Effect of Nutrient Induced Salinity on Growth, Membrane Permeability, Nitrate Reductase Activity, Proline Content and Macronutrient Concentrations of Tomato Grown in Greenhouse

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Abstract—A greenhouse experiment was conducted to investigate the effects of different types of nutrients induced salinity on the growth, membrane permeability, nitrate reductase activity, proline content and macronutrient concentrations of tomato plants. The plants were subjected to six different treatments: 1 (control) containing basic solution, 2 basic solution+40mM of NaCl, 3 basic solution+40 mM of KNO_3, 4 basic solution+20 mM of $Ca(NO_3)_2.4H_2O$, 5 basic solution+20 mM of $Mg(NO_3)_2.6H_2O$ and 6 basic solution+20 mM of KNO_3+5 mM of $Ca(NO_3)_2.4H_2O$+5 mM of $Mg(NO_3)_2.6H_2O$. Membrane permeability was increased significantly only with addition of NaCl, and then decreased to its lower level with addition of $Ca(NO_3)_2.4H_2O$ and $Mg(NO_3)_2.6H_2O$. Proline accumulation were followed the same trend of results when they had been exposed to NaCl salinity. Nitrate reductase activity (NRA) was significantly affected by addition of different types of nutrient induced salinity.

Keywords—Membrane Permeability, Nitrate Reductase Activity, Nutrient induced salinity, Proline.

I. INTRODUCTION

IT is well known that under conditions of arid and semi-arid regions the soil salinization is becoming a major issue limiting plant growth and productivity. Also salinity is becoming more of a problem in some humid coastal areas due to greater salt water intrusion into the fresh ground water [1]. High salt concentrations in the rhizosphere change the nutritional requirements of plants in relation to the type and rate of fertilizers used [2]. Response to the increased salt concentrations may differ considerably among plant species as a function of their inherent salt tolerance [3].

Most of studies confirmed that crop performance adversely affected by salinity regardless to the salt source. The nutrient induced salinity causes nutritional disorders in most of plant species. These disorders may result from the effect of salinity on nutrient availability, competitive uptake, transport, or partitioning within the plant [4]. Nevertheless, the nutrient induced salinity is a major environmental stress in irrigated land that adversely affects plant metabolism and growth.

Salinity or in other words the nutrient induced salinity in irrigated land has a negative effect on tomato yield because it decreases fruit weight [5] and marketable yield [6]. Yield reductions caused by salinity occur on an estimated 30 % of all irrigated land in the United States, with the percentage increasing to 50 % worldwide [7]. Salinity affects plant physiology through changes in the water and ionic status of the cells [8]-[9].

Salt stress has been linked to macro-nutrient deficiencies, for example, high NaCl concentration induced calcium and nitrogen deficiencies in tomato [2]. In some horticultural crops [10] reported that salinity can directly affect nutrient uptake, such as sodium (Na) reduces potassium (K) uptake or chloride (Cl^-) reduces nitrate $(NO_3)^-$ uptake. On the other hand all salinity effect may not be negative; salinity may have some favourable effects of yield quality and disease resistance [11]. Reference [4] reported that under saline conditions, membrane permeability was markedly affected by high salt concentration. Calcium as a major nutrient is well known to have regulatory roles in metabolism [12], and sodium ions may compete with calcium ions for membrane-binding sites. Therefore, it has been hypothesized that high calcium levels can protect the cell membrane from the adverse effects of salinity [13]. According to reference [14], Ca plays a crucial role in controlling cell membrane permeability and selectivity. In salinity induced Ca deficiency, ion uptake and osmoregulation of plants is imbalanced, and ion toxicity, osmotic stress and nutritional disruption occurs.

Nitrate reductase located in the cytoplasm converts nitrate (NO_3) to nitrite (NO_2) and nitrite reductase localized in the plastids reduces the nitrite (NO_2) to ammonia [15]. Thus ammonia can be delivered either from nitrate reduction, uptake of ammonia, or photorespiration. There is a substantial volume of evidence to show that nitrate reductase activity is

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subjected to inhibition following the application of a range of environmental stresses [16]-[17].

