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Effect of Exogenous Morphogenetic Signals on Differentiation *in Vitro* and Secondary Metabolite Formation in the Genus *Hypericum*

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ABSTRACT

The use of *in vitro* culture of *Hypericum* spp. as an experimental system with prospective biotechnological application was triggered by discovering of new activities of hypericin and its derivatives. At the end of the 1990s it was evident that along with anti-depressive effects of *Hypericum* extracts, there are other very important activities such as anticancer and antiviral which rate the extract and/or some of the individual constituents to the leading herbal products in the world. From among the representatives of this extensive genus, complex research, including *in vitro* culture and biotechnology, was performed only with *H. perforatum.* Besides the knowledge we have from this model *Hypericum* species, there are some partial but promising results from other species of the genus which can be considered as candidates for further investigations and possible future application. This review summarises recent knowledge on some fundamental aspects on hormonal or hormone-like regulation of plantlet differentiation of *Hypericum* species *in vitro*, morphogenetic programmes leading to organogenesis, ways of enhancing biosynthesis of profiling secondary metabolites by plant growth substances and/or elicitors and ways of small-scale production of plantlets biomass. To-date results favour differentiated tissues for further studies of biosynthesis of secondary metabolites due to presence of morphological structures serving for their accumulation although promising studies were performed with dedifferentiated cell and callus cultures as well.

Keywords: embryoid, plant growth substances, shoot and root development

Abbreviations: **ADE**, adenine; **BAP**, 6-benzylaminopurine; **2,4-D**, 2,4-dichlorophenoxyacetic acid; **IAA**, indole-3-acetic acid; **IBA**, indole-3-butyric acid; **2iP**, 6-(γ, γ dimethylallylamino)-purine; **KIN**, kinetin; **NAA**, naphthalene-1-acetic acid; **RAPD**, random amplified polymorphic DNA; **TDZ**, thidiazuron

CONTENTS

INTRODUCTION ... 62
EFFECT OF EXOGENOUS SIGNALS ON MORPHOGENETIC RESPONSE AND BIOSYNTHETIC POTENTIAL OF *HYPERICUM* SPP. CELLS AND TISSUES CULTURED *IN VITRO* ... 63
Exogenous signals in shoot and root development ... 63
Regulation of dedifferentiation ... 63
Stimulation of biosynthetic potential of cell and tissue cultures by elicitors ... 64
PATTERNS OF *IN VITRO* REGENERATION IN THE GENUS *HYPERICUM* ... 66
SMALL-SCALE CULTURE OF DIFFERENTIATED STRUCTURES IN BIOREACTORS ... 66
FUTURE PROSPECTS ... 67
CONCLUSIONS ... 68
ACKNOWLEDGEMENTS ... 68
REFERENCES ... 68

INTRODUCTION

The genus *Hypericum* is the largest in the Hypericaceae family containing more than 460 species (Robson 2006) belonging to 30 sections. The species are characterized by an extensive infraspecific and interspecific variation regarding distribution, habit, morphology, way(s) of reproduction, basic chromosome number, ploidy level and potential to synthesize bioactive substances. Some *Hypericum* species are distributed worldwide but some are endemic. The habit of the species range from small herbs to about 10 m tall trees with an extensive morphological variation. Different *Hypericum* species reveal high plasticity of reproduction and coexistence of several ways of seed formation even on individual plant (Matzk *et al*. 2001, 2003). The basic chromosome number within the genus forms a descending series from 12 to 7 (possibly to 6) (Robson and Adams 1968). Tetraploidy has been recorded on the basic chromosome numbers x = 8, 9 and 10 but not on x = 7 or definitely on x = 12; and higher degrees of polyploidy are associated with the largely apomictic *H. perforatum* (2n = 32, 48) and its hybrid with *H. maculatum* (2n = 32, 40, 48) (Robson 1981). Great variation occurs also in ability to synthesize bio-active substances; some of them, namely hypericins, are unique in the plant kingdom. It is assumed that the representatives of about 60 percent of *Hypericum* sections are able to produce these secondary metabolites (Robson 2003) which contribute to wide spectrum of pharmacological effects. Besides the use of *Hypericum* species as valuable medicinal plants, some of them are also popular

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Invited Review

horticultural crops.

These all makes the genus a fascinating subject for biotechnological investigations. Most of the information we have on regulation of morphogenesis *in vitro* comes from *Hypericum perforatum* as a model with the initial studies in the 1990s (Čellárová *et al*. 1992; Zdunek and Alfermann 1992). As shown later, there are some other species with promising results for prospective biotechnological use (reviewed by Čellárová 2003). This review summarizes the current status of knowledge on the role of exogenous signals in morphogenesis and organogenesis in *Hypericum* spp. under *in vitro* conditions.

EFFECT OF EXOGENOUS SIGNALS ON MORPHOGENETIC RESPONSE AND BIOSYNTHETIC POTENTIAL OF *HYPERICUM* SPP. CELLS AND TISSUES CULTURED *IN VITRO*

Exogenous signals in shoot and root development

Phytohormones and their synthetic analogues represent the most important exogenous factors in regulation of morphogenesis *in vitro*. Cytokinins are involved in regulation of many aspects of plant development. It is evident that their resulting effect depends on many other signals, hormonal and environmental, perceived by plant cell (D´Agostino and Kieber 1999; Brault and Maldiney 1999). Cytokinins are required for proliferative shoot apical meristem activity and are known to promote outgrowth of dormant axillary buds (reviewed by Werner and Schmülling 2009). Some exogenously added cytokinins and/or their synthetic analogues were repeatedly proved to be effective in multiple shoot apical meristems development in several representatives of the genus *Hypericum*. 6-benzylaminopurine (BAP) alone in concentration ranging between 10^{-7} to 10^{-4} M or in combination with low auxin concentrations were effective for shoot differentiation from various explants of *H. perforatum* (Čellárová *et al.* 1992; Zdunek and Alfermann 1992; McCoy and Camper 2002; Santarém and Astarita 2003; Gadzovska *et al*. 2005; Franklin and Dias 2006; Karppinen *et al*. 2007) *H. erectum* (Yazaki and Okuda 1990), *H. canariense* (Mederos *et al*. 1997), *H. foliosum* (Moura 1998), *H. bupleuroides* (Çirak *et al*. 2007), *H. triquetrifolium* (Karakas *et al*. 2009; Namli *et al*. 2009), *H. maculatum*, *H. annulatum*, *H. tomentosum*, *H. monogynum*, *H. kalmianum* and *H. canariense* (Čellárová, unpubl.) (**Fig. 1B, D, G, J, L, M**). BAP or meta-topolin treatments successfully induced callus capable of regeneration and shoot differentiation in *Hypericum* sp. hybrid (Meyer *et al*. 2009). In addition to the morphogenetic role of BAP, several reports indicate that manipulation with concentration of this cytokinin in the culture medium can optimize hypericin production by shoot cultures of hypericin-producing *Hypericum* species *in vitro* (Gadzovska *et al*. 2005; Karakas *et al*. 2009). Smith *et al*. (2002) found that cytokinin supplementation of shoot culture medium resulted in a proliferation of abundant leaf glands with enhanced levels of hypericin as compared to *H. perforatum* controls. Increasing levels of BAP in *H. perforatum* shoot cultures stimulated also formation of another bioactive compound hyperforin (Charchoglyan *et al*. 2007). On the contrary, Murch *et al*. (2000) did not find BAP effective for *in vitro* regeneration of shoots from hypocotyl explants of *H. perforatum*. They developed an effective regeneration system using thidiazuron (TDZ) supplementation followed by culture on basal medium. These authors provided later the first evidence about the physiological role of mammalian neurohormones melatonin and serotonin in morphogenesis of *H. perforatum*. Increased endogenous levels of serotonin corresponded with increased rate of shoot formation and elevated endogenous levels of melatonin correlated with *de novo* root formation (Murch *et al*. 2001). High TDZ concentrations were effective not only for enhancing the number of clustering shoots in *H. perforatum* but also for hypericin and pseudohypericin production (Liu *et al.* 2007). TDZ had no positive effect on hyperforin accumulation (**Table 1**). For stem explants of *H. humifusum* and *H. canariense* 2iP exhibited an inductive effect on multiple shoot formation (Čellárová, unpubl.) (**Fig. 1C, K**).

