Analysis of true chalcone synthase from *Humulus lupulus* L. and biotechnology aspects of medicinal hops

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ABSTRACT

The complete sequence hop gene, which corresponds to true chalcone synthase (EC 2.3.1.74), was amplified using a combination of PCR, RT PCR and inverse PCR methods and cloned from Czech Osvald's clone 72. The gene designated *chs*_H1 was found to be specifically expressed on glandular trichomes, whereas negligible level of specific mRNA was found in leaves. Thus, *chs*_H1 may co-determine biosynthesis of prenylated chalcones, compounds valuable as anti-cancer and antiproliferative components of lupulin. It was shown by the comparative analyses and by the structure modelling that the true hop chalcone synthase differs from previously described CHS-like protein, phlorisovalerophenone synthase, which is involved in biosynthesis of bitter acids. Several hop cultivars were analysed for the presence of genes homologous to *chs*_H1 using *chs*_H1 cDNA as probe. 2-4 *Hin*dIII specific genomic fragments were detected by Southern blots, depending on cultivar. More detailed analysis revealed at least six homologous genes in Osvald's 72 hop, suggesting a great potential of this hop also as a genetic source for modern biotechnology. High level of xanthohumol in some Osvald's 72-derived hybrid cultivars like cv. Sládek, as well as a comparatively high level of prenylated flavonoids in some comprehensive hops, suggest also the presence of additional, regulatory genes co-determining levels of prenylated flavonoids valuable for medicinal hops.

Keywords: phlorisovalerophenone synthase; prenylflavonoids; anti-cancer compounds; DNA polymorphism; hop genome

Together with bitter acids and volatile oils, prenylated flavonoids (Stevens et al. 1997) and in particular, prenylated chalcones (Etteldorf et al. 1999) constitute complex spectrum of secondary metabolites forming lupulin. This spectrum is relatively stable in different hop varieties to such an extent that it is considered to be a stable genetic trait (Menary and Doe 1983, Burkhart 1986). Prenylated chalcones like xanthohumol, isoxanthohumol, 8-prenylnaringenin and 6-prenylnaringenin have been investigated as potential antiproliferative and anti-cancer compounds (e.g. Miranda et al. 1999, Miranda 2000). Some of prenylflavonoids have potential to act as phytoestrogens and could be beneficial to health by reducing osteoporosis and cardiovascular disease (Milligan et al. 2000). Taken together, it seems likely that prenylflavonoids could be considered in future as valuable for medicinal hops and beer.

The presence of prenylflavonoids in hop cones suggest the key role of enzyme with true chalcone synthase (EC 2.3.1.74) activity, which catalyses with high kinetics the production of chalcone by the condensation of three molecules of malonyl-CoA and one of p-coumaroyl-CoA. CHS activity was detected in protein extracts from hop cones by Zuurbier et al. (1995), however, corresponding gene(s) encoding true hop CHS has not yet been characterised. Recently cloned phlorisovalerophenone synthase (VPS) was the first CHS homologue isolated from hop *Humulus lupulus* cones. This enzyme was proven to be involved in the first steps of biosynthesis of hop bitter acids (Paniego et al. 1999, Okada and Ito 2001). However, because there is either no or very low true CHS activity caused by this enzyme (Paniego et al. 1999), it has to be assumed that true chalcone synthase(s), if expressed in glandular tissue, has to fulfil this function.

In this study we aimed to look for true *chs* homologue(s) from *H. lupulus* valuable in relation to the biotechnology of so-called medicinal hops. We concentrated mainly to soft-aromatic, semi-early, red-bine Osvald's clone 72 as one by the law-protected (č. 97/1996 Sb.) Czech commercial materials, which is often used in the traditional recipes by the brewing industry i.e. without significant loss of phenylflavonoids due to hop extraction technology. We describe here the complete sequence of true hop chalcone synthase that shows specific expression in glandular trichomes. Corresponding cDNA is now available for modern alternative biotechnology approaches to ensure high yield of prenylflavonoids according to modification principles similar to those described previously for artificial marking of bohemian hops (Matoušek et al. 2000).

