

A grid of glass jars containing plant explants in culture medium. The jars are arranged in rows and columns, with some jars in the foreground showing more detail of the plant tissue and the clear liquid medium. The background is slightly blurred, emphasizing the jars in the foreground.

05_Mikropropagace Množení *in vitro*

Mgr. Hana Cempírková, Ph.D.

Rostlinné explantáty

Definice mikropropagace

- **Micropropagation is the true-to-type propagation of a selected genotype using *in vitro* culture techniques.**
- **Micropropagation is a method to produce genetically identical plantlets by using tissue culture techniques**
- **High yield plant production, grown in a nutrient rich gel under sterile lab conditions.**

[R. Fenwick 2004]

Micropropagation is an advanced vegetative propagation technology for producing a large number of genetically superior and pathogen-free transplants in a limited time and space.

Výhody množení *in vitro*

- malý rozměr řízku
- vysoký množitelský koeficient
- zkrácení množitelského cyklu
- možnost použití netradičních orgánů
- možnost načasování na určitý termín
- dobrý zdravotní stav (ozdravování)

Typy regeneračních procesů

(Němec 1905)

restituce = náhrada odňaté části - meristémy
(meriklonové množení)

reprodukce = regenerace z již existujících základů

regenerace *de novo* = odvozování přes kalus, v něm
diferenciace adventivních pupenů

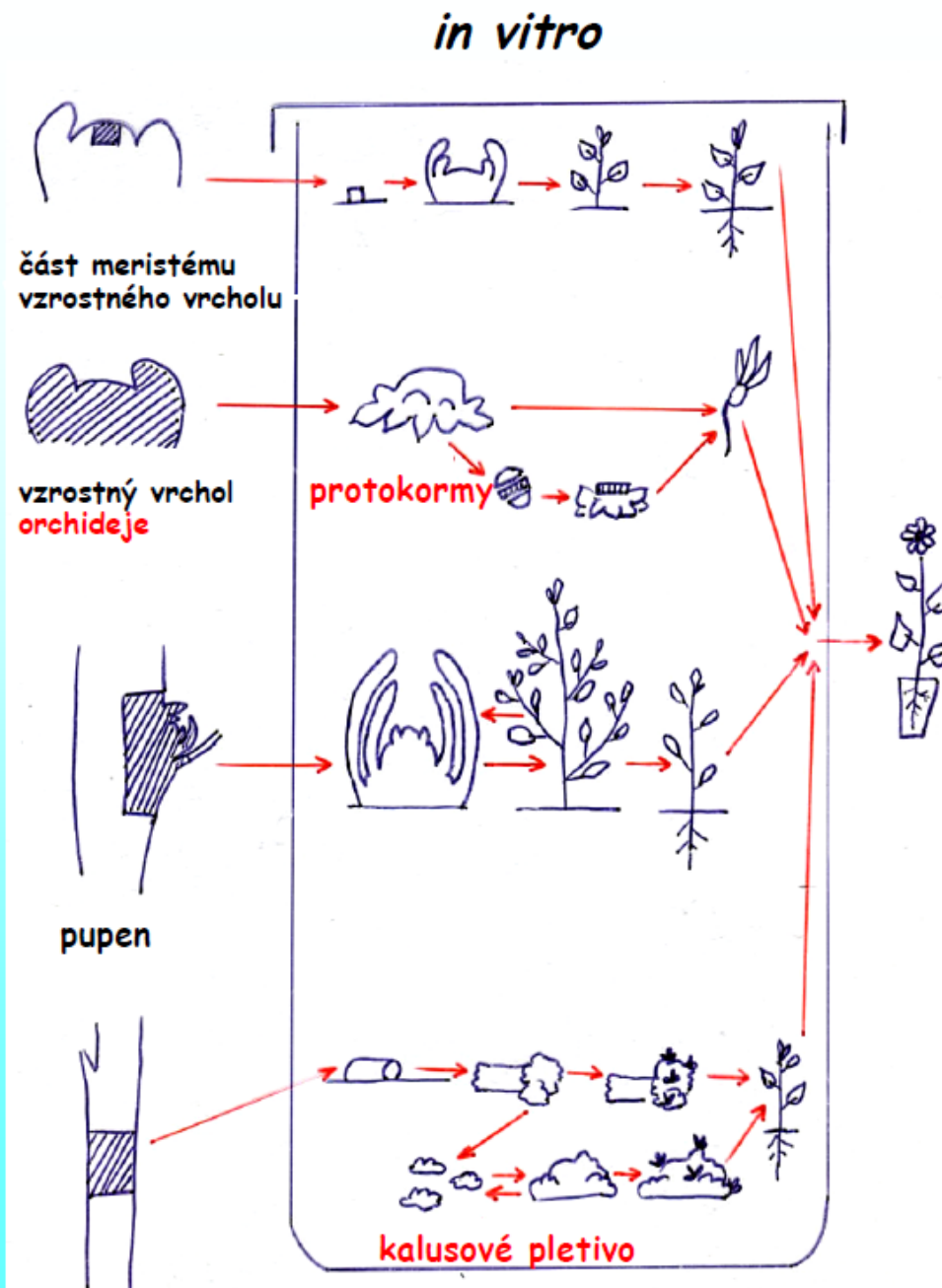
viz schéma: Opatrný (1988)

Opatrný 1988

restituce = náhrada odňaté části - **meristémy** (meriklonové množení)

reprodukce = regenerace z již existujících základů **nodální segmenty**

regenerace de novo = odvozování přes kalus **internodální segmenty, diferencované orgány**



