ANTIMUTAGENICS EFFECTS OF STIGMASTEROL ON TWO SALT STRESSED *Lupinus termis* CULTIVARS

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*L*upinus termis L. is recommended as staple food and as a medicinal plant by health organizations (Leterme, 2002). It's seed have a nutritional quality and could be an important source of protein and oil. Lupine seeds contain 33-40% crude protein, 5-13% oil (Písaříková, and Zralý, 2009; Hassan *et al.*, 2012). Lupin seed oil is suitable for utilization in different food products due to presence of high antioxidants content of α- and δ-tocopherol (Alamri, 2012).

Salinity is one of the most important abiotic stresses that manipulate crop productivity and has been described as one of the most significant threats to agriculture. Increased salinization of arable land is prospective to have destructive universal effects, generating 30% land loss within the next 25 years and up to 50% by the year 2050 (Munns and Tester, 2008). Salinity adversely affects on seed germination and growth (Dash and Panda, 2001; Ashraf *et al.*, 2002), enzyme activity (Seckin *et al.*, 2009), DNA and RNA (Rong-Chao *et al.*, 2007) and mitosis (Tabur and Demir, 2010). Electrophoretic protein pattern induced by different factors was used by many authors as a good candidate for studying and monitoring changes in gene expression between the treated plants (Majoul *et al.*, 2000). It is well certified that various environmental stresses cause serious modifications in gene expression of plants (Soussi *et al.*, 2001). Significant number of genes that are transcriptional activated in plants during stress condition has been identified (Matos *et al.*, 2001).

Phytohormones play serious roles in stress adaptation. It is thought that the repressive effect of salinity on seed germination and plant growth could be associated with a drop in endogenous levels of phytohormones (Jackson, 1997; El-Saeid *et al.*, 2011; Vaurasi and Kant, 2016). Some researchers have used plant growth regulators (PGRs) for reducing or exterminate the negative effects of salinity (Kabar, 1987; El-Mashad and Mohamed, 2012). Phytohormones are linked with the control of cell cycle machinery (Hirotomo and Masaaki, 2014). Stigmasterol is a type of brassinosteroids (group of phytohormones) and is the precursor of numerous secondary metabolites (Genus, 1978). Sterols carry out a substantial role in plant development (Abdel-Wahed, 2001). A number of studies have provided evidence that fluctuation in the stigmasterol ratio plays a role in restraint to biotic and abiotic stresses (Arnqvist *et*

al., 2008). Exogenous stigmasterol applications have an effective role to enhance the salt tolerance of crops and eventually improve crop productivity under high salinity (El-Mashad and Mohamed, 2012).

The root tips are often the first to be exposed to chemicals spread in soil and water. Examination of the root tip established a rapid and sensitive method for environmental monitoring. Cytological studies will donate detailed information on qualitatively and quantitatively harmful effects at the microscopic level. Among the test systems appropriate for toxicity monitoring, the *Allium* test is well known (Grant, 1982). Genotoxicity of different chemicals can be modulated by growth hormones and bio-compounds which reduce the frequency of chromosomal aberrations (Morsi *et al.*, 2016). Therefore, the aim of the present study was to assess the effect of stigmasterol on some growth parameters and protein banding pattern on two salt-stressed *Lupinus termis* cultivars and to examine the utility of stigmasterol as a plant regulatory substance to increase the salt tolerance of *Lupinus termis* cultivars. Also, this study demonstrates the anti-mutagenicity effect of stigmasterol and suggests that it has a potential as an anti-mutagenic agent.

MATERIALS AND METHODS

Seeds of *Lupinus termis* (L). cultivars Giza 1 and Giza 2 and *Allium cepa* bulbs were obtained from the Agricultural Research Center, Giza, Egypt. Stigmasterol was purchased from MP Biomedicals LLC, Illkirch, France.

Seeds of each lupine cultivar were surface sterilized with 1% sodium hypochlorite for 5 min. and then cleaned with distilled water. The seeds were then soaked for 24 hrs in either distilled water (control) or 500 μM freshly prepared stigmasterol solution. Then the seeds were divided into three groups. The first one was taken for germination percentages. The second group was taken for growth parameters. The third sets of produced seedlings were used for estimation of protein banding patterns.

Mitotic analysis

Six sets of rooted healthy and uniform *Allium cepa* bulbs were immersed in freshly prepared concentrations of control (H_2O), 100, and 200 mM NaCl, 500 μM stigmasterol, 100 mM NaCl+500 μM stigmasterol, and 200 mM NaCl+500 μM stigmasterol) for 24 hours; control roots were treated with tap water. Roots (1-2 cm) long were fixed in Carnoy solution (3 ethanol: 1 glacial acetic acid) for 24 hours then stained with basic Fuchsin. Cytological preparations were carried out using Feulgen squash technique. The frequencies of the mitotic index and the total abnormalities and the different types of chromosomal abnormalities were recorded.

