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The Effect of Sodium Ions on the Anatomical Structure and Growth of Early *Simmondsia chinensis* (Link) C.K. Schneid. Roots under Reduced Water Potential and Temperature

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THIS STUDY deals with the effects of tri-factorial interaction on some traits (elongation; weight and biomass accumulation efficiency of roots) and anatomical features of seedling *Simmondsia chinensis* roots. The obtained data indicated that, the moderate sodicity affected the root length which was enhanced by the sub optimal and optimal temperatures with low salinity stress. The decreased root elongation was inhibited under supra optimal temperature with decreased Ψ_s. Statistically, the Ψ_s had a dominant role while temperature effect was secondary on the root length and weight. It was found that, the temperature ranges from 20 to 28 was favorable for well development of the root anatomical structures and the root hairs formation, particular at low osmotic stress and moderate sodicity. Adversely, the elevated temperature had a deleterious effect on the root circulation which caused a wider vascular cylinder with narrower root cortex, as well as a distortion {lysis} of parenchymatous cells. The effect of the investigated factors and their combinations on the efficiency of cortical layer and xylem vessels was discussed.

Keywords: Osmotic water potential, Root growth, *Simmondsia chinensis,* Sodicity, Temperature.

Introduction

Simmondsia chinensis (Jojoba) is one of the economic oleaginous plants which are widespread in hot desert areas. In arid and semi-arid regions, the salinization process caused by high evaporation, water scarce and rarely rainfall. Therefore, the soil salinity inhibits plant growth and development, through complex traits that include osmotic stress, ion toxicity, mineral deficits, and biochemical defects (Aboualhamed & Loutfy, 2020; Khatab et al., 2021). Hence, salinity affected the early seedling establishment and plant development, through complex of root features such as morphological and anatomical structure, as well as some physiological activities. Also, salinity, by osmotic and specific ion toxic effects, inhibits the maintenance of necessary nutrient levels essential for plant growth, ultimately limiting the absorbance surface of root and seedling growth (Abari, 2011).

Under saline conditions, the reduction of seedling root lengths was a common phenomenon in many plants (Ebeed et al., 2019; Osman et al., 2019; Budran et al., 2023), because roots were the first organs exposed to soil environments. Consequently, the extension of root systems was critical for many plants to maintain cellular hydration by avoiding water deficit (Huang, 2008; Wu, 2013). Therefore, increased salinity in the root medium had a negative effect on the cell ultra-structures and tissues of plants (Koyro, 2002). Moreover, the morphological and anatomical modifications in many plant roots under salinity were detected that included decrease in root biomass, root length, cortical parenchyma thickness, and vascular cylinder diameter (Céccoli et al., 2011). Also, the changes in the root anatomy under salinity might determine the salinity tolerance level of the plant. Accordingly, the increase in root diameter and number of root hairs were more pronounced in the sensitive genotypes under salt application (Karjunita et al., 2019). In desert habitats, soil temperature, salinity and sodium ion stresses expressing familiar rather common

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edaphic problems (El Sharkawi et al., 2012). The interactions between salinity and soil temperature may had significant ecological implications in terms of time of plant growth under natural conditions (Jaleel et al., 2007). Hence, the increased hydraulic resistance with increased diameter of vessel results in lowering early water use by plants growing on shortage soil water or with increase in temperature (Khan & Ungar, 1998).

This investigation deals with the effects of osmotic water potential, sodicity, temperature and their combinations on the root length, biomass accumulation efficiency and the anatomical features in *S. chinensis* shrub. The obtained data were evaluated by suitable statistical analysis to understand the role of the previous mentioned factors on the increasing root efficiency through increasing the absorbing surfaces and improvement of the vascular system (xylem) conductivity.

Materials and Methods

The investigation was carried out on the seedling roots of *Simmondsia chinensis* (Link) Schneider (family Simmondiaceae). This shrub (Jojoba) was characterized as a desert economic, medical, wild, evergreen and perennial. It was originated in the Sonoran Desert of Northern Mexico and the United States (Phillips, 2000). *S. chinensis* seeds were collected from the shrubs cultivated in eastern desert areas of Egypt.

