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RESEARCH REPORT

Comparative effects of mineral fertilizer and digestate on growth, antioxidant system, and physiology of lettuce under salt stress

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Abstract

Salt stress in plants presents a major challenge to future agricultural production. Digestate has various effects on plant growth, but little information is available on its effects on the antioxidant system and physiological characteristics of lettuce under salt stress. In this study, the impacts of mineral fertilizer and digestate application on edible parts of lettuce were compared under three salinities. Experimental treatments comprised application of two types of fertilizer (mineral fertilizer and digestate) and three NaCl concentrations (0, 3, and 7.5 dS m^{-1}). High NaCl concentrations resulted in significantly lower photosynthesis, growth, and physiological indices compared with those under no NaCl addition. However, under the 7.5 dS m^{-1} NaCl condition, digestate application (DA) increased the fresh weight (42%), dry weight (27%), photosynthetic pigment contents and photosynthesis (20%) of lettuce compared with that under mineral fertilizer application (MFA). Accumulation of reactive oxygen species was markedly lower, and the membrane stability index was therefore higher, under DA compared with under MFA within the same salinity level. Lipid peroxidation was lower under DA compared with under MFA in all salinity treatments. Salt stress up-regulated the antioxidant system and DA further increased the enzymatic and non-enzymatic antioxidant capability compared with that under MFA. In addition, the total water use was lower and water-related indices, such as water use efficiency of fresh weight, water use efficiency of dry weight and relative water content, were higher under DA compared with under MFA. The application of digestate instead of mineral fertilizer could be a promising practice to alleviate the negative impact of salt stress on the productivity and physiological characteristics of lettuce plants.

Keywords Antioxidant system · Digestate · Growth · Lettuce · Physiological characteristics · Salt stress

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1 Introduction

Lettuce (*Lactuca sativa* L.) is among the most economically significant leafy vegetable crops cultivated worldwide. Lettuce is rich in several nutritive and healthful compounds, such as minerals, protein, and carbohydrates (Usue et al. 2015), and provides a pleasant flavor to salads and other dishes. In addition, lettuce contains various antioxidants, such as ascorbic acid (AsA), phenols, and carotenoids, and thus its regular consumption can improve antioxidant status and prevent cardiovascular disease (Nicolle et al. 2004). However, lettuce plants are sensitive to moderate salinity (Andriolo et al. 2005; Neocleous et al. 2014).

Anaerobic digestate is proposed to be a cleaner and more efficient organic fertilizer for agricultural use. Digestate can be sustainably used as a substitute to mineral fertilizer (Plaimart et al. 2021). After anaerobic digestion, some nutrients from the feedstock remain in the digestate, such as potassium, nitrogen and phosphorus (Bolzonella et al. 2018). In addition, the digestate is enriched in microelements (such as boron, copper, manganese and zinc), which are vital for plant growth but are not usually incorporated into the majority mineral fertilizers (Wang et al. 2019; Ivanchenko et al. 2021). Digestate is also considered to be an environmentally friendly soil amendment for management of salinization (Hamid et al. 2021). Bioactive substances, such as saccharides, vitamins, organic acids, and phytohormones (e.g., indoleacetic acid and gibberellins), in digestate can promote plant growth and mitigate plant exposure to salt stress (Yu et al. 2010; Nkoa 2014; Panuccio et al. 2019). Digestate can not only provide substances of mineral nutrients but also hinders soil degradation attributed to salinity and confers several benefits simultaneously on the physical and chemical properties of the soil, such as enhanced carbon isolation and soil water-holding capacity (Jabeen et al. 2017; Cristina et al. 2020).

Salt stress severely limits horticultural productivity and plant growth. Salinity is a severe impediment to agricultural production worldwide (Haddadi et al. 2016). Currently, salinization affects approximately 74% of agricultural land worldwide and over-salinization affects more than 397 million ha of soil worldwide (Gong et al. 2013; Orosco-Alcalá et al. 2021). Salt stress adversely affects many metabolic processes in plants and leads to a reduction in biomass accumulation. Salt stress also induces hyperionic and hyperosmotic effects, resulting in elevated accumulation of reactive oxygen species (ROS) (Elsawy et al. 2018). Plants possess a complex defensive system of antioxidants, including antioxidative defense enzymes and non-enzymatic antioxidant substances, to alleviate the impacts of ROS. Antioxidative defense enzymes (e.g., catalase [CAT], ascorbate peroxidase [APX], peroxidase [POD], and superoxide dismutase [SOD]) and non-enzymatic antioxidant substances (e.g., AsA, glutathione [GSH], carotenoids, and phenolics including flavonoids and tocopherols) in plants are efficient scavengers of ROS and suppressants of lipid peroxidation, and hence are extremely relevant in relieving salt stress (Arora et al. 2020).

A variety of methods in plants are effective to reduce salt stress, such as application of organic and mineral amendments, phytohormones, nano-based products, etc. Application of organic matter in digestate can improve the cation exchange capacity and fertility of saline soils (Saqib et al. 2017). Most studies involving digestate application (DA) have been conducted on non-salinity-affected soils (Lee et al. 2018; Cheong et al. 2020). However, the few studies of DA to saline soils suggested that digestate may have a favorable impact on plant growth and the physicochemical characteristics of these problematic soils (Jabeen et al. 2017; Saqib et al. 2017; Hamid et al. 2021). The interaction between DA and the antioxidative defense system is not well understood. However, DA may alleviate the detrimental impacts of salt stress on plants on account of the high organic matter content in digestate and promotion of salt uptake capacity (Nkoa 2014; Panuccio et al. 2019; Hamid et al. 2021).

The usage of digestate as a fertilizer has received increased attention recently (Cheong et al. 2020). Little literature is available regarding the effect of digestate amendment on the antioxidant system of plants under salt stress. In previous studies, the influence of digestate as a soil fertilizer was studied. However, the comparative salt stress response of lettuce to mineral fertilizer and digestate remains unknown. In the present experiment, the effect of DA was investigated under different NaCl concentrations in edible parts of lettuce. The objective was to investigate the response to application of two fertilizer types (mineral fertilizer and digestate) on lettuce grown in non-saline and saline soil by analyzing the growth and antioxidative defense system. In addition, we explored the differences in water-relation indices, photosynthesis, oxidative stress, and lipid peroxidation among the treatments.

