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Molecular and Biochemical Analysis of OsF_3H Gene Expressed in Yeast Regulating Kaempferol and Quercetin

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[Introduction]

Kaempferol and quercetin are the essential plant secondary metabolites confer huge biological functions regarding plant defense system. Due to low level of production in plant, use of microbial factory for biosynthesis of these compounds is a promising strategy. In plants kaempferol and quercetin biosynthetic pathway is well developed and synthesize in phenylpropanoid pathway from aromatic amino acids like phenylalanine and tyrosine. Naringenin is the main intermediate compound responsible for various flavonoids depends on the enzymes. In case of kaempferol and quercetin biosynthesis, flavanone 3-hydroxylase (F_3H) uses naringenin as a substrate and converts it into dihydrokaempferol and further into dihydroquercetin. Flavonol (kaempferol & quercetin) are synthesizing from dihydrokaempferol and dihydroquercetin catalyze by flavonol synthase. Naringenin conversion to different flavonoids is due to the hydroxyl group addition to different position of the compound depending on the enzyme.

[Materials and Methods]

In this study the biosynthesis pathway of kaempferol and quercetin was constructed in S. cerevisiae using naringenin as a substrate. Flavonol 3-hydroxylase (F_3H) from rice was cloned into pRS42K yeast using BamH1 and Xho1 restriction enzymes. E. coli, DH5α and S. cerevisiae D452-2 were used in this study and transformation to yeast were carried out through Lithium acetate/single stranded carrier DNA/ Polyethylene glycol (LiAc/SScarrierDNA/PEG) method. Protein expression was confirmed via western blotting and synthesis of kaempferol and quercetin were confirmed through TLC, NMR and HPLC.

[Results and Discussion]

In this study full ORF region of OsF_3H gene were ligated into pRS42k expression vector in between PGK1 promoter and CYC1 terminator. Transformation and ligation were confirmed by plasmid isolation and digestion with related restriction enzyme. Transformation to yeast was confirmed through colony PCR. Thin layer chromatography is one of the most important evidence to confirm the presence of related compound in the extract. Sample were clearly separated with toluene: ethyl acetate: formic acid (7:3:0.5) mobile phase and Rf of related compounds were found similar with standard. Activity of OsF_3H was determined by level of recombinant protein expression among the three time points via western blotting. Our result confirmed that empty vector dose not produced the target protein similar to transformed strain which shows lack of F_3H activity. Structural identification of related compound was verified by NMR and the result illustrated only quercetin presence while lack of kaempferol possibly due to low concentration. Our further HPLC analysis shows that by using naringenin as a substrate, the recombinant yeast expressing OsF_3H gene successfully metabolized naringenin directly into kaempferol and quercetin. The control strain having empty vector show lack of kaempferol and quercetin accumulation which indicates that flavanone 3-hydroxylase is responsible for naringenin metabolism to both compounds. It is strong evidence that spontaneous conversion of naringenin to kaempferol and quercetin did not tack place. We presumed that it is novel finding which hypothesized that OsF_3H gene is responsible for catalyzing naringenin metabolism directly into kaempferol and quercetin without association of FLS, which is contradictory with other researchers.

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