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Cloning and Molecular Analysis of the Regulatory Factor *HlMyb1* in Hop (*Humulus lupulus* L.) and the Potential of Hop To Produce Bioactive Prenylated Flavonoids

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The concentrations of prenylated chalcones and bitter acids were analyzed in Czech hop varieties. The highest levels of (xanthohumol + desmethylxanthohumol) (0.97%, m/m) and of total bitter acids (17.19%, m/m) were observed for cv. Agnus. The concentration ratios of bitter acids to prenylated chalcones varied depending on the genotype, thereby suggesting genetic determination by different set(s) of structural and regulatory genes. Promoter elements of the *chs_H1* gene encoding a “true” chalcone synthase, a candidate gene to co-determine the biosynthesis of prenylated chalcones, were analyzed, and several boxes for *cis*-regulatory elements including *Myb* transcription factors were discovered. A cDNA library was established from glandular tissue-enriched cones of cv. Osvald’s clone 72 and used to screen for *Myb* regulatory elements. The cDNA of the first *Myb* regulatory factor from hop, called *HlMyb1*, was cloned and analyzed. The *HlMyb1* open reading frame encodes 272 amino acids (29.8 kDa), and the protein showed highest homology to the light-regulated factor *AtMyb68* from *Arabidopsis thaliana* within the *Myb* domain, whereas there was no significant homology with known MYB proteins outside this domain. Unlike *AtMyb68*, which is expressed in mature leaves, *HlMyb1* is strongly expressed in hop inflorescences and could participate in the regulation of developmental processes involved in the production of hop cones and bioactive secondary metabolites.

KEYWORDS: Hop; prenylated flavonoids; transcription factors; hop cDNA library; biosynthesis

INTRODUCTION

Hop has been known in traditional medicine for a long time, and many authoritative books and pharmacopoeia of medieval times have described various bioactivities of hop (*Humulus lupulus* L.). Recent findings on particular medicinal properties of hop secondary metabolites indicate a potential role of the hop plant as a most interesting “molecular factory”. Several studies have particularly focused on prenylated flavonoids that constitute a subclass of polyphenols characterized by the presence of a 3-methylbut-2-enyl substituent, generally referred to as a prenyl group, on a chalcone or flavan-4-one nucleus. Hop proved to be a rich source of prenylated flavonoids, and accumulation in the lupulin glands of female hop cones is prominent. 8-Prenylnaringenin, a prenylflavanone, is the most potent phytoestrogen currently known, at least 100-fold more active than the isoflavones present in soy and red clover (*1*). Xanthohumol, a prenylated chalcone, has been proven to be a fascinating cancer-chemopreventive compound exhibiting a broad spectrum of inhibition mechanisms at all stages of carcinogenesis (*2*, *3*). The main prenylated chalcones in the lupulin glands are xanthohumol (X; up to 1.3%, m/m, of the dry weight of a hop cone) and desmethylxanthohumol (DMX; up to 0.2%). These prenylated chalcones are prone to undergo a Michael-type cycloaddition leading to isoxanthohumol (from X) and a mixture of 8-prenylnaringenin and 6-prenylnaringenin (from DMX). This series of prenylated flavonoids presents intriguing challenges not only to control the interconversions, but, even more interesting, to acquire information on gene expression, because the compounds differ by methylation of one phenolic functionality. In this context, it should be extremely rewarding to being able to unravel the pathway to DMX, which

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is the precursor to 8-prenylnaringenin and, hence, must be considered a pro-estrogen. The formation of X and DMX during hop growing has been documented previously (*4*). It was observed that the compounds are present from the onset of flowering and accumulate during development from female inflorescences to cones. Interestingly, each individual hop variety showed specific features, for example, the mass ratios of DMX to X varied from 0.13 in Wye Target (a bitter hop) to 1.00 in Wye Challenger (an aroma hop). It was also reported that leaves of fully grown hops contain low but detectable levels of the prenylated chalcones.

