

Turkish Journal of Agriculture and Forestry

http://journals.tubitak.gov.tr/agriculture/

Research Article

Turk J Agric For (2022) 46: 28-37 © TÜBİTAK doi:10.3906/tar-2104-81

Biochar mitigates salt stress by regulating nutrient uptake and antioxidant activity, alleviating the oxidative stress and abscisic acid content in cabbage seedlings

Melek EKİNCİ¹, Metin TURAN², Ertan YILDIRIM^{1,*}

¹Department of Horticulture, Faculty of Agriculture, Atatürk University, Erzurum, Turkey ²Department of Genetic and Bioengineering, Faculty of Engineering and Architecture, Yeditepe University, İstanbul, Turkey

Received: 27.04.2021	٠	Accepted/Published Online: 17.11.2021	•	Final Version: 09.02.2022
----------------------	---	---------------------------------------	---	---------------------------

Abstract: A pot experiment was conducted to investigate the growth, physiology, and biochemistry of cabbage seedlings in response to salinity stress with biochar. The study was conducted as factorial completely randomized design with two factors; salinity levels (0 and 150 mM NaCl) and biochar doses (weighed at the rate of 0%, 2.5%, and 5% by soil weight). Growth parameters such as stem diameter, leaf area, shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight of cabbage seedlings treated with 150 mM NaCl were lower than the control. Biochar amendments improved the growth parameters of cabbage seedlings under salt stress and normal conditions compared to the control. Salinity stress conditions induced the increase in malondialdehyde (MDA), hydrogen peroxide (H₂O₂), proline, and sucrose content while biochar lowered the concentration of these parameters. Salinity stress conditions caused decrease in the plant nutrient element content of leaf and root of cabbage seedlings except for Na and Cl while amendment of biochar into soil media enhanced the plant nutrient element content of leaf and root of cabbage seedlings. Salinity increased the Na and Cl content, whereas biochar decreased Na and Cl content of cabbage seedlings. In conclusion, biochar mitigated the negative impact of salinity stress on cabbage seedlings by reduction of Cl and Na concentration, and reactive oxygen species (ROS) production, regulating abscisic acid (ABA) content, antioxidant enzyme activity and plant nutrient element content.

Key words: Biochar, biochemical characteristics, cabbage, salinity stress

1. Introduction

Salinity is a major problem in arid and semiarid regions where rainfall is not sufficient and available water is insufficient (Rao et al., 2006). It causes important problems especially in agriculture today, and if precautions are not taken, it will be one of the biggest problems in the production of agricultural products in the future. Twentythree percent of 15 million ha cultivated land is noted as saline (Dajic, 2006).

Salinity stress firstly affects the root system, causing osmotic stress and decreasing water presence. It then causes ion toxicity due to nutrient imbalances in the cytosol in the long term (Acosta-Matos et al., 2017). Salinity inhibits shoot development and growth via disrupting photosynthesis, decreasing turgor of tissues and osmoregulation, downregulating of shoot growth, and defecting mineral supply of the plant (Dajic, 2006). Plant growth is affected by cellular responses to the osmotic effects and growth is decreased because of the toxic effects of accumulated salts under salinity (Zhu, 2007). Plants develop a tolerance mechanism to salinity with many morphological, physiological, and

adaptation of plants to salt stress. While focusing on the identification and breeding of varieties that are tolerant to salt stress, researchers also focus on different external applications against abiotic stresses such as salt stress. One of these applications is to protect the plants from salt stress damage by treating with soil conditioner such as biochar which ameliorates effects of salt stress on plant. Biochar, a carbon-rich material developed as a result of burning the material without oxygen or with a limited amount of oxygen, reduces the negative effect of salt stress on plants. Biochar improves biomass and yield, and increase nutrient intake, photosynthesis and gas exchange properties of saltstressed plants (Ali et al., 2017). Furthermore, biochar improves physical, biochemical and biological properties of soil. And it also reduces Na uptake and increased K uptake under abiotic stress conditions (Chaganti and Crohn, 2015). Alleviation of salt stress damage with biochar has been reported in vegetable species such as bean (Farhangi-Abriz and Torabian, 2018a), potato (Akthar et al., 2015a),

biochemical changes (Acosta-Motos et al., 2017). Also,

there is a complex network of different mechanisms in the



^{*} Correspondence: ertanyil@atauni.edu.tr

soybean (Farhangi-Abriz and Torabian, 2018b), and tomato (Usman et al., 2016; She et al., 2018) under salt stress. White cabbage (Brassica oleracea var. capitata L.) is a relatively salt-tolerant crop (Sahin et al., 2018). Earlier studies reported that salinity stress conditions negatively influenced the growth of cabbage (Magio et al., 2005; Sahin et al., 2018). According to our best knowledge, there was no research focused on the influence of biochar treatments on the growth, physiological, and biochemical properties in cabbage seedlings under salinity stress conditions. Thus, this study focused to evaluate the potential of biochar in enhancing the physiological and biochemical attributes of salinity tolerance in cabbage. In this study, the effects of biochar applied to the soil on cabbage seedlings under salt stress were examined in terms of morphological, physiological, and biochemical properties.

