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The effects of drought, salt and combined stresses on ion exchanges of eggplant (*Solanum melongena* L.) seedlings

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ABSTRACT

In this study, ion exchanges in eggplant plants exposed to drought, salt, and combined stress were researched. While drought-stressed plants were irrigated at 60% FC, salt-stressed plants were irrigated with water containing 50 mM sodium chloride (NaCl). Plants under combined stress were irrigated with water containing 50 mM sodium chloride (NaCl) at 60% FC. The plants remained under stress conditions for 90 days, after which they were harvested and evaluated for their ion content. Ca^{2+}, K^{+} and Mg^{2+} contents in the shoot and root decreased significantly under drought, salt, and combined stresses. The most severe losses were detected in plants grown under combined stress. However, while Na accumulations increased under stress, these increases were more pronounced in the root under combined stress. K^{+}/Na^{+} and Ca^{2+}/Na^{+} ratios in the shoot and root under salt and combined stress were found to be lower than those under drought stress. In all stress conditions, especially K^{+}/Na^{+} and Ca^{2+}/Na^{+} ratios in the root showed significant decreases compared to the control. These findings showed that when drought and salt stress conditions were separately applied, Ca^{2+}, K^{+} and Mg^{2+} accumulations in the shoot were less. On the other hand, the combination of drought and salt increased the ion losses in each stress factor more.

1. Introduction

Drought and salinity are considered the most important abiotic stress factors that negatively affect plant production. Agricultural drought is expressed as the lack of sufficient moisture for the plant to grow and develop in the root zone. Salinity is another source of abiotic stress that negatively affects plant growth and crop yield, especially in arid and semi-arid regions (Kiran et al. 2019). Insufficient precipitation, high evaporation, natural salt rocks, saline irrigation water and insufficient drainage lead to soil salinity. Many products of economic importance can be significantly affected by drought and salinity. Morpho-physiological and biochemical changes occur in plants that are faced with drought and salinity problems (Munns and Tester 2008), as a result, plant growth is inhibited, and many losses, such as yield and quality decline, may occur as a result of metabolic damage (Abobatta 2019). Although there is a strong relationship between soil moisture and macro and micro nutrients that affect plant growth, the mobility of mineral elements and their uptake by the plant are prevented in soils with low soil moisture (Al-Kaisi et al. 2013). As the salt content in the soil increases, the plant's water uptake from the soil decreases, and the ion toxicity and nutrient uptake resulting from the decrease in the osmotic potential of the soil solution negatively affect plant growth (Parvaiz and Satyawati 2008). In addition, the uptake of nutrients by the roots and their transmission to the shoots are reduced due to active transport and membrane permeability, which deteriorates with the effect of physiological drought caused by salinity (Alam 1999). Salty conditions; Ca^{2+}, Na^{+}, K^{+}, Mg^{2+} and Cl^{-} can cause ion toxicity, nutrient imbalances may occur due to the competition of Na^{+} and Cl^{-} with nutrients such as K^{+}, Ca^{2+} and NO_3^{-}. High Na^{+} and Cl^{-} accumulations in plants under salinity stress may impair nutrient ion activities by causing high Na^{+} Na^{+}/Ca^{+2} and Na^{+}/K^{+} ratios. (Singh et al. 2014). As a result, changes may occur in the physicochemical and metabolic properties of the plant (Zhao et al. 2021).

Eggplant, which is widely grown in open and greenhouse agriculture in our country; although it varies genotypically, it is a type of vegetable that is generally moderately sensitive to salinity and drought (Ghaemi and Rafiee 2016; Brenes et al. 2020). In this plant species, drought and salinity stresses can be seen together and serious yield losses can occur. The effect of ion exchanges is also very important in the emergence of these losses. The aim of this study is to determine the effects on drought and salt stress alone and in combination on the ion exchanges of eggplant plant.

