EFFECT OF NaCI AND CaCI₂ SALTS ON SEED GERMINATION AND SEEDLING GROWTH OF Capsicum annuum L.

Arafa, A.A.; M.A. Khafagy; A. M. Abo El-Kheer; R.A. Fouda and M.F. El-Banna

Dept. of Agric. Botany, Fac. of Agric., Mansoura University, Egypt.

ABSTRACT

To compare the effect of NaCl, CaCl₂ and their combinations on germination and early seedling growth stages of Capsicum annuum L., two-separated experiments were laid out at Laboratory of the Agric. Botany Dept., Fac. of Agric., Mansoura Univ., Egypt, during the growing season 2008. Results indicated that significant increases were recorded in percentage of germination (GP), germination performance index (GPI), as well as seedling fresh and dry weights, seedling length, water content, catalase (CAT) activity and photosynthetic pigments concentrations (chlorophyll a, b and total chlorophylls as well as carotenoids) under the low level (2000 ppm) of NaCl or CaCl2 and their combination(1:1). Increasing salt concentration in nutrient cooper solution caused significant decrease in all of these parameters. The great reduction occurred under high salinity level of NaCl (4000 ppm). However, seedling water content (WC) was not-significantly decreased. In addition, CaCl2 at 4000 ppm increased significantly the total carotenoids as compared with NaCl. Meanwhile, peroxidase (POD) activity increased significantly with increasing salinity levels from 2000 to 4000 ppm of both applied salinity types. Moreover, POD activity under NaCl levels showed a marked increase followed by NaCl+ CaCl2 (1:1) and CaCl2 at 4000

Keywords: Capsicum annuum L., salinity, germination, seedling growth, catalase, peroxidase, photosynthetic pigments.

INTRODUCTION

Sweet pepper (*Capsicum annuum* L.) is among the most important crops for the world human nutrition. The arid and semi-arid conditions as well as less availability of fresh water have inflicted the saline conditions in these provinces and are threatening the productivity of this crop, which is considered as moderately sensitive to salt stress (Lycoskoufis et *al.*, 2005). In general, seed germination and early seedling growth are considered as the most sensitive stages to salinity stress (Ashraf and Foolad, 2005) in most of the crops than the growth of established. Seed germination and seedling growth of sweet pepper like other crops, were negatively affected by salinity stress (Khan *et al.*, 2006 and Bassuony *et al.*, 2008).Germination and emergence of pepper seeds are also slow and non-uniform under normal as well as stress conditions (Demir and Okcu, 2004). Soil salinity, if not properly managed, causing decrease in germination rate and germination percentage of pepper seeds.

The salt damage to the seed germination is attributed to various factors such as reduction in water availability, changes in mobilization of stored reserves and affecting structural organization of proteins (Almansouri *et al.*, 2001). The seeds require higher amount of water uptake during the germination under the salt stress due to the accumulation of the soluble

solutes around the seeds, which increases the osmotic pressure. This causes excessive uptake of the ions which results in toxicity in the plant. Moreover, water potential gradient (reduced water availability) between the external environment and the seeds also inhibits the primary root emergence (Eneas Filho et al., 1995 and Delachiave and DePinho, 2003). The most important process that is affected in plants, growing under saline conditions, is photosynthesis. Reduced photosynthesis under salinity is not only attributed to stomata closure leading to a reduction of intercellular CO2 concentration, but also to non-stomata factors. There is strong evidence that salt affects photosynthetic enzymes, chlorophylls and carotenoids (Stepien and Klobus, 2006). In addition, salinity causing disturbance of membrane integrity (Hasegawa et al., 2000), activities of enzymes and damaged photosynthetic components (Winston, 1990). An important cause of this damage is production of reactive oxygen species (ROS) (Smirnoff, 1993). Moreover, plants have the ability to scavenge/detoxify ROS by producing different types of antioxidants (enzymatic and non-enzymatic). Enzymatic antioxidants such as catalase (CAT), peroxidase (POD), (Prochazkova and Wilhelmova, 2007 and Ashraf, 2009). Therefore, the present study was planned to determine the effects of NaCl, CaCl2 salinity and their combinations on sweet pepper germination.

MATERIALS AND METHODS

The experiment was carried out in the Laboratory of the Agricultural Botany Dept., Fac. of Agriculture, Mansoura Univ. during the growing season 2008, to study the effect of NaCl or CaCl₂ and their combination (1:1 w/w) on germination and early seedling growth stages of sweet pepper (*Capsicum annuum* L. cv. Orlando), a hybrid 'California Wonder'. The seeds used in this investigation were secured from the Gohara Co. Cairo, Egypt. Salinity stress was induced by Sodium Chloride (NaCl), Calcium Chloride (CaCl₂) and their rcombination, NaCl: CaCl₂ (1:1 w/w) from EL-Gomhoria Co., Egypt and were used at the concentrations of 2000 and 4000 ppm each (Table 1).

Table (1): The Molarity (Mol), Electrical Conductivity (E.C.) and pH values for different nutrient solutions.

Nutrient solution (N.S.) Ppm		N.S.+ NaCl		N.S.+ CaCl ₂		N.S.+ {NaCl+CaCl ₂ } (1:1 w/w)			
		2000 NaCl	4000 NaCl	2000 CaCl ₂	4000 CaCl ₂	2000(NaCl+CaCl ₂)		4000 (NaCl+CaCl₂)	
						1000 NaCl	1000 CaCl ₂	2000 NaCl	2000 CaCl ₂
Mol (M)	0 (Control)	3.4×10 ⁻²	6.9×10 ⁻²	2.0×10 ⁻²	3.6×10 ⁻²	1.7×10 ⁻²	0.9×10 ⁻²	3.4×10 ⁻²	2.0×10 ⁻²
Ec dSm ⁻¹	2.00	5.42	8.42	4.59	7.60	5.08		8.08	
рН	5.50	5.77	5.80	5.19	5.30	5.45		5.34	

