

ORIGINAL ARTICLE

**The salicylic acid effect on the tomato (*lycopersicum esculentum* Mill.)
germination, growth and photosynthetic pigment under salinity stress
(NaCl)**

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Soil salinity is a serious environmental problem that has negative effect on plant growth, production and photosynthesis. Fresh and dry plant weights decreases with salinity treatments. The very important role of salicylic acid (SA) in response to different stress and modification and decline damages due to stresses has established in different studies. In this research tomato seeds planted in pots containing perlite in a growth chamber under controlled conditions of $27\pm 2^{\circ}\text{C}$ and $23\pm 2^{\circ}\text{C}$ temperature, 16h lightness and 8h darkness respectively, 15 Klux light intensity and 75% humidity; NaCl concentration of 0, 25, 50, 75 and 100 mM and salicylic acid concentration of 0, 0.5, 1 and 1.5 mM were used in the form of factorial experiment in a complete randomized design (CRD). Results show that germination was decreased with salinity increasing. At low levels of salinity, SA leads to decrease in germination and had no effect in high levels of salinity. The length of shoot were not effected by salinity but decrease with increase in SA concentration. Low salinity concentrations led to significant increase in root length and high concentrations don't have significant difference with control. SA also had no effect on it. The highest amount of a, b, c and total chlorophyll and carotenoid was show in 50 mM salinity levels.

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Salinity is an important ecological factor because limits nutrition production (Yokoi et al., 2002). High salinity, influences soil penetration decline

soil water potential and leads to physiological drought and lead to dead in worst condition (Yusuf et al., 2007). The most important agent of soil

salinity is sodium chloride therefore the study about it is necessary (Dantas et al., 2005). Germination assessment of control and exposed to salt seeds are used as a sign of some species and varieties resistance to salinity. Root and shoot length are most important parameter for salinity stress. Salinity stress inhibits plants growth (The length and fresh weight of root and shoot) but root length is more influenced than shoot length (Jamil et al., 2006). Soil salinity is reported as a destroyer of photosynthetic organization in several levels like pigments, stomata operation, gas exchange, thylakoid membrane structure and operation, electron conduction and enzyme (Lakshima et al., 1996; Roger et al., 1998). SA is a plant phenol and is used for rising plant resistance to undesirable effects of biotic and abiotic stresses and participates in regulation of plant physiological stages. SA has significant effect on different aspects of plant life like plant growth and development, photosynthesis, evaporation, ion transmission and absorption; also causes to special changes in leaf anatomy and chloroplast structure (Sakhabutdinova et al., 2003). In this research the effect of salinity and SA on germination, growth and photosynthetic pigments was studied on tomato plant that is relatively sensitive plant to salinity.

MATERIALS AND METHODS

Seed tomato germination study

At first germination papers were put in Petri dishes and were sterilized in oven with 180°C temperature. Then seed sterilized with sodium hypo chloride and seeds with same size were chose and soaked in SA concentrations for 12h and 60 seeds were transferred with sterile pincers and specific space into Petri dishes and closed the door of Petri dishes; then were placed in germinator (made of Tehran GROUC company) in 30±2°C temperature and 16/8

hours photoperiod lightness and darkness respectively.

Germination percentage Account

About 72h later, germinated seeds in every petri were counted and the percentage of seeds germination were accounted with use of relation 1.

Germination percentage = $\frac{\text{number of germinated seeds}}{\text{number of whole seeds}} \times 100$

Planting

At first, the seeds were disinfected with hypo chloride sodium, and then 5 seeds were planted in each pot containing perlite and kept in a growth chamber under controlled conditions of 27 ±2°C, 23 ±2°C temperatures, 16 h lightness, 8 h darkness, respectively, 15 Klux light intensity and 75% humidity. Then the pots were irrigated with deionized water and nutrient solution, with 6.5 pH every two day for one month. NaCl factor at 5 levels including 0, 25, 50, 75, 100 mM and salicylic acid treatment at 4 levels including 0, 0.5, 1 and 1.5 mM were used. The experiment was performed as factorial in the form of completely random plan (CRD Design) with 3 repetitions (60 pots). Salicylic acid treatments were sprayed on the leaves every two days for two weeks, and then different levels of salinity factor were used every two days with nutrient solution for 15 days.

Measurement of root and shoot length to sample were harvested of each pot (120 sample, totally). The root and shoot lengths were measured with ruler. The average of root and shoot lengths were account in this 2 samples and were reported in centimetre.