Root formation in *in vitro* cultures occurs either spontaneously due to sufficient endogenous auxin level or can be induced by exogenously supplied culture medium with auxins or their synthetic analogues. They regulate diverse responses in plant development. Large number of auxin-regulated genes is involved in transcription regulation or RNA metabolism through a complex network (Huang *et al*. 2008). The roots in *Hypericum* species cultured *in vitro* differentiate on basal medium lacking auxins, on half-strength medium with or without auxins or under effect of exogenously added indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) or naphthalene-1-acetic acid (NAA) as reported for *H. perforatum* (Čellárová and Kimáková 1999; Pasqua *et al*. 2003; Zobayed and Saxena 2003; Wojcik and Podstolski 2007; Goel *et al*. 2009), *H. bupleuroides* (Çirak *et al*. 2007) or *H. canariense* (Mederos Molina 1991) (**Table 1**). Exogenous supplementation of culture media with auxins or auxinoids does not always lead to root formation only. While root culture of *H. tomentosum* derived from root cuttings on MS medium with IAA promotes proliferation of adventitious roots, in other species like *H. maculatum, H. annulatum or H. pulchrum* the effect of exogenously added IAA or IBA resulted in root proliferation from which multiple shoot differentiated spontaneously without cytokinin supplementation (Čellárová, unpubl.) (**Fig. 1A, E, F, H, I**).

In all these experiments different plant parts were used for shoot or root differentiation and multiplication. From among the explants most of investigators favour root cuttings as the most responsive to exogenous morphogenetic signals. Franklin and Dias (2006) concluded that regeneration response in *H. perforatum* is clearly plant growth regulator-driven and explants-dependent phenomenon.

Regulation of dedifferentiation

Most of data on isolation of callus and cell suspension cultures within the genus *Hypericum* come from *H. perforatum* and some other representatives able to synthesize valuable substances. For *H. perforatum* in most cases supplementation of media with either a balanced level of auxins and cytokinins or auxins, especially 2,4-dichlorophenoxyacetic acid (2,4-D) and NAA exceeding cytokinin content was beneficial for callus induction and proliferation (Čellárová and Kimáková 1999; Pretto and Santarem 2000; Bais *et al*. 2002; Pasqua *et al*. 2003). Surprisingly, Gadzowska *et al*. (2005) isolated friable callus from *H. perforatum* on the medium with lower concentrations of cytokinin BAP ranging between 4.4 to 8.9 μM. These results indicate a crucial role of genotype in type of morphogenetic response within a particular species. Higher concentrations up to 22.2 μM led to compact callus formation. These compact calli were subjected to analyses of the content of hypericin and pseudohypericin. While the content of the former remained unchanged, the latter was affected by BAP. Induction effect on callus proliferation by 2,4-D was published also for *H. brasiliense* (Cardoso and Oliviera 1996) and its combination with NAA for *H. patulum* (Ishiguro *et al*. 1999). Kartnig *et al*. (1996) reported on the isolation of callus and cell suspension cultures of seven *Hypericum* species (*H. maculatum*, *H. tomentosum*, *H. bithynicum*, *H. glandulosum*, *H. balearicum*, *H. olympicum* and *H. perforatum*) under the same culture conditions using BAP and NAA with an aim to ascertain the ability to accumulate some secondary metabolites, especially hypericin and pseudohypericin and flavonoids (**Tables 1, 2**). Although these findings indicate that even undifferentiated cell cultures contain hypericins which are accumulated and/or synthesized *in planta* in specialized dark gland structures, their continuous production by cell cultures where these structures are absent is questionable.