2 Materials and methods

2.1 Digestate acquisition

Digestate was collected from a 10^5 L digester (Fig. 1a) fed with cattle manure collected from a farm located on the campus of Hokkaido University, Hokkaido, Japan. The digester was operated steadily at a loading rate of 4,000 L d^{-1} to produce 80–120 m^3 biogas containing 60–65% methane.

Fig. 1 Experimental environment used in the study. (a) Anaerobic digester; (b) transplanted lettuce in pots in the summer of 2021

Table 1 Characterization of the digestate used in this study

Digestate					
pH	EC (dS m^{-1})	Ash (%)	C (g L^{-1})	OM (%, dw)	N (g L^{-1})
8.85	12.23	1.02	18.99	60.74	1.99
K_2O (g L^{-1})	P_2O_5 (g L^{-1})	MgO (g L^{-1})	CaO (g L^{-1})	N-NH_4^+(g L^{-1})	N-NO_3^- (mg L^{-1})
4.58	0.86	0.44	1.15	1.05	1.81

Table 2 Partial elemental profile of mineral fertilizer and digestate in the quantity applied during this study

Treatment	N (mM)	P (mM)	K (mM)	Ca (mM)
MFA	143	116	62	15
DA	143	13	100	20

MFA: application of mineral fertilizer; DA: application of digestate

3 Plant materials and experimental design

Lettuce 'Grand Rapids' seeds were purchased from the Sakata Seed Corporation Ltd. (Yokohama, Japan). The experiment was conducted in the summer of 2021 (22 May to 13 July; Fig. 1b) under natural conditions in a plastic-film greenhouse on the campus of Hokkaido University (43°4′ N, 141°20′ E; 20 m above sea level). The seeds were washed and soaked in warm water at 55°C for 5 min and then wrapped in wet gauze to accelerate germination for 48 h at 25°C. After germination, the seeds were sown in a seeding tray with 60 cells and two seeds per cell on 24 May. Commercial nutrition soils were used as seedling medium and purchased from the Iris Ohyama Corporation Ltd. (Sendai, Japan). Uniform healthy seedlings were selected at 20 days after sowing (DAS) and randomly transplanted into 3-L commercial plastic pots (diameter 18 cm, height 16 cm).

A pot experiment was performed using a completely randomized factorial design with four replications (four pots per replication). Ninety-six 3-L pots were each filled with soil with a field water-holding capacity of 22.94%. The field capacity of the soil was measured using the descriptive method of Mehdizadeh et al. (2020). The experimental treatments comprised two types of fertilizer (mineral fertilizer and digestate) and three salinities (0, 3.0, and 7.5 dS m^{-1}). Mineral fertilizer (NPK 10-18-12) was purchased from the Hokuren Fertilizer Co., Ltd. (Sapporo, Japan). Characterization of the digestate is summarized in Table 1, Supplementary Table S1 and Fig. S1. For each fertilizer application as a soil drench, either 100 mL digestate or 2 g of mineral fertilizer dissolved in 100 mL pure water was applied to the respective pots with a same nitrogen dosage (Table 2). After the final irrigation session, the fertilizer treatments were applied in the late afternoon at 20 and 36 DAS. An application of 100 mL was less than the volume pre-determined gravimetrically to be the soil saturation volume of a 3-L pot. Three salinities were applied by supplying NaCl in the irrigation water. Over the entire experimental period, irrigation was provided to maintain the soil water content at field capacity. All plants were irrigated with tap water at 17:00–19:00 daily before 25 DAS. The salinity treatments were applied from 26 to 50 DAS. Each pot was weighed daily to confirm the plant water status and to guide the irrigation volume required following the method of Liu et al. (2021). The difference in weight between the day before around 18:00 and the day around 18:00 was recorded as the irrigation volume per day during the salinity treatment period. In addition, throughout the experimental period, on each day a known volume of water was placed in a small evaporator comprising a round metal basin (diameter 20 m, height 10 cm) and, after 24 h, the remaining water volume was measured with a measuring cup. The reduction in water volume was recorded as the daily evaporation. The daily evaporation and temperature during the growth period are shown in Supplementary Fig. S2. All plants were harvested at 50 DAS.

4 Sampling and analytical methods

4.1 Growth parameters

At 50 DAS, the photosynthetic rate (P_n) was measured from 10:00 to 11:00 using a mini plant photosynthesis meter (miniPPM-300, EARS, Delft, The Netherlands) before harvest. The second, third, and fourth fully expanded leaves were selected for the photosynthesis measurements, and measurements were taken three times under about 1000 μmol m^{-2} s^{-1} PPFD illumination and 30 °C average temperature on each leaf sheet. Leaves were assessed in position near the major veins, and the mean value was calculated as the P_n of each plant. Three plants were selected randomly per treatment for measurement.

After harvest, the edible parts of six plants per treatment were excised. The edible parts of three plants were immediately weighed to record the fresh weight (FW) using an electronic scale (XS204V, Mettler Toledo, Greifensee, Switzerland). To measure the mean dry weight (DW), these three plant parts were dried at 75 °C to a constant weight in a drying oven (WFO-420 W, EYELA, Tokyo Rikakikai Co., Ltd., Tokyo, Japan). The other three plants were used to measure physiological indices.

5 Water use efficiency and relative water content

The water use efficiency (WUE; g L^{-1}) was determined following Barbieri et al. (2012) using formula S1. The relative water content (RWC; %) was determined using the method of Galmés et al. (2007) and calculated with formula S2.

6 Photosynthetic pigment contents

Chlorophyll (Chl), comprising Chl *a* and Chl *b*, and carotenoid contents were measured using the methods of Lichtenthaler (1987) and Popescu et al. (2017). These were extracted from fresh leaf tissues with 95% ethanol and magnesium oxide. The absorbance was calculated with a spectrophotometer (UV/VIS-560, Jasco Co., Tokyo, Japan) at 665, 646, and 470 nm.

7 Superoxide anion radical, lipid peroxidation, and membrane stability index

Superoxide anion radical ($O_2^{\bullet-}$) concentration was measured using the described method of Rauckman et al. (1979) (see S-A1). Malondialdehyde (MDA) content was determined in accordance with the method of Li (2000) (see S-A2). The membrane stability index (MSI; %) was measured in accordance with the procedure of Sairam et al. (1997) and calculated using formula S3.

8 Antioxidant enzyme activity

For enzyme extraction, frozen samples in edible parts of lettuce were homogenized with 100 mM phosphate buffer (pH 7.0) containing 1% PVPP (w/v) at 4 °C and centrifuged for 10 min at 15,000 × *g*. The extract solution was used to measure enzyme activities.

