations. For yield measurements, samples were collected on the 150th day after sowing.

Determination of H_2O_2 and Lipid peroxides

H_2O_2 and lipid peroxides were extracted in trichloroacetic acid (0.1%, w/v) and centrifuged at 12,000 ×*g* for 15 min at 4°C. The assay of H_2O_2 was performed in potassium phosphate buffer (10mM, pH 7.0) containing 1M KI and absorbance was measured at 390nm (Alexieva et al., 2001). Lipid peroxides were assayed as malondialdehyde (Heath & Packer, 1968). Malondialdehyde amount was calculated by using an extinction coefficient of $155mM^{-1}$ cm^{-1}.

Determination of ascorbate and glutathione

Ascorbate was extracted in 62.5mM phosphoric acid and centrifuged at 12000 ×*g* for 20min then loaded onto ion exclusion column (300 x 7.8mm) and eluted with 4.5mM H_2SO_4 at a flow rate of 0.5mL min^{-1}. The elution of ascorbate was detected at 245nm (Ahn et al., 1999). Glutathione was extracted in trichloroacetic acid (5%, w/v) containing 10mM EDTA and centrifuged at 12000 ×*g* for 15min. Glutathione was assayed in 100mM phosphate buffer, pH 6.8 containing 10mM EDTA, 1mM 1-chloro-2,4-dinitrobenzene and 1.0U equine glutathione-S-transferase and incubated at 35ºC for 30min. The absorbance at 340nm was recorded before commencing the reaction and after the reaction had run to completion (Anderson & Gronwald, 1991).

Assay of antioxidant enzymes activity

Plant tissues were homogenized in sodium phosphate buffer (50mM, pH 7.0) containing 2mM EDTA and 5mM β-mercaptoethanol and centrifuged at 12,000 ×g at 4°C for 10min. Catalase activity was measured by following the consumption of H_2O_2 at 240nm in sodium phosphate buffer (50mM, pH 7.0) containing 10mM H_2O_2 and the protein extract (Aebi, 1984). Guiacol peroxidase activity was measured in sodium phosphate buffer (50mM, pH 6.9) containing 3.2mM guaiacol, 0.4mM H_2O_2 and protein extract, the mixture was allowed to stand for 3min then absorbance was measured at 470nm (Chance & Maehly, 1955). Ascorbate peroxidase activity was determined using the spectrophotometric method where the rate of decrease in absorbance of ascorbate during its oxidation is measured at 290nm (Nakano & Asada, 1981). Glutathione reductase activity was measured by following the oxidation of NADPH at 340nm for 3min using extinction coefficient of $6.22mM^{-1}$ cm^{-1} (Schaedle & Basshan, 1977). Glutathione peroxidase activity was assayed indirectly, as described by Dixon et al. (1998). The reactions contained 1.2mM cumene hydroperoxide, 125mM KPO_4 (pH 7.0), 1.25mM EDTA, 1.25mM sodium azide,1.0mM GSH, 0.25mM NADPH, 0.6 units of yeast glutathione reductase (Sigma, Type III) and 25mg of enzyme extract in a final volume of 1ml. After incubation for 10min at 25°C with all ingredients except cumene hydroperoxide, the reaction was initiated with cumene hydroperoxide. Disappearance of NADPH (NADP formation) at 340nm ($E = 6.2mM^1$ cm^1) was corrected for-non-enzymatic controls. Rubisco was extracted with Tris-HCl (20mM, pH 8.0) containing 10mM magnesium chloride, 10mM sodium bicarbonate, 5 mM dithiothreitol, 1mM EDTA, 0.002% Hibitane, and 1% PVP. Rubisco activity was assayed in Hepes (50mM, pH 7.8) containing 10mM sodium bicarbonate, 0.66mM Ru-1,5-P, 5mM ATP, 20mM magnesium chloride, 0.2mM NADPH, 5mM creatine phosphate, 2.0U creatine phosphokinase, 2.8 U glyceraldehydes-3-phosphate dehydrogenase, and 2.0U phosphoglycerate kinase (Keys & Parry, 1990).