The mature seeds of investigated species were pretreated and sterilized with sodium hypochlorite solution (5% for 10 minutes), thoroughly washed, and embedded on chemically pure filter paper in sterilized glass petri dishes 15 cm in diameter. Each dish contained 10 seeds to which was added 20 ml. of treatment solution (of a certain Ψs and SAR), which was found adequate to support the seeds during the period of investigation as the dishes were always covered during incubation.

Adjustments of simulated osmotic water potential, Ψs and sodicity

The effect of decreased osmotic water potential (Ψs) on germination process was simulated by using sodium chloride + calcium chloride solutions as substrate media for germinating seeds. The presence of $CaCl_2$ in the incubated media with Na Cl could ameliorate the inhibitory effect of Na ion toxicity. Solutions having different water potentials, ψs , were prepared by dissolving certain amounts of sodium chloride (Na Cl) and calcium chloride ($CaCl_2$) in water, according to preconstructed calibration curves, with different sodium adsorption ratio (SAR) . The treatment solutions were prepared thus of certain levels of treatment combinations. Seeds were exposed to the following range of osmotic water potentials (ψs): 0 (control), -0.3, -0.7, and-1.1 MPa (El-Sharkawi *et al*., 2012). Another series of SAR (5%; 12.5% and 20%) at the same varies levels of osmotic water potential were prepared according to Lagerwerff & Eagle (1961). Sets of 4 Petri-dishes were randomly assigned to each osmotic potential level with (ψs+ SAR) or without SAR, and then incubated at the specific temperatures as explained before. Seed incubation was terminated after 15 days of sowing, a period long enough to cover any delay of germination due to stress especially at low water potential levels or extreme temperature treatments. Therefore, treatment combinations of this investigation were covered the three factors used (osmotic water potential, SAR and temperature).

Adjustments of incubation temperatures

Incubators with air circulation which allow control of temperature between 20°C and 36°C were used in testing temperature effect on germination. The incubators were kept constantly dark during the incubation period. The tests were run at: 20°C, 28°C and 36°C.

Measurements and calculations of root elongation and accumulation efficiency:

At the end of the incubation period, some criteria were done at various treatments for successful germination. The parameters of seedling roots were comprised:

Root elongation

These criteria of embryonic axis elongations were important for seedling establishment under natural habitat conditions. The Elongation of root was measured (cm) at different treatment levels.

Biomass accumulation efficiency of roots

Changes in dry weight of roots at various treatments will indicative the degree of buildup of materials in the root. Therefore, dry weight (oven-dry at 80^{0}C) of the seedling roots was determine {mg} at different treatment combinations. The assessment of biomass accumulation efficiency refers to the dry biomass building up which was corresponding to the root elongation. Therefore, the expressed accumulation efficiency was calculated as: mg dry Wt. cm^{-1} length (Farghali et al., 2022).

Preparation of root samples for anatomical examination

Root samples of seedling used were chosen at certain levels of treatment combinations. This cover segments of *Simmondsia chinensis* roots at temperature ranges from (20^{0}C, 28^{0}C and 36^{0}C) at various *Ψs* and SAR treatments. The root segments of freshly germinated seedlings were taken and fixed in 70% ethanol. After fixation, the root segments were dehydrated with graded ethanol series, cleared in xylol and finally, embedded in paraffin wax. By using microtome serial transverse sections (approximately thick) were cut, mounted on clean slides and deparaffinized thrice in xylol for 9 minutes (3min interval), followed by absolute ethanol for 5min. The sections of root samples were then double stained with saffranin and light green for digital microscopic (Olympus C×41) examination (Sass, 1961). The examined root sections were recorded in digital computerized camera (Olympus SC30).

Statistical analysis

Statistical inferences necessary to evaluate the effects and relative roles (shares) of single factors and of their interactions on root elongation. The significant effects of single factors and their interactions were determined by analysis of variance (Ostle, 1963). Based on the significance status, the magnitudes of the relative effect of each single factor and its interaction was determined as percentage (Share percent) by using SPSS program (2016), which is considered a test used to indicate the degree of control of each factor and its interaction on the tested parameters according to Ploxinki (1969), and applied by El-Sharkawi & Farghali (1985).

