

INFLUENCE OF SALICYLIC ACID & HUMIC ACID ON SALINITY STRESS TOLERANCE
DURING SEED GERMINATION OF (*LENS CULINARIS* MEDIK)

Hamide Fatemi¹, Atefe Ameri¹, Samane Mohammadi¹, Alireza Astarvae²

1- Ferdowsi university of Mashhad, Agriculture Faculty, horticulture Science Department, Mashhad, Iran

2- Associate Professor, Department of Soil Science, College of Agriculture, Ferdowsi University of Mashhad, Iran

ABSTRACT: Optimal germination and plant establishment is an important problem for agricultural productivity in arid and semi-arid areas. Priming is an approach for increasing plant establishment in undesirable conditions. In order to evaluate effects of different levels of humic acid and salicylic acid on root and shoot development of *Lens culinaris* Medik, an experiment was conducted in 2012 at Faculty of Agriculture, Ferdowsi University of Mashhad. Results showed that humic acid and salicylic acid significantly improvement factors under salinity. All salinity levels decreases the all factors. The results of this study reveal that various concentrations of SA and HU had significant effects on germination percentage and radicle.

KEYWORD: Salt stress, Growth, Lentil, Radicle, Priming.

INTRODUCTION

Plants, growth and production are affected by natural stresses in the form of biotic and abiotic stresses, inversely. The abiotic stress causes loss of hundred million dollars annually, because of reduction and loss of products ([Mahajan and Tuteja, 2005](#)). Stress consists of water stress, salinity, low temperature stress and etc. Salinity is one of main problem in Iran and others country. The high salinity of the soil affected the soil penetration, decreased the soil water potential and finally caused physiological drought ([Yusuf et al., 2007](#)). Salinity levels can adversely affect agronomic and physiological attributes in crops ([Cicek and Cakirlar, 2007](#); [Gómez-Pando et al., 2010](#)). Among many approaches/strategies used to combat salinity stress, exogenous application of plant growth regulators has received considerable attention ([Afzal et al., 2005](#); [Dolatabadian et al., 2008](#)). There are a lot of chemical compound for decreasing effect of stress.

Salicylic acid (SA) is known as an endogenous growth regulator of phenolic type distributing in a wide range of plant species, which induces biotic and abiotic stress tolerance in crops ([Conrath et al., 2002](#); [Nowak and Pruski, 2004](#); [Pal et al., 2006](#); [Hayat et al., 2007](#); [Horvath et al., 2007](#); [Janda et al., 2007](#); [Sakhanokho and Kelley, 2009](#); [Joseph et al., 2010](#); [Ghorbani Javid et al., 2011](#)). The role of salicylic acid in seed germination ([Cutt and Klessig, 1992](#)), enzymatic activity ([Dolatabadian et al., 2008](#)),

photosynthetic rate ([Khan et al., 2003](#)), uptake and transport of ions ([Harper and Balke, 1981](#); [Afzal et al., 2005](#)), and plant growth and yield ([Hussein et al., 2007](#)) have been described. Salicylic acid is mediated in photosynthesis ([Khan et al., 2003](#); [Cag et al., 2009](#)), transpiration, stomatal regulation, nutrient uptake and transport ([Gunes et al., 2005](#)), flowering, inhibition of fruit.

Humic acid is a major element of humic substances which are the main organic constituents of soil, (humus), peat, coal and ocean water. HA present a random polymeric, amorphous structure formed by polyaromatic building blocks bridged to each other by ester, ether and C links and carrying variable parts of carboxyl, hydroxyl, amino and other hydrophilic groups ([Andreux, 1996](#)). Humic substances (humic and fulvic acids) constitute 65.70% of the organic matter in soils, and are the subject of study in various areas of agriculture, such as soil chemistry, fertility, and plant physiology as well as environmental sciences, because the multiple roles played by these materials can greatly benefit plant growth ([Knicker et al., 1993](#); [Friedel and Scheller, 2002](#)). [Bohme and ThiLua \(1997\)](#) reported that humic acid had beneficial effects on nutrient uptake by plants, and was particularly important for the transport and availability of micro- nutrients. The effects of humic acids derived from different organic wastes on seedling growth of tomato in some growth media were investigated by [David et al.,](#)

(1994); Loffredo *et al.*, (1997) and Atyeh *et al.*, (2002). In order to determine the effect of Salicylic acid and humic acid on factors of lentil (*Lens esculenta* moench) an experiment was conducted. This variety cultured in Iran very much.

