# Effects of Response of Ocimum basilicum to the interactive effect of salicylic acid and salinity stress

**Maryam Delavari[1], Shekoofeh Enteshari[1]* and Khosrow Manoochehri Kalantari[2,3]**

*1. Biology Department, Payam Noor University, I. R. Iran*
*2. Biology Department, Shahid Bahonar University of Kerman, , Kerman, Iran*
*3. International Center for Science, High Technology & Environmental sciences, Kerman, Iran*

## Abstract

In this study, the role of salicylic acid pre-treatment (0.01, 0.1 mM was investigated) in inducing salt tolerance in sweet basil. Results showed germination percentage, length of shoot and root, fresh and dry weight of leaf, and photosynthetic pigments were decreased in response to high salinity but the lipid peroxidation, coefficient allometry, and soluble sugars increased. In plants pre-treated with salicylic acid (especially 0.01 mM) the adverse effects of salinity on all measured parameter were alleviated.

*Keywords:* salicylic acid; salinity; MDA; reducing sugars; photosynthetic pigments; fresh and dry weight; *Ocimum basilicum*

**Delavari, M., Sh. Enteshari** and **Kh. Manoochehri Kalantari.** 2014. 'Effects of Response of *Ocimum basilicum* to the interactive effect of salicylic acid and salinity stress'. *Iranian Journal of Plant Physiology* 4 (2), 983-990.

## Introduction

Salinity is the major stress factor which limits crop plants cultivation, especially in developing countries. The adverse effect of salinity on plants may lead to disturbances in plant metabolism, which consequently lead to a reduction of the plant growth and productivity (Greenway and Munns, 1980; Sharma and Hall, 1991; Allakhverdiev et al., 2000). The strategy for osmotic adjustment varies from one plant to another as well as in tissues (Shaddad et al., 1990; Dat et al., 1998).

During long-term exposure to salinity, plants experience ionic stress, which can lead to premature senescence of adult leaves due to accumulation of toxic $Na^+$ and $K^+$ depletion (Al-Karaki et al., 2000; Demiral et al., 2005). This results in a reduction in the photosynthetic area available to support continued growth (Sultana et al., 1999). Salt also affects photosynthetic components such as chlorophylls and carotenoids. Changes in these parameters depend on the severity and duration of stress (Misra et al., 1997) and on plant species (Dubey et al., 1994). High concentrations of salts also disrupt homeostasis in water potential and ion distribution in plants.

At the cellular level, osmotic stress causes alterations in membrane Lipid composition and properties. It has been postulated that at least part of the induced leakiness of membrane is caused by lipid

*Corresponding author
*E-mail address:* sh_enteshari@yahoo.com
Received: April,2013
Accepted: January, 2014

peroxidation resulting from uncontrolled ROS increase (Rodriques-Rosales et al., 1999). Measurement of thiobarbituric acid reacting substances (TBARs) concentration such as malondialdehyde (MDA) is routinely used as an index of lipid peroxidation under stress conditions (Gapinska et al., 2008).

Many trials have been made to help the plants to overcome these disturbances using various treatments in the laboratory, for future application in the field. It has been reported that the exogenous application of SA may influence a range of diverse processes in plants, including seed germination (Cutt and Klessig, 1992), stomatal closure (Larque-Saaveda, 1979), membrane permeability (Barkosky et al., 1993), and photosynthetic and growth rate (Khan et al., 2003). Khodary (2004) found that SA treatment increased the chlorophyll and carotenoids content in Maize plants. SA is also known as an important signal molecule for modulating plant responses to environmental stress (Senaratna et al., 2000)

The present study was conducted to assess if the exogenous application of SA could ameliorate the adverse effect of salinity on *Ocimum basilicum* plants. For this purposes germination, Growth Characteristics, lipid peroxidation, and carbohydrates in roots and shoots of sweet basil plants were measured.

