Journal of Stress Physiology & Biochemistry, Vol. 7 No. 1 2011, pp. 55-65 ISSN 1997-0838

ORIGINAL ARTICLE

EFFECT OF DROUGHT STRESS AND ITS INTERACTION WITH ASCORBATE AND SALICYLIC ACID ON OKRA (*Hibiscus esculents* L.) GERMINATION AND SEEDLING GROWTH

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Received January 28, 2011

Effect of drought stress was investigated at water potentials of-0.2 &-0.4 MPA (by using PEG 6000) on okra germination and seedling growth. In this study, percent germination, average time necessary for germination in day, radical and plumule length, fresh and dry weight of okra seedlings were measured. Effect of ascorbate and salicylic acid and interaction of these tow substances were investigated on germination and seedling growth under normal condition and dry conditions. Drought inhabits from germination and seedling growth in this plant. Ascorbate and salicylic acid increase germination and decrease of average time necessary for germination under drought conditions. The seeds that were treated by ascorbate and salicylic acid, radical and plumule length, fresh and dry weight of radical and plumule were increased. Results showed that ascorbate and salicylic acid decrease effects and damages of drought stresses on okra germination and seedlings growth. In general, adding salicylic acid and ascorbic acid significantly relieved the harsh effects of drought on okra germination and growth parameters and it seems that ascorbate and salicylic acid were able to enhance the tolerant ability of the plant to drought stress.

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Key words: Drought stress; okra; Germination; Seedling; Ascorbate; Salicylic acid

Despite, water is one of abundant compounds in earth and 2/3 of earth surface is covered by water, but water shortage is limitative to produce agriculture products in the world. Drought is one of the major physical parameter of an environment, which determines the success or failure of plants establishment (Gamze et al., 2005). Drought is the most important limiting factor for crop production and it is becoming an increasingly severe problem in many regions of the world (Passioura, 1996 and Passioura, 2007). Generally drought stress occurs when the available water in the soil is reduced and atmospheric conditions cause continuous loss of water by transpiration or evaporation. (Khaje Hosseini et al., 2003). The plants under dry condition change their metabolism to overcome the changed environmental condition. The complexity response of the plant to the drought stress could be justified. Seed germination is one of the most important phases in the life cycle of plant and is highly responsive to existing environment. The study of drought tolerance during germination early and late growth of plants is important for determining dry limits at each developmental phase. Drought decrease germination and seedling growth, and this are one important case to produce crops (Gamze et al., 2005). In 2 decades past, scientists have considered to physiological treatment that causes improvement seedling germination. Plants produce proteins to reaction biotic and a biotic stresses that were induced by some phytohormones such as salicylic acid (SA) and Ascorbic acid (As) (Davis, 2005). These compounds can decrease drought effects in plants under stress. Salicylic acid is an endogenous growth regulator from group phenol compound, that in different of process in plants is operative such as seed germination, stomata else, absorption and transfer ions. Salicylic acid is a conservative compound of some biological stresses and it is important molecular signal for adjustment plants reaction to environmental stresses. Salicylic acid is a plant phenol, and today it is in use as internal regulator hormone, because its role in the defensive mechanism against biotic and a biotic stresses has been confirmed. Ascorbic acid as (vitamin) is a non enzymatic compound that is resisted plant to stresses by reduction oxygenic free radicals constituted stress duration. The Drought stress decrease starch metabolism in cotyledons and decrease of transformation sucrose. (Inze and Montago, 2000). One mechanisms utilized by the plants for overcome the water stress effects might be via accumulation of compatible osmolytes, such as proline. Seeds of cowpea (*Vigna unguiculata* L. Walp.) were tested for salt and drought tolerance at germination, seedling emergence and early seedling growth in NaCl and PEG-8000 solutions of different osmotic potentials (0, −0.2, −0.4, −0.6, and −0.8 MPa). Results showed that germination and emergence rate were delayed by both solutions. Seedling growth was reduced by both stresses, but NaCl usually caused less damage than PEG to cowpea seedlings, suggesting that NaCl and PEG acted through different mechanisms (Murillo et al., 2002).