Proline is known to accumulate in many plant species under a broad range of stress conditions such as water shortage, salinity, and high-radiation intensity [18]-[19]. The stress state of a tomato plant is considered to be reflected by the proline content of leaves because this is closely related to their relative water content [20] and their osmotic and water potential.

No specific data exist to our literature knowledge about the effects of type of macronutrient induced salinity on the growth, membrane permeability, nitrate reductase activity, proline content and macronutrient concentrations of tomato plant. The objective of this study is to determine the influence of salinity induced by macronutrients at similar level of salt concentrations on the above mentioned plant parameters comparatively with NaCl salinity in tomato grown under greenhouse conditions.

II. MATERIALS AND METHODS

The experiment was carried out under the greenhouse conditions in the department of Soil Science, Faculty of Agriculture, University of Ankara. Tomato (*Lycopersicon esculentum* L) seeds were germinated in seedling trays filled with 1:1 v/v mixture of peat and perlite on 07th of April. After germination the seedlings were then transplanted in plastic pots holding 1.5 L of perlite. The plants were grown from 15 May to 9 July for a period of 55 days with a non-recirculating nutrient film technique at a rate of 200 mL of nutrient solutions applied per day per pot.

Six different treatments were established depending on the nutrient solution applied to the pots. Basic solution (BS) containing 15 mM nitrogen was prepared from commercial fertilizer (20+20+20 plus the essential trace elements). The basic solution was then referred as treatment(1) or control. The compositions of nutrient induced salinity solutions of the other five treatments were prepared as shown in Table I. The pH of the basic nutrient solution in treatment (1) or control was maintained at 6.5 and EC = 1.0 dSm^{-1}. The average pH value of the other treatments was 6.2, whereas their average EC value was 5.68 dSm^{-1}.

In vivo membrane permeability, proline accumulation and nitrate reductase activity (NRA, EC 1.6.6.1), were assayed in fresh plant material sampled 54 d after transplanting.

For the measurement membrane permeability the method of reference [21] was followed. A portion of fresh material at the middle of the leaves from the plants was weighed into a glass beaker containing reverse osmosis water. The beaker was immersed at 30°C for 3 h, and the conductivity of the solution was measured with a hand-held conductivity meter (Hanna Instruments Co. Ltd.). After boiling the samples for 2 min, their conductivity was then measured again when the solution was cooled to room temperature. The percentage of electrolyte leakage (EC) was calculated as Percent EC= (C1/C2) × 100, where C1 and C2 are the electrolyte conductivities measured before and after boiling, respectively.

Nitrate reductase activity was determined by the method of reference [22] with some modifications. Leaf samples were collected and immediately cooled in ice. Leaves were cut into small 3-4 mm pieces including mid-rib, and 500 mg samples were placed in test tubes containing 10 mL of buffer solution, prepared by dissolving 0.2 M potassium nitrate (KNO_3) in 0.1 M mono potassium phosphate (KH_2PO_4) and adjusting the pH to 7.5. The leaf pieces were vacuum infiltrated in vacuum desiccators. Thereafter, samples were incubated in the dark at 25°C for 1 h. By heating the test tubes in the boiling water bath for 1 min, the reaction was stopped. To estimate the amount of nitrite formed, 1 mL of each of 1% sulfanilamide in 1 N hydrochloric acid (HCl) and 0.02% naphtylethylene diamine dihydrochloride were added and the test tubes vortexed. Absorbance of the resulting solution was recorded at 540 nm with a Shimadzu UV-VIS 1201 spectrophotometer. Concentration of nitrite was calculated by drawing a calibration curve of nitrite. Enzyme activity was expressed as μmol of nitrite formed per gram fresh weight per hour.

