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ALLEVIATION OF OSMOTIC STRESS DURING SEED GERMINATION IN SOYBEAN BY A-TOCOPHEROL AND ASCORBIC ACID

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ABSTRACT

The present experiment was carried out to minimize the harmful effects of water stress induced by NaCl or polyethylene glycol 6000 on soybean germination percentage and seedling growth (fresh and dry weights as well as seedling length) through presoaking seeds in AsA or α-Toco at 50 and 100ppm. The low levels of NaCl(-2 bar) increased germination percentage and seedling fresh and dry weights significantly, but PEG 6000 (-2 bar) was no significant differences, whereas increasing NaCl or PEG 6000 concentration up to -4 bar caused a decrease in this respect. The great reduction occurred under high level of NaCl or PEG (-6 bar) as compared to control. Presoaking seeds in AsA or α-Toco were markedly increased soybean germination percentage and seedling growth. In addition, ascorbic acid at 50 ppm and α-tocopherol at 100 ppm was more effective under NaCl(salinity stress) than PEG 6000 (drought stress). The low levels (-2 bar) of NaCl or PEG were no significant differences in seedling length, while this parameter was significant decrease at -4 bar of water potential and these decrease was frequently dependent on NaCl and PEG concentration . Generally, both AsA and α-Toco counteracted the harmful effect of stress on soybean seedling weight (fresh and dry) against different stress levels. In addition, α-Toco was more effective than AsA to reduce the effect of stress on seedling fresh and dry weights.

Keywords: Soybean, seed germination, polyethylene glycol (PEG 6000), NaCl, ascorbic acid (AsA) and α-tocopherol (α-toco).

INTRODUCTION

Soybean, *Glycine max (L.) Merr* is one of the most important agricultural plants and attains the first place in the world about the cultivated area and amount of production. In addition, it is considered as a good source of protein (38 to 40 %.) and oil (18 to 20%). It use as a livestock feed and as an oil crop for cooking oil, margarines, and other edible products. In Egypt, it is not feasible to expand the area for such valuable crop due to high competition since cotton, rice and maize. It is considered a sensitive species to the several abiotic stress mainly during seed germination (Van Heerden and Krüger,2002).Water stress resulting from drought and salinity are widespread problems around the world (Soltani *et al,*2006). Very often salinity and drought are coupled to each other and affect more than 10 percent of arable land (Bray *et al.,* 2000). Salinity and drought stresses are physiologically related, because both induce a water deficit or osmotic stress and most of the mbolic responses of the affected plants are similar to some extent (Katerji *et al.,* 2004). Many investigators have been used NaCl and PEG to determine the effect of salt and drought stresses on seed germination and seedling growth of tomato (Fellner and Sawhney, 2001);phaseolus (Jeannette *et al.,* 2002); cowpea (Murillo-Amador *et al.,* 2006) and sugar beet (Jafarzadeh and Aliasgharzad, 2007). To minimize the effects of oxidative stress, plant cells have evolved a complex antioxidant system, which is composed of low-molecular mass antioxidants as well as ROS-scavenging enzymes (Apel and Hirt, 2004). One approach for inducing oxidative stress tolerance would be to increase the cellular level of the various non-enzymatic antioxidants such as tocopherols and ascorbic acid(El–Bassiouny and Bakh, 2005 and El Tohamy and El Gready, 2007 and Bassuony *et al*., 2008).Exogenous application of ascorbic acid and α-tocopherol to higher plants has positive effects in overcoming the adverse effects of salt or draught stress. The aim of this experiment was carried out to evaluate the effect of AsA and α-Toco on soybean seed germination and subsequent seedling growth under water potential of NaCl or polyethylene glycol 6000.

MATERIALS AND METHODS

Three drought stresses with different osmotic potentials of -2, -4 and-6 bars were arranged as described by Michel and Kaufmann (1973). Salt concentrations that had the same osmotic potentials of -2, -4 and-6 bars (electrical conductivities of the solutions were 4.5, 8.8 and 12.7 dS m-1, respectively) were adjusted using NaCl (Coons *et al.,* 1990). Distilled water served as a control .A homogenous lot, healthy and almost uniform size, of soybean seeds surface sterilized by soaking in 0.01% mercuric chloride for 3 minutes, then repeatedly washed with distilled water and divided into 5 groups. The first group of seeds was soaked (6 hours) in distilled water to serve as control, and the remaining 4 groups were separately soaked for 6 hours in aqueous solutions of AsA, at 50 or 100 ppm and α-Toco at 50 or 100 ppm.

