Complementation analysis of hop transcription factors using *Arabidopsis thaliana* genes in transient system and in transgenotes

Kocábek, Tomáš and Matoušek, Jaroslav

Biology Centre of the Academy of Sciences of the Czech Republic, v. v. i., Institute of Plant Molecular Biology, Branišovská 31, CZ-370 05 České Budějovice, Czech Republic, kocabek@umbr.cas.cz

Several hop genes which are involved in the phenylpropanoid pathway responsible for the production of prenylated chalcones (e.g. xanthohumol) with significant bioactivity and anticancerogenic properties have been cloned so far. These include genes encoding for the crucial enzymes of this pathway, like chalcone synthases (*chs*_H1) or o-methyltransferase (OMT) and putative genes encoding for their regulators, transcription factors (**TF**s). The genes for TFs belong to the families of **Myb** (*Hl*Myb1, *Hl*Myb2, *Hl*Myb3, *Hl*Myb7), **bZip** (*Hl*bZip1, *Hl*bZip2), **bHLH** (*Hl*bHLH1, *Hl*bHLH2) and **WD**R (*Hl*WD40_1) classes.

Three basic strategies to understand the role of the cloned TFs in the phenylpropanoid pathway have been approached.

1. Evaluation of the promoter activity in the presence or absence of cloned TFs.
2. Overexpression of the hop TF genes
3. Possible complementation of known *Arabidopsis* mutant genes either with significant homology or involved in similar pathways as hop genes.

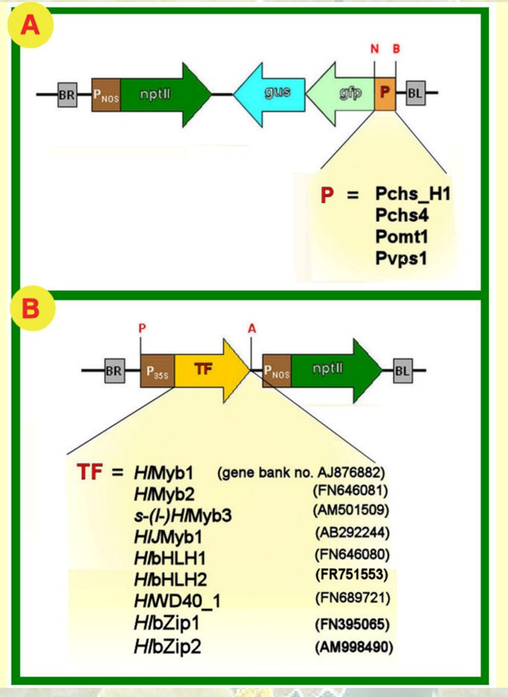


Fig. 1: A – *Promoters of genes crucial in the hop phenylpropanoid pathway were fused with GUS marker gene to perform transient expression studies of their activation with cloned hop transcription factors (TFs)*

B – hop *TF genes were cloned under control of constitutive promoter 35S into Agrobacterium tumefaciens vectors and their overexpression in transgenotes was studied.*

1

TRANSIENT EXPRESSION STUDIES

The transient expression studies were performed to test whether our cloned TFs are able in vivo activate the expression of selected hop genes (chs_H1, chs4, omt1). Their promoters linked to GUS reporter gene were *Agrobacterium* infiltrated together with genes for cloned TFs into *N. benthamiana* leaves and the GUS enzyme activity was evaluated. The strongest response was achieved with the *chs*_H1 promoter.

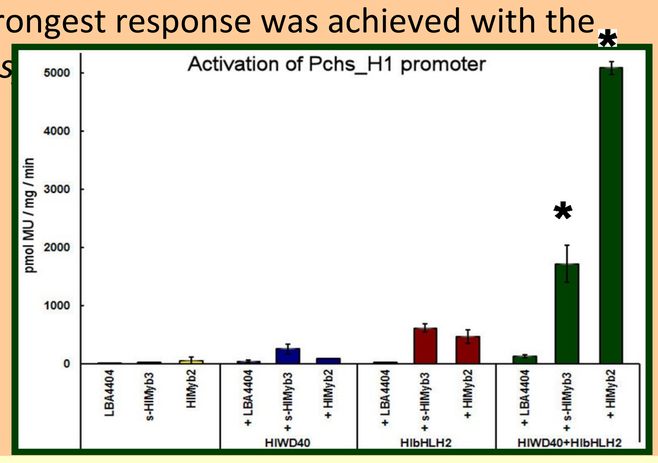


Fig. 2: *Activation of Pchs_H1 by hop TF and TFs complexes. Activation by full HlM2W1H2 and HlM3W1H2 complexes is marked by asterisks.*

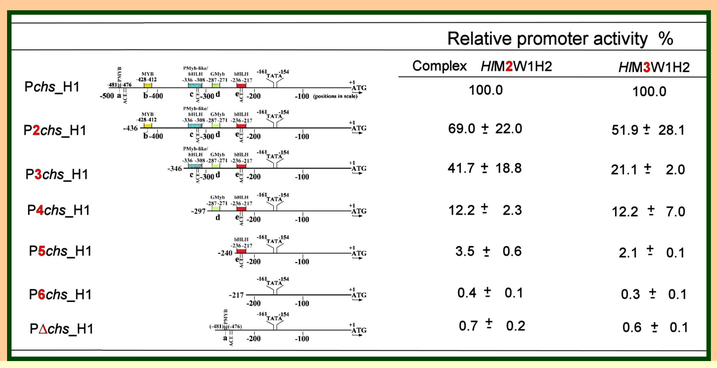


Fig. 3: *Variants of chs_H1 promoter with marked potential TF binding sites and GUS activity relative to maximum enhancement with full length chs_H1 promoter.*

CONCLUSION

Transcription factors from the hop genome form specific complexes which are able to activate significantly *chs*_H1 promoter. The interplay and regulation of expression of these TF complexes could co-determine the rate of accumulation of valuable metabolites of lupulin.

