

Article

Evaluation of the Effects of Magnetically Treated Saline Water on Physiological, Antioxidant and Agronomic Traits of Jojoba [*Simmondsia chinensis* (Link) Schneider]

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Abstract: Salinity poses a serious challenge to agriculture across the globe. In the Middle East, countries such as Saudi Arabia are facing potential problems of salinity due to the use of processed saline Red Sea water for agriculture. To tackle this challenge, the current study was conducted with the objective of assessing the effects of magnetically (1.80 mT) treated normal agriculture water (NW = 2.11 DSm⁻¹) and different concentrations of Red Sea water (RSWC1 = 5.61 DSm⁻¹ and RSWC2 = 7.01 DSm⁻¹) on the physiological traits (chlorophyll, photosynthesis rate, transpiration rate, stomatal conductance and membrane damage), antioxidant enzymes (superoxide dismutase, catalase and peroxidase), proline and agronomic characteristics (germination percentage, germination rate, shoot length and root length) of jojoba (*Simmondsia chinensis*) seedlings. The experiment was set in a glasshouse with three replicates, using RCBD with two factorial arrangements. The data were collected and subjected to statistical analysis using statistix8.1 and R-program. All magnetically treated concentrations of saline water showed significant improvements in all traits compared with their respective controls, except proline, membrane damage (MD) and germination rate (GR). However, the response of these all traits was more significant at NW compared with RSWC1 and RSWC2. Furthermore, correlation, PCA and heat map analysis revealed that all traits are significantly interlinked in determining the jojoba response to different concentrations of salinity, both in the presence and absence of MF.

Keywords: jojoba; salinity; SEM; antioxidant; proline

1. Introduction

Abiotic stress, for instance, salinity, is a potential threat to worldwide agriculture. Although various breeding strategies have been employed to impart crops with potential tolerance against abiotic stresses, desired results have not been attained yet [1]. To meet the challenge of salinity, various engineering and reclamation techniques have been used but have only provided temporary solutions [2]. Therefore, it is necessary to look for alternate tools for addressing this problem in more cost-effective way [3]. Salinity is a growing challenge in the regions where the water table is either shallow or high in saline content [1]. Moreover, the increasing pace of salinization is forcing the farmers to use innovative techniques of crop production. In this respect, the beneficial effects of magnetic field (MF) have been reported in a number of farming practices [4]. The magnetic treatment of water positively impacts root growth of many plant species. Besides, application of water treated with MF increases agronomic yield of strawberry and tomato [5]. Furthermore, application of MF not only influences seed germination percentage (GP) and germination rate (GR), but also promotes various agronomic parameters including root and shoot growth [6–8]. Correspondingly, dormant seeds treated with magnetized water display a significant increase in germination rate (GR) and seedling growth in barley, wheat, beans, corn, tomato, fruit trees and other plant species, as reviewed by Sarraf et al. [4].

The application of MF with saline water is an environmentally friendly and inexpensive technique that can enhance water quality and crop productivity [9]. The MF can improve the basic properties of water such as surface tension, ionic strength and pH, that enhance vibration and polarization of water molecules resulting in an increase in cellular water uptake [10]. Many researchers have reported the positive impact of MF on the activities of different antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) in plant cells [11–13]. Treatment of saline water with MF also induces greater plant resistance under stress conditions, which can be attributed to the improvement in antioxidant systems [14]. Furthermore, MF treatment also accelerates the mobilization of nitrogen in plants. In addition, pretreatment of irrigation water with MF enhances the formation of photosynthetic pigments that leads to increased light absorption, resulting in high photosynthesis (Pn), transpiration (Tr) and stomatal conductance (Gs) [8,15]. In this regard, *Simmondsia chinensis* (link) Schneider, commonly known as Jojoba, is an eminent oil seed crop of economic value inhabiting arid and semiarid regions across the globe [16,17]. The jojoba plant is well-adapted to dry and warm climatic conditions, and is cultivated in the areas with severe water scarcity where conventional farming methods are not viable [18]. This shrub, being drought and high-temperature tolerant, can be considered as an economical oil seed crop in desert regions of Saudi Arabia. Moreover, as a halophyte, this crop is slightly tolerant to salinity, therefore available saline water can be used as an option [19]. Additionally, the Middle East in particular is a region of water scarcity, where treated sea water is a sole source of irrigation, with high contents of salinity making it unfit for agricultural uses [3]. Moreover, an extensive area of Saudi Arabia exhibits conditions where mineral weathering and poor-quality water are aggravating the problem of salinity [20]. Therefore, a good option is to pretreat the water with MF for its potential application in boosting agriculture. In this context, the current study aims to comparatively evaluate the effects of magnetically treated, different dilutions of Red Sea water and normal irrigation water (used in the region) on physiological, antioxidant and agronomic traits of jojoba plants.

2. Materials and Methods

This study was conducted in the growth chamber at the Department of Arid Land Agriculture, Faculty of Meteorology, Environment and Arid Land Agriculture, King Abdulaziz University, Jeddah, Saudi Arabia. The trireplicate experiment was conducted on jojoba (*Simmondsia chinensis* (link) Schneider) in RCBD design using a two factorial arrangement, with magnetic field (MF) as one factor and saline water type as another factor.

2.1. Crop Husbandry

Ten seeds of jojoba were sown in 2 L pots filled with 75% fresh soil and 25% peatmoss. For each treatment, three pots were used and kept under controlled 30/20 °C day and night temperature, respectively. The pots were watered according to field capacity once every two days before germination, while after germination they were watered once every four days. For minimizing the effect of positional error, the pots were randomized on an alternate basis.

