ARAUCARIA EXCELSA L. VITROCULTURES INITIATION

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Abstract: Araucaria excelsa L. is a well-known conifer, mostly used as an indoor ornamental plant. For the initiation of Araucaria excelsa L. vitrocultures, we have studied the reactions of explants, in the presence of different growth regulators, added in the aseptic nutritive media. We have prelevated apexes from a unique plant and used them as biological material. The explants were sterilized and inoculated on BM media, with and without growth regulators. This experiment, which lasted for 90 days, has brought forth the following conclusions: On V0 (control variant—BM without growth regulators), the inoculs have presented a very week regenerative capacity; the best medium for elongation of Araucaria excelsa L. was V2 (BM with 2 mg/l BA + 2 mg/l NAA); the most ramifications and buds can be obtained if using V4 experimental variant (BM with 0.5 mg/l NAA + 0.5 mg/l KIN); at any experimental variant the rhysogenesis wasn’t observed.

Keywords: Araucaria excelsa L., vitroculture, growth regulators, conifer

Introduction

Araucaria excelsa L. is a beautiful conifer from Canar and Mader Irelands, and commonly cultured to decorate different indoor and outdoor places. The multiplication of this tree is problematical [4]; therefore the “in vitro” micropropagation remains a good alternative to be studied.

The purpose followed on this experiment was the Araucaria excelsa L. vitroculture initiation and its evolution during 90 days observation.

Material and methods

For this research we have collected 2 cm length apexes from a 2 m height adult Araucaria excelsa L. plant, which is founded in the glasshouse of University of Oradea. The apexes were prelevated from basal zone of crown and sterilized in 96° alcohol for 1 minute submersion, followed by a Natrium Hypochloride 0.8%, for 15 minutes resubmersion. After these were done, the biological material was washed, more times, in sterile water [1].

In aseptic environment, the resulted pieces were shorted at 1 cm length and so the inoculs were obtained. They were inoculated (fig.1) on 5 different variants of nutritive media. The control experimental variant have consisted in Araucaria excelsa L. apexes, placed on Murashige and Skoeg (1962) nutritive standard medium [3], abbreviated here BM (basic medium). The other variants have contained, in addition, different growth regulators.

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The Murashige & Skoog (1962) [4] mineral medium, which have consisted in macroelements, FeEDTA, Heller microelements, vitamins (B6, B1 and PP), m-inositol, sucrose and agar, was used as basic medium (BM). In this mixture, growth regulators were added, as following:

- \( V_0 \) – (control variant) – BM without growth regulators;
- \( V_1 \) – BM with 2 mg/l BA + 2.5 mg/l IBA;
- \( V_2 \) – BM with 2 mg/l BA + 2 mg/l NAA;
- \( V_3 \) – BM with 0.5 mg/l KIN + 2.5 mg/l IBA;
- \( V_4 \) – BM with 0.5 mg/l NAA + 0.5 mg/l KIN;

The growth media were sterilized at 121°C, during 30 minutes [2]. After their cooling, in the sterile room, we proceeded to inoculate the minicuttings, one piece per culture recipient, and place them on shelves, at 20-22°C, under fluorescent white light, at 1700 lux, with a 16h light/24h photoperiod.

**Results and discussions**

The *Araucaria excelsa* L. vitroplantlets evolution has been observed during 90 days, and the watched parameters were noted and compared.

At 30 days after inoculation, the *Araucaria excelsa* L. vitroplantlets have presented stagnation, their height modification being mostly insignificant (fig.2, fig.4). The highest elongation was founded on \( V_2 \) medium (BM with 2 mg/l BA + 2 mg/l NAA). No buds or ramifications were observed. The rhysogenesis was missing, too.

We didn’t find any infection on cultures, but some necroses have occurred in a few growth recipients, at all experimental variant, excepting \( V_3 \) (BM with 0.5 mg/l KIN + 2.5 mg/l IBA), where all plantlets have survived. The lowest survival level (84.61%) was observed on \( V_0 \) (control variant), where the growth regulators were missing (fig.3).

