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Responses of the Tomato (*Lycopersicon esculentum* Mill.) Plant to Exposure to Different Salt Forms and Rates

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Abstract: We aimed to investigate the effects of NaCl and Na_2SO_4 on seed and pollen germination of tomato (*Lycopersicon esculentum* Mill.) in vitro. In addition, the effects of NaCl, Na_2SO_4, and $CaCl_2$ on yield and quality, plant growth, some physiological parameters, and the distribution of mineral composition in greenhouse grown tomato plants were investigated. Seed germination was affected by high salinity treatments (MS and 1/2 MS). Pollen germination and pollen tube length were significantly affected by salt forms and doses. Pollen germination was blocked by the above doses of 50 mM NaCl and 30 mM Na_2SO_4. In the greenhouse experiment, with increasing concentration of all forms of salt, stomatal density, chlorophyll content, plant growth, and yield decreased. Reductions were higher in fruit yield and stomatal density in the NaCl treatment than those in Na_2SO_4 and $CaCl_2$ treatments. Membrane permeability was impaired with increases in all 3 forms of salt concentrations, but the effect of NaCl treatment on membrane permeability was more striking compared to the other salt forms. Proline accumulation increased with increasing salt concentrations. The K and N concentrations decreased with increases in all 3 types of salt concentrations. Concentration of Ca decreased with increasing NaCl and Na_2SO_4, but increased with $CaCl_2$ salt concentrations. The growth and yield reduction under both NaCl and Na_2SO_4 stress may be due to the combined effects of lower rates of Ca, K, and N, and excess accumulation of Na, while in the $CaCl_2$ experiment the growth reduction may be related to lower rates of K and N and the high rate of Ca.

Key Words: Tomato, salinity, seed and pollen germination

Farklı Tuz Formu ve Oranlarına Maruz Kalan Domates Bitkisinin Yanıtı

Özet: Serada yetiştirilen Target F1 domates çeşidinin verimi, kalitesi, mineral beslenmesi ve bazı fizyolojik özellikleri üzerine NaCl, Na_2SO_4 ve $CaCl_2$'nin etkisi ve in vitro deneme ile de tohum ve polen çimlenmesi üzerine NaCl ve Na_2SO_4'ün etkisi araştırılmıştır. Tohum çimlenmesi hem yüksek tuz dozlarından hem de MS ortamının tam veya yarı güçlü (1/2 MS) olmasından etkilenmiştir. Polen çimlenmesi ve pollen tüp uzunluğu da tuz formu ve dozlarından etkilenmiş ve 50 mM NaCl, 30 mM Na_2SO_4 dozundan yüksek dozlarda polen çimlenmesi görülmemiştir. Sera denemesinde, tuz formlarının dozlarındaki artış ile klorofil kapsamı, stoma yoğunluğu, bitki gelişimi ve verim azalmıştır. Stoma yoğunluğu ve verimdeki azalma NaCl uygulamasında daha belirgindir. Membran geçirgenliği tüm tuz konsantrasyon ve formları ile artmış, ancak en çarpıcı etki NaCl'de gözlenmiştir. Artan tuz konsantrasyonları hem prolin birikimine neden olmuş hem de bitkinin K ve N kapsamlarını azaltmıştır. Bitkinin Ca kapsamı, NaCl ve Na_2SO_4 uygulamalarıyla azalırken $CaCl_2$ uygulamasıyla artmıştır. NaCl ve Na_2SO_4 stresi altında bitki gelişmesi ve verimdeki azalma Ca, K ve N'un düşük oranlarının kombine etkisi ve aşırı Na birikiminden kaynaklanmıştır.

Anahtar Sözcükler: Domates, tuzluluk, tohum ve pollen çimlenmesi

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Introduction

Soil salinity is a major problem in both arid and semi-arid regions of the world for agriculture production (Epstein et al., 1980). In these regions, the shortage of water and hot, dry climates often result in high concentrations of salts in soils, which limit crop production. Salinity is a complex environmental constraint that presents 2 main components: an osmotic component due to the decrease in the external osmotic potential of the soil solution, and an ionic component linked to the accumulation of ions that become toxic at high concentrations (mainly Na, Cl, SO_4, CO_3, and HCO_3), and a stress-induced decrease in the content of essential elements, such as K and Ca. A salt-induced increase in the endogenous polyamine or proline content has been reported in various species such as tomato (Aziz et al., 1999), sorghum and maize (Erdei et al., 1996), and mung bean (Friedman et al., 1989).

