Optimisation of a Real Time RT-PCR Protocol for the Analysis of Gene Expression in Hop Tissues

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Introduction & Aim

Hop (*Humulus lupulus* L.) is a dioecious, perennial, climbing plant, included in the family Cannabinaceae. Female hop plants are cultivated in most temperate regions of the World for the extraction of secondary metabolites. Important secondary metabolites produced by hop include α-acids and β-acids and essential oils which are used in beer brewing as well as the prenylated chalcones, Xanthohumol and Desmethylxanthohumol which exhibit interesting bioactive properties. Xanthohumol, which is the most abundant prenylchalcone present in hop cones, inhibits carcinogenesis at all stages[1]. Desmethylxanthohumol serves as a pro-estrogen, since it can isomerize to 8-prenylnaringenin, the most potent phytoestrogen currently known[2]. One of the most important limiting factors for the development of commercial products which exploit these properties is the relatively low amounts of these components which are present in hop cones. Our objective in this study is to unravel the genes which influence the biosynthesis of these two compounds in hop. Here we report on the development of a Real Time RT-PCR protocol for the analysis of the expression profiles of previously identified hop candidate genes (De Keukeleire et al.[3]). Four Housekeeping genes (HG) are evaluated.

Materials and Methods

Plant Materials

Tissues of the hop varieties Whitbread Golding Variety (WGV) and Admiral (AD), found to possess contrasting characteristics for the accumulation of prenylchalcones in a previous study (De Keukeleire et al.[4]) were used. Female organs were collected at three different dates, corresponding to three stages of development, from the appearance of inflorescences (stage 1) to the formation of small hop cones (stage 2) and mature hop cones (stage 3) (Fig.1). In addition, leaf material of each variety was collected at the time of hop harvest. Samples were immediately immersed in liquid nitrogen and stored at -80 °C until RNA extraction.

Figure 1: Developmental stages of hop cones sampled in this study. Three developmental stages, from flowering up to full developed cones were analyzed.

RNA Isolation and Quantification

Total RNA of these 8 samples was extracted with RNeasy Midi Kit (Qiagen, Hilden, Germany). Total RNA was quantified with a spectrophotometer (GeneQuant, Pharmacia Biotech, Roosendaal, The Netherlands) at wavelengths of 260 and 280 nm. RNA integrity was verified by running samples on 1.5% agarose gels and staining with SYBRgold (Molecular Probes, Leiden, The Netherlands).

Real-Time RT-PCR

DNAse treatment, cDNA synthesis, primer design and SYBR Green I RT-PCR were carried out as described in ref. 6 using a ABI PRISM 7000 Real-Time PCR System. Four housekeeping genes were evaluated as internal control genes: NADH, 7sl RNA, chlorophyl-a/b binding protein and Histone H3. The relative expression of a putative HEN1 gene was quantified using these housekeeping genes. The assay included (in duplicate): a standard curve of eight serial dilutions of cloned PCR products of each housekeeping gene and HEN1, a no template control (NTC) and the eight samples of each test cDNA. The results were exported as tab-delimited text files and imported into geNorm[5] for further analysis. This allowed us to identify the most stably expressed control genes in this set of tissues, and to determine the minimum number of genes required to calculate a reliable normalization factor.

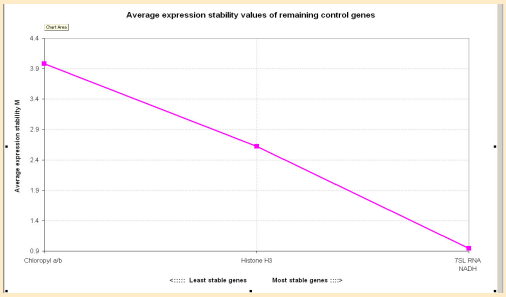

Figure 2: GeNorm output for evaluation of the best HG out of the four candidate HG tested . According to these results, 7sl RNA and NADH are the best to use for normalization.

Results & Discussion

Housekeeping genes

Primer pairs were designed for the four housekeeping genes described in M&M. The expression level of these genes was determined in 8 hop samples. Data analysis using geNorm software[5] identified the two housekeeping genes 7sl RNA and NADH as the most suitable for the tissues analysed (Fig. 2), **M value** is an estimation of the average expression stability of a set of genes. A small M value indicates stable expression[5]. Normalization factors were calculated using these two housekeeping genes.

The expression level of HEN1 is genotype-dependent

7sl RNA and NADH were used to normalize the expression level of HEN1, as illustrated in (Fig. 3). The expression of this gene seems to be variety dependent. This gene was expressed both in somatic and reproductive tissues (hop cones). In hop cones, the lowest expression values were recorded for Stage 2.

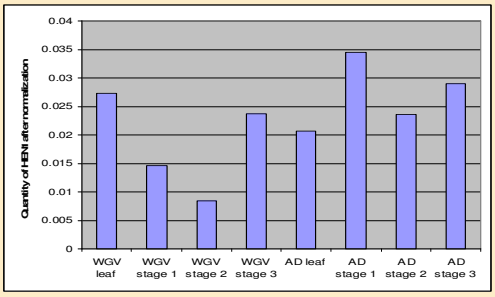

Figure 3: The relative expression levels of HEN1 is variety-dependent.

Conclusion

The choice of stably expressed internal control genes to normalize the mRNA fraction is crucial in the design of Real Time RT-PCR experiments. In this study the analysis of four housekeeping genes allowed us to select the two most stably expressed in hop leaves and cones. This is a prerequisite for accurate RT-PCR expression profiling of the candidate genes identified in hop, as demonstrated here for HEN1.

References

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