## Materials and Methods

### Plant material and culture conditions

A pot experiment was conducted at the growth chambers in International center for science, High Technology and Environmental sciences, Kerman, Iran. Seeds of *Ocimum basilicum* (provided by Yasateb Research Center, Isfahan, Iran) were preliminary screened for germination soaked in 100 and 200 mM NaCl with various concentrations of salicylic acid (0,01, 0.1, 0, 0.5, 1, 1.5, 2, and 3 mM) to obtain the optimum response of germination. The concentrations of 0.01 and 0.1 mM were selected.

Salicylic acid was dissolved in deionized water and the pH was adjusted to 5.5 ± 0.2 with KOH (1 N). The seeds were soaked for 6 h in the dark at 25 °C, in SA 0.01 and 0.1 mM or in deionized water as control. Twenty-five seeds were then germinated in Petri dishes (9 cm diameter) provided with two filter paper saturated with deionized water, 100 and 200 mM NaCl. The seeds were kept under aseptic conditions for 1 day in the dark and 5 days in 16 h/8 h light/dark cycle, with a light intensity of 350 µmol $m^{-2}$ $S^{-1}$ and a relative humidity of 65 % at 25 ˚C.

Three replicates were prepared for each treatment. The seeds considered to be germinated after the radicle emerged from the caryopses. The final percentage of germination was recorded after a period of 5 days in those seeds that were germinated in deionized water. For pot experimented, seeds were sowed in plastic pot (15 Cm) filled with perlite and grown under controlled conditions (light/ dark regime of 16/8 h at 25/20 ˚C, relative humidity of 65 -75%, photosynthetic photon flux density of (PAR) 350 µmol $m^{-2}S^{-1}$) After germination, seedlings were watered regularly with half strength long Ashton solution. Irrigation was regularly done at intervals according to weather conditions to keep the moisture content of the soil to field capacity. Plants were foliar sprayed with 0.01 and 0.1 mM SA five times a day and control plants were sprayed with distilled water and the volume of the spraying solution was maintained just to cover completely the plant's foliage till drip. Then salt stress treatment was imposed by adding 100 and 200 mM NaCl in the pot for five days intervals, the control groups were just irrigated with water.

Plants were harvested after 7 days of salinity treatment. Plants were taken at random and divided into separate leaf and root fractions. The fresh leaves and shoots were weighed, and root lengths were measured. The samples were then dried in an oven at 70 ˚C for 72 h, and the dry weights were determined. At harvest, roots, shoots, and leaves were washed quickly with deionized water and blotted dried on filter paper and then stored –20 °C for further analyses.

## Chlorophyll extraction and measurement

Chlorophyll concentration was determined from fully expanded leaves. A fresh leaf sample of 0.1 g was ground and extracted with 5 ml of 8 % (V/V) acetone in the dark (Arnon et al., 1949). The mixture was filtered and absorbance was determined at 645, 663, and 450 nm (S2100 Arragy UV visible spectrophotometer). Concentration of chlorophyll a (Chl. a), chlorophyll b (Chl. b), and carotenoids were estimated by the equations of Wittham et al. (1971).

## Lipid peroxidation

A decomposition product of poly unsaturated fatty acids was utilized as a biomarker for lipid peroxidation (Mittler, 2002). The level of lipid peroxidation was quantified by measuring the amount of malondialdehyde (MD), which is determined by thiobarbituric acid (TB) reaction as described by Madhava Rao and Sresty (2000).

## Reducing sugars content

Reducing sugars content were measured based on Somogyi –Nelson (19532).

## Statistical analysis

All analyses were done on a completely randomized design and the data obtained were subjected to one-way analyses of variance (ANOVA) and the mean differences were compared by least significant differences (L. S. D test). The experiments were repeated twice with three replicates (n=3) with the probability level set at $p \leq 0.05$.

