Australian Journal of Crop Science AJCS
AJCS 5(13):1858-1867 (2011) ISSN:1835-2707

Stigmasterol seed treatment alleviates the drastic effect of NaCl and improves quality and yield in flax plants

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Abstract

A few years of stigmasterol research brought into light several vital functions of this new group of phytohormone in plant growth and development. New discoveries of the physiological properties of stigmasterol allow us to consider it as a highly promising, environmentally-friendly natural substance suitable for a wide application in plant protection and yield promotion in agriculture. In the present work we studied the effect of flax seed soaking in stigmaterol (200 ppm) on growth, hormonal contents, total phenol, phenylalanin ammonia lyase (PAL) activity, in addition to fiber composition, yield components and the quality of the yielded seeds and fibers of *Linum usitatissimum* L.cv. Sakha 2 plants grown under different salinity levels. We proved that stigmasterol is phytohormone with a pleiotropic effect. It has a promotive effect on growth, hormonal contents, PAL activity, fiber quality, seed oil content and all the yield characteristics under normal conditions of growth. Moreover, stigmasterol improves the nutritional value and the economic importance of the flax seed oil by increasing linolenic acid and oleic acid contents. It also helped to overcome stress provoked by NaCl as we reported that seed soaking in stigmasterol gives plants some halophytic characteristics which enable the plant to partially alleviate the drastic effect of all the used concentrations of NaCl.

Keywords: Fatty acids; growth; lignin; *Linum usitatissimum*; phenylalanine ammonia lyase; phytohormone; salt stress.
Abbreviations: ABA- Abscisic acid; ALA- Alpha-linolenic acid; DEGS- Diethylene glycol succinate; GA_3- Gibberellic acid; GC- Gas chromatography; IAA- Indole-3-acetic acid; LA- Linoleic acid; PAL- Phenylalanine ammonia lyase.

Introduction

Flax (*Linum usitatissimum* L.) is a dicotyledonus plant from the family Linaceae. It is an important source of natural fibers and industrial oil and has the potential of meeting edible oil and protein deficiencies (Green and Marshall, 1984). Flax contains a mixture of fatty acids. It is rich in polyunsaturated fatty acid, particularly alpha-linolenic acid (ALA), the essential omega-3 fatty acid and linoleic acid (LA), the essential omega-6 fatty acid. These two polyunsaturated fatty acids are essential for humans because our bodies cannot manufacture them and we must consume them in our diets (Morris, 2003). Omega-3 and -6 fatty acids play a crucial role in brain function as well as normal growth and development. They have also become popular because they may reduce the risk of heart disease. In addition many studies indicate that they have beneficial effects for different diseases such as osteoporosis, depression, schizophrenia, bipolar disorder, skin disorder, inflammatory bowel disease and breast cancer, to mention but few (Galli and Risé, 2009; Sarris et al., 2009). In Egypt, flax is cultivated for a dual purpose (seeds for oil and stem for fiber). The cultivated area through the last 20 years was decreased from 60.000 to 30.000 feddan due to the great competition of other economic winter crops resulting in a gap between production and consumption. Therefore, it is necessary to increase flax productivity per unit area which could be achieved by using high yielding and stress tolerant cultivars (Ibrahim, 2009). Among the stresses, salinity has emerged as one of the most serious factors limiting productivity of agriculture crops as well as claiming a substantial farmable area (Purty et al., 2008). Salinity affects about 7% of the world total area (Flowers et al., 1997). The percentage of cultivated land affected by salt is even greater, with 23% of the cultivated land being saline and 20% of the irrigated land suffering from secondary salinization. Furthermore, there is also a dangerous trend of a 10% per year increase in the saline area throughout the world (Amor et al., 2005). The imposing of salinity in the root medium is responsible for reduction in leaf area (Neuman et al., 1988), decrease in growth (Fung et al., 1998) and the cause of disturbances in the photosynthetic process (Singh et al., 1996). In addition, the interactions of salts with mineral nutrition may result in nutrient imbalances and deficiencies which can ultimately lead to plant death as a result of growth arrest and molecular damage (Yamaguchi and Blumwald, 2005). One of the best solutions is to use saline soil, effectively, for improved salt tolerant crops. For this purpose different approaches were adapted and, among those, one is the exogenous application of plant growth regulators (Ashraf and Fooland, 2007). Stigmasterol is a structural component of the lipid core of cell membranes and is the precursor of numerous secondary metabolites, including plant steroid hormones, or as carriers in acyl, sugar and protein transport (Genus, 1978). Sterols play an important role in plant development including cell expansion, vascular differentiation, etiolation and reproductive development (Abd El-Wahed et al., 2001). Similar to the effect of brassinosteroids, both typical sterols (sistosterol and