Measurement of photosynthetic pigments; Chlorophyll and carotenoid based on Lichtenthaler, 1987 method

0.2 g of leaf and root fresh tissue were weighted by laboratory subtle scale (satrius) BP211D model with 0.0001g accuracy. Each sample was grinded

with 80% acetone in a china mortar, then its content were filtered by watman paper number 1. after that another 5 ml acetone were added to it and the solution volume became 15 ml. this solution contains a and b chlorophyll and carotenoid. 3ml of this solution were poured in covet. The solution absorption were determined in 663.2, 646.8 and 470 nm by spectrophotometer system. 80% acetone were used as blank solution for spectrophotometer setting. Concentration of this pigments were accounted with relation 2.

$$\text{Chl.}_a(\mu\text{g. ml}^{-1}) = 12.5A_{663.2} - 2.79 A_{646.8}$$

$$\text{Chl.}_b(\mu\text{g. ml}^{-1}) = 21.51A_{646.8} - 5.1 A_{663.2}$$

$$\text{Chl.}_{\text{Total}}(\mu\text{g. ml}^{-1}) = \text{Chl.}_a + \text{Chl.}_b$$

$$\text{Car}(\mu\text{g. ml}^{-1}) = (1000A_{470} - 1.8\text{Chl}_a - 85.02\text{Chl}_b) / 198$$

Relation 2: formula of a,b and total chlorophylls and carotenoids measurement

Concentrations were determined according to microgram per mili liter plant extraction. results of photosynthetic pigments, measurement were account according to milligram per fresh weight gram (mg/g fw).

Statistical analysis

In this study, the total number of experiments was done in different stages in completely randomized design with 3 repetitions; and the htest considers the reciprocal effect of Salicylic acid and salinity on different parameters as factorial. The levels of 0, 25, 50, 75, 100 mM of salinity were used and the levels of Salicylic acid were 0, 0.5, 1, 1.5 mM. The comparison of means was done with Duncan test to SPSS 12.0 software in probability level of 1%. For drawing graph, we used Excel 2003 software.

RESULTS AND DISCUSSION

Germination percent

According to fig1 germination was decreased, with salinity increasing. So that, 0 mM salinity level , has highest germination percentage and in 100 mM

salinity level, germination became zero. In 0, 50, 75 and 100 mM salinity levels, SA doesn't create any significant change but in 25 and 50 mM salinity levels, SA decreased germination.

Shoot length

Only SA could affect on shoot length and shoot length was decreased with SA increasing (Fig. 2). Fig. 3 shows tomato plants at the end of treatment period.

Root length

Only salinity could have significant effect on root length. 25 and 50 mM salinity concentrations caused to significant effect on root length (Fig. 4).

Chlorophyll a

Only salinity could affect on chlorophyll a. according to Fig 5, 50 mM salinity concentration has most amount of chlorophyll a. other levels don't have significant difference with each other.

Chlorophyll b

Only simple effects of salinity and SA on Chlorophyll b were significant. 50mM salinity level had highest Chlorophyll b amount and other levels don't have significant difference (Fig. 6a)

SA in 1 and 1.5 concentrations caused increase and decrease in Chlorophyll a respectively. other levels had no significant difference with each other (fig.6b).

Total chlorophyll

According to fig.7a, 50mM salinity concentration had significant increase and other levels had no significant deference relatively. 1.5mM SA concentration led to increase in total chlorophyll and 1mM SA concentration caused it (fig. 7b).

Total chlorophyll

According to fig.7a, 50mM salinity concentration had significant increase and other levels had no significant deference relatively. 1.5mM SA concentration led to increase in total

chlorophyll and 1mM SA concentration caused it (fig. 7b).