MATERIAL AND METHODS

Plant material, DNA isolation and Southern blot analysis

Czech semi-early red-bine hop (*Humulus lupulus*) Osvald's clone 72 and the following hop cultivars Sládek, Premiant, Yeoman, Southern Brewer, Galena, Eroica, Taurus, and Brewers Gold were analyzed in our experiments. Preparation of glandular tissue from hop cones was performed similarly as described by Okada and Ito (2001).

Genomic DNA from leaves was isolated as described by Tai and Tanksley (1991). Southern analyses were perfomed by using protocol for Qiabrane Nylon Plus membrane (Qiagen, Hilden, Germany) and hybridisation was carried out according to Church and Gilbert (1984) using [α-^{32}P]dCTP-labelled probes.

PCR and RT PCR amplifications, cloning and sequencing

All primer positions are derived from a complete *chs*_H1 sequence, which was submitted during our work to GeneBank database under ACAJ304877. Original PCR fragment which was used as specific probe was amplified from Osvald's hop genome using primers CHSJ3 (5'ATGATGTACCAACAAGGTTG3') and CHSJ4 (5'GTCTCAACAGTGAGTCCAGG3') derived from multiple sequence alignments of true chalcone synthases available in the GeneBank. This fragment covered positions 1154-1824 of *chs*_H1 gene. Forward primer CHSJ6 (5'GAGCACAAAACTGAGCTCAAGG3') and reverse primer CHSJ5 (5'GCATGTAACGCTTTCTAATCATGG3') were used for analysis of intron length polymorphism in chalcone synthase genes. A complete coding region was amplified by RT PCR using primers CHS_H1 Nde (5'AGGACATATGGTTACCGTCGAGGAA3') and CHS_H1Bam (5'CTAGGATCCCACACTGTGAAGCAC3') (position 489-1852) and the full length of *chs*_H1 gene was amplified using CHSH1PROM (5'GATCACGACCGTCCATTCT3') and CHSH13'END (5'GAAATTGACCTTTACTCCAAA3') primers. Quantitative RT PCR (Q RT PCR) was performed using total RNA purified by Qiagen and treated with DNAse I. Primers A1 (5'ATCACTGCCGTCACTTTC3') and A2 (5'AAATAAGCCCAGGA ACATC3') were combined to amplify specific *chs*_H1 cDNA product for quantification. Primers AV5' (5'ATCACAGCTTGTATCTTT3') and AV3' (5'TTTATTCGGGTCCT TGTAG3') were used for Q RT PCR of VPS.

If not stated otherwise, Pwo polymerase (Angewandte Gentechnologie Systeme GmbH, Germany) was used for PCR reactions. In a typical experiment we used the following amplification conditions: 2 min at 94°C, 35 × (30 s at 94°C, 30 s at 52°C, 60 s at 72°C), 10 min at 72°C. RT PCR reactions were performed using Titan One Tube RT PCR system (Roche Molecular Biochemicals). If not stated otherwise, reverse transcription run for 30 min at 48°C. After 2 min denaturation at 94°C the polymerase chain reaction started 30 s at 94°C, 30 s at 55°C and 60 s at 68°C for 31 cycles.

DNA and cDNA fragments were cloned in the vector pCR-Script SK(+) (pCR-Script Cloning Kit, Stratagene). Automatic sequencing was performed with an ALF II system (Amersham-Pharmacia) using a sequencing kit with Cy5-labelled standard primers.

GeneBank database sequences and computing

For comparative analyses we selected sequence data for alfalfa *chs*2 chalcone sythase and phlorisovalerophenone synthase from the GeneBank database (e.g. Benson et al. 1993) under accession numbers L02902 and AB047593, respectively.

Comparative protein analysis of the 3-D structures of CHS_H1 and VPS was performed using SWISS-MODEL Version 36.0002 (Peitsch 1995, 1996). The theoretical structures were portrayed against the template of CHS2 of *Medicago sativa* (PDB ID 1BI5). This structure was determined by Ferrer et al. (1999) using crystallography. Alignments of 3-D structures and structural analyses were performed using Swiss-PdbViewer v3.7b2 (Guex and Peitsch 1997).