Growth parameters

The first group of pre-soaked seeds was placed in 9 cm Petri dishes containing double layer of Whatman No.1 filter paper. Distilled water or test solutions were added to each Petri dish. Three replicate

were used in each treatment. The number of germinated seeds was recorded on the ten day and germination percentage (GP %) was calculated according to the given formula (Bybordi, 2010):

$$GP \% = n \div N \times 100$$

Where: n is the number of germinated seeds after ten days and N is the number of all seeds.

The second group of pre-soaked seeds was sown in plastic pots containing garden soil. Fifteen seeds /treatment (control and 500 µM stigmasterol treatment) were sown in each pot and three replicates were used for each treatment. Pots were maintained in a green house under natural light conditions with an 8h photoperiod and average 30/20 ± 2°C day/night temperatures. Fifteen days after sowing, the control seedling and that treated with stigmasterol were subjected to the desired salinization levels (100 or 200 mM NaCl). Thus, the different treatments were as follows: control (H$_2$O), 100 and 200 mM NaCl, 500 µM stigmasterol, 100 mM NaCl+500 µM stigmasterol, and 200 mM NaCl+500 µM stigmasterol. Samples from each treatment were collected at 21 days-old plants to determine some growth parameters (root and shoot length).

Protein electrophoresis

Gel electrophoresis SDS-PAGE was carried out with gel slabs according to the method of Laemmli (1970). Protein bands were stained with Coomassie blue R-250 by standard techniques. The gel was scanned using Gel pro-Analyzer.

Statistical analysis

All cytological data were statistically analyzed using t-test. Score was taken from 9 roots (3 roots/replicate) for each treatment.

RESULTS AND DISCUSSION

Mitotic analysis

The data obtained from the cytological analysis of *Allium cepa* root tips treated with NaCl and stigmasterol is summarized in (Table 1 and Fig. 1-a). One of the major effects of NaCl on root tips of *Allium cepa* is its influence on the rate of cell division. Mitotic index was reported to be a good indicator to access the cytotoxic level, whereas chromosomal aberration was used to test mutagenicity of chemicals in cells (Akinboro *et al.*, 2011; Leme and Marin- Morales, 2009). Salinity caused an inhibitory effect on the process of cell division. The reduction of mitotic index (MI) was significantly decreased and was found to be directly proportional to the concentration of NaCl used. Treatment root tip cells for 24 h with 200 mM NaCl caused highly significant inhibition in cell division and drastic drop in the mitotic index which was recorded (0.21%). On the other hand, roots treated with 100 mM NaCl showed significant depressive effect on the mitotic index (5.88%) as compared with control which recorded 7.08%, these results indicate that NaCl has mitostatic effect at this treatment. Mitotic inhibition may be due to arrest the cells at G1 phase and so suppressing DNA synthesis (Schneiderman *et*

al., 1971), or arrest the cell in G2, preventing the cell from entering mitosis (Van't Hof, 1968). The entry of cells from G2 into mitosis is regulated by cyclin dependant kinase CDK/cyclin B while cyclins (D and E) can move cells from G1 into S phase (Fabian- Marwedel *et al*., 2002).

On the other hand, treatment of the roots with 500 μM stigmasterol showed an increase in MI (10.84%) as compared with the control. The present data indicated that stigmasterol had obviously antimutagenic effects against NaCl which induced inhibition of cell division (Table 1). It is evident from the present results that, treatment with NaCl (100 or 200 mM) combined with stigmasterol (500 μM) showed strong improvement of MI compared with the same concentrations without stigmasterol. The 100 mM NaCl together with stigmasterol (500 μM) showed significant increase in MI, the percentage reached 6.91% compared with 5.88% without stigmasterol (Table 1). On the other hand, the depression of MI was still observed but intensity of reduction was much less in the concentration (200 mM NaCl) together with (500 μM) stigmasterol when compared to the same concentration without stigmasterol. Hu *et al*. (2000) showed that Brassinolide (BL) induces the transcription of cyclin genes (CycD3, D-type cyclin) which activate cell division. This supports the findings of Miyazawa *et al.* (2003) who found that BL promoted cell division of *Nicotiana tabacum*. Because BL-treated cells responded quickly with a significant increase in the number of pro-

phases and telophases, this would indicate an alteration of the cell cycle with more cells entering and exiting mitosis than in the controls. William *et al.* (2007) stated that low doses of BL (0.005 ppm) nearly doubled the mean root length and the MI% over that of controls. Mary-Paz *et al.* (2011) stated that brassinosteroids (BRs) play a regulatory role in the control of cell-cycle progression and differentiation in the *Arabidopsis* root meristem.

