Australian Journal of Crop Science AJCS
AJCS 8(12):1615-1624 (2014) ISSN:1835-2707

Effects of salt stress on growth, antioxidant enzyme activity and some other physiological parameters in jojoba [*Simmondsia chinensis* (Link) Schneider] plant

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Abstract

Reports show that salinity is a major problem that negatively affects agricultural activities all over the world. Although the importance of jojoba as a new industrial crop is known, but information concerning the relation between salt stress and physiological parameters such as antioxidant enzyme activity as well as membrane damage in jojoba has not been reported yet. This study was carried out to investigate the effect of different salinity concentrations i.e. 0, 4, 6, 8. 10 and 12 dSm^{-1} NaCl on plant growth, leaf measurements, antioxidant enzyme activity and some biochemical and mineral accumulation of jojoba (*Simmondsia chinensis* (Link) Schneider) plant grown in saline-alkaline soil. Salinity treatments significantly decreased plant height, number of both branches and leaves compared to the control. Salinity stress significantly reduced leaf area, stomatal density, relative water content (RWC), leaf chlorophyll content, N^{+}, PO_4^{-3}, K^{+}, Ca^{+2} and Mg^{+2}, compared to control. Meanwhile, sodium, chloride and total soluble sugars were significantly increased with increasing salinity concentration and the homeostasis of minerals was disturbed. The Na^{+}:K^{+} ratio was increased with increasing salinity level. Membrane permeability, proline accumulation and antioxidant enzymes activities (SOD, CAT and POD) were increased in salt stressed plants. The increment of both antioxidant enzyme activities and proline accumulation may suggest that they are involving as a part of the defense against salt stress in jojoba plant.

Keywords: salinity, jojoba, growth parameters, antioxidant enzymes, membrane damage, proline, nutrient status.
Abbreviations: RWC_ Relative water content, TSS_Total Soluble Sugars, ANOVA_Analysis of variance, dSm^{-1}_deciSiemens per meter, SOD_superoxide dismutase, CAT_catalase, POD_peroxidase, NaCl_Sodium Chloride, EC_ Electric Conductivity

Introduction

The sustainability of agriculture production in many areas of the world including North and South America, Asia, Europe, Australia and Africa is at risk due to soil salinization (Rengasamy, 2006). Jojoba (*Simmondsia chinensis* (Link) Schneider), belongs to Simmondsiaceae family, is a relatively new crop that is adapted to hot and dry climates. It is a new industrial crop being grown commercially in hot arid and semiarid regions as it is considered to tolerate fairly high levels of salinity and water stress and; therefore, has a better chance for economic and agricultural success (Botti et al., 1998). Jojoba has become an attractive alternative crop because of the promising commercial applications for its seed oil in cosmetics and the useful properties of the liquid wax obtained from the seeds (Brown et al., 1996).

Salinity is a major problem that negatively affects agricultural activities in many regions in the world and salinity problems increase with increasing salt concentration in irrigation water (Abdel Latef, 2010). Moreover, salinity reduces the productivity of agricultural land and threatens the agricultural sustainability (Mckee et al., 2004). Despite the necessity of chloride as a micronutrient for all higher plants and as mineral nutrient for many halophytes, salt accumulation may convert agricultural areas in unfavorable environments, reduce local biodiversity, limit growth and reproduction of plants, which may lead to toxicity in non salt-tolerant plants, known as glycophytes (Ashraf and Harris, 2004; Parida and Das, 2005). The effects of salinity are generally summarized as water stress, salt stress and stress due to ionic imbalance (Greenway and Munns, 1980).

Sodium chloride (NaCl) is the most commonly encountered source of salinity (Li et al., 2006). In our previous experiments, we observed that jojoba seedlings tolerated salinity to 6 dSm^{-1} level at the first year of cultivation. Therefore, we expected that it will tolerate higher levels of NaCl at advanced growth stages. Benzioni et al. (1996) reported that jojoba has been developed reasonably well under salinities of 4 dSm^{-1} or at 8 dSm^{-1}. Exposure of plants to extreme conditions such as high salinity causes a diverse set of physiological, morphological and developmental changes (Jampeetong and Brix, 2009). Salinity adversely affects the vegetative growth characteristics and dry weight (Gunes et al., 2007; Chookhampaeng et al., 2008; Shoresh et al., 2011) and leaf area (Khalid and Cai, 2011; Abdel Latef and Chaoxing, 2011).

Relative water content (RWC) in leaves is known as a method to determine the plant water status, reflecting the metabolic activity in tissues (Flower and Ludlow, 1986). The reduction in RWC is an indicator for leaf turgor loss that resulted in limited water availability for the cell extension process (Katerji et al., 1997). Similar reports have been

published in many plant species under salinity stress conditions (Shalhevet, 1993; Thind and Malik, 1988; Srivastava et al., 1998; Gadallah, 2000; Tuna et al., 2008).

Salinity stress also affected some physiological parameters such as chlorophyll content, total soluble sugars and proline content. In this regard, several authors indicated that the chlorophyll content was significantly decreased as a result of salt stress (Tuna et al., 2008; Khalid and Cai, 2011; Shoresh et al., 2011; Abdel Latef and Chaoxing, 2011; Celik and Atak, 2012). On the other hand, salt stress affected proline content and total soluble sugars in an opposite manner. The accumulation of proline in leaves (Eraslan et al. 2007; Tuna et al. 2008; Heidari and Jamshid, 2011; Celik and Atak, 2012) and total soluble sugars (Chookhampaeng et al., 2008; Khalid and Cai, 2011) have been reported under salt stress.

