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Effects of individual and combined effects of salinity and drought on physiological, nutritional and biochemical properties of cabbage (*Brassica oleracea* var. capitata)

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ABSTRACT

To understand the effects of salt and drought stress factors on the growth, physiological and biochemical responses of cabbage (*Brassica oleracea* var. capitata), a greenhouse experiment was conducted with different levels of salinity (S0: tap water, S1: tap water containing extra 75 mM dose of NaCl, and S2: tap water containing extra 150 mM dose of NaCl), irrigation quantity (W0: Full-irrigation, W1: irrigation with 80% of the W0, and W2: irrigation with 60% of the W0), and their combinations. The results showed that antioxidant activity, proline and sucrose contents increased under both salinity and drought stress as well as their combination. Moreover, oxidative damage indicating parameters such as electrical leakage (EL), malondialdehyde (MDA), and hydrogen peroxide (H_2O_2) increased as well. Increased level of salinity and drought stress caused a decrease in chlorophyll content (SPAD), leaf relative water content (LRWC), stomatal conductance (g_s), net photosynthetic activity (A_n), intercellular CO_2 content (Ci) and transpiration rate (Tr). We observed that proline and sucrose contents could not stimulate the growth of plant under increased levels of salinity and drought stress. Individual drought and salt stress conditions have negatively affected plant growth including the shoot, root fresh and dry weights when applied separately. On the other hand, the combination of drought and salinity enhanced the adverse effects of each stress factor.

1. Introduction

Salinity and drought are the most common environmental factors that suppress plant growth and yield in agricultural production (Khan et al., 2017). The area affected with drought is approximately 40% of the world's available land. Additionally, the climate change which may lead to extreme temperatures is predicted to cause severe prolonged drought in some areas (Zhang et al., 2014). Even worse, fresh accessible water is scarce in many parts of world and it is not shared out equally across the world. There are nearly 900 million people worldwide, who still do not have access to safe water, and almost half the population of the developing world does not have access to safe fresh water (Corcoran et al., 2010). Using of the low-quality water in agriculture is a strategy required considering insufficient fresh water resources. Salinity is considered one of the major factors among the environmental factors repressed the agricultural production in worldwide after the drought. Salt-affected lands in Europe are mainly located in the Mediterranean countries and estimated as one to 3 million hectares (Ladeiro, 2012).

Drought stress induces a set of physiological and biochemical reactions in plants and is one of the most complex abiotic stress factors in envoronment. (Khan et al., 2017). Salt stress is a composite process that limits the usable water content with its osmotic effect and causes the ionic content to reach to the toxic level. The major secondary effects caused by salinity is synthesis of reactive oxygen species (ROS) that damage DNA, protein, chlorophyll and membrane function, which can be counted as the restriction of photosynthesis and limitation of the K uptake, metabolic toxicity and cell death (Culha and Cakırlar, 2011). The rate of photosynthesis is reduced as mainly by stomatal closure due to increasing abscisic acid (ABA) in plant cells, membrane damage, and disturbed activity of various enzymes under drought conditions (Farooq et al., 2012). Water stress assists the formation of reactive oxygen species such as hydrogen peroxide (H_2O_2) (Parida and Das, 2005; Das and Uprety, 2006). Another indicator of membrane damage is the increase of malondialdehyde (MDA) amount, the last product of lipid peroxidation in membranes. There are numerous studies showing that drought leads to lipid peroxidation measured by MDA in plant tissues

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(Yildirim et al., 2015; Tiryaki, 2016; Samancıoğlu et al., 2016). Moreover, high contents of Na^+ and Cl^- under salinity stress repressed nutrient-ion activities as it disturbs the nutrient ratios by producing extreme ratios of Na^+/Ca^{+2} and Na^+/K^+ (Singh et al., 2014).

Plant hormonal and signaling components under various abiotic stress conditions provide various protection mechanisms to manage stress (Pastori and Foyer, 2002). Proline and soluble sugars assist the removal of free radicals from the cells, by increasing osmotic concentration to limit stress effects on physiological functions such as stomata opening and photosynthesis (Tiryaki, 2016). High antioxidant activity can avoid cell death and improve stress tolerance (Khan et al., 2017). To prevent oxidative damage, plants improve their antioxidant enzyme activities such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) (Das and Uprety, 2006).

Abiotic stress imposed by either drought or salinity brings about severe growth retardation in many plants. Cabbage (*Brassica oleracea* var. capitata) is one of the important vegetable crops contributing to human nutrition and it can be considered as sensitive or moderately tolerant to abiotic stress conditions such as salinity and drought (Zhang et al., 2014; Beacham et al., 2017). Most of the plants are subject to either drought or salinity problems and in most cases, these stress factors exist together in arid regions. Current literature on physiological and growth responses of many plants under different intensities of combined drought and salt stress is still inadequate. The data on plant growth, biochemical and physiological responses of cabbage caused by the salinity-drought stress is rather scarce. Considering these aspects, the aims of the study are to (1) determine growth performance of cabbage plant in different salinity-drought levels and (2) describe its physiological and biochemical responses.

2. Material and methods

2.1. Plant material and growth conditions

Cabbage (*Brassica oleracea* var. capitata cv. Yalova 1) was used as plant material in the experiment. Cabbage seeds were firstly sown into the multi-celled trays filled with peat. About one month later, the homogenous and healthy seedlings were transferred into 2.5 L pots as a seedling for each pot. The pots were filled with mix of loamy soil, sand and solid cattle manure with a volume ratio of 2:1:1. Bulk density of the mix media in the pots was approximately 1.3 g cm^{-3}. The pots were placed randomly on benches in a greenhouse with temperature and humidity controlled, which belongs to Agricultural Faculty of Ataturk University in Erzurum, Turkey. The average minimum temperature in greenhouse was 14.4 °C and the average maximum temperature was 32.9 °C during the growing period. The average air humidity was 25 ± 5% at the same period. The total number of pots was 160, comprising four replications of each treatment, 5 plants for each replication.