Apikální stonkový meristém

listová primordia

izolovaný „meristém“



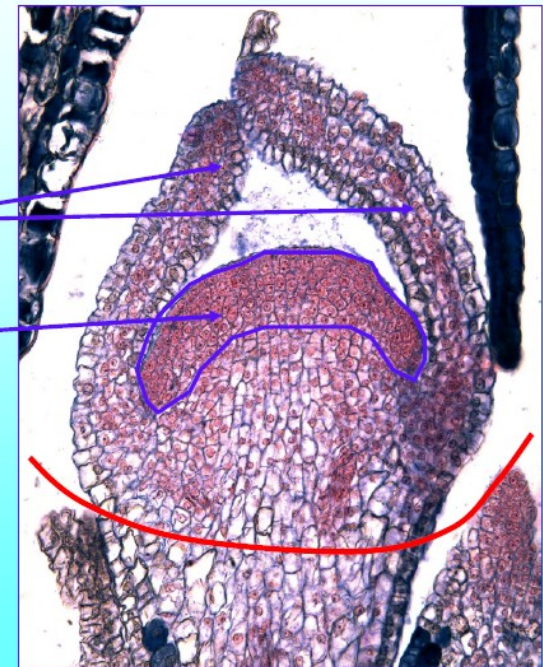
vlastní meristém

Apex stonku *Begonia rex*

listová primordia

apikální meristém

izolovaný „meristém“



Typy mikropropagace

- 1) **meristémové množení** (apikální nebo axilární meristémy)
- 2) **tvorba adventivních prýtů** (z již diferencovaných orgánů, např. listy, internodia, listy cibulových hlíz, květenství, dělohy... - přes kalus nebo přímo)
- 3) **kalusové, suspenzní a protoplastové kultury**
- 4) **somatická embrya a umělá semena**

Stadia mikropropagace *in vitro*

Murashige (1974)

Debergh *et* Maene (1981)

0. příprava explantátu - ovlivnění mateřské rostliny

I. iniciace

II. propagace (množení)

II.a elongace (prodlužování) prýtů

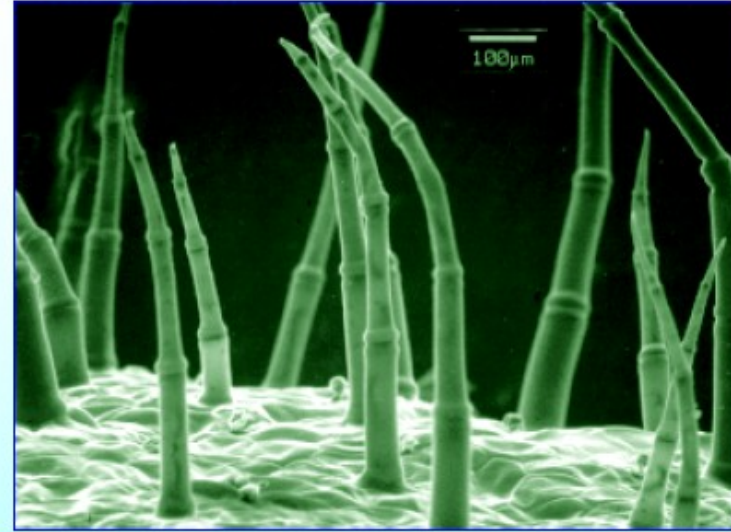
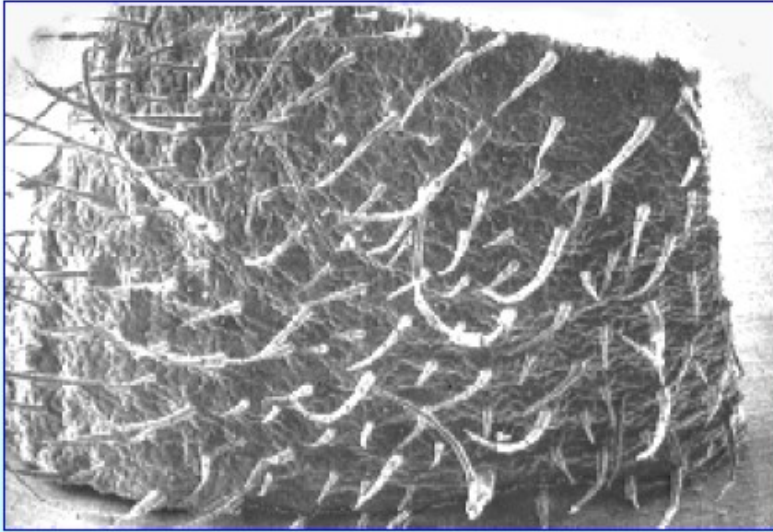
III. zakořeňování

IV. Převádění do nesterilních podmínek *ex vitro*

aklimatizace na nesterilní podmínky (nižší vzdušnou vlhkost, větší kolísání teplot, normální osvětlení)

Stadia mikropropagace *Saintpaulia ionantha* Wendl.

I.



II.



III.



Stage I
Establishment of Explants



Explant Source -
soft wood shoots



Shots are
established
in culture
2-3 months



Cycle 1
Development
of small
clusters of
shoots



Cycle 2
Small clumps
of shoots
increase
2 months



Cycle 3 - n
Normal
increase
2 months

Stage III
Rooting

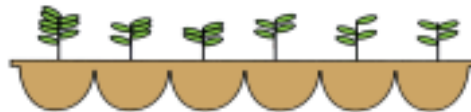


In-vitro
rooted in culture
1 month



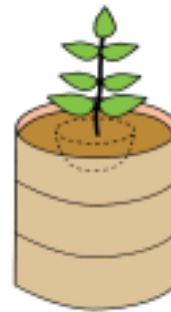
Ex-vitro
rooted directly in soil
1-2 months

