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SCIENTIFIC ARTICLE

Control of shoot-tip necrosis during *Argylia radiata in vitro* multiplication

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Abstract

Argylia radiata is an herbaceous perennial plant native to northern Chile and a representative species of the "Blooming Desert". Due to its showy flowers and other morphological characteristics, *A. radiata* has great ornamental potential. In earlier work, a deep morpho-anatomical description was made, but the micropropagation protocols, which could be used for commercial purposes, are not known. Previous assays showed that cytokinin supplementation improves the multiplication rate but produces shoot-tip necrosis in the microplants. To avoid it, different modifications of the growth medium were tested, including calcium nitrate supplementation; increasing in agar concentration; indole-3-butyric acid enrichment; and change of the basal medium formulation. The effect of these changes over the damage level, number of shoots, multiplication rate, plant height (cm), fresh weight and dry weight (g), and water content (%) of the microplants were evaluated. The use of McCown Woody Plant formulation as basal medium showed the best effect, reducing the damage level and improving the multiplication rate. Additionally, IBA supplementation was effective in reducing necrotic damage. However, 0.1 mg L^{-1} of IBA significantly decreased the multiplication rate, while 0.01 mg L^{-1} led to a higher multiplication rate than that of plants grown in the control medium. In conclusion, the use of McCown Woody Plant medium and IBA supplementation should be considered in commercial *A. radiata* micropropagation.
Key words: Bignoniaceae, Chilean blooming desert, growth medium, plant tissue culture.

Resumo

Controle da necrose apical durante a multiplicação *in vitro* de *Argylia radiata*

Argylia radiata é uma planta herbácea perene nativa do norte do Chile e uma espécie representativa do "Deserto Florido". Por suas flores vistosas e outras características morfológicas, *A. radiata* possui grande potencial ornamental. Em trabalhos anteriores, uma descrição morfoanatômica profunda foi feita, mas os protocolos de micropropagação, que poderiam ser usados para fins comerciais, não são conhecidos. Ensaios anteriores mostraram que a suplementação de citocinina melhora a taxa de multiplicação, mas produz necrose apical nas microplantas. Para evitá-lo, diferentes modificações do meio de crescimento foram testadas, incluindo suplementação de nitrato de cálcio; aumento da concentração de ágar; enriquecimento de ácido indol-3-butírico; e mudança da formulação do meio basal. Foi avaliado o efeito dessas mudanças sobre o nível de dano, número de brotações, taxa de multiplicação, altura da planta (cm), massa fresca e massa seca (g) e teor de água (%) das microplantas. O uso da formulação McCown Woody Plant como meio basal apresentou o melhor efeito, reduzindo o nível de danos e melhorando a taxa de multiplicação. Além disso, a suplementação com IBA foi eficaz na redução dos danos necróticos. No entanto, 0,1 mg L^{-1} de AIB diminuiu significativamente a taxa de multiplicação, enquanto 0,01 mg L^{-1} levou a uma taxa de multiplicação maior do que as plantas cultivadas no meio controle. Em conclusão, o uso do meio McCown Woody Plant e suplementação com IBA deve ser considerado na micropropagação comercial de *A. radiata*.
Palavras-chave: Bignoniaceae, cultura de tecido vegetal, deserto florido do Chile, meio de crescimento.

Introduction

Argylia radiata (L.) D. Don is a perennial plant that is a member of the *Bignoniaceae* family and is native to arid areas of northern Chile, between the Atacama and Coquimbo regions. It is also part, and a representative species, of the "Blooming Desert" (Morales-Tapia et al., 2020), a phenomenon defined as the appearance of a great number of plants that cover plains and hills that are usually devoid of vegetation (Carevic, 2016). This understudied species could be a new option for ornamental crops due to

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its green-blue foliage and multiple floral stems with 20 to 50 beautiful trumpet flowers. The color of the flowers ranges from white to deep red through tones of yellow, orange and pink. The extremely challenging conditions in which these plants grow could make this species an interesting option for low water usage landscaping. As part of the domestication process of *A. radiata*, micropropagation could be a tool to help its breeding program because tissue culture allows for the rapid propagation of outstanding phenotypes. In previous trials, it was possible to micropropagate *A. radiata* on Murashige and Skoog medium without growth regulators. However, the observed multiplication rates were not satisfactory. For that reason, supplementation with different kinds of exogenous cytokinins was evaluated. The addition of 0.5 mg L^{-1} of 6-benzylaminopurine (BAP) in the growth medium increased the multiplication rates significantly. Nevertheless, its use also generated shoot-tip necrosis (STN), reducing the quality of the plant material (Morales, 2019).