Quantification of lupulin compounds

Hop resins composition and level of xanthohumol was determined by high pressure liquid chromatography (HPLC) according to *EBC 7.7* procedure (1997) on HPLC column Nucleosil RP C_{18} (Machererey Nagel, Düren, Germany, 5 mm, 250 × 4.6 mm) using liquid chromatograph Shimadzu LC-10A (Tokyo, Japan). Quantification was performed using external standard *ICE 2*.

Gel electrophoresis of DNA fragments and other methods

Temperature-gradient-gel electrophoreses (TGGE) were performed in preparative 5% acrylamide gels (Riesner et al. 1989) using the prototype system (Biometra Biomedizinische Analytik GmbH). The gels contained 19:1 acrylamide:bisacrylamide (w/w), 7 M urea and were buffered with 17.8 mM Tris-borate (0.2 × TBE). Gene introns were analyzed in 6% acrylamide gels buffered by 1 × TBE pH 8.3 (PAGE).

Acrylamide gels were stained for nucleic acids with $AgNO_3$ according to the method described by Schumacher et al. (1986). All autoradiograms were scanned and quantified using STORM device and ImageQuaNT software (Molecular Dynamics, USA).

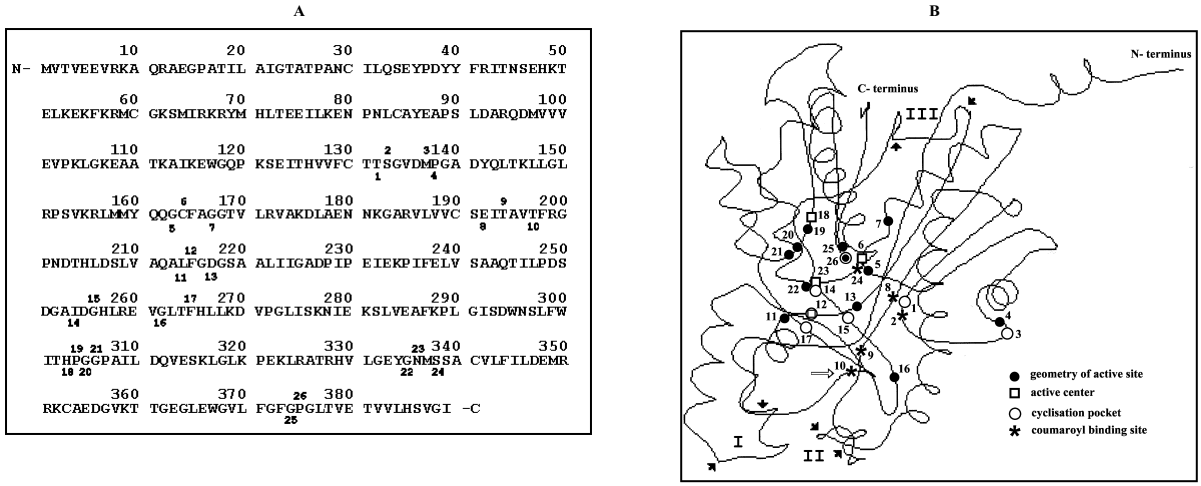

Figure 1. Amino acid sequence (A) and structure model of CHS_H1 presented as a single-line ribbon monomer (B); essential residues forming geometry of active site, active center, cyclisation pocket and coumaroyl binding site were identified by the analogy to alfalfa chalcone synthase crystallography (Ferrer at al. 1999) and mapped on the structure B, using swiss viewers as described in Material and Methods; the same conserved aa residues are idexed in sequence A from N to C terminus; the positions of largest structural deviations of CHS_H1 from VPS are marked by the filled arrows and numbered as I, II and III; position of T197, where no homology was found in VPS is indicated by a white-hollow arrow