Yield measurements

After seed maturity, on the 150th day after sowing, the numbers of capsules per plant and the numbers of seeds per capsule were counted. Oil of the yielded seeds was extracted by grinding flax seeds with *n*-hexane (60–80°C) as a solvent using mortar and pestle as described in the A.O.A.C. (1975). The conversion of seed oil into fatty acid methyl ester was performed for gas chromatography for determination of fatty acid composition. As described by Danish & Nizami

(2019), an aliquot of oil was mixed with methanolic NaOH (0.5 N), then heated for 3min at 60°C. After cooling, 10mL isooctane were added and agitated well. After settling down, the upper layer was mixed with Na_2SO_4. The determinations of fatty acid composition were performed by injecting fatty acid methyl ester onto gas chromatography-Flame Ionization Detector (Hewlett Packard, 6890), then the peak areas were related to that of authentic standards of the different fatty acids. Gas chromatography apparatus is equipped with Flame Ionization Detector, the oven and injector temperatures were 220 and 240°C, respectively. The oven program initial temp was 140°C for 5min with a rate of 4°C min^{-1}. The carrier gas was N at flow of 1mL min^{-1}. The column was 50% Cyanopropyl methylpolysiloxane, 30m DB-23, 0.32mm ID, 0.25µm film thickness. The syringe size was 10µL and the injection volume was adjusted at 3µL.

Statistical analysis

A twice repetition was performed in the design of this work. The design of the experiment consisted of 60 pots (3 set treatments) x (10 replications) x (2 repetitions) and demonstrated as a complete randomized block. Values were calculated as mean±SD of n= 6 except for fatty acid determinations where n= 3. SPSS 18.0 was used for performing one-way analysis of variance-least significant differences of data for each parameter separately of all treatments at every experimental time.

Results

The results show that drought provoked significant decreases in height of shoots and length of roots. Similar decreases were shown in fresh weights of both shoots and roots in comparison to control values during the entire experimental period (Fig. 1). The decreases augmented with the elapse of time. Meanwhile, dry weight of shoot seemed to be unaffected by drought, however, decreases were detected in root dry weight. Nonetheless, seed priming with stigmasterol greatly counterbalanced the drought-induced reductions in growth parameters, the effects of drought were highly retracted reaching mostly to control values. The retraction was more detected in height of shoots and in fresh and dry weights of roots than in length of roots and fresh weight of shoots.

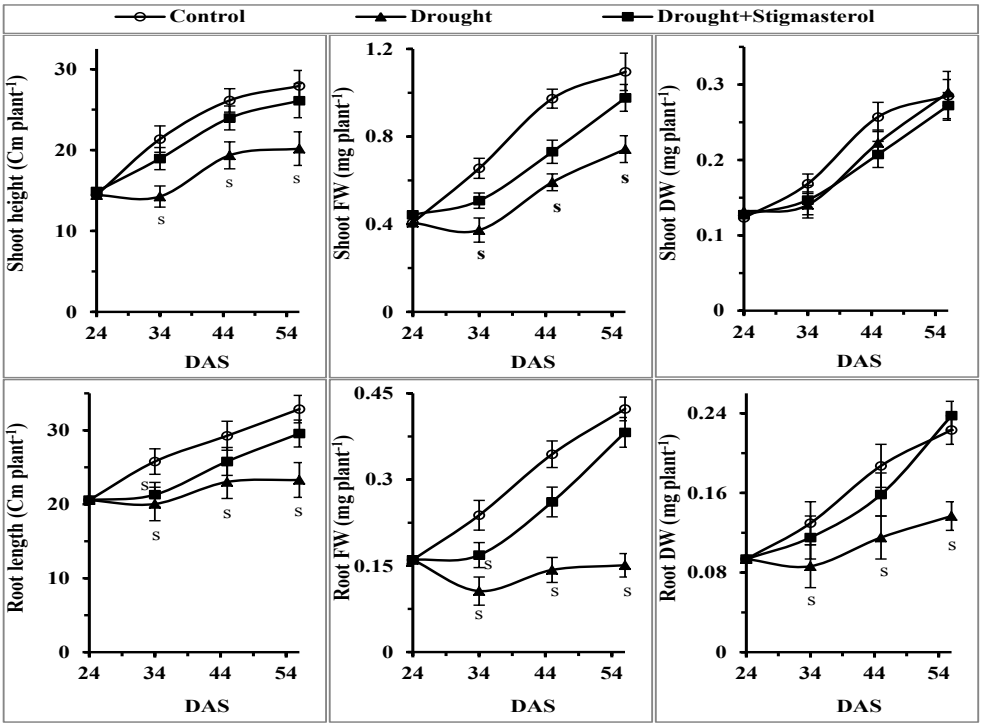

Fig. 1. Influence of soaking flax seeds in 100ppm stigmasterol on the effects of drought imposed by withholding water up to the 56th day after sowing (DAS) on growth parameters of 24-day-old flax seedlings [Values are means±SD (n= 6). One–way ANOVA-LSD was performed at P≤ 0.05. The letter's' means significant difference from the untreated control]

In the same pattern, water content of shoot and root was significantly dropped; meanwhile, the number of leaves was reduced by drought relative to control (Fig. 2). Nonetheless, presoaking of seeds in stigmasterol counterbalanced the drop in water content and, moreover, overcame the reduction of leaf number.

