The efficiency of PVS eradication depends on genotype, conditions of thermotherapy and size of excised meristem tip. Failure of ELISA to detect PVS in the first regenerants was probably related to inoculum size which was represented by amount of infected tissue on excised meristem tip. The concentration of PVS in the first regenerants was likely lower than the detection limit of ELISA. However, the size of the meristem played an important role on the differentiation rate and the virus eradication effect.

References

BANTTARI, E. E. - ELLIS, P. J. - KHURANA, S. M. P. 1993. Management of diseases caused by viruses and viruslike pathogens. In: ROWE, R. C. Potato health management. St. Paul, Minnesota: APS Press, 1993, p. 127-134.
BARKER, H. - WEBSTER, K. D. - REAVY, B. 1993. Detection of potato virus Y in potato tubers: a comparison of polymerase chain reaction and enzyme - linked immunosorbent assay. Potato Res., vol. 36, 1993, p. 13-20.
CLARK, M. F. - ADAMS, A. N. 1977. Characteristics of microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol., vol. 34, 1977, p.475-483.
KOTKAS, K. - ROSENBERG, V. 1999. Disease eradication and propagation of the initial seed potato material in Estonia. Potato Res., vol. 42, 1999, p.577-583.
McKENZIE, D. J. - TREMAINE, J. H. - STACE-SMITH, R. 1989. Organization and interviral homologies of the 3´-terminal portion of potato virus S RNA. Acta virol., vol. 70, 1989, p.1053-1063.
PAWLIKOWSKI, K. - KUNZE, R. - DE VRIES, S - BISSELING, T. 1994. Isolation of total, poly(A) polysomal RNA from plant tissues. In.: STANTON, S. B. - SCHILPEROORT, R. A.: Plant Molecular Biology Manual. Dortrecht: Kluwer Academic Publisher, 1994, D5 p.1-13.
ROSENBERG, V. R. 1985. Vozmožnosti povyšenia effektivnosti metoda verchušečnoj meristemy dľa ozdorovlenia semennogo kartofeľa. In: Zaščita rastenij v respublikach Pribaltiki i Belurusii (Tezisy dokladov naučno-praktičeskoj konferencii - časť I). Tallin, 1985, p.233-234.

INDIRECT REGENERATION OF *HYPERICUM PERFORATUM* L. UNDER *IN VITRO* CONDITIONS

Milan BEŽO, Veronika ŠTEFÚNOVÁ
Slovak University of Agriculture in Nitra, Faculty of Agronomy, Department of Genetics and Plant Breeding

Summary

Hypericum perforatum L. is a traditional medical plant. Naphtodiantrones are considered to be one of the most important secondary metabolites. There are possibilities to expand genetic variability using modern biotechnology methods. The callus induction was observed on the MS medium supplemented with 2,4- dichlorophenoxyacetic acid (2,4-D). The callus growth is possible to induce with supplementation of cytokinins (kinetin – KIN, 6-benzylaminopurine – BAP) and auxins (2,4-D) to the medium. The best callus growth, from the point of size, was observed on the medium supplemented with 1 mg.l^{-1} KIN + 1 mg.l^{-1} 2,4-D (10 %) and 1 mg.l^{-1} BAP + 1 mg.l^{-1} 2,4-D (12,7 %). The most intensive regeneration (> 20 regenerants from callus) were observed on the medium supplemented with 0,1 mg.l^{-1} indole-3-butyric acid (IBA) + 5 mg.l^{-1} BAP (7,7 %), 0,1 mg.l^{-1} IBA + 5 mg.l^{-1} BAP + 80 mg.l^{-1} adenin (6,7 %), 0,1 mg.l^{-1} IBA + 5 mg.l^{-1} KIN (5,6 %) + 80 mg.l^{-1} adenin, 3 (5,1 %) and 5 mg.l^{-1} BAP (4,1 %).

***Key words*:** *Hypericum perforatum*, *in vitro* cultivation, callus formation, regeneration

Introduction

Hypericum perforatum L. is a traditional medicinal plant, which received great attention in recent years (http://www.admin.ch/bbw/abstracts/abstr-99/abstracts/cost/c97.0068.html, 2001). *Hypericum perforatum* L. as well as its extracts are used in the treatment of psychovegetative disorders and minor depressions. These activities are attributed among others to hypericin and compounds similar to hypericin (Biza et al., 1999). Despite of these activities, naphtodiantrones – hypericin and similar compounds – are responsible for anti-viral and anti-retroviral activity (Büter et al., 1998).
It is important to cultivate the genotypes with higher content of desired compounds. The genotype of cultivated plants of *Hypericum perforatum* present a key factor for an economically successful cultivation (Büter et al., 1998).
There is a possibility to utilize the modern biotechnology methods for potential creation of new genotypes, which may have higher content of desired compounds.
The aim of our study was to ulitilze modern biotechnology methods for expanding genetic variability using:

- callogenesis,

- indirect regeneration of *Hypericum perforatum* L. plants via callus cultures.