stigmasterol) involved in the regulatory function of plant development, affected gene expression involved in cell expansion and cell division, vascular differentiation and other diverse developmental programs (Rao et al., 2002; Sasse, 2003). Stigmasterol has anti-inflammatory effects; it inhibited both acute inflammation and chronic inflammation (Gómez et al., 1999). Sterols like stigmasterol have also been recommended for their cholesterol lowering abilities, although more study is needed to determine which compounds perform this function and how they work in the body (Hoffman, 2003). Interestingly, stigmasterol also exhibit bacteriostatic or bactericidal activity against a broad range of gram-positive and gram-negative organisms, as well as *Candida albicans* (Barel et al., 1991). A number of studies have provided evidence that fluctuation in the stigmasterol/sitosterol ratio plays a role in response to biotic and abiotic stresses. (Arnqvist et al., 2008). In this respect, Griebel and Zeier (2010), working on *Arabidopsis thaliana*, found that, upon inoculation with pathogenic microbes, plants induce an array of metabolic changes that, potentially, contribute to induced resistance. When analyzing leaf lipid composition during the *Arabidopsis thaliana–Pseudomonas syringae* interaction, they found that accumulation of the phytosterol stigmasterol is a significant plant metabolic process that occurs upon bacterial leaf infection. Stigmasterol is synthesized from β-sitosterol by the cytochrome P450 CYP710A1 via C22 desaturation. However, the role of stigmasterol in plants during stress is still poorly understood. The objective of this study was to investigate the effect of seed soaking in stigmasterol on growth and yield of flax plants grown under saline conditions in order to highlight the possible mechanisms by which stigmasterol increases plant stress tolerance. As the cost of stigmasterol is brought down to affordable levels, the present research theme may contribute greatly to the usage of stigmasterol in agriculture production as well as to overcome the threat of salinity to crop production around the world.

Results and discussion

Molecular and physiological studies have determined that plant hormones and abiotic stresses have interactive effects on a number of basic biochemical and physiological processes, leading up to regulation of plant growth and development. Various strategies have been considered or employed to maximize plant growth and productivity under environmental stresses such as salt stress. A fundamental approach is to develop salt-tolerant plants through genetic means. An alternative and technically simpler and safer approach is to induce salt tolerance through exogenous application of certain plant growth regulating compounds (Abdel Kader et al., 2010; Ashraf et al., 2010). In the present work the effect of salt stress on flax plants and the role of stigmasterol, as a promising plant regulating substance, in increasing plant tolerance was investigated.

Growth parameters

It has been found in the present work that the examined growth parameters; shoot length, root length, area of leaves per plant, fresh and dry weights of shoots and roots of *Linum usitatissimum* plants L.cv. Sakha 2 were significantly reduced in response to treatment with 100, 150 and 200 mM NaCL (Table 1), the reduction was directly proportion to the applied concentration of NaCl, reaching maximum reduction at 200 mM NaCl in shoot length (34.14%), root length (35.78%), area of leaves (62.98%), fresh and dry weights of shoots (67.6 % and 62.22%, respectively) and fresh and dry weights of roots (65.71% and 28.26, respectively), as compared with those of untreated control plants. Many authors reported that salt stress inhibits the growth of several species, including *Vicia faba* (Cordovilla et al., 1994), *Gylcine max* (Durand and Lacan, 1994), tobacco (Reda et al., 1998), *Oryza sativa* (Lutts et al., 1996), cotton plants (Brugnoli and Björkman, 1992), *Zea mays* (Hassanein et al., 2009). The fresh and dry masses of the plants were decreased gradually by increasing the applied concentration of NaCl (Fariduddine et al., 2009). Sobrado and Turner (1986) observed that the reduction in dry matter of *Helianthus* species under stress was mainly due to decrease in the rate of leaf expansion and assimilation per unit leaf area. It was proved that salinity caused an osmotic inhibition of water absorption (Mekki and Orabi, 2007) and increased the amount of work necessary to counteract osmotic and ionic stress for normal cellular maintenance; as a consequence, these are leaving less energy for growth requirements (El-Saidi, 1997). Ashraf et al. (1998) showed that plant under salinity might have less dry weight because of stunted growth, reduction in cell division, ion toxicity and reduction in plant turgor potential which may reduce both cell production and cell expansion and, hence, reduce plant growth and productivity (Saffan, 2008; Aldesuquy et al., 2009). The earliest plant response to salt stress is a reduction in the rate of leaf surface expansion, followed by cessation of expansion as the stress intensifies (Parida and Das, 2005). The leaf area, as well as its fresh and dry masses, decreased significantly in response to stress induced by salinity. Reduction in leaf area by salinity is an important cause of reduction in crop yield through the reduction of photosynthesis (Rucker et al., 1995). Application of stigmasterol in the present work improved the growth of *Linum usitatissimum* plants by causing significant increases in the values of shoot length, area of leaves per plant, fresh and dry weights of shoots as compared with the corresponding controls (Table 1). This is, probably, by increasing the efficiency of water uptake and utilization, enhancing cell division and/or cell enlargement, resulting in longer shoots and roots and increasing leaf area which, consequently, increased the fresh and dry matter of root and shoots, presumably, as a result of larger surface area available for anabolic activities. Similar results were obtained by Abd El-Wahed et al. (2001), working on wheat, who mentioned that both stigmasterol and spermidine caused stimulation of vegetative growth characteristics (plant height, leaf area, plant fresh and dry weight) and net assimilation rate of maize and vascular bundles differentiation of wheat. Similar to the effect of brassinosteroids, both typical (sitosterol and stigmasterol) and atypical sterols play a regulatory function in plant development (He et al., 2003).