The presence of various prenylated flavonoids in hop cones suggests the involvement of an enzyme with chalcone synthase specificity (EC 2.3.1.74), designated "true" chalcone synthase for simplicity (*5*), which catalyzes efficiently the biosynthesis of chalcones by condensation of three malonyl-CoA moieties and *p*-coumaroyl-CoA. CHS activity has, indeed, been detected in protein extracts from hop cones by Zuurbier et al. (*6*) and the corresponding candidate enzymes have been characterized in our previous work (*5*, *7*). Herein, we described the gene encoding a "true" chalcone synthase, called CHS_H1. Moreover, an oligofamily of *chs_H1*-related sequences has been detected in hop genomes. Subsequently, catalytic properties of recombinant CHS_H1 and other CHS-like enzymes from hop were studied by Novák et al. (*8*). For CHS_H1, a high affinity for *p*-coumaroyl-CoA ($K_M = 40.9 \pm 0.1$, $V_{lim} = 256.5 \pm 28.1$) has been observed in our work. The V_{lim} value for CHS_H1 was 44-fold higher than the value for the recombinant valerophenone synthase (VPS), described originally by Okada and Ito (*9*) as an enzyme possibly involved in the biosynthesis of bitter acids. The kinetic parameters show that CHS_H1 catalyzes the formation of chalconaringenin at a much higher rate than VPS. Moreover, recombinant CHS_H1, similarly to VPS, can utilize isovaleryl and isobutyryl CoA substrates, albeit at a low rate, and could be involved in the biosynthesis of bitter acids (*8*). Similar observations have recently been published by Okada et al. (*10*).

Developmentally regulated and tissue-specific expression of CHS homologues from hop (*8*) suggests the presence of specific *cis*- and possibly *trans*-regulatory elements in the hop genome that determine the potential of hop to synthesize valuable compounds in the lupulin glands. Detailed analysis of hop genomes for *chs* homologues (*6*, *7*) indicated that the differences on the level of CHS regulation rather than the number of *chs* genes or alleles determine varying levels of prenylated chalcones and bitter acids in different hop cultivars. From the fact that *chs* homologues are involved in the first committed step in the formation of bitter acids and in the biosynthesis of prenylated chalcones in hop, it seems reasonable to compare homologous regulatory systems, known in the regulation of phenylpropanoid biosynthesis, to screen for specific hop regulatory factors expressed in the lupulin glands. It has been found that regulatory genes controlling anthocyanin pigmentation (like the originally described B and R locuses in maize) are functionally conserved in higher plants (*11*) and that the key function is determined by MYB proteins. It is known that *R2R3 Myb* factors form a typical plant-specific group of transcriptional regulators that participate in various processes including plant morphogenesis [see, e.g., Kranz et al. (*12*)].

In this study, we characterized the first *Myb* regulatory element isolated from hop cones via cDNA library screening. Sensitive analytical approaches were applied to analyze the tissue- and genotype-specific potential of several important Czech hop varieties to produce, in cones and leaves, prenylated flavonoids, primarily chalcones, and bitter acids having potent bioactivities. Furthermore, the possible involvement of a characterized regulatory element is discussed.

MATERIALS AND METHODS

Plant Sampling and Analysis of Secondary Metabolites. Czech semi-early red-bine hop (*H. lupulus* L.) cv. Osvald's clone 72 and the hop varieties Sládek, Premiant, and Agnus were analyzed for secondary metabolites. To avoid differences in conditions with respect to climate and soil, both full-grown hop cones and leaves were collected in the experimental hop garden of the Hop Research Institute, Žatec, Czech Republic, during hop harvest. The cones were dried by lyophilization. Samples were transferred to round-bottom flasks and lyophilized for 36 h [Heto Lyolab 3000 from Heto-Holten, Allerod, Denmark, in combination with a DUO 5 Pfeiffer vacuum pump (from Pfeiffer Vacuum, Asslar, Germany)]. The temperature of the condenser was −50 °C and a pressure of 10^{-5} atm was obtained. Samples of dried hop cones (from 500 mg to 1 g) and dried leaves (1 g) were ground, suspended in methanol (20 mL) containing formic acid (0.01%), vortex-mixed (1 min), and shaken for 1 h. A sample of the supernatant (~1 mL) was withdrawn and filtered over a 0.2 μm filter (regenerated cellulose, 13 mm; Alltech, Lokeren, Belgium) prior to analyis by high-performance liquid chromatography (HPLC). The analytical procedure has been described previously (*4*). In short, HPLC was performed using a Waters 2695 Alliance Separations module equipped with a C18 reversed-phase column. Peaks were identified by comparison of the retention times with those of authentic reference compounds, as well as by inspection of the respective UV spectra. A mixture of hop bitter acids, more specifically α-acids and β-acids, of well-known composition [ICE-2, International Calibration Extract-2 (Labor Veritas, Zurich, Switzerland): 14.45% cohumulone, 34.94% (humulone + adhumulone), 12.92% colupulone, and 12.02% (lupulone + adlupulone)], served as an external standard to quantify α-acids and β-acids. Desmethylxanthohumol (DMX) and xanthohumol (X) were isolated as described previously (*4*). Injections were done in twofold for three preparations of each sample, and standard deviations were calculated on the basis of peak area integration.

