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Article

Micropropagation of *Nolana intonsa* as a tool for its domestication and conservation

Micropropagação de *Nolana intosa* como ferramenta para sua domesticação e conservação

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Abstract: A complete protocol of *Nolana intonsa* I. M. Johnst, micropropagation was developed. The culture media used were WPM, DKW, and MS as control. DKW medium produced the best results over *in vitro* development of *N. intonsa*, generating a significant increase in the multiplication rate, lateral shoots formation, height, and general quality of the plantlets. In the case of fresh weight, dry weight, and water content, no differences were observed between MS and DKW, but a significant decrease in these parameters was produced when the WPM medium was used. Subsequently, when DKW was defined as basal medium, the effect of auxin supplementation was also evaluated. The addition of IBA generated a desirable effect over the *in vitro* plantlets. The addition of 0.05 mg L^{-1} of IBA improve the multiplication rate, the shoot's development, plant's height, and reduced in a significant way the damage to the plantlets. Fresh weight, dry weight, and water contents also showed a significant increase when IBA supplementation was added to the DKW medium. The plants where acclimatization of the plantlets was possible, obtaining finished pot plants capable of growth under greenhouse conditions. The development of a micropropagation protocol of *N. intonsa* could help the conservation and sustainable utilization of this endemic Chilean species.
Keywords: chilean flora, new ornamental, plant tissue culture, *Solanaceae.*

Resumo: Um protocolo eficiente de micropropagação foi desenvolvido para *Nolana intosa* I. M. Johnst. Os meios de cultura utilizados foram WPM, DKW e MS como controle. O meio DKW produziu os melhores resultados sobre o desenvolvimento *in vitro* de *N. intonsa*, gerando aumento significativo a taxa de multiplicação, formação de brotos laterais, altura e qualidade geral das plântulas. No caso de peso fresco, peso seco e teor de água, não foram observadas diferenças entre MS e DKW, e redução significativa nesses parâmetros foi produzida quando o meio WPM foi usado. Posteriormente, quando DKW foi definido como meio basal, o efeito da suplementação de auxina também foi avaliado. A adição de AIB gerou um efeito favorável sobre as plântulas *in vitro*. A adição de 0,05 mg L^{-1} de AIB melhorou a taxa de multiplicação (organogênese), o desenvolvimento da parte aérea, a altura da planta e reduziu de forma significativa os danos nas mudas. O peso fresco, o peso seco e os teores de água também mostraram um aumento significativo quando a suplementação de AIB foi adicionada ao meio DKW. Por fim, foi possível a aclimatação das mudas, obtendo-se plantas de vasos prontas capazes de crescer em casa de vegetação. O desenvolvimento de um protocolo de micropropagação de *N. intonsa* pode auxiliar na conservação e utilização sustentável desta espécie endêmica do Chile.
Palavras-chave: cultura de tecidos vegetais, flora chilena, nova ornamental, *Solanaceae.*

Introduction

Nolana intonsa I. M. Johnst. is an endemic shrub native from the North of Chile which can grow as an annual or perennial plant according to the environmental conditions. It belongs to the *Solanaceae* family, and is one of the 49 species of the genus *Nolana* described for Chile (Douglas and Freyre, 2010).

Although this species is not included in the Chilean official System of Conservation Status, there is growing concern about its survival. *N. intonsa* has a very limited distribution, inhabiting only the fog oases of the Tarapacá region in the north of Chile, such as Punta Gruesa (20°22'S and 70°09'W, 800 m.a.s.l.), Alto Patache (20°48'S and 70°09'W, between 200 to 800 m.a.s.l.), and Punta Lobos (21°02'S and 70°09'W, 500 m.a.s.l.) (Mesa et al., 1998). Additionally, in recent years, our field observations show that about 82% of the seeds present an absence of embryo (unpublished data). All the above, along with projections of less rainfall and increasing temperatures in Chile towards the end of the 21st century (Araya-Osses et al., 2020), put at risk the conservation of this species in its natural habitat. On the other hand, this species has great ornamental potential, due to its compact and creeping growth, pubescent and succulent leaden green leaves, violaceous flowers with dark purple center, and its abundant and long blooming period when small amounts of water are available (Douglas and Freyre, 2010). This last aspect is very relevant. Today, water efficiency is a major issue for horticulture, especially in arid and semi-arid areas, so the development of new ornamental crops which have lower water demands could be an alternative for saving water in landscaping (Elansary and Salem, 2015; Mayer et al., 2015; Hernández et al., 2016; Sánchez-Blanco et al., 2019). Under these circumstances, plant tissue culture could provide a new tool to support the conservation of this plant, allowing the massive propagation of plant material for research, commercial use, and repopulation of natural habitats (Werden et al., 2020). At the same time, micropropagation could play a relevant role in the domestication of *N. intonsa* as an ornamental crop.