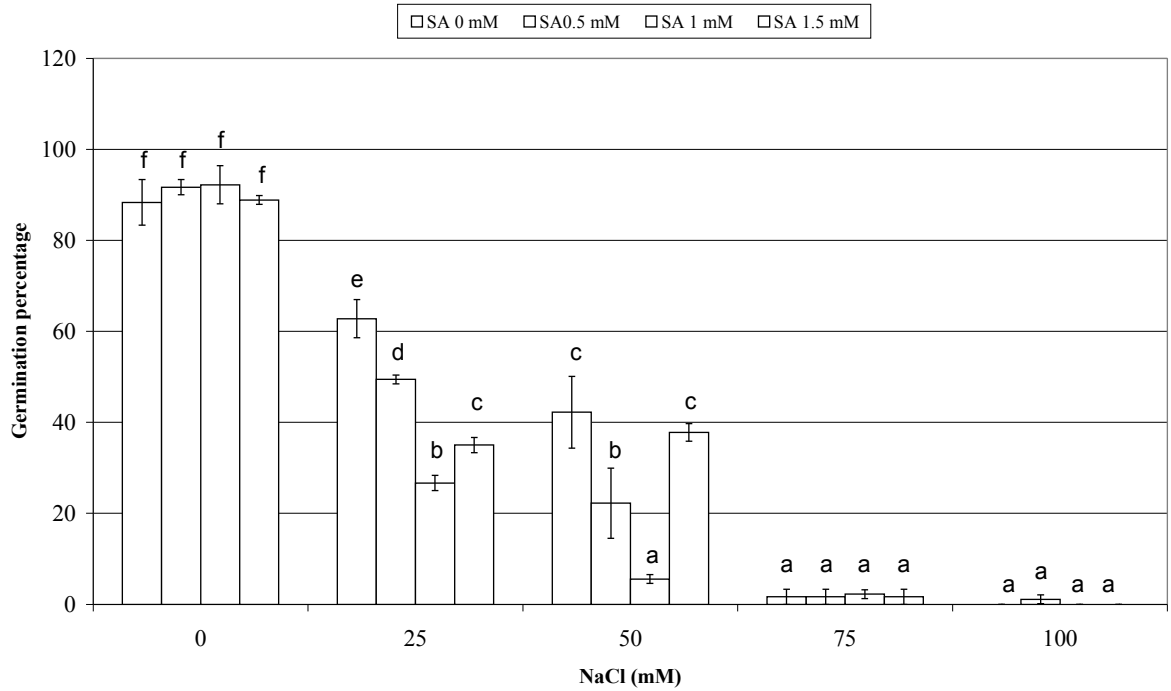


Fig. 1. Effect of NaCl salinity and SA on the germination percentage of tomato seeds

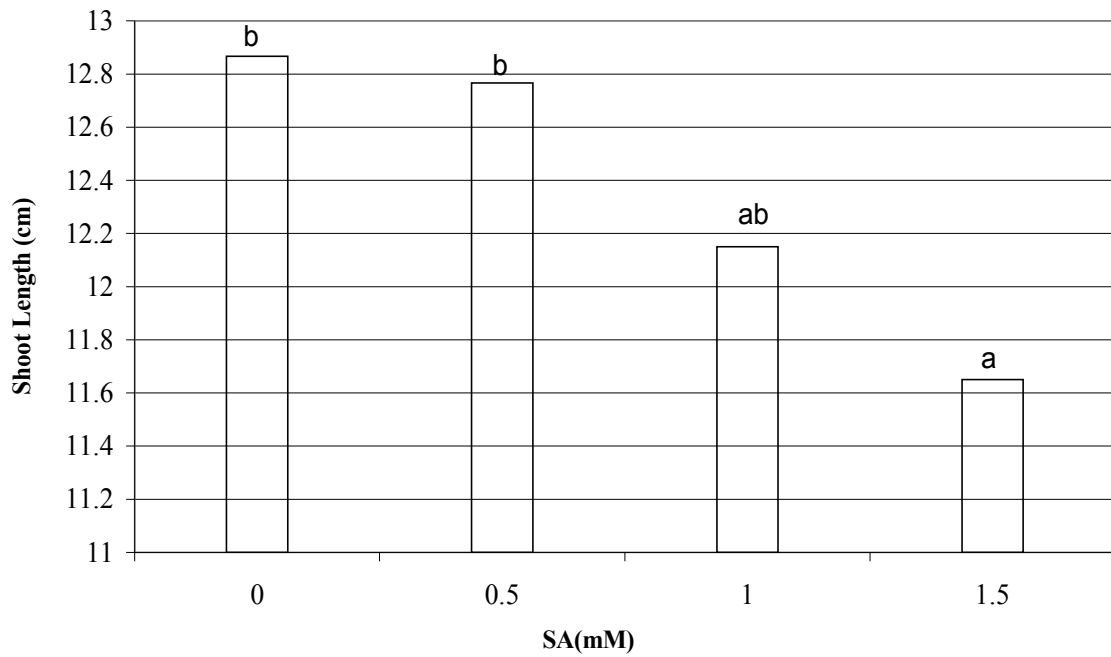


Fig.2. Effect of different SA concentrations on shoot length

SA 0 mM



SA 0.5 mM



SA 1 mM



SA 1.5 mM



0 mM 25mM 50mM 75mM 100mM

Fig.3. Effect of different treatment of NaCl and SA on tomato growth at end of treatment period

