



Interactive effects of salinity and drought stress on photosynthetic characteristics and physiology of tomato (*Lycopersicon esculentum* L.) seedlings

Selda Ors^a, Melek Ekinici^b, Ertan Yildirim^{b,*}, Ustun Sahin^a, Metin Turan^c, Atilla Dursun^{b,d}

^a Department of Agricultural Structures and Irrigation, Erzurum, Turkey

^b Department of Horticulture, Erzurum, Turkey

^c Yeditepe University, Engineering Faculty, Department of Genetics and Bioengineering, Istanbul, Turkey

^d Kyrgyz-Turkish Manas University, Department of Horticulture and Agronomy, Bishkek, Kyrgyzstan

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ABSTRACT

Sufficient quantity and quality of water are essential for vegetable production.

Evaluating the individual and combined effects of drought and salt stress on plant establishment may help inform management of plant production in terms of stabilizing yield. This study investigates the effects of drought and salt stress on plant growth, nutrition elements, and select physiological parameters of tomato (*Lycopersicon esculentum* L. cv. H2274) through a pot experiment under greenhouse conditions. Drought stress was applied to tomato seedlings at 3 different levels of irrigation: D0 (control), D1, and D2 at 100%, 75%, and 50% field capacity, respectively. Salinity stress was applied at 3 different doses S0, S1, and S2 at 0 mM, 100 mM, and 150 mM NaCl, respectively. The effects of salinity, drought and their co-occurring stresses on growth, physiological and photosynthetic properties of tomato seedlings were statistically significant. Photosynthetic rate (P_n), stomatal conductance (g_s), chlorophyll reading value (CRV), and plant dry weight (DW) decreased with salt and drought treatments. However, electrolyte leakage (EL) of the leaves increased with increasing salt and drought treatments. Fresh weight (FW) of the tomato seedlings decreased 67% under the 150 mM NaCl treatment (S-2), and decreased 69% under severe drought (D2); however, the interactive effects of these two treatments together (S2D2) resulted in an 80% decrease in FW. Furthermore, dry weight (DW) decreased 80% in the S2D2 treatment compared to the control. In general, both drought and salinity altered the mineral nutrient composition by decreasing N, P, K, Fe, Ca and Z content of the tomato seedlings, but Na, B and Cl content increased. These results show that effects of salinity and drought on tomato seedlings were less detrimental for plant establishment than the combined effects of the two stresses together.

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1. Introduction

Vegetables are crucial for human nutrition and health as they provide vital minerals vitamins, antioxidants, plant sterols and fibers (Dalal et al., 2006). Drought and salinity are the most common coexistent, abiotic stress factors, impacting crop yield and productivity from the first stage of plant development. The yield and quality of vegetables are adversely affected by drought and salinity stress (Maggio et al., 2005). Water is indispensable for successful growth of vegetables; as such, providing the optimal quantity and quality of water to the plant is crucial. Significant periods of drought and highly saline conditions can result in permanent damage to the plant including disruption of stem and root development, as well as decrease in leaf number and width. In addition, the decrease in the water potential in the cells can lead to a

slowdown in the development of the plant. Many studies have shown drought stress can damage growth, metabolic processes and photosynthetic characteristics of plants (Liu et al., 2016; Ors et al., 2016; Sahin et al., 2018; Ekinici et al., 2020).

Salt stress is one of the major environmental conditions that usually occurs in arid and semi-arid climates and reduces plant yield. Low precipitation, high evaporation, salty irrigation water, and poor water use management lead to salinity stress in agricultural areas. Most plants are sensitive to salinity, and as such either cannot survive under highly saline conditions, or try to adapt by reducing their yield (Sahin et al., 2018). As one of the abiotic stresses, salinity also severely restricts crop productivity worldwide. Salt stress was previously stated as a major affecting factor on absorption of water and nutrients by elevating intracellular concentrations of salts to toxic levels on the plant (Munns and Tester, 2008). Salinity affects plant physiology at both the whole plant as well as at cellular levels through osmotic and ionic stress. High salt concentrations can

* Corresponding author.

E-mail address: ertanyil@atauni.edu.tr (E. Yildirim).

adversely affect seed germination, seedling growth, vegetative growth, flowering and fruit set, subsequently reducing economic yield and quality (Arora et al., 2008).