STN is a physiological disorder observed in the micropropagation of many plants (Surakshitha et al., 2019; Silva et al., 2020). It begins with browning injuries over the shoot tips followed by basipetal necrosis in the plantlets. The necrotic damage gradually moves to the base of the plants, producing senescence of apical buds and shoots. In severe cases, all plant tissue is affected leading to the death of plants (Surakshitha et al., 2019). STN can be produced by different factors, such as mineral deficiency, a lack vitamins and growth regulators, ethylene, hyperhydricity, agar concentration, explant age, successive subcultures, sugar sources, accumulation and oxidation of phenolic compound in the plants tissue, endophytic contamination, and even over supplementation with gibberellic acid in some cases (Bairu and Kane, 2011; Osabe et al., 2012; Wen et al., 2016; Al-Aizari et al., 2020; Cheong et al., 2020; Silva et al., 2020; Thakur et al., 2021). To avoid the occurrence of this disorder, changes in medium components such as calcium supplementation, an increase in agar concentration, indole-3-butyric acid (IBA) addition and changes in basal mediums were tested during *A. radiata in vitro* multiplication stage.

Materials and Methods

Plant material and pre-culture

The used plantlets were obtained from the initiation of herbaceous shoots which were collected from Bahía Inglesa, in North of Chile (27°07'32" S; 70°48'53" W). The sterilization process started by dipping the shoots in fungicide solution (Captan 20 g L^{-1}) for 30 minutes. Then, they were passed through 70% (v v^{-1}) ethanol solution and washed in a 20% (v v^{-1}) solution of commercial bleach for 20 minutes. After that, the material was rinsed three times with sterile water for 5 min. The explants were cut, eliminating all damaged tissue. Finally, nodal sections were planted in MS basal medium (Duchefa Biochemie B.V. catalog code M0222). The medium was supplemented with 30 g L^{-1} of sucrose and 6.5 g L^{-1} of agar, pH was adjusted to 5.8 through a solution of potassium hydroxide 8.0 N. After 8 weeks, when lateral shoots were completely expanded, they were cut and isolated in fresh medium. The obtained plantlets were kept in *in vitro* conditions for 5 months. They were transferred to fresh medium every 5 weeks.

Detail of the treatments

The composition of the control medium was MS basal medium, including vitamins, supplemented with 0.5 mg L^{-1} of BAP, 6.5 g L^{-1} of agar, and 30 g L^{-1} of sucrose, at pH 5.8. To reduce STN incidence, 4 experiments were done to adjust the supplementation of calcium, the amount of agar, auxin concentration, and type of basal medium.

The first experiment corresponds to calcium supplementation. MS basal medium was supplemented with three concentrations of calcium nitrate [$Ca(NO_3)_2$]. 0.3, 0.6, 0.9 g L^{-1} were tested. The second experiment was related to the amount of agar. The control medium contained 6.5 g L^{-1} agar, and 8.0 and 10.0 g L^{-1} agar were used as treatments. The third experiment was in regard to auxin concentration, three concentrations of IBA were tested, adding 0.01, 0.05, and 0.1 mg L^{-1}. The control medium was kept free of IBA. Finally, the fourth experiment was run testing different basal mediums. The control is based on MS medium, Lloyd & McCown medium (WPM) and DKW/Juglans medium (DKW), with their respective vitamins, were evaluated (Lloyd and McCown, 1980; Driver and Kuniyuki, 1984). Both mediums were supplied by Duchefa Biochemie B.V. (WPM catalog code M0219 and DKW catalog code D0247). For all experiments, the amount of BAP, sucrose concentration, and pH were maintained.

Evaluated parameters

To evaluate the experiments, sixty explants were used for each treatment, with 10 plants per glass jar. The used glass jars had a total capacity of 200 cc. A transparent polypropylene sheet was used as lid, which was fixed to the jars through an elastic band. After 5 weeks inside the growth room (23 ± 2 °C, 40% humidity and 16 hours of light), the damage level, number of shoots, multiplication rate, and plant height (cm) of 50 plants were evaluated. The damage level was defined on a scale from 0 to 5, 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, it is not possible to obtain explants from them (Morales, 2019). The fresh weight (g), dry weight (g), and water content (%) of 10 plants were also determined. Dry weight was obtained by weighing the plantlets after dehydration in a drying oven for 48 h at 70 °C. The water content was calculated using fresh and dry weight.

Statistical analysis

The normality of the data was evaluated through the Shapiro-Wilks test, which indicated that the obtained data did not follow a normal distribution, therefore they were analyzed with the Kruskal-Wallis test.

Results

The results show that some of the modifications made to the growth media, significantly improved the quality of the plantlets, while others produced a negative response. The assessment of the different experiments was mainly based on the occurrence of necrotic injuries. However, other parameters related to the efficiency of *A. radiata* micropropagation were also evaluated (Table 1, 2, 3, 4).