Every group divided into seven sub-groups .The sub-group transferred to sterile Petri dishes (11 cm diameter) containing two layers of the filter papers (Whitman No. 1).The first sub-group was moisture with 10 ml of distilled water (control). Three sub-groups (salinity stress) were salinized with 10 ml distilled water added with NaCl at 4.5, 8.8 and 12.7 dS m-1for treatments (S1, S2 and S3, respectively). Solutions (10 cm3) containing different concentrations of polyethylene glycol (PEG 6000) with different osmotic potentials of -2, -4, and -6 bars (D1, D2 and D3, respectively),were added separately in the remainder three sub-groups (drought stress).Seeds of soybean allowed germinating at about 25±2C in the dark. Petri dishes tightly sealed with the impermeable colorless film in order to avoid water losses during the incubation. Thiram added to the solutions at a concentration of 0.2% (w/v) to control the fungi infection. Seeds considered to have germinated when shoot extended to more than 2mm from the seeds. After 10 days final germination, seedlings harvested and washed with water after harvest. The experiment was repeated two times and arranged in a complete randomized block design with 3 replicates and 10 seeds per replicate. The following data recorded:

1) Germination percentage was measured according to the ISTA rules (ISTA, 1999).
2) Seedling Shoot and root lengths (mm) was measured on ten seedlings randomly taken from each replicate and the mean length of seedlings was calculated.
3) Seedling Shoot and root fresh weights (mg) were measured on ten seedlings randomly taken from each replicate (The harvested plants were partitioned into roots and shoots).
4) Seedling shoot and root dry weights (mg) measurement used the same seedlings taken for the determination of fresh weight after were placed in paper bags and dried at 80^0 C to constant weight in an oven, then the plant parts were weighed again.

RESULTS AND DISCUSSION

Germination Percentage (G %):

Data in Fig (1) and plates (1&2) revealed that soybean seed germination percentage under low levels (-2 bar) of NaCl increased significantly, but PEG 6000 drought stress (-2 bar) had no significant differences. It was observed that seed germination percentage was decreased gradually with increasing NaCl or PEG 6000 concentration up to -4 bar (8.8 dS m-1) and decreasing water potentials of the media. Moreover, the great reduction occurred under high level of NaCl or PEG (-6 bars).

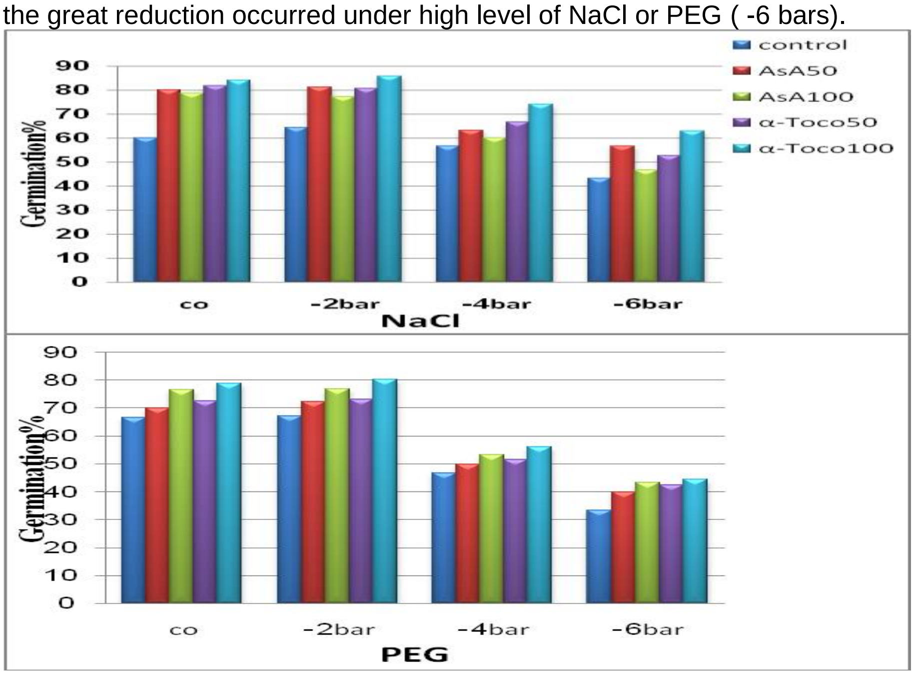

Fig. (1): Effect of presoaking in ascorbic acid and α- tocopherol on germination percentage of soybean germinated at different osmotic potentials of NaCl and PEG.(after7 days from sowing).

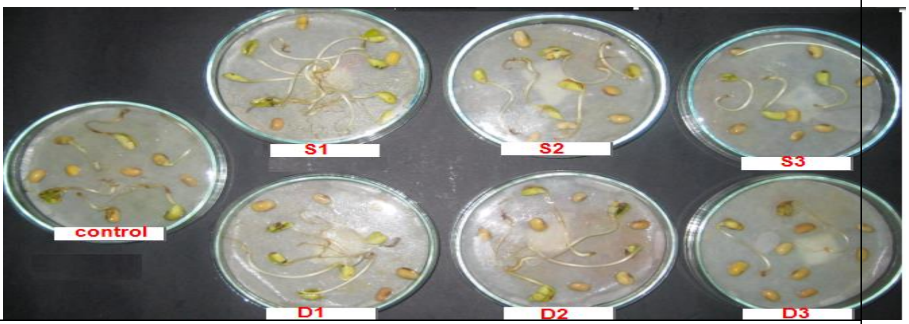

Plate (1): Seed germination of soybean at different osmotic potentials of NaCl and PEG.(after7 days from sowing).

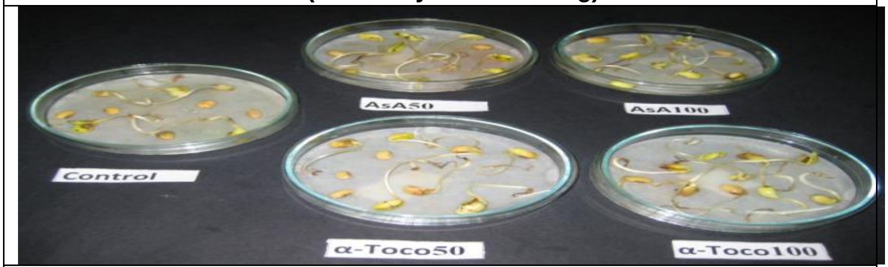

Plate (2): Seed germination of soybean presoaking in ascorbic acid and α- tocopherol (after7 days from sowing).