Materials and Methods:

Investigation of germination and seedlings growth:

In this stage, germination slip set into plate, that diameter it is 10 centimeters, seeds were disinfected by sodium hippo chloride 1% at time 5 minute. Then seeds treated 1mM, 1.5mM (SA) Salicylic acid and 1mM Ascorbic acid (AS) and participant 1Mm salicylic acid with 1mM Ascorbic acid and 1.5mM salicylic acid with 1mM Ascorbic acid at time 12 hours. Then seeds isolated from solutions and dried in shade, the duration of this stage was 1 hour. Then 20 treated seeds were set in aside plates and per plates spotted 3 repeats. After this 12 ml from these solutions added to any treats. Plates set in germinator (Model: 1CH, RH), for 20°C during 16 hours for day and for 16°C during 8 hours with humidity 25% for night. Number of germinated seeds was counted for 21 days with 3 replications. (Shah et al., 2002). Then germination parameters were calculated from below equations (Shakirova and Sahabutdinova., 2003).

$$MGT = \frac{N_1 T_1 + N_2 T_2 + \ldots\ldots}{N}$$

MGT= time average for germination.
N_1=number of germinated seeds at one time
N_2=number of germinated seeds at two time
T=Time
N=sum of germinated seeds

GP= (N*/N). 100

GP= percent of Germination.
N=number of total seeds.
N^*= number of germinated seeds.

For implementation of drought stress used polyethylene glycol (PEG 6000). For implementation of osmotic stress used 2 different Concentration of PEG (-0.2 and -0.4 MPa). Furthermore strilled water as control was used. Length of radical and plumule measured by millimeteric rule and for measuring of fresh and dry weight of radical and plumule were used to milligram from balance (Sartorius model: BPSIID) by accuracy 0.001 gram.

Statistical Analysis:

In this study, the total number of experiments was done in different stages in completely randomized design with 3 repetitions; Variance Analysis (F Test) was done for investigation of the reciprocal effect of Salicylic acid and Ascorbic acid with drought stress on different parameters as factorial trial. The levels of control, 1mM Salicylic acid (sa1), 1.5mM Salicylic acid (sa2), 1Mm Ascorbic acid (as), participant 1Mm salicylic acid with 1Mm Ascorbic acid (sa1+as) and 1.5mM salicylic acid with 1mM Ascorbic acid (sa2+as) were used and the levels of drought were control, 0.2 MPa PEG and 0.4 MPa PEG. The comparison of means was done with LSD test to SPSS 14.0 software in probability level of 5%. For drawing graph, we used Excel 2003 software.

Results:

Data of this investigation showed that germination percent of osmotic potential 0.4 MPa decreased in comparison to control, but at osmotic potential 0.2 MPa didn’t significant decrease (Fig.1). Average time necessary for germination in day didn’t change at all during drought stresses in comparison to control, in while that maximum of germination average in day was dependent to seeds to 0.4 MPa osmotic potential (Fig.2). Salicylic acid induced reduction of germination percent and average time necessary for germination in day. Maximum of reduction observed in 1.5mM concentration of salicylic acid, but ascorbic acid improved germination percent, average time necessary for germination in day. Joint treatment of salicylic acid and ascorbic acid caused decreased germination percent and increased average time necessary for germination in day. In seedlings that were treated by salicylic acid and ascorbic acid and joint treatment these 2 compounds, increased radical and plumule length, fresh and dry weight of radicle and plumule. Drought stress caused reduction of fresh and dry weight and length of radical and plumule in comparison to seedling control. (Fig.3-Fig.8). In seedlings that were treated by different concentrations salicylic acid, Ascorbic acid and joint treatment these 2 compounds were increased radical and plumule length in compared to control.