Material and methods

The seeds of *Hypericum perforatum* L. were obtained from the Regional Research Institute of Agroecology in Michalovce in Slovak Republic.
The plants, grown under *in vitro* conditions on a basal MS medium (Murashige, Skoog, 1962) were the primary explantats for callogenesis. The leaf and stem segments were placed on the MS medium supplemented with 2,4-D (1, 3 and 5 mg.l^{-1}). The calluses were transferred on the medium supplemented with other plant growth regulators (Table 1) after one month of cultivation. The phenotypic variability was evaluated using classification according to Bežo (1995) after one month of cultivation.
The calluses were primary explantats for indirect regeneration. Calluses were transferred on the medium supplemented with plant growth regulators (Table 2) after one month of cultivation on the medium for callus growth. The regeneration was evaluated after one month of cultivation.
Obtained data were processed using statistical program STATGRAPHICS. The tissue cultures were cultivated at 22 – 24 °C, with a 16 hours light photoperiod under 3000 lx irradiance.

Table 1 The content of plant growth regulators supplemented in the medium for callus growth

Medium	Plant growth regulators
1	1 mg.l^{-1} KIN + 1 mg.l^{-1} 2,4-D
2	5 mg.l^{-1} KIN + 5 mg.l^{-1} 2,4-D
3	1 mg.l^{-1} BAP + 1 mg.l^{-1} 2,4-D
4	5 mg.l^{-1} BAP + 5 mg.l^{-1} 2,4-D
5	5 mg.l^{-1} 2,4-D

Table 2 The content of plant growth regulators in the medium for indirect regeneration

Medium	Plant growth regulators	Medium	Plant growth regulators
1	1 mg.l^{-1} KIN	8	1 mg.l^{-1} IBA
2	5 mg.l^{-1} KIN	9	0,1 mg.l^{-1} IBA + 5 mg.l^{-1} KIN
3	1 mg.l^{-1} BAP	10	0,1 mg.l^{-1} IBA + 5 mg.l^{-1} BAP
4	5 mg.l^{-1} BAP	11	0,1 mg.l^{-1} IBA + 5 mg.l^{-1} BAP + 80 mg.l^{-1} adenin
5	0,1 mg.l^{-1} NAA	12	0,1 mg.l^{-1} IBA + 5 mg.l^{-1} KIN + 80 mg.l^{-1} adenin
6	1 mg.l^{-1} NAA	13	Control
7	0,1 mg.l^{-1} IBA		

Abbreviations; 2,4-D – 2,4-dichlorophenoxyacetic acid, BAP – 6-benzylaminopurine, KIN – kinetin, IBA – indole-3-butyric acid, NAA – α-naphtalene acetic acid, MS – Murashige, Skoog (1962) medium

Results

The phenotypic variability of calluses was evaluated. According to the descriptor (Bežo, 1995), the form, consistency, structure, colour, shape, exterior and size of calluses were assessed. The statistical agreement was confirmed between the variants of medium for callogenesis induction and form, consistency, structure and surface of calluses. The statistical agreement was confirmed also between the variants of medium for growth of calluses and form, consistency, structure and surface of calluses. The statistical agreement was not confirmed between colour (χ^2 – 69,76; f – 12; P – 0,00 %), shape (χ^2 – 22,53; f – 6; P – 0,00 %) and size (χ^2 – 43,04; f – 6; P – 0,00 %) of calluses and medium for callogenesis induction. The statistical agreement was not confirmed likewise between colour (χ^2 – 194,85; f – 12; P – 0,00 %), shape (χ^2 – 62,38; f – 8; P – 0,00 %) and size (χ^2 – 160,46; f – 8; P – 0,00 %) of calluses and medium for growth of calluses. The best callus growth, from the point of size, was observed on the medium supplemented with 1 mg.l^{-1} KIN + 1 mg.l^{-1} 2,4-D (10 %) and 1 mg.l^{-1} BAP + 1 mg.l^{-1} 2,4-D (12,7 %).
The indirect regeneration was assessed as a number of regenerants from callus cultures according to scale for classification (Štefúnová, 2000). The most intensive regeneration (> 20 regenerants from callus) were observed on the medium

supplemented with 0,1 mg.l^{-1} IBA + 5 mg.l^{-1} BAP (7,7 %), 0,1 mg.l^{-1} IBA + 5 mg.l^{-1} BAP + 80 mg.l^{-1} adenin (6,7 %), 0,1 mg.l^{-1} IBA + 5 mg.l^{-1} KIN (5,6 %) + 80 mg.l^{-1} adenin, 3 (5,1 %) and 5 mg.l^{-1} BAP (4,1 %). The statistical agreement between medium for indirect regeneration and degree of regeneration was not confirmed (χ^2 – 224,04; f – 36; P – 0,00 %).

Discussion

The importance of *Hypericum perforatum* L. has an increased tendency in recent years, especially because of the anti-viral and anti-retroviral activity (Čellárová, 1995).