A homogenous lot of sweet pepper seeds were placed in 100 ml beakers and 20 ml of 1% sodium hypochlorite was added for sterilization. These were left in the solution for 5 min followed by washing under running tap water and ionized water twice, then soaked (24 hours) in distilled water. After soaking, the sterilized seeds were divided into 7 sets (7 salinity levels), then placed in glass Petri dishes (11 cm) (25 seeds/dish) with a double layer

of Whatman No. 1 filter paper. The first set was moistened with 10 ml nutrient Cooper solution(table 2), (Cooper, 1979) E.C., ($2.0~\rm dSm^{-1}$) served as control. The six remainder sets were salinized with 10 ml nutrient solution adding salts (Table 1). Measuring the electrical conductivity by digital conductivity meter Lutron CD-4301. The dishes were left in an incubator in the dark for seed germination at $25 \pm 2^{\circ}$ C and 90% relative humidity, then the dishes were covered with aluminum foils for darkens. In order to avoid water losses, 5 ml of the nutrient solution were added to Petri dishes, every 5 days. Thiram was added to the solution at a concentration of 2% (w/v) to control the fungi infection. The experiment was repeated two times and arranged in a completely randomized block design with three replicates.

Table (2): Weights (g) of pure substances to be dissolved in 1000 liters of water to give the theoretically ideal concentrations (Cooper, 1979).

(000)					
Substance	Formula	Weight			
Potassium dihydrogen Phosphate	KH ₂ PO ₄	263			
Potassium Nitrate	KNO ₃	583			
Calcium Nitrate	Ca(NO ₃) ₂ . 4H2O	1003			
Magnesium Sulphate	MgSO ₄ . 7H ₂ O	513			
EDTA Iron	CH ₂ .N(CH ₂ .COO) ₂] ₂ Fe Na	79.0			
Manganous Sulphate	MnSO ₄ .H ₂ O	6.10			
Boric Acid	H ₃ BO ₃	1.70			
Copper Sulphate	CuSO ₄ .5H ₂ O	0.39			
Ammonium Molybdate	(NH ₄) ₆ Mo ₇ O ₂₄ .4H ₂ O	0.37			
Zinc Sulphate	ZnSO ₄ .7H ₂ O	0.44			

Germination percentage was recorded every 24 h for 11 days (ISTA, 1999). Germination performance index (GPI) was calculated according to the formula: GPI= GP/MGT; where GP is germination percentage and MGT is mean time to germination in days (Pill and Fieldhouse, 1982).In the second set of this experiment, the Petri dishes were placed under the laboratory condition at $20 \pm 2^{\circ}$ C under light (40 W fluorescent tubes) for greening. After 14 days(,end of germination), the following data were recorded:

- a. Seedling fresh and dry weight (mg/10 seedling).
- b. Seedling length (cm).
- **c.** The seedling water content (WC) expressed as (mg g⁻¹) was calculated from FW and DW values (Song and Fujiyama, 1998) using the following equation: WC = [(FW-DW)/FW] × 1000 .Where WC is the water content (mg g⁻¹); FW and DW are the fresh weight (mg seedling⁻¹) and dry weight (mg seedling⁻¹) of the seedlings plant, respectively.
- d. Enzymatic activity: The enzyme extraction was done as recommended by Maxwell and Bateman (1967). One gram of fresh sample (cotyledons) was ground with 10 ml 0.1 M Na-phosphate buffer at pH 7.1 in a lab mortar. The homogenate was transferred to centrifuge tubes and was centrifuged at 4C for 15 min at 15000 rpm in laboratory refrigerated

centrifuges Model SIGMA 4K15. The supernatant was made up to a known volume with the same buffer and used for enzyme assay.

- **d.1. Peroxidase activity (POD) (EC1.11.1.7):** The enzymatic activity of POD was determined with a (Spekoll 11) spectrophotometer. The activity of POD was determined according to the method described by Allam and Hollis (1972). This method depends on measuring the oxidation of pyrogallol to pyrogallin in the presence of hydrogen peroxide (H_2O_2) at 425 nm. The sample cuvette contained 500 μ l of 0.1 M potassium phosphate buffer (pH 7.0) + 300 μ l of 0.05 M pyrogallol (6.3 g/L.) + 100 μ l of 1.0% H_2O_2 + 100 μ l enzyme extract. Readings were recorded every 30 seconds for 5 minutes at $27\pm2^{\circ}C$. The activity was expressed as ΔA_{410} g⁻¹min⁻¹.
- **d.2. Catalase activity (CAT) (EC 1.11.1.6):** The enzymatic activity of CAT was measured according to Aebi (1984). About 3 ml reaction mixture containing 1.5 ml of 100 mM potassium phosphate buffer (pH 7.0), 0.5 ml of 75 mM H_2O_2 , 0.05 ml enzyme extraction and distilled water to make up the volume to 3 ml. The reaction started by adding H_2O_2 and a decrease in absorbance was recorded at 240 nm for 1 min. Enzyme activity was computed by calculating the amount of H_2O_2 decomposed. Each enzyme activity was expressed as enzyme unit per gram fresh weight of leaf.
- e. Photosynthetic pigments (mg/g FW): Fresh leaf samples (0.05 g) were extracted by methanol for 24 h at laboratory temperature after adding a trace from sodium carbonate (Robinson et al., 1983), then Chlorophyll a, b and carotenoids were determined spectrophotometrically (Spekol II) (at wave lengths 452, 650, 665 nm). The quantities of total chlorophylls, chlorophyll a, b and carotenoids concentration (mg/g) in leaves were determined by the equations proposed by Mackiny (1941).

Total Chlorophyll = $(25.5*E_{650} + 4*E_{665})/5$ Chlorophyll a = $(16.5*E_{665} - 8.3*E_{650})/5$

Chlorophyll b = $(33.8 \times E_{650} - 12.5 \times E_{665})/5$

Carotenoids = (4.2 * E_{452.5}) - (0.0264 *Chl. a) - (0.496 *Chl. b)/5

Statistical analysis: The obtained data were subjected to statistical analysis of variance according to Gomez and Gomez (1984).

