49c The Study of Benzylisoquinoline Alkaloids Production by Analysis of Biosynthetic Pathway in *Eschscholtzia Californica*

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Industrial application of plant cell system is limited by contamination of culture during long period and low yields of target compounds. These limitations are occurred by little characterization and optimization of plant cell systems. As a result, general strategies for achieving high productivity are lacking. If the liming step of biosynthetic production pathway can be found, we can determine how to knock out side-pathway of unwanted compound or how to activate the liming step from precursors to sanguinarine. California poppy(Eschscholtzia californica Cham.) produces a variety of benzylisoquinoline alkaloids of pharmaceutical importance, including the sanguinarine, macarpine and chelerythrine. Sanguinarine has been used a traditional drug which has antimicrobial activity with broad range spectrum. Benzylisoquinoline alkaloids biosynthesis begins with a network of decarboxylations, hydroxylations and deanminations that convert L-tyrosine to both dopamine and 4-hydroxyphenylacetaldehyde. And biosynthetic pathway includes several enzyme steps and reactions until (S)-reticuline, which is branch-point intermediate from (L)-tyrosine to sanguinarine and morphine. Several reactions from L-tyrosine to (S)-reticuline are involved by specific enzymes related with sanguinarine biosynthetic pathway, for example BBE, CYP80B1, CNMT and so on. Elicitor treatment is a tool for boosting productivity of sanguinarine and expanding limiting step in biosynthetic pathway. We activated all secondary metabolisms by the addition of yeast extracts to cultured cells in mid-exponential growth phase. Elicitor-treated cells showed higher productivity of sanguinarine and sanguinarine derivatives than untreated cells. We revealed major differences on the profiles of metabolites production and mRNA expression level of several genes related with biosynthetic pathway between control(unelicited) cells and elicited cells. Also cell transformation for modulating expression level of enzymes related with biosynthetic pathway was used to study important upstream metabolic processes. It gave us important information on bottleneck in sanguinarine production pathway and will provide insight into the complex regulation of benzylisoquinoline alkaloids.