http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS
Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2019.412.418

Research Article

Effect of Proline on Growth and Nutrient Uptake of *Simmondsia chinensis* (Link) Schneider under Salinity Stress

[1]Saqer Alotaibi, [2,3]Esmat Ali, [1,4]Hadeer Darwesh, [1,5]Abo-Taleb Ahmed and [1,5]Emad Al-Thubaiti

[1]Department of Biotechnology, Faculty of Sciences, Taif University, Taif, Saudi Arabia
[2]Department of Biology, Faculty of Sciences, Taif University, Taif, Saudi Arabia
[3]Department of Horticulture, Faculty of Agriculture, Assiut University, Egypt
[4]Department of Medicinal and Aromatic Plants, Horticulture Institute, Agricultural Research Center, Egypt
[5]General Department of Education, Ministry of Education, Taif, Saudi Arabia

Abstract

Background and Objective: *Simmondsia chinensis* (Link) Schneider grows as an important economic and medical plant in deserts. It suffers from salt stress during the first period of growth despite having to endure it after an advanced age. More than 30% of irrigated lands worldwide are destructively impacted by salt stress, which enormously influences the growth and productivity of several crops worldwide. Proline (Pro) aggregation has been correlated with salt tolerance. This treatise was conducted to evaluate the impact of Pro on the negative effects of salinity. **Materials and Methods:** In this experiment, sodium chloride (NaCl) (5 and 10 dS m^{-1}) and Pro treatments (10 and 20 mM) were examined and then growth parameters, relative water content (RWC), chlorophyll and inorganic ion contents of jojoba plant were determined. **Results:** Salt stress significantly minimized the growth parameters (i.e., plant height, branch number/plant, leaf number/plant and dry weight), RWC, chlorophyll and N^+ and K^+ contents, whereas Na^+ and Cl^- contents showed the opposite manner. **Conclusion:** Contrariwise, when Pro was applied at 10 and 20 mM, the adverse effects of salt stress on the previous parameters were mitigated; 20 mM Pro treatment showed superior effects compared with 10 mM treatment.

Key words: Jojoba plant, proline, salt stress, RWC, chlorophyll, inorganic ions

Citation: Saqer Alotaibi, Esmat Ali, Hadeer Darwesh, Abo-Taleb Ahmed and Emad Al-Thubaiti, 2019. Effect of proline on growth and nutrient uptake of *Simmondsia chinensis* (Link) Schneider under salinity stress. Pak. J. Biol. Sci., 22: 412-418.

Corresponding Author: Saqer Alotaibi, Department of Biotechnology, Faculty of Sciences, Taif University, Taif, Saudi Arabia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Several countries look forward to planting *Simmondsia chinensis* (Link) Schneider, a relatively new industrial crop, to address overproduction and low price of other conventional crops[1]. Jojoba has attracted much attention in recent years because of its ability to produce odourless oil that contains triglycerides and exhibits unique physical and chemical properties[2,3]. This oil is used in cosmetic industries as an anti-foaming agent, in pharmaceutical industry and in the paint, food product and automobile industries[4,5]. Its seeds contain simmondsin, an important compound. Simmondsin content is an important seed attribute because it makes the seed commercially valuable for food production. Jojoba plantations now have a good chance for agricultural and economic success[6]. Thus, jojoba contributes to economic productivity especially that it is resistant to abiotic stresses[7,8]. Findings show that jojoba is suitable for commercial production in high salinity regions. Abiotic stresses, especially, salinity, is a major risk that passively influences agricultural activities and this problem is worsened by the increasing salt levels in irrigation water[9,10]. Furthermore, salt stress reduces crop productivity and menaces the sustainability of agricultural lands[11]. During salt stress, the associated reduction in plant growth is usually linked to osmotic potential in soil solution alongside root waistband[12]. Sodium chloride (NaCl) is the most common cause of salt stress[13]. Studies show that in the first period of cultivation, jojoba can endure up to 6 dS m^{-1} salt stresses, although its growth is negatively influenced relative to that of the unstressed plants, they can tolerate higher levels of salt. In *Salvinia natans*, exposure to high salt levels results in a diverse set of physiological and morphological changes[14]. Growth parameters are adversely impacts by salt stress[15-18]. Moreover, RWC is renowned as an execution to realize the water status in plant, reverberating the cell metabolic activity[19]. Ali and Hassan[20] revealed that low RWC indicates low leaf turgor, leading to restricted availability of water that is needed for various cell processes. Comparable studies have been done for other plants grown under salt stress[21]. These studies show that salt stress treatments decrease chlorophyll contents in jojoba leaves[15,16,20]. Regarding the impacts of salt treatments on nutrients, the amounts of N, K and Mg in leaves are reduced by salt stress; however, Na and Cl levels increase[10,15,18].

