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In vitro vegetative micropropagation of purple coneflower (*Echinacea purpurea* L.)

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SUMMARY

The application of medicinal plants has become into prominence in last decades and expectedly will widen on.

Elemental expectation that industrial production of medicinal plant products need to be continuous and consistent but it can't be assured because of field-grown plants exposed to environmental factors.

Nowadays permanent basic commodity supply is covered by *in vitro* micropropagation. By the help of this method sterile, homogeneous and quantity of plant material can be produced without reference to seasons and climate, correspondent agent-content is guaranteed.

In the course of micropropagation of purple coneflower, regeneration capacity of shoot tip, leaf section and the base of leaf stick was examined by different cytokinin concentrations. The degree of sterility was established with seed-coat and after removal of seed-coat. It could be defined that sterilization without seed-coat was more successful.

The sterile seeds were placed on MS basal medium and were incubated in a growth room. The developed plants with 6–8 leaves provided the explants for the experiments. The explants were placed on MS medium supplemented with different concentrations of 6-benzylaminopurine (BAP). Medium without plant growth regulators was used as a control. After 9 weeks incubation were the experiments evaluated.

The regeneration of shoot tips were the most successful, the best development was observed on the medium containing 0.5 mg/l BAP. In case of regeneration from leaf explant was observed that absence of auxin and cytokinin supplement alone didn't allow of differentiation of new plant.

Regeneration induced from the base of leaf stick proved unavailable.

Keywords: *Echinacea purpurea* L., purple coneflower, *in vitro* vegetative micropropagation, 6-benzylaminopurine.

Introduction

The application of medicinal plants has become into prominence in last decades and expectedly will widen on. The active compounds of several medicines and specifics with curativ effect are provided by natural, synthetic and semisynthetic plant materials. The increased demand of remedies made from medicinal plants has entailed the expansion of volume of cultivation of medicinal plants though paralell with it the quality-insurance of produced plant materials has become very important, too. The quality and active content of field-grown plant products depend on environmental factors, applied cultivation technology and suitable primary-processing performed by producer in large scale. Elemental expectation that industrial production of medicinal plant products need to be continuous and consistent (*Petri* 2006).

Nowadays permanent basic commodity supply is covered by *in vitro* micropropagation. By the help of this method sterile, homogeneous and quantity of plant material can be produced without reference to seasons and climate, correspondent agent-content is guaranteed.

The purple coneflower is a wide-applied medicinal plant, it can be used by treatments of several diseases and health care, it's colorful application possibility suits the present's requirements (*Csupor* 2007).

The object of this study is to establish wich section of plant and wich cytokinin concentration is the most suitable to obtain intact plant by examination of plant regeneration induced from shoot tip, leaf section and the base of leaf stick with different cytokinin concentrations as well as characteristics of the micropropagation of *Echinacea* can be determined namely such as the effect of the surface sterilization, the mass of plant material producated during unit of time and the numbers of shoots, leaves and roots produced by regenerated plants. Considering the specificities of the micropropagation of purple coneflower a method can be elaborate wich can provide the necessary basic material in required quality and quantity for manufacturing of *Echinacea* products. The improvement of quality and reliability of medicinal plant products can result in further increase of the use of natural materials.

The characterization of purple coneflower (Echinacea purpurea L.)

The genus *Echinacea* belongs to the family of *Asteraceae* and to the order of *Asterales.* The genus *Echinacea* is represented by 11 species wich are native in the USA and in central Canada. Nowadays therapeutic importance of three species is known, these are *Echinacea purpurea* L., *Echinacea angustifolia* L. and *Echinacea pallida* L., *Echinacea purpurea* L. is the most widely cultivated medicinal species of the genus.