Plants strive to adapt to physiological and metabolic changes with minimal damage to their growth and development under environmental stress conditions such as drought and salinity. Due to the low availability of water resources under periods of drought, the potential uses of low quality water resources in agriculture has come to the attention of the researchers. Under the matrix and osmotic stress sources the plant is exposed to, it is a matter of curiosity how much decrease in yield will occur when exposed to each stress factor separately and/or together (Ors and Suarez, 2017).

Earlier studies have reported that combined effects of individual stresses including drought and salinity were more detrimental than the separate, individual effects of each stress on crops for plant growth and yield (Hanci and Cebeci, 2015; Khan et al., 2016; Caliskan et al., 2017; Ors and Suarez, 2017; Sahin et al., 2018). Though responses of various plants to salinity and drought have similar patterns, their thresholds to stress vary from one plant to another. To minimize negative effects, plants adapt to drought and salt stress by decreasing physiological and morphological functions (Ge et al., 2012; Liu et al., 2011). For instance, tomato, one of the most cultivated vegetable species in the world, is known to be moderately tolerant to salinity but susceptible to severe damage under drought conditions. Thus, water quality and quantity are important for yield and quality of tomato cultivation (Machado and Serralheiro, 2017; Cui et al., 2020).

Studies have shown both salt and drought conditions adversely affected growth and modulated some physiological and biochemical properties of tomato (Zhou et al., 2017; Tanveer et al., 2020); however, the combined effects of these conditions on tomato have not yet been subject to much research. Plant response to drought and salt stress is complex, with varying tolerance levels specific to each plant. Tolerance mechanisms should therefore be carefully investigated, understood and evaluated before large-scale applications are implemented. The purpose of this study is to gain additional insight on both the individual and combined effects of drought and salt stress on the growth, physiological and nutrient element content of tomato during its early vegetative phases.

2. Materials and methods

A pot experiment was conducted with tomato (*Lycopersicon esculentum* L. cv. H2274) in the greenhouse at Ataturk University, Erzurum, Turkey. Greenhouse temperature and humidity conditions were controlled via a sensor set to a range from an average temperature of $25(\pm 2)$ °C during the day to $18(\pm 2)$ °C during the night. Humidity was maintained at around $40(\pm 5)\%$.

Seeds were sown in multiple pots inside peat. Seedlings at the three to four true-leaf stage were transplanted into pots (14 L, $72 \times 19.5 \times 17.5$ cm) in substrate consisting of 50% soil, 25% cattle manure, and 25% sand. Each pot contained four plants, and drought treatments were applied 4 weeks after sowing at the four to five-leaf stage. Treatment was replicated with three pots, and the total four pots were randomly arranged. Salt treatments were prepared with 0 (S0), 100 (S1) and 150 (S2) mM of NaCl. Salt treatments were applied as irrigation water at three-day intervals until harvest. Salts were added in 3 equal increments over a period of 5 days to avoid osmotic shock to the seedlings.

Pots were irrigated at different levels based on 100% (control, D0), 75% (D1) and 50% (D2) of water to reach field capacity. To determine the water retained at field capacity, the pot was saturated with tap water and the top of the pot was covered with a plastic sheet in order to prevent evaporation. Field capacity was assumed to be the water content of the growing media in the pot after drainage stopped (roughly 30 h), measured by a Wet-2 sensor (wet sensor, type WET-2, Delta-T Device Ltd, Cambridge, England). Drought treatments in

the pots were maintained by checking soil moisture using a Wet-2 moisture sensor calibrated for the experimental soil and pot size. The water content at field capacity of the growing media was $0.45 \text{ m}^3 \text{ m}^{-3}$. Control drought treatments (100%) were irrigated to maintain this field capacity throughout the growing period.

A portable Li-Cor 6400 Photosynthesis System Photosynthetic was used to measure photosynthetic rate (P_n) and stomatal conductance (g_s) of plants at the third, fully expanded upper leaves along the right abaxial side of the leaf lamina. Measurements were taken between 10:00–11:00 am, one week before the harvest. The Li-Cor 6400 unit was set at the following: $1100 \mu\text{mol m}^{-2} \text{ s}^{-1}$ leaf chamber PAR (photosynthetic active radiation); 1.7 to 2.6 kPa leaf to air vapor deficit pressure; $20\text{--}22$ °C leaf temperature; and, $400 \mu\text{mol mol}^{-1}$ chamber CO_2 .