## Results

## Influence of SA on seed germination in saline medium

To document the influence of this molecule at the level of seed germination, in *Ocimum basilicum L.CV* seeds germinated under salt stress conditions with or without SA, addition of 200 mM NaCl reduced percentage of seed germination (Fig. I). Under normal conditions, maximum germination (94.66 %) was achieved in those seeds which were either primed with 0.1 mM salicylic acid or distilled water and this was statistically similar to all other remaining treatments. When seeds were treated with 200 mM NaCl, SA significantly improved germination (Fig. I).

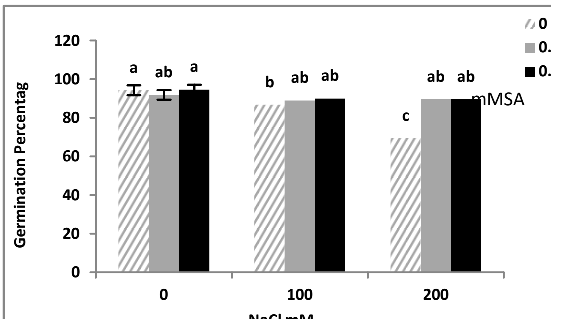


Fig. I. Effect of salicylic acid on seed germination percentage of *Ocimum basilicum* under saline conditions.

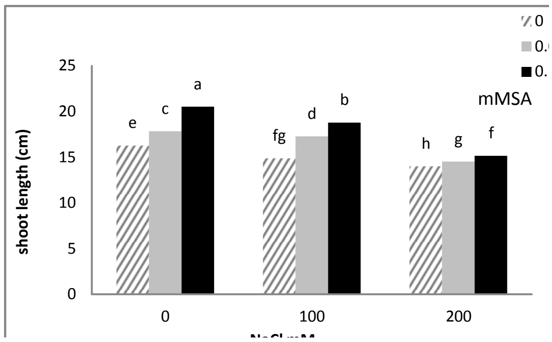


Fig. II. Effect of salicylic acid on shoot length of *Ocimum basilicum* under saline conditions.

## Growth parameters

Spraying with SA showed a significant effect on shoot and root lengths and ratio under saline conditions (Figs. II and III). Fig. (II) shows the effects of NaCl on shoot length of *Ocimum basilicum* under increasing salinity. Shoot length decreased as salinity increased and maximum shoot length was achieved in seeds primed with 0.1 mM salicylic acid.

Priming with 0.1 mM SA could only improve root length at 100 mM salinity but

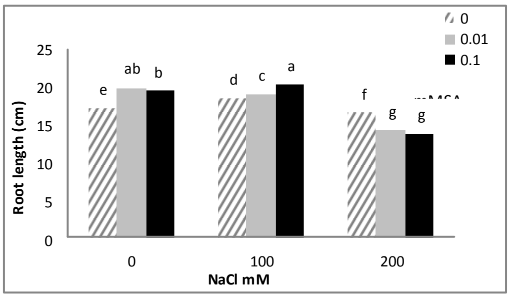


Fig. III. Effect of salicylic acid on root length of Ocimum basilicum under saline conditions.

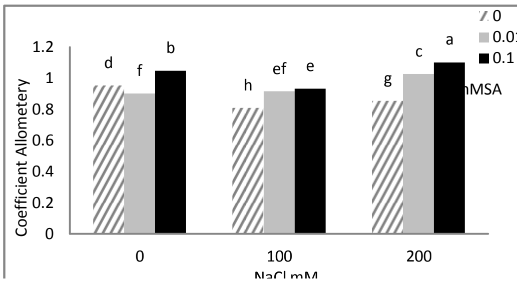


Fig. IV. Effect of salicylic acid on Coefficient Allometery of Ocimum basilicum under saline conditions.

