

RESEARCH

Open Access



Salinity tolerance improvement of in vitro propagated *Paulownia tomentosa* using proline

Nora Muhammad Youssef*, Khaled Ismail Hashish and Lobna Salah Taha

Abstract

Background: *Paulownia tomentosa* has been used as an ornamental plant and is still vastly used for this objective and can be utilized for the production of energy, wooden building materials, and paper pulp. The aim was to improve the in vitro propagation ability under salinity stress.

Methodology: This experiment was conducted on *Paulownia tomentosa* at Tissue Culture Technique Lab., Central Laboratories, Department of Ornamental Plants and Woody Trees, National Research Centre (NRC), Egypt, to examine the micropropagation behavior using different plant growth regulators (BA, Kin, and IBA) and improve the in vitro propagation ability of plant under the effect of salinity levels (500, 1000, 2000, and 4000 ppm) using two concentrations of proline (0.2 and 0.4 g/l).

Results: For in vitro propagation behavior, the best results for both shooting and rooting behaviors were obtained when MS culture medium was supplemented with BA at 0.2 mg/l, and Kin and IBA at 0.1 mg/l. For the improvement of the in vitro propagation ability under salinity stress, the survival was 100% when the shootlets of *Paulownia* grown on MS culture medium supplemented with 0.5 g NaCl plus 0.2 or 0.4 g/l proline and 1 g NaCl plus 0.2 g/l proline which were similar to control treatment.

Conclusion: Micropropagation ability of *Paulownia tomentosa* under salinity stress was optimized when the explants were cultured on MS medium supplemented with BA at 0.2 mg/l, and Kin and IBA at 0.1 mg/l using proline at 0.2 g/l. This study will help the producers to cultivate *Paulownia* trees in saline soils up to 1000 ppm such as some new lands which are not suitable for growing other crops.

Keywords: Paulownia, Micropropagation, Salinity, and Proline

Background

Paulownia trees belong to Scrophulariaceae family involve nine species of fast-growing trees that are native to China and East Asia (Ayan et al. 2006). *Paulownia tomentosa* has been entered into the USA and Europe as an ornamental plant and is still vastly used for this objective. The average height of trees reaches 12 m and 13.4 cm of average diameter through 7 years in Bulgaria (Kalmukov 1995). The trees can reach up to 30 m at

maturity (Bonner 1995). This high yielding tree can be utilized for the production of energy, wooden building materials, and paper pulp (Bergmann and Moon 1997).

The environmental deterioration causes biotic and abiotic stresses in plants and restricted their growth and development (Shao and Chu 2005). Among the abiotic stresses, salinity and drought are the main stresses that damage crop worldwide (Vinocur and Altman 2005). Rising salinity causes severe harm to plants, including growth inhibition, necrosis, impaired metabolism, and reduction of production and quality (Sivritepe and Eris 1999). Salinity stress cause accumulation of reactive oxygen species (ROS) (O₂, superoxide radicals; OH, hydroxyl radical;

* Correspondence: norayousif20@yahoo.com

Ornamental Plants and Woody Trees Department, National Research Centre, Dokki, Giza, Egypt

H₂O₂, hydrogen peroxide; and ¹O₂, singlet oxygen), producing an oxidative stress. Oxidative stress can damage DNA, lipid peroxidation, and inactivate enzymes (Gill and Tuteja 2010).

However, sugars, sugar alcohols, and several other molecules as amino acids, organic acids, or inorganic ions have the ability to improve salt tolerance mechanisms (Munns 2005). Proline acid is a compatible osmolyte that accumulates in many crops under salt stress (Munns and Tester 2008). Proline helps to protect cells of the plant from the negative effects of salt by preserving the osmotic balance, scavenging reactive oxygen species (ROS), and stabilizing subcellular structures, such as proteins and membranes (Ashraf and Foolad 2007). Many reports show the effective role of exogenous proline in osmoregulation of several plant species under salt stress (Lone et al. 1987; Tal and Katz 1980; Wyn et al. 1977).

Plant tissue culture provides beneficial information to illustrate plant response to salt stress. Micropropagation provides greater control than in vivo growth conditions and has the advantage of small scale with monitoring responses of shoot and root in the presence of imposed stress (Shibli et al. 1992). The micropropagation technique of *Paulownia* species offers a rapid means of producing woody biomass and planting stock for afforestation, and it is an effective method to maintain the genetic gain (Park and Bonga 1992). The success of consecutive micropropagation for many woody plants could be affected by different factors such as plant growth regulators which are the most important ones (Taha et al. 2008).