Stage IV
Acclimatization



Plants established in individual cells.
3 - 4 weeks under mist

Total growth period in cells = 3 months

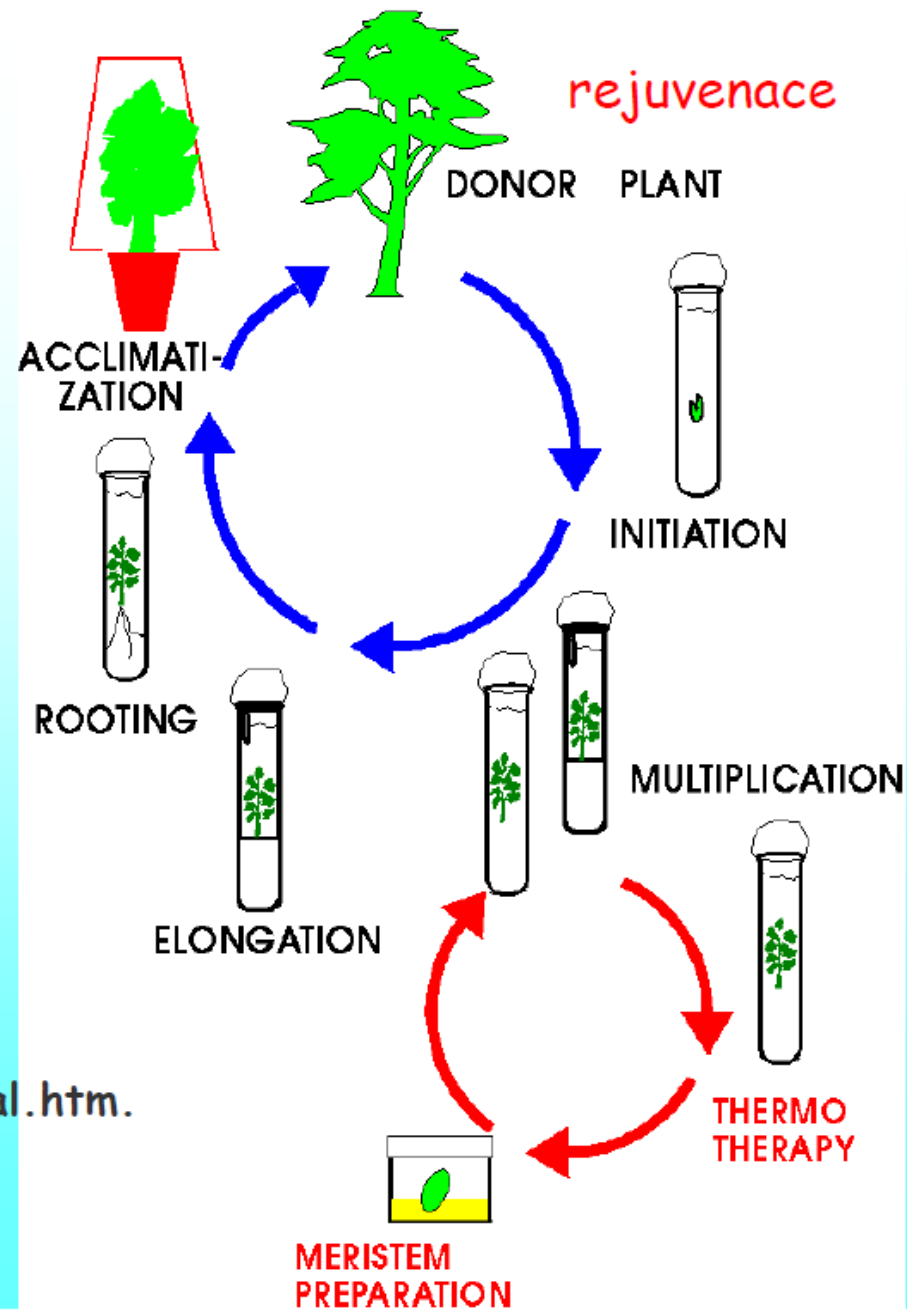


1 gal. cans



Saleable plant

fáze mikropropagace dřevin



www.boku.ac.at/iam/poster/revital.htm

Rejuvenace dřevin

- [Methods Mol Biol.](#) 2013;11013:383-95. doi: 10.1007/978-1-62703-074-8_30.
- **In vitro rejuvenation of woody species.**
- [Read PE¹](#), [Bavougian CM](#).
- [Author information](#)
- **Abstract**
- Juvenility and phase change in woody plant species exert profound impacts on plant morphology and the ability of explants to be successfully propagated in vitro. Morphological characteristics such as leaf shape modifications, thorniness, and the inability to initiate flowers are associated with juvenility. Physiological maturity, that is the ability to reproduce sexually, is reached by many woody species only after many years of juvenile growth. As a result, micropropagation of woody species has historically been difficult with many plant species proving to be exceedingly **recalcitrant**. The importance of juvenility and its impact on successful vegetative reproduction in vitro has therefore received much research attention. In vitro technologies that have been demonstrated to induce **rejuvenation** include meristem culture, chemical treatments, pruning and hedging, forcing new growth, and taking advantage of epicormic buds, grafting and micrografting, and somatic embryogenesis. Applications of these technologies are discussed in this chapter.

Meristémové kultury a termoterapie



Kombinace *in vitro* termoterapie a meristémové kultury - nejúčinnější metoda eliminace virů. Aktivně rostoucí rostlinný materiál je umístěný do termoterapeutické komory.

Expozice 3 týdny nebo déle, fotoperioda 16 h 38°C, 8 h tma 36°C.

Teplota a doba expozice jsou limitovány tolerancí rostliny (závisí na druhu a varietě).

I. stadium: Iniciace mikropropagace

1. výběr materiálu:

zdravotní stav matečných rostlin

ontogenetické stáří (rejuvenilizace)

vliv genotypu

2. desinfekce

3. výběr typu explantátu

4. přítomnost **cytokininu a auxinu** v médiu

II. Stadium mikropropagace: propagace

médium se sníženou koncentrací regulátorů
nebo bez nich, možný přídavek giberelinů

Opakované rozdělování vzniklých prýtů **není**
neomezené: většinou 10 - 15 pasází

pak je nutné nové založení kultury

možné problémy:

habituaace

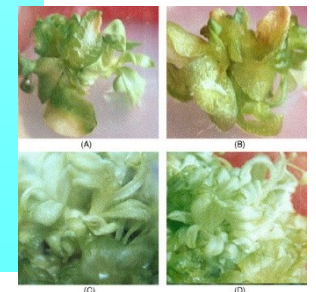
vitřifikace

Kultura pokračuje ve vývoji i přes nepřítomnost
auxinů nebo cytokininů

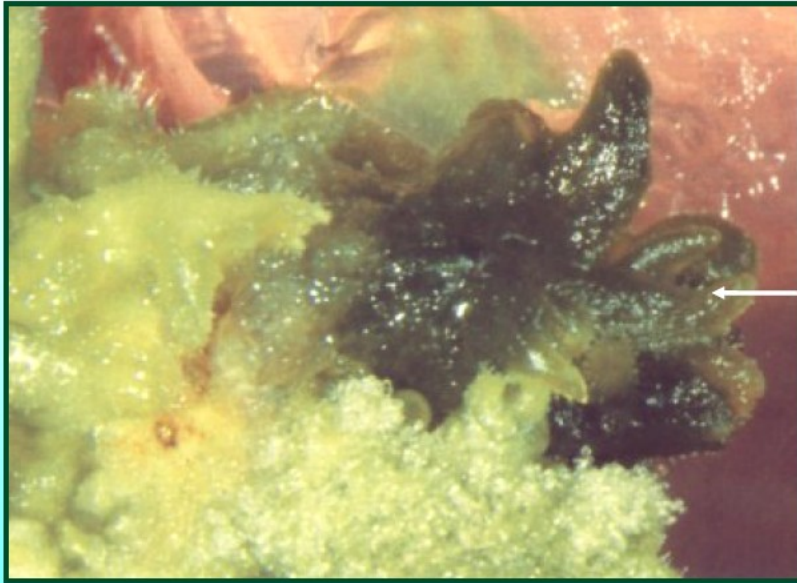
(hyperhydratace) – převodnění pletiv

Habituation

- Habituation is now defined more generally as a stable heritable loss in the requirement of cultured plant cells for growth factors.
- Habituation is when a culture continues to develop in the absence of auxin or cytokinin.