The leaf greenness of the tomato plants was determined by a portable chlorophyll meter (SPAD-502; Konica Minolta Sensing, Inc., Japan) during the gas exchange measurements and given as leaf chlorophyll reading values (CRV) (Shams et al., 2016).

At end of the experiment (30 days after planting seedling in pot) all plants were harvested. Fresh weight of the plants were measured by an electronic balance (CX-600), placed in an oven at 68 °C for 48 h, then measured again for dry weight.

During harvest, 10 leaf discs (10 mm in diameter) were put in 50 mL glass vials to measure electrolyte leakage (EL). Vials were then filled with 30 mL of distilled water and allowed to stand in the dark for 24 h at room temperature. Vials were heated in a temperature-controlled water bath at 95 °C for 20 min, and the EC (EC1) of the bathing solution was obtained at the end of the incubation period. EC (EC2) was measured again after vials were then cooled to room temperature. EL was then calculated as a percentage of EC_1/EC_2 .

Samples of the tomato leaves for mineral analysis were dried at 68 °C for 48 h in an oven and then ground. To determine the total N, the Kjeldahl method was used with a Vapodest 10 Rapid Kjeldahl Distillation Unit (Gerhardt, Konigswinter, Germany). An inductively coupled plasma spectrophotometer (Optima 2100 DV, ICP/OES; Perkin-Elmer, Shelton, CT) was used to determine tissue K, P, Mg, Na, Fe, Zn, B, Ca and Cl (Mertens, 2005a; 2005b).

The experimental design was a completely randomized experiment with three replications. The SPSS program was used to evaluate the data. Data were subject to analysis of variance (ANOVA) to compare the effects of irrigation levels and NaCl applications. The differences among the means were compared using Duncan multiple range tests.

3. Results

The individual and combined effects of salt and drought stresses on plant growth and physiological parameters of tomato were statistically significant (Table 1). Both salinity and drought significantly decreased photosynthetic rate (P_n) individually; however, a greater decrease was observed under saline conditions while 100 and 150 NaCl levels were relatively similar. The combination of the two stresses together resulted in greater decrease in P_n than on their own, as shown by the lowest P_n obtained from S2D2 levels. Stomatal conductance (g_s) of tomato seedlings decreased statistically with salinity stress, but the combined effect of salinity and drought on stomatal conductance was not significant (Table 1).

CRV showed significant decrease under both salinity and drought individually; however, the decrease caused by drought was smaller compared to the decrease caused by salinity. The greatest decrease was observed by a combination of the two stress factors. The highest decrease of CRV was obtained from S2D2. The differences in CRV found statistically significant, it changed slightly for D1, but decreased sharply under D2 (Table 1). Salt and drought-stressed plants had higher EL values than control plants. Combined effects of salt and drought stresses on EL were more pronounced than individual effects (Table 1). Plant fresh weight and dry weight (FW and DW) showed significant decrease with salinity and drought. Plant fresh weight decreased roughly 80%

Table 1

Tomato plant growth and some physiological responses to salinity and drought stress conditions.

Drought Salt	D0 P _n (μmol m ⁻² s ⁻¹)	D1	D2	Mean	SXD
S0	5.91 a*	5.26 a	3.63 b	4.93 A**	<i>p</i> <0.05
S1	3.60 b	2.26 c	2.56 c	2.81 B	
S2	3.59 b	1.95 d	1.82 d	2.45 B	
Mean	4.36 A	3.16 B	2.67 C		
	g_s (mmol m⁻² s⁻¹)				
S0	0.06 ^{ns}	0.05	0.04	0.05 A**	<i>p</i> >0.05
S1	0.04	0.04	0.03	0.04AB	
S2	0.03	0.03	0.03	0.03B	
Mean	0.04^{ns}	0.04	0.03		
	CRV (SPAD)				
S0	40.96 a***	38.05 ab	34.95 b	37.99 A***	<i>p</i> <0.05
S1	26.84 c	24.29 c	21.42 c	24.18 B	
S2	24.87 c	22.75 c	20.35 c	22.66 B	
Mean	30.89 A	28.36 AB	25.57 B		
	FW (g plant⁻¹)				
S0	20.81 a***	16.99 b	6.36 cd	14.72 A***	<i>p</i> <0.001
S1	9.22 c	7.93 cd	6.40 cd	7.85 B	
S2	6.66 cd	6.30 cd	4.15 d	5.70 C	
Mean	12.23 A***	10.41 B	5.64C		
	DW (g plant⁻¹)				
S0	2.58 a***	1.86 b	0.75 c	1.73 A***	<i>p</i> <0.001
S1	1.07 c	1.00 c	0.79 c	0.95 B	
S2	0.85 c	0.76 c	0.55 d	0.72 B	
Mean	1.50 A***	1.21 B	0.70 C		
	EL (%)				
S0	53.85 c***	71.66 b	73.69 b	66.40 B***	<i>p</i> <0.05
S1	76.61 ab	76.20 ab	79.52 ab	77.44 A	
S2	84.20 a	81.74 ab	86.47 a	81.13 A	
Mean	71.55 B	76.53 AB	79.89A		