Paulownia tree is considered as very sensitive to salinity. The aim of the research was to optimize a micropropagation protocol using different plant growth regulators (BA, Kin, and IBA) and improving plant ability to salinity tolerance so that it can be cultivated in the new lands which are not suitable for growing other crops.

Materials and methods

The experimental work was conducted on *Paulownia tomentosa* during the years 2018 and 2019 at the Tissue Culture Technique Lab., Central Laboratories, Department of Ornamental Plants and Woody Tress, National Research Centre (NRC), Egypt, to evaluate and improve the in vitro propagation ability of plant under the effect of salinity stress.

Explant source and disinfection

Nodal explants of *Paulownia tomentosa* at the Faculty of Agriculture, Ain Shams University, Shobra El-Khyema, Egypt, were taken and washed in septal soap, then rinsed with running tap water for 1 h. The surface disinfection of explant was done under aseptic condition using 70% ethanol for 1 min, followed by 15% (v/v) of Clorox (NaOCl 5.25%) for 10 min, followed by mercuric chloride at 0.2%

(w/v). The explants were rinsed three times with sterile distilled water after each disinfection treatment.

Culture medium

Explants were cultured on MS medium (Murashing and Skoog, 1962) supplemented with 2.5% sucrose and 0.7% agar (w/v) prior to autoclaving at 1.2 kg/cm² for 15 min. The pH of the culture medium was adjusted to 5.8. Culturing was done in 200 ml glass jars containing 25 ml of the medium.

For evaluation of in vitro propagation behavior, plant growth regulators were added to MS culture medium at different concentrations:

Benzlaminopurine (BAP) at 0.2 and 0.4 mg/l with or without Kin at 0.1 mg/l and indole-3-butyric (IBA) at 0.1 or 0.2 mg/l.

For evaluation of in vitro propagation ability under salinity stress, the in vitro growth (shooting and rooting) behavior was examined through culturing the nodal explants which were obtained from the most suitable multiplication medium (MS + 0.2 mg/l BA + 0.1 mg/l Kin + 0.1 mg/l IBA) under different concentrations of NaCl (0.0, 500, 1000, 2000, 4000 ppm). Two concentrations of proline (0.2 and 0.4 g/l) were examined to improve the in vitro propagation ability under salinity stress.

Incubation conditions

Cultures were incubated under controlled conditions in the growth chamber. The incubation temperature was 24 ± 2 °C controlled by a “power” air conditioner. The photoperiod was 16-h light/8-h darkness, controlled automatically. Illumination intensity was 3000 lux from cool fluorescent lamps.

Chemical analysis

Photosynthetic pigments

Plant material after multiplication stage was collected, and photosynthetic pigments (chlorophyll a and b) as well as carotenoids were determined in shootlets tissues as mg/100 g fresh weight by using spectrophotometer, according to the procedure achieved by Saric et al. (1967).

Proline determination

The proline was determined according to Carrilo et al. (2011). A 500-μl ethanolic extract (80% ethanol) or 100 μl of 5-2-1-0.5 and 0.2-mM proline standard solution was completed with up to 400 μl of ethanol: water (40:60 v/v) is added to 1 ml of reaction mixture (ninhydrin 1% (w/v) in acetic acid 60% (v/v), ethanol 20% (v/v)) and incubated at 95 °C for 20 min. After that, the mixture was cooled at room temperature and centrifuged 1 min at 10000 rpm. The supernatant was read at 520 nm with UV-spectrophotometer.

Proline in nmol.mg⁻¹ FW or in μmol.g⁻¹ FW = (Abs_{extract} - blank) / slope × Vol_{extract} / Vol_{aliquot} × 1 / FW

Experimental design and data analysis

The data were analyzed through analysis of variance ANOVA, and the treatments' means were compared for significance by Duncan's new multiple range test at 0.05% level of probability (Duncan 1955) using COSTATV-63.