The ions leakage is a well-known parameter for determination of oxidative damage to membranes (Lu et al., 2008), which expresses membrane dysfunction as the increase in permeability and leakage of electrolytes from the cell. Salt stress increased electrolyte leakage as well as membrane permeability (Gunes et al., 2007; Tuna et al., 2008; Shoresh et al., 2011) and membrane damage was more pronounced in NaCl treatment (Eraslan et al., 2007). The effects of salt stress on mineral content of leaves have been studied in a range of plant species. Salinity decreased N^{+}, PO_4^{-3}, K^{+}, Ca^{+2} and Mg^{+2} concentrations; however, it increased the accumulation of Na^{+} and Cl^{-} in leaves (Grattan and Grieve, 1999; Gunes et al., 2007; Giri et al., 2007; Tuna et al., 2008; Khalid and Cai, 2011). It is widely accepted that competition occurred between Na^{+} and K^{+} leads to a reduction of internal K^{+} level at high external level of NaCl and; hence, the Na^{+}:K^{+} ratio is increased under salinity stress (Tuna et al., 2008; Shoresh et al., 2011).

Salinity induces oxidative stress in plants (Hajiboland and Joudmand, 2009). Exposure of plants to salinity is known to induce formation of reactive oxygen species (ROS), which are involved not only in damage mechanisms but also in cell growth processes (Bernstein et al., 2010). Plants employ different mechanisms and repair systems that can reduce the oxidative damage caused by ROS (Abdel Latef, 2010). The most common mechanism for detoxifying ROS synthesized during stress response is the induction of ROS-scavenging enzymes, such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX) (Agarwal and Pandey, 2004; Chookhampaeng et al., 2008; Bernstein et al., 2010; Abdel Latef and Chaoxing, 2011). There are no established criteria concerning the response of antioxidant defense system to stress factors in plants since some investigators reported that the antioxidant enzyme activity is increased and in some cases decreased when plants exposed to salt stress (Porce et al., 2003; Hajiboland et al., 2010). In addition, enzyme activity is also used as an indicator for the salinity stress (Agarwal and Pandey, 2004). Hence, the mechanisms that reduce ROS and increase antioxidant enzyme system in plants have important roles in imparting tolerance in plants under environmental stress conditions (Abd El-baky et al., 2003). The effects of salt stress on antioxidant responses have been studied in a range of plant species. The salinity-induced changes in activities of antioxidant enzymes are well documented (Eraslan et al., 2007; Tuna et al., 2008; Bernstein et al., 2010; Abdel Latef and chaoxing, 2011; Heidari and Jamshidi, 2011). However, to our knowledge, information concerning the relation between salt stress and antioxidant enzyme activity as well as membrane damage in jojoba has not been previously reported.

In the present investigation, an attempt was made to study the effects of salt stress on growth and physiological parameters such as chlorophyll, total soluble sugars, proline content, membrane permeability and antioxidant enzyme activity of jojoba plant. Moreover, relative water content and mineral accumulation of leaves were also investigated.

Results

Vegetative growth affected by salinity

Data presented in Table 1. show that jojoba growth was negatively affected by different salinity treatments. The plant height, branch number/plant, plant fresh and dry weights were significantly decreased with increasing salinity levels relative to control. The shortest plants with the lowest branch number were obtained by the highest salinity level (12 dSm^{-1}). This treatment also resulted in the lightest fresh and dry weights of jojoba plants. On the other hand, control treatment recorded the highest values of the previous growth characteristics.

Relation between leaf measurements and salinity

The results indicate that leaf number and its area were significantly reduced by applying different salinity treatments compared with the control (Table 2). The increase of the salinity concentration, the decrease of leaf number and its area was observed. The lowest leaf number as well as its area (24.63 and 2.44 cm^2) was recorded by the highest salinity level. However, control plants resulted in the highest values in this respect (39.74 and 4.87 cm^2). There were no significant differences between control and 4 dSm^{-1} treatment. There was significant effect of salinity levels on stomatal density of jojoba leaves (Table 2). All salinity levels significantly and gradually decreased stomatal density compared to the control, which recorded the maximum values in this parameter.

Effect of salinity on relative water content (RWC %)

RWC was gradually decreased with increasing salinity concentrations (Fig. 1). A sharp decrease was observed especially with the highest salinity level; however, the water content was maintained by untreated control. The differences were significant between control and higher salinity levels (8, 10 and 12 dSm^{-1}). However, there were no significant differences between control and 4 or 8 dSm^{-1} treatments at 0.05 level.

Response of membrane permeability to salinity

Fig. 2 clearly indicates that membrane permeability of jojoba leaves was significantly increased as a result of applying different salinity treatments. The lowest electrolyte leakage was observed in untreated leaves. However, the highest value in this respect was obtained by the highest salinity level.

Proline content as affected by salinity

Salinity stress stimulated the accumulation of proline in jojoba leaves in the current study (Fig. 3). The proline content was gradually increased with increasing salinity levels. The differences between any salinity treatment and control were significant except for 4 dSm^{-1} treatment at 0.05 % level.

Table 1. Plant height, branch number as well as fresh and dry weight of jojoba plant as affected by different salinity levels applied as NaCl (dSm^{-1}).

NaCl (dSm^{-1})	Plant height (cm)	Branch number/plant	FW/plant (g)	DW/plant (g)
Control	47.52 ± 0.56 a	3.36 ± 0.11 a	41.66 ± 0.23 a	14.72 ± 0.05 a
4	46.37 ± 0.33 b	3.11 ± 0.09 b	39.43 ± 0.04 b	13.84 ± 0.09 b
6	42.67 ± 0.44 c	2.89 ± 0.08 b	35.72 ± 0.34 c	11.86 ± 0.09 c
8	38.82 ± 0.35 d	2.42 ± 0.14 c	30.18 ± 0.10 d	11.13 ± 0.05 c
10	36.34 ± 0.42 e	2.17 ± 0.10 d	28.57 ± 0.12 e	9.37 ± 0.06 d
12	35.22 ± 0.45 f	1.86 ± 0.13 e	26.42 ± 0.12 f	8.69 ± 0.06 e

Values (mean ± S.D.) are the average of two independent experiments (n = 12). Means in the same column followed by different letters are significantly different of each other at 0.05 level.