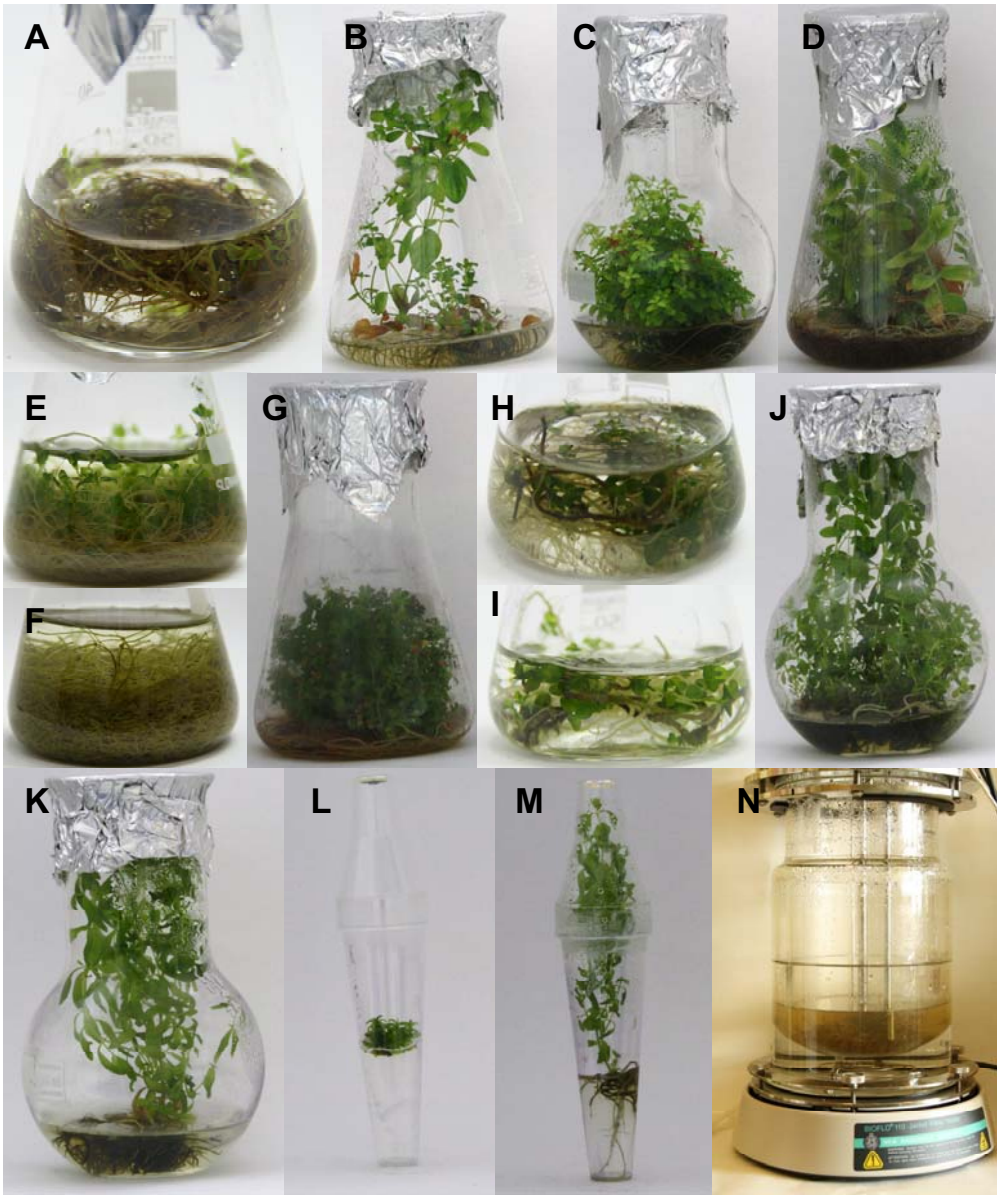

Fig. 1 (**A**) *Hypericum maculatum* L. root explants cultured in liquid MS medium supplemented with 1 mg.l^{-1} IBA. (**B**) *Hypericum maculatum* L. root explants cultured in liquid MS medium supplemented with 1 mg.l^{-1} BAP. (**C**) *Hypericum humifusum* L. stem cuttings cultured on MS medium supplemented with 1 mg.l^{-1} 2iP. (**D**) *Hypericum annulatum* Morris.: morphogenetic response of root explants cultured on MS medium supplemented with 1 mg.l^{-1} BAP. (**E**) *Hypericum annulatum* Morris.: morphogenetic response of stem explants cultured in liquid MS medium supplemented with 0.1 mg.l^{-1} IBA. (**F**) *Hypericum tomentosum* L.: root culture derived from root cuttings cultured in liquid MS medium supplemented with 0.1 mg.l^{-1} IAA. (**G**) *Hypericum tomentosum* L.: plants regenerated from leaf explants cultured on MS medium with 1.0 mg.l^{-1} BAP. (**H**) *Hypericum pulchrum* L.: root explants cultured in liquid MS medium with 1.0 mg.l^{-1} IAA. (**I**) *Hypericum pulchrum* L.: root explants cultured in liquid MS medium with 1.0 mg.l^{-1} IBA. (**J**) *Hypericum monogynum* L.: regeneration from stem explants cultured on MS medium with 0.1 mg.l^{-1} BAP. (**K**) *Hypericum canariense* L.: regeneration from stem explants cultured on MS medium with 0.1 mg.l^{-1} 2iP. (**L**) *Hypericum kalmianum* L.: beginning of regeneration from leaf explants cultured on MS medium with 1.0 mg.l^{-1} BAP. (**M**) *Hypericum canariense* L.: regeneration from leaf explants cultured on MS medium with 1.0 mg.l^{-1} BAP. (**N**) *Hypericum tomentosum* L.: root culture in MS hormone-free medium in bioreactor. All photos: Matúš Skyba.

Stimulation of biosynthetic potential of cell and tissue cultures by elicitors

The exogenous application of biotic or chemical elicitors to the cell cultures is used for enhancement of biotechnological production of secondary metabolites. The first report showing the stimulatory effect of mannan, an elicitor from yeast on hypericin production by shoot cultures of *H. perforatum* was published by Kirakosyan *et al*. (2000b). Later, Bais *et al*. (2002) examined the effect of jasmonic acid, salicylic acid and fungal cell wall elicitors from *Phytophtora cinnamoni* on production of hypericins by *H. perforatum* cell cultures. They found that only jasmonic acid stimulated growth of cells under dark conditions and increased accumulation of hypericin in the cells grown in the dark compared to elicited cell cultures grown in the light and their respective controls. Charchoglyan *et al*. (2007) have proved that elicitation by jasmonic acid, methyl jasmonate and mannan did not trigger accumulation of hyperforin in *H. perforatum* calli but stimulated accumulation of hyperforin and secohyperforin in morphogenic cultures due to differentiation of translucent glandular structures as accumulation sites which are not present in callus cultures. Stimulating effect of methyl jasmonate and its analogue 2, 3-dihydroxypropyl jasmonate on production of hypericins and hyperforin in microshoots of *H. perforatum* and *H. sampsonii* was recorded by Liu *et al*. (2007) although the former inhibited biomass production especially in *H. perforatum*. Along with stimulating effect of methyl jasmonate on the production of hypericin and hyperforin, Pavlik *et al*. (2007) used inactivated culture of *Agrobacterium tumefaciens* which exhibited similar effect (**Table 3**).

Table 1 Morhogenetic response of some *Hypericum* species on PGRs (arranged according to alphabetical order of the species).