Results

In vitro propagation behavior

The results presented in Table 1 and Fig. 1 show the in vitro shooting and rooting ability of *Paulownia tomentosa* under the effect of different plant growth regulators (BA, Kin, and IBA) concentrations. The best results for both shooting and rooting behaviors were obtained when MS culture medium was supplemented with BA at 0.2 mg/l, at Kin and IBA at 0.1 mg/l which lead to the highest survived explants (100%), number of shootlets (5.67), longest shootlets (6.96 cm), and highest rooting percent (98.33%) as compared to control which was MS medium without growth regulators and caused lowest values. The data also revealed that using of low concentration of BA (0.2 mg/l) combined with Kin (0.1 mg/l) and IBA at 0.1 or 0.2 mg/l were favored for improving the in vitro propagation ability of *Paulownia* comparing with other treatments. This indicates the role of all used plant growth regulators for attaining the internal hormone balance that led to the best results.

Means within a column having the same letters are not significantly different according to Duncan's multiple range test (DMRT) at 5% level

Effect of salinity stress on micropropagated plants

The growth characters as survival, shoot number, shoot length, and leaves' number, also the rooting parameters as rooting percentage, roots number, and root length of *Paulownia tomentosa* were significantly affected by the NaCl treatments. As shown in Table 2, all in vitro

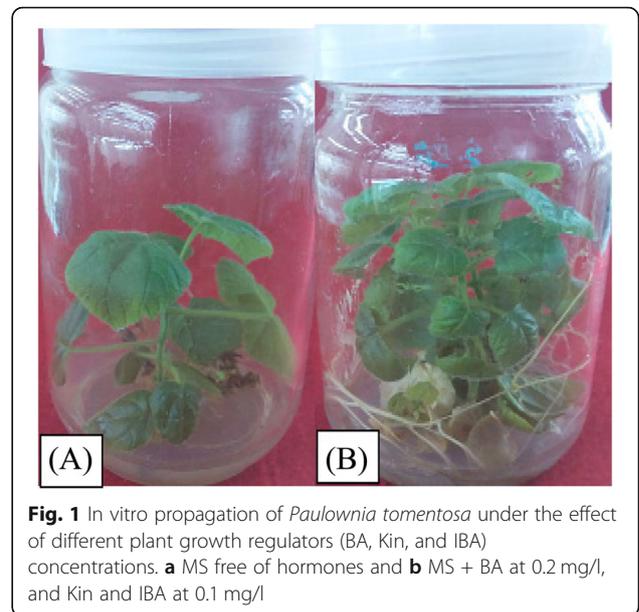


Fig. 1 In vitro propagation of *Paulownia tomentosa* under the effect of different plant growth regulators (BA, Kin, and IBA) concentrations. **a** MS free of hormones and **b** MS + BA at 0.2 mg/l, and Kin and IBA at 0.1 mg/l

growth characters were decreased with increasing NaCl concentrations from 500 to 4000 ppm. The 500 ppm NaCl did not reduce the survival (100%) and produced an increase of the shoot number (5.67) as compared to control. At 4000 ppm NaCl, the number of shootlets, shoot length, and leaves' number was adversely affected and gave only half values approximately of that of control, whereas the survival percentage was decreased to 61%. The rooting parameters were decreased significantly from 500 and 1000 ppm NaCl and were absent at 2000 and 4000 ppm NaCl. Concentrations of NaCl at 500 and 1000 ppm resulted in a significant reduction in rooting percentage (22.22 and 11.11 %), roots number (0.67 and 0.33), and roots' length (11.78 and 5 mm), respectively, compared with control treatment; however, no rooting occurred when explants were grown on 2000 and 4000 ppm of NaCl.

Means within a column having the same letters are not significantly different according to Duncan's multiple range test (DMRT) at 5% level

Table 3 demonstrates the effect of proline on alleviation of salinity stress on the micropropagated plants, with increasing salinity concentrations using different proline level, and all parameters were reduced. The survival was 100 % when the shootlets of *Paulownia* grown on MS culture medium supplemented with 0.5 g NaCl plus 0.2 or 0.4 g proline and 1 g NaCl plus 0.2 g proline which were similar to control treatment. Applying 0.5 g of NaCl plus 0.2 proline gave the best values after the control for each of the number of shootlets/explant, length of shootlets, and number of nodes/shootlet (1.33, 57.92, and 41.33 mm, respectively). All previous parameters were declined sharply with increasing salinity levels.