2.2. Treatment Application and Data Collection

Three different levels of saline water, such as normal water used in local agriculture (NW) [2.11 DSm⁻¹] and two red sea water dilutions (RSWC1 and RSWC2) [10% sea water (5.61 DSm⁻¹) and 12.5% sea water (7.01 DSm⁻¹)], with and without magnetic field (MF) of 180 millitesla (mT) [7] were applied to jojoba plants following the aforementioned schedule. Data for physiological parameters, antioxidant enzymes and proline were recorded from randomly selected plants on weekly basis starting from physical maturity until the seedling attained the age of two months. Among agronomic parameters, shoot length (SL) and root length (RL) were recorded when the seedling attained the age of two

months, while the data for germination percentage (GP) and germination rate (GR) were recorded after germination.

2.3. Estimation of Physiological Parameters

Total chlorophyll content was estimated by using the method followed by Mahmood et al. [21]. Further, for the estimation of stomatal conductance (Gs), photosynthesis rate (Pn) and transpiration rate (Tr) Syrus 3 upgraded model SC-1, 2011 (Decagon Devices, 2011) was used. Membrane damage (MD) percentage was recorded using the protocol followed by Sairam et al. [22].

2.4. Estimation of Antioxidant Enzymes Activity and Proline

The activities of antioxidant enzymes such as SOD, CAT and POD were estimated using the protocol followed by Djanaguiraman et al. [23]. For the estimation of SOD activity, superoxide dismutase activity assay kit (Sigma-Aldrich, USA, Product number CS0009-1KT) was used according to the manufacturer's instruction. The activity of CAT was measured using a catalase assay kit (Sigma-Aldrich, USA, Product number 219265-1KIT) following the instructions given. Furthermore, a peroxidase activity assay kit (Elabscience, USA, Product number E-BC-K227-S) was used to determine the activity of POD. Furthermore, proline quantity was estimated on account of its reactivity with ninhydrin by using a UV-Vis spectrophotometer (Spectroquant, Germany, Product number 173018).

2.5. Estimation of Agronomic Parameters

Agronomic parameters, such as germination, were calculated in percentage (GP) using the percentage formula. Furthermore, germination rate (GR) was estimated in days, while shoot length (SL) and root length (RL) were calculated in centimeters using scale.

2.6. Statistical Analysis

The data recorded were subjected to analysis of variance (ANOVA) at a 5% probability level, using computer-based software Statistix 8.1 (McGraw-Hill 2008). Furthermore, correlation, principal component analysis (PCA) and heatmap analysis were executed in RStudio version 1.3.959 (RStudio Team 2020), using the PerformaceAnalytics, FactoMineR, factoextra, devtools, ggplot2, ggpubr, gplots and pheatmap packages of R version 4.1.0 (R Core Team 2021).

2.7. Scanning Electron Microscopy

The selected leaf samples from the jojoba plants watered with different concentration of magnetically treated water were placed in EDX system of scanning electron microscope for generating the SEM spectrographs. The peaks in the spectrograph indicate the assimilated elements in leaves. In the spectrograph, peak height helped in elemental quantification while peak position helped in elemental identification. Furthermore, the SEM was already equipped with autoquantification and autoidentification features.

3. Results

3.1. Physiological Parameters

The application of magnetic field (MF) significantly differentiated the impacts of saline water treatments on all physiological parameters such as total chlorophyll, stomatal conductance (Gs), photosynthesis rate (Pn), transpiration rate (Tr) and membrane damage (MD) compared with the control (Figure 1). Application of magnetized water at all levels of salinity made dramatic improvements to chl, Pn, Tr and Gs, although improvement was the highest at NW while the lowest at RSWC1 (Figure 1a–e). Contrarily, compared with the control, magnetized water resulted in a significant reduction in membrane damage under all treatments of saline water, however this reduction was more dramatic for NW and least dramatic for RSWC1 (Figure 1d).