The SOD activity was measured at 560 nm sustained to prevent photoreduction of nitro blue tetrazolium (NBT) by about 50% according to S-A3 following the method of Li (2000). The CAT activity was determined as the decrease in absorbance at 240 nm according to S-A3 following the method of Aebi (1984) due to the decline of extraction of H_2O_2. The POD activity was measured as the increase in absorbance at 470 nm according to S-A3 following the method of Urbanek et al. (1991) due to the guaiacol oxidation. The APX activity was measured according to S-A3 following the method of Nakano et al. (1981), which depends on the decrease in absorbance at 290 nm as ascorbate was oxidized.

9 Non-enzymatic antioxidant components

The AsA content was determined using the molybdenum blue colorimetric method (Bajaj and Kaur 1981) according to S-A4. The GSH content was determined following the method of Li (2000) according to S-A4. The total phenolic content (TPC) was determined using the Folin–Ciocalteu method according to S-A4 in accordance with the procedure described by Rajapaksha and Shimizu (2020).

9.1 Statistical analysis

The values are presented as the mean ± SE of three biological replicates ($n = 3$). The data were subjected to two-way ANOVAs using SPSS 25.0. In addition, one-way ANOVAs were performed followed by Tukey's post-hoc tests at $p < 0.05$ to assess the significance of differences among the means. All figures were generated with OriginPro 2021.

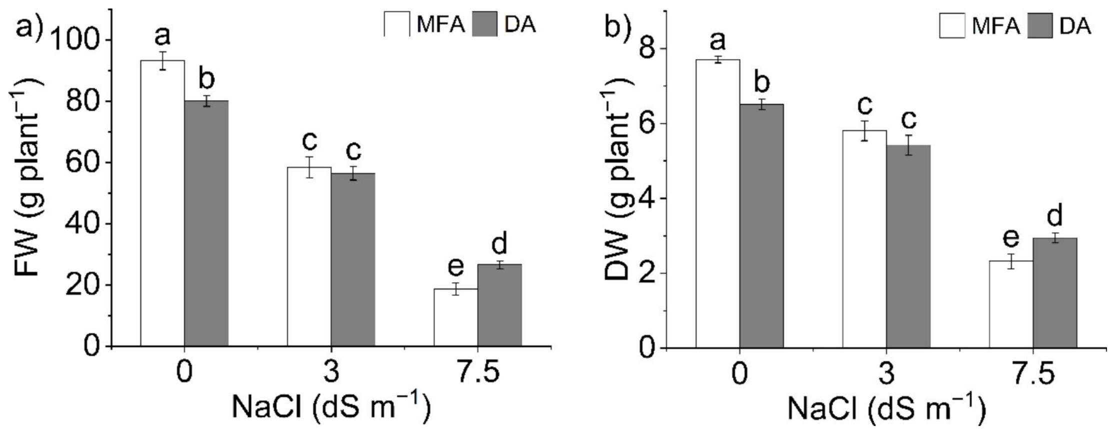

Fig. 2 Fresh weight (FW; a) and dry weight (DW; b) in response to application of two fertilizers (mineral fertilizer [MFA] and digestate [DA]) as affected by salt stress (0, 3, and 7.5 dS m^{-1} NaCl) in edible parts of lettuce plants. Means (± SE, n = 3) with the same letter in the same graph are not significantly different from each other ($p < 0.05$)

10 Results

10.1 Plant growth

Plant FW and DW, under both MFA and DA treatments, decreased with increasing NaCl concentration (Fig. 2). Under the non-saline condition, the FW and DW of edible parts of lettuces were higher under MFA than under DA. In contrast, DA markedly increased FW (42%) and DW (27%) compared with MFA under the 7.5 dS m^{-1} NaCl treatment. Fertilizer treatment did not significantly affect ($p > 0.05$) the FW or DW of the edible parts of lettuce plants under the 3 dS m^{-1} NaCl treatment.

11 Water-relation indices

Water relations were affected by salt stress during plant development (Fig. 3). The WUE_{FW}, WUE_{DW}, and RWC in edible parts of lettuce plants decreased with increasing salinity; however, DA-treated plants were less severely affected than MFA-treated plants by salt stress ($p < 0.05$; Fig. 3a–c). Specifically, the WUE_{FW}, WUE_{DW}, and RWC in edible parts of the plants were reduced by 29%, 15%, 13% and 17%, 2%, 9% ($p < 0.05$) under 3 dS m^{-1} NaCl treatment compared with levels under the non-saline condition under MFA and DA, respectively. Under the 7.5 dS m^{-1} NaCl treatment, these indices declined more substantially. Inconsistent with the reduction in WUE, the total water use was significantly higher ($p < 0.05$) throughout the growth period in MFA-treated plants compared with in DA-treated plants under the same salinity (Fig. 3d). Furthermore, with increasing duration of salt exposure, DA markedly reduced the volume of water consumption compared with MFA, and the water consumption declined with increasing salinity under both MFA and DA treatments (Fig. 3e).

12 Photosynthetic pigment contents and P_n

Salt stress reduced the concentrations of photosynthetic pigments and P_n (Fig. 4). Compared with MFA treatment, lower contents of Chl *a* and Chl *b* were observed under DA treatment in the non-saline condition (Fig. 4a,b). However, these contents were 10% and 11% higher ($p < 0.05$), respectively, in the DA treatment compared with in the MFA treatment under 7.5 dS m^{-1} NaCl. The Chl *a* content in DA-treated plants was elevated visibly ($p < 0.05$), whereas the Chl *b* content showed no significant difference, under the 3 dS m^{-1} NaCl treatment compared with that of MFA-treated plants. The carotenoids content in DA-treated plants was considerably higher compared with that of MFA-treated plants (Fig. 4c), increasing by 1%, 7%, and 20% under 0, 3, and 7.5 dS m^{-1} NaCl, respectively. The P_n decreased with increasing NaCl concentration (Fig. 4d). In addition, P_n differed significantly ($p < 0.05$) between fertilizer treatments only under the 7.5 dS m^{-1} NaCl concentration; at this concentration, P_n was higher (20%) under DA than under MFA.

