Gene Expression and Identification of Full-length cDNA sequences of hop (*Humulus lupulus* L.) candidate genes of the prenylflavonoid pathway

Lina Maloukh [1,2], Jaroslav Matousek [3], Paul D.Matthews [4], Axel Schwekendiek [5], Oliver Yu [6], Erik Van Bockstaele [1,2] & Isabel Roldán-Ruiz [1]

[1] Institute for Agricultural and Fisheries Research, Unit Plant, Growth and Development, Caritasstraat 21, 9090 Melle, Belgium.
[2] Ghent University, Faculty of Bioscience Engineering, Department of Plant Production, Coupure Links 653, 9000 Ghent, Belgium.
[3] Biology Centre of the ASCR, v.v.i. Institute of Plant Molecular Biology, Branišovská 31, 370 05 České Budějovice, Czech Republic.
[4] S.S. Steiner Inc, 305 N. 2nd Ave, Yakima, WA 98902, USA.
[5] Department of Biology, 107 McCollum Science Hall, Cedar Falls , Iowa 50614-0421, USA.
[6] Donald Danforth Plant Science Center, St Louis, Missouri, USA.

e-mail: Lina.Maloukh@ilvo.vlaanderen.be

Introduction

Hop (*Humulus lupulus* L.) has the property to synthesize bioactive compounds that accumulate in peltate trichomes. Compounds such as Desmethylxanthohumol (DMX) and Xanthohumol (X), two of the products of the phenylpropanoid pathway, can have applications in human health[1]. To better understand the working of this pathway, we isolated hop Cinnamic acid 4- hydroxylase (C4H) and 4- Coumarate:CoA ligase (4CL) homologues using Rapid Amplification cDNA-ends (RACE)[2]. We also demonstrate that the enzymes coded by the two cloned genes display the predicted activities, using a yeast heterologous system. The expression of these genes in vegetative and reproductive hop tissues, studied using Real Time-PCR, supported their implication in the synthesis of DMX and X. At this moment, gene transfer approaches are being used to explore the function Iof these two genes in hop.

Materials and Methods

Plant materials

The hop cultivars Whitebread Golding, Admiral and Wye Challenger were used. Mature cones and leaves of the three genotypes were collected on September 8th, 2006 at the farm of Joris Cambie (Poperinge, Belgium). Samples were immediately immersed in liquid nitrogen and stored at -80 °C until RNA extraction or biochemical analysis.

RNA extraction, cDNA synthesis and Real Time RT-PCR

Total RNA was extracted following the protocol of De Keukeleire et al. (2006)[3]. Single-strand cDNA was used in Real Time RT-PCR reactions following the protocol described by De Keukeleire et al. (2006)[3] and Maloukh et al. (2009)[5]. PCR primer pairs were designed using short sequence tags of the two genes (C4H and 4CL) identified in a cDNA-AFLP experiment as template.

Testing the Functionality

The functionality of the cloned sequences was tested using a yeast heterologous system. The two genes were first transformed into a donor vector and then into the yeast destination vector PYEST DEST 52 (Invitrogen). The two gene-constructs (4CL-PYEST DEST 52 and C4H-PYEST DEST 52) were then transferred in to *Saccharmoyces cerevisiae* strain Wat11 independently. The yeast cultures were fed with the corresponding substrates and HPLC was used to follow-up the rate of either substrate disappearance of product appearance.

Cloning into Gateway overexpression vectors

The full length cDNA of the two genes was cloned into the Gateway overexpression vector PK7WG2D (Invitrogen). Transformation experiments according to Horlemann et al. (2003)[4] are currently going on

