

## Salicylic acid mitigates the effects of drought and salinity on nutrient and dry matter accumulation of Linseed

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### Abstract

Salicylic acid can mitigate the impacts of salinity and drought stresses in crops. To study the effects of salicylic acid under iso-osmotic drought and salt stresses on nutrients absorption and growth of linseed (*Linum usitatissimum* L.), an experiment was conducted based on a completely randomized design with three replications in Yasouj University in 2016. Treatments included salinity and drought with similar osmotic potentials (-2, -4, -7 and -9 bar) in 8 levels and control that applied in Hoagland solution. The second factor was salicylic acid (0 and 0.5 mM). Salinity and drought applied using sodium chloride and polyethylene glycol 6000, respectively. The results showed that root and shoot dry weight and Fe, Zn, Mn and Ca content decreased by increasing salinity and drought compared to the control, however salicylic acid application significantly increased these traits, especially under salinity conditions. Na content of Shoot significantly raised by increasing salinity compared to the control and salicylic acid decreased it. Drought, and also salicylic acid, had no significant effect on shoot and Na content of root. Shoot and root dry weights were more significantly affected by drought than salinity in mild stress levels, but the effect of drought and salinity were the same at high levels. Generally it was found that the negative effects of drought were more destructive than salinity at lower osmotic potentials but at higher osmotic potentials impact of drought and salinity was the same indicating the ionic toxicity of salinity at high Na stress. Salicylic acid significantly mitigated the negative impacts of osmotic stress especially under salinity conditions.

**Keywords:** Calcium, Iso-osmotic, Potassium, Sodium, Zinc

### Introduction

Linseed (*Linum usitatissimum* L.) is one of the important oil plants all over the world and is an annual herb with erect stems from the family Linaceae. Linseed originated in India and was first domesticated in the so-called "Fertile Crescent" (Jacobsz and van der Merwe, 2012). It is rich in poly unsaturated fatty acids, particularly omega-3 fatty acid. South west Asia including Iran, is considered as the center of diversity of linseed. Linseed cultivation has been common in Iran since ancient times, and nowadays, it is still used as a sub-culture (Omidbeigi *et al.*, 2001).

Drought and salinity are two most important environmental stresses which occur concurrently in arid and semiarid regions (Slama *et al.*, 2008). Large areas of the planet are affected by high levels of drought and salinity that damage the production of the crop plants (FAO, 2017).

Drought constitutes a limiting factor in the successful production of crop plants around the world

and has adverse effects on plant growth and development and other metabolic processes (Osugwu *et al.*, 2010). In this regard Attarzadeh *et al.* (2016) reported that drought stress increased root dry weight and also affects nutrient absorption, for example, leading to a reduction in shoot and root potassium content.

Salinity is a common environmental challenge of the world and one of the major problems that limit agricultural production. Salinity stress also causes several chemical, physiological and morphological changes in plants. Plant response to salinity depends on plant species, developmental stage, severity and duration of stress (Manchanda and Garg, 2008). The two main threats imposed by salinity are induced by osmotic stress and ionic toxicity associated with excessive Cl<sup>-</sup> and Na<sup>+</sup> uptake, leading to Ca<sup>2+</sup> and K<sup>+</sup> deficiency and other nutrient imbalances. Also, salinity disrupts mineral absorption by interfering the activity of carriers and ion channels in the root such as K<sup>+</sup> selective

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channels (sodium competition with potassium), inhibition of root growth by  $\text{Na}^+$  or  $\text{Na}^+$  impact on soil structure that reduces the absorption of water and minerals. Salinity stress also causes a significant decrease in Mg and Mn content (Talei *et al.*, 2012). Thus, salinity stress either promotes or inhibits the uptake of some nutrients depending on plant species. The results of one study on wheat showed that the salinity stress increased the sodium content of the shoot and root and decreased the content of potassium (Attarzadeh *et al.*, 2016). They also reported that negative effects of salinity stress were more than that of drought and eventually reduced the production of dry matter in the shoot. However, water deficit stress led to increasing root growth and finally increased the dry weight of root.

Osmotic stress can be investigated using polyethylene glycol (PEG), which is the most widely used osmoticum to study the water status of plants (Chen *et al.*, 2010). Salinity stress also is amplified in the laboratory conditions using one or a combination of more than one salts (i.e., NaCl, KCl,...).

Some of the chemicals, such as salicylic acid (SA), as signal molecules have beneficial effects on plant growth and development. SA (2-hydroxybenzoic acid) is a phenolic compound and one of the plant hormones that is found in all plant organs, and when the cells, organs, or the whole plant are exposed to non-biological or biological stress, the concentration of this hormone increases. SA has been shown to significantly reduce ionic leakage and accumulate toxic ions in the plant (Krantev *et al.*, 2008). Jayakannan *et al.* (2013) reported that the pretreatment of Arabidopsis with SA decreased NaCl induced  $\text{K}^+$  efflux from roots while the SA-untreated plants exhibited large  $\text{K}^+$  efflux upon exposure to salt stress. They also stated that the SA pretreated plants had the higher cytosolic  $\text{K}^+/\text{Na}^+$  ratio required for normal cell function under salt stress.

There are scattered studies in connection with the effect of drought stress, salinity stress and SA on linseed. Considering that concurrent analysis of the effects of salinity and drought on the absorption of nutrients and growth of linseed have not been studied, and also regarding the importance of the regulating role of SA in coping plants with stresses, especially drought and salinity, it is expected that its application can be somewhat mitigated the effects of drought and salinity. Therefore, the aim of this study was to compare the uptake of nutrients under drought and salinity stress, with similar osmotic potential, conditions and to investigate the effect of SA on linseed nutrients uptake.

## Materials and Methods

**Experimental design and culture conditions:** This study was carried out as a factorial arrangement in a completely randomized design with three replications in the greenhouse of the Faculty of Agriculture of Yasouj University, Yasouj, Iran in 2016, to compare the nutrients uptake of linseed under drought and salinity

stresses and SA foliar application.

The first factor consisted of the osmotic potential, equal to -2, -4, -7 and -9 bar, including four levels of salinity stress (using NaCl) and four levels of drought stress (with PEG 6000) and the control which applied in Hoagland solution. The second factor was SA foliar application including zero (control) and 0.5 mM.

