EEET ECOLOGICAL ENGINEERING & ENVIRONMENTAL TECHNOLOGY

Ecological Engineering & Environmental Technology 2021, 22(5), 55–64
https://doi.org/10.12912/27197050/139408
ISSN 2719-7050, License CC-BY 4.0

Received: 2021.06.12
Accepted: 2021.07.10
Published: 2021.08.01

Salts Induced Salinity and in Vitro Multiplication of *Paronychia argentea*

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ABSTRACT
Paronychia argentea is a wild herb plant with a high medicinal value. *P. argentea* plant is a neglected herb that grows without any attention in terms of research for cultivation and propagation. The conventional propagation methods of *P. argentea* by seeds and cutting are not preferred due to low germination percentage and cutting rooting problems. As a substitute for seed propagation, effective micropropagation protocols were developed. Using 0.6 mg/L 6-Benzylaminopurine (BAP), maximum of 3.90 (shoot/explant) was produced on Murashige and Skoog (MS) medium. The *in vitro* growth was significantly decreased with increasing NaCl concentration. The potassium (K) and nitrogen (N) concentrations in *P. argentea* plantlets decreased significantly under NaCl treatment. The level of protein in leaf tissue generally decreased with increased NaCl concentrations in the medium. The proline content in *P. argentea* plantlets increased significantly along with NaCl concentrations. An increase in NaCl concentration in the medium resulted in an increase in total soluble solids (TSS) in plant tissue. Moreover, as salinity level concentration increased, relative water content was decreased. High NaCl was significantly affected the *in vitro* growth of plants. It was shown that *in vitro P. argentea* plantlets could be tolerant to *in vitro* salinity.

Keywords: micropropagation, salinity, *Paronychia argentea,* protein, proline.

INTRODUCTION

Medicinal plants have been considered as a significant basis of medication for many years. Plants have been used as sources of new synthetic compounds and treatment agents (Craker et al., 2003). In Jordan, medicinal plants have been widely used in traditional medicines. The practices of using medicinal plants are deeply rooted in Jordan culture and are considered highly valuable, being preserved and respected throughout all groups of people from high income to low income groups (Hung and Chi 2014). Therefore, for better living conditions, modern medicines and functional food sources derived from medicinal plants are in high demand.

Paronchia argentea is a wild medicinal plant that is commonly named (Rejelel Al-Hamama). It belongs to the Caryophyllaceae family. It grows in uncultivated field margins and field borders (Braca et al., 2008). It consists of important secondary metabolites, which have been used in folk medicine (Atta *et al.* 2013). *P. argentina* is commonly used to treat a variety of ailments, including kidney stones, urinary tract infections, gastro-intestinal conditions, as well as colds and fevers. (Noubani et al., 2006). The aerial parts of this plant are

utilized for the treatment of abdominal pain, respiratory infections and as an anti-stress agent (Zama et al., 2007). However, the demand for *P. argentea* has increased, which requires improving mass production for *P. argentea* by *in vitro* propagation methods.

The tissue culture methods provide a long-term production mechanism for this important plant, which could be used in medical research and industry in the future (Shatnawi et al., 2007; Shatnawi 2011; 2013; Al-Ajlouni et al., 2015). The in vitro culture of *P. argentina* is capable of resolving the propagation issues. It ensures mass plant material production without jeopardizing (endangering) natural resources, as well as improving and conserving this plant. Tissue culture has been used widely in different plant species, including: *Capparis spinose* (Al-Mahmoud et al., 2011), *Ficus carica* (Shatnawi et al., 2019), *Ruta graveolens* (Al Ajlouni et al., 2015), and *Moringa peregrine* (Alrayes et al., 2016).

Salinity is an environmental stress that could decrease crop productivity (Lee et al., 2005; Othman et al., 2006; Al Tawaha and Al Al-Ghzawi 2013; Ackin and Yalcin 2016; Al-Tawaha et al. 2018; AL-Issa et al., 2020). Moreover, Petropouplo *et al.* (2017) indicated that the plants exposed to adverse abiotic stresses showed varied effect on development and absorption. In addition, plants may change their characteristic by decreased cell elongation, enhanced division, and changing cell identification. Thus, this study was conducted to develop a reliable and simple method for *in vitro* propagation of *P. argentea* and to study the growth response of *P. argentea* to *in vitro* salinity induced stress.

MATERIAL AND METHODS

In vitro plant

In vitro microshoots of *P. argentea* were obtained from Plant Biotechnology Laboratories/Faculty of Agriculture, Jordan University, Amman, Jordan.

Shoot multiplication

The method developed by Shatnawi et al. (2010c) was used to multiply microshoots. Explants were subdivided on the MS medium (Murashige and Skoog 1962). For shoot proliferation, the microshoots were cultured on the MS medium supplemented with different concentration of 6-Benzylaminopurine (BAP), kinetin, or adenine at 0.0, 0.3, 0.6, 1.2, 1.8, or 2.0 mg/L. 80 mL of the medium was distributed into a 250 mL Duran flask. The media were hardened by using agar agar at 8.0 g/L. The media were sterilized using the autoclaved for 20 min at 121 ºC. Microshoots were incubated at 24 ± 2 ºC for 16 h. photoperiod and photosynthetic photon flux density (PPFD) of 50 µmol $m^{-2}s^{-1}$ supplied by cool white fluorescent lamps. Each flask containing three microshoots and seven replicates was used for each treatment. After five weeks, the data were collected on number of new shoots, shoot length, fresh and dry weight".