2.2. Irrigation water treatments

Cabbage plants were irrigated with different NaCl concentrations (0 mM for S0, 75 mM for S1, and 150 mM for S2) during the growth period. Salts in the treatments were added gradually to avoid osmotic shock to the seedlings. The first irrigation was performed with the dose of 50 mM of NaCl for all treatments, and later increased to 75 mM for S1 and S2. The highest level of salinity was obtained after using irrigation water of 100 mM NaCl and finally 150 mM NaCl for S2 treatment. The final EC levels of irrigation waters were 0.245 dS m^{-1} for S0 (tap water), 5.7 dS cm^{-1} for S1 and 11.82 dS cm^{-1} for S2 treatments. Irrigations were applied intervals of three days. Irrigation quantities applied to the plant pots were adjusted as the volumetric by using a portable moisture meter (HH2 Moisture Meter, WET Sensor, Delta-T Devices, Cambridge, England). In order to manage irrigation applications, first, the moisture meter was calibrated for the growing media used in the experiment, and then the volumetric moisture amount retained in the field capacity of the media was determined. Irrigation quantity applied to the control treatment (full-irrigated; W0) was equal to the required water that current soil moisture to reach to the field capacity. In the other two irrigation treatments irrigation quantities were adjusted at a rate of 80% (W1) and 60% (W2) of the W0 treatment. Treatments used in the experiment were S0W0, S0W1, S0W2, S1W0, S1W1, S1W2, S2W0, S2W1 and S2W2.

The evapotranspiration of cabbage plants was calculated using the water balance method given the equation below (Allen et al., 1998).

$$ET = IR - D \pm \Delta S,$$

where, ET is the crop evapotranspiration, IR is the irrigation quantity, D is the drainage loss from pot bottom, and ΔS is the media moisture change during growing period. The units for all parameters are mm. Drainage loss was considered zero as it was not observed.

2.3. Chlorophyll readings and leaf area

The area of the cabbage leaf was measured with a leaf area meter (LI-3100, LICOR Lincoln, NE, ABD) at harvest. A chlorophyll meter (SPAD-502, Konica Minolta Sensing, Inc., Japan) was used to measure the green color of three youngest fully expanded leaves as SPAD.

2.4. Measurement of electrolyte leakage (EL)

For measurement of electrolyte leakage, 10 leaf discs (10 mm in diameter) from the young fully expanded leaves from two plants per replicate were placed in 50-mL glass vials and rinsed with distilled water to remove the electrolytes released during the leaf disc excision. Vials were then filled with 30 ml of distilled water and allowed to stand in the dark for 24 h at room temperature. The EC (EC1) of the bathing solution was determined at the end of the incubation period. Vials were heated in a temperature-controlled water bath at 95°C for 20 min and then cooled to room temperature, and the EC (EC2) was measured. Electrolyte leakage was calculated as a percentage of EC1/EC2 (Shi et al., 2006).

2.5. Leaf relative water content (LRWC)

LRWC was measured according to González and González-Vilar (2001). Three young fully expanded leaves were first removed from stem and immediately weighed to determine the FW. Leaves, then, were floated in distilled water inside a closed petri dish in order to determine the turgid weight (TW). At the end of the imbibition periods when a steady state was achieved, eaves were placed in an oven at 70 °C for 48 h to obtain DW. Values of FW, TW and DW were used to determine leaf LRWC (%) using the following equation: LRWC = [(FW-DW)/(TW-DW)]x100

2.6. Photosynthetic activity

Photosynthetic rate (A_n), intercellular CO_2 content (Ci), stomatal conductance (g_s) and transpiration rate (Tr) of the plants were measured on the third fully expanded upper leaves along the right abaxial side of the leaf lamina from each plant between 10:00 am and 11:00 am using a portable Li-COr 6400 Photosynthesis System (LI-COR, Lincoln, USA) one week before the harvest. Measurement conditions were: leaf chamber PAR (photosynthetically active radiation), 1100 µmol m^{-2} s^{-1}; leaf to air vapor deficit pressure, -1.7 to -2.6 kPa; leaf temperature 20–22 °C and chamber CO_2 400 µmol mol^{-1}(Ors et al., 2016).

2.7. Harvest and growth parameters

Forty days after transplanting, five plants from each replicate were

harvested, and stem diameter, plant height, leaf number, shoot fresh -dry weight and root fresh - dry weight per plant were determined. The roots were carefully harvested from the pots, and gently washed to remove the media. Maximum attention was paid to avoid root loss. The plant material for dry weight was dried at 70 °C for 48 h. For analysis of the contents of proline, sucrose, MDA, H_2O_2 and antioxidant enzyme activity, plant samples from each replication were randomly selected. Approximately 20 g of fresh leaves selected from the middle section of the plants were frozen in liquid nitrogen and then stored at -70 °C for analysis. Four laboratory replicates were used.

2.8. Lipid peroxidation (measurement of malondialdehyde -MDA) and hydrogen peroxide (H_2O_2)

Lipid peroxidation was defined by the content of MDA. 0.2 *g* sample of frozen leaves was grounded to a fine powder with liquid nitrogen and extracted with 3 ml of cold ethanol. The crude extract preparation was centrifuged at 12,000 *g* for 20 min A mixture of trichloroacetic acid (TCA), thiobarbituric acid, butylated hydroxytoluene and an aliquot of supernatant was heated and the reaction was stopped quickly by placing the mixture in an ice bath. The cooled mixture was centrifuged, and the absorbance of the supernatant was measured at 400, 500 and 600 nm. Thiobarbituric acid-reactive substances were measured as MDA, a degraded product of the lipid. The concentration of MDA was determined from the absorbance, by using an extinction coefficient of 155 mmol l^{-1} cm^{-1}.