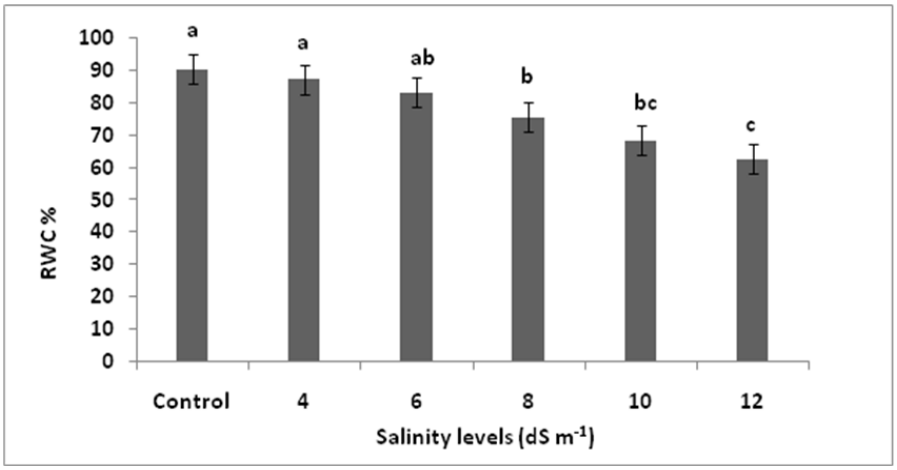

Fig 1. Effect of salt stress on relative water content (RWC) of jojoba leaves. The values (mean ± S.E.) are the average of two independent experiments (n = 12). Bars had different letters are significantly different of each other at 0.05 level.

Table 2. Leaf number per jojoba plant, leaf area (cm^2) and stomatal density as affected by different salinity levels applied as NaCl (dSm^{-1}).

NaCl (dSm^{-1})	Leaf number/plant	Leaf area (cm^2)	Stomatal density
Control	39.74 ± 0.84 a	4.87 ± 0.15 a	83.27 ± 0.47 a
4	36.56 ± 0.43 b	4.82 ± 0.13 a	81.35 ± 0.40 b
6	32.18 ± 0.25 c	3.92 ± 0.29 b	79.62 ± 0.35 b
8	28.41 ± 0.44 d	3.35 ± 0.12 c	75.24 ± 0.42 c
10	26.35 ± 0.35 e	2.92 ± 0.24 d	73.53 ± 0.44 d
12	24.63 ± 0.41 f	2.44 ± 0.32 e	71.18 ± 0.41 e

Values (mean ± S.D.) are the average of two independent experiments (n = 12). Means in the same column followed by different letters are significantly different of each other at 0.05 level.

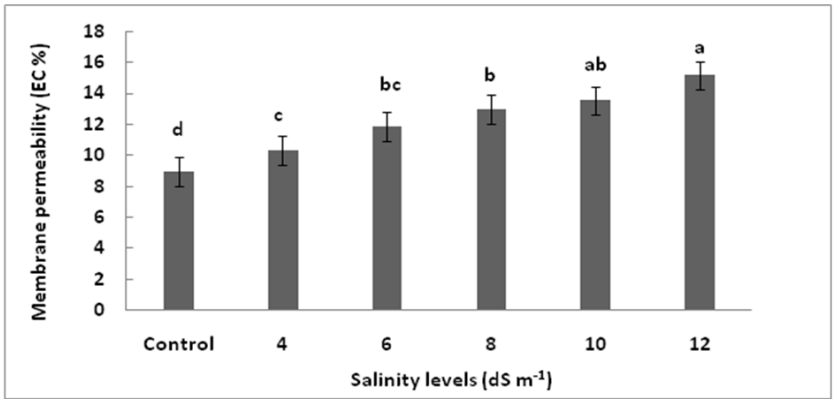

Fig 2. Effect of salt stress on membrane permeability of jojoba leaves. The values (mean ± S.E.) are the average of two independent experiments (n = 12). Bars had different letters are significantly different of each other at 0.05 level.

Impact of salinity on total chlorophyll content

As shown in Fig. 4, the total chlorophyll content was gradually decreased with increasing salinity concentration. The highest chlorophyll content was observed in untreated plants; meanwhile, the lowest value was recorded by 12 dSm^{-1} salinity treatment.

Relation between total soluble sugars and salinity

Total soluble sugars were increased by saline irrigation water. All salinity levels induced total soluble sugars compared with the control (Fig. 5). Increasing salinity concentrations led to a significant and a gradual increase in total soluble sugars in jojoba leaves and reached its maximum value by applying the highest salinity concentration.

Antioxidant enzymes activities as affected by salinity

Fig. (6) reveals that SOD, CAT and POD enzyme activities were stimulated as a result of salt stress. All salinity levels used in this experiment induced the activity of the previous enzymes, compared to control. The highest salinity level showed the highest stimulation effect; however, untreated plants recorded the lowest values in this concern.