A total of 108 experimental units (plastic pots of 20 cm in diameter and 30 cm in height) were filled by washed sand and disinfected by autoclaving. In each pot, 20 seeds were sown at a depth of one cm. One day after the emergence of seedlings, the pots were irrigated with a quarter-volume and then one week after, irrigated with half-volume Hoagland solution (Movahhedi Dehnavi *et al.*, 2017). Thinning was done to 10 plants per pot.

**Salinity and drought applying:** With the gradual increase of sodium chloride and polyethylene glycol in Hoagland solution, salinity and drought were started and the SA foliar application was done at the 4-leaf stage. Water osmotic potential by NaCl was calculated according to equation 1 (Taiz and Zeiger, 1991):

$$\Psi = -CIRT \quad \text{:(Eq 1)}$$

Where C is NaCl concentration (M), I is the ionization coefficient (2 for NaCl), R is the gas constant, T is the solution temperature ( $^{\circ}\text{K}$ ), and  $\Psi$  is the osmotic potential (bar).

Calculating the required PEG 6,000 was done as described by Michel and Kaufmann (1973). The application of drought and salinity treatments continued until the end of the vegetative stage. SA foliar application (0 and 0.5 mM) was applied one time just before applying salinity and drought treatments so that the surface of the plant was fully wetted.

### Sample preparation and nutrients measurement:

Twenty days after the beginning of the stress treatments, root and shoot samples were taken from each pot to measure potassium, sodium, iron, zinc and manganese content, and also shoot and root dry weight. Samples were oven dried at  $70^{\circ}\text{C}$  for 48 hrs. and weighted. Dry ash method was used to measure micro and macro elements. 0.5 gr of each sample was burned at  $470^{\circ}\text{C}$  for 4 hours. Then, 10 ml HCl 2N was added. The total volume of the solution reached 50 ml by adding distilled water after passing the filter paper. This extract was used to measure K, Na, Fe, Zn, Ca and Mn. Total potassium and sodium were determined by the flame photometer, Jen Way PFP7, and calcium, zinc, and manganese with atomic absorption spectrometry (Hitachi Z-2300).

**Statistical Analysis:** Statistical analysis of the experimental data was done by SAS version 9.1.3 software. Figures depicted by excel 2010 and mean comparison showed by the standard error on figures.

## Results

The interaction of osmotic potential and SA on all traits except root dry weight was significant ( $P < 0.01$ ), however the main effects of osmotic potential and SA

**Table 1- Mean squares obtained from variance analysis of measured traits in linseed under different levels of SA, salinity and drought**

| SOV                          | df | Shoot potassium | Root potassium | Shoot sodium | Root sodium | Shoot iron | Root iron          | Shoot zinc | Root zinc  |
|------------------------------|----|-----------------|----------------|--------------|-------------|------------|--------------------|------------|------------|
| Salicylic acid (SA)          | 1  | 2053**          | 1111**         | 20611**      | 3.2**       | 0.002**    | 0.06 <sup>ns</sup> | 0.001**    | 0.0024**   |
| Osmotic Potential (OP)       | 8  | 3216**          | 1774**         | 55192**      | 44.4**      | 0.003**    | 0.23**             | 0.002**    | 0.0046**   |
| SA*OP                        | 8  | 347**           | 284**          | 3991**       | 2.84**      | 0.000099** | 0.019**            | 0.000099** | 0.000056** |
| Error                        | 53 | 44.81           | 19.4           | 194          | 0.35        | 0.00001    | 0.00074            | 0.000013   | 0.000016   |
| Coefficient of Variation (%) | -  | 6.32            | 4.18           | 8.83         | 10.16       | 6.6        | 12.82              | 10.84      | 9.98       |

ns, \* and \*\*Non-significant and significant at  $P \leq 0.05$  and  $P \leq 0.01$  respectively

**Continue of table 1-**

| SOV                          | df | Shoot manganese | Root manganese | Shoot calcium | Root calcium | Shoot dry weight | Root dry weight | Root to shoot ratio |
|------------------------------|----|-----------------|----------------|---------------|--------------|------------------|-----------------|---------------------|
| Salicylic acid (SA)          | 1  | 0.0022**        | 0.0002**       | 0.11**        | 0.05**       | 0.0016**         | 0.003**         | 0.32 <sup>ns</sup>  |
| Osmotic Potential (OP)       | 8  | 0.0018**        | 0.0038**       | 0.19**        | 0.168**      | 0.0036**         | 0.002**         | 0.59**              |
| SA*OP                        | 8  | 0.0015**        | 0.000069**     | 0.0063**      | 0.0098**     | 0.00007**        | 0.00001**       | 0.16**              |
| Error                        | 53 | 0.000025        | 0.00036        | 0.00046       | 0.0016       | 0.00001          | 0.00002         | 0.38                |
| Coefficient of Variation (%) | -  | 18.1            | 16.3           | 8.1           | 19.54        | 9.98             | 11.6            | 33.8                |

ns, \* and \*\*Non-significant and significant at  $P \leq 0.05$  and  $P \leq 0.01$  respectively

on the root dry weight was significant (Table 1).

**Shoot and root potassium content:** In both consumption and non-consumption of SA conditions, the amount of shoot and root potassium of linseed at different levels of NaCl stress treatments showed a significant decrease compared to non-stress treatment (control) (Fig. 1 and 2). However, in all NaCl levels, potassium in the SA treatment was higher. With increasing drought stress, from -2 to -7 bar the amount of potassium in the shoot and root increased. However, there was no significant difference between consumption and absence of SA. Then, in severe drought stress (-9 bar), the amount of potassium in the shoot decreased (Fig. 1 and 2). This decrease was higher in SA intake treatment.

**Shoot and root sodium content:** In both consumption and non-consumption of SA conditions, the shoot sodium content of linseed at different levels of salt stress treatment showed a significant increase compared to non-stress treatment (control) (Fig. 3). However, the shoot sodium content at all salinity levels, especially high salinity levels, was lowered with SA. In fact, the use of SA seemed to reduce the effect of salinity stress. However, drought stress and SA associated with did not have a significant effect on the sodium content of the shoot and the amount of shoot sodium at all levels of stress was equal to Hoagland's control.

With increasing drought stress from -2 to -4 bar, no significant increase was observed in root sodium content, but increasing more drought stress (-7 to -9 bar), the sodium content of the root increased. SA had

no significant effect of root Na content under both NaCl and PEG conditions.