RESULTS AND DISCUSSION

1. Germination percentage: The data illustrated in Figures (1, 2) revealed that low levels (2000 ppm) of all of salinity types NaCl, CaCl₂ and their combinations(1:1) increased significantly germination percentage (GP) and germination performance index (GPI). In addition, seeds germinated under NaCl combined with CaCl₂ (1:1) (82.7 %) resulted in a greater germination percentage followed by CaCl₂ and NaCl (81.3 %) as compared to control (74.7 %). The stimulating effects of low level of salinity on GP and GPI may be resulted from the beneficial effect of low Cl⁻ concentration on activities of enzymes (ATPase and α -amaylase) which hydrolyse starch to sugars (Metzler, 1977) and dependent the growth of the embryonic axis on the transfer of storage materials from the cotyledons (Glenn *et al.*, 1999). In addition, Na⁺ is an essential micronutrient for some of the C₄ photosynthetic plants, which import pyruvate into mesophyll chloroplasts by a Na⁺/pyruvate co- transporter (Ohnishi *et al.*, 1990).

The data reveal that GP decreased gradually with increasing salinity from 2000 to 4000 ppm and the great reduction occurred under high salinity level of NaCl (54.7 %). The inhibitory effect of high salinity levels of NaCl and NaCl+ CaCl2, may be due to increasing osmotic potential which hinder water uptake, an essential initiating step that activates a number of metabolic processes necessary for germination (Kayani et al., 1990) and/or facilitate the intake of ions in sufficient amounts to be toxic for the embryonic activities (Ayers et al., 1952) and/or the influence of the cations more than anions of acid radical used, Cl- is the most toxic acid and Na+ is the most toxic base (Bewley and Black, 1982) and/or reduced water availability between the external environment and the seeds also inhibit the primary root emergence (Enéas, Filho et al., 1995) and/or contact of the seeds with high concentrations of Na⁺ and Cl⁻ ions (Almodares et al., 2007) and/or altering hormonal balances and decreasing endogenous cytokinins biosynthesis and auxins productions (Schmidt, 2005) and/or increasing ABA (Roy et al., 1995) and/or effects of salts on the enzymes activities for hydrolysis, translocation of severe hydrolysis products from the storage organs to embryo axis(Ungar, 1995) and/or altered amaylase (Kocacaliskan and Kabar, 1990), protease (Prisco and Vierira, 1976) and RNAase (Gomes Filho and Sodek, 1988), and/or decreasing amaylase while increasing phenol oxidase activities (Kord and Khalil, 1995).

Generally, this differential behavior of sweet pepper seeds germination according to the salt types are presumably due to the fact that the same concentration of salt generate different osmotic potentials and the osmotic effect may well have a greater influence on germination than specification toxicity (Ungar, 1996).

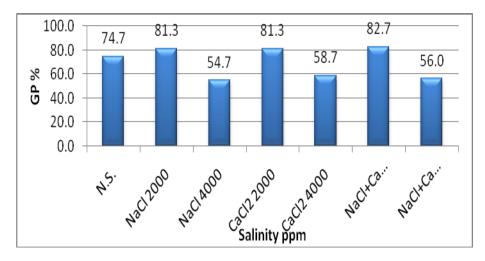


Figure (1): Germination percentage (GP %) of sweet pepper under normal or saline condition (NaCl, CaCl₂ and their combinations) after 11 days from sowing.

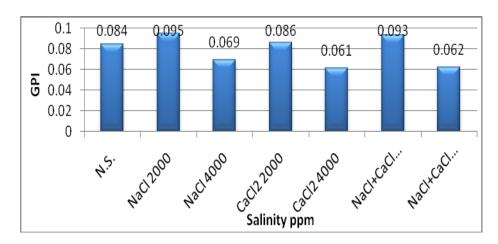


Figure (2): Germination performance index (GPI) of sweet pepper under normal or saline condition (NaCl, CaCl₂ and their combinations) after 11 days from sowing.

2. Seedling growth: The data illustrated in Figures (3-6) and shown in Plate (1) indicate that fresh and dry weights, seedling length as well as water content after 14 days were increased significantly under low levels 2000 ppm of all salinity types. On the other hand, the increasing salt concentration in nutrient Cooper solution caused significant decrease in fresh and dry weights as well as seedling length but seedling water content showed no-significant effect. The great reduction occurred under high salinity level of NaCl. In the present investigation, Reduced seedling length under saline condition may be due to accumulation of toxic ions, that facilitates the intake ions in sufficient amounts to be toxic for the embryonic activities due to the influence of the cations more anions, the entry of ions to the seeds that might have been toxic to the embryo or the developing seedlings (Almodares et al., 2007) and/or inhibition of the uptake of several essential nutrients causing nutritional or ionic imbalance (Taamalli et al., 2004) and/or disturbance in metabolic metabolism leading to an increase in phenolic compounds (Ayaz, et al., 2000) and/or which led to decreasing both cell division and cell elongation.

Salinity induced osmotic cell enlargement depending on soluble accumulation and its effect on cell size and number of cells per unit area (Greenway, 1963). The bad effects of salinity on seedling shoot and root length may be due to the negative effects of salinity on meristematic cell division and elongation as well as root penetration (Hatung, 2004) and due to reduced cell division or cell enlargement caused by salinity stress (Hawker and Walker, 1978).

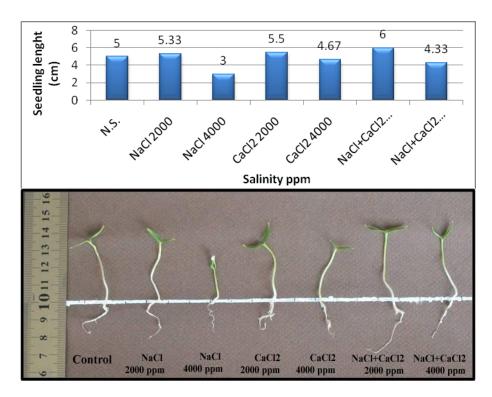


Figure (3): Plate (1) Seedling length (cm) of sweet pepper under normal or saline condition (NaCl, CaCl₂ and their combinations) after 14 days from sowing.

