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# *THE INITIATION OF LOTUS (Nelumbo nucifera) VITROCULTURE*

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## INTRODUCTION

The *"in vitro"* micropropagation of the plants from the spontaneous flora, presenting a lot of advantages. The mentioned technique offer the possibility to obtain an amazing samples number, same individuals or approximately identical explants derived from the mother plant, which stay to the base of the *"in vitro"* cultures initiation. On the other hand, these vitrocultures can be stored and preserved easily in restricted spaces with minimum efforts, which can serve to repopulation with species of some areas, where the vegetation was compromised because of an ecological accident.

This technique represent the unique modality of the plants multiplication which cannot be multiplied on sexual way (from seeds), finally of this limiting possibilities of these to form seeds, most from these species can be aseptic, through the impossibility to reach the complete maturity stage because of climatic conditions. In the case of the species with the lower regenerative capacity and germination process, the vitrocultures of different embrionar fragments can serve to the cloning of respective plants, similarly, exist species which have embryos coming in a very long rest period (latency), making impossible the germination. To these species, the initiation of some organs culture it was difficult, because the plant secrete phenolic substances, which oxide some components of the medium culture, preventing the development of vitrocultures, finally, leading to necrosis. In this situation, the adding of some antioxidant substances in the medium culture can prevent the apparition of the mentioned phenomenon (*ZIV M.1991).*

This paper work have the purpose, beside the establishment of a vitroculture of *Nelumbo nucifera*, the finding of an feasible method of initiation of an aquatic species vitroculture, with an fruition perspective in the future of an aseptic culture of an endemic species of *Nympheea lotus termalis*, protected b law, as a natural monument, from the Peţea lack and Băile 1 Mai from Oradea.

**Lotus (*Nelumbo nucifera).* Botanical descriptions**

The *LOTUS (Nelumbo nucifera)* derive from *Magnoliophyta*, *Magnoliopsida* class, *Magnoliidae* subclass, *Nymphaeales* ord., *Nymphaeaceae* fam.

Is an aquatic plant, originally from India and Indonesia, can reach 50 cm height, her waist depend from the bog deepness. The radicular system of lotus is steady fixed in the mud from the water deepness, the plant has a long petiole which on this are attached the leaves and the flowers. The leaves are big, circular, which growing up to the water surface. The flowers are pink, long petiolated and reach almost 1 m in height above the water glisten. These flowers will open in the early morning and can exceed the 20 cm in diameter. The fruit is a poricide conic capsule, with some incorporated seeds in some orifices. (figure 1***)***.

***Fig. 1*** – Imagines with Lotus plants in the nature

The Lotus flowering in the whole year.

The fruits, the seeds, young leaves, sometimes 2 m in diameter and the rhizomes of this plant are eatable.

In the Asian kitchen, the petals are used in soups, or as garnish. The seeds can be eating as toasted seeds, and the rhizomes can be boiled.

Parts from lotus are used as well in the traditional medicine, because have benefic effects against diarrhea, hemorrhoids treatments, and the seeds are used against fever and insomnia.

The flowers are used in the treatment of syphilis, and the capsules have a hemostatic role. The sepals in wine or in tea have painkiller effects.

## MATERIAL AND METHOD

For the initiation of the *Lotus* vitroculture, we had used as explants derived from petiole. Limb and from immature seeds of *Lotus* derived from the thermal water basin from Baile Felix Station. The vegetal material was transported in the laboratory; it was washed with tap water for 10 minutes, than from petioles and from the central portion of the limb was dimensioned slices with 2 cm diameter, which were submersed in 70°C ethilic alcohol, for 1 minute. Rinsing the vegetal material in sterile water, in abundance made the obliteration of the alcohol scent. For the normal sterilization of the vegetal material was proceeded for 8-10 minutes in Clorox 100%, using 3-4 drops of Tween, after this operation was rinsed for 5 minutes, many times with sterile warm water. At the final of this operation, the vegetal material was placed on filtered aseptic paper, in sterile Petri dishes.