Meanwhile, drought significantly enhanced H_2O_2 accumulation in flax all over the experimental time in comparison to values of the normally grown plants, over aggregation was high on the 34th day after sowing then became steady thereafter (Fig. 3). Also, malondialdehyde was significantly accumulated in response to drought and increased as time elapsed. Contrarily, drought led to a marked drop of ascorbate and glutathione contents, the decrease was higher in glutathione than in ascorbate. However, seed pretreatment with stigmasterol highly suppressed the accumulation of H_2O_2 and malondialdehyde during the entire experimental period leading to comparable values with control. Moreover, elevations of the contents of ascorbate and glutathione were induced by stigmasterol to reach mostly those of the control. Despite this induction, values remained lower than control but with no significant differences. However, the magnitude of glutathione induction was more than ascorbate.

As depicted in Fig. 4, significant inhibition of catalase, guiacol peroxidase, ascorbate peroxidase and glutathione reductase activities were induced due to drought stress in relation to control values. The inhibitions were sharp during the first 10 days of stress and continued consistent thereafter. As a whole, soaking of seeds with stigmasterol caused significant increases in the enzyme activities comparing to the stressed plants. These increases rendered the activity values very close to those of the respective control on the 56th day after sowing.

In addition, glutathione peroxidase and Rubisco were highly inhibited in the drought-stressed samples as compared to control; the effect was great from the onset of treatment and continued consistently all over the experimental time (Fig. 5). Nonetheless, soaking of seed with stigmasterol greatly relieved the activities of glutathione peroxidase and Rubisco, the relief was greater for glutathione peroxidase than Rubisco (Fig. 5).

Yield analysis indicates that plants subjected to water stress produced lesser numbers of capsules and also lesser number of seeds in capsules than the yield of the normally grown control plants (Fig. 6). However, the capsule and seed numbers of plants derived from seeds soaked in stigmasterol were very close to those of control. It is clear also that drought significantly decreased oil content of seeds as well as the percentages of fatty acids in the oil. However, the yielded seeds of plant samples derived from stigmasterol soaking treatment produced more oil content than the yield of the stressed plants to reach nearly the control values. In addition, the percentages of fatty acids of the yielded seeds were increased and even became higher than control (Fig. 6).

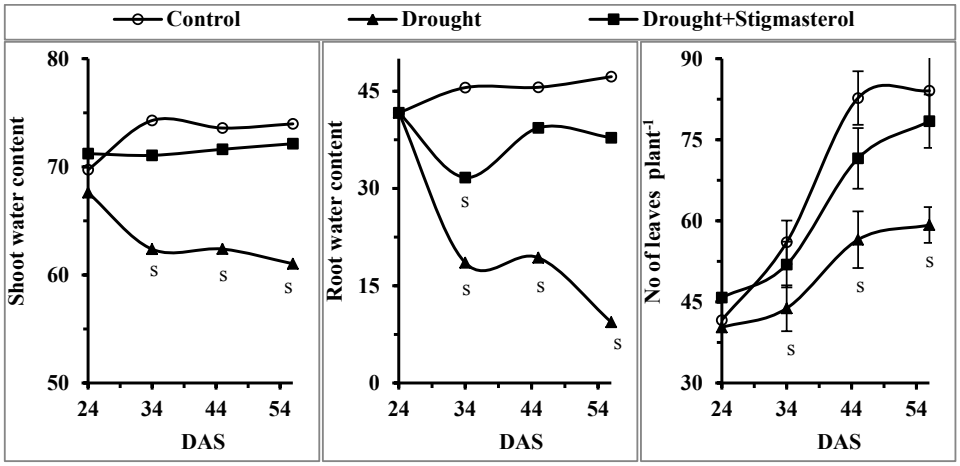

Fig. 2. Influence of soaking flax seeds in 100ppm stigmasterol on the effects of drought imposed by withholding water up to the 56th day after sowing (DAS) on the number of leaves and water content of 24-day-old flax seedlings [Values are means±SD (n= 6). One–way ANOVA-LSD was performed at P≤ 0.05. The letter 's' means significant difference from the untreated control]

