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Planta Med 2016; 81(S 01): S1-S381
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Abstracts

Georg Thieme Verlag KG Stuttgart · New York

Role in terpenoid biogenesis of plant growth regulators in Artemisia alba Turra shoot cultures

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Congress Abstract | Full Text

Artemisia alba Turra is characterized by a variability of the terpenoid components of its essential oil, due to environmental, climatic and genetic factors[1]. Previously we established that auxin and cytokinin treatments affected its essential oil profile *in vitro* [2]. Here, relations between its terpenoid profile, thylakoid and chloroplast morphology and photosynthetic apparatus structure with endogenous cytokinin levels were studied, in response to exogenous plant growth regulators (PGRs) treatments. It was established that irrespectively of the different indole-3-butyric acid (IBA) and benzyladenine (BA) modifications applied, essential oils of the aerials of *A. alba* demonstrated two general terpenoid profile types based on their monoterpenoid/sesquiterpenoid ratios. Thus, plants with well-expressed rooting and normally represented chloroplast morphology (PGR-free control and IBA treated) expressed elevated levels of primary metabolites – 81.4% (0.5 mg/l IBA) and 144% (1.0 mg/l IBA) of bioactive cytokinins sum, while in plants with suppressed rooting (0.5 mg/l IBA + 0.2 mg/l BA and 1.0 mg/l IBA + 0.2 mg/l BA) this parameter dropped to 51.8% and 63.3%, respectively (sum of bioactive CKs in controls was accepted as 100%). Similar dependencies were observed for secondary metabolites. Monoterpenoid/sesquiterpenoid ratio dropped more than twice in the second group of plants, where also alterations of PSII and chloroplast were recorded. Knowing that monoterpenoid biogenesis is spatially bound to chloroplasts in the plant cell [3], the observed relations might be an indication of the possible mediatory role of endogenous CKs in terpenoid biogenesis by means of affecting thylakoid morphology and alteration of the efficacy of biogenetic pathways, spatially situated in the chloroplast.

Acknowledgements: Bulgarian-Swiss Research Programme (BSRP, grant No. IZEBZ0_142989), Joint Scientific Research Project between CAS and BAS (reg. No. 29), Czech Science Foundation (16 – 14649S).

Keywords: *Artemisia alba* shoot cultures, plant growth regulators, endogenous cytokinins, terpenoid profile, chloroplast.

References:

[1] *Radulović N, Blagojević P.* Volatile profiles of *Artemisia alba* Turra from contrasting serpentine and calcareous habitats. Nat Prod Commun 2010; 5: 1117 – 1122

[2] *Danova K, Todorova M, Trendafilova A, Evstatieva L.* Cytokinin and auxin effect on the terpenoid profile of the essential oil and morphological characteristics of shoot cultures of *Artemisia alba*. Nat Prod Commun 2012; 7: 1 – 2



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Abstracts

Georg Thieme Verlag KG Stuttgart · New York

HPTLC and HPTLC-coupled Bioassays for Bioprocess Control of Medicinal Plant In Vitro Cultures from the Balkan region

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› Further Information

Congress Abstract | Full Text

An *in vitro* germplasm collection has been established for the delivery of biologically active compounds of medicinal and aromatic plants of the Balkans. Representatives of the species *Pulsatilla montana, Pulsatilla slaviankae, Pulsatilla halleri* and *Clinopodium vulgare* have been subjected to a media optimization study by means of alteration of plant growth regulators treatments. The aim of this study was to demonstrate the suitability of HPTLC phytochemical fingerprints combined with the assessment of antioxidant (DPPH and β-Caroteen/Linolenic Acid Bleaching HPTLC assay) as well as optimized Xanthine Oxidase [1] and Lipase [2] inhibiting HPTLC assays (enzymatic bioautography). For *Pulsatilla* species, a mixture of ethyl acetate, formic acid, acetic acid and water (68/8/6/18) and for *Clinopodium vulgare* a slight modification (68/6/8/18) has been identified for optimal separation. Chlorogenic acid and rutin were applied as references. Total polyphenolic content [3] of the cultures was assessed spectrophotometrically as a sum parameter for one class of target secondary metabolites. Phytochemical fingerprints with adequate derivatisation (Flavonoids and Anisaldehyde-Sulfuric acid) demonstrate visual differences in number and nature of in vitro formed substances as well as differences in colour fluorescence at 366nm or visible zones in white light. Compared to control vouchers from ex situ samples exhibiting also yellow zones, *in vitro* cultures of *Pulsatilla* sp. show only a large number of blue phenolcarbonic acids zones. In vitro cultures of *C. vulgare* exhibit also two faint yellow zones in *in vitro* samples, one at the Rf value of rutin. Inhibition zones of oxidant and enzyme reactions corresponding to phytochemical zones at the comparable Rf heights render valuable rapid and cost effective data and facilitates the control and optimization of the plant in vitro culture processes. *C. vulgare* culture showed compounds with Xanthine Oxidase and Lipase inhibition. In conclusion, the adapted HPTLC separation for *Pulsatilla* sp and *Clinopodium vulgare* combined with rapid activity screening demonstrate the potential of HPTLC and its hyphenated bioassay methods as a relatively easy and feasible in-process control tool. The obtainable data is the basis for further optimization.