Physiological responses to NaCl

Growth *in vitro*

The methods used in this study were according to the methods developed on *Chrysanthemum morifolium* by Shatnawi *et al.* (2010). Microshoots, 15 mm in length, were cultured on the MS medium enhanced with 0.6 mg/L BAP, and accompanied with different concentration of NaCl (0, 40, 80, 120, 160 or 200 mM. After five weeks, the data on number of new shoots, shoot length, fresh and dry weight were collected.

Chlorophyll and carotenoid content

After growing for five weeks on MS media containing salts, pigments were assessed. A 0.1 g fresh weight of leaves was tested per replicate. Following the method of De Filippis *et al*. (1981), the samples were extracted by using acetone. Using a pestle and mortar, a 0.1 g shoot fresh weight was homogenized in 2.0 ml of 80% acetone (v/v). The obtained solution was pipetted into 2.0 ml microtubes and centrifuged at 15000 g for two min. The floatable solution was collected with a Pasteur pipette, and topped up to 3.0 ml in a 10 ml calculating tube with extra 80% acetone. At 480, 510, 626, 645, 649, 663, and 665 nm absorbance, the absorption spectra were calculated by a spectrophotometer, (as a reference, 80% acetone was used). According to Anderson and Boardman (1964), the chlorophyll *a*, and chlorophyll *b* contents were studied, whereas according to Duxbury and Yentsch (1956) carotenoids content was assessed.

Mineral composition

The plant samples were dried for 24 hours at 80 °C and then ground to determine the nitrogen, sodium, and potassium content. Total nitrogen was determined using the Micro-Kjeldahl

digestion procedure. A plant sample dry weight of 0.5 g was placed in test tubes. Ten g of mixture (1M K_2SO_4 + 1M $CuSO_4.5H_2O$) and 20 ml of concentrated H_2SO_4 were added to each test tube. The test tubes were then placed in the digester (Buchi Digest Automat K-438) for 135 min at 400 ºC. The samples were cooled down and then placed in the distiller (Buchi Autokjeldahl Unit K-370) to evaluate nitrogen according to Bremner *et al*. (1992). For evaluation, the crude protein content in the microshoots the total nitrogen was multiplied by 6.25 (Balman and Smith 1993). The Na and K concentrations were calculated using a flame photometer. The plant samples were turned to ash in a Thermolyne muffle furnace at 500–550 ºC for 20–24 hours (6000 Furnace). A total of 10 ml of 2N HCl was supplemented to the samples and gently heated for 7-10 min at 75–80 ºC. The solution was filtered and diluted to 50 ml with distilled using Whatman No. 42 filter paper. Using Flame photometer 410, the Na and K concentrations were determined after calibration with different concentrations of either Na or K solutions (Chapman and Pratt 1961).

Statistical analysis

Treatments were organized in a fully randomized design in each experiment (CRD). Each treatment was replicated three times in the shoot multiplication experiments. According to Tukey's HSD, the analysis of variance (ANOVA) was performed, and mean separation was tested at a 0.05 probability level. The data were statistically analyzed using the SPSS (2017) analysis system.

RESULTS

In vitro shoot proliferation of *P. argentea*

Effect of BAP

In vitro propagation showed that BAP, adenine, and kinetin have increased shoot formation of new microshoots (Table 1). When explants were inoculated on MS media containing 0.6 mg/L BAP, 3.9 microshoots were produced (Table 1, Fig. 1). Moreover, using BAP at 1.8 mg/L did not increase

Table 1. Effect of different BAP concentrations on *in vitro* growth of *Paronychia argentea* after five week growth periods

BAP (mg/L)	Number of new shoots	Microshoot length (cm)	Fresh weigh (g)	Dry weight (g)
0.0	1.2a	1.90a	0.09a	0.018a
0.3	1.6a	2.34a	0.09a	0.025a
0.6	3.9b	2.34a	0.09a	0.025a
1.2	1.6a	2.02a	0.12a	0.026a
1.8	1.3a	2.21a	0.12a	0.022a

Figure 1. Multiple microshoots formation of *Paronychia. argentea.* Extensive microshoot formation grown on MS containing 0.3 mg/L BAP. Bar represents 6 mm

the formation of new microshoots (1.30) (Table 1). With the use of BAP treatment (0.0–1.8 mg/L), the shoot length ranged from (1.9 to 2.21 cm). The highest microshoots (2.34 cm) were obtained on the MS media supplemented with 0.3 to 0.6 mg/L BAP. Fresh and dry weight displayed no significant differences at all treatment tested (Table 1). Using the MS medium containing 1.2 mg/L BAP a dry weight of 0.026 g was reported (Table 1).

The different adenine concentrations added in the MS medium was found to affect the formation of microshoots (Table 2). At 0.3 mg/L, it proved to be better compared with other adenine concentrations (2.30 microshoots) (Table 2). Using the MS medium containing 1.2 or 1.8 mg/L adenine, microshoot length of 2.03 cm was produced. Fresh weight was significantly higher on the MS medium added with 1.2 or 1.8 mg/L. On the other hand, using 0.6 and 1.2 mg/L adenine produced maximum dry weight (Table 2).

