

Metabolic Engineering of Lignan Biosynthesis in *Forsythia* Cell Culture

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Lignans are a large class of secondary metabolites in plants, with numerous biological effects in mammals, including anti-tumor and anti-oxidant activities. Sesamin, the most abundant furofuran-class lignan in sesame seeds (*Sesamum* plants), is produced by the cytochrome P450 enzyme CYP81Q1 from the precursor lignan, pinoresinol. In contrast, *Forsythia* plants produce dibenzylbutyrolactone-class lignans, such as matairesinol, from pinoresinol via the catalysis of pinoresinol/lariciresinol reductase (PLR) and secoisolariciresinol dehydrogenase. Here we present the engineering of lignan biosynthesis in *Forsythia* cell suspension cultures for the development of an efficient production method of beneficial lignans. A suspension cell culture prepared from leaves of *Forsythia koreana* produced lignans, mainly pinoresinol and matairesinol glucosides, at levels comparable to that obtained from the leaves. In an attempt to increase the pinoresinol content in *Forsythia*, we generated a transgenic cell line overexpressing an RNA interference (RNAi) construct of *PLR* (*PLR*-RNAi). Down-regulation of *PLR* expression led to a complete loss of matairesinol and an accumulation of approximately 20-fold pinoresinol in its glucoside form in comparison with the non-transformant. Moreover, the *Forsythia* transgenic cells co-expressing *CYP81Q1* and *PLR*-RNAi exhibited production of sesamin as well as accumulation of pinoresinol glucoside. These data suggest *Forsythia* cell suspension to be a promising tool for the engineering of lignan production. To the best of our knowledge, this is the first report on transgenic production of an exogenous lignan in a plant species.

Fig. 1

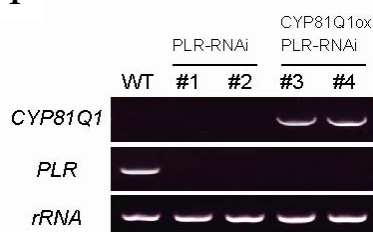


Fig. 2

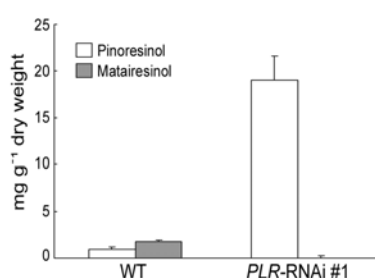


Fig. 3

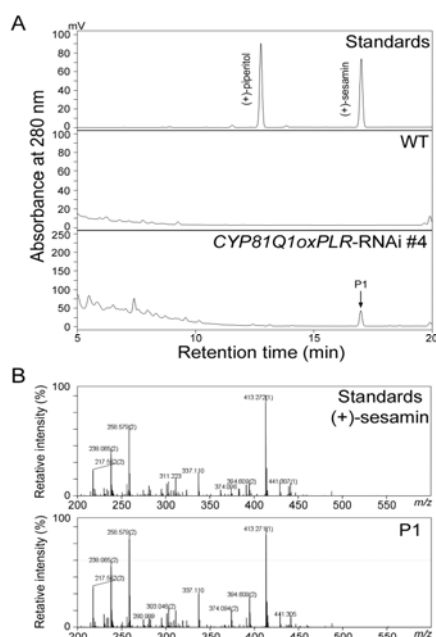


Fig.1: Generation of transgenic *Forsythia* cell lines. Transcript accumulation of the wild-type (WT) and transgenic cell lines (*PLR*-RNAi #1, #2 and *CYP81Q1*ox*PLR*-RNAi #3, #4) assay by RT-PCR. **Fig.2:** Analysis of the lignans produced in the *PLR*-downregulated cell lines. The content of matairesinol was reduced to non-detectable levels, and approximately 20-fold pinoresinol in its glucoside form was accumulated in comparison with the wild-type. **Fig.3:** The double transgenic lines newly accumulated a lignan product. (A)HPLC analysis of the resultant lignan extracts demonstrated that a product was eluted at a retention time of 16.9 min, which is identical to the retention time of the standard sesamin. (B)LC-MS analysis revealed that the product completely corresponded to the calculated mass of the standard sesamin.