Foliar application of Pro significantly increases both chlorophyll and carbohydrate contents in salt-stressed jojoba plants relative to the unstressed plants. Similarly, Pro treatment increases the photosynthetic activity and leaf water content in salt-stressed *Olea europaea* plant[22]. This effectiveness of Pro has motivated researchers to use Pro for improving growth and chlorophyll contents of lupine varieties grown under salt stress[18,23]. Regarding the influence of Pro treatments on nutrients, Sobahan *et al.*[24] observed that Pro represses Na^+ uptake and raises K^+ content in rice exposed to salt stress. Furthermore, Pro counters the deleterious impacts of salt in melon plants by enhancing the stability of plasma membrane, resulting in increased Ca^{2+}, N and K^+ levels and reduced Na^+ levels in leaves[25]. Zheng *et al.*[26] also found that exogenous supplementation of Pro (10 mM) improves growth and increases Pro and K^+ levels but decreases Na^+ in leaves of salt-stressed *Eurya emarginata*. Also, Lone *et al.*[27] revealed that Pro significantly diminishes Na^+ and Cl^- levels in barley shoots exposed to high salinity, thereby augmenting salt stress tolerance; this cumulative enhancement is attributed in membrane stability. In barley, Pro instantaneously reduces Na^+-induced K^+ efflux[28]. Although the mitigating effect of Pro against negative effects of salinity were previously studied in some plants, its alleviating effect on medicinal and aromatic plants is yet to be investigated. Given the economic value of jojoba plant being an important medicinal plant, it is a suitable plant for this experimental research. To our knowledge, scarce literature was available concerning this subject. Therefore, this project aims to investigate the effect of exogenous Proline treatments on morpho-physiological characteristics, that is, the growth characters and chlorophyll and nutrient contents in leaves, to understand the possible techniques regarding salt stress mitigation in jojoba plant. However, to our best knowledge, the relationship between salt stress and Pro treatments in jojoba has not yet been investigated.

MATERIALS AND METHODS

Plant materials: A pot experiment was performed in a greenhouse in Taif University, Saudi Arabia during spring/summer seasons 2018/2019. Healthy seeds of *S. chinensis* (Link) Schneider of uniform sizes were selected and sown directly in plastic pots (30×20 cm) filled with local (sandy) top soil. The physical and chemical properties of this soil were as follows: sand, 79.11%; silt, 9.21%; clay, 11.68%; pH, 7.72; EC, 2.03 dS m^{-1}; OM, 0.16% and total N, P, K of 0.19, 0.053 and 0.047%, respectively. After 2 weeks of germination, the plants were thinned into two in each pot or replicate. A constant dose (3 g/pot) of compound fertilizer (NPK, 17:17:17) was used for all pots according to the manufacturer's protocol. Night/day temperatures of 16/20°C and relative humidity of 67-79% were maintained during the experiment.

Treatments: NaCl (5 and 10 dS m^{-1}) was used to induce salt stress. It was used to prepare a solution that simulates the salt stress levels in irrigation water. Appropriate amounts of NaCl were mixed with distilled water and then detected by a portable EC meter instrument. All pots were irrigated with tap water and maintained under greenhouse condition. Thirty days after being planted, the plants were exposed to salt stress every 7 days; however, all pots were leached by tap water every 15 days to prevent salt build-up during the salt stress treatment period. After 2 weeks of salt stress treatment, Pro (10 and 20 mM) was sprayed on leaves until run off. Foliar application was performed once a week (four times in a month) for 2 months and Tween 80 (0.5% v/v) was used as surfactant. The control was treated with distilled water, with Tween 80 as surfactant. The pots were designed in a completely randomized design with a two-way factorial experiment involving four replicates, each containing four pots[29].

Growth parameters: The growth parameters determined in this experiment include plant height (cm), branch number/plant (g), leaf number/plant (g) and dry weight (g). At the end of the experiment, the plants were removed from their pots and then dried at 70°C for 48 h to determine the dry weight (g).