The general survival percents were pretty good, the best being founded on \( V_3 \) (BM with 0.5 mg/l KIN + 2.5 mg/l IBA) (fig.3).
Fig. 2 *Araucaria excelsa* L. cultures after 30 days from inoculation (V₀ (control variant) – BM without growth regulators, V₁ – BM with 2 mg/l BA + 2.5 mg/l IBA, V₂ – BM with 2 mg/l BA + 2 mg/l NAA, V₃ – BM with 0.5 mg/l KIN + 2.5 mg/l IBA, V₄ – BM with 0.5 mg/l NAA + 0.5 mg/l KIN)

Fig. 3. The survival percent of *Araucaria excelsa* L. plantlets, at 30 days after inoculation
At 60 days after inoculation the best elongation was observed also on V_2 medium (BM with 2 mg/l BA + 2 mg/l NAA) (fig.5, fig.6), but the ramifications were more on V_4 medium (BM with 0.5 mg/l NAA + 0.5 mg/l KIN), (fig.5, fig.7).

Fig. 5. *Araucaria excelsa* L. cultures after 60 days from inoculation (V_0 (control variant) – BM without growth regulators, V_1 – BM with 2 mg/l BA + 2.5 mg/l IBA, V_2 – BM with 2 mg/l BA + 2 mg/l NAA, V_3 – BM with 0.5 mg/l KIN + 2.5 mg/l IBA, V_4 – BM with 0.5 mg/l NAA + 0.5 mg/l KIN)
Fig. 6. The vitroplantlets elongation at 60 days after inoculation on aseptic media.

Fig. 7. The vitroplantlets stalk ramification at 60 days from inoculation.

The 90th day of this experiment has revealed us that in the V2 medium (BM with 2 mg/l BA + 2 mg/l NAA) the vitroplantlets were 30% taller than those from the control variant (BM without growth regulators) (fig.8, fig.9). The most ramifications were found again at V4 experimental variant (BM with 0.5 mg/l NAA + 0.5 mg/l KIN) (fig.8, fig.10).

The control experimental variant V0 (BM without growth regulators) hasn’t manifested any ramification (fig.8, fig.10).

No one of experimental variants has manifested rhysogenesis.
Fig. 8. *Araucaria excelsa* L. cultures after 90 days from inoculation (V₀ (control variant) – BM without growth regulators, V₁ – BM with 2 mg/l BA + 2.5 mg/l IBA, V₂ – BM with 2 mg/l BA + 2 mg/l NAA, V₃ – BM with 0.5 mg/l KIN + 2.5 mg/l IBA, V₄ – BM with 0.5 mg/l NAA + 0.5 mg/l KIN)

Fig. 9. The vitroplantlets elongation at 90 days after inoculation on aseptic media
According to this research, the initiation of Araucaria excelsa L. viticulture is possible, and this is a useful tool for micropropagation of this ornamental conifer specie.

On V₀ (control variant– BM without growth regulators), the inoculs have presented a very week regenerative capacity.

No ramifications were observed on standard MS medium (V₀).

The best medium for elongation of Araucaria excelsa L. was V₂ (BM with 2 mg/l BA + 2 mg/l NAA).

The most ramifications and buds can be obtained if using V₄ experimental variant (BM with 0.5 mg/l NAA + 0.5 mg/l KIN).

90 days seems to be a to short vitroculture period, for rhysogenesis occurrence.

These positive results stimulate us to go forward with the experiments concerning Araucaria excelsa L. “in vitro” micropropagation.

**Abreviations:** MS – Murashige&Skoog (1962), BM – basic medium, BA – benzyladenine; IBA – β–indolilbutilic acid; NAA – α-naftilacetic acid; KIN – kinetin

**REFERENCES**


