

Tracking Growth, Biochemical Responses, and Ion Uptake in *Paronychia Argentea* Micro Plants Grown in Vitro under Salinity Induced Environment

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ABSTRACT

Paronychia argentea is a wild medicinal herbaceous herb that contains many secondary metabolites. It grows wild in Jordan under harsh high salinity soil conditions. This study was conducted to investigate the effect of salinity stress on growth, biochemical responses, and ion uptake of in vitro *Paronychia argentea* micro plants. Our results revealed a clear reduction in all growth parameters in response to an increase in salinity level used (0, 25, 50, 100, 200 mM). When the growth medium was supplemented with 200 mM NaCl; the fresh and dry weights were reduced by 40% compared with the control treatment. Moreover, micro plant uptake of phosphorus calcium and potassium ions decreased with increasing salinity levels. The Mg⁺⁺ content was increased by 10% at the 25 mM level of NaCl compared with control, while it declined by 20% at 200 mM of NaCl. On the other hand, Na⁺ uptake increased with increasing salinity levels and reached a maximum value of (18328 ppm/D. W) at 200 mM NaCl. Moreover, proline content increased in response to salinity level to record maximum value (80.8 µmole/g) at 200 mM NaCl compared to (30.4 µmol/g) obtained in the control. Quercetins content in *P. argentea* micro plants was increased by 1.7-fold in concentration in response to salinity at 50 mM of NaCl, while an about 7-fold increase in the concentration of isorhamnetin content was obtained due to salinity at 100 mM.

Keywords: Biochemical responses, In vitro, Micro plant, Nutrient uptake, Salinity.

INTRODUCTION

Soil salinity is one of the most constraints that threaten Agriculture in the Middle East and is considered a serious

problem limiting the productivity of crops (Shrivastava and Kumar, 2015). Many reasons can cause high soil salinity including, poor quality of irrigation water, high temperatures, low rainfall precipitations, and high evaporation rates. The growth and development of plants are influenced by salinity levels in the soil, and

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consequently, plants were classified based on their adaptive evolution of salinity into halophytes (salt-tolerant plants) and glycophytes (sensitive-salt plants) (Gupta and Huang 2014). Unfortunately, most crops belong to this second category, which made salinity is one of the most environmental stresses that affect crop productivity worldwide (Gupta and Huang 2014). Plant tolerance to salinity stress includes biochemical and metabolic pathways, complex physiological traits, and gene networks or molecular mechanisms (Munns and Tester, 2008). A comprehensive realization of how plants respond to salinity stress is of immense importance to developing salt-tolerant species in salt-influenced areas (Gupta and Huang, 2014).

Plant tissue culture techniques can offer a good option to produce whole plants in a short period for better testing, selecting, studying, and understanding the diverse environmental conditions like salinity in isolation of all other external environmental factors (Nagella and Murthy, 2011). The physiological responses to salt stress in vitro grown plants are similar to those in vivo plants under similar stress conditions (Shibli and Al-Juboory, 2002). Therefore, plant tissue culture is beneficial for understanding stress tolerance mechanisms in plants and its impact on secondary metabolites production (Shibli et al., 1992; Nagella and Murthy, 2011). Proline content was measured as an indication of the salt tolerance of the plant. Proline is one of the most abundant natural amino acids and is one of the most common to accumulate in plants under abiotic environmental stress as such as drought, salinity, high or low temperatures, or nutrient shortage. However, the high accumulation of proline in the roots and leaves of plants is a signal of a physiological response caused by osmotic stress like salinity and drought. (Aleksza, *et al*, 2017; Szabados and Savoure).

Paronychia argentea is a perennial medicinal herb that belongs to the family of Caryophyllaceae, and commonly named as silver nail root or silvery whitlow (Abuhamdah, 2013) and is vastly distributed throughout Jordan, Syria,

Palestine, Libya (Mukassabi et al., 2012), and Egypt (Gad et al., 2012), where it grows wild under harsh environmental conditions of high temperatures, low rainfall precipitations, high evaporation rates, and saline soil.

This herb is reported to contain many secondary metabolites like flavonoids including mainly Isorhamnetin, Quercetins, and luteolin (Adjadj and Djarmouni, 2018). Quercetins is considered as the main type of flavonoids (Bors, et al. 1990), that have many biological functions including protecting against lipid peroxidation, anticancerous, anti-inflammatory actions, and antioxidant benefits (Arya, et al., 2008) Isorhamnetin is naturally presented in different kinds of plants and has many biological functions including anti-inflammatory and oxidation activities and it can decrease cancer risk and control the growth of many kinds of cancer (Zuo, et al 2011).

P. argentea is popularly known as Arabic tea and is commonly used in folk medicine in the region (Adjadj and Djarmouni, 2018) for the treatment of bladder and prostate diseases, and abdominal ailments (Bouanani et al., 2010) in addition to kidney stones, diabetes and heart pains (Afifi et al., 2005).

In this study, growth, biochemical responses, and ion uptake in *P. argentea* were investigated under -salinity induced in vitro conditions. This study was conducted to evaluate the in vitro growth of *P. argentea* micro-plants and quercetin and isorhamnetin production under different NaCl concentrations.

Materials and Methods

Mother stock establishment: Pre-established in vitro grown micro shoots of *P. argentea* by (Al Qudah et al., 2014 & Al Enizi 2020) was transferred with 2.0 cm length onto solid free-hormone Murashige and Skoog (MS) media (Murashige and Skoog, 1962) consisting of 4.43 g/L of MS premix salt (Sigma Aldrich Murashige and Skoog Basal salt mixture + vitamins) and 30 g/L sucrose.