**Shoot and root iron content:** In the conditions of consumption and non-consumption of SA, the iron content of shoot was significantly decreased at different levels of salinity and drought stress treatments than control (Fig. 5). Similar to the shoot iron, under SA consumption and non-consumption, the content of root iron decreased significantly with increasing salt and drought stress levels compared to the non-treated (control) stress (Fig. 6). But the amount of shoot iron in SA intake was higher in all stresses, and for root, SA increased iron content only at mild stresses. Negative and significant correlations (Table 2) between the root iron and sodium content ( $r^2 = -0.87$  \*\*) emphasize this issue.

**Shoot and root zinc content:** In both the conditions of consumption and non-consumption of SA, salinity and drought stress levels significantly decreased shoot and root zinc content (Fig. 7 and 8). The consumption of SA increased zinc levels at all levels of salinity and drought. In mild and moderate salinity and drought stresses (-2 and -4 bar) the negative effect of salinity stress was more than drought stress, but in severe stresses (-7 and -9 bar), the impact of salinity and drought were almost the same.

**Shoot and root manganese content:** In both conditions of consumption and non-consumption of SA, shoot and root manganese content significantly decreased compared to the non-stress treatment (control) (Fig. 9 and 10). At all levels of salinity and drought stresses, shoot and root manganese content was

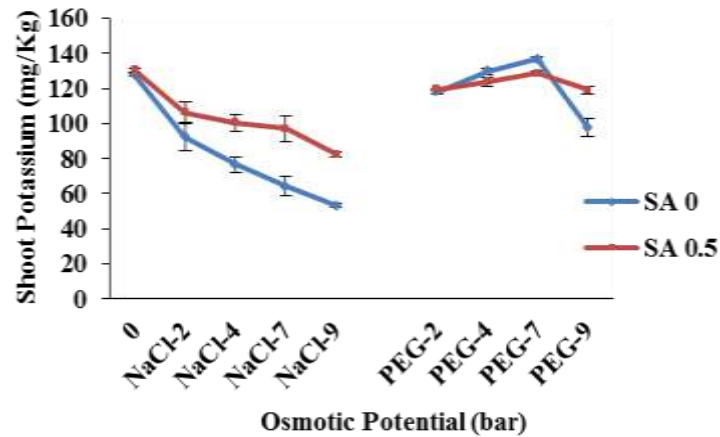


Fig. 1- Effect of of different levels of salinity and drought and SA on shoot potassium content of linseed. Bars show the standard error

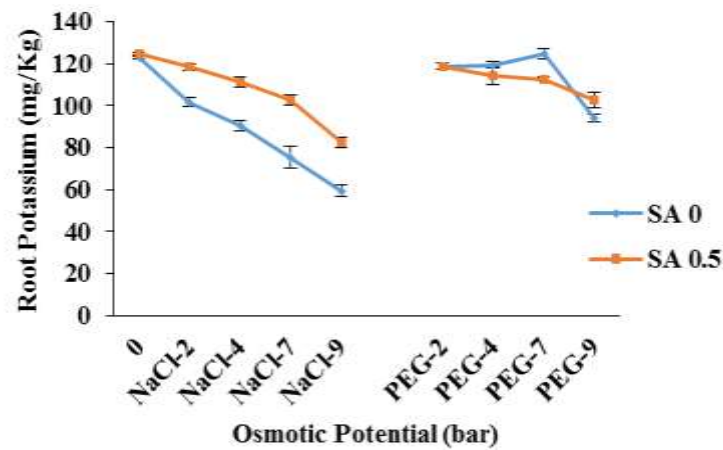


Fig. 2-. Effect of different levels of salinity and drought and SA on root potassium content of linseed. Bars show the standard error

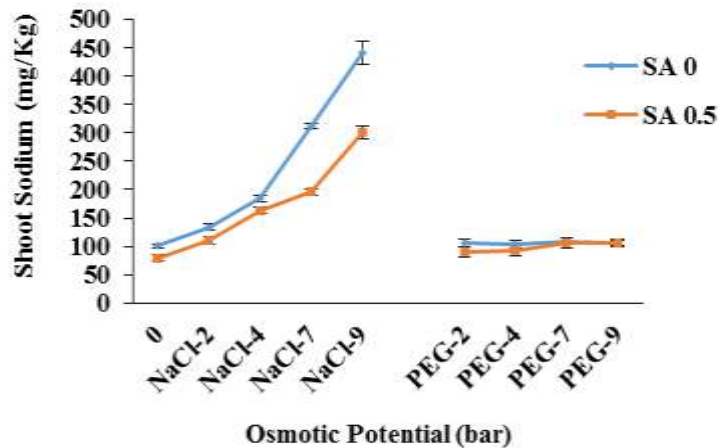


Fig. 3- Effect different levels of salinity and drought and SA on shoot sodium content of linseed. Bars show the standard error

higher with SA, except for the -7 and -9 bar of drought for root manganese, which no significant difference between consumption and absence of silicic acid was seen.

**Shoot and root calcium content:** In both conditions of consumption and non-consumption of SA, salinity

and drought levels significantly reduced shoot and root calcium compared to the non-stress treatments (Fig. 11 and 12). Of course, this decline for root calcium was not significant at low levels of salinity. At all levels of salinity and drought, the calcium content of the shoot and root was higher with SA, except for the -7 and -9

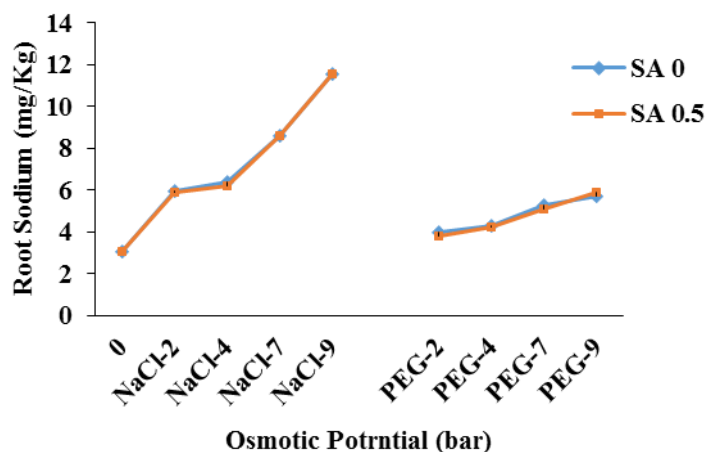


Fig. 4- Effect of different levels of salinity and drought and SA on root sodium content of linseed. Bars show the standard error