Treatment of *Allium cepa* root cells with different concentrations of NaCl for 24 h induced a wide range of mitotic abnormalities (Table 1 and Fig. 1-b). The types of mitotic abnormalities noticed in the present study were, disturbed chromosomes, sticky, laggard, bridges and C-Metaphase. The percentage of chromosomal aberrations increased with increasing the concentration of NaCl. Such percentage reached 24.48 and 96% after treatment with 100 and 200 mM NaCl, respectively compared with 0.62% for the control. Treatment *Allium cepa* roots with stigmasterol showed considerable decreases in the percentage of mitotic abnormalities (Table 1). The most common type of abnormalities observed with NaCl treatments was stickiness. The percentage of metaphase and ana-telophase stickiness reached to 84.62% and 72.73% after treatment with 200 mM NaCl, respectively. Stickiness may result from physical adhesion involving mainly the proteinaceous matrix of chromatin material (Lamsal *et al*., 2010). Induction of chromosomal and chromatin bridges at anaphase and telophase stages was also

observed after treatment with NaCl. The percentage of bridges reached to 27.27% with 200 mM NaCl. Sifa (2005) declared that chromosome bridges might be due to chromosomal stickiness then failure of anaphase separation. Considerable percentages of disturbed mitotic phases were induced by treatment with NaCl. Disturbed phases may be attributed to disruption in the mechanism of chromosomes movement and the orientation of these chromosomes at the equatorial plate (Shehata *et al*., 2000). Lagging chromosomes were noticed in the different mitotic stages. The percentage of lagging metaphase reached to 2.75% with 200 mM NaCl/500μM stigmasterol. The induction of lagging chromosomes could be attributed to disturbance in the mechanism of chromosomes movement (Pandey and Upadhyay, 2010).

The total number of aberrations was significantly reduced in root tip cells pretreated with stigmasterol. Our results showed that administration of stigmasterol ameliorated chromosome aberrations induced by NaCl at all tested doses. This ameliorative effect by stigmasterol might have resulted from enhancement of detoxification pathways those converted reactive compounds to less toxic and more easily excreted products. Vardhini and Rao (2003) stated that stigmasterol treatment reduced the adverse effects of salt stress on plants. This study revealed that stigmasterol has antimutagenic potential against NaCL induced chromosomal aberrations in *Allium cepa* root meristematic cells. Zhiponova *et al.* (2013) stated that

brassinosteroid (BR) hormones control plant growth through acting on both cell expansion and division. They stated that stigmasterol treatment might play a key role in providing stress tolerance by stimulation of the antioxidant system as a stress protection mechanism. Behzad (2014) investigated that growth hormone has antioxidant activity and increases plant tolerance against stress such as salinity, pathogens, and UV rays. In line to this, earlier reports regarding moderate antimutagenic activity of many agents (Akinboro and Bakare, 2007; Sameer, 2008; Ifeoluwa and Adekunle, 2013; Selestin *et al*., 2013).

Finely the present results indicated that the administrations of growth regulator such as stigmasterol were very helpful in minimizing the mito-inhibition and aberration effect induced by salinity. However, the definite molecular mechanisms of antimutagenic effects or antigenotoxicity of stigmasterol in *A. cepa* root meristem cells need further investigations.

Changes in percentage of germination and some growth parameters

It is obvious from (Table 2 and Fig. 2-a) that, salt stress caused reduction in germination percentage of *Lupinus termis* L. (cv. Giza 1 and cv. Giza 2) seeds. The effect of NaCl on mitotic activity is accompanied by a reduction in the germination rate of seedlings (Tables 1 and 2). This reduction appears to be concentration-dependent. The results indicated that

cv. Giza 2 had the ability to tolerate the increasing of salt stress (100 mM and 200 mM) than cv. Giza 1. Also, the results showed that, stigmasterol treatment can alleviate the adverse effect of NaCl by increasing the germination percentages of the tested seeds. On the other hand, treatment seeds with stigmasterol showed a considerable increase in germination percentage. Combination of NaCl (100 mM and 200 mM) with stigmasterol antagonized the inhibition effect of NaCl and caused increase in the germination percentage in both cultivars. These results are in agreement with that obtained by Rao *et al.* (2002), who reported that the exogenous applications of stigmasterol enhanced the germination percentage of many leguminous crops under salinity stress. Also, El-Mashad and Mohamed (2012) demonstrated that germination percentage reduced by salt but the exogenous brassinolide application significantly counteracted this inhibition. Numerous trails have been made to improve the salinity tolerance of different crops by traditional breeding programs, but commercial success has been limited thus far. Exogenous stigmasterol application has been submited not only as a convenient approach for unveiling its role in the salinity response, but it is also considered to be an effective approach to enhance the salt tolerance of crops and eventually improves crop productivity under high salinity. Stigmasterol treatment to salt stressed rice plants enhanced antioxidant activities helped to decrease oxidative damage in stigmasterol-treated rice (*Oryza sativa* L.) plants (Anuradha and Rao 2001). The data