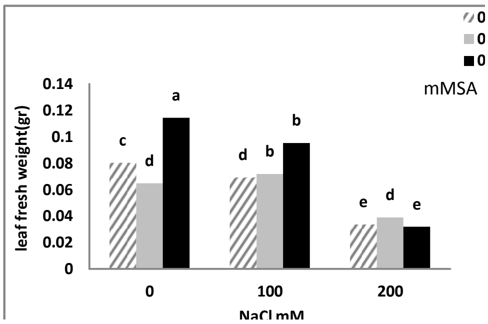


Fig. V. Effect of salicylic acid on leaf fresh weight of *Ocimum basilicum* under saline conditions.

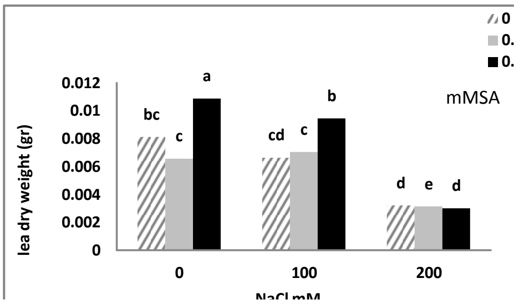


Fig. VI. Effect of salicylic acid on leaf dry weight of *Ocimum basilicum* under saline conditions.

priming with SA could not improve root length at 200 mM NaCl (Fig III). Root to shoot ratio (Coefficient allometer) was significantly increased in sweet basil when seedlings were sprayed with 0.1 mM salicylic acid (Fig. IV). Also, SA treatments had significant effects only on fresh and dry weight of leaf when salt concentration was not too much. Leaf fresh weight significantly increased at 100 mM salinity when seedlings were sprayed with 0.1 mM SA but in high salinity SA had no effect (Fig. V).

Maximum leaf dry weight was attained due to spraying with 0.1 mM SA which significantly increased leaf dry weight in those plants which were treated with 100 mM NaCl (Fig. VI).

## Photosynthetic pigments (Chlorophyll a, b and carotenoids) content

Analysis of variance of photosynthetic pigments (Chlorophyll a, b and carotenoids) is shown in (Figs VII, VIII, and IX. Salinity decreased all pigments and salicylic acid significantly increased photosynthetic pigments (Chlorophyll a and b carotenoids).

## Lipid peroxidation

The contents of lipid peroxidation in basil roots and shoots were assessed by MDA measurement. It was observed that salinity significantly increased MDA content in both root and shoot of basil plants. Treatment with SA, decreased lipid peroxidation based on MDA measurement only in the salt stressed plants. SA treatment decreased MDA content compared with the corresponding control (Figs. X and XI).

## Reducing sugars content

Reducing sugars content in leaf increased under 100mM NaCl treatment. But in plants treated with SA and 100 Mm NaCl, reducing sugars decreased significantly.

Reducing sugars content in roots of plants treated with 100 Mm NaCl increased significantly. On the other hand, in salt-stressed plants treated with SA, this parameter increased significantly (Fig. XIII). Finally, in plants only treated with SA, reducing sugars content of roots reduced significantly (Fig. XII).

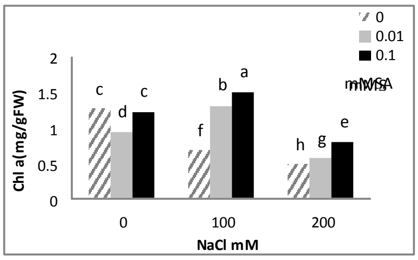


Fig.VII. Effect of salicylic acid on Chlorophyll a of *Ocimum basilicum* under saline conditions

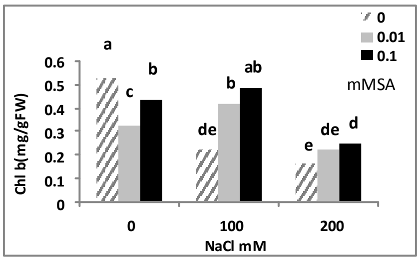


Fig.VIII. Effect of salicylic acid on Chlorophyll b of *Ocimum basilicum* under saline conditions