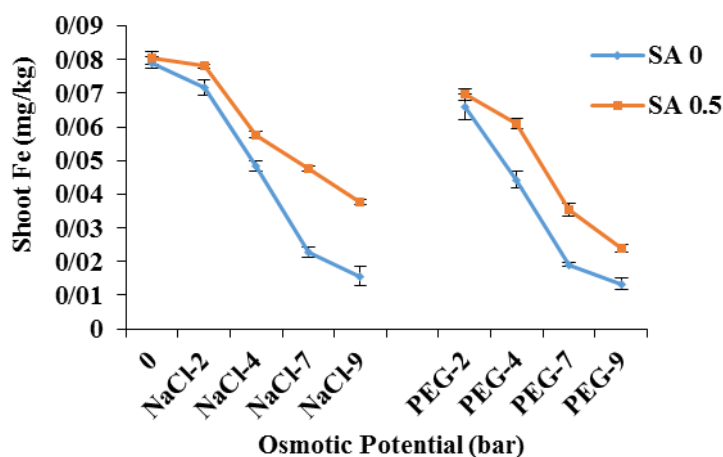


Fig. 5- Effect of different levels of salinity and drought and SA on shoot iron content of linseed. Bars show the standard error

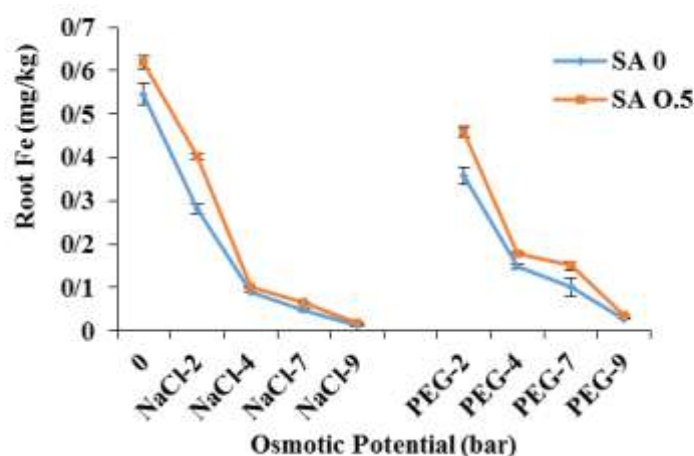


Fig. 6- Effect of different levels of salinity and drought and SA on root iron content of linseed. Bars show the standard error.

bar for root calcium, which did not show significant differences between consumption and absence of SA.

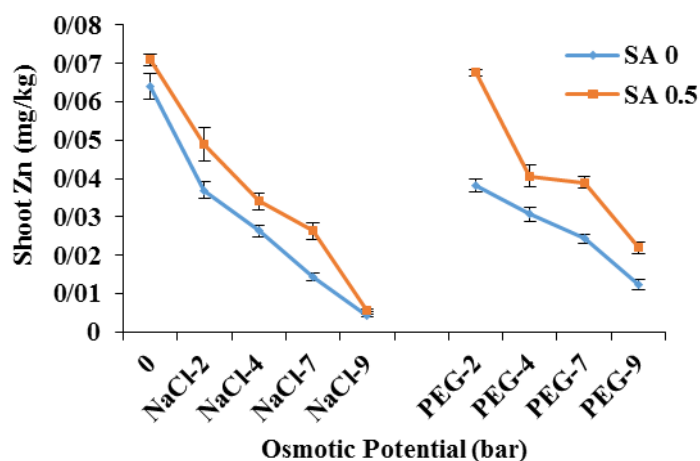
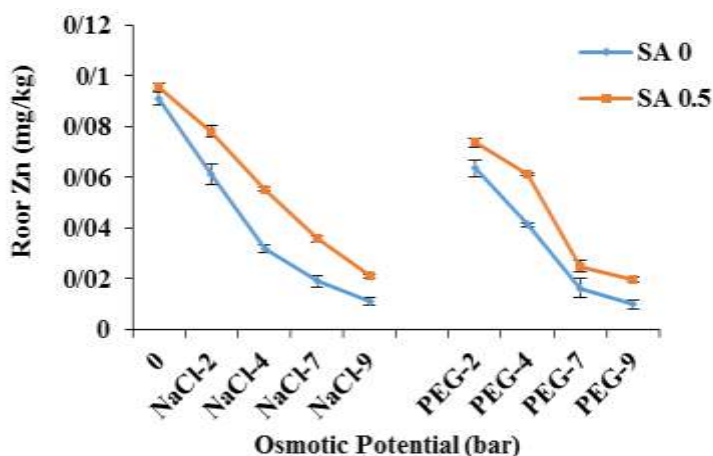
**Shoot dry weight:** In both consumption and non-consumption of SA conditions, shoot dry weight of linseed had a significant decrease from the levels of

salinity and drought stresses than non-stress treatment (control) (Fig. 13). SA had a significant effect on shoot dry weight in all salinity and drought levels.

**Root dry weight:** With increasing salinity and drought, root dry weight decreased significantly (Fig.

**Table 2- Pearson correlation coefficient for measured traits in linseed under different levels of SA, salinity and drought**