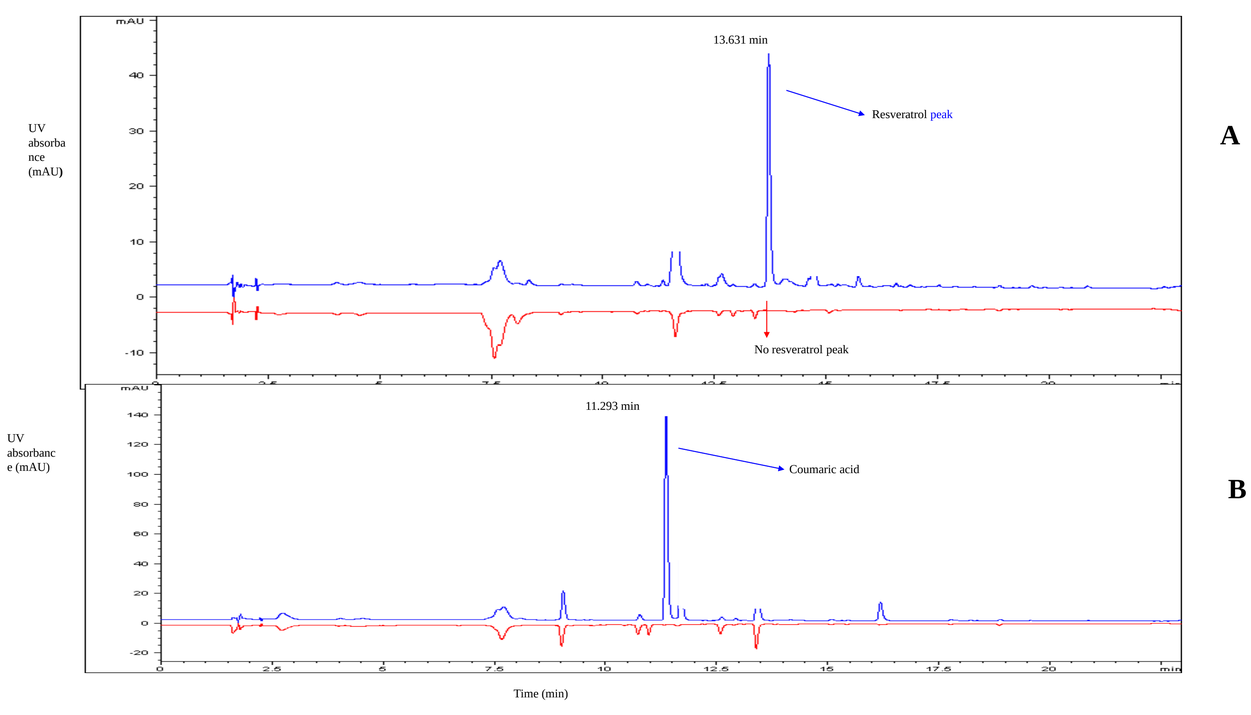

Figure 2: A: HPLC result for C4H; B: HPLC result for 4CL

Results

The data were normalized using the housekeeping genes and methods described in Maloukh et al (2009). A positive correlation was found between the abundance of C4H transcript and the accumulation of DMX and X. A negative correlation was found between the abundance of 4CL transcript and DMX and X. (Fig.1).