Tomato (*Lycopersicon esculentum* Mill.) is moderately tolerant to salinity (1.3 dS m^{-1} < electrical conductivity of the saturated soil extract (EC) < 6 dS m^{-1}) and is typically cultivated in regions that are exposed to soil salinization (Cuartero and Fernandez, 1999). Many crop plants including tomato are susceptible to cell damage from high salinity and can survive only with decreased yields.

The effect of salinity on the nutrient composition of plant tissues, especially concentrations of Ca and K, has been extensively investigated, and several researchers have proposed that the detrimental effects of salinity on plant growth may occur through an ionic imbalance, particularly of Ca and K (Cerda et al., 1995).

An accumulation of ions in plant tissues can affect membrane selective permeability, thus altering the uptake of ions as was demonstrated in rice plants (Khan et al., 1997). Chloride ions have been reported to be more toxic than Na in some species of woody plants (Shannon et al., 1994). However, because relatively few studies have focused on the effects of Na_2SO_4 on tomato plant growth, the effects of the SO_4 anion are much less studied. Because of limited information and apparent contradictions in the literature concerning the effects of high sulfate and chloride on plants (Rogers et al., 1998), this area of research requires further investigation. Although there are tolerance differences among the varieties and species of plants with regards to salinity in soil and plant growth, Na_2SO_4 is more toxic than NaCl. On the other hand, $CaCl_2$ is less toxic as it includes Ca (Güner, 1971).

Plant tissue culture is a technique useful for plant nutrition research and monitoring stress tolerance (such as salinity and pathogen) of plant species over a short time period. New varieties of a species in particular can be tested in plant tissue culture to examine responses to different environmental conditions. In vitro culture techniques are reported to be reliable and quick methods for examining salinity stress (Cano et al., 1998; Altan and Bürün, 2005). The effects of NaCl on some plants have been studied using in vitro techniques (Demir and Kocaçalışkan, 2002). Tissue culture methods are also reported for efficient studies on pollen germination (estimation of micro gametophyte response) (Azarov et al., 1990). For this reason, in the present experiment, an in vitro experiment was designed for quick evaluation of tomato in response to different salt forms and rates of exposure.

The objectives of this study were (1) to determine the effects of NaCl, Na_2SO_4, and $CaCl_2$ salinity on fruit yield, plant growth, membrane permeability, chlorophyll content, and mineral composition of tomato in greenhouse experiments; (2) to test the effects of both NaCl and Na_2SO_4 on the germination of seeds and pollen in vitro; and (3) to compare results obtained in short-term experiments (in vitro tissue culture) with those obtained from long-term experiments (greenhouse).

Materials and Methods

Experiment I: In vitro culture

Seed germination

Seeds of tomato *Lycopersicon esculentum* cultivar Target NF_1 from De Ruiter Seeds Netherland were germinated in a culture medium of Murashige-Skoog (MS) (1962). For each treatment 20 seeds were used and the experiment was conducted as 2 repetitions. MS medium in full and half strengths (1/2 MS) was used without plant growth regulator culture medium, and was supplemented with sucrose and agar at 30 and 6 g l^{-1}, respectively. The pH of the medium was adjusted to 5.8 and was prepared according to Franklin and Dixon (1994). Firstly, in the first experiment of in vitro seed germination, seeds were germinated in MS and 1/2 MS medium containing 0, 40, 80, and 120 mM Na_2SO_4 and

0, 50, 100, and 150 mM NaCl. Then, in the second experiment of in vitro seed germination, seeds were germinated in 1/2 MS medium containing 0, 20, 40, 60, 80, and 120 mM Na_2SO_4 and 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, and 150 mM NaCl. For seed disinfection, seeds were surface sterilized for 5 min in a 2.25% solution of sodium hypochloride and rinsed in sterile distilled water modified from Van den Bulk et al. (1990) was used. Culture vessels were incubated at 26 ºC with 16 h/8 h (day/night) photoperiod and 28.4 µmol m^{-2} s^{-1} light intensity.