13 $O_2^{\bullet-}$ concentration, MDA content, and MSI

Salt stress conditions increased $O_2^{\bullet-}$ and MDA concentrations compared with the corresponding non-saline condition. In this study, under the 3 dS m^{-1} NaCl concentration, the increase in concentrations of $O_2^{\bullet-}$ and MDA was 57.2% and 27.6% in MFA- and DA-treated plants, respectively, resulting in a 6% decrease in the MSI, compared with the corresponding non-saline condition (Fig. 5). Under exposure

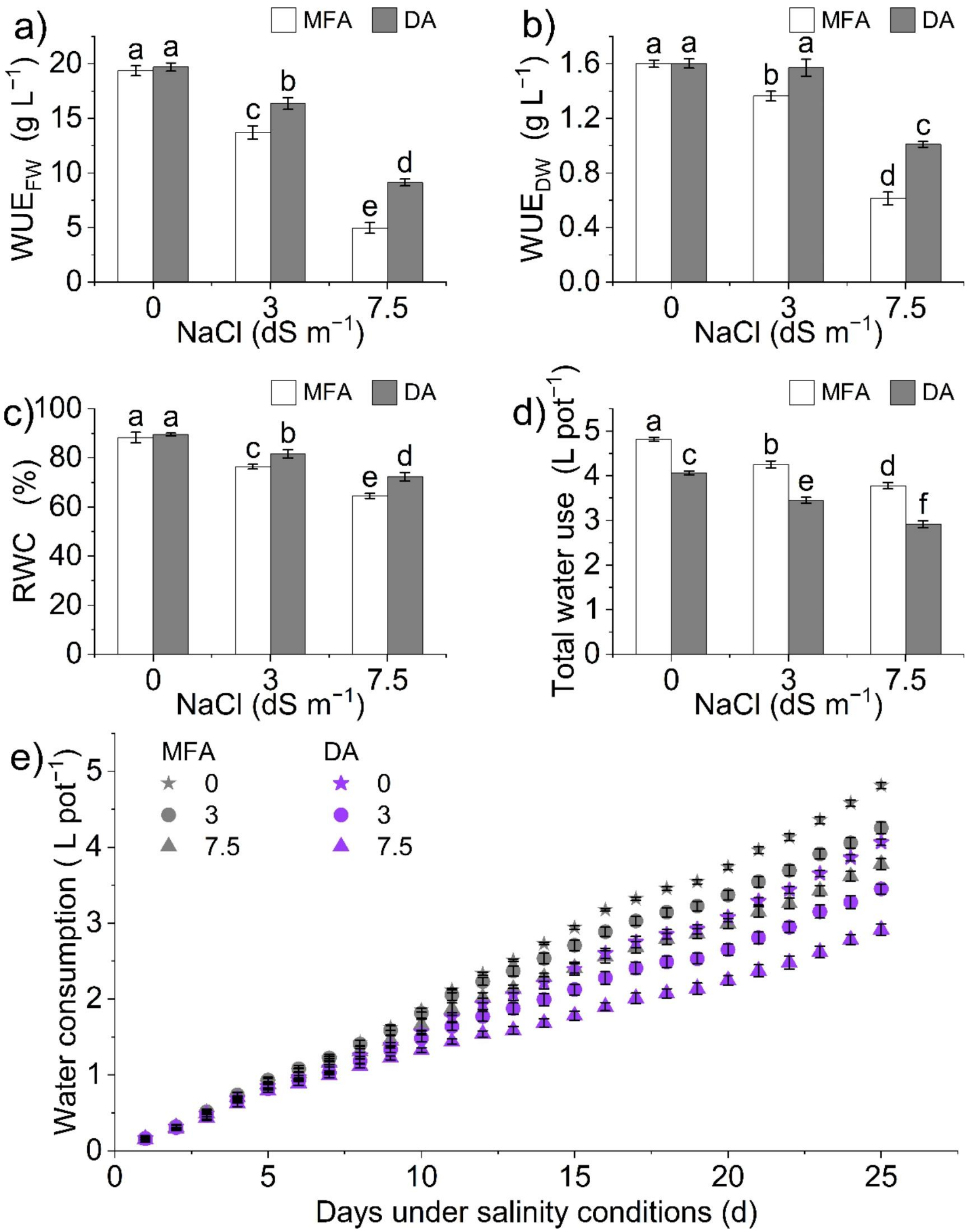

Fig. 3 Fresh-weight water use efficiency (WUE_{FW}; a), dry-weight water use efficiency (WUE_{DW}; b), relative water content (RWC; c), total water use (d), and water consumption in response to two fertilizers (mineral fertilizer [MFA] and digestate [DA]) as affected by salt stress (0, 3, and 7.5 dS m^{-1} NaCl) in edible parts of lettuce during the salinity treatment period. Means (± SE, n = 3) with the same letter in the same graph are not significantly different from each other ($p < 0.05$)

to 7.5 dS m^{-1} NaCl, $O_2^{\bullet-}$ and MDA increased further and MSI decreased further under both MFA and DA treatments. However, the $O_2^{\bullet-}$ and MDA concentrations were higher and the MSI was lower in MFA-treated plants compared with in DA-treated plants at the same salinity.

14 Activities of antioxidant enzymes

The activities of SOD, CAT, POD, and APX in the edible parts of lettuces were markedly elevated under both fertilizer treatments under salt stress (Fig. 6). In addition, DA