2. Materials and methods

2.1. Plant material and setting up the trial

The study was carried out in greenhouse conditions where temperature and humidity conditions (day-night temperature: 18-22-26/22-26°C, relative humidity 50-55%) are automatically provided. Eggplant (*Solanum melongena* L.) seeds of the Kemer variety were germinated in a medium containing vermiculite and perlite (1:1, v/v) (March 18). 30 days after planting (Day after planting-DAS), the seedlings reaching 3-4 true leaves were transferred to pots (diameter: 25 cm, depth: 22 cm) with medium textured soil (electrical conductivity (EC): 1.28 dS m^{-1}, soil reaction (pH): 7.75, soil organic matter: 0.54%, soil reaction (pH): 7.75, soil organic matter: 0.54%, available phosphorus: 3.60%, total nitrogen: 0.18%, available potassium 0.86%). It was ensured that there were 8 seedlings in each pot. For each matter, 3 pots were used and a total of 48 pots were studied in 4 replications. Before planting, chemical fertilizers were applied to the seedlings according to the soil analysis results (100 mg kg^{-1} N, 25 g kg^{-1} P and 100 mg kg^{-1} K). All pots were watered at field capacity (FC) until stress treatments began. The amount of irrigation water was determined by considering the pot weight. Pots were weighed every three days and the missing water was made up to FC level. For the determination of FC; first, the sample pots were kept in a container filled with water until the saturation point was reached for 48 hours. Afterwards, the pots (covered with plastic covers) were kept for the gravity water to drain and then weighed and accepted as 100% FC.

2.2. Drought, salt and combined stress treatments

Drought, salt, and combined stress treatments were initiated at 37 DAS and lasted up to 90 DAS. Plants belonging to the drought stress treatment were kept at 60% FC, while control plants were irrigated at FC level. For this, 60% of the water given to the control pots was given to the pots in which drought stress was applied. Moisture lacking in the control was completed to FC. In the salt stress treatment, plants were irrigated with water containing 50mM sodium chloride (NaCl) during the study. For this, the missing moisture in the pot was completed to FC with water containing 50 mM NaCl. The plants in the combined stress application were irrigated with water containing 50mM NaCl at 60% FC. That is, 60% of the water supplied to the control was given as water containing 50 mM NaCl.

2.3. Mineral element analysis

At the end of the study (90 DAS), the plants removed from each application were divided into shoot and root parts. After washing with tap water and distilled water, they were dried at 65°C until they reached a constant weight and were then ground. For K, Ca, Mg and Na analysis, 250 mg of the ground leaf sample was first burned with nitric acid (HNO_3) in a microwave device and then the samples were transferred to a 50 ml erlenmeyer (Kacar and Inal 2008). Total K^+ and Na^+ in the obtained extracts were determined by reading with a Jenway PFP 7 Flamephotometer device. Ca^{2+} and Mg^{2+} were measured by reading with a Varian 720-ES ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometer) (Kacar and Inal 2008).

2.4. Statistical analysis

The study was carried out in 4 replications according to the randomised plot design. The obtained data were subjected to analysis of variance. SPSS 11.0 software program was used for statistical evaluations. Principal component analysis (PCA) was performed using the XLSTAT program (Addisonsoft XLSTAT, Paris) to determine relationships between the parameters.

3. Results and Discussion

The responses of eggplant plants in terms of Ca^{2+}, K^+, Mg^{2+} and Na^+ contents in the shoot and roots were found to be statistically significant ($P<0.01$) (Table 1 and Table 2). Ca^{2+}, K^+ and Mg^{2+} concentrations in root and shoot decreased importantly in the drought, salt and combined stresses compared to the control. The highest values in terms of Ca^{2+} concentration; while it was determined under drought and salt stresses (1.58% and 1.66%), combined stress (1.48%) gave the lowest value. Root Ca^{2+} content in the roots was similar to shoot Ca^{2+} content in the shoots under stress conditions, with the lowest Ca^{2+} accumulation under combined stress conditions (0.76%). Ca^{2+} accumulation was higher under drought and salt stress (0.89% and 0.84%). Yuan-Yuan et al. (2009) stated that the deterioration of chloroplasts under drought stress may decrease Ca^{2+} accumulation. It is also known that salt stress causes Na^+ accumulation in plants and decreases the Ca^{2+} ratio (Nengfei et al. 2010). It has also been emphasized in previous

Table 1. Effect of drought, salt and combined stress on Ca^{2+} and K^+ contents of shoot and root (mean±SE) in *S. melongena*. Duncan test ($P<0.01$) was used for differentiation among treatments mean ($n= 4$)

Treatment	Ca^{2+} (%)		K^+ (%)	
	Shoot	**Root**	**Shoot**	**Root**
Control	1.96±0.05^a	1.17±0.04^a	1.56±0.04^a	1.70±0.05^a
Drought stress	1.58±0.02^b	0.89±0.04^b	1.40±0.03^b	0.99±0.06^b
Salt stress	1.66±0.04^b	0.84±0.02^b	1.38±0.05^b	0.73±0.05^c
Combined stress	1.48±0.08^c	0.76±0.04^c	0.97±0.03^c	0.56±0.02^d
Significance of treatments	**	**	**	**