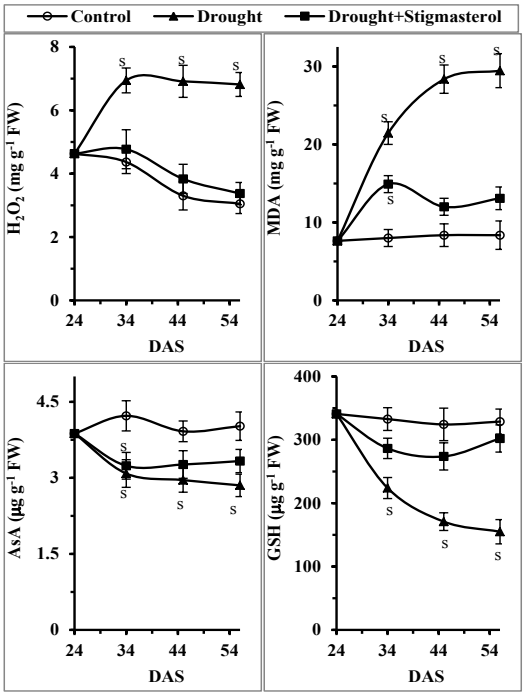

Fig. 3. Influence of soaking flax seeds in 100ppm stigmasterol on the effects of drought imposed by withholding water up to the 56th day after sowing (DAS) on contents of H_2O_2, malondialdehyde (MDA), ascorbic acid (AsA) and reduced glutathione (GSH) of shoots of 24-day-old flax seedlings [Values are means±SD (n= 6). One–way ANOVA-LSD was performed at P≤ 0.05. The letter 's' means significant difference from the untreated control]

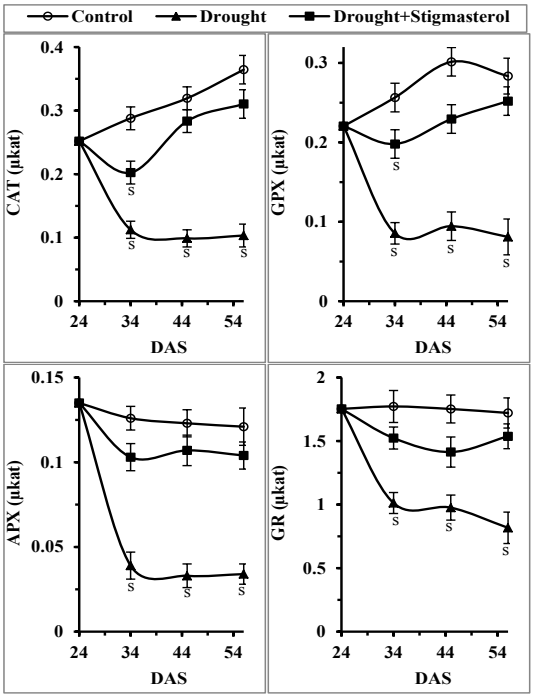

Fig. 4. Influence of soaking flax seeds in 100ppm stigmasterol on the effects of drought imposed by withholding water up to the 56th day after sowing (DAS) on the activities of catalase (CAT), guiacol peroxidase (GPX), ascorbic peroxidase (APX) and glutathione reductase (GR) of shoots of 24-day-old flax seedlings [Values are means±SD (n= 6). One–way ANOVA-LSD was performed at P≤ 0.05. The letter 's' means significant difference from the untreated control]

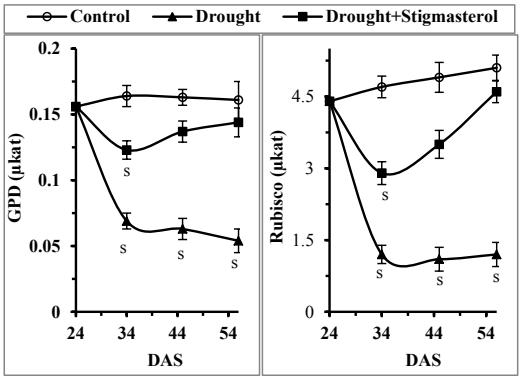

Fig. 5. Influence of soaking flax seeds in 100ppm stigmasterol on the effects of drought imposed by withholding water up to the 56th day after sowing (DAS) on the activities of glutathione peroxidase (GPD) and ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) of 24-day-old flax seedlings. [Values are means±SD (n= 6). One–way ANOVA-LSD was performed at P≤ 0.05. The letter 's' means significant difference from the untreated control]

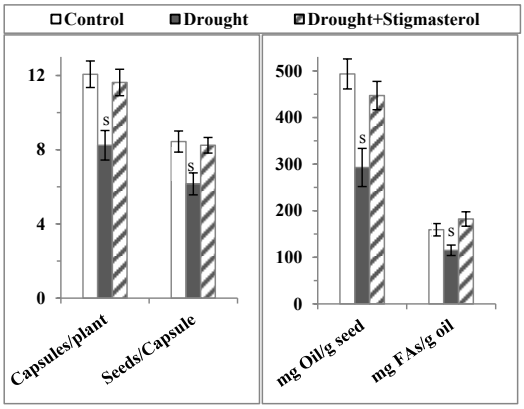

Fig. 6. Influence of soaking flax seeds in 100ppm stigmasterol on the effects of drought imposed by withholding water on yield (the numbers of capsules and seeds, and the contents of oil and fatty acids of the yielded plants) [Values are means±SD (n= 6). One–way ANOVA-LSD was performed at P≤ 0.05. The letter 's' means significant difference from the untreated control]