provided evidence that stigmasterol treatment reduced the adverse effects of salt stress on *Lupinus termis* L. plants, and might play a key role in providing stress tolerance by excitation of the antioxidant system as a stress protection mechanism.

Results presented in (Table 2 and Fig. 2-b) demonstrated that stigmasterol treated lupine seeds displayed the highest growth rate expressed as shoot and root length. Reversibly, salinity decreased all growth increments as compared to control. The combined effect of salinity and stigmasterol caused significant increase in growth parameters (shoot and root lengths). Stigmasterol treated plants (cv. Giza 2 and cv. Giza 1) irrigated with 100 mM NaCl resulted in better shoot and root growth (15.02 and 12.52%) and (4.11 and 3.18%), respectively than 200 mM salinized plants (11.62 and 10.62%) and (3.12 and 2.68%), respectively. Also, salinized plants (100 and 200 mM NaCl) showed suppressed plant growth but in case of 200 mM NaCl, the growth parameters showed highly significant change than 100 mM NaCl when compared with control. These data agree with those of El-Khallal (2001) who reported that pretreatment of pea seedling with stigmasterol alleviated the deleterious effect of NaCl on the plant growth. Takatsuto *et al.* (1983) found that BL applied to the roots of radish or tomato seedlings promoted the elongation of the hypocotyls. Mussig *et al.* (2003) found that low concentrations of BRs activate root elongation in *Arabidopsis* plants up to 50%. Retardation of plant growth may be the result of inhibition of growth regula-

tors or the delay in mitotic division. Padmavahti *et al.* (1992) relate seedling growth retardation to germination injury and the production of chromosomal abnormalities in dividing cells. Mendhulkar (1993) attributed plant growth inhibition to disturbances in natural growth regulators and mitotic chromosomal irregularities as additional factor.

SDS-PAGE protein analysis

The effect of applied salt stress (100 and 200 mM NaCl) on the protein profiles of *Lupinus termis* L. cultivars Giza 1 and Giza 2 in the absence or presence of stigmasterol are shown in (Table 3 and Fig. 3). In the present study, salinity stress induced a considerable variation in the protein patterns among different *Lupinus termis* L. cultivars. This alteration has been manifested as the novel expression of some polypeptides; the absence of others and over expression of a third class of polypeptides. In addition, the generated distinct protein bands within each cultivar appeared to be responsible, to a great extent, for the tolerance and/or the susceptibility of such cultivars to salt stress. The soluble protein profiles of the two *Lupinus termis* L. cultivars comprise five common monomorphic major bands in cultivars Giza 2 (108, 85, 58, 34 and 30 KDa) and 3 bands in cultivars Giza 1 (230, 108 and 46 KDa). The main polypeptide bands are located between 16 and 230 KDa. In cv. Giza 2 treatment with 100 mM NaCl or 200 mM NaCl only lead to produce three new protein bands (46, 17 and 16 kDa). In case of cv. Giza 1 treatment with 100 mM

NaCl only lead to produce one new protein band (17 kDa), while in case of 200 mM NaCl treatment only three new bands appeared (30, 17 and 16 kDa). Several of new proteins which are synthesized in response to environmental stress have been reported as stress-proteins in plants (Hoyos and Zhang, 2000). Many of these proteins were suggested to preserve the cell against the adverse effect of salt stress. Kawasaki *et al*. (2001) found that protein turnover in stressed plants were detected at early time, followed by the induction of known stressed-responsive transcripts within hours, and the creation of transcripts for defense-related function later. Arora *et al.* (2000) stated that water stress induces the accumulation of stress responsive proteins belonging to dehydrin group (25-60 KDa) and aquaporins (25-30 KDa). Such newly- synthesized proteins help the plant to act as defense responses against abiotic and biotic responses (Fauteux *et al.*, 2006). Salinity treatment was found to induce the disappearance of the protein bands with a molecular weight of 48, 96 and 230 KDa in cultivar Giza 2 and 38, 48 and 85 KDa in cultivar Giza 1 under NaCl treatment. In the present study, the *Lupinus* cultivar Giza 2 which is claimed to be salt-tolerant appears to be the least affected by salinity with regard to growth and protein pattern (Tables 2 and 3). The levels of proteins differ in salt tolerant and salt-sensitive genotypes when they are subjected to salinity stress (Dubey and Rani, 1989). Majoul *et al.* (2000) study the effect of salt-stress on polypeptide profiles of wheat salt-tolerant and salt sensitive cultivars. They reported