RWC: RWC was determined using the following relationship according to Weatherley[30]:

$$\text{RWC} = \frac{W_{fresh}-W_{dry}}{W_{turgid}-W_{dry}} \times 100$$

where, W_{fresh} is the fresh weight, W_{turgid} is the turgid weight after saturation with distilled water for 24 h at 4°C and W_{dry} is the dry weight.

Chlorophyll content: Random leaf samples were segregated for chlorophyll determination. Extraction in acetone was repeated until all pigments were extracted. The absorbance of the extracts was determined according to the procedure used by Sadasivam and Manikam[31]. The chlorophyll content was expressed as mg g^{-1} FW.

Inorganic ion content: Wet digestion of dried jojoba leaves (0.5 g) was performed by using the sulphuric and perchloric acid method as described by Jackson[32], Piper[33] and Chapman and Pratt[34] for determining the inorganic ion contents (i.e., N, P, Na and Cl).

Statistical analysis: Data were analysed using the MSTATC program. ANOVA (Two way) was used to compare means. Means were separated using Duncan's multiple range test at 0.05 significance level. Data were presented as Means±SD.

RESULTS

Impact of pro application on growth parameters of salt-stressed jojoba plants: Salt significantly influenced plant height, branch and leaf numbers/plant and dry weight of jojoba plant. Data show that the effects of salinity during the vegetative growth phase was additive, but this was not true as regards the effects of salinity on shoot height. Plants grew normally under salt stress-free condition. Averaged data show that the shortest plants were those subjected to salt stress treatments (Table 1 and Fig. 1). Among the salt-stressed plants, those subjected to 10 dS m^{-1} NaCl were shorter than those subjected to 5 dS m^{-1} NaCl. By contrast, Pro treatments

Table 1: Effects of Pro on plant height (cm), branch number/plant and leaf number/plant of *Simmondsia chinensis* (Link) Schneider plant grown under salt stress conditions.

Treatments	Plant height (cm)	Branch number/plant	Leaf number/plant
5 dS m^{-1}			
Control	21.18±0.48^{b}	2.23±0.62^{b}	27.24±0.85^{b}
10 mM Pro	23.57±0.39^{a}	3.72±0.25^{a}	28.41±0.69^{a}
20 mM Pro	26.18±0.42^{a}	3.98±0.50^{a}	31.57±0.86^{a}
10 dS m^{-1}			
Control	17.26±0.36^{d}	1.58±0.64^{c}	21.23±0.74^{c}
10 mM Pro	19.32±0.27^{b}	1.74±0.21^{c}	23.25±0.87^{d}
20 mM Pro	20.46±0.34^{c}	1.89±0.62^{c}	25.47±0.96^{b}

Values are Means±SD (n = 8), means within a column with different letters are significantly different from each other according to Duncan multiple range test at p = 0.05

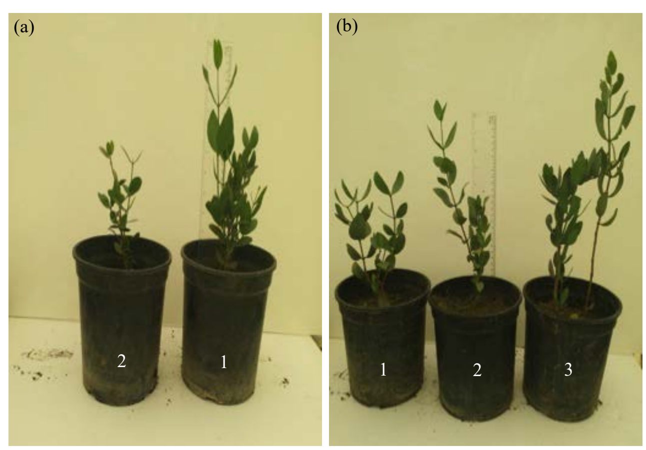

Fig. 1(a-b): (a) Salt stress treatments; 1 (5 dS m^{-1}), 2 (10 dS m^{-1}) and (b) Pro treatments; 1 (0 Pro; control), 2 (10 mM Pro), 3 (20 mM Pro)