Species	Culture medium	Growth regulators	Physical conditions	Morphogenetic response	Reference
H. androsaemum	MS	4.57 × 10^{-6} M IAA and 2.32 × 10^{-6} M KIN	16-h photoperiod, 52 µmol.m^{-2}.s^{-1}, 24 ± 2ºC	shoots	Guedes *et al*. 2003
H. balearicum	½ MS	10^{-6} M BAP and 10^{-7} M NAA	continuous light 27 µmol.m^{-2}.s^{-1} 24 ± 2ºC	callus, cell cultures	Kartnig *et al.* 1996
H. bithynicum	½ MS	10^{-6} M BAP and 10^{-7} M NAA	continuous light 27 µmol.m^{-2}.s^{-1} 24 ± 2ºC	callus, cell cultures	Kartnig *et al*. 1996
H. brasiliense	MS	none		multiple shoots	Cardoso and de Oliveira 1996
H. brasiliense	MS/B5	4.5 – 9.0 × 10^{-6} M 2,4-D		callus	Cardoso and de Oliveira 1996
H. canariense	MS	BAP and NAA	16-h photoperiod, 20 µmol.m^{-2}.s^{-1} 24ºC	shoots	Mederos Molina 1991
H. canariense	½ MS	IBA or NAA	16/8 h photoperiod, 20 µmol.m^{-2}.s^{-1} 24ºC	roots	Mederos Molina 1991
H. canariense	MS, B5, WPM, QL.4	10^{-7} M BAP and 10^{-8} M NAA	16-h photoperiod, 20 µmol.m^{-2}.s^{-1}, 24 ºC	shoots	Mederos *et al*. 1997
H. erectum	LS	10^{-5} M BAP and 10^{-5} M IAA	dark, 25ºC	callus	Yazaki and Okuda 1990, 1994
H. erectum	LS	10^{-5} M BAP and 10^{-5} M IAA	dark, 25ºC	shoot primordia	Yazaki and Okuda 1990, 1994
H. erectum	LS	10^{-5} M BAP and 10^{-5} M IAA	dark, 25ºC	etiolated multiple shoots	Yazaki and Okuda 1990, 1994
H. erectum	LS	10^{-5} M BAP and 10^{-5} M IAA	12-h photoperiod, 81 µmol.m^{-2}.s^{-1} 25ºC	green multiple shoots	Yazaki and Okuda 1990, 1994
H. frondosum	MS	5 × 10^{-6} M BAP or meta topolin and 3.75 × 10^{-6} M IAA	dark, 23 ± 2ºC	regenerative callus and shoots	Meyer *et al*. 2009
H. galioides	MS	5 × 10^{-6} M BAP or meta topolin and 3.75 × 10^{-6} M IAA	dark, 23 ± 2ºC	regenerative callus and shoots	Meyer *et al*. 2009
H. glandulosum	½ MS	10^{-6} M BAP and 10^{-7} M NAA	continuous light 27 µmol.m^{-2}.s^{-1} 24 ± 2ºC	callus, cell cultures	Kartnig *et al*. 1996
H. kalmianum	MS	5 × 10^{-6} M BAP or meta topolin and 3.75 × 10^{-6} M IAA	dark, 23 ± 2ºC	regenerative callus and shoots	Meyer *et al*. 2009
H. maculatum	½ MS	10^{-6} M BAP and 10^{-7} M NAA	continuous light 27 µmol.m^{-2}.s^{-1} 24 ± 2ºC	callus, cell cultures	Kartnig *et al*. 1996
H. maculatum	MS	2.46 x 10^{-6} M 2iP and 0.89 x 10^{-6} M BAP and 2.69 x 10^{-7} M NAA	16-h photoperiod, 25 µmol.m^{-2}.s^{-1} 25 ± 1ºC	multiple shoots	Bacila *et al.* 2010
H. maculatum	MS	5.71 x 10^{-6} M IAA	16-h photoperiod, 35 µmol.m^{-2}.s^{-1} 25 ± 1ºC	elongating shoots and rooting	Bacila *et al.* 2010
H. olympicum	½ MS	10^{-6} M BAP and 10^{-7} M NAA	continuous light 27 µmol.m^{-2}.s^{-1} 24 ± 2ºC	callus, cell cultures	Kartnig *et al*. 1996
H. perforatum	LS	4.4 × 10^{-7} - 4.4 × 10^{-6} M BAP	16-h photoperiod, 65 µmol.m^{-2}.s^{-1} 25-29ºC	green multiple shoots	Čellárová *et al*. 1992, 1994
H. perforatum	LS	4.6 × 10^{-7} - 2.3 × 10^{-6} M KIN	16-h photoperiod, 6.75 µmol.m^{-2}.s^{-1} 25-29ºC	shoots	Čellárová *et al*. 1992
H. perforatum	LS	4.9 × 10^{-7} - 2.5 × 10^{-6} M 2iP	16-h photoperiod, 6.75 µmol.m^{-2}.s^{-1} 25-29ºC	shoots	Čellárová *et al*. 1992
H. perforatum	LS	4.6 × 10^{-6} M KIN and 5.4 × 10^{-7} M NAA	16-h photoperiod, 65 µmol.m^{-2}.s^{-1} 25-29ºC	green multiple shoots	Čellárová *et al*. 1992
H. perforatum	½ MS	10^{-6} - 10^{-5} M BAP and 10^{-7} - 10^{-6} M IAA	continuous light 40 µmol.m^{-2}.s^{-1} or continuous dark 26ºC	shoots	Zdunek and Alfermann 1992
H. perforatum	½ MS	10^{-6} M BAP and 10^{-7} M NAA	continuous light 27 µmol.m^{-2}.s^{-1} 24 ± 2ºC	callus, cell cultures	Kartnig *et al*. 1996
H. perforatum	LS	0.57 × 10^{-6} – 10^{-4} M IAA	16-h photoperiod, 25 µmol.m^{-2}.s^{-1} 22ºC	roots	Čellárová and Kimáková 1999
H. perforatum	LS	0.49 × 10^{-6} – 10^{-4} M IBA	16-h photoperiod, 25 µmol.m^{-2}.s^{-1} 22ºC	roots	Čellárová and Kimáková 1999
H. perforatum	LS	0.45 × 10^{-6} – 10^{-4} M 2,4-D	16-h photoperiod, 25 µmol.m^{-2}.s^{-1} 22ºC	callus	Čellárová and Kimáková 1999
H. perforatum	LS	0.54 × 10^{-6} – 10^{-4} M NAA	16-h photoperiod, 25 µmol.m^{-2}.s^{-1} 22ºC	callus	Čellárová and Kimáková 1999
H. perforatum	LS	0.44 × 10^{-6} – 10^{-4} M BAP	16-h photoperiod, 25 µmol.m^{-2}.s^{-1} 22ºC	multiple shoots	Čellárová and Kimáková 1999
H. perforatum	LS	0.49 × 10^{-6} – 10^{-4} M 2iP	16-h photoperiod, 25 µmol.m^{-2}.s^{-1} 22ºC	shoots	Čellárová and Kimáková 1999
H. perforatum	LS	0.46 × 10^{-6} – 10^{-4} M KIN	16-h photoperiod, 25 µmol.m^{-2}.s^{-1} 22ºC	multiple shoots	Čellárová and Kimáková 1999
H. perforatum	LS	0.74 × 10^{-6} – 10^{-4} M ADE	16-h photoperiod, 25 µmol.m^{-2}.s^{-1} 22ºC	shoots	Čellárová and Kimáková 1999
H. perforatum	MS	5 × 10^{-6} M TDZ	16-h photoperiod, 40-60 µmol.m^{-2}.s^{-1} followed by 30-35 µmol.m^{-2}.s^{-1} 24ºC	shoots	Murch *et al*. 2000
H. perforatum	MS	0.44 × 10^{-6} – 10^{-5} M BAP	continuous light, 30 µmol.m^{-2}.s^{-1} 26 ± 2ºC	shoots	Pretto and Santarém 2000
H. perforatum	MS	0.45 × 10^{-6} – 10^{-5} M 2,4-D	continuous dark or light, 30 µmol.m^{-2}.s^{-1} 26 ± 2ºC	callus	Pretto and Santarém 2000
H. perforatum	MS	0.46 × 10^{-6} – 10^{-5} M KIN	continuous dark or light, 30 µmol.m^{-2}.s^{-1} 26 ± 2ºC	callus	Pretto and Santarém 2000
H. perforatum	MS	0.49 × 10^{-5} M IBA	continuous light, 30 µmol.m^{-2}.s^{-1} 26 ± 2ºC	roots	Pretto and Santarém 2000

Table 1 (Cont.)