*: *p*<0.05; **: *p*<0.01; ***: *p*<0.001; ns: *p*>0.05 Data followed by a different letter were significantly different according to Duncan's Multiple Range Test.

S0: 0 mM NaCl, S1: 100 mM NaCl, S2: 150 mM NaCl.

D0: 100% irrigation-control, D1: 75 irrigation, D2: 50% irrigation.

P_n: Photosynthetic rate, g_s: stomatal conductance, EL: electrolyte leakage.

CRV: chlorophyll reading value, FW: fresh weight, DW: dry weight.

with severe level of drought (D2), and a similar decrease was observed under the highest salinity (S2). The greatest decline of FW and DW were obtained from S2D2 levels. Both drought levels and salt levels have significantly reduced FW and DW (Table 1).

The individual and combined effects of salt and drought stresses on nutrient content of tomato seedlings were statistically significant (Table 2). The content of N in the plant showed significant decrease with salinity and drought. An even greater decrease observed under the two stresses together (S2D2), with the greatest decline of N at 1.79%. The interaction between salt and drought treatments were found to be statistically significant. Effects of salt and drought stress as well as their combined effects on K content of tomato seedling were statistically significant. The content of K in the plant showed significant decrease under salinity and drought respectively. The two stress factors together (S2D2) resulted in the greatest decrease of K by 16,510.00 mg/kg. The individual and combined effects of salt and drought stress on P content of tomato seedling were statistically significant (*p*<0.001). Both salt and drought treatments on their own resulted in lower P content than the control treatments, but combined treatments (S2D2) resulted in the greatest decline in P content (1199.00 mg/kg). Similar decreasing results on Mg, Fe, Ca and Zn content of tomato seedling were also observed. The individual and combined effects of salt and drought were statistically significant. On the other hand, the content of Na, Cl and B of tomato seedling increased with stress especially under salt treatment. The highest increase of Na (617.50 mg/kg), B (32.22 mg/kg) and Cl (34.79 mg/kg) was obtained from S2D2 levels. Individual and combined effects of salt and drought stress on Na, Cl and B content of tomato seedling were statistically significant (*p*<0.001). It was determined that the decrease in the content of N, P, K, Fe and Zn were prominently higher

(24%, 24%, 23%, 19% and 15% respectively) in severe drought treatment (D2) compared to the decrease (19%, 20%, 17%, 19% and 24% respectively) in the highest salt treatment (S2). The combined stress treatments (S2D2) caused more reduction in the content of N, P, K, Mg, Fe and Zn (36%, 27%, 34%, 2%, 31% and 27% respectively). However, the combined stress treatments (S2D2) increased as a ratio of 88%, 55%, 18% and 133% in the content of Na, B, Ca, and Cl respectively.

4. Discussion

The individual and combined effects of salt and drought stress on plant growth properties and plant nutrient content of tomato seedlings were statistically significant (*p*<0.001, *p*<0.05; Table 1 and Table 2). FW and DW of the tomato seedlings decreased both under salt-only and drought-only treatments. However, combined effects of these two treatments together had cumulative impact on the measured parameters (Table 1). These results show that the individual effects of salt and drought on tomato seedlings were less detrimental for plant establishment than additive effects of individual stresses. Salinity and drought stress have previously been reported to adversely affect shoot and root development, leaf area and number, root length, fresh and dry weights of plants (Ors and Suarez 2017; Sahin et al., 2018). Studies have reported that plant growth of onions (Hanci and Cebeci, 2015) and soybeans (Khan et al., 2016) were adversely affected when salinity and drought conditions are applied separately or together. In fact, previous studies have shown that abiotic stress factors of drought and salinity reduce the root and leaf growth and adversely affect plant growth and development (Dolferus, 2014; Farooq et al., 2009; Sahin et al., 2018). In line with our findings, Ahmed et al. (2013) reported that drought and salinity stress combined have more negative effects on barley development than individual stress conditions. Plants grown under drought and saline conditions resulted in water loss in the cell, degradation of plasma membrane and release of hydrolytic enzymes which, in turn, lead to the degradation of cytoplasm and an overall slowdown in growth and reduction in turgor (Kusvuran, 2010; Ahmed et al., 2013). Therefore, plant growth and development are expected to be limited at various levels for each plant under stress conditions. Similar to our results, Al-Omran et al. (2005), Amer (2011) and El-Mageed and Semida (2015) stated that stress conditions such as salinity and drought negatively affected growth and physiological characteristics of various plants.