Then cultures were kept under growth room conditions (24 ± 2 °C under a daily light regime of 16-h, 8-h dark).

Sub-culturing of micro shoots was performed every 3-4 weeks by transferring 2.0 cm micro shoots into fresh MS media described above under completely sterile conditions (laminar air-flow cabinet) till enough mother stock was obtained for the experiments.

Effect of NaCl level on growth of *P. argentea* micro plants grown in vitro under salinity induced environment:

Plant materials were subcultured onto full strength MS media plus 30 g/L sucrose and supplemented with NaCl at different concentrations (0, 25, 50, 100, and 200 mM). Five micro shoots (1.0 cm) were transferred into each flask randomly before being incubated under growth room conditions described earlier. Each treatment was replicated three times. Data was collected for the shoot growth parameter (shoot length, number of shoots, and root length) after 4 weeks of culturing.

Effect of NaCl level on ion uptake of *P. argentea* micro plants grown in vitro under salinity induced environment:

Ion analysis was experimented for potassium (K), magnesium (Mg), phosphorus (P), sodium (Na), and calcium (Ca) content, as ion analysis was performed for in vitro micro plants grown under different salinity stress levels (0, 25, 50, 100, and 200 mM) for four weeks. Samples of different dried plant materials were weighed and were placed in 100 ml beakers. About 5 ml of concentrated nitric acid (HNO₃) and 1.5 ml of perchloric acid (HClO₄) were added to each 0.5 g of the dried plant samples (Jones, 1984). Then the samples were heated on a hot plate until the production of red NO₂ fumes was stopped. After cooling the samples (at room temperature), 5 ml of diluted (1:1) of HCl were added and finally diluted with distilled water up to 25 ml solution. The samples

were transferred to a 50 ml volumetric flask and the ions were measured using an Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES; model optima 4300, Australia).

Effect of NaCl level on biochemical responses of *P. argentea* micro plants grown in vitro under salinity induced environment

Proline Accumulation: Free proline accumulation was determined using Bates protocol (Bates et al., 1973). Samples of the fresh micro plants (each weighed 0.5 g) were placed in 10 ml of 3% sulfosalicylic acid. The samples were then filtrated twice using Wattman filter paper. About 3 ml of supernatant were mixed with 3 ml of glacial acetic acid and 3 ml of acid ninhydrin solution (1.25 g ninhydrin and 30 ml of glacial acetic acid and 20 ml of 6 M phosphoric acid) in a test tube, the mixture was then boiled for 1 hour to allow color development and then the absorbance at OD520 of the solution was measured by using a BIO-RAD UV/Visible Spectrophotometer (Smart Spec™ Plus spectrophotometer, USA) and compared against with a toluene solution treated as blank. Using a standard stock solution (100 µg), serial dilutions (0, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µmole/ml) was prepared and the proline content was weight basis as follow:

Proline in µmol g⁻¹ FW = (OD_{extract} – blank)/Slop × Vol_{extract}/Vol_{aliquot} × 1/FW)

Where FW is the fresh weight of the sample in g, OD₅₂₀ is the optical density and Vol is the final volume of the aliquot.

Quercetins and Isorhamnetin: *P. argentea* micro plants cultured at different NaCl levels (0, 25, 50, 100, 200 mM) were analyzed after 4 weeks for their contents of Quercetins and Isorhamnetin. Plant materials were collected and dried-oven at 50 °C for 2 days, then grounded using mortar and pistil. After that, 2 g was taken from the dried matter of each treatment and soaked in 20 ml of pure ethanol with 5% of glacial acetic acid. The samples were then placed on a shaker at a slow speed (140

rpm) for at least 72 hours at room temperature (Sait et al., 2015). The plant extract will have purified with nano-filtration and centrifuged at 10,000 rpm for 10 min, and the resulting supernatant will have pooled, filtered, and dried in a rotary evaporator at 70 °C. Next, the dried extract was resuspended in the mobile phase for HPLC analysis.

Preparation of quercetins and isorhamnetin stock solutions and working standards: Quercetins stock solution (40 ppm) was prepared by weighing 2.0 mg of quercetins in a 5 ml volumetric flask, dissolved, and completed up to the volume by methanol HPLC grade. The prepared stock solution was stored at 4°C in dark. Working solutions were prepared by serially diluting stock solutions using the mobile phase at concentrations of 25, 12.5, 6.25, 3.125, 1.5, 0.78, 0.39, 0.19, 0.09, 0.049 ppm. Fresh working standards were prepared on daily basis. The calibration curve was constructed by performing a linear regression analysis of the peak area versus the quercetins concentration as shown.

Isorhamnetin stock solution (1000 ppm) was prepared by weighing 5 mg of isorhamnetin in a 5 ml volumetric flask, dissolved, and completed up to the volume by methanol HPLC grade. The prepared stock solution was stored at 4°C in darkness. Working solutions were prepared by serially diluting stock solutions using the mobile phase at concentrations of 20, 10, 5, 2.5, 1.25, 0.625, 0.3125, 0.156, 0.78, 0.039 ppm. Fresh working standards were prepared on daily basis. The calibration curve was constructed by performing a linear regression analysis of the peak area versus the isorhamnetin concentration. Where y is the peak area, x is the isorhamnetin concentration in ppm.