2. Materials and methods

The study was conducted as a pot experiment under controlled greenhouse conditions (average temperature 30 °C and humidity was 60%). Cabbage (*Brassica olerecae* var. capitata cv. Yalova1) seeds were sown in multicelled trays with peat and seedlings were transplanted into 2-L pots after 30 days.

The study was conducted as factorial completely randomized design with two factors; salinity levels (0 and 150 mM NaCl) and biochar doses (weighed at the rate of 0%, 2.5%, and 5% by soil weight). There were three replications and 6 pots for each repeat. The soil used in study was collected from the Ap horizon (0–30 cm) of uncultivated field. The collected soil samples were airdried for 1 week and then sieved through a 2 mm mesh to exclude the large debris and gravel. The physical and chemical properties of the examined soil samples are shown in Table 1.

2.1. Biochar application

Three biochar doses as 0% control (B0), 2.5% (B1), and 5% (B2) were applied by weight. For control treatment, only soil was filled in the pots. The biochar used in the study was produced by the Synpet (Synpet Technologies, İstanbul, Turkey and New York, USA) using the thermal conversion process (TDP). Information on this process and the content of biochar has been summarized in three stages in the following. 1st stage reactor (Depolymerization): 60% sewage sludge and 40% domestic wastes coming from the feed and mixing tank to the 1st stage reactor were kept at 150 °C under 5-8 bar pressure to separate inorganic and organic polymers. 2nd stage reactor (hydrolysis): by adding some more water to the aqueous stock material coming to this stage, using the reactive feature of water at high temperature (250 °C) and pressure (50 bar), solid and liquid phases are separated. The solid phase, which water was removed by filtration, was concentrated in the evaporator. 3rd Stage reactor (cracker): The concenter solid intermediate transported to this reactor broken down into short hydrocarbon chains at high temperature (550 °C), from which energy products (renewable natural gas and renewable crude oil) and biochar were obtained. Chemical properties of the biochar are presented in Table 2.

2.2. Salinity applications

Two salinity levels treatments (0 mM NaCl: S0 and 150 mM NaCl: S1) were used in the study. The salinity treatments were established by adding 0 and 150 mM of NaCl to irrigation water and their electrical conductivities were 0.61 and 10.21 dS m^{-1} , respectively. Salinity treatments were initiated 7 days after seedling transplanting with an increase of 25 mM NaCl to avoid an osmotic shock for plants. Plants were irrigated with a half-strength Hoagland solution at 10-day intervals. At the end of the study, EC values were measured of soils treated with different

Table 1. Physical and chemical properties of the starting soils.

Properties	Value	Properties	Value
Sand (%)	33.40	P (mg kg ⁻¹ dw)	3.20
Silt (%)	29.70	K (cmolc kg ⁻¹ dw)	2.55
Clay (%)	36.90	Ca (cmolc kg ⁻¹ dw)	14.40
pH (1:2,5 w/s)	7.30	Mg (cmolc kg ⁻¹ dw)	1.28
EC (micromhos cm ⁻¹)	115.00	Na (cmolc kg ⁻¹ dw)	0.20
CaCO ₃ (%)	2.80	B (mg kg ⁻¹ dw)	0.07
Organic matter (%)	0.96	Cu (mg kg ⁻¹ dw)	0.80
$NH_4 - N (mg kg^{-1} dw)$	1.70	Fe (mg kg ⁻¹ dw)	6.54
NO_3 -N (mg kg ⁻¹ dw)	0.89	$Zn (mg kg^{-1} dw)$	0.17
		Mn (mg kg ⁻¹ dw)	0.30

Properties	Analysis results	Properties	Analysis results
pН	7.8	Cu (mg kg ⁻¹)	393
EC (dS m ⁻¹)	0.38	Ni (mg kg ⁻¹)	310
Total (humic + fulvic) (%)	4.9	Zn (mg kg ⁻¹)	1187
Organic Nitrogen (%)	1.6	Cr (mg kg ⁻¹)	449
C (%)	21.54	Mn (mg kg ⁻¹)	549
H (%)	1.26	K (mg kg ⁻¹)	10290
N (%)	1.38	P (mg kg ⁻¹)	22980
O (%)	2.1	Mg (mg kg ⁻¹)	7372
Pb (mg kg ⁻¹)	162	Ca (mg kg ⁻¹)	57500
Cd (mg kg ⁻¹)	10	Fe (mg kg ⁻¹)	25680

Table 2. Chemical characteristics of biochar produced from urban wastes.

salinity levels. Under 150 mM NaCl, average EC values of 0%, 2.5% and 5% biochar treatments were 5.76 ± 0.34 , 4.48 \pm 0.63, and 4.9 \pm 0.24, respectively. Biochar treatments significantly reduced EC values of soil compared to the control.