As support for future conservation and domestication projects, an efficient protocol of micropropagation was developed, for which three basal mediums, the effect of auxins supplementation, and acclimatation process of *N. intonsa* plantlets were tested.

Materials and Methods

Herbaceous shoots were collected from one single plant of *N. intonsa* that was gathered from Punta Patache Oasis (around 65 km south of Iquique, Región de Tarapacá, North of Chile), transplanted to a 4 L pot, and maintained in unheated greenhouse conditions for six months. The used substrate was peat, sand, and perlite in a 2:1:2 ratio (Fig. 1).

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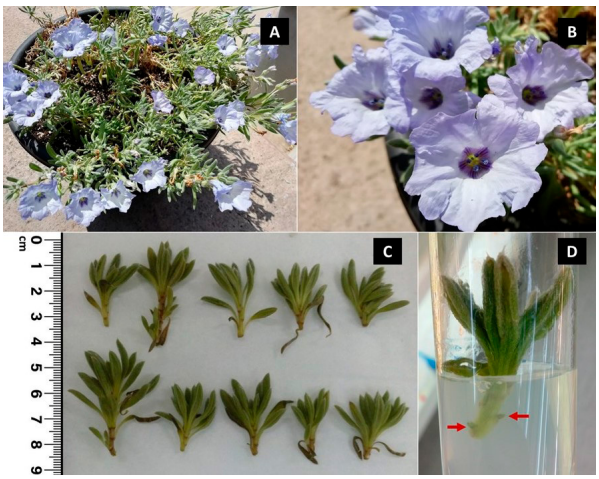

Fig. 1. Mother plant growing in a 15 L pot. It is possible observe the growth habit and flower detail of *Nolana intonsa*. (A) General view of the plant growing in a 15 L pot. (B) Close up of the flowers. It is possible observe the dark purple center of the flower. (C) Herbaceous shoots collected from a one single plants before been washed. (D) Initiated shoot after 10 days of the washing. Red arrows mark the sprouted buds.

For *in vitro* initiation, a standard disinfection protocol was used (Morales, 2019). Twenthy herbaceous shoots of 2 to 3 cm long were submerged in a fungicide solution with 20 g L^{-1} of Captan (Captan 83 WP, Adama Makhtesmim Ltd.) and 10 g L^{-1} of fosfetil aluminum (Aliette® 80% WP, Bayer CropScience S.A.) for 30 minutes. Under the flow cabinet, the fungicide solution was removed, and the shoots were washed with sterilized water to eliminate the remains. Next, the shoots were transferred into sterilized glass jars. Subsequently, a 70% (v/v) ethanol solution was added, and the shoots were gently dipped for 10 seconds. The nodal sections were immersed in 1% chlorine hypochlorite solution plus 1 mL L^{-1} of Tween20 for 20 minutes. Following this step, the material was rinsed three times with sterile water for 5 minutes. After the sterilization, damaged tissue was removed, and the explants were planted individually in glass tubes with 10 mL of MS basal growth medium (Duchefa Biochemie B.V., catalog code M0222) (Murashige and Skoog, 1962), supplemented with 1.0 mg L^{-1} of BAP, 30 g L^{-1} of sucrose and 7.0 g L^{-1} of agar (Duchefa Biochemie B.V., catalog code P1001), where the pH had been adjusted to 5.8 (Morales and Montañola, 2018) (Fig. 2). The cultures of the initiated buds were maintained during 5 weeks in growth chamber with 16 hours photoperiod of light (regular LED tubes of 18 W, cold light, brand D. R. L. Lighting), at 23 ± 2 °C and around 40% humidity.

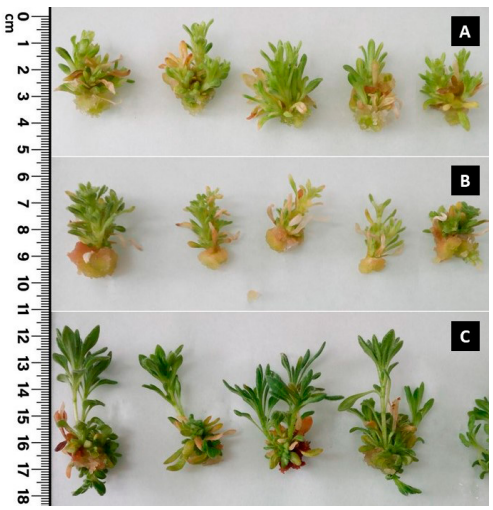

Fig. 2. *In vitro* development of *N. intonsa* plantlets in different basal mediums. (A) Plantlets cultivated in MS medium. (B) Plantlets cultivated in WPM medium. (C) Plantlets cultivated in DKW medium.