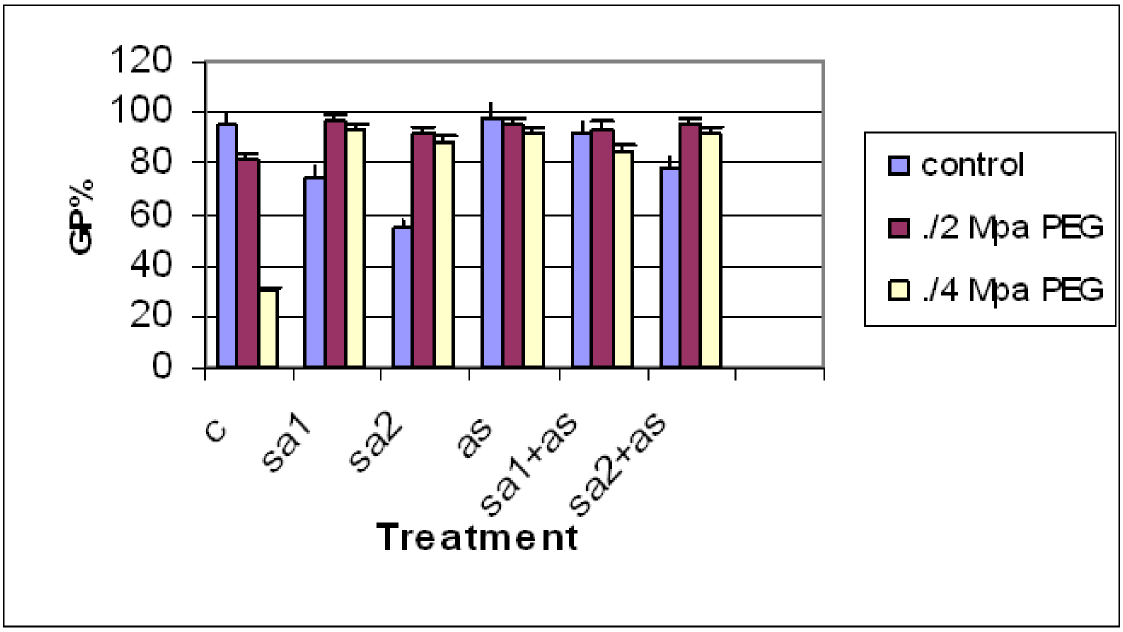

Fig. 1. Effect of drought stress, salicylic acid and ascorbic acid on the germination percentage

C: control, sa1: Salicylic acid 1mM, sa2: Salicylic acid 1.5mM, As: ascorbic acid 1mM, MPa PEG: deal of PEG at mega Pascal unit.

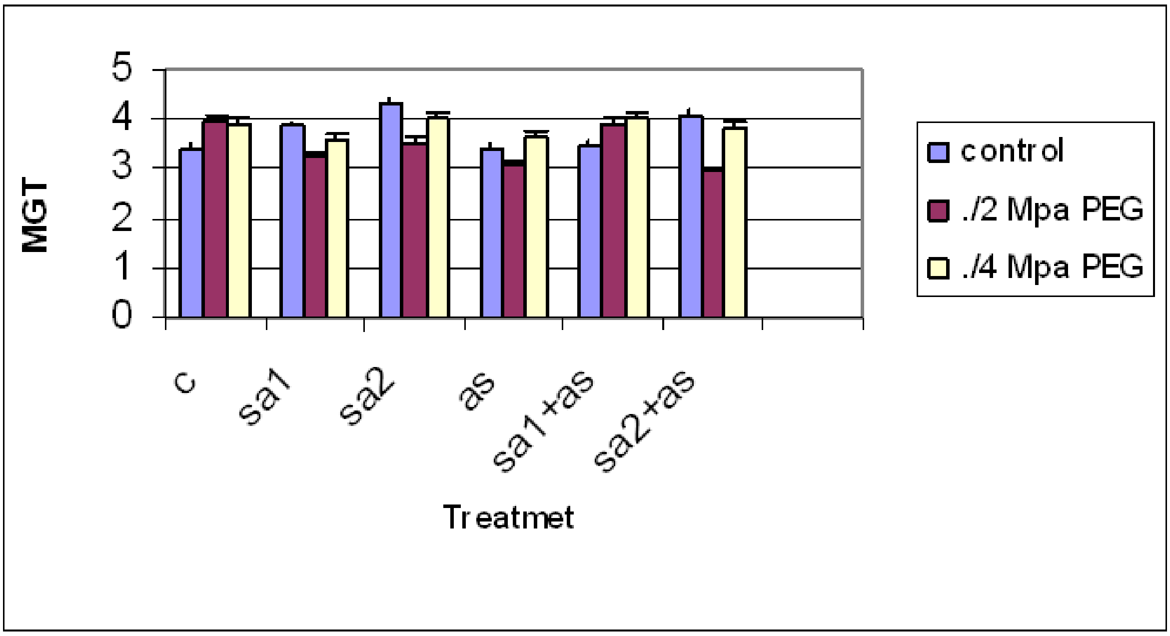

Fig. 2. Effect of drought stress, salicylic acid and ascorbic acid on the time average for germination

C: control, sa1: Salicylic acid 1mM, sa2: Salicylic acid 1.5mM, As: ascorbic acid 1mM , MPa PEG: deal of PEG at mega Pascal unit.