H_2O_2 was determined according to Velikova et al. (2000). Leaf tissues (200 mg) were homogenized in 2 ml of 0.1% (w/v) TCA solution on ice. The homogenate was centrifuged at 12,000 *g* for 15 min, and 0.4 ml of the supernatant was added to 0.4 ml of 10 mmol l^{-1} potassium phosphate buffer, pH 7.0 and 0.8 ml of 1 mol l^{-1} KI. The absorbance of the supernatant was measured at 390 nm. The content of H_2O_2 was calculated by comparing with a standard calibration curve previously made using different concentrations of H_2O_2.

2.9. Carbohydrate (sucrose)

Concentration of sucrose was assayed according to the method of Liu and Huang (2000). A 0.1 g r dry samples were incubated in 10 ml 0.1 M phosphate buffer (pH 5.4) for 24 h at 22° C. Supernatant samples (0.2 mL) were mixed with distilled water and 1.0 ml invertase (10 U Ml^{-1}), and then incubated in a water bath for 1 h at 50° C. The difference in decreasing sugar content between the incubation solution with and without invertase was used to calculate sucrose content (Ting, 1956).

2.10. Proline

A 50 mg of frozen leaf sample was powdered with liquid nitrogen and extracted with a pestle and mortar with 4.5 ml of 5-sulfosalicylic acid 3% in an ice bath. The homogenates were filtered with a filter paper (#2). Two ml of filtrate was reacted with 2 ml acid-ninhdrin and 2 ml of glacial acetic acid in a test tube for 1 h at 100 °C, and the reaction terminated in an ice bath. The filtrates were used for the analysis. Proline concentration was assayed spectrophotometrically at 520 nm (Bates, 1973).

2.11. Assay of antioxidant enzyme activity

In brief, for SOD, POD and CAT activities, the frozen cabbage leaves were homogenized in 5 ml of 100 mM phosphate buffer (pH 7.0) containing 1% (w/v) PVPP and all processes were proceed at 4 °C. The homogenate was centrifuged at 15,000× g for 15 min and the supernatant fraction was directly examined for enzyme activities.

CAT activity was analyzed based on the rate of hydrogen peroxide decomposition according to the method (Abedi and Pakniyat, 2010). The CAT activity was determined by a decrease in reaction mixture absorbance at 240 nm that was caused by adding H_2O_2. The reaction mixture contained 50 mM phosphate buffer (pH 7.0), 10 mM H_2O_2 and 100-µL extract. The activity was computed oxidation extinction coefficient of 39.4 mM cm^{-1} for H_2O_2.

POD activity was measured to base its capability to turn guaiacol into tetraguaiacol at 436 nm according the method of (Angelini et al., 1990).

(SOD) activity is based on the determination of inhibition in the photochemical diminution of nitroblue tetrazolium at 560 nm according to the method by (Abedi and Pakniyat, 2010). The total SOD activity was determined by monitoring the prevention of the depletion of p-nitro-blue tetrazolium chloride (NBT). 200 µl of the reaction mixture (50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, 63 µM NBT, 50 µM riboflavin, 13 mM methionine and 50 µL of plant extract) were placed in wells of a 96-well microplate under a 40 W fluorescent lamp. After 8 min of lightening, the absorbance was read at 560 nm A non-illuminated reaction mixture, which is conducted in the same manner, was used as blank. One unit of SOD was determined as the amount of the enzyme, which have produced a 50% inhibition of the sNBT reduction.

2.12. Mineral analysis

To determine the mineral concentrations in the cabbage leaves from each plot, samples were oven-dried at 68 °C for 48 h and ground. The Kjeldahl method and a Vapodest 10 Rapid Kjeldahl Distillation Unit (Gerhardt, Konigswinter, Germany) were used to determine total N (Bremner, 1996). Macro- (P, K, Ca, Mg and Na) and microelements (Fe, Zn, Cl, B and Si) were determined after wet digestion of dried and ground subsamples using a HNO_3- H_2O_2 acid mixture (2:3 v/v) with three steps in a microwave (Bergof Speedwave Microwave Digestion EquipmentMWS-2) (Mertens, 2005a). Tissue P, K, Ca, Mg, Na, Cl, Fe, Zn, B and Si were determined with an inductively coupled plasma spectrophotometer (Optima 2100 DV; Perkin-Elmer, Shelton, CT) (Mertens, 2005b).

2.13. Statistical analysis

The experiments were repeated twice, there were no significant differences by the experiments. The statistical analysis was made using SPSS. The experimental design was hierarchical with respect to two factors arranged in a completely randomized design with four replications per treatment and 5 plants per replicate. The first factor (NaCl levels) had three levels (0, 75 and 150 mM), and the second one (Irrigation levels) had three levels (100%, 80 and 60). Data was subjected to analysis of variance (two-way ANOVA) to compare the effects of salt stress and irrigation level treatments. Means were separated by Duncan's multiple range tests (DMRT) (SPSS, 2010). The correlation analysis was made to determine the relationship between the parameters investigated.

3. Results and discussion

3.1. Plant growth

Plant growth properties were affected from both salinity and irrigation quantities. All vegetative parameters such as plant height, stem diameter, leaf area, number of leaves, fresh and dry shoot and root weights in all treatments are significantly lower than the control treatment (S0W0) (Table 1). Cumulative effects of increasing the salinity and reducing the irrigation quantity have resulted in higher decreases in vegetative growth of cabbage. Plant heights in the S0W1, S0W2, S1W0, S1W1, S1W2, S2W0, S2W1, and S2W2 treatments were lower by 7.1, 20.0, 20.3, 34.1, 37.7, 35.6, 38.4, and 39.1%, respectively compared to the S0W0 values. Lesser stem diameter values of 6.9, 8.4,

Table 1
Cabbage growth parameters under different salinity-drought levels.