2.3. Harvest, growth properties, measurements

In the study, morphological parameters such as stem diameter, plant height, leaf area, shoot fresh and dry weight, root fresh and dry weight of the cabbage seedlings were measured after 50 days after planting. Plant height was determined by measuring the part from the soil level to the growth tip of the plant. The total leaf area of a plant was determined by the leaf area meter (CI-202 Portable Laser Leaf Area Meter by CID Bio-Science, USA). The fresh weights of the above-ground parts (shoot) and roots of the harvested plants were determined, and then they were dried at 67 °C until they reached a constant weight and their dry weight was determined by weighing on a precision scale. Dried shoot and root samples were ground and used for mineral analysis and mineral analysis was determined as per the Mertens (2005a; 2005b) method. Hydrogen peroxide (H₂O₂), malondialdehyde (MDA), proline, sucrose, and antioxidant enzyme (catalase (CAT) and superoxide dismutase (SOD)) analyses were performed on fresh leaf samples according to Shams et al. (2019). Leaf relative water content (LRWC) was determined according to Ors et al. (2021). To determine chlorophyll a, chlorophyll b, and total chlorophyll content of plants were measured at 663 and 645 nm by a microplate spectrophotometer. For ABA analysis, extraction and purification processes were executed as described by Kuraishi et al. (1991) and the ABA was analyzed by HPLC using a Zorbax Eclipse-AAA C-18 column (Agilent 1200 HPLC) and absorbance of 265 nm in UV detector (Ekinci et al., 2014).

The data obtained from the study arranged according to the factorial completely randomized design was analyzed using the SPSS program. Data were subjected to variance analysis, and differences of means were determined by Duncan multiple comparison test.

3. Results

3.1. Growth parameters

The overall results obtained from the present study indicate that application of the biochar leads to recovery of the plants from the salt stress as shown in Tables 3–7; Figures 1.

Growth parameters such as stem diameter, leaf area, shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight of cabbage seedlings treated with 150 mM NaCl were significantly lower than that of the control. However, biochar improved the stressed parameters of cabbage seedlings. Specifically, the fresh and dry weight of roots treated with 2.5% biochar was more than 64% and 200% compared to the control, while those of shoot fresh and dry weight was more than 412% and 373% compared with the control. Moreover, biochar amendments improved the growth parameters of cabbage seedlings under nonsaline conditions compared to the control. Our findings represent that significant interactions between salinity and biochar were observed on all growth parameters investigated (Table 3 and 4).

3.2. Chlorophyll content and LRWC

Salinity stress (150 mM NaCl) in cabbage seedlings led to a significant decline in LRWC, chlorophyll a, chlorophyll b, and total chlorophyll up to 40%, 22%, 26%, and 24%, respectively, as compared to the nonsaline control condition. Both doses of biochar elevated these parameters up to the values from the nonsaline control condition. Interactions between salinity and biochar were significant for LRWC, chlorophyll a, chlorophyll b, and total chlorophyll (Table 5).

Salt (mM NaCl)	Biochar (% based on soil weight)	Stem diameter (mm)	Plant height (cm)	Leaf area (cm²/plant)
	0	2.03 ± 0.05e	7.84 ± 0.11c	$107.35 \pm 8.00e$
0	2.5	3.28 ± 0.18 a	15.51 ± 1.92a	308.90 ± 1.59b
0	5	2.99 ± 0.06 b	$14.30 \pm 0.50a$	387.86 ± 15.80a
	0	$1.77 \pm 0.04 \text{ f}$	6.18 ± 0.17d	85.01 ± 3.87f
150	2.5	2.19 ± 0.02 d	9.74 ± 0.82b	$224.18 \pm 14.87c$
150	5	2.44 ± 0.10 c	8.55 ± 0.11bc	206.41 ± 4.27d
S		***	***	***
В		***	***	***
SXB		***	**	***

Table 3. The effect of biochar on stem diameter, plant height, and leaf area of cabbage seedlings under salinity conditions.

Means followed by a different letter are significantly different according to Duncan's multiple range test. ***: $p \le 0.001$, **: $p \le 0.01$, S: salt, B: biochar

Table 4. The effect of biochar on shoot fresh	and dry weight; root fresh and dry	y weight of cabbage seedlin	gs under salinity conditions.

Salt (mM NaCl)	Biochar (% based on soil weight)	Shoot fresh weight (g/plant)	Shoot dry weight (g/plant)	Root fresh weight (g/plant)	Root dry weight (g/plant)
	0	$1.40 \pm 0.03d$	$0.22 \pm 0.01e$	0.51 ± 0.04de	$0.06 \pm 0.003 d$
0	2.5	12.94 ± 0.60b	1.27 ± 0.05a	1.59 ± 0.06a	0.17 ± 0.004 a
0	5	14.06 ± 0.78a	$1.16 \pm 0.07b$	1.30 ± 0.20b	0.14 ± 0.026b
	0	1.23 ± 0.05d	$0.15 \pm 0.01 f$	$0.42 \pm 0.01e$	$0.03 \pm 0.002e$
150	2.5	6.30 ± 0.31c	$0.71 \pm 0.02c$	$0.69 \pm 0.06c$	$0.09 \pm 0.011c$
150	5	5.86 ± 0.05c	0.64 ± 0.04 d	0.59 ± 0.03cd	0.07 ± 0.006cd
S		***	***	***	***
В		***	***	***	*
SXB		***	***	***	**

Means followed by a different letter are significantly different according to Duncan's multiple range test. ***: $p \le 0.001$, **: $p \le 0.01$, *: $p \le 0.05$, S: salt, B: biochar

3.3. Sucrose, proline, H₂O₂, and MDA

A considerable increase in sucrose, proline, H_2O_2 , and MDA content of 43%, 31%, 15%, and 11% was observed under 150 mM NaCl as compared to normal conditions. A significant interaction effect of salinity x biochar was recorded on sucrose, proline, H_2O_2 , and MDA; however, exogenous biochar treatment significantly lowered sucrose, proline, H_2O_2 , and MDA content by 33%, 14%, 49%, and 18% in cabbage seedlings as compared to the control under saline conditions. Five percent biochar was the most effective in lowering sucrose, H_2O_2 , and MDA content (Table 6).