Yield components

Yield is a result of the integration of metabolic reactions in plants; consequently any factor that influences this metabolic activity at any period of plant growth can affect the yield (Ibrahim and Aldesuquy, 2003). Thus, in the present investigation all the applied concentrations of NaCl caused a marked decrease in all the investigated yield characteristics (number of capsules per plant, number of seeds per plant, dry weight of each capsule, seed index, weight of yielded seeds per plant and seed yield (kg/hectare) in *Linum usitatissimum* plant (Table 1). This was in agreement with the results of Ahmed (2009) who found that salinized mung bean plants showed a decrease in seed yield per plant, associated with a reduced number of seeds per pod and 100 seeds weight. He

Table 1. Effect of different concentrations of NaCl either alone or in combination with stigmasterol on growth parameters, yield components and seed oil percentage of *Linum usitatissimum* L. plants. Each value is a mean of 5 replicates.

Growth parameters								
Treatment	NaCl mM	Shoot length (cm)	Root length (cm)	Area of leaves per plant (cm^2)	Shoot weight (g) Fresh	Shoot weight (g) Dry	Root weight (g) Fresh	Root weight (g) Dry
Reference controls	0.0	58.00 b	10.90 a	37.06 d	1.426 c	0.450 b	0.046 abc	0.070 ab
	100	50.80 c	8.64 bc	40.95 c	0.860 e	0.264 d	0.042 ab	0.060 abc
	150	43.20 e	7.50 bc	24.28 f	0.800 f	0.216 e	0.037 bc	0.046 c
	200	38.20 g	7.00 c	13.72 h	0.460 g	0.170 f	0.033 c	0.024 d
Stigmasterol 200 ppm	0.0	60.40 a	10.90 a	55.78 a	1.980 a	0.538 a	0.051 a	0.080 a
	100	52.25 c	9.10 b	50.79 b	1.903 b	0.453 b	0.049 ab	0.076 a
	150	46.60 d	7.90 bc	32.50 e	1.040 d	0.310 c	0.041 ab	0.050 bc
	200	40.80 f	7.90 bc	18.45 g	0.870 e	0.210 e	0.036 c	0.047 c
L.S.D. at 5 %		1.731	1.750	2.141	0.027	0.021	0.012	0.021

Yield components and seed oil percentage										
Treatment	NaCl mM	No. of flowers per plant	No. of capsules per plant	No. of seeds per plant	Dry wt. of each Capsule	Seed index (Wt. of 1000 seeds,g)	Wt. of yielded seeds per plant (g)	Seed yield (Kg/ hectare)	Seed oil percent-age (%)	Seed oil yield (Kg/hecta re)
Reference controls	0.0	7.0 b	6.2 c	41.29 c	0.036 c	7.99 c	0.3302 c	186.833 c	33.39	62.383 c
	100	5.4 c	4.6 d	23.73 e	0.026 cd	5.00 e	0.1186 e	67.238 e	33.70	22.66 e
	150	3.6 e	2.7 f	12.82 g	0.020 de	3.20 g	0.0410 g	23.355 g	30.46	7.114 g
	200	1.4 g	1.2 g	2.40 h	0.010 e	0.75 h	0.0048 h	7.076 h	27.89	1.976 h
Stigmasterol 200 ppm	0.0	7.4 a	7.1 a	65.32 a	0.068 a	11.2 a	0.7314 a	414.024 a	35.21	145.779 a
	100	7.2 ab	6.8 a	54.40 b	0.055 b	9.00 b	0.4900 b	277.405 b	35.57	98.679 b
	150	4.2 d	4.0 e	27.32 d	0.028 cd	6.00 d	0.1640 d	92.690 d	34.04	31.557 d
	200	3.2 f	2.7 f	15.28 f	0.025 cd	3.90 f	0.0600 f	35.381 f	34.25	12.119 f
L.S.D. at 5 %		0.362	0.318	2.372	0.011	0.117	0.001	3.862	-	1.507

Means with the same letter are not significantly different.