Salinity level	Drought level	Plant height cm	Stem diameter mm	Leaf area cm^2	Number of leaves	Fresh shoot weight g	Dry shoot weight g	Fresh root weight g	Dry root weight g
S0	W0	28.91 a	6.66 a	353.4 a	10.22 a	49.18 a	7.33 a	13.24 a	1.09 a
	W1	26.87 b	6.20 bc	324.9 b	8.89 b	34.65 b	5.54 b	7.90 b	0.64 b
	W2	23.12 c	6.10 bc	234.3 c	7.89 c	26.50 c	3.79 c	7.15 c	0.47 c
S1	W0	23.05 c	6.21 b	245.5 c	8.67 b	26.57 c	3.88 c	6.49 d	0.41 d
	W1	19.05 d	6.06 c	222.3 d	6.67 d	21.67 d	2.65 d	6.63 d	0.39 d
	W2	18.02 d	5.52 d	215.7 de	6.33 d	16.05 f	2.19 e	4.65 e	0.33 e
S2	W0	18.61 d	4.66 e	204.3 ef	7.44 c	18.17 e	2.77 d	3.71 f	0.30 f
	W1	17.82 d	4.23 f	198.3 f	6.56 d	14.48 f	1.87 f	3.35 fg	0.28 f
	W2	17.62 d	4.17 f	163.5 g	6.11 d	10.66 g	1.73 f	3.14 g	0.22 g

The means marked with different lower case in each column differ meaningfully ($P < 0.001$). S0: tap water with low salinity (0.245 dS m^{-1}); S1: tap water containing extra 75 mM dose of NaCl; S2: tap water containing extra 150 mM dose of NaCl; W0: Full-irrigation; W1: irrigation with 80% of the W0; W2: irrigation with 60% of the W0.

6.8, 9.0, 17.1, 30.0, 36.5, and 37.4% in the S0W1, S0W2, S1W0, S1W1, S1W2, S2W0, S2W1, and S2W2 treatments than the one in the S0W0 were observed. Leaf area values in the S0W1, S0W2, S1W0, S1W1, S1W2, S2W0, S2W1, and S2W2 treatments decreased by the ratio of 8.1, 33.7, 30.5, 37.1, 38.9, 42.2, 43.9, and 53.7%, respectively. Number of leaves in the S0W1, S0W2, S1W0, S1W1, S1W2, S2W0, S2W1, and S2W2 treatments were lesser by 13.0, 22.8, 15.2, 34.7, 38.1, 27.2, 35.8, and 40.2% than the one in the S0W0 treatment. There were a significant ($P < 0.01$) negative linear correlations between salinity-drought stress levels and plant height ($r = 0.930$), stem diameter ($r = 0.943$), leaf area ($r = 0.918$), number of leaves ($r = 0.873$), fresh shoot weight ($r = 0.928$), and fresh root weight ($r = 0.908$). Jamil et al. (2006) has also determined negative relationships between salinity stress and plant height, fresh root weight and fresh shoot weight in cabbage.

The higher plants had thicker stem, the more leaf number and leaf area. Noticeable decreases in the plant height, stem diameter, leaf area and number of leaves due to increased salinity-drought stress resulted in significantly lesser plant weights (Table 1). Previous studies have pointed out that salt and drought stress conditions had a negative effect on growth of a lot of crops (Ekinci et al., 2012, 2015; Yildirim et al., 2015; Sahin et al., 2015; Ors et al., 2016; Shams et al., 2016). The linear correlation ($r = 0.964$) between plant height and fresh shoot weight was statistically significant ($P < 0.01$). The linear correlations for stem diameter-fresh shoot weight ($r = 0.817$), leaf area-fresh shoot weight ($r = 0.966$), and number of leaves-fresh shoot weight ($r = 0.951$) also was found statistically significant ($P < 0.01$). Maintaining shoot and root growth is important under salt and drought stress because the roots absorb water and minerals from soil and transfer to the other plant parts by shoot. The changes in fresh shoot and root weights have showed similar trend, both decreasing with the increased salinity-drought stress (Table 1). The linear correlation ($r = 0.973$) between fresh shoot and fresh root weights has been found statistically significant ($P < 0.01$). Maggio et al. (2005) expressed that drought stress causes a considerable decrease in cabbage shoot/root ratio, which also indicates that it is a controversial issue under salinity. Furthermore, Samancıoğlu et al. (2016) suggested that lower irrigation levels decreases the growth parameters of cabbage seedlings.

Considering all plant vegetative parts investigated, our findings showed that the reductions in plant growth based on increased salinity-drought stress were obvious. The relative plant fresh and dry shoot weights were decreased 46.11–48.29% with severe drought stress (S0W2), and decreased 63.05- 62.21% with severe salt stress (S2W0) compared to the control (S0W0), respectively. However, combined effects of these two treatments together (S2W2) caused 78.32-76.39% decrease in fresh and dry shoot weights, respectively. Savvides et al. (2015) expressed that the plant growth was inhibited under high salinity via increasing of osmotic pressure and toxicity of Na and Cl. Therefore, it could be point out that the reason for reduced plant growth in present study may be salinity toxic effects and reduced water uptake due to elevating matrix and osmotic potentials in soil from the salinity and drought. Similarly, our findings showed that the leaf water contents decreased and the leaf Na and Cl contents increased with the increased the salinity and drought stress (Fig. 1 and Table 2). There are many studies reporting the decline in different plant parts under salinity and drought conditions. Jamil et al. (2005, 2007) has determined a significant decrease in plant height, shoot and root fresh weights, leaf area and number of leaves in cabbage plants as the salinity level increases. Maggio et al. (2005) found significant reductions for leaf area and dry matter accumulation in cabbage plants under the salinity and drought conditions. Sanoubar et al. (2016) has indicated that decreased shoot and root fresh weights and leaf area in cabbage plants by the salinity. Sarker et al. (2014) observed significant reductions in cabbage plant height, shoot and root fresh weights at high salinity levels. de Oliveira et al. (2013) suggested that salt stress in plants is more severe than water stress because salt stress occurs from both osmotic stress due to low water potentials and salt-specific effects. Plants simultaneously exposed to drought and salt stress had a more reduced growth compared with controls. Similarly, Manuchehri and Salehi (2014); Álvarez and Sánchez-Blanco (2015) and Tavousi et al. (2015) reported that combined effect of salt and drought conditions have more negative impact on plant growth than their individual effects.