II. stadium mikropropagace



adventivní
prýty

Debergh *et al.*

mikropropagace melounu *Cucumis melo* L.

mikropropagace ananasu



III. Stadium mikropropagace: zakořeňování

- snížení koncentrace minerálních solí
- možná indukce auxinem (pulsní - vysokou koncentrací nebo dlouhodobější působení nízkou koncentrací) pro tvorbu funkčních kořenů = vhodnější tekuté médium
- absence mykorrhizy inokulace kulturami hub



Mikropropagace - stadium III. zakořeňování



mikropropagace *Lilium*
katalog firmy



**mikropropagace
*Malus***

Aklimatizace

problémy:

nedostatečně vyvinutá kutikula (vosky)

nefunkční stomata

odumírají kořeny vyvinuté *in vitro*



Snižování relativní vlhkosti *in vitro* - „bottom cooling“

Zvyšování vlhkosti - tunely nebo mlžení

Zabránění infekci po převodu:

pečlivé odstranění zbytků agaru

desinfekce substrátu - Previcur-N (0,15-0,25%)

Aklimatizace *Saintpaulia ionantha* Wendl.

skleněné akvárium



regenerované rostliny



Aklimatizace

sadbovače s nezakořeněnými
mikrořízky přeneseny do mlžné
komory ve skleníku



vyvíječ mlhy

<http://instruct1.cit.cornell.edu/courses/hort400/raspberry/stageIV.html>

Nevýhody množení *in vitro*

- možnost nežádoucího zvětšení variability (tzv. somaklonální variabilita)
- nebezpečí genetické degradace
- protokoly nejsou optimalizované pro všechny druhy
- problémy s vitrifikací a habituací
- pracnost a energetická náročnost - cena

Habituace = forma zkušenosti, která vede k vymizení reakce živočicha nebo rostliny na neškodný, dlouho opakovaný podnět nebo skupinu podnětů = **snížení odpovědi**

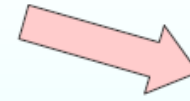
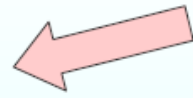
Vitrifikace („hyperhydricity“)

Symptomy vitrifikace nemusí být vždy viditelné pouhým okem. Viditelné příznaky se mohou objevit u citlivějších taxonů nebo v případě nepříznivějších podmínek.

Příklady nepříznivých podmínek:

- příliš vysoká koncentrace cytokininu
- vysoká kapacita retence vody
- příliš těsně uzavřené kultivační nádoby
- příliš nízká koncentrace gelujících látek (agar)

Znaky vitrifikace



morfologické

anatomické

biochemické

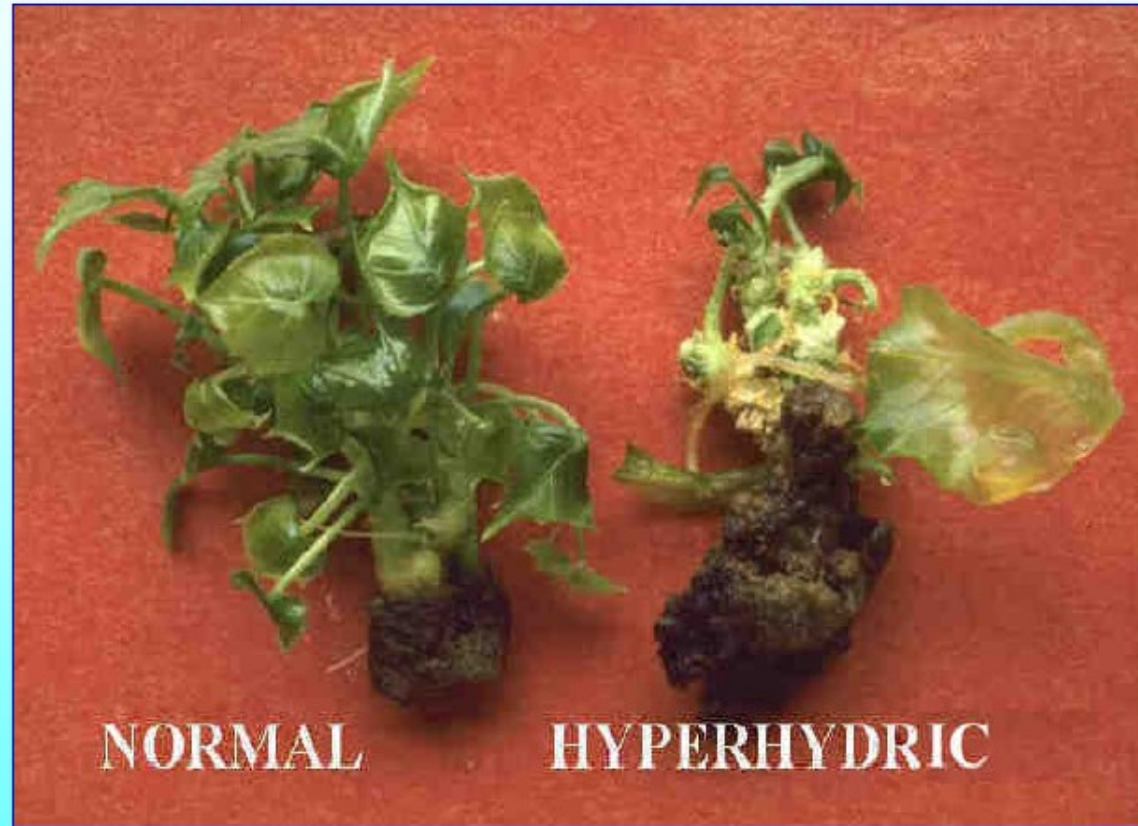
1. kratší internodia
2. tvorba růžic
3. průhledné, křehké a zkroucené listy
4. abnormální barva
5. slepené jehlice u konifer

1. velké interceluláry
2. hypolignifikace
3. redukovaný vývoj cévního systému
4. defektní epidermis
5. změněné ukládání vosků
6. snížená funkce průduchů

žádné
obecné
závěry

Debergh et al.