Results

The effect of osmotic water potential, temperature, sodicity and their combinations on the morphological and anatomical features of *S. chinensis* roots were illustrated in Figs. 1, 2 and Photo Plates (I- VI).

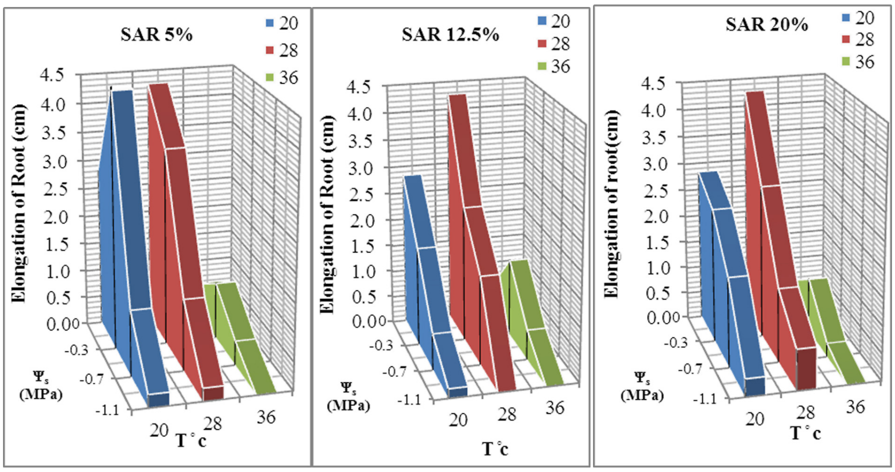

Fig. 1. Average values of root elongation in *Simmondsia chinensis* under different factors of osmotic water potential {Ψ_s} ,temperature and sodicity{SAR}

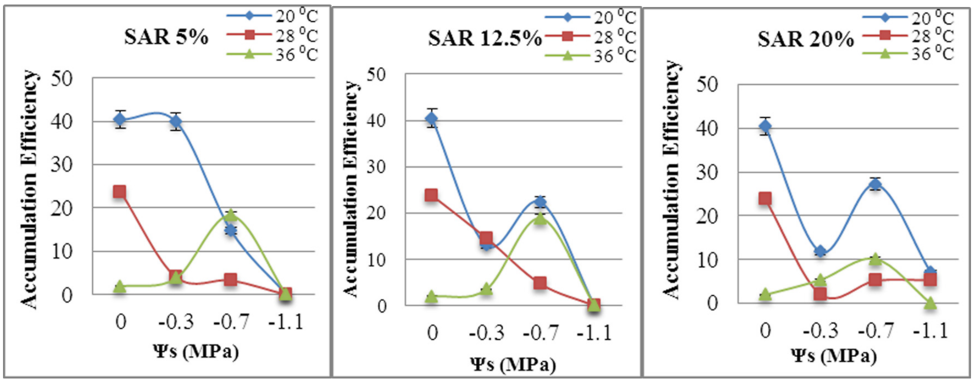

Fig. 2. Average values of biomass accumulation efficiency of radicle {mg dry Wt. cm^{-1} length} of n *S. chinensis* under different levels of Ψ_s (MPa), temperature (°C) and sodicity (SAR %)

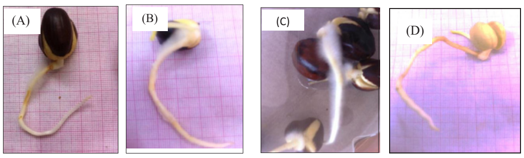

Photo Plate (I). Root hairs in *S. chinensis* grown under optimal temperature {28°C} at moderate ψs {=-0.3 to -0.7MPa} and SAR range 5 -20%, [(A) Control, 28°C; (B) -0.3MPa, SAR= 5%, 28°C; (C) -0.3MPa, SAR=12.5%, 28°C; (D) -0.7MPa, SAR=12.5%, 28°C]