The culturing period is 21 days for seed germination in vitro in nutrition medium. At the end of 21-day periods, the tubes were emptied and the length of the seedlings growing from the germinated seeds was measured from the soil line to the apical shoot apex and the leaf number of each seedling was determined.

Pollen germination

For the pollen germination we used control plants grown in the greenhouse experiment. Pollen grains were collected between 0900 and 1000 hours from freshly opened flowers in the fourth and fifth inflorescence and were spread on the germination medium. The composition of the medium used for pollen germination was 15% sucrose, 60 mg l^{-1} H_3BO_3, and 1% agar (Song et al., 1999). For the test of pollen germination, NaCl and Na_2SO_4 were used at the concentrations of 0, 10, 20, 30, 40, 50, 100, and 150, and 0, 10, 20, 30, 40, 80, and 120 mM, respectively. They were incubated for 24 h in a dark incubator at 26 °C. After 24 h, germination percentage of pollen and pollen tube length were determined. Pollen grains were counted as germinated when the pollen tube length exceeded the pollen diameter. Pollen tube length was recorded for at least 30 randomly selected pollen tubes in each replicate.

Experiment II: Greenhouse trial

An experiment was conducted under greenhouse conditions in Ortaca, Muğla, from January to April 2005 with the tomato cv. Target. Temperature was controlled using heaters early in the growing season and fans from the end season with the aim of keeping the daytime temperature at 20-25 °C. Night temperature was maintained above 10 °C. Relative humidity was kept between 50% and 60%. Three tomato seeds were sown directly in each plastic pot containing 8 kg of a peat, perlite, and sand mixture in equal volumetric ratios. After germination, the plants were thinned to one per pot. The nutrient solution was composed of the following individual nutrients (mM): N (19.2), P (1), K (6), Ca (5), Mg (2), S (2), Fe (0.05), Mn (0.01), B (0.05), Cu (0.003), Zn (0.0007), and Mo (0.001). The pH of the nutrient solution was adjusted before each use to 5.6 ± 0.2 with a minimum volume of 0.1 mM KOH. Treatments were initiated 20 days after germination. Salinity treatments were applied through a nutrient solution containing 30, 60, and 90 mM NaCl; 20, 40, and 60 mM Na_2SO_4; or 20, 40, and 60 mM $CaCl_2$. As it is thought that NaCl, Na_2SO_4, and $CaCl_2$ can be found in different amounts in soils where the culture plant is grown, 3 different forms of salt were used to compare the levels of detrimental effects.

Each treatment was replicated 3 times and each replicate included 5 plants. Three plants per replicate were harvested (April, 2005) after fruit set to assess biomass, membrane permeability, relative water content, chlorophyll content, proline content, and mineral nutrients. The remaining 2 plants in each replicate were grown for 4 further weeks to determine fruit yield. At the harvest time, 6 or 7 inflorescence of each plant was formed, and then at the third inflorescence the fruits were used for quality and yield analysis. For the purpose of quality analysis, only mature fruits were evaluated.

Chlorophyll content was determined by Strain and Svec (1966). Electrolyte leakage was assessed as described by Lutts et al. (1996) using young leaf disks (9 leaf disks from the fifth and sixth leaves per replicate). Subsequently, electrolyte leakage was calculated as electrolyte leakage (%) = $(L_t / L_0) \times 100$. Proline concentration was determined according to Bates et al. (1973). Relative water content (RWC) was calculated based on the methods from Yamasaki and Dillenburg (1999). Values of FM (fresh mass), TM (turgid mass), and DM (dry mass) were used to calculate RWC using the following equation: RWC (%) = [(FM - DM) / (TM - DM)] × 100. Stomatal density was determined from randomly chosen leaves collected from the mid-section of the plant, in order to minimize age effects. For dry weight determinations and chemical analyses, 3 randomly selected plants per replicate were divided into leaves, stems, and roots, and dried in an oven at 70 °C for 2 days to determine dry weights and elemental concentrations. Chemical analyses were carried out on a dry weight basis. N in samples was determined by Kjeldahl method (Kacar, 1972). Ground samples were dry-ashed at 550 °C for 4

h, mixed with 2 M hot HCl, filtered, and then made up to a final volume of 50 ml with distilled water. Ca, K, and Na concentrations were determined in these sample solutions using an Eppendorf flame photometer (Chapman and Pratt, 1982). Two-way analysis of variance (ANOVA) and regression analysis were performed using SAS (Statistical Analysis Software). The means and calculated standard errors are reported. Significance was tested at 5% level.