The reduced seedling FW and DW under salt stress conditions could be attributed to the physiological drought induced by the low water potential and osmotic adjustments 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) and/or osmotic adjustment needed to keep root water potential lower than that of the external medium, energy must be expended to create such osmotic adjustment and this may lead to seedling growth reduction (Yeo, 1983) and /or might be attributed to the osmotic effect resulting from salt stress which causes disturbances in water balance and inhibits apical growth and internal hormonal imbalance (Younis et al., 2003 and Abo Shama and Hegazy, 2009) and/or inhibits cytokinins biosynthesis and hormonal unbalances, reducing water content and some plant nutrients uptake as well as biosynthesis of α -tocopherol , ascorbic acid and net photosynthetic rate accompanied with high respiration rate were also reported under stress conditions (Tripathi et al., 2007) and/or may be due to toxic effects unbalanced nutrient uptake by the seedlings (Hajibagheri et al., 1989) and/or decreases in water content (WC) have been communicated for many seedlings growing under salinity (Meloni et al., 2008).

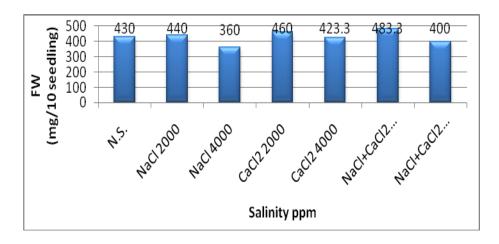


Figure (4): Fresh weight (FW) (mg/10 seedling) of sweet pepper under normal or saline condition (NaCl, CaCl₂ and their combinations) after 14 days from sowing.

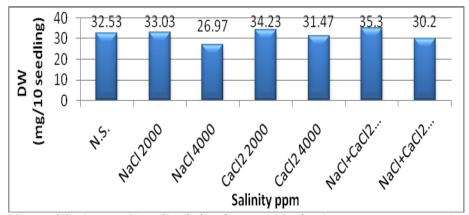


Figure (5): Dry weight (DW) (mg/10 seedling) of sweet pepper under normal or saline condition (NaCl, CaCl₂ and their combinations) after 14 days from sowing.

The WC, although a convenient and widely used methods of assessing plant water status, is not a useful indicator of turgor in salt-treated plants undergoing osmotic adjustment. In most plants, especially halophytes, the solute content of cells at high salinity is higher than in non-saline condition, due largely to accumulation of ions (e.g. Na⁺ and Cl⁻) and organic solutes. Therefore, during the rehydration to establish WC, the higher solute content in salt-treated than in untreated cotyledons causes a greater water uptake in the former than the latter. Thus, this fact results in an apparently low RWC under salinity (Munns *et al.*, 2006).

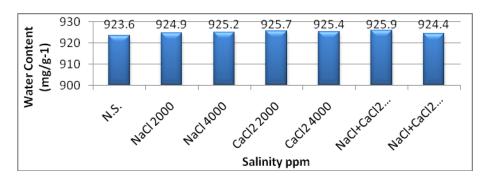


Figure (6): Seedling water content (WC) (mg/g⁻¹) of sweet pepper under normal or saline condition (NaCl, CaCl₂ and their combinations) after 14 days from sowing.

3. Peroxidase (POD) and Catalase (CAT) activity: The data illustrated in (Figure, 7) indicate that all applied salinity types increased significantly POD activity of sweet pepper seedling and high level of salinity was more effective in this respect. Moreover, POD activity under NaCl stress showed a marked increase followed by NaCl+ CaCl₂ (1:1) and CaCl₂.

Concerning the CAT activity (Figure, 8) under low level of all applied salinity types (2000 ppm) increased significantly CAT activity and NaCl+CaCl₂ (1:1) proved to be more effective in this respect., followed by CaCl₂ and NaCl. On the other hand, increasing salinity levels to 4000 ppm decreased significantly CAT activity and the great reduction occurred under NaCl stress. Salt stress produced ROS is a common phenomenon which can interact with a number of cellular molecules and metabolites, thereby leading to a number of destructive processes causing cellular damage (Ashraf, 2009), cell signaling, gene regulation, senescence, programmed cell death, pathogen defense, and others (Gechev *et al.*, 2006).

In this study, the antioxidant enzymes POD and CAT were increased under NaCl salinity (Figures, 7, 8) and further enhanced due to CaCl2 treatment. These results are in agreement with those reported by Jaleel et al., (2007). The plants defend against these reactive oxygen species by induction of activities of certain antioxidative enzymes such as catalase (CAT), peroxidase (POD) (Mittova et al., 2003). Catalase is specific to a great extent for H₂O₂, and remove excess H₂O₂ before it can leak out into other parts of the cell (Ali and Alqurainy, 2006). The high concentration of H₂O₂ in tissues was mainly scavenged by CAT which led to a low level of H₂O₂, while low concentration of H₂O₂ was mainly scavenged by POD during the period of oxidation of relative substances. When POD and CAT were consistent and in harmony with one another, free radicals from ROS in plants could be kept at a low level which exerted the plant growth and metabolize naturally (Jiang, 1999). In addition, Turhan et al., (2006) proposed that the POD activity was increased coordinately in response to salt stress. In addition, Li (2009) revealed that, on tomato seedling the CAT activity increased under (100 mM NaCl), but the CAT activity decreased under 200300 mM NaCl. Moreover, Wang *et al.* (2009) on alfalfa, found that salinity stress increased CAT activity. On the other hand, Noreen and Ashraf (2009) revealed that salt stress enhanced the activities of POD while, decreased the CAT activity in pea. Also, Hassanein *et al* (2009) found that, activity level of POD enzyme progressively increased with increasing salinity levels, while the behavior of CAT activity showed an opposite response. In addition, Gadalla (2009) found that NaCl reduced the activity of CAT and POD.