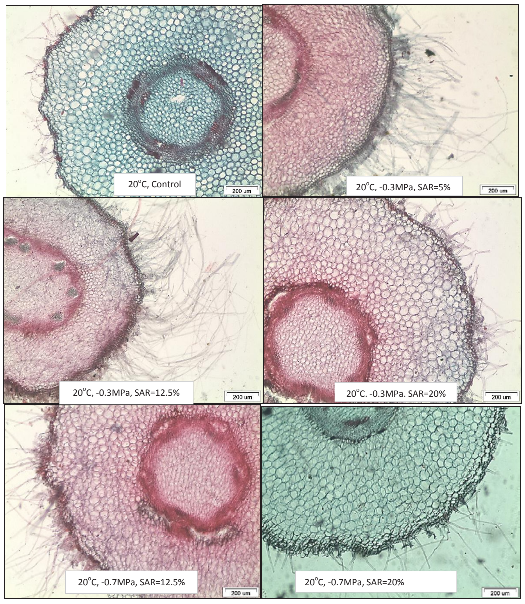

Photo Plate (II). T.S. in roots of *S. chinensis* incubated at 20°C at different ψs and SAR levels

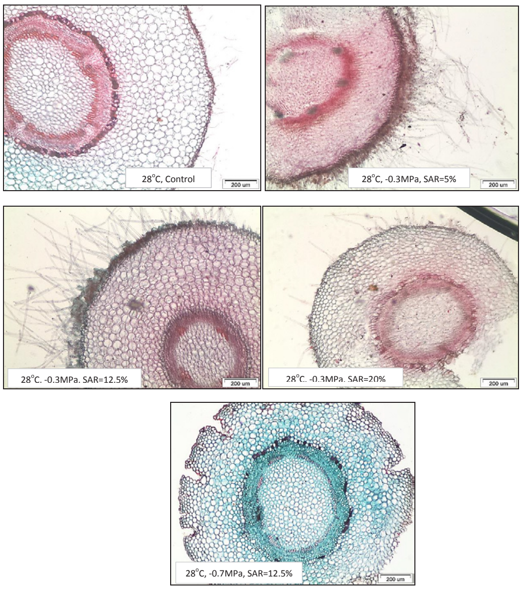

Photo Plate (III). T.S. in roots of *S. chinensis* incubated at 28°C at different ψs and SAR levels

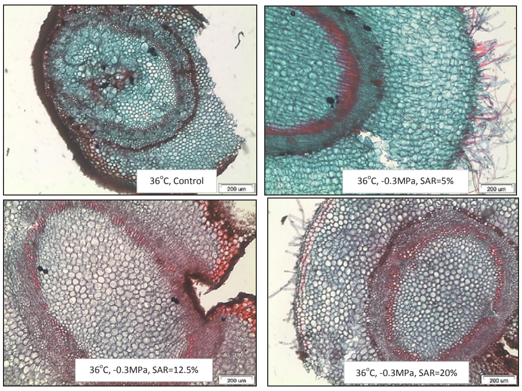

Photo Plate (IV). T.S. in roots of *S. chinensis* incubated at 36°C at different ψs and SAR levels

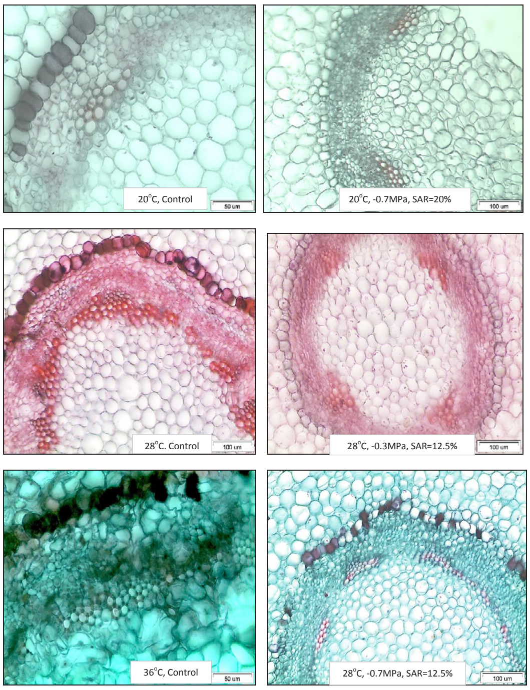

Photo Plate (V). T.S. in root endodermal layer of *S. chinensis* at control and ψs under different temperatures with SAR levels