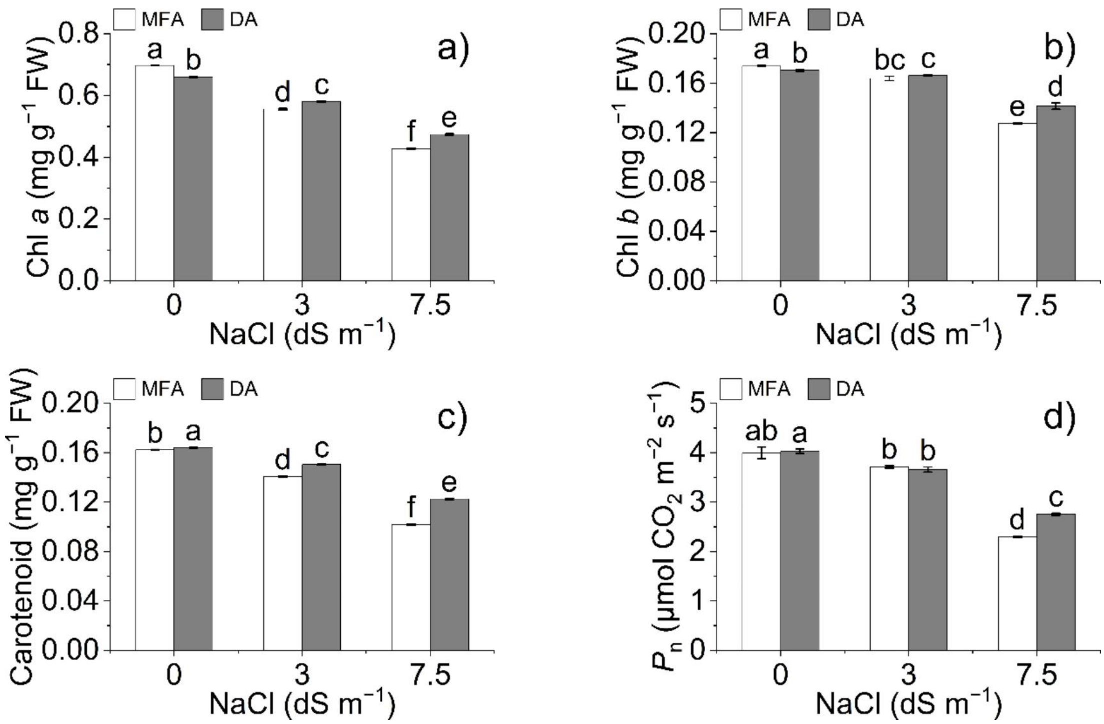

Fig. 4 Contents of chlorophyll *a* (a), chlorophyll *b* (b), and carotenoids (c), and net photosynthetic rate (P_n; d) in response to two fertilizers (mineral fertilizer [MFA] and digestate [DA]) as affected by salt stress (0, 3, and 7.5 dS m^{-1} NaCl) in edible parts of lettuce. Means (± SE, n = 3) with the same letter in the same graph are not significantly different from each other ($p < 0.05$)

treatment increased the activities of antioxidant enzymes compared with the MFA treatment under the same salinity. The activities of SOD and CAT in the non-saline condition were 36% and 74% ($p < 0.05$) higher in DA-treated plants than in MFA-treated plants (Fig. 6a,b). In contrast, the activities of POD and APX were not significantly different ($p > 0.05$) between the two fertilizer sources under the non-saline condition (Fig. 6c,d).

15 Non-enzymatic antioxidants

Treatment with DA significantly increased ($p < 0.05$) the AsA, GSH, and TPC contents compared with those of MFA-treated plants under the same salinity (Fig. 7). Compared with the MFA treatment, DA treatment increased accumulation of these antioxidants by approximately 11%, 10%, and 27% for AsA, 24%, 38%, and 11% for GSH, and 20%, 10%, and 16% for TPC under 0, 3, and 7.5 dS m^{-1} NaCl, respectively. The GSH and TPC contents increased with increasing salinity (Fig. 7b,c). In contrast, AsA content increased from the 0 to 3 dS m^{-1} NaCl treatment but decreased from the 3 to 7.5 dS m^{-1} NaCl treatment under both MFA and DA (Fig. 7c).

16 Discussion

Soil salinity represents a major challenge for agricultural production in all climates worldwide (Evelin et al. 2019). The current study evaluated differences in the edible parts of lettuce in response to salt stress between MFA and DA treatments. The results revealed that salt stress had a detrimental effect on accumulation of biomass in lettuce. The studies of Usue et al. (2015) and Shams et al. (2016) demonstrated similar results. Biomass is an optimal plant index for evaluation of various stresses, and its accumulation indicates the life-sustaining activities of plants. In the present study, biomass accumulation under DA was obviously lower than that observed under MFA in the non-saline condition (Fig. 2). Similar results have been reported using digestate originating from poultry manure and food waste as a replacement for mineral fertilizer in leafy vegetables (Wang et al. 2019; Cheong et al. 2020). We consider that digestate may be an unbalanced fertilizer because the lack of phosphorus in the digestate limited the growth of lettuce (Table 2). Li et al. (2016) also reported a deficiency of phosphorus in digestate applied as fertilizer. In addition, comparisons of DA and MFA based on an equivalent nitrogen dose have shown that DA provides lower fertilizer nitrogen amounts than mineral fertilizers (Quakernack et al. 2012). It was notable that, especially under the 7.5 dS m^{-1} NaCl concentration, the FW