In this study, photosynthetic rate and stomatal conductance of tomato seedlings decreased significantly under both saline and drought conditions (Table 1). Photosynthesis is one of the main complex processes affected by salinity and drought stress (Chaves et al., 2009). Studies have reported that both these stresses can adversely affect photosynthetic activity in plants (Farooq et al., 2009; Xu and Lescovar, 2015; Ors et al., 2016; Ekinici et al., 2020). Sanoubar et al. (2016) reported that salt stress reduces the efficiency of leaf photosynthesis (Tr, g_s and P_n) in cabbage, while Ashraf (2004) stated high salt stress may reduce photosynthesis due to stoma limitation. Drought leads to stagnation in plant growth, causing cell division to cease the expansion of meristem tissues, resulting in reduced photosynthesis. In addition, drought stress reduces photosynthetic activity in plant tissues due to the imbalance between capture and use of light (Noctor and Foyer, 1998). Vermeulen et al. (2007) reported that drought stress adversely affected plant growth, increased leaf temperature, and closed the stomata of the tomato plant. Similarly, earlier studies showed salt stress decreased the photosynthetic characters of plant (Zapata et al., 2003; Pérez-López et al., 2013).

In our study, CRV of tomato seedlings grown under drought and saline conditions decreased. This reduction was greater when both stresses were applied together (Table 1). Previous studies have suggested reductions in chlorophyll amount may be caused by damage to chlorophyll membranes. For instance, the amount of chlorophyll in pepper was reported to have reduced under salt and/or drought stress (Okunlola et al., 2017).

Table 2
Tomato mineral content responses to salinity and drought stress conditions.

Drought Salt	D0 N (%)	D1	D2	Mean	SXD
S0	2.81a ***	2.40 b	2.21 d	2.47 A***	$p < 0.001$
S1	2.43 b	2.16 e	2.13 e	2.24 B	
S2	2.26 c	1.93 f	1.79 g	1.99 C	
Mean	2.50 A***	2.16 B	2.04 C		
	K (mg/kg)				
S0	25,171.50 a ***	22,232.50 c	21,055.00 d	22,819.67A***	$p < 0.001$
S1	23,888.50 b	20,015.50 e	19,450.00 f	21,118.00 B	
S2	20,898.50 d	17,999.50 g	16,510.00 h	18,469.33 C	
Mean	23,319.50 A***	20,082.50 B	19,005.00 C		
	P (mg/kg)				
S0	1649.00 a ***	1394.50 b	1333.00 b	1458.83 A***	$p < 0.001$
S1	1397.50 b	1239.00 c	1248.50 c	1295.00 B	
S2	1333.00 b	1209.50 c	1199.00 c	1247.17 C	
Mean	1459.83 A***	1281.00 B	1260.17 B		
	Mg (mg/kg)				
S0	1306.00 abc ***	1376.00 b	1366.50 ab	1349.50 B*	$p < 0.001$
S1	1383.00 a	1255.00 c	1165.00 d	1267.67 A	
S2	1294.00 bc	1268.50 c	1278.50 bc	1280.33 B	
Mean	1327.67^{NS}	1299.83	1270.00		
	Na (mg/kg)				
S0	327.00 g ***	443.00 d	427.00 e	399.00 B***	$p < 0.001$
S1	355.50 f	453.00 d	483.00 c	430.50 B	
S2	443.00 d	555.50 b	617.50 a	538.67 A	
Mean	375.17 C***	483.83 B	509.17 A		
	Fe (mg/kg)				
S0	54.00 a ***	48.21 c	43.35 d	48.52 A***	$p < 0.001$
S1	51.22 b	40.72 e	39.02 f	43.65 B	
S2	43.67 d	38.23 fg	37.17 h	39.69 C	
Mean	4963 A***	42.39 B	39.84 C		
	Zn (mg/kg)				
S0	15.16 a ***	13.40 bc	12.80 c	13.79 A***	$p < 0.001$
S1	13.84 b	11.88 d	10.73 e	12.15 A	
S2	11.45 de	11.06 e	11.03 e	11.18 B	
Mean	13.48 A***	12.11 B	11.52 C		
	B (mg/kg)				
S0	20.74 f ***	24.34 d	27.72 c	24.27 C***	$p < 0.001$
S1	22.73 e	28.17 bc	29.16 b	26.69 B	
S2	26.91 c	32.84 a	32.33 a	30.69 A	
Mean	23.46 C***	28.45 B	29.74 A		
	Ca (mg/kg)				
S0	11,332.00 c ***	10,287.00 e	10,323.00 e	10,647.33C***	$p < 0.001$
S1	11,321.50 c	10,944.50 d	10,343.50 e	10,869.33 B	
S2	12,200.00 b	12,655.00 a	11,163.00 cd	12,006.00 A	
Mean	11,617.83 A	11,295.50 B	10,609.83 C***		
	Cl (mg/kg)				
S0	14.90 g ***	16.29 f	25.62 d	18.94 C***	$p < 0.001$
S1	19.23 e	28.24 c	30.67 b	26.05 B	
S2	27.67 c	34.43 a	34.79 a	32.30 A	
Mean	20.60 C***	26.32 B	30.36 A		