Once the new shoots were obtained, they were cut and transferred to fresh medium. These plantlets were maintained in tissue culture conditions for 3 months. The plants were divided and transferred to fresh medium every 4 weeks, placing 5 plantlets per jar. The used glass jars had 30 mL of growth medium and a total capacity of 200 cc.

For the micropropagation trials, three different basal mediums were tested: MS medium, Lloyd and McCown (WPM), and DKW/Juglans (DKW), all of them with their respective vitamins (Murashige and Skoog, 1962; Lloyd and McCown, 1980; Driver and Kuniyuki, 1984), plus 1.0 mg L^{-1} of BAP; 30 g L^{-1} of sucrose; and 7.0 g L^{-1}; pH 5.8. When the effect of the basal medium was established, the response of the plantlets to IBA enrichment was evaluated. The control was not enrichment with IBA, the basal DKW medium supplemented with 1.0 mg L^{-1} of BAP, 30 g L^{-1} of sucrose, and 7.0 g L^{-1} was used as control, while the three concentrations 0.01, 0.05, and 0.1 mg L^{-1} of IBA were tested. The pH of all mediums was adjusted to 5.8, and the conditions of the growth chamber were kept (23 ± 2 °C, around 40% humidity, and 16 hours of light). For all the experiments, the effect of the treatments was evaluated after keeping the plants in the same growth medium for three culture cycles.

For each experiment 60 plantlets were assessed, evaluating the multiplication rate (units), the number of shoots (units), height (cm), damage level, fresh weight (g), dry weight (g), and water content (%). To establish the damage level, a scale from 0 to 5 was used according Morales (2019), where 0 = no damage; 1 = light damage, less than 25% of the tissue shows damage; 2 = medium damage, 25% to 50% of the plant tissue shows necrotic injuries; 3 = severe damage, 50% to 75% of the tissue has necrotic damage; 4 = serious damage, over 75% of the plant tissue shows necrotic injuries; 5 = dead plants. Water content (%) was obtained by comparison of fresh weight (g) and dry weight (g) of plantlets after dehydration in a drying oven at 70 °C for 48 hours.

Rooting and acclimation processes were performed at the same time, using solid peat-based substrate, and following a conventional strategy. One hundred and twenty plantlets, around 2 cm long, were conditioned removing lateral shoots and leaving only one growth axis. These were treated in fungicide solution (Captan 83 WP 20 mg L^{-1}) for 10 minutes. Auxin containing powder was applied in the base of the plants (Keri Root $^{M.R.}$, NAA 4.000 ppm), and planted in peat. The seedling trays (alveoli of 8 mL) were cover with a transparent polyethylene terephthalate lid (PET plastic) and kept inside growth chamber (23 ± 2 °C, 40% humidity and 16 hours of light). After one month, the plants were transferred to an unheated greenhouse, where the seedling trays were gradually opened during a period of 30 days to avoid dehydration problems during the rooting and acclimatization of the plants. After that, when the plants were completely rooted and acclimated, they were transplanted into a seedling tray with alveoli of 37 mL.

Shapiro-Wilks test was used to evaluate the normality of the data. This indicated that obtained data did not follow a normal distribution, therefore, Kruskal-Wallis test was utilized for statistical analysis of the data.

Results

Regarding the initiation process, the disinfection protocol got good results, with 71,88% of the initiated plantlets were sterilized correctly, exhibiting new shoots regeneration a few weeks later. About the treatments of basal mediums and IBA supplementation, the obtained data is shown in the graphics of figures 3 and 4, respectively (Figs. 3, 4).