Analysis of Quercetins and Isorhamnetin using (Reversed-phase (RP) Chromatography): The samples were eluted through (RP) C18 column using an isocratic mobile phase comprising 40% from (0.5%) phosphoric acid: 60% acetonitrile and 5.0 ml THF of pH 2.4, at room temperature with a sustainable flow rate. The samples

were injected and the analysis was carried out at 210 nm as described by Sait et al., (2015). The peaks appear at 3.2 min for quercetins and 4.3 min for isorhamnetin.

Statistical Analysis: All the treatments described above were arranged in a completely randomized design (CRD). For experiments of NaCl effect on growth parameters, each treatment was replicated three times (three flasks per treatment), and each flask contained five micro plants, and the mean was calculated for each flask. For the determination of ions, proline, (quercetins and isorhamnetin), three replicates were taken from each treatment and the mean was calculated for each replicate. The collected data were statistically analyzed using the SPSS analysis system. Mean separation and standard errors were calculated at a probability level of 0.05 according to Tukey's HSD.

Results

Effect of NaCl level on growth of *P. argentea* micro plants grown in vitro under salinity induced environment

The obtained results for NaCl impacts on growth indicated that increasing NaCl level in the media had negatively affected most growth parameters in *P. argentea* micro shoots (Table 1, Figure 1-2). Moreover, the severity of the adverse effects of NaCl on growth increased significantly with increasing the salt level in the media (Table 1).

For shoot length, a decline happened gradually in shoot length with increasing the salt concentration. At 25 and 50 mM; the shoot length was reduced to 3.1 and 2.4 cm; respectively. On the other hand; a decline was observed at salinity levels of 100 and 200 mM NaCl, with (1.6 and 1.2 cm; respectively) of shoot length. compared to (4.7 cm) obtained in the control treatment (Table 1, Figure 1). Similarly, the number of newly developed micro shoots had also decreased with an increasing salt level in the media, where a maximum number of micro shoots were

recorded in the control (2.5) followed by 25 mM treatment with a mean value of 1.6 (Table 1).

Moreover, root growth declined with increasing NaCl level in the media compared to the control treatment (Table 1). The longest roots were recorded in the control treatment (4.4 cm), while the shortest roots (0.6 cm) were obtained at a NaCl level of 100 mM. On the other hand, root formations were stopped at the maximum salinity level of 200 mM (Table 1).

Meanwhile, shoot fresh and dry weights were decreased by salinity levels. The highest values for shoot fresh weight (8.7 g) were obtained under the control (non-saline) conditions, while the fresh weight tended to decline with increasing NaCl level in the media and recorded a minimum value of (5.2 g) at 200 mM NaCl (Figure 2). A similar trend was also found in dry weight results, as the highest shoot dry weight (1.8 g) was recorded in the control treatment, while the lowest shoot dry weight (1.1 g) was obtained at 200 mM (Figure 2). As we can see from Table 1; the salinity does that *P. argentea* can tolerate before significant reductions in the shoot or root growth were observed at 50 mM of NaCl.

Effect of NaCl level on ion uptake of *P. argentea* micro plants grown in vitro under salinity induced environment

Obtained data revealed a decline in phosphorus content in response to NaCl in the media (Figure 3 A), where maximum phosphorus content was obtained under the control level (1114 ppm/D. W), while it continued to decline as NaCl increased in the media to reach a minimum level of (938 ppm/D.W) at 200 mM (Figure 3 A). Moreover, Mg^{++} content increased at salinity level of 25 mM level (535 ppm/D. W) compared to the control (480 ppm/D. W), while it tended to decline at higher NaCl levels to reach the lowest value (391 ppm/D.W) of Mg^{++} at 200 mM NaCl (Figure 3 B).

Meanwhile, increasing the NaCl levels from 0 to 200 mM NaCl resulted in an increase of Na^{+} content (Figure 3 C), and Na^{+} content reached the maximum value (18328

ppm/D. W) at 200 mM compared to (1124 ppm/D. W) obtained with the control. The content of Ca^{++} was reduced by 20% at NaCl concentration of 200 mM compared with control (Figure 3 D). A similar trend was also found in K^{+} results, as K^{+} content was declined in response to salinity stress. About 30 % reduction in K^{+} level at 200 mM when compared with the control (Figure 3 E)

Effect of NaCl level on biochemical responses of *P. argentea* micro plants grown in vitro under salinity induced environment

Proline Content: Proline content in *P. argentea* in vitro stressed micro plants was affected by salinity stress, where proline level tended to increase with increasing NaCl concentration in the (Figure 6). The highest proline contents (80.0 and 80.8 $\mu\text{mol/g}$) were obtained at 100 mM and 200 mM of NaCl levels compared to only (30.4 $\mu\text{mol/g}$) proline recorded in the control treatment contents (Figure 4).

Quercetins and Isorhamnetin: Data revealed that quercetin content in *P. argentea* micro plants was increased by 1.7-fold in concentration in response to salinity at 50 mM of NaCl. (Figure 5). Interestingly, no quercetins were traced at either 100 mM or 200 mM NaCl level (Figure 5).

Moreover, an about 7-fold increase in the concentration of isorhamnetin content was obtained due to salinity at 100 mM (Figure 5). Meanwhile, isorhamnetin declined significantly in micro plants exposed to 200 mM NaCl to reach (0.9 mg/g) (Figure 5).