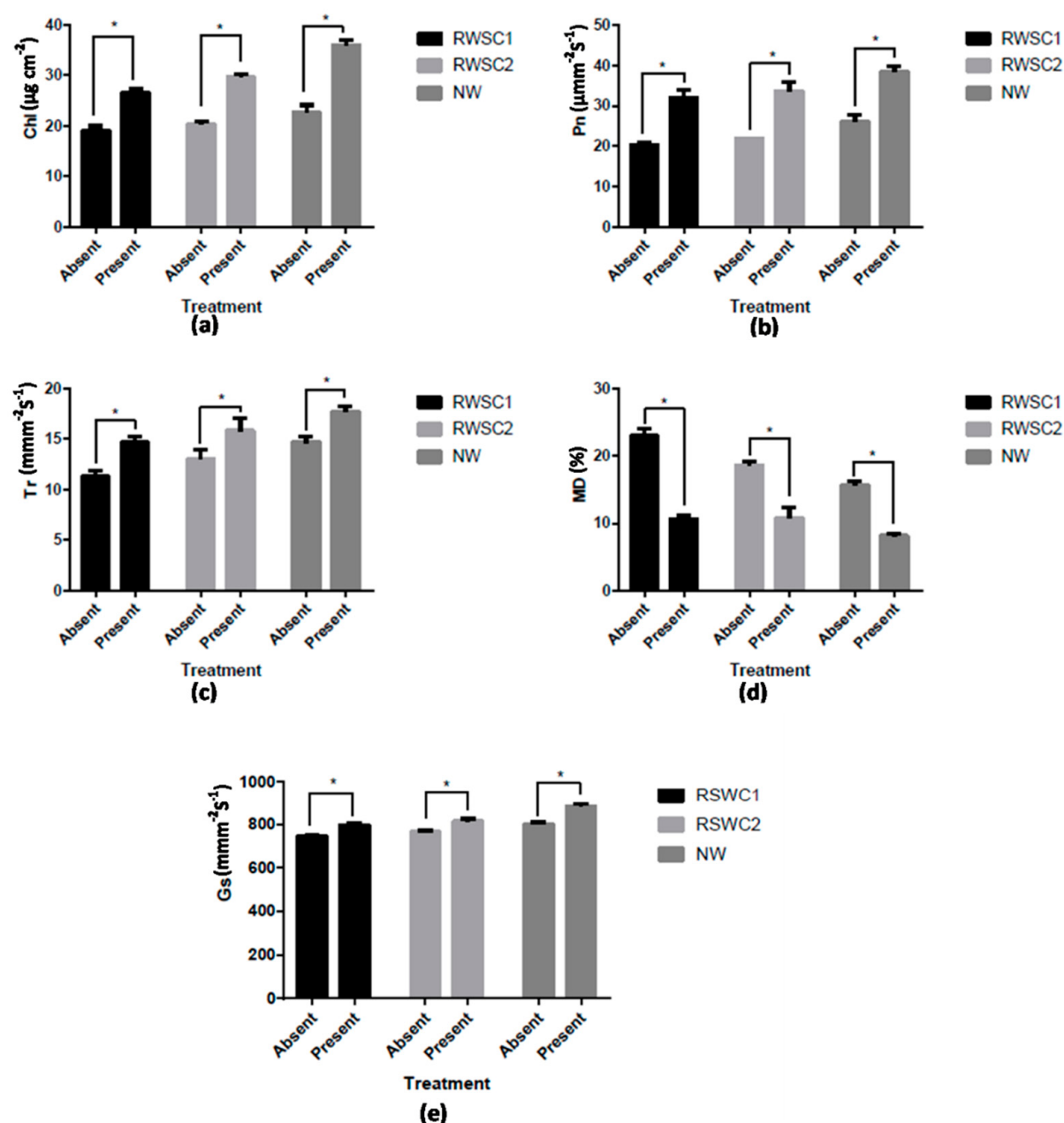


Figure 1. Effect of normal irrigation water (NW) and different Red Sea water concentrations (RSWC1 and RSWC2) on different physiological parameters of Jojoba under the presence and absence of magnetic field. Pn, photosynthesis rate; Tr, transpiration rate; MD, membrane damage; Gs, stomatal conductance. Values shown are mean estimates averaged on different days from seedling stage to two months during trireplicate experiments at $p \leq 0.05$.

3.2. Antioxidant Enzymes and Proline Contents

Presence of MF showed statistically significant changes in the effects of saline water treatments on antioxidant enzymes SOD, CAT and POD, in addition to proline, compared with the control (Figure 2). Under MF, all treatments of saline water depicted significant increase in the activity of antioxidant enzymes SOD, CAT and POD, however this increase was maximum at NW while minimum at RSWC1 (Figure 2a,b,d). On the other hand, compared to control, all saline water concentrations treated with MF showed significant decreases in proline content of jojoba leaves, which was the highest at NW while the lowest at RSWC1 (Figure 1c).

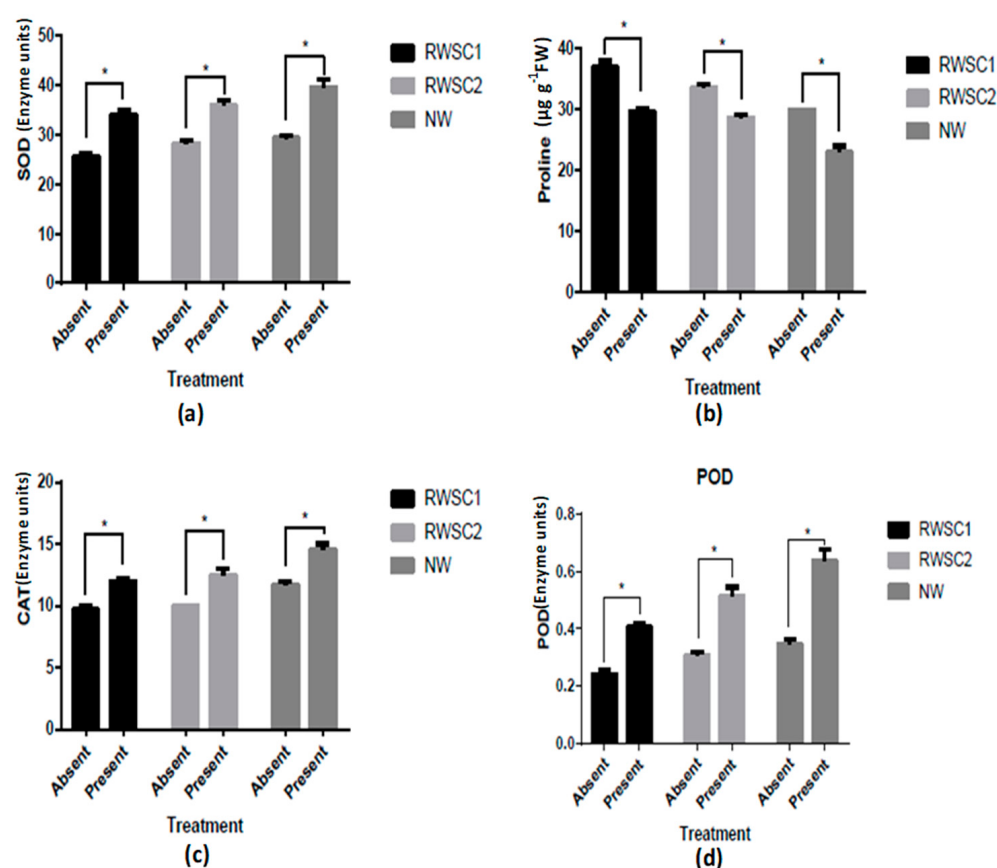


Figure 2. Effect of normal irrigation water (NW) and different Red Sea water concentrations (RWS1 and RWS2) on different antioxidant enzymes and proline of Jojoba under the presence and absence of magnetic field. SOD, superoxide dismutase; CAT, catalase; POD, peroxidase. Values shown are mean estimates averaged on different days from seedling stage to two months during trireplicate experiment at $p \leq 0.05$.