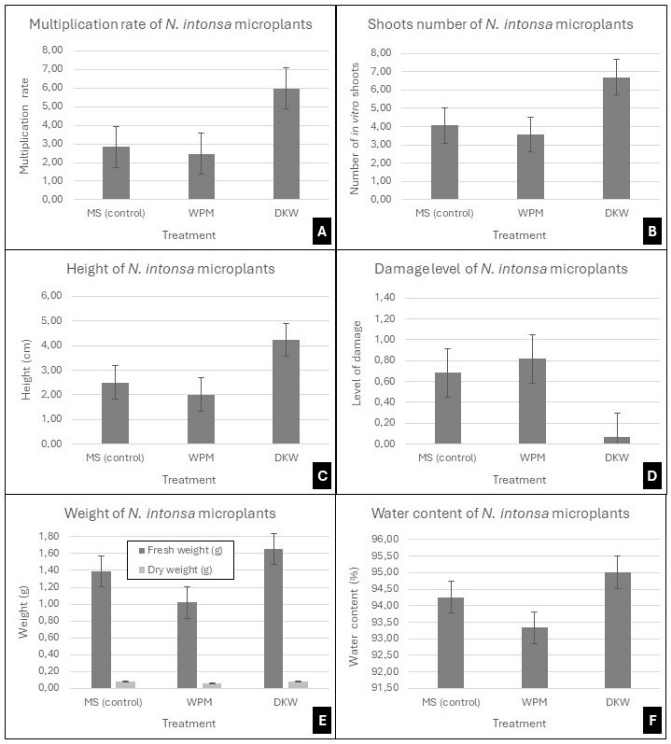

Fig. 3. Respond of *Nolana intonsa* microplants to three different basal mediums: MS, WPM, and DKW. (A) Effect of the basal medium over the multiplication rate of *N. intonsa* microplants. (B) Effect of the basal medium over the regeneration of new shoots of *N. intonsa* microplants. (C) Effect of the basal medium over the height of the *N. intonsa* microplants. (D) Effect of the basal medium over damage level of the microplants. (E) Effect of the basal medium over the fresh and dry weight, of *N. intonsa* microplants. (F) Effect of the basal medium over the water content over the microplants.

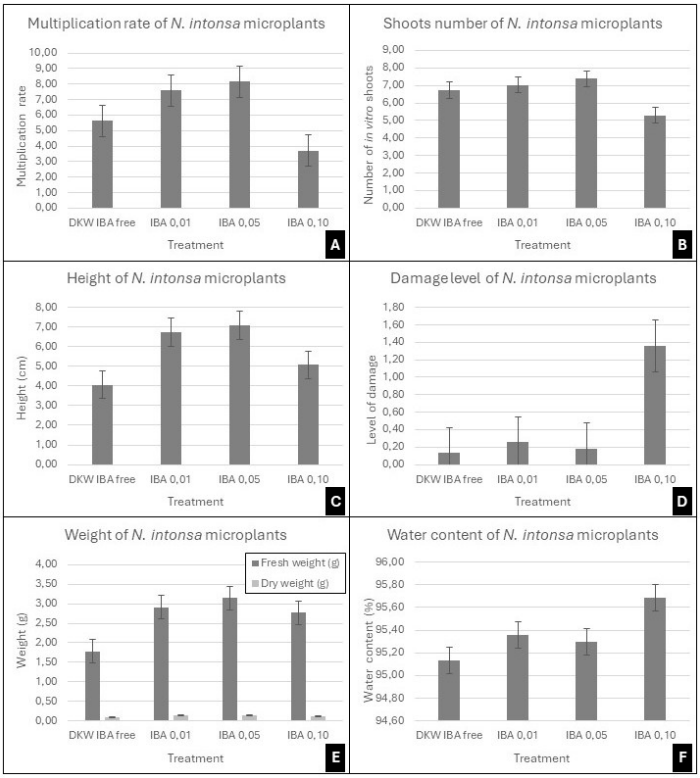

Fig. 4. Respond of *Nolana intons*a microplants to different supplementations of IBA in the growth medium: 0.01 mg L-1, 0.05 mg L-1, and 0.10 mgL-1. (A) Effect of IBA supplementation over the multiplication rate of *N. intonsa* microplants. (B) Effect of IBA supplementation over regeneration of new shoots of *N. intonsa* microplants. (C) Effect of IBA supplementation over the height of *N. intonsa* microplants. (D) Effect of IBA supplementation over the level of damage of N. intonsa microplants. (E) Effect of IBA supplementation over the fresh and dry weight of *N. intonsa* microplants. (F) Effect of IBA supplementation over the water content of *N. intonsa* microplants.

The use of DKW produced a notorious improvement in the quality of the plantlets (Fig. 2). This medium showed the highest values of multiplication rate (5.98), number of shoots (6.68), and plant height (4.24 cm). No significant differences in multiplication rate and number of shoots were observed between MS and WPM, but the plants cultivated in WPM medium were significantly smaller (2.01 cm). The damage level of DKW was the lowest (0.07), while MS and WPM did not show significant differences between them. Fresh weight, dry weight, and water content had no significant differences between DKW and MS, but plantlets cultivated in WPM medium had the lowest values.