For determination of proline content, 500 mg leaf samples cut into small 3-4 mm pieces (excluding mid-rib) and cooled in ice, placed into tubes with 10 mL of distilled water. The tubes were kept for 30 min in a boiling water bath to extract hotwater soluble compounds and then cooled to room temperature. Proline in the water extracts was determined by the ninhydrin method [23], improved to avoid interference with concentrated sugars [24]. One mL ninhydrin reagent was added to an aliquot of extract. The mixture was boiled for 30 min in a water bath, and then cooled in an icewater bath. The chromophore formed was extracted with 2.5 mL of toluene by vigorous shaking for about 20 s. Absorbance of the resulting organic phase was measured at 520 nm with a Shimadzu UV-

TABLE I
COMPOSITIONS OF THE NUTRIENT INDUCED SALINITY SOLUTIONS APPLIED TO PLANTS AS REPRESENT IN 6 DIFFERENT TREATMENTS

Treatments	Contents of nutrient induced salinity solution
1	BS* (Control)
2	BS* + 40mM of NaCl
3	BS* + 40 mM of KNO_3
4	BS* + 20 mM of $Ca(NO_3)_2.4H_2O$
5	BS* + 20 mM of $Mg(NO_3)_2.6H_2O$
6	BS* + 20 mM of KNO_3 + 5 mM of $Ca(NO_3)_2 .4H_2O$ + 5 mM of $Mg(NO_3)_2 6H_2O$

BS* Basic Solution containing 15 mM nitrogen prepared from commercial fertilizer (20+20+20 plus the essential trace elements)

VIS 1201 spectrophometer. Calibration curves were made with L-proline as a standard.

All plants were harvested with a knife by cutting at the joint point of fine roots to the main stem after eight weeks of seedlings transplantation. The plants were weighed for the fresh weight determination. After washing, the plants dry blotted on paper towel and dried at $65^{\circ}C$ in a ventilated oven until reaching constant weight. Then, the plants weighed again for the dry weights.

Kjeldahl N was determined by Kjeldahl digestion according to the method of reference [25]. Dried plant samples (0.25 g) were wet-ashed on a block digester (Kjeldatherm, Gerhardt GmbH) with the aid of concentrated sulphuric acid (H_2SO_4), salicylic acid, and salt mixture. The wet ashed plant materials were distilled on a distillation apparatus (Vapodest 20, Gerhardt GmbH). Then the distillates were titrated against 0.05 N H_2SO_4. The plant contents of nitrate were determined by colorimetric method as described by reference [26] and Chloride (Cl⁻) as described by reference [27]. For the measurement of P, K, Ca, and Mg contents of the plants, the dried and finely ground (40 mesh) plant tissue was dry-ashed in a muffle furnace at 500◦C for 5 h, dissolved in 5 mL of 2 M nitric acid (HNO_3), and finally diluted to 25 mL with reverse osmosis water. Extracts were filtered and stored in plastic vials until analyzed. In the extract solutions, K and Na were determined by flame photometer (Jenway PFP7, ELE Instrument Co. Ltd), and P by the colorimetric phosphomolybdate method of reference [28] by using a Shimadzu UV-VIS 1201 spectrophotometer. Calcium and Mg were determined by atomic absorption spectrophotometer (Analyticjena, AAS Vario 6). Sulfur was determined by using C-S determinator (Eltra CS 500).

Yield and chemical data were processed statistically by analysis of variance (ANOVA) to determine if significant differences were present among treatments. The means of treatments were tested for statistically significant differences with Duncan's Multiple Range Test (P = 0.05).

III. RESULTS AND DISCUSSION

The results obtained from Table II showed the relation between the six different treatments as sources of salinity on some plant parameters such as fresh weight, dry weight, membrane permeability, proline content and nitrate reductase activity. Statistical analysis indicated that there were significant differences (P = 0.05) among all the treatments as different source of salinity when compared with control. Fresh and dry weights of plant are markedly affected by the addition of the type of macronutrient solution as a fertilizer.