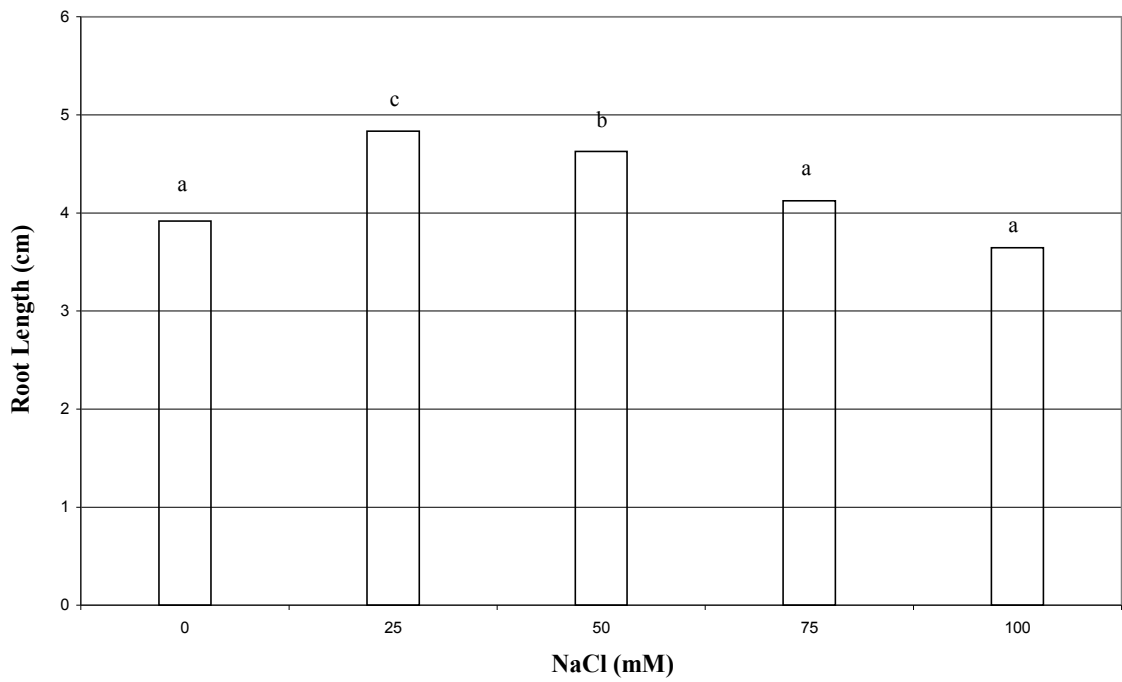


Fig.4. Effect of different NaCl concentrations on root length

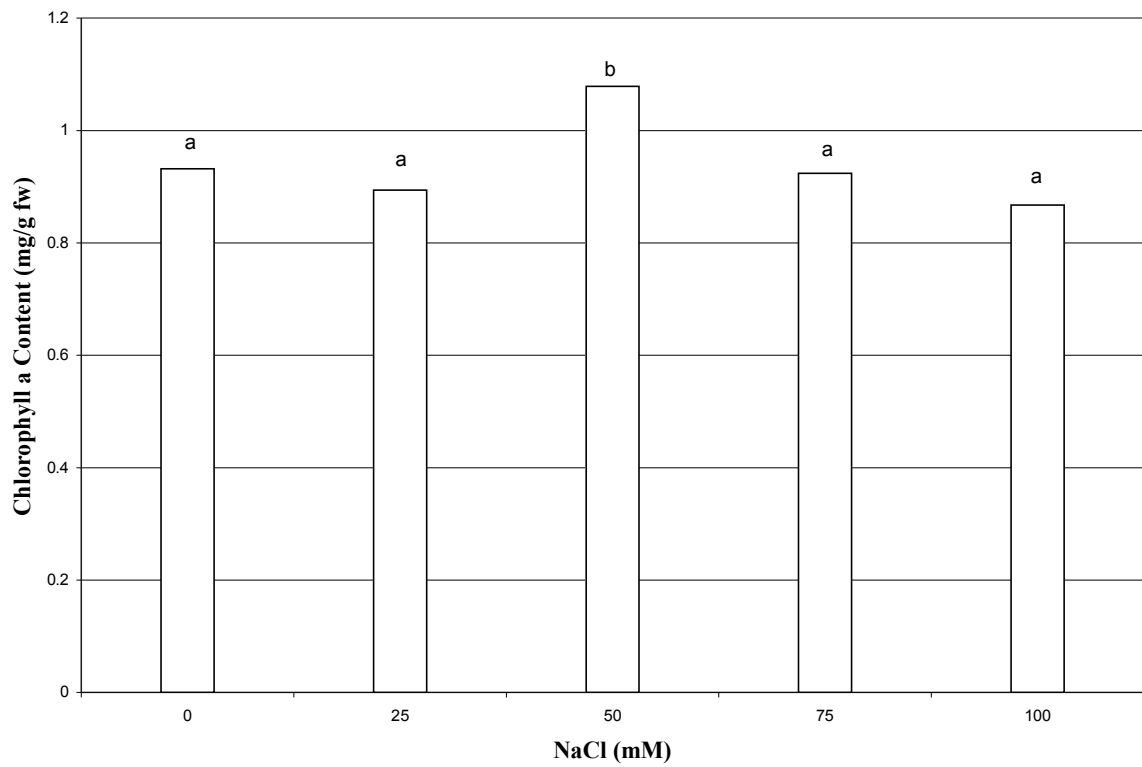


Fig.5. Effect of different NaCl concentrations on chlorophyll a content

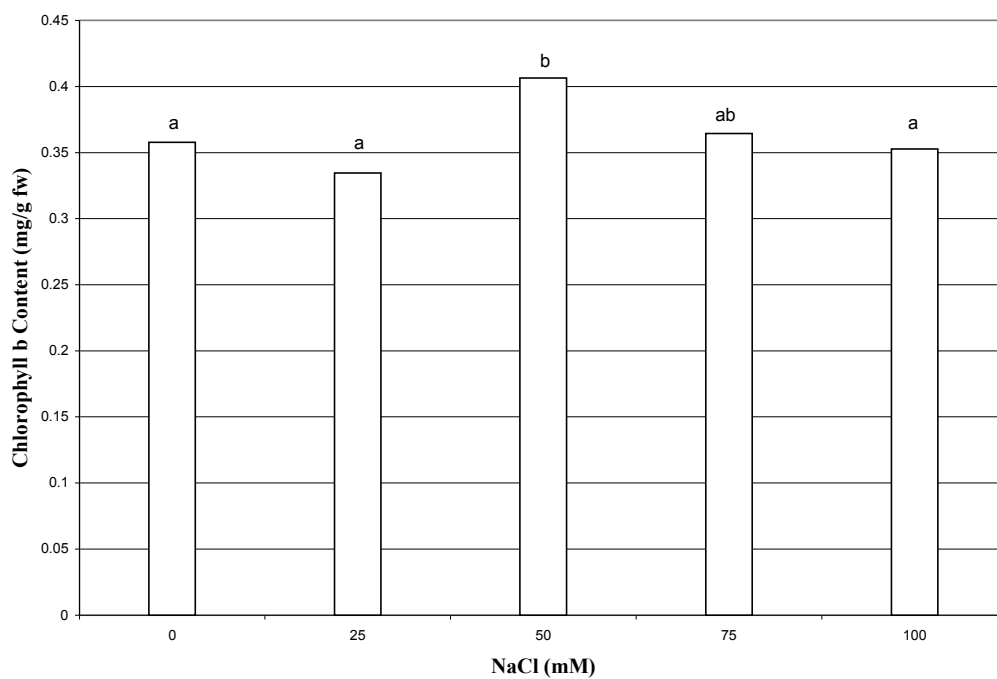


