

## Effects of NaCl on some physiological and biochemical responses in two cultivars of *L. usitatissimum*

Leila Amraee<sup>1,2</sup>, Fatemeh Rahmani<sup>1,2\*</sup>, Babak Abdollahi Mandoulakani<sup>3</sup>

<sup>1</sup> Department of Biology, Faculty of Sciences, Urmia University, Urmia, Iran

<sup>2</sup> Institute of Biotechnology, Urmia University, Urmia, Iran

<sup>3</sup> Department of Plant Breeding and Biotechnology, Faculty of Agriculture, Urmia University, Urmia, Iran

(Received: 1/07/2018-Accepted: 27/11/2018)

### Abstract

*Linum usitatissimum* L. is one of the economically important oilseed crops and salt stress is one of the major environmental challenges affecting several physiological processes in plants. The present investigation was carried out to study the effects of NaCl on some physiological and biochemical responses in two cultivars of *L. usitatissimum*. Three week-old plants were exposed to salt stress (0 and 150 mM) for 21 days. Results showed that salinity enhanced proline and flavonoid contents and total antioxidant capacity in flax cultivars. Exposure to NaCl (150 mM) did not alter levels of oxidative stress parameters, such as Malondialdehyde (MDA) and other aldehydes, content of phenol and POD enzyme activity in TN-97-28 cultivar while increased these parameters in TN-97-106 cultivar. On the other hand, there was no change in the soluble protein and K<sup>+</sup> contents in TN-97-28 cultivar in response to salt stress whereas these contents decreased in TN-97-106 cultivar under salinity. Contents of Na<sup>+</sup> and Cl<sup>-</sup> were increased in NaCl-treated flax plants. Antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) were declined and increased in TN-97-28 and TN-97-106 cultivars, respectively. It might be concluded that various cultivars have different mechanisms to diminish negative impacts of salinity stress.

**Keywords:** Antioxidant enzymes, Flax, Lipid, NaCl, Proline, Protein

### Introduction

Flax plant (*Linum usitatissimum* L.), a cool temperate annual herb with erect stems, is one of the economically important oilseed crops. The increasing demand for flax is mostly due to the high amount of  $\alpha$ -linolenic rich oil, protein, lignans and fiber (El-Bassiouny and Sadak, 2015). Moreover, utilization of the flax oil or seeds for cooking has been reported to have beneficial effects on cardiovascular health and in the treatment of neurological and hormonal disorders, inflammatory diseases and certain cancers (Patil *et al.*, 2015).

One of the major challenges in plant physiological studies is to increase the plant fertility under harmful environmental conditions (Yu *et al.*, 2014; Patil *et al.*, 2015). Salinity is a major problem which limits agricultural production in arid and semiarid regions and leads to delayed germination and emergence, low seedling survival, irregular crop stands and lower yield due to abnormal morphological, physiological and biochemical changes (El-Bassiouny and Sadak, 2015). Based on the soil map of the world, 831 million hectares are affected by saline-alkaline stress. Of these, the area of saline soils was 397 million hectares in comparison

to 434 million hectares of alkaline soils (Yu *et al.*, 2014). Based on recent investigation, Iran has 6.8 million hectares of saline lands after India and Pakistan (Ranjbar and Pirasteh-Anosheh, 2015).

Salinity stress, similar to many abiotic stress factors, stimulates the generation of reactive oxygen species (ROS), such as a singlet oxygen, superoxide anion, hydrogen peroxide and hydroxyl radical. Salt and osmotic stresses produce ROS that can cause oxidative stress in plants leading to membranes damages and other essential macromolecules such as photosynthetic pigments, proteins, DNA and lipids (El-Bassiouny and Sadak, 2015; Ahmad *et al.*, 2016). Antioxidant defense system is one of the defense mechanisms against free radical induced by oxidative stress. Superoxide dismutase, catalase and ascorbate peroxidase are enzymatic antioxidants which are induced and scavenge reaction oxygen species (Parvaiz and Satyawati, 2008; Razavizadeh and Rostami, 2013). On the other hand, secondary metabolites such as phenolic and flavonoid compounds are accumulated in the plants under salt stress to alleviate the salinity-induced oxidative stress. Phenolic compounds have different biological activities,

\*Corresponding Author, Email: f.rahmani@urmia.ac.ir

they are well-known as powerful radical scavengers and ions chelators, and are crucial to attract pollinators through flower pigmentation, plant growth and reproduction (Minh *et al.*, 2016; Bettaieb *et al.*, 2017).

In most plants, accumulation of osmolytes, such as proline, is an efficient strategy in response to salt stress. The type and number of proline accumulated, differ among different plant species. Proline accumulation might be used as a selection index of salt tolerant varieties by means of interfering in osmotic adjustment (Ahmad *et al.*, 2016). In addition to osmotic role, proline has been shown to stabilize proteins and membranes, protects plants against free radical-induced damages, maintains appropriate NADP<sup>+</sup>/NADPH ratios (Matysik *et al.*, 2002). Proline considered as a carbon and nitrogen storage component, buffer of cytosolic pH, cell redox balance status and finally, signal of stress adaptive responses (Jaarsma *et al.*, 2013; Tavakoli *et al.*, 2016).

In various plant species, peroxidation of membrane lipids by ROS is an indication of membrane damage and leakage under salt stress conditions (Bazrafshan and Ehsanzadeh, 2016). Malondialdehyde (MDA) content, a product of lipid peroxidation, is considered as the general indicator of oxidative damage and cell membrane stability which has been widely considered to differentiate salt-tolerant and -sensitive cultivars (De Azevedo Neto *et al.*, 2006). It appears that little information is available regarding the effect of salinity stress on growth, physiology and biochemistry of different flax cultivars (Patil *et al.*, 2015).

The objective of this study was to quantify the responses of two *L. usitatissimum* cultivars to salinity. The effects of salt stress on the soluble protein, proline and total phenolic and flavonoid contents, antioxidant enzymes activities, total antioxidant capacity, ion homeostasis and membrane lipid peroxidation were evaluated in two *L. usitatissimum* cultivars in order to better understand the differences for response to salt stress.