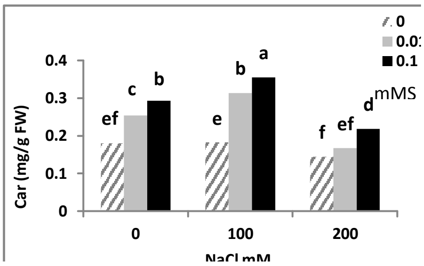


Fig.IX. Effect of salicylic acid on carotenoids of *Ocimum basilicum* under saline conditions

## Discussion

Seed germination is the most important stage in plant establishment in saline soils. In this study we observed significant difference in the seed germination rate of basil under 200 mM NaCl. These results are in agreement with those obtained by Sarin and Narayanan (1968) showing that in wheat, seed germination was significantly decreased by salinity (Sarin and Narayanan., 1968). It may be that NaCl reduced the rate of germination due to the reduced water potential and the resulting slower rate of imbibition (Afzal et al., 2006). In the present study, priming the seeds with various concentrations of salicylic acid proved to be effective in inducing salt tolerance

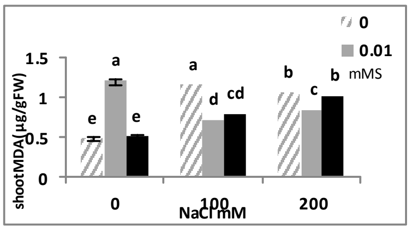


Fig.X. Effect of salicylic acid on shoot MDA of *Ocimum basilicum* under saline conditions

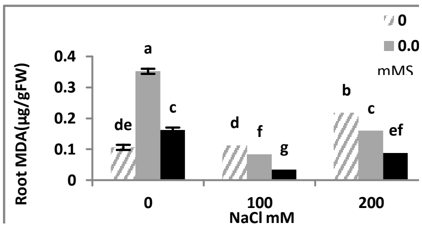


Fig. XI. Effect of salicylic acid on root MDA of Ocimum basilicum under saline conditions

at the seed germination stage in basil plants.

The results related to germination percentage can be compared to earlier finding. For example, El-Tayeb et al. (2005) found an improvement in germination of these seeds pre-treated with SA solution compared with un-treated seeds. These results are consistent with those of Rajasekaran et al. (2002) and Shakirova et al. (2003) who showed a promotion in seed germination with SA application.

Fresh and dry weights of leaf decreased drastically under salinity condition compared with non-saline condition. These results are similar to those reported by Kaydan et al. (2007) and Afzal et al. (2005) who found that dry weight was reduced by salt stress in wheat.

Dry weights of seedling was decreased due to salinity stress but in seedlings raised from seeds primed with SA dry weight was improved as compared to non SA treated plants under non salinity and salinity conditions. This may indicate that treatment of seedling with SA exhibited a significant increase in salt tolerance. This result was similar to the studies of El-Tayeb et al. (2005) who reported that SA pre-treatment increased dry weight in the stressed barley seedlings.

In our experiment under the influence of salinity the photosynthetic pigments greatly

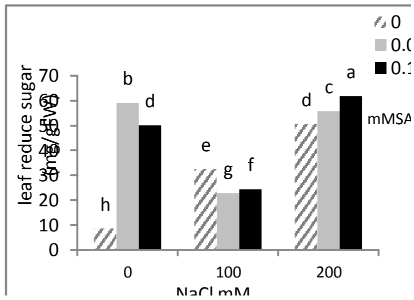


Fig.XII.Effect of salicylic acid on leaf reduce sugar of *Ocimum basilicum* under saline conditions.