Calcium nitrate supplementation

The results of the first experiment related to calcium supplementation (Table 1), resulted in a worse response than that from the control medium. The necrotic damage shown by the plantlets from the calcium treatments was greater than the injuries observed in the plantlets from the control medium (Figures 1B, C, D). Neither the number of shoots nor the multiplication rate were neither better than the control response. Even the result of supplementation with 0.3 g L^{-1} of $Ca(NO_3)_2$ was significantly worse than that of the control medium for both parameters. 0.6 and 0.9 g L^{-1} of $Ca(NO_3)_2$ improved the height of the plants. Regarding fresh weight, dry weight, and water content (%), no differences between the control and the treatments were observed.

Table 1. *In vitro* growth parameters of *A. radiata* cultivated on growth medium supplemented with different amounts of calcium nitrate ($Ca(NO_3)_2$).

Treatment	Damage level [a, b]	Number of shoots [b]	Multiplication rate [b]	Plant height (cm) [b]	Fresh weight (g) [c]	Dry weight (g) [c]	Water content (%) [c]
Control	2.15[a]	5.71[a]	3.00[a]	4.20[a]	0.83[ab]	0.07[a]	91.53[a]
0.3 g L^{-1}	2.92[b]	4.75[b]	1.80[b]	4.38[a]	0.66[b]	0.08[a]	89.45[a]
0.6 g L^{-1}	2.74[b]	5.89[a]	2.72[a]	5.03[b]	0.68[b]	0.05[a]	92.48[a]
0.9 g L^{-1}	2.79[b]	5.58[a]	2.56[a]	5.01[b]	0.91[a]	0.08[a]	94.26[b]

Different letters indicate significant differences between groups according to Kruskal-Wallis test ($p \leq 0.05$); [a] Numeric scale from 0 to 5, where 0 = no damage, plants have no necrotic tissue and 5 = dead plants, it is not possible to obtain new explants from them; [b] Average, n=60; [c] Average, n=10.

Increasing in agar concentration

In the second experiment (Table 2), which is related to the amount of agar, the damage level was not significantly different between them and the control medium (Figures 1E, F). Nevertheless, a slight reduction in necrotic injuries was observed when 8 g L^{-1} of agar was used. No differences between the control and the treatments were observed in the number of shoots, multiplication rate, fresh weight, dry weight, and water content. Regarding plant height, the treatment of 10 g L^{-1} of agar produced taller plants than those from the control medium (4.71 ± 0.92 vs 4.20 ± 0.96 cm).

Table 2. *In vitro* growth parameters of *A. radiata* cultivated on growth media at different agar concentrations.

Treatment	Damage level [a, b]	Number of shoots [b]	Multiplication rate [b]	Plant height (cm) [b]	Fresh weight (g) [c]	Dry weight (g) [c]	Water content (%) [c]
Control	2.15[ab]	5.71[a]	3.00[a]	4.20[b]	0.83[a]	0.07[a]	91.53[a]
8 g L^{-1}	1.77[a]	6.03[a]	3.30[a]	4.35[ab]	0.78[a]	0.18[a]	83.85[a]
10 g L^{-1}	2.29[b]	5.57[a]	2.75[a]	4.71[a]	0.65[a]	0.05[a]	91.70[a]

Different letters indicate significant differences between groups according to Kruskal-Wallis test ($p \leq 0.05$); [a] Numeric scale from 0 to 5, where 0 = no damage, plants have no necrotic tissue and 5 = dead plants, it is not possible to obtain new explants from them; [b] Average, n=60; [c] Average, n=10.