explained that, delayed maturity due to salt stress pushes the plant to desiccation stress causing shriveled seeds. The decrease in yield and yield components in different crops under similar conditions has also been reported by many workers (Afroz et al., 2005; Arbona et al., 2005; Sohrabi et al., 2008; Aldesuquy et al., 2009). It could be suggested that the observed decrease in the yield components in the present work could be attributed to the detected reduction in leaf area which may affect the photosynthetic efficiency of the leaves and, hence, the carbohydrate contents of the plants. Similarly, the reduction in yield of soybean plants was attributed to the decrease in photosynthetic rate, carbohydrate accumulation, nitrogenase activity and consequently protein synthesis and accumulation in seed yield (Van Hoorn et al., 2001). The reduction in the yield could be also attributed to the accumulation of toxic ions, impaired uptake of essential nutrients and/or damage in cellular organelles (Torres-Schumann et al., 1989). Furthermore, crop growth reduction due to salinity is generally related to the osmotic potential of the root-zone soil solution. This will lead to certain phonological changes and substantial reduction in productivity (Abou-Hadid, 2003). The decrease in harvest index with the increase of salinity was mainly due to the severe inhibitory effects on fertility (Abdullah et al., 2001). The results clearly indicated that application of stigmasterol has positive effects on yield and yield components of *Linum usitatissimum* plants as compared with the corresponding salt stressed control plants. Stigmasterol treatment was found to enhance a partial alleviation of the harmful effects on the yield caused by salt stress. Worthy to mention is that the increases in the yielded seeds per hectare in response to stigmasterol application were estimated by 121.6%, 312.57%, 296.88% and 400% in plants treated with 0.0, 100, 150, 200 mM NaCl, respectively, over those of the reference control plants. In this connection, Abd El-Wahed et al. (2000) found that, maize yield and its components (ear length, ear diameter, number of grain rows/ear, weight of 100 grains, shelling percentage and grain yield/plant) were significantly increased for plants which were twice sprayed by the stigmasterol, especially at 80 mg/l. Moreover, Abd El-Wahed et al. (2001) found that sitosterol application significantly increased spikelet number/spike and 1000 grain weight of wheat. He also recorded that plant yield and grains number/row in maize plant increased with increasing sitosterol concentrations. Previously, Helmy et al. (1997) found that brassinosteroid application significantly increased growth and yield characters of broad bean. El-Greedly and Mekki (2005) also stated that the increase in seed/plant of the two sesame cultivars and the increase in number and weight of capsules as well as 1000-seed weight at high stigmasterol concentration (200 ppm) might be due to the increment of growth regulators which improved photosynthetic activities, consequently, this beneficially affects the number and weight of capsules and seed yield.

Seed oil contents

In the present work, stigmasterol treatment was found to significantly increase seed oil contents of flax plants subjected to salt stress as compared with untreated salt stressed plants (Table 1). Interestingly, stigmasterol caused a total alleviation of the inhibitory effect of 100 mM NaCl on the seed oil yielded (kg/hectare) of flax plants and even increased it over those of unstressed control plants; the magnitude of such increase was estimated by 58.18%. Hence, it was proved that if the flax seed soaked in

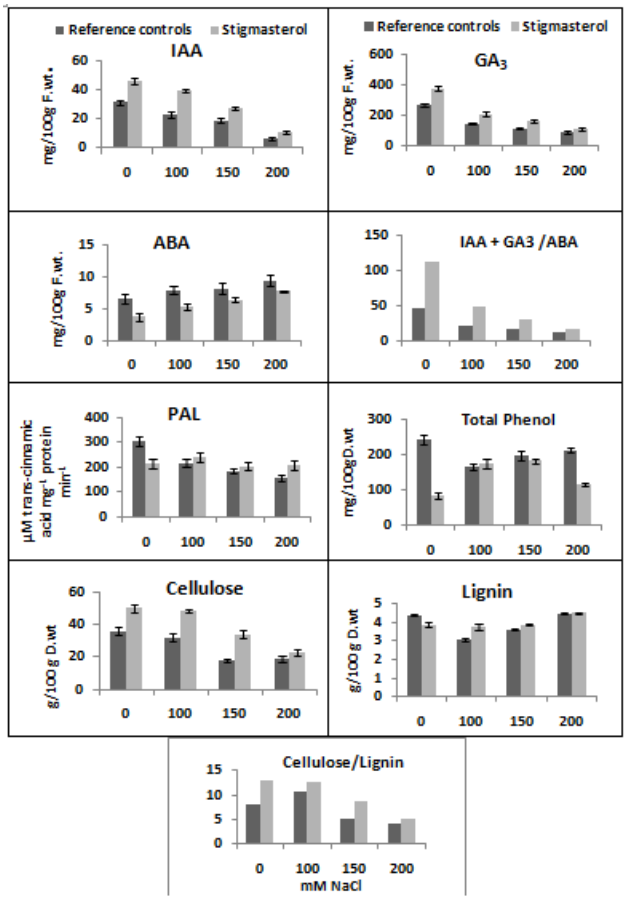

Fig (1a). Effect of different concentrations of NaCl either alone or in combination with stigmasterol on hormone contents, the activity of phenylalanine ammonia lyase and total phenol contents of shoot (at the vegetative stage), and fiber composition of *Linum usitatissimum* L. plant.