Purple coneflower is 80–150 cm tall. It's roots are composed of filament-like side-roots. The coneflower's stalk is cylindrical with antocyanic spots. It's leaves are dark-green, shaggy and broad-spear. The stock leaves are 25–35 cm long, 7–12 cm wide, the stalk leaves are smaller and longish. The flower is cone-shaped, it blooms from Juny to September. The crop of *Echinacea* is brown-grey, cornered, the mass of thousand seeds is 3.8–4.5 g (*Bernáth* 2000).

Echinacea species are firstly used by indigenous Americans for the treatment of infections, inflammations, colds, toothaches and snake bites. In view of the efficient results the application of coneflower has become general soon (*Koroch et al.* 2002). In some countries it's suitability is also exemined by the treatments of cancer and AIDS.
The demand for *Echinacea* products increased in the end of 1990's significantly. Currently, medicinal plant products are prepared from field-grown crops (*Choffe et al.* 2000). Numerous problems exist with preparations that have raised concerns about the quality of *Echinacea* products (*Consumer Reports* 2000).
The quality of *Echinacea* and medicinal plant preparations in general can be seriously compromised by contamination of fungi, bacteria and environmental pollutants (*Laughlin* and *Munro* 1982), adulteration with the wrong plant species (*Betz* 1998, *Slifman et al.* 1998) and considerable variation can be found in the content of medicinally active constituents (*Murch et al.* 2000).

In vitro vegetative micropropagation

The *in vitro* vegetative micropropagation is one of the sections of plant biotechnology. It can be regard as clone-technique, because the organogenesis sets out from somatic cells so the genotype of descendants is unchanged (*Dudits* and *Heszky* 2003).
The *in vitro* propagation is always carried out among sterile and controlled conditions. It's economical importance that plant materials can be produced in large quantity and in the same quality in shorter time than by field production.
Micropropagation is a complex process, five phases can be distinguished:

1. phase of preparation (promoting the success of surface sterilization),
2. phase of starting (establishing sterile culture),
3. phase of propagation (propagating culture),
4. phase of elongation and rooting (lengthening of shoots, inducing of rooting),
5. phase of acclimatization (accustoming in greenhouse) (*Jámborné* and *Dobránszki* 2005).

Materials and methods

Establishment of sterile culture

The seeds of purple coneflower can be obtained in commercial trade. After selecting healthy seeds physical contamination was removed under running tap water. After that seeds were soaked in 70% ethanol for 1 minute then in sodium-hypochlorite solution for 15 minutes. Removal of remains of disinfectant was accomplished by 6–7 rinses with sterile deionized water. Then seed coats were removed and seeds were disinfected again with sodium-hypochlorite solution. After that seeds were rinsed again with deionized water 6–7 times. The surface sterilized seeds were placed in plastic vessels containing MSØ basal medium (*Murashige* and *Skoog* 1962) in a laminar flow bench. The vessels were sealed and placed into a growth room with 16 hours photoperiod at 22–24 °C for two weeks. After 14 days 64 seeds weren't infected from 98 seeds.

Effect of different growth regulator-concentrations

Plantlets cultured on MSØ medium provided explants for investigation of the effect of different 6-benzylaminopurine (BAP) concentrations. The tip of shoots, leaf sections and the base of leaf sticks were used as explants. Medium without plant growth regulators was used as a control.
Isolated shoot tips, leaf sections and bases of leaf sticks were transferred to basal medium alone or in combination with different concentrations (0.5; 1; 3; 5 mg/l) of BAP.
All treatments consisted of 7 explants and were incubated in a growth room. Evaluation of treatments was carried out after 9 weeks of incubation.

Determination of fresh mass and of regeneration of shoot and of root

Plantlets were removed from medium and fresh mass was measured after drying up on an analytical scale. Numbers of shoots, leaves and roots per plant was determined at this time, too. Data were analyzed statistically by analysis of variance with the level of significance set at 5%.

RESULTS

Effect of surface sterilization

In case of removal of seed coats effectiveness of sterilization was 65%. If seed coats weren't be removed sterility % was 35%. The deficiency of sterility was probably caused by pathogens and pollutants being found in the gaps of seed coat.