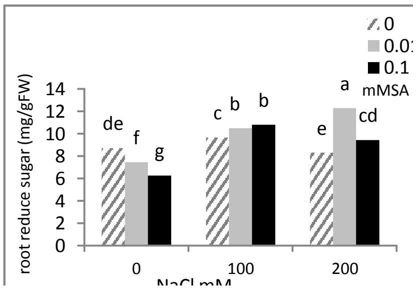


Fig.XIII. Effect of salicylic acid on root reduce sugar of Ocimum basilicum under saline conditions

decreased. Dela-Rosa and Maiti (1995) also observed that chlorophyll biosynthesis decreased in sorghum plants under salt conditions. Zhou et al. (1999) reported that photosynthetic pigments were increased in corn with SA application. Moreover, Khan et al. (2003) showed that SA increased photosynthetic rate in corn and soybean. In this experiment we also observed when the seedlings were sprayed with 0.1 mM SA solution, they exhibited higher chlorophyll content.

Salinity stress enhanced free radicals levels in plants when the damage of membranes was investigated by monitoring MDA content. The data showed that lipid peroxidation in both shoots and roots increased as the stress level raised. These results are in agreement with those of Bor et al. (2003) who found that salt stress increases the Lipid peroxidation in the leaves of two beet species. In this experiment spraying seedlings with SA led to a significant decrease in the level of lipid peroxidation in those seedlings which were under salinity stress (100 and 200 mM NaCl). Similarly, Tari et al. (2002) found a significant decrease in the concentration of TBA-reactive compounds of salinity stressed tomato plants. Similarly, Popova et al. (2003) showed that pre-treatment with SA decreased the level of lipid peroxidation induced by paraquat oxidative stress in barley plants.

Shakirova et al. (2003) showed that SA – induced increase in ABA might contribute to a pre-adaptation of plants to stress. In fact, ABA is known to have a key role in the induction of the synthesis of a range of stress proteins (Leung et al 1998).

*Ocimum* plants subjected to NaCl salinity showed a progressive increase in reducing sugar content as the salinity level increased. SA treatment is shown to cause a significant decrease in sugars content in the leaves associated with an increase in roots of normal and stressed plants. Likewise, Maria et al. (2000) and Khodary (2004) also reported a similar decrease in soluble sugar content associated with an increase in polysaccharides in NaCl-stressed maize plants with SA application. In this study there was also a possibility that SA application may activate the metabolic consumption of reducing sugars to form new cell constituents as a mechanism to stimulate the growth of Ocimum plants. It is also assumed that SA treatments in leaves may inhibit polysaccharide-hydrolyzing enzymes and/or accelerate the incorporation of sugars into polysaccharides.

## Conclusion

The differences observed in growth parameters under stress showed that *Ocimum basilicum* is tolerant to low salinity stress (100 mM NaCl). This species probably maintains the osmotic adjustment with accumulation of $Na^+$ indicated by $Na^-$ compartmentation of $Na^+$ and up–regulation of enzymatic anti-oxidant systems.

SA is also able to ameliorate the effect of salinity on this plant and can be used to improve production of agriculture plants under harsh conditions.

## References


**Aberg, B.** 1981. 'Plant growth regulators XLI Mono substituted benzoic acid' .*Swed .J . Agric .Res,* 11 :93-105.

**Afzal I., S. M. A. Basra, M. Faroog** and **A. Nawaz** .2006. ' Alleviation of salinity stress in spring wheat by hormonal priming with ABA, salicylic acid and ascorbic acid '. *Int J Agr Biol.* 1: 23-28.

**Afzal, I., S. Maqsood ,A. Basra , N. Ahmed and M. Farooq** .2005. 'Optimization of hormonal priming techniques for alleviation of salinity stress in wheat (*Triticum aestivum* L.) *'Caderno de Pesquisa Ser.Bio Santa Cruz do Sul* ,17.

**Al-Karaki G.N**. 2000. 'Growth, water use efficiency, and sodium and potassium acquisition by tomato cultivars grown under salt stress'. *J Plant Nutr* 23(1):1- 8.