3.2. Leaf gas exchanges and plant physiological and biochemical responses

Irrigation water salinity and quantity noticeably affected the chlorophyll content as the SPAD, leaf relative water content (LRWC), stomatal conductance (g_s), net photosynthetic activity (A_n), intercellular CO_2 content (Ci), and transpiration rate (Tr) (Fig. 1). Decreased SPAD values linearly reduced photosynthetic activity due to high positive correlation between SPAD and A_n ($r = 0.830$). Sim et al. (2015) reported that leaf chlorophyll content is highly correlated with A_n and leaf N content. Similarly, Rostamikia et al. (2016) and Fageria (2014) indicated the expressive relationship between A_n and leaf N content that incorporates contributing the formation of chlorophyll content. Our findings also showed significant ($P < 0.01$) linear positive correlation ($r = 0.905$) between A_n and the leaf N content given in Table 2. Photosynthesis is one of the main complex processes affected by salinity and drought (Chaves et al., 2009). Therefore, lowering photosynthesis with an increase of salinity-drought stress has decreased plant growth (Table 1). The results of this study revealed strong ($P < 0.01$) linear correlations between plant height and A_n ($r = 0.935$) and leaf area and A_n ($r = 0.924$). The increase of water deficit and salinity similarly reduced LRWC, g_s and Tr values. There was a linear ($P < 0.01$) positive correlation between the g_s and LRWC ($r = 0.776$). Chartzoulakis et al. (2002) also expressed that there is a relationship between stomatal

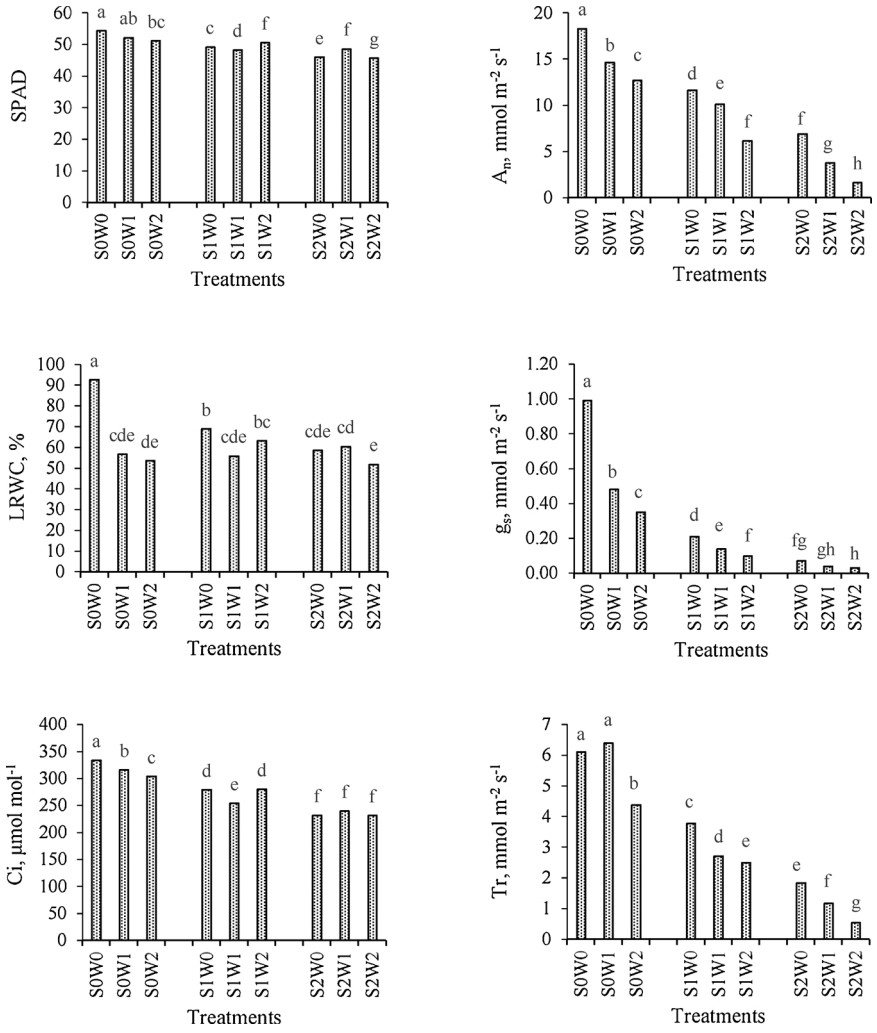

Fig. 1. Chlorophyll content as the SPAD, leaf relative water content (LRWC), stomatal conductance (g_s), net photosynthetic activity (A_n), intercellular CO_2 content (Ci), and transpiration rate (Tr) values in cabbage plants under different salinity-drought levels. The means marked with different lower case in each graph differ meaningfully ($P < 0.001$). S0: tap water with low salinity (0.245 dS m^{-1}); S1: tap water containing extra 75 mM dose of NaCl; S2: tap water containing extra 150 mM dose of NaCl; W0: full-irrigation; W1: irrigation with 80% of the W0; W2: irrigation with 60% of the W0.

function and leaf water content. Sanoubar et al. (2016) found lower values in leaf gas exchange parameters (Tr, g_s and A_n) of cabbage with increased salt content. Ashraf (2004) indicated that high salinity stress might reduce photosynthesis due to stomatal limitation. However, Xu and Leskovar (2014) determined no-significant changes in A_n, g_s and Tr in cabbage during early development under deficit irrigation.