Related to IBA supplementation trials (Fig. 5), significant differences were observed between the control and treatments. In the case of the multiplication rate, 0.05 mg L^{-1} of IBA produced a significant effect, showing the highest value (8.16). No significant differences were observed between the control and the medium supplemented with 0.01 mg L^{-1} of IBA, while the plantlets cultivated in medium with 0.1 mg L^{-1} had the lowest multiplication rate (3.70).

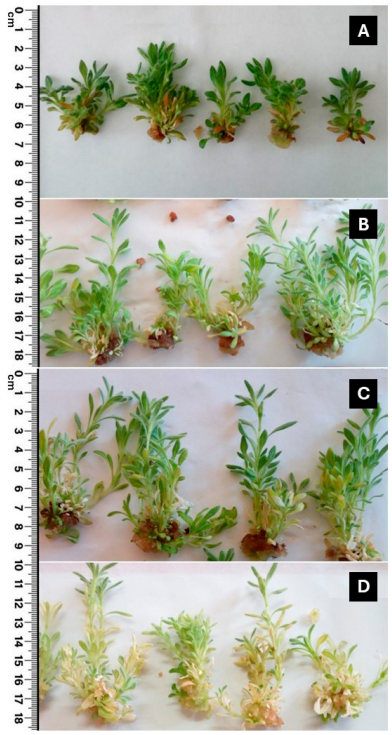

Fig. 5. Growth of the plantlets cultivated in multiplication mediums supplemented with IBA. (A) Control medium, without IBA supplementation. (B - D) plantlets cultivated in multiplication medium supplemented with 0.01, 0.05, and 0.1 mg L^{-1} of IBA respectively.

Regarding to the number of shoots, the treatment of 0.1 mg L^{-1} of IBA had a significant effect compared with the other two concentrations and showed the smallest shoots regeneration of 5.30 shoots per plantlet. This treatment was not significantly different from the control. Regarding the plant height, 0.01 and 0.05 mg L^{-1} of IBA produced an increase in the plantlets size of 6.74 and 7.04 cm, respectively, while 0.1 mg L^{-1} was not significantly different from the control.

Concerning the level of damage, only 0.1 mg L^{-1} was significantly different, showing the worst results, 1.36 on the damage scale. The rest of the IBA treatments were not different from the control, exhibiting levels lower than 1 on the damage scale.

Relative to fresh weight and dry weight, IBA treatments were significantly different from the control. It showed the lower values for both parameters. Additionally, IBA supplementation produced an increase in the water content of the plantlets. This increment, although small, was significantly higher in the three treatments, reaching 0.33% to 0.67% more water than the control.

Rooting and acclimation were obtained after 30 days. All plantlets developed roots and only 8 plants died during the acclimation process, obtaining a survival rate of 93.33% (Fig. 6).

Fig. 6. Acclimatization of *Nolana intonsa* micro-shoots. (A) Shoots were individualized and placed in covered seedling trays for one month. (B) After the first month in the growth chamber (23°C ± 2°C and 16 light hours) the rooted plantlets were moved to a greenhouse and gradually uncovered (30 days). (C) After 60 days of acclimatization, the fully rooted plants were transferred to a seedling tray with alveoli of 37 mL.

Discussion

N. intonsa is an extremophile and endemic plant from the North of Chile, exposed to various problems that can endanger the survival of the species. *N. intonsa* faces significant risks to its conservation due to its extremely restricted distribution, severe seed sterility issues, and reduced water availability resulting from climate change (Muñoz et al., 2016; Moreira-Muñoz et al., 2022). Micropropagation has emerged in the last years as a critical tool for plants conservation efforts (Werden et al., 2020; Twaij et al., 2020; Kulak et al., 2022), especially on species where it is not possible to conserve seeds because it is not possible to obtain viable seeds like in the case of *N. intonsa*, hence, developing a micropropagation protocol for this species could help, not only for preservation but also for its research and future domestication as an ornamental crop.

The results indicate that the composition of the basal medium would be playing a significant role in the development of the plantlets. While MS and WPM mediums allowed the regeneration of new shoots, when DKW medium was used, the obtained growth and the quality of the plantlets were far superior (Fig. 2), even when this medium was formulated for micropropagation of woody plants (Faria et al., 2019; Phillips and Garda, 2019).

