INCREASING THE POLYPHENOL AND FLAVONOID CONTENTS IN HOP CELL CULTURES THROUGH ELICITATION

Pšenáková, I., Michalková, I., Faragó, J.

Department of Biotechnology, Faculty of Natural Sciences, University of SS. Cyril and Methodius in Trnava, Slovak Republic

INTRODUCTION

Hop (*Humulus lupulus* L.) is a well known crop important for the brewing industry. Recently, it has been recognized also as a plant having very interesting chemical composition, that renders it potentially usable for food industry and medical applications (Chadwick *et al*, 2006, Stevens *et al*, 1997). Especially, hop polyphenols and flavonoids are standing in the centre of research interest. Plant tissue cultures, notably callus and cell suspension cultures, provide a convenient model system to study the biosynthesis of biologically active hop compounds, as well as a potentially reliable tool for their production.

The aim of our study was to study the possibility of increasing the accumulation of polyphenols and flavonoids in hop cell suspension cultures using the elicitation strategy. Jasmonic acid (JA), a plant growth regulator, playing a key role in responses of plant cells to exogenous signals from the surrounding environment, was chosen as the elicitor of secondary metabolism. (Felton et al, 1999)

MATERIAL AND METHODS

Plant material

Cell suspension cultures were established in two hop genotypes, K-72/6/13 and PRM/3, derived from the cultivars Saaz (clone Osvald's clone 72) and Premiant using a meristem culture technique to eradicate viruses. Callus cultures were initiated by plating leaf segments (LS) and internodal segments (StS), excised from *in vitro* maintained shoot cultures, onto two culture media B2D2 (MS salts + WS vitamins + 2 mg/l 6-benzylaminopurine [BAP] + 2 mg/l 2,4-dichlorophenoxyacetic acid [2,4-D]) and B2N2 (MS salts + WS vitamins + 2 mg/l 6-benzylaminopurine [BAP] + 2 mg/l a-naphthylacetic acid [NAA]), Callus proliferation was induced in conditions of photoperiod (FP, 16 h light/8 h dark) or in continuous dark (T). After 4 weeks of cultivation, calli were subcultured onto media B1D1 and B1N1.

Cell suspension cultures (CSCs) were established by placing 8-weeks-old calli into 40 ml of B1D1 or B1N1 liquid media in 100ml Erlenmeyer flasks.

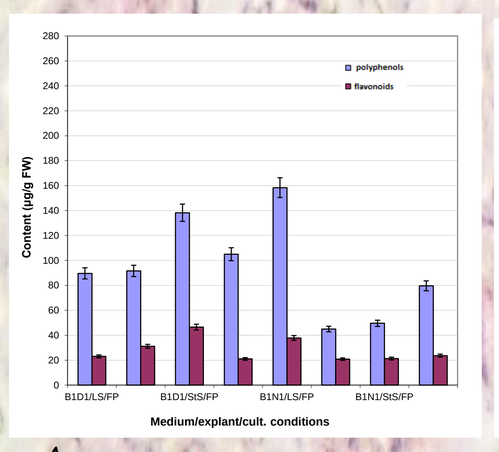
Seven days after the CSCs establishment, the elicitor – jasmonic acid – was added to the liquid cultures in final concentrations of 0 (control), 20, 40, and 100 mg/l. The CSCs were agitated on a rotary shaker (rpm = 120), and incubated in PP or D conditions at 23°C.

Analytical methods

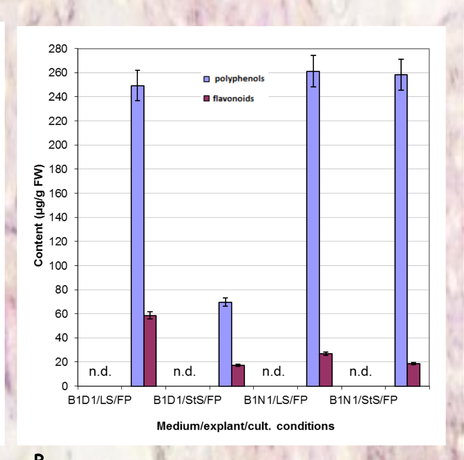
Total polyphenol (TPC) and flavonoid (TFC) contents in CSCs were determined spectrophotometrically using the methods of Singleton & Rossi (1965), and Rakotoarison et al. (1997).

RESULTS AND DISCUSSION

TPC and TFC in callus cultures differed greatly, depending on the genotype, explant type, culture medium composision, and culture conditions. In calli, the TPC ranged between 49.6-261.1 µg/g FW. The content of flavonoids varied between 17.2-58.5 µg/g FW (Fig. **1A**, **1B**).