| Traits               | 1      | 2      | 3      | 4      | 5     | 6     | 7     | 8     | 9     | 10    | 11    | 12    | 13    | 14 |
|----------------------|--------|--------|--------|--------|-------|-------|-------|-------|-------|-------|-------|-------|-------|----|
| 1-Shoot K            | 1      |        |        |        |       |       |       |       |       |       |       |       |       |    |
| 2-Root K             | .87**  | 1      |        |        |       |       |       |       |       |       |       |       |       |    |
| 3- Shoot Na          | -.84** | -.87** | 1      |        |       |       |       |       |       |       |       |       |       |    |
| 4-Root Na            | -.73** | -.83** | .86**  | 1      |       |       |       |       |       |       |       |       |       |    |
| 5- Shoot Fe          | .36**  | .58**  | -.48** | -.61** | 1     |       |       |       |       |       |       |       |       |    |
| 6-Root Fe            | .55**  | .63**  | -.54** | -.70** | .61** | 1     |       |       |       |       |       |       |       |    |
| 7-Shoot Zn           | .63**  | .75**  | -.66** | -.77** | .81** | .89** | 1     |       |       |       |       |       |       |    |
| 8-Root Zn            | .44**  | .64**  | -.52** | -.68** | .82** | .94** | .79** | 1     |       |       |       |       |       |    |
| 9-Shoot Mn           | .54**  | .65**  | -.57** | -.67** | .82** | .78** | .78** | .86** | 1     |       |       |       |       |    |
| 10-Root Mn           | .43**  | .63**  | -.53** | -.72** | .91** | .79** | .85** | .94** | .86** | 1     |       |       |       |    |
| 11- Shoot Ca         | .44**  | .65**  | -.55** | -.70** | .95** | .79** | .85** | .95** | .88** | .95** | 1     |       |       |    |
| 12- Root Ca          | .35**  | .55**  | -.55** | -.63** | .85** | .59** | .77** | .85** | .79** | .86** | .92** | 1     |       |    |
| 13- Shoot Dry matter | .23**  | .46**  | .36**  | .55**  | .89** | .72** | .78** | .90** | .76** | .86** | .88** | .73** | 1     |    |
| 14- Root Dry matter  | .56**  | .70**  | -.63** | -.74** | .90** | .84** | .91** | .93** | .88** | .90** | .92** | .88** | .86** | 1  |

\*\* significant  $P \leq 0.01$ **Fig. 7- Effect of different levels of salinity and drought and SA on shoot zinc content of linseed. Bars show the standard error****Fig. 8- Effect of different levels of salinity and drought and SA on root zinc content of linseed. Bars show the standard error**

14). It seemed that in higher water potentials, with increasing salt stress, root dry weight showed a higher reduction compared to the drought stress conditions.

With the applying SA, an increasing trend was observed in the root dry weight of linseed (Fig. 15). In fact, SA reduced the negative effects of drought and

salinity stress on root dry weight.

**Root to shoot dry weight ratio:** In both consumption and non-consumption of SA conditions, there were no significant differences in root to shoot dry weight ratio from salinity stress (Fig. 16). However, with increasing drought stress to -7 bar, root to shoot

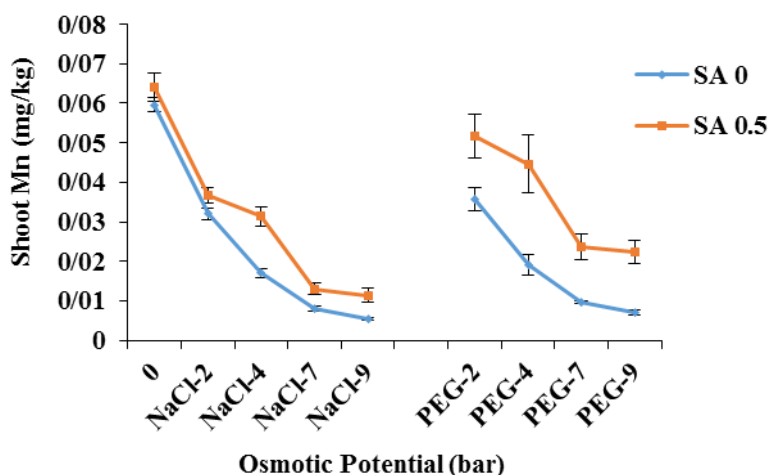


Fig. 9- Effect of different levels of salinity and drought and SA on shoot manganese content of linseed. Bars show the standard error

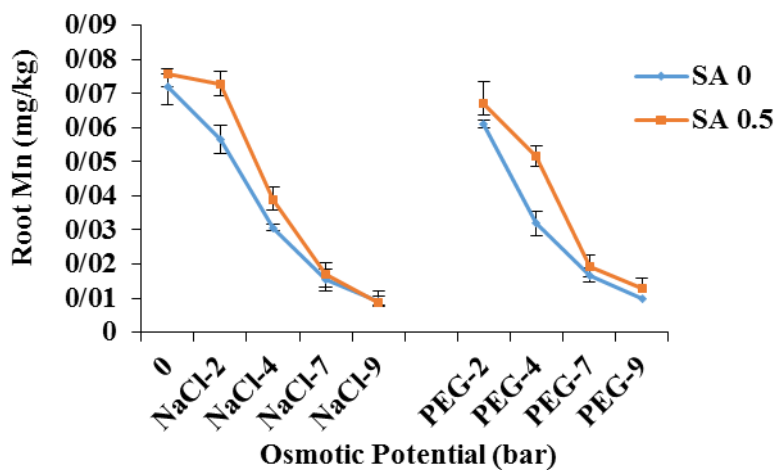


Fig. 10- Effect of different levels of salinity and drought and SA on root manganese content of linseed. Bars show the standard error

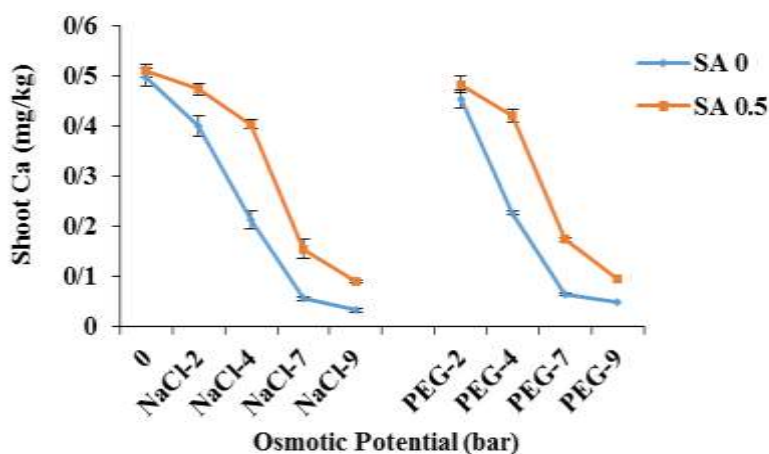


Fig. 11- Effect of different levels of salinity and drought and SA on shoot calcium content of linseed. Bars show the standard error

with increasing drought stress to -7 bar, root to shoot dry weight ratio increased, and with the stress increased to -9 bar, the ratio decreased. In fact, in mild to moderate stress (-2 to -7 bar), the negative effect of

drought stress on the shoot was more than the root, but in severe stress (-9 bar) the negative effect of drought stress on the root was higher.