The composition of fatty acids of the yielded seeds is depicted in Table 1. As a whole, the fatty acids palmitic, stearic, oleic, linoleic and α-linolenic comprised the core bulk of the fatty acids in control samples, α-linolenic acid was detected in higher amounts followed by oleic and linoleic acid whist palmitic and stearic acids were the least. Due to drought stress, the contents of α-linolenic, oleic and linoleic acids were greatly decreased whereas palmitic and stearic acids were increased. Moreover, drought stress resulted in disappearance of some fatty acids namely, heptadecenoic (17:1), eicosatrienoic (C20:3ω6), elaidic (C18:1), linolelaidic (C18:2), eicosatrienoic (C20:3), docasadienoic (C22:2) and docosahexaenoic acid (C22:6). On the contrary, drought generated the fatty acids behenic and lignoceric. Nonetheless, plants generated from seeds soaked in stigmasterol showed elevated levels of α-linolenic, oleic and linoleic acid relative to the stressed plants while palmitic and stearic acids were decreased, these levels became close to control. On the other hand, fatty acid composition of these plants exerted the same pattern of disappearance of heptadecenoic, elaidic and linolelaidic acid as induced by drought whereas eicosatrienoic, docasadienoic and docosahexaenoic acids were continued as control. As a whole, water stress decreased the sum of ω-3, ω6, ω-9, monounsaturated, polyunsaturated and total unsaturated fatty acids. On the contrary, drought increased only the saturated fatty acids. These alterations were highly counterbalanced in plants generated from seeds soaked in stigmasterol such that increases were detected in ω-3, ω-6 and ω-9 as well as unsaturated concomitant with retractions of saturated fatty acids.

TABLE 1. Influence of soaking flax seeds in 100ppm stigmasterol on the effects of drought imposed by withholding water on fatty acid composition of the yielded seeds, concentration (mg g^{-1} oil) and percentage

Fatty acids	Concentration			%		
	C	D	D+S	C	D	D+S
C14:0 Myristic	0.06±0.01	3.62±0.41*	0.19±0.02	0.04	3.14	0.11
C16:0 Palmitic	8.51±0.95	18.36±1.89*	15.64±1.67*	5.35	15.95	8.58
C16:1 (ω9) Palmitoleic	0.12±0.01	2.88±0.31*	1.58±1.48*	0.08	2.50	0.87
C17:0 Heptadecanoic	0.10±0.01	8.11±0.77*	1.75±0.19*	0.06	7.05	0.96
C17:1 cis-Heptadecenoic	0.07±0.01	ND	ND	0.04	0.00	0.00
C18:0 Stearic acid	8.67± 0.79	9.90±1.01	13.40±1.13*	5.45	8.60	7.35
C18:1 (ω9) trans-9-Elaidic	0.11±0.01	ND	ND	0.07	0.00	0.00
C18:1 (ω9) Oleic	32.20±3.37	16.62±1.54*	34.08±2.98	20.22	14.44	18.70
C18:2 (ω6) trans-Linolelaidic	0.32±0.02	ND	ND	0.20	0.00	0.00
C18:2 (ω6) Linoleic	24.16±2.06	13.64±1.41*	26.99±2.87	15.18	11.85	14.81
C18:3 (ω3) a-Linolenic	78.82±6.64	35.47±4.11*	79.01±9.88	49.51	30.82	43.35
C18:3 (ω6) g-Linolenic	0.38±0.05	0.92±0.08*	1.47±0.13*	0.24	0.80	0.81
C20:0 Arachidic	0.30±0.04	0.86±.09*	1.46±0.15*	0.19	0.75	0.80
C22:0 Behenic	ND	1.86±0.19*	0.46±0.06*	ND	1.62	0.25
C24:0 Lignoceric	ND	2.86±0.32*	1.46±0.16*	ND	2.48	0.80
C20:3 (ω3) cis-11,14,17-Eicosatrienoic	2.86±0.26	ND	1.92±0.21	1.80	0.00	1.05
C22:2 (ω6) cis-13,16-Docasadienoic	1.16±0.12	ND	1.32±0.12	0.73	0.00	0.73
C22:6 (ω3) cis-4,7,10,13,16,19-Docosahexaenoic	1.35±0.11	ND	1.51±0.16	0.85	0.00	0.83
Sum of Omega-3 (n-3)	78.82	35.47	79.01	49.51	30.82	43.35
Sum of Omega-6 (n-6)	24.16	13.64	26.99	15.18	11.85	14.81
Sum of Omega-9 (n-9)	32.20	16.62	34.08	20.22	14.44	18.70
Saturated Fatty Acids	17.34	44.71	32.90	10.89	38.84	18.05
Monounsaturated Fatty Acids	32.50	19.50	35.66	20.42	16.94	19.57
Polyunsaturated fats Fatty Acids	109.05	50.04	112.23	68.50	43.47	61.58
Total Unsaturated Fatty Acids	141.56	69.54	147.89	88.92	60.42	81.15
Total Fatty Acids	159.2	115.1	182.2			