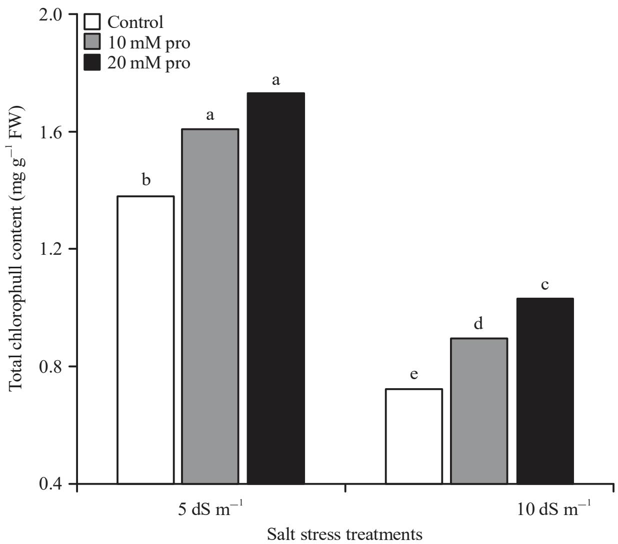

Fig. 2: Impact of Pro on chlorophyll content of *Simmondsia chinensis* (Link) Schneider plant grown under salt stress

Bars have different letters are significantly differ from each other according to Duncan multiple range test at p = 0.05 (n = 8)

Table 2: Pro effects on dry weight (g/plant) and RWC of *Simmondsia chinensis* (Link) Schneider plant grown under salt stress conditions

Treatments	Dry weight (g/plant)	RWC (%)
5 dS m^{-1}		
Control	31.27 ± 1.41^{c}	72.39 ± 2.24^{c}
10 mM pro	33.47 ± 1.69^{b}	79.51 ± 2.64^{b}
20 mM pro	34.98 ± 1.57^{a}	86.38 ± 3.29^{a}
10 dS m^{-1}		
Control	26.12 ± 1.89^{f}	69.32 ± 1.98^{c}
10 mM pro	28.56 ± 1.68^{e}	75.42 ± 2.31^{b}
20 mM pro	29.14 ± 1.15^{de}	80.59 ± 2.63^{a}

Values are Means±SD (n = 8), means within a column with different letters are significantly different from each other according to Duncan multiple range test at p = 0.05

Table 3: Pro effects on inorganic nutrients of *Simmondsia chinensis* (Link) Schneider plant grown under salt stress conditions

Treatments	N (%)	K (%)	Na (mg g^{-1})	Cl (mg g^{-1})
5 dS m^{-1}				
Control	2.85 ± 0.10^{c}	2.92 ± 0.18^{c}	0.82 ± 0.09^{d}	9.23 ± 0.42^{d}
10 mM Pro	2.96 ± 0.09^{b}	3.04 ± 0.16^{b}	0.63 ± 0.07^{e}	7.36 ± 0.36^{e}
20 mM Pro	3.32 ± 0.12^{a}	3.14 ± 0.14^{a}	0.47 ± 0.05^{f}	7.89 ± 0.28^{f}
10 dS m^{-1}				
Control	2.24 ± 0.18^{f}	2.56 ± 0.24^{f}	1.23 ± 0.013^{a}	13.87 ± 0.34^{a}
10 mM Pro	2.32 ± 0.14^{e}	2.74 ± 0.12^{e}	1.12 ± 0.04^{b}	12.08 ± 0.37^{b}
20 mM Pro	2.56 ± 0.13^{d}	2.83 ± 0.15^{d}	1.04 ± 0.07^{c}	10.17 ± 0.27^{c}

Values are Means±SD (n = 8), means within a column with different letters are significantly different from each other according to Duncan multiple range test at p = 0.05

significantly promoted plant height and the highest value was recorded with 20 mM treatment. Branch number/plant and leaf number/plant progressively decreased with increasing salt stress level (Table 1). The opposite trends were observed with Pro treatment, indicating the mitigating effects of Pro counter the negatively effects of salt stress. Branch and leaf numbers/plant gradually increased as Pro levels increased from 10-20 mM. Furthermore, salt stress considerably influenced the dry mass of jojoba plant; the mean dry weight (g/plant) significantly decreased under 5 and 10 dS m^{-1} NaCl treatments (Table 2). The higher level of salt stress, the lower dry weight/plant. Consequently, the lowest weight was recorded under the 10 dS m^{-1} NaCl treatment. By contrast, exogenous application of Pro promoted the development of heavier shoots and the highest value was recorded under 20 mM Pro treatment.