Microshoot multiplication could be achieved from the explants of *P. argentea* inoculated on the MS medium with different kinetin concentrations (Table 3). The effect of kinetin on *P. argentea* shoot induction *in vitro* showed statistically significant differences among treatments on the number of microshoots, shoot length, fresh and dry weights (Table 3). Kinetin at 1.2 mg/L effectively induced 2.65 microshoots (Table 3). Higher levels of kinetin 1.8 mg/L were significantly less effective (p<0.05) regarding microshoot multiplication, compared with 1.2 mg/L. Maximum fresh weight was recorded at 1.2 mg/L (0.120 g). In addition, at 1.2 mg/L kinetin maximum dry weight (0.0.035 g) was recorded (Table 3).

In vitro plant growth

Table 4 shows the impact of different NaCl concentrations on length of shoot, shoot number, fresh (g), and dry weight (g) after five week growth

Table 2. Effect of different adenine concentrations on *in vitro* growth of *Paronychia argentea* after five week growth periods

Adenine (mg/L)	Number of new microshoots	Microshoot length (cm)	Fresh weigh (g)	Dry weight (g)
0.0	1.22a	1.90a	0.092a	0.020a
0.3	2.30c	2.90c	0.085a	0.025b
0.6	1.47b	1.85b	0.085a	0.035c
1.2	1.57b	2.03a	0.120b	0.035c
1.8	1.24a	2.03a	0.110b	0.025b

Table 3. Effect of different kinetin concentrations on *in vitro* growth of *Paronychia argentea* after five week growth periods

Kinetin (mg/L)	Number of new microshoots	Microshoot length (cm)	Fresh weigh (g)	Dry weight (g)
0.0	1.22a	1.20a	0.090a	0.020a
0.3	1.92b	1.40b	0.090a	0.020a
0.6	1.87b	1.40b	0.095a	0.025a
1.2	2.65c	1.99c	0.120b	0.035b
1.8	2.35c	2.04c	0.110b	0.020a

Table 4. Effect of different NaCl concentrations on shoot length, number of shoots, fresh and dry weight of *Paronychia argentea* after five week growth in the MS medium containing 0.2 mg/L BAP

NaCl concentration (mM)	Length of shoot (cm)	Shoot number	Fresh weight (g)	Dry weight (g)
0.0	2.61d	3.90e	0.090b	0.015b
40	2.11bc	2.85e	0.089b	0.015b
80	1.90c	2.33d	0.092b	0.015b
120	1.60b	1.90c	0.092b	0.015b
160	1.50b	1.60b	0.070a	0.010a
200	1.20a	1.12a	0.070a	0.090a

periods in the MS media containing 0.6 mg/L BAP. The maximum shoot length of *P. argentea* decreased significantly with NaCl applications (Table 4). With the increase in the NaCl levels, the number of shoots, fresh weight, and dry weight decreased significantly. The NaCl levels from 120 to 200 significantly reduced all growth parameters. The maximum shoot length was obtained with the control (2.61 cm) compared to other NaCl treatments. Furthermore, NaCl increased fresh weight and dry weight significantly (Table 4).

Chlorophyll and nutrient content

The chlorophyll content decreased significantly with increased NaCl concentrations (Table 5). A decrease in the photosynthetic pigment content of *P. argentea* was observed with increasing NaCl treatment.

Total chlorophyll significantly decreased with increasing NaCl concentration in the medium from 40 to 200 mM. As NaCl increased in the medium, the chlorophyll *a* and *b* content decreased significantly (Table 5). Moreover, NaCl significantly decreased carotenoid (µg/g FW) in the *P. argentea* plants. Maximum carotenoid at 0.0 mM was 40.1 µg/g FW, while at 200 mM NaCl, carotenoid was equal to 17.1 µg/g FW. Furthermore, maximum Carot-Chloro value (0.187) was recorded at 80 mM NaCl (Table 5).

The K and N concentrations in *P. argentea* were significantly decreased under different NaCl treatments compared with control plants (Table 6). Na increased with higher NaCl treatments (Table 6). The exposure to 40 to 80 mM NaCl did not cause significant differences in the K and N content in the microshoot. Na increased along with the NaCl concentrations, compared with the control treatment (Table 1). In turn, as the NaCl level concentration increased, N decreased significantly (Table 6). NaCl applied to the *in vitro* grown plants caused a noticeable difference in proline concentration on *in vitro* plantlets (Fig. 2). With increasing NaCl concentrations in the media, the proline content was increased. At 200 mM NaCl concentration, the high value of proline was recorded. However, using 120 or 160 mM NaCl did not show any significant difference in the proline content (Fig. 2). Increasing NaCl significantly raised the content total soluble solids in *P. argentea* (Fig. 3). Maximum value was recorded at 200 mM NaCl (12.8), while control treatment (0.0 NaCl) recorded (2.8) (Fig. 3).