- C, control; D, drought; D+S, drought + stigmasterol.
- Fatty acid values are means ± SD (n= 3).
- Values followed by an asterisk are significantly different at P≤ 0.05 with respect to untreated control.

Discussion

The results showed that drought markedly reduced growth parameters of flax could result from retardation of cell elongation, cell volume and eventually cell growth. Like other abiotic stresses, drought results in some malfunctions in plant metabolism which would drastically influence growth, development and productivity of plants (Kapoor et al., 2020). Accordingly, cell turgor maintenance is essential for the plants to survive and also for the assistance to grow well under stress (Shao et al., 2008). A positive relationship between CO_2 fixation and fresh weight generation was observed (Nemat Alla & Hassan, 2020). However, seed priming with stigmasterol greatly counterbalanced the drought-induced reductions in growth parameters, these magnitudes became very close to that of the control. Concurrently, stigmasterol was found to play a regulatory function for plant development (He et al., 2003). Of the important roles played for in development plants are the expansion of cells and vascular differentiation (Rao et al., 2002). Hartmann (2009) concluded that phytosterols are biogenic precursors for several components essential for plants to grow well particularly membrane permeability and modulation of enzyme activity. He indicated that stigmasterol might be specifically required for cell proliferation. Bassiouny et al. (2014) indicated that stigmasterol has a positive impact on *Vicia faba* plants growth and yield subjected to salt stress.

Water stress overproduced some reactive oxygen species (ROS) ranges when plants undergo normal respiratory and photosynthetic processes and also with the photorespiration (Taylor et al., 2003). In plants, the overproduction of ROS takes place usually during the normal metabolic processes in most organelles; nevertheless, the systems protecting the plants can be influenced with the exposure to the environmental stress and so resulting in oxidative stress (Berni et al., 2019) due to the generation of ROS (Mittler, 2002; Nemat Alla et al., 2008). ROS toxicity issued from the damage of biomolecules, like lipids forming their peroxides, proteinous molecules then denaturizing them as well as DNA and RNA leading to mutation with a consequent damage of cellular membranes and organelles (Quiles & Lopez, 2004). In the present results, H_2O_2 and malondialdehyde were greatly accumulated in response to drought. The increases in H_2O_2 and malondialdehyde are involved as biochemical changes induced during water loss causing oxidative damage in stressed plants (Mittler, 2002). However, stigmasterol diminished H_2O_2 and malondialdehyde accumulation concomitant with the alleviation of dehydration effect on growth parameters, such changes could conclude an improvement in plant metabolism (Nemat Alla & Hassan, 2020).

The exposure of the plant to stress, could lead to excess of ROS production with a consequence disruption of lipids *via* oxidation causing production of highly reactive lipid peroxidation-derived molecules. The increased H_2O_2 and malondialdehyde confirm the existence of a status of oxidative stress due to drought; however, these states of stress could be overcome by the plant due to the presence of the endogenous mechanisms to resist stresses (Hassan et al., 2020; Kapoor et al., 2020; Loutfy et al., 2020; Nemat Alla & Hassan, 2020). Antioxidants, either non-enzymatic (ascorbate and glutathione), or enzymatic (catalase, guiacol peroxidase, ascorbate peroxidase, glutathione reductase and glutathione peroxidase) have efficiency in ROS scavenges. Ascorbate is one of the most powerful antioxidant (Nemat Alla & Hassan, 2020). The ability to donate electrons causes ascorbate essential for ROS detoxification. Ascorbate has the ability to eliminate many forms of ROS as well as the reduction of H_2O_2 forming H_2O through the activity of ascorbate peroxidase (Borella et al., 2019). Moreover, glutathione, the key antioxidative element for plants, participates in ROS scavenging through ascorbate-glutathione cycle (Gill & Tuteja, 2010). Therefore, the decrease of ascorbate and glutathione by drought could indicate the impact of water stress on the antioxidant system with a consequent retardation in the scavenging of ROS. The decreased glutathione content might be because of its oxidation to oxidized glutathione (Nakano & Asada, 1981). Consistently, salicylate and ascorbate alleviate drought stress in maize due to the increase in antioxidative capacity (Loutfy et al., 2020). Nonetheless, the nonenzymatic antioxidants were elevated in plants derived from seeds soaked in stigmasterol in coincidence with retraction in H_2O_2 and malondialdehyde concluding the repair of the antioxidant system by stigmasterol. So, stigmasterol could lead to improvement in the antioxidant system for

increasing ROS scavenging and so drought tolerance.

