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Discovery and Validation of a Novel Step Catalyzed by *OsF$_3$H* in the Flavonoid Biosynthesis Pathway

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[Introduction]

Kaempferol and quercetin are the essential plant secondary metabolites that confer huge biological functions in the plant defense system. These metabolites are produced in low quantities in plants, therefore engineering microbial factory is a favorable strategy for the production of these metabolites.

[Materials and Methods]

In this study, biosynthetic pathways for kaempferol and quercetin were constructed in *Saccharomyces cerevisiae* using naringenin as a substrate. Flavanol 3-hydroxylase (F_3H) from rice was cloned into pRS42K yeast vector using BamH1 and Xho1 restriction enzymes. Transformation to yeast was carried by Lithium acetate/single stranded carrier DNA/polyethylene glycol (LiAc/SScarrierDNA/PEG) method.

[Results and Discussion]

The results elucidated a novel step for the first time in kaempferol and quercetin biosynthesis directly from naringenin catalyzed by flavonol 3-hydroxylase (*F$_3$H*). *F$_3$H* gene from rice was cloned into pRS42K yeast episomal plasmid (YEP) vector using *Bam*H1 and *Xho*1 restriction enzymes. We analyzed our target gene activity in engineered and in empty strains. The results were confirmed through TLC followed by Western blotting, nuclear magnetic resonance (NMR), and LC-MS. TLC showed positive results on comparing both compounds extracted from the engineered strain with the standard reference. Western blotting confirmed lack of *Oryza sativa* flavonol 3-hydroxylase (*OsF$_3$H*) activity in empty strains while high *OsF$_3$H* expression in engineered strains. NMR spectroscopy confirmed only quercetin, while LC-MS/MS results revealed that *F$_3$H* is responsible for naringenin conversion to both kaempferol and quercetin. These results concluded that rice *F$_3$H* catalyzes naringenin metabolism via hydroxylation and synthesizes kaempferol and quercetin.

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