Table 1 In vitro propagation ability of *Paulownia tomentosa* effecting by plant growth regulators

Character PGR (mg/l)			Survival %	Shootlets number/explant	Shootlets length (cm)	Rooting%
BA	Kin	IBA				
0	0	0	33.33 e	1.00 e	3.23 fg	50.00 f
0.2	0	0	66.67 d	1.33 de	3.33 f	28.83 g
0.4	0	0	33.33 e	0.97 e	2.80 g	25.33 h
0.2	0	0.1	75.00 c	1.83 cd	4.83 d	86.67 e
0.4	0	0.2	76.33 d	1.60 cde	4.16 e	67.33 e
0.2	0.1	0.1	100.00 a	5.67 a	6.96 a	98.33 a
0.2	0.1	0.2	86.67 b	4.33 b	6.10 b	89.33 b
0.4	0.1	0.1	72.67 cd	3.67 b	5.40 c	82.00 d
0.4	0.1	0.2	75.00 c	2.33 c	5.10 cd	80.00 d

Table 2 In vitro shooting and rooting behaviors of *Paulownia* under the effect of salinity stress

Salinity (ppm)	Survival%	Shootlets number/explant	Shootlet length (mm)	Nodes number /shootlet	Rooting%	Roots number	Root length (mm)
0.00	100 a	5.00 a	26.28 a	30.50 a	100 a	8.50 a	23.33 a
500	100 a	5.67 a	23.36 b	18.67 c	22.22 b	0.67 b	11.78 b
1000	88.89 ab	3.67 b	17.47 cd	14.33 d	11.11 b	0.33 b	5.00 bc
2000	77.78 ab	3.67 b	18.59 c	20.67 b	0.0	0.0	0.0
4000	61.11 b	2.33 c	15.42 d	15.00 cd	0.0	0.0	0.0

The shootlets were produced roots even applying 1 g of sodium chloride and using the higher concentration of it prevents root formation. These results indicate that *Paulownia* plants can grow at 1000 ppm of NaCl, but the higher concentration of NaCl causes poor growth or death. The use of proline with salt stress improved in vitro growth slightly.

Means within a column having the same letters are not significantly different according to Duncan's multiple range test (DMRT) at 5% level

Photosynthetic pigments under salinity stress

Figure 2 indicates the effect of salt stress by applying different levels of sodium chloride on the chlorophyll content of the *Paulownia* leaves under study, including chlorophyll a and b. The results show a reverse relationship between salt level and chlorophyll a and b contents. Whenever the level of NaCl was increased, chlorophyll content was decreased. Chlorophyll a and b reach to the lowest contents (71.42 and 25.51 mg/100 g F.W. at 4000 ppm of NaCl) compared to control (271.90 and 175.90 mg/100 g F.W., respectively). A statistical analysis demonstrates that the observed differences were significant. By following carotenoids content through the exposure of the *Paulownia* explants to salt stress, it appears from Fig. 2 that salt stress (NaCl) was an inhibiting agent for the formation of carotenoids inside the stressed leaves, where the carotenoids' content was decreased. The least carotenoids content was appeared with the level 4000 ppm of NaCl (54.80 mg/100 g F.W.), whereas the highest content

(606.7 mg/100 g F.W.) appeared in control leaves. There were gradual decreases with increasing salinity levels.

Photosynthetic pigments under different salinity levels and proline concentrations

Data illustrated in Fig. 3 mention the role of proline concentration in accumulated pigments content in shootlets of *Paulownia* that grown under salinity stress. The highest chlorophyll a and b contents (528.4 and 273.1 mg/100 g F.W.) were obtained in shootlets grown on MS culture medium supplemented with 0.5 g NaCl plus 0.2 g proline followed by the treatment of NaCl 1.0 g plus 0.2 g proline which caused also the highest carotenoids content (397.1 mg/100 g F.W.) comparing with other treatments. This indicates the role of proline when it was used at low concentration in increasing chlorophyll content under salinity stress.

Proline content under salinity stress

The data presented in Fig. 4 shows the response of proline content in the shootlets of *Paulownia* to salt stress. The content of proline ranged between 0.277 and 0.443 nmol/mg. The salinity treatments increased the content of proline as compared with the control. A slight increase was recorded with the low concentrations of NaCl (500 and 1000 ppm), then marked increases were recorded using NaCl at 2000 and 4000 ppm. Where the proline content reached its maximum value (0.443 nmol/mg) when the explants were grown on the highest concentration of NaCl, compared to that for the untreated explants.