Salt stress reduces CO_2 supply to the leaf and leads to a production of unstable reactive oxygen species that disrupt normal metabolism through oxidative damage (Sanoubar et al., 2016). Our results might indicate that the increased Ci values could create a damage in plant metabolism, causing less growth under salinity and drought stress (Table 1). Chaves et al. (2009) indicated that salinity and drought reduce CO_2 diffusion through the stomata. Similarly, we observed a significant ($P < 0.01$) linear correlation ($r = 0.874$) between the g_s and Ci.

The highest EL, MDA and H_2O_2 values were determined in the S2W2 treatment and the S0W0 treatment had the lowest values (Fig. 2). General trend showed that EL, MDA, H_2O_2 values increased with the increase of the salinity and drought. Positive linear correlations between salinity-drought stress levels and EL ($r = 0.944$), MDA ($r = 0.895$) and H_2O_2 ($r = 0.922$) were found ($P < 0.01$). Khan et al. (2017) expressed that drought stress increased H_2O_2 and EL contents in brassica seedlings. Damage in leaves caused by the drought stress resulted in a leakage of electrolytes from cell membranes (Masoumi et al., 2010). Ekinci et al. (2015) reported that EL increased with drought for spinach. Numerous studies have shown that H_2O_2 is one of the mobile forms of reactive oxygen species under stress conditions and H_2O_2 at lesser contents enhances plant resistance to abiotic stresses (Khan et al., 2017). Das and Uprety (2006) found H_2O_2 and MDA accumulation in brassica species under moisture stress. Yan (2016) found that MDA contents increased as the salinity contents increase. Elevating MDA and EL results in an oxidative damage that troubles the membrane system and reduces photosynthesis and respiration (Bai et al., 2006). Our study findings showed significant ($P < 0.01$) negative linear correlations between A_n and EL ($r = 0.949$) and MDA ($r = 0.879$).

The highest superoxide dismutase (SOD), catalase (CAT) and, peroxidase (POD) activities in cabbage plants were found under the highest salinity and drought conditions (Fig. 3). Although the enzymes activities decreased with an increase of drought in non-saline conditions, they also increased with an increase of drought under saline conditions. Therefore, no-meaningful positive correlations were obtained between salinity-drought stress levels and SOD ($r = 0.324$), CAT ($r = 0.470$), and POD contents ($r = 0.081$). The plants develop antioxidant defense systems to protect themselves against the destructive effects of oxidative stress caused by the drought and salinity. Therefore, higher antioxidant enzyme activities (SOD, CAT, and POD) were observed in

Table 2
Mineral contents in leaves and roots of cabbage under different salinity-drought levels.

Salinity level	Drought level	N %	P mg kg^{-1}	K mg kg^{-1}	Ca mg kg^{-1}	Mg mg kg^{-1}	Na mg kg^{-1}	Cl mg kg^{-1}	B mg kg^{-1}	Fe mg kg^{-1}	Zn mg kg^{-1}	Si mg kg^{-1}	K/Na	Ca/Na
						Leaf								
S0	W0	2.75 a	1634 a	25810 a	13012^{ns}	1366 a	331.7 d	15.4 c	25.9 bcd	57.0 a	16.2 a	11.8 ^{ns}	77.9a	39.2ns
	W1	2.50 c	1442 b	22144 c	10388	1142 cde	303.9 d	15.4 c	23.8 cd	51.6 b	13.6 b	11.0	73.0b	34.2
	W2	2.24 d	1433 b	21239 d	10441	1101 def	316.6 d	16.4 c	23.6 d	48.7 b	13.3 b	10.9	67.1c	33.0
S1	W0	2.58 b	1461 b	23665 b	11972	1220 b	425.2 c	20.7 b	28.1 ab	51.7 b	12.0 c	11.1	55.7d	28.2
	W1	2.20 d	1166 d	20036 e	11693	1131 c-f	519.2 b	18.8 b	27.5 abc	39.6 cd	11.2 cd	10.4	38.6ef	22.5
	W2	2.25 d	1157 d	19280 f	11376	1153 cd	548.5 b	20.5 b	29.2 ab	41.8 c	10.7 d	10.6	35.3fg	20.7
S2	W0	2.22 d	1293 c	20932 d	10391	1159 c	523.8 b	25.1 a	28.1 ab	41.3 c	12.0 c	11.3	40.0e	19.8
	W1	1.95 e	1068 e	17203 g	10721	1078 f	583.5 a	24.4 a	30.6 a	37.9 d	10.7 d	11.1	29.5h	18.4
	W2	1.87 f	1033 e	17055 g	9763	1089 ef	529.3 b	26.3 a	27.9 ab	36.8 d	10.3 d	10.3	32.2 g h	18.4
						Root								
S0	W0	2.75 a	1617 a	25810 a	20191 a	1366 a	331.7 g	15.4 d	48.9 a	257.0 a	56.2 b	27.1 ab	77.9a	60.9a
	W1	1.72 c	986.9 b	15804 d	14797 d	1065 c	551.5 f	21.4 c	38.3 c	238.0 b	63.9 a	25.2 bc	28.7	26.9b
	W2	1.50 d	964.8 bc	15557 de	14849 d	1070 c	548.2 f	22.4 c	38.2 c	185.3 d	54.3 bc	24.5 c	28.4	27.2b
S1	W0	1.78 b	1016 b	17408 b	16968 b	1196 b	1010 e	28.9 ab	44.6 ab	219.5 c	52.8 bcd	27.7 a	17.2c	16.8c
	W1	1.49 d	933.2 cd	15153 e	13793 e	1036 c	1291 c	26.7 ab	42.7 bc	178.5 de	51.4 cd	24.4 c	11.7de	10.7de
	W2	1.49 d	907.5 d	14228 f	13310 ef	1030 c	1359 b	28.9 ab	45.0 ab	180.0 de	53.1 bcd	26.5 abc	10.5e	9.8e
S2	W0	1.52 d	837.5 e	16593 c	15513 c	1025 c	1191 d	26.2 b	44.6 ab	187.7 d	49.5 d	27.2 ab	13.9d	13.0d
	W1	1.27 e	758.3 f	13016 g	12954 f	1059 c	1418 a	29.8 ab	44.6 ab	166.0 ef	52.3 bcd	24.5 c	9.2e	9.1e
	W2	1.28 e	716.8 f	13053 g	12079 g	1052 c	1351 b	30.2 a	40.8 bc	163.1 f	49.8 d	25.6 abc	9.7e	8.9e