Effect of 6-benzylaminopurine on fresh mass

At the examination of treatments after 9 weeks of culture could be observed that the most intensive developement was showed by plantlets derived from shoot tip explants. Development of plantlets cultured on basal medium without growth regulator was the slightest while the effect of BAP in concentrations 0.5 and 3 mg/l was the most advantageous.
Callus formation and occasionally shoot initiation was observed in case of regeneration from leaf explant. At concentrations 0 and 0.5 mg/l of BAP leaf segments became swollen and brown.
Plant differentiation wasn't achieved in the course of experiments derived from the base of leaf sticks in any concentrations of BAP. Explants became brown and didn't show any development (*Table 1.*).

Table 1. Effect of BAP on fresh mass of *Echinacea purpurea* L. shoot tip and leaf explants after 9 weeks of culture (g)

Treatments	**Shoot tip explants** (g)	**Leaf explants** (g)
0 BAP	0.38	0.000
0.5 mg/l BAP	1.02	0.000
1 mg/l BAP	0.68	0.005
3 mg/l BAP	1.54	0.304
5 mg/l BAP	0.21	0.246
$SD_{5\%}$	0.47	0.073

Effect of 6-benzylaminopurine on shoot organogenesis

The effect of the medium containing 0.5 mg/l BAP was the most advantageous for the formation of shoots. In this case every plantlets showed an intensive development but shoot organogenesis was achieved with other concentrations of BAP, too (*Figure 1.*).

Figure 1. Effect of BAP on shoot organogenesis of *Echinacea purpurea* L. shoot tip explant after 9 weeks of culture

In case of leaf explants shoot organogenesis was observed at concentrations 3 and 5 mg/l of BAP. At this concentrations green calli were developed, shoot organogenesis was observed to develop from the margins of leaf explants *(Table 2.).*

Table 2. Effect of BAP on shoot organogenesis of *Echinacea purpurea* L. shoot tip and leaf explants after 9 weeks of culture (numbers of shoots)

Treatments	Shoot tip explants (numbers of shoots)	Leaf explants (numbers of shoots)
0 BAP	2.71	0.00
0.5 mg/l BAP	5.71	0.00
1 mg/l BAP	3.14	0.00
3 mg/l BAP	4.29	1.29
5 mg/l BAP	3.43	0.14
$SD_{5\%}$	2.31	1.17

Effect of 6-benzylaminopurine on leaf formation

The best results were achieved at concentration 0.5 mg/l of BAP, where the average number of differentiation of leaves was 24 per plant. In this case leaves were smaller than at concentrations inducing less leaves. The effect of concentration 1 mg/l of BAP was the slightest.

At the development induced from leaf explants leaf differentiation was achieved at concentrations 3 and 5 mg/l of BAP (*Table 3.*).

Table 3. Effect of BAP on leaf organogenesis of *Echinacea purpurea* L. shoot tip and leaf explants after 9 weeks of culture (numbers of leaves)

Treatments	Shoot tip explants (numbers of leaves)	Leaf explants (numbers of leaves)
0 BAP	10.71	0.00
0.5 mg/l BAP	23.86	0.00
1 mg/l BAP	9.57	0.00
3 mg/l BAP	13.29	3.14
5 mg/l BAP	14.43	0.29
$SD_{5\%}$	10.34	2.77

Effect of 6-benzylaminopurine on root organogenesis

Root formation was observed at concentrations 0 and 0.5 mg/l of BAP. Higher concentration of BAP caused inhibition of root initiation *(Table 4., Figure 2.).* Considering that neither media contained auxin the endogenous auxin production resulted in root formation. Root organogenesis on leaf explants wasn't achieved.

