

Comparative Physiological and Growth Responses of Tomato and Pepper Plants to Fertilizer Induced Salinity and Salt Stress under Greenhouse Conditions

Figen Eraslan^{1*}, Aydın Güneş², Ali İnal², Nuray Çiçek², Mehmet Alpaslan²

¹Süleyman Demirel University, Faculty of Agriculture, Soil Science Department, Isparta.

*eraslan@ziraat.sdu.edu.tr

²Ankara University, Faculty of Agriculture, Soil Science Department, Ankara

ABSTRACT

Fertilizer induced salinity adversely affects plant growth through its ionic and osmotic effects as in ordinary salinity caused by toxic ions (Na, Cl, etc.). In this study, to determine the ionic and osmotic effects of fertilizer induced salinity and NaCl salinity on growth, ascorbic acid, proline and hydrogen peroxide (H₂O₂) accumulation and stomatal resistance (SR), relative water content (RWC), malondialdehyde (MDA) contents of tomato and pepper plants subjected to different treatments (i.e. control, 40 mM NaCl salinity and excess fertilizer salinity) were investigated under greenhouse condition. The results of this study indicated that similar to NaCl salinity, fertilizer induced salinity significantly reduced the fresh and dry weights of tomato and pepper plants. Relative water content of the plants was decreased by NaCl salinity. Both NaCl and fertilizer induced salinity caused significant increases in proline, MDA, ascorbic acid and H₂O₂ accumulation, and stomatal resistance of the plants. Salinity achieved by NaCl and fertilizer altered plant growth and plant physiological processes ionically and osmotically in a similar manner.

Key words: ascorbic acid, malondialdehyde, proline, relative water content, H₂O₂, stomatal resistance

INTRODUCTION

Salinization plays a major role in soil degradation. It affects 19.5% of irrigated land and 2.1% of dry land agriculture existing on the globe (FAO, 2000). In many crop production areas, use of low quality water for irrigation and application of excess amounts of mineral fertilizer are the major reasons for increased salinity problem in cultivated soils. Due to very rapid accumulation of salts in soil under greenhouse conditions, salinity problem is also a critical constraint to vegetable production (Shannon and Grieve, 1999). Salinity effects are more conspicuous in arid and semiarid regions, where limited rainfall, high evapotranspiration and high temperature associated with poor water and soil management contribute to the salinity problem and become of great importance for agriculture production in these regions.

Salinity stress depresses plant growth and development at different physiological levels. The reduction in plant growth by salinity stress might be related to adverse effects of excess salt on ion homeostasis, water balance, mineral nutrition and photosynthetic carbon metabolism (Zhu, 2001;

Munns, 2002). The mechanisms by which salt stress damage plants are still a discussing matter due to very complex nature of the salt stress in plants.

Salt stress tolerance in plants is a complex phenomenon that may involve developmental changes as well as physiological and biochemical processes. Salinity can damage the plant through its osmotic effect, which is equivalent to a decrease in water activity, through specific toxic effects of ions and by disturbing the uptake of essential nutrients (Marschner, 1995; Dorais et al., 2001). In general, enzymes and metabolic activities in plants are highly influenced by both amount and type of salts. Salt resistance includes both avoidance and tolerance mechanisms (Levitt, 1980). The former may operate through active extrusion of ions by specific pumps, passive exclusion of ions due to membrane impermeability, or dilution by rapid growth associated with an increase in water content. An unavoidable consequence of growth in a solution containing a high salt concentration is the development of osmotic stress, which is followed by a loss of turgor. Tolerance to osmotic stress may operate either through dehydration tolerance, which permits the cell to survive without growing when the turgor decreases, or by avoiding dehydration through osmoregulation, which includes an increase of solute concentration in the cell and consequently rehydration. The solutes may be either salt ions, which can be sequestered in the vacuole and osmotically balanced by organic solutes in the cytoplasm, or organic substances. The latter occurs when salt ions are prevented from entering the cells (Tal, 1984). Salt-sensitive in which group most agricultural and horticultural crops belong respond to salinity with profound decrease in vegetative and reproductive growth (Greenway and Munns, 1980). These responses are highly due to osmotic effects. The inability of osmoregulation may result from either an insufficient uptake of salt ions or a lack of synthesis of organic solutes, which leads to growth disruption occasioned by reduced water uptake as well as reduced water potential in the soil, resulting to physiological water stress. In addition, salinity can cause injury by inorganic ions, which are absorbed by the cell, are not compartmentalized, and are accumulated at toxic levels in plant tissues. (Wahome, 2003; Neocleous and Vasilakakis, 2007).