In aseptically conditions, in the functioning box with laminar sterile air flux, it has been proceed the detach from the level of sterilized vegetal material of the necrosis parts and the sectioning of the futures clones.

The basic medium culture (BM), it was consist from. Macroelements and FeEDTA Murashige-Skoog (1962), microelements Heller, a mineral mix to this was added the vitamins: pyridoxine HCl, thiamin HCl and nicotinic acid (1 mg/l each), mezzo– inositol - 100 mg/l, sucrose - 20 g/l, with or without agar -10 g/l, the pH of the medium was adjusted to 5.7, for autoclaving.

To this basic medium have been added phytohormones, consisting the following experimental variants:

- $V_0$ – BM witness lot (control) – without growth regulators,
- $V_1$ – *agarised* BM, by adding 1ml/l K and 1ml/l NAA;
- $V_2$ – *liquid* MB, by adding1ml/l BA and 1ml/l NAA. where:
  - K – kinetin;
  - NAA –α–naphtilacetic acid;
  - BA – bensiladenine;
  - IAA –ß indolilacetic acid.

So, the initiation of the *Nelumbo nucifera* vitrocultures were made in solid and liquid medium cultures too.

The sterilization of these medium cultures, being proportioned in these flasks, was autoclaved at 121ºC, for 30 minute. After cooling of this medium culture, it has been proceed to the inoculation of the explants in the sterile room, in the horizontal box with laminar sterile air flux.

After inoculation, the recipients with explants (covered with colorless and transparent polyetilen, the flasks were locked up with rubber band) and the were placed on shifts, at 20-24ºC temperature, to 16 hours light / 24h at 1700 lucks intensity light, in white light emitted by fluorescent tubes.

After the vegetal material sterilization, respectively of those petiole and limb fragments, these were fragmented in 7-10mm diameter portions and were inoculated on culture medium, being in contact with the medium culture surface. In the case of the seeds was proceed to the remove of the external layer and those were fragmented in two pieces, in longitudinal position, followed by those inoculation on the above mentioned variants ($V_0$, $V_1$, $V_2$).

The fragmented seeds were inoculated, in such as the embryo was in direct contact with solid medium culture ($V_0$ and $V_1$) by submersing in the case of the liquid medium culture ($V_2$).

Weekly was made observation to the level of the regenerated vitrocultures from the various types of explants and from the inoculated seeds on the aseptic medium, following those evolutions, was determined: the length of the regenerated plantlets from minicuttings, risogenesis, the length of stems, and that petiole, and also the number of leaves and their dimension.

The values were calculated mathematic and the resulted values were related to the registered results to the witness lot ($V_0$) considered as reference values 100%, the medium cultures being without growth hormones.

## RESULTS AND DISCUSSION

From the made observation in the evolution process of those vitrocultures, it was consisted that in the all case of the variants, the regeneration and the growth was unequal, reflecting a difference of the clones reactivity in the types of the growth hormones present in the medium culture.

We had also got some negative results, observing that the majority of the petiole and limb type clones were necrotic.

In figure 2 are represented graphically the biometric values media of the *Nelumbo nucifera* vitroplantlets at *1, 2, 3 and 4 weeks*, after the explants inoculation on aseptic medium.

To the realized vitrocultures from fragmented seeds on median line through half pieces dimensioned, after *1 week* (see figure 3) it has been observed that in the both solid and liquid medium culture was resulted clones from the center of the embryos, generating stems between 0, 5-3cm.