Table 3 Alleviation of salinity stress on the micropropagated *Paulownia* under the effect of proline concentration

Character treatment	Survival %	Shootlets number/explant	Shootlet length (mm)	Nodes number/shootlet	Rooting%	Roots number	Root length (mm)
Control	100.00 a	2.44 a	51.67 a	46 a	100 a	7.25 b	115 a
0.5 g NaCl + 0.2 g proline	100.00 a	1.61 b	57.92 a	41.33 b	100 a	12 a	108.3 a
0.5 g NaCl + 0.4 g proline	100.00 a	1.50 bc	27.00 b	12.67 c	55.67 b	6.98 b	90.00 a
1.0 g NaCl + 0.2 g proline	100.00 a	1.33 bcd	27.11 b	12.33 cd	22.22 b	0.67 c	11.78 c
1.0 g NaCl + 0.4 g proline	89.00 a	1.28 bcde	20.11 bc	12.00 cd	66.67 a	7.33 b	51.67 b
2.0 g NaCl + 0.2 g proline	78.00 ab	1.17 cde	19.11 bc	12.00 cd	0	0	0
2.0 g NaCl + 0.4 g proline	61.33 b	1.11 de	15.00 c	9.17 cde	0	0	0
4.0 g NaCl + 0.2 g proline	38.67 c	1.00 de	15.33 c	8.67 de	0	0	0
4.0 g NaCl + 0.4 g proline	22.00 c	0.92 e	15.33 c	6.67 e	0	0	0

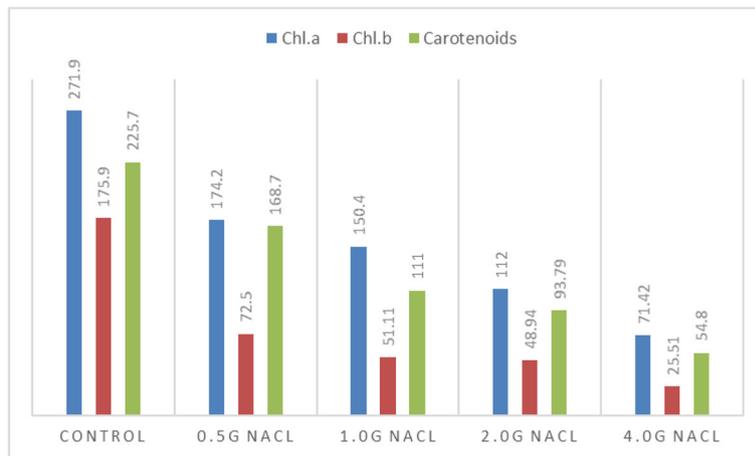


Fig. 2 Photosynthetic pigments contents (mg/100 g F.W.) in *Paulownia tementosa* shootlets effecting by different salinity levels

Proline content under salinity stress and proline concentration

Figure 5 shows that the highest proline content (0.377 nmol/mg) resulted in shootlets grown on MS culture medium supplemented with 4 g NaCl plus 0.2 g proline followed by those obtained from NaCl 2.0 g plus 0.2 proline which gave 0.335 nmol/mg as compared to control which gave the lowest value (0.121 nmol/mg).

Discussion

In this study, the response of in vitro propagation could be improved by adding plant growth regulators (PGR) to the culture medium (Table 1). Confirmed results pointed out that different concentrations of BAP, Kin, or IBA showed a significant effect on bud sprouting of *Paulownia kawakemii* during three repeated subcultures (Abd El-Kader

and Abou El-Ghit 2015). They mentioned also that adding IBA to the culture medium had a promotion effect on bud break, especially with a low concentration of BAP. The inhibition of adventitious meristem elongation could reduce the number of shoots as a result of using a high concentration of BAP (Borchetia et al. 2009).