RWC: RWC in leaves was significantly and progressively reduced when salinity doses were raised from 5-10 dS m^{-1} (Table 2). The RWC was 69.32 and 72.39% under 10 and 5 dS m^{-1} treatments, respectively. By contrast, Pro treatments have had positive effect on RWC of jojoba leaves, that is, RWC under any water stress level was improved when Pro treatment was performed.

Chlorophyll contents: Jojoba plants exposed to salt stress showed significantly reduced chlorophyll content while chlorophyll contents under 5 and 10 dS m^{-1} treatments significantly differed. On the contrary, Pro treatment significantly increased chlorophyll content of leaves relative to control. Moreover, foliar application of Pro demonstrated an alleviation effect on chlorophyll content (Fig. 2). Chlorophyll content was higher under 20 mM treatment than under 10 mM treatment.

Nutrient contents: The nutrient contents of jojoba leaves varied as a result of salt stress treatments (Table 3). NaCl treatments significantly decreased N and K contents. An opposite trend was recorded as regards to Na^{+} and Cl^{-} contents. N and K contents significantly increased under 10 and 20 mM Pro treatments, whereas Na^{+} and Cl^{-} levels were decreased relative to the control.

DISCUSSION

In the current study, Pro was exogenously applied to mitigate the counteractive effects of salt stress on jojoba plant. Intracellular mechanisms, such as compartmentation of solutes and ions and osmotic adjustment in salt-tolerant plants, may promote growth and survival under salt stress[35]. Jojoba is salt tolerant especially in advanced growth stage;

therefore, one would expect that growth of a juvenile plant is reduced under salt stress. Our results show that the growth parameters (i.e. plant height, branch number, leaf number and dry weight) of salt-stressed plants were reduced especially under high salt stress dose. In particular, branch number/plant was drastically reduced under this condition. These consistent with the findings of Hassan and Ali[10]. Several studies reported that plant dry weight decreases under salt stress[36]. Our results are in agreement with this finding particularly with that obtained by Moghimi and Ghavami[37] and Mbadi *et al.*[38] on *Calendula officinalis*, by Hassan *et al.*[39] on *Rosmarinus officinalis* and by Hassan and Ali[10] on jojoba, wherein they demonstrated that salt stress seriously affects plant growth and development. Salt stress limits plant growth primarily due to ion imbalances and osmotic stress[36]. Further, Benzioni *et al.*[7] revealed that jojoba plant could overcome on salt stress through increasing water uptake for diluting the high intracellular salt levels. This phenomenon is supported by the considerable increase in succulence of jojoba leaves in response to salinity. Exposure to high salt stress causes diverse physiological and developmental changes in plants[14]. Thus, salt stress adversely influences plant growth[15,40,41].

Exogenous application of Pro effectively mitigated the unfavorable effects of salt stress and then promoted plant growth. Pro helps plants to neutralize the adverse effects of different abiotic stresses[42]. Rady *et al.*[23] have found that exogenous application of Pro enhances growth, productivity and anatomy of lupine varieties grown under salt stress. RWC in leaves is renowned as a restraint to realize the water status in plant, reverberating the leaf metabolic activity[19]. Salt stress usually results in drought stress; water deficiency is manifested in tissue dehydration and its impact could be evaluated by determining RWC, which represents the status of water level in cells. In our research, RWC reduction was observed during exposure to salt stress. Salt stress causes water deficiency in plants, which in turn is indicated by stomata closure in leaves. A reduced RWC is an indication of low turgor, resulting in restricted water availability for cell extension practicability[10]. Similar findings for another salt-stressed plant species have been reported by Tuna *et al.*[21]. In our study, RWC significantly increased with foliar application of 10 or 20 mM Pro compared with the control. Notably, RWC reduction in jojoba leaves was more retarded in Pro-treated plants. Pro restores the RWC in *Arabidopsis thaliana* plants treated exogenously with Pro[43]. Moreover, our results show that N and K contents of jojoba leaves were significantly reduced, whereas Na and Cl contents showed the opposite trend. More studies reported that salt stress reduces leaf nutrient contents, such as N, P, K, Ca and Mg levels, whereas salt stress increases Na^+ and Cl^- levels in leaves[10,15,18,21]. Thus, it could be suggested that Pro-mediated partial closing of stomata is responsible for maintaining high RWC under salt stress. Furthermore, enhancing the photosynthetic system is a logical reason to promote growth as manifested in increase in branch and leaf number and in dry mass, ultimately increasing plant yield. The prime role of Pro as a cellular osmoprotectant in salt tolerance possibly allows date palms to tolerate the unfavourable impacts of salt stress[44]. This could be due to the direct osmotic pressure or a different signalling pathway induced by excessive NaCl levels in plant. Djibril *et al.*[45] revealed that Pro is directly associated with NaCl treatments. In addition, salt stress treatment promotes Pro content by 2.0-fold in roots and by 6.3-fold in leaves[44]. From the obtained results of this study, it is useful and recommended by carrying more scientific research on other medicinal and aromatic plants grown under different environmental stresses and not salt stress only because they represent important plants in the pharmaceutical industry and cosmetics industries as well as other industrial uses of these plants.