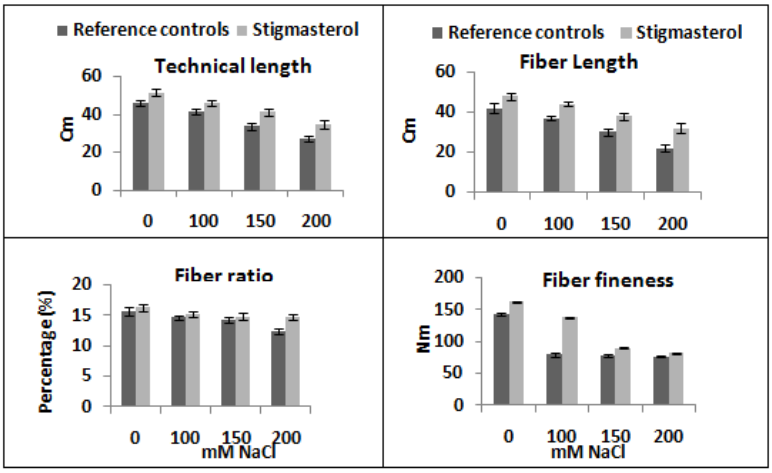

Fig (1b). Effect of different concentrations of NaCl either alone or in combination with stigmasterol on technical characteristics of fiber of *Linum usitatissimum* L. plant.

stigmasterol before sowing, the resulting plant could be cultivated in a land subjected to up to 100mM NaCl without any loss in the seed oil contents of the plants. In this respect, Abd El-Wahed et al. (2000) found that the oil percentage of maize grains was increased as the number of sprays and concentrations of stigmasterol increased. In addition, Bekheta et al. (2003) concluded that application of stigmasterol, which exerts their effect on the level of gibberellins metabolism, might increase the accumulation of essential oil. Moreover, Vardhini and Rao (1998) stated that exogenous application of brassinosteroids resulted in enhanced yield and also fat content.

Endogenous phytohormones

It appears that, from the obtained results that, salt stress led to sharp changes in the balance of endogenous hormones; it has an inhibitory effect on the detected amounts of IAA and GA_3 and a stimulatory effect on ABA contents of flax shoot as compared with untreated plants. The effect of salinity either stimulatory or inhibitory is directly proportion to the applied concentration of NaCl (Fig. 1a). These results are in agreement with those obtained by Hassanein (1999) working on rice plant and El-Bassiouny (2005) working on wheat plant and Hassanein et al.(2009) working on *Zea mays*.It should be evident that growth regulator balance (IAA+GA_3/ABA) can be changed at high salinities, and this effect can be partially alleviated with the application of exogenous growth promoter substances (Debez et al., 2001). Stigmasterol, in combination with the different salinity levels (0.0, 100, 150, 200 mM NaCl), was found to significantly increase IAA contents by 147.28%, 173.25%, 142.11% and 175.00% and GA_3 levels by 138.69%, 140.94%, 141.026% and 120.43% as compared with salt stressed plant untreated with stigmasterol. In addition, a significant decrease was detected in ABA content in response to seed soaking in stigmasterol; and is worthy of mention that stigmasterol caused a complete alleviation of the inhibitory effect of up to 150 mM NaCl in case of IAA and up to 100 mM NaCl in the case of GA_3. The increase in IAA and GA_3 may lead to enhancement of enzyme activity and, in turn, to an increase of the metabolic compounds which can also explain the increased growth parameters in stigmasterol-treated plants, as compared with untreated ones. In this respect, Bekheta et al. (2003) reported that foliar application of stigmasterol on thyme plants resulted in an increase of endogenous amounts of growth regulators such as GA_3 and IAA. Also, they indicated that the application of stigmasterol exert its effect on the level of gibberellins metabolism which might increase the accumulation of essential oil. In this respect, Shunquan et al. (2001) showed that spermidine and stigmasterol treatment enhanced phytohormones, which can play an important role as signals and regulators of growth and development of plants. In addition, El-Greedly and Mekki (2005) studied the response of two maize varieties (Giza-32 and Shandweel-3) to different concentrations of stigmasterol and stated that in both cultivars application of 150 or 200 ppm of stigmasterol had the highest GA_3, IAA and lowest ABA compared to the treatments by 100 ppm and/or to untreated plants.

Phenylalanine ammonia lyase activity, total phenol contents and fiber composition