Effect of salinity on mineral content

The chemical analysis based on dry weight of jojoba leaves as affected by salinity concentrations was presented in Table 3. The obtained results indicate that N^{+}, PO_4^{-3}, K^{+}, Ca^{+2} and Mg^{+2} contents were significantly decreased using different salinity levels, compared with the control. The highest contents of the previous elements were observed in untreated plants; however, the lowest values were recorded by the highest salinity concentration, in which differences among treatments were significant at 0.05 level. Increasing salinity concentration gradually increased Na^{+} and Cl^{-} contents and reached their maximum values using 12 dSm^{-1} treatment and the differences were significant at 0.05 level. Otherwise, the Na^{+}:K^{+} ratio was gradually increased with increasing NaCl level. The highest value was obtained by applying 12 dSm^{-1} treatment (Table 3).

Discussion

In the current study, the growth of jojoba plant was negatively affected by salt stress. The plant height, branch number, leaf number, plant fresh and dry weights, leaf area were gradually decreased with increasing salinity concentrations (Tables 1 and 2). These negative effects of salt stress may be due to reduction of both cell division and cell enlargement (Yasseen et al., 1987). Otherwise, inhibition of shoot growth has been considered a whole plant adaptation to salt stress (Mulholland et al., 2003; Qaderi et al., 2006). The reduction of growth due to inadequate water uptake is a common indicator of salt stress (Munns, 2002; Borsani et al., 2003). The suppression of growth under salt-stress may be also due to direct effects on ion toxicity especially Na^{+} and Cl^{-} or indirect effects of saline ions that cause soil/plant osmotic imbalance (Abdel Latef, 2010; Hajiboland et al., 2010). These results support those obtained by Chookhampaeng et al. 2008; Shoresh et al. 2011; Abdel Latef and Chaoxing, 2011; Khalid and Cai, 2011). On the other hand, osmotic stress caused by salinity was reported not to be the limiting factor for plant growth reduction but rather salt toxicity (Roshandel and Flowers, 2009). Although jojoba plant is considered tolerant to high levels of salinity (Botti et al., 1998), the growth reduction observed by salinity may be due to the age of seedlings used in this study as one year old seedlings was cultivated in saline-alkaline soil. Interestingly, stomatal density was decreased with increasing salinity levels, compared to control. The reduction of stomatal density could be explained through the reduction of leaf area as our data indicated in Table 2. Botti et al. (1998) reported that the stomatal density and leaf area were decreased with increasing salinity levels. This reduction may be occurred to make an adaptation to salt and inhabitation of its uptake. The RWC in leaves was dramatically decreased by increasing salt stress compared to the control (Fig. 1). RWC is considered as an important parameter for water statues. Hence, the reduction in water content under salt stress was reflected in decreasing the previously mentioned growth parameters in current study. Katerji et al. (1997) reported that the decrease in RWC indicated a loss of turgor that resulted in limited water availability for the cell extension process. The reduction in RWC may be occurred as a result of lower water availability under stress conditions (Shalhevet, 1993), or root systems, which are not able to compensate for water lost by transpiration through a reduction of the absorbing surface (Gadallah, 2000). These results are in accordance with the findings of Tuna et al. (2008) who reported that the RWC was significantly decreased with increasing salinity levels.

The decrease of chlorophyll as a result of applying salinity treatments may be due to one or more of the following reasons: (1) the formation of proteolytic enzymes such as chlorophyllase, which is responsible for the chlorophyll degradation (Sabater and Rodriguez, 1978), (2) damaging to the photosynthetic apparatus (Yasseen et al., 1987), (3) the suppression of specific enzymes that are responsible for the synthesis of photosynthetic pigments (Murkute et al., 2006), (4) the salt-induced water stress reduction of chloroplast stoma volume and regeneration of reactive oxygen species which play an important role in the inhibition of photosynthesis seen in salt-stressed plants (Allen, 1995), (5) a reduction in the uptake of minerals i.e. Mg needed for chlorophyll biosynthesis (Sheng et al., 2008), (6) or membrane deterioration (Ashraf and Bhatti, 2000). Our results support the last two reasons because we observed a significant decrease in Mg^{+2} (Table 3) and increase in membrane permeability (Fig. 2) under salt stress. These results are in agreement with many authors who revealed that, the total chlorophyll content of leaves was reduced under salinity (Tuna et al., 2008; Shoresh et al., 2011; Abdel Latef and Chaoxing, 2011; Celik and Atak, 2012). The obtained results showed a significant increase of total soluble sugars in salt stressed plants. This increment may be occurred in order to regulate the osmotic potential under salt stress (Teixeira and Pereira, 2007). Increasing total soluble sugars was increased possibly to sustain metabolism, prolong energy supply and for better recovery after stress relieve (Slama et al., 2007). In addition, the accumulation of total soluble sugars by salt stress has been attributed to impaired carbohydrate utilization (Munns and Jermaat, 1986). These results are comfortable to the others of Chookhampaeng et al. (2008) and Khalid and Cai (2011) who reported that salinity stress increased the activity of sucrose phosphate synthase; the key enzyme in the sucrose synthesis pathway, consequently, the total soluble sugars was increased.

Table 3. Jojoba leaf mineral content as affected by different salinity levels applied as NaCl (dSm^{-1}).