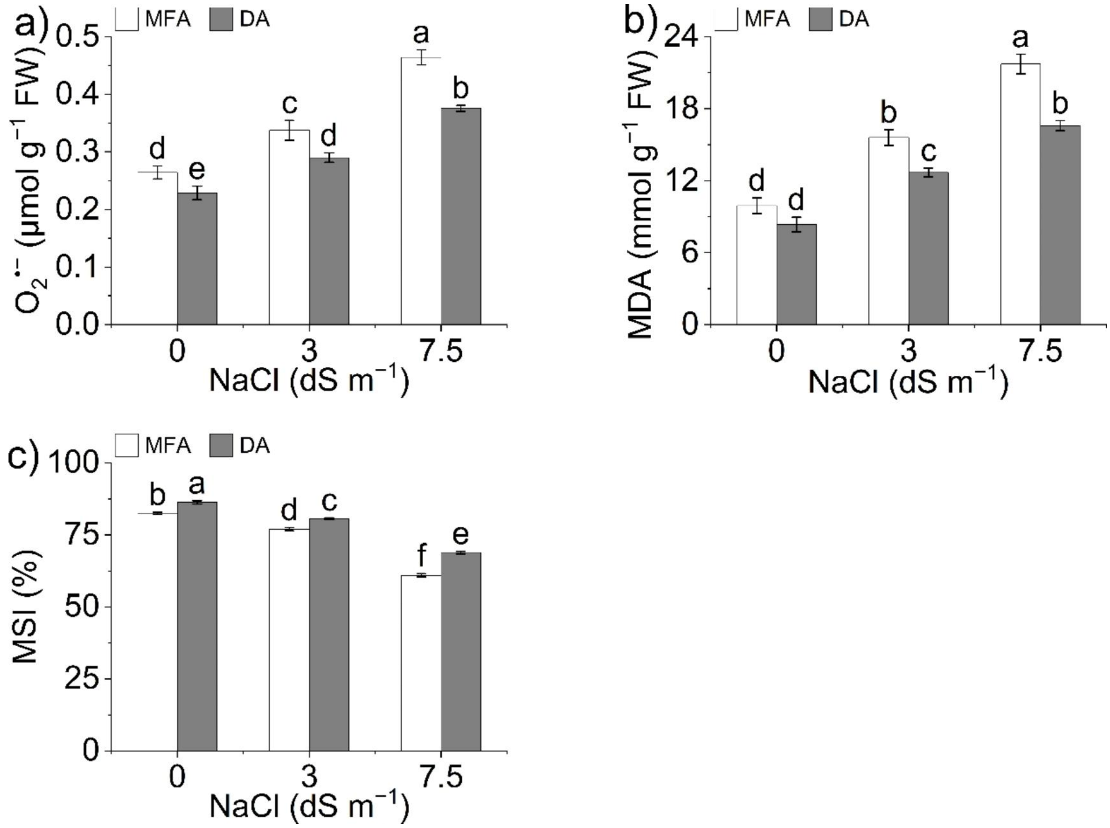

Fig. 5 Contents of superoxide anion radical ($O_2^{\bullet -}$; a) and malondialdehyde (MDA; b), and membrane stability index (MSI; c) in response to two fertilizers (mineral fertilizer [MFA] and digestate [DA]) as affected by salt stress (0, 3, and 7.5 dS m^{-1} NaCl) in edible parts of lettuce. Means (± SE, n = 3) with the same letter in the same graph are not significantly different from each other ($p < 0.05$)

and DW of DA-treated plants were markedly greater than those of MFA-treated plants. The reduced biomass under the 3 dS m^{-1} NaCl concentration was ameliorated by DA because the element imbalance in the digestate limited plant growth. The FW and DW biomasses under the 3 dS m^{-1} NaCl concentration were inconsistent with those under the non-saline condition. The DA treatment ameliorated the negative consequences of salt stress by promoting biomass accumulation under the different salinities; DA under 7.5 dS m^{-1} NaCl achieved superior performance compared with MFA in this regard. Hamid et al. (2021) reported that DA enhanced the K^+/Na^+ ratio in sunflower, improved plant growth, and declined the harmful effects of salinity in saline soil (EC ≤ 8 dS m^{-1}).

Water uptake by plants is lowered due to high soil salinity concentration (Phogat et al. 2018). Consistent with this phenomenon, the total water use was lower during the growth period under saline conditions (Fig. 3). Saline irrigation water negatively affects plant growth due to lower soil osmotic potential, leading to lower RWC and plant dehydration (Maggio et al. 2004; Larbi et al. 2020). The current results demonstrated that DA improved the WUE of FW and DW biomass (WUE_{FW} and WUE_{DW}) under salt stress compared with those under MFA, including at 3 and 7.5 dS m^{-1} NaCl concentrations (Fig. 3a,b). WUE decreased with increasing salt concentration. However, different crops have different salinity thresholds. The WUE of bell pepper under saline conditions (EC ≤ 8 dS m^{-1}) (Orosco-Alcalá et al. 2021) is similar to that of lettuce recorded in the current work. In contrast, wheat WUE was higher under a saline condition (EC ≤ 8 dS m^{-1}) than under a non-saline condition (Khataar et al. 2018), which contrasted with the present results for lettuce under salt stress (EC ≤ 8 dS m^{-1}), because the salinity threshold of wheat is about 8 dS m^{-1} and wheat WUE declined with increasing salt concentration (EC ≥ 8 dS m^{-1}). A reduction in RWC denotes loss of turgor with a limited supply of water for cell expansion. In the current study, salinity decreased the RWC in edible parts of lettuce plants under both MFA and DA. Kaya et al. (2007) reported a reduction in RWC in salt-stressed melon plants compared with in unstressed melon plants. The decrease in RWC under DA was significantly greater than that under MFA at the 3 and 7.5 dS m^{-1} NaCl concentrations (Fig. 3c). Therefore, DA was associated with a superior water retention in soil. Similar to most organic fertilizers, digestate has a high organic matter content and improves plant water