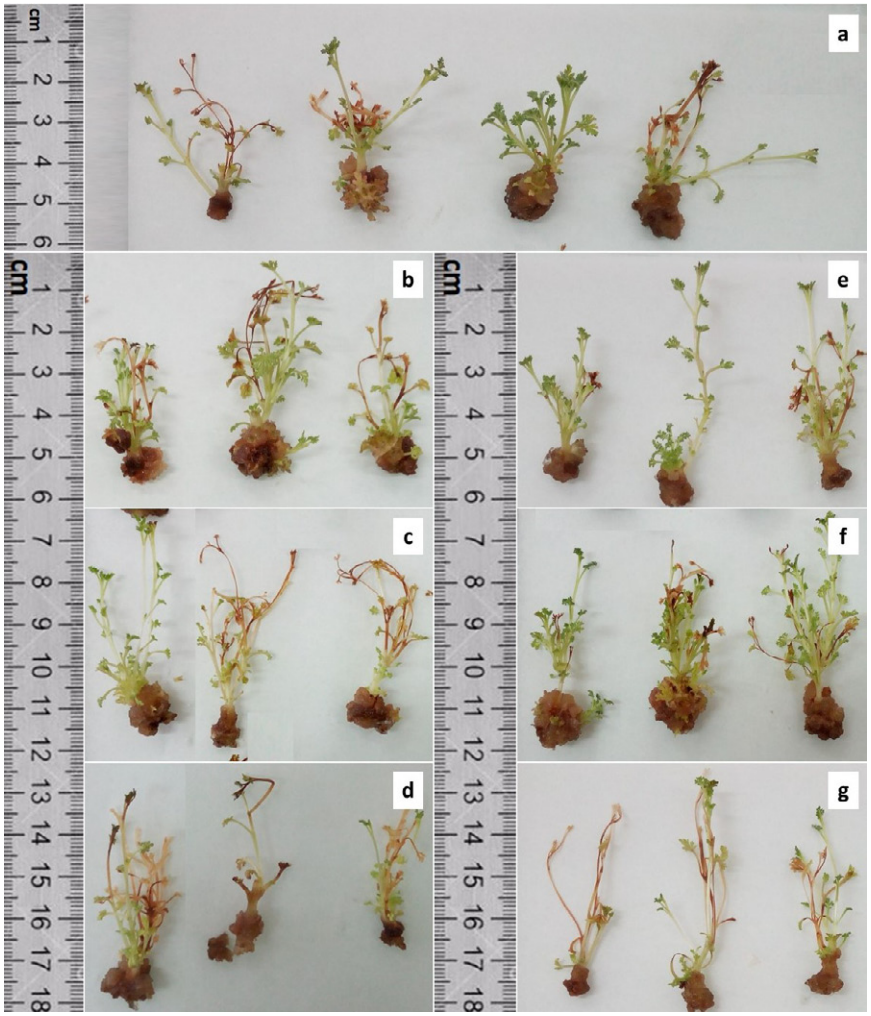

Figure 1. Development of shoot tip necrosis (STN) with different modifications of *Argylia radiata* growth medium. (a) Control medium, MS basal medium, supplemented with 0.5 mg L^{-1} of BAP and 30 g L^{-1} of sucrose, and pH 5.8. (b-d) Calcium supplementation treatments, 0.3, 0.6, and 0.9 g L^{-1} of $Ca(NO_3)_2$, respectively. (e-f) Changes in agar concentration, 8 and 10 g L^{-1}, respectively. (g) Plants cultivated on DKW basal medium.

IBA enrichment

About the third experiment (Table 3), IBA treatments reduced STN development. A trend of damage reduction with increasing IBA concentration was observed. In terms of the multiplication rate, supplementation with 0.01 mg L^{-1} improved this parameter with respect to the control medium, while the result of supplementation with 0.05 mg L^{-1} was not different from that of the control, and 0.1 mg L^{-1} of IBA induced a significant reduction in the multiplication rate (Figures 2C, D, E). Regarding plant height, 0.01 and 0.05 mg L^{-1} IBA produced taller plants than the control. The plant height after supplementation with 0.1 mg L^{-1} IBA was not significantly different than that from the control medium. This effect was most likely the result of apical dominance induced by the application of exogenous auxins, which reverses its response in high concentrations such as 0.1 mg L^{-1}. The results for fresh weight, dry weight and water content did not show differences between the control medium and IBA treatments.

Table 3. *In vitro* growth parameters of *A. radiata* cultivated on growth medium supplemented at three concentrations of IBA.

Treatment	Damage level [a, b]	Number of shoots [b]	Multiplication rate [b]	Plant height (cm) [b]	Fresh weight (g) [c]	Dry weight (g) [c]	Water content (%) [c]
Control	2.15[c]	5.71[a]	3.00[b]	4.20[b]	0.83[a]	0.07[a]	91.53[a]
0.01 mg L^{-1}	0.75[b]	4.37[b]	3.87[a]	5.18[a]	0.67[a]	0.05[a]	91.91[a]
0.05 mg L^{-1}	0.85[b]	3.65[b]	3.06[b]	5.29[a]	0.74[a]	0.06[a]	91.66[a]
0.1 mg L^{-1}	0.42[a]	2.32[c]	2.29[c]	4.41[b]	0.64[a]	0.06[a]	90.16[a]

Different letters indicate significant differences between groups according to Kruskal-Wallis test ($p \leq 0.05$); [a] Numeric scale from 0 to 5, where 0 = no damage, plants have no necrotic tissue and 5 = dead plants, it is not possible to obtain new explants from them; [b] Average, n=60; [c] Average, n=10.