Results

Experiment I: In vitro culture

Germination of seeds in vitro was negatively affected by NaCl or Na_2SO_4 supplementation in MS medium. The highest germination percentage and seedling height ratio in vitro were in the control treatment. This was 63.5% in MS and 100% in 1/2 MS. In addition, seed germination ratio was higher in 1/2 MS medium than in MS medium at both NaCl and Na_2SO_4 concentrations (Table 1).

Furthermore, when 1/2 MS medium was used, germination was observed up to 100 mM NaCl and 80 mM Na_2SO_4, at a lower rate, but much greater than that obtained in MS with the same range of NaCl and Na_2SO_4. Due to the very low germination rate and no growth at 100 mM NaCl and 80 mM Na_2SO_4, seedling height and numbers of leaves were not determined (Table 1). The numbers of leaves did not differ significantly between MS and 1/2 MS. The numbers of leaves were higher in control MS. However, in the case of adding salt, they were higher in 1/2 MS grown plants. However, the effect of NaCl and Na_2SO_4 on the leaf number was significant ($P < 0.05$) (Tables 1 and 2).

In terms of seed germination percentage, seedling height, and leaf numbers, differences observed among control and salinity levels were significant ($P < 0.05$) (Table 2).

Pollen germination and pollen tube length were decreased when both NaCl and Na_2SO_4 concentrations were increased to 50 and 30 mM, respectively, and when concentrations exceeded those doses of both salts germination did not occur (Table 3). The effects of both NaCl and Na_2SO_4 on pollen germination were significant ($P < 0.05$).

Table 1. Seed germination, seedling height and leaves numbers of tomato seedlings, grown in MS and $^1/_2$ MS medium with various levels of NaCl and Na_2SO_4.

Treatments	Medium	Seed germination (%)	Seedling height (cm)	Numbers of leaves
Control	MS	63.5 bc	5.5 b[1]	4.6 a
	$^1/_2$ MS	100.0 a	9.7 a	3.6 b
NaCl Treatments (mM)				
50	MS	34.0 de	4.6 b	2.10 c
	$^1/_2$ MS	72.0 b	5.7 b	2.85 bc
100	MS	0	-	-
	$^1/_2$ MS	16.0 e	-	-
150	MS	0	-	-
	$^1/_2$ MS	0	-	-
Na_2SO_4 Treatments (mM)				
40	MS	44.0 d	4.5 b	2.6 bc
	$^1/_2$ MS	80.0 b	5.3 b	3.5 b
80	MS	0	-	-
	$^1/_2$ MS	14.0 e	-	-
120	MS	0	-	-
	$^1/_2$ MS	0	-	-

[1]Within each column, the same letter indicates no significant difference among treatments ($P < 0.05$)

Table 2. Seed germination, seedling height and leaves numbers of tomato seedlings grown in $^1/_2$ MS medium with various levels of NaCl and Na_2SO_4.

Treatments	Seed germination (%)	Seedling height (cm)	Numbers of leaves
Control	100.0 a	9.7 a	3.6 a
NaCl Treatments (mM)			
10	95.7 a[1]	7.32 b	3.52 a
20	76.2 c	6.25 bc	3.16 a
30	87.0 b	5.95 bc	2.96 a
40	86.4 c	5.40 c	2.95 ab
50	72.0 c	5.70 c	2.85 ab
60	55.0 e	3.66 c	1.66 b
70	65.0 d	3.13 cd	1.45 b
80	45.0 f	3.05 cd	1.33 b
90	22.7 g	2.37 cd	1.00 b
100	16.0 g	-	-
150	-	-	-
Na_2SO_4 Treatments (mM)			
20	92.0 a	6.90 a	3.65 a
40	80.0 b	5.30 b	3.50 a
60	75.0 b	5.05 c	1.55 b
80	14.0 c	-	-
120	-	-	-

[1]Within each column, the same letter indicates no significant difference among treatments ($P < 0.05$)

Table 3. Pollen germination and pollen tube length in different levels of salt.