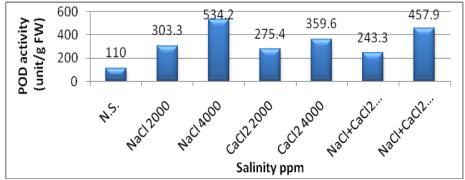


Figure (7): Peroxidase activity (POD) (unit/g FW) of seedling sweet pepper as affected by salinity (NaCl, CaCl₂ and their combinations) after 14 days from sowing.

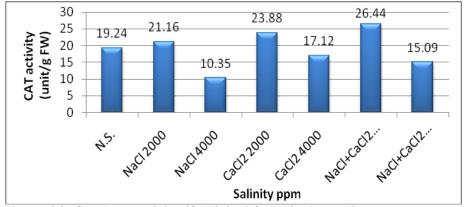


Figure (8): Catalase activity (CAT) (unit/g FW) of seedling sweet pepper as affected by salinity (NaCl, CaCl₂ and their combinations) after 14 days from sowing.

5. Photosynthetic pigments concentrations

The data illustrated in Figures (9-12) clearly show that low salinity level (2000 ppm) of all applied salinity types {NaCl, CaCl₂ and their combinations1:1 (w:w)} caused a high significant increase in the photosynthetic pigments concentrations (chlorophyll a, b and total chlorophylls as well as carotenoids). In addition, NaCl+CaCl₂ (1:1) caused a greater increase in photosynthetic pigments concentrations followed by CaCl₂

and NaCl. The stimulating effects of low salinity level may be resulted from the effect of low concentration of Cl⁻ on photosynthesis and enzymes activity and chloride acts a-cofactor of an NH₂oH sensitive, Mn-containing, O₂-evolving enzyme (Kelley and Izawa, 1978) and Cl⁻ was involved in the splitting of water molecules in photosystem II (Izawa *et al.*, 1969).On the other hand, photosynthetic pigments concentrations decreased gradually with increasing salinity levels from 2000 to 4000 ppm. The great reduction in photosynthetic pigments occurred under NaCl at high salinity levels (4000 ppm). While, CaCl₂ at 4000 ppm increased significantly the total carotenoids as compared with other salinity types. These results are in agreement with those recorded by Parida *et al.* (2004)who reported that photosynthetic rate increased at low salinity level and decreased at the higher ones.

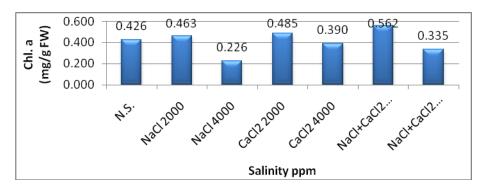


Figure (9): Chlorophyll a concentration (mg/g FW) of seedling sweet pepper as affected by salinity (NaCl, CaCl₂ and their combinations) after 14 days from sowing.

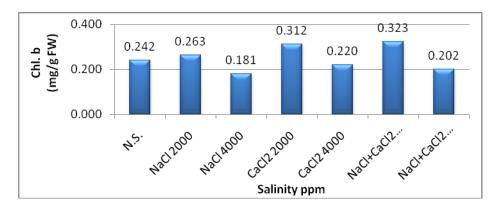


Figure (10): Chlorophyll b concentration (mg/g FW) of seedling sweet pepper as affected by salinity (NaCl, CaCl₂ and their combinations) after 14 days from sowing.

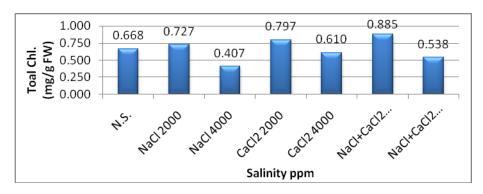


Figure (11): Total chlorophyll concentration (mg/g FW) of seedling sweet pepper as affected by salinity (NaCl, CaCl₂ and their combinations) after 14 days from sowing.

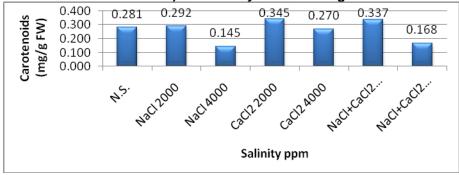


Figure (12): Carotenoids concentration (mg/g FW) of seedling sweet pepper as affected by salinity (NaCl, CaCl₂ and their combinations) after 14 days from sowing.

The reduction in photosynthetic pigments concentrations under high salinity levels may be due to inhibitory effect of chloride on the activity of Fe containing enzymes, cytochrome oxidase which may decrease the rate of chlorophyll, biosynthesis and their accumulation (Helaly *et al.*, 1984) and/or enhancing the activity of chlorophyll degrading enzyme chlorophyllase (Mishra and Sharma, 1994) and/or oxidation of chlorophyll and decreased its concentration (Pell and Dann, 1991) and /or the toxic action of NaCl on the biosynthesis of pigments, increasing their degradation and/or maintaining damage of the chloroplast thylakoid (Hashem, 2000).

Concerning CaCl₂ at 4000 ppm it is observed that, carotenoids concentration was increased as compared to control (Figure 12). These results may be due to the fact that Ca²⁺ play a critical role in light harvesting complex assembly and function (Munné-Bosch *et al.*, 1999) and/or antioxidants under salt stress (Vaidyanathan *et al.* 2003).

It could be concluded that low salinity level irrespective its type had a stimulative effect on the studied parameters and the higher ones showed the opposite effect in this respect.