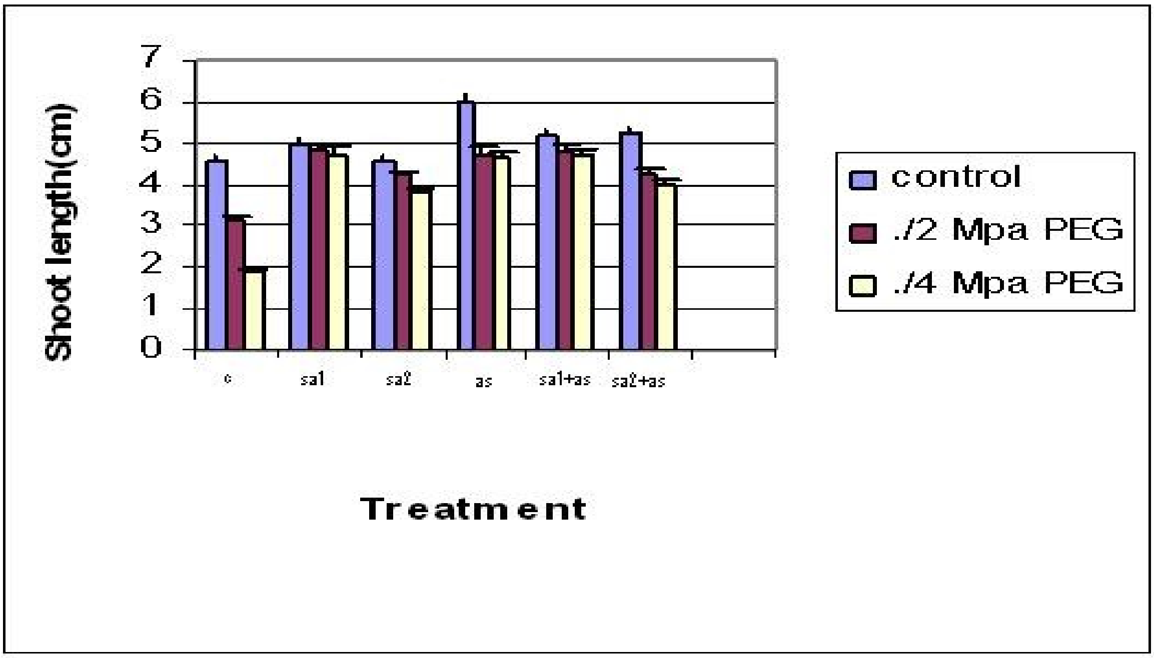

Fig. 3. Effect of drought stress, salicylic acid and ascorbic acid on the shoot length

C: control, sa1: Salicylic acid 1mM, sa2: Salicylic acid 1.5mM, As: ascorbic acid 1mM, MPa PEG: deal of PEG at mega Pascal unit.