As shown in Figure 4, high protein content was obtained after five weeks of *in vitro* growth periods at low NaCl treatments. Increasing the

Table 5. Influence of different NaCl concentrations on chlorophyll and carotenoid pigments of the *Paronychia argentea* plantlets after five weeks growth on the MS medium containing 0.2 mg/L BAP

NaCl concentration (mM)	Chlorophyll a (µg/g FW)	Chlorophyll b (µg/g FW)	Tot. Chlorophyll (µg/g FW)	Carotenoid (µg/g FW)	Carot-Chloro ratio
0.0	120c	100f	220f	40.1f	0.182a
40	112c	80e	192e	35.0e	0.182a
80	100b	72d	160d	30.0d	0.187c
120	85b	66c	142c	26.2c	0.184b
160	66a	54b	120b	22.2b	0.185b
200	55a	44a	94a	17.2a	0.182a

Table 6. Influence of different NaCl concentrations on the sodium, potassium and nitrogen content in the *Paronychia argentea* plantlets after five week growth on the MS medium containing 0.2 mg/L BAP

NaCl concentraion (mM)	Na%	K%	N%
0.0	0.25a	5.01e	6.83e
40	1.20b	4.79e	6.70e
80	1.91b	4.26e	5.90d
120	3.10c	3.15c	5.30c
160	4.15d	2.81b	4.21b
200	4.35d	1.18a	3.90a

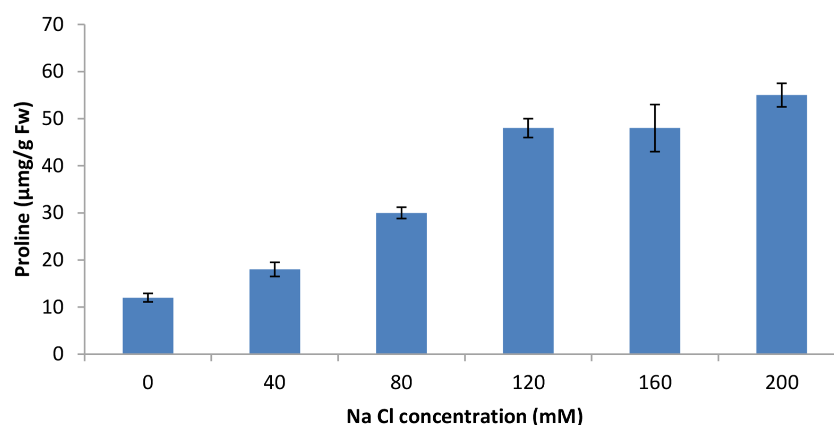

Figure 2. Impact of different NaCl concentrations on the proline content in the *Paronychia argentea* plantlets after five weeks on the MS media containing 0.2 mg/L BAP

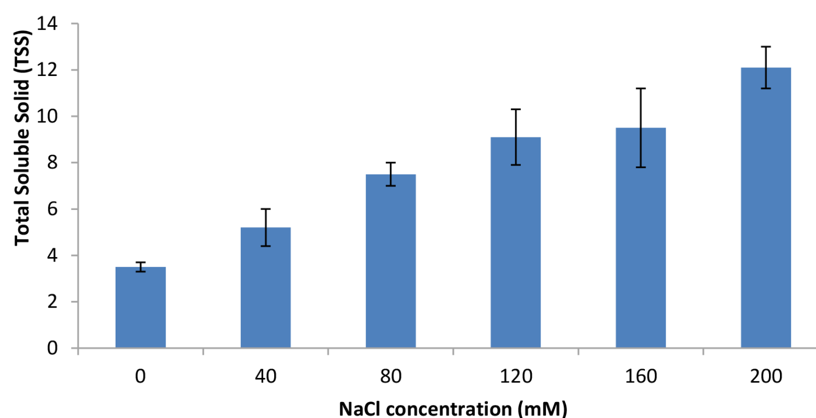

Figure 3. Impact of different NaCl concentrations on the total soluble solids (TSS) content on *in vitro Paronychia argentea* plantlets after five weeks on the MS media containing 0.2 mg/L BAP

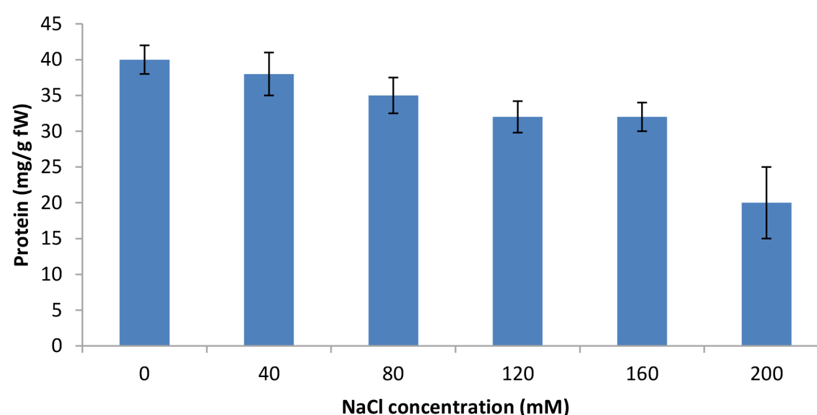

Figure 4. Impact of different NaCl concentrations on the protein content in the *Paronychia argentea* plantlets after five week growth on the MS media containing 0.2 mg/L BAP