**Allakhverdiev S.I., A. Sakamoto. Y. Nishiyama , M. Inaba** and **N. Murata** .2000.' Ionic and osmotic effects of NaCl-induced inactivation of photosystems I and II in *Synechococcus* sp'. *Plant Physiol*. 123: 1047-1056.

**Barkosky R.R.** and **F. A. Einhellig** .1993.' Effects of salicylic acid on plant water relationship'. *J . Chem .Ecol* .19 :237-247.

**Bor, M., F. Ozdemir** and **I. Turkan**. 2003. 'The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet (*Beta vulgaris* L.) and wild beet (*Beta maritime* L. ).*Plant Sci.* 164: 77-84.

**Cutt J.R.** and **D. F. Klessig** .1992.' Salicylic acid in plants :A changing perspective'. *Pharmaceu . Technol* .16 :25-34.

**Dash, M.** and **S. K. Panda** .2001. 'Salt stress induced changes in growth and enzyme activities in germination *Phaseolus mungo* seeds'. *Biol Plant* 44(4): 587-589.

**Dat J.F., H. Lopez-Delgado , C.H. Foyer** and **I.M. Scott** .1998. 'Parallel changes in $H_2O_2$ and catalase during thermo-tolerance induced by salicylic acid or heat acclimation in mustard seedlings'. *Plant Physiol*,116: 1351-1357.

**Dela-Rosa I.M.** and **R. K. Maiti** .1995.' Biochemical mechanism in glossy sorghum lines for resistance to salinity stress'. *J Plant Physiol*. 146:515–519.

**Demiral T.** and **I. Turkan** .2005. 'Comparative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance'. *Environ. Exp. Bot*. 53: 247-257.

**Dubey R. S.** 1994. 'Protein synthesis by plants under stressful conditions'. In: Pessaraki M, (Ed), Hand book of plant and crop stress. Marcel Dekker, New York : 277-299.

**El-Tayeb MA.** 2005.' Response of barley grains to the interactive effect of salinity and salicylic acid '.*Plant Growth Regul*. 45: 215 – 224.

**Gapinska M.** and **M. Sklodowska** .2008. 'Effect of short- and long-term salinity on the activities of antioxidative enzymes and lipid peroxidation in tomato roots'. *Acta Physiol Plant* 30: 11-18.

**Greenway H.** and **R. Munns** .1980.' Mechanism of salt tolerance in non-halophytes' *.Annu Rev. Plant Physiol.* 31:149-190.

**Kaydan D, M. Yagmur** and **N. Okut** .2007.' Effects of salicylic acid on the growth and some physiological characters in salt stressed wheat (*Triticum aestivum* L.). *Tarim Bilimleri Dergisi* ,13 (2): 114-119.

**Khan W, B. Prithiviraj** and **P. Smith** .2003. 'Photosynthetic responses of corn and soybean to foliar application of salicylates'. *J Plant Physiol*.20: 1-8.

**Khodary ,SEA** .2004.' Effect of salicylic acid on the growth, photosynthesis and carbohydrate metabolism in salt-stressed maize plants'. *Int J Agri. Biol.* 6: 5-8.

**Larque-Saavedra, A**. 1978.'The anti -transpirant effect of acetylsalicylic acid on *Phaseolus vulagaris* L '.*Physiol. Plant*. 43 :126-128.

**Leung J.** and **J. Giraudat** .1998.' Abscisic acid signal transduction'. *Annu Rev Plant Mol Biol* 49: 199-222.

**Madhava Rao K.V.** and **T.V.S. Sresty** .2000. 'Antioxidative parameters in the seedlings of pigeon pea *(Cajanus cajan* L. *Millspaugh)* in response to Zn and Ni stresses'. *Plant Sci* .157: 113-128.

**Maria EB, D.A. José , C.B. Maria** and **P.A. Francisco** .2000. 'Carbon partitioning and sucrose metabolism in tomato plants growing under salinity'. *Physiol .Plant.* 110 :503-511.