In addition, soybean germination percentage under drought resulted to a greater followed by salinity under the same level of water potential. These results agree with those of Almansouri *et al*. (2001); Soltani *et al*.(2006) and Kalefetoglu *et al.* (2009).Salinity and draught inhibited the germination percentages by causing a complete inhibition of the enzyme germination process or delaying the germination of seeds but do not prevent germination. Higher germination percentages obtained from NaCl compared to those of PEG at the same concentrations proved that the adverse effect of PEG on the germination was due to an osmotic effect rather than specific ion accumulation. Seeds germinated better in NaCl than PEG at the equivalent water potential, possibly due to the uptake of Na+ and Cl- ions by the seed, maintaining a water potential gradient allowing water uptake during seed germination. With no toxicity effect of PEG reported (Khajeh-Hosseini *et al.,* 2002), the lower germination percentage obtained from PEG compared with NaCl suggests that adverse effects of PEG on germination were due to osmotic effect rather than specific ion accumulation. Chartzoulakis and Klapaki (2000) affirmed that growth medium salinity or drought could affect the seed germination by decreasing the ease of uptake water. Mehra *et al,* (2003) proposed that the PEG molecules did not enter the seed and hence once water potential of the seed and around were in equilibrium the seed would not continue to imbibe. Foolad and Lin (1997) concluded that the osmotic rather than ionic effects of the medium mainly affected the tomato seed germination. These results agree with those given by Murillo-Amador *et* *al.* (2002), who observed that NaCl had a lesser effect on the germination and seedling growth of cowpea than did PEG and Sadeghian and Yavari (2004)stated that seedling growth was severely diminished by water stress in sugar beet. At the same water potential, the lower mean germination time in NaCl than in PEG could be explained by more rapid water uptake in NaCl solutions. Khajeh- Hosseini *et al.* (2002) found faster germination in NaCl in soybean.. At the same water potential, the higher final germination in NaCl than in PEG could be explained by more rapid water uptake in NaCl solutions and achievement of a moisture content that allowed germination. Moreover, Salinity inhibits seed germination through accumulation of toxic ions and/or reduced water uptake, which arrested radical emergence (Begum, *et al*., 1992). Furthermore, excess of Na+ might cause problems with membranes, enzyme inhibition, disturbance in embolism which disorganize cell division, elongation and structure (Abo-Kassem, 2006).

Water and salt stresses are correlated, as excess of soluble salts reduces soil water potential and, thus, hinders water absorption by seeds and plants in general. Water is one of the most important environmental factor influencing seed germination as it triggers the germination process and remains involved, direct or indirectly, in all other subsequent stages of plant metabolism. It decisively participates in enzymatic reactions metabolite solvency and transportation, and is a reagent itself in the hydrolytic digestion of proteins, carbohydrates and lipids from seed reserve tissues. The physical process of water uptake leads to the activation of metabolic processes as the dormancy of the seed is broken following hydration. During seed germination, one of the major steps is the mobilization of carbohydrates or proteins reserves from the cotyledons to the embryonic axis (Prisco, *et al.,* 1975). Drought and salinity stress may inhibit germination of seeds by its effect on the activity of the enzymes responsible for the mobilization of the carbohydrates or protein reserves of the cotyledons. Low water potential in soil medium caused by either soil salinity or water deficit, leading to late and inadequate germination and associated failure of stand establishment adversely affects crop productivity (Willenborg *et al.,* 2005).

The decrease in the germination percentage due to ascending concentration of PEG-6000 in the growth medium was due to less availability of water during imbibitions, because seed germination mostly depends upon this process (Ashraf and Naqvi ,1995). Moreover, Sajjan *et al*.(1999) observed a gradual decrease in germination percentage of sunflower with increase in concentration of external osmotic. The inhibitory in germination percentage with decreasing water potential observed in this experiment, possibly due to the uptake of Na+ and Cl- ions by the seed, maintaining a water potential gradient allowing water uptake during seed germination. With no toxicity effect of PEG reported (Khajeh-Hosseini *et al,* 2002), the lower germination percentage obtained from PEG compared with NaCl suggests that adverse effects of PEG on germination were due to osmotic effect rather than specific ion accumulation and probably was caused by the low hydraulic conductivity of the environment, where PEG 6000 makes water unavailable to seeds, affecting the imbibitions process of the seed which is fundamental for germination .The lowest germination percentage was observed at -4 bar stress level. This larger reduction with PEG solution could be attributed to high viscosity, where solubility and diffusion of oxygen were reduced compared to water. A decrease in water potential gradient between seeds and their surrounding media adversely affects seed germination.