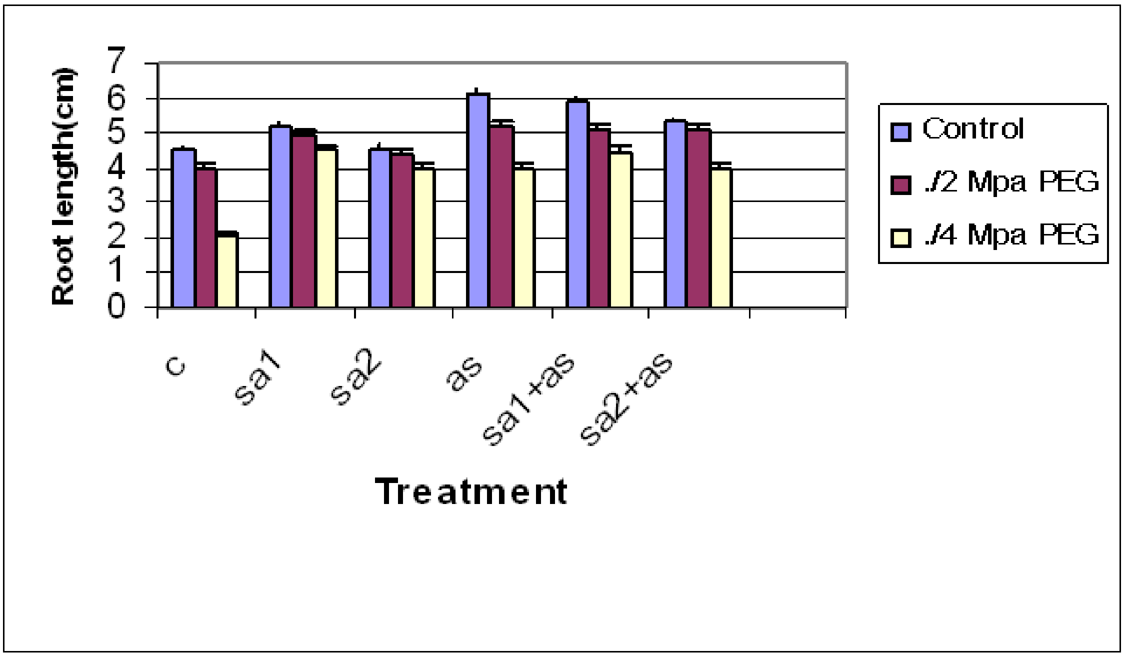

Fig. 4. Effect of drought stress, salicylic acid and ascorbic acid on the root length

C: control, sa1: Salicylic acid 1mM, sa2: Salicylic acid 1.5mM, As: ascorbic acid 1mM, Mpa PEG: deal of PEG at mega Pascal unit.

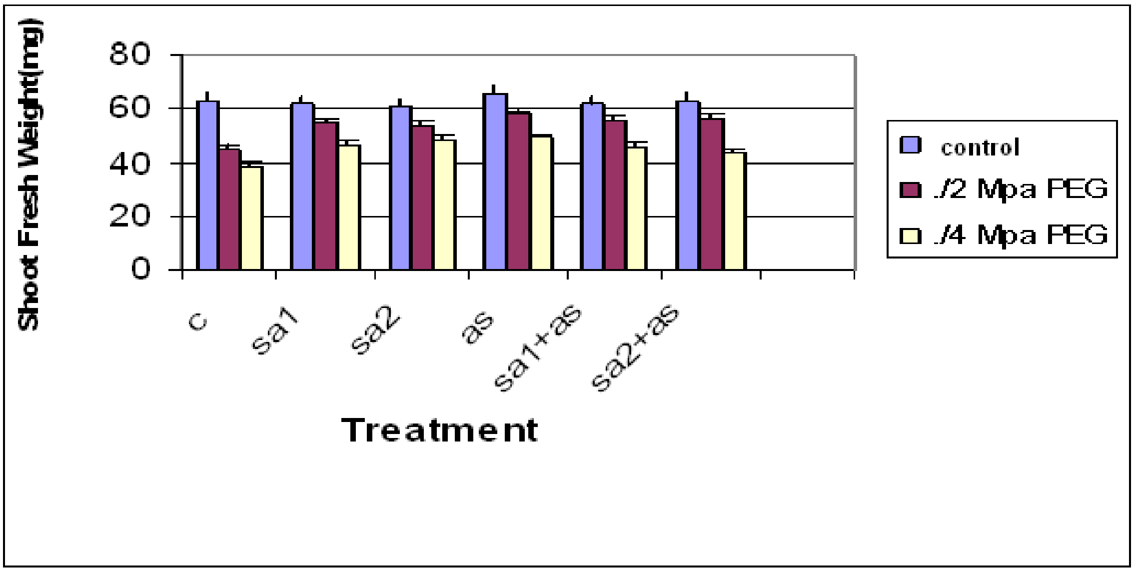

Fig. 5. Effect of drought stress, salicylic acid and ascorbic acid on the fresh weight of plumule (Shoot)

C: control, sa1: Salicylic acid 1mM, sa2: Salicylic acid 1.5mM, As: ascorbic acid 1mM, Mpa PEG: deal of PEG at mega Pascal unit.