RESULTS AND DISCUSSION

Sequential and structural features of true chalcone synthase from hop

We used primers derived from multiple sequence alignment of sixteen true *chs* genes in order to amplify chs-specific sequence from hop. By the use of PCR and subsequently RT PCR and inverse PCR we isolated a complete sequence of gene designated *chs*_H1 (GeneBank AC: AJ304877). 399 aa (42.5 kDa) CHS_H1 protein (Figure 1) shows 93% homology with alfalfa CHS2 and 100% identity in topology of catalytical center to the alfalfa true-chalcone synthase, which was analysed by crystalography (Ferrer et al. 1999). This identity included all 26 conserved amimo acid residues forming the geometry of the active site, active center, coumaroyl binding site and cyclisation pocket. In contrast to this identity, structural modelling (Figure 1) revealed clear differences between CHS_H1 and VPS homologue described previously (Paniego et al. 1999, Okada and Ito 2001). The major differences between CHS_H1 and VPS included residues T132, T197 of the catalytical center and three structural deviations localised rather on the periphery of the protein structure (Figure 1). According to our homology plots, conserved CHS-specific residue T132 within the cyclisation pocket corresponds to G134 in VPS. T197, which contributes to the coumaroyl binding site in chalcone synthases, has no homologous position in VPS. This amino acid difference is consistent with the different initial capture specificity of VPS, which utilises isovaleryl-CoA or isobutyryl-CoA as substrate components, instead of 4-coumaroyl-CoA, to which it has either no or low affinity [see Paniego et al. (1999) for substrate specificities]. This assumption is strongly supported by recent work published by Jez et al. (2000), who performed site-specific mutagenesis of several plant-specific polyketide synthases including position of T197 and found differences in substrate specificities of corresponding mutants. Based on these analyses one has to conclude that hop CHS_H1 or closely related homologues are the candidates to be involved in production of prenylated chalcones, providing that they are expressed in glandular trichomes of hop, whereas other, more distantly related homologues like VPS, are predominantly involved in biosynthesis of other secondary metabolites of lupulin. The biggest structural loops designated I, II and III (Figure 1) which were detected on aligned structures (not shown), are obviously due to the presence of non homologous amino acid residues in these regions. According to our comparisons, non-homologous residues at CHS/VPS positions from N45/K47 to K49/M51 should contribute to differences within loop I, where a β turn motif is predicted in CHS, and an α helix is formed in VPS. Loop II at CHS/VPS positions from N82/H84 to A88/A90 shows an α helix in CHS, while a β turn-like structure is predicted in VPS. Loop III indicates some structural differences in the region extending from I229/D231 to I236/I239 on the CHS/VPS alignment. These differences include the prediction of a longer α helix predicted in CHS as compared to VPS. These bigger structural deviations between CHS_H1 and VPS do not apparently include the predicted catalytic domains. However, it is possible that some of these differences include structural domains responsible for the interaction of CHS_H1 or VPS with various proteins and forming different protein complexes. It is known that CHS functions in cells as a homodimer (for review see Martin 1999), which interact with other fla-

