

## **TRANSCRIPTIONAL CO-REGULATION OF TERPENE BIOSYNTHETIC GENES ENHANCE SYNTHESIS OF ANTITUMORAL DITERPENES IN *SALVIA SCLAREA* HAIRY ROOTS**

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*anti-tumoral diterpenes, Salvia sclarea, hairy roots, metabolic engineering*

The roots of *S. sclarea* are rich in abietane diterpenoids (e.g. aethiopinone, 1-oxoaethiopinone, salvipisone, and ferruginol), with known antibacterial, antifungal, and sedative pharmacological properties. More recently, this class of tricyclic diterpenoids has raised much attention for its cytotoxic activity against human leukemic and solid tumor cell lines (Vaccaro et al., 2014). Elicitation of *S. sclarea* hairy roots with methyl-jasmonate (MJ) enhances significantly the total content of this class of compounds (about 20-fold over the control hairy roots), due to transcriptional up-regulation of several genes belonging to the plastidial MEP-dependent terpenoid pathway, from which diterpenes derive. In an effort to identify transcription factors (TFs) that might regulate in a coordinate manner the expression of key genes of the MEP-dependent pathway, the promoter region of different MEP-pathway biosynthetic genes was scanned and it was found that they contain either G-box (typical binding site of Myc2 transcription factor) and/or W-box (binding site of the WRKY transcription factor family). Four different TFs (WRKY18, WRKY40, WRKY60 and Myc2) from *A. thaliana* containing MJ-RE motifs in the promoter regions were selected and over-expressed in *S. sclarea* hairy roots.

Quantitative RT-PCR analysis of independent over-expressing lines revealed that AtWRKY18 and AtMyc2 TFs were the most efficient in inducing the expression of several MEP-pathway biosynthetic genes. Targeted metabolic analysis (HPLC-DAD) revealed also that the content of abietanic diterpenes was 3-5-fold higher in transgenic hairy roots compared to control root lines transformed with the empty vector. Beside confirming that the overexpression of transcription factors is a successful strategy to push the metabolic flux towards the synthesis of the compounds of interest, the identification of these two TFs add novel information on the regulatory control of the MEP-dependent terpenoid pathway.

Vaccaro MC, Malafronte N, Alfieri M, De Tommasi N, Leone A, 2014. Enhanced biosynthesis of bioactive abietane diterpenes by overexpressing *AtDXS* or *AtDXR* genes in *Salvia sclarea* hairy roots.

Plant Tissue and Organ Culture. DOI 10.007/s11240-014-0514-4.