MATERIAL AND METHODS

This experiment was carried out at the Department of Horticulture, Faculty of Agriculture, and University of Mashhad, Iran. Adas seed were obtained from Native variete of Mashhad. Healthy seeds of similar size were used. The experiment was arranged in a completely randomized design (CRD) with three replication and 20 seeds per replication, in two steps. Seed were soaked in solutions of 2 and 4 mM or salicylic acid and 500 and 1000 ppm for 12 hours in dark. Seed were washed three times in distilled water. Then, seeds were germinated in Petri dishes (9 cm diameter) on double layers of filter paper (whatman). Level of salinity were 0(control), 2, 4, 6 dS m⁻¹ of NaCl (99.1%) salt. The germination data were recorded for a

continuous period of seven days. The treatment include: percentage germination (G%), fresh weight (FW), dry weight (DW) and length of radical(LR), and length of stem. Statistical analyses were performed using PROC GLM of SAS (SAS Inst. Inc., 1996) to determine treatment effects. When F value was significant, a multiple means comparison was performed using LSD tests at a P value of 0.05.

RESULT AND DISCUSSION

Analysis of variance of factors showed that significant differences existed in G, LR but not in LS, FW, and DW by Salicylic acid. G, LR, DW and LS were affected by level of salinity and the significant SA* Salinity effect was observed on all factors (Table 1).

The statistically evaluated results of all factor were presented in table 2 .Results obtained indicated effect of HU was significant differences for G, LR, and LS. Salinity and interaction of HU* Salinity effect were observed on all factors.

Table1: Analysis of variance traits under study

SV	DF	M.S				
		G%	LR	LS	FW	DW
SA	2	20.52*	4.18*	0.33 ^{ns}	0.001 ^{ns}	0.001 ^{ns}
salinity	3	1568.55**	25.58**	2.81**	0.002 ^{ns}	0.003 ^{ns}
SA*salinity	6	85.27**	2.65*	2.44**	0.002*	0.002*
Error	24	21.91	0.85	0.030	0.000	0.002

Ns: no significant

*: significant at the 0.05 level of probability according to LSD test.

** : significant at the 0.01 level of probability according to LSD test.

DW: dry weight, **FW** fresh weight, **LR:** length of radicle; **LS:** length of stem, **G:** germination percentage

Table2: Analysis of variance traits under study

SV	DF	M.S				
		G%	LR	LS	FW	DW
HU	2	309.8**	6.37**	6.13**	0.002 ^{ns}	0.0006 ^{ns}
salinity	3	991.2**	14.27**	3.30**	0.0057**	0.003*
HU*salinity	6	42.33**	3.09**	2.52**	0.005**	0.002*
Error	24	18.26	0.94	0.28	0.001	0.002

Ns: no significant

*: significant at the 0.05 level of probability according to LSD test.

** : significant at the 0.01 level of probability according to LSD test.

DW: dry weight, **FW** fresh weight, **LR:** length of radicle; **LS:** length of stem, **G:** germination percentage

All factor decrease with increase of salt in two experiments. All salinity levels decreases the radicle length, however in radicle length production the highest radicle length was obtained by control in two experiments. But salicylic acid and Humic acid causes balance and increases. The results also revealed that humic acid was the best treatment the root length and 1000 ppm humic acid had the radicle length (6.03cm), and salicylic acid (4.89cm). The interaction effect of salt stress and Humic Acid and salicylic acid highly significant in radicle length and was showed in figure 1 and 2

respectively. Root length is one of the most important characters for stress because roots are in contact with soil and absorb water from soil. For this reason, root length provides an important clue to the response of plants to stress.

The concentration of salt showed that there were significant differences among the stem length. The highest stem length was found in control in both experiments. The concentrations of acid salicylic revealed that there was no significant difference among the stem length, but it showed that there was a significant difference

in concentration humic acid. The highest length was found in 500 ppm humic acid treatment. Interaction HU and SA with salt stress also had great influences on the stem length, with the increase in salt stress, stem length were also increased in 500 ppm HU* 6 dS m⁻¹ NaCl and 2mM AC* 6 dS m⁻¹, respectively.