Discussion

Effect of NaCl level on growth of *P. argentea* micro plants grown in vitro under salinity-induced environment: Our results revealed that all growth parameters in *P. argentea* micro shoots were decreased in response to the addition of NaCl (Tables 1, Figure 1). Moreover, the decrease in growth parameters (shoot, and

root lengths in addition to a number of shoots) became more as NaCl concentration increased (Tables 1, Figure 1). Generally, salinity was described as a direct cause of the reduction in plant growth and yield due to the accumulation of Na⁺ and Cl⁻ ions in plant cells that would prevent further cell elongation and division activities (Azizi et al., 2011). Our results were in complete agreement with Ahmad et al., (2017) and Li et al. (2014), who reported a significant reduction in growth parameters in vitro grown cultures of *Pisum sativum* and *Dianthus superbus* in response to adding NaCl to the culture media. Moreover, unlike the results of NaCl treated micro plants, the control in our experiments recorded the highest fresh weight in *P. argentea* (Figure 2). This can be referred to as the low amount of water available for micro plants absorption due to the presence of NaCl in the media, which reduced water uptake in addition to the continuous dehydration of plant cells due to high salt accumulation inside the cells. Zaki and Yokoi (2016) confirmed that fresh weight on tomatoes was reduced in response to salinity stress which would indicate a direct relationship between the salinity concentration and growth reduction. According to our findings, a reduction in dry weight occurred when NaCl concentration increased in the culture media (Figure 2). Similar results were reported in *Dianthus barbatus* in vitro grown under salinity conditions (Azizi et al., 2011). According to Akula and Ravishankar (2011), when plants grow under salinity stress conditions nutrient uptake tends to decline as a result of low water uptake in the cytoplasm and this will reduce cell growth which can be translated into a decrease in the synthesis and accumulation of dry matter.

Nutrient uptake in *P. argentea*: The obtained results indicated a reduction in P, Ca⁺⁺, Mg⁺⁺, and K⁺ uptake compared to high accumulation of Na ions in response to NaCl level in the media (Figure 3). However, the decline in K⁺ in our results would indicate that this plant is salt-sensitive (Maathuis and Amtmann, 1999). A reduction in

the concentrations of P, Ca⁺⁺, and K⁺ uptake in response to salinity was reported in many plant species. For example, a decline in roots uptake and shoot translocation of P was found in strawberry plantlets grown under high salinity-induced conditions (Demiral et al., 2017). Moreover, uptake of Na ions was reported to compete with K⁺ in many plants such as soybean (*Glycine max* L.) (Weisany et al., 2014) and sorghum (*Sorghum bicolor*) (Colmer et al., 1996) upon exposure to salt stress. Meanwhile, ion uptake of K⁺, Ca²⁺, and Mg²⁺ was found to decrease in six olive cultivars grown in NaCl enriched mineral solution, while P was not affected (Loupassaki et al., 2002).

Proline Content: In this study, the amount of proline in *P. argentea* was increased as salinity level increased, where proline content was maximized at 200 mM NaCl (Figure 4). Proline is well known as one of the most important physiological strategies in plant defense and protection against different abiotic stresses especially salinity (Kavi Kishor et al., 2014; Ashraf et al., 2012). Our data were in full agreement with (Reddy et al., 2015), who reported an increase in proline amounts in *Sorghum bicolor* grown in vitro under NaCl stress. Also, our findings agreed with Ghoulam et al., (2002), who reported that increasing salts in the planting media of sugar beet resulted in increasing the amount of proline in the leaves. Also, Bolarin et al., (1995) reported an increase in proline amounts in tomato leaves when grown under salt stress environment.

Quercetins and Isorhamnetin: Quercetins and isorhamnetin contents in the in vitro grown micro plants of *P. argentea* were affected by the different levels of NaCl (Figure 5). Maximum quercetin content (3.2 mg/g) was obtained at 50 mM NaCl treatment, while the highest isorhamnetin value (6.3 mg/g) was recorded at 100 mM salt level (Figure 5).

The addition of NaCl to the culture media was reported to increase and enhance the production of different forms of secondary metabolites in various species of in vitro grown plants (Akula and Ravishankar 2011; Naik and Al-Khayri 2016) as a protective response to both biotic and abiotic stress conditions (Rejeb et al., 2014). For example, salinity stress resulted in increasing the number of phenolic compounds in *Fagopyrum esculentum* (Lim, *et al.*, 2012). Also, elevated levels of NaCl up to 200 mM were found to increase contents of gossypol in cotton by 26.8–51.4%, while flavonoids increased by 22.5–37.6% at 7–28 days after salt stress (Wang et al, 2015). However, based on our results for quercetins and isorhamnetin in *P. argentea*, adding NaCl up to a certain level to the media can be used for enhancing the production of these two valuable medicinally important compounds in vitro.

Conclusions

In this study, responses of the in vitro grown *P. argentea* micro plants to the addition of different levels of NaCl to the media were investigated in terms of growth, ions uptake, in addition to proline, quercetins, and isorhamnetin content. Our results showed that *P. argentea* showed a salt-tolerant behavior at 25 mM. Our results indicated that the highest quercetin content was obtained at 25 mM of NaCl; while isorhamnetin was increased at 100 mM. In general, preliminary results of our study indicate that; 25 mM of NaCl was the best concentration for *P. argentea* growth and secondary metabolites production. However, more research is still needed for a better understanding of *P. Argentina's* behavior under salt stress conditions. Also, more research is needed to find other means for enhancement of the production of quercetins and isorhamnetin in *P. argentea* in a way that wouldn't expose

plant growth to decline to insure obtaining a constant supply of plant material.