NaCl concentrations in the culture medium significantly decreased the protein content in the *in vitro* plantlets (Fig. 4). The level of protein in leaf tissue generally decreased with increased NaCl level in the medium (Fig. 4). The protein content in medium supplemented with 40 mM NaCl was (38.3 mg/g FW), while by using 160 mM in the medium, it dropped to (25 mg/g. FW). Moreover, the protein content was highly reduced at high NaCl (160 mM) (Fig. 4). As the NaCl concentration in the medium increased, the relative water content (RWC) was decreased. Maximum RWC

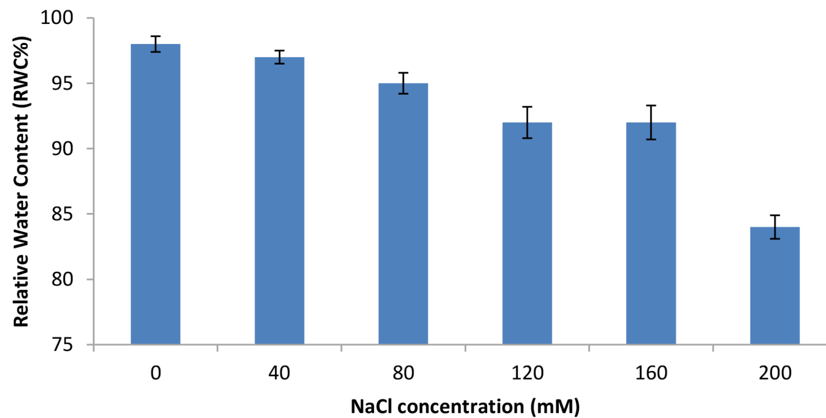

Figure 5. Impact of different NaCl concentrations on the relative water content (RWC) in the *Paronychia argentea* plantlets after five week growth on the MS media added with 0.2 mg/L

(96.89) was recorded when MS media added with 0.0 mM NaCl, which was significantly different from 160 mM NaCl (82.7%) (Fig. 5).

DISCUSSION

In vitro propagation

The current results show that BAP, adenine, and kinetin have significantly affected the number of newly developed microshoots, length, fresh and dry weights of *P. argentea* plantlets (Table 1, 2, or 3). BAP was found to enhance the number of newly developed microshoots and length at 0.6 mg/ (Tables 1, 2 or 3). In addition, the obtained results show that BAP at a level of 0.3–0.6 mg/L produced the highest shoot number. Meanwhile, BAP had no significant effect on fresh and dry weights (Table 1). The current results are similar to the previous studies on *Moringa peregrine* by Alrayes *et al.* (2016). BAP was the most appropriate cytokinin for shoot multiplication compared with adenine or kinetin. Furthermore, Poudel *et al.* (2018) reported that 1.0 mg/L BAP and 1.0 mg/L IBA was very effective for shoot proliferation of *Amomum subulatum*. In addition, on lavender, the maximum number of newly developed microshoots was developed on the MS media containing 0.1 mg/L BAP (TienVinh *et al*. 2017). These results are similar to the previous findings on *Achillea millefolium* and *Stevia rebaudiana* (Shatnawi et al., 2011; 2013). Therefore, BAP was utilized for micropropagation of many plant species because of its capability for enhanced formation of shoot.

The current study indicates that the *in vitro* shoot formation of *P. argentea* is strongly dependent on BAP. In this study, BAP showed to be beneficial for the *P. argentea* shoot formation because of its remarkable capability to prompt shoot formation compared with kinetin and adenine. Naeem *et al*. (2004) reported that that kinetin enhanced shoot elongation by blocking shoot extension. In the current study, adenine and kinetin was noticed to slightly improve shoot multiplication compared with the control (Table 2 and 3). Meanwhile, shoot length had increased significantly. However, adenine and kinetin were reported to enhance growth by increasing shoot length rather than expansion, which was proven by Naeem *et al*. (2004). Meanwhile, enhancement of shooting is governed by the specific cytokinin type and level added to the culture media, in addition to plant species and explant type used (Al-Mahmood *et al*. 2012).

Salinity

Responses of i*n vitro* microshoots of *P. argentea* to different NaCl concentrations

Plant growth

Salinity is one of the main environmental constraints in different essential areas of the world. Salinity influences plant metabolism, such as the uptake of certain essential nutrients, enzyme activity, photosynthesis, and osmotic adjustment; therefore, it affects the growth of most plants (Kaur and Kumar 2017; Jiang *et al*. 2017). Salinity affects photosynthesis, the respiration rate of plants, seed germination, development, growth, and survival percentages (Kaur and Kumar 2017).

Haq *et al*. (2007) reported that the tissue culture technique is a useful tool to study salt tolerance or avoid it at the cellular level, and it can be considered as a simple method of salinity study.