REFERENCES

- Aboshama, H.M.S. and Hegazy, A.E. 2009. *In vitro* screening and production of salt tolerant *Citrus volkamariana* plant. J. Agric. Sci. Mans. Univ., 34 (10): 10115-10133.
- Aebi, H. 1984. Catalase in vitro. Methods Enzymol., 105: 121-126.
- Ali, A.A. and Alqurainy, F. 2006. Activities of antioxidants in plants under environmental stress. In: Motohashi, N. The lutein-prevention and treatment for diseases. India: Transworld research network, p. 187-256.
- Allam, A.I. and Hollis, J.P. 1972. Sulfide inhibition of oxidases in rice roots. Phytopathology, 62: 634-639.
- Almansouri, M.; Kinet, M. and Lutts, S. 2001. Effect of salt and osmotic stresses on germination in durum wheat (*Triticum durum* Desf.). Plant and Soil, 231: 243-254.
- Almodares, A.; Hadi, M.R. and Dosti, B. 2007. Effects of salt stress on germination percentage and seedling growth in sweet sorghum cultivars. J. Biological Sciences., 7 (8): 1492-1495.
- Ashraf, M. 2009. Biotechnological approach of improving plant salt tolerance using antioxidants as markers. Biotechnology Advances, 27: 84-93.
- Ashraf, M. and Foolad, M.R. 2005. Pre-sowing seed treatment a shotgun approach to improve germination, plant growth, and crop yield under saline and non-saline conditions. Advances in Agronomy, 88: 223-271.
- Ayaz, F.A.; Kadioglu, A. and Turgut, R. 2000. Water stress effects on the content of low molecular weight carbohydrates and phenolic acids in *Ctenanthe setosa* (Rose.) Eichler. Can. J. Plant Sci., 80: 373-378.
- Ayers, A.D.; Brown, J.W. and Wideigh, C.H. 1952. Salt tolerance of barley and wheat in soil plots receiving several salinization regime. Agro. J., 44: 307-310.
- Bassuony, F.M.; Hassanein, R.A.; Baraka, D.M. and Khalil, R.R. 2008. Physiological effects of nicotinamide and ascorbic acid on *Zea mays* plant grown under salinity stress ii-changes in nitrogen constituents, protein profiles, protease enzyme and certain inorganic cations. *Aust.* J. Basic and App. Sci., 2(3): 350-359.
- Bewley, J.D. and Black, M. 1982. Physiology and biochemistry of seed in relation to germination. *Vol. 2* Springer Verlag, Berlin.
- Churchli, K.A. and Sze, H. 1984. Anion-sensitive, H⁺ pumping ATPase of oat roots. Plant Physiol., 76: 490-497.
- Cooper, A. 1979. The ABC of NFT. Growers Books, London, P. 59.
- Cramer, G.R. 1992. Kinetics of maize leaf elongation. 2-Response of a Naexcluding cultivar and a Na-including. Exp. Bot., 43: 857-864.

- Demir, I. and Okcu, G. 2004. Aerated hydration treatment for improved germination and seedling growth in aubergine (*Solanum melongena*) and pepper (*Capsicum annuum*). Annals of Applied Biology, 144: 121-123.
- Delachiave, M.E.A. and De Pinho, S.Z. 2003. Scarification, temperature and light in germination of *Senna occidentalis* seed (Caesalpinaceae). Seed Sci. and Technology, 31(2): 225-230.
- Enéas, Filho, J.; Oliveira Neto, O.B.; Prisco, J.T.; Gomes Filho, E. and Monteiro, C. 1995. Effects of salinity in vivo and in vitro on cotyledonary galactosidases from *Vigna unguiculata* (L.) Walp. during seed germination and seedling stablishment. Revista Brasileira de Fisiologia Vegetal, 7 (2): 135-142.
- Gadalla, S.F. 2009. The roles of ascorbic acid and α -tocopherol in minimize of salt-induced wheat leaf senescence. J. Agric. Sci. Mans. Univ., 34 (11): 10645-10661.
- Gechev, T.S.; Breusegem, F.V.; Stone, J.M.; Denev, I. and Laloi, C. 2006. Reactive oxygen species as signals that modulate plant stress responses and programmed cell death. Bio. Essays, 28:1091-1101.
- Glenn, E.; Brown, J. and Blumwald, E. 1999. Salt tolerance and crop potential of halophytes. Crit. Rev. Plant Sci., 18: 227-255.
- Gomes Filho, E. and Sodek, L. 1988. Effect of salinity on ribonuclease activity of *Vigna unguiculata* cotyledons during germination. J. Plant Physiology, 132: 307-311.
- Gomez, K.A. and Gomez, A.A. 1984. Statistical Procedures for Agriculture Research. John Wiley and Sons, Inc. New York.
- Greenway, H. 1963. Plant responses to saline substrates. III. Effect of nutrient concentration on the growth and ion uptake of *Hordeum vulgare* during NaCl stress. Aust. J. Biol. Sci., 16: 616-619.
- Hajibagheri, M.A.; Yeo, A.R.; Flowers, T.J. and Collins, J.C. 1989. Salinity resistance in *Zea mays* fluxes of potassium, sodium and chloride, cytoplasmic concentrations and microsomal membrane lipids. Plant, Cell and Env.., 12: 753-757.
- Hasegawa, P.M.; Bressan, R.A.; Zhu, J.K. and Bohnert, H.J. 2000. Plant cellular and molecular responses to high salinity. Annu. Rev. Plant Physiol. Plant Mol. Biol., 51: 463-499.
- Hashem, H.A., 2000. Molecular physiological studies on heat shock protein expression in a stressed plant. M.Sc. Thesis, Fac. Sci. Ain Shams Univ., Cairo, Egypt.
- Hassanein, R.A.; Bassuony, F.M.; Baraka, D.M. and Khalil, R.R. 2009. Physiological effects of nicotinamide and ascrobic acid on *zea mays* plant grown under salinity stress. 1-changes in growth, some relevant metabolic activities and oxidative defense systems. Res. J. Agric. and Biolo. Sci., 5(1): 72-81.
- Hatung, W. 2004. Plant Response to Stress: Abscisic Acid Fluxes. Marcel Dekker. Inc., New York.
- Hawker, J.S. and Walker, R.R. 1978. Effect of sodium chloride on expansion rates and invertase activity of leaves. Aust. J. Plant Physiol., 5: 73-80.