It was observed that, phenylalanine ammonia lyase (PAL) activity in flax leaves decreased gradually by increasing the applied concentration of NaCl and such reduction was concomitant with a decrease in the leaf total phenols contents, as compared with the untreated control plant. This could be explained by the fact that L-PAL is considered as a key enzyme of phenolic biosynthesis. In this respect, Schovánková and Opatová (2011), working on apples after fungal infections, found a very good correlation between the activity of PAL and total phenol content. Leaf phenolic contents are important protective components of plant cells. The potential of phenolics to act as an antioxidant is mainly due to their properties to act as hydrogen donators, reducing agents and quenchers of singlet O_2 (Zhang and Wang, 2001). The synthesis of phenolics is, generally, affected in response to different biotic/ abiotic stresses including salinity (Parida et al., 2004). Dkhil and Denden (2010) reported that, the total phenolics content of germinated okra sprouts were accumulated, by increasing NaCl concentration from 60-100 mM. On the other hand, reduced phenolic contents were observed in *Cynara cardunculus* leaves under saline conditions (Falleh et al., 2008). Perhaps, high accumulation of phenolics at the reproductive stage occurs, due to their putative role in reproduction (Bravo, 1998). Furthermore, Hichem et al. (2009) reported that such variation in the concentration of leaf phenolics within a plant under salt stress in relation to leaf age, may be due to the reflection of different requirements for counteracting abiotic stresses at different growth stages. Interestingly, the results show that although stigmasterol generally stimulated the activity of PAL in *Linum usitatissimum* plants by 10.28%, 9.95%, and 32.58 % in plants treated with 100, 150 and 200 mM NaCl, respectively, as compared with the reference controls, it causes a reduction in total phenol contents of flax leaves as compared with plants untreated with stigmasterol. It is possible that new phenols are rapidly oxidized by oxidative enzymes, such as polyphenol oxidase or peroxidase and similar results were obtained by Jones (1984) and Slatnar et al. (2010). In addition, PAL is not committed exclusively to phenol. PAL catalyzes the first reaction in the biosynthesis from phenylalanine of a wide variety of phenylpropanoid natural products including lignin, flavonoid pigments, and phytoalexins (Liang et al., 1989). This is in agreement with our results as we found that stigmasterol has a promotive effect on lignin contents of flax stem fiber as compared with untreated control plant (Fig. 1a), thus stigmasterol enable the treated plants to acquire some resistance by increasing lignin contents. Many authors suggest a positive correlation between plant resistance and lignin contents; Tronchet et al. (2010), working on *Arabidopsis* and Umesha and Kavitha (2011), working on tomato. The adverse effect of salt stress on cellulose content and cellulose/lignin ratio in fibers of *Linum usitatissimum* plants were also partially alleviated in response to stigmasterol treatment, thus, stigmasterol enables the treated plants to acquire some halophytic characteristics.

Technical characteristics of fiber

Fibers quality (fiber length, fiber percentage and fiber fineness) of *Linum usitatissimum* plants were found to decrease with the increase in the applied concentration of NaCl as compared with those of reference controls (Fig. 1b). Similar results were obtained by El Hariri et al. (2010) who proved that salt stress induced reduction in growth, yield components and fiber yield in flax plants as compared with those of control plants. Stigmasterol in the present work was found to have an enhancement effect on the fiber quality of flax, as compared with that of the untreated control plants. In this respect, stigmasterol treatment was shown to induce, in most cases, significant increases in fiber length, fiber percentage and fiber fineness by 14.2, 4.41 and 13.4%, as

Table 2. Effect of different concentrations of NaCl either alone or in combination with stigmasterol on fatty acid composition (%) of seeds of *Linum usitatissimum* L.cv. Sakha 2 .

Treatment/ Fatty acids	0.0 mM NaCl (control)	100 mM NaCl	150 mM NaCl	200 mM NaCl	Stigmasterol 200 ppm	Stigmasterol +100 mM NaCl	Stigmasterol +150 mM NaCl	Stigmasterol +200 mM NaCl
Lauric C12:0	3.65	6.71	7.29	4.12	2.27	4.56	2.80	2.66
Myristic C14:0	13.09	32.29	32.94	27.63	10.80	20.09	20.69	10.52
Palmitic C16:0	8.21	6.27	5.47	6.65	8.24	8.41	7.69	7.75
Stearic C18:0	3.59	1.74	1.52	4.79	4.37	3.36	2.76	11.17
Oleic C18:1	12.53	9.36	8.53	6.16	14.01	13.27	12.22	11.75
Linoleic C18:2	10.52	11.76	12.14	19.95	11.00	10.88	10.51	12.40
Linolenic C18:3	43.92	28.08	24.12	22.88	47.61	37.2	25.18	35.40
Arachidic C20:0	1.73	1.3	3.56	0.757	1.2	0.971	2.72	5.01
Total known	97.24	97.51	95.57	92.94	99.6	98.74	94.57	96.66
Total unknown	2.76	2.49	4.43	7.06	0.4	1.26	5.43	3.34
unsaturated	66.97	49.2	44.79	48.99	72.72	61.35	57.9	59.55
saturated	30.27	48.31	50.78	43.95	26.88	37.39	36.66	37.11
Ratio of unsaturated/ saturated	2.21	1.02	0.882	1.115	2.71	1.64	1.58	1.6

compared with those of the untreated control plants respectively. In addition, stigmasterol was found to alleviate the adverse effect of different concentrations of salinity. Such effect was much more pronounced by the combined effect of stigmasterol and 200 mM NaCl. Stigmasterol, in this treatment, caused highly significant increases in fiber length and percentages estimated as 45.45% and 18.4%, respectively, over those of untreated salt stressed plants. These results, collectively, suggest the economic value of stigmasterol treatment in both normal and stress conditions.