Under oxidative stress conditions such as salinity, plants produce active oxygen species (AOS). To scavenge AOS; plants have evolved specific defense tactics involving both enzymatic and non-enzymatic antioxidant mechanisms. Several antioxidant enzymes participate in the detoxification of AOS. Superoxide dismutases react with superoxide radicals ($O_2^{\bullet-}$) to produce H_2O_2 (Bowler et al., 1992). In the absence of natural scavengers such as catalase and peroxidase, H_2O_2 accumulates in tissues to high levels. Accumulation of free proline is a typical response to salt stress. When exposed a high salt content in the soil (leading to water stress), many plants accumulate high amounts of proline, in some cases several times the sum of all the other amino acids (Mansour, 2000). Proline has been found to protect cell membranes of onion against salt injury (Mansour, 1998). Malondialdehyde (MDA) content, a product of lipid peroxidation, has been considered an indicator of oxidative damage (Zhua et al. 2004).

The aim of this work was an effort to investigate the ionic and osmotic effects of NaCl and fertilizer induced salinity to physiological, biochemical and growth parameters in tomato and pepper plants.

MATERIALS and METHODS

Growth Condition and Treatments

To determine the ionic and osmotic effects of salt stress on growth and physiological parameters of tomato (*Lycopersicon esculentum* L.) and pepper (*Capsicum annuum* L.) plants under stress conditions (NaCl and fertilizer induced salinity), an experiment was conducted in greenhouses under natural light conditions at the Faculty of Agriculture, Ankara university. For this aim, plants were grown three different treatments as a follow:

a) Control Treatment: Plants were grown with basic nutrient solution (BNS) contained as a mg kg⁻¹; 263 KH₂PO₄, 583 KNO₃, 1003 Ca(NO₃)₂ 4H₂O, 513 MgSO₄ 7H₂O, 171 Fe-EDDHA, 6.1 MnSO₄ H₂O, 0.39 CuSO₄ 5H₂O, 0.37 (NH₄)₆Mo₇O₂₄ 4H₂O, 0.33 ZnSO₄ 7H₂O and 1.7 H₃BO₃

b) NaCl Treatment: BNS + 40 mM NaCl

c) Exceed Fertilizer Treatment: Basic nutrient solution (BNS) concentration increased three folds in order to achieve fertilizer induced salinity.

Tomato and pepper plants were grown in a glasshouse under natural light conditions. Three-week-old seedlings were transplanted at a rate of one plant per pot filled with 5000 g of air-dried soil. Some characteristics of the soil were as follows: texture loam, calcium carbonate (CaCO₃) 6.02%, pH (1:2.5 water) 7.68, EC 0.2 dS m⁻¹, organic matter 0.76%, total N 0.20%. The concentration of ammonium acetate (NH₄Oac)-extractable K, Ca and Na were as follows (mg kg⁻¹) 378, 4800 and 60 respectively. Sodium bicarbonate (NaHCO₃)-available P was 14.25 mg kg⁻¹, and DTPA-extractable Zn, Fe, Cu and Mn were as follows (mg kg⁻¹); 0.57, 5.24, 0.48 and 33 respectively. After one day seedling transplantation, treatments were applied to have divided three for seedlings adaptation to stress conditions. For fresh matter used for assay, samples were taken fully matured leaves from tomato and pepper plants chosen at random. All the measurements with fresh matter were carried out during the last week before harvesting. After the 60 d of growing period, all plants were harvested with a knife by cutting at the joint point of fine roots to the main stem. The rest of the plants were weighed for the fresh weight determination. They were then dried in an air-forced oven at 65 °C until constant mass was reached, and then weighed dry weight determination.

