Hop stunt viroid (HSVd) disease causes alteration of expression of hop transcription factors from MYB, bHLH and WRKY families

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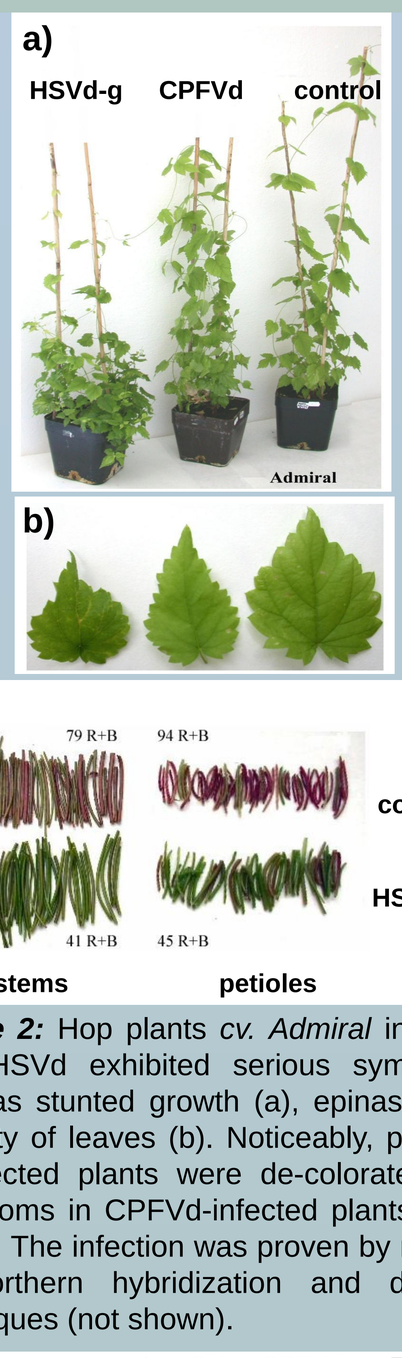

Figure 2: Hop plants *cv. Admiral* infected with HSVd exhibited serious symptoms such as stunted growth (a), epinasty and rugosity of leaves (b). Noticeably, petioles in infected plants were de-colorated (c). Symptoms in CPFVd-infected plants were milder. The infection was proven by means of Northern hybridization and dot-blot techniques (not shown).

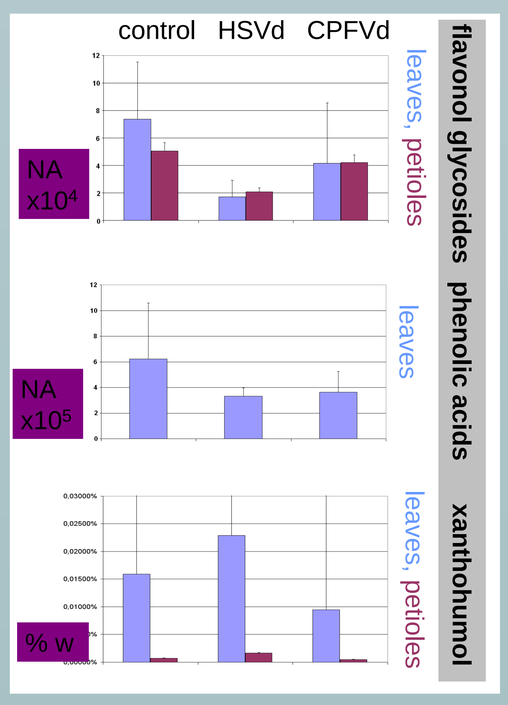

Figure 3: Changes in secondary metabolites composition according to HPLC analyses. Several metabolites were roughly identified and sorted to biochemical groups (e.g. flavonol glycosides, phenolic acids) according to their retention time and absorption spectra. In infected plants, shifts in some secondary metabolites spectra are observed compared to healthy controls. NA= normalized area.

References:

Diermann 2010, BIOL. CHEM. 391:1379. Matoušek *et al.* 2003, *PLANT SOIL ENVIRON.* 49:168. Matoušek *et al.* 2005, *J. AGRIC. FOOD CHEM.* 53:4793. Matoušek *et al.* 2007, *J. AGRIC. FOOD CHEM.* 55:7767.

Introduction

Hop stunt viroid (HSVd) is a serious pathogen that has been emerging as a threat for hop-gardens in Japan, USA, China, and potentionally dangerous in central Europe, for it is latently residing in grapewines and could be transmitted to hops (Matoušek *et al.* 2003). HSVd is also remarkable for being simple in structure (***Figure 1***), but complex in induction of symptoms. The pathogenesis of this non-coding, circular and highly complementary ssRNA molecule has not been clearly explained, though involvement of viroid-derived small RNAs (vd-sRNAs) is considered to be plausible.

In this process, vd-sRNAs target complementary host mRNAs for degrading. If a regulatory protein expression is targeted, also downstream genes might be affected (e.g. Diermann *et al.* 2011). By disbalancing the expression of various target transcription factors (TFs), the symptoms in our model hop *cv.* Admiral could be induced (***Figure 2***). In addition to stunted growth and leaf rigidity of plants infected by HSVd-g (AC: E01844.1), we observed shifts in the levels of secondary metabolites (***Figure 3***, unpublished) and changes in petiole colouration.