Treatments	Pollen germination (%)	Pollen tube length (µm)
Control	53.96 a[1]	160.22 a
NaCl Treatments (mM)		
10	30.21 b	52.35 b
20	39.47 b	57.61 b
30	25.00 c	32.38 c
40	18.30 c	30.25 c
50	12.14 d	23.94 d
100	-	-
150	-	-
Na_2SO_4 Treatments (mM)		
10	33.58 b	41.56 bc
20	14.46 cd	14.47 e
30	1.59 e	0.52 f
40	-	-
80	-	-
120	-	-

[1]Within each column, the same letter indicates no significant difference among treatments ($P < 0.05$)

Experiment II: Greenhouse trial

Both NaCl and Na_2SO_4 treatments reduced plant dry weight, chlorophyll contents, stomatal density, and relative water content (RWC) in the leaves. However, $CaCl_2$ treatment increased RWC and reduced chlorophyll content and stomatal density and did not change plant dry weight except for the 60 mM $CaCl_2$ treatment, which reduced dry weight compared to control plants (Table 4). Reductions in plant dry weight and numbers of stomata were lower in 60 mM $CaCl_2$ than in other treatments of NaCl and Na_2SO_4, but reductions in total chlorophyll contents were higher in the $CaCl_2$ treatment.

Electrolyte leakage and proline content were increased in the leaves of tomato plants grown in high levels of all 3 forms of salts compared to the control, untreated plants. The highest NaCl treatment had a more striking effect on membrane permeability than the other 2 forms of salt. Furthermore, increases in proline content were higher in NaCl than the other forms of salt (Table 5).

Fruit yield per plant and average fruit weight decreased in the plants grown under all experimental conditions with the 3 forms of the salts tested, according to increasing concentration. The highest reduction in fruit yield was in the 90 mM NaCl treatment (Table 5).

Na concentration in plant tissues increased with increasing levels of both NaCl and Na_2SO_4 but it did not change with $CaCl_2$ treatment. The K and N concentrations decreased with increases in any of the 3 types of salt concentrations, but root N concentrations increased at the highest $CaCl_2$ dose. Concentration of Ca decreased with increasing NaCl and Na_2SO_4, but increased as a result of $CaCl_2$ salt concentrations (Table 6).

Table 4. Effects of different levels of NaCl, Na_2SO_4, and $CaCl_2$ on dry weight (DW) (g $plant^{-1}$), chlorophyll content (Chl) (mg kg^{-1}), relative water content (RWC) (%), and stomatal density (units mm^{-2}) for tomato grown in greenhouse.

Treatments	Shoot DW	Root DW	Total plant DW	Chl a	Chl b	Total Chl	Stomatal density	RWC
NaCl Treatments (mM)								
Control	135.2 a[1]	19.55 a	154.7 a	1150 a	490 a	1640 a	169 a	78.7 a
30	109.6 b	19.57 a	129.2 b	920 b	460 ab	1380 b	150 ab	76.2 b
60	102.8 c	18.61 a	121.4 c	870 c	450 b	1320 c	135 b	76.0 b
90	96.2 d	17.05 b	113.2 d	760 d	400 c	1160 d	115 c	72.6 c
Na_2SO_4 Treatments (mM)								
Control	135.2 a[1]	19.55 a	154.7 a	1150 a	490 a	1640 a	169 a	78.7 a
20	110.2 b	19.67 a	129.9 b	1060 b	510 a	1570 b	152 ab	76.2 b
40	106.7 c	18.05 b	124.7 c	890 c	380 b	1270 c	134 b	72.5 c
60	99.8 d	19.03ab	118.8 d	790 d	240 c	1130 d	128 b	71.2 d
$CaCl_2$ Treatments (mM)								
Control	135.2 a[1]	19.55 c	154.7 a	1150 a	490 a	1640 a	169 a	78.7 c
20	136.9 a	21.03 b	157.9 a	1130 a	480 a	1610 a	170 a	80.4 b
40	132.3 b	22.19 a	154.5 a	820 b	370 c	1190 c	158 ab	83.5 a
60	122.0 c	22.80 a	144.8 b	880 b	440 b	1320 b	141 b	84.3 a

[1]Within each column, the same letter indicates no significant difference among treatments ($P < 0.05$)

Table 5. Effects of different levels of NaCl, Na_2SO_4, and $CaCl_2$ on electrolyte leakage (%), proline content (µmol g^{-1} FW), yield (kg $plant^{-1}$), numbers of fruit (fruit no $plant^{-1}$), and fruit weight (g $fruit^{-1}$) for tomato grown in greenhouse.