REFERENCES

- Abuhamdah, S., Abuhamdah, R., Al-Olimat, S., & Chazot, P. (2013). Phytochemical investigations and antibacterial activity of selected medicinal plants from Jordan. *European Journal of Medicinal Plants*, 3
- Adjadj, M., & Djarmouni, M. (2018). Study of the Antioxidant Property and Xanthine Oxidase Inhibitory Activity of Various Extracts from the Algerian Medicinal Plant *Paronychia argentea* L. *Pharmacognosy Communications*, 8(1), 49-53.
- Afifi, F. U., Al-Khalidi, B., & Khalil, E. (2005). Studies on the in vivo hypoglycemic activities of two medicinal plants used in the treatment of diabetes in Jordanian traditional medicine following intranasal administration. *Journal of ethnopharmacology*, 100(3), 314-318.
- Ahmad, F., Singh, A., & Kamal, A. (2017). The ameliorative effect of salicylic acid in salinity stressed *Pisum sativum* by improving growth parameters, activating photosynthesis, and enhancing the antioxidant defense system. *Biosci Biotech Res Comm*, 10, 481-489.
- Akula, R., & Ravishankar, G. A. (2011). Influence of abiotic stress signals on secondary metabolites in plants. *Plant signaling & behavior*, 6(11), 1720-1731
- Al Qudah, T., Engelmann, F., Saifan, S., and Shibli, R. Effect of liquid nitrogen (LN) exposure on survival of selected plant species from Jordan. BOT-ERA "Reinforcing cooperation between the Royal Botanic Garden of Jordan and ERA". FP7: THEME [INCO.2011- 6.2 INCO.2011- 6.2 KBBE]. WP2 Twinning, Topic B: Genetic Resource Diversity and Biotechnology. Royal Botanic Garden-Jordan, Hamdi Mango Research Center Jordan, IRD Montpellier-France. 2014
- Aleksza, D., Horváth, G. V., Sándor, G., and Szabados, L. (2017), Proline accumulation is regulated by

- transcription factors associated with phosphate starvation. *Plant Physiology* 175(1): 555-567.
- Al Enizi, A., Shibli, R. A., Tahtamouni, R. W., Al-Qudah, T. S., & Abu-Iramaileh, B. (2020). In Vitro Propagation and Enhancement of Quercetins and Isorhamnetin Production in Wild *Paronychia argentea* L. *Jordan Journal of Pharmaceutical Sciences*, 13(1):65-75.
- Arya, D., Patni, V., and Kant, U. (2008), In vitro propagation and quercetin quantification in callus cultures of Rasna. *Indian Journal of Biotechnology*, 7: 383-387.
- Ashraf, M. A., Ashraf, M., & Shahbaz, M. (2012). Growth stage-based modulation in antioxidant defense system and proline accumulation in two hexaploid wheat (*Triticum aestivum* L.) cultivars differing in salinity tolerance. *Flora-Morphology, Distribution, Functional Ecology of Plants*, 207(5), 388-397
- Azizi, M., Chehraz, M., & Zahedi, S. M. (2011). Effects of salinity stress on germination and early growth of sweet William (*Dianthus barbatus*). *Asian Journal of Agricultural Sciences*, 3(6), 453-458.
- Bates, L. S., Waldren, R. P., & Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. *Plant and Soil*, 39(1), 205-207
- Bolarin, M. C., Santa-Cruz, A., Cayuela, E., & Perez-Alfocea, F. (1995). Short-term solute changes in leaves and roots of cultivated and wild tomato seedlings under salinity. *Journal of Plant Physiology*, 147(3-4), 463-468.
- Bors, W., Heller, W., Michel, C., and Saran, M. (1990). Flavonoids as antioxidants: Determination of radical-scavenging efficiencies. *In Methods in enzymology* 186: 343-355.
- Bouanani, S., Henchiri, C., Migianu-Griffoni, E., Aouf, N., & Lecouvey, M. (2010). Pharmacological and toxicological effects of *Paronychia argentea* in experimental calcium oxalate nephrolithiasis in rats. *Journal of ethnopharmacology*, 129(1), 38-45.
- Colmer, T. D., Fan, T. W. M., Higashi, R. M., & Läuchli, A. (1996). Interactive effects of Ca^{2+} and NaCl salinity on the ionic relations and proline accumulation in the primary root tip of *Sorghum bicolor*. *Physiologia Plantarum*, 97(3), 421-424.
- Demiral, M. A. (2017). Effect of salt stress on the concentration of nitrogen and phosphorus in root and leaf of the strawberry plant. *Eurasian Journal of Soil Science*, 6(4), 357-364
- Gad, M. R. M., El-Hadidy, M. E., & El-Nabarawy, A. A. (2012). Comparative study on the adaptation of some natural plants grown under macronutrients limitation at North Sinai sand dunes (Egypt). *Annals of Agricultural Sciences*, 57(1), 81-90.
- Ghoulam, C., Foursy, A., & Fares, K. (2002). Effects of salt stress on growth, inorganic ions, and proline accumulation in relation to osmotic adjustment in five sugar beet cultivars. *Environmental and Experimental Botany*, 47(1), 39-50
- Gupta, B., & Huang, B. (2014). Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization. *International journal of genomics*, 2014:1-18
- Jones Jr J B. (1984) Plants. In *Official Methods of Analysis of the Association of Official Analytical Chemists*. Ed. S Williams. pp 38-64. Association of Official Analytical Chemists, Arlington, Virginia 22209, USA.
- Kavi Kishor, P. B., & Sreenivasulu, N. (2014). Is proline accumulation per se correlated with stress tolerance or is proline homeostasis a more critical issue? *Plant, cell & environment*, 37(2), 300-311.
- Li, T., Hu, Y., Du, X., Tang, H., Shen, C., & Wu, J. (2014). Salicylic acid alleviates the adverse effects of salt stress in *Torreya grandis* cv. *Merrillii* seedlings by activating photosynthesis and enhancing antioxidant systems. *PLOS One*, 9(10), e109492.
- Lim, J. H., Park, K. J., Kim, B. K., Jeong, J. W., & Kim, H. J. (2012). Effect of salinity stress on phenolic compounds and carotenoids in buckwheat (*Fagopyrum esculentum* M.) sprout. *Food Chemistry*, 135(3), 1065-1070
- Loupassaki, M. H., Chartzoulakis, K. S., Digalaki, N. B., & Androulakis, I. I. (2002). Effects of salt stress on the