that the protein patterns for control and salt-stressed seedlings were qualitatively similar. Treatments of seeds with stigmasterol induced the appearance of some new protein bands in seedling of the *Lupinus termis* L. cultivars. Also, the polypeptides which have disappeared in salinized seedling returned to appear when those seedlings are treated with stigmasterol (Table 3). The cv. Giza 2 showed to be more tolerant than cv. Giza 1, these may be due to the ability of cv. Giza 2 to produce more new protein bands under treatment with stigmasterol than cv. Giza 1 can not to do so. Also, treatment the seeds with stigmasterol resulted in an increased intensity of most polypeptide bands which already apparent in the control (Fig. 3). The induction of new bands and increase in the intensity of some original bands, indicate that the stigmasterol has a qualitative and quantitative changes on the protein components of the plants. It is obvious that treatment with (100 mM NaCl/500 μM stigmasterol) and (200 mM NaCl/500 μM stigmasterol) stimulated the production of two new bands with molecular weights 17 and 96 kDa in the seedlings of the cv. Giza 1 and five new bands with molecular weights 16, 17, 38, 46 and 80 kDa in the seedlings of the cv. Giza 2. Phytohormones are vital for the capability of plants to adapt to abiotic stresses by mediating a wide range of adaptive responses (Peleg and Blumwald, 2011). They often quickly modify gene expression by stimulate or preventing the degradation of transcriptional regulators through the proteasome system (Santner and Estelle, 2010). The induction of par-

ticular protein bands among the other ones in cv. Giza 2 seedlings and their absence in cv. Giza 1 may be the incidental factor of amplifying the signal transduction pathway of the endogenous plant hormones within the tissues of cv. Giza 2. This would enable such cultivar to promote its cell division machinery and operating the mechanism of gene regulation efficiently resulting in production of certain types of functional proteins having the capability to defeat the inimical effects of salinity (Fu-Ping *et al*., 2010).

SUMMARY

The present work was carried out to examine the effect of exogenous application of stigmasterol on mitotic cell division; some growth parameters and protein banding pattern using two salt stressed *Lupinus termis* cultivars (cv. Giza 1 and Giza 2). On the other hand, the mutagenic and the antimutagenic effect of stigmasterol were studied using *Allium cepa* assay. The results of germination percentage revealed that cv. Giza 2 had higher response to the interaction between salinity and stigmasterol than cv. Giza 1. Also, highly significant inhibition in growth parameters (shoot and root growth) by increasing the salinity stress in Giza 1 than Giza 2 and this inhibition was significantly alleviated with stigmasterol treatment. Salinity induced a considerable variation in the protein patterns among these cultivars. These changes have been appeared in the novel expression of some polypeptides, the absence of the other and the over expression of a third class of pol-

ypeptides. Treatment with sodium chloride (100 and 200 Mm) has mitoclassic impact on cell division. Few types of mitotic abnormalities were induced in different treatments. Stigmasterol treatments were minimize the inhibition effect of NaCl and showed a considerable increase in the mitotic index. This study detected that stigmasterol has antimutagenic effect against sodium chloride that induced chromosomal aberrations in *Allium cepa* root meristematic cells.

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Table (1): Percentage of mitotic index, metaphase and ana-telophase abnormalities and different types of mitotic abnormalities after treating *Allium cepa* root tips with sodium chloride with or without stigmasterol for 24 h.

Treatment mM NaCl /µM stigmasterol.	(%) of Mitotic index	% of different types of Metaphase abnormalities				% of different types of Ana-telophase abnormalities				% of total abnormalities
		Stic.	C-M	Lag.	Dist.	Stic.	Brid.	Lag.	Dist.	
200/0 NaCl	0.21 ± 0.25**	84.62	0.00	0.00	15.38	72.73	27.27	0.00	0.00	96.00 ± 0.23**
200/500 NaCl + stigmasterol	4.01 ± 0.54*	27.52	36.70	2.75	9.17	13.70	0.00	27.39	36.30	48.50 ± 0.62*
100/0 NaCl	5.88 ± 0.23*	33.94	9.78	0.00	9.78	13.24	2.21	0.00	11.03	24.48 ± 0.56**
100/500 NaCl + stigmasterol	6.91 ± 0.21*	14.29	7.14	3.57	8.57	3.59	5.13	10.28	2.56	15.03 ± 0.45*
0/500 stigmasterol	10.84 ± 0.62*	3.08	3.08	3.08	4.62	2.76	1.38	2.76	2.76	6.67 ±0.21**
0/0 (H$_2$O) control	7.08 ± 0.27	0.00	0.00	0.00	1.25	0.00	0.00	1.09	0.00	0.62 ± 0.87

