Results in the *vitro* propagation acclimatization of *Pontederia lanceolata*

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Summary: This paper gives an outline of micropropagation of *Pontederia lanceolata*. *Pontederia l.* is a widely used aquatic plant, therefore there is an increasing demand for them, which can be satisfied only by in vitro culture. Research was carried out to find the best nutrient media conditions for micropropagation and acclimatisation of *Pontederia lanceolata*.

Introduction

The aim of the research was to find the best solution for micropropagation of *Pontederia lanceolata,* it is a perennial, herbaceous aquatic plant, which has been used widely in the last few years. It can be propagated by seeds and by shoots. But these methods are not able to satisfy the demand for this plant. Therefore a new method of propagation is needed, which can be the micropropagation. There aren't available any published references about in vitro culture of *Pontederia lanceolata,* as it is quite a new field of interest. Some scientists in the United States, in England and in Asia are engaged in working out the complete technology for micropropagation of *Pontederia lanceolata.* No results are publicly accessible yet.

Material and methods

Pontederia lanceolata (Pontederia cordata var. lancifolia) a perennial, emergent, aquatic plant is a member of the *Pontederiaceae* family. The genus *Pontederia* comprises five species, which are distributed throughout the tropical, subtropical and temperate regions of America. Pickerelweed is one of the more common aquatic plants in Florida. It is found in all parts of Florida. *Pontederia lanceolata* has a floral tube that is persistently pubescent with glandular hairs. The leaves are narrowly to broadly lanceolate with bases typically unlobed. Its flowers are blue to blue purple. It ranges from South America and the West Indies to Tennessee, including the South Eastern United States. There is expanding market for *Pontederia l.* The following facts point out the importance of *Pontederia l.:* their increased use in a wetland and lake restoration projects as biological filters, and for aesthetic purposes in retention and water garden aquascaping.

Culture media and disinfecting method used

Shoot tips of *Pontederia lanceolata* were used for micropropagation. During the research nutrient media having different components were tried out. A large number of substances and even mixtures of substances were added to the nutrient media.

Basic components of nutrient media

- Macro- and micro salt: The Murashige and Skoog micro- and macro-element mixture was used in half concentration.
- Distilled Water
- Agar: Here 7.5 mg/l Difco Bacto agar was used.
- Sugar: Refined household saccharose was applied 20g/l.
- Adjustment of the pH to 5.7-5.8 was done before autoclaving, adjustments should be carried out after autoclaving, too.
- Regulators: used as follows

Regulators used

- Auxins-IAA was added to the nutrient media at concentration of 0.1 mg/l. It was used to stimulate elongation, division of cells, swelling of tissues, formation of adventitious roots and to inhibit adventitious shoots.
- Cytokinins-Kinetin was used at concentrations of 3-4-5 mg/l and BA at concentrations of 4-5 mg/l. The nutrient media in that we put kinetin were called K3, K4 and K5, the ones in that we put BA were called N4 and N5 *(Table 1).*

Table 1 Regulators used in research.

Regulators used			
Concentration of kinetin	Code of media	Concentration of BAP	Code of media
3 mg/l	K3	4 mg/l	N4
4 mg/l	K4	5 mg/l	N5
5 mg/l	K5		

Vitamins – four different kinds of vitamins were used:

- Inositol, 50 mg/l
- Nicotinic acid, 0.25 mg/l
- Pyridoxin and 0.25 mg/l
- Tyamin. 0.25 mg/l

Other substances used:

- Adeninesulphate – stimulates adventitious shoot formation. 80 mg/l
- Antibiotics – to eliminate the internal infections.
- Quartz sand.

The physical qualities of Nutrient media were gelled media, agitated liquid media and so-called "sanded" media (*Table 2*). Gelled and agitated liquid media are very common used media. With "sanded" media the imitation of the natural conditions of *Pontederia lanceolata* was attempted, soil for quartz sand and water for liquid media was substituted.

Finally, perlit and peat was applied during the acclimatisation.

Table 2 The combination of nutrient media used in the research (Physical qualities and composition).