cDNA library, PCR and RT PCR Amplifications, Cloning, Sequencing, and Northern Blot Analysis. RNA for the cDNA library was isolated from glandular tissue-enriched hop cones using the Concert-plant RNA purification reagent (Invitrogen, Carlsbad, CA). mRNA fractions were prepared using polyT immobilized on magnetic beads (Dynal, Oslo, Norway) according to the manufacturer's protocol. Five micrograms of poly(A) RNA was used for the preparation of the library (cDNA synthesis kit from Stratagene, La Jolla, CA) according to the protocol. cDNA was ligated into the Uni-ZAP-XR vector (lambda phage vector) in an oriented manner.

The following primers derived from conserved motifs of *Arabidopsis thaliana* Myb genes *PAP1* and *PAP2* (see below), designated 5′CPAP (5′AAAAGTTGTAGATTAAGATGG3′) and 3′CPAP (5′CAATTAAAGACCACCTATTCCC3′), respectively, were used to amplify an authentic *Myb* cDNA fragment from hop as a template to screen for the 5′ portion of *Myb* sequences. A new *Myb* 5′ primer (5′GTCACCTACAGCTAACCACAAAG 3′), derived from the 5′ end of the *Myb* cDNA, was then combined with the M13 forward primer to screen for full-length *HlMyb1* cDNA from the cDNA library. The cDNA fragment was isolated from the gel by the use of the Qiagen gel extraction kit (Qiagen, Hilden, Germany) and treated with *Xho*I before cloning in the vector pCR-Script SK(+) (pCR-Script cloning kit from Stratagene) and sequencing. Automatic sequencing was performed with an ALF II system (Amersham Pharmacia Biotech, Freiburg, Germany) using a sequencing kit with Cy5-labeled standard primers.

If not stated otherwise, Pwo polymerase (Angewandte Gentechnologie Systeme, Heidelberg, Germany) was used for PCR reamplifications. In a typical experiment, the following amplification conditions were used: 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.

For Northern blot analysis, total RNA was isolated from 100 mg of hop tissue by the RNeasy Plant Total RNA kit (Qiagen). RNA samples of 35 μg each were separated on a formaldehyde-denaturating agarose

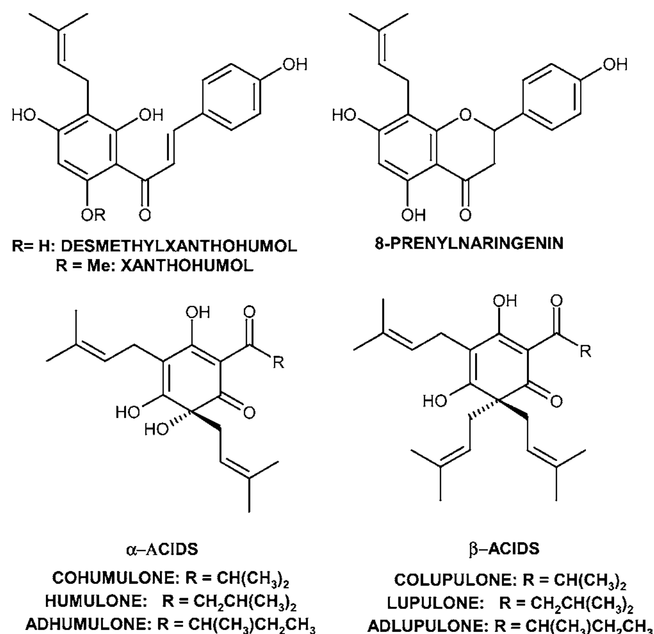

Figure 1. Structures of desmethylxanthohumol (DMX), xanthohumol (X), 8-prenylnaringenin, α-acids, and β-acids.