NaCl (dSm^{-1})	N (%)	P (%)	K (%)	Ca (mgg^{-1})	Mg (mgg^{-1})	Na (mgg^{-1})	Cl (mgg^{-1})	Na: K %
Control	2.76 ± 0.14 a	0.46 ± 0.01 a	2.65 ± 0.16 a	17.96 ± 0.46 a	0.74 ± 0.03 a	0.32 ± 0.06 f	6.34 ± 0.32 f	1.21 ± 0.01f
4	2.42 ± 0.10 b	0.41 ±0.03 b	2.58 ± 0.19b	17.64 ± 0.39 a	0.69 ± 0.05 b	0.54 ± 0.08 e	7.52 ± 0.45 e	2.09 ± 0.01 e
6	2.13 ± 0.08 c	0.37 ± 0.02 c	2.43 ± 0.17c	17.21 ± 0.32 b	0.65 ± 0.04 c	0.78 ± 0.11 d	8.89 ± 0.33 d	3.21 ± 0.02 d
8	1.98 ± 0.13 d	0.33 ± 0.03 d	2.31 ± 0.11d	17.04 ± 0.52 b	0.60 ± 0.09 d	0.92 ± 0.06 c	9.76 ± 0.34 c	3.98 ± 0.02 c
10	1.74 ± 0.17 e	0.29 ± 0.02 e	2.21 ± 0.07 e	16.87 ± 0.52 c	0.55 ± 0.08 e	1.05 ± 0.04 b	11.45 ± 0.40 b	4.75 ± 0.04 b
12	1.63 ± 0.23 f	0.21 ± 0.03f	2.07 ± 0.22 f	15.42 ± 0.57 d	0.52 ± 0.06 f	1.19 ± 0.05 a	14.22 ± 0.33 a	5.75 ± 0.04 a

Values (mean ± S.D.) are the average of two independent experiments (n = 12). Means in the same column followed by different letters are significantly different of each other at 0.05 level.

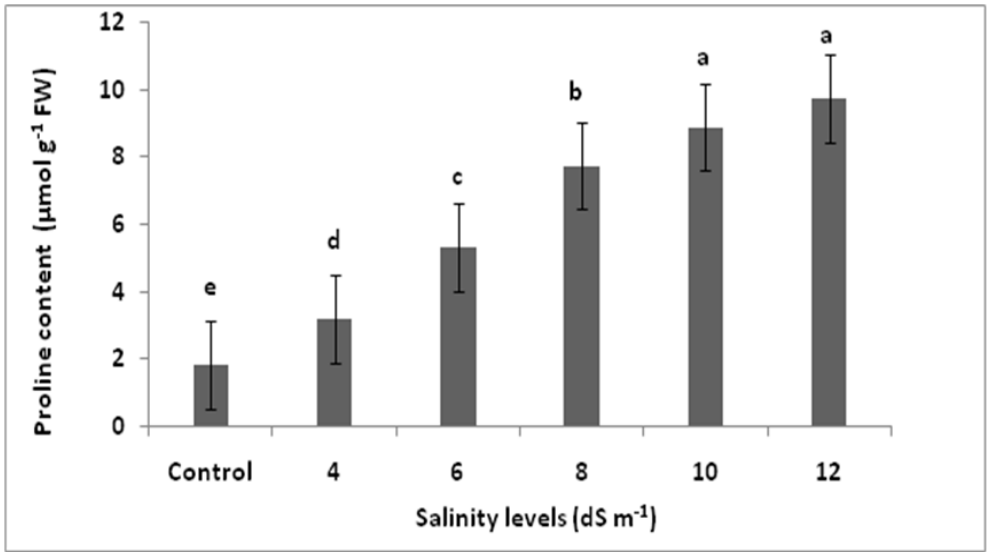

Fig 3. Effect of salt stress on proline content of jojoba leaves. The values (mean ± S.E.) are the average of two independent experiments (n = 12). Bars had different letters are significantly different of each other at 0.05 level.

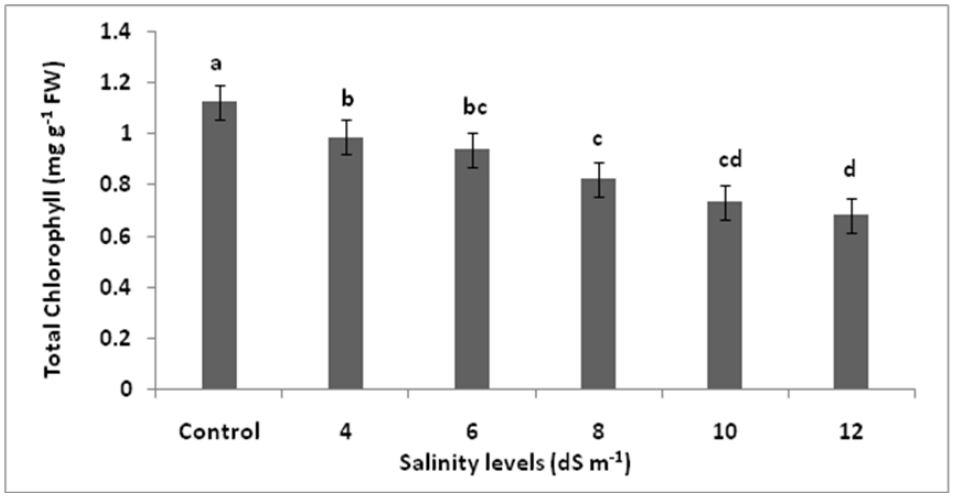

Fig 4. Effect of salt stress on total chlorophyll content of jojoba leaves. The values (mean ± S.E.) are the average of two independent experiments (n = 12). Bars had different letters are significantly different of each other at 0.05 level.