CONCLUSION

Salt stress reduces jojoba plant growth, chlorophyll content and leaf nutrient contents except for Na^+ and Cl^- which showed the opposite manner. Exogenous application of Pro can mitigate these negative effects of salt stress and consequently improve plant growth as well as prevent chlorophyll degradation and ion homeostasis, which are possibly the mechanisms leading to salt stress alleviation in jojoba. From the obtained results of this study, it is useful and recommended to carry out more physiological studies on other medicinal and aromatic plants grown under not only salt stress but different environmental stresses because they offer prominent sources in serious industrial applications such as the pharmaceutical and cosmetics industries.

SIGNIFICANCE STATEMENT

The current study is the first research which is interested in investigating the effects of Pro to alleviate the adverse effects of salt stress on Jojoba. Moreover, This study discovered the role of pro on alleviating the negative effects of salinity and that can be beneficial for using salt water in irrigation more areas and consequently increasing the agriculture area around the world. This study will help the researchers to uncover the critical areas of mitigating the negative effects of different environmental stresses not only salt stress that many researchers not able to explore. Thus a new theory on motivating salinity effects may be arrived at several researchers.

ACKNOWLEDGMENT

Dr. Saqer Alotaibi is thankful to the support of Deanship of Scientific Research, Taif University, Kingdom of Saudi Arabia for funding the Future Researcher Program in the project No. 1-439-6091 under the project titled ''Bioinformatics, Molecular and Physiological Studies on Jojoba and Arabidopsis plants for Economically Agricultural Purposes within Neom's Project".