3.4. CAT and SOD activity

Salinity stress conditions significantly elevated the activity of antioxidant enzymes. NaCl-stressed plants had more

CAT and SOD activity up to 18% and 30% compared to the control. Supplemental 2.5% biochar was found to be effective in decreasing CAT and SOD under salinity stress. Five percent biochar amendment further decreased the SOD activity at 150 mM NaCl. A significant interaction effect of salinity x biochar was recorded on CAT and SOD activity (Table 7).

3.5. ABA content

ABA content of cabbage seedlings significantly increased with salinity stress. However, biochar amendment into soil declined the content ABA content of cabbage seedlings by a ratio of 38% under salinity stress conditions. Furthermore, biochar treatments also decreased the ABA content of cabbage seedlings under normal conditions compared to the control (Table 7).

Salt (mM NaCl)	Biochar (% based on soil weight)	LRWC (%)	Chlo-a (mg/g)	Chlo-b (mg/g)	Chlo-T (mg/g)
	0	65.02 ± 0.81a	$0.32 \pm 0.01b$	$0.19 \pm 0.02b$	$0.51 \pm 0.03b$
0	2.5	63.47 ± 0.94a	0.60 ± 0.01a	$0.32 \pm 0.03a$	0.92 ± 0.02a
0	5	64.87 ± 3.20a	0.61 ± 0.02a	$0.32 \pm 0.02a$	0.92 ± 0.01a
	0	38.74 ± 1.41c	$0.25 \pm 0.01b$	$0.14 \pm 0.02c$	0.39 ± 0.01c
150	2.5	47.28 ± 1.71b	0.60 ± 0.01a	0.31 ± 0.02a	0.92 ± 0.01a
150	5	45.72 ± 3.42b	$0.62 \pm 0.03a$	0.32 ± 0.01a	0.93 ± 0.02a
S		***	***	*	***
В		*	***	***	***
SXB		**	***	*	***

Table 5. The effect of biochar on LRWC, chlorophyll a, chlorophyll b, and chlorophyll total of cabbage seedlings undersalinity conditions.

Means followed by a different letter are significantly different according to Duncan's multiple range test. ***: $p \le 0.001$, *: $p \le 0.05$, S: salt, B: biochar

Salt (mM NaCl)	Biochar (% based on soil weight)	H ₂ O ₂ (mmol kg ⁻¹)	MDA (mmol kg ⁻¹)	Proline (mmol kg ⁻¹)	Sucrose (%)
	0	6.03 ± 0.82 b	419.26 ± 13.46 b	0.016 ± 0.0003 c	0.21 ± 0.005 d
0	2.5	6.26 ± 0.18 b	337.73 ± 32.97 d	0.014 ± 0.0002 d	0.22 ± 0.012 cd
0	5	4.39 ± 0.19 c	396.48 ± 17.05 bc	0.017 ± 0.0005 bc	0.24 ± 0.013 c
	0	6.95 ± 0.14 a	466.08 ± 7.80 a	0.021 ± 0.0012 a	0.30 ± 0.018 a
150	2.5	4.57 ± 0.06 c	424.56 ± 6.58 b	0.018 ± 0.0005 b	0.20 ± 0.013 d
150	5	3.53 ± 0.32 d	381.79 ± 14.61 c	0.018 ± 0.0007 b	0.27 ± 0.009 b
S		***	***	***	***
В		***	***	***	***
SXB		***	***	***	***

Table 6. The effect of biochar on H₂O₂, MDA, proline, and sucrose content of cabbage seedling under salinity conditions.

Means followed by a different letter are significantly different according to Duncan's multiple range test. ***: $p \le 0.001$, S: salt, B: biochar

3.6. Leaf and root mineral content

Figures 2 and 3 reveal that leaf and root mineral concentrations of cabbage seedlings were influenced by biochar and salinity treatments. Na and Cl concentrations of leaf and root increased, while the other elements' concentrations of leaf and root decreased at 150 mM NaCl compared to nonsaline conditions. Biochar amendment declined Na and Cl contents but elevated the other elements' conditions. Generally, 5% biochar amendment was found to be more effective in increasing plant nutrient element content under saline conditions.