The means marked with different lower case in each column differ meaningfully ($P < 0.001$). ns: non-meaningful. Explanations of the abbreviations are as shown in Table 1.

higher levels of the oxidative damage. Some previous studies reported increased antioxidant enzyme (SOD, CAT, POD) actiivities in brassica species under drought or salinity stress (Das and Uprety, 2006; Abedi and Pakniyat, 2010; Yan, 2015). However, our lower plant growth results indicated that the increase of antioxidant enzymes production could not prevent oxidative damage. Similarly, Parida and Das (2005) expressed that deteriorated balance between the production of reactive oxygen species and the antioxidants quenching activity in the plants exposed to the abiotic stress conditions such as drought and salinity is because of the oxidative damage.

In our experiment, the results showed enhanced proline and sucrose contents in plants subjected to salinity-drought stress (Fig. 4). Proline and soluble sugars are the key osmolytes providing osmotic adjustment (Valentovič et al., 2006). In response to water deficit and salinity stress, plants accumulate large quantities of proline and sucrose (Hayat et al., 2012; Krasensky and Jonak, 2012). Accumulation of proline and sucrose in the present study has been well correlated with salinity-drought stress levels ($r = 0.896$ for proline, and $r = 0.861$ for sucrose). Krasensky and Jonak (2012) expressed strong relationship between proline content and stress tolerance. Our results are consistent with the previous studies reporting the increased proline and sucrose contents in response to drought or salinity stress. Khan et al. (2017) determined higher proline contents in drought stressed brassica seedlings. Yan (2015) found increased proline and sugar contents in Chinese cabbage seedlings with increasing of water stress. Yan (2016) showed that increasing salinity increased proline content in Napa cabbage cultivars. Parida and Das (2005) reported that the sucrose and proline accumulate increases under salt stress in many plants.

3.3. Accumulation of minerals in plant leaves and roots

Macro mineral contents (N, P, K, Ca, and Mg) in both leaves and roots were the highest at S0W0 treatment (Table 2). Macro mineral contents decreased with increasing of salinity-drought stress. There were high negative correlations between the salinity-drought stress levels and leaf N, P, K, Ca, and Mg contents, and the correlation coefficients were determined as 0.888, 0.912, 0.877, 0.572, and 0.646, respectively. The significant relations were also found between the stress levels and root N, P, K, Ca, and Mg contents, correlation coefficients were obtained as 0.763, 0.807, 0.715, 0.750, and 0.622, respectively. The water and mineral uptake decrease due to rising matrix and osmotic potential under drought and salinity. Ion homeostasis degraded under the stress conditions (Parida and Das, 2005). Moreover, Maksimovic and Ilin (2012) reported that water stress disturbs nitrogen metabolism in plant tissues and excess salinity disturbs protein synthesis as well. Generally, excess of salts leads to a decline in P content in the tissues of plants. This can be attributed to the activity of ions-antagonists (Kochian, 2000). Chakraborty et al. (2016) found lower N contents in all plant parts in Brassica plants under salinity stress. They discussed that the reduction in N uptake of plants could be due to high

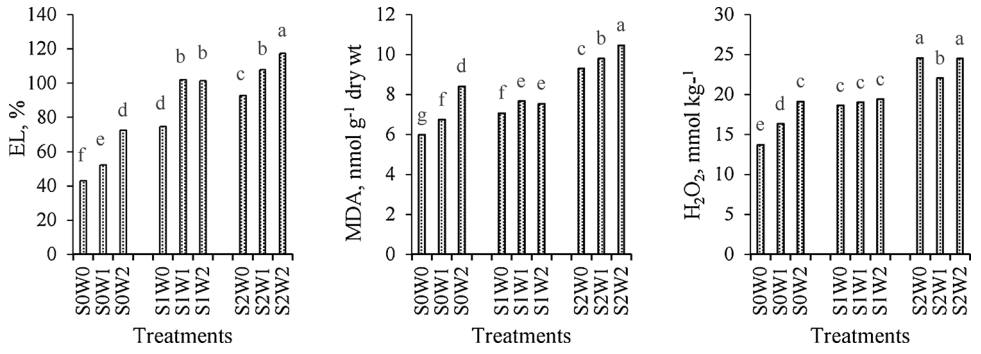

Fig. 2. Electrical leakage (EL), malondialdehyde (MDA), and hydrogen peroxide (H_2O_2) values in cabbage plants under different salinity-drought levels. The means marked with different lower case in each graph differ meaningfully ($P < 0.001$). Explanations of the abbreviations are as shown in Fig. 1.