* = Significant change   ** = Highly significant change

Table (2): Changes in some growth parameters after treating two *Lupinus* - cultivars with NaCl with or without stigmasterol treatmrnt.

Treatment mM NaCl /μM stigmasterol.	Germination (%)		Shoot Growth (%)		Root Growth (%)	
	Giza 1	Giza 2	Giza 1	Giza 2	Giza 1	Giza 2
200/0 NaCl	63.32 ± 0.43**	76.67 ± 0.27**	7.35 ± 0.23**	7.84 ± 0.6 3**	2.05 ± 0.36**	2.66 ± 0.21**
200/500 NaCl + stigmasterol	80.32 ± 0.58**	83.33 ± 0.47*	10.62 ± 0.91*	11.62 ± 0.12*	2.68 ± 0.56*	3.12 ± 0.21*
100/0 NaCl	76.33 ± 0.25**	93.34 ± 0.34*	9.01 ± 0.42**	11.01 ± 0.1 2*	2.67 ± 0.34*	4.17 ± 0.21
100/500 NaCl + stigmasterol	86.66 ± 0.47*	95.00 ± 0.17*	12.52 ± 0.84*	15.02 ± 0.68*	3.18 ± 0.82*	4.11 ± 0.21*
0/500 stigmasterol	100.00 ± 0.56	100.00 ± 0.12	15.53 ± 0.62	16.53 ± 0.57	4.30 ± 0.68	5.01 ± 0.21
0/0 (H_2O) control	100	100	11.5 ± 0.36	13.00 ± 0.35	4.14 ± 0.21	4.51 ± 0.21

* = Significant change   ** = Highly significant change

Table (3): SDS-PAGE analysis of protein patterns of *Lupinus termis* cv. Giza 2 (A) and Giza 1 (B) after treatment with different concentrations of NaCl/stigmasterol.

Band no.	MW KDa	cv. Giza 2						cv. Giza 1					
		L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12
1	230	1	0	1	0	1	1	1	1	1	1	1	1
4	108	1	1	1	1	1	1	1	1	1	1	1	1
5	96	1	0	0	1	1	0	0	0	0	1	0	1
6	85	1	1	1	1	1	1	1	1	0	0	1	1
7	80	0	0	0	0	0	1	1	1	1	1	1	0
8	58	1	1	1	1	1	1	1	1	1	0	0	1
9	48	1	0	0	1	0	1	1	0	0	1	1	1
10	46	0	1	1	1	1	1	1	1	1	1	1	1
11	38	0	0	0	1	1	0	1	0	1	1	1	1
12	34	1	1	1	1	1	1	1	1	1	0	1	0
13	30	1	1	1	1	1	1	0	0	1	0	0	0
15	17	0	1	1	1	1	1	0	1	1	1	1	0
16	16	0	1	1	0	1	0	0	0	1	0	0	0
Total No=16		8	8	9	10	10	10	9	8	10	8	9	8
a		-	3	3	3	4	3	-	1	3	2	1	1
b		-	3	2	1	1	1	-	2	2	3	1	2