3.3. Agronomic Parameters

All saline water treatments significantly altered the values of agronomic parameters such as GP, GR, SL and RL under the presence of magnetic field (Figure 3). In the presence of MF, the parameters GP, SL and RL demonstrated maximum increase, however this increase was the highest for NW and the lowest for RWS1 (Figure 3a,c,d). Additionally, under all treatments of magnetized saline water, jojoba seeds took less days to germinate compared with their respective controls, however this reduction in days was the highest for NW and the lowest for RWS1 (Figure 1b).

3.4. Correlation Analysis

All traits showed significant correlations with each other under the influence all treatments. During paired combination, all traits varied in same direction except proline, GR and MD, which varied in the opposite direction (Table S1). Traits such as Chl, Gs, Pn, Tr, SOD, CAT, POD, GP, SL and RL depicted positive association during pairing, however, this pairing was statistically more significant during the presence of MF compared to the absence of MF. On the other hand, the traits proline, GR and MD showed positive correlation but negative correlation with aforementioned traits, although this association was comparatively weak in the presence of MF compared to the absence of MF (Table S1). Furthermore, all traits illustrated statistically significant correlation under all treatments of saline water, however this correlation was comparatively stronger at NW compared to RWS1 and RWS2 (Table S2). Overall, the parameters Chl, Gs, Pn, Tr, SOD, CAT, POD, GP, SL and RL showed positive paired correlation, while proline, GR and MD depicted

positive paired correlation among themselves and negative correlation with the rest of the traits (Table S2).

3.5. PCA and Heatmap Analysis

PCA analysis of different concentrations of saline water revealed significant dispersion of traits at NW when compared to RSWC1 and RSWC2 (Figure 4). Additionally, all traits showed clear dispersion during both the presence and the absence of MF (Figure 5). Furthermore, the heatmap summarized the responses of physiological traits, antioxidant enzymes and agronomic traits to the presence and absence of MF (Figure 6). In the context of response to the presence and absence of MF and the association of traits, the heatmap divided the concentrations into two dendrograms. RSWC1 formulated one dendrogram cluster while RSWC2 and NW formulated another dendrogram cluster (Figure 6). All traits illustrated differential levels of association varying from positive to negative extremes for all concentrations of salinity under the absence and presence of MF (Figure 6).

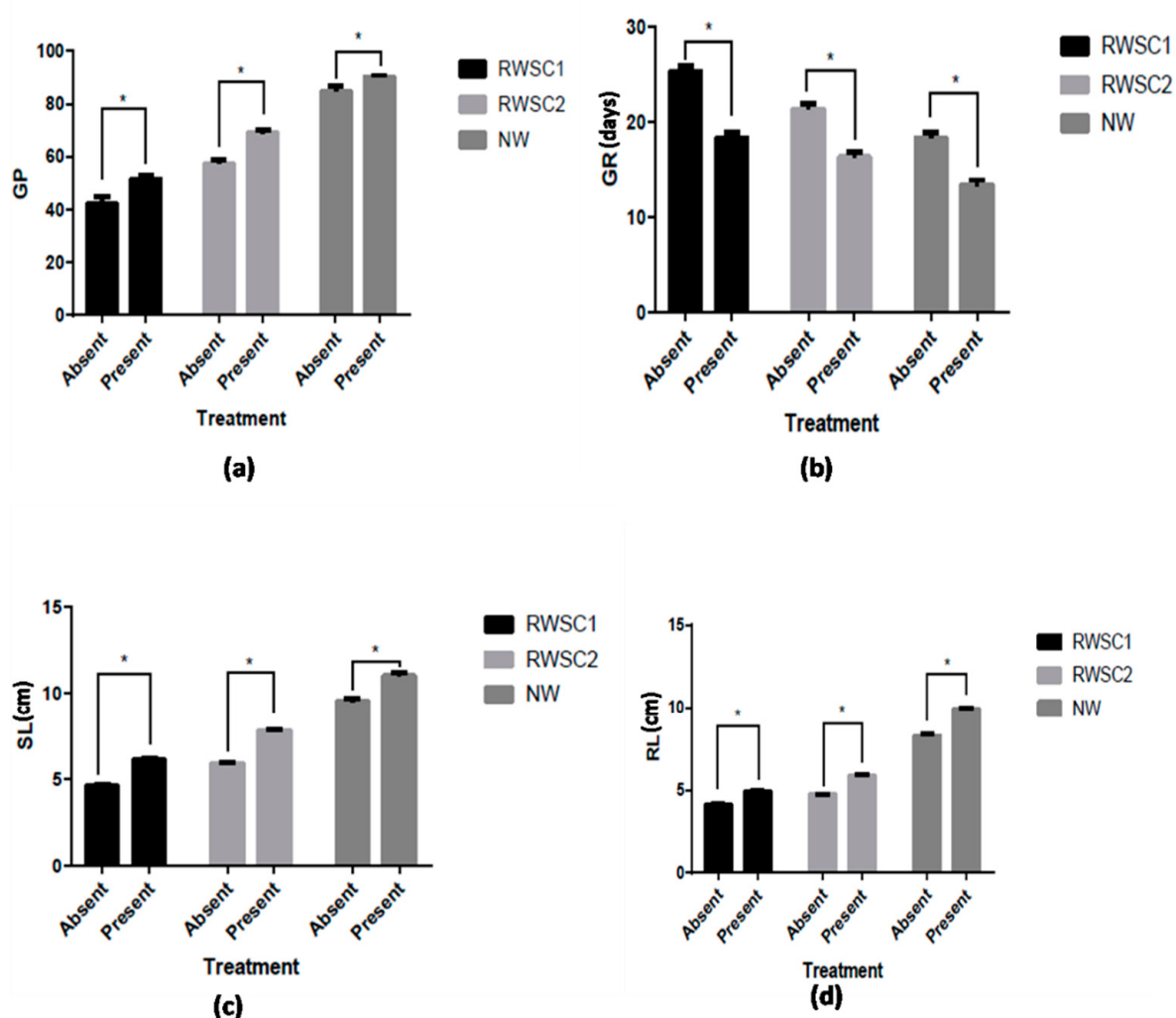


Figure 3. Effect of normal irrigation water (NW) and different Red Sea water concentrations (RSWC1 and RSWC2) on different agronomic parameters of jojoba under the presence and absence of magnetic field. GR, growth rate; GP, germination percentage; SL, shoot length; RL, root length. Values shown are mean estimates averaged from randomly selected plants during trireplicate experiment at $p \leq 0.05$.