**Misra A.N., S. M. Sahu , M. Mishra, P. Singh ,I. Meera , N. Das, M. Kar** and **P. Sahu** .1997.

'Sodium chloride induced changes in leaf growth and pigment and protein contents in two rice cultivars'. *Biol Plant* 39: 257-262.

**Popova L., E. Ananieva , V. Hristova , K. Christov , K. Georgieva , V. Alexieva** and **Z.H. Stoinova**. 2003.' Salicylic acid-and metyl jasmonate-induced protection on photosynthesis to paraquat oxidative stress' . *Bulg .J Plant Physiol.* Special Issue 133–152.

**Rajasekaran L.R., A. Stiles , M. A. Surette, A. V. Sturz, T.J. Blake, C. Caldwell** and **J. Nowak** .2002. 'Stand establishment technologies for processing carrots: Effects of various temperature regimes on germination and the role of salicylates in promoting germination at low temperatures'. *Canadian J of Plant Sci*, 82: 443-450.

**Raskin, I** .1992. 'Role of salicylic acid in plants'. *Annu Rev Plant Physiol Plant Mol Boil* 43: 463 – 739.

**Rodriquez-Rosales M.P., L. Kerkeb, B. Bueno** and **J. P. Donaire**. 1999. 'Changes induced by NaCl in lipid content and composition, lipoxygenase, plasma membrane $H^+$-ATPase and antioxidant enzyme activities of tomato *(Lycopersicum esculantum* Mill*)* calli'. *Plant Sci.* 143: 143-150.

**Sarin M.N.** and **A. Narayanan** . 1968. 'Effects of soil salinity and growth regulators on germination and seedling metabolism in wheat'. *Physiol Plant* 21: 1201-1209.

**Senaratna T., D. Touchell, E. Bunn** and **K. Dixon** .2000.' Acetyl salicylic acid (Asprin) and salicylic acid induce multiple stress tolerance in bean and tomato plants' *.Plant Growth Regul.* 30 :157-161.

**Shaddad M. A., A. F. Radi ,A. M. Abdel-Rahman** and **M.M. Azooz** .1990.' Response of seeds of *Lupinus termis* and *Vicia faba* to the interactive effect of salinity and ascorbic acid or pyridoxine'. *Plant Soil* ,122 :177-183.

**Shakirova F.M.,A. R. Sakhabutdinova ,M.V. Bozrutkova, R. A. Fatkhutdinova** and **D. R. Fatkhutdinova** .2003. 'Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity'. *Plant Sci*. 164: 317-322.

**Sharma, P.K.** and **D. O. Hall**. 1991' .Interaction of salt stress and photoinhibition on photosynthesis in barley and sorghum'.*J . Plant Physiol* .138,614-619.

**Sultana N., T. Ikeda** and **R. Itoh** .1999. 'Effect of NaCl salinity photosynthesis and dry matter accumulation in developing rice grains'. *Environ Exp. Bot*. 42(3):211-220.

**Tari I,J. Csiszar, G. Szalai, F. Horvath, A. Pecsvaradi , G. Kiss, A. Szepsi, M. Szabo** and **L. Erdei** .2002.'Acclimation of tomato plants to salinity stress after a salicylic acid pre-treatment'. *Acta Biologica Szegediensis* ,46 (3-4): 55-56.

**Witham F.H.,D. R. Blaydes** and **R. M. Devlin** .1971. 'Experiments in plant physiology'. Van Nostrand Reinhold New York 1-11.

**Zhou X.M., A. F. Mackenzie, C. A. Madramootoo** and **D. L. Smith** .1999. Effects of stem-injected plant growth regulator, with or without sucrose, on grain production, biomass and photosynthetic activity of field-grown corn plants'. *J Agronomy and Crop Sci* 183:103-110.