Příklad jasných vizuálních symptomů vitrifikace
u *Oreopanax nymphaefolia*



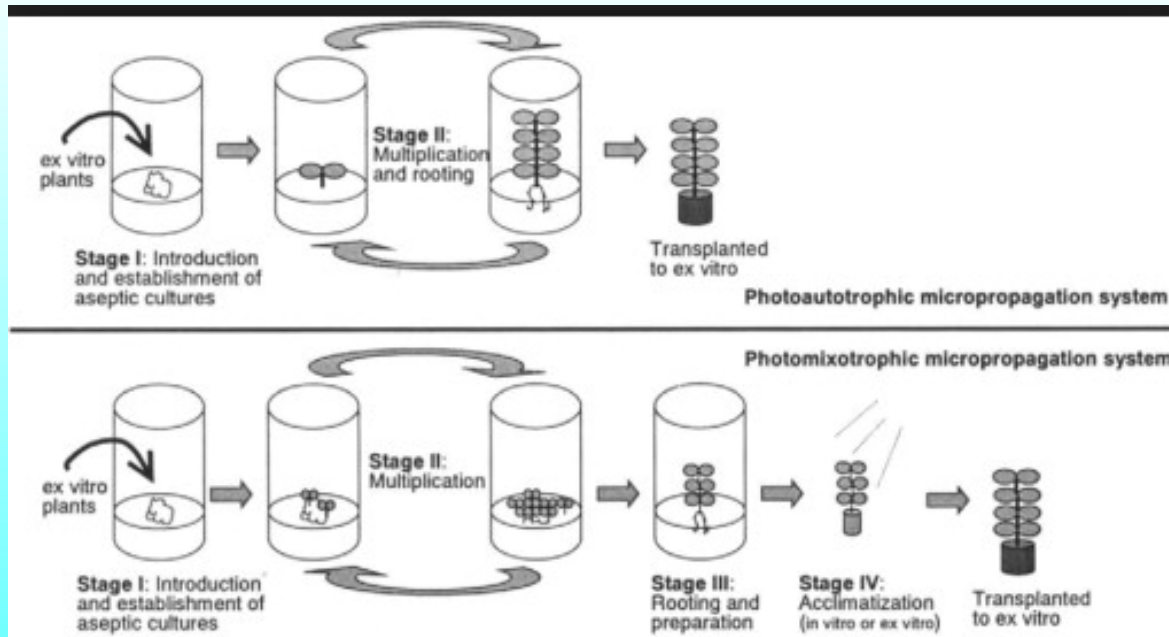
Debergh *et al.*

Srovnání atmosféry ve skleníku a kultivační nádobě

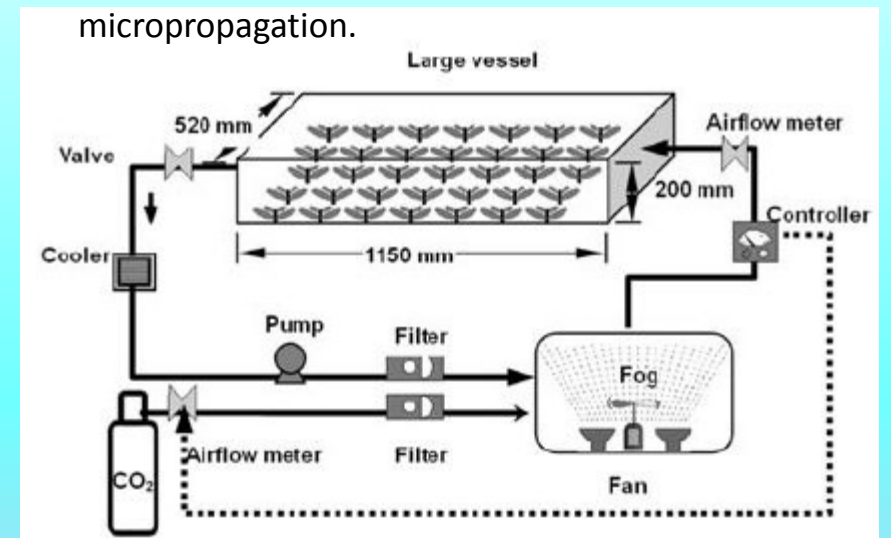
složka	skleník	kultivační nádobá
O_2	22 %	až 4 %
N_2	77 %	až 87 %
CO_2	365 - 1000 ppm	0,1 – 0,2 %
vodní pára	60-85 %	± 100 %
ethylen	5 ppb - 100 ppb	větší než 2 ppm

Fotoautotrofní mikropropagace

- bez dodání exogenních organických látek (cukry, vitamíny...)