REFERENCES

1. Halawa, S.M., A.M. Kamel and S.R.A. El-Hamid, 2007. Chemical constituents of jojoba oil and insecticidal activity against *Schistocerca gregaria* and biochemical effect on albino rats. J. Egypt. Soc. Toxicol., 36: 77-87.
2. NRC., 1985. Jojoba: New Crop for Arid Lands, New Raw Material for Industry. National Academy Press, Washington, DC., USA., Pages: 113.
3. Brown, J., J. Arquette and J. Reinhardt, 1996. Jojoba esters; a new family of cosmetic emollients. Proceedings of the 9th International Conference on Jojoba and its Uses and 3rd International Conference on New Industrial Crops and Products, September 25-30, 1994, Catamarca, Argentina, pp: 100-103.
4. Bhatia, V.K., 2001. Potential of jojoba oil for new energy applications. Proceedings of the National Seminar on Jojoba, February 19-20, 2001, Jaipur, India.
5. Khattab, E.A., M.H. Afifi and G.A. Amin, 2019. Significance of nitrogen, phosphorus and boron foliar spray on jojoba plants. Bull. Natl. Res. Centre, Vol. 43. 10.1186/s42269-019-0109-7.
6. Botti, C., D. Palzkill, D. Munoz and L. Prat, 1998. Morphological and anatomical characterization of six jojoba clones at saline and non-saline sites. Ind. Crops Prod., 9: 53-62.
7. Benzioni, A., A. Nerd, Y. Rosenartner and D. Mills, 1992. Effect of NaCl salinity on growth and development of jojoba clones: I. Young plants. J. Plant Physiol., 139: 731-736.
8. Wang, X., F. Gao, J. Bing, W. Sun and X. Feng *et al*., 2019. Overexpression of the Jojoba aquaporin gene, *ScPIP1*, enhances drought and salt tolerance in transgenic *Arabidopsis*. Int. J. Mol. Sci., Vol. 20, No. 1. 10.3390/ijms20010153.
9. Ali, E.F., A.F. Ebeid, R.M. Sayed and H.H. Hammad, 2011. Response of some woody trees seedlings to saline irrigation water. Assiut J. Agric. Sci., 42: 302-316.
10. Hassan, F. and E. Ali, 2014. Effects of salt stress on growth, antioxidant enzyme activity and some other physiological parameters in jojoba [*Simmondsia chinensis* (Link) Schneider] plant. Aust. J. Crop Sci., 8: 1615-1624.
11. McKee, K.L., I.A. Mendelssohn and M.D. Materne, 2004. Acute salt marsh dieback in the Mississippi River deltaic plain: A drought-induced phenomenon? Global Ecol. Biogeogr., 13: 65-73.
12. Abou-Hadid, A.F., 2003. The use of saline water in agriculture in the near East and North Africa region: Present and future. J. Crop Prod., 7: 299-323.
13. Li, X.G., F.M. Li, Q.F. Ma and Z.J. Cui, 2006. Interactions of NaCl and Na_2SO_4 on soil organic C mineralization after addition of maize straws. Soil Biol. Biochem., 38: 2328-2335.
14. Jampeetong, A. and H. Brix, 2009. Effects of NaCl salinity on growth, morphology, photosynthesis and proline accumulation of *Salvinia natans*. Aquat. Bot., 91: 181-186.
15. Khalid, K.A. and W. Cai, 2011. The effects of mannitol and salinity stresses on growth and biochemical accumulations in lemon balm. Acta Ecol. Sinica, 31: 112-120.
16. Ali, E.F., F.A.S. Hassan and O.M. El-Zahrany, 2012. Planting of jojoba for oil production under salt and water stress in Taif region. Aust. J. Applied Basic Sci., 6: 358-371.
17. Ali, E.F., S.A. Bazaid and F.A.S. Hassan, 2014. Salinity tolerance of taif roses by gibberellic acid (GA_3). Int. J. Sci. Res., 3: 184-192.
18. Ali, E.F. and F.A.S. Hassan, 2018. β-Aminobutyric acid raises salt tolerance and reorganises some physiological characters in *Calendula officinalis* L. plant. Annu. Res. Rev. Biol., 30: 1-16.
19. Flower, D.J. and M.M. Ludlow, 1986. Contribution of osmotic adjustment to the dehydration tolerance of water-stressed pigeon pea (*Cajanus cajan* (L.) millsp.) leaves. Plant Cell Environ., 9: 33-40.
20. Ali, E.F. and F.A.S. Hassan, 2014. Alleviatory effects of salt stress by mycorrhizal fungi and gibberellic acid on chamomile plant. Int. J. Sci. Res., 3: 109-118.
21. Tuna, A.L., C. Kaya, M. Dikilitas and D. Higgs, 2008. The combined effects of gibberellic acid and salinity on some antioxidant enzyme activities, plant growth parameters and nutritional status in maize plants. Environ. Exp. Bot., 62: 1-9.
22. Ahmed, C.B., S. Magdich, B.B. Rouina, S. Sensoy, M. Boukhris and F.B. Abdullah, 2011. Exogenous proline effects on water relations and ions contents in leaves and roots of young olive. Amino Acids, 40: 565-573.
23. Rady, M.M., R.S. Taha and A.H.A. Mahdi, 2016. Proline enhances growth, productivity and anatomy of two varieties of *Lupinus termis* L. grown under salt stress. S. Afr. J. Bot., 102: 221-227.
24. Sobahan, M.A., C.R. Arias, E. Okuma, Y. Shimoishi and Y. Nakamura *et al*., 2009. Exogenous proline and glycinebetaine suppress apoplastic flow to reduce Na^+ uptake in rice seedlings. Biosci. Biotechnol. Biochem., 73: 2037-2042.