Treatments	Electrolyte leakage	Proline content	Fruit numbers	Fruit weight	Fruit yield
NaCl Treatments (mM)					
Control	11.2 c[1]	10.1 b	34 a	151 a	5.14 a
30	11.9 c	10.7 b	36 a	99 b	3.55 b
60	21.5 b	19.6 a	34 a	87 c	2.99 c
90	33.1 a	18.4 a	25 b	79 c	1.97 d
Na_2SO_4 Treatments (mM)					
Control	11.2 c[1]	10.1 b	34 b	151 a	5.14 a
20	12.2 c	9.4 b	35 b	100 b	3.51 b
40	19.8 b	13.9 a	41 a	80 c	3.30 b
60	26.8 a	14.8 a	35 b	65 d	2.29 c
$CaCl_2$ Treatments (mM)					
Control	11.2 c[1]	10.1 b	34 b	151 a	5.14 a
20	13.9 b	17.9 b	34 b	114 b	3.90 b
40	23.3 a	20.1 a	44 a	84 c	3.70 b
60	25.2 a	17.2 b	34 b	84 c	2.86 c

[1]Within each column, the same letter indicates no significant difference among treatments ($P < 0.05$)

Table 6. Effects of different levels of NaCl, Na_2SO_4, and $CaCl_2$ on Na, K, Ca, and N concentrations (% dry weight) in leaves and roots of tomato grown in greenhouse.

Treatments	Leaf Na	Root Na	Leaf K	Root K	Leaf Ca	Root Ca	Leaf N	Root N
NaCl Treatments (mM)								
Control	0.0165c[1]	0.64 b	1.36 a	0.74 a	3.61 a	0.92 a	1.78 a	1.40 a
30	0.091 c	0.76 b	1.27 a	0.8b a	3.55 a	0.71 c	1.72 b	1.19 c
60	0.35 b	1.57 a	1.15 b	0.55 b	3.59 a	0.73 c	1.67 b	1.24 b
90	0.48 a	1.52 a	1.07 b	0.66 ab	2.75 b	0.79 b	1.58 c	1.22 b
Na_2SO_4 Treatments (mM)								
Control	0.016 d[1]	0.64 c	1.36 a	0.74 a	3.61 a	0.92 a	1.78 a	1.40 a
20	0.15 c	1.33 b	1.43 a	0.46 b	3.56 a	0.56 b	1.51 b	1.24 c
40	0.26 b	1.51 b	1.44 a	0.38 c	3.33 b	0.57 b	1.40 c	1.18 d
60	0.71 a	1.8 a	1.16 b	0.47 b	2.71 c	0.54 b	1.49 b	1.30 b
$CaCl_2$ Treatments (mM)								
Control	0.016b[1]	0.64 a	1.36a	0.74b	3.61c	0.92c	1.78a	1.40b
20	0.028a	0.45 c	1.24b	0.56c	3.82b	0.99c	1.50b	1.13d
40	0.017b	0.54 b	0.87c	0.92a	5.15a	1.67a	1.30c	1.31c
60	0.012b	0.56 b	0.64d	0.74b	5.11a	1.55a	1.26c	1.70a

[1]Within each column, the same letter indicates no significant difference among treatments ($P < 0.05$)

Discussion

In vitro culture

In vitro plant tissue culture has been determined to be a useful and quick tool to evaluate salt tolerance. There are many studies about this issue that were carried out through various tissue culture methods (Bhatia et al., 2004). Germination of seeds in in vitro culture conditions has been reported in many crops. Most of these studies were on salt stress and factors affecting seed germination (Amini and Ehsanpour, 2006; Pence et al., 2006).