Table 4. Effect of BAP on root organogenesis of *Echinacea purpurea* L. shoot tip explants after 9 weeks of culture (numbers of roots)

Treatments	**Shoot tip explants** (numbers of roots)
0 BAP	1.71
0.5 mg/l BAP	2.00
1 mg/l BAP	0.00
3 mg/l BAP	0.00
5 mg/l BAP	0.00
SD5%	1.52

Figure 2. Effect of BAP on root organogenesis of *Echinacea purpurea* L. shoot tip explant after 9 weeks of culture

Discussion

On the evidence of the experiments can be established that shoot tip proved to be the most suitable for explant at the concentration 0.5 mg/l of BAP due the micropropagation of *Echinacea purpurea* L. At this section of plant every concentration of growth regulator induced organogenesis.

Leaf explants incubated on basal medium supplemented with higher concentrations of cytokinin (3 and 5 mg/l of BAP) demonstrated callus formation. In case of lower concentrations or absence of cytokinin organogenesis wasn't achieved. It can be concluded that absence of auxin and cytokinin supplement alone didn't allow of differentiation of new plant. Regeneration induced from the base of leaf stick proved unavailable.

Experiments were accomplished on medium containing cytokinin only. Taking the obtained results into consideration performing of an experiment tending to determination of an optimal auxin–cytokinin combination can be proposed.
Developing of ideal regeneration system of *Echinacea purpurea* L. the micropropagation of this medicinal plant can become more successful and economical if obtaining of an optimal (maximal) number of plants can be certained where deterioration of quality can be still eliminated.

A bíbor kasvirág (*Echinacea purpurea* L.) *in vitro* vegetatív mikroszaporítása

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Összefoglalás

A gyógy- és illóolajos növények felhasználása az utóbbi évtizedekben ismét előtérbe került és várhatóan tovább bővül.
Alapvető elvárás, hogy a növényi alapanyagú termékek ipari előállítása folytonos, kiegyensúlyozott termelés során történjen. Ezt a környezeti tényezők által befolyásolt mezőgazdasági tevékenység során termesztett növényekkel kevésbé lehet biztosítani.
A permanens alapanyag-ellátást ma már sok helyütt *in vitro* (steril) mikroszaporítással biztosítják. E módszer segítségével laboratóriumi körülmények között nagy mennyiségű, steril és homogén nyersanyag állítható elő évszaktól és éghajlattól függetlenül, a faj- és fajtaazonosság, a megfelelő hatóanyag-tartalom garantálható.
A bíbor kasvirág mikroszaporítása során különböző citokinin-koncentrációk mellett vizsgáltuk a hajtáscsúcs, a levélszegment és a levélnyél alapjának regenerálódó képességét. Meghatároztuk a magok sterilitásának mértékét maghéjat tartalmazó és a maghéj eltávolítása után végzett fertőtlenítés esetén. Megállapítható, hogy a sterilizálás sikeresebbnek bizonyult a maghéj eltávolítása után.
A steril felszínű magokat MSO táptalajra helyeztük és tenyészszobában inkubáltuk. Az így nyert 6–8 leveles növények szolgáltatták az inokulumokat a további kísérletek folytatásához. Az izolátumokat különböző koncentrációjú benzil-amino-purint tartalmazó táptalajokra helyeztük és 9 hetes inkubáció után értékeltük ki a kísérleteket.
A növényi részek közül a hajtáscsúcs regenerációja bizonyult a legsikeresebbnek, a legideálisabb fejlődést 0,5 mg/l BAP hormonkoncentráció jelenlétében mutatta.

A levélszegmentből indukált fejlődés esetén megállapítható, hogy a citokinin egyedüli adagolása és az auxin hiánya nem teszi lehetővé új növény differenciálódását.
A levélnyél alapjából indukált regeneráció kizárólag citokinint tartalmazó közegben sikertelennek bizonyult.
Kulcsszavak: *Echinacea purpurea* L., bíbor kasvirág, *in vitro* vegetatív mikroszaporítás, 6-benzil-amino-purin.

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