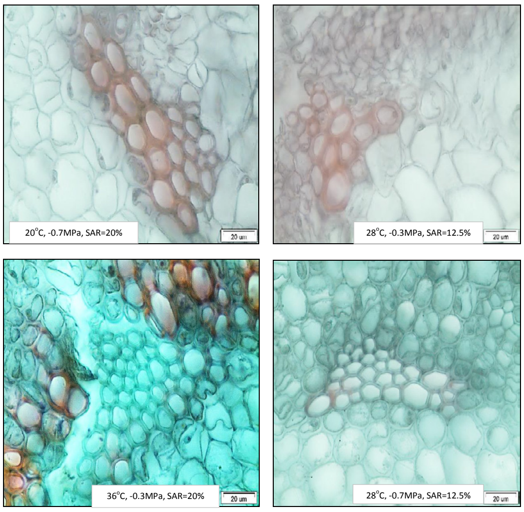

Photo Plate (VI). T.S. in root xylem elements of *S. chinensis* at different moderate osmotic stress; SAR and temperatures

Root elongation

The root elongation in *S. chinensis* was affected by the investigated factors (Fig. 1). The elongation of roots was enhanced by low SAR levels under relatively high Ψ_s. It seems to be that, the high water potential (0 and -0.3 MPa) had a boosting effect on the root elongation under sub optimal and optimal temperature with 5% SAR level. A maximum rate of root elongation {3.55cm and 4.27cm) was detected at 20 and 28^{o}C, respectively. The same was true with optimal temperat8ure at SAR range 12.5% -20% levels. Regardless of SAR levels, the reduction of root length was taken place under supra-optimal temperature, particularly with water stress. Commonly, the length of the early roots were gradually decreased with reduced water potential (Ψ_s =-0.3 to -01.1 MPa). Accordingly, the effect of Ψ_s, temperature and their interaction were significant on the root length. Hence, the osmotic water potential was played a dominant role, the temperature had a subdominant role on root length and root fresh weight of *S. chinensis*. The dry weight of the root was mainly affected by the Ψ_s, whereas the (Ψ_s x T) interaction had the secondary role (Table1).

Biomass accumulation efficiency of root

The sub optimal temperature had a boosting effect on the accumulation efficiency of roots which was reached to a maximum (40.4 mg dry Wt.cm^{-1}) under relatively high Ψ_s levels (Fig. 2). The same was true with the optimal temperature of the same Ψ_s levels. However, the reduced water potential induced an increase in the root biomass efficiency at SAR 5% under temperature range 20 - 36 ^{o}C, as well as at 20% sodicity and 28 ^{o}C. The building up efficiency in *S. chinensis* roots was affected by supra optimal temperature under low and moderate SAR levels. At 36^{o}C a peak of accumulation efficiency was showed at Ψs= -0.7MPa different of SAR levels.

Development of root hairs

The root hairs were greatly affected by the investigated factors and their combinations. The low and moderate levels of sodicity (5-12.5%) stimulated the root outgrowth, particularly under low salinity stress with mild incubated temperatures (20-28^{o}C).Furthermore, the optimal temperature had a boosting effect on the plentiful root hairs along the different root regions under SAR 12.5% level (Photo Plate I). Under SAR 5% and 20%, the abundant root hairs where showed at the upper root portion at the same temperature of high Ψ_s level. While, the decreased Ψ_s were inhibited the formation of the root hairs with increasing SAR and temperature levels.