In the present study, seed germination was adversely affected by both types of salt added to the medium. As MS nutrition medium includes nutrients in the form of salt, the highest negative effect was observed in complete MS medium. This situation is clearly seen in the control (Table 1). Ordinarily, seeds do not require supplied nutrient elements in high concentrations in growth medium for germination and it is expected that these nutrients added to the medium can be toxic. These findings are in agreement with those reported by Bürün and Çoban (2002), who stated that 1/2 MS was more amenable for proper germination of seeds. In this study, tomato seed germination percentages were determined on MS medium. Germination percentages were lower in MS medium than in 1/2 MS medium. Besides seed germination, seedling height and leaf number parameters were also negatively affected by the gradual increase in salt (Table 2). In the seedling growth in MS medium including 50, 100, 150, and 200 mM NaCl, significant decreases were observed, and seedling height and leaf number decreased with the increase in salt concentration (Shibli et al., 2007).

Both seeds and pollen are physiologically sensitive towards toxic pollutants during germination (Tuna et al., 2002). In the present experiment, pollen germination and pollen tube length were decreased with increasing doses of NaCl and Na_2SO_4. However, Na_2SO_4 treatments, pollen germination, and pollen tube length were blocked by the highest rate (Table 3). Microgametophyte response in vitro might be possible to select resistant pollen grains in vitro. Azarov et al. (1990) studied the effects of 0.3% and 0.5% NaCl on pollen germination and pollen tube growth of different races of *Arabidopsis thaliana* grown in vitro and found that there were differences in responses to salinity among different races of *Arabidopsis thaliana.* The results of the present experiment are in agreement with those reported by Martinez-Palle et al. (1995), who described pollen germination in pistachio (*Pistachia vera*) occurring in in vitro culture medium containing NaCl up to 50 mM NaCl and up to 30 mM Na_2SO_4.

Greenhouse trial

It is well known that one of the first plant responses to salinity stress is a reduction in leaf growth rate with associated reductions in leaf area available for photosynthesis. Subsequently, excessive accumulation of salts can lead to death of tissues, organs, and whole plants (Munns and Termaat, 1986). Moreover, since plant growth is a result of massive and irreversible expansion of young cells produced by ongoing meristematic divisions, salinization can inhibit both cell division and cell expansion in growing tissues of roots, stems, and leaves (Zidan et al., 1990).

It has been reported that Na_2SO_4 reduced the shoot dry weight of tomato (İnal, 2002). Biomass production of the plants was inhibited by salinity. Increasing NaCl and Na_2SO_4 doses decrease the total plant dry matter content equally; however, $CaCl_2$ treatment led to a very small decrease at 60 mM dose (Table 4).

Salt treatment induced a reduction in leaf RWC. While NaCl and Na_2SO_4 treatments resulted in a decrease in RWC, $CaCl_2$ treatment led to an increase in RWC (Table 4). The decrease in RWC indicated a loss of turgor that resulted in limited water availability for the cell extension process (Katerji et al., 1997). The decrease in RWC under salinity stress and decrease in chlorophyll content due to salinity have already been reported in tomato (Romero-Aranda et al., 2001). Chlorophyll contents have been suggested as one of the parameters of salt tolerance in crop plants (Hernandez et al., 1995).

The membrane permeability parameter was included in order to have information on the membrane stability and thereby on the relative ion content in the apoplastic space. In a saline environment, plants take up excessive amounts of Na at the cost of K and Ca. High Na/Ca and Na/K ratios in a saline nutrient solution may cause an increase in membrane permeability, and this may result in the passive accumulation of Na^+ and Cl^- in the roots and shoots of salt-stressed plants (Lutts et al., 1996).

All 3 forms of salt treatment induced significant increases in electrolyte leakage in the stressed plants compared with those in the control plants (Table 5).

Similar results were obtained by Lutts et al. (1996) and Kaya et al. (2002), who reported that high salt concentrations increased the membrane permeability of sensitive rice varieties, and strawberry and cucumber plants, respectively.

In the present study, because of this mechanism described above, a high level of proline was accumulated in the leaves of tomato plants grown under high salinity. This may be an adaptive strategy against osmotic stress. Proline accumulation has been shown to be a late adaptive response in plant tissues under salt stress. It has been proposed recently by Hare and Cress (1997) to have a multi-component effect in stress tolerance. When the smallest doses of all 3 salt forms are compared to the control, no significant difference is observed among the proline contents of the leaves. With the increase in salt doses, the proline contents of the leaves increase (Table 5).