## Materials and methods

**Plant materials:** The seeds of two *L. usitatissimum* cultivars, TN-97-28 (Var. *usitatissimum*, Zabol) and TN-97-106 (Var. *humil*, Meshgin Shahr, Ardabil) were obtained from Agricultural Research Center of West Azerbaijan Province, Urmia, Iran. TN-97-28 and TN-97-106 cultivars have been identified as moderate and high cold tolerant varieties, respectively (Ghoreishi *et al.*, 2017). The experiment was conducted as a completely randomized design in a growth chamber at Institute of Biotechnology of Urmia University. The surface of the seeds was sterilized in 75% ethanol for 2 mins. and 50% sodium hypochlorite for 5-10 mins., and washed with sterile distilled water 4-6 times. After germination in petri dish, seedlings were cultivated in 15 cm × 15 cm pots in peat moss and perlite 3-1 (v/v). The growth chamber was set at 250 μmol m<sup>-2</sup> S<sup>-1</sup> light intensity, photoperiod of 16 hrs. light and 8 hrs. darkness and temperature of 23 ± 1°C with 80–90 %

relative humidity. After three weeks, flax plant were divided into two groups and subjected to two salinity levels for 21 days: 1- plants watered with NaCl (0 mM), 2- plants supplied with NaCl (150 mM). At the end of treatment, electrical conductivity (EC) of 1.0 and 17 (dS m<sup>-1</sup>) was calculated for drainage water of pots treated with NaCl (0 mM) and (150 mM), respectively. The experiment was performed in triplicate and each replicate contained 15 seedlings. At the end of the experiment, samples were collected and frozen in liquid nitrogen and then transferred to -80°C until analyses.

### Protein content and antioxidant enzymes activity:

Enzyme extractions were carried out at 4°C according to Azarmehr *et al.* (2013) method. Plant tissues were frozen in liquid nitrogen and ground with an ice-cold pestle and mortar, and then extracted in 50 mM buffer (Tris-HCl, pH 7.5). The homogenate was centrifuged two times at 12000 rpm at 4°C for 20 mins., and at 9000 RPM at 4°C for 15 mins. The supernatant was collected and used for protein and enzyme assays. Total soluble proteins were determined in triplicate for each sample, and then the concentration of the proteins was calculated by BSA standard curve (Azarmehr *et al.*, 2013).

SOD activity was measured according to the protocol of Azarmehr *et al.* (2013) at 560 nm. one unit of SOD was defined as the amount of enzyme that produced 50% inhibition of NBT reduction, and SOD specific activity was expressed as units mg<sup>-1</sup> protein (Azarmehr *et al.*, 2013).

The reaction mixture for POD enzyme activity contained 50 mM phosphate buffer (pH 6.6), guaiacol (1%), H<sub>2</sub>O<sub>2</sub> (0.3%) and enzyme extract in a total volume of 1 mL. Guaiacol as the substrate and increase in the absorbance, due to the oxidation of guaiacol, was measured at 470 nm for 1min (Altın *et al.*, 2017).

CAT activity was determined by measuring the consumption of H<sub>2</sub>O<sub>2</sub> at 240 nm for 1 min. A reaction mixture (Azarmehr *et al.*, 2013) and 100 μl of enzyme extract was used.

The activity of APOX was determined by following the decrease of ascorbate and measuring the change in absorbance at 290 nm for 1 min based on Karimi and Khataee (2012) method. The decrease in ascorbate concentration was followed as a decline in the optical density at 290 nm (Karimi and Khataee, 2012).

### Phenolic content and total antioxidant capacity:

The total phenol content was determined according to Heimler *et al.* (2009) method with 70% ethanol overnight extract (Heimler *et al.*, 2009). The extracts were used for determination of total phenol and flavonoid. Total phenol contents were determined by spectrophotometer (Bechman DU530) at 760 nm, using the folin–Ciocalteu reagent (Lippi *et al.*, 2011) and gallic acid was used to develop standard curves. Total flavonoid contents were measured according to Beketov *et al.* (2005) method at 414 nm using quercetin calibration curve (Beketov *et al.*, 2005).

DPPH and FRAP methods were used, in order to

evaluate the antioxidant activity of the samples. FRAP assay is a non-specific method and rely on the reduction of TPTZ (2,4,6-tri-pyridyl-s-triazine)-Fe<sup>3+</sup> complex to TPTZ-Fe<sup>2+</sup> form, with total reducing power of all the antioxidant substances found in the test solution. One-tenth mL of sample was mixed with 2.9 mL FRAP reagent and the absorbance was measured at 593 nm after the mixture was held at 37°C for 10 mins. (Li *et al.*, 2014).

The antioxidant capacity of flax leaf methanol extracts was determined by DPPH radical scavenging assay described by Hatamnia *et al.* (2014). Absorbance of the solution at 515 nm was measured. The test was carried out in triplicate and the radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration by following formula:

$$\text{RSA}\% = (A_0 - A_1) / A_0 * 100$$

Where A<sub>0</sub> is the absorbance of control reaction (containing all reagents except the extract) and A<sub>1</sub> is the absorbance of solution when the sample extract has been added.

**Proline content:** Proline content in the stem tissues was extracted and analyzed according to the method described by Cha-Um and Kirdmanee (2009). Fifty-milligram fresh stem materials were ground in a mortar with liquid nitrogen. The homogenate powder was mixed with 1 mL aqueous sulfosalicylic acid (3 % w/v). After centrifugation, supernatant was reacted with an equal volume of glacial acetic acid and ninhydrin reagent (1.25 mg ninhydrin in 30 mL of glacial acetic acid and 20 ml 6 M H<sub>3</sub>PO<sub>4</sub>) and incubated at 95°C for 1 hrs. The reaction was terminated placing in an ice bath. The reaction mixture was vigorously mixed with 2 mL toluene. After warming at 25°C, the chromophore was measured at 520 nm. L-proline was used as a standard. The method was calibrated for each determination with standard proline solutions within the detection range of the method (0-10 µg. ml<sup>-1</sup>) (Cha-Um and Kirdmanee, 2009).

**Ion contents:** For measurement of ion concentration, 50 mg of the powdered dry matter of the aerial organ was boiled in 5 mL of distilled water for one hour at 100°C using ben murray. This hot water extract was cooled and filtered using filter paper. filtered extracts were used to measure the amount of Na<sup>+</sup> and K<sup>+</sup> contents by flame photometer (Fater electronic 405, Iran). 0.5 mL aliquots were analyzed to measure tissue Cl<sup>-</sup> concentration using chloride analyzer (Model 926, Sherwood scientific, UK) (Hatamnia *et al.*, 2013).