Fatty acids composition

Gas liquid chromatographic analysis cleared that eight fatty acids could be detected in *Linum usitatissimum* seeds oil. These acids are named Lauric (12:0), Myristic (14:0), Palmitic (16:0), Steric (18:0) and Arachidic (20:0) as saturated fatty acid and Oleic (18:1), Linoleic (18:2) and Linolenic (18:3) as unsaturated fatty acids (Table 2). Total saturated fatty acids percentage ranged from 26.88-50.78% and total unsaturated fatty acids ranged from 44.79-72.72%. Total unsaturated/total saturated fatty acids ratio ranged from 0.882 to 2.7. All the applied concentrations of NaCl were found to cause a marked reduction in unsaturated fatty acids content accompanied with a marked increase in saturated fatty acids content as compared with untreated control plants. The most affected unsaturated fatty acid was linolenic acid which decreased by 36.07%, 45.08% and 47.91% in response to 100, 150 and 200 mM NaCl, respectively, as compared with control plants. On the other hand, Myristic acid (C14:0) was the most increased saturated fatty acid in response to different concentrations of NaCl, the highest value obtained in response to 150 mM NaCl and was estimated by 151.64%, as compared with the control value. This indicates that mystiric acid may play an important role in salt tolerance mechanism of plants. In this respect, Ishitani et al. (2000) reported evidence for an essential role of protein N-myristoylation (refers to the covalent attachment of myristic acid, by an amide bond to N-terminal glycine residue of a nascent polypeptide) in plant salt tolerance. The effect of stigmasterol treatment was found to be contrary to that of salinity; as marked increases were observed in unsaturated fatty acid contents, as compared with the reference control plants. In this respect, oleic acid was increased by 11.81%, 41.77%, 43.26% and 47.57% and linolenic acid by 8.4%, 32.48%, 45.85% and 54.72%, as compared with plant treated with 0.0, 100, 150 and 200 mM NaCl alone, respectively. Similar results were obtained by Abd El-Wahed and Gamal El-Din (2004). Putanam et al. (1990) stated that the good quality of sunflower oil is, principally, due to its high level of unsaturated fatty acids. The increase in oleic acid and linolenic acid, in response to stigmasterol treatment, increases the nutritional value and the economic importance of the flax seed oil, as the flax seed oil health benefits are, primarily, due to it being the highest food source of crucially needed omega 3 fatty acids. There is a terribly widespread deficiency of the omega 3 source essential Fatty Acid (Alpha Linolenic Acid) and the omega 3 fatty acids that are derived from it. Oleic acid (omega 9) has also received a great attention lately, due to its ability to lower blood pressure and the level of cholesterol in the body, being rich in antioxidants that help in fighting the effects of free radicals in the body. It also boosts the immune system, reduces the inflammation of joints and other complications related to arthritis, reducing the resistance of insulin, thereby, improving glucose (blood sugar) maintenance (Thompson and Cunnane, 2003; Berab et al., 2006). El –Lethy et al.(2010) found that foliar application of putrescine, stigmasterol or α-tocopherol, significantly, affected growth criteria and seed yield of flax plant and linolenic acid was found to be the main fatty acid in the seeds of all treatments under study.

Materials and Methods

Flax seeds

Pure strain of flax seeds (*Linum usitatissimum* L.cv. Sakha 2) was obtained from Agriculture Research Center, Giza, Egypt.

Chemicals

Stigmasterol was purchased from MP Biomedicals, LLC. France.

Growth condition and treatment

A pot experiment was carried out under natural conditions, in the green house of Benha University Faculty of Science, Department of Botany. Plastic pots (40 cm in diameter and 25

cm in depth) were used and contained 20 kg of a mixture of clay-sand (2:1 w/w) soil. Phosphorus and potassium fertilizations were added before sowing at a rate of 6.0 and 3.0 g/pot in the form of calcium superphosphate (15.5 % P_2O_5) and potassium sulphate (48 % K_2O), respectively. Seeds were surface sterilized with 0.1 % mercuric chloride for 5 min and washed thoroughly with several changes of sterile distilled water. They were then soaked overnight (12 hours) in either (i) distilled water or (ii) 200 ppm of freshly prepared stigmasterol solution. Fifteen seeds of each treatment were sown in each pot at 3 cm depths. After emergence, the seedlings were thinned to 10 healthy seedlings per pot. Pots were maintained in a green house under natural conditions of light with a 8 hours photoperiod and average 25/10 °C ± 3 day / night temperature. After 20 days from sowing, seedlings were subjected to the desired salinization levels (0, 100, 150 and 200 mM NaCl) at 70% of soil of water holding capacity. Thereafter, the plants were irrigated with water and the pots were kept at 70% soil water holding capacity till the end of the experimental period. The experimental plants were left to grow under the different salinization levels and stigmasterol treatments until the harvest. Ten replicates (planted pots), from each level of treatments, were considered. Samples were collected at the vegetative stage (40 days old plants) to determine phytohormones, phenylalanine ammonia lyase activity and total phenol contents. Whereas, samples were collected from plants at fruiting stage (100 days old plants) for assessment of growth parameters (shoot and root length, area of leaves, fresh and dry weights of shoot and roots). The area of leaves per plant (cm^2) were determined by multiplying length x width x 0.75 (Quarrie and Jones, 1979). Finally, 125 days old plants were harvested to determine fiber quality and composition, yield components, oil percentage and fatty acid composition of the yielded seed oil.

