



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 (H₂O₂) 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 CO₂ 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 environment. (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 (H₂O₂) (Parida and Das, 2005; Das and Upreti, 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; Samancioğlu 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 $\text{Na}^+/\text{Ca}^{+2}$ 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 Upreti, 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 $25 \pm 5\%$ 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).

$$\text{ET} = \text{IR} - \text{D} \pm \Delta\text{S},$$

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, leaves 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: $\text{LRWC} = [(\text{FW}-\text{DW})/(\text{TW}-\text{DW})] \times 100$

2.6. Photosynthetic activity

Photosynthetic rate (A_n), intercellular CO_2 content (C_i), stomatal conductance (g_s) and transpiration rate (T_r) 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), $1100 \mu\text{mol m}^{-2} \text{ s}^{-1}$; leaf to air vapor deficit pressure, -1.7 to -2.6 kPa; leaf temperature $20\text{--}22^\circ\text{C}$ and chamber CO_2 $400 \mu\text{mol mol}^{-1}$ (Ors et al., 2016).

2.7. Harvest and growth parameters

Forty days after transplanting, five plants from each replicate were

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