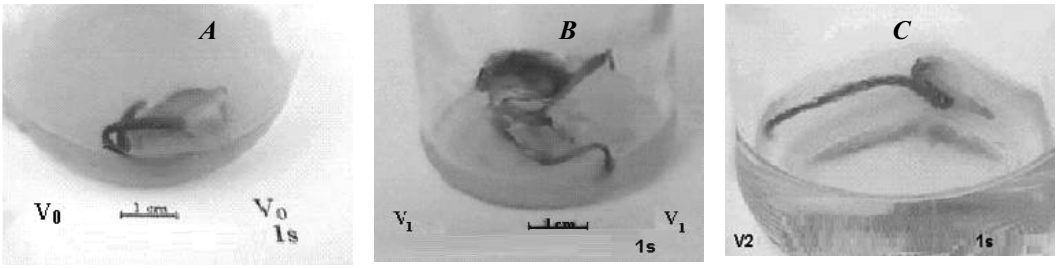


***Fig. 3*** – Vitroplantlets of lotus (Nelumbo *nucifera)* at *1 week*, inoculated on a basic medium culture BM (solid) without growth hormones – witness lot (variant $V_0$ - A), respectively on a basal medium culture BM (solid) by adding 1mg/l K and 1mg/l NAA (variant $V_1$ - B) and a basal medium BM (liquid) by adding 1mg/l BA and 1mg/l IAA (variant $V_2$ - C).

The *lotus* vitroplantlets, in the *first week* (see figure 3A) after the aseptically cultures initiation, the not generated roots at those all variants.

In the *number of the stem, petioles and leaves*, to all variants, the regenerative processes were near to those on the witness lot consisted as 100%. The higher values of the *stems length* was present on the variant $V_2$ (BM b adding 1mg/l BA and 1mg/l IAA), the increases being 133, 33% comparatively to the witness lot. Also, the *root length* of the *Nelumbo nucifera* vitroplantlets the presented maximal values of 433, 33% on the variant $V_2$ (BM (liquid medium) b adding of 1mg/l BA and 1mg/l IAA), and on the variant $V_1$ (BM (solid medium) by adding 1 mg/l K and 1mg/l NAA, it was a decrease of 10% comparatively to the witness lot $V_0$. They have not formed leaves on no experimental variant.

After *2 weeks* (see figure 4) after vitroculture initiation, on the variant $V_1$ (solid BM with 1mg/l K and 1mg/l NAA) it has been observed the apparition of roots of those petioles, and even some leaves too, and on the variant $V_2$ (liquid BM with 1mg/l BA and 1mg/l IAA) it has been observed that from 2 fragmented embryos it has developed a stem with 1-3cm length.

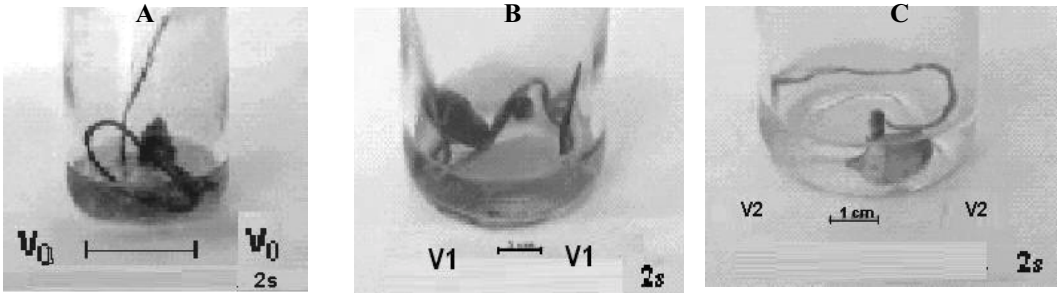


***Fig. 4*** – Vitroplantlets of lotus (*Nelumbo nucifera) at 2 weeks*, inoculated on a basal medium BM (solid medium) without growth hormones - witness lot (variant $V_0$ - A), respectively on basal medium BM (solid medium) by adding 1mg/l K and 1mg/l NAA (variant $V_1$ –B) and basal medium BM (liquid medium) by adding 1mg/l BA and 1mg/l IAA (variant $V_2$ – C)

At *2 weeks* after the inoculation of the *Lotus* vitroplantlets (see figure 4B) on the variant $V_1$ (solid medium BM with 1mg/l K and 1mg/l NAA) the explants presented a high risogenesis process then the other experimental variants, an increase of 300% to the witness lot.