gel and blotted onto a nylon membrane. Prehybridization and hybridization were carried out using a formamide-based (pre)hybridization buffer at 42 °C. The final washing was performed in (0.5× SSC + 0.1 SDS) at 55 °C for 30 min. The probes for the detection of *HlMyb1* and 7SL RNA (*13*) were labeled using the Redivue [α-^{32}P]dCTP 3000 Ci·mmol^{-1} Rediprime II random prime labeling system (Amersham Pharmacia Biotech, Freiburg, Germany). The autoradiograms were scanned using a STORM device and ImageQuaNT software (Molecular Dynamics, Sunnyvale, CA).

GenBank Database Sequences and Computing. For phylogenetic comparisons, the following plant transcription factors were selected from the GenBank and EMBL databases (AC numbers given after commas): *AtMyb68* from *A. thaliana*, AF062901; light-regulated *Myb* factor from *A. thaliana*, AC006922_38; *AtMyb75* (*PAP1*) *Myb* factor from *A. thaliana*, AF325123; *AtMyb90* (*PAP2*) *Myb* factor from *A. thaliana*, AF325124; *AtMyb4* from *A. thaliana*, AB005889; c-myb-like transcription factor *MYB3R-1* from *A. thaliana*, AF188677; *AtMyb2* from *A. thaliana*, AB052239; *AtbZIP* transcription factor from *A. thaliana*, AJ419852; *Mybc05* transcription factor from *Perilla frutescens*, AB111049; *c1* locus *Myb* homologue from *Zea mays*, M37153; *PcMyb1* from *Petroselinum crispum*, U67134; *GhMyb 3* from *Gossypium hirsutum*, AF377308; *GhMyb 5* from *G. hirsutum*, AF377316; *Myb*-related transcription factor *cpm5* from *Craterostigma plantagineum*, U33916; *Myb*-related transcription factor mixta-like 1 from *Antirrhinum majus*, AJ006292; P-like *Myb* factor from *Oryza sativa,* AP005495. The *Myb* factor from *H. lupulus* described in this study, named *HlMyb1*, has AC AJ876882. The sequence of the *chs*_H1 gene is described in the EMBL database under AC AJ304877.

Sequence data analysis was carried out with the computer program DNASIS, version 2.5. Protein sequence alignments were performed using the CLUSTAL W program with amino acid (aa) classes described by Bork et al. (*14*). Analysis of the three-dimensional structure of the R2R3 part of the MYB protein was done using SWISS-MODEL (*15*). Alignments of three-dimensional structures and structural analyses were performed using RasWin v. 2.6.4 and Swiss-PdbViewer v3.7b2 (*16*).

RESULTS AND DISCUSSION

Quantification of Prenylated Chalcones in Generative and Somatic Tissues of Hop and Factors Involved. The main interest of this study is focused on prenylated chalcones (**Figure 1**) in hop. A detailed analysis performed previously using five hop cultivars, Wye Challenger, Wye Target, Golding, Admiral, and Whitbread Golding, with an optimized gradient HPLC procedure (*4*) led to the conclusion that prenylated chalcones, mainly DMX and xanthohumol X, were present from the onset of flowering not only in female hop cones but also in male inflorescences, albeit in low concentrations. During the development from female inflorescences to cones, levels of these compounds gradually increased; each hop variety exhibited individual accumulation rates. It has been described in parallel that, in genotypes showing a high content of bitter acids (α-acids and β-acids, see **Figure 1**), the levels of chalcone synthase mRNA remained more elevated during later stages of development than in cultivars with low contents of bitter acids (*8*). These observations confirm the complex developmental and genotype-dependent differences in accumulation of these secondary metabolites in hop. The results of the quantitative analyses of four Czech hop varieties (**Table 1**) showed that cv. Agnus, having the highest level of total bitter acids (sum of α-acids and β-acids = 17.19%, m/m), contained also the highest concentration of prenylated chalcones (sum of DMX and X = 0.97%, m/m).