Plant Measurement and Statistical Analyses

Stomatal Resistance Measurements

Stomatal resistance (SR) of the plants was measured by a ΔTAP4 Porometer (DELTA-T DEVICES, UK). The outer leaves were used in the SR measurements. Measurements were made on

three leaves in each plant in the morning (10.30–11.30 a.m.) at a steady photon flux density ($>250 \text{ mmol m}^{-2} \text{ s}^{-1}$), while leaf temperature varied between 18 and 20 °C.

Determination of H₂O₂, Proline and Ascorbic Acid

Hydrogen peroxide was estimated with titanium reagent (Terenashi et al., 1974). Titanium dioxide (1 g) and 10 g of potassium sulfate were mixed, and boiled with 150 mL of concentrated sulfuric acid for 2 h on a hot plate. The digested mixture was cooled and diluted to 1.5 mL with distilled water, and used as titanium reagent. Sample preparation and H₂O₂ estimation was performed as described by Mukherjee and Choudhuri (1983). Leaf material (0.5 g) was homogenised in 10 mL of cold acetone (90% v/v). The homogenate was filtered through Whatman no. 10 filter paper. Titanium reagent (4 mL) was added to the filtrate, followed by 5 mL of concentrated ammonium solution to precipitate the peroxide– titanium complex. The reaction mixture was centrifuged in a refrigerated centrifuge for 5 min at 10,000g, 4°C, and the supernatant was discarded and the precipitate was dissolved in 10 mL of 2M H₂SO₄. It was re-centrifuged to remove the undissolved material and absorbance was recorded at 415 nm. Concentration of H₂O₂ was determined using a standard curve plotted with known concentration of H₂O₂.

Proline was determined as described by Bates et al., (1973). Leaf tissues (250 mg) were rinsed three times with distilled water and the stoppered tubes with 10 ml water placed in a boiling water bath for 10 min to extract the hot water-soluble compounds. An aliquot of water extract was treated with ninhydrin reagent. Toluene phase was decanted and the absorbance was recorded at 520 nm. The concentration of proline was calculated from a standard curve plotted with known concentration of L-proline as standard.

Ascorbic acid was estimated as described by Mukherjee and Choudhuri (1983). Leaf tissue (250 mg) was extracted with 10 ml of 6% TCA. An aliquot (4 ml) of the extract was mixed with 2 ml of 2% dinitrophenyl hydrazine (in acidic medium) followed by the addition of 1 drop of thiourea (in 70% ethanol). The mixture was boiled for 15 min in a water bath, and after cooling to room temperature, 5 ml of 80% (v/v) (0°C) H₂SO₄ was added to the mixture at 30°C (in an ice bath). The absorbance was recorded at 530 nm. The concentration of ascorbic acid was calculated from a standard curve plotted with known concentration of ascorbic acid.

Determination of Lipid Peroxidation and Relative Water Content (RWC)

The level of lipid peroxidation was measured in terms of malondialdehyde (MDA) content, a product of lipid peroxidation, leaf sample (0.5 g) was homogenised in 10 mL of 0.1% trichloro acetic acid (TCA). The homogenate was centrifuged at 15,000g for 5 min. To 1.0 mL aliquot of the supernatant 4.0 mL of 0.5% thiobarbituric acid (TBA) in 20% TCA was added. The mixture was heated at 95 °C for 30 min and then quickly cooled in an ice bath. After centrifugation at 10,000 g for 10 min, the absorbance of supernatant was recorded at 532 nm. The value for non-specific absorption

at 600 nm was subtracted. The amount of MDA–TBA complex (red pigment) was calculated from the extinction coefficient $155 \text{ (mM}^{-1} \text{ cm}^{-1}\text{)}$ (Hodges et al., 1999).

All spectrophotometric measurements were carried out by spectrophotometer (Shimadzu UV-VIS, Japan).

For relative water content (RWC) determinations, the samples were also weighed immediately as fresh weight (FW), and then floated on distilled water for 4 h. The turgid leaves were then rapidly blotted to remove surface water and weighed to obtain turgid weight (TW). The leaves were dried in the oven at $60 \text{ }^{\circ}\text{C}$ for 24 h and then dry weight (DW) obtained. The RWC was calculated by formulae given in (Dhanda and Sethi, 1998): $\text{RWC (\%)} = (\text{FW} - \text{DW})/(\text{TW} - \text{DW}) \times 100$.