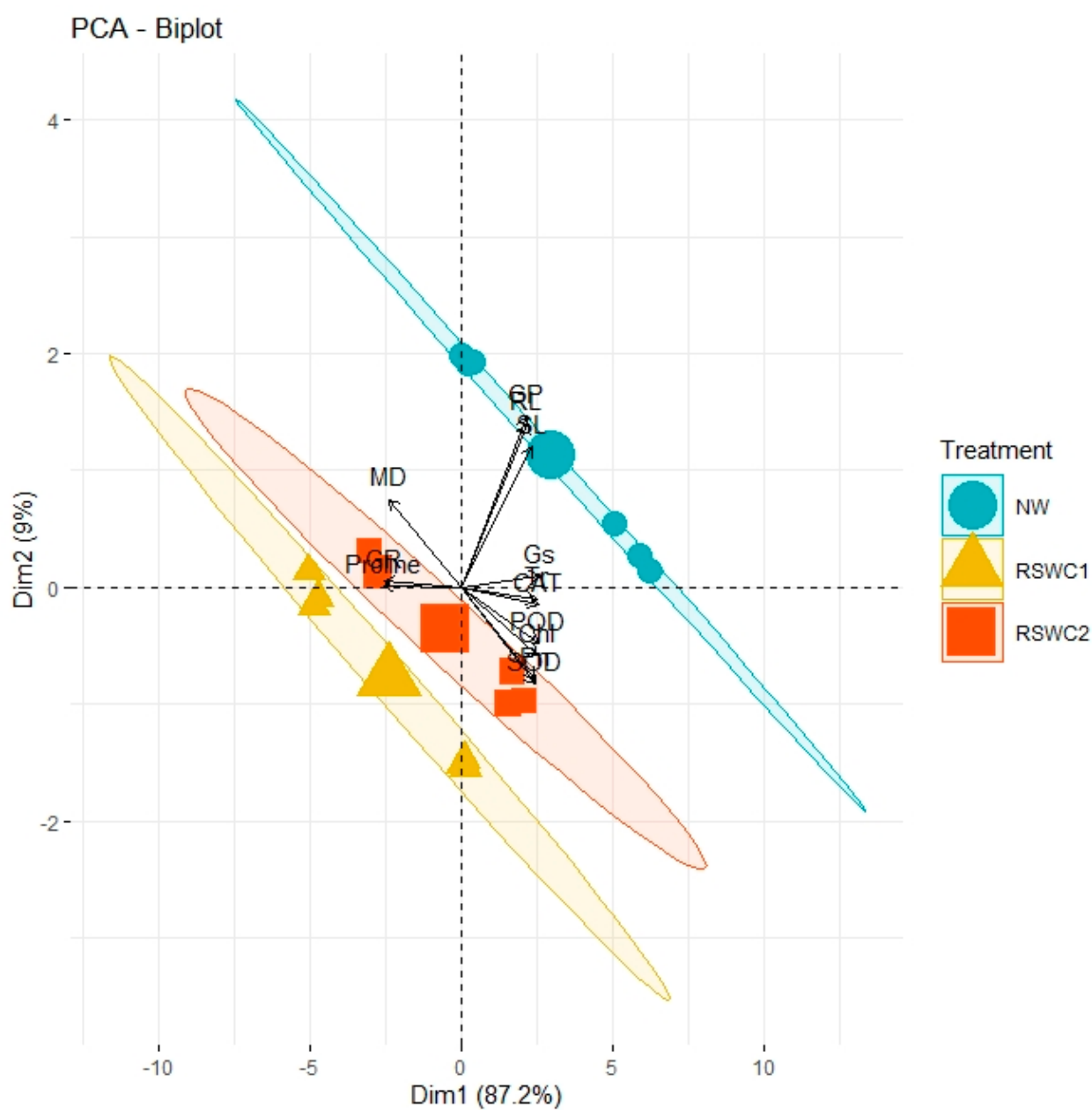


Figure 4. PCA biplot of physiological traits, antioxidant enzymes and agronomic parameters grouped with respect to their similarity and dissimilarity at different dilutions of irrigation water. The vectors drawn from biplot origin indicate positive and negative correlation among traits. Gs, stomatal conductance; Tr, transpiration rate; MD, membrane damage; Pn, photosynthesis rate; SOD, superoxide dismutase; CAT, catalase; POD, peroxidase; GR, germination rate; GP, germination percentage; SL, shoot length; RL, root length.