25. Kaya, C., A.L. Tuna, M. Ashraf and H. Altunlu, 2007. Improved salt tolerance of melon (*Cucumis melo* L.) by the addition of proline and potassium nitrate. Environ. Exp. Bot., 60: 397-403.
26. Zheng, J.L., L.Y. Zhao, C.W. Wu, B. Shen and A.Y. Zhu, 2015. Exogenous proline reduces NaCl-induced damage by mediating ionic and osmotic adjustment and enhancing antioxidant defense in *Eurya emarginata*. Acta Physiol. Plant., Vol. 37, No. 9. 10.1007/s11738-015-1921-9.
27. Lone, M.I., J.S.H. Kueh, R.G.W. Jones and S.W.J. Bright, 1987. Influence of proline and glycinebetaine on salt tolerance of cultured barley embryos. J. Exp. Bot., 38: 479-490.
28. Cuin, T.A. and S. Shabala, 2005. Exogenously supplied compatible solutes rapidly ameliorate NaCl-induced potassium efflux from barley roots. Plant Cell Physiol., 46: 1924-1933.
29. Snedecor, G.W. and W.G. Cochran, 1980. Statistical Methods. 7th Edn., Iowa State University Press, Iowa, USA., ISBN-10: 0813815606, Pages: 507.
30. Weatherley, P.E., 1950. Studies in the water relations of the cotton plant. I. The field measurement of water deficits in leaves. New Phytol., 49: 81-97.
31. Sadasivam, S. and A. Manikam, 1992. Biochemical Methods for Agriculture Sciences. Wiley Eastern Ltd., New Delhi, Pages: 187.
32. Jackson, M.L., 1973. Soil Chemical Analysis. Prentice-Hall of India Pvt. Ltd., New Delhi, India, Pages: 498.
33. Piper, C.S., 1967. Soil and Plant Analysis. 2nd Edn., Asia Publishing House, Bombay, India.
34. Chapman, D.H. and P.F. Pratt, 1961. Methods of Analysis of Soils, Plant and Water. University of California Press, Riverside, CA., USA., Pages: 309.
35. Girija, C., B.N. Smith and P.M. Swamy, 2002. Interactive effects of sodium chloride and calcium chloride on the accumulation of proline and glycinebetaine in peanut (*Arachis hypogaea* L.). Environ. Exp. Bot., 47: 1-10.
36. Romero-Aranda, R., T. Soria and S. Cuartero, 2001. Tomato plant-water uptake and plant-water relationships under saline growth conditions. Plant Sci., 160: 265-272.
37. Moghimi, S.M. and S.H. Ghavami, 2015. Effect of zeolite and salinity on growth indices of marigold (*Calendula officinalis* L.). Sci. J., 36: 641-644.
38. Mbadi, S.H., Z.T. Alipour, H. Asghari and B. Kashefi, 2015. Effect of the salinity stress and Arbuscular Mycorhizal Fungi (AMF) on the growth and nutrition of the Marigold (*Calendula officinalis*). J. Biodivers. Environ. Sci., 6: 215-219.
39. Hassan, F.A.S., E.F. Ali and O.M. El-Zahrany, 2013. Effect of amino acids application and different water regimes on the growth and volatile oil of *Rosmarinus officinalis* L. plant under Taif region conditions. Eur. J. Scient. Res., 101: 346-359.
40. Abdel Latef, A.A.H. and H. Chaoxing, 2011. Effect of arbuscular mycorrhizal fungi on growth, mineral nutrition, antioxidant enzymes activity and fruit yield of tomato grown under salinity stress. Scientia Horticulturae, 127: 228-233.
41. Shoresh, M., M. Spivak and N. Bernstein, 2011. Involvement of calcium-mediated effects on ROS metabolism in the regulation of growth improvement under salinity. Free Radic. Biol. Med., 51: 1221-1234.
42. Mansour, M.M.F. and E.F. Ali, 2017. Glycinebetaine in saline conditions: An assessment of the current state of knowledge. Acta Physiol. Plant., Vol. 39. 10.1007/s11738-017-2357-1.
43. Fu, Y., H. Ma, S. Chen, T. Gu and J. Gong, 2018. Control of proline accumulation under drought via a novel pathway comprising the histone methylase CAU1 and the transcription factor ANAC055. J. Exp. Bot., 69: 579-588.
44. Yaish, M.W., 2015. Proline accumulation is a general response to abiotic stress in the date palm tree (*Phoenix dactylifera* L.). Genet. Mol. Res., 14: 9943-9950.
45. Djibril, S., O.K. Mohamed, D. Diaga, D. Diegane, B.F. Abaye, S. Maurice and B. Alain, 2005. Growth and development of date palm (*Phoenix dactylifera* L.) seedlings under drought and salinity stresses. Afr. J. Biotechnol., 4: 968-972.