However, the correlation between the accumulation of bitter acids and prenylated chalcones is imperfect, as can be deduced from the concentration ratios of bitter acids to prenylated chalcones. This ratio was 11.58 for cv. Sládek and 26.65 for cv. Premiant, both being hybrid cultivars originating from cv. Osvald's clone 72 (ratio of 15.86). This observation is consistent with a previous suggestion (*7*) that the levels of bitter acids and prenylated chalcones are probably differentially co-determined involving various *chs* families and their regulation. In accordance with this, data obtained from the screening of cDNA spectra and molecular hybridization showed that there is a differential expression of *chs*_H1 and *vps* alleles or genes in hop inflorescences and during cone development (data not shown). The enzyme CHS_H1 is most probably involved in the accumulation of prenylated chalcones, and the expression of the *chs*_H1 oligofamily co-determines the potential to produce these compounds in hop, although the competitive pathway that leads to the biosynthesis of anthocyanins has to be considered as well (*8*).

The *chs*_H1 mRNA has been detected in various tissues, but concentrations found in cones (*6*) were several hundred fold more elevated. Our previous results (*4*) confirm that prenylated chalcones can be detected in somatic hop tissue, however, at very low concentrations. As is obvious from inspection of **Figure 2**, the levels of prenylated chalcones are 1000-fold to several hundred fold lower in leaves than in hop cones depending on the genotype. The ratios are in the range of differences observed in levels of *chs*_H1 mRNA in somatic tissues with respect to concentrations found in hop cones (*6*).

The developmental and tissue-specific differences suggest a potential involvement of various regulatory factors driving the specific expression of hop CHS homologues. Indeed, according to analysis of the promoter *chs*_H1 sequence AC AJ304877, there are motifs of CHS, H, G, and tissue-specific boxes (*6*). In addition, at positions 19−25 (**Figure 3**), a specific box is observed fitting to the consensus CCT/AACC for the binding site of the *Myb* homologue that has been described as an activator of the flavonoid biosynthetic gene subset (*17*). At positions 163−171, a *Myb* box fitting to the consensus A/CAC-CTAAA/CC known for the *A. thaliana Myb4* gene (*18*) is observed (**Figure 3**). This factor has the ability in *A. thaliana* to modulate the phenylpropanoid biosynthetic pathway (*19*). Some *cis*-regulatory elements such as H and G boxes have been found also in the promoter part of the *vps* gene (*20*). The existence of various *cis*-sequence motifs in the promoter region

Table 1. Quantification of Desmethylxanthohumol (DMX) and Xanthohumol (X), Cohumulone, (Humulone + Adhumulone), Total α-Acids, Colupulone, (Lupulone + Adlupulone), and Total β-Acids in Cones of Four Selected Czech Hop Varieties

secondary metabolite	%, m/m			
	Premiant	Sládek	Osvald's clone 72	Agnus
DMX	0.086 ± 0.003	0.077± 0.002	0.092 ± 0.006	0.107 ± 0.012
X	0.378 ± 0.015	0.491 ± 0.019	0.351 ± 0.024	0.860 ± 0.104
cohumulone	1.599 ± 0.072	0.880 ± 0.002	0.864 ± 0.061	3.844 ± 0.016
(humulone + adhumulone)	6.987 ± 0.283	2.326 ± 0.009	2.535 ± 0.166	8.133 ± 0.018
total α-acids	8.586 ± 0.355	3.206 ± 0.010	3.399 ± 0.227	11.977 ± 0.034
colupulone	1.557 ± 0.075	1.740 ± 0.034	1.461 ± 0.099	2.888 ± 0.007
(lupulone + adlupulone)	2.223 ± 0.101	1.631 ± 0.041	2.163 ± 0.133	2.328 ± 0.008
total β-acids	3.780 ± 0.175	3.371 ± 0.076	3.625 ± 0.232	5.216 ± 0.015