However, the drop of catalase activity could lead to the accumulation of H_2O_2 such that to reach the toxic level Gill and Tuteja, 2010). Under stress conditions, inactivation of catalase is linked to H_2O_2 accumulation. Indeed, Lee et al. (2001) concluded that stress preferentially enhances H_2O_2 content but decreases catalase which is related with either photo inactivating the enzyme or prevention of new synthesis of enzymes (Jang, 2004). Not only the catalysis of catalase could decrease H_2O_2 levels in plant cells but also the activities of guiacol peroxidase and ascorbate peroxidase might lead to further decreases in H_2O_2 levels. Consequently, rises in membrane stability and CO_2 fixation could result as high H_2O_2 levels directly inhibit CO_2 fixation (Yamazaki et al., 2003). So the inhibited guiacol peroxidase and ascorbate peroxidase activities by drought would leave H_2O_2 to accumulate to toxic levels. On the other hand, presoaking of seeds with stigmasterol resulted in relief of the enzyme activities to be more efficient in scavenging of H_2O_2. This relief was coincided with the elevated nonenzymatic antioxidants and concomitant with the retracted accumulation of ROS concluding a repair in the antioxidant system. Moreover, drought caused a significant inhibition in glutathione reductase activity confirming the drop in glutathione content while the increased glutathione reductase activity by stigmasterol was in concomitant with the an elevation in glutathione level. So, the increase in the enzymatic and nonenzymatic antioxidants in response to stigmasterol might indicate existing efficiently system for ROS homeostasis to protect plants through reducing the oxidation impacts. Moreover, the antioxidant system is participated for plants to tolerate drought stress (Gill & Tuteja, 2010; Nemat Alla & Hassan, 2020).

In addition, glutathione peroxidase and Rubisco were highly inhibited in drought-stressed samples. However, stigmasterol rendered them to become close to control. This would indicate the rise of glutathione peroxidase amount and in Rubisco because of stigmasterol treatment to improve antioxidant and photosynthesis (Vu et al., 2008; Nemat Alla & Hassan, 2020), respectively. Rubisco is a stromal protein which catalyses CO_2 fixation in photosynthesis and carbon oxidation in photorespiration. Rubisco breakdown has been detected during and after phases of stresses (Thoenen et al., 2007; Feller et al., 2008). In accordance, wheat Rubisco was dropped due to drought stress while exogenously applied spermine and putrescine elevated its content (Hassan et al., 2020). These findings support the degradation of glutathione peroxidase and Rubisco by drought and might be occurred for the other enzymes too (Vu et al., 2008; Nemat Alla & Hassan, 2020), the effects that were counterbalanced by stigmasterol. This degradation would decrease the enzyme concentration with a consequent delay in the catalytic efficiency by drought; nonetheless, a relief was induced by stigmasterol. As a whole, the effects of drought on growth, oxidative stress indices and antioxidants would consequently affect the plant productivity.

Yield analysis demonstrated that the capsules as well as the yielded seeds numbers were significantly retarded by water stress by about 32 and 27%, respectively; however, these decreases were retracted by stigmasterol to 4 and 2%, respectively. Also, drought dropped oil content and the percentages of total fatty acid composition by about 41 and 28%, respectively; while stigmasterol led to slight decrease in oil content by only 9% and even increases were detected in total fatty acid composition by 15% as compared to untreated control. In fact, water stress reduces the plant growth parameters and retards harvest index with a subsequent yield reduction. Thus, the plant yield could result from integrating of several metabolic processes; therefore, yield is influenced by factors which disrupt these processes. So, the detected declines of yield herein could result from growth cessation beside the reduction in antioxidant system which leaves the plant suffering from stress. Nonetheless, stigmasterol improved yield as did also for growth, ROS homeostasis, antioxidant and Rubisco.