- Helaly, M.N.; Salama, A.M. and Arafa, A.A. 1984. Effects of salinity on growth mineral constituents, water fractions and endogenous growth substances in Horse bean plants. J. Agric. Sci. Mans. Univ., 9 (2): 251-264.
- ISTA, 1999. International rules for seed testing. Seed Science and Technology, 27: 1-333.
- Izawa, S.; Heath, R.L. and Hind, G. 1969. The role of chloride ion in photosynthesis. III. The effect of artificial electron donors upon electron transport. Biochem. Biophys. Acta., 180: 388-398.
- Jaleel, C.A.; Manivannan, P.; Sankar, B.; Kishorekumar, A. and Panneerselvam, R. 2007. Calcium chloride effects on salinity-induced oxidative stress, proline metabolism and indole alkaloid accumulation in Catharanthus roseus. C. R. Biologies, 330: 674–683.
- Jiang, M.Y. 1999. Generation of OH and oxidation injury of plants under the condition of water stress. Acta Botanica Sinica., 41 (3): 229-234.
- Kayani, S.A.; Naqvi, H.H. and Ting, I.P. 1990. Salinity effects of germination and mobilization of reserves in jojoba seed. Crop Sci., 30: 704-708.
- kelley, P.M. and Izawa, S. 1978. The role of chloride ion in photosystem II. I. Effects of chloride ion on photosystem II electron transport and on hydroxylamine inhibition. Biochem. Biophys. Acta, 502: 198-210.
- Khan, M.M.A.; Ahmed, Z.; and Hameed, A. 2006. Effect of sea salt and L-ascorbic acid on the seed germination of halophytes. J. Arid Environments, 67: 535-540.
- Kocacaliskan, I. and Kabar, K. 1990. Effect of salinity on polyphenol oxidase during seed germination. Doga Tr. J. Bot., 15: 41-49.
- Kord, A.A. and Khalil, M.S. 1995. Salinity stress and enzymatic activities during seed germination. Egypt. J. Physiol. Sci., 19: 225-265.
- Li, Y. 2009. Physiological responses of tomato seedlings (*Lycopersicon Esculentum*) to salt stress. Modern Applied Sci., 3: 171-176.
- Lycoskoufis, I.H.; Savvas, D. and Mavrogianopoulos, G. 2005. Growth, gas exchange and nutrient status in pepper (*Capsicum annuum* L.) grown in recirculating nutrient solution as affected by salinity imposed to half of the root system. Scientia Hort., 106: 147-161.
- Mackiny, G. 1941. Absorption of light by chlorophyll solution. J. Biol. Chem., 140: 315-322.
- Maxwell, D.P. and Bateman, D.F. 1967. Changes in the activities of some oxidases in extracts of *Rhizoctonia* infected bean hypocotyl in relation to lesion maturation. Phytopathology, 57: 132.
- Meloni, D.A.; Gulotta, M.R. and Martínez, C.A. 2008. Salinity tolerance in *Schinopsis quebracho* colorado: seed germination, growth, ion relations and metabolic responses. J. Arid Env., 72: 1785-1792.
- Metzler, D.E. 1977. Biochemistry the Chemical Reactions of Living Cells. Academic press, New York, pp 357-370.
- Mishra, S.N. and Sharma, I. 1994. Putrescine as a growth inducer and as a source of nitrogen for mustard seedlings under sodium chloride salinity. Indian J. Exp. Physiol., 32: 916-918.

- Mittova, V.; Tal, M.; Volokita, M. and Guy, M. 2003. Upregulation of the leaf mitochondrial and peroxisomal antioxidative systems in response to salt induced oxidative stress in the wild salt-tolerant tomato species *Lycopersicon pennellii*. Plant Cell Environ., *26*: 845-856.
- Munné-Bosch, S.; Schwarz, K. and Alegre, L. 1999. Enhanced formation of α-tocopherol and highly oxidized abietane diterpenes in water-stressed rosemary plants. Plant Physiol., 121: 1047-1052.
- Munns R.; James, R.A. and Laüchi, A. 2006. Approaches to increasing the salt tolerance of wheat and other cereals. J. Exp. Botany, 57: 1025-1043.
- Noreen, Z. and Ashraf, M. 2009. Assessment of variation in antioxidative defense system in salt-treated pea (*Pisum sativum*) cultivars and its putative use as salinity tolerance markers. J. Plant Physiol., 166: 1764-1774.
- Ohnishi, J.; Flugge, U.I.; Heldt, H.W. and Kanai, R. 1990. Involvement of Na⁺ in active uptake of pyruvate in mesophyll chloroplasts of some C4 plants: Na⁺/Pyruvate co-transport. Plant Physiol., 94: 950-959.
- Parida, A.K.; Das, A.B. and Mittra, B. 2004. Effects of salt on growth, ion accumulation, photosynthesis and leaf anatomy of the mangrove, *Bruguiera parviflora*. Trees-Struct. Funct., 18: 167-174.
- Pell, E.J. and Dann, M.S. 1991. Multiple stress-induced foliar senescence and implication for whole plant longevity. In A.H. Mooney, V.E. Vinner, E.J. Pell, E. Chu, eds, Response of plants to multiple stresses. Academic Press Inc., pp 189-204.
- Pill, W.G. and Fieldhouse, D.G. 1982. Emergence of pregerminated tomato seed stored in gels up to twenty days at low temperatures. J. of the Am. Soc. Hort. Sci., 107: 722-725.
- Prisco, J.T. and Vierira, H.F. 1976. Effect of NaCl salinity on nitrogenous compounds and protease during germination of *Vigna Sinesis* seeds. Physiol. Plant. 36: 317-320.
- Prochazkova, D. and Wilhelmova, N. 2007. Leaf senescence and activities of the antioxidant enzymes. Biol. Plant., 51: 401-406.
- Robinson, S.P.; Downton, W.J.S. and Millhouse, J.A. 1983. Photosynthesis and ion content of leaves and isolated chloroplasts of salt-stressed spinach. Plant Physiol., 73: 238-242.
- Roy, D.; Basu, N.; Marik, R. and Banerjee, S.K. 1995. Changes in levels of endogenous gibberellins and abscisic acid in salt-sensitive germinating rice by NaCl-salinity. Plant Physiol. Biochem. New Delhi, 22 (2): 200-202.
- Schmidt, R.E. 2005. Biostimulants function in turfgrass nutrition. Ph.D. Emeritus Virginia Tech.
- Schwarz, M. 1985. The use of saline water in hydroponics. Soilless Cult., 1: 25-34.
- Smirnoff, N. 1993. The role of active oxygen in the response of plants to water deficit and desiccation. New Phytol., 125: 27-58.
- Song, J.Q. and Fujiyama, H. 1998. Importance of Na content and water status for growth in Na-salinized rice and tomato plants. Soil Sci. Plant Nutr., 44: 197-208.

