

Transcriptional regulation mechanism of the biosynthesis of linalool, a monoterpenoid compound from *Osmanthus fragrans*

Yan-gang Lan¹ Yan Xiang*

(Anhui Provincial Key Laboratory of Forest Resources and Silviculture, Anhui Agricultural University, Hefei 230036, China)

Abstract: *Osmanthus fragrans* (*Osmanthus fragrans* (Thunb.) Lour.) is an important woody aromatic plant in China. It is colorful and fragrant in its flowering period, which is not only of high ornamental value, but also one of the main sources of natural fragrances. It has high economic value in perfume, cosmetics, condiments and other industries and food industries. Floral fragrance is one of the most important ornamental traits of garden plants, which determines people's liking of garden landscape. Floral fragrance can not only improve the ornamental value of garden plants, but also floral products play an important role in food, daily chemical and medical care. As the most important characteristic aroma substance of *O. fragrans*, linalool, a single terpene compound, exists widely in various varieties of *O. fragrans*, and is the main aroma component of *O. fragrans* essential oil. At present, researchers have used multi-omics technology to explore the metabolism of linalool in *O. fragrans* from the level of gene expression and metabolites, and preliminarily identified the terpenoid synthase and its regulatory genes involved in the biosynthesis of linalool in *O. fragrans*. However, the molecular regulatory network and the signal pathway involved in the synthesis of linalool in *O. fragrans* are still unclear.

In our laboratory, a terpene synthase gene *OfTPS2*, which was highly expressed in full flowering, was identified by transcriptome sequencing of samples from *O. fragrans* flowers at different stages of flower development. In vitro enzyme activity analysis and transient transformation experiment of tobacco confirmed that *OfTPS2* was linalol synthase gene. Based on this, this study used bioinformatics and molecular biology methods to identify upstream transcription factors of linalool synthase gene *OfTPS2* and study their regulatory mechanisms, and further studied the interaction regulatory mechanisms of key transcription factors and their interacting proteins. The main results were obtained as follows:

1. By analyzing the transcriptome data of *O. fragrans* flowers in different tissues and at the flower stage downloaded from the database, multiple transcription factors that potentially regulate the linalool synthase gene *OfTPS2* were screened. Y1H, luciferase reporter gene detection and EMSA experiments showed that R2R3-MYB transcription factor OfMYB21 activated its expression by binding to *OfTPS2* promoter MYB transcription factor site;

2. OfMYB21 has transcriptional autoactivation activity and functions as a homologous dimer. Functional analysis of transgenic *Arabidopsis thaliana* and tobacco plants with transient expression and allogeneic overexpression of *OfMYB21* showed that OfMYB21 positively regulated the biosynthesis of linalool by up-regulating the expression of linalool synthase gene in transgenic plants.

3. The results of Y2H, BiFC and Pull down experiments show that OfMYB21 transcription factor interacts with

jasmonic acid (JA) signal factors OfJAZ2 and OfJAZ3 in vivo and in vitro. The detection of promoter activation effect and competitive EMSA experiments showed that OfJAZ3 formed transcription complex by interacting with OfMYB21, thereby inhibiting OfMYB21 transcriptional regulation of linalool synthase gene *OfTPS2*.

4. The transient expression and allogeneic overexpression of OfJAZ3 gene in *Arabidopsis thaliana* and tobacco plants showed that OfJAZ3 negatively regulated the biosynthesis of linalool in transgenic plants by inhibiting the expression of linalool synthase gene.

5. Exogenous MeJA treatment promoted OfJAZ3 protein degradation and hindered OfJAZ3's inhibition of *OfTPS2* expression, while MG132 treatment inhibited MeJA-induced OfJAZ3 protein degradation. Further studies found that OfJAZ3 interacts with the E3 ubiquitin ligase OfCOI1 of *O. fragrans*. These results suggest that the stability of OfJAZ3 protein is regulated by the ubiquitin-proteasome system mediated by jasmonic acid signaling, indicating that jasmonic acid signaling pathway plays an important regulatory role in mediating the synthesis of linalool in *O. fragrans*.

In summary, this study identified the R2R3-MYB transcription factor OfMYB21 regulating the monoterpene linalool synthase gene *OfTPS2* of *O. fragrans*, clarified the biological function and molecular mechanism of OfMYB21 regulating linalool synthesis, and further discovered the interacting protein OfMYB21, OfJAZ3. The molecular mechanism of OfMYB21-OfJAZ3 transcription complex mediated the synthesis of linalool from *O. fragrans* and its involved signaling pathway were studied. The results of this study analyzed the key signal pathways regulating the enrichment of aromatic substance linalool in *O. fragrans* flowers, and provided an important theoretical basis for mining the germplasm resources of *O. fragrans* flowers, developing high-quality *O. fragrans* varieties and regulating the synthesis efficiency of terpenoid compounds.