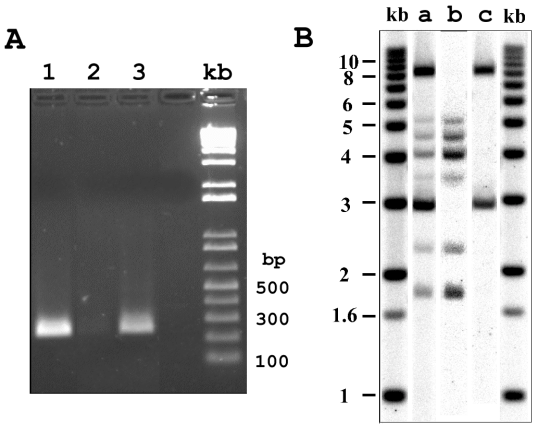

Figure 2. An example of quantitative RT PCR of hop *chs*_H1 mRNA (A) and Southern blot analysis of *chs*_H1-related genes (B); RNA was isolated from different tissues at stage of maturating cones and subjected to RT PCR; PCR products were analysed in 2% agarose gels and quantified using STORM in the volume units which characterise pixel intensities (U) within the zone; 1-peltate glands, detection of *chs*_H1 mRNA, 2-young leaves, detection of *chs*_H1 mRNA, 3-peltate glands, detection of *vps* mRNA; DNA on Southern blots DNA was isolated from Osvald's clone 72, digested with *Bst*Y I, electrophoresed, blotted and probed with probes specific for complete cds (a), exon 1 (b) or exon 2 (c); probes are positioned on *chs*_H1 sequence as shown in Figure 4 lanes kb, marker DNA (1 kb ladder, BRL)

vonoid enzymes (Burbulis and Winkel-Shirley 1999). Similar protein-protein interactions can also be assumed for the VPS homologue.

Expression of true chalcone synthase in glandular tissue

The major question, which has to be addressed, concerns the specific expression of CHS_H1 in glandular tissue. Originally we found low levels of corresponding mRNA in young female inflorescences as well as in different somatic tissues (not shown). In further experiments the specific mRNA expression has been detected in glandular trichomes (Figure 2A). Moreover, it was found that the levels of *chs*_H1 mRNA are quite comparable with transcription of previously described VPS (Okada and Ito 2001). The relative levels of *chs*_H1 mRNA were assessed according to the accumulation of 250 bp fragment using A1 and A2 primers (Figure 2A). In maturating cones, about 1000 times higher mRNA level was detected than in young leaves, where the specific signal at this ontogenetic stage was hardly detectable (Figure 2A). In conclusion, these results suggest that there is strong tissue specific regulation of the *chs*_H1 gene. Once expression of *chs*_H1 is clearly induced in glandular tissue, one has to suggest unambiguously the responsibility of *chs*_H1 for true chalcone synthase activity, as detected in glandular tissue by Zuurbier et al. (1995), as well as the involvement of CHS_H1 in biosynthesis of prenylated flavonoids.

According to our unpublished results, the main characteristics of promoter regulatory elements of *chs*_H1 (see GeneBank, sequence AJ304877) are the presence of CHS and H and G boxes which are known for light regulated, and stress-, and pathogen-inducible genes (for reviews see Martin 1993, Rushton and Somssich 1998). The characteristics of *chs*_H1 mRNA expression suggests tissue specificity, which is often observed for CHS isoforms encoded by *chs* gene families (e.g. Koes et al. 1989, Johzuka-Hisatomi et al. 1999, Durbin et al. 2000, Hirner and Seitz 2000). It is also possible that the TACPyAT motif of organ-specific expression (van der Meer et al. 1992), which was found in *chs*_H1, is responsible for the differences in mRNA levels that we observed by quantitative RT PCR. If so, then the promoter element(s) of *chs*_H1 gene is of interest to be used in biotechnology experiments to achieve overexpression of prenylated flavonoids in medicinal hops, although it is possible that additional regulatory genes are involved in regulation of true chalcone synthases in different hop genotypes.

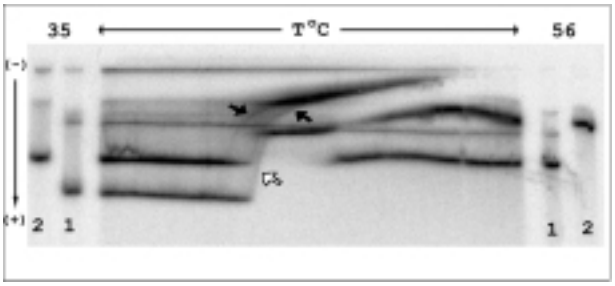

Figure 3. Analysis of *chs*_H1-specific PCR fragments on TGGE; DNA from clone *chs*_H1 (1) was loaded as the first sample; after 15 min of electrophoresis at 10°C, DNA amplified from genome of Osvald's hop 72 was loaded (2) as the second sample; then the temperature gradient 35–56°C was switched on and the electrophoresis continued until the tracking dye reached the bottom edge of the gel; the gel was transblotted onto Nylon membrane, hybridised to *chs*_H1-specific probe and visualised using STORM devise as described in Material and Methods; the melting curves of two genomic sequence variants are indicated by the black-filled arrows; the melting curve of cloned sequence variant is indicated by a white-hollow arrow