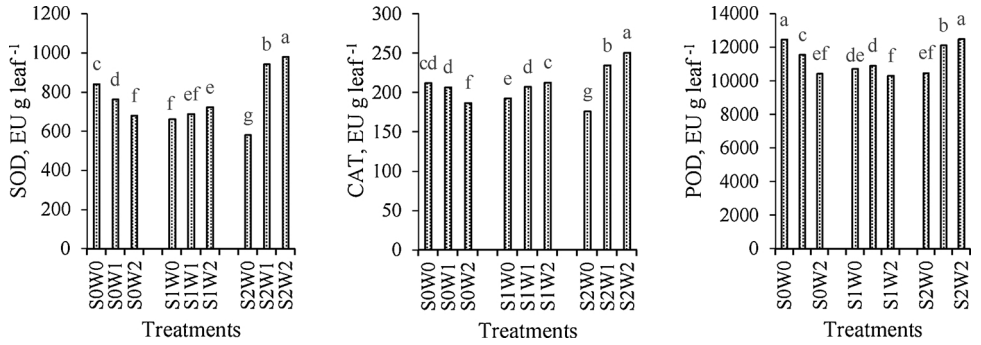

Fig. 3. Superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) activities in cabbage plants under different salinity-drought levels. The means marked with different lower case in each graph differ meaningfully ($P < 0.001$). Explanations of the abbreviations are as shown in Fig. 1.

Cl content under salinity conditions. Similarly, we determined strong linear negative correlations between leaf N and leaf Cl contents ($r = 0.740$), and root N and root Cl contents ($r = 0.832$).

Higher salinity resulted in higher Na and Cl contents in leaves and roots. Conversely, Ca, Mg and K contents decreased with an increase of stress levels. Similarly, Parida and Das (2005) and Maksimovic and Ilin (2012) expressed that the increased content of NaCl causes Na and Cl accumulation in plants and a decline of Ca, Mg and K. Purty et al. (2008) determined that Na content in various genotypes of Brassica increased with salinity stress, whereas K content decreased. As the Na content increases, while the Na uptake increases, K decreases and consequently the Na/K balance is disturbed (Tester and Davenport, 2003). High K/Na and Ca/Na ratios under saline conditions are important selection criteria for salt tolerance in plants (Ashraf, 2004). However, our findings showed that, the ratios of K/Na and Ca/Na in leaves and roots decreased with an increase of salinity-drought stress (Table 2). The negative linear correlation between Na and K contents was determined in leaves ($r = 0.749$) and roots ($r = 0.721$). The linear correlation coefficients for the negative relationship between Ca and Na contents were 0.219 in leaves and 0.734 in roots.

Full irrigation under non-saline conditions caused higher Fe, Zn contents in leaves and roots. The lowest Fe and Zn contents were determined in the highest salinity-drought stress (Table 2). In the drought stress, the B content in leaves was significantly higher in the highest saline conditions than the non-saline conditions. However, full irrigation with fresh water caused higher B accumulation in roots. The highest drought stress under non-saline conditions resulted in lowest B contents in both leaves and roots (Table 2). Although Hu and Schmidhalter (2005) expressed the micronutrients might have lesser importance for providing plant resistance to drought and salinity compared to macronutrients, silicon (Si) provides tolerance against salinity and drought due to its maintaining a high stomatal conductance and transpiration rate (Rios et al., 2017). However, in present study, Si contents in leaf and root were not changed regarding to salinity-drought stress levels. Talei et al. (2012) indicated that the uptake of some micro minerals in the saline conditions may change depending on plant species. Chakraborty et al. (2016) observed a meaningful reduction in Fe and Zn contents in leaf, stem and root in seven Brassica cultivars with increased levels of salinity.

4. Conclusions

Our results indicate that physiological adaptation mechanisms may significantly diverge under the salinity and drought stress. Individual drought and salt stress conditions negatively affected plant growth.However, the combination of drought and salinity magnifies the adverse effects of each stress factor.

The antioxidant enzyme activities and osmotically active substances were increased under the drought and salt stress conditions. Therefore, in this study, we concluded that common mechanisms that contributed to tolerate both salinity and drought stress in the cabbage could be antioxidant activity and osmotic adjustment. However, improvement of antioxidant enzyme activities and osmolyte content in cabbage could not be enough to stimulate plant growth against increase of salinity-drought stress. Decrease of the ratios of Ca/Na and K/Na, stomatal conductance, and photoshentetic activity with an increase of salinity-drought stress showed that cabbage was very sensitive to the combined effect of drought and salinity stress.

As a general suggestion, it could be concluded that the photosynthetic activity would be a more effective selection criterion among the observed parameters under salinity-drought stress due to strong linear correlation between the photosynthetic activity and cabbage plant growth. High level of salinity and drought conditions would seriously jeopardize the cultivation of cabbage. Observed mechanisms definitely require additional research to identify novel strategies in improving cabbage plant growth under salinity-drought stress.

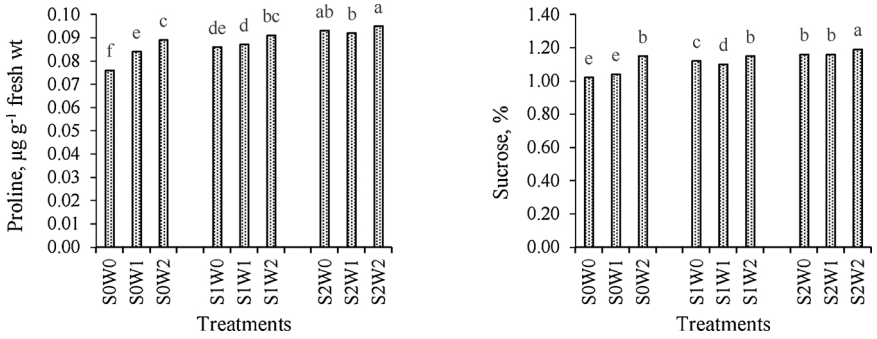

Fig. 4. Proline and sucrose contents in cabbage plants under different salinity-drought levels. The means marked with different lower case in each graph differ meaningfully ($P < 0.001$). Explanations of the abbreviations are as shown in Fig. 1.

Author contributions

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

Conflicts of interest

The authors declare no competing financial interest.

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