The growth and fruit yield reduction under both NaCl and Na_2SO_4 stress may be due to the combined effects of lower rates of Ca, K, and N, and excess accumulation of Na, while in the $CaCl_2$ experiment the growth reduction may be related to lower rates of K and N and the high rate of Ca (Tables 5 and 6). For horticultural and commercial practice, the fruit yield and quality reduction according to NaCl salinity have been investigated (Saied et al., 2005). As can be seen in Table 5, when the control is compared with the highest doses, the smallest decrease in the fruit yield is observed in $CaCl_2$ treatment. Another interesting point worth considering when compared to the control is the increase in the number of fruits and the direct relationship between the increase in fruit number and yield in 40 mM Na_2SO_4 and 40 mM $CaCl_2$ treatment. At 60 mM dose, a significant decrease was observed in both the number of fruits and the yield.

Among the culture plants, tomato is within the group that is moderately sensitive to the salt and in soils having nearly 5-7.5 dS m^{-1} EC the yield exhibits a decrease by 25%-45%. EC values in NaCl, Na_2SO_4, and $CaCl_2$ 40 mM concentrations are 4.76, 7.02, and 7.22 dS m^{-1}, respectively. Although $CaCl_2$ has the highest EC value, it causes the least detrimental effect; this is because of the fact that it includes Ca^{2+} in ion form and the toxicity index of Ca is very low. As seen in Table 5, among the 3 salt forms, the highest decrease in the fruit yield is observed for NaCl at 90 mM, and for Na_2SO_4 and $CaCl_2$ at 60 mM concentrations. This situation shows that, independent of the decrease in the number of fruits in inflorescence, the decrease in the yield is directly or indirectly connected with the detrimental effects of the salt.

Nutrient imbalance can develop in salt-stressed plants in different ways. Salinity stress under certain experimental conditions may curtail or promote nutrient uptake by plant species by affecting the mobility of a nutrient within the plant or by increasing the nutrient requirement by plants in the cells. The simultaneous presence of salts and nutrient elements in the root zones can influence nutrient uptake by plants and thereby affect their chemical composition. Synergistic and antagonistic effects may increase or decrease the intensity of these processes (Syed, 1997).

With regards to the tolerance of culture plants against salt, Na/K and Na/Ca ratios are of great importance. Salt tolerant plants include less active Na yet more K and Ca in their content with the help of ion selection mechanisms. As can be calculated from Table 6, in the group exposed to NaCl, the Na/K ratio at 0, 30, 60, and 90 mM is 0.012, 0.07, 0.30, and 0.44, respectively, and the Na/Ca ratio is 0.0045, 0.025, 0.097, and 0.174, respectively. Na/K and Na/Ca ratios increase with increasing amount of salt. High ratios affect the metabolism and physiology of the plant negatively (Cuin et al., 2003).

Salinity dominated by Na salts not only reduces Ca availability, but reduces Ca transport and mobility to growing regions of the plant, which affects the quality of both vegetative and reproductive organs. It has been reported that high NaCl concentrations induced K deficiencies in a broad range of crops such as spinach (Chow et al., 1990), tomato (Lopez and Satti, 1996), and maize (Botella et al., 1997). Furthermore, high salinity induces N deficiency in tomato (Pessarakli and Tucker, 1988) and lettuce and cabbage (Feigin et al., 1991).

Conclusion

All 3 forms of salt tested decreased plant growth, some physiological parameters, and fruit yield of tomato. However, NaCl and Na_2SO_4 were more detrimental than $CaCl_2$ on plant growth and fruit yield. Both growth and fruit yield reductions under both NaCl and Na_2SO_4 at their highest concentrations may be due to the combined effects of lower rates of Ca, K, and N, and excess

accumulation of Na. In the $CaCl_2$ experiment, the growth reduction may be related to lower rates of K and N, and the high rate of Ca.

This study was carried out in the Muğla region, which represents 10% of the total greenhouse potential in Turkey and which produces nearly 15% of the country's tomatoes. In greenhouse soils, there is the potential for the formation of Cl and SO_4 type salinity due to the rising ground waters and excessive use of fertilizers. Excessive salinity results in quality loss and accordingly economic losses. The results obtained in the present study show the negative effects of both SO_4 and Cl type salinity on seed or pollen germination, plant growth, and yield. It is suggested to use Ca fertilization and to take necessary precautions to prevent salinity in greenhouses to alleviate the detrimental effects of Na.

These results are useful to agronomists to predict sowing rates depending upon saline conditions.

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