Fig.6a. Effect of different NaCl concentrations on chlorophyll b content

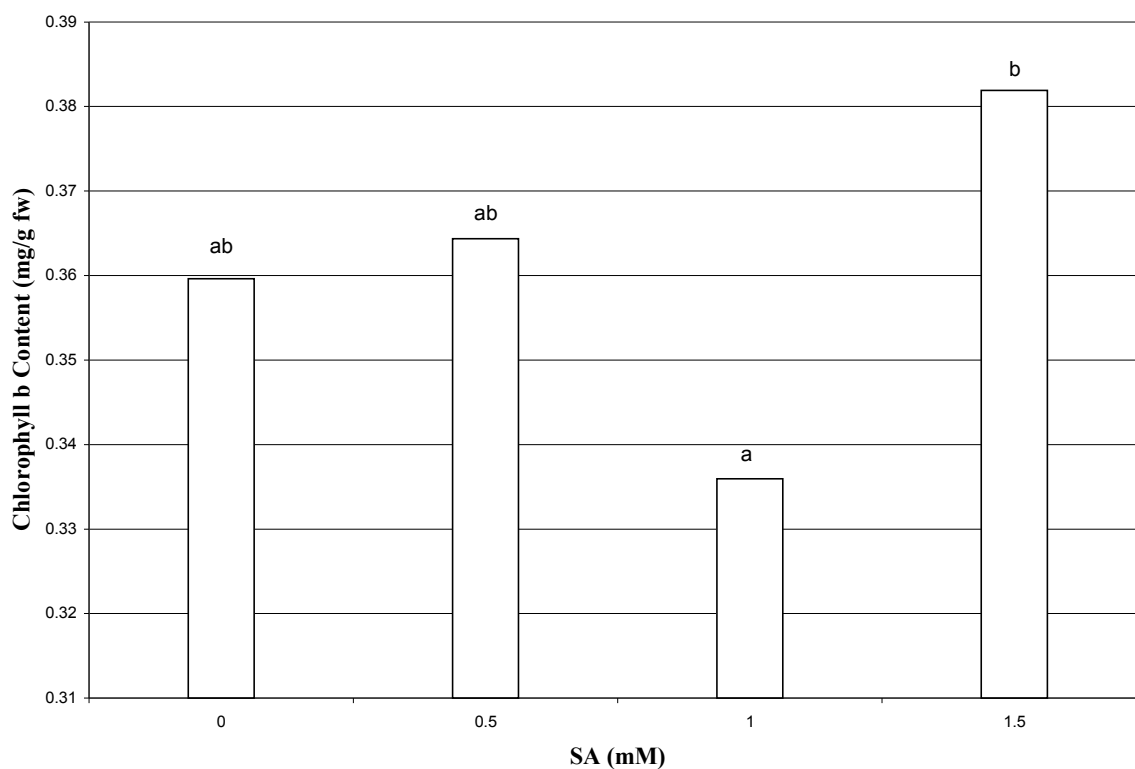


Fig.6b. Effect of different SA concentrations on chlorophyll b content

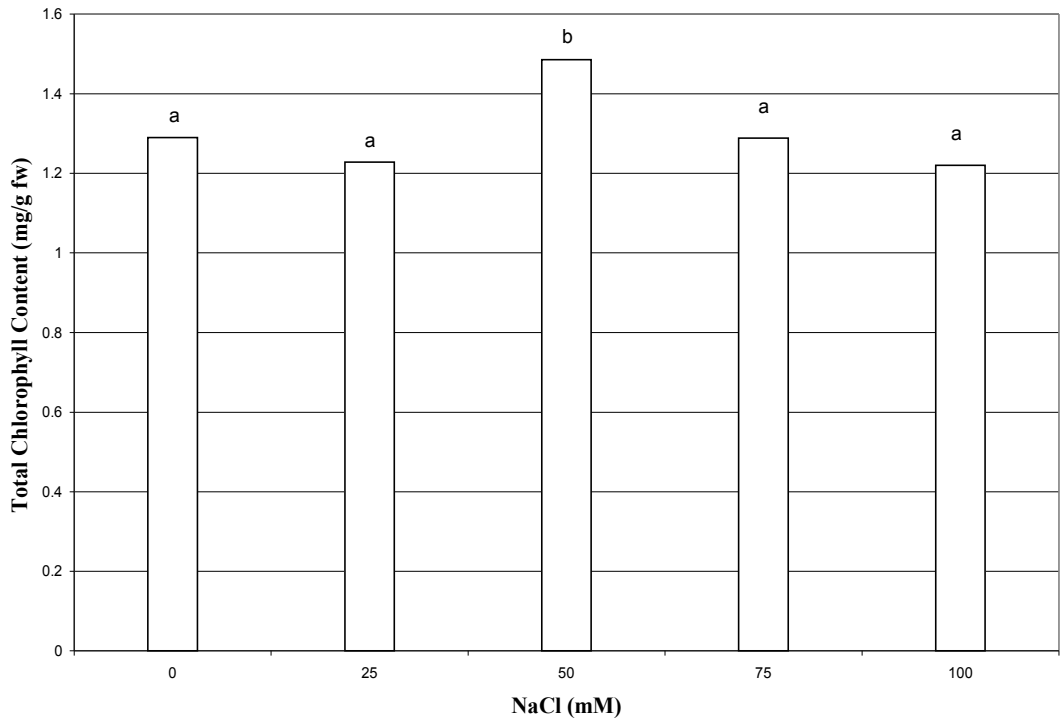


Fig.7a. Effect of different NaCl concentrations on total chlorophyll content

