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 α-acids and ß-acids, however, another group of compounds present in hops, such as prenylated chalcones, xanthohumol, and desmethylxanthohumol, 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.

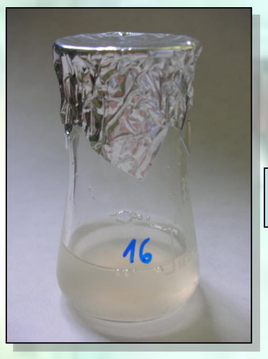
MATERIAL AND METHODS

For optimization of the *in vitro* system, we studied the effect of growth regulators (BAP + NAA or 2,4-D), culture conditions (continual dark *vs*. photoperiod of 16 h light/8 h dark), explant type (internodal segments *vs.* leaf segments) and genotype (K-31/3/7, K-70/4/1 and Lučan4/3) on callus culture of hops.

Field grown plants: genotypes K-31/3/7; K-70/4/1 a Lučan/4/3

In vitro shoot cultures: established and maintained in *in vitro* coditions; medium W_0A (according to Faragó et al., 2008)

Callus cultures induced fro *in vitro* maintained shoot cultures: shoot internodal segments and leaf blade segments; media B2N2 and B2D2



Cell suspension cultures established from callus cultures derived from internodal and leaf segments; media B1N1 and B1D1

Spectrophotometric measurement of polyphenols: spectrophotometer UV VIS Genesys 10UV

Callus cultures were established from *in vitro* grown shoot cultures of three genotype (K-31/3/7, K-70/4/1 and Lučan 4/3 from indernodal- or leaf segments and cultured on MS medium supplemented with 2.0mg/l BAP and 2.0 mg/l NAA or 2.0mg/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 expresed as gallic acid equivalent (GAE) respectively as quercetin equivalent (Singleton & Rossi, 1965).

Tab. 1 Effect of genotype, explant type, cultivation conditions and plant growth regulators on frequency of callogenesis (%C), callus induction rate (I_K), callus fresh weight (FWc), frequency of shoot regeneration ($\%R_{Sh}$) and root formation ($\%R_{RT}$), and root induction rate (I_{Rt}) in three genotypes of hops in *in vitro* culture

Genotype	Explant type	Cultivation conditions	Culture medium	%C [%]	I_K	FWc [mg]	$\%R_{Sh}$ [%]	$\%R_{Rt}$ [%]	I_{Rt}
K-70/4/1	shoot internodal segments	dark	B2D2[1]	100	2	32,5	0	0	0
			B2N2[2]	100	3	57,1	0	0	0
		photoperiod[3]	B2D2	100	3,667	52,9	0	0	0
			B2N2	100	1,417	34,2	4,17	0	0
	leaf-base segments	dark	B2D2	100	3,083	5,4	0	0	0
			B2N2	100	3,583	98,7	0	25	0,83
		potoperiod	B2D2	100	3,333	79,0	0	0	0
			B2N2	100	1,167	16,3	0	0	0
K-31/3/7	shoot internodal segments	dark	B2D2	100	2,167	32,2	0	0	0
			B2N2	100	3,25	51,4	0	0	0
		photoperiod	B2D2	100	3,917	81,9	0	0	0
			B2N2	100	1,667	30,1	4,17	0	0
	leaf-base segments	dark	B2D2	100	2,667	37,4	0	0	0
			B2N2	100	3,261	42,9	0	21,7	0,26
		photoperiod	B2D2	100	2,905	48,3	0	0	0
			B2N2	100	3,25	66,8	0	0	0
Lučan/4/3	shoot internodal segments	dark	B2D2	100	2,167	27,1	0	0	0
			B2N2	100	2,75	39,7	0	0	0
		photoperiod	B2D2	100	3,5	6,3	4,17	0	0
			B2N2	95,83	1,458	72,7	0	0	0
	leaf-base segments	dark	B2D2	100	1,75	36,9	0	0	0
			B2N2	100	3,348	46,8	0	13	0,09
		photoperiod	B2D2	95,83	2,542	9,5	0	0	0
			B2N2	100	1,583	23,5	0	0	0

[1]B2D2 = MS + 2 mg/l BAP + 2 mg/l 2,4-D; [2]B2N2 = MS + 2 mg/l BAP + 2 mg/l NAA; [3] 16h light (2500-3000 lx)/8 h dark