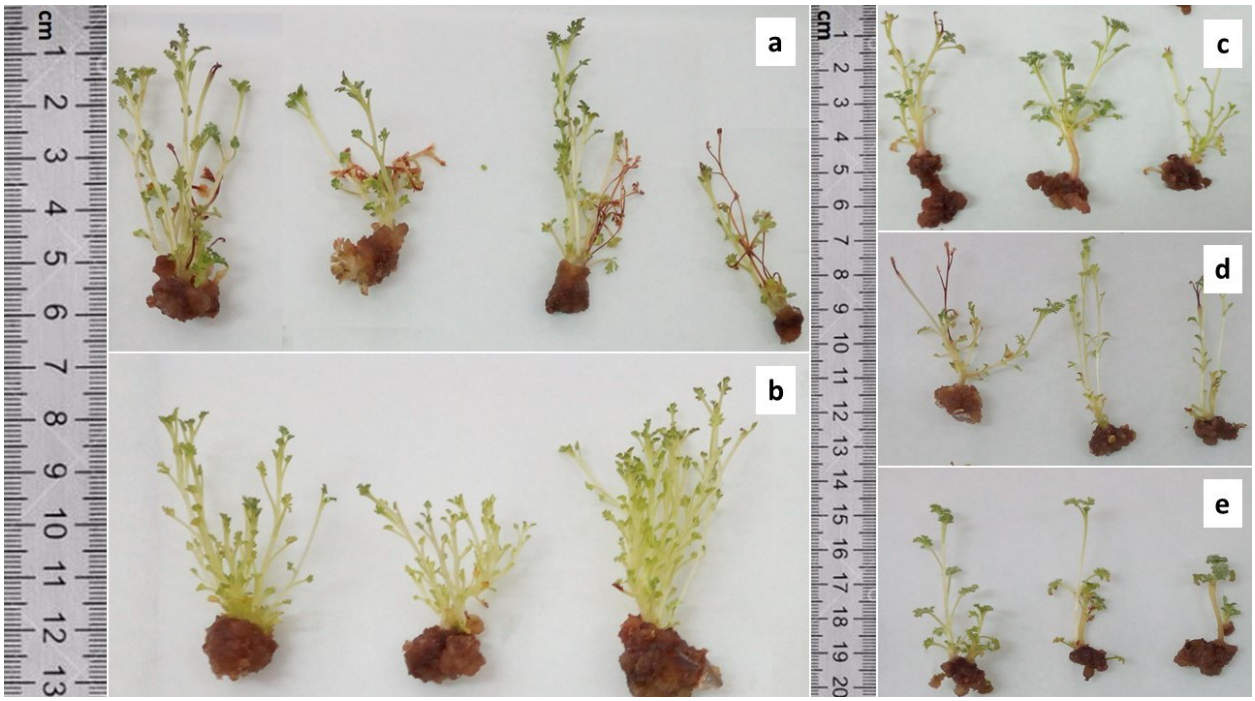

Figure 2. Reduction of shoot tip necrosis (STN) in microplants of *Argylia radiata* cultivated on different growth media. (a) Control medium, MS basal medium, supplemented with 0.5 mg L^{-1} of BAP and 30 g L^{-1} of sucrose, and pH 5.8. (b) WPM medium as basal growth medium. Its use produced no necrotic injuries. (c-e) IBA treatments, 0.01, 0.05, and 0.1 mg L^{-1}, respectively. A reduction in necrotic injuries and a decrease in the number of lateral shoots were observed.

Change of the basal medium formulation

Regard to the results of the fourth experiment (Table 4), the effect of WPM basal medium was the best of all treatments, observing a significant reduction in necrotic injuries compared to the control (Figure 2B). WPM showed a damage level of 0.15, while in the control medium the observed damage level was 2.15. No differences were observed in the incidence of STN between the control and the DKW medium treatment (Fig. 1G). The number of shoots did not show any differences between the treatments, but the multiplication rate showed an increase in the WPM medium. This was caused by the reduction in STN, which permitted a higher quantity of healthy tissue to be used as explants. Regarding plant height, DKW medium produced taller plants than the control and WPM media. The fresh weight, dry weight, and water content did not show any differences between plants grown in the control and the treatment media.

Table 4. *In vitro* growth parameters of *A. radiata* cultivated on different basal media.