The *length of the roots* have reached equal values with the witness lot to those vitroplantlets on the variant $V_1$ (BM solid with 1mg/l K and 1mg/l NAA), and on the variant $V_2$ (BM liquid with 1mg/l BA and 1mg/l IAA) had presented a decrease of 70%. Also, neither at *2 weeks* after inoculation, the vitroplantlets had no generated other leaves or petioles, just only those initially presented, only the *stem length* of the vitroplantlets on the variant $V_1$ (BM (solid medium, with 1mg/l K and 1mg/l NAA) had presented a increase of 126,77% and on the variant $V_2$ (BM (liquid medium) with 1mg/l BA and 1mg/l IAA) a increase of only

65% comparatively to the witness lot. The *petioles length* was considerably grown to the vitroplantlets on the variant $V_1$ (BM (solid medium) with 1mg/l K and 1mg/l NAA) with 310%, respectively with 216, 77% on the variant $V_2$ (liquid BM with 1mg/l BA and 1mg/l IAA).

To all variants of culture, the vitroplantlets in the both experimental variants had presented a decrease of 50% comparatively to the witness lot $V_0$.

At *3 weeks* after inoculation (see figure 5), on the variant $V_1$ (BM solid with 1mg/l K and 1mg/l NAA) was observed that the number of roots and also the number of petioles and stems it was increased, the petioles length being higher on the liquid medium culture, on the variant $V_2$ (BM with 1mg/l BA and 1mg/l IAA).

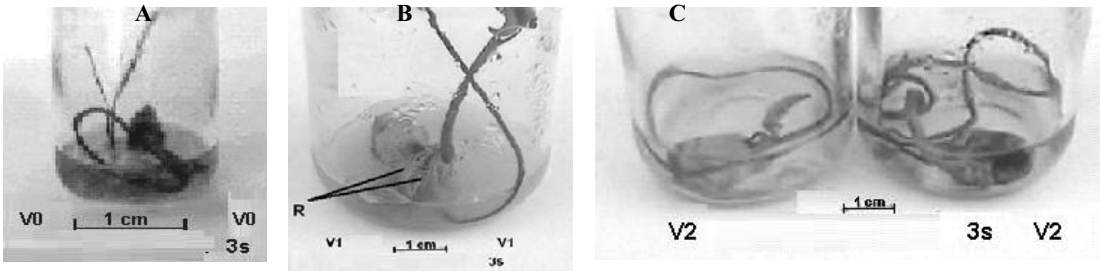


***Fig. 5 –*** *Nelumbo nucifera* vitroplantlets inoculated on solid BM MS without growth hormones - lot witness (variant $V_0$ - A), respectively on solid BM MS by adding 1mg/l K and 1mg/l NAA (variant $V_1$ - B), liquid BM MS by adding of 1mg/l IAA and 1mg/l BA (variant $V_2$ - C).

The risogenesis has registered increases of 250% to the *medium length of the roots* on $V_1$ (BM solid with 1mg/l K and 1mg/l NAA) and 70% to the variant $V_2$ (BM (liquid) with 1mg/l IAA and 1mg/l BA); also on the *number of roots*, this being 100% on the variant $V_1$ (BM solid with 1mg/l K and 1mg/l NAA) and 400% to the variant $V_2$ (BM (liquid) with 1mg/l BA and 1mg/l IAA). Neither of the experimental variants $V_1$ and $V_2$ at *3 weeks*, after inoculation of the *lotus* vitroplantlets, the *stem length* of the vitroplantlets it has registered increases of 100% to the variant $V_1$, respectively a increase of only 50% to the variant $V_2$ comparatively to the witness lot, and they had no generated other stems.