Salt stress caused a significant reduction in shoot fresh weights. But, growth was not appreciably promoted due to exogenous application of salicylic acid and Humic Acid under both salt-stressed and non-stressed conditions. The interaction effect of salt stress and Humic Acid and salicylic acid highly significant in fresh weights, and highest fresh weight was recorded in control *2mM SA and control*1000 ppm HU.

The results of this study reveal that various concentrations of SA and HU had significant effects on germination percentage. It was observed that, there was a decrease in germination percentage due to salinity stress increment and maximum germination percentage was delayed. The highest and the lowest germination percentage were observed in 500 ppm HU, 4mM AC and control, respectively. Under conditions of the highest salinity stress was not recorded between two concentrate of HU, but 2mM of SA was showed highest germination percentage. But HU is better than SA in the highest salinity. According to a fact, decrease of seed germination under stress conditions is due to occur of some metabolic disorders.

The positive effect of SA on morphological attributes such as multiplication rate and fresh weight of shoot tips is of interest, as some reports on in vitro water stress tolerance studies suggest that multiplication rate and fresh weight not only adversely affected by water stress but also positively correlate with drought tolerance ([Mohamed *et al.*, 2000](#); [Gopal and Iwama, 2007](#)). Humic acid application to plant growth media increased the growth of both shoots and roots significantly. Our results were in agreement with those reports.

Moreover, the positive influences of humic acids on plant growth and productivity, which seem to be concentration-related, could be mainly due to hormone-like activities of the humic acids through their involvement in cell respiration, photosynthesis, oxidative phosphorylation, protein synthesis, antioxidant and various enzymatic reactions. The effects of humic acid application on ion uptake appear to be more or less selective and variable, relative to their concentration. With calcium, applications affected N, Ca and S contents of shoot, and N and

K contents of root, but the effects of humic acid application were more pronounced than those of calcium application in both seedling performance and plant nutrient uptake.

REFERENCES

- Afzal I, Basra SMA, Ahmad N Farooq M. Optimization of hormonal priming techniques for alleviation of salinity stress in wheat (*Triticum aestivum* L.). *Caderno de Pesquisa Ser. Bio., Santa Cruz do Sul* 2005;17:95-109.
- Andreux F. Humus in world soils. In: Piccolo A. (Ed.). *Humic substances in terrestrial ecosystems*. Elsevier, Amsterdam, the Netherlands 1996;pp:45-100.
- Atiyeh RM, Edwards CA, Metzger JD, Lee S, Arancon NQ. The influence of humic acids derived from earthworm-processed organic wastes on plant growth. *Bioresource Technology* 2002;84:7-14.
- Bohme M, ThiLua H. Influence of mineral and organic treatments in the rhizosphere on the growth of tomato plants. *Acta Horti* 1997;450:161-168
- Cag S, Cevahir-oz G, Sarsag M, Goren-Saglam N. Effect of Salicylic acid on pigment, protein content and peroxide activity in excised sunflower cotyledons. *Pak J Bot* 2009;41(5):2297-2303.
- Cicek N, Cakirlar H. Effects of salt stress on some physiological and photosynthetic parameters at three different temperatures in six soya bean (*Glycine max* L. Merr.) cultivars. *J Agron Crop Sci* 2007;194:34-36.
- Conrath U, Pieterse CMJ, Mauch - Mani B. Priming in plant - pathogen interactions. *Trends Plant Sci* 2002;7(5):210-216.
- Cutt JR, Klessig DF. Salicylic acid in plants. A changing perspective. *Pharmaceutical Tech* 1992;16:25-34
- David PP, Nelson PV, Sanders DC. Humic acid improves growth of tomato seedlings in solution culture. *J Plant Nutri* 1994;17:173-184.
- Dolatabadian A, Sanavy SAMM Chashmi NA. The effects of foliar application of ascorbic acid (vitamin C) on antioxidant enzymes activities, lipid peroxidation and proline accumulation of canola (*Brassica napus* L.) under conditions of salt stress. *J Agron Crop Sci* 2008;194:206-213.
- Friedel JK, Scheller E. Composition of hydrolysable amino acids in soil organic matter and soil microbial biomass. *Soil Biol Biochem* 2002;34:315-325.
- Ghorbani Javid M, Sorooshzadeh A, Moradi F, Modarres Sanavy SAM, Allahdadi I. The role