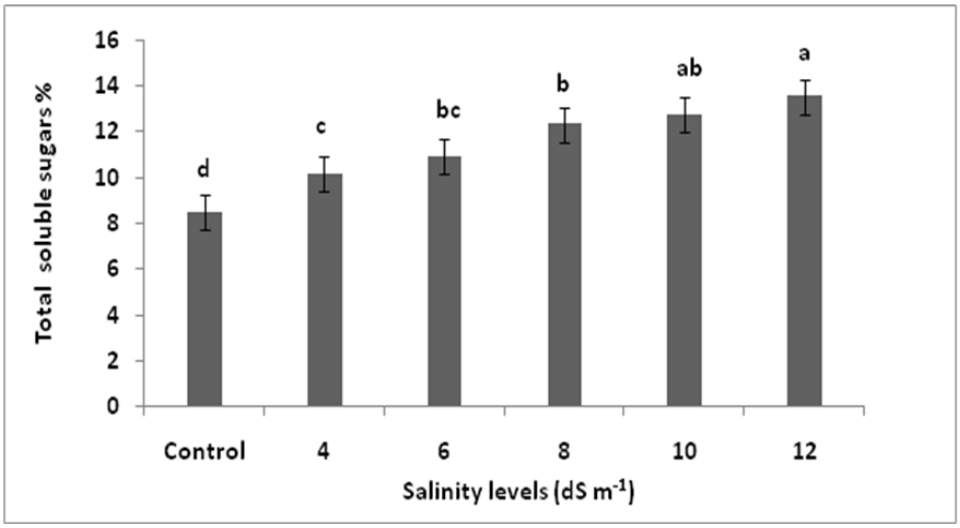

Fig 5. Effect of salt stress on total soluble sugars of jojoba leaves. The values (mean ± S.E.) are the average of two independent experiments (n = 12). Bars had different letters are significantly different of each other at 0.05 level.

In this study, the increment of proline content observed by salinity application is considered a physiological response of plants under salt stress. Proline has a function of osmotic adjustment in plants; however, it protects membranes and enzymes against oxidative stress (Agrawal and Panday, 2004). It has been widely reported that proline may play a role in stress adaptation within the cell (Gilbert et al., 1998). Chookhampaeng et al. (2008) stated that the accumulation of nitrogen-containing compatible solutes including proline is known to function in osmotic adjustment, protection of cellular macromolecules from damage by salts, storage of nitrogen and scavenging of free radicals. Such proline accumulation as a result of salt stress is well-documented (Eraslan et al., 2007; Tuna et al., 2008; Heidari and Jamshid, 2011; Celik and Atak, 2012). In this study, it was observed that N^{+}, PO_4^{-3}, K^{+}, Ca^{+2} and Mg^{+2} contents were reduced. However, Na^{+} and Cl^{-} were increased by salt stress (Table 3). The reduction in nitrogen may be due to the competition between NO_3^{-}/Cl^{-} at the sites for ion transport (Cram, 1983) due to membrane depolarisation caused by sodium accumulation (Suhayda et al., 1990). Moreover, reduction of PO_4^{-3} uptake in saline soils was attributed to precipitation of H_2PO_4 with Ca^{+2} ions in soil and of K^{+} and Ca^{+2} to a competition with Na^{+2} (Marschner, 1995). Our data showed that salt stress caused by NaCl induced a progressive absorption of Na^{+} and Cl^{-} in jojoba leaves, agreeing with Turan et al. (2007). The accumulation of NaCl disturbed the homeostasis not only Na^{+} and Cl^{-} but also essential cations such as K^{+} and Ca^{+2} (Tattini et al., 2002; Roussos et al., 2007). The reduction of K^{+} could be explained through the competition exists between Na^{+} and K^{+} leading to a reduced level of internal K^{+} at high external NaCl concentration (Botella et al., 1997). This can explain why the Na^{+}: K^{+} ratio was increased in our study (Table 3) which reflects the growth reduction in our results. In addition, decreasing the Mg content in leaf led to a reduction in chlorophyll content. It has been reported that sodium ions may compete with calcium ions for membrane binding sites and consequently the Ca^{+2} content was reduced under salt stress.

In this experiment, the membrane permeability was measured by determining electrolyte leakage. The membrane permeability of jojoba leaves was significantly increased as a result of salt stress relative to the control (Fig. 2). It has been