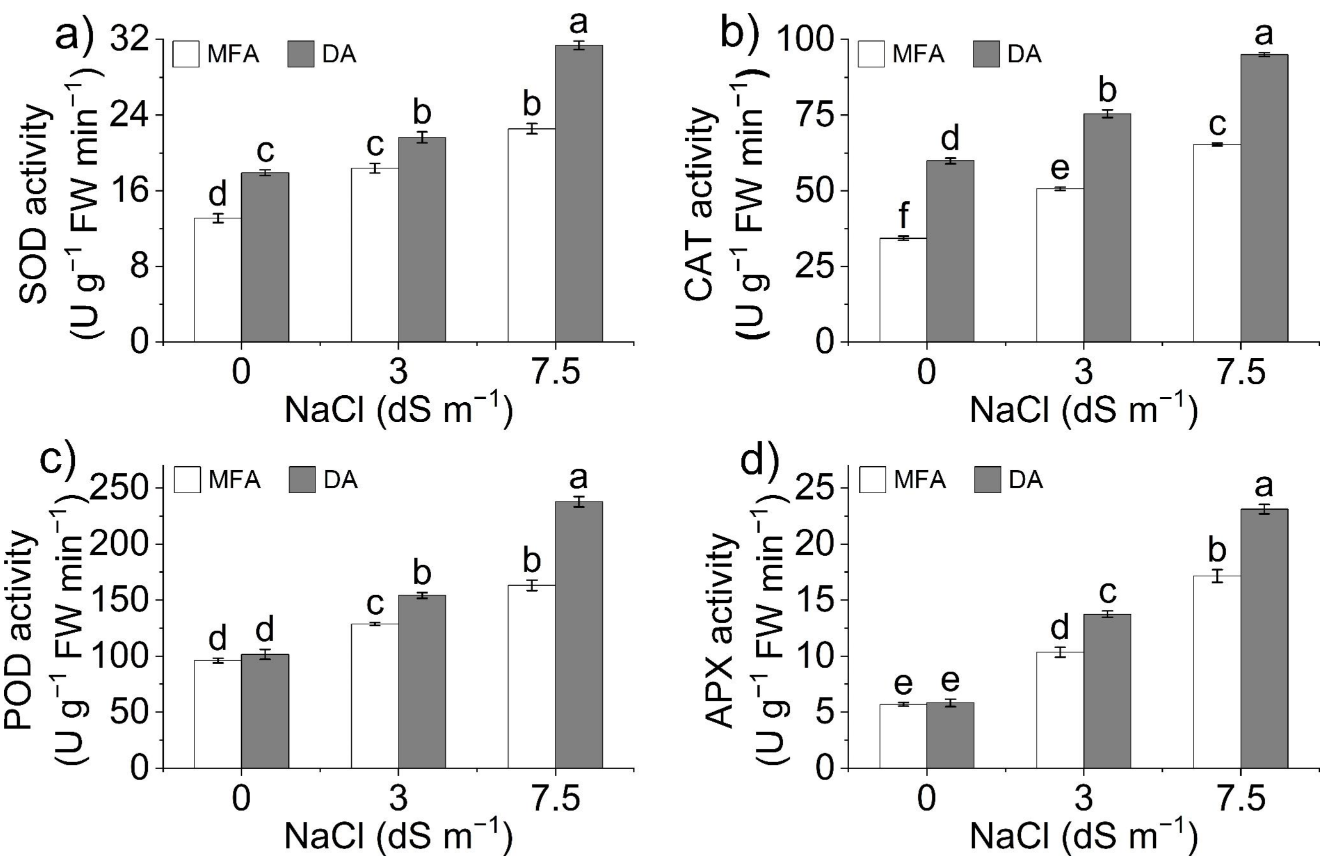

Fig. 6 Activity of superoxide dismutase (SOD; a), catalase (CAT; b), peroxidase (POD; c), and ascorbate peroxidase (APX; d) in response to two fertilizers (mineral fertilizer [MFA] and digestate [DA]) as affected by salt stress (0, 3, and 7.5 dS m^{-1} NaCl) in edible parts of lettuce. Means (±SE, n=3) with the same letter in the same graph are not significantly different from each other ($p<0.05$)

relations during growth (Jabeen et al. 2017). In addition, DA resulted in relatively lower integrated total water use than MFA in the growth period when the field capacity was used to determine the irrigation requirement. This may be attributed to the improvement in soil properties resulting from the micronutrients and high organic matter content in digestate (Cristina et al. 2019). In short, the results of the current study support the notion that DA improves the water relations of lettuce under salt stress. Hamid et al. (2021) reported that water use and salt stress tolerance of plants might be increased by DA. The present results corroborate this suggestion.

Reduction in photosynthetic pigment contents of salt-stressed plants (Fig. 4) inhibits chlorophyll synthesis and activity of enzymes implicated in carbohydrate metabolism (Castañares and Bouzo 2019). Chlorophyll is the most pivotal pigment for photosynthesis, hence a decrease in chlorophyll content will decrease photosynthesis. The current study indicated that DA suppressed chlorophyll degradation under 7.5 dS m^{-1} NaCl. Decreased plant biomass under salt stress is often a direct result of photosynthesis inhibition (Gong et al. 2013). The leaf P_n was lowest in MFA-treated lettuce under the 7.5 dS m^{-1} NaCl concentration (Fig. 4d), consistent with the lowest biomass. Under 3 dS m^{-1} NaCl, some aspects of chlorophyll synthesis were promoted by DA compared with MFA, such as Chl *a* accumulation. Application of certain mineral nutrients, such as calcium, magnesium, zinc, and manganese, can promote chlorophyll synthesis and alleviate salt stress (Bohn et al. 2004; Li et al. 2017; Nadeem et al. 2020). In the current study, calcium and microelement concentrations in lettuce were not measured under DA and MFA, but DA provided higher calcium and potassium concentrations than MFA at the same nitrogen dose (Table 2). A variety of microelements are present in digestate (Nkoa 2014; Wang et al. 2019; Ivanchenko et al. 2021). In addition, the results of Wang et al. (2019) indicated that the calcium content in lettuce leaves under DA treatments were significantly higher than those under MFA. Larbi et al. (2020) reported similar findings in which supplementary potassium and calcium mitigated salt stress. Therefore, DA improved chlorophyll synthesis in lettuce compared with MFA, which may reflect that digestate provides certain amounts of calcium and micronutrients.

Cell membrane damage is among the first subcellular impacts of salt stress. The loss of cellular membrane integrity and stability is caused by lipid peroxidation (Castañares

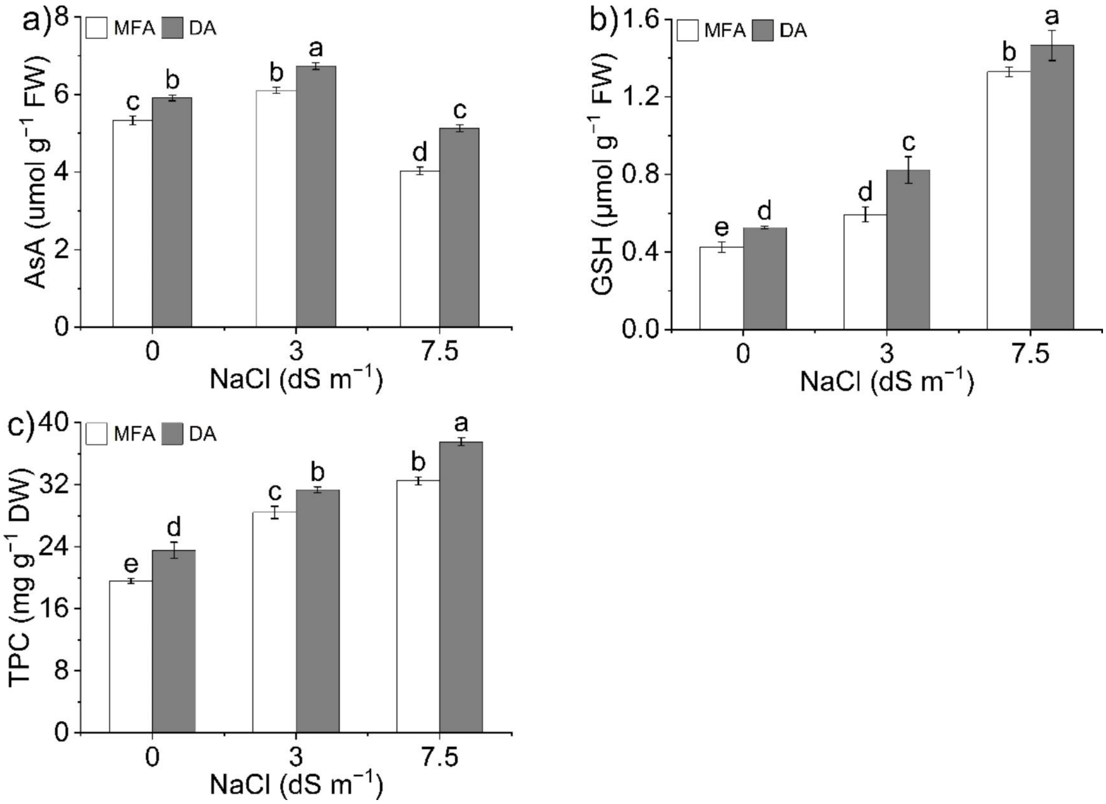

Fig. 7 Ascorbic acid (AsA; a), glutathione (GSH; b), and total phenolic content (TPC; c) in response to two fertilizers (mineral fertilizer [MFA] and digestate [DA]) as affected by salt stress (0, 3, and 7.5 dS m^{-1} NaCl) in edible parts of lettuce. Means (± SE, n = 3) with the same letter in the same graph are not significantly different from each other ($p < 0.05$)