***P 0.01*

Table 2. Effect of drought, salt and combined stress on Mg^{2+} and Na^+ contents of shoot and root (mean±SE) in *S. melongena*. Duncan test ($P<0.01$) was used for differentiation among treatments mean ($n= 4$)

Treatment	Mg^{2+} (%)		Na^+ (%)	
	Shoot	**Root**	**Shoot**	**Root**
Control	0.46±0.01^a	0.41±0.01^a	0.49±0.02^c	0.10±0.01^d
Drought stress	0.39±0.01^b	0.33±0.01^c	0.50±0.01^c	0.35±0.02^c
Salt stress	0.40±0.01^b	0.36±0.01^b	0.62±0.04^b	1.14±0.10^b
Combined stress	0.37±0.01^c	0.31±0.01^d	0.72±0.03^a	1.53±0.07^a
Significance of treatments	**	**	**	**

***p 0.01*

studies that NaCl reduces the plant's Ca^{2+} uptake in the plants and transport, causes Ca^{2+} deficiency and ion imbalance in the plant (Kiran et al. 2015; Hand et al. 2017). In this study, only drought and salt stress decreased Ca^{2+} concentrations in the shoot and roots. The fact that Na^{+} accumulation was higher, especially in the root, in the combined stress environment led to a decrease in the uptake of Ca^{2+} in the root and its transmission to the stem. Thus, the combined effect of drought and salt stress heightened Ca^{2+} losses.

When the change in K^{+} concentrations in the trunk under stress conditions was examined; The highest K^{+} accumulation occurred under drought and salt stress (1.40% and 1.38%). This was followed by the combined stress with a value of 0.97 (Table 1). Roots accumulated more K^{+} under drought stress than combined and salt stress (0.99%). This was followed by salt and combined stress with a value of (0.73% and 0.56%, respectively) (Table 1). Potassium is of great importance for the osmotic potential of the cell to increase and water intake to take place. Nasri et al. (2008) determined that there was a decrease in K^{+} concentration in the plant body as a result of drought stress in watermelon; It has been reported that potassium is effective in the opening and closing of stomata, photosynthetic effect and maintaining water balance. In the study, K ion losses in the root are relatively higher than in the trunk under all stress conditions. It has been reported that there is an antagonistic relationship between Na^{+} and K^{+}, and that K^{+} uptake can be prevented due to competition with Na^{+} (Levitt 1980). Also, it has been reported that salt stress may cause a decrease in K^{+} uptake in shoot and/or root in different plant species (Yong et al. 2014; Turhan et al. 2020), and that more K^{+} may accumulate in the shoot than in the root (Jalali-Honarmand et al. 2014). In addition, the increase in Na^{+} and the limitation in the uptake of K^{+} under salt stress conditions, K^{+} uptake is limited in the combined stress environment due to the inability to take water into the shoot under drought stress.

While drought, salt stress and combined stress caused decreases in the amount of Mg^{2+} in shoot and root, statistical differences emerged between applications. When the values measured in terms of Mg^{2+} amount in the shoot were examined, the highest Mg^{2+} values were determined in plants under salt and drought stress (0.49% and 0.39%, respectively) (Table 2). Plants under combined stress faced the highest Mg^{2+} loss (0.37%) (Table 2). Similarly, Mg^{2+} losses in the root were highest under combined stress (0.31%) (Table 2). Plant roots under salt stress were able to preserve the amount of Mg^{2+}, keeping the Mg^{2+} loss at a limited level (36%) (Table 2). Mg^{2+} is part of photosynthesis as a chlorophyll component and activator. Decreased water uptake and delivery due to drought and salinity also led to less Mg^{2+} accumulation under combined stress. In addition, the decrease in Mg^{2+} concentration in the shoot may be the reason for the deterioration in photosynthesis. Similar results were also reported by Liu et al. (2020) under salt stress conditions. On the other hand, under combined stress; the reduction in Mg^{2+} content appeared more severely under the combined stress due to the reduction in water potential and ion toxicity.