Several are the reasons that could be producing this response. DKW and MS have more nitrogen than WPM. While the WPM has 5.0 mM of NH_4 and 9.7 mM of NO_3, DKW has around 3.5 times more (17.7 mM of NH_4 and 34.4 mM of NH_3). This lack of nitrogen in the WPM medium is consistent with the growth shown for the plantlets cultivated in it. Those plants exhibited chlorosis and less growth while those grown in the DKW medium looked greener and bigger. On the other hand, the MS medium has close to 16% more NH_4 and 15% more NO_3 than DKW. It has been reported that MS medium, although widely used in the micropropagation of different herbaceous species, it can cause growth problems due to excess ammonium (Phillips and Garda, 2019).

Sulfur (SO_4^-) is another element which could play a significant role. The content of SO_4^- in DKW medium is around 7 times higher that MS and WPM (12.3 mM versus 1.7 mM from MS and 1.6 mM from WPM). Sulfur is the fourth essential element in plants, part of several biomolecules such as proteins, vitamin, chlorophyll, coenzyme A, phytochelatins, S-adenosyl-methionine, and a variety of secondary metabolites. Sulfur also plays an important role in oxidative stress control through the activation of enzymes relative to ROS-scavenge (Wu et al., 2020; Narayan et al., 2022; Cao et al., 2023).

Another important difference is the calcium (Ca^{+2}). DKW medium has three times the amount of Ca^{+2} than MS and WPM mediums (9.3 mM versus 3.0 mM in MS and WPM). Calcium is an essential element with bioavailability problems due to its low mobility inside the plant. It is common to observe calcium deficiency problems during tissue culture of some species, even when the media are enriched with this element (Machado et al., 2014; Thakur et al., 2021).

Other elements that are in higher concentration in the DKW medium are phosphorus (PO_4^-) (2.0 mM versus 1.3 mM in MS and WPM); magnesium (Mg^+) two times more (3.0 mM versus 1.5 mM in MS and WPM); copper (Cu^{+2}) ten times more in DKW (1.0 mM versus 0.1 in MS and WPM); zinc (Zn^{+2}) close to two times more (57.2 mM in DKW versus 29,9 mM in MS and WPM); manganese (Mn^{+2}) close to double in DKW than MS (198 versus 100 mM) and 4.4 times more in comparison with WMP (44.8 mM); and molybdenum (MoO_4^-) (1.6 mM in DKW and 1.0 in MS and WPM). In addition, DKW is the only one medium that has nickel (Ni^+), although at a very low concentration (Murashige and Skoog, 1962; Driver and Kuniyuki, 1984; Morales-Tapia and Gambardella, 2022).

Vitamins concentration also has some changes. DKW has a double concentration of nicotinic acid (8.12 mM versus 4.06 mM in MS and WPM) and close to 20 times more thiamine than MS and 2 times more than WPM (5.93, 0.3, and 2.96 mM respectively).

Regarding IBA supplementation, its use during the multiplication stage improved the growth of the plantlets. This effect is not new, and it has been reported in other plants (Sofian et al., 2018; Ahmed et al., 2021; Mohamad et al., 2022; Morales-Tapia and Gambardella, 2022). In this case, the addition of the IBA in the growth medium (0.01 and 0.05 mg L^{-1}) produced an increment in the multiplication rate of the plantlets caused by the increase in the number of lateral shoots and the augmentation in the height of the plants. On the other hand, when 0.1 mg L^{-1} of IBA was used, a significant reduction in the multiplication rate was observed. That was produced by the fall of the number of lateral shoots developed and by the reduction in the height of the plantlets, triggering a decrease in the number of obtained nodal sections. The use of exogenous auxins induces apical dominance, so the decrease in the number of lateral shoots when 0.1 mg L^{-1} dose of IBA was used was an expected response.

Finally, rooting and acclimatization using micro-shoots obtained directly from the multiplication stages showed good results. This is a common practice during micropropagation of many species and helps to reduce the use of resources related to *in vitro* rooting how growth mediums, labor, and space in the growth chamber, among others.

Conclusions

Micropropagation of *N. intonsa* is possible using DKW as the base medium. Even though this medium is used for the tissue culture of woody species, when *N. intonsa* was cultivated in it the best results were observed, generating higher quality plants and high multiplication rate. On the other hand, the addition of small amounts of IBA to the culture medium during the multiplication stage also had a positive effect on the development of the plants. Having a micropropagation protocol could help in the conservation and domestication of *N. intonsa*, facilitating the obtaining of plant material for reforestation, research, and commercialization, without affecting wild populations with the extraction of plants or seeds. Additionally, having *in vitro* culture techniques can facilitate the process of plant breeding, since we can apply techniques such as polyploidization and mutagenesis, in addition to enabling the rapid propagation of outstanding genotypes.