In SA consumption treatment relative to no-

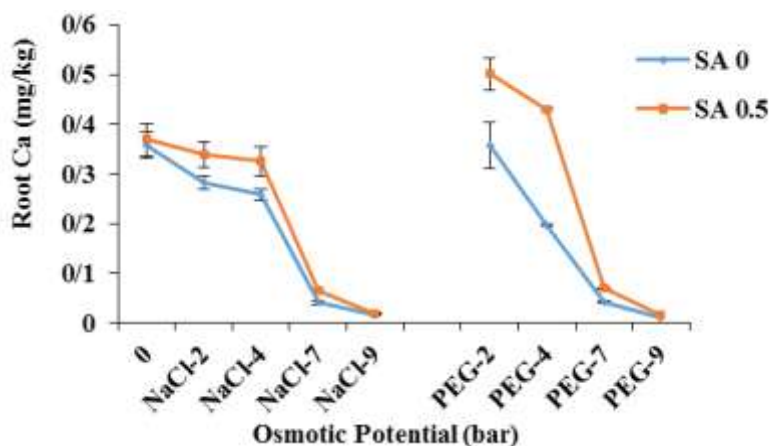


Fig. 12- Effect of different levels of salinity and drought and SA on root calcium content of linseed. Bars show the standard error

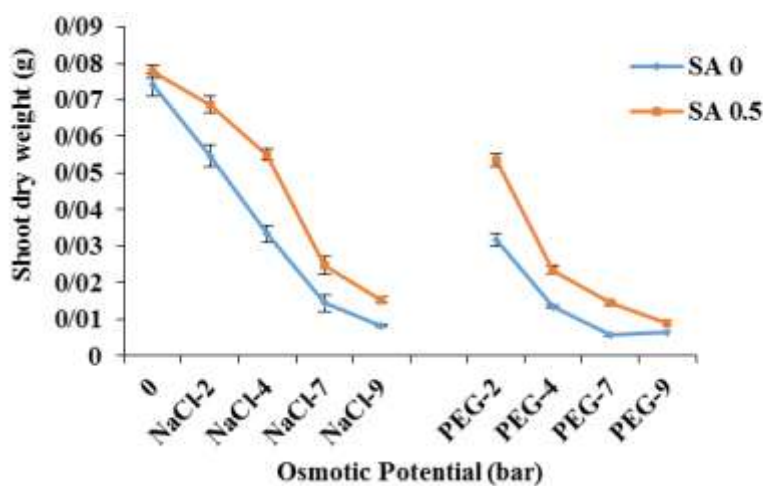


Fig. 13- Effect of different levels of salinity and drought and SA on shoot dry weight of linseed. Bars show the standard error

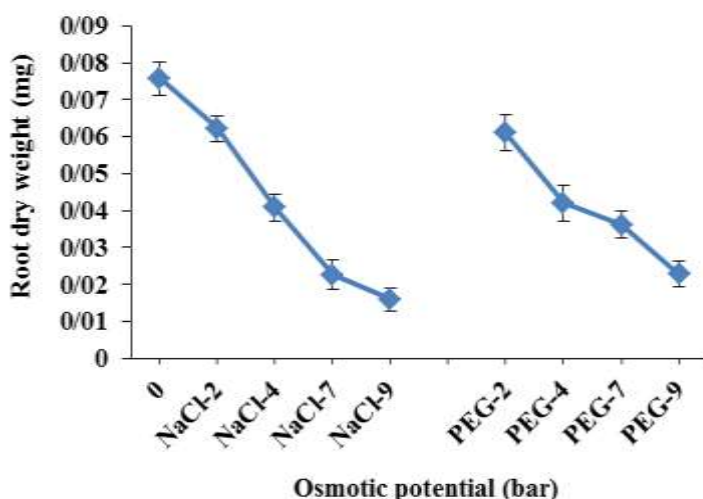


Fig. 14- Effect of different levels of salinity and drought on root dry weight of linseed. Bars show the standard error

consumption, there was not a significant difference in root to shoot dry weight ratio by increasing salinity stress (Fig. 16). However, with increasing drought stress to -7 bar root to shoot dry weight ratio decreased by SA consumption and then increased at -9 bar. In fact, the

consumption of SA prevented severe root depletion in severe drought stress.

#### Discussion

Potassium is one of the vital elements required for plant



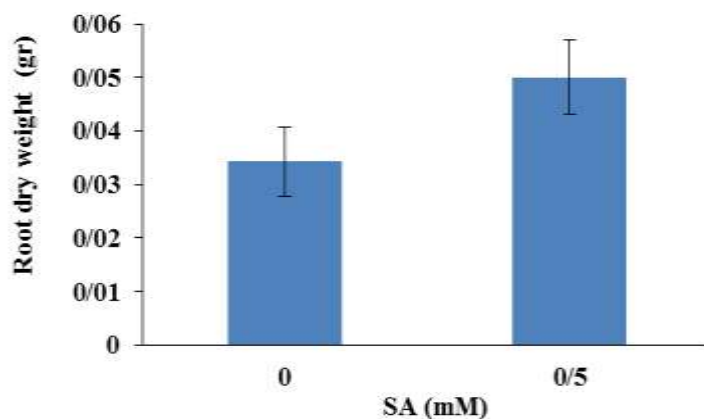


Fig. 15- Effect of SA on root dry weight of linseed. Bars show the standard error