Water deficient conditions induced with PEG and NaCl decreased germination percentage at the highest concentrations due to water stress. This was because the increase of NaCl and PEG concentrations lower water uptake by seed resulting a decreases in germination. Therefore, the decrease in water potential gradient between seeds and their surrounding media by the effects of PEG 6000 and NaCl adversely affects seed germination. Dodd and Donovan (1999) reported that conditions with higher NaCl contents and water deficient condition reduces germination due to limited water uptake by the seeds. The reduction in seed germination percentage was higher in PEG compared to NaCl, that is, at the equivalents of osmotic potential, seed germinated is better in NaCl than in PEG. Moreover, at the same osmotic potentials, germination starts earlier and higher germination percentage is obtained with NaCl (Figure 1). The explanation of the higher inhibitory effectsof PEG than NaCl lies in ion or solute entry into the seed (Alam *et al,* 2002). Especially, the accumulation of Na+ by the imbibing seed embryo functions to promote a water potential gradient between the embryo and substrate, and maintain water uptake during seed germination (Dod and Donovan, 1999). Almansouri *et al.* (2001) reported that moderate stress intensities only delayed germination; whereas the highest concentration of NaCl and PEG reduced final germination percentages. Elevated salinity slows down water uptake by seeds, thereby inhibiting their germination. NaCl was found to be inhibitorier to water uptake, especially at high concentrations, than iso-osmotic solutions of PEG. The first phase of water uptake by the seeds involves movement of water into the free space (apoplast) and does not depend on the osmotic potential of the surrounding solution (Simon, 1984). The second slower linear phase of water uptake involves the movement of water across cell membranes into the cells of the seeds and is determined by the difference between the osmotic potential of the seed and that of the medium (Bewley and Black, 1982).Unlike PEG, NaCl may readily cross the cell membrane into the cytoplasm of the cells unless an active metabolic pump prevents accumulation of the ions. In some cases, NaCl in the cytoplasm can result in toxic accumulation of a particular ion or decreased availability of some essential nutrients (Werner and Finkelstein, 1995). Specific ion toxicity of the Na+ and Cl− ions on the cell membrane, cytoplasm and}or nuclei of the cells of halophyte seeds may partly be responsible for the fact that NaCl is more inhibitory to the three processes than iso-osmotic concentrations of PEG. Moreover, the presence of Na+ and Cl− ions in the cells may induce changes in protein activity because ions affect the structure of the hydration water, which surrounds the protein molecule (Waisel, 1972). Moreover, Al-Taisan *et al*. (2010) showed that different responses to water stress induced by PEG and NaCl. However, seedling growth was more sensitive to NaCl than was germination responses to water stress induced by PEG. However, seedling growth was more sensitive to NaCl than was germination. Results also indicated that seed germination of *Phaseolus divisum* is less sensitive to osmotic potential indicating that the seeds of the species are efficient in osmotically adjusting to soluble salts. Garg (2010) revealed that the germination and early seedling growth of *Phaseolus mungo* varieties significantly differed for salt and drought(Polyethylene glycol 6000) stress and both NaCl and PEG inhibited germination and seedling growth in both varieties, but the effects of NaCl compared to PEG was less on germination and seedling growth. All varieties were able to germinate at all NaCl levels without significant decrease in germination, while a drastic decrease in germination was recorded at -6 and -8 bars of PEG. It was concluded that inhibition in germination at equivalent water potential of NaCl and PEG was mainly due to an osmotic effect rather than salt toxicity. In the same table, presoaking seeds in AsA or α-Toco were markedly increased soybean germination percentage. In addition, ascorbic acid at 50 ppm and α-tocopherol at 100 ppm was more effective under NaCl, but ascorbic acid at 100 ppm and α-tocopherol at 100 ppm under PEG 6000 drought stress. As regard to the interactions, between NaCl or PEG 6000 and AsA or α-Toco, showed a significant increased in germination percentage under NaCl and PEG 6000 levels. In addition, the interaction between ascorbic acid at 50 ppm and α-tocopherol at 100 ppm and stress levels showed that maximum of percentage of germination as compared with control.