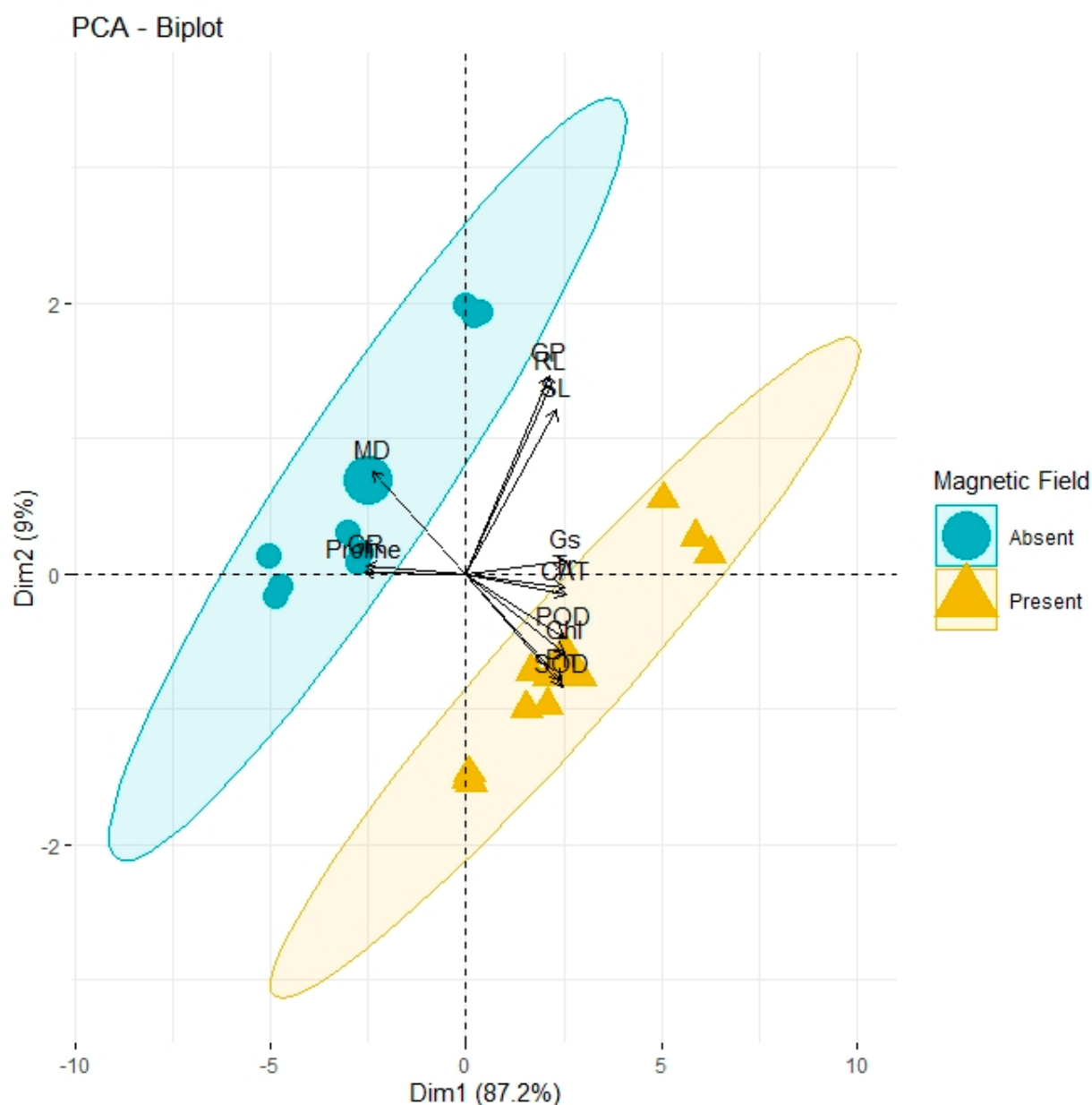


Figure 5. PCA biplot of physiological traits, antioxidant enzymes and agronomic parameters grouped with respect to their similarity and dissimilarity during absence and presence of magnetic field. The vectors drawn from biplot origin indicate positive and negative correlation among traits. Gs, stomatal conductance; Tr, transpiration rate; MD, membrane damage; Pn, photosynthesis rate; SOD, superoxide dismutase; CAT, catalase; POD, peroxidase; GR, germination rate; GP, germination percentage; SL, shoot length; RL, root length.