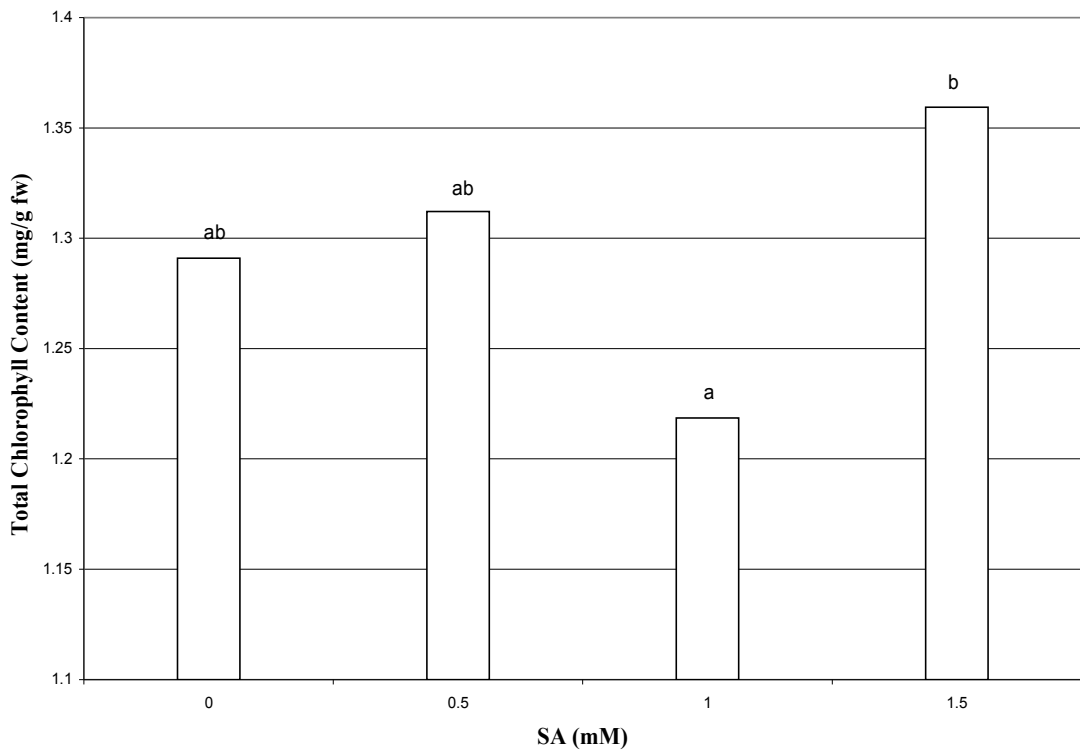


Fig.7b. Effect of different SA concentrations on total chlorophyll content

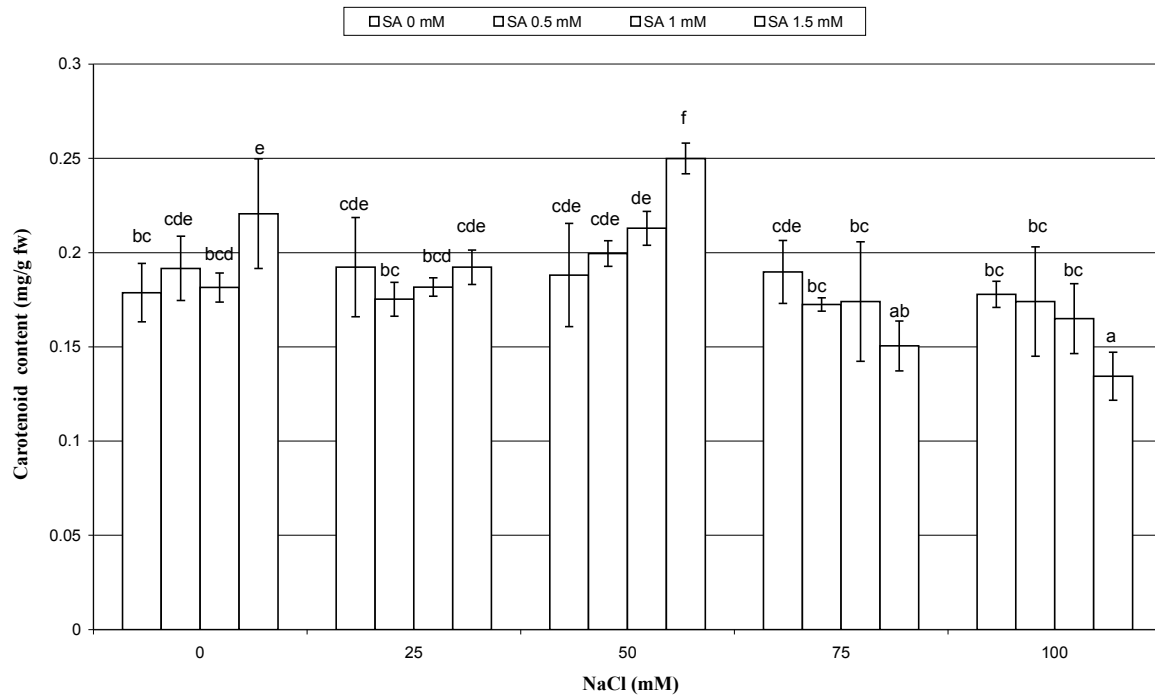


Fig.8. Effect of NaCl salinity and SA on carotenoid content

Carotenoid

Fig.8 shows that in high salinity levels (75 and 100 mM), carotenoid content decreased. 50 mM salinity level has the most carotenoid content. In 0 and 50 mM salinity levels, SA caused to increase and in 75 and 100 mM salinity levels caused to decrease carotenoid content.