TABLE 1. F values and sharing % for the effects of osmotic water potential (Ψs) ,temperature(T.), sodicity{SAR} and their interactions on the root dry weight and length in *S. chinensis* plant

Parameter		Root L.		Root fresh Wt.		Root dry Wt.	
Source of variance	**df**	**F**	**Sharing (%)**	**F**	**Sharing (%)**	**F**	**Sharing (%)**
Ψs	3	48.57**	49.5	57.67**	51.0	3.92*	23.0
T.	2	39.81**	27.1	47.02**	27.7	1.12	4.4
SAR	2	1.00	0.7	1.63	1.0	0.37	1.5
Ψs x T.	6	7.49**	15.3	5.04**	8.9	3.11**	36.5
Ψs x SAR	6	1.22	2.5	4.46**	7.9	1.18	13.9
T. x SAR	4	1.11	1.5	0.73	0.9	0.72	5.7
Ψs x T. x SAR	#	0.84	3.4	0.78	2.8	0.64	15.0

*Significant at 0.05 confidence level ** Significant at 0.01 confidence level

Anatomical features in the root structure

The significant structural changes induced in the early root tissues were mainly concerned to root piliferous layer, cortical with endodermal cells and vascular cylinder (Exclusive phloem). In general, the sub optimal and optimal temperature had a regular circulation on the T.S. of root with Ψ_s = -0.3 MPa at different SAR levels (Photo Plate II - IV). Adversely, the supra optimal temperature exhibited extreme distortion of the root sections under low Ψ_s at all SAR levels. Additionally, the oblong and irregular discontinuous shape of wide pith and narrow cortex in roots were existed, especially under moderate SAR levels. Commonly, the low Ψs and high sodicity induced an increased in vascular cylinder diameter compared to that of the cortex which was narrow with increasing temperature. At the same treatments of Ψs and sodicity, the exo-dermal layer was formed and accompanied by crushed or absence of the root hairs under supra optimal temperature. Apparently, the presence of SAR 5% and 12.5% stimulated the root hairs which were well developed under the sub optimal and optimal temperature with low salinity stress.

The endodermis was well developed under low levels of salinity stress and SAR with temperature range 20 -28 °C. Also, at sub optimal temperature colorless endodermal cells was showed under moderate Ψ_s levels {0-0.7MPa} and SAR =20%. Whereas, at optimal temperature the same was true under Ψ_s =-0.3MPa and SAR=12.5%. The pigmentation {Brown color} of the endodermal cells was observed in the control of different tested treatments that may be due to the presence of secondary compounds e.g., phenols. This phenomenon was also showed with relatively elevated temperature {36°C}. Similarly, the supra optimal temperature caused a distortion and deformation of the endodermal cells. While, at the optimal temperature the coloration of endodermal cells were variable under high SAR levels (Photo Plate V).

The photo plate {VI} illustrated the changes in the vascular cylinder, particular xylem elements under the investigated factors. In unstressed roots, the number of xylem bundles was four arches at sub optimal temperature. While the increased salinity, sodicity and temperature induced an increase in xylem arches number (Photo Plates II-IV). Clearly, the changed temperatures had a remarkable effect on the vessel's lignification, numbers and diameter, which had a crucial role on the axial and radial water flow capacity. Conspicuously, the sub optimal temperature exerted a few xylem vessels with wide and thin lignification, particularly at control. With increasing incubation root temperature, a numerous xylem vessels were observed with narrow and thick lignification under low Ψ_s and moderate SAR levels. While mean, the supra optimal temperature had a boosting effect on the supporting secondary elements. In general, the vascular cylinder had a regular circulation in optimal response to sub and optimal temperature at various treatments. Hence, the xylem tissue was compacted, and root anatomical integrity was quite preserved. At different osmotic stress levels, the circulation of the vascular cylinder was absent and oblong deformed take place with supra optimal temperature. At the same elevated temperature, the distortion {lysis} of parenchymatous cells was detected under different Ψ_s and SAR levels which may be due to high respiration.