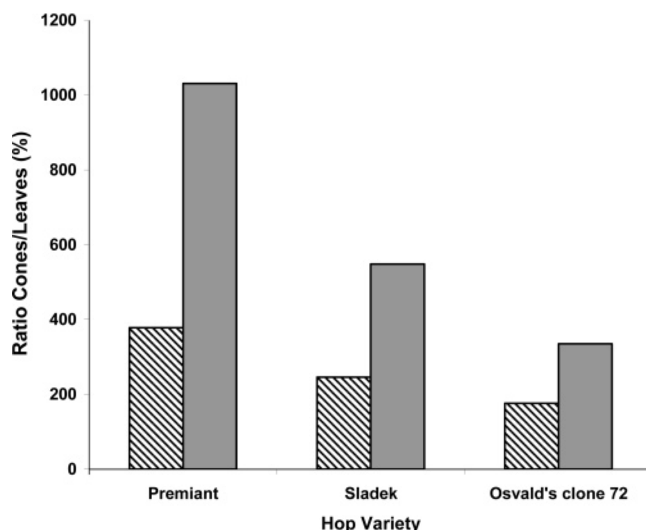

Figure 2. Relative levels of (desmethylxanthohumol and xanthohumol) and bitter acids in hop cones and leaves of three selected hop genotypes, Premiant, Sládek, and Osvald's clone 72. Quantification (%, m/m) of (desmethylxanthohumol + xanthohumol) (striped bars) and total bitter acids (sum of humulone, adhumulone, cohumulone, lupulone, adlupulone, and colupulone) (solid bars) was performed for leaves and cones (for details, see Materials and Methods). The concentration ratios of cones to leaves are expressed as percentages.

of hop *chs* homologues suggests a combinatorial interaction between various MYB, MYC, and B/HLH proteins, as has been observed for different biochemical pathways in plants [see, e.g., Singh (*21*)].

Isolation, Sequential Analysis, and Expression of Transcription Factor *HlMyb1* from Hop. Because of the direct evidence of the involvement of *Myb* R2R3 factors in the regulation of the phenylpropanoid biosynthesis pathway [see, e.g., Stracke et al. (*22*)], we aimed initially at screening for *Myb*-related hop sequences using PCR-based methods and a hop-specific cDNA library from glandular tissue-enriched hop cones as a source of a PCR template. The expression library contained 1.2×10^6 pfu. Randomly selected clones were analyzed by PCR with primers corresponding to flanking sequences of cloned inserts, which ranged from 0.8 to 2 kb (**Figure 4**). Using a blue-white color selection system, the background was estimated, and it was found that <0.5% of recombinant phages contained no cDNA insert. From the cDNA library, 8.4×10^5 clones were used. Approximately 2×10^3 of the clones hybridized with *chs* homologous sequences, suggesting an enrichment of the cDNA library for *chs* sequences that corresponds to the fact that this library was established from hop tissue enriched lupulin glands. Conserved motifs of two specific R2R3 genes from *A. thaliana*, PAP1 (AC: AF325123) and PAP2 (AC: AF325124), were used

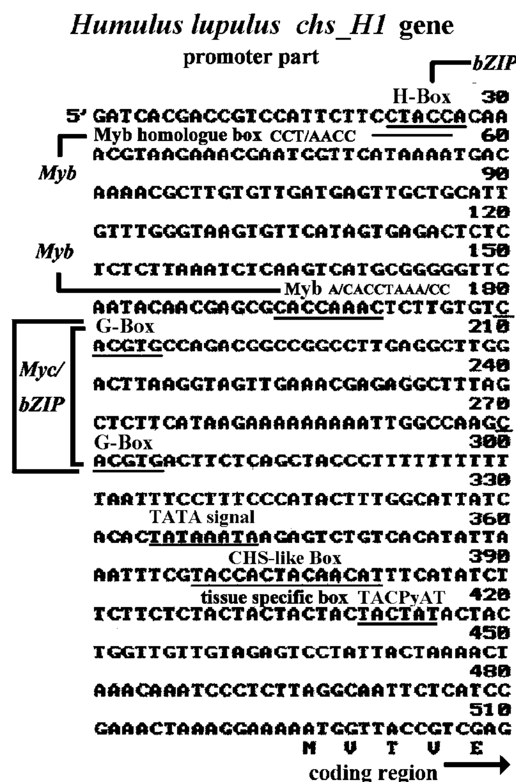

Figure 3. Cladogram of the promoter part of the *chs*_H1 gene. The *chs*_H1 promoter is numbered according to the sequence submitted to the EMBL database under AC AJ304877. The translation starting point is indicated by the arrow. Putative regulatory elements include TATA signal, CHS-like box, tissue-specific box (TACPyAT), and G, H, and Myb boxes. Binding of various predictable regulatory factors is shown schematically.