Yazaki et al. (1990) present the callus initiation of the *Hypericum erectum* on the cultivation medium supplemented with combination of auxin and cytokinin plant growth regulators – IAA (10^{-5} mol.dm^{-3}) + BAP (10^{-5} mol.dm^{-3}). According to Brutovská et al. (1994) it is possible to initiate callus culture of *Hypericum perforatum* L. from the leaf segments on the medium with growth regulators – NAA (0,2 mg.l^{-1}) + KIN (0,2 mg.l^{-1}) + 2,4-D (0,2 mg.l^{-1}). Karting et al. (1996) found callus formation of seven species of the genus *Hypericum* on the medium supplemented with BAP (10^{-6} mol.dm^{-3}) + NAA (10^{-7} mol.dm^{-3}) from the sterile seedlings. Dias et al. (1998) present callus cultures creation from the stem segments.

According to our results it is evident, that the initiation of the callus formation is possible on the medium with 2,4-dichlorophenoxyacetic acid from the leaf or stem segments. The growth of callus cultures was observed on the medium supplemented with auxin and cytokinin (1 : 1); KIN (1 mg.l^{-1}) + 2,4-D (1 mg.l^{-1}), KIN (5 mg.l^{-1}) + 2,4-D (5 mg.l^{-1}), BAP (1 mg.l^{-1}) + 2,4-D (1 mg.l^{-1}), BAP (5 mg.l^{-1}) + 2,4-D (5 mg.l^{-1}). The necrosis of callus cultures were observed on the medium with 2,4-D (5 mg.l^{-1}).

The most intensive regeneration was observed on the medium supplemented with BAP (1 and 5 mg.l^{-1}), IBA (0,1 mg.l^{-1}) + BAP (5 mg.l^{-1}), IBA (0,1 mg.l^{-1}) + BAP (5 mg.l^{-1}) + adenin (80 mg.l^{-1}) and IBA (0,1 mg.l^{-1}) + KIN (5 mg.l^{-1}) + adenin (80 mg.l^{-1}). Čellárová (1997) found indirect regeneration from the green compact calluses on the medium with variable concentration of BAP.

References

BEŽO, M. 1995. Analýza fenotypovej variability explantátovývh kultúr pomocou počítačového zobrazenia a klasifikátora. In: Biotechnologické metódy v šľachtení rastlín. Zborník referátov zo IV. vedeckej konferencie BIOS′ 95. Nitra : VES VŠP v Nitre, 1995, s. 5-14.

BIZA, B. – MICHELITSCH, A. – WURGLICS, M. et al. 1999. Determination of hypericin in pharmaceutical preparations by diferential pulse polarography. In: http://www.biozentrum.uni-frankfurt.de/DPhG/doktorandentagung/abstracts/Biza.html. (1999-08-05)

BRUTOVSKÁ, R. – ČELLÁROVÁ, E. – DAVEY, M. R. et al. 1994. Stimulation of Multiple Shoot Regeneration from Seedling Leaves of Hypericum perforatum L. by Pluronic F-68. In: Acta Biotechnol. vol. 14, 1994, no. 4, p. 347-353.

BÜTER, B. – ORLACCHIO, C. – SOLDATI, A. et al. 1998. Significance of Genetic and Environmental Aspects in the Field Cultivation of Hypericum perforatum. In: Planta Medica, vol. 64, 1998, p. 431-437.

ČELLÁROVÁ, E. – BRUTOVSKÁ, R. – DAXNEROVÁ, Z. et al. 1997. Correlation between Hypericin Content and the Ploidity of Somaclones of Hypericum perforatum L. In: Acta Biotechnol. vol. 17, 1997, no. 1, p. 83-90.

ČELLÁROVÁ, E. – KIMÁKOVÁ, K. – DAXNEROVÁ, Z. et al. 1995. XIV Hypericum perforatum (St. John′s Wort): In vitro Culture and the Production of Hypericin and Other Secondary Metabolites. In: Biotechnology in Agriculture and Forestry, vol. 33, 1995, p. 261-274.

DIAS, A. C. P. – BARBERÁN, F. A. T. – FERREIRA, M. F. 1998. Unusual flavonoids produced by callus of Hypericum perforatum. In: Phytochemistry, vol. 48, 1998, no. 7, p. 1165-1168.

KARTNING, T. – GOBEL, I. – HEYDEL, B. 1996. Production of Hypericin, Pseudohypericin and Flavonoids in Cell Cultures of Various Hypericum Species and their Chemotypes. In: Planta Medica, vol. 62, 1996, p. 51-53.

MURASHIGE, J. – SKOOG, F. 1962. A revised medium for rapid growth and bis assay with tobacco tissue cultures. In: Physiol. Plant. 1962, p. 15.

ŠTEFÚNOVÁ, V. 2000. Kultivácia druhu ľubovník bodkovaný (*Hypericum perforatum* L.) *in vitro* : Diplomová práca. Nitra : SPU v Nitre, 2000. 65 s.

YAZAKI, K. – OKUDA, T. 1990. Procyanidins in callus and multiply shoot cultures of Hypericum erectum. In: Planta Med., vol. 56, 1990, p. 490-491.

http://www.admin.ch/bbw/abstracts/abstr-99/abstracts/cost/c97.0068.html (02.03.2001)