**Lipid peroxidation:** Lipid peroxidation was determined in terms of MDA content in 0.05 g of leaf fresh weight using the thiobarbituric acid (TBA) method described by Meir *et al.* (1992) employing slight modifications. The absorbance of the colored reaction product was determined at 455 nm for aldehydes (which are products of lipid peroxidation) and at 532 nm for MDA. The measurements were corrected for non-specific turbidity by subtracting the absorbance at 600 nm. For calculations, an extinction coefficient of 0.457

× 10<sup>5</sup> Mcm<sup>-1</sup> was used at 455 nm for five aldehydes (1-propanal, 1-butanal, 1-hexanal, 1-heptanal, and 1-propanal-dimethylacetal), and the MDA content was calculated using an extinction coefficient of 1.56 × 10<sup>5</sup> Mcm<sup>-1</sup> at 532 nm (Meir *et al.*, 1992).

**Statistical analysis:** All parameters assayed, were measured in triplicate. The experimental design was completely randomized factorial and data subjected to analysis of variance using SAS 9.2. The mean separations were performed by Duncan's multiple range tests with the same software. Significance was determined at P ≤ 0.01 and the results were expressed as mean values and standard error (SE) of the means.

## Results

### Protein content and antioxidant enzymes activity:

The level of protein in TN-97-106 cultivar was recorded higher than TN-97-28 cultivar under NaCl (0 mM). However, NaCl (150 mM) had no significant impact on content of protein in TN-97-28 cultivar while decreasing it (41%) in TN-97-106 cultivar (Table 1). In TN-97-28 cultivar, the activity of POD enzyme did not change in response to salinity. On the other hand, TN-97-106 cultivar showed induction in POD enzyme activity (2.6 fold) under 150 mM NaCl (Table 1).

Imposing three week old flax plants to 150 mM NaCl reduced SOD enzyme activity (13%) in TN-97-28 cultivar, whereas increased it (34%) in TN-97-106 cultivar. The CAT enzyme activity decreased (25%) in TN-97-28 cultivar whereas increased (3.2 fold) in TN-97-106 cultivar under salinity. Activity of APOX enzyme did not show any significant changes under salt stress in both cultivars (Table 1).

### Phenolic and flavonoid contents and total antioxidant capacity:

Based on our results, TN-97-28 and TN-97-106 cultivars responded differently for phenol content under NaCl (150 mM) (Table 2). No significant change was observed in TN-97-28 cultivar while salinity stress increased phenol content (94%) in TN-97-106 cultivar. The content of flavonoid was significantly increased in TN-97-28 (29%) and in TN-97-106 (43%) cultivars in response to salt stress (Table 2).

The results showed that NaCl (150 mM) caused an increase in total antioxidant capacity in flax cultivars (Table 2). Based on the results of FRAP assay, total antioxidant capacity was increased in TN-97-106 cultivar (43%) but no significant change was detected in TN-97-28 cultivar in response to salinity (Table 2). On the other hand, based on DPPH method, total antioxidant content was induced in NaCl treated TN-97-28 (3 fold) and TN-97-106 (5 fold) cultivars.

**Proline content:** The results showed that proline content was significantly increased under NaCl (150 mM) treatment compared to the control groups with 10 and 26-fold induction in TN-97-28 and TN-97-106 cultivars, respectively (Table 2).

**Ion contents:** Application of 150 mM NaCl significantly elevated the content of Na<sup>+</sup> (4.5 and 6 fold) and Cl<sup>-</sup> (2.7 and 2.8 fold) in TN-97-28 and TN-97-106

**Table 1. Soluble protein content and enzymes activity (peroxidase (POD), superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX)) in TN-97-28 and TN-97-106 *Linum usitatissimum* L cultivars under 0 and 150 mM NaCl.**