Species	Culture medium	Growth regulators	Physical conditions	Morphogenetic response	Reference
H. perforatum	MS	10^{-6} M BAP and 10^{-7} M NAA	continuous illumination 40 μmol.m^{-2}.s^{-1} 26 ± 1ºC	callus	Kirakosyan *et al*. 2000a
H. perforatum	MS	0.9 × 10^{-6} M 2,4-D and 0.1 × 10^{-6} M KIN	dark, 25 ± 1ºC	callus	Bais *et al*. 2002
H. perforatum	MS	5.0 × 10^{-6} M IAA or IBA	16/8 h photoperiod, 20-25 μmol.m^{-2}.s^{-1} 24ºC	roots	Zobayed and Saxena 2003
H. perforatum	MS	5.0 × 10^{-6} M TDZ	16-h photoperiod, 20 μmol.m^{-2}.s^{-1} 24ºC	multiple shoots	Zobayed and Saxena 2003
H. perforatum	MS	4.5 × 10^{-6} M BAP and 5.0 × 10^{-8} M NAA	16-h photoperiod, 30 μmol.m^{-2}.s^{-1} 25 ± 2ºC	multiple shoots	Santarém and Astarita 2003
H. perforatum	MS	0.44 – 8.90 × 10^{-6} M BAP	16-h photoperiod, 50 μmol.m^{-2}.s^{-1} 26 ± 1ºC	shoots	Gadzovska *et al*. 2005
H. perforatum	MS	14.8 - 22.2 × 10^{-6} M BAP	16-h photoperiod, 50 μmol.m^{-2}.s^{-1} 26 ± 1ºC	callus	Gadzovska *et al*. 2005
H. perforatum	MS	2.2 × 10^{-6} M BAP and 5.7 × 10^{-6} M IAA	16-h photoperiod, 25 μmol.m^{-2}.s^{-1} 25ºC	shoots	Franklin and Dias 2006
H. perforatum	MS	8.8 × 10^{-6} M BAP and 2.85 × 10^{-6} M IAA	16-h photoperiod, 25 μmol.m^{-2}.s^{-1} 25ºC	callus	Franklin and Dias 2006
H. perforatum	MS or B5	5.8 × 10^{-6} M 2,4-D and 1.34 × 10^{-6} M NAA	16-h photoperiod, 70 μmol.m^{-2}.s^{-1} 26 ± 1ºC	proembryogenic mass	Pasqua *et al*. 2008
H. perforatum	MS or B5	none	16-h photoperiod, 70 μmol.m^{-2}.s^{-1} 26 ± 1ºC	embryoids developed from proembryogenic mass	Pasqua *et al*. 2008
H. perforatum	MS	3.0 × 10^{-6} M TDZ and 2.0 × 10^{-6} M IBA	16-h photoperiod, 70 μmol.m^{-2}.s^{-1} 26 ± 1ºC	shoots	Pasqua *et al*. 2008
H. perforatum	MS	19.7 × 10^{-6} M IBA or 22.8 × 10^{-6} M IAA	14-h photoperiod, 20 μmol.m^{-2}.s^{-1} 25 ± 2ºC	roots	Goel *et al*. 2009
H. perforatum	MS	9.3 × 10^{-6} M KIN	14-h photoperiod, 20 μmol.m^{-2}.s^{-1} 25 ± 2ºC	shoots	Goel *et al*. 2009
H. perforatum	½ MS	0.46 × 10^{-6} M KIN and 4.9 × 10^{-6} IBA	dark, 25 ± 1ºC, bioreactor airflow rate 400 ml/min	adventitious roots	Cui *et al*. 2010
H. retusum	MS	2.22 × 10^{-6} M BAP	16-h photoperiod, 40 μmol.m^{-2}.s^{-1} 25 ± 2ºC	shoots	Namli *et al*. 2010
H. tomentosum	½ MS	10^{-6} M BAP and 10^{-7} M NAA	continuous light 27 μmol.m^{-2}.s^{-1} 24 ± 2ºC	callus, cell cultures	Kartnig *et al*. 1996
H. triquetrifolium	MS	5.67 × 10^{-6} M TDZ and 2.85 × 10^{-6} M IAA	16-h photoperiod, 54 μmol.m^{-2}.s^{-1} 27 ± 4ºC	shoots	Oluk and Orhan 2009
H. triquetrifolium	MS	5.71 × 10^{-6} M IAA	16-h photoperiod, 54 μmol.m^{-2}.s^{-1} 27 ± 4ºC	roots	Oluk and Orhan 2009
H. triquetrifolium	MS	8.90 × 10^{-6} M BAP	continuous light, 25 ± 2ºC	shoots	Karakas *et al*. 2009

MS - Murashige-Skoog culture medium (1962); LS - Linsmaier-Skoog culture medium (1965); B5 - Gamborg culture medium (1968); WPM - Lloyd-McCown culture medium (1981); QL.4 – (Mederos 1981)

PATTERNS OF *IN VITRO* REGENERATION IN THE GENUS *HYPERICUM*

Regeneration of plants from *in vitro* cultured explants can proceed in two specific ways, from either unipolar or bipolar structures. Exogenous signals promoting shoot and root differentiation are described in more detail above. Here I focus mainly on somatic embryogenesis. The first note on somatic embryogenesis came from the paper of Franklin and Dias (2006) who tried to elucidate the specific pathways of regeneration in cultures originated from root explants of *H. perforatum* on culture medium supplemented with BAP and IAA. They found that regeneration can occur by both, organogenesis and somatic embryogenesis. The shoots were initiated from globular structures attached to the explants while somatic embryos developed from those detached from the explants. However, the cotyledonary embryos failed to establish root system. Later, Pasqua *et al*. (2008) obtained somatic embryos and normal shoots from leaf-derived callus as a result of separate morphogenetic programmes. While the former were obtained on medium supplemented with 2,4-D, NAA and kinetin (KIN), the latter differentiated on the medium supplemented with TDZ combined with IBA. However, the efficiency of embryos development remained very low. Early-stage embryo-like structures were observed also in callus cultures of Balkan endemic *H. rumeliacum* cultured on medium supplemented with BAP and NAA (Danova *et al*. 2010). These preliminary results indicate that some *Hypericum* species possess endogenous potential for different morphogenetic programmes but proper exogenous signals for normal embryo conversion should be uncovered.

SMALL-SCALE CULTURE OF DIFFERENTIATED STRUCTURES IN BIOREACTORS

The use of large-scale cultures for production of uniform and high-yielding plants requires optimization of culture system for proliferation of explants followed by shoot regeneration. The first attempt to develop an optimized protocol for the *in vitro* multiplication of *H. perforatum* in bioreactor was made by Zobayed and Saxena (2003). Based on test of regeneration potential of different tissues they selected root explants for bioreactor culture which regenerated *de novo* shoots under the effect of TDZ. More than 95% of the regenerated plantlets were successfully adapted to greenhouse conditions. Similar approach for the same species was recently reported by Goel *et al*. (2009). The authors used culture of adventitious roots regenerated from shoot-derived callus on medium supplemented with IAA and IBA. Shoots proliferated from excised roots on medium supplemented with KIN which was even more efficient than BAP or TDZ. These authors assessed clonal fidelity of regenerated plants by RAPD analysis and found that more than 50% of the assessed regions were polymorphic. Taking into account that only small part of the genome is assessed by the RAPD primers, the micropropagated plants can be considered as variable. However, variability is a common feature of *H. perforatum* grown in natural habitats. The *in vitro* culture can contribute to some extent to this physiological variation. An opposite approach for *H. perforatum* was recently applied by Cui *et al*. (2010). They used adventitious roots originated from leaf explants on MS medium supplemented with IAA for culture in a balloon type bubble bioreactor. The biomass of adventitious roots which were cultured in a bioreactor under optimised culture conditions,

Table 2 Production of secondary metabolites by different cultures of some *Hypericum* species (arranged according to alphabetical order of the species).