In this study, with applying AsA or α-Toco and evaluation of its interaction with different stress levels due to NaCl and PEG 6000, germination percentage of soybean increased. It seems that in addition to increasing osmotic stress, higher PEG or NaCl concentration produced more reactive Oxygen Species (ROS) and its progressive increase during germination and oxidative damage. In our study, presoaking soybean seeds on AsA or α-Toco as an important antioxidative along with coordinate induction of the antioxidative mechanisms could scavenge more reactive Oxygen Species produced under stress conditions and improved germination process. So, the efficacy of AsA increased soybean seed germination percentage (Figs 1). Reactive Oxygen Species generation in the embryonic axis of germinating seeds at the onset of germination suggests a risk of oxidative damage at that stage of development (Puntarulo *et al.,* 1988). It requires a coordinated series of events during dehydration that are associated with preventing oxidative damage and maintaining the native structure of macromolecules and membranes (Hoekstra *et al.,* 2001). AsA is a key factor in ROS detoxification (Tommasi *et al*., 2001). Because cell division and cell expansion require AsA and these two processes are fundamental for seedling development, AsA could also be involved directly or by maintaining the opportune cellular redox balance in the process responsible for rendering the reserve substances available for the germinating embryos (Tommasi, *et al.*, 2001). The early availability of ascorbic acid (AsA) is necessary to meet the large demands for AsA by the restored cell metabolism and because AsA is, in some way, required to elicit AsA peroxidase. It has Arrigoni *et al*. (1992) reported that decreases in AsA peroxidase occurrence was correlated with the onset of a biochemical pathway leading to morphological anomalies of seedlings and to the loss of seed germination capacity. Therefore, in the present study, it is possible to conclude that while oxidative stress increased due to rising PEG, AsA with PEG solutions could have increased the redox capacity. This event has apparently supported germination and cell division and expansion in drought stressed treatments and growth process. In addition, AsA and tocopherols are also lipophilic antioxidants (Falk, *et al.,* 2003). Simontacchi *et al* (1993), shown that the α-tocopherol content increased in soybean embryonic axes upon imbibition and post-germination under oxidative stress. Therefore, it could be concluded that using exogenous AsA would probably protect germination of soybean against lipid peroxidation of membrane in storage period. Since AsA could be considered as the best supporter for seed tocopherol storage and germination process might be protected with high confidence against ROS oxidative damage, control of oxidative stress would be an important ability of plant to tolerate drought stress. In the present study, concurrent with increasing stress levels delay germination in the low water potential media compared to the control treatment (Fig 1).Presence of AsA provoked acceleration in the germination process. In regards to the results of this study, it is believed that presence of exogenous AsA affected the biochemical processes of germination; because the enzymes involved have ranges of action that will accelerate seed germination, (Bewley and Black, 1982).Ascorbic acid is one of the most powerful antioxidant (Smirnoff and Wheeler, 2000). The ability to donate electrons makes ascorbic acid the main ROS-detoxifying compound in aqueous phase. Ascorbic acid can directly scavenge superoxide hydroxyl radicals and singlet oxygen and reduce H_2O_2 to H_2O via ascorbate peroxidase reaction (Noctor and Foyer, 1998). Ascorbic acid regenerates tochopherol providing membrane protection (Thomas *et al.,* 1992). In addition, ascorbic acid carries out a number of non-oxidant functions in the cell. It has been implicated in the regulation of cell division, cell cycle progression from G1 to S phase (Smirnoff, 1996) and cell elongation (De Tullio *et al.,* 1999); and Shabala *et al.* (2000) showed an amazing capacity for recovery and germination after treatment with ascorbic acid or α-toco (Table 1). The strategy of osmotic adjustment in salinized soybean seedlings treated with AsA or α-Toco might be mediated by accumulation of some compatible solutes (amino acids, sugars or proline) acting as osmolytes.

Seedling length:

Data in Fig (2&3) revealed that low level (-2 bar) of NaCl or PEG had no significant differences in seedling length compared to untreated plants. While, this parameter was significant decrease at -4 bar (8.8 dS m-1) of water potential and these decrease was frequently dependent on NaCl and PEG concentration. The results are in agreement with Rahman *et al.* (2001) and Shawky,(2003), they revealed that root length was severely more suppressed than shoot length. In addition, soybean seedling shoot length under PEG resulted to a greater decreased than NaCl under the same level of water potential. Inhibition of NaCl was less than that of PEG. Our results confirm the findings of Khajeh-Hosseini *et al.*(2002) in soybean and those of

Murillo-Amador *et al*, (2002) in cowpea. On the other hand, seedling shoot and root length were decreased gradually with increasing NaCl or PEG 6000 concentration up to - 4 bar (8.8 dS m^{-1}). Moreover, the great reduction occurred under high level of NaCl or PEG at -6 bars.The results of this study are in agreement with the observations of Bahaji *et al.* (2002) and Azooz *et al.,* 2004). Exposure plants to draught or salt stress usually begins with the exposure of the roots to that stress. Salt stress leads to changes in growth, morphology and physiology of the roots that water change and ion uptake and production of signals (hormones) that can communicate information to the shoot. Salinity may cause water deficit at the root zone similar to that produced by drought. The inhibition of root growth by salinity stress may be due to the reduction in the length of root tip elongation (Abraham and Kiran, 2003). Exposure plants to draught or salt stress usually begins with the exposure of the roots to that stress. Salt stress leads to changes in growth, morphology and physiology of the roots that water change and ion uptake and production of signals (hormones) that can communicate information to the shoot. Salinity may cause water deficit at the root zone (Zidan *et al ,*1990) and might come from the marked lowering of root radial hydraulic conductivity that occurs with salinization (Azaizeh *et al*., 1992) but, the drastic decline in conductivity, water movement into cells was still sufficiently rapid so as not to restrict their rate of expansion (Neumann *et al.,*1994) and decreasing ATP-dependent proton expulsion at the root cell plasmalemma (Spanswiek ,1981) as well as reduces the ability of plants to take up water, and this quickly causes reduction in growth rate, the initial reduction in seedling shoot growth is probably due to hormonal signals generated by the roots (Munns ,2002). The results of this study are in agreement with the observations of Zhu *et al*. (2007) stated that under drought conditions, the roots or radicals will develop faster than the hypocotyls to acclimatize with the water stress.

In addition, osmotic stress had more inhibiting influence on hypocotyls growth than radicals growth (Fig2&3) and as has been also reported by other studies (Huang and Redmann, 1995;Foolad, 1996 and Bayuelo-Jimenez *et al*., 2002).It has been reported that PEG-induced osmotic stress can cause hydrolysis of storage compounds that further lower the internal osmotic potentials of the seed (Hampson and Simpson, 1990).