Amount of true-*chs* genes in hop and genetic potential for production of prenylated chalcones in medicinal hops

It is known that *chs* genes are usually organised in small multigene families. Different levels of prenylflavonoids in different hops as summarised in Table 1, could be determined by the additive effect(s) of multiple alleles of the same gene or closely related gene homologues. TGGE analysis of DNA fragments corresponding to exon 2 (see Material and Methods) derived from genome of Osvald's 72 was performed in order to assay amount of sequence variants of cloned *chs*_H1 gene. As depicted in Figure 3, at least two thermodynamically different sequence variants were detectable by TGGE. The cloned variant was more thermostable, having Tm = 43.2°C, whereas less thermostable variant showed Tm = 42.6°C.

Table 1. Analysis of DNA variability of *chs*_H1-related genomic sequences and content of selected lupulin components in several hop cultivars

Os	Sl	Pr	Y	SB	E	G	T	BG
RFLP of *chs*_H1-related genes (*Hind*III fragments*, length in kb)								
11.5	11.9	11.9	13.5	11.9	13.5	11.9	13.5	12.2
9.6	9.6	9.6	9.8	7.8	11.6	9.6	11.9	9.2
8.5	6.9	6.9	8.6		9.8	7.8	9.8	7.7
5.5			5.3		7.6			
Intron length polymorphism **(amount of variable introns of *chs* genes)								
6	5	4	6	2	2	4	6	2
The mean value of α bitter acids (% d.w.)								
5.3	6.2	9.6	–***	–	–	12.2	14.9	8.8
The mean value of xanthohumol (% d.w.)								
0.39	0.66	0.40	–	–	–	0.50	0.96	0.70
Ratio α acids/xanthohumol								
13.6	9.4	23.7	–	–	–	24.4	15.5	12.5

* as determined by Southern blot analysis
** as determined by PAGE of PCR fragments
*** not determined
Cultivars: Os – Osvald's clone 72, Sl – Sládek, Pr – Premiant, Y – Yeoman, SB – Southern Brewer, E – Eroica, G – Galena, T – Taurus, BG – Brewers Gold

The same shape of the melting curves on TGGE and low difference in the melting points suggest that these two variants represent most probably the allelic forms of the same gene, which differ by individual point mutation(s). In order to assess the total amount of *chs*_H1-related genes in hop, Southern blot analyses were performed using DNA from several hop genotypes (Table 1) and hybridisation probe, which clearly discriminated between *chs* and *vps* genes (not shown). These results clearly showed 2-4 *chs*_H1 specific *Hin*dIII fragments in different genotypes ranging from 13.5 to 5.3 kb. This RFLP spectrum clearly differed from spectrum of *vps* genes, where only two genes were predicted in different cultivars (not shown). The variability of *chs*_H1 related genes was also proved by PCR analysis of intron regions (Table 1). In this case, specific primers were derived from exon 1 and exon 2 of *chs*_H1 and used to amplify intron regions. Surprisingly, no uniform intron sequence was revealed and instead, 2-6 polymorphic fragments ranging from 260 to 400 bp were detected for different cultivars (Table 1). These fragments hybridized strongly to the *chs*_H1 probe and showed similar distributions even when different combinations of nested primers were used for PCR (not shown), suggesting *chs*-specific PCR prod-