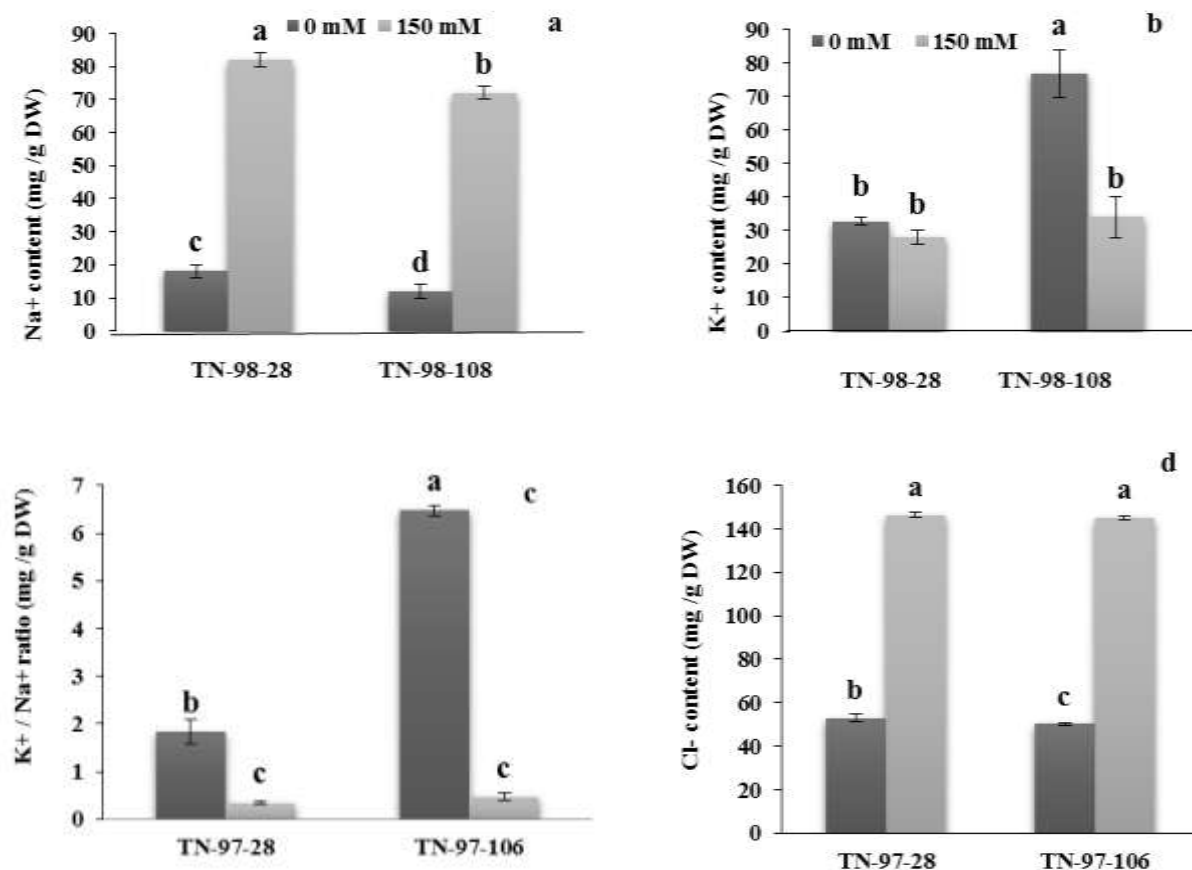
Cultivars	NaCl (mM)	Soluble protein content (mg/g fw)	POD activity ( $\mu\text{mol}/\text{mg pr min}$ )	SOD activity (U/mg pr)	CAT activity ( $\mu\text{mol}/\text{mg pr min}$ )	APOX activity ( $\mu\text{mol}/\text{mg pr min}$ )
TN-97-28	0 mM	7.57 $\pm$ 0.11 <sup>b</sup>	8.70 $\pm$ 1.99 <sup>ab</sup>	27.9 $\pm$ 1.30 <sup>a</sup>	13.5 $\pm$ 0.47 <sup>a</sup>	13.6 $\pm$ 1.88 <sup>ab</sup>
	150 mM	8.76 $\pm$ 0.27 <sup>b</sup>	11.42 $\pm$ 0.63 <sup>a</sup>	24.1 $\pm$ 0.96 <sup>b</sup>	10.1 $\pm$ 0.43 <sup>b</sup>	10.7 $\pm$ 1.69 <sup>b</sup>
TN-97-106	0 mM	12.6 $\pm$ 1.47 <sup>a</sup>	4.46 $\pm$ 0.43 <sup>b</sup>	16.8 $\pm$ 2.02 <sup>c</sup>	2.86 $\pm$ 0.73 <sup>c</sup>	14.9 $\pm$ 1.67 <sup>ab</sup>
	150 mM	7.40 $\pm$ 1.42 <sup>b</sup>	11.8 $\pm$ 3.99 <sup>a</sup>	22.7 $\pm$ 1.39 <sup>b</sup>	9.20 $\pm$ 1.57 <sup>b</sup>	22.8 $\pm$ 7.02 <sup>a</sup>

Means followed by the different letter are significantly different according to Duncan's multiple range test ( $P \leq 0.01$ ).

**Table 2. Phenol and flavonoid contents, FRAP assay and DPPH radical scavenging (%) in TN-97-28 and TN-97-106 *Linum usitatissimum* L cultivars under 0 and 150 mM NaCl.**

Cultivars	NaCl (mM)	Phenolic contents (mg/g fw)	Flavonoid contents (mg/g fw)	FRAP assay ( $\mu\text{g}/\text{g fw}$ )	DPPH radical scavenging (%)	Proline content ( $\mu\text{mol}/\text{g FW}$ )
TN-97-28	0 mM	9.3 $\pm$ 0.43 <sup>b</sup>	2.8 $\pm$ 0.02 <sup>c</sup>	15.4 $\pm$ 0.76 <sup>b</sup>	2.7 $\pm$ 0.93 <sup>c</sup>	0.62 $\pm$ 0.10 <sup>c</sup>
	150 mM	9.8 $\pm$ 0.61 <sup>b</sup>	3.6 $\pm$ 0.13 <sup>b</sup>	16 $\pm$ 0.11 <sup>b</sup>	8.7 $\pm$ 1.15 <sup>b</sup>	6.3 $\pm$ 0.49 <sup>b</sup>
TN-97-106	0 mM	7.1 $\pm$ 0.63 <sup>c</sup>	2.9 $\pm$ 0.23 <sup>c</sup>	13.9 $\pm$ 0.46 <sup>b</sup>	2.5 $\pm$ 1.03 <sup>c</sup>	0.51 $\pm$ 0.12 <sup>c</sup>
	150 mM	13.8 $\pm$ 0.31 <sup>a</sup>	4.2 $\pm$ 0.10 <sup>a</sup>	20 $\pm$ 1.00 <sup>a</sup>	13.7 $\pm$ 0.06 <sup>a</sup>	13.4 $\pm$ 1.93 <sup>a</sup>

Means followed by the different letter are significantly different according to Duncan's multiple range test ( $P \leq 0.01$ ).



**Fig. 1. Ion contents in TN-97-28 and TN-97-106 *Linum usitatissimum* L cultivars under 0 and 150 mM NaCl: Na<sup>+</sup> content (a) K<sup>+</sup> content (b) K<sup>+</sup> / Na<sup>+</sup> ratio (c) and Cl<sup>-</sup> content (d). Means followed by the different letter are significantly different according to Duncan's multiple range test ( $P \leq 0.01$ ).**

cultivars, respectively. In response to NaCl (150 mM), the content of K<sup>+</sup> decreased in TN-97-106 cultivar (55%), but no significant change was detected in TN-

97-28 cultivar (Fig. 1). The K<sup>+</sup> / Na<sup>+</sup> ratio was significantly reduced in TN-97-28 (81%) and TN-97-106 (92%) cultivars under imposition of NaCl (150mM)