In conclusion, soybean is more sensitive to osmotic stress at germination stage. However, at early growth stage both salt-induced osmotic stress and Na toxicity reduced growth. Root length is an important trait against drought stress in plant varieties; in general, variety with longer root growth has resistant ability for drought (Leishman and Westoby, 1994).In the present study, decreases in the external osmotic potential caused a reduction in seedling growth. Although, the increasing concentrations induced water stress leading to decrease in root length and shoot length .This reduction in root and shoot length was lower in NaCl than those of draught stress conditions .

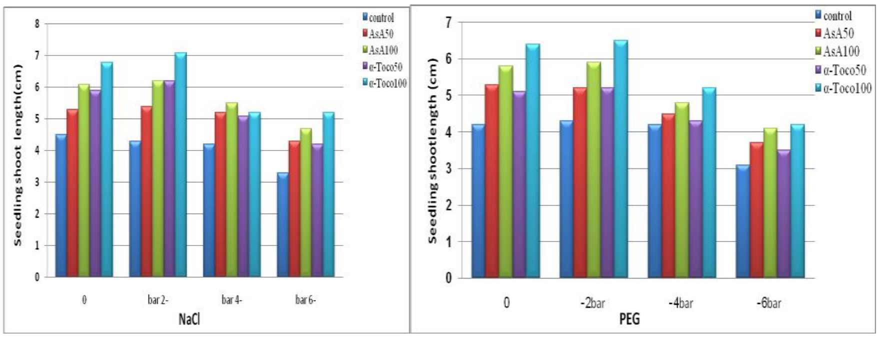

Fig. (2): Effect of presoaking in ascorbic acid or α tocopherol on soybean seedling shoot length(cm) germinated at different osmotic potentials of NaCl and PEG(after10 days from sowing).

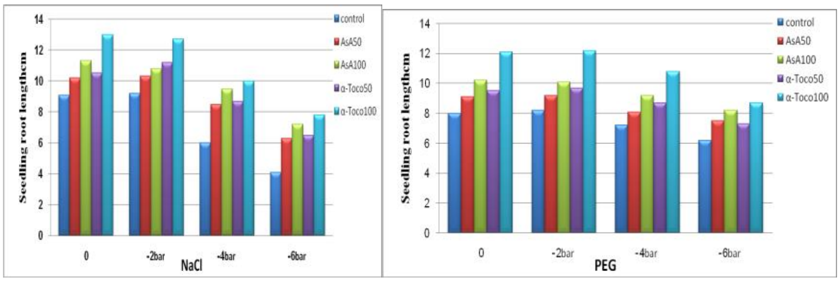

Fig. (3): Effect of presoaking in ascorbic acid or α tocopherol on soybean seedling root length(cm) germinated at different osmotic potentials of NaCl and PEG(after10 days from sowing).

Roots play an important role in plant survival during periods of drought (Hoogenboom *et al.,*1987) and also drought resistance is characterized by an extensive root growth and small reduction of shoot growth in drought stressed conditions (Guoxiong *et al.,* 2002). PEG and NaCl solutions caused a growth reduction in shoots of soybean seedling with rising osmotic stress (Atak *et al.,* 2006). Although the seedling weights decreased with the effects of NaCl and PEG, large seeds had higher dry root weights, even in NaCl and PEG increased concentrations. The stimulation effect of low salinity level on seedling length may be resulted from the beneficial effect of low concentration of chloride on many physiological processes as photosynthesis and osmoregulators. The observed reduction in both seedling shoot height and root length in the present study under moderate and high levels of NaCl or PEG (- 4 and - 6 bars) may be due to the expenditure of energy on the synthesis of organic or inorganic solutes for osmotic adjustment rather than for growth (El-Banna, 1985) energy must be expended to create such osmotic adjustment and this may lead to growth reduction (Yeo, 1983). The harmful effect of salinity on seedling length may be due to the suppressing effects of salinity on both meristematic cell division and elongation as well as root penetration (Bernstein, 1971), and of turgor (Eisa, 1999) and accumulate high levels of salt and an osmotic adjustment is needed to keep root water potential lower than that of the external medium, and high respiration rate (Burchett *et al,* 1989). The chemical potential of the saline solution initially establishes a water potential imbalance between the apoplast and symplast that leads to turgor decrease and may be due to the bad effects of salinity on meristematic cell division and elongation as well as root penetration (Hatung ,2004) and/or inhibited apical growth in plants as well as internal hormonal imbalance (Younis *et al,* 2003). Moreover, the redaction in seedling growth caused by salinity or draught may be attributed mainly to the osmotic stream, which reduced availability and uptake of water and essential nutrients (Neumann, 1997), as well as the excessive accumulation of both toxic ions i.e. Na+ and intermediate compounds such as reactive oxygen species (Rodriguez *et al.,* 2004) which cause damage to DNA, lipid and proteins and consequently a decrease in plant growth. The main inhibitory effect of salinity on seedling growth has been attributed to osmotic inhibition of the absorption of available water, specific ion effect causing excessive accumulation of Na+ or Cl- or in adequate uptake of an essential nutrient, hormonal imbalance and accumulation of toxic intermediate products as free radical oxygen (Roy *et al.,* 1995).

