

## Integrated transcriptome and metabolome analysis reveals the podophyllotoxins accumulation and formation mechanisms in *Juniperus sabina* L. needles

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**Abstract:** Podophyllotoxin is an important secondary metabolite because of its remarkable anti-cancer and anti-Condyloma acuminatum activities. *Juniperus sabina* L. (*J. sabina*) has been proven as a new plant source of podophyllotoxins. However, the mechanism of podophyllotoxins accumulation and synthesis in *J. sabina* is unclear, which greatly limits their applications. Based on this, transcriptomic and metabolomic analyses were performed on different developmental stages of *J. sabina* needles to determine the molecular mechanism of the biosynthesis of podophyllotoxins in this study. Firstly, 1022 compounds were identified from metabolomic analysis of *J. sabina* needles at different developmental stages, which contained 8 podophyllotoxins. The total lignans and podophyllotoxin contents were determined by HPLC, clarifying that May to September was the critical period for the accumulation of podophyllotoxins in *J. sabina* needles. Transcriptome and metabolic analysis showed that 47 DEGs encoded 16 key enzymes involved in podophyllotoxin biosynthesis, among which 8 key enzyme genes PAL, HCT, CCR, C4H, DIR, PLR, OMT and CYP82 were identified. Furthermore, JsPLR was proven as a key rate-limiting enzyme gene, with overexpression significantly increasing matairesinol and podophyllotoxin content. Subcellular localization results indicated that JsPLR is a cytoplasmic localization gene. The results of qRT-PCR study indicated that the differential expression of JsPLR gene might be a key factor leading to the differential accumulation of podophyllotoxin. Overall, the study laid a theoretical foundation for revealing the accumulation and formation mechanisms of podophyllotoxins and the comprehensive development and utilization of *J. sabina* resources.

**Keywords:** *Juniperus sabina* L.; Podophyllotoxins; Accumulation; Transcriptome; Metabolome

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