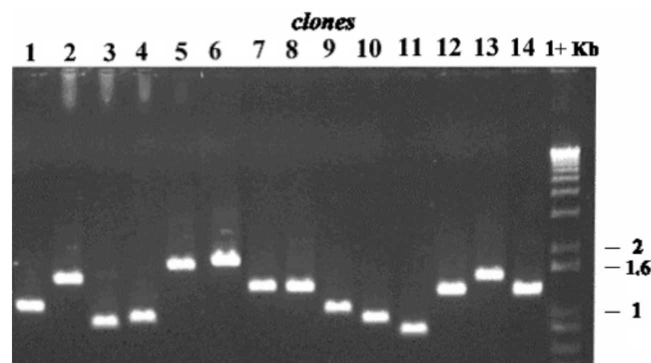

Figure 4. PCR analysis of individual recombinant phages of the hop cDNA library using universal and reverse primers (for details, see Materials and Methods).

to amplify a specific 160 bp *Myb* fragment from hop (data not shown). This fragment was used to screen for the 5′ portion of

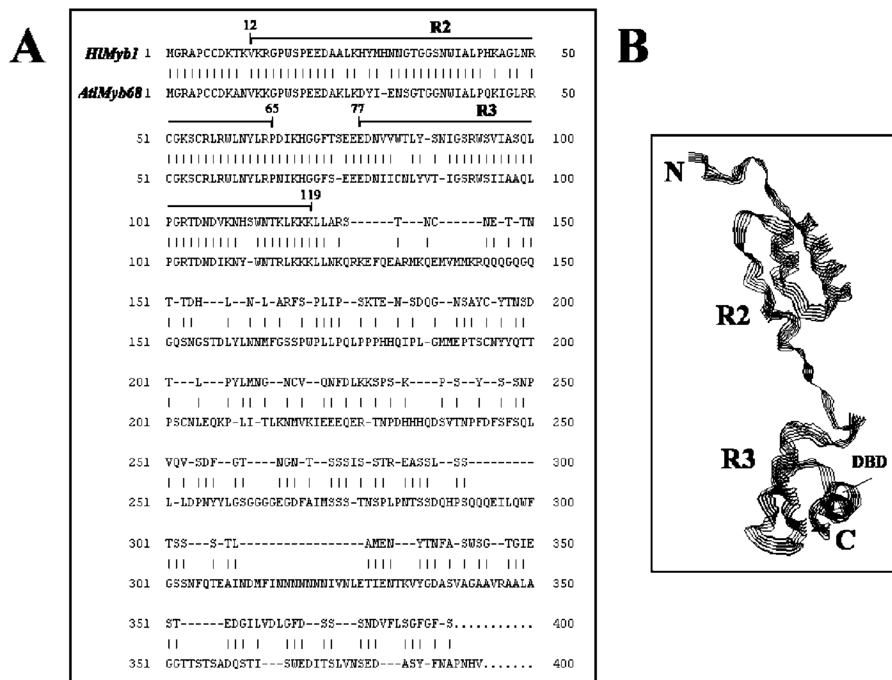

Figure 5. Alignment of amino acid sequences of selected *Myb* factors (**A**) and the structure of *HlMyb1* presented as a strands model (**B**). *HlMyb1* is compared with *AtMyb68* from *A. thaliana* (AC AF062901) showing the highest homology. Predicted R3R3 domains are shown on the *HlMyb1* amino acid sequence at positions 12–65 and 77–119, respectively. The *HlMyb1* structure in the R2R3 part of the protein is viewed using RasWin v. 2.6.4., as described under Materials and Methods. DBD designates DNA-binding domain.

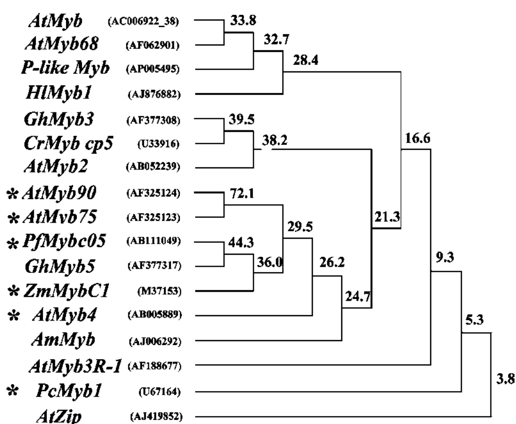

Figure 6. Cluster analysis for deduced amino acid sequences of selected plant R2R3 *Myb* factors designated by the accession numbers from the EMBL database (see Materials and Methods). The position of *HlMyb1* is shown. Asterisks designate proteins for which the involvement in the regulation of phenylpropanoid biosynthesis has been proven. Trees were generated with the Clustal W option of DNASIS (Hitachi). Homology percentages are shown. The *AtbZIP* transcription factor from *A. thaliana* (AC AJ419852) was chosen for rooting the dendrogram. Percentages of amino acid identity are included in the phylogenetic tree.