Presoaking seeds in AsA and α-Toco enhance seedling growth by direct effects of the vitamins on the mbolism and/ or act as coenzymes and/ or constituents of enzymes cofactor and/or functions as growth regulators of hormone precursors (Oertli, 1987) and/or antioxidative properties and probably also yet unknown modes of actions (Bayer and Schmidt, 1991). The improving effect of AsA on seedling growth because AsA is reviewed as a natural antioxidant compound may be accumulated in all plants to millimolar, under normal and stress conditions. AsA is a major primary antioxidant (Nijs and Kelley, 1991), plays an important role in preserving the activity of enzymes (Padh, 1990). In addition, AsA is a small, water-soluble antioxidant molecule, which acts as a primary substrate in the cyclic pathway for enzymatic detoxification of hydrogen peroxide; it acts directly to neutralize superoxide radicals (Noctor and Foyer ,1998) and regulates implicated in regulation of cell division (Smirnoff, 1996) and cell wall expansion and cell elongation (El-Yazal, 2007).Concerning the interactions, between salinity levels and application of AsA and α-Toco, it was found that these treatments decreased seedling length when compared with untreated plants. Both AsA and α-Toco at high concentrations (100ppm) increased significantly seedling shoot length. Moreover, AsA or α-Toco at 100 ppm reduce the harmful effect of NaCl and PEG on seedling growth .in addition, AsA was more effective than α-Toco to reduce the harmful effects of salinity on the root length. Generally, AsA or α-Toco counteracted the harmful effects of water stress resulting from drought and salinity on seedling length.

Seedling fresh and dry weight

Data in Fig (4&5) indicate the effect of of NaCl or PEG and AsA or α-Toco as well as their interactions on seedling fresh and dry weight.

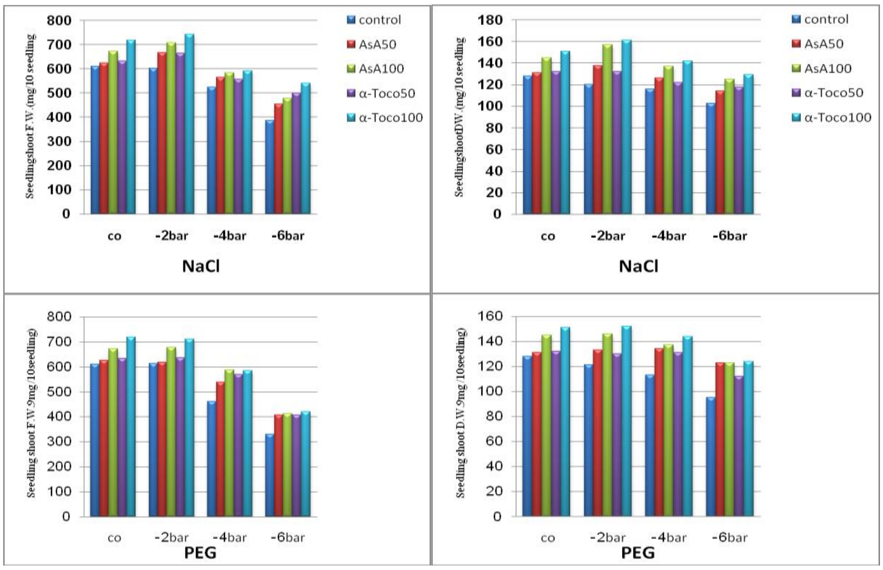

Fig. (4): Effect of presoaking in ascorbic acid or α tocopherol on soybean seedling shoot fresh and dry weight (mg/10seedling) germinated at different osmotic potentials of NaCl and PEG (After10 days from sowing).

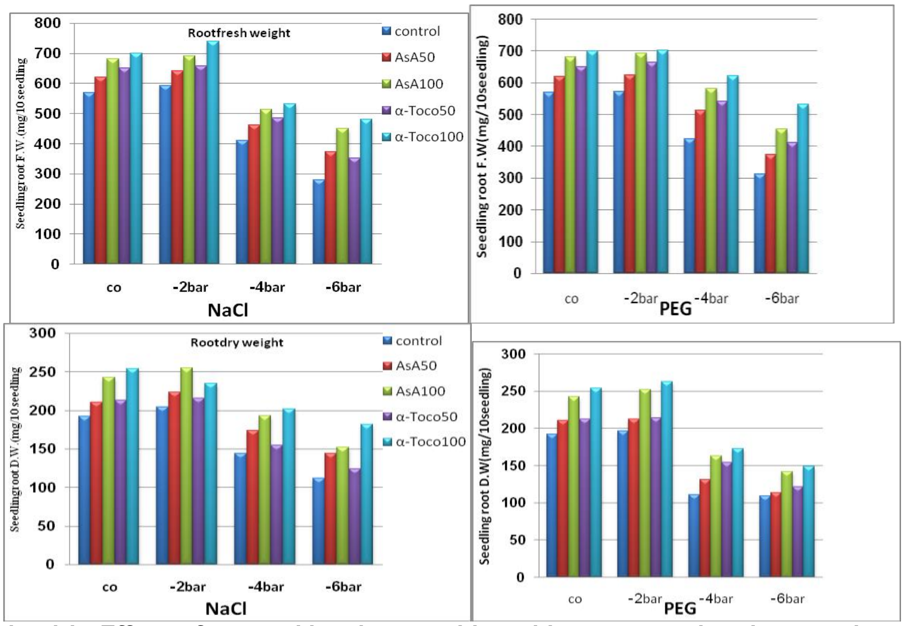

Fig. (5): Effect of presoaking in ascorbic acid or α tocopherol on soybean seedling root fresh and dry weight (mg/10seedling) germinated at different osmotic potentials of NaCl and PEG (After10 days from sowing).

