Three new CYP450 from *Betula platyphylla* Suk. with 28 oxidation function catalyze the conversion of lupineol to betulinic acid

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Abstract

Birch(*Betula platyphylla* Suk.) bark contains important pentacyclic triterpenes as betulin and betulinic acid, which play important functions in anti-tumor and anti-HIV.Cytochrome P450 monooxygenase(CYP450) is essential for the diversification and functional modification of the triterpene skeleton.In this study, five new *CYP450* genes were cloned from birch with ORF lengths of 1284bp, 1533bp, 1188bp, 1704bp, and 1539bp, respectively.Phylogenetic tree analysis shown that five *BpCYP450* genes are located in five subfamilies, named *CYP94B89*, *CYP89S1*, *CYP97B62*, *CYP86B54*, and *CYP86A182*.The expression characteristics of five *CYP450* genes in different tissues and their responses to different stresses(MeJA, SA, GA_3, ABA, ethylene, and mechanical damage) were significantly different, among which *CYP89S1*, *CYP97B62* and squalene epoxidase(*BpSE*) and dammarenediol synthase(*BpDS*) were highly expressed in leaves.*CYP89S1*, *CYP97B62*, and *CYP86A182* genes are induced by MeJA and significant synergistic expression effects with lupeol synthase(*BpW*).*CYP89S1*, *CYP97B62*, *CYP86A182* have C-28 oxidation function and catalyzing the conversion of lupeol to betulinic acid.Among them, *CYP97B62* gene has the highest catalytic efficiency, increasing the content of betulinic acid by 1136%.In addition, co expression of *BpMYB21* and *CYP86A182* can significantly enhance the conversion and synthesis efficiency of betulinic acid in tobacco(*Nicotiana tabacum* L.), and *CYP89S1* can enhance salt and alkali resistance in yeast(*Saccharomyces cerevisiae*)

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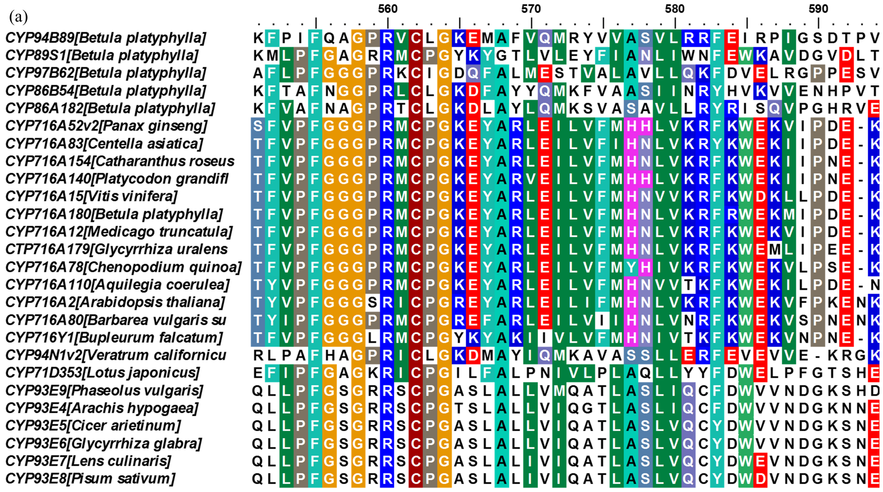
(a)



(b)