Extraction, separation and estimation of growth regulating substances

The method of extraction was, essentially, similar to that adopted by Shindy and Smith (1975) as described by Hassanein et al. (2009). To estimate the amounts of acidic hormones IAA, ABA and GA_3, the plant hormone fractions and standard ones were methylated according to Vogel (1975) to be ready for GC analysis. A flame ionization detector was used for identification and determination of acidic hormones using a Hewlett Packard Gas Chromatography (5890). The chromatography was fitted and equipped with HP-130 m x 0.32 mm x 0.25 µm capillary column coated with methyl silicone. The column oven temperature was programmed at 10°C/min from 200°C (5 min) to 260°C and kept finally to 10 min. Injector and detector temperature were 260°C and 300°C, respectively. Gases flow rates were 30, 30, 300 cm/sec for N_2, H_2 and air, respectively, and flow rate inside column was adjusted at 2 ml/min. Standards of IAA, GA_3, and ABA were used.

Estimation of total phenol

Total phenols were determined in leaves according to the method described by Malik and Singh (1980) using the Folin-Ciocalteau reagent. The absorbance was read at 650 nm.

Determination of Phenylalanine Ammonia Lyase (PAL) activity

PAL activity (EC 4.3.1.5) was measured according to the method of Brueske (1980). The absorbance was measured at 290 nm and the reaction rate was expressed as µM trans-cinnamic acid mg^{-1} protein min^{-1}.

Determination of seed oil content

The method adopted for extraction of the oil content of an oleaginous material was that described by Meara (1955). After extraction, the extract was quantitatively transferred to a weighed flask and the solvent was evaporated using an electric fan. The last traces of the solvent and moisture were removed by heating the flask at 100 °C under reduced pressure. The flask was then allowed to cool in a desiccator and reweighed to the nearest mg and the increase in weight was considered equivalent to the weight of oil in the sample.

Identification, fractionation and quantification of different fatty acids

Methylation of fatty acids for gas-liquid chromatography analysis was performed in the manner adopted by Sink et al. (1964). Then, 1 µl of fatty acid methyl ester was injected into a 6 feet x 1/8 inch internal diameter column packed with 20 % diethylene glycol succinate (DEGS) on chromos orb 60-80 mesh by using Hewlett-Packard (model : HP – GC – MS) according to the following conditions; GLC Model Shimadzu-8 A (PFE), F.I.D. detector, Column 5 % DEGS on 80/100 Chromo Q. Chart speed : 2.5 mm / min., H2 flow rate : 75 ml / min., N2 flow rate : 20 ml / min., Air flow rate : 0.5 ml / min. and Sensitivity : 32 X 10^2. The fatty acid composition of the samples was expressed as a percentage of the total fatty acids resolved.

Estimation of cellulose and lignin

Cellulose was estimated according to the method described by Updegroff (1969), and lignin was estimated according to Lin and Kao (2001).

Technical characteristics of fibers and yield attributes

* Technical length of the main stem (cm); from the cotyledonary node till the beginning of apical branching zone of the main stem.
* Seed index**;** is the weight of 1000 seeds (Gregory, 1988).
* After harvesting and removing the capsules from plants, a retting process took place at Faculty of Science, Benha University. Straw was arranged in bundles and put in retting basins and soaked in water for about 12 hours. After soaking, the water was changed, to leach out all of the soluble materials. The retting period was about one week. The degree of water temperature during the retting process ranged from 28°C to 30ºC and the acidity was pH 6-7. The retted straw was washed with water and finally dried in air. Thus, the fibers were easily extracted from above the woody part of the stem. The following fiber characters were studied:

- Fiber length (cm); fiber ribbons of 10 plants from each sub-plot were extracted individually and the mean length of each ribbon was measured.
- Fiber ratio was calculated as follow; fiber ratio = weight of fiber/weight of plant X 100
- Fiber fineness (N.m) determined using Radwan and Momtaz (1966) method, according to the following formula,

N.m = N X L / W

Where: N = Number of fibers, L = Length of fibers in cm, W = Weight of fibers in g.

Statistical analysis

The experiment was set up in a completely randomized design. The mean values of growth parameters and yield components were calculated from five replicates and all other mean values in the study were calculated from three replicates. All data were analyzed statistically by one-way ANOVA using the Statistical Package for Social Science (SPSS) program and significant differences among treatment means were calculated by Duncan's multiple range test (p= 0.05). The bars in all figures represent standard deviations of the replicates from the means.

Conclusion

From the above mentioned data, it could be concluded that stigmasterol might play a role in plant physiology through affecting flax plant growth and productivity, phytohormones content, PAL activity, lignin and cellulose contents of flax fiber and fatty acids composition. In addition, stigmasterol treatment was found to be valuable from the economic point of view if it was applied on plants grown under normal or stress conditions. Further studies should be undertaken to supply more information about the mode of action of stigmasterol on molecular bases.

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