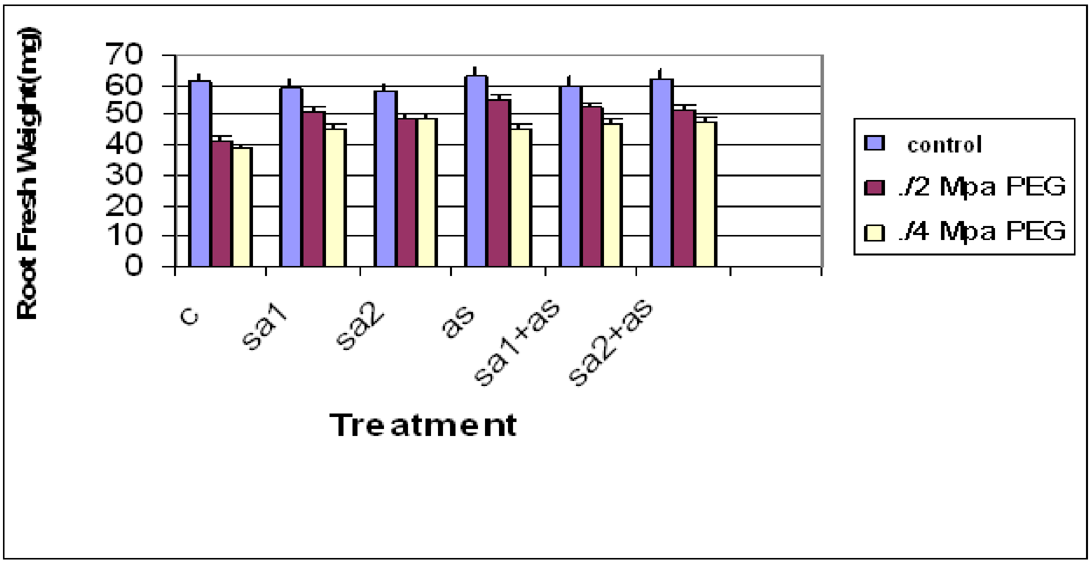

Fig. 6. Effect of drought stress, salicylic acid and ascorbic acid on the fresh weight of radical (Root)

C: control, sa1: Salicylic acid 1mM, sa2: Salicylic acid 1.5mM, As: ascorbic acid 1mM, Mpa PEG: deal of PEG at mega Pascal unit.

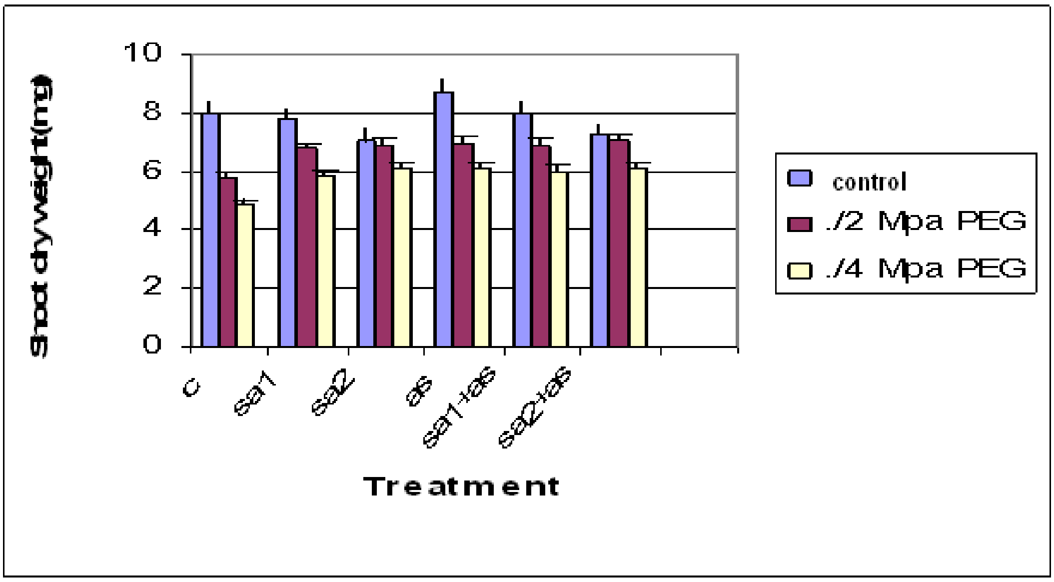

Fig. 7. Effect of drought stress, salicylic acid and ascorbic acid on the dry weight of plumule(Shoot)

C: control, sa1: Salicylic acid 1mM, sa2: Salicylic acid 1.5mM, As: ascorbic acid 1mM, Mpa PEG: deal of PEG at mega Pascal unit.