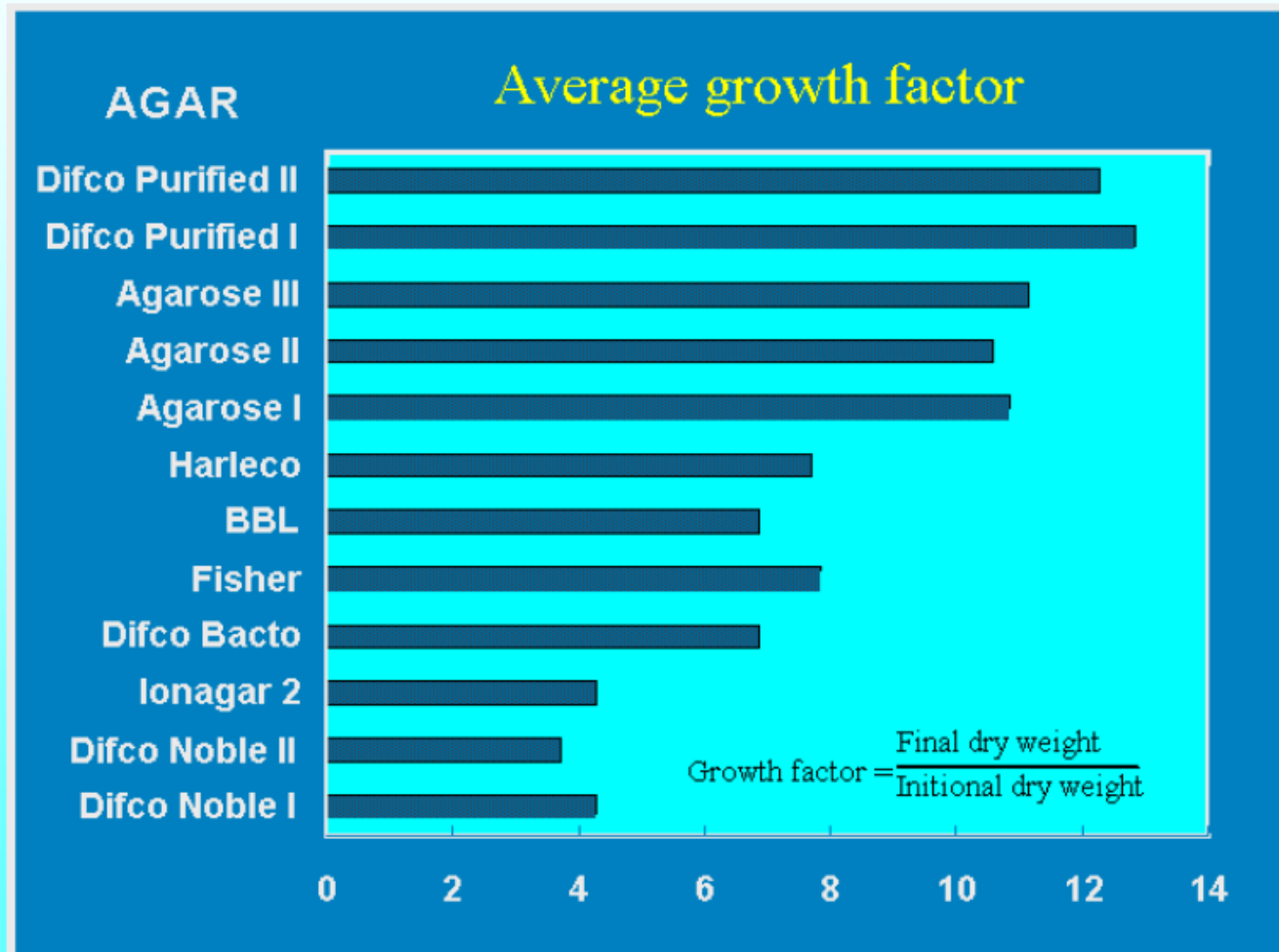
A schematic diagram of the forced ventilation unit for supplying CO₂-enriched, humidified, and cooled air for photoautotrophic micropropagation.



Growth promotion of Gerbera plantlets in large vessels by using photoautotrophic micropropagation system with forced ventilation, PROPAGATION OF ORNAMENTAL PLANTS 5(4):179-185

Vliv různých druhů agaru na růst apikálních prýtů *Picea abies*

(koncentrace agaru 1%) (Romberger and Tabor, 1971)



Komerční mikropopagace

- <http://www.micropropagation-services.co.uk/>
- <http://bestcarnivorousplants.com/dionaea/>
- <http://www.monfori.com/>



Rostlinné taxony v komerčních laboratořích

COST meeting 1992, Dijon

Rod	Počet laboratoří
<i>Prunus</i>	107
<i>Ficus</i>	82
<i>Philodendron</i>	56
<i>Spathiphyllum</i>	46
<i>Nephrolepis</i>	44
<i>Rosa</i>	41
<i>Syngonium</i>	37
<i>Malus</i>	35
<i>Orchidaceae</i>	31
<i>Solanum</i>	30
<i>Gerbera, Begonia, Fragaria</i>	26

Innovative techniques of micropropagation

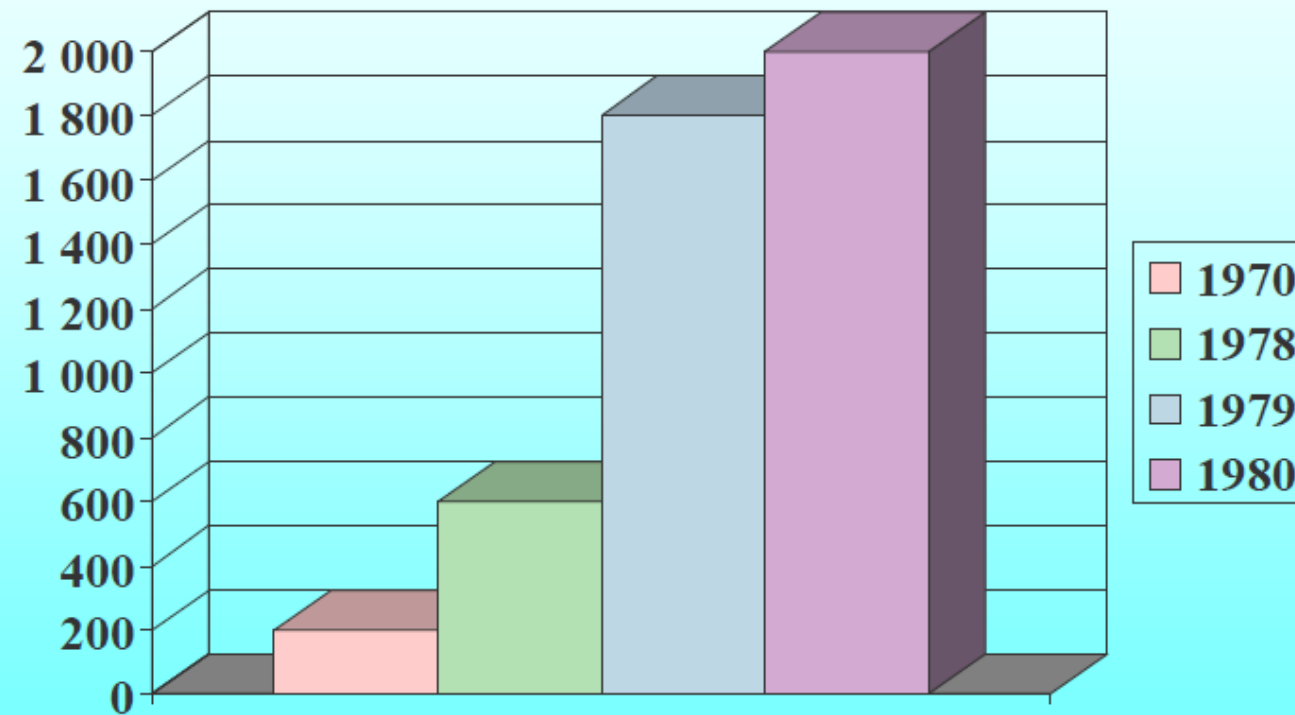


National Research Council of Italy
Trees and Timber Institute



- <http://www.ivalsa.cnr.it/en/research/innovative-techniques-of-micropropagation-in-vitro-propagation.html>
- almost 25 millions the plants produced by micropropagation
- many **fruit** species, but also **ornamental** plants and some **vegetables**
- the optimization of specific micropropagation protocols in **semi-solid media**, and the **development of innovative tissue culture systems**, such as **somatic embryogenesis**, the production and regeneration of encapsulated explants (**synthetic seeds**), the liquid culture in **temporary immersion system (TIS)**