Na^{+} concentration in shoots and roots of eggplant plants under all stress treatments increased compared to the control (Table 2). Eggplant plants under stress applications, showed similar responses in terms of Na^{+} concentration in shoot and root. Accordingly, the plants that limited Na^{+} uptake to their shoots and roots the most were those under drought stress (0.50% and 0.35%, respectively) (Table 2). However, Na^{+} accumulation was higher in shoots and roots under salt and combined stress (for salt stress; 0.62% and 1.14, for combined stress; 0.72% and 1.53) (Table 2). It has been emphasised by many researchers that there may be an increase in Na^{+} amounts in root under drought and/or salt stress conditions (Chen et al. 2011; Hand et al. 2017; Dugasa et al. 2019). In the study, Na^{+} accumulation in the trunk was found to be high under all stress conditions. Thus; it is seen that the development of plants that accumulate Na^{+}, which they take with their roots, at toxic level by being transmitted to the shoot, is adversely affected (Alian et al. 2000).

Plants need to continue to be fed with K^{+} and/or Ca^{2+} while keeping their Na^{+} intake limited; this is an important feature that contributes to the high salt stress tolerance of plants. In this respect, K^{+}/Na^{+} and Ca^{2+}/Na^{+} ratios are considered to be an indicator of the preferences between K^{+} and Ca^{2+} with Na^{+}. In the study, the changes in K^{+}/Na^{+} and Ca^{2+}/Na^{+} ratios were found to be significant ($P<0.01$) (Table 3). In the shoot, the highest K^{+}/Na^{+} ratio was determined under drought and salt stress (2.80 and 2.23 respectively), and the lowest ratio was determined under combined stress (1.35) (Table 3). In terms of K^{+}/Na^{+} ratio in the root, the highest ratio was again determined in drought stress (2.82), while they remained relatively low (0.65 and 0.37 respectively) under salt and combined stress (Table 3). The fact that Na^{+} ion accumulation was higher than K^{+} under salt stress caused a decrease in the K^{+}/Na^{+} ratio. This was especially evident in the combined stress environment and roots. Due to the similarity of their ionic diameters and electrical charges, the Na^{+} ion competed with the K ion and prevented the uptake of this ion. High K^{+}/Na^{+} ratios are used as a reliable parameter for the determination of drought and/or salt tolerance in crop plants such as tomato (Dasgan et al. 2002), tobacco (Ahmed et al. 2013), wheat (Kumar et al. 2018). However, in plants under stress Ca^{2+}/Na^{+} ratios were higher in the shoot than in the root. The highest rate was determined in the trunk under drought stress (3.16). Similarly, the highest Ca^{2+}/Na^{+} ratio in the root occurred under drought stress (2.54). The lowest Ca^{2+}/Na^{+} ratios were determined in shoot (2.06) under the combined stress conditions and in root (0.74 and 0.50 respectively) under the salt and combined stress conditions. Na^{+} ions under the salt and combined

Table 3. Effect of drought, salt and combined stress on K^{+}/Na^{+} and Ca^{2+}/Na^{+} of shoot and root (mean±SE) in *S. melongena*. Duncan test ($P<0.01$) was used for differentiation among treatments mean ($n=4$)

Treatment	K^{+}/Na^{+}		Ca^{2+}/Na^{+}	
	Shoot	Root	Shoot	Root
Control	3.17±0.13^{a}	17.00±0.98^{a}	3.99±0.22^{a}	11.70±1.00^{a}
Drought Stress	2.80±0.15^{b}	2.82±0.20^{b}	3.16±0.14^{b}	2.54±0.16^{b}
Salt stress	2.23±0.19^{c}	0.65±0.06^{c}	2.68±0.18^{c}	0.74±0.09^{c}
Combined stress	1.35±0.08^{d}	0.37±0.01^{c}	2.06±0.17^{d}	0.50±0.04^{c}
Significance of treatments	**	**	**	**

***P 0.01*

stress also decreased in Ca^{2+} ion concentrations such as K^+ in the root. This led to significant decreases in the Ca^{2+}/Na^+ ratio. Drought and, indirectly, the decrease in water potential under salt stress adversely affected the transport of Ca ions in the xylem (Kiegle et al. 2000). Our findings are similar to Sahin et al. (2018).