Discussion

Successful establishment of early plant growth in natural habitats was mainly depending on the strategies of root development in the soil medium environments. Several stresses in the soil environment affected the shrub and tree roots such as osmotic water potential, sodicity and temperature. In the present work, the morphological and anatomical structure of the *S. chinensis* roots was not only affected by the single factors, but also by their combinations. Apparently, the optimal temperature had a boosting effect on the root length under various SAR levels, particularly under low salinity stress. Likewise, the sub optimal temperature affects the root lengths at low SAR levels. This means that, low to moderate salinity had a stimulating effect on the root growth of xero-halophytic species (Céccoli et al., 2011). Adversely, the supra optimal temperature had negative effects on the root length which was tended to a low value with decreased ψs at different SAR levels. In general, the5% SAR level exerted a high root length at temperature range 20 to 28°C. Regardless of SAR, the root length was gradually decreased with increasing salinity stress. Consequently, plants in dry lands usually allocate more biomass to roots to increase water uptake (Padilla & Pugnaire, 2007). Statistically, the ψs; temperature and their interaction had a highly significant effect on the length, fresh weight (also, ψs xSAR interaction) and dry weight of the roots.

Whereas, the ψs was played the major role on the root length and fresh weight, the temperature had a secondary role. These results were also indicated by El Sharkawi et al. (2010) in the early roots of some xerophytes. On the other hand, dry weight of the root was greatly affected by the (ψs x T.) interaction followed by the ψs.

The true root growth was indicated the degree of buildup material in relation to the root length (Farghali et al., 2022). The present investigation emphasized that, the biomass accumulation efficiency of *S. chinensis* roots was exerted a high value under a sub optimal temperature with high ψs levels. The same was true at optimal temperature with control. It was found that, the salinity and temperature interact in their control of root growth with greatest inhibition due to salinity at the extreme limits of tolerance to temperature (Gulzar & Khan, 2001). Obviously, a lowest values of accumulation efficiency was detected under supra optimal temperature, especially with high SAR and salinity levels. Additionally, a moderate ψ_s was exerted a high efficiency at different SAR levels. Commonly, the low ψs was decelerated the biomass accumulation efficiency at different levels of SAR and temperatures.

The anatomical structure of roots such as number of xylem vessels, lignification of vessels, root cortex width, and formation of root hairs plays an essential function in the acquiring water and nutrients of roots (Acosta-Motos et al., 2017). In this respect, the development of root hairs in S. chinensis was variable at different investigated treatments. Conspicuously, sub optimal and optimal temperatures were stimulated root hair formations under low and moderate SAR levels with relatively high ψ_s. In contrary, the decreased ψ_s delayed the root hair formations with supra optimal temperature. This means that, the slightly changes in soil temperature (compared to air temperature) was an advantage for the plentiful root hairs. Furthermore, the abundant root hairs played a crucial role in the uptake of water vapor dispersed in the soil atmosphere (Un-capillary spaces); particularly in plants as S. chinensis were grown under arid and semi-arid regions. Additionally, at certain levels of sodicity and salinity stress increases root branches and plentiful root hairs led to increase water absorption surface, ultimately maintain seedling growth under arid and semi-arid conditions (El-Sharkawi et al., 1999).

In this respect, the anatomical structure of roots was greatly affected by the combinations between the temperature, sodicity and reduced water potential. It was found that, the cortex thickness or cortical area in the root tip decreased as the NaCl concentration increases (Karjunita et al., 2019). Conspicuously, the low and moderate temperatures had additive effects in the uniform and regular circulation of root structure, with narrow vascular cylinder and wide cortex at different levels of ψs and SAR. Adversely, the elevated temperature of incubated roots exhibited an irregular circulation and distortion with oblong deformation of roots. Hence, it was found that, the supra optimal temperature caused an increase in vascular cylinder diameter with narrow cortical layer at different sodicity and ψs levels. Moreover, the increase in the ratio between root vascular cylinder and root cortex diameter in jojoba plants would indicate a better adaptation to saline stress with a larger root vascular tissue diameter (Gonzalez et al., 2021).