4. Discussion

Salinity is an important problem that causes a serious decrease in crop production. Soil salinity negatively affects the growth, yield, quality, and productivity of many crops. As a matter of fact, it was shown in our study that salinity stress negatively influenced the growth of cabbage seedlings (Tables 3–7; Figure 1). The prohibitory effects of salinity on cabbage seedlings were previously reported by Sahin et al. (2018). This inhibition in growth due to salinity stress could be attributed to reduce water availability, nutrient disorders, and Na toxicity. The findings of the study indicated that salinity stress caused

EKİNCİ et al. / Turk J Med Sci

Salt (mM NaCl)	Biochar (% based on soil weight)	CAT (EU gr leaf ⁻¹)	SOD (EU gr leaf ⁻¹)	ABA (ng/gDW)
	0	36.86 ± 2.29 b	134.65 ± 1.38 d	424.08 ± 3.92 b
0	2.5	35.33 ± 1.45 b	117.74 ± 2.95 e	305.23 ± 29.34 d
0	5	37.35 ± 1.31 b	131.37 ± 3.29 d	313.37 ± 16.83 d
	0	43.43 ± 2.37 a	175.48 ± 3.05 a	586.64 ± 13.48 a
150	2.5	37.98 ± 0.32 b	160.07 ± 5.12 b	365.50 ± 6.61 c
150	5	43.59 ± 2.24 a	145.55 ± 2.52 c	367.51 ± 11.56 c
S	·	***	***	***
В		**	***	***
SXB		*	***	***

Table 7. The effect of biochar on CAT, SOD, and ABA of cabbage seedlings under salinity conditions.

Means followed by a different letter are significantly different according to Duncan's multiple range test. ***: $p \le 0.001$, **: $p \le 0.01$, *: $p \le 0.05$, S: salt, B: biochar

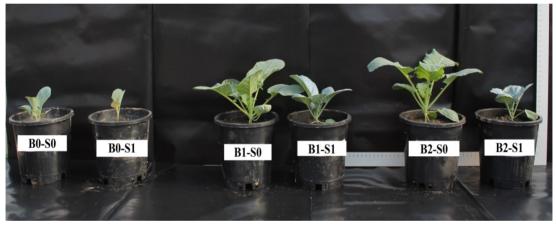


Figure 1. The effect of biochar on cabbage seedling under salt stress. S0: 0 mM NaCl, S1: 150 mM NaCl, B0: 0% biochar (based on soil weight), B1: 2.5% biochar (based on soil weight), B2: 5 % biochar (based on soil weight).

significant decreases in chlorophyll a, chlorophyll b, and total chlorophyll, which are compatible with those of Sahin et al. (2018). The devaluation of the chlorophyll content under salinity stress could be attributed to the inhibition of chlorophyll synthesis and its degradation due to oxidative stress (Santos, 2004). Salt stressed plants had less LRWC value than those of the control plants. This situation could be attributed to the osmotic stress originated by NaCl (Füzy et al., 2008).

Our findings pointed out that salinity stress conditions induced oxidative stress in cabbage seedlings as indicated in MDA and H_2O_2 levels (Table 6). Salinity stress conditions results in the accumulation of ROS like H_2O_2 (Yan et al., 2018). ROS damage nucleic acids and proteins, and cause lipid peroxidation. Lipid peroxidation is considered to be the most harmful process known in all living organisms. Lipid peroxidation occurs when ROS levels above the threshold are reached (Montillet et al., 2005). Proline and sucrose contents of salt stressed cabbage seedlings were greater than those of nonstressed cabbage seedlings. Osmotic regulation is one of the most important mechanisms by which plants tolerate salinity stress by maintaining cell water content and turgor (Sahin et al., 2018). Proline is considered to act as an osmoprotectant, an inhibitor of lipid peroxidation and ROS scavenger (Trovato et al., 2008).

Salinity tolerance can be attributed to enhancing antioxidant enzyme activity, thus decreasing oxidative damage. In the study, salinity stress conditions enhanced SOD and CAT activity (Table 7). SOD and CAT have been shown to be the defense enzymes, scavenging of O2⁻ radicals into H_2O_2 which is further detoxified to water (Mittler, 2002). The findings of the study show that salinity stress conditions elevated the ABA content of the cabbage seedlings, which is an important stress response hormone (Table 7). ABA plays a crucial role in the plants'

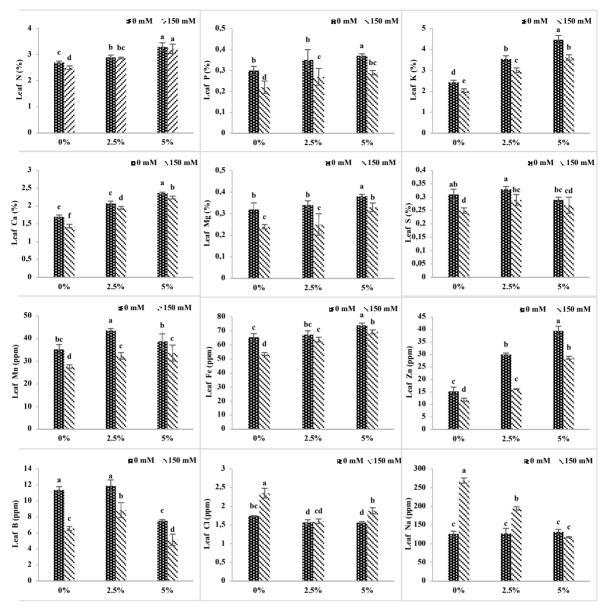


Figure 2. The effect of biochar on leaf mineral content of cabbage seedlings under salinity conditions. Means followed by a different letter are significantly different according to Duncan's multiple range test ($p \le 0.001$).

tolerance to salt. Salinity stress conditions enhance ABA accumulation, activating sucrose nonfermenting 1-related protein kinases (Yu et al., 2020).