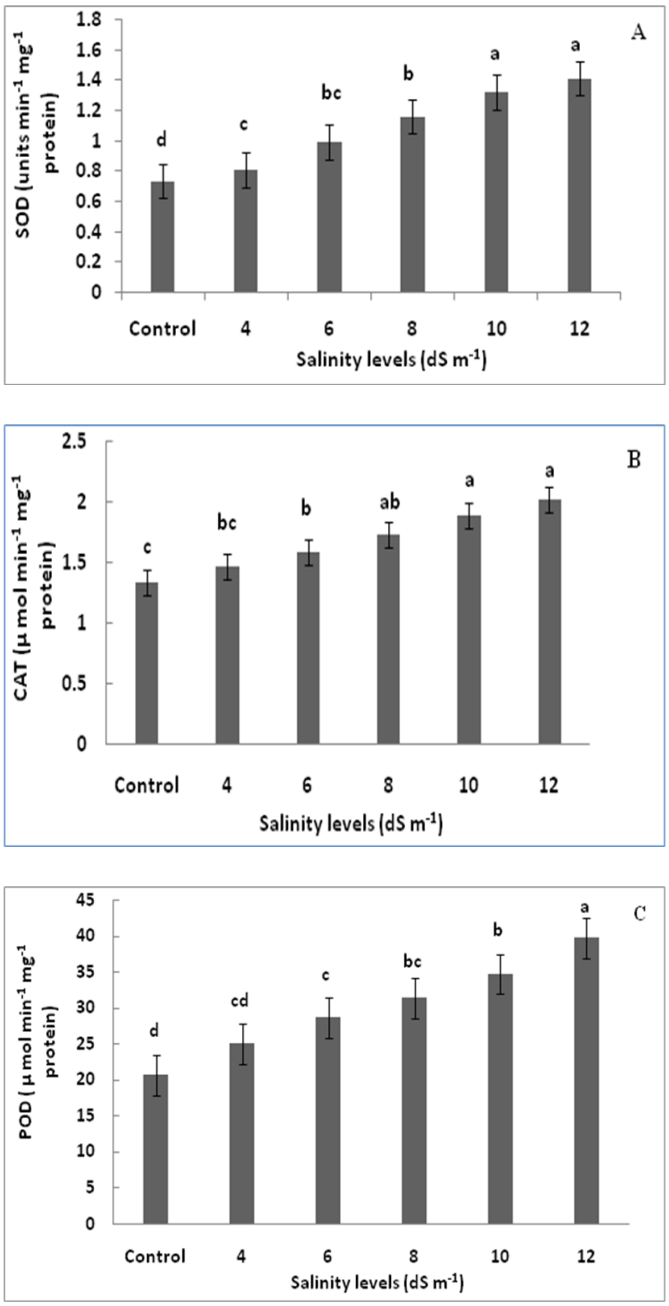

Fig 6. Effect of salt stress on the activity of antioxidant enzymes, A: superoxide dismutase (SOD), B: catalase (CA) and C: peroxidase (POD) of jojoba plant. The values (mean ± S.E.) are the average of two independent experiments (n = 12). Bars had different letters are significantly different of each other at 0.05 level.

reported that calcium significantly improved the membrane stability (Shoresh et al., 2011). This could be why membrane permeability is impaired in leaves of salinity-stressed plants, where Ca^{+2} level is lower. To the best of our knowledge, these results are the first report of jojoba leaf membrane damage caused by salt stress. Similar trends have been observed in other plants (Eraslan et al., 2007; Gunes et al., 2007; Tuna et al., 2008; Shoresh et al., 2011). The antioxidant enzyme activities (CAT, SOD and POD) were increased with increasing salinity levels, compared with the control (Fig. 6). Under salinity stress, which being considered as an oxidative stress, plants produce reactive oxygen species (ROS), which are harmful to plant growth due to their detrimental effects on the sub cellular components and metabolism of the plant, leading to the oxidative destruction of cells and finally cause deterioration of membrane lipids, leading to increased leakage of solutes from membranes (Mishra and Choudhuri, 1999). Moreover, there is evidence that salt stress can induce oxidative stress due to generation of ROS, including single oxygen, superoxide anion, hydrogen peroxide and hydroxyl radical (Gill and Tuteja, 2010; Malik et al., 2011). As a result of ROS production, plant cell has to activate the antioxidant defense system including enzymatic antioxidant to scavenge ROS. The SOD is a major scavenger which catalyzes the dismutation of superoxide, which could cause membrane damage, to hydrogen peroxide and oxygen. Meanwhile, hydrogen peroxide is also toxic and has to be scavenged by CAT or POD to water and oxygen (Sairam et al., 2005). Hence, the mechanisms that reduce reactive oxygen species (ROS) and increase antioxidant enzyme system in plants have important roles in imparting tolerance in plants under environmental stress conditions (Abd El-baky et al., 2003).

On the other hand, it was recently demonstrated that ROS is also involved in growth processes (Rodriguez et al., 2002; Foreman et al., 2003). The ROS was suggested playing a role in cell expansion and this may explain the growth reduction caused by salt stress as we reported in our results. Consequently, under salinity stress, plants compromise the need of ROS for growth to overcome the possible oxidative damage induced by salinity (Bernstein et al., 2010). In the same line, Bernstein et al. (2010) reported that because ROS are required for cell expansion, the higher increase in SOD and APX activities in the growing leaf cells that resulted in reduction of ROS content under salinity could lead to the inhibition of cell growth under salinity. It was reported that high peroxidase activity is correlated with the reduction of plant growth and this increment may play an important role as defense against salt stress (Agarwal and Pandey, 2004). The increment in antioxidant enzyme activity under salt stress in jojoba has not been previously reported. However, it has been reported to increase in *Cassia angustifolia* plants (Agarwal and Pandey, 2004), *Beta maritima* and *Beta vulgaris* cv. Ansa (Bor et al., 2003), lettuce (Eraslan et al., 2007), maize (Bernstein et al., 2010), pearl millet (Heidari and jamshidi, 2011) and tomato (Abdel Latif and Caoxing, 2011) subjected to salt stress.

Materials and Methods

Plant material

This study was carried out at Biology Department, Faculty of Science, Taif University, Saudi Arabia during 2013 and 2014 seasons seasons to investigate the effect of salt stress on plant growth, leaf measurements and chemical constituents of jojoba leaves (*Simmondsia chinensis* (Link) Schneider). One year jojoba seedlings were used in this experiment. Homogenous seedlings were chosen and potted into the plastic pots (30 x 20 cm). The physical properties of soil used in this study were (sand, 82.40 %, silt 7.10 % and clay 10.50 %) and chemical properties were (pH, 8.37, EC, 2.33 dsm^{-1}, OM, 0.11 %, Total $CaCO_3$, 0.98 %, Na^+, 3.67 ($meqL^{-1}$), Ca^{+2}, 44.75 ($meqL^{-1}$), SO_4^{-2}, 47.55 ($meqL^{-1}$), HCO_3, 2.33 ($meqL^{-1}$), Cl^-, 0.67 ($meqL^{-1}$), total N^+, PO_4^{-3}, K^+ were 0.17,0.036 and 0.043 %, respectively).

Salinity treatments

Salinity treatments used in this experiment were 0, 4, 6, 8, 10 and 12 dSm^{-1} NaCl. To prepare irrigation water with different salinity levels, NaCl salt was used. The salinity levels were obtained by addition of appropriate amount of NaCl to water and were adjusted by a portable EC meter instrument. Plants were subjected to saline irrigation water every 14 days and pots were flushed out with saline water at the middle of that period to ensure homogeneity of salinity and to prevent the induction of salt build up. Salinity treatments were continued for six months. Irrigation started with 4 dS m^{-1} saline water and was gradually increased until reaching the exact salinity level to prevent shock to plants. The experimental design was complete randomized contained six treatments with six replicates each.