Consequently, we examined several hop TFs cloned in our laboratory for possibly altered expression as a response to viroid infection. These TFs are lupulin gland-specific and putatively connected with lupulin metabolites biosynthesis, partly via regulation of flavonoid pathway key enzyme, chalcon synthase *chs*_H1 (*HlbHLH2*, *HlMyb1* described in Matoušek *et al.* 2005, and *HlMyb3* described in Matoušek *et al.* 2007). Others are related to factors involved in stress (*HlMyb4* and *HlMyb5*), or in pathogen resistance (*HlWRKY75*).

Aim of work:

Quantitative analysis of putative hop transcription factors (TFs) expression in infected vs. healthy control plants.

Quantitative analyses of hop TFs expression

On the basis of altered metabolites production, we expected some of the recently cloned hop cone-specific TFs to have altered expression as a response to viroid infection. Using quantitative RT-PCR we found several of these TFs to be expressed differentially in infected and control plants (***Figure 4***).

Various TFs from MYB family were observed to have increased mRNA levels, most importantly *HlMyb3* having 5-fold higher expression in symptomatic petioles and leaves. On the contrary, we observed 8-fold decrease of *HlbHLH2* (AC:FR751553) mRNA in both leaves and petioles. The observation is remarkable, since we assume that HlbHLH2 and HlMyb3 form a ternary complex with HlWD-40_1. According to our transient expression experiments, this complex is highly potent to activate chalcon synthase promoter (*chs*_H1) *in planta* (see oral presentation of Matoušek *et al.*). Such a serious disbalance in the complex components might also affect other genes unknown to date.

Direct interaction of HlMyb3 with the promoter sequence of *chs*_H1 is implicated from electromobility shift assays (***Figure 5***). Functional analyses of novel hop Myb and WRKY TFs mentioned above are in progress to provide deeper insight into regulation of lupulin metabolome during HSVd infection as well as in healthy plants.

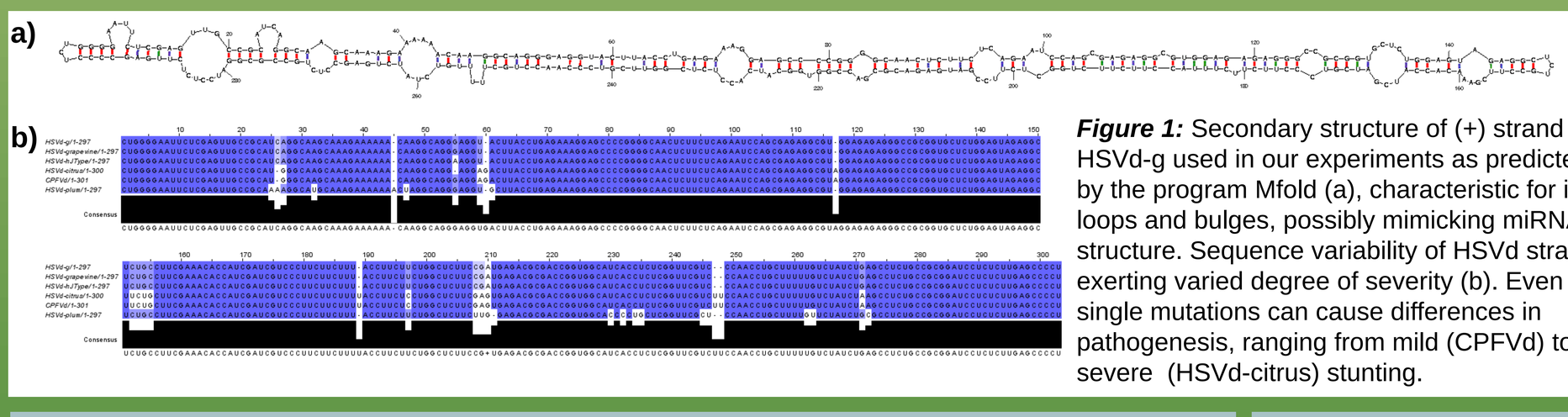

Figure 1: Secondary structure of (+) strand HSVd-g used in our experiments as predicted by the program Mfold (a), characteristic for its loops and bulges, possibly mimicking miRNA structure. Sequence variability of HSVd strains exerting varied degree of severity (b). Even single mutations can cause differences in pathogenesis, ranging from mild (CPFVd) to severe (HSVd-citrus) stunting.

Figure 4: Changes in expression of putative hop TFs as measured by qRT-PCR. The analyses were carried out in HSVd- and CPFVd-infected plants from leaf and petiole tissue. The data come from two independent experiments with reactions run in doublets. On the vertical axis is the relative mRNA level to GAPDH as internal control („housekeeping gene"). Error bars represent the standard deviation of measured levels.

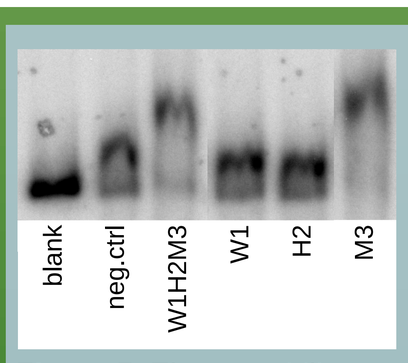

Figure 5: Electromobility shift assay (EMSA) of hop TFs putatively involved in *chs*_H1 regulation. The assay was carried out using crude protein extracts from plants transiently expressing TFs either single contructs of *HlWD-40_1* (W1), *HlbHLH2* (H2), and *s-HlMyb3* (M3), or the combination of these three constructs (W1H2M3).

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