Evelin et al. (2019) suggested that the negative impacts of salinity stress on plant growth can be attributed to nutrient element imbalances. In the present study, we have concluded that contents of N, P, K, Ca, Mg, Fe, etc. in leaf and root of cabbage seedlings were lower at 150 mM NaCl than those of the control conditions. Conversely, salt-stressed plants had more Na and Cl contents (Figures 2 and 3). This situation led to the ionic imbalance and spoiled photosynthetic activity and other metabolic processes of the plant (Ramoliya et al., 2004). Salinity stress conditions have been reported to enhance Na and Cl contents of the root and leaves in plants, reducing plant nutrient elements such as K and NO₃ uptake (Evelin et al., 2019).

Biochar is a pyrolysis product derived from organic materials and is used to increase plant growth and development. Biochar is produced for the purpose of improving carbon sequestration and soil properties (Lehmann and Joseph, 2009). Biochar, which is exactly stable in soil, could reduce salinity stress impacts (Thomas et al., 2013). Akhtar et al. (2015a) suggested that biochar increases plant growth and photosynthetic activity of crops under salinity stress. In the present study, biochar

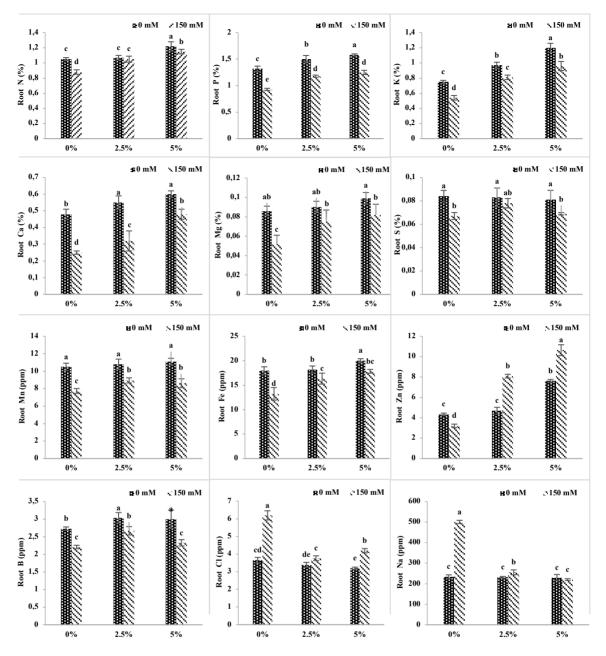


Figure 3. The effect of biochar on root mineral content of cabbage seedlings under salinity conditions. Means followed by a different letter are significantly different according to Duncan's multiple range test ($p \le 0.001$).

amendment into soil positively influenced growth parameters of cabbage seedlings (Figure 1; Tables 3 and 4). Biochar amendment can ameliorate salinity stress by improving soil physical, chemical and biological characteristics (Sun et al. 2016; Kim et al. 2016). Luo et al. (2017) reported that biochar addition to saline soil enhanced organic matter and cation exchange capacity (CEC) in the soil. In another study, Usman et al. (2016) showed that biochar improved growth and yield of tomato plants under salinity stress. Biochar reduced CAT and SOD activity, proline, sucrose, H_2O_2 , and MDA contents in leaf of cabbage seedlings under salinity stress conditions (Tables 6 and 7). Organic amendments can mitigate deleterious impacts of salinity stress by modulating the antioxidant enzyme activity in crops (Tartoura et al., 2014). In fact, Kim et al. (2016) showed that biochar declined ascorbate peroxidase (APX) and glutathione reductase (GR) activities of *Zea maize* under salinity conditions. Similarly, Farhangi-Abriz and Torabian (2017) showed that biochar treatments lowered antioxidant enzyme activities and oxidative stress in salt stressed bean plants. They also suggested that beneficial effects of biochar can be due to decreased MDA and H₂O₂ content.

Biochar amendment decreased the contents of ABA under salinity stress conditions (Table 7). In the present experiment, biochar enhanced shoot and root growth and decreased Na concentrations of cabbage seedlings compared to the nonbiochar treatment. Incorporation of biochar into salt-affected soil could mitigate salinity stress in cabbage seedlings by decreasing Na uptake, thus alleviating salinity stress. This can lead to decrease the ABA content of cabbage seedlings under salt stress. Similarly, Farhangi-Abriz and Torabian (2018a) pointed out that biochar alleviated the negative effects of salt stress on bean seedlings by reducing Na concentration and ABA content.