Discussion

Germination

Salinity hinders seed germination. One of the most worldwide spread methods for determination of plant tolerance to salts is the germination percentage in salt solutions (dantas et al., 2005). Salt affects seed germination via osmotic stress (jamil et al., 2006). There are many reports about delay and decrease in various plants germination under salt stress (puppala et al., 1999) and (volkmar and miller, 2001). Study of various cultivar germination cowpea (*Vigna unguiculata* L.) under salt stress shows that

germination of Pitiúba, Epacé 10 and Canapu seeds decreased, respectively, 6.1, 10.7 and 25% when treated with NaCl 100 mol m⁻³ (dantas et al., 2005). This research shows that germination was decreased with salinity increasing, and SA decreased germination in 25 and 50 mM salinity levels. The most important enzymes in plant seed germination inductance is α -amylase that its action is influenced by salt stress. Ion such as Na⁺ bonds to active place of this enzyme leads to decreased activity and destroyed structure of this enzyme (saboury and karbassi, 2000). It is also assumed that in addition to toxic effects of certain ions, higher concentration of salt reduces the water potential in medium with hinders water absorption by germination seeds and thus reduces germination (jamil et al., 2006). SA is an effective inhibitor of ethylene biosynthesis and do it via effect on ACC-synthetase enzyme (Raskin et al., 1992). Since ethylene manner as a germination stimulator in many seeds are defined, the absorbed

decrease in germination percentage in this research may be result of ethylene biosynthesis inhibition. In study of salt effect on sugar beet, cabbage and amaranth, specific difference were absorbed in germination beginning and end times. SA is germination stimulator (sakhabutdinova et al., 2003). Other researches show that salicylates don't have increasing effect on germination but Pretreating seeds with salicylates such as acetylsalicylic acid, 2,6-dihydroxybenzoic acid and salicylic acid all promote germination at a limiting temperature of 5°C (Rajasekaran et al., 2002).

Shoot and root length

Salinity and water deficiency led to decrease plant metabolic activities and finally decrease plant growth (hossein et al., 2007). Exogenous salicylic application in S24 wheat cultivar, increase growth and neutralize salt stress while there are no increase in growth with SA application in MH-29 durum wheat cultivar (arfan et al., 2006). SA led to ABA level increase in plant under salt stress and decrease stress harmful effect on growth (inal ,2002). Salt stress leads to decrease root and shoot length in sugar beet and amaranth but root length is effected more than shoot length. The reduction in root and shoot development may be due to toxic effects of the NaCl used as well as unbalanced nutrient uptake by the seedlings (jamil et al., 2006). pretreatment of wheat plants with SA increased the level of cell division within the apical meristem of seedling roots which caused an increase in plant growth (sakhabutdinova et al., 2003). In this research only SA could have significant effect on shoot length so with SA increasing, shoot length decreased, that shows salinity in this concentration and during treatment couldn't effect on shoot length. Only salinity could have effect on root length so 25mM and 50mM salinity concentrations cause to shoot significant

increase. Salt stress leads to decrease of green been length and a pretreatment of SA, significantly increases it (Stanton, 2004). SA significantly increase plant growth either in stress and without stress conditions. This effect probably related to SA inhibition of Cl^- and Na^+ absorption and its help for Mg,Fe,Mn,N and Cu absorption or results from its effect on lipid peroxidation and membrane permeability (Gunes et al., 2007).

Pigment contents

Photosynthesis is a key line in plant that influence by salt stress. Salinity leads to decrease chlorophyll content that is because of increase chlorophylls activity and digression of metabolic machine toward producing nitrogen compound such as proline because glutamate is subscriber precursor of chlorophyll and proline biosynthesis and salt leads to more activity of proline synthesis line. Decrease of chlorophyll a content is more than chlorophyll b that showed more sensitive of chlorophyll a (Alpaslan et al., 1996). H_2O_2 and OH^- are responsible for injurious effect of salt stress on chlorophyll content and chloroplast ultra structure (Yamane et al., 2004). During water stress brought about by salt stress, reduction of chloroplast stromal volume and generation of reactive oxygen species (ROS) are also thought to play important roles in inhibiting photosynthesis (Borsani et al., 2001). SA cause to pigment increasing and in some plants high SA concentration cause to chlorophyll content decreasing. SA-pretreatment improved the photosynthetic efficiency of tomato plants under salt stress (Szepesi, 2006). salicylic acid enhanced photosynthetic rate.

However, chlorophyll did not altered in both soybean (a C3 plant) and corn (a C4 plant) (Hussein et al., 2007). SA-Pretreated plants maintained high levels of non enzymatic antioxidants, such as carotenoids and a polyamine, in the shoots under salt stress (Szepesi, 2006). In this research, the highest

amount of a,b and total chlorophylls were showed in 50 mM salinity level. 1 and 1.5 mM SA concentrations respectively cause to decrease and increase in chlorophyll amount. Carotenoid amount also was least in high salinity levels (75 and 100 mM) and highest carotenoid amount was in 50 mM salinity concentration and SA in all of salinity levels unless 50 mM leads to decrease in carotenoid amount. Stress with effect on lipo-oxygenase and peroxidase enzymes cause to chlorophyll catabolism induction and SA decrease activity of this enzymes. Decrease of chlorophyll content in high SA concentration probably is because of ABA accumulation. There are reports about beta-carotene degeneration because of salt stress (Trebitch et al., 1993).

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