- concentration of nitrogen, phosphorus, potassium, calcium, magnesium, and sodium in leaves, shoots, and roots of six olive cultivars. *Journal of Plant Nutrition*, 25(11), 2457-2482.
- Maathuis, F. J., & Amtmann, A. N. N. A. (1999). K⁺ nutrition and Na⁺ toxicity: the basis of cellular K⁺/Na⁺ ratios. *Annals of botany*, 84(2), 123-133.
- Mukassabi, T. A., Ahmidat, G., Sherif, I. M., Elmogasapi, A., & Thomas, P. A. (2012). Checklist and life forms of plant species in contrasting climatic zones of Libya. *Biological Diversity and Conservation*, 5(3), 1-12.
- Munns, R., & Tester, M. (2008). Mechanisms of salinity tolerance. *Annual Review of Plant Biology*, 59, 651-681.
- Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum*, 15(3), 473-497
- Nagella, P., & Murthy, H. N. (2011). Effects of macroelements and nitrogen source on biomass accumulation and withanolide-A production from cell suspension cultures of *Withania somnifera* (L.) Dunal. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 104(1), 119-124
- Naik, P. M., & Al-Khayri, J. M. (2016). Abiotic and biotic elicitors-role in secondary metabolites production through in vitro culture of medicinal plants. *Abiotic and biotic stress in plants-recent advances and future perspectives*, 247-277
- Reddy, P. S., Jageswar, G., Rasineni, G. K., Maheswari, M., Reddy, A. R., Varshney, R. K., & Kishor, P. K. (2015). Proline over-accumulation alleviates salt stress and protects photosynthetic and antioxidant enzyme activities in transgenic sorghum [*Sorghum bicolor* (L.) Moench]. *Plant Physiology and Biochemistry*, 94, 104-113
- Rejeb, I., Pastor, V., & Mauch-Mani, B. (2014). Plant responses to simultaneous biotic and abiotic stress: molecular mechanisms. *Plants*, 3(4), 458-475.
- Sait, S., Hamri-Zeghichi, S., Boulekbache-Makhlouf, L., Madani, K., Rigou, P., Brighenti, V., ... & Pellati, F. (2015). HPLC-UV/DAD and ESI-MSn analysis of flavonoids and antioxidant activity of an Algerian medicinal plant: *Paronychia argentea* Lam. *Journal of pharmaceutical and biomedical analysis*, 111, 231-240.
- Shi, H., & Zhu, J. K. (2002). Regulation of expression of the vacuolar Na⁺/H⁺ antiporter gene AtNHX1 by salt stress and abscisic acid. *Plant molecular biology*, 50(3), 543-550
- Shibli, R. A., & Al-Juboory, K. (2002). Comparative Responses of "NABALI" Olive micro shoot, callus, and suspension cell cultures to salinity and water deficit. *Journal of plant nutrition*, 25(1), 61-74
- Shibli, R. A., Smith, M. A. L., & Spomer, L. A. (1992). Osmotic adjustment and growth responses of three *Chrysanthemum morifolium* Ramat. cultivars to osmotic stress-induced in vitro. *Journal of plant nutrition*, 15(9), 1373-1381.
- Shrivastava, P., & Kumar, R. (2015). Soil salinity: a serious environmental issue and plant growth-promoting bacteria as one of the tools for its alleviation. *Saudi journal of biological sciences*, 22(2), 123-131.
- Szabados, Laszlo, and Arnould Savoure (2010), Proline: a multifunctional amino acid. *Trends in Plant Science* 15(2): 89-97.
- Wang, Q., Eneji, A. E., Kong, X., Wang, K., & Dong, H. (2015). Salt stress affects secondary metabolites of cotton in relation to gene expression responsible for aphid development. *PloS one*, 10(6), e0129541.
- Weisany, W., Sohrabi, Y., Heidari, G., Siosemardeh, A., & Badakhshan, H. (2014). Effects of zinc application on growth, absorption, and distribution of mineral nutrients under salinity stress in soybean (*Glycine max* L.). *Journal of plant nutrition*, 37(14), 2255-2269
- Zaki, H. E., & Yokoi, S. (2016). A comparative in vitro study of salt tolerance in cultivated tomato and related wild species. *Plant Biotechnology*, 16-1006.
- Zuo, A., Yanying, Y., Li, J., Binbin, X., Xiongying, Y., Yan, Q., and Shuwen, C. (2011), Study on the relation of

structure and antioxidant activity of isorhamnetin,
quercetin, phloretin, silybin, and phloretin isonicotinic

hydrazone. *Free Radicals and Antioxidants*, 1(4): 39-47.



Figure 1. Effect of different levels (0, 25, 50, 100, and 200 mM) of NaCl on micro shoot length of *P. argentea*.