Acknowledgements: Swiss National Science Foundation in the Framework in the Bulgarian-Swiss Research Program (BSRP, grant No. IZEBZO_142989; DO2 – 1153); World Federation of Sciences – Bulgarian National Scholarship Programme for young researchers.

Keywords: HPTLC, bioprocess control, bioassay-coupled-HPTLC, bioautography, *in vitro* plant culture.

References:

[1] *Ramallo IA, Zacchino SA, Furlan RLE.* A Rapid TLC Autographic Method for the Detection of Xanthine Oxidase Inhibitors and Superoxide Scavengers. Phytochem Anal 2006; 17: 15 – 19

[2] *Hassan AMS.* TLC Bioautographic Method for Detecting Lipase Inhibitors. Phytochem Anal 2012; 23: 405 – 407

[3] *Saeed N, Khan MR, Shabbir M.* Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L BMC Complement Altern Med 2012; 12: 221 – 233



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Abstracts

Georg Thieme Verlag KG Stuttgart · New York

Biotechnological yield of phytopharmaceuticals of valuable Balkan medicinal plants

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Further Information

Congress Abstract | Full Text

In vitro germplasm collection has been developed for the controlled delivery of biologically active compounds of medicinal and aromatic plants of the Balkans. Representatives of the *Hypericum* and *Pulsatilla* genera, *Sideritis scardica, Inula britannica, Artemisia alba* were collected from Bulgaria [1]. Overall 71 shoot accessions in solid medium, 8 genetically non-transformed roots and 8 suspension lines in liquid culture were developed. *In vitro* culture modifications were performed based on vitamin content, plant growth regulators, agar supplementations, active charcoal and light regime treatments. Total polyphenolic contents were assayed spectrophotometrically. HPTLC fingerprinting coupled with DPPH assays was applied in order to assess secondary metabolite and active compound content. Plant extracts were purified and compounds with phytopharmaceutical potential identified by chromatographic and spectroscopic techniques, respectively. Essential oils were prepared by micro-steam distillation of fresh material, and characterized by GC-MS chromatography. Based on the conducted optimizations, three different *in vitro* systems were established for production of essential oils with modified terpenoid profile for *A. alba* (with high, medium or low values of the monoterpenoid/sesquiterpenoid ratio in the oils); high mountainous *H. richeri* was selected as a superior producer of hypericins and the hypericin non-producing *H. calycinum* – as a producer of polyphenolics with radical scavenging activity *in vitro*; biotechnological delivery of sesquiterpene lactones gailiardin and britannin of *I. britannica* was obtained. The obtained processes were suggested for up-scale in RITA temporary immersion bioreactor system.

Acknowledgements: Swiss National Science Foundation in the Framework in the Bulgarian-Swiss Research Program (BSRP, grant No. IZEBZO_142989; DO2 – 1153); World Federation of Sciences – Bulgarian National Scholarship Programme for young researchers.

Keywords: Balkan medicinal and aromatic plants, *in vitro* germplasm collection, secondary metabolites, RITA temporary immersion system.

References:

[1] *Todorova M, Trendafilova A, Krumova S, Idakieva K, Genova V, Markovska Y, Raynova Y, Evstatieva L, Wolfram E, Danova K.* Interdisciplinary interaction for the biotechnological development of Balkan medicinal plant species. Proceedings book of the Seminar of Ecology 2014, 24 – 25 April, Union of Scientists, Bulgaria 2015; 95 – 98