In a study conducted by Wang and Xu (2013), it was shown that while the soil pH increased in pots with adding biochar, the soil salt content decreased depending on the biochar dose. Akhtar et al. (2015b) found that biochar amendment enhanced the tolerance of plants to salinity stress and alleviated the negative effects of salt in their study. Researchers found that biochar reduced Na intake of plants due to its high adsorption capacity, osmotic stress has decreased due to the increase in water content of soils, and released plant nutrients such as K, Ca, and Mg in soil solution. Our findings indicated that biochar amendment decreased Na and Cl contents of leaf and root of cabbage seedlings under salinity stress conditions

References

- Acosta-Motos JR, Ortuño MF, Bernal-Vicente A, Diaz-Vivancos P, Sanchez-Blanco MJ et al. (2017). Plant responses to salt stress: Adaptive mechanisms. Agronomy 7: 18. doi: 10.3390/ agronomy7010018
- Akhtar SS, Andersen MN, Liu F (2015a). Biochar mitigates salinity stress in potato. Journal of Agronomy and Crop Science 201: 368-378.
- Akhtar SS, Andersen MN, Liu F (2015b) Residual effects of biochar on improving growth, physiology and yield of wheat under salt stress. Agricultural Water Management 158: 61-68.
- Ali S, Rizwan M, Qayyum MF, Ok YS, Ibrahim M et al. (2017). Biochar soil amendment on alleviation of drought and salt stress in plants: A critical review. Environmental Science and Pollution Research 24 (14): 12700-12712.
- Chaganti VN, Crohn DM (2015). Evaluating the relative contribution of physiochemical and biological factors in ameliorating a saline-sodic soil amended with composts and biochar and leached with reclaimed water. Geoderma 259: 45-55.

(Figures 2 and 3). Similarly, Usman et al. (2016) indicated that biochar enhanced P, K, Fe, Mn, Zn, and Cu contents in salt-stressed tomato plants. Earlier studies reported that biochar amendments decreased Na content and increased the contents of N, K, P, Ca, Mg, etc. of several crops under salinity stress conditions (Lashari et al., 2015; Akhtar et al., 2015b; Hammer et al., 2015; Kim et al., 2016). The authors indicated that biochar could be influential in reducing Na by increasing the other plant nutrient elements in salinity conditions. Moradi et al. (2019) suggested that biochar treatments lowered Na concentration of the soil under salinity conditions due to the high biochar potential for sodium absorption.

5. Conclusion

Our results revealed that salinity increased proline, sucrose, H_2O_2 , MDA, and Na contents, and the activity of CAT and SOD, and ABA contents; whereas it decreased plant growth, chlorophyll content, LRWC, and plant nutrient content in cabbage seedlings. Biochar could contribute to protecting cabbage seedlings against NaCl stress by alleviating the oxidative stress, ABA content, and Na uptake. Therefore, biochar can be used in salt-affected soils to enhance growth of cabbage seedlings. Especially, biochar can have the potential to be widely used in crop production.

Acknowledgments

We are very grateful to Synpet (Synpet Technologies, İstanbul, Turkey and New York USA) for biochar.

- Dajic Z (2006). Salt stress. In: Rao KVM, Raghavendra AS, Reddy KJ (editors). Physiology and Molecular Biology of Stress Tolerance in Plants. Springer, pp.41-100.
- Ekinci M, Turan M, Yildirim E, Güneş A, Kotan R et al. (2014). Effect of plant growth promoting rhizobacteria on growth, nutrient, organic acid, amino acid and hormone content of cauliflower (*Brassica oleracea* L. var. botrytis) transplants. Acta Scientiarum Polonorum Hortorum Cultus 13 (6): 71-85.
- Evelin H, Devi TS, Gupta S, Kapoor R (2019). Mitigation of salinity stress in plants by arbuscular mycorrhizal symbiosis: current understanding and new challenges. Frontiers in Plant Science 10: 470.
- Farhangi-Abriz S, Torabian S (2017). Antioxidant enzyme and osmotic adjustment changes in bean seedlings as affected by biochar under salt stress. Ecotoxicology and Environmental Safety 137: 64-70.
- Farhangi-Abriz S, Torabian S (2018a). Biochar increased plant growth-promoting hormones and helped to alleviates salt stress in common bean seedlings. Journal of Plant Growth Regulation 37 (2): 591-601.