The experiments were designed as completely randomized with four replications. The experimental data were analyzed by ANOVA and the differences were compared by Least Significant Difference Test (LSD) at alpha 0.05.

RESULTS and DISCUSSION

Plant Growth

The ionic and osmotic effects of NaCl and fertilizer induced salinity on fresh and dry weight (g) of tomato and pepper plant are shown in Table 1. In tomato plant, NaCl and fertilizer induced stress caused to decrease plant fresh and dry weight creating significant differences compared to controls. In pepper plant, NaCl salinity significantly decreased fresh and dry weight, fertilizer induced salinity also decreased fresh and dry weight, however, was not found statistically significant compared to control. The growth reduction in fresh and dry weights of tomato plant occurred at approximately 80% and 73% decreases with NaCl treatment compared to control, and approximately 55% and 57% decreases with fertilizer induced salinity, respectively. The growth reduction in fresh and dry weights of pepper plant occurred at approximately 57% decreases with NaCl treatment compared to control, and approximately 27% and 18% decreases with fertilizer induced salinity, however, was not found statistically significant compared to control. The suppression of plant growth under saline conditions may either be due to osmotic reduction in water availability, which was supported by stomatal resistance values of the plants, or to excessive ions accumulation in plant tissues.

Salinity-induced growth reduction has been well documented in several plant species by many researchers; red raspberry (Neocleous and Vasilakakis, 2007), tomato and cucumber (Alpaslan and Gunes, 2001), lettuce (Eraslan et al., 2007a), pepper (Aktas et al., 2006), tomato (Maggio et al., 2007), and carrot (Eraslan et al., 2007b).

Proline, Lipid Peroxidation (MDA) and Ascorbic Acid Concentrations

The effect of NaCl and fertilizer induced salinity on proline, lipid peroxidation (MDA) and ascorbic acid concentrations of tomato and pepper plant are given in Table 2. Both NaCl and fertilizer

induced salinity significantly increased proline concentration of tomato and pepper plants. Understanding the biosynthesis, degradation, transport and role of proline during stress and signaling events that regulate stress induced accumulation is vital in developing plants for stress tolerance. An increased proline level is a common response of plants to stress treatments by reported that Jaleel et al. (2007), Eraslan et al. (2007a and 2007b), and Turan and Aydin (2005).

Table 1. The effect of NaCl and fertilizer induced salinity on fresh and dry weight (g) of tomato and pepper plants

Treatment	Fresh weight (g)	Dry weight (g)
Tomato		
Control	212.1 a ^a	28.30 a
NaCl	41.1 c	7.54 b
Fertilizer	94.3 b	12.16 b
F-test	**	**
Pepper		
Control	40.23 a	5.36 a
NaCl	16.98 b	2.26 b
Fertilizer	29.14 a	4.35 a
F-test	**	*

*: p<0.05, **: p<0.01, ^a: values with the same letter are not statistically significant (P<0.05)

Lipid peroxidation (MDA concentration) of the tomato leaves increased statistically significant with fertilizer induced stress, but MDA concentration of the pepper leaves not affected by NaCl and fertilizer induced stress (Table 2). The increases in MDA concentration can be correlated with the accumulation of ions and active oxygen species (AOS) production under salt stress. This is in agreement with the result of Jaleel et al. (2007), and Gunes et al. (2007).

Table 2. The effect of NaCl and fertilizer induced salinity on proline (mmol kg⁻¹ FW), lipid peroxidation (MDA, nmol g⁻¹ FW) and ascorbic acid (mmol kg⁻¹ FW) concentrations of tomato and pepper plants

Treatment	Proline (mmol kg ⁻¹ FW)	MDA (nmol g ⁻¹ FW)	Ascorbic Acid (mmol kg ⁻¹ FW)
Tomato			
Control	2.17 b ^a	4.90 b	10.66 b
NaCl	6.27 a	6.65 b	11.38 b
Fertilizer	6.23 a	9.42 a	14.73 a
F-test	**	**	**
Pepper			
Control	1.54 b	7.55	14.83
NaCl	2.92 a	8.45	17.51
Fertilizer	3.50 a	8.04	19.02
F-test	**	ns	ns

*: p<0.05, **: p<0.01, ns: non significant, ^a: values with the same letter are not statistically significant (P<0.05).

