## Summary

*Salvia miltiorrhiza* Bunge is one of the most popular plants of traditional Chinese medicine. Its therapeutic properties are due to the ability to biosynthesise more than two hundred bioactive compounds, including tanshinones with multidirectional effects. It has been proven that the rate of tansinone biosynthesis depends largely on a 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR) from the mevalonic acid pathway. To date, five *S. miltiorrhiza HMGR* gene sequences (*HMGR–HMGR4*) have been identified, of which the *HMGR4* gene has not yet been studied.

One of the most important issues of molecular biology is to understand the mechanisms of regulation of gene activity from transcription to post-translational modification of proteins and at the level of epigenetics, as well as the ability to influence these processes. In plants, a key step in this regulation occurs during transcription, where the promoter plays an important role. Within the promoter, the assembly of the preinitiation complex necessary for the initiation of gene transcription takes place. Moreover, motifs (TFBSs) recognised by transcription factors (TFs) (activators or repressors) and binding sites for microRNAs (miRNAs) are located here. Hence, the analysis of the promoter structure, the proteins and RNA interacting with it is fundamental to understanding the mechanisms regulating the expression of the studied gene.

In the present study, a new *S. miltiorrhiza HMGR4* promoter sequence was subjected to a series of *in silico* analyses. The results indicated the presence of a TATA box, tandem repeat, pyrimidine-rich sequence in the 5′UTR, 5369 potential TFBSs and 365 interacting TFs, 12 binding sites for mature miRNAs, and the absence of CpG islands. According to literature data, genes with promoters of similar structure are stimulated by stress and extracellular factors, and their activity is highly variable.

The evaluation of TFBSs from the proximal *HMGR4* promoter and interacting TFs indicated that light, salicylic acid (SA), bacterial infection, auxins, abscisic acid, and gibberellins may be mainly involved in the regulation of *S. miltiorrhiza HMGR4* gene expression. The conducted experiments confirmed the effects of gibberellic acid ($GA_3$), indole-3-acetic acid (IAA), and SA on its activity.

Analysis performed with the Pathway System tool revealed the existence of interactions between some of the TFs potentially binding to the *S. miltiorrhiza HMGR4* promoter, i.e. SVP−AGL18−SPL3, PDF2−ATML1−ANL2, EIL3−EIL1−EBP, ATHB13−HB-1, NAC3 with RD26 and ZF2. These interactions were predominantly related to the presence of binding sites for a given TF in the promoter of another TF. In addition, it has been shown that some TFs can form dimers, which increases the specificity and affinity of binding to the promoter and allows for more precise control of gene expression. This included factors interacting with the proximal part of the

studied *HMGR4* promoter representing the following families: HD-ZIP (ATML1, PDF2, HDG1), WRKY (WRKY2, WRKY14, WRKY45, WRKY57, WRKY69) and DOF (DOF5.4).

Analyses performed with the Common TFs and FrameWorker tools revealed the presence of multiple common TFBSs and their frameworks within the *S. miltiorrhiza HMGR1*, *HMGR2* and *HMGR4* promoters. The evaluation of TFs binding to these TFBSs indicated the possibility of co-regulation of controlled genes in response to abiotic factors (auxins, gibberellins, abscisic acid, SA, jasmonic acid, brassinosteroids, light, water deprivation, salt stress, cold, phosphate deficiency) and biotic factors (bacteria, fungi, viruses) and during root, stem, leaf and flower organogenesis.

In the *S. miltiorrhiza HMGR4* promoter sequence, 65 sites for TFs with a confirmed positive effect on the biosynthesis of tanshinones were detected, i.e. BHLH6, BHLH74, BZIP20, WRKY2 and WRKY61. In a study with *S. miltiorrhiza* plants transformed with a pRI201-AN-HMGR4 overexpression construct, there was a significant increase in the content of all tested tanshinones in roots (from 0.44 to 5.39 mg/g DW) and tanshinone IIA (TIIA) in stems and leaves relative to control. The results confirm for the first time not only the involvement, but also the important role of the *S. miltiorrhiza HMGR4* gene in the biosynthesis of these secondary metabolites. However, *HMGR4* overexpression did not change the characteristic organ-dependent pattern of tanshinone biosynthesis. Roots remained the main source, while trace amounts were found in stems and leaves. Furthermore, the addition of $GA_3$ to transformed *S. miltiorrhiza in vitro* root culture significantly increased cryptotanshinone (by 0.79 mg/g DW) and TIIA (by 88.1 µg/g DW) levels in comparison to untreated roots. This presumably occurred as a result of strong induction of the expression of some key enzyme(s) involved in the terminal stage of CT and TIIA biosynthesis.

In conclusion, the presented *HMGR4* promoter structure and the detected potential TF−promoter, miRNA−promoter and TF−TF interactions reveal a fragment of the complex network of relationships determining the expression of the *HMGR4* gene and other *S. miltiorrhiza* genes.