The endodermal cells played an important role in the controlling of water transport from the cortex to vascular cylinder. It was found that, the endodermal cells coloration was detected in Simmondsia seedlings under control condition (Unstressed plants) at various experimental factors. The same was detected with elevated temperature at different SAR and ψs levels. This pigmentation {Brown color} of the endodermal cells may be due to the formation of secondary compounds (e.g., phenols). Under low salinity with low and moderate SAR levels the colorless of endodermal cells was released at sub optimal and optimal temperatures (20°C to 28°C).This indicated that, the presence of sodium ions at certain level may be beneficial in the remove of endodermal phenolic compounds, ultimately facilitated the radial water flow capacity with moderate soil temperatures.

Under salinity and drought stress reduced xylem area, was a critical adaptation, as it was characterized by numerous, narrower, and longer vessels, reducing damage caused by an embolism (Kondoh et al., 2006; Slima et al., 2021). In the present paper, the anatomical adaptation and increased lignification of xylem were induced in roots under temperature, water stress and sodicity. Apparently, the sub optimal temperature exerted a few xylem vessels with wide and thin lignification, particularly at control. With increasing root temperature, a numerous xylem vessels were detected with narrower and thicker lignification under low Ψ_s with low and moderate SAR levels. While mean, the supra optimal temperature had a boosting effect on the formation of secondary elements and increased in number of narrower vessels in each root arches. Also, the presence of fibers around vessels contributes to cavitation resistance (Jacobsen et al., 2005). This could be interpreted as an adaptation to facilitate excess of water transport under water and heat stress conditions (El-Sharkawi et al., 1999; Masrahi, 2020). Furthermore, the xylem vessels of the old roots were embedding in fiber tissues, participated in the protection of water columns from embolism (Abd Elhalim et al., 2016). Accordingly, many adaptational features were endogenously exerted by S. Chinenmsis roots in order to maintain water flow through xylem vessels under drastic conditions in arid and semi-arid regions.

Conclusion

Generally, it could be concluded that, the anatomical structure of *Simmondsia chinensis* roots was well developed under moderate temperatures which interacted with low salinity and moderate sodicity levels. This means that, the combinations of the mentioned factors had a stimulated effect on the root hair formations, consequently increasing the water absorption efficiency. Moreover, the transported water in xylem vessels was increased in the axial and radial water flow capacity. Finally, temperature, Ψ_s and their interaction had a main role on the root extension and weight which played a crucial role on the growth of *S. chinensis* under hot desert conditions.

Ethics approval: Not applicable

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تأثير أيونات الصوديوم على التركيب التشريحي ونمو الجذور المبكرة لنبات الجوجوبا تحت نقص الجهد المائي ودرجة الحرارة

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تعاملت هذه الدراسة مع تأثير التفاعل لثلاثة عوامل على بعض الخصائص (الاستطالة؛ الوزن وكفاءة التراكم) والتركيب التشريحي لجذور بادرات نبات الجوجوبا. وقد اكدت النتائج أن الصوديومية المتوسطة كان لها تأثير على طول الجذر والذي استحث بدرجات الحرارة المثلى وتحت المثلى مع نقص الجهد المائي. ونقص استطالة الجذور كان مثبطا تحت درجة الحرارة فوق المثلى مع نقص الجهد المائي الأسموزي. إحصائيا، كان للجهد المائي الأسموزي الدور السائد ولدرجة الحرارة الدور الثانوي على طول ووزن الجذر. لقد وجد أن مدى درجات الحرارة من 20 ^{0}م إلى 28 ^{0}م كان ملائما ومطورا جيدا للتركيب التشريحي وتكوين الشعيرات الجذرية؛ خصوصا عند الضغط الأسموزي المنخفض والصويومية المتوسطة. عكس ذلك كان لدرجات الحرارة المرتفعة تأثير ضار على تدوير الجذور والتي تسببت فى اسطوانة وعائية واسعة مع ضيق فى القشرة وتشويه في الخلايا البرانشيمية، تأثير العوامل المنفردة والمشتركة على طبقة القشرة والاوعية الخشبية تم مناقشتها.