Species	Type of culture	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	Reference
H. androseamum	cell suspensions															+							Dias *et al*. 2000
	calli																						
H. androseamum	shoot cultures																			+			Guedes *et al*. 2003
H. balearicum	cell cultures						+	+	+	+	+	-	+	+	+								Kartnig *et al*. 1996
H. bithynicum	cell cultures						+	+	+	+	+	+	+	+	+								Kartnig *et al*. 1996
H. erectum	callus	+	+	+	+	+	t																Yazaki and Okuda 1990, 1994
H. erectum	shoot primordia	+	+	+	+	+	t																Yazaki and Okuda 1990, 1994
H. erectum	etiolated multiple shoots	+	+	+	+	+	t																Yazaki and Okuda 1990, 1994
H. erectum	green multiple shoots	+	+	+	+	+	+																Yazaki and Okuda 1990, 1994
H. glandulosum	cell cultures						+	+	+	+	+	+	+	+	+								Kartnig *et al*. 1996
H. maculatum	cell cultures						+	+	+	+	+	+	+	+	+								Kartnig *et al*. 1996
H. olympicum	cell cultures						-	-	-	-	-	-	+	-	-								Kartnig *et al*. 1996
H. patulum	cell suspension cultures															+							Ishiguro *et al*. 1996, 1999
H. perforatum	shoot cultures													+	+								Zdunek and Alfermann 1992
H. perforatum	cell cultures						+	+	+	+	+	+	+	+	+								Kartnig *et al*. 1996
H. perforatum	callus																+						Dias *et al*. 1998
H. perforatum	cell cultures															+							Dias *et al*. 1999, 2000
H. perforatum	cell cultures													t	t								Kirakosyan *et al*. 2000a
H. perforatum	shoot cultures													+	+								Kirakosyan *et al*. 2000a
H. perforatum	cell cultures													+									Bais *et al*. 2002
H. perforatum	callus													t									Santarém and Astarita 2003
	shoots													+									
	plantlets													+									
H. perforatum	cell cultures													+	+			+					Kirakosyan *et al.* 2004
	callus cultures													+	+			+					
	shoot cultures													+	+			+					
H. perforatum	shoots													+	+								Gadzovska *et al*. 2005
	calli													+	+								
	plantlets													+	+								
H. perforatum	shoot cultures																	+					Charchoglyan *et al.* 2007
H. perforatum	shoot cultures													+	+								Kornfeld *et al*. 2007
H. perforatum	calli															+							Mulinacci *et al*. 2008
	regenerated shoots															+							
H. perforatum	adventitious roots													+							+	+	Cui *et al*. 2010
H. perforatum	cell suspensions																		+				Piekoszewska *et al*. 2010
H. tomentosum	cell cultures						+	-	-	-	-	-	+	+	+								Kartnig *et al*. 1996
H. triquetrifolium	shoot cultures													+									Karakas *et al*. 2009

t trace; + secondary metabolite present; - secondary metabolite not present; 1 (-)-epicatechin; 2 procyanidin B2; 3 procyanidin C1; 4 cinnamtannin A2; 5 hyperin; 6 quercitrin; 7 rutin; 8 hyperoside; 9 isoquercitrin; 10 quercetin; 11 I3,II8-biapigenin; 12 amentoflavone; 13 hypericin; 14 pseudohypericin; 15 xanthone; 16 luteolin derivatives; 17 hyperforin and derivatives; 18 arbutin; 19 essential oil; 20 flavonoids; 21 phenolics

i.e. half strength MS medium supplemeted with KIN and IBA, increased over a 5 week subculture almost 18-fold. Moreover, the roots were capable of synthesis not only flavonoids, phenolics and chlorogenic acid but also hypericin in surprisingly high amount (**Table 2**). An example of biorector culture of *H. tomentosum* adventitious roots derived from root cuttings on MS medium supplemented with IAA and further cultured in MS hormone-free medium is shown in **Fig. 1N** (Čellárová, unpubl.).

FUTURE PROSPECTS

The genus *Hypericum* is, as to the number and variability of species, very extensive. Despite that there are only few representatives that have been successfully introduced into *in vitro* culture. Almost all we know about exogenous stimuli taking part in regulation of morphogenetic responses comes from *H. perforatum* which can be considered as a model. The study of *H. perforatum* in detail including *in vitro* culture and biotechnology was triggered by significant progress in the field of a study the photodynamic activity of hypericin and its derivatives with potential use in photodynamic therapy of cancer. Along with these naphthodianthrones, it is evident that there are other constituents such as phloroglucinols and flavonoids which contribute to the synergic effect of the *Hypericum* extract. Many of the fundamental aspects such as biosynthesis of these valuable constituents, association of the bio-active substances with unique morphological structures, genes and the respective enzymes involved in biosynthetic pathway and others remain partially or completely uncovered so far.

Introduction of other *Hypericum* species into *in vitro* culture, especially those with qualitative differences in biosynthetic potential, determination of exogenous signals needed for differentiation and/or dedifferentiation, production of biomass of plants and/or embryoids rather than cell cultures due to presence of special morphological structures for accumulation/biosynthesis of the valuable secondary metabolites seems to be essential for further progress in this area.

Search for genes coding for key enzymes of the biosynthetic pathways leading to formation of valuable compounds and knowledge on their regulation are an essential prerequisites for their potential biotechnological production. For studying the gene function and manipulating the host genome an efficient transformation system is required.

Table 3 Stimulation of secondary metabolites synthesis in *Hypericum* species by elicitors.

Species	Type of elicitor	Type of culture	Effect on product	Reference
H. perforatum	mannan	shoot cultures	stimulation of hypericin and pseudohypericin production	Kirakosyan *et al*. 2000b
H. perforatum	yeast extract	shoot cultures	inhibition of hypericin and pseudohypericin production	Kirakosyan *et al*. 2000b
H. perforatum	β-1,3,-glucan	shoot cultures	lower stimulation of pseudohypericin production, no effect on hypericin content	Kirakosyan *et al*. 2000b
H. perforatum	pectin	shoot cultures	lower stimulation of pseudohypericin production, no effect on hypericin content	Kirakosyan *et al*. 2000b
H. perforatum	jasmonic acid	cell suspension cultures	increased hypericin content under dark conditions	Walker *et al.* 2002
H. perforatum	salicylic acid	cell suspension cultures	no stimulatory effect	Walker *et al.* 2002
H. perforatum	fungal cell wall elicitors from *Phytophthora cinnamoni*	cell suspension cultures	no stimulatory effect	Walker *et al.* 2002
H. perforatum	methyl jasmonate, inactivated *A. tumefaciens* culture	plantlets	Stimulation of hypericin and hyperforin production	Pavlik *et al.* 2007
H. perforatum	methyl jasmonate	micro-shoots	enhanced production of hypericin, pseudohypericin and hyperforin	Liu *et al*. 2007
H. perforatum	2,3-dihydroxypropyl jasmonate	micro-shoots	enhanced production of hypericin, pseudohypericin and hyperforin	Liu *et al*. 2007
H. sampsonii	methyl jasmonate	micro-shoots	enhanced production of hypericin, pseudohypericin and hyperforin	Liu *et al*. 2007
H. sampsonii	2,3-dihydroxypropyl jasmonate	micro-shoots	enhanced production of hypericin, pseudohypericin and hyperforin	Liu *et al*. 2007

Those we have at present are either not sufficiently reproducible or lack regeneration capability of complete plants. Along with candidate genes involved in the pathways of bio-active substances, there is a need for prospective manipulation of apomixis genes; some *Hypericum* species may serve as a suitable host for such studies.

Despite all these limitations, there is a chance to develop tissue or cell culture systems for commercial production. Among them a continuous biomass production of high-yielding *Hypericum* spp. tissues and organs or embryoids for making artificial seeds can be taken into account. Further development of effective cryopreservation protocols for *Hypericum* spp. would be very helpful in establishment of gene bank of elite genotypes.