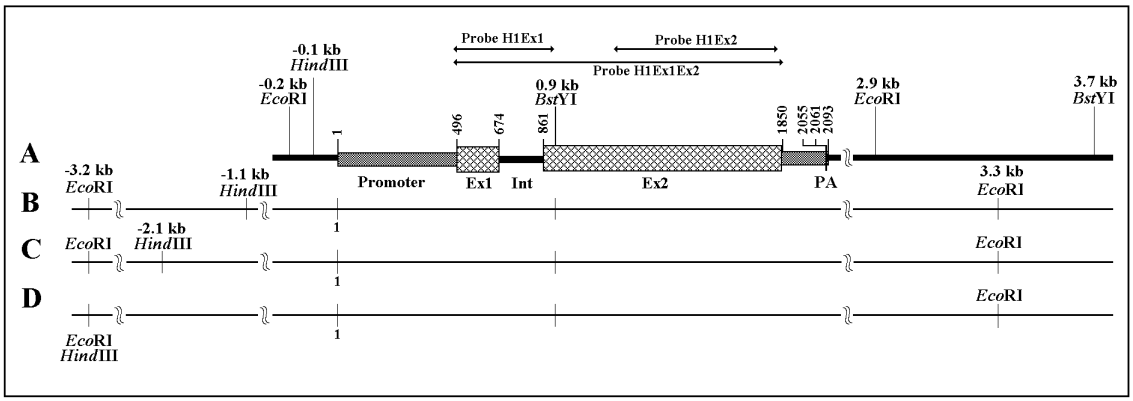

Figure 4. Schematic drawing of genomic organisation of *chs*_H1 gene and alternative mapping of restriction sites within genomic fragments hybridising to chalcone synthase specific probes; the scheme of cloned *chs*_H1 gene (position 1-2093 according to sequence AJ304877) is depicted within suppositious fragment A; positions of the restriction sites are given in kb relative to the first nucleotide of *chs*_H1, and were deduced for alternative restriction maps A, B, C and D from Southern blot analyses of Osvald's 72 genome; positions of individual chalcone synthase probes are shown on the top of the scheme; Ex1, Int, Ex2 and PA designate exon 1, intron, exon 2, and polyadenylation signal, respectively

ucts. Considering the RFLP and PCR analysis of *chs* introns, one have to assume with exception for cv. Southern Brewer, the presence from 3 to 6 *chs*_H1-related genes.

A single cleavage of genomic DNA with *Eco*RI (not shown) was considered for the alternative mapping of *chs*_H1-related fragments of Osvald's 72 DNA. Two fragments having 3.1 and 6.5 kb were obtained after the *Eco*RI cleavage. Four *Hin*dIII fragments hybridizing to exon 2 probe and having 11.5, 9.6, 8.5 and 5.5 kb (Table 1), as well as four *Dra*I fragments having 14.1, 11.8, 2.5 and 2.4 kb, were detected on Southern blots, suggesting the presence of at least four genes closely related to *chs*_H1 in the genome of Osvald's 72. However, double cleavage of DNA with *Eco*RI and *Dra*I revealed five specific bands of 12.5, 3.4, 2.3, 2.2 and 2.1 kb hybridizing to the exon 2 probe (not shown) and six distinct bands were found on the autoradiograms, where DNA was cleaved with *Bst*YI and hybridized to the exon 1-specific probe (Figure 2B). These results suggest that at least 6 sequences related to *chs*_H1 are present in the Osvald's hop genome and that some of them are arranged in tandem. For simplicity, the tandem arrangement(s) was not considered on the scheme presented in Figure 4, where four suppositious maps based on 3.0, 4.4, 5.4 and 6.9 kb *Eco*RI/*Hin*dIII fragments are depicted. It is probable that introns of some of these sequences differ within a narrow range of about 150 bp, as follows from the PCR analysis. Some differences in the intron sequences are also probable from the double *Eco*RI/*Dra*I cleavage, where at least three fragments having 2.3, 2.2 and 2.1 kb would map an AT rich (TTT↓AAA) *Dra*I restriction site close to or into the intron, providing that a 2.9 kb downstream *Eco*RI site is considered for this calculation (Figure 4). The same would be true if a 3.4 *Eco*RI/*Dra*I fragment and *Eco*RI at a 3.3 kb downstream position was considered. At the same time, although the *chs*_H1 intron represents an AT rich sequence (see GeneBank sequence AJ304877), there is not a *Dra*I site detectable by the sequence analysis within the *chs*_H1 sequence. This means that sequences other than *chs*_H1 intron sequences must present in some of the *chs*_H1-related genes. The oligofamily of *chs*_H1 related genes in Osvald's 72 hop, suggests a great potential of this hop also as a genetic source of true chs-related sequences.