Growth characters

The growth characters taken in this experiment were plant height (cm), number of main branches/plant as well as fresh and dry weights per plant (g).

Leaf measurements

Data recorded concerning the leaf were number of leaves/plant, leaf area (cm^2) and stomatal density (number/mm^2). In order to determine leaf area, blade area was measured using digital image analysis according to the method of Matthew et al. (2002). Digital image of the leaf blade was created in digital format using a Hewlett- Packard scanner (Hewlett Packard, Cupertino, CA). The image was scanned at dot per inch (100 dpi), the blade area was measured using public domain software (scion image version 4.02). Stomatal density (number/mm^2) was measured according to the method as described by (Botti et al., 1998).

Relative water content (RWC)

Leaf RWC was determined and calculated from the following relationship: $(W_{fresh} - W_{dry}) / (W_{turgid} - W_{dry}) \times 100$, where W_{fresh} is the sample fresh weight, W_{turgid} is the sample turgid weight after saturating with distilled water for 24 h at 4 °C, and W_{dry} is the oven-dry (70 °C for 48 h) weight of the sample (Weatherley, 1950).

Chlorophyll content

Randomly samples of fresh leaves were taken from the middle part of stem for chlorophyll determination. Chlorophyll content was determined according to Sadasivam and Manickam (1992) by spectrophotometer (Pharmacia, LKB-Novaspec II and calculated as (mgg^{-1} FW).

Total soluble sugars (TSS)

Total soluble sugars were determined in leaf samples according to the method of Dubois et al. (1956).

Proline determination

The free proline content was determined as described by Bates et al. (1973). Frozen leaf tissue (0.5 g) was homogenized with 10 mL of 3 % sulfosalicylic acid at 4 °C. Then, the obtained extract was filtered with Whatman No. 2. Mixture of 2 mL of filtrate, 2 mL of acid-ninhydrin, and 2 mL of glacial acetic acid were mixed in a test tube and incubated at 100 °C for 1 h. The reaction was terminated on ice, and the reaction mixture was then extracted with 4 mL of toluene. The chromophore-containing toluene was separated from the hydrated phase. The absorbance at 520 nm was spectrophotometrically determined with toluene as the blank. The proline concentration was calculated based on a standard curve and was expressed as µmol g^{-1} FW.

Membrane permeability

Membrane permeability of the excised leaves was measured at the end of the experiment (Yan et al. 1996). Fresh part from the middle of leaves was weighed into a glass beaker containing reverse osmosis water. The beakers were immersed at 30 ± 1˚C for 3 h, and then the conductivity of the solution was measured with a conductivity meter. The conductivity was measured again after boiling the samples for 2 min when the solution was cooled to room temperature. The percentage of electrolyte leakage was calculated using the equation, $EC\ \% = (C_1/C_2) \times 100$, since C_1 and C_2 are the electrolyte conductivities measured before and after boiling, respectively.

Antioxidant enzyme activity

To obtain the enzyme extract for antioxidant enzymes determination, the method of Hassan and Mahfouz (2012) was used. The resulting supernatant was used as an enzyme extract to determine superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) activities. Soluble protein contents of the enzyme extract were assayed according to the method of Bradford (1976).

SOD (Ec 1.15.1.1) activity was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT). SOD activity was expressed as SOD units min^{-1} mg^{-1} protein. One unit of SOD was considered to be the amount of enzyme required to inhibit NBT reduction by 50 % as described by Giannopolitis and Ries (1977) by measuring the absorbance at 560 nm by a spectrophotometer (type GBC, UV/VIS 916).CAT (Ec 1.11.1.6) activity was spectro-photometrically estimated by method of Clairbone (1985), following the disappearance of H_2O_2 at 240 nm. The level of enzyme activity was expressed as µ mol min^{-1} mg^{-1} protein.

POD (Ec 1.11.1.7) activity was tested according to Shanon et al. (1966). Sodium acetate buffer (0.1 M) and 0.5 % guaiacol were added to the enzyme extract. The reaction was started with 0.1 % H_2O_2. The rate of change in absorbance was spectrophotometrically measured at 470 nm and the level of enzyme activity was expressed as µ mol min^{-1} mg^{-1} protein.

Leaf mineral content

Nitrogen, phosphorus, potassium, calcium, magnesium, sodium and chloride were determined in dried leaf samples, digested using sulphuric and perchloric acids method according to the methods described by Black et al. (1965), Jackson (1978) and Johnson and Ulrich (1959).

Statistical analysis

The experiment was performed twice in the two successive seasons of 2011 and 2012 with 6 replicates each. The two experiments had qualitative and quantitative similar trend. Where indicated, the results are expressed as mean values (± SE) from two experiments (*n = 12*). The results of two experiments were pooled and the analysis of variance

(ANOVA) was performed using MSTAT program, USA. Means were separated using Duncan's multiple range test at a significance level of 0.05.

Conclusion

From the results of this study, it could be concluded that salinity stress significantly reduced jojoba growth measurements, stomatal density, relative water content (RWC), leaf chlorophyll content, all compared to the control. Membrane permeability, proline accumulation and antioxidant enzymes activities (SOD, CAT and POD) were increased in salt stressed plants. The increment of both antioxidant enzymes activities and proline accumulation may suggest that they are involving as a part of the defense against salt stress in jojoba plant. Moreover, salinity treatments significantly increased Na^+: K^+ ratio and consequently the homeostasis of minerals was disturbed.

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