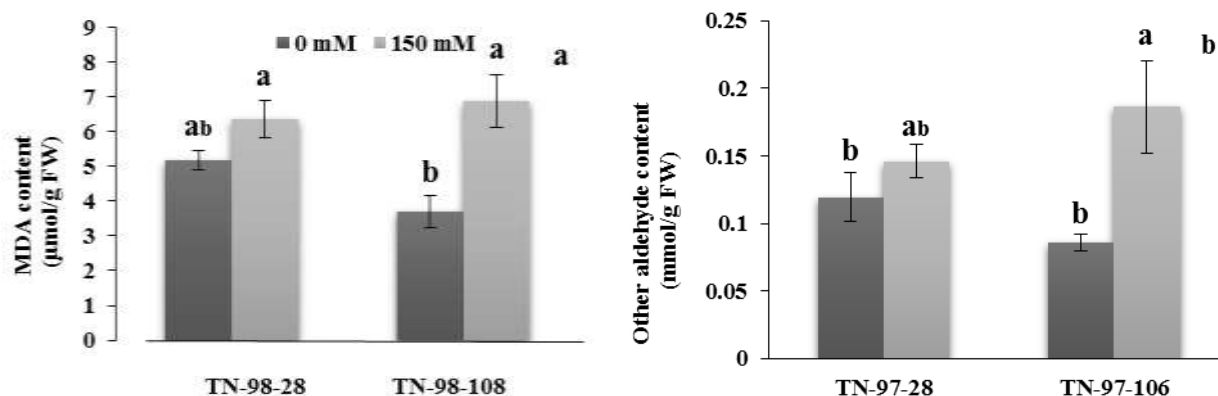


Fig. 2. Lipid peroxidation in TN-97-28 and TN-97-106 *Linum usitatissimum* L cultivars under 0 and 150 mM NaCl: Malondialdehyde (MDA) content (a) and other aldehydes content (b). Means followed by the different letter are significantly different according to Duncan's multiple range test ( $P \leq 0.01$ ).

(Fig. 1).

**Lipid peroxidation:** MDA and other aldehydes levels did not show any significant change in TN-97-28 cultivar under salt treatment compared to control groups (Fig. 2). Contents of MDA and other aldehydes showed 85% and 2.16-fold induction in TN-97-106 cultivar under salinity (Fig. 2).

## Discussion

The pattern of soluble protein changes was evident among *L. usitatissimum* cultivars in reaction to NaCl treatment (Table 1). In our study, TN-97-28 cultivar showed no significant change in soluble protein in response to NaCl treatment while the content of soluble protein significantly decreased in TN-97-106 cultivar. The present result was in accordance with Dasgupta *et al.* (2010), who experimentally reported that the total protein content decreased in five mangroves under salinity. Protein pattern has a dual reaction under salinity in plants. Salt stress reduces the total protein content, and also commences the synthesis of other specific proteins necessary for tolerating the effect of salinity through engaging ABA (Arefian *et al.*, 2014). The degradation of protein content in saline environment might be due to the enhanced activity of protease. Degraded proteins may supply storage form of nitrogen which plays a vital role in osmotic adjustment under stress (Dasgupta *et al.*, 2010; Jamil and Rha, 2013).

Under salinity stress, formation and accumulation of reactive oxygen species can be induced and its over accumulation results in oxidative damage of membrane lipids, proteins and nucleic acids (Abdelgawad *et al.*, 2016). In higher plants, enzymatic and non-enzymatic antioxidant defense systems participate in elimination of ROSs (Razavizadeh and Rostami, 2013). Antioxidant enzymes such as CAT, SOD and POD are usually considered as the key enzymes of antioxidant defense of the plants (Zhang *et al.*, 2013). SOD is the major scavenger of superoxide ( $O_2^-$ ) to form  $H_2O_2$  and  $O_2$ , and plays an important role in defense activity against the cellular damage caused by environmental stress.

CAT is common enzyme found in nearly all living organisms exposed to oxygen, catalyzing the dismutation of hydrogen peroxide into oxygen and water, and is known as a mediator of oxidative damage (Giannakoula *et al.*, 2010). APOX is considered to be a key antioxidant enzyme in plants (Kachout *et al.*, 2013). Under our investigation, differences in protective enzyme activities were known for two studied flaxseed cultivars (Table 1). A large number of studies on various species have indicated that salt stress alters the amount and the activity of the enzymes involved in ROS scavenging (Meratan *et al.*, 2008). In agreement with our findings for TN-97-28, SOD activity decreased in two corn cultivars (Carrasco-Rios and Pinto, 2014). Our results for TN-97-28 cultivar also in accordance with increase of SOD activity in cotton plants (Kachout *et al.*, 2013) under salinity. In the present study, reduction in CAT activity was observed in TN-97-28 cultivar, while the TN-97-106 cultivar showed enhancement of CAT activity under salinity. Previous study has shown that salt preferentially decreases the CAT in rice leaf (Lee *et al.*, 2001) and cotton plants (Kachout *et al.*, 2013). Abdelgawad *et al.* (2016) showed that the activity of CAT enzyme significantly increased in roots and shoots of maize seedlings in response to NaCl (150 mM). Similar to our result for TN-97-28 cultivar, the activity of POD enzyme did not show any change under NaCl (75 and 150 mM) (Abdelgawad *et al.*, 2016). On the other hand, activity of POD enzyme was significantly elevated in TN-97-106 cultivar which was in parallel with Weisany *et al.* (2012) result. The results have indicated that activity of APOX enzyme did not change under NaCl (150 mM) in two flax cultivars. Previous studies indicated different records of APOX enzyme activity in various species. Decline in APOX enzyme activity has been reported in cotton plant under salt stress (Kachout *et al.*, 2013), Whereas Weisany *et al.* (2012) indicated elevation of APOX enzyme activity in *Glycine max* L. plant as a result of salinity imposition. In agreement with our findings, NaCl treatment did not display any significant effect on APOX enzyme activity in canola plants

(Razavizadeh and Rostami, 2013). Meratan *et al.* (2008) reported that the diversity in number and intensities of antioxidative enzymes isoforms could be involved in salinity tolerance of the species (Meratan *et al.*, 2008). Perhaps, TN-97-28 and TN-97-106 cultivars have different isoforms of antioxidative enzymes and various responses to salt stress.