- Stepien, P. and Klobus, G. 2006. Water relations and photosynthesis in *Cucumis sativus* L. leaves under salt stress. Biol. Plant., 50: 610-616.
- Taamalli, W.; Abz, L.; Youssef, N.B.; Miled, D.D.B. and Zarrouk, M. 2004. Lipid breakdown in sunflower (*Helianthus annuus* L.) seeds during post germinative growth under salt-stress. Rivista Italian delle Sostanze Grasse, 81: 90-97.*
- Tripathi, S.B.; Gurumurthi, K.; Panigahi, A.K. and Shaw, B.P. 2007. Salinity induced changes in proline and betaine contents and synthesis in two aquatic macrophytes differing in salt tolerance. Biol. Plant., 51: 110-115.
- Turhan, E.; Karni, L.; Aktas, H.; Deventurero, G.; Chang, D.C.; Bar-Tal, A. and Aloni, B. 2006. Apoplastic anti-oxidants in pepper (*Capsicum annuum* L.) fruit and their relationship to blossom-end rot. J. Hort. Sci. and Biot., 81 (4): 661-667.
- Ungar, I.A. 1995. Seed germination and see-bank ecology in halophytes. In: Kigel J, Galili G, eds. Seed development and seed germination. New York: Marcel Dekker, 599-628.
- Ungar, I.A. 1996. Effect of salinity on seed germination, growth and ion accumulation of *Atriplex patula* (Chenopodiaceae). Am. J. Bot., 83: 604-607.
- Vaidyanathan, H.; Sivakumar, P.; Chakrabarty, R. and Thomas, G. 2003. Scavenging of reactive oxygen species in NaCl-stressed rice (*Oryza sativa* L.) differential response in salt-tolerant and sensitive varieties. Plant Sci., 165: 1411-1418.
- Wang, W.B.; Kim, Y.H.; Lee, H.S.; Kim, K.Y.; Deng, X.P.; Kwak, S.S. 2009. Analysis of antioxidant enzyme activity during germination of alfalfa under salt and drought stresses. Plant Physiol. and Bioch., 47: 570-577.
- Winston, G.W. 1990. Physio-chemical basis for free radical formation in cells; production and defenses, pp. 57-86. *In*: R. G. Alscher and J. R. Cummings (eds.). Stress responses in plants. Adaptation and acclimation mechanisms. Wiley-Liss, New York.
- Yeo, A.R. 1983. Salinity resistance: physiologies and prices. Physiol. Plant., 58: 214-222.
- Younis, M.E.; El-Shahaby, O.A.; Nemat Ally, M.M. and El-Bastawisy Zeinab, M. 2003. Kinetin alleviates the influence of waterlogging and salinity in *Vigna sinensis* and *Zea mays*. Agronome, 23: 277-285.

^{*} In French with English summary

تأثير ملحي كلوريد الصوديوم والكالسيوم علي إنبات ونمو بادرات الفلفل الحلو عرف مدف أبوالخير، عرف محمد أبوالخير، مضان عبدالله محمد أبوالخير، مضان عبدالمنعم فوده و مصطفي فؤاد البنا قسم النبات الزراعي - كلية الزراعة - جامعة المنصورة - مصر

لمقارنة تأثير ملوحة كلوريدي الصوديوم والكالسيوم وكذلك مخلوطهم علي إنبات ونمو بادرات الفلفل الحلو أجريت تجربتان منفصلتان في معمل قسم النبات الزراعي كلية الزراعة جامعة المنصورة مصر عام ٢٠٠٨. وأوضحت النتائج إلي حدوث زيادة معنوية في كل من نسبة الانبات، دليل نسبة الانبات، الوزن الطازج والجاف وطول البادرات، المحتوي المائي للبادرات، نشاط إنزيم الكاتاليز وكذلك تركيز صبغات البناء الضوئي (كلوروفيل أ، ب والكلوروفيل الكلي ، الكاروتينات) وذلك تحت مستوي الملوحة المنخفض (كلوروفيل أ، ب في المليون من كل من كلوريدي الصوديوم والكالسيوم وكذلك مخلوطهم بنسبة (وزن:وزن).

أدي زيادة تركيز الملح في المحلول المغذي الي حدوث نقص معنوي في كل الصفات السابقة، وكان كلوريد الصوديوم ٤٠٠٠ جزء في المليون الأكثر تأثيراً. ولكن كان النقص غير معنوي بالنسبة للمحتوي المائي للبادرات. بالإضافة إلي ذلك أدي كلوريد الكالسيوم بتركيز ٤٠٠٠ جزء في المليون إلي حدوث زيادة معنوية بالنسبة لتركيز الكاروتينات وذلك بالمقارنة بالأملاح الأخري.

بينما أدي زيادة تركيز الملح من ٢٠٠٠ إلي ٤٠٠٠ جزء في المليون إلي زيادة نشاط إنزيم البيروكسيديز. علاوة على ذلك كان كلوريد الصوديوم الأكثر تأثيراً يليه مخلوط الأملاح (١:١).

قام بتحكيم البحث

أ.د / سمير محمد عبد الجواد سلامة أ.د / السيد حسن عسكر

كلية الزراعة – جامعة المنصورة مركز البحوث الزراعية