- of phytohormones in alleviating salt stress in crop plants. *Aust J Crop Sci* 2011;5(6):726-734.
- Gómez-Pando LR, Álvarez-Castro R, Eguiluz-de la Barra A. Effect of Salt Stress on Peruvian Germplasm of *Chenopodium quinoa* Willd.: A Promising Crop. *J Agron Crop Sci* 2010;196:391-396
- Gopal J, Iwama K. In vitro screening of potato against water stress mediated through sorbitol and polyethylene glycol. *Plant Cell Rep* 2007;26:693-700.
- Gunes A, Inal A, Alpaslan M, Cicek N, Guneri E, Eraslan F, Guzelordu T. Effects of exogenously applied salicylic acid on the induction of multiple stress tolerance and mineral nutrition in maize (*Zea mays* L.). *Arch Agro Soil Sci* 2005;51:687-695.
- Harper JP, Balke NE. Characterization of the inhibition of K⁺ absorption in oat roots by salicylic acid. *Plant Physiol* 1981;68:1349-1353.
- Hayat S, Ali B, Ahmad A. Salicylic acid: Biosynthesis, metabolism and physiological role in plants. Springer, Dordrecht, The Netherlands 2007;pp:1-14.
- Horvath E, Szalai G, Janda T. induction of abiotic stress tolerance by salicylic acid signalling. *J Plant Growth Reg* 2007;26:290-300.
- Hussein MM. Balbaa LK, Gaballah MS. Salicylic acid and salinity effects on growth of maize plants. *Res. J Agric Biol Sci* 2007;3:321-328.
- Janda T, Horvath E, Szalai G, Paldi E. Role of salicylic acid in the induction of abiotic stress tolerance. In salicylic acid a plant hormone. Springer link 2007;pp:295.
- Joseph B, Jini D, Sujatha S. Insight into role of exogenous salicylic acid on plants growth under salt environment. *Asian J Crop Sci* 2010;2(4):226-235.
- Khan W, Prithviraj B, Smith DL. Photosynthetic responses of corn and soybean to foliar application of salicylates. *J Pl Physiol* 2003;160:485-492.
- Knicker H, Frund R, Ludemann HD. The chemical nature of nitrogen in native soil organic matter. *Naturwissenschaften* 1993;80:219-221.
- Loffredo E, Senesi N, Dorazio V. Effects of humic acids and herbicides, and their combinations on the growth of tomato seedlings in hydroponics. *Zeitschrift Fur Pflanzenernahrung und Bodenkunde* 1997;160:455-461.
- Mahajan S, Tuteja N. Cold, Salinity and Drought stresses: An overview. *Biochem Biophys* 2005;444:139-158.
- Mohamed MAH, Harris PJC, Henderson J. In vitro selection and characterisation of a drought tolerant clone of *Tagetes minuta*. *Plant Sci* 2000;159:213-222.
- Nowak J, Pruski K. Priming tissue cultured propagules. In Low cost options for tissue culture technology in developing countries. Proceedings of a technical meeting organized by the joint FAO/IAEA division of nuclear techniques in food and agriculture, Vienna, Australia 2004;pp:68-81.
- Pal M, Horvath E, Janda T, Paldi E, Szalai G. Physiological changes and defense mechanisms induced by cadmium stress in maize. *J Plant Nutr Soil Sci* 2006;169:239-246.
- Sakhanokho HF, Kelley RY. Influence of salicylic acid on in vitro propagation and salt tolerance in *Hibiscus acetosella* and *Hibiscus moscheutos* (cv 'Luna Red'). *Afr J Biotechnol* 2009;8(8):1474-1481.
- Shakirova FM, Sakhabutdinova AR, Bezrukova MV, Fathudinova RA, Fathudinova DR. Changes in hormonal status of wheat seedlings induced by Salicylic acid and salinity. *Plant Sci* 2003;164:317-322.
- Yusuf M, Hasan SA, Ali B, Hayat S, Fariduddin Q, Ahmad A. Effect of salicylic acid on salinity induced changes in *Brassica juncea*. *J Integr Plant Biol* 2007;50(9):1096-1102.