Effect of NaCl on plant pigment

In the current study, all growth parameters of *P. argentea* were significantly decreased with high NaCl concentrations (Table 4 and 5). With increasing salinity levels, shoot number, fresh and dry weights decreased significantly (Table 4). Moreover, higher salinity concentrations reduced the chlorophyll *a*, chlorophyll *b,* and carotenoid contents in the *P. argentea* plantlets (Table 5). Accumulation of ions of various NaCl concentrations in the *P. argentea* plantlets causes a reduction in the chlorophyll *a* and *b* content which is probably due to accumulated ions of various salts in the plants which may affect the biosynthesis of chlorophyll. Zhao *et al*. (2007) revealed that salinity affects the chlorophyll content through reducing the synthesis of chlorophyll or by acceleration its degradation.

The main effect of salinity related to the decrease availability of water as well as the toxic influence of salt ions that are amenable to salinization. Similarly to the obtained results, a previous study by Ackin and Yalcin (2016), indicted that high NaCl concentration decreased total chlorophyll pigments and carotenoid. Carotenoids protect plasma membrane lipids from oxidative stress when plants are grown under stressful conditions (Conceição Gomes *et al*. 2017). Moreover, under NaCl stress, plants may exhibit a set of physiological alterations enabling the plants to resist severe salinity. At 80 mM, the maximum anthocyanin content was recorded, but at higher concentrations, it showed a remarkable decrease.

Salinity and nutrient uptake

The K and N concentrations in the *P. argentea* plantlets decreased significantly under NaCl treatments (Table 6). Haq *et al*. (2007) stated that NaCl affects the biochemical processes leading to decrease in the K^+ content and increases in Na^+ and Cl^-. Al-Khayri (2002), reported that at high salinity levels, there was a negative effect on *in vitro* grown plantlets. In addition, Mosavi *et al*. (2018) stated that increased Na and Cl ions in the protoplasm cause ion imbalance and phosphorylation effects in the respiratory chain producing very small amounts of energy; therefore, nitrogen assimilation and protein metabolism are disturbed, and the accumulation of diamines and polyamines occurs. In addition, Amiro and Qados (2010) reported that salinity decreased the N, P, and K contents in plant tissues and significantly increased the Na, Cl, Ca, and Mg contents. Petropoulos *et al*. (2017) reported that *Cichorium spinosum* showed significant changes in fatty acids, minerals, ascorbic acid, macro and micro-nutrients, sugars, and tocopherols as a result of salinity.

Proline

In the current study, the proline content in the *P. argentea* plantlets increased significantly with increasing NaCl concentrations (Fig. 2). A similar result was obtained in tomato plants (Qaryouti 2001). Furthermore, Yaish (2015) and Abbas (2016) showed that proline was accumulated as a response to NaCl stress. The proline accumulation in the plant refers to physical responses resulting from abiotic and biotic stress in plants.

Protein content

Figure 4 shows that level of protein in leaf tissue generally decreased with increasing NaCl concentrations in the medium. Reduction of the protein content under elevated salinity was correlated with low nitrate reeducates activity and a reduction in plant growth (Silveira *et al*. 2001). Furthermore, significant reductions in the protein content under high salinity were also observed in tomato and rice (Abdalah *et al*. 2016). In the current study, increased protein content coincides with the increase in salinity levels. Moreover, the results obtained are in agreement with previous findings by Sibole *et al*. (2003) on *Medicago citrna*. This study confirmed that NaCl treatments reduced the protein content on the *in vitro* plant tissues.

Total Soluble Solids (TSS)

Increasing the NaCl concentrations in the media significantly increased total soluble solids in the leaves of *P. argentea* (Fig. 3). TSS increased in plant tissue along with the NaCl concentrations in the medium. Similar to the obtained finding, Qaryouti (2001) indicated that increased NaCl concentrations in the medium raised total soluble solids of tomato.

Relative water content (RWC)

As salinity level increased, the relative water content significantly decreased (Fig. 5). This might be due to the decrease in water flow from the root to the shoot. Similar results were obtained by Siddiqi and Ashraf (2008) on safflower cultivars. The *in vitro* culture of *P. argentea* can solve propagation problems, it guarantees mass production of plant material without menacing natural resources; moreover, it can improve and conserve this plant. This finding has led to a sudden rise in demand for such herbal medicines plant. Simple and reliable methods from the current study have been achieved for the *P. argentea* plant. Moreover, the *in vitro* growth was significantly decreased with increasing NaCl concentration.

CONCLUSIONS

With increasing NaCl concentration, the in vitro growth was significantly reduced. The concentrations of potassium (K) and nitrogen (N) in the *P. argentea* plantlets decreased significantly after NaCl treatment. The protein levels in leaf tissue generally decreased as the NaCl concentrations in the medium increased. With increasing NaCl concentrations, the proline content in the P. argentea plantlets increased significantly. A rise in total soluble solids (TSS) in plant tissue was observed when the NaCl concentration in the medium was increased. Furthermore, as the salinity level increased, the relative water content decreased. In general, it can be concluded that high NaCl significantly affected the *in vitro* growth of plants. *P. argentea* showed that *in vitro* the *P. argentea* plantlets could be tolerant to *in vitro* salinity.