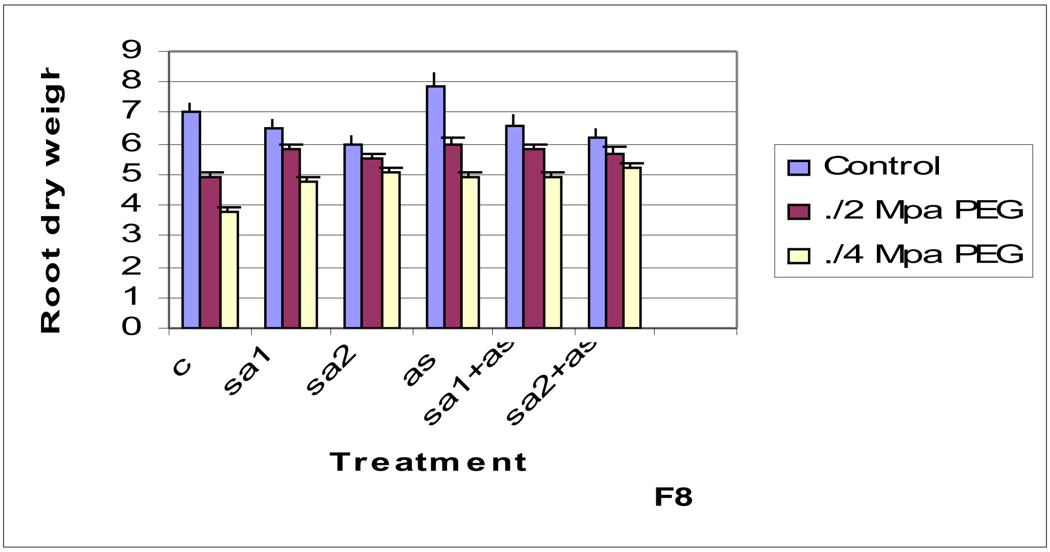

Fig. 8. Effect of drought stress, salicylic acid and ascorbic acid on the dry weight of radical (Root)

C: control, sa1: Salicylic acid 1mM, sa2: Salicylic acid 1.5mM, As: ascorbic acid 1mM. , Mpa PEG: deal of PEG at mega Pascal unit.

Discussion:

Drought, salinity, temperature stresses are decreased germination percent (Shakirova and Sahabutdinove., 2003). Stresses of high severity in addition germination reduction are decreased germination rate, seedling emersion and vigor (Liusia et al., 2005). Drought is one of the serious a biotic stresses in most countries of the world. About 36% of the land area worldwide is classed as arid to semi- arid, receiving only 13 cm to 78 cm of rainfall annually defined as a period without significant rainfall. The soil and water engineering methods increase farm production in the damaged soil by drought. It was reported the PEG and NaCl decreased germination in pea, but effect of PEG on germination is more than NaCl (Gamze et al., 2005). Murillo et al., (2002) observe that seed germination and seedling growth in legumes decreased under condition drought treat by PEG. Sadeghian and Yavari (2004) showed that drought stress cause reduction of seedlings growth in beet too. Gamze (2005) in his investigation on pea, find that decrease germination percent (GP) by increase of drought stress (to PEG), increase average time necessary for germination in day, decrease radical and plumule length, fresh and dry weight of radical and plumule. In this research drought caused reduction of germination rate and seedlings growth in okra. Ascorbic acid and salicylic acid are 2 compounds that able to decreased harmful effective drought stress, on germination percent, radical and plumule length, fresh and dry weight of radical and plumule in different stages germination and seedling growth (Smironoff., 1996). Ascorbic acid is one of the best identified non-enzymatic compounds as antioxidant that plant bearing is increased to oxidative stresses (Smironoff., 1996). Hamad and Hamada reported that treat of wheat seeds to ascorbic acid decreased bad effectives drought stress on fresh and dry weight of radical and plumule. It is reported that ascorbate and salicylic acid caused reduction of effectives salinity stress on germination and seedlings growth of wheat, in concentrations: 50ppm, 100ppm, 200ppm (Irfanazel et al., 2006). Concerning the effect on salicylic acid on germination, there is different and opposite reports. Wu et al. (1998) reported that some of phenol compounds haven't effete on germination. In other hand, salicylic acid interferes and spoils of oxidative damages in duration germination, at concentrations over of 1 mM (Lopez et al., 1999).

Rajasekaran et al., (2002) reported that salicylic acid haven't intensification effect on germination. It was reported that between salicylic acid and absysic acid signal there are correlation on germination (Perales et al., 2005). Zhang reported that salicylic acid has an inhibitor role in ethylene biosynthesis (Zhang et al.,2002). Because ethylene role was defined as germination stimulant in many of seeds, probably observed reduction in germination percent may be from inhibition of ethylene biosynthesis. Concerning the role of salicylic acid on growth parameters there is antithesis observes. It was reported that treatment by salicylic acid increase cell division on apical meristem of wheat seedling, and reduced of plant growth improve under condition drought stress (Shah et al., 2002). It was reported that salicylic acid decreased toxity from heavy metal stress in barely seedlings and seedlings growth increased under treated by heavy metal (Metwally et al., 2003). It was reported that salicylic acid regulate cell extension, division and death. In fact between growth and senescence create a balance (Zhang et al., 2002).