In order to counter the damaging effects of ROS, plants produce a variety of antioxidant metabolites, including phenol and flavonoid compounds. Phenolic molecules are constitutive compounds in all higher plants with free radical quenching activity due to their redox properties along with metal chelation, hydrogen donation and singlet oxygen quenching (Taarit *et al.*, 2012; Abideen *et al.*, 2015). Moreover, vast evidence showed that flavonoids in higher plants have the potential to serve as antioxidants in response to stressed environments including salinity (Zhou *et al.*, 2018). In the present study, total phenol did not change in TN-97-28 cultivar and increased in TN-97-106 cultivar in response to salt stress (Table 2). However, the content of flavonoid was elevated in both cultivars under salinity. The enhancement in the synthesis of flavonoids and phenolics of flaxseed plants varieties might be the adaptive mechanism under salt stress. In previous reports, induction of phenolic compounds was observed in different plants under salinity, including *Garden cress* (Ahmed *et al.*, 2012), *Salvia officinalis* (Taarit *et al.*, 2012) and *Olea europaea* L. (Petridis *et al.*, 2012) plants. However, the accumulation of phenolic compounds under salinity stress may vary in different plant species (Zhou *et al.*, 2018).

Antioxidant activity was determined by scavenging DPPH radical and FRAP assay. DPPH is a stable free organic radical that receive the hydrogen radical to become a constant diamagnetic molecule: yellow-colored diphenylpicrylhydrazine (Taarit *et al.*, 2012). The FRAP assay, a simple direct test of antioxidant capacity is the antioxidant ability to reduce a ferric complex to the ferrous form (Sharma *et al.*, 2014). The data indicated that salinity stimulated the increase of antioxidant capacity in both cultivars according to DPPH method (Table 2). Based on FRAP assay, total antioxidant capacity did not alter in response to salinity in TN-97-28 cultivar while was increased in TN-97-106 cultivar under salt stress (Table 2). These capacities could be directly related with the measures of phenolic compounds because of their free radical scavenging capacities (Table 2). The Significant correlation between FRAP and DPPH assays and total phenolic content was previously observed in three halophytes (*Salsola baryosma*, *Trianthema triquetra*, *Zygophyllum simplex*) under salinity (Sharma *et al.*, 2014). Moreover, total phenolic content was significantly correlated with antioxidant capacity in maize varieties (Hichem *et al.*, 2009) and some Chinese medicinal plants (Wong *et al.*, 2006).

Numerous studies have linked the accumulation of proline to salt stress as a particular osmolyte in plants

under reduced water levels which assists the plants to preserve cell turgor (Marin *et al.*, 2010). It is also hypothesized that proline, besides being an osmolyte, is also involved in scavenging free radicals and protects plant cell against adverse effect of salt by maintaining osmotic balance. In the present study, two *L. usitatissimum* cultivars showed increase in proline content in NaCl treated flaxseed compared to the control groups (Table 2). The highest accumulation was observed in TN-97-106 (25 fold) cultivar compared to TN-97-28 (10 fold) cultivar (Table 2). Similar results have been reported in rice and sorghum grown under salt stress (De Lacerda *et al.*, 2005; Demiral and Turkan, 2005). Although it is generally agreed upon that proline accumulation is important for plants to tolerate environmental stresses, a good correlation between proline accumulation and stress tolerance is not always obvious (Jaarsma *et al.*, 2013).

In this work, NaCl (150 mM) significantly altered shoot  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$  concentration (Fig. 1).  $\text{Na}^+$  and  $\text{Cl}^-$  contents sharply increased in the shoots of both flaxseed plants under high level of salinity. Similar results have been found in a number of previous studies as in *Suaeda fruticosa* L. (Khan *et al.*, 2000) and *Shepherdia argentea* (Qin *et al.*, 2010) plants. Salt stress significantly alters uptake and absorption rates of all mineral nutrients and could lead to problems of decline in leaf function and ionic imbalance and toxicity. Excessive  $\text{Na}^+$  accumulation can cause a range of osmotic and metabolic problems for plants. Potassium is essential plant macro-nutrient that has a major role in the regulation of water in plants (osmo-regulation), enzyme activity, cell expansion, neutralization of non-diffusible negatively charged ions and membrane polarization. The toxic effects of  $\text{Na}^+$  have been greatly related to its competition with  $\text{K}^+$  in order to bind to essential sites for cellular functions (Qin *et al.*, 2010).

Peroxidation of membrane lipids has been associated with cell damages caused by different biotic and abiotic stresses and MDA, a lipid peroxidation product, has been used as an appropriate biomarker to evaluate the level of free radicals in living cells (Arefian *et al.*, 2014; Bazrafshan and Ehsanzadeh, 2016). However, it is not clear whether the saturation of membrane lipids has an adaptive value, if there is a correlation between salt tolerance and a specific class of lipids, or if it is just a negative effect of salinity (Carrasco-Rios and Pinto, 2014). In the current study, MDA and other aldehyde contents were significantly accumulated in TN-97-106 cultivar under NaCl (150 mM) (Fig. 2). In agreement with our result, content of MDA increased in cotton plants (Zhang *et al.*, 2013) and also in two Turfgrass species (Xu *et al.*, 2013) under salinity.

## Conclusion

Unlike TN-97-106 cultivar, SOD enzyme activity was significantly decreased in NaCl treated TN-97-28 plants and consequently reduction in CAT enzyme activity was observed. Moreover, parameters such as proline,

phenol and flavonoid contents, and antioxidant capacity were significantly lower in TN-97-28 cultivar compared to TN-97-106 cultivar under salinity. However, the data presented above demonstrated better responses in protein content and ion homeostasis in TN-97-28 cultivar compared to TN-97-106 cultivar under salt stress. Finally, lipid peroxidation was significantly lower in TN-97-28 cultivar compared to TN-97-106

cultivar under salinity. In conclusion, probably these two cultivars have different mechanisms to counter with damaging effects of salinity.

#### Acknowledgment

This research was supported by Urmia University and Institute of Biotechnology of Urmia University.