CONCLUSIONS

This review attempts to summarize the results of biotechnology of *Hypericum* spp. achieved especially over the last decade. From these results it can be concluded:

i) Despite positive achievements on regulation of morphogenesis and organogenesis in some *Hypericum* species, especially for *H. perforatum*, the number of other representatives of the genus containing valuable compounds which were successfully introduced into *in vitro* culture so far with an aim of future biotechnological production is still limited;
ii) In general, cytokinins and cytokinin-like compounds have been proved as effective not only for shoot induction and proliferation but indirectly also for production of hypericins;
iii) It is likely that in isolation of cell and callus cultures genotype has a decisive role; for different genotypes diverse exogenous hormone-like signals were inductive;
iv) Elicitation as a mean of increasing secondary metabolite biosynthesis seems to be more applicable for differentiated aerial plant parts than for cell suspension cultures or calli;
v) In *H. perforatum*, and, possibly in some other species as well, differentiation of plantlets *in vitro* can be realized either via shoot or root meristemoid or somatic embryos formation although the conditions for complete conversion of the latter into plantlets failed;
vi) All these results are promising for their prospective use in small-scale production of plantlets in bioreactor.

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REFERENCES

Bacila I, Coste A, Halmagyi A, Deliu C (2010) Micropropagation of *Hypericum maculatum* Cranz, an important medicinal plant. *Romanian Biotechnological Letters* **15**, 86-91

Bais HP, Walker TS, McGrew JJ, Vivanco JM (2002) Factors affecting growth of cell suspension cultures of *Hypericum perforatum* L. (St. John's wort) and production of hypericin. *In Vitro Cellular and Developmental Biology – Plant* **38**, 58-65

Brault M, Maldiney R (1999) Mechanisms of cytokinin action. *Plant Physiology and Biochemistry* **37**, 403-412

Cardoso NA, de Oliviera DE (1996) Tissue culture of *Hypericum brasiliense* Choisy: Shoot multiplication and callus induction. *Plant Cell, Tissue and Organ Culture* **44**, 91-94

Čellárová E (2003) Culture and biotechnology of *Hypericum*. In: Ernst E (Ed) *Hypericum. The Genus Hypericum*, Taylor and Francis, London and New York, pp 65-76

Čellárová E, Kimáková K, Brutovská R (1992) Multiple shoot formation and somaclonal variation of regenerants in *Hypericum perforatum* L. *Acta Biotechnologica* **12**, 445-452

Čellárová E, Daxnerová Z, Kimáková K, Halušková J (1994) The variability of hypericin content in the regenerants of *Hypericum perforatum*. *Acta Biotechnologica* **14**, 267-274

Čellárová E, Kimáková K (1999) Morphoregulatory effect of plant growth regulators on *Hypericum perforatum* L. seedlings. *Acta Biotechnologica* **19**, 163-169

Çirak C, Ayan AK, Kevseroglu K (2007) Direct and indirect regeneration of plants from internodal and leaf explants of *Hypericum bupleuroides* Gris. *Journal of Plant Biology* **50**, 24-28

Charchoglyan A, Abrahamyan, A, Fujii I, Boubakir Z, Gulder TAM, Kutchan TM, Vardapetyan H, Bringmann G, Ebizuka Y, Beerhues L (2007) Differential accumulation of hyperforin and secohyperforin in *Hypericum perforatum* tissue cultures *Phytochemistry* **68**, 2670-2677

Cui XH, Chakrabarty D, Lee EJ, Paek KY (2010) Production of adventitious roots and secondary metabolites by *Hypericum perforatum* L. in a bioreactor. *Bioresource Technology* **101**, 4708-4716

D'Agostino IB, Kieber JJ (1999) Molecular mechanisms of cytokinin action. *Current Opinion in Plant Biology* **5**, 359-364

Danova K, Čellárová E, Macková A, Daxnerová Z, Kapchina-Toteva V (2010) *In vitro* culture of *Hypericum rumeliacum* Boiss. and production of phenolics and flavonoids . *In Vitro Cellular and Developmental Biology - Plant* **46**, 422-429

Dias ACP, Tomás-Barberán FA, Fernandes-Ferreira M, Ferreres F (1998) Unusual flavonoids produced by callus of *Hypericum perforatum*. *Phytochemistry* **48**, 1165-1168

Dias ACP, Seabra RM, Andrade PB, Fernandes-Ferreira M (1999) The development and evaluation of an HPLC-DAD method for the analysis of the phenolic fractions from *in vivo* and *in vitro* biomass of *Hypericum* species. *Journal of Liquid Chromatography and Related Technologies* **22**, 215-227

Dias ACP, Seabra RM, Andrade PB, Ferreres F, Fernandes-Ferreira M (2000) Xanthone biosynthesis and accumulation in calli and suspended cells of *Hypericum androsaemum. Plant Science* **150**, 93-101

Franklin G, Dias ACP (2006) Organogenesis and embryogenesis in several *Hypericum perforatum* genotypes. *In Vitro Cell and Developmental Biology – Plant* **42**, 324-330

Gadzovska S, Maury S, Ounnar S, Righezza M, Kaščáková S, Refregiers M, Spasenoski M, Joseph C, Hagége D (2005) Identification and quantification of hypericin and pseudohypericin in different *Hypericum perforatum* L. *in vitro* cultures. *Plant Physiology and Biochemistry* **43**, 591-601

Guedes AP, Amorim LR, Vicente AMS, Ramos G, Ferreira MF (2003) Essential oils from plants and *in vitro* shoots of *Hypericum androseamum* L. *Journal of Agricultural and Food Chemistry* **51**, 1399-1404

Goel MK, Kukreja AK, Bisht NS (2009) *In vitro* manipulations in St. John's wort (*Hypericum perforatum* L.) for incessant and scale up micropropagation using adventitious roots in liquid medium and assessment of clonal fidelity using RAPD analysis. *Plant Cell, Tissue and Organ Culture* **96**, 1-9

Huang YC, Chang YL, Hsu JJ, Chuang HW (2008) Transcriptome analysis of auxin-regulated genes of *Arabidopsis thaliana. Gene* **420**, 118-124

Ishiguro K, Fukumoto H, Suitani A, Nakajima M, Isoi K (1996) Prenylated xanthones from cell suspension cultures of *Hypericum patulum*. *Phytochemistry* **42**, 435-437

Ishiguro K, Oku H, Isoi K (1999) *Hypericum patulum: In vitro* culture and production of xanthones and other secondary metabolites. In: Bajaj YPS (Ed) *Biotechnology in Agriculture and Forestry 43, Medicinal and Aromatic Plants XI*, Springer, Heidelberg, pp 199-212

Karakas O, Toker Z, Tilkat E, Ozen HC, Onay A (2009) Effect of different concentrations of benzylaminopurine on shoot regeneration and hypericin content in *Hypericum triquetrifolium* Turra. *Natural Product Research* **23**, 1459-1465

Karppinen K, Hokkanen J, Tolonen A, Mattila S, Hohtola A (2007) Biosynthesis of hyperforin and adhyperforin from amino acid precursors in shoot cultures of *Hypericum perforatum*. *Phytochemistry* **68**, 1038-1045

Kartnig T, Göbel I, Heydel B (1996) Production of hypericin, pseudohypericin and flavonoids in cell cultures of various *Hypericum* species and their chemotypes. *Planta Medica* **62**, 51-53

Kirakosyan A, Vardapetyan H, Charchoglyan A (2000a) The content of hypericin and pseudohypericin in cell cultures of *Hypericum perforatum. Russian Journal of Plant Physiology* **47**, 270-273