In the case of the *number of petioles and leaves*, to both experimental variants it has been registered increases of 100% to the variant $V_1$, and to the variant $V_2$ it was 200%. The *medium length of petioles* had reached extreme values comparing to those registered vitroplantlets grown on the medium without growth hormones, respectively the witness lot ($V_0$), the most higher values were presented on the variant $V_2$ (liquid BM with 1mg/l IAA and 1mg/l BA) an increase of 1350% and on the variant $V_1$ (solid BM with 1mg/l K and 1mg/l NAA) an increase of 1100%. The *leaf length* had reached higher values of 130% on the variant $V_2$ comparatively to the witness lot $V_0$.

At *4 weeks* after inoculation (see figure 6), the *lotus* vitroplantlets cultured on the $V_1$ variant (solid BM with 1mg/l K and 1mg/l NAA) it has been observed a higher risogenesis then to those *lotus* explants cultured on the variant $V_2$ (liquid medium with 1mg/l BA and 1mg/l IAA). In fact, the *number of petioles and leaves* was increased, the *petioles length* being higher on the liquid medium culture, on variant $V_2$ (medium with 1mg/l BA and 1mg/l IAA).

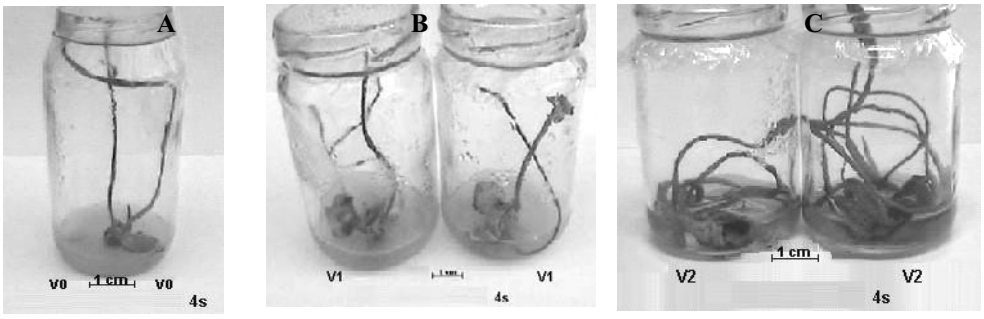


***Fig. 6 –*** Vitroplantlets of *Nelumbo nucifera* at 4 weeks, inoculated on basal (solid) medium MS without growth hormones – witness lot (variant $V_0$ - A), respectively on basal (solid) medium MS by adding 1mg/l K and 1mg/l NAA (variant $V_1$ - B); R - roots, basal (liquid) medium MS by adding 1mg/l IAA and 1mg/l BA (variant $V_2$ - C).

The *number of roots* it was increased on both variants $V_1$ and $V_2$, and on variant $V_1$ (medium with 1mg/l K and 1mg/l NAA) the increases were higher with 1500%, and on variant $V_2$ (liquid BM with 1mg/l BA and 1mg/l IAA) it was registered an increase of 700% comparatively to the witness lot $V_0$, the *length of roots* being the same. They had not generated other stems or petioles, and it was not been observed any ramification on those, also they stagnated.

The *petioles length* it had very increased, presenting an increase of 2400% at variant $V_2$ (liquid BM with 1mg/l BA and 1mg/l IAA) and at the variant $V_1$ (solid BM with 1mg/l K and 1mg/l NAA), the increase being 1700%. With all of this, they had not generated new leaves, only the limb dimensions have reached very huge values, at the variant $V_1$ it was 1600% and on the variant $V_2$ the value was 2500% comparatively to the witness lot.

At the both variants we get very good results to the *length of petioles* (10-25cm) and *of the leaves* (0.5-2cm) to all variants of medium cultures.

## CONCLUSIONS

The clones were consisting from petioles and leaves fragments, on all types of tested medium cultures they were necrotic.