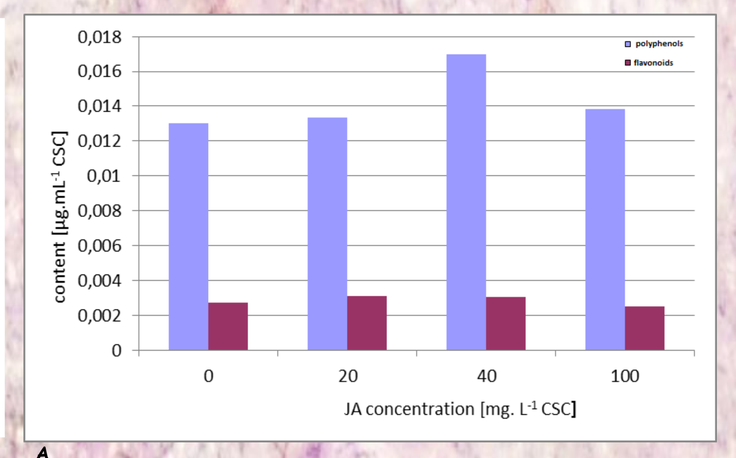


A

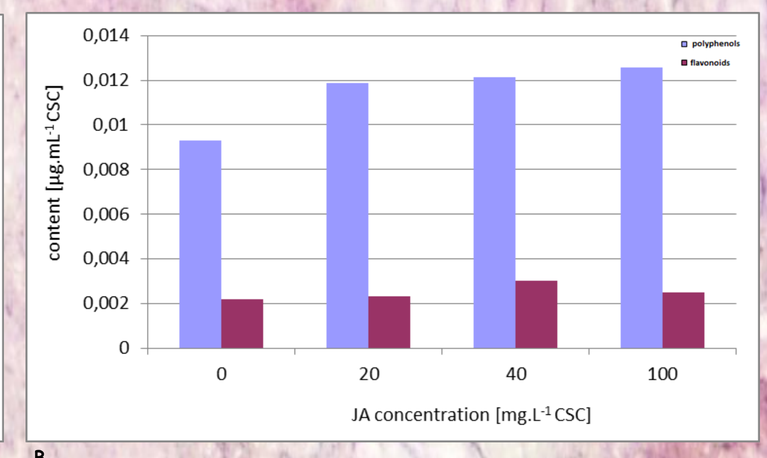


B

Fig. 1: Comparison of content of polyphenols and flavonoids (µg.g^{-1} fresh weight) in *in vitro* callus cultures of two genotypes common hops, K-72/6/13 (**A**) and PRM / 3 (**B**), induced from leaf (LS) and the internodal (StS) segments grown in continuous dark conditions (T) and photoperiod (FP) onto medium B1D1 or B1N1 after 54 days of cultivation. n.d. - data not available.



A



B

Fig. 2: Comparison ofcontent of polyphenols and flavonoids (µg. mL^{-1} CSC) in *in vitro* cell suspension cultures of two genotypes common hops, K-72/6/13 (A) and PRM / 3 (B), depending on the concentraton of added jasmonic acid (JA)

In cell suspension cultures, JA in the whole concentration range stimulated the accumulation of polyphenols and flavonoids (Fig. **2A**, **2B**). In the case of polyphenol production, 17-31% increase was achieved in media containing JA at higher concentrations (40-100 mg/l). The JA-induced increase in flavonoid accumulation was lower (9-23%) and shifted to the lower concentrations (20-40 mg/l) of elicitor in media.

CONCLUSION

The supplementation of culture media with jasmonic acid proved to be a useful strategy to increase the polyphenol and flavonoid production in hop cell suspension cultures.

REFERENCES

Felton, G.W.; Korth, K.L.; Bi, J.L.; Wesley, S.V.; Huhman, D.V.; Mathews, M.C.;Murphy, J.B.; Lamb, C.; Dixon, R.A.: Inverse relationship between systemic resistance of plants to microorganisms and to insect herbivory. In *Current Biol.*, ISSN 0960-9822, 1999, vol. 9, no. 6, p. 317-320.

Chadwick, L.R.; Pauli, G.F.; Farnsworth, N.R.: The pharmacognosy of *Humulus lupulus* L. (hops) with an emphasis on estrogenic properties. In *Phytomed.*, ISSN: 0944-7113, 2006, 13, 1-2, s. 119-131.

Rakotoarison, D.A.; Greisser, B.; Trotin, F.; Brunet, C.; Dine, T.; Luyckx, M.; Vasseur, J.; Cazin, M.; Cazin, J.C.; Pinkas, M.: Antioxidant activities of polyphenolic extracts from flowers, *in vitro* callus and cell suspension cultures of *Crataegus monogyna*. In *Pharmazie*, ISSN 0031-7144, 1997, 52, s. 60-63.

Singleton, V.L., Rossi, J.A.: Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. In *American Journal of Enology and Viticulture*, ISSN 0002-9254, 1965, 3, 16, s. 144-158.

Stevens, J.F.; Miranda, C.L.; Buhler, D.R.; Deinzer, M.L.: Chemistry and biology of hop flavonoids. In *Journal of the American Society of Brewing Chemists,* ISSN 0361-0470, 1998, 56, 4, s. 136-145.

Acknowledgements. This study was partially supported by project **VEGA 1/0635/13** ***Hop as an important source of phytomedicinally active compounds***