#### References

- Abdelgawad, H., Zinta, G., Hegab, M. M., Pandey, R., Asard, H. and Abuelsoud, W. (2016) High salinity induces different oxidative stress and antioxidant responses in maize seedlings organs. *Frontiers in Plant Science* 7: 276.
- Abideen, Z., Qasim, M., Rasheed, A., Adnan, M. Y., Gul, B. and Khan, M. A. (2015) Antioxidant activity and polyphenolic content of *Phragmites karka* under saline conditions. *Pakistan Journal of Botany* 47: 813-818.
- Ahmad, P., Latef, A. A. A., Hashem, A., Abd\_Allah, E. F., Gucel, S. and Tran, L. S. P. (2016) Nitric oxide mitigates salt stress by regulating levels of osmolytes and antioxidant enzymes in chickpea. *Frontiers in Plant Science* 7: 347.
- Ahmed, A. R., Gabr, A. M. M., AL-Sayed, H. M. and Smetanska, I. (2012) Effect of drought and salinity stress on total phenolic, flavonoids and flavonols contents and antioxidant activity in in vitro sprout cultures of garden cress (*Lepidium sativum*). *Journal of Applied Sciences Research* 8: 3934-3942.
- Altın, S., Tohma, H., Gulcin, I. and Koksall, E. (2017) Purification, characterization, and inhibition sensitivity of peroxidase from wheat (*Triticum sativum* ssp. vulgare). *International Journal of Food Properties* 20: 1949-1959.
- Arefian, M., Vessal, S. and Bagheri, A. (2014) Biochemical changes in response to salinity in chickpea (*Cicer arietinum* L.) during early stages of seedling growth. *Journal of Animal and Plant Sciences* 24: 1849-1857.
- Azarmehr, B., Karimi, F., Taghizadeh, M. and Gargari, S. L. M. (2013) Secondary metabolite contents and antioxidant enzyme activities of *Cichorium intybus* hairy roots in response to zinc. *Journal of Medicinal Plants and By-Products* 2: 131-138.
- Bazrafshan, A. H. and Ehsanzadeh, P. (2016) Evidence for differential lipid peroxidation and antioxidant enzyme activities in *sesamum indicum* L. genotypes under nacl salinity. *Journal of Agricultural Science and Technology* 18: 207-222.
- Beketov, E. V., Pakhomov, V. P. and Nesterova, O. V. (2005) Improved method of flavonoid extraction from bird cherry fruits. *Pharmaceutical Chemistry Journal* 39: 316-318.
- Bettaieb, e. p., Rebey, I., Bourgou, S., Marzouk, B., Fauconnier, M. L. and Ksouri, R. (2017) Salinity impact on seed yield, polyphenols composition and antioxidant activity of Fennel (*Foeniculum vulgare* Mill) extracts. *Journal of New Sciences* 3: 2610-2619.
- Carrasco-Rios, L. and Pinto, M. (2014) Effect of salt stress on antioxidant enzymes and lipid peroxidation in leaves in two contrasting corn, 'Lluteno' and 'Jubilee'. *Chilean Journal of Agricultural Research* 74: 89-95.
- Cha-Um, S. and Kirdmanee, C. (2009) Effect of salt stress on proline accumulation, photosynthetic ability and growth characters in two maize cultivars. *Pakistan Journal of Botany* 41: 87-98.
- Dasgupta, N., Nandy, P., Tiwari, C. and Das, S. (2010) Salinity-imposed changes of some isozymes and total leaf protein expression in five mangroves from two different habitats. *Journal of Plant Interactions* 5: 211-221.
- De Azevedo Neto, A. D., Prisco, J. T., Eneas-Filho, J., De Abreu, C. E. B. and Gomes-Filho, E. (2006) Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. *Environmental and Experimental Botany* 56: 87-94.
- De Lacerda, C. F., Cambraia, J., Oliva, M. A. and Ruiz, H. A. (2005) Changes in growth and in solute concentrations in sorghum leaves and roots during salt stress recovery. *Environmental and Experimental Botany* 54: 69-76.
- Demiral, T and Turkan, I. (2005) Comparative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance. *Environmental and Experimental Botany* 53: 247-257.
- El-Bassiouny, H. and Sadak, M. S. (2015) Impact of foliar application of ascorbic acid and  $\alpha$ -tocopherol on antioxidant activity and some biochemical aspects of flax cultivars under salinity stress. *Acta Biologica Colombiana* 20: 209-222.
- Ghoreishi, M., Rahmani, F., Mandoulakani, B. A., and Gortapeh, A. H. (2017) Impact of variety on resistance to cold stress at physiological levels in *Linum usitatissimum*. *Plant Omics* 10: 269-276.
- Giannakoula, A., Moustakas, M., Syros, T. and Yupsanis, T. (2010) Aluminum stress induces up-regulation of an efficient antioxidant system in the Al-tolerant maize line but not in the Al-sensitive line. *Environmental and Experimental Botany* 67: 487-494.
- Hatamnia, A. A., Abbaspour, N. and Darvishzadeh, R. (2014) Antioxidant activity and phenolic profile of different parts of bene (*Pistacia atlantica* subsp. kurdica) fruits. *Food Chemistry* 145: 306-311.