REFERENCES

1. Abdallah, S.H., Aung, B., Amyot, L., Lalin, I., Lachaal, M & Bouraui K.N. 2016. Salt stress (NaCl) affects plant growth and branch pathways of carotenoid and flavonoid biosyntheses in *Solanum nigrum.* Acta Physiol. Plan 38(3), 1-13.
2. Akcin, A., & Yalcin, E. 2016. Effect of salinity stress on chlorophyll, carotenoid content, and proline in *Salicornia prostrata* Pall. and *Suaeda prostrata* Pall. subsp. prostrata (Amaranthaceae). Braz. J. Bot 39 (1), 101–106.
3. Al-Ajlouni, Z., Abbas, S., & Shatnawi M. 2015. *In vitro* propagation, callus induction, and evaluation of active compounds *Ruta graveolens*. J. Food, Agric. Environ 13 (2), 101-106.
4. Al-Khayri, J.M. 2002. Growth, proline accumulation and ion content in sodium chloride-stressed callus of date palm. In vitro Cell. Dev. Biol. Plant 38, 79-82.
5. AL-Issa, R.M.S., Odat, N., Qrunfleh, I., Hasan, M., & Al-Tawaha, A.R. 2020. The impact of NaCl on different genotypes of tomato (Solanum Lycopersicon Mil) on germination, some physiology characteristics and gene expression. Eurasian Journal of Biosciences, 14(2), *4467-4470*.
6. Al-Mahmood, H., Shatnawi, M., Shibli, R., Makhadmeh, I., Abubaker, S., & Shadiadeh A. 2012. Clonal propagation and medium-term conservation Capparis spinosa: A medicinal plant. J. Med. Plant. Res 6 (22, 3826-3836.
7. Alrayes, L.M., Al Khateeb, W.M., & Shatnawi M.A. 2016. Clonal propagation and antibacterial activity of Moringa peregrina (Forssk) Fiori plant. J. Adv. Biotechno 6 (1), 787-797.
8. Al-Tawaha, A .M. and Al-Ghzawi, A. 2013.Effect of Chitosan coating on seed germination and salt-tolerance of lentil (Lensculinaris L.) Res. on Crops14 (2), 489-491.
9. Al-Tawaha, A.R., Turk, M.A., Al-Tawaha, A.R.M., Alu'datt, M.H ., Wedyan, M., Al-Ramamneh, E. Al-D.M & Hoang, A.T. 2018. Using chitosan to improve growth of maize cultivars under salinity conditions. *Bulg. J. Agric. Sci.*, 24 (3), 437–442
10. Amiro, M.S, & Qados, A. 2010. Effect of salt stress on plant growth and metabolism of bean plant Vicia faba (L.). J. Saudi Soc. Agric. Sci 10, 7-15.
11. Anderson, J.M & Boardman, N.K. 1964. Studies on the greening of dark grown bean plants II. Development of photochemical activity. Australian J Biol Sci 17, 93-101.
12. Atta, E., Nassar, A., Hasan, N., & Hasan N.A. 2013. New Flavonoid Glycoside and Pharmacological Activities of Pteranthus dichotomus Forssk. Rec. Nat. Prod 7, 69-79.
13. Braca, A., Bader, A., Siciliano, T., & De Tommasi, N. 2008. Secondary metabolites from Paronychia argentea. Magn. Reson. Chem 46, 88–93.
14. Chapman, H.D., & Pratt, P.F. 1961. Method of analysis for soil plants and water. University of California. pp, 169-170.
15. Conceição., Gomes, MA., Pestana, I.A., Santa-Catarina, C., Hauser-Davis R.A., & Suzuki, M.S 2017. Salinity effects on photosynthetic pigments, proline, biomass and nitric oxide in *Salvinia auriculata* Aubl. Acta Limnol. Bras 29, 9-13.
16. Craker, L.E., Gardner, Z., & Etter, S.C. 2003. Herbs in American fields: A horticultural perspective of herb and medicinal plant production in the United Sates. 1903–2003. HortScience 38, 977–983.