Treatment	Damage level [a, b]	Number of shoots [b]	Multiplication rate [b]	Plant height (cm) [b]	Fresh weight (g) [c]	Dry weight (g) [c]	Water content (%) [c]
Control	2.15[b]	5.71[a]	3.00[b]	4.20[b]	0.83[a]	0.07[a]	91.53[ab]
WPM	0.15[a]	5.85[a]	7.09[a]	4.54[b]	1.12[a]	0.07[a]	93.59[b]
DKW	2.67[b]	4.72[a]	2.45[b]	5.33[a]	1.03[a]	0.12[a]	82.56[a]

Different letters indicate significant differences between groups according to Kruskal-Wallis test ($p \leq 0.05$); [a] Numeric scale from 0 to 5, where 0 = no damage, plants have no necrotic tissue and 5 = dead plants, it is not possible to obtain new explants from them; [b] Average, n=60; [c] Average, n=10.

Discussion

The occurrence of necrotic damage during plant *in vitro* multiplication is commonly associated with nutrient deficits, especially calcium and boron, but it is actually the result of a complex set of factors. Vitamin and plant regulator deficiency, mineral toxicity, ethylene, the age of the explants, hyperhydricity, and agar concentrations have been described as possible causes of necrotic injuries. Even continuous subcultures could produce STN (Thakur and Kanwar, 2011). The increase of cytokinin concentration in the growth medium has been also reported as one of the causes of necrotic injuries occurrence in different species (Ruffoni and Savona, 2013; Surakshitha et al., 2019; Park et al., 2020). This is the case of *A. radiata* (Morales, 2019).

Calcium supplementation it has been reported to avoid STN during plant micropropagation. Ca^{+2}, can activate enzymes which reduce the accumulation of polyphenolic compound in the plant tissues, decreasing the occurrence of STN (Wen et al., 2016; Silva et al., 2020; Thakur et al., 2021). In the case of the first experiment, the addition of calcium nitrate produced a worse response than the control medium. This may be an effect of the increase in nitrate levels, which could be toxic for *A. radiata*. Moreover, in many cases, the problem might be related to nutrient absorption and translocation rather than a deficit. There is a limit to the amount of calcium to add to the medium, beyond which a toxicity problem could develop due to the overaccumulation of calcium because of its limited absorption. The intake of calcium by plantlets is preferably located in their basal and middle areas, while apices accumulate less calcium, producing damage to this tissue (Machado et al., 2014). In our case, the supplementation with calcium nitrate showed a negative response of all experiments, generating a high level of shoot tip necrosis. It is likely that *A. radiata* plantlets are more sensitive to nitrate, which could produce a toxic effect on them.

Related to the second experiment, agar essays did not produce significant differences in necrotic damage. Despite this, 8 g L^{-1} showed a mild reduction in the STN, making it a good option to be used as the standard agar concentration for *A. radiata* micropropagation. Additionally, 10 g L^{-1} of agar induced an increase in the height of the plants. An increment in the agar concentration limits the movement of medium components, including growth regulators (Avestan et al., 2017). This could lead to lower absorption of BAP, increasing internal levels of auxins, and leading to more apical dominance and an increase in plant height. This is a similar effect to that produced by the application of exogenous auxins.

Regard the third experiment, IBA treatments significantly reduced the necrotic damage to the plantlets, but the shoot regeneration was also reduced. This is an expected result because IBA supplementation induces apical dominance and reduces lateral shoot emission (Kaviani and Negahdar, 2017). Despite this, 0.01 mg L^{-1} of IBA produced a higher multiplication rate than that observed with the control medium. The decrease in necrotic damage induced by the IBA supplementation compensated for the reduction in the number of shoots and allowed the plants to obtain a better multiplication rate. Less necrotic damage translates to healthier tissue available to be used as explants. The rest of the IBA treatments did not improve the multiplication rate. The positive effect of auxins supplementation over plantlets regeneration has been reported to different species (Hesami et al., 2017; Sofian et al., 2018).

In the case of the fourth experiment, WPM basal medium produced the best response on *A. radiata*, drastically reducing the necrotic damage to the tissues and improving the multiplication rate. This medium has been successfully used on many species, generally woody plants, but some herbaceous and semi-woody species have also been successfully micropropagated in this medium (Kang et al., 2018; Restrepo-Osorio et al., 2020).

Necrosis reduction could be explained by the amounts and sources of essential elements that are contributed by the WPM basal medium. MS and WPM mediums have almost the same content of calcium, around 3 mM in WPM, but MS supplies a higher amount of nitrogen, which also has been reported as one of the causes of STN (Pêgo et al., 2014). MS medium has four times more of NH^{+4} (20.61 vs 5.00 mM), and NO_3^- (39.41 vs 9.71 mM) (Silva et al., 2020). Another explanation for the different results between the mediums is the major concentration of chloride ions supplied by MS medium (5.98 mM vs 1.3 mM). That extra amount of chlorine could be toxic for *A. radiata*.