- Hatamnia, A. A., Abbaspour, N., Darvishzadeh, R., Rahmani, F. and Heidari, R. (2013) Effect of salt stress on growth, ion content and photosynthesis of two oriental tobacco (*Nicotiana tabacum*) cultivars. *International Journal of Agriculture and Crop Sciences* 6: 757-761.
- Heimler, D., Isolani, L., Vignolini, P. and Romani, A. (2009) Polyphenol content and antiradical activity of *Cichorium intybus* L. from biodynamic and conventional farming. *Food Chemistry* 114: 765-770.
- Hichem, H., Mounir, D. and Naceur, E. (2009) Differential responses of two maizes (*Zea mays* L.) varieties to salt stress: Changes on polyphenols composition of foliage and oxidative damages. *Industrial Crops and Products* 30: 144-151.
- Jaarsma, R., De Vries, R. S. and De Boer, A. H. (2013) Effect of salt stress on growth, Na<sup>+</sup> accumulation and proline metabolism in potato (*Solanum tuberosum*) cultivars. *Plos One* 8: 60183.
- Jamil, M. and Rha, E. S. (2013) NaCl stress-induced reduction in growth, photosynthesis and protein in mustard. *Journal of Agricultural Science* 5: 114.
- Kachout, S. S., Hamza, K. J., Bouraoui, N. K., Leclerc, J. C. and Ouerghi, Z. (2013) Salt-induced changes in antioxidative enzyme activities in shoot tissues of two Atriplex varieties. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 41: 115-121.
- Karimi, F. and Khataee, E. (2012) Aluminum elicits tropane alkaloid production and antioxidant system activity in micropropagated *Datura innoxia* plantlets. *Acta Physiologiae Plantarum* 34: 1035-1041.
- Khan, M. A., Ungar, I. A. and Showalter, A. M. (2000) The effect of salinity on the growth, water status, and ion content of a leaf succulent perennial halophyte, *Suaeda fruticosa* (L.). *Forssk. Journal of Arid Environments* 45: 73-84.
- Lee, D. H., Kim, Y. S. and Lee, C. B. (2001) The inductive responses of the antioxidant enzymes by salt stress in the rice (*Oryza sativa* L.). *Journal of Plant Physiology* 158: 737-745.
- Li, D., Luo, Z., Mou, W., Wang, Y., Ying, T. and Mao, L. (2014) ABA and UV-C effects on quality, antioxidant capacity and anthocyanin contents of strawberry fruit (*Fragaria ananassa* Duch.). *Postharvest Biology and Technology* 90: 56-62.
- Lippi, G., Chance, J. J., Church, S., Dazzi, P., Fontana, R., Giavarina, D., Grankvist, K., Huisman, W., Kouri, T., Palicka, V. and Plebani, M. (2011) Preanalytical quality improvement: from dream to reality. *Clinical Chemistry and Laboratory Medicine* 49: 1113-1126.
- Marin Velazquez, J. A., Andreu Puyal, P., Carrasco A. and Arbeloa Matute, A. (2010) Determination of proline concentration, an abiotic stress marker, in root exudates of excised root cultures of fruit tree rootstocks under salt stress. *Revue des Regions Arides* 24: 722-727.
- Matysik, J., Alia Bhalu, B. and Mohanty, P. (2002) Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. *Current Science* 10: 525-32.
- Meir, S., Philosoph-Hadas, S. and Aharoni, N. (1992) Ethylene-increased accumulation of fluorescent lipid-peroxidation products detected during senescence of parsley by a newly developed method. *Journal of the American Society for Horticultural science* 117: 128-132.
- Meratan, A. A., Ghafari, S. M. and Niknam, V. (2008) Effects of salinity on growth, proteins and antioxidant enzymes in three Acanthophyllum species of different ploidy levels. *Journal of Agricultural Science and Technology* 33: 1-8
- Minh, L. T., Khang, D. T., Ha, P. T. T., Tuyen, P. T., Minh, T. N., Quan, N. V. and Xuan, T. D. (2016) Effects of salinity stress on growth and phenolics of rice (*Oryza sativa* L.). *International Letters of Natural Sciences* 57: 1-10.
- Parvaiz, A. and Satyawati, S. (2008) Salt stress and phyto-biochemical responses of plants-a review. *Plant Soil and Environment* 54: 89-99
- Patil, N. M., Datir, S. S. and Shah, P. V. (2015) Salt-induced physiological and biochemical changes in two varieties of *Linum usitatissimum* L. *International Journal of Current Microbiology and Applied Sciences* 4: 296-304.
- Petridis, A., Therios, I., Samouris, G. and Tananaki, C. (2012) Salinity-induced changes in phenolic compounds in leaves and roots of four olive cultivars (*Olea europaea* L.) and their relationship to antioxidant activity. *Environmental and Experimental Botany* 79: 37-43.
- Qin, J., Dong, W. Y., He, K. N., Yu, Y., Tan, G. D., Han, L., Dong, M., Zhang, Y. Y., Zhang, D., Lil, A. Z. and Wang, Z. L. (2010) NaCl salinity-induced changes in water status, ion contents and photosynthetic properties of *Shepherdia argentea* (Pursh) Nutt. seedlings. *Plant Soil Environment* 56: 325-332.
- Ranjbar, G. and Pirasteh-Anosheh, H. (2015) A glance to the salinity research in Iran with emphasis on improvement of field crops production. *Iranian Journal of Crop Sciences* 17.
- Razavizadeh, R. and Rostami, F. (2013) Changes in growth and antioxidant capacity of canola by salinity and salicylic acid under in vitro. *International Research Journal of Applied and Basic Sciences* 4: 4093-4101.
- Razavizadeh, R. and Rostami, F. (2013) Changes in growth and antioxidant capacity of canola by salinity and salicylic acid under in vitro. *International Research Journal of Applied and Basic Sciences* 5: 192-200.
- Sharma, V. and Ramawat, K. G. (2014) Salt stress enhanced antioxidant response in callus of three halophytes (*Salsola baryosma*, *Trianthema triquetra*, *Zygophyllum simplex*) of Thar Desert. *Biologia* 69: 178-185.



- Taarit, M. B., Msaada, K., Hosni, K. and Marzouk, B. (2012) Physiological changes, phenolic content and antioxidant activity of *Salvia officinalis* L. grown under saline conditions. *Journal of the Science of Food and Agriculture* 92: 1614-1619.
- Tavakoli, M., Poustini, K. and Alizadeh, H. (2016) Proline accumulation and related genes in wheat leaves under salinity stress. *Journal of Agricultural Science and Technology* 18: 707-716.
- Weisany, W., Sohrabi, Y., Heidari, G., Siosemardeh, A. and Ghassemi-Golezani, K. (2012) Changes in antioxidant enzymes activity and plant performance by salinity stress and zinc application in soybean (*Glycine max* L.). *Plant Omics* 5: 60.
- Wong, C. C., Li, H .B., Cheng, K. W. and Chen, F. A. (2006) systematic survey of antioxidant activity of 30 chinese medicinal plants using the ferric reducing antioxidant power assay. *Food Chemistry* 97: 705–711.
- Xu, R., Yamada, M. and Fujiyama, H. (2013) Lipid peroxidation and antioxidative enzymes of two turfgrass species under salinity stress. *Pedosphere* 23: 213-222.
- Yu, Y., Huang, W., Chen, H., Wu, G., Yuan, H., Song, X., Kang, Q., Zhao, D., Jiang, W., Liu, Y. and Wu, J. (2014) Identification of differentially expressed genes in flax (*Linum usitatissimum* L.) under saline–alkaline stress by digital gene expression. *Gene* 549: 113-122.
- Zhang, L., Zhang, G., Wang, Y., Zhou, Z., Meng, Y. and Chen, B. (2013) Effect of soil salinity on physiological characteristics of functional leaves of cotton plants. *Journal of Plant Research* 126: 293-304.
- Zhou, Y., Tang, N., Huang, L., Zhao, Y., Tang, X. and Wang, K. (2018) Effects of salt stress on plant growth, antioxidant capacity, glandular trichome density, and volatile exudates of *Schizonepeta tenuifolia* briq. *International Journal of Molecular Sciences* 19: 252.