Produkce sazenic *in vitro* (x 1000) Firma Miyoshi (Japonsko)



Laminární boxy komerční laboratoře



katalog firmy

Kultivace - komerční laboratoře



Search results - hana.cempirkov... | Diskuze - Rodina.cz | micropropagation - Hledat Goo... | micropropagation statistics - Hle... | Micropropagation: From Labora... | micropropagation asia - Hledat... | +

https://financialtribune.com/articles/economy-business-and-markets/17287/micropropagation-from-laboratory-to-market

Apilicace | Coprint s.r.o. | Ekologické stelivo A... | Kompas - interaktiv... | Skvělé rohlíky | Ovocný koláč ze za... | Donutky | Edukativní online hr... | French Toast Roll-U... | Dětská BMI kalkula... | www.gerontologie... | Plant Biotechnolog... | Biotechnologie a G...

FINANCIAL TRIBUNE
FIRST IRANIAN ENGLISH ECONOMIC DAILY

TUESDAY March 19 2019

NATIONAL ENERGY ECONOMY BUSINESS & MARKETS AUTO TRAVEL SCI-TECH

Economy, Business And Markets | May 20, 2015 19:43

Micropropagation: From Laboratory to Market

he agriculture sector is constantly looking for innovative and modern techniques to increase crop efficiency and produce high quality and safe food, feed, and fiber at affordable prices. Recently, tissue culture micropropagation is increasingly used to reproduce crops that are difficult to propagate by conventional methods such as seeding or cutting. Micropropagation is the practice of rapidly multiplying stock plant material to produce a large number of progeny plants, using modern plant tissue culture methods.

Tissue culture micropropagation is a collection of techniques used to maintain or grow plant cells, tissues, or organs under sterile conditions on a nutrient culture medium of known composition. Success in producing large numbers of plantlets requires the most sterile facility, an ideal cultural media, and an experienced expert to oversee stringent protocol. An article by Persian daily Forsat-e Emruz investigates the advantages and challenges of investing in the science-based industry.

Advantages and Limitations

Tissue culture micropropagation offers many unique advantages over other propagation methods, including:

- Production of more robust plants, leading to accelerated growth compared to similar plants produced by conventional methods;
- Producing rooted plantlets ready for growth, saving time for the commercial grower when seeds or

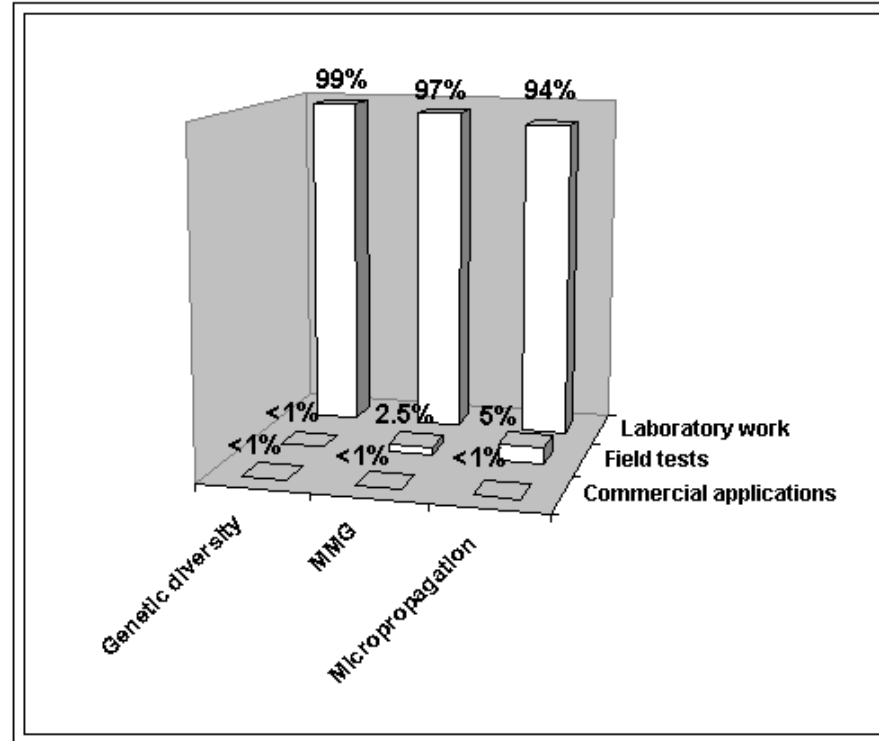
advertise with us

Today's Top Stories

14:21 19.3.2019

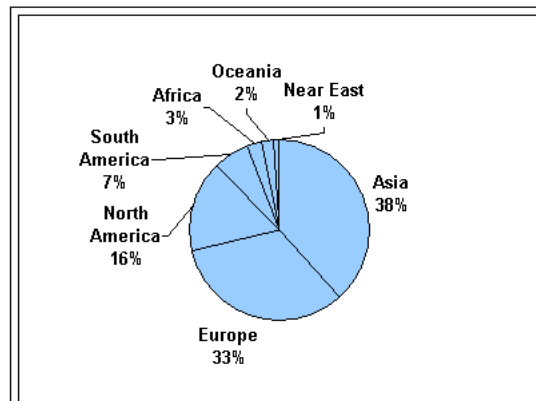
Despite the many advantages, **high production costs, labor-intensive process, requirement of sophisticated facilities and skills** and the **vulnerability of progeny plants to infections** are among major limitation in the use of micropropagation. Unavailability of immediate market for the produced crops and the **high cost of export and transportation** are among other limitations while considering investment in the sector. Experts believe micropropagation is not economically feasible when practiced in small areas, suggesting the **establishment of large-scale micropropagation plants for generating reasonable profits.**

Figure 2.1.5. Distribution of reported forest biotechnology activities, excluding genetic modification, by category and applications (laboratory studies, field trials and commercial deployments)¹



¹MMG: mapping, marker-assisted selection and genomics.