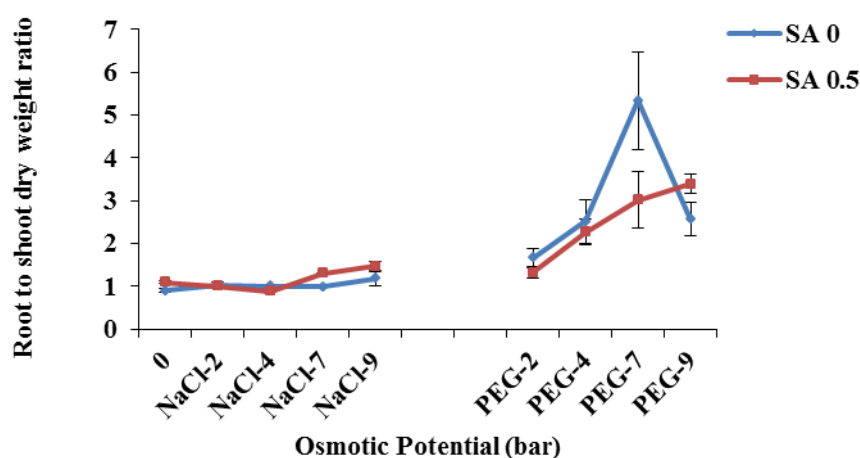


Fig. 16- Effect of different levels of salinity and drought and SA on root to shoot dry weight ratio of linseed. Bars show the standard error

growth and physiology and also has a regulatory function in several biochemical processes related to protein synthesis, carbohydrate metabolism, and enzyme activation. Several physiological processes depend on K, such as stomatal regulation and photosynthesis (Hasanuzzaman *et al.*, 2018). In salinity conditions that sodium overcomes potassium, potassium deficiency occurs in the plants. Under salinity stress, the osmotic effect and ion toxicity inhibits plant root growth, which decreases nutrient uptake and translocation, especially that of  $K^+$  (Wang *et al.*, 2013).

Increasing potassium uptake in the plant by increasing drought stress is due to the regulation of osmotic pressure and the role of potassium ion in stomatal control. By increasing the intensity of drought stress, the overall growth of the plant, including the absorption capacity of the roots, decreases and cannot absorb potassium from the soil colloids. It seems that the negative effect of salt stress on potassium adsorption was more than drought stress. Under salt stress, K helps to maintain ion homeostasis and to regulate the osmotic balance. Under drought stress conditions, K regulates the stomatal opening and helps plants adapt to water deficits. A close relationship between the K nutritional status and plant drought resistance has been

demonstrated (Hasanuzzaman *et al.*, 2018). Potassium mediated the xylem hydraulic conductance and maintained cell turgor, stomatal movement, and sufficient gas exchange as part of the drought adaptation because these events help to maintain water balance in plants (Oddo *et al.*, 2011). Figure 1 illustrates the involvement of K in plant tolerance under drought stress.

In general, oxidative stress, which is one of the effects of salinity and drought stress on plants, causes lipid peroxidation and damages the cell membrane. Peroxidation also disrupts the function of the membrane, resulting in leakage of potassium from the cell (Ferreira-Silva *et al.*, 2008), which eventually results in the reduction of potassium content under stress conditions. Salinity induces membrane depolarization and decreases the membrane integrity, which results in  $K^+$  leakage through depolarization-activated outward-rectifying  $K^+$  channels (Shabala and Cuin, 2008). On the other hand, studies have shown that SA prevents damage to membrane fatty acids, decreases membrane permeability and protects membranes during osmotic stress in plants, which results in a reduction of potassium leakage from the cell. It seems that the abundance of sodium ion in conditions of salt stress in

nutrient solution increases its absorption by the root and increases its concentration and decreases other ions, especially potassium. The negative and significant correlation of potassium with the sodium content of root ( $r^2 = -0.83$  (table 2)) also confirms this issue.

Under salinity stress, endogenous level of SA increased along with the increase in the activity of the SA biosynthetic enzyme in rice seedling (Sawada *et al.*, 2006). Jayakannan *et al.* (2013) also proved that SA application increased the potassium content under salinity conditions and reduced sodium accumulation in the root of Arabidopsis. They also showed that SA improves salinity tolerance in Arabidopsis by restoring membrane potential and preventing salt-induced  $K^+$  loss via a guard cell outward rectifying  $K^+$  channel.

For almost all terrestrial plants,  $Na^+$  is not essential for either growth or development or reproduction. An exception is a subgroup of C4 plants for which  $Na^+$  has been shown to be essential (Maathuis, 2013). The sodium ion ( $Na^+$ ) competes with  $K^+$  for major binding sites during key metabolic processes in the cytoplasm, with these binding sites including both low-affinity and high-affinity transporters; this competition disturbs the plant metabolism (Wang *et al.*, 2013). According to our findings, shoot sodium content in salt stress treatments increased more than drought stress treatments. Similar to our results, Lokhande *et al.* (2010) reported that sodium content was 183% higher in salt stress than the control, but only 67% increasing observed in drought stress treatments.

It seems that the negative effect of salinity stress on root sodium content was higher than drought stress, and drought stress had an impact on root sodium content only at high levels (severe stress). Plants can increase sodium absorption to increase the water absorption capacity of the soil to regulate the cell and tissue osmotic adjustment under drought stress.

The sodium content of the shoot was several times greater than the root at all levels of salinity and drought, showing that linseed is unable to keep sodium in the roots, and the higher transfer of sodium absorbed to the shoot indicates that the plant can not tolerate salinity.

In this experiment salinity and drought significantly decreased shoot and root Fe, Zn and Mn content and SA could mitigate this reduction, although the response to SA was different for each nutrient and NaCl and PEG levels. Shoot and root Fe, Zn and Mn content was more in lower NaCl levels compared to the lower PEG levels, however at higher osmotic levels the shoot and root Fe, Zn and Mn content were the same, indicating that the impact of drought is more than salinity in lower osmotic potentials. It seems that at higher NaCl level ion toxicity was added to the osmotic potential of NaCl. The negative and significant correlation was observed between root and shoot Fe, Zn and Mn and sodium content (Table 2), also emphasizes this issue. It seems that decreasing water flow due to drought stress as well as reducing root dry weight in this experiment is due to diminished absorption of micronutrients by the plant.

Positive and significant correlations between root and shoot dry weights and root and shoot Fe, Zn and Mn content also confirms this result (Table 2). Iron is the third most limiting nutrient for plant growth and metabolism, primarily due to the low solubility of the oxidized ferric form in aerobic environments (Samaranayake *et al.*, 2012). Reports on the influence of salinity and drought on the Fe concentration in plants are inconsistent. Iron is deposited in the form of iron sulfate under saline conditions and the ability to transfer and move it in the soil decreases (Soltani *et al.*, 2009).

It seems that the use of SA in stress conditions by increasing plant Fe, Zn and Mn content contributes to stress modulation. Since SA has slightly reduced the sodium content of the shoot, it can be concluded that SA, by reducing the accumulation of toxic ions in the plant cells, somewhat mitigates salinity and drought stresses by better absorption of micronutrients.