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Author Contribution

PMT and JH: obtaining and maintenance of the *in vitro* and *ex vitro* plant material. Also, they developed the experimental work. **PMT, JH, DV, and KV**: experimental design, data analysis, and writing of the article.

Conflict of Interest

The authors declare that they have no potential conflict of interest in the submitted work.

Data Availability Statement

Data will be made available on request.

References

AHMED, Z.S.; SALIM, A.M.; ALLAWI, A.K. Effect of growth regulators (BA, IBA) on micropropagation of *Gardenia jasminoides* L. *in vitro***. International Journal of Agricultural and Statistical Sciences**, v.17, p.181–186, 2021. https://connectjournals.com/03899.2021.17.181

ARAYA-OSSES, D.; CASANUEVA, A.; ROMÁN-FIGUEROA, C.; URIBE, J.M.; PANEQUE M. Climate change projections of temperature and precipitation in Chile based on statistical downscaling. **Climate Dynamics**, v.54, p.4309-4330, 2020. https://doi.org/10.1007/s00382-020-05231-4

CAO, Y.; MA, C.; YU, H.; TAN, Q.; DHANKHER, O.P.; WHITE, J.C.; XING, B. The role of sulfur nutrition in plant response to metal(loid) stress: Facilitating biofortification and phytoremediation. **Journal of Hazardous Materials**, v.443, p.130283, 2023. https://doi.org/10.1016/j.jhazmat.2022.130283

DOUGLAS, A.C.; FREYRE, R. Floral development, stigma receptivity and pollen viability in eight Nolana (*Solanaceae*) species. **Euphytica**, v.174, p.105–117, 2010. https://doi.org/10.1007/s10681-010-0145-8

DRIVER, J.A.; KUNIYUKI, A.H. *In vitro* propagation of Paradox Walnut root stock. **HortScience**, v.19, p.507–509, 1984. https://doi.org/10.21273/HORTSCI.19.4.507

ELANSARY, H.O.; SALEM, M.Z.M. Morphological and physiological responses and drought resistance enhancement of ornamental shrubs by trinexapac-ethyl application. **Scientia Horticulturae**, v.189, p.1-11, 2015. https://doi.org/10.1016/j.scienta.2015.03.033

FARIA, D.V.; CORREIA, L.N.F.; MATOS, E.M.; SOUZA, M.V.C.; BATISTA, D.S.; COSTA, M.G.C.; PAIVANETO, V.B.; XAVIER, A.; HERNÁNDEZ, A.; MIRANDA, M.D.; ARELLANO, E.C.; DOBBS, C. Landscape trajectories and their effect on fragmentation for a Mediterranean semi-arid ecosystem in Central Chile. **Journal of Arid Environments**, v.127, p.74-81, 2016. https://doi.org/10.1016/j.jaridenv.2015.10.004

KULAK, V.; LONGBOAT, S.; BRUNET, N.D.; SHUKLA, M.; SAXENA, P. *In vitro* technology in plant conservation: relevance to biocultural diversity. **Plants**, v.11, p.1-20, 2022. https://doi.org/10.3390/plants11040503

LLOYD, G.; MCCOWN, B. Commercially-feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture. **Proceedings of the International Plant Propagator's Society**, p.421-427, 1980.

MACHADO, M.P; SILVA, A.L.L.; BIASI, L.A.; DESCHAMPS, C.; BESPALHOK FILHO, J.C.; ZANETTE, F. Influence of calcium content of tissue on hyperhydricity and shoot-tip necrosis of *in vitro* regenerated shoots of *Lavandula angustifolia* Mill. **Brazilian Archives of Biology and Technology**, v.57, p.636-643, 2014. http://dx.doi.org/10.1590/S1516-8913201402165

MAYER, P.; LANDER, P.; GLENN, D. Outdoor water use: abundant savings, scarce research. **Journal - American Water Works Association**, v.107, p.61-66, 2015. https://doi.org/10.5942/jawwa.2015.107.0029

MESA, A.; MUÑOZ, V.; PINTO, R. Presencia de *Nolana adansonii* (Roemer y Schultes) Johnst. y *Nolana intonsa* Johnst. (*Nolanaceae*) en el desierto costero de Iquique, Norte de Chile. **Noticiario Mensual del Museo Nacional de Historia Natural**, v.333, p.3-7, 1998.