From the clones constituted from half parts of immature seeds, they regenerated in the *first weeks* after inoculation.

The best results in the case of the seeds were obtained on the liquid medium culture MS consisted from BM by adding of 1mg/l BA and 1mg/l IAA (variant $V_2$), starting from the *2 week* after inoculation.

At the end of this experiment, we had deduced that on the solid medium culture MS (variant $V_1$), by adding of 1mg/l BA and 1mg/l IAA, the vitroplantlets had a very good risogenesis comparatively to the other variants of media culture, and on the liquid medium culture MS (variant $V_2$) they had generated a big number of petioles and leaves

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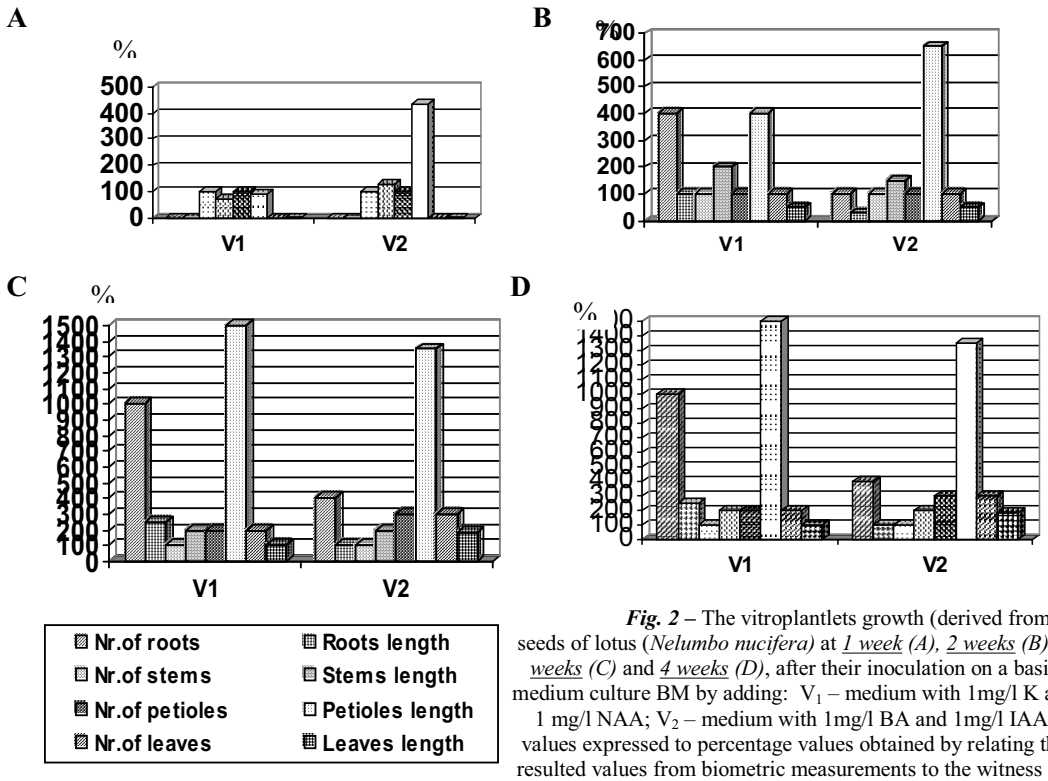


***Fig. 2*** – The vitroplantlets growth (derived from seeds of lotus (*Nelumbo nucifera)* at *1 week (A), 2 weeks (B), 3 weeks (C)* and *4 weeks (D)*, after their inoculation on a basic medium culture BM by adding: $V_1$ – medium with 1mg/l K and 1 mg/l NAA; $V_2$ – medium with 1mg/l BA and 1mg/l IAA, values expressed to percentage values obtained by relating the resulted values from biometric measurements to the witness lot ($V_{0)}$), values considered as 100%.