Concerning the effect of salinity stress on in vitro propagation ability (Table 2), explants' growth of apple rootstock was severely affected by salinity treatments. High salinity gave the reduction in shoot number, shoot length, rooting percentage, root number, and root length (Bahmani et al. 2012). Shahid et al. (2011) noticed the reduction in the length of the shoot with an increasing level of NaCl. This might be explained as the inadequate photosynthesis caused by stomatal closure and the reduction of carbon assimilation rate under salt stress

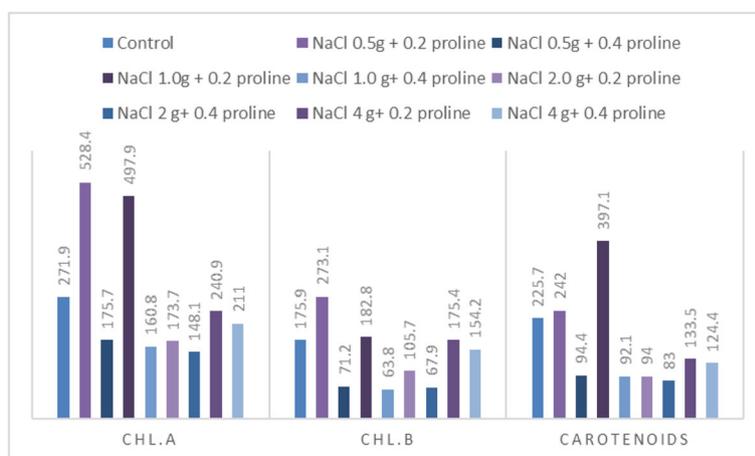


Fig. 3 Photosynthetic pigments contents (mg/100 g F.W.) in *Paulownia tementosa* shootlets under different salinity levels and proline concentration

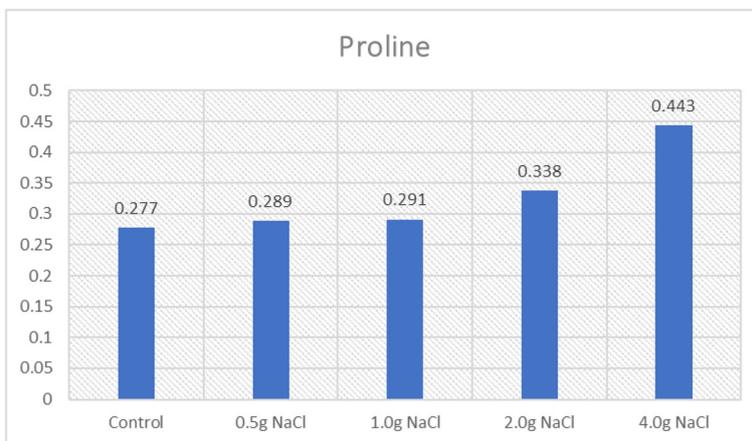


Fig. 4 Proline content (nmol/mg) in *Paulownia* shootlets effecting by different salinity levels

(Ben Ahmed et al. 2009). Miladinova et al. (2013) reported that the leaf number, root, and stem length in *Paulownia* plants were reduced with increasing salinity concentration.

Concerning the results in Table 3, using proline with salt stress improved in vitro growth slightly. Proline is a predominant organic molecule that acts as an intercessor of osmotic adjustment under salinity stress, a sink for energy, a stabilizer of sub-cellular structures, and even a stress linked signal. It is also contributed to cell osmoregulation and protection of proteins during dehydration, and under stress conditions, it can act as an enzymatic regulator (Rontain et al. 2002).

As results in Figs. 2 and 3, the pigments (chlorophyll a, b, and β-carotene) in *Paulownia imperialis* leaves were significantly reduced when exposed to a high level of NaCl (Astorga and Meléndez 2010).

The response of proline to salt stress (Figs. 4 and 5) may due to these osmoprotectants that acts an important role in decrease stress-induced cellular acidification and osmotic adjustments and stabilizes sub-cellular structures for recovery (Tan et al. 2008). Recent studies also suggested that the protective role of proline depends on protecting the protein turnover machinery against stress damage and upregulating stress-protective proteins (Khedr et al. 2003).

Conclusion

MS culture medium that was supplemented with BA at 0.2 mg/l, and Kin and IBA at 0.1 mg/l gave the best results for both shooting and rooting behaviors of *Paulownia tomentosa*. The plantlets were able to grow in a saline condition (1000 ppm), and the growth could be improved slightly using proline.