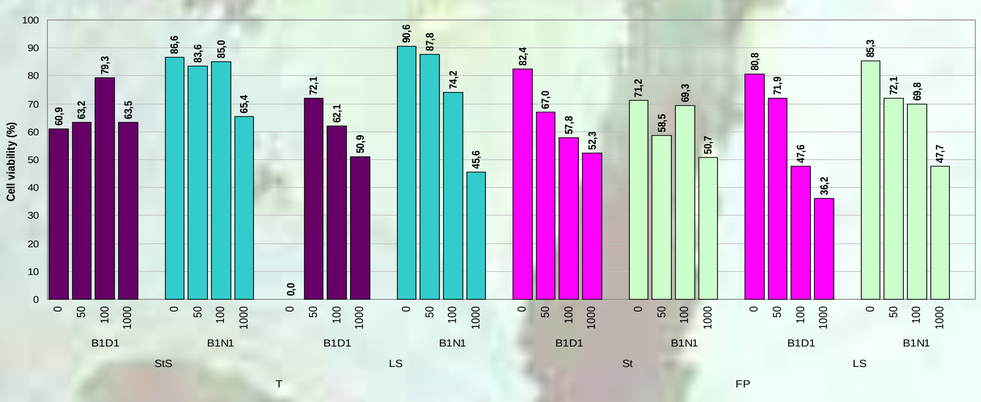


Fig. 1 Effect of pectinase addition into culture medium on the viability of cells of hop genotype K-70/4/1 in cell suspension culture

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 favourable, comparing to photoperiod, where higher frequency of necrosis of calli occured.

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 internode-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.l-1 of gallic acid equivalent (GAE) in cell suspension cultures and 76.6-158.5 mg.l-1 GAE in callus cultures in comparison to 121.4 mg.l-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 cultured in dark conditions.

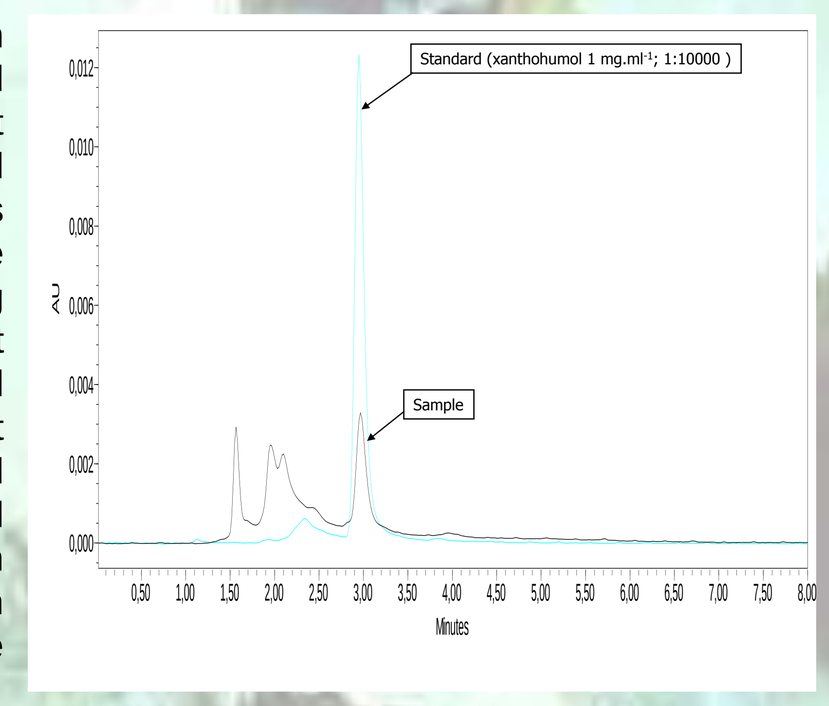


Fig. 2 HPLC analysis of presence of xanthohumol in culture medium of cell suspension culture of hop genotype K-70/4/1. Flow rate 0.8ml.min^{-1}; Mobile phase : methanol:water=9:1; absorption at 370 nm; Injection volume 5 µl; Time of analysis: 8 min; Column: Nucleodur Sphinx RP 5µm (150/4.6mm); Detector: Waters 2487 Dual λ Absorbance Detector; Pump: Waters 1525 Binary HPLC Pump

CONCLUSIONS

In the last century and even in recent years several researchers focused an increasing interest on *Humulus lupulus* L. and its compomenets 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

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