POLYPHENOL AND FLAVONOID CONTENTS OF HOP CALLUS AND CELL SUSPENSION CULTURES

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INTRODUCTION
It is well recognized that plants are a rich source of commercially important secondary metabolites. Hop (Humulus lupulus L.) is traditionally known as an essential ingredient in most beers. Secondary metabolites of hops important for the brewing of beer include a-cids and b-hexos, however, another group of compounds present in hops, such as prenylated chalcones, xanthohumol, and desmethyloxanthohumol, were recently found to exhibit interesting bioactive properties. The increased demand for medicinally important secondary metabolites increases the pressure to produce these compounds via alternative ways, especially using cell/tissue cultures and transgenic plants, respectively. The aim of our study was to establish a convenient in vitro system, based on the induction of callogenesis and establishment of cell suspension culture in hops for chemical analyses of constituents of in vitro cultures and for potential production of interesting flavonoids in in vitro culture systems.

MATERIAL AND METHODS
For optimization of the in vitro system, we studied the effect of growth regulators (BAP + NAA or 2,4-D), culture conditions (continuous dark vs. photoperiod of 16 h light/8 h dark), explant type (intercalary segments vs. leaf segments) and genotype (K-31/3/7, K-70/4/1 and Lučan-4) on callus culture of hops.

Callus cultures were established from in vitro grown shoot cultures of three genotype (K-31/3/7, K-70/4/1 and Lučan-4) from intercalary-leaf segments and cultured on MS medium supplemented with 2.0 mg/l BAP and 2.0 mg/l NAA or 2.0 mg/l BAP and 2.0 mg/l 2,4-D (MURASHIGE & SKOOG, 1962). Callus cultures were developed in the dark or photoperiod (16 h light/8 h dark) at 23±1°C during 12 weeks. Cell suspension cultures were established from stabilized callus cultures in liquid MS media containing 1.0 mg/l BAP with combination of 1.0 mg/l NAA or 1.0 mg/l 2,4-D. The total polyphenol and flavonoid contents were determined by spectrophotometric measurements of the methanol extract. The absorbance were measured at 765 nm respectively at 405 nm. The total polyphenol content was expressed as gallic acid equivalent (GAE) respectively as quercetin equivalent (Singleton & Rossi, 1965).

RESULTS
Callus induction rate was independent of explant type and it was the highest on MS+2,4-D media in photoperiod and on MS+NAA in dark conditions. For maintenance of calli, culture in dark was more favorable, comparing to photoperiod, where higher frequency of necrosis of calli occurred.

Cell suspension cultures were established from stabilized callus cultures in liquid MS media containing 1.0 mg/l-1 BAP with combination of 1.0 mg/l-1 NAA or 1.0 mg/l-1 2,4-D. Cell suspension cultures derived from both the types of explants showed higher biomass accumulation (FW and DW) in conditions of photoperiod. Cell proliferation was higher in both culture conditions in cultures derived from intercalary-derived calli. Higher biomass accumulation was observed on media with NAA in comparison with media with 2,4-D. The viability of cells (assessed as % of TTC-positive cells) depended on the concentration of pectinase added to liquid media to liberate cells from cell clumps and ranged from 60.9-90.6 % in media without pectinase to 36.2-65.4 % in media with 1000 μl pectinase.g-1 tissue FW.

Content of total polyphenols depended on the type of in vitro culture and ranged 60.5-137.1 mg.g-1 of gallic acid equivalent (GAE) in cell suspension cultures and 76.6-158.5 mg.g-1 GAE in callus cultures in comparison to 121,4 mg.g-1 GAE in the source shoot cultures of hops. Using HPLC analysis, we were able to detect also a production of xanthohumol in cell suspension cultures of hops. The highest production of xanthohumol was observed in cell suspension cultures established from leaf segment-derived calli in medium containing 1.0 mg.l-1 BAP in combination with 1.0 mg.l-1 2,4-D without pectinase and cultivated in dark conditions.

CONCLUSIONS
In the last century and even in recent years several researchers focused an increasing interest on Humulus lupulus L. and its components for their biological activities.

The use of cell suspension cultures of hops might provide clues for the biosynthesis of these compounds as well as they can be potentially used for their production. There is still a lot of work to be done in order to achieve a reliable stabilized in vitro culture system for potential production of interesting flavonoids.

References