Figure 2.1.13A. Distribution of micropropagation activities by region of activity



<http://www.fao.org/3/ae574e/AE574E06.htm>

Micro-propagation a tool for commercialization (Taiwan story)

According to Taiwan Today, Jan 1, 2016

- The nation's orchids are exported to 36 countries in Northern America, Northern Europe and South Africa.
- This country began the export with US \$ 23 million in 2004 but in 2015 they have exported with worth US \$ 130 million.
- They are selling a Phalaenopsis or moth orchid in Dubai at the price of US \$ 1,000.
- Won the bid to host the 23rd World Orchid Conference in 2020.

What may be the reason for this achievement ?

- According to John Feng, CEO of SOGO TEAM CO LTD., they are exploiting micro-propagation as a technique in mass production as well as in variety improvement.
- In I-Hsin Biotechnology Corp's Tissue Culture Lab only, they have conducted 12,000 breeding experiments which have yielded 2,300 Phalaenopsis varieties.
- This all is being possible through tissue culture.

Commercial Production of Ornamental Tropical Foliage Plants: Microp

- <https://edis.ifas.ufl.edu/ep520>



SUCCULENT TISSUE CULTURE

A new way of producing cacti and other succulent plants

HOME

Introduction.

We welcome you to the website of Succulent Tissue Culture (S.T.C.). Robert Wellens started in 1995 STC which has grown to a medium-sized tissue culture laboratory mainly focussed on the in vitro multiplication of rare and endangered succulent plants, which include many plants like Haworthia, Aloe, Gasteria but also Echeveria, Dracaena, Bulbine, Agave, Yucca and many more succulent plants.

Beside the production of rare succulents, STC acts as a research-partner in developing new protocols enhancing ornamental plant breeding. STC has a state-of-the-art cleanroom facility and produces rare and endangered plants, some even being extinct in their natural habitat.

Tissue culture techniques offer us a range of different approaches to establish a reliable in vitro protocol to multiply all sorts of succulent plants. Robert and his wife Teresa are daily working on improving standard protocols, inventing new ways of production technology and implementing these to produce supreme collectors items.

Our techniques vary from single axillary plant reproduction to organogenesis and embryogenesis on calli from a specific range of (succulent) plants. Mutation-induction, haploid- and tetraploid-induction and all sorts of specific techniques are used to produce wonderful one-of-a-kind supreme collectors items mainly in the field of variegated succulent plants.

Our aim is to provide interested collectors with extremely rare plants on one hand and to protect against illegal collection and plant destruction in habitat on the other hand. With the use of tissue culture there is less need to collect habitat plants and in this way STC hopes to contribute to keep the world heritage of succulent plants existing.

NEWEST ITEM

Gasteria nitida v. armstrongii cv. Giant
[70114]
7 cm pot

Go to the webshop

LOGIN

Username
Password

Log in

Forgot password?
Create an account

NEWSLETTER

Sign Up

Sign out


SHOPPING BASKET

You have 0 item(s) in your shoppingbasket

Total: € 0,-

place order

We accept
PayPal



***In vitro* propagation of endangered *Mammillaria* genus (Cactaceae) species and genetic stability assessment using SSR markers, [In Vitro Cellular & Developmental Biology - Plant](#) October 2018, Volume 54, [Issue 5](#), pp 518–529**



- <https://www.succulent-tissue-culture.com/EN/home>

Table 3. Projected multiplication rate of banana under of tissue culture from a single explant

Particulars Stage		Duration (days)	No. of plants
Initiation		25	1
Subculture stages	1 st	50	3
	2 nd	75-80	12
	3 rd	100-110	48
	4 th	125-130	192
	5 th	175-180	760
	6 th	200-210	3,040
	7 th	225-230	12,160
Rooting		255-260	11-12,000
Primary hardening		270-280	11,500-11,000*
Secondary hardening		310-320	10,000-10,500**

*Success depends on the sophistication of the hardening structure

**Somaclones and off-types constitute the major discards

Příklady využití in vitro rostlin v ČR

- <http://zahradaweb.cz/rozmnozovani-okrasnych-drevin-mikropropagaci/>
- <http://www.janholub.cz>
- <http://www.profiplants.cz/kategorie/akvarijni-rostliny>
- <http://www.lesaktualne.cz/vyzkum/vedci-prispivaji-k-udrzeni-biologicke-rozmanitosti-drevin>
- <http://www.agris.cz/clanek/190191>
- **Plant culture is used commercially**
- Culturing plants in the laboratory has become common practice, and this technique is used commercially to produce plants for the agricultural and horticultural industries. You may be surprised to learn that most of our **native carnivorous plants** that can be purchased legally are the products of tissue culture. It is illegal to collect most species in the wild, so producers pay for licenses to collect a limited number of specimens that they then use to establish tissue culture lines. Periodically, some of the descendent cultures from a line are subjected to growth regulators which cause them to develop into young plants that can be sold.



Micropropagation

Asbl CEDEVIT

Mme Etienne



Faites des Sciences... à l'Embarcadère du Savoir !

19 - 25 mars 2007 dans le cadre du Printemps des Sciences
Pôle moyen d'Enseignement Supérieur et Universitaire


<https://www.amazon.com/Microclone-Tissue-Culture-Starter-Kit/dp/B01M7NDE10>

Garden Tales | Online hra zdarma x Amazon.com : Microclone Plant | x +

amazon.com/Microclone-Tissue-Culture-Starter-Kit/dp/B01M7NDE10

Aplikace E-volby a hlasování Coprint s.r.o. Ekologické stelivo A... Twitter for Scientists Antarctic Biosciences Přítomnost lidí na...

Patio, Lawn & Garden > Gardening & Lawn Care > Indoor Gardening & Hydroponics



Microclone Plant Tissue Culture Starter Kit

Brand: Microclone Starter Kit

★★★★☆ 6 ratings

- BAY HYDRO LLC Ships in 24 HOURS, Don't believe the Estimated Date, As we are "SUPER FAST"

Roll over image to zoom in