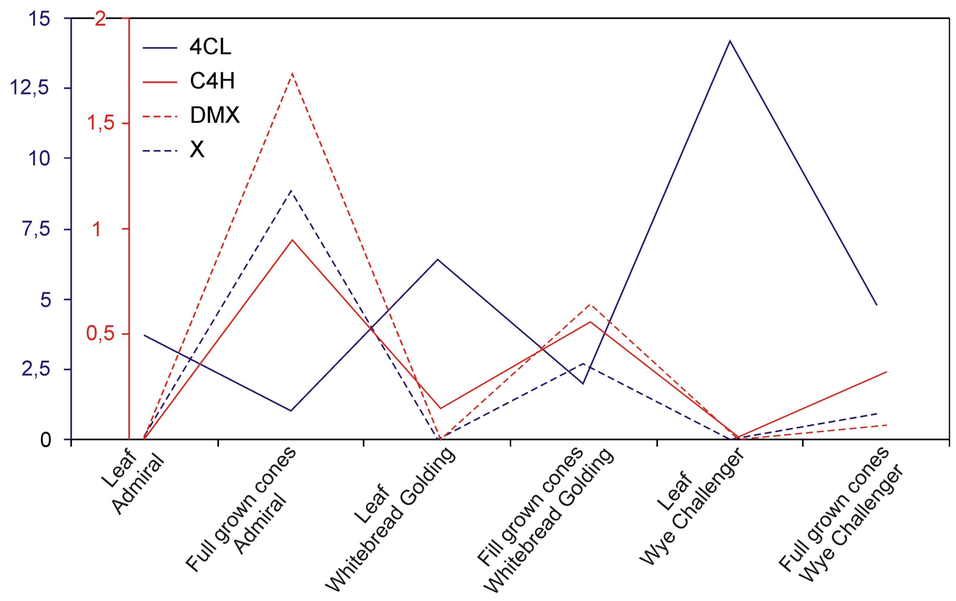

Figure 1: Normalized expression of 4CL and C4H and DMX and X concentration in leaves and mature cones of three cultivars.

The functionality of the 4CL (FJ617540) and C4H (FJ617541) cDNAs described in Maloukh et al (2009)[2] was tested in yeast. This displays that the enzymes coded by the two clones genes C4H and 4CL display the predicted activities (Figure 2).
Transformation experiments are currently going on using the cultivar Tettnanger. In figure 3 shoots produced on calli and a callus displaying GFP activity are shown.

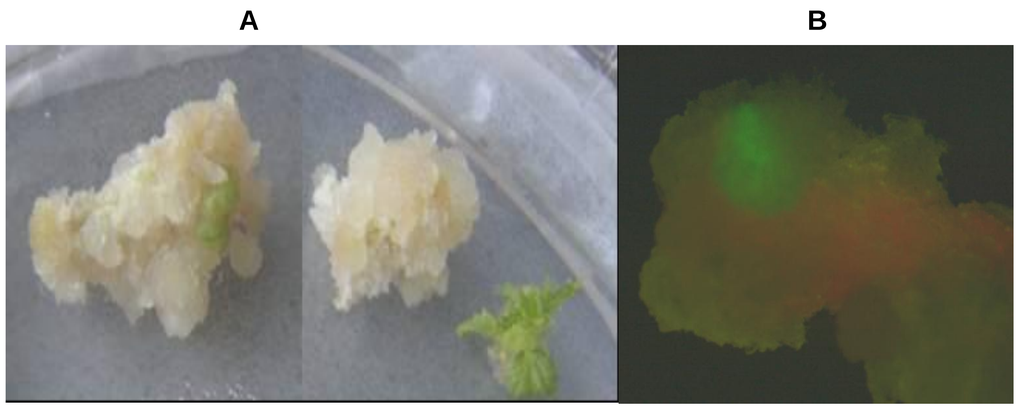

Figure 3: A: Two-week old calli formation; B: GFP signal in callus tissue.

References

1. Stevens JF, Page JE (2004). Xanthohumol and related prenylflavonoids from hops and beer: to your good health.Pytochemistry 65 (10):1317-1330; 2. Maloukh L, De Keukeleire J, Matousek J, Matthhews P.D, Schwekendiek A, Van Bockstaele E, Roldan Ruiz-I (2009). Identification of full-length cDNA sequences of Hop (*Humulus lupulus* L.) candidate genes of the prenylflavonoid pathway.Submitted for publication to Horticulturae (ISHS); 3. De Keukeleire J, Roldán-Ruiz I, Van Bockstaele E, Heyerick A, De Keukeleire D (2006). Efficient extraction of high-quality total RNA from various hop tissues (*Humulus lupulus* L.). Preparative Biochemistry and Biotechnology 36 (4): 355-362; 4. Horlemann C, Schwekendiek A, Höhnle M, Weber G (2003). Regeneration and Agrobacterium-mediated transformation of hop (*Humulus lupulus* L.). Plant Cell Rep. 22: 210-217; 5. Maloukh L, Matousek J, Van Bockstaele E, Roldan Ruiz-I (2009). Housekeeping gene selection for Real Time-PCR in Female Hop (*Humulus lupulus* L.)Tissues. Journal of Plant Biochemistry and Biotechnology 18(1):53-58.