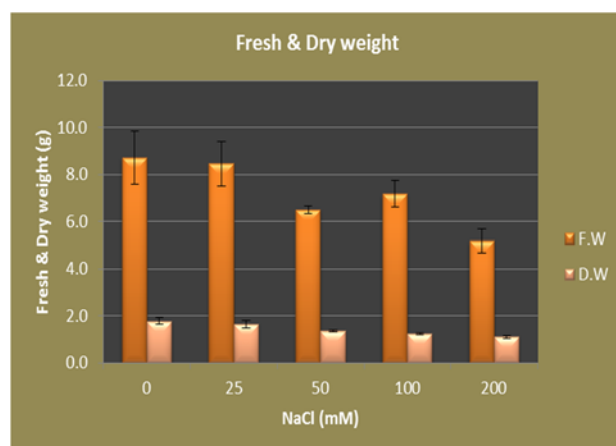
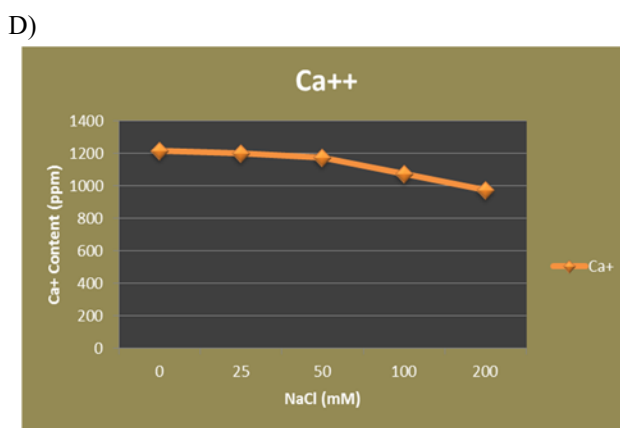
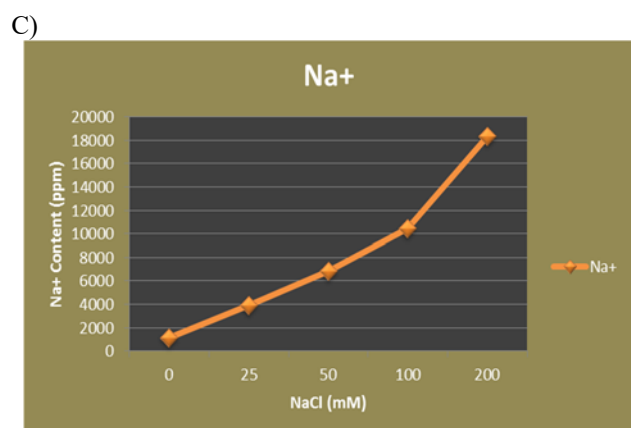
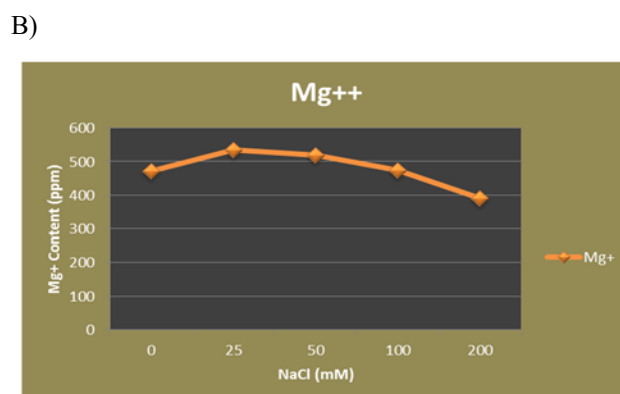
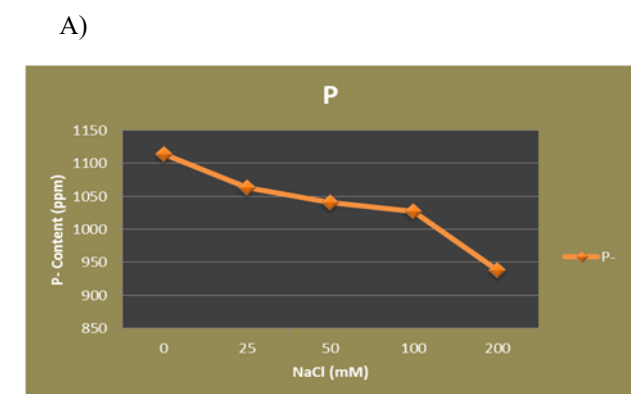


Figure 2: Effect of different levels of NaCl on the fresh and dry weight of *P. argentea* micro shoots. Values represent means \pm SE. Control represents NaCl free MS media + 30 g sucrose



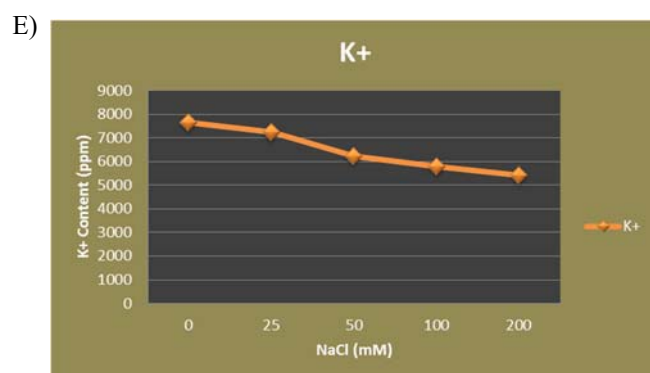


Figure 3: Effect of different levels of NaCl on minerals (P-, Mg+2, Na+1, Ca+2, K+1) contents (ppm/D.W) in *P. argentea* micro shoots; in the Figure (A-E); respectively. Control (0) represents NaCl free MS media plus 30 g sucrose