Principal component analysis (PCA) was carried out to determine the connection between the ion contents determined in the shoot and root of the plants. The relationships between the variables are shown with a biplot (Figure 1). According to biplot analysis, two principal components accounted for 97.74% of the total variation. PCA shows that there is a significant and positive relationship between drought stress and K^+-Shoot and K^+/Na^+-Shoot (as shown in the ellipse in Figure 1). As a matter of fact, plants tend to accumulate K^+ in their shoots as opposed to Na^+. Kumar et al., (2018) reported that plant tissues tend to accumulate enough K^+, which can reduce the toxic effects of Na^+. A similar correlation was observed between drought stress and Ca^{2+}/Na^+-Shoot. Combined stress (DS+SS) according to PCA had significant positive correlations with Na^+-Root and Na^+-Shoot. The short distance to the Na^+-Root and Na^+-Shoot variables of the combined stress (DS+SS,) indicates the strong increase in Na^+ accumulation in the root and shoot under combined stress conditions. Indeed, strong increases in Na^+ accumulation in plant roots and leaves have been reported under combined applications of drought and salt stress (Dugasa et al. 2019). Regarding the correlations between the examined variables; a strong negative correlation was observed between Na^+-Root and K^+/Na^+-Shoot (a 180 degree angle). More Na^+ accumulation in the root under stress caused a decrease in the K^+ concentration in the shoot and a decrease in the K^+/Na^+ ratio due to the increase in Na^+ conduction. This indicates that plant tissues may sometimes not tend to accumulate enough K^+ that can effectively reduce the toxic effects of Na^+. There was also a strong positive correlation between Mg^{2+}-Root and Mg^{2+}-Shoot (a rather narrow angle). A similar correlation was observed between Ca^{2+}-Shoot and Ca^{2+}-Root. As a matter of fact, it is known that Mg^{2+} and Ca^{2+} accumulations in the shoot are related to their concentrations in the root. Kumar et al. (2018) reported a similar relationship. In addition, there were strong and positive correlations between K^+/Na^+-Root and Ca^{2+}/Na^+-Root. This may be due to increases in both K^+/Na^+-Root and Ca^{2+}/Na^+-Root due to low Na^+ concentrations compared to high K^+ and Ca^{2+} concentrations in the roots of stressed plants.

4. Conclusions

The results of the study revealed that ion exchanges can differ significantly in eggplant plants under drought and salt stress. While the K^+, Ca^{2+} and Mg^{2+} accumulations in shoot and root decreased under the salinity and drought stress, the Na^+ accumulations increased significantly. However, the combined application of salinity and drought strengthened the negative effects of individual stress factors. The significant decrease in K^+/Na^+ and Ca^{2+}/Na^+ ratios with the combined effect of drought and salt stress indicated that eggplant is sensitive to the combined effects of salt and drought stress. Eggplant cultivation will be significantly restricted under drought and salinity conditions, which are highly likely to be seen together. This study, which examines the changes in the ion uptake mechanism in eggplant, is important for determining valid strategies that may be effective in improving the growth of eggplants under combined stress conditions. However, in order to better understand the ion uptake mechanism under combined stress conditions, it is recommended to examine the eggplant plant in its advanced developmental stage and under continuous/temporary stress conditions.

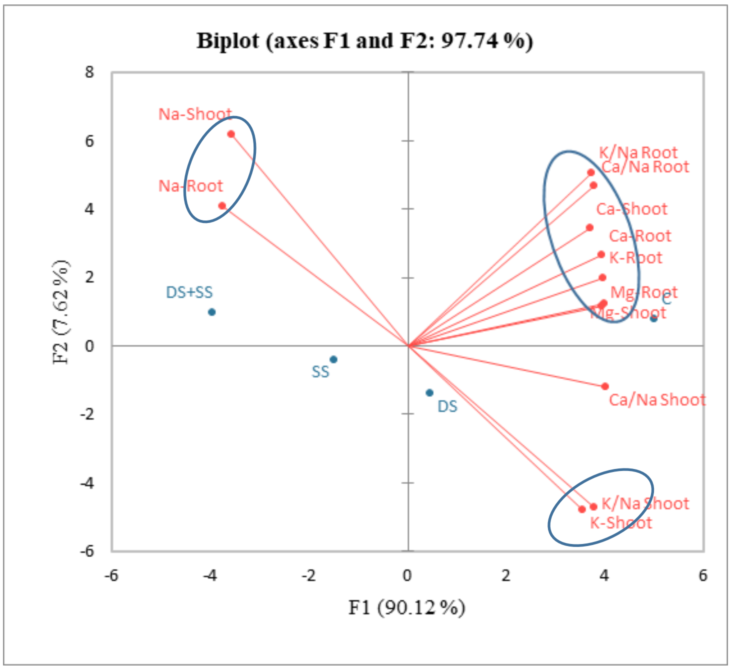

Figure 1. Biplot on ion contents in shoot and root of eggplant plants under drought (DS), salt (SS) and combined (DS+SS) stress.

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