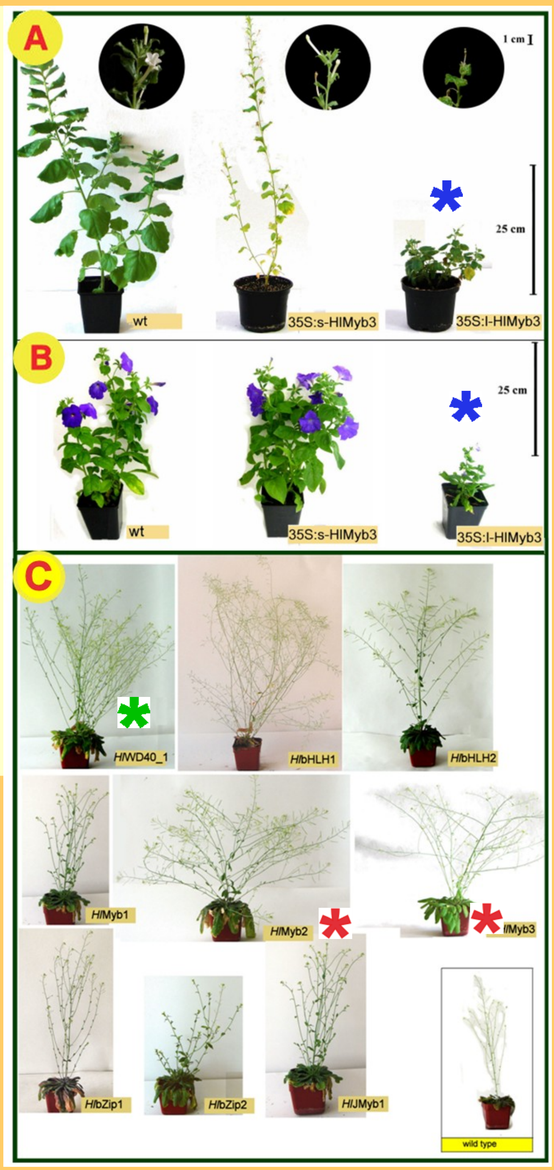
2

OVEREXPRESSION OF HOP GENES FOR TRANSCRIPTION FACTORS

Because the hop transformation is labour intensive and time consuming (still in progress to get transgenic cones with lupulin glands), we performed such analysis also on model plants *Arabidopsis thaliana, Nicotiana benthamiana* and *Petunia hybrida*.

Fig. 4: *Transgenic N. benthamiana* **A**, *P. hybrida* **B** *and A. thaliana* **C** *plants overexpressing hop lupulin gland-specific transcription factors.*

Several morphological changes, such as enhanced shoot branching ✱ *(A. thaliana with s-HlMyb3 or HlMyb2), dwarfism* ✱ *(N. benthamiana or P. hybrida with l-HlMyb3) or gigantism* ✱ *(A. thaliana with HlWD40_1 gene) were observed.*



CONCLUSION

TFs isolated from hop genome are able to bind to promoter targets also in heterologous genomes and their overexpression can influence the phenotype of heterologous transgenotes.

3

COMPLEMENTATION STUDIES

Several known *Arabidopsis* mutants of TF genes can be used in attempt to complement their function with our cloned hop TFs.

Table1: *Preliminary results of the analysis of complementation of Arabidopsis mutants with overexspressed hop genes*

gene class	hop gene	*Arabidopsis* gene	phenotype	complemented
	chs_H1	*tt4*	yellow seeds	yes
Myb	*Hl*Myb1, s-HlMyb3	*flp*	stomata with 4 guard cells	no
		gl1	trichomeless	no
	l-*Hl*Myb3	*as1*	asymetric leaves	yes
bZip	HlbZip1	*hy5*	long hypocotyl	no
bHLH	*Hl*bHLH1	*gl3*	trichomeless	no
		tt8	yellow seeds	yes
WD repeat	*Hl*WD40_1	*ttg1*	yellow seeds, trichomeless	yes

Fig. 5: *Complementation of the Arabidopsis WD40 gene ttg1. Trichomeless plants with yellow seeds were transformed with HlWD40_1 gene . Leaves of the transformed plants contained many trichomes and the seeds showed brown colour due to presence of the anthocyanin in the seed coat.*

CONCLUSION

TFs isolated from hop genome are able to complement inactivated genes in *Arabidopsis* genome and thus, they might share similar function in similar pathways as in model plants.

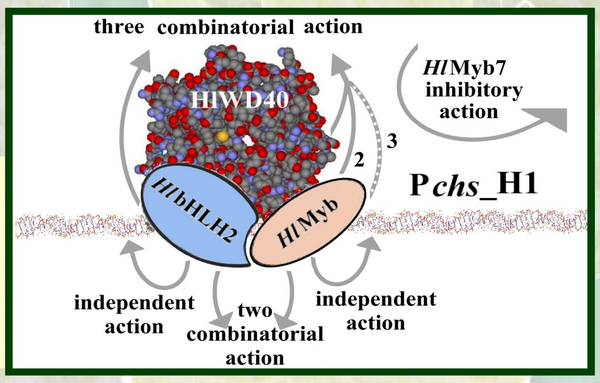


Fig. 6: *Based on the results, mainly of the transient expression studies, a hypothetical model of the action of the hop MWH (Myb + WD40 + bHLH) complexes on chs_H1 promoter has been created.*

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