17. De Filippis, L.F., Hampp, R., Ziegler, H. 1981. The effect of sub-lethal concentrations of zinc, cadmium and mercury on Euglena growth and pigments. Z. Pflanzenphysiol 101, 37-47.
18. Duxbury, A.C., & Yentsch, C.S. 1956. Plankton pigment monographs. J. Mar. Res 15, 92-101.
19. Hung, T,N., & Chi, V.L. 2014. Country status report on medicinal and aromatic plants in Vietnam. In expert consultation on promotion of medicinal and aromatic plants in the Asia-Pacific Region. pp. 218–226.
20. Jiang, Y., Ding, X., Zhang, D., Deng, Q., Yu, C.L., Zhou, S., & Hui, D. 2017. Soil salinity increases the tolerance of excessive sulfur fumigation stress in tomato plants. Enviromen. Exp. Bot. J 133, 70–77.
21. Kaur, G., & Kumar, A. 2017. Effect of salinity stress on plant growth, chlorophyll content and carotenoids of Coriander (*Coriandrum sativum* L.) cultivars. Int. J. Curr. Res 9, 57536-57544.
22. Mosavi, N., Ebadi, M., Khorshidi, M., Masoudian, N., & Hokmabadi, H. 2018. Study of some physiological characteristics of potato tissue under salinity stress. Int. J. Farm. Alli. Sci 7(1), 1-5.
23. Murashige, T., & Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant 15, 473-479.
24. Naeem, M., Bhatti, I., Hafeez, R, & Ashraf, Y. 2004. Effect of some growth hormones (GA_3, IAA and kinetin) on the morphology and early or delayed initiation of Bud of Lentil (*Lens culinaris*). Pak. J. Botnol 36 (4), 801-809.
25. Noubani, R., Abu Irmaileh, B., & Afifi, F. 2006. Folk utilization of traditional medicinal plants among rural population in Wadi Mujib–Jordan. Jordan Med. J 40 (4), 232-240.
26. Lee, K.D., Tawaha A.R.M. & Supanjani, 2005. Antioxidant status, stomatal resistance and mineral composition of hot pepper under salinity and boron stress. Bioscience Research, 2(3), 148-154
27. Petropoulo, S.A., Levizou, E., Ntatsi, G., Fernandes, A., Petrotos, K., Akoumianakis, K., Barros L, & Ferreira, I.C. 2017. Salinity effect on nutritional value, chemical composition and bioactive compounds content of Cichorium spinosum L. Food Chem 214, 129–136.
28. Poudel, K., Praski, K., & Shestha, D. 2018. Micropropagation and acclimatization of large Cardamom (*Amomum subulatum* Roxb.). Turkish J. of Agri. Nat. Sci 5(3), 231–235.
29. Qaryouti, M.M., Qawasmi, W., Hamdan, H., & Edwan, M. 2001. Influence of NaCl salinity stress on yield, plant water uptake and drainage water of tomato grown in soilless culture. Acta Hortic 747, 539-544.
30. Othman, Y., Al-Karaki, G., Al-Tawaha, A.R. & Al-Horani, A. 2006. Variation in germination and ion uptake in barley genotypes under salinity condition. World Journal of Agricultural Sciences, 2(1), 11-15.
31. Shatnawi, M.A., Shibli, R.A., Qrunfleh, I., Bataeineh, K., & Obeidat M. 2007. In vitro propagation and cryopreservation of Prunus avium using vitrification and encapsulation dehydration methods. J. Food, Agri. Environ 5 (2),204-208.
32. Shatnawi, M., Al-Fauri, A., Megdadi, H., Al-Shatnawi, M.K., Shibli, R.A., Abu-Romman, S., & Al-Ghzawi, A. 2010. In vitro multiplication of Chrysanthemum morifolium Ramat and its responses to NaCl induced salinity. Jordan J. of Biol. Sci 3 (3), 101-110.
33. Shatnawi, M.A. 2011. Multiplication and cryogenic storage of Artemisia herba-alba: A medicinal plant. J. Food, Agri. Environ 9, 340-344.
34. Shatnawi, M.A. 2013. Multiplication and cryopreservation of Yarrow (*Achillea millefolium* L., Astraceae). J. Agric. Sci. Technol 15, 163-173.
35. Shatnawi, M.A., Shibli, R.A., Shahrour, W.G., Al-Qudah, T.S., & Abu-Zahra, T. 2019. Micropropagation and conservation of Fig (*Ficus carica* L.). J. Adv. Agric 10,1669-1679.
36. Shibli, R.A., Kushad, M., Yousef, G.G., & Lila, M.A. 2007. Physiological and biochemical responses of tomato microshoots to induce salinity stress with associated ethylene accumulation. Plant Growth Regul 51, 159-169.
37. Siddiqi, E.H., Ashraf, M., Al-Qurainy, F., & Akram, N.A. 2011. Salt induced modulation in inorganic nutrients, antioxidant enzymes, proline content and seed oil composition in safflower (*Carthamus tinctorius* L.). J. Sci. Food Agric 91,2785–2793.
38. Sibole, J .V., Cabot, C., Poschenreder, C, & Barcelo, J. 2003. Efficient leaf ion partitioning, an overriding condition for abscisic acid controlled stomatal and leaf growth responses to NaCl salinization in two legumes. J. Exp. Bot 54 (390), 2111–2119.
39. Silveira, J.A.G, Melo, A.R.B, Viégas, R.A, & Oliveira, J.T.A. 2001. Salinity-induced effects on nitrogen assimilation related to growth in cowpea plants. Exp. Exp. Bot 46(2),171-179.
40. SPSS. 2017. Complex samples, SPSS INC., Chicago ILL: USA.
41. Tien., Vinh, D., Hoa, M.T., Khai, P.C., & Minh T.V. 2017. Micropropagation of lavender (*Lavandula angustifolia*). J. Innov. Pharm Biol. Sci 4 (2), 7-11.
42. Yaish, M.W. & Kumar P.P. 2015. Salt tolerance research in date palm tree (*Phoenix dactylifera* L.), past, present, and future perspectives. Front. Plant Sci. 6, 348-352.
43. Zama, D., Tebibel, Z., Benayssa, W., Benayache, F., Benayache, S., & Vlietinc A.J. 2007. Chlorpyrifos-induced oxidative stress and tissue damage in the liver, kidney, brain and fetus in pregnant rats: The protective role of the butanolic extract of Paronychia argentea L. Indian J. of Pharmacol 39, 145-150.
44. Zhao, G.Q., Ma, B.L, & Ren, C.Z. 2007. Growth, gas exchange, chlorophyll fluorescence and ion content of naked oat in response to salinity. Crop Sci 47, 123-131.