a: indicates appearance of new bands and b: disappearance of normal bands.
Lane 1, 7: Control (Giza 1, 2)   Lane 2, 8: 100 mM NaCl (Giza 1, 2)
Lane 3, 9: 200 mM NaCl (Giza 1, 2)   Lane 4, 10: 500 μM stigmasterol (Giza 1, 2)
Lane 5, 11: 100 mM NaCl + 500 μM stigmasterol (Giza 1, 2)
Lane 6, 12: 200 mM NaCl + 500 μM stigmasterol (Giza 1, 2)
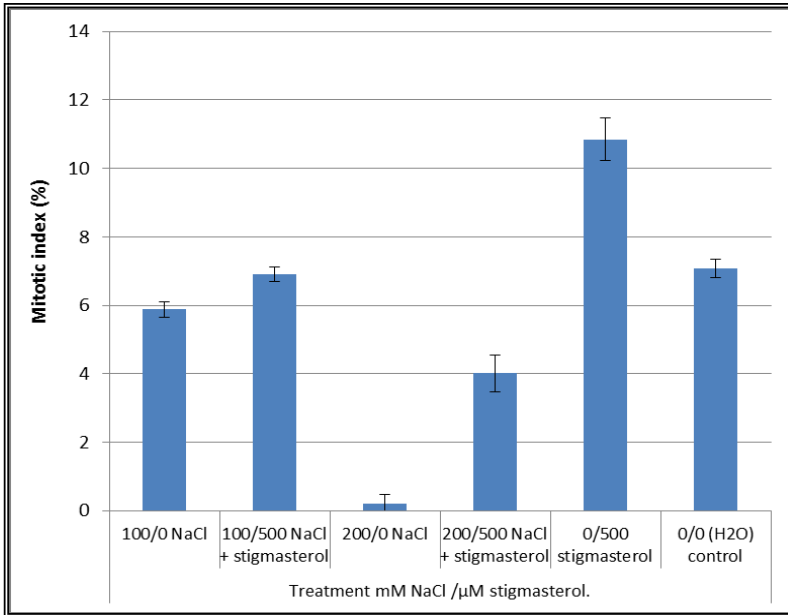
Fig. (1-a): Changes in the mitotic index (MI) after treating *Allium cepa*
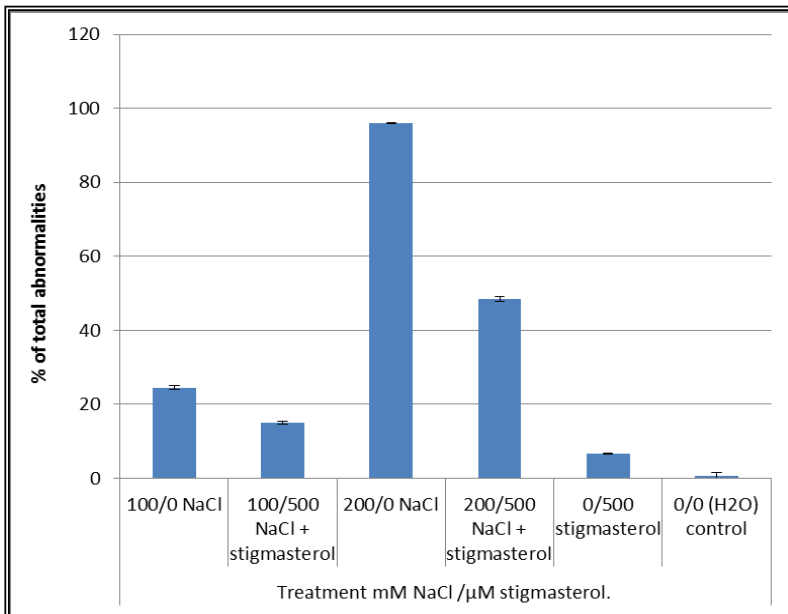root tips with sodium chloride/stigmasterol.

Fig. (1-b): Frequency of total mitotic abnormalities after treating *Allium
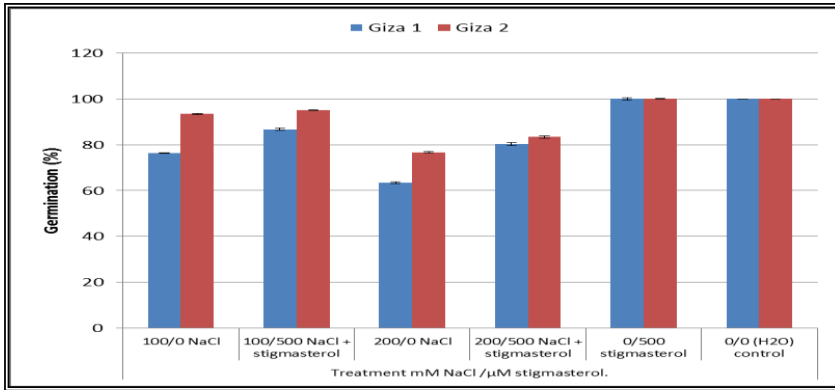cepa* root tips with sodium chloride/stigmasterol.