Data revealed that the low levels (-2 bar) of NaCl increased significantly soybean seedling fresh and dry weight, but PEG 6000 drought stress (-2 bar) was no significant differences in seedling fresh and dry weights. It was observed that seedling fresh and dry weight was decreased gradually with increasing NaCl or PEG 6000 concentration up to -4 bar (8.8 dS m-1) and decreasing water potentials of the media. Moreover, the great reduction occurred under high level of NaCl or PEG (-6 bars) compared to untreated plants.

PEG and NaCl solutions increased in soybean seedling root dry matter with rising osmotic stress (Leinhos *et al,* 1996 and Atak *et al,* 2006).The effect of AsA or α-Toco on seedling fresh as well as dry weights was vary to antioxidants types and its concentrations as shown in Fig (4). Both AsA and α-Toco applications increased seedling fresh weight. α-Toco at 100ppm gave the highest values in this concern. The inhibitory effects of stress on soybean seedling fresh and dry weight add more support to the ubiquitous findings of, Demiral and Türkan(2006). The reduced seedling growth under salt stress conditions could be attributed to the physiological drought induced by the low water potential of the soil solution and osmotic adjustments in plants as a result of increased ionic concentration in their cells, which result in deformation of macromolecules by disrupting their shell or bound water (Schwarz, 1985).The basis of limitation of seedling growth under saline conditions is complex. It has been suggested that the decrease in growth is caused by a diversion of the plant assimilates from growth to maintenance (Poljakoff-Mayber and Gale, 1975), e.g. to meet the higher energy requirement of various mechanisms involved in adjustment to unfavorable ionic conditions (Helal and Mengel, 1981) or for compartmentation secretion and repair of cellular damage used by high salinity (Penning de Vries, 1975). Moreover, the effect earlier investigations (Abraham and Kiran ,2003 ; Azooz *et* al,2004)of salinity on plant growth is complex syndrome that involves osmotic stress, ion toxicity and mineral deficiency (Munns *et al.,* 2000), ion and hormonal imbalance or a combination of these factors (Kurth *et al.*,1986).Generally, both AsA and α-Toco counteracted the harmful effect of stress on soybean seedling weight (fresh and dry). In addition, α-Toco was more effective than AsA to reduce the effect of stress on seedling fresh and dry weights.

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تقليـل الضـغط الاسـموزى أثنـاء إنبـات بـذور فـول الصـويا بواسـطة الفـاتوكوفيرول وحمض الاسكوربيك

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اجريت هذه التجربة بغرض تقليل الاثر الضار لنقص الماء الناتج عن الملوحة (كلوريد الصوديوم) والجفاف (البولي ايثيلين جلايكول٦٠٠٠) على نسبةالانبات ونمو البادرات نبات فول الصويا وذلك بنقع البذور فى الفاتوكوفيرول وحمض الاسكوربيك بتركيزى٥٠و١٠٠جزء فى المليون لكل منهم. ولقد اوضحت الدراسة ان الضغط الاسموزى المنخفض من الملوحة (bar 2-) يزيد معنويا من نسبة الانبات ووكذلك يحسن من نمو البادرات(طول البادرة و الوزن الطازج والجاف)بينما الضغط الاسموزى المنخفض من البولي ايثيلين جلايكول ليس لة تاثيرمعنوى، بينما زيادة الضغط الاسموزى حتى (bar 4-) تؤدى الى نقص فى نسبة الانبات وكذلك نمو البادرات ويعتمد ذلك على الملوحة (كلوريد الصوديوم) أوالجفاف (البولي ايثيلين جلايكول) ،وكان الضغط الاسموزى (bars 6-) اكثر تأثيرا مقارنة بالبذور غير المعاملة(الكنترول).كما أثبتت الدراسة أن نقع البذور فى الفاتوكوفيرول وحمض الاسكوربيك كانت فعالة فى زيادة نسبةالانبات ،تحت الظروف الطبيعية ،حيث ان اكبر نسبة انبات فى البذور التى نقعت فى حمض الأسكوربيك (٥٠ جزء فى المليون) والفاتوكوفيرول (١٠٠جزء فى المليون) كان لكل منهما تاثيرا معنويا فى وجودالملح وغيرمعنويا فى وجود البولي ايثيلين جلايكول.. كماان الضغط الاسموزى المنخفض من الملوحة(bar٢-) والبولي ايثيلين جلايكول لم تظهر اختلافات فى طول البادرة. عموما، الفاتوكوفيرول وحمض الاسكوربيك لة تأثير فى تقليل الاثر الجهاد المائى على نسبة إنبات ونمو البادرات . كما أوضحت نتائج التجربة أن حمض الأسكوربيك يقلل الاثر الضار لملوحة كلوريد الصوديوم على نسبةالانبات ونمو بادرات فول الصويا. ولقد كان الفاتوكوفيرول الاكثرتأثير من حمض الاسكوربيك فى تقليل الاثر الاجهاد المائى على الوزن الطازج و الجاف للبادرات .

قام بتحكيم البحث

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