*, $p < 0.05$; ***, $p < 0.001$; ns: $p > 0.05$ Data followed by a different letter were significantly different according to Duncan's Multiple Range Test.

S0: 0 mM NaCl, S1: 100 mM NaCl, S2: 150 mM NaCl.

D0: 100% irrigation-control, D1: 75% irrigation, D2: 50% irrigation.

EL values increased under both drought and salt stress. Increased EL values of tomato seedlings were more pronounced when both stresses were applied together (Table 1). This increase in EL value may have resulted from damage in the cell membrane caused by stress conditions. Masoumi et al. (2010) supports this finding, reporting that drought stress caused damage to the leaves and, as a result, allowed electrolytes to leak through the cell membranes. Similar findings show that the increase in EL occurs as a result of drought stress in spinach (Ekinci et al., 2015). Our results are also in accordance with findings by Shams et al. (2016) who found that salt stress increased EL in lettuce.

Both drought and salt stress decreased nutrient content of tomato leaves, while irrigation with saline water increased Na and Cl content. (Table 2). Similarly, drought and salt stress have been reported to reduce nutrient content in other studies (Ekinci et al., 2015; Sahin et al., 2018; Yildirim et al., 2015). Drought stress causes an

inadequate intake of plant nutrients and nutrient deficiency in plants; therefore it is reported to cause disruptions in enzyme activities other than biochemical events such as respiration and photosynthesis, resulting in disruption of osmotic balance and in plant growth and development (Kusvuran, 2010; Rizwan et al., 2015; Sahin et al., 2018). The amount of Na in the leaves increased due to increased NaCl doses in irrigation waters. Due to the increased Na uptake in the leaves, K, P and N uptake, especially Ca, is adversely affected as a result of competition. This is due to antagonism between Na and other elements (Fageria, 2001).

Conclusions

The adverse effects of salinity and drought stress on tomato plant growth are well known; however, the combined effects of these constraints on tomato have not been subject to much research. In this

study we evaluated salt and/or drought stress factors on growth, plant nutrition elements and some physiological properties of tomato at stage of seedling. Both salinity and drought stress conditions had negative effects on several features such as plant growth, photosynthetic activity, nutrient element content and chlorophyll amount in tomato seedlings, with increasing effect in treatments where the two stress factors were applied together. This experiment should be repeated under field conditions, and further investigations on the response to stress factors from tomato after the seedling period will be valuable to help inform future management decisions under these conditions.

Author contributions

Ertan Yildirim, Selda Ors and Melek Ekinci designed the experiments; Melek Ekinci, Atilla Dursun and Ustun Sahin conducted the experiments; Ertan Yildirim, Metin Turan, Atilla Dursun and Metin Turan analyzed the results; Ertan Yildirim, Selda Ors, Ustun Sahin and Melek Ekinci wrote the manuscript.

Declaration of Competing Interest

The authors declare no competing financial interest.

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