Fig. (2-a): Changes of germination percentage in response to stigmasterol treatment of *Lupinus termis* (cv. Giza 1 and cv. Giza 2) plants subjected to salt stress.
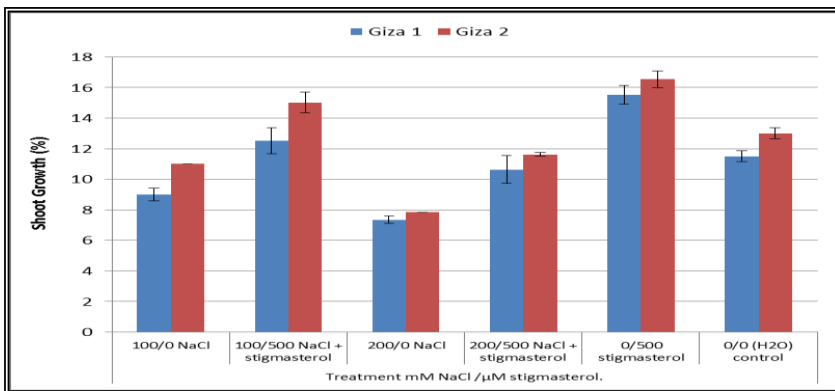

Fig. (2-b): Changes of shoot length in response to stigmasterol treatment of *Lupinus termis* (cv. Giza 1 and cv. Giza 2) plants subjected to salt stress.
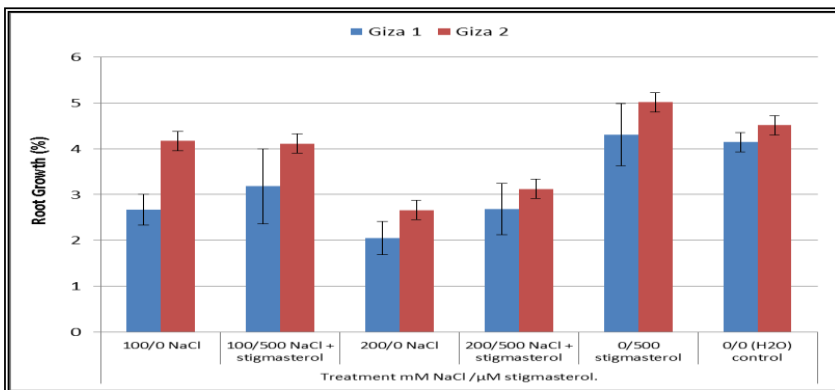

Fig. (2-c): Changes of root length in response to stigmasterol treatment of *Lupinus termis* (cv. Giza 1 and Giza 2) plants subjected to salt stress.
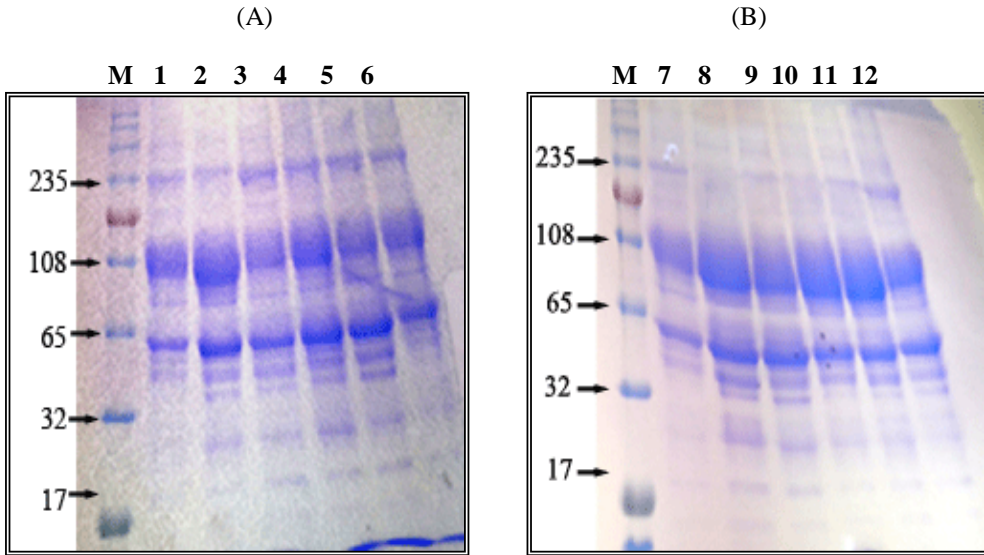
(A)   (B)

Fig. (3 A and B): SDS-PAGE analysis of protein patterns of *Lupinus termis* cv. Giza 1 (B) and Giza 2 (A): after treatment with different concentrations of NaCl/stigmasterol.

Lane 1, 7: Control (Giza 1, 2)   Lane 2, 8: 100 mM NaCl (Giza 1, 2)

Lane 3, 9: 200 mM NaCl (Giza 1, 2)   Lane 4, 10: 500 µM stigmasterol (Giza 1, 2)

Lane 5, 11: 100 mM NaCl + 500 µM stigmasterol (Giza 1, 2)

Lane 6, 12: 200 mM NaCl + 500 µM stigmasterol (Giza 1, 2)