In general, basal media for woody plant propagation have higher concentrations of magnesium, iron, and manganese, which could be necessary for the correct development of microplants of *A. radiata*. WPM contributes higher amounts of sulfate than MS medium, which could be another explanation for the best result shown by the plants cultivated in WPM basal medium.

DKW medium did not show a better result than the control medium (MS). As mentioned previously, calcium deficit is one of the most common causes of STN in plant tissue culture (Thakur et al., 2021). For that reason, the use of DKW medium, which provides higher amounts of calcium than MS basal medium, could have been a good alternative to reduce necrotic damage. Despite this, the incidence of STN was not significantly different between both mediums.

Regard to vitamin concentrations, MS and WPM mediums show the same concentrations of glycine, mio-Inositol, nicotinic acid, pyridoxine, and thiamine, while DKW has the double of nicotinic acid and thiamine, and it has not pyridoxine in its formulation. These differences could be also played a role in the observed responses.

Regarding fresh weight, dry weight, and water content (%), no significant differences were observed between the control and the experiment. It could be that the number of plants measured during the trials (n=10) was not enough to detect the effects of the medium changes on the biomass accumulation of the plantlets.

Conclusions

The use of WPM as a basal medium showed the best results, achieving a significant decrease in the occurrence of STN and an increase in the multiplication rate. IBA supplementation also achieved a significant reduction in apical necrosis. On the other hand, calcium nitrate supplementation and increased agar concentration did not produce a reduction in the necrosis injuries. The obtained results allow suggesting a protocol for the future commercial propagation of *A. radiata*, which would be WPM basal medium, including its vitamins, supplemented with 0.01 mg L^{-1} of IBA, 0.5 mg L^{-1} of BAP, 8 g L^{-1} of agar, and 30 g L^{-1} of sucrose, at a pH of 5.8. Despite this, it is necessary to continue the tests to see the effect of new modifications in the culture medium over *A. radiata* micropropagation and its later acclimatization.

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Author Contribution

PMT: He developed the experimental work, data analysis, and supported the writing of the article. **MG:** She helped with the experimental design of the trials, analysis of results and writing of the manuscript.

References

AL-AIZARI, A.A.; AL-OBEED, R.S.; MOHAMED, M.A.H. Improving micropropagation of some grape cultivars via boron, calcium and phosphate. **Electronic Journal of Biotechnology**, v.48, p.95-100, 2020. https://doi.org/10.1016/j.ejbt.2020.10.001

AVESTAN, S.; NASERI, L.; BARKER, A.V. Evaluation of nanosilicon dioxide and chitosan on tissue culture of apple under agar-induced osmotic stress. **Journal of Plant Nutrition**, v.40, n.20, p.2797-2807, 2017. https://doi.org/10.1080/01904167.2017.1382526

BAIRU, M.W.; KANE, M.E. Physiological and developmental problems encountered by *in vitro* cultured plants. **Plant Growth Regulation**, v.63, n.2, p.101-103, 2011. https://doi.org/10.1007/s10725-011-9565-2

CAREVIC, F. El desierto florido: alternativas para su aprovechamiento sustentable. **Idesia**, v.34, p.3-6, 2016. https://doi.org/10.4067/S0718-34292016000100001

CHEONG, E.J.; NA, M.; JEONG, U. The effect of endophytic bacteria on *in vitro* shoot growth of *Prunus yedoensis* and its identification and elimination. **In Vitro Cellular and Developmental Biology-Plant**, v.56, p.200-206, 2020. https://doi.org/10.1007/s11627-019-10018-2

DRIVER, J.A.; KUNIYUKI, A.H. *In vitro* propagation of Paradox Walnut root stock. **HortScience**, v.19, n.4, p.507-509, 1984.

HESAMI, M.; DANESHVAR, M.H.; LOTFI, A. *In vitro* shoot proliferation through cotyledonary node and shoot tip explants of *Ficus religiosa* L. **Plant Tissue Culture and Biotechnology**, v.27, n.1, p.85-8, 2017. https://doi.org/10.3329/ptcb.v27i1.35017

KANG, Y.; LEE, K.; CHOI, J.; KOMAKECH, R.; MIN, J.; JU, S.; KIM, S.W.; YOUN, C.; KIM, Y.G.; MOON, B.C. Maximizing seedling and root tuber production in *Polygonum multiflorum* for use in ethnomedicine. **South African Journal of Botany**, v.119, p.119-131, 2018. https://doi.org/10.1016/j.sajb.2018.08.016

KAVIANI, B.; NEGAHDAR, N. Propagation, micropropagation and cryopreservation of *Buxus hyrcana* Pojark., an endangered ornamental shrub. **South African Journal of Botany**, v.111, p.326-335, 2017. https://doi.org/10.1016/j.sajb.2017.04.004