In general, the amount of the Fe and Zn in the root was much higher than that of the shoot, which indicates the low efficiency of linseed in the translocation absorbed by the root to the shoot. However, root and shoot Mn content was the same. The relatively high sodium concentrations or the limited availability of water for the plant, due to the high amounts of soluble salts, is likely to be responsible for reducing zinc concentrations in tissues under salt stress (Tavallali *et al.*, 2009). Our results (Fig. 7 and 8) are in agreement with this report for both shoot and roots. It seems that the negative effects of drought stress and salinity on zinc content decreased SA consumption relative to non-consumption of SA.

Our results are in agreement with previous studies; for example, Mn was significantly lowered in shoots and roots in both salt-drought-treated plants.

It seems that the negative effects of drought stress and salinity on root dry weight with increasing consumption of SA have decreased compared to the non-consumption of SA. Considering the positive and significant correlation between root and shoot dry weight with the Mn, Zn and Fe content in this study, it can be concluded that SA consumption increased the absorption of these elements.

The reason for the decrease of calcium content in plant organs under salt stress conditions is the antagonistic effect of plant-absorbed sodium and calcium. The increasing of salinity level decreased shoot  $K^+$  and  $Ca^{2+}$  concentrations due to  $Na^+/K^+$  and  $Na^+/Ca^{2+}$  antagonism (Tuna *et al.*, 2008). Similar to our findings, Talei *et al.* (2012) reported that by increasing salinity and sodium accumulation, absorption of  $Ca^{2+}$  was reduced.

The negative and significant correlations (Table 2) between the content of shoot calcium with the shoot sodium content ( $r^2 = -0.55^{**}$ ) also confirms this, and the reduction of sodium content in SA-treated plants can increase the calcium content of the shoot.

It seems that the impacts of drought and salinity have decreased by SA consumption compared to the

non-consumption of SA. Khan *et al.* (2010) also showed that the treatment of mungbean plant with 0.50 mM SA reduced the sodium content and increased the calcium content in salt stress conditions.

In mid salinity stresses, calcium, possibly as an essential nutrient, is required to regulate osmotic adjustment in cell vacuoles, and in severe stresses, antagonistic effects of sodium and calcium have prevented calcium absorption. Drought stress reduces the absorption of nutrients by reducing transpiration, active transmission system, membrane permeability and root absorption capacity. In this research, reducing the absorption of calcium can be attributed to decreasing the solubility and availability of calcium, reducing transpiration, and growth and development of root system under soil moisture deficit conditions (Hopkins and Henr, 2009).

Studies have shown that SA can stimulate membrane integrity by inhibition of lipid peroxidation (Gunes *et al.*, 2007). On the other hand, the role of ATPase is known in the transport of several ions through the plasma membrane. Therefore, the role of SA in increasing uptake of elements such as calcium under stress conditions can be justified.

By Comparison of similar levels of salinity and drought, it can be said that drought effect on shoot dry weight of linseed was more severe than salinity, but at the osmotic potential of -9 bar, the severity of drought and salinity on shoot dry weight was the same. Therefore, the osmotic effect on the linseed shoot dry weight was greater than that of ionic toxicity. Flax can withstand lower levels of salt stress by possibly uptaking more nutrients and osmotic regulation, better than lower levels of drought stress. Yang *et al.* (2010) have reported similar growth inhibition kinetics upon exposure of cultured cells to high levels of NaCl in *Nitraria tangutorum* callus. The main causes of dry matter decline in many plants are the reduction of photosynthetic surfaces and excessive consumption of energy to control and to reduce the effect of salt stress by osmotic adjustment to maintain cellular turgor (Kabiri *et al.*, 2012). In salinity conditions, several factors such as reduction of the photosynthesis rate, increasing permeability, and degradation of cell membranes, reducing the available water availability of plants and accumulation of Na<sup>+</sup> in leaves were the main causes of weight loss under the salt stress (Hajiaghayi-Kamrani *et al.*, 2013).

It seems that the negative effects of salinity and drought stress on shoot weights decrease with SA consumption in comparison to non-consuming

conditions. Shoot dry weight in SA treated treatments was higher than that in without SA. Also, the positive effect of SA on shoot dry weight was more in salinity stress than in drought stress. Based on the findings of this study, the SA applied by improving the uptake of shoot potassium (Fig.1) reducing the shoot sodium accumulation (Fig.3) and thus preventing damaging effects of sodium such as membrane degradation, cell destruction, and reduced leaf area caused an increase in shoot dry weight.

Therefore, the effect of ionic toxicity on root dry weight of linseed was more than the osmotic effect. Reducing the roots growth may be due to the effect of ionic toxicity and imbalance in the absorption of nutrients caused by salt stress. Alternatively, the ability of the root system to control the ions entering the shoot for survival of the plant under salt stress conditions is another reason (Belkheiria and Mulas, 2013). The negative and significant correlation between root dry weight and root sodium content ( $r^2 = 0.74^{**}$ ) also confirms this issue. Reduction in the growth of the roots due to low water supply includes the root characteristics especially root density, root length and root thickness. The root system, which enhances the ability of a plant to absorb water, is a major adaptation mechanism for drought. Similar to the dry weight of the shoot, root dry weight was also affected by excessive and decreased accumulation of sodium and potassium, respectively (Fig.1 and Fig.3). A linear decline in root dry weight of linseed correlated with increasing salinity levels recorded in our experiment.

## Conclusion

Our results revealed that osmotic potentials created by NaCl and PEG, reduced root and shoot phosphorus, iron, manganese, zinc and calcium concentrations, and also decreased root and shoot dry matter. SA could mitigate these reductions. Comparing the iso-osmotic potentials showed that at lower osmotic potentials, impact of PEG on root and shoot dry weight is higher but, at higher ones the effects of NaCl and PEG is the same, representing that ionic toxicity adds to the osmotic potential at higher NaCl concentration. This trend is also observed for root and shoot nutrients uptake except for Ca, so that the impact of PEG and NaCl is almost the same. SA can also reduce the sodium absorption under NaCl salinity conditions but not under PEG. Apparently Na<sup>+</sup> and Cl<sup>-</sup> absorption at lower NaCl osmotic potentials can help linseed to tolerate salinity.

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