Salinity achieved by NaCl as shown in treatment (2) was significantly (P=0.05) decreased the amount of fresh and dry weights of plant. High amounts of fresh and dry weights were observed in treatment (4) and (6) . That means the addition of fertilizer or salt containing Ca (NO_3)$_2$ increase the fresh and dry weights of tomato plant.

The result from this study showed that the addition of NaCl, KNO_3 and $Mg(NO_3)_2$ as source of salinity induced by nutrients adversely affect the tomato plants growth. Also, it was noticeable that the salinity induced by nutrients containing monovalent cations such like Na^+ and K^+ adversely affected the plant growth than that by the divalent ones.

According to the results obtained from Table II membrane permeability of tomato plants was increased to 86.49 % (EC) only with addition of NaCl as a source of salinity, and then decreased to its lower level with addition of $Ca(NO_3)_2.4H_2O$ and $Mg(NO_3)_26H_2O$ to 67.42 and 65.81% (EC) respectively. Whereas, the addition of KNO_3 and $KNO_3 + Ca(NO_3)_2.4H_2O + Mg(NO_3)_26H_2O$ in treatment(3) and (6) showed no significant differences in membrane permeability when compared with control. It is well noticed that salt solution dominant by divalent cations such as Ca^{++} which has regulatory roles in metabolism has positive effect on membrane permeability and can protect the cell membrane from the adverse effects of NaCl salinity in tomato plants [12].

The results showed in Table II indicated that proline contents in plants tissue were significantly (P= 0.05) affected by the type of nutrient induced salinity. Although effects of different stresses on leaf proline accumulations have been reported by references [29]-[30]. However [31] reported that without specifying the elements, macronutrients caused increases in the proline accumulation in lettuce, no data exist to our knowledge about the effects of type of macronutrient induced salinity on proline accumulation in tomato plants.

According to the type of salinity, particularly NaCl, the proline content was significantly increased to its higher level 9.45 µmol g^{-1} fw. These findings were in line with [32]. The findings of [29] showed that proline increased by NaCl stress and its accumulation was markedly enhanced with the addition of Cl as $CaCl_2$ and KCl. Proline accumulation may reduce stress-induced cellular acidification and proline itself may act as a substrate for respiration which might produce energy needed for recovery from stress.

Whereas, the other types of macronutrient induced salinity in treatment (3), (4), (5) and (6) were showed a very marginally increase in proline contents (7.64, 6.29, 6.48 and 6.86 µmol g^{-1} fw) respectively, when compared to the control (4.40 µmol g^{-1} fw) and there was no significant differences among these treatments.

According to the results showed in Table II, nitrate reductase activity (NRA) was affected by the different types of nutrients induced salinity. Treatment (4) and (6) which containing $Ca(NO_3)_2$ as a source of nutrient induced salinity showed higher values of NRA 0.76 and 0.77 µmol g^{-1} fw h^{-1}, respectively. Whereas the lower value of NRA (0.20 µmol g^{-1}

TABLE II
FRESH AND DRY WEIGHTS, MEMBRANE PERMEABILITY, PROLINE CONTENT AND NITRATE REDUCTASE ACTIVITY OF TOMATO PLANTS AS AFFECTIVE BY DIFFERENT TREATMENTS OF NUTRIENT INDUCED SALINITY

Treatments	Fresh weight (g)	Dry weight (g)	Membrane permeability (EC, %)	Proline content $\mu mol\ g^{-1}$ fw	Nitrate reductase activity $\mu mol\ g^{-1}$ fw h^{-1}
1	106.6 b	11.46 b	75.77 b	4.40 c	0.35 ab
2	80.85 c	6.69 c	86.49 a	9.45 a	0.20 b
3	49.54 d	5.40 c	72.79 b	7.63 b	0.44 ab
4	125.32 ab	16.53 a	67.42 c	6.29 b	0.76 a
5	63.65 cd	7.38 c	65.81 c	6.48 b	0.61 ab
6	142.0 a	16.29 a	73.41 b	6.86 b	0.77 a

Means followed by the same letter in the same column do not differ significantly at (0.05) probability. Using Dancan's Multiple Range Test

fw h^{-1}) was obtained when NaCl salinity in treatment(2) was applied. However, all the treatments were not significantly different when they compared with control.

