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Regenerative capacity and phenolic acids productivity of different types of *Inula britannica* *in vitro* root culture systems

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The flowers of the medicinal plant *Inula britannica* L. (Asteraceae) have been used in Traditional Chinese Medicine, alongside with *Inula japonica* Thunb. (Asteraceae), for the treatment of tracheitis, bronchitis, hepatitis and alimentary tract neoplasm and its dried roots and leaves – for knife wounds, furunculosis and cough [1]. Britannin and gaillardin productivity of shoot cultures of the plant has been previously investigated [2]. Here, the effect of plant growth regulators (PGR) on the regenerative capacity of leaf, stem and root explants has been studied and further on genetically non-transformed root cultures were developed and maintained in liquid medium supplemented with PGR. The identification and semi-quantification of phenolic acids was performed by means of HPTLC and UPLC techniques. Results were compared between root samples derived from whole plant grown sterile in the basic Murashige and Skoog culture medium and the roots grown in the PGR supplemented liquid systems. It was established that roots maintained in liquid culture medium supplemented with 0.01 mg/l benzyl adenine (BA) in combination with 0.5 mg/l naphthaleneacetic acid (NAA) or indole-3-butyric acid (IBA) displayed over five times higher levels of 3,5-dicaffeoylquinic and chlorogenic acids as compared with the roots derived from the sterile whole plant. In addition, NAA supplementation, as compared with IBA and cultivation at 16/8 h photoperiod, as compared with dark growth, enhanced the production of the polyphenolic acids *in vitro*. Being ubiquitous in the plant kingdom and especially abundant in roots, due to their allelopathy functions, caffeoylquinic acid compounds also represent interest in human medicine because of their potent antioxidant, anti-inflammatory and anti-spasmodic activities. The present findings might be further used for the targeted scale-up delivery of phytopharmaceuticals of *in vitro* root systems of *I. britannica*.

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1. Jun-Ping Xu (2017) In: Cancer Inhibitors from Chinese Natural Medicines. CRC Press, Taylor & Francis Group, LLC, p. 456.
2. A. Trendafilova, M. Todorova, V. Ivanova, D. Antonova, D. Dimitrov, E. Wolfram, K. Danova (2016) Effect of *in vitro* culture on the content of biologically active sesquiterpene lactones in *Inula britannica* shoots. CIPAM 2016, 29 May -1 June, Coimbra, Portugal.

Relations between hydrogen peroxide and polyphenolics levels in *Sideritis scardica* in vitro cultures

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In vitro culture of the Balkan endemic *Sideritis scardica* Griseb., Lamiaceae was initiated. Stock shoots were maintained on the Murashige and Skoog basic medium (MS), supplemented with Gamborg vitamins. Multiplication was achieved on media treated with benzyl adenine (BA) and naphthaleneacetic acid (NAA). Total phenolic and flavonoids content and hydrogen peroxide were assayed colorimetrically and low molecular antioxidants (ascorbate and glutathione) and antioxidant enzymes (ascorbate peroxidase, APx, EC 1.11.1.11; glutathione reductase, GR, EC 1.8.1.7; quaiacol peroxidase; GPOX, EC 1.11.1.7; catalase, CAT, EC 1.11.1.6 and phenylalanine amonalyase, PAL, EC 4.3.1.24) - spectrophotometrically. Growth on the MS medium resulted in low multiplication, high extent of necrosis *in vitro* and allowed only a short sub-culture period. Supplementation of plant growth regulators (PGR) to the medium increased significantly the explants' survival rate, multiplication index and subculture period and stimulated the production of total phenolic and flavonoid compounds. At all PGR supplementations both hydrogen peroxide and phenolic/flavonoid levels were increased as compared with the PGR-free control. Interestingly, when high auxin concentration, exceeding the ones of cytokinin, was applied (0.2 mg/l and 0.5 mg/l BA with 1.0 mg/l NAA), the activities of CAT, GPOX, APx (and lowered AsA which is the substrate for APx) and PAL were increased, correspondingly to the maximal levels of phenolics and flavonoids, as compared with all other treatments and the control. On the contrary, when 0.2 mg/l BA combined with 0.02 mg/l and 0.5 mg/l NAA were applied, in spite of the high phenolic/flavonoid levels, low CAT, PAL activity and maximal hydrogen peroxide levels were established. Hydrogen peroxide has a varied role in maintenance of the physiological status of the plant organism. On one hand its elevated levels are a consequence of the damaging impact of reactive oxygen species, but on the other it is also a messenger for induction of the plant's antioxidant defense and a mediator for establishment of resistance of the plant to environmental stress stimuli (1). Thus, a qualitative identification of the components with phenolic and flavonoid character in the different treatments is in process, which would provide more understanding about their role for maintaining the physiological fitness of the plant *in vitro*.

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1. Orozco-Cárdenas ML, Narváez-Vásquez J, Ryan CA (2001) Hydrogen peroxide acts as a second messenger for the induction of defense genes in tomato plants in response to wounding, systemin, and methyl jasmonate. *The Plant Cell*, 13: 179-191.