LLOYD, G.; MCCOWN, B. Commercially feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot tip culture. **Combined Proceedings, International Plant Propagators' Society**, v.30, 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, n.5, p. 636–643, 2014. http://dx.doi.org/10.1590/S1516-8913201402165

MORALES-TAPIA, P.; CABRERA-BARJAS, G.; GIORDANO, A. Polyphenolic distribution in organs of *Argylia radiata*, an extremophile plant from Chilean Atacama Desert. **Natural Product Research**, v.35, n.21, p. 4143-4147, 2020. https://doi.org/10.1080/14786419.2020.1739678

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

OSABE, K.; KAWANABE, T.; SASAKI, T.; ISHIKAWA, R.; OKAZAKI, K.; DENNIS, E.S.; KAZAMA, T.; FUJIMOTO, R. Multiple mechanisms and challenges for the application of allopolyploidy in plants. **International Journal of Molecular Sciences**, v.13, n7, p.8696-8721, 2012. https://doi.org/10.3390/ijms13078696

PARK, K.; JANG, B.K.; LEE, H.M.; CHO, J.S.; LEE, C.H. An efficient method for *in vitro* shoot-tip culture and sporophyte production using *Selaginella martensii* spring sporophyte. **Plants**, v.9, n.2, p.235, 2020. https://doi.org/10.3390/plants9020235

PÊGO, R.G..; PAIVA, P.D.O.; PAIVA, R. Micropropagation protocol for *Syngonanthus elegans* (Bong.) Ruhland: an ornamental species. **Acta Scientiarum Agronomy**, v.36, n.3, p.347-353, 2014. https://doi.org/10.4025/actasciagron.v36i3.17946

RESTREPO-OSORIO, C.; GIL-CORREAL, A.; CHAMORRO-GUTIÉRREZ, L.; RAMÍREZ-RÍOS, V.; ÁLVAREZ, J.C.; VILLANUEVA-MEJÍA, D. Efficient direct shoot organogenesis and genetic stability in micropropagated sacha inchi (*Plukenetia volubilis* L.). **BMC Research Notes**, v.13, n.1, p.1-7, 2020. https://doi.org/10.1186/s13104-020-05257-1

RUFFONI, B.; SAVONA, M. Physiological and biochemical analysis of growth abnormalities associated with plant tissue culture. **Horticulture Environment and Biotechnology**, v.54, n.3, p.191-205, 2013. https://doi.org/10.1007/s13580-013-0009-y

SILVA, J.A.T.; NEZAMI-ALANAGH, E.; BARREAL, M.E.; KHER, M.M.; WICAKSONO, A.; GULYÁS, A.; HIDVÉGI, N.; MAGYAR-TÁBORI, K.; MENDLER-DRIENYOVSZKI, N.; MÁRTON, L.; LANDÍN, M.; GALLEGO, P.P.; DRIVER, J.A.; DOBRÁNSZKI, J. Shoot tip necrosis of *in vitro* plant cultures: a reappraisal of possible causes and solutions. **Planta**, v.252, n.47, p.1-35, 2020. https://doi.org/10.1007/s00425-020-03449-4

SOFIAN, A.A.; PRIHASTANTI, E.; 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, n.2, p. 379387, 2018. https://doi.org/10.15294/biosaintifika.v10i2.14977

SURAKSHITHA, N.C.; SOORIANATHASUNDARAM, K.; GANGA, M.; RAVEENDRAN, M. Alleviating shoot tip necrosis during *in vitro* propagation of grape cv. Red Globe. **Scientia Horticulturae**, v.248, p.118-125, 2019. https://doi.org/10.1016/j.scienta.2019.01.013

THAKUR, A.; KANWAR, J.S. Effect of phase of medium, growth regulators and nutrient supplementations on in vitro shoot-tip necrosis in pear. New Zealand. **Journal of Crop and Horticultural Science**, v.39, n.2, p.131-140, 2011. https://doi.org/10.1080/01140671.2011.559254

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, n.2, p.238–246, 2021. https://doi.org/10.1007/s40502-021-00580-6

WEN, S.S.; CHENG, F.Y.; ZHONG, Y.; WANG, X.; LI, L.Z.; ZHANG, Y.X.; QIU, J.M. Efficient protocols for the micropropagation of tree peony (*Paeonia suffruticosa* "Jin Pao Hong", *P. suffruticosa* "Wu Long Peng Sheng", and *P.* ×l*emoinei* 'High Noon') and application of arbuscular mycorrhizal fungi to improve plantlet establishment. **Scientia Horticulturae**, v.201, p.10-17, 2016. https://doi.org/10.1016/j.scienta.2016.01.022