On the other hand, the impact of drought on fatty acid composition of yielded oil showed great counterbalances regarding the plant yield derived from seeds soaked in stigmasterol. The percentages of the bulk fatty acids in the oil (ω9 oleic, ω6 linoleic and ω3 linolenic) decreased by drought by 48, 44 and 55%, respectively; however, stigmasterol augmented these percentages to become very close to the control values. In this account, Danish & Nizami (2019) reported that

the core bulk fatty acids in flaxseed oil are palmitic, stearic acid, ω9 oleic, ω6 linoleic and ω3 linolenic acid comprised about 99% of seed oil, however, these fatty acids comprised about 96% of seed oil in the present results. The present findings clearly declare that drought not only decreased oil content but also lowered its characteristics. Nevertheless, the use of stigmasterol improved –to great extent- oil yield quantity and quality. Stigmasterol overcame the impact of drought on the unsaturated fatty acids and even induced some rises. These findings indicate that stigmasterol improved the characteristics of oil of the drought stressed plants and moreover alleviated the drought-induced drops of ω3, ω6 and ω9. Generally, oil quality is measured depending on the essential fatty acid contents (Johnson & Bradford, 2014); however, combining water stress with plant hybrid might achieve some characteristics of oil quality suitable for several industrial purposes.

Conclusion

Soaking of flax seeds in stigmasterol mitigated the drastic effects of drought on the plant growth, antioxidant levels, indices of oxidative stress, capsule number, seed number of capsules, yield oil content, fatty acids content and fatty acid composition. Moreover, stigmasterol improved the quantity and quality of the oil yield, raised the antioxidants and lowered the oxidative stress indices indicating efficiency in antioxidant system and ROS homeostasis. Such efficiency was synchronized with the alleviation of the deleterious effects of drought and concomitant with the production of improvement in oil yield and fatty acid composition concluding that stigmasterol alleviated the deleterious impacts of drought and improved oil yield through modulating the efficiency of antioxidant and ROS homeostasis.

Conflict of interests: The authors declare no conflict of interest.

Authors contribution: Nemat Hassan and Zeinab El-Bastawisy have conceived and designed the experiments. Inas Budran and Ebtisam El-Harary have performed the experiments, result analysis. Nemat Hassan and Mamdouh Nemat Alla have performed manuscript drafting and prearranging of the manuscript. The ultimate manuscript was read and established by Nemat Hassan and Mamdouh Nemat Alla.

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المعاملة بستيجماستيرول تخفف من التأثير السلبي للجفاف على الكتان من خلال تعديل توازن الأكسدة والاختزال

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تهدف البحث إلى دراسة تخفيف التأثير السلبي للجفاف على نمو الكتان من خلال نقع البذور في ستيجماستيرول. فقد تم استنبات بذور الكتان في الأصص ثم تم تطبيق المعاملة بالجفاف في اليوم الرابع والعشرين ثم أخذت العينات حتى اليوم السادس والخمسين بعد الانبات لقياس معايير النمو والشقوق الحرة ومضادات الأكسدة وإنزيم روبيسكو. وعند نضج البذور تم إجراء قياسات تحليل المحصول (عدد الكبسولات، عدد البذور، محصول الزيت ومكونات الأحماض الدهنية). وأوضحت النتائج أن الجفاف تسبب في حدوث انخفاضات كبيرة في معايير النمو وكميات حمض الأسكوربيك والجلوتاثيون ولكنه أدى إلى ارتفاع في كميات بيروكسيدات الدهون وفوق أكسيد الهيدروجين بالتزامن مع تثبيط كبير في نشاط إنزيمات الكتاليز، جوياكول بيروكسيديز، أسكوربات بيروكسيديز، جلوتاثيون ريدكتيز، جلوتاثيون بيروكسيديز وروبيسكو. كما أظهر تحليل المحصول انخفاضًا في عدد الكبسولات وعدد البذور، وكمية الزيت وكذلك محتويات ومكونات الأحماض الدهنية. وعلى العكس من ذلك، فقد قلل ستيجماستيرول من التأثيرات السلبية للجفاف على معايير النمو ومضادات الأكسدة وإنزيم روبيسكو كما أدى لجعل محتويات بيروكسيدات الدهون وفوق أكسيد الهيدروجين مقاربة لقيم العينات الضابطة. في نفس الوقت، تحسنت إنتاجية النبات من الزيت وكذا مكونات الأحماض الدهنية بالزيت بالتزامن مع كفاءة مضادات الأكسدة والتوازن التأكسدي لأنواع الأكسجين النشط. وخلصت نتائج الدراسة إلى أن ستيجماستيرول قد خفف من التأثيرات السلبية الجفاف على نبات الكتان كما أنه قد حسن من إنتاج الزيت ومكوناته من الأحماض الدهنية وذلك من خلال تعديل فعالية مضادات الأكسدة والتوازن التأكسدي في النبات.