Acknowledgments:

This study has been supported by the International Center for Science, High Technology & Environmental Sciences, Kerman, Iran.

References

Afzal I., Basra S.M.A., Farooq M., Nawaz A. (2006) Alleviation of salinity stress in spring wheat by hormonal priming with ABA, salicylic acid and ascorbic acid. *Int. J. Agric. Biol.*, **8**: 23–28

Davis P.J. (2005) Plant hormones: biosynthesis, signal transduction, action. Springer; 3rd edition. Kluwer Academic Publishers. Netherlands. 700 p.

Gamze O., Mehmet D.K., Mehmet A. (2005) Effects of salt and drought stresses on germination and seedling growth of pea (*Pisum sativum L.)*. *Turk. J. Agric*. **29,** 237-242.

Hamad A., Hamada A. (2001) Grain soaking presowing in ascorbic acid or thiamin versus the adverse effects of combined salinity and drought on wheat seedlings. in *Proceedings of the 12th International Congress on Photosynthesis* (Melbourne, Australia, Brisbane, Australia, August 18-23, August 18-23, 2001), CSIRO Publishing, S15-005 .

Inze D., Montage M.V. (2000). Oxidative Stress in plant. *Curr. Opin. Biotechnol*. **6**: 153- 158.

Khajeh Hosseini M., Powell A.A., Bingham I.J. (2003) The interaction between salinity stress and seed Vigor during germination of soybean seed. *Seed Sci. Technol*, **1**, 715-725.

Liusia J., Penuelas J., Munne B. (2005) Sustained accumulation of Methylsalicylate alters antioxidant protection and reduces tolerance of holmoak to heat stress. *Physiologia Plantarum* .**124**, 353-361.

Lopez M., Humara J.M., Casares A., Majada J. (1999) The effect of temperature and water stress on laboratory germination of eucalyptus globules. *Plant Physiol*. **94**, 1616-1624.

Metwally A., Finkemeier I., Georgi M., Dietz K.J. (2003) Salicylic acid alleviates the cadmium toxicity in Barley seedlings. *Plant Physiol*. **132**, 272-281.

Murillo A., Lopez B.R., Aguilar C., Kaya T., Larrinaga A., Flores H. (2002) Comparative effects of NaCl and Polyetylene glycol on germination, emergence and seedling growth of cowpea. *J Agron Crop Sci*. **188**, 235-247

Passioura J.B. (1996) Drought and drought tolerance. *Plant Growth Regulation*. **20**(2), 79-83.

Passioura J.B. (2007). The drought environment: physical, biological and agricultural perspectives. *J. Exp. Bot.* **58**(2), 113-117.

Perales L., Arbona V., Cadenas A.G., Cornego M., Sanz A. (2005) A relationship between tolerance to dedyhidration of rice cell lines and ability for ABA synthesis under stress. *Plant Physiol and Biochem*. **43,** 786-792.

Rajasekaran L., Stiles R., Surette A., Sturz M.A., Blake A.V., Caldwell T.J., Nowak J. (2002) Stand establishment technologies for prossesing Carrots: effects of various temperature regimes on germination and the role of salicylates in promoting germination at low temperatures. *Can J Plant Sci*. **82,** 443-450.

Sadeghian S.Y., Yavari N. (2004) Effect of water – deficit stress on germination and early seedling growth in sugar beet. *J Agron Crop Sci*, **190**(2), 138-144.

Shah F.S., Watson C.E., Cabera E.R. (2002). Seed vigor testing of subtropical Corn hybrids . *Research Rep*. **23**(2), 56-68

Shakirova F.M., Sahabutdinova D.R. (2003) Changes in the hormonal status of Wheat seedling induced by salicylic acid and salinity. *Plant Sci*, **164,** 317-322.

Smironoff N. (1996) The function and metabolism of ascorbic acid in plant. *Annals of Bot*., **78,** 661-669.

Wu L., Guo X., Harivandi M.A. (1998) Allelopatic effects of phenolic acids detected in buffalo grass (*Buchloe dactyloides*) clippings on growth of annual. Bluegrass (*poa annua*) and buffalo grass seedling. *Environ Exper Bot*. **39,** 159-167.

Zhang W., Curtin C., Kikuchi M., Franco C. (2002) Integration of Acid and light irradiation for enhancement of anthocyanin biosynthesis in *Vitis inifera* suspension cultures .*Plant Sci*. **162**, 459-468.