The comparison of levels of α-acids and xanthohumol in selected cultivars (Table 1) shows that there is not strict correlation between levels of these two compounds, as can be judged from high variability of α-acids/xanthohumol ratios, ranging from 9 to 24. For instance, some differences were found in cvs. Sládek (high level of xanthohumol) and Premiant (low level), both hybrid cultivars originating from Osvald's hop. This observation is consistent with the conclusion described here that the levels of bitter acids and prenylated flavonoids are co-determined by different *chs*-related gene families. Based on this fact, it can be assumed that the independent breeding schemes can be considered either for high levels of bitter acids or for prenylflavonoids. In addition, there is not strict correlation between levels of xanthohumol and amount of *chs*_H1-specific genomic fragments detectable by RFLP and intron analysis (Table 1). Therefore, it seems likely that the level of prenylflavonoids is co-determined by the additional regulatory gene(s) rather than by the simple additive effects of *chs*_H1-related gene family. Regulatory genes involved in biosynthesis of lupulin compounds and in particular, of prenylflavonoids are of interest for hop industry and biotechnology. Although such hop genes remain to be identified and cloned, the genetic background involved in regulation of true *chs*-genes is known for anthocyanin biosynthesis pathway (Dooner et al. 1991). Finally, it can be suggested as an alternative that the Osvald's 72 *chs*_H1 gene could be used for a genetic modification of hops by the transformation with artificial expression constructs in order to achieve high level of prenylflavonoids valuable for medical purposes.

Acknowledgments

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ABSTRAKT

Analýza pravé chalkonsyntázy druhu *Humulus lupulus* L. a aspekty biotechnologie ozdravného chmele

S použitím metod PCR, RT PCR a inverzní PCR byla z českého chmele Osvaldův klon 72 připravena a klonována úplná sekvence genu kódujícího pravou chalkonsyntázu (EC 2.3.1.7). Bylo zjištěno, že se uvedený gen nazvaný *chs*_H1 specificky exprimuje v žláznatých trichomech, kdežto v listech chmele bylo zjištěno pouze zanedbatelné množství specifické mRNA. Na základě tohoto zjištění lze předpokládat, že *chs*_H1 je genem, který spoluurčuje biosyntézu prenylovaných chalkonů, cenných antiproliferativních a protirakovinných látek, jež jsou součástí lupulinu. Srovnávací analýzou a strukturním modelováním bylo prokázáno, že se pravá chalkonsyntáza chmele významně liší od CHS-podobného proteinu, jež byl publikován dříve a identifikován jako phlorisovalerophenonsyntáza, která se účastní biosyntézy hořkých kyselin. S použitím cDNA jako hybridizační sondy bylo na přítomnost homologů *chs*_H1 analyzováno několik odrůd chmele. V závislosti na odrůdě byly detegovány pomocí Southernovy analýzy 2-4 specifické genomové *Hin*dIII fragmenty. Detailnější analýza odhalila přinejmenším šest homologických *chs* genů u Osvaldova klonu 72, což ukazuje na značný potenciál tohoto chmele rovněž jako genového zdroje pro moderní biotechnologie. Na základě vysoké hladiny xanthohumulu u některých hybridních odrůd

odvozených z Osvaldova klonu 72, jako např. u odrůdy Sládek, a rovněž na základě poměrně vysoké hladiny prenylovaných flavonoidů u některých vysokoobsažných chmelů lze předpokládat přítomnost doplňujících regulačních genů, které kodeterminují hladinu prenylovaných flavonoidů potenciálně cenných pro tzv. ozdravné chmele.

Klíčová slova: phlorisovalerophenonsyntáza; prenylované flavonoidy; protirakovinné látky; DNA polymorfismus; chmelový genom

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