The combination of nutrient media		
Gelled	Liquid	"Sanded"
K3	K3	K3
K4	K4	K4
K5	K5	K5
N4	N4	N4
N5	N5	

Process of the experiment

Before the sterilisation the small shoot tips were washed and cleaned thoroughly, which are an apical or lateral shoot meristems with a leaf primordia or leaves. After this sterilisation was carried out. The shoot tips were dipped into 70% alcohol for a few seconds to eliminate air bubbles. Then they were sterilised for 10 minutes in 1% NaOCl containing a few drops of TWEEN 20. Finally the shoot tips were dipped for a few seconds into $HgCl_2$. This is exceedingly toxic substance for plants so it was used in a concentrations of 0.1–0.5%, plants were rinsed in distilled water three times.

After all these steps the sterilised shoot tips were inoculated onto the nutrient media consisting malachite-green, which provided to be unsatisfactory. In the following the shoot tips were inoculated onto the nutrient media N4 and N5. These media were tried out with the combination of the three types of physical nutrient media. This was the first part of the experiment.

In the second part the shoot tips were inoculated onto the nutrient media K3, K4 and K5. Combinations with every single physical nutrient media were tried out.

In the final step the acclimatisation took place by allowing the in vitro rooted plants to gradually get used to in vivo conditions. The plantlets were transferred to peat and perlit. During acclimatisation plants were exposed to the same irradiance and temperature conditions, but different water conditions. Half of the plants were placed into 2-3 cm. Deep water and the rest were watered only every other day.

Results

During the research the length and number of shoots and roots were measured. The data were processed with the Tukey-Kramer statistical method.

Effects of the regulators on Pontederia lanceolata

The shoot tips on N4 and N5 nutrient media began to grow, sprout and develop. Among the nutrient media with different regulators content the N4 nutrient media produced far more shoots than N5. In the case of N5 the shoots were higher but the rate of death was higher, too.

The plantlets inoculated from N4 and N5 onto K3, K4 and K5 nutrient media produced shoots and adventitious roots. K4 proved to be the best from these three concentrations.

Effects of the physical quality of nutrient media

Among the three types of nutrient media "sanded" media proved to be the best. In our opinion "sanded" media imitated the natural conditions of *Pontederia lanceolata* very well. (*Figure1–4*)