MOHAMAD, M.E.; AWAD, A.A.; MAJRASHI, A.; ESADEK, O.A.A.; EL-SAADONY, M.T.; SAAD, A.M.; GENDY, A.S. *In vitro* study on the effect of cytokines and auxins addition to growth medium on the micropropagation and rooting of *Paulownia* species (*Paulownia* hybrid and *Paulownia tomentosa*). **Saudi Journal of Biological Sciences**, v.29, p.1598-1603, 2022. https://doi.org/10.1016/j.sjbs.2021.11.003

MORALES-TAPIA, P.; GAMBARDELLA, M. Control of shoot-tip necrosis during *Argylia radiata in vitro* multiplication. **Ornamental Horticulture**, v.28, p.423-430, 2022. https://doi.org/10.1590/2447-536x.v28i4.2536

MORALES, P. *Argylia radiata* micropropagation, a biotechnological tool to domesticate a new ornamental crop. **Acta Horticulturae**, v.1240, p.69-72, 2019. https://dpi.org/10.17660/ActaHortic.2019.1240.11

MORALES, P.; MONTAÑOLA, M.J. Development of *Nolana carnosa* micropropagation protocol. **Acta Horticulturae**, v.1224, p.229-234, 2018. https://doi.org/10.17660/ActaHortic.2018.1224.31

MOREIRA-MUÑOZ, A.; PALCHETTI, M.V.; MORALES-FIERRO, V.; DUVAL, V.S.; ALLESCH-VILLALOBOS, R.; GONZÁLEZ-OROZCO, C.E. Diversity and conservation gap analysis of the Solanaceae of Southern South America. **Frontiers in Plant Science**, v.13, p.1-7, 2022. https://doi.org/10.3389/fpls.2022.854372

MUÑOZ, R.C.; QUINTANA, J.; FALVEY, M.J.; RUTLLANT, J.A.; GARREAUD, R. Coastal clouds at the Eastern Margin of the Southeast Pacific: Climatology and trends. **Journal of Climate**, v.29, p. 4525-4542, 2016. https://doi.org/10.1175/JCLI-D-15-0757.1

MURASHIGE, T.; SKOOG, F. A revised medium for rapid growth and bioassay with tobacco tissue cultures. **Physiologia Plantarum**, v.15, p.473-497, 1962. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

NARAYAN, O.P.; KUMAR, P.; YADAV, B.; DUA, M.; JOHRI, A.K. Sulfur nutrition and its role in plant growth and development. **Plant Signaling and Behavior**, e2030082-3, 2022. https://doi.org/10.1080/15592324.2022.2030082

PHILLIPS, G.C.; GARDA, M. Plant tissue culture media and practices: an overview. **In Vitro Cellular and Developmental Biology - Plant**, v.55, p.242–257, 2019. https://doi.org.10.1007/s11627-019-09983-5

SÁNCHEZ-BLANCO, M.J.; ORTUÑO, M.F.; BAÑON, S.; ÁLVAREZ, S. Deficit irrigation as a strategy to control growth in ornamental plants and enhance their ability to adapt to drought conditions. **Journal of Horticultural Science and Biotechnology**, v.94, p.137-150, 2019. https://doi.org.10.1080/14620316.2019.1570353

SOFIAN, A.A.; S. PRIHASTANTI, S.; SUEDY, S.W.A. Effect of IBA and BAP on shoot growth of Tawangmangu Tangerine (*Citrus reticulate*) by In-Vitro. **Biosaintifika: Journal of Biology & Biology Education**, v.10, p.379–387, 2018. https://doi.org/10.15294/biosaintifika.v10i2.14977

THAKUR, M.; SHARMA, V.; LUHARCH, R. Propagation of plum (*Prunus salicina* L.) cultivar Frontier *in vitro* through control of shoot tip necrosis (STN) and validation of genetic integrity using ISSR markers. **Plant Physiology Reports**, v.26, p.238–246, 2021. https://doi.org/10.1007/s40502-021-00580-6

TWAIJ, B.M.; JAZAR, Z.; HASAN, M.N. Trends in the use of tissue culture, applications and future aspects. **International Journal of Plant Biology**, v.11, p.8385, 2020. https://doi.org/10.4081/pb.2020.8385

WERDEN, L.K.; SUGII, N.C.; WEISENBERGER, L.; KEIR, M.J.; KOOB, G.; ZAHAWI, R.A. *Ex situ* conservation of threatened plant species in island biodiversity hotspots: A case study from Hawaii. **Biological Conservation**, v.243, p.108435, 2020. https://doi.org/10.1016/j.biocon|.2020.108435

WU, Z.; NAVEED, S.; ZHANG, C.; GE, Y. Adequate supply of sulfur simultaneously enhances iron uptake and reduces cadmium accumulation in rice grown in hydroponic culture. **Environmental Pollution**, v.262, p.114327, 2020. https://doi.org/10.1016/j.envpol.2020.114327