and Bouzo 2019). In the current study, the MSI was higher under DA compared with under MFA in the same salinity (Fig. 5c). This response likely strengthened the cellular membranes by reducing water deficiency and decreasing ion leakage (Tabaei et al. 2000). Presumably, this reinforcement of cellular membrane stability aids in absorption of essential minerals and hence improved plant growth. Salt stress can cause enhanced lipid peroxidation (Meloni et al. 2003), and MDA is a result of oxidative stress as the final product of lipid peroxidation. Inhibition of oxidative stress can be prevented by reducing MDA levels (Koca et al. 2007). In the current results, MDA contents in the edible parts of lettuce plants were lower under DA compared with under MFA in the non-saline condition and salt stress conditions (Fig. 5b), indicating that DA inhibited membrane damage. These findings are concordant with the result of Ali et al. (2019). Damage to membrane lipids is usually provoked by oxidants, including $O_2^{\bullet-}$ (Elsawy et al. 2018), which explains why $O_2^{\bullet-}$ was observed in all lettuce plants. $O_2^{\bullet-}$ accumulation was substantially higher in leaves of MFA-treated lettuce than in those of DA-treated plants under the same salinity (Fig. 5a). The high $O_2^{\bullet-}$ concentration in lettuce tissue might explain the higher MDA content and lower MSI observed in the MFA treatment compared with in the DA treatment.

To relieve oxidative stress, the ROS-scavenging system in plants include enzymes and non-enzymatic antioxidant components. Antioxidant enzymes (e.g., SOD, CAT, POD, and APX) have an efficient scavenging effect on ROS. Enhanced activities of these enzymes are commonly associated with decreased oxidative damage; thus, these enzymes could mitigate salt stress (Barbieri et al. 2012). Antioxidant enzymes in plants frequently operate in concert to achieve ROS detoxification. The action of SOD is enabled to scavenge $O_2^{\bullet-}$, which is the most abundant component of ROS (Elsawy et al. 2018). Hence, SOD represents the main line of defense to remove ROS. Subsequently, the H_2O_2 generated by dismutation of $O_2^{\bullet-}$ must be eliminated because its accumulation readily causes oxidative damage. At this point, other antioxidant enzymes (e.g., CAT, POD, and APX) are vital as defense against oxidative stress. These enzymes play an influential role in scavenging H_2O_2, which is produced under salt stress (Gong et al. 2013; Elsawy et al. 2018). In the current research, activities of antioxidant enzymes increased with increasing salinity under both fertilizer applications. It should be noted that SOD and CAT activities under the non-saline condition and salt stress conditions were higher with DA than with MFA, whereas fertilizer treatment did not significantly affect activities of GPX or APX under the non-saline condition (Fig. 6). Antioxidant enzymatic activities of plants exhibit varying sensitivities and responses to digestate treatments (Aihemaiti et al. 2019). The current results confirmed that SOD and CAT are more responsive to DA than other antioxidant enzymes in lettuce. Therefore, we speculated that DA can alleviate the harmful effect of biomass accumulation and lipid peroxidation in lettuce induced by salt stress, which is presumably mediated by the increase in antioxidant enzymatic activities to various degrees.

The non-enzymatic components under salt stress are also important elements of antioxidant defense systems. In the current research, the concentrations of AsA, GSH, and TPC in edible parts of lettuce were relatively higher under DA compared with under MFA (Fig. 7). These findings are similar to the results of Panuccio et al. (2019), who reported that digestate enhances phenol and flavonoid contents in cucumber. Under salt stress, plants produce non-enzymatic antioxidant substances to scavenge ROS. A previous study (Ashraf et al. 2008) showed that the antioxidant capability of plants is directly associated with salt tolerance. In addition, a previous study of lettuce observed markedly higher radical-scavenging capability in plants containing high contents of antioxidant substances (GSH and TPC) (Shams et al. 2016). As an essential non-enzymatic scavenger of ROS, AsA effectively plays an influential role to repair the damage induced by salt stress. A previous study indicated that DA enhances the AsA content in lettuce leaves (Wang et al. 2019). In the present work, the AsA content increased initially and thereafter reduced with increasing salt concentration, but the AsA content under DA was always higher than that under MFA. This is consistent with the findings of Gong et al. (2013), who observed that GSH content rose consistently with increasing NaCl concentration, whereas AsA content increased under a low NaCl concentration and decreased under higher NaCl concentrations. The present results indicate that DA may contribute to the mitigation of oxidative damage induced by ROS by promoting the actions of antioxidant enzymes and non-enzymatic components in edible parts of lettuce.

17 Conclusion

In the present study, lettuce plants did not grow optimally under irrigation to field capacity following application of digestate instead of mineral fertilizer because biomass in the edible parts of the plants were reduced. However, DA led to superior salt tolerance compared with MFA under the salinity treatments. Our study revealed that DA ameliorated most of the variables studied under NaCl stress. In particular, the amelioration was more pronounced in plants exposed to 7.5 dS m^{-1} NaCl stress. Therefore, the present results corroborated the presence of a window within which DA is potentially beneficial to lettuce for overcoming salt stress. In conclusion, we found that DA alleviated, at least during the short term, the salt stress in lettuce. This could be explained by the reduced oxidative stress and increased photosynthetic pigment contents and water relations, which were conferred by the increased activities of antioxidant enzymes and non-enzymatic substances in edible parts of lettuce plants. Our results indicate that application of digestate instead of mineral fertilizer offers potential for growth of lettuce in saline soils.

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Authors' contributions FQWL designed the experiment and wrote the manuscript; FQWL and YHY determined the experimental indicators and analyzed the data; FQWL, PXG, YI, and RSN were involved in the pot experiment; NS revised the manuscript, and supervised and provided funding.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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