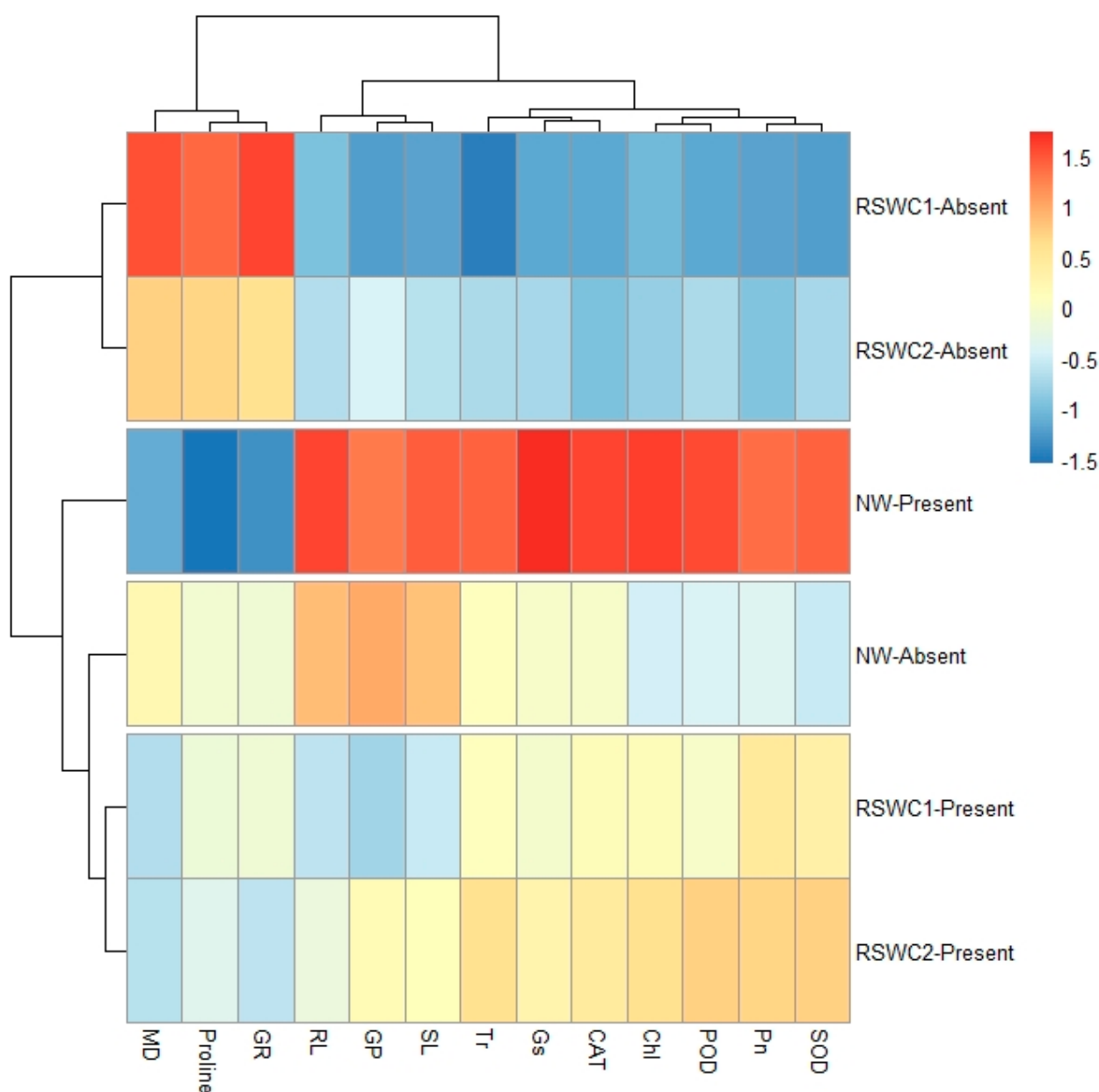


Figure 6. Heat map cluster chart illustrating the responses of physiological traits, antioxidant enzymes and agronomic characteristics to different concentrations of irrigation water (NW, normal irrigation water; RSWC1, Red Sea water concentration 1; RSWC2, Red Sea water concentration 2) under the presence and absence of magnetic field. The color bands express the varying association of traits. Gs stomatal conductance; Tr, transpiration rate; MD, membrane damage; Pn, photosynthesis rate; SOD, superoxide dismutase; CAT, catalase; POD, peroxidase; GR, germination rate; GP, germination percentage; SL, shoot length; RL, root length.

3.6. SEM-based Micrographs

The SEM micrographs attained at all concentrations of magnetically treated water revealed visible differences in the assimilation of elements in the leaves of jojoba (Figure 7). The peaks in spectrographs revealed clear differences in the counts of elements, that showed each concentration behaves differently under the influence of MF. Moreover, the broader peak area at NW reflected the highest assimilation of nutrients compared to RSWC1 and RSWC2.

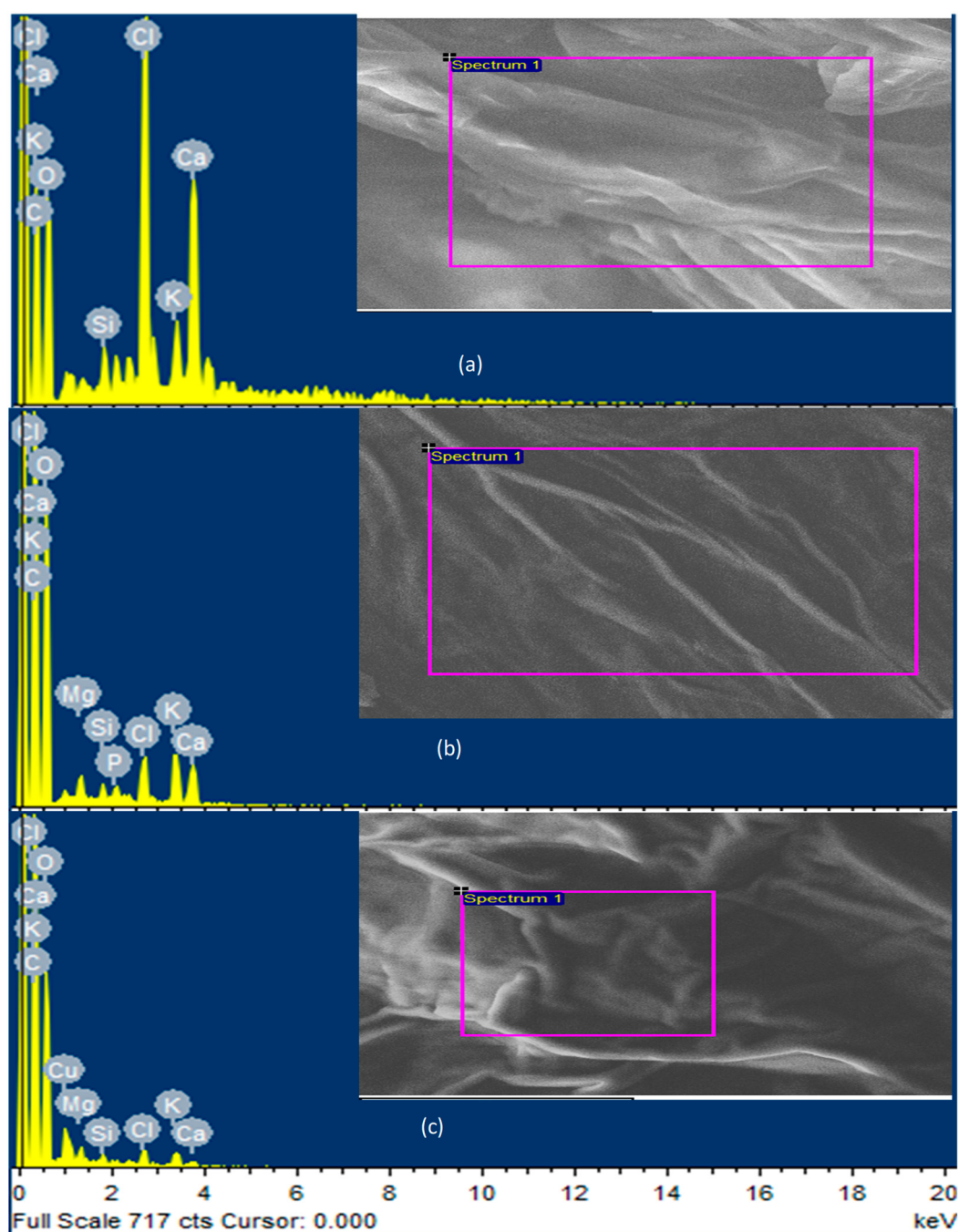


Figure 7. Scanning electron micrographs showing the elemental distribution in the leaves of Jojoba under different concentrations of saline water in the presence of MF. (a) NW [2.11 DSm^{-1}] (b) RSWC1 [10% sea water (5.61 DSm^{-1})] (c) RSWC2 [12.5% sea water (7.01 DSm^{-1})].