Table III showed the effects of macronutrient induced salinity on total nitrogen, nitrate-nitrogen, phosphorus, potassium, calcium, magnesium, sodium, and chloride contents of tomato plants. The values represent the means of 4 replicates as percentage of plant dry weight.

The plant content of total nitrogen was significantly (P= 0.05) different throughout the treatments when compared with the control, the lower values of total N content was observed when NaCl was applied. This result is in line with [33] who applied the same salt to cucumber plants. Whereas, the higher values of N content was obtained with addition of salts that containing $Ca(NO_3)_2$ as shown in treatment(4) and (6).

The results obtained from Table III also showed that the concentrations of P, K, Ca, Mg, Na, Cl and NO_3-N in plant material were markedly affected by the different source of salinity treatments. The concentration of each element is

The lowest value of P concentration was observed with treatment (3) when applying KNO_3 whereas in the same treatment K concentration recorded the highest value. Higher values of Ca (38.0 g kg^{-1}) and Mg (34.60 g kg^{-1}) concentrations in plant material were observed in treatment (4) and (5) respectively. It was markedly observed that higher values of Na and Cl concentrations were obtained when the plant treated with NaCl. Also it was noticed that the concentrations of NO_3 in plant follow the same trend of N results only there was no significant difference between the treatment(2) and the control.

IV. CONCLUSION

The influences of some different nutrient induced salinity, at the similar concentrations, on some tomato plant parameters were investigated. In general nutrient induced salinity containing $Ca(NO_3)_2$ positively affected most of the plant parameters examined whereas NaCl salinity negatively affected the same plant parameters. NaCl salinity was

TABLE III
TOMATO PLANTS CONTENTS OF TOTAL NITROGEN, PHOSPHORUS, POTASSIUM, CALCIUM, MAGNESIUM, SODIUM, CHLORIDE, AND NITRATE-NITROGEN, AS AFFECTED BY DIFFERENT TREATMENTS OF NUTRIENT INDUCED SALINITY

Treatments	TN	P	K	Ca	Mg	Na	Cl	NO_3-N
				g kg^{-1} dry matter				
1	43.00 bc	19.90 a	47.38 c	11.15 bc	4.60 d	2.30 b	4.25 b	3.40 c
2	37.25 d	15.45 bc	23.93 d	11.15 bc	4.65 d	5.75 a	48.13 a	0.45 c
3	41.50 c	6.13 d	127.38 a	8.73 c	6.13 d	1.62 bc	2.13 c	9.73 b
4	55.25 a	12.73 c	24.85 d	38.00 a	16.03 c	0.75 c	1.00 c	16.85 a
5	46.50 b	16.73 b	14.08 e	5.68 d	34.60 a	0.97 bc	2.88 bc	11.15 b
6	52.00 a	14.93 bc	59.18 b	13.25 b	27.18 b	0.86 bc	2.63 bc	15.83 a

Means followed by the same letter(s) in the same column do not differ significantly at (0.05) probability. Using Dancan's Multiple Range Test

markedly increased in plant material when the added salt solution comprising of the same element. Addition of NaCl as source of salinity decrease the concentration of P and K in plant when compared with control and these findings were in line with other workers like [34]-[35] who linked NaCl stress with macro-nutrient deficiencies, high NaCl concentration has been shown to induce phosphorus and potassium deficiencies in tomato.

significantly decreased the amount of fresh and dry weights of plant. Whereas high amounts of fresh and dry weights were observed when addition of fertilizer or salt containing $Ca(NO_3)_2$. Membrane permeability and proline content of tomato plant were increased significantly and reached their higher values only with addition of NaCl as a source of salinity, in contrast the nitrate reductase activity showed the lowest values within the NaCl salinity.

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