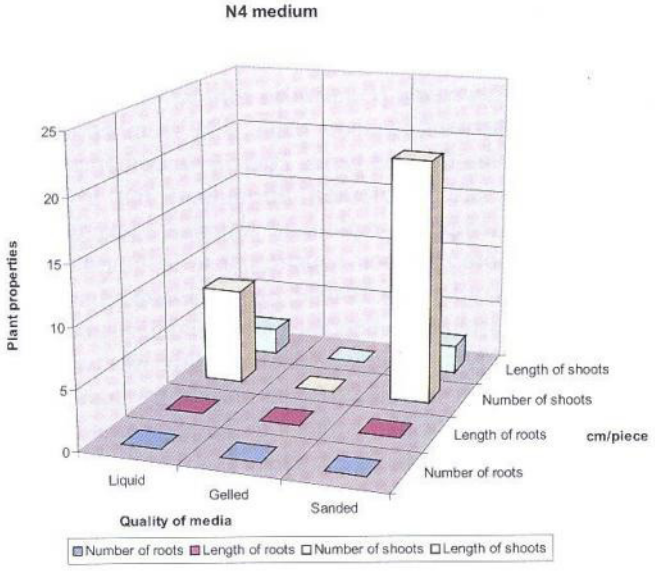

Figure 1 Results achieved on nutrient medium N4

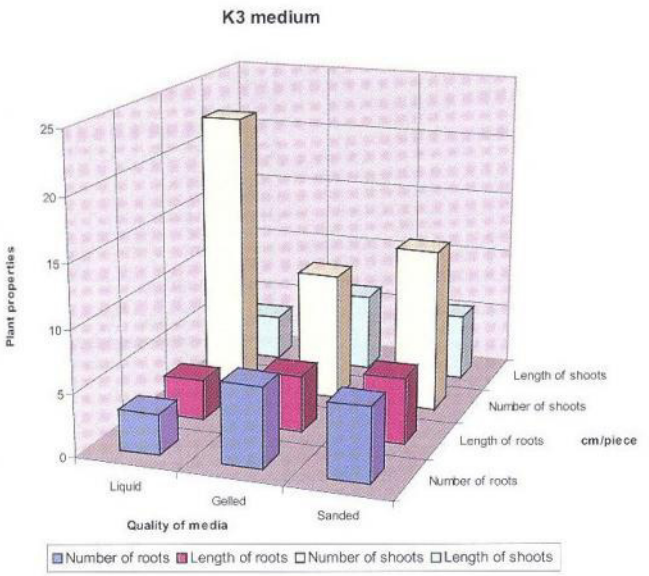

Figure 2 Results achieved on nutrient medium K3

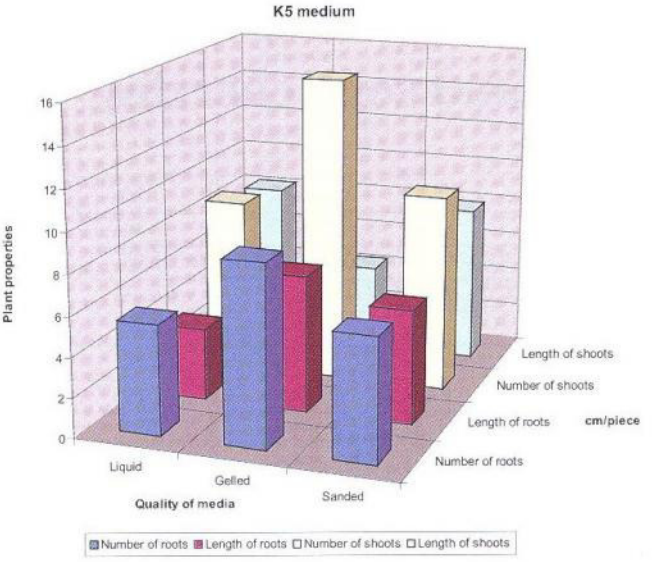

Figure 4 Results achieved on nutrient medium K5

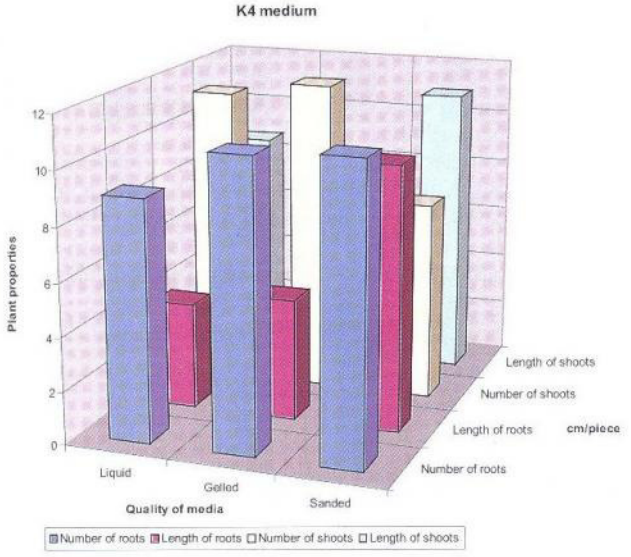

Figure 3 Results achieved on nutrient medium K4

Effects of different media during acclimatization

Peat turned out to be the most satisfactory among the two types of media. On perlit the roots became thinner and they were undeveloped, whereas plants placed into 2-3 cm. depth water grew better and higher, and developed thicker and longer roots. Special attention to the depth of water had to be paid. If the water was too deep the plants were submerged, and drown in absence of oxygen (*Figure 1–4*).

To start a new in vitro culture N4 "sanded" nutrient medium is suitable for the nutrient medium for stage 3 (inoculation and subculturing). Not the length of the shoots, but the number and quality of shoots is of importance and it was observed on N4 nutrient medium. In the case of K4 shoots and roots developed satisfactorily in consequence of the best auxin/cytokinin ration. This K4 "sanded" nutrient medium is suitable for the nutrient medium of stage 4 (Adventitious roots development).

Conclusion

The first steps of the micropropagation of *Pontederia lanceolata* are successfully accomplished. The N4 and K4 nutrient media can be applied for subculturing and rooting the *Pontederia lanceolata*. Unfortunately comparison of the results with other researches could not be carried out, because of the lack of publicly accessible papers or articles dealing with this subject (the reason for this is probably the young state of this field and commercial interests).

Further researches should be done in connection with the concentration of BA or kinetin in nutrient media. Maybe other kind of auxin or cytokinin could be added into nutrient media. An additional point for investigation could be the profitability of the technology.

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