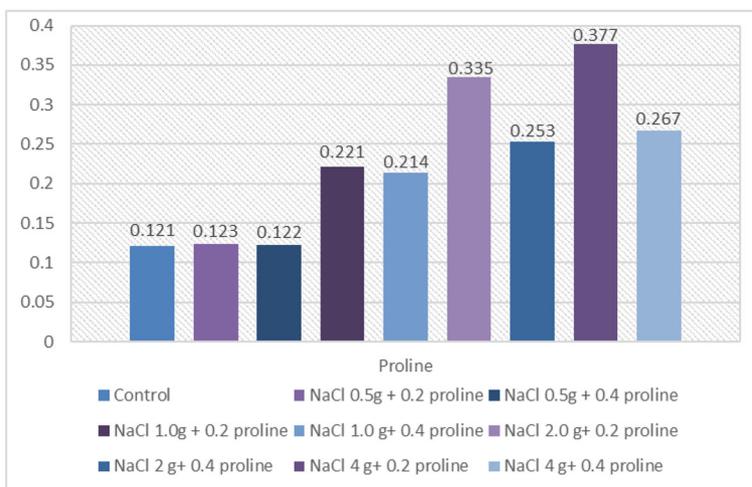


Fig. 5 Proline content (nmol/mg) in *Paulownia* shootlets effecting by different salinity levels and proline concentrations

Acknowledgements

The authors are greatly thankful to the National Research Centre, 33 El Bohouth st. (formal El Tahrir st.), Dokki, Giza, Egypt, P.O.12622, to provide the funding credit of this work.

Authors' contributions

NMY and LST performed the in vitro experiment, analyzed the data, and contributed to writing and reviewing the paper. Kh I H performed a part of in vitro experiment. The authors read and approved the final manuscript.

Authors' information

Dr. Nora Muhammad Youssef Researcher at the Department of Ornamental Plant and Woody Trees, Agricultural and Biological Res. Division, National Research Centre (NRC), P.O. Box 12622 Dokki, Giza, Egypt.

Dr. Khaled Ismail hashish assistant prof. at the Department of Ornamental Plant and Woody Trees, Agricultural and Biological Res. Division, National Research Centre (NRC), P.O. Box 12622 Dokki, Giza, Egypt.

Dr. Lobna Salah Taha prof. at the Department of Ornamental Plant and Woody Trees, Agricultural and Biological Res. Division, National Research Centre (NRC), P.O. Box 12622 Dokki, Giza, Egypt.

Funding

This work was supported and funded by the National Research Centre.

Availability of data and materials

The datasets generated and/or analyzed during the current study are included in this published.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

Received: 22 October 2019 Accepted: 25 May 2020

Published online: 05 June 2020

References

- Abd El-Kader EM, Abou El-Ghit HM (2015) In vitro growth behavior of Paulownia kawakamii hybrid under nutrient media and plant growth regulators effect. *Biolife* 3(4):910–916
- Ashraf M, Foolad MR (2007) Roles of glycinebetaine and proline in improving plant abiotic stress tolerance. *Environ Exp Bot* 59:206–216
- Astorga GA, Meléndez LA (2010) Salinity effects on protein content, lipid peroxidation, pigments, and proline in Paulownia imperialis (Siebold & Zuccarini) and Paulownia fortunei (Seemann & Hemsley) grown in vitro. *Electronic Journal of Biotechnology* 13(5):1–15
- Ayan S, Sivacioglu A, Bilir N (2006) Growth variation of Paulownia Sieb. and Zucc. species and origins at the nursery stage in Kastamonu-Turkey. *J Environ Biol* 27:499–504
- Bahmani R, Gholami M, Mozafari AA, Alivaisi R, (2012) Effects of salinity on in vitro shoot proliferation and rooting of apple rootstock MM.106. *World Applied Sciences Journal* 17(3):292-295
- Ben Ahmed C, Ben Rouina B, Sensoy S, Boukhriss M, Abdullah FB (2009) Saline water irrigation effects on antioxidant defense system and proline accumulation in leaves and roots of field-grown olive. *J Agric Food Chem* 57: 11484–11490
- Bergmann BA, Moon HK (1997) In vitro adventitious shoot production in Paulownia. *Plant cell reports* 16:315–319
- Bonner FT (1995) Paulownia tomentosa (Thunb.) Sieb. & Zucc. Ex Steud, royal Paulownia. In: Burns, R.M. and Honcala, B.H. eds. *Silvics of North America, Hardwoods Agric Handbk* 654 Washington DC. USDA Forest Service 2:501–502
- Borchetta S, Das SC, Handique PJ, Das S (2009) High multiplication frequency and genetic stability for commercialization of the three varieties of micropropagated tea plants (Camellia spp.). *Scientia Horticulturae* 120(4):544–550
- Duncan DB (1955) Multiple range and multiple F-tests. *Biometrics* 11:1–42

- Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry* 48(12):909–930
- Kalmukov K (1995) Influence of primary density upon the process of growth and development of some fast-growing tree species used for biomass. In: Ed. V.N. Brezin 70 Years Forestry education in Bulgaria. Jubilee Sciences Session, 7–9. VI., Sofi a, v. I, pp. 129–137.
- Khedr AH, Abbas MA, Wahid AA, Quick WP, Abogadallah GM (2003) Proline induces the expression of salt-stress-responsive proteins and may improve the adaptation of *Pancreaticum maritimum* L to salt stress. *J Exp Bot* 54:2553–2562
- Lone MI, Kueh JS, Wyn, R.J. Bright SJ (1987) Influence of proline and glycinebetaine on salt tolerance of cultured barley embryos. *Journal of Experimental Botany* 38:479-490
- Miladinova K, Ivanova K, Georgieva T, Geneva M, Markovska Y (2013) The salinity effect on morphology and pigments content in three paulownia clones grown ex vitro. *Bulgarian Journal of Agricultural Science* 19(2):52–56
- Munns R (2005) Genes and salt tolerance: bringing them together. *Tansley Rev New Phytol* 167:645–663
- Munns R, Tester M (2008) Mechanisms of salinity tolerance. *Annu Rev Plant Biol* 59:651–681
- Murashing T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. plant.* 15:473–497
- Park YS, Bonga JM (1992) Conifer micropropagation: its function in tree improvement programs. In: Ahuja MR (ed) *Micropropagation of woody plants*. Ed. MG. Ahuja, Kluwer Academic, Dordrecht, pp 457–470
- Rontain D, Basset G, Hanson AD (2002) Metabolic engineering of osmoprotectant accumulation in plants. *Metab Eng* 4:49–56
- Saric MR, Kastrori-Cupina T, Gergis I (1967) Chlorophyll determination Univ. Unoven Sadu-Praktikum is Kiziologize Bilika-Beograd, Haucua Anjiga 215
- Shahid MA, Pervez MA, Balal RM, Ahmad R, Ayyub CM (2011) Salt stress effects on some morphological and physiological characteristics of okra (*Abelmoschus esculentus* L.). *Soil Env* 30: 66
- Shao HB, Chu LY (2005) Plant molecular biology in China: opportunities and challenges. *Plant Molec Biol Rep* 23:345–358
- Shibli RA, Spomer LA, Smith MAL (1992) Osmotic adjustment and growth response of three *Chrysanthemum Morifolium* Ramat. Cultivars to Osmotic Stress Induced in vitro. *J Plant Nutr* 15:1373–1381
- Sivritepe N, Eris A (1999) Determination of salt tolerance in some grapevine cultivars (*Vitis vinifera* L.) under in vitro conditions. *Turk J Biol* 23:473–485
- Taha LS, Soad MM Ibrahim and Farahat MM (2008). A micropropagation protocol of Paulownia kawakamii through in vitro culture technique. *Australian Journal of Basic and Applied Sciences*, 2(3): 594- 600
- Tal M, Katz A (1980) Salt tolerance in the wild relatives of the cultivated tomato: the effect of proline on the growth of callus tissue of *Lycopersicon esculentum* and *L. peruvianum* under salt and water stress. *Z Pflanzenphysiol Bd* 98:283–288
- Tan J, Zhao H, Hong J, Han Y, Li H, Zhao W (2008) Effects of exogenous nitric oxide on photosynthesis, antioxidant capacity and proline accumulation in wheat seedlings subjected to osmotic stress. *World J Agric Sci* 4:307–313
- Vinocur B, Altman A (2005) Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. *Curr Opinion Biotechn* 16:123–132
- Wyn RG, Storey R, Leigh RA, Ahmad N, Pollard A (1977) A hypothesis on cytoplasmic osmoregulation. In: *Regulation of Cell Membrane Activities in Plants*. (Eds): E. Marr & O Ciferri Elsevier/North-Holland Biomedical Press Amsterdam 121–136

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.