- Farhangi-Abriz S, Torabian S (2018b). Biochar improved nodulation and nitrogen metabolism of soybean under salt stress. Symbiosis 74 (3): 215-223.
- Füzy A, Tóth T, Biró B (2008). Soil-plant factors, others than the type of salt-specific anions are affecting the mycorrhiza colonisation of some halophytes. Community Ecology 9 (1): 125-130.
- Hammer EC, Forstreuter M, Rillig MC, Kohler J (2015). Biochar increases arbuscular mycorrhizal plant growth enhancement and ame- liorates salinity stress. Applied Soil Ecology 96: 114-121.
- Kim HS, Kim KR, Yang JE, Ok YS, Owens G et al. (2016) Effect of biochar on reclaimed tidal land soil properties and maize (*Zea mays* L.) response. Chemosphere 142: 153-159.
- Kuraishi S, Sakurai N, Eun JS, Sugiyama K (1991). Effect of brassinolide on levels of indoleacetic acid and abscisic acid in squash hypocotyls: possible application for preventing fruit abortion. In: Cutler HG, Yokota T, Adam G (editors). Brassinosteroids. Chapter 28: 312-319. doi: 10.1021/bk-1991-0474.ch028
- Lashari MS, Ye Y, Ji H, Li L, Kibue GW et al. (2015). Biochar-manure compost in conjunction with pyroligneous solution alleviated salt stress and improved leaf bioactivity of maize in a saline soil from central China: a 2-year field experiment. Journal of the Science of Food and Agriculture 95: 1321-1327.
- Lehmann J, Joseph S (2009). Biochar systems. Biochar for environmental management. Science and Technology 147-168.
- Lou Z, Sun Y, Bian S, Baig SA, Hu B et al. (2017). Nutrient conservation during spent mushroom compost application using spent mushroom substrate derived biochar. Chemosphere 169: 23-31.
- Maggio A, De Pascale S, Ruggiero C, Barbieri G (2005). Physiological response of field-grown cabbage to salinity and drought stress. The European Journal of Agronomy 23: 57-67.
- Mertens D (2005a). Plants preparation of laboratory sample. In: Horwitz W, Gw L (editors) Official methods of analysis. 18th ed. Gaithersburg (MD): AOAC, pp. 1-2.
- Mertens D (2005b). Metal in plants and pet foods. In: Horwitz W, Gw L (editors) Official Methods of Analysis. 18th ed. Gaithersburg (MD): AOAC, pp. 3-4.
- Mittler R (2002). Oxidative stress, antioxidants and stress tolerance. Trends in Plant Science 7 (9): 405-410.
- Montillet JL, Chamnongpol S, Rustérucci, C, Dat J, Van De Cotte B et al. (2005). Fatty acid hydroperoxides and H₂O₂ in the execution of hypersensitive cell death in tobacco leaves. Plant Physiology 138 (3): 1516-1526.
- Moradi S, Rasouli-Sadaghiani MH, Sepehr E, Khodaverdiloo H, Barin,M (2019). Soil nutrients status affected by simple and enriched biochar application under salinity conditions. Environmental Monitoring and Assessment 191 (4): 1-13.
- Ors S, Ekinci M, Yildirim E, Sahin U, Turan M et al. (2021). Interactive effects of salt and drought stress on photosynthetic characteristics and physiology of tomato (*Lycopersicon esculentum* L.) seedlings. South African Journal of Botany 137: 335-339.

- Ramoliya PJ, Patel HM, Pandey AN (2004). Effect of salinization of soil on growth and macro-and micro-nutrient accumulation in seedlings of *Salvadora persica* (Salvadoraceae). Forest Ecology and Management, 202 (1-3): 181-193.
- Rao KVM, Raghavendra AS, Reddy KJ (2006). Physiology and Molecular Biology of Stress Tolerance in Plants. Springer.
- Sahin U, Ekinci M, Ors S, Turan M, Yildiz S et al. (2018). Effects of individual and combined effects of salinity and drought on physiological, nutritional and biochemical properties of cabbage (*Brassica oleracea* var. capitata). Scientia Horticulturae, 240: 196-204.
- Santos CV (2004). Regulation of chlorophyll biosynthesis and degradation by salt stress in sunflower leaves. Scientia Horticulturae 103 (1): 93-99.
- Shams M, Ekinci M, Ors, S, Turan M, Agar G et al. (2019). Nitric oxide mitigates salt stress effects of pepper seedlings by altering nutrient uptake, enzyme activity and osmolyte accumulation. Physiology and Molecular Biology of Plants 25: 1149-1161. doi: 10.1007/s12298-019-00692-2
- She D, Sun X, Gamareldawla AH, Nazar EA, Hu W et al. (2018). Benefits of soil biochar amendments to tomato growth under saline water irrigation. Scientific Reports 8 (1): 1-10.
- Sun J, He F, Shao H, Zhang Z, Xu G (2016). Effects of biochar application on Suaedasalsagrowthandsaline soilproperties. Environmental Earth Sciences 75: 1-6.
- Tartoura KA, Youssef SA, Tartoura ESA (2014). Compost alleviates the negative effects of salinity via up-regulation of antioxidants in *Solanum lycopersicum* L. plants. Plant Growth Regulation 74: 299-310.
- Thomas SC, Frye S, Gale N, Garmon M, Launchbury R et al. (2013). Biochar mitigates negative effects of salt additions on two herbaceous plant species. Journal of Environmental Management 129: 62-68.
- Trovato M, Mattioli R, Costantino P (2008). Multiple roles of proline in plant stress tolerance and development. Rendiconti Lincei 19 (4): 325-346.
- Usman ARA, Al-Wabel MI, Abdulaziz AH, Mahmoud WA, El-Naggar, AH et al. (2016). Conocarpus biochar induces changes in soil nutrient availability and tomato growth under saline irrigation. Pedosphere 26 (1): 27-38.
- Wang G, Xu Z (2013). The effects of biochar on germination and growth of wheat in different saline-alkali soil. Asian Agricultural Research 5 (11): 116.
- Yan Y, Pan C, Du Y, Li D, Liu W (2018). Exogenous salicylic acid regulates reactive oxygen species metabolism and ascorbate– glutathione cycle in *Nitraria tangutorum* Bobr. under salinity stress. Physiology and Molecular Biology of Plants 24 (4): 577-589.
- Yu Z, Duan X, Luo L, Dai S, Ding Z, Xia G (2020). How plant hormones mediate salt stress responses. Trends in Plant Science 25 (11): 117-1130. doi: 10.1016/j.tplants.2020.06.008
- Zhu JK (2007). Plant salt stress. Encyclopedia of Life Sciences, John Wiley & Sons, Ltd.