Kirakosyan A, Hayashi H, Inoue K, Charchoglyan A, Vardapetyan H (2000b) Stimulation of the production of hypericins by mannan in *Hypericum perforatum* shoot cultures. *Phytochemistry* **53**, 345-348

Kirakosyan A, Sirvent TM, Gibson DM, Kaufman PB (2004) The production of hypericins and hyperforin by *in vitro* cultures of St. John's wort (*Hypericum perforatum*). *Biotechnology and Applied Biochemistry* **39**, 71-81

Kornfeld A, Kaufman PB, Lu CR, Gibson DM, Bolling SF, Warber SL, Chang SC, Kirakosyan A (2007) The production of hypericins in two selected *Hypericum perforatum* shoot cultures is related to differences in black gland structure. *Plant Physiology and Biochemistry* **45**, 24-32

Liu XN, Zhang XQ, Sun JS (2007) Effects of cytokinins and elicitors on the production of hypericins and hyperforin metabolites in *Hypericum sampsonii* and *Hypericum peforatum*. *Plant Growth Regulation* **53**, 207-214

Matzk F, Meister A, Brutovská R, Schubert I (2001) Reconstruction of reproductive diversity in *Hypericum perforatum* L. opens novel strategies to manage apomixis. *The Plant Journal* **26**, 275-282

Matzk F, Hammer K, Schubert I (2003) Coevolution of apomixis and genome size within the genus *Hypericum*. *Sexual Plant Reproduction* **16**, 51-58

McCoy JA, Camper ND (2002) Development of a micropropagation protocol for St. John's wort (*Hypericum perforatum* L.). *HortScience* **37**, 978-980

Mederos Molina S (1991) *In vitro* growth and multiplication of *Hypericum canariense* L. *Acta Horticulturae* **289**, 133-134

Mederos S, San Andrés L, Luis JG (1997) Rosmanol controls explants browning of *Hypericum canariense* L. during the *in vitro* establishment of shoots. *Acta Societatis Botanicorum Poloniae* **66**, 347-349

Meyer EM, Touchell DH, Ranney TG (2009) *In vitro* shoot regeneration and polyploid induction from leaves of *Hypericum* species. *HortScience* **44**, 1957-1961

Moura M (1998) Conservation of *Hypericum foliosum* Aiton, an endemic Azorean species, by micropropagation. *In Vitro Cellular and Developmental Biology – Plant* **34**, 244-248

Murch SJ, Choffe KL, Victor JMR, Slimmon TY, KrishnaRaj S, Saxena PK (2000) Thidiazuron-induced plant regeneration from hypocotyl cultures of St. John's wort (*Hypericum perforatum*, cv 'Anthos'). *Plant Cell Reports* **19**, 576-581

Murch SJ, Campbell SSB, Saxena PK (2001) The role of serotonin and melatonin in plant morphogenesis: Regulation of auxin-induced root organogenesis in *in vitro*-cultured explants of St. John's wort (*Hypericum perforatum* L.) *In Vitro Cellular and Developmental Biology – Plant* **37**, 786-793

Namli S, Toker Z, Isikalan C, Ozen HC (2009) Effect of UV-C on production of hypericin in *Hypericum triquetrifolium* Turra grown under *in vitro* conditions. *Fresenius Environmental Bulletin* **18**, 123-128

Oluk EA, Orhan S (2009) Thidiazuron induced micropropagation of *Hypericum triquetrifolium* Turra. *African Journal of Biotechnology* **8**, 3506-3510

Pasqua G, Avato P, Monacelli B, Santamaria AR, Argentieri MP (2003) Metabolites in cell suspension culture, calli, and *in vitro* regenerated organs of *Hypericum perforatum* cv. Topas. *Plant Science* **165**, 977-982

Pasqua G, Santamaria AR, Caniato R, Filippini R (2008) Somatic embryogenesis and shoot regeneration from leaf derived callus of *Hypericum perforatum* var. *angustifolium* (sin. Fröhlich) Borkh. *Plant Biosystems* **142**, 106-110

Pavlik M, Vacek J, Klejdus B, Kuban V (2007) Hypericin and hyperforin production in St. John's wort *in vitro* culture: Influence of sucrose, polyethylene glycol, methyl jasmonate, and *Agrobacterium tumefaciens*. *Journal of Agricultural and Food Chemistry* **55**, 6147-6153

Piekoszewska A, Ekiert H, Zubek S (2010) Arbutin production in *Ruta graveo*lens L. and *Hypericum perforatum* L. *in vitro* cultures. *Acta Physiologiae Plantarum* **32**, 223-229

Pretto FR, Santarém ER (2000) callus formation and plant regeneration from *Hypericum perforatum* leaves. *Plant Cell, Tissue and Organ Culture* **62**, 107-113

Robson NKB (1981) Studies in the genus *Hypericum* L. (Guttiferae). 2. Characters of the genus. *Bulletin of the British Museum of Natural History (Botany)* **8**, 55-226

Robson NKB (2003) *Hypericum* botany. In: Ernst E (Ed) *Hypericum. The Genus Hypericum*, Taylor and Francis, London, pp 1-22

Robson NKB (2006) Studies in the genus *Hypericum* L. (Clusiaceae).1. Section 9. *Hypericum sensu lato* (part 3): subsection 1. *Hypericum* series 2. *Senanensia*, subsection 2. *Erecta* and section 9b. *Graveolentia. Systematics and Biodiversity* **4**, 19-98

Robson NKB, Adams P (1968) Chromosome number in *Hypericum* and related genera. *Brittonia* **20**, 95-106

Santarém ER, Astarita LV (2003) Multiple shoot formation in *Hypericum perforatum* L. *Brazilian Journal of Plant Physiology* **15**, 43-47

Smith MAL, Kobayashi H, Gawienowski M, Briskin DP (2002) An *in vitro* approach to investigate medicinal chemical synthesis by three herbal plants. *Plant Cell, Tissue and Organ Culture* **70**, 105-111

Walker TS, Bais HP, Vivanco JM (2002) Jasmonic acid-induced hypericin production in cell suspension cultures of *Hypericum perforatum* L. (St. John's wort). *Phytochemistry* **60**, 289-293

Werner T, Schmülling T (2009) Cytokinin action in plant development. *Current Opinion in Plant Biology* **12**, 527-538

Wojcik A, Podstolski A (2007) Leaf explants response in *in vitro* culture of St. John's wort (*Hypericum perforatum* L.) *Acta Physiologiae Plantarum* **29**, 151-156

Yazaki K, Okuda T (1990) Procyanidins in callus and multiple shoot cultures of *Hypericum erectum. Planta Medica* **56**, 490-491

Yazaki K, Okuda T (1994) *Hypericum erectum* Thunb. (St. John's Wort): *In vitro* culture and production of procyanidins. In: YPS Bajaj (Ed) *Biotechnology in Agriculture and Forestry 26*, *Medicinal and Aromatic Plants* VII, Heidelberg, pp 167-178

Zdunek K, Alfermann AW (1992) Initiation of shoot organ cultures of *Hypericum perforatum* and formation of hypericin derivatives. *Planta Medica* **58 (Suppl)**, 621-622

Zobayed SMA, Saxena PK (2003) *In vitro*-grown roots: A superior explant for prolific shoot regeneration of St. John's wort (*Hypericum perforatum* L. cv 'New Stem') in a temporary immersion bioreactor. *Plant Science* **165**, 463-470