4. Discussion

Salinity is hitherto considered as a major constraint impairing crop production in different regions of the world. For mitigating its impacts, various land reclamation and modulation techniques have been used that have proved to be expensive to some extent. In this regard, the current study explored the impact of MF in reducing the damaging impact of salinity on jojoba plants. In fact, MF has a tendency to improve various basic properties of water, such as surface tension, pH and polarization, leading towards increased water uptake by plants [10]. Therefore, the application of magnetized water positively impacts various attributes of plants such as seed germination, root length, shoot length,

Pn, Gs, Chl and antioxidant enzymes, as proved by various studies [7,9,24,25]. In the current study, MF correspondingly improved aforementioned traits in jojoba (Figure 1). Application of magnetized water improves the photosynthetic efficiency by enhancing the level of photosynthetic pigments such as chlorophyll [24]. Therefore, increased Pn is associated with stomatal opening that accelerates other linked traits such as Gs and Tr, as reported in the current study (Table S1). Additionally, a high level of salinity disturbs the osmotic balance within plant cells, leading to proline accumulation and more electrolyte leakage owing to cell membrane damage (MD) [1]. Likewise, the current study reported high level of MD in jojoba plants watered with high levels of salinity such as RSWC1 (Figure 1). Moreover, application of MF to saline water readjusts its osmotic balance and polarity, reducing its deleterious impacts to some extent [4]. Correspondingly, the current study reported significant reduction in MD for all levels of saline water treated with MF (Figure 1).

In addition to this, many studies have explored the effects of MF on the activities of antioxidant enzymes, for instance SOD, CAT and POD, in plant cells. In this regard, Bhardwaj et al. [10] reported an increase in the activities of SOD by 8%, and CAT by 83% in cucumber (*Cucumis sativus* L.) under MF compared to control. An analogous experiment was conducted on different plant species such as maize (*Zea mays*) [12] and artichoke (*Cynara scolymus*) [11], which found significant increases in the activities of antioxidant enzymes (CAT, SOD and POD) under saline water integration with MF. Similar to their findings, the current study reported a dynamic increase in the activities of antioxidant enzymes SOD, CAT and POD due to MF compared to control (Figure 2). Correspondingly, Hajnorouzi et al. [13] reported the alleviation of excessive ROS in maize seedlings when they were treated with magnetized water, however contrarily, they reported a decline in the activity of SOD. Similarly, Çelik et al. [26] found an increase in the activities of enzymes SOD and CAT in plant leaves due to MF treatment of irrigation water. In fact, salinity stress causes oxidative stress in plants, leading to ROS production causing MD and electrolyte leakage due to membrane lipid peroxidation [27–30]. However, MF tends to impede the damage of salinity by triggering the activities of antioxidant enzymes that not only hamper the production of ROS and free radicals, but also protect MD by resisting lipid peroxidation [25]. Likewise, the current study noticed that negative association between antioxidant enzymes and MD increased with elevation in the levels of salinity (Table S2). However, this association weakened in the presence of MF compared with the control (Table S1).

On the other hand, water treated with MF can increase various plant agronomic parameters including GP, GR, SL and RL [31,32], as reported in current study (Figure 3). In fact, MF alters some basic properties of water including surface tension, polarity, ionic strength and pH, intensifying its uptake by the plant. In this regard, Hirota et al. [33] found increases in GP and GR of cucumber seeds when supplemented with magnetically treated water when compared with a control. Likewise, the current study recorded dynamic improvement in GP and GR of jojoba seeds when watered with magnetic saline water when compared with a respective control (Figure 3). In addition to this, Fernandez et al. [34] reported a consistent increase in growth-associated traits such as RL and SL in onion seedlings when they were irrigated with magnetically treated water. The current study confirmed their findings by reporting increases in SL and RL of jojoba seedlings in the presence of MF when compared to the control (Figure 3).

A high level of salinity has the tendency to generate ROS within plant cell, leading to chlorophyll deterioration and increased MD that results in reduced Pn, Gs and Tr, causing a decrease in growth of SL and RL, as reported in the present study, particularly at RSWC1 (Figure 4, Table S2). However, magnetic treatment of water impedes the negative impact of salinity, that, in consequence, accelerates the activities of antioxidant enzymes SOD, CAT and POD, leading to more ROS scavenging [26], reduced MD, less proline [30] and increased Pn, Gs and Tr [24], resulting in high RL and SL [32], as confirmed by the present study (Figure 5 and Table S1). The current study disclosed remarkable impact on the

association of physiological traits, antioxidant enzymes and agronomic parameters on all three treatments of salinity, as confirmed by a heat map (Figure 6) clustered diagram, however this association was more dramatic at NW compared to RSWC1 and RSWC2. Moreover, impact of MF on elemental distribution (Figure 7) in the leaves of jojoba further authenticated its dynamic role in improving plants' nutrient profiles. Therefore, the current study concluded that magnetic treatment of existing saline water in Saudi Arabia or Red Sea water dilution can be an effective tool for boosting agriculture to some extent.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/su132313199/s1>, Table S1: Significance of correlation among different traits of jojoba during presence and absence of MF, Table S2: Significance of correlation among different traits of jojoba during different concentrations of saline water.

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Conflicts of Interest: The authors declare no conflict of interest.

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