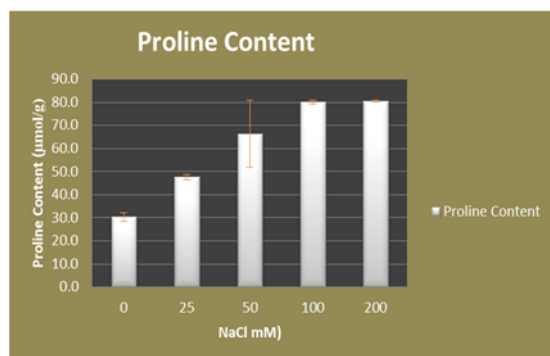


Figure 4: Effect of different levels of NaCl on the proline content (µmol /g F.W) of *P. argentea* micro shoots. Values represent means \pm SE. Control (0) represents NaCl-free MS media plus 30 g sucrose.

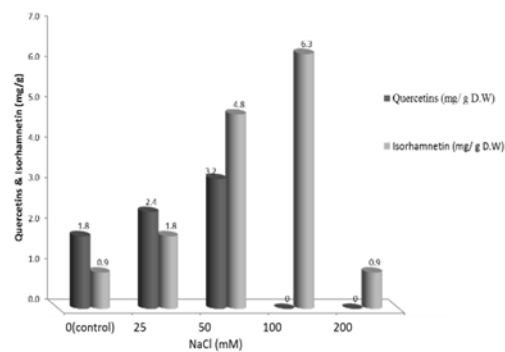


Figure 5: Effect of different levels of NaCl on Quercetins and Isorhamnetin content (mg/g D.W) of *P. argentea* micro shoots.

Table 1: Effects of different levels of NaCl on the growth parameters of *P. argentea* micro shoots.

NaCl levels (mM)	Shoot length (cm)	Shoot number	Root length (cm)
0 (control)	4.7 ± 0.25a*	2.5±0.07a	4.4±0.24a
25	3.1 ± 0.09b	1.6±0.20a	2.6±0.05b
50	2.4 ± 0.07c	0.0±0.00b	2.6±1.21c
100	1.6 ± 0.08d	0.3±0.33b	0.6±0.25d
200	1.2 ± 0.03d	0.0±0.00b	0.0±0.00d
Mean Square	5.637	3.824	9.120
F Value	118.021	40.971	9.644

*Values represent means ± SE. Means with different letters are significantly different according to Tukey HSD range test at $P \leq 0.05$. Control represents NaCl free MS media + 30 g sucrose.

تتبع النمو والاستجابات البيوكيميائية وامتصاص الأيونات في سويقات نبات رجل الحمام المزروعة داخل

الأنابيب في بيئة مزودة بالملوحة

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ملخص

نبات رجل الحمام هو نبات طبي بري عشبي يحتوي الكثير من المركبات الثانوية، وينمو نبات رجل الحمام في الأردن في ظل ظروف التربة شديدة الملوحة، وأجريت هذه الدراسة لبحث تأثير إجهاد الملوحة على النمو والاستجابات البيوكيميائية وامتصاص الأيونات لسويقات نبات رجل الحمام المزروعة داخل الأنابيب، وأظهرت نتائجنا انخفاضاً واضحاً في جميع معايير النمو استجابةً لزيادة مستويات الملوحة المستخدمة (0، 25، 50، 100، 200 ملي مولار). وعندما تم تزويد وسط النمو بـ 200 ملي مولار من كلوريد الصوديوم؛ انخفض الوزن الرطب والجاف بنسبة 40% مقارنة بالمعاملة المرجعية. علاوة على ذلك، انخفض امتصاص النباتات داخل الأنابيب لأيونات الأملح P و Ca ++ و K + مع زيادة مستويات الملوحة، وتمت زيادة محتوى Mg ++ بنسبة 10% عند مستوى 25 ملي مولار من كلوريد الصوديوم مقارنة بالمعاملة المرجعية، بينما انخفض بنسبة 20% عند مستوى 200 ملي مولار من كلوريد الصوديوم، ومن ناحية أخرى زاد امتصاص الصوديوم مع زيادة مستويات الملوحة ووصل إلى قيمة قصوى 18328 جزء في المليون/ وزن جاف، عند 200 ملي مولار من كلوريد الصوديوم، وعلاوة على ذلك، زاد محتوى البرولين استجابة لمستوى الملوحة ليسجل قيمة قصوى (80.8 ميكرو مول / جرام) عند 200 ملي مولار من كلوريد الصوديوم مقارنة بـ (30.4 ميكرو مول / جرام) في المعاملة المقارنة، وتمت زيادة محتوى مادة الكيروتستين في سويقات نبات رجل الحمام داخل الأنابيب بمقدار 1.7 ضعفاً استجابةً للملوحة عند 50 ملي مولار من كلوريد الصوديوم، وبينما تم الحصول على زيادة بمقدار 7 أضعاف في تركيز محتوى الأيزورهمانتين بسبب الملوحة عند 100 ملي مولار.

الكلمات الدالة: الاستجابات البيوكيميائية، داخل الأنابيب، النبتة الدقيقة، امتصاص المغذيات، الملوحة.