Myb sequences from the cDNA library. Finally, a primer covering the 5′ end of the *Myb* sequence (see Materials and Methods) was combined with the M13 forward primer to amplify the full-length *Myb* cDNA from the cDNA library.

Isolated cDNA encoded for a typical R2R3 MYB protein, named *HlMyb1*, having 272 amino acids with an apparent molecular mass of 29.8 kDa and a p*I* 7.57. Homology searching using WU-Blast2 showed the highest homology with the light-regulated *Myb* factor from *A. thaliana AtMyb68* (AC: AF062901) (**Figure 5A**) and with another light-regulated *Myb* factor from this species (AC: AC006922_38). The homology with *AtMyb68* reached 87% within the MYB protein domain (79% amino acid identity), whereas outside the domain, in the C-terminal part of the proteins, a significant homology was not apparent (**Figure 5A**). The exact functions of *A. thaliana Myb68* and of the light-regulated *Myb* transcription factor are not known. A structural model was predicted for the R2R3 protein domain of the HlMYB1 protein with a total surface area of 7756 Å (**Figure 5B**), showing typical helix−turn−helix (HTH) structural elements. The conserved motifs of the DNA-binding domain could be localized by analogy with other MYB proteins at positions 102−119 (RTDNDVKNHSWNTKLKKK) (**Figure 5B**).

Homology comparisons with selected *Myb* factors from other plants (**Figure 6**) show that *HlMyb1* forms an independent cluster with the light-regulated *A. thaliana Myb* factors and the P-type R2R3 *Myb* factor from *Oryza sativa*, whereas some of the known light-regulated factors that are involved in the induction of the phenylpropanoid pathway, such as PAP1 and PAP2 proteins of *A. thaliana* (*23*) or the C1 protein of *Z. mays* (*24*), occurred in separate clusters, suggesting that *HlMyb1* is not closely sequence-related to these factors. However, according to complex comparisons made by Kranz et al. (*12*), various *Myb* factors involved in the regulation of the phenylpropanoid pathways occurred in different clusters. Although the actual role of *HlMyb1* is not known, its expression pattern could contribute to insight into its function. Northern blot analyses (**Figure 7**) proved that *HlMyb1* is strongly expressed in hop female inflorescences, as well as in male inflorescences, whereas low expression was detected in young cones and almost no expression signal was observed on Northern blots for the RNA sample from mature cones and leaf tissues. The hybridization signals on the Northern blot were quite specific even after washing of the membrane at high-stringency conditions in 0.1 SSC and 60 °C (not shown), suggesting detection of homologous sequences. The band of ∼1 kbp corresponds to the predictable mRNA length including the coding sequence and noncoding regions isolated from the cDNA library. These results suggest a possible role of this factor in switching some developmental processes

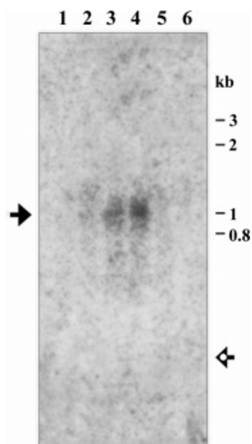

Figure 7. Northern blot analysis of total RNA isolated from mature cones (lane 1), young cones (lane 2), female flowers (lane 3), male flowers (lane 4), young leaves (lane 5), and old leaves of hop (lane 6). A probe derived from the cDNA of *HlMyb1* was used for hybridization. The position of specific bands is indicated by the filled arrow. Positions of the stained marker bands are indicated on the side, and the position of hop 7SL RNA having ~300 bases (not shown) is indicated by the hollow arrow.

in hop inflorescences that may be important for subsequent steps of cone formation. A functional analysis is necessary to verify this possibility. Unlike the sequence-related factor *AtMyb68* from *A. thaliana* that accumulates in mature leaves (*12*), *HlMyb1* accumulated strongly in flowers.

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