The ascorbic acid concentrations of tomato plants increased significantly with fertilizer induced salinity compared to control. As for ascorbic acid concentrations of pepper plants increased with salt treatment, however this increase was found not to be significant (Table 2). Ascorbic acid, a non-enzymatic antioxidant, is associated with H_2O_2 scavenging via APX (Sairam et al., 1998). A role for increased ascorbic acid content in amelioration of oxidative stress has also been reported by Sairam et al. (2005) and Panda and Upadhyay (2003).

Relative Water Content (RWC), Hydrogen Peroxide (H_2O_2) Concentration and Stomatal Resistance (SR)

The ionic and osmotic effects of NaCl and fertilizer induced salinity on relative water content (RWC), hydrogen peroxide (H_2O_2) concentration and stomatal resistance (SR) of tomato and pepper plant are presented in Table 3. Both NaCl and fertilizer induced salinity were not affected statistically significant relative water content of tomato and pepper plants. Neocleous and Vasilakakis (2007) have reported that RWC was decreased only for higher salt concentration. Sairam et al. (2002) and Ghoulam et al. (2002) have concluded that salt treatment induced a reduction in leaves RWC.

Hydrogen peroxide (H_2O_2) concentration in the leaves of tomato and pepper plant significantly increased both NaCl and fertilizer induced salinity. Fertilizer induced salinity more increased than NaCl salinity H_2O_2 concentration of pepper plant (Table 3). Several studies have stated that H_2O_2 can act as a mobile signal, alerting the plant to various biotic and abiotic threats (Neill et al. 2002). Zhua et al. (2004) have reported that salt stress significantly increased H_2O_2 content in the leaves of cucumber plant. Similar reports were also given by Sairam et al. (2002), and Eraslan et al. (2007a).

Leaf stomatal resistance considerably increased with the both treatment in the leaves of tomato and pepper plant. NaCl salinity more increased than fertilizer induced salinity stomatal resistance in the leaves of tomato plant (Table 3). According to Gunes et al. (1996), measurement of leaf stomatal resistance provides determining the degree of stress in plants under salt stress. Stomatal resistance of the plants increased with the increasing NaCl in the nutrient solution that could be explained by the reduction of water use efficiency under saline condition. In our previous studies, leaf stomatal resistance increased under saline condition to have been reported (Eraslan et al. 2007a, 2007b).

As a conclusion, the results of this study showed that similar to NaCl salinity, fertilizer induced salinity significantly reduced the fresh and dry weights of tomato and pepper plants. Both NaCl and fertilizer induced salinity caused significant increases in proline, MDA, ascorbic acid and H_2O_2 accumulation, and stomatal resistance of the plants. Relative water content of the plants was decreased by NaCl salinity, but, was not found statistically significant. Salinity achieved by NaCl and fertilizer altered plant growth and plant physiological processes ionically and osmotically in a similar manner.

Table 3. The effect of NaCl and fertilizer induced salinity on relative water content (RWC, %), hydrogen peroxide (H₂O₂, mmol kg⁻¹ FW) concentration and stomatal resistance (SR, s cm⁻¹) of tomato and pepper plants

Treatment	RWC (%)	H ₂ O ₂ (mmol kg ⁻¹ FW)	SR (s cm ⁻¹)
Tomato			
Control	84.0	14.92 b ^a	2.06 b
NaCl	77.4	22.70 a	8.01 a
Fertilizer	84.4	23.79 a	4.98 ab
F-test	ns	*	*
Pepper			
Control	84.6	16.63 c	3.62 b
NaCl	81.2	21.14 b	14.13 a
Fertilizer	83.7	27.99 a	14.62 a
F-test	ns	**	**

*: p<0.05, **: p<0.01, ns: non significant, ^a: values with the same letter are not statistically significant (P<0.05)

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