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애기장대 전사인자 AtMYB12 에 의한 일반메밀 모상근에서의 루틴 증가

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Enhancement of rutin in *Fagopyrum esculentum* hairy root culture by the Arabidopsis transcription factor AtMYB12

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실험목적 (Objectives)

Transcription factors might have different specificities for target genes in different plants; we have expected that it is possible to accumulate high level of rutin in buckwheat by the overexpression of *AtMYB12* and *AtMYB75/PAP1*. In this study, the hairy root culture system was used to observe the activity of transcription factors. Using transgenic hairy roots of buckwheat, we showed that flavonoid biosynthetic genes are regulated by transcription factors.

재료 및 방법 (Materials and Methods)

○ 실험재료

-Dehulled seeds of common buckwheat
-The seeds were germinated in a growth chamber at 25°C

○ 실험방법

-Total RNA extraction and quantification of gene expression
-Plasmid construction for transformation of buckwheat hairy roots
-Transformation of buckwheat hairy roots
-Quantitative analysis of rutin using high-performance liquid chromatography (HPLC)

실험결과 (Results)

Explants of the stems buckwheat were infected with *Agrobacterium rhizogenes* strain R1000 that harbored the pPAP1, pAtMYB12 or pGUS (β-glucuronidase, for control hairy roots) binary vector.

AtMYB12 was strongly overexpressed in AtMYB12-overexpressed hairy root lines with relative expression levels ranging from 35- to 60-fold above that of GUS-overexpressed hairy root lines. The flavonoids biosynthetic genes showed a correlation to the AtMYB12 expression level. The expression level of flavonoids biosynthetic genes (*FePAL*, *FeC4H*, *Fe4CL1*, *Fe4CL2*, *FeCHS*, *FeCHI*, *FeF3H*, *FeF3´H*, *FeFLS1*, and *FeFLS2*) was in general increased in response to elevated

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AtMYB12 expression. However, the expression level of *FeDFR* and *FeANS* was more or less unaffected by changes in AtMYB12 expression, although *FeDFR* expression level wass lightly increased in AtMYB12-overexpressed hairy root lines (**Fig. 1**). The flavonoids biosynthetic genes were seemed that not correlated to the PAP1 expression level. The expression level of all flavonoids biosynthetic genes analyzed in this study was not changed in PAP1-overexpressed hairy root lines.

To investigate the role of AtMYB12 and PAP1 on the level of rutin, the AtMYB12- and PAP1- overexpressed hairy roots were analyzed by HPLC (**Fig. 2**). High level of rutin was detected in the hairy roots lines expressing AtMYB12 compare to the control hairy roots expressing GUS. AtMYB12-overexpressed hairy root lines produced rutin ($0.53 - 0.88 \mu g \cdot mg^{-1}$) 2.2 - 3.7 times more than the GUS-control hairy root line. In contrast, no significant difference could be detected for the HPLC analysis between control and PAP1-overexpressed hairy.

* 시험성적

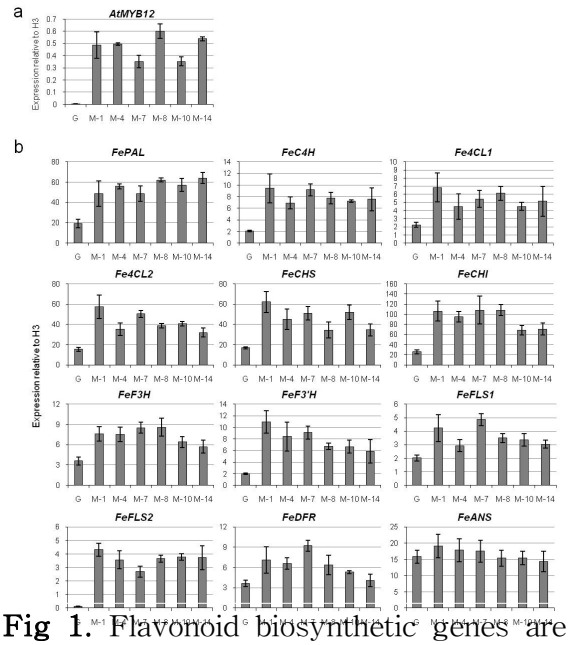

Fig 1. Flavonoid biosynthetic genes are target of AtMYB12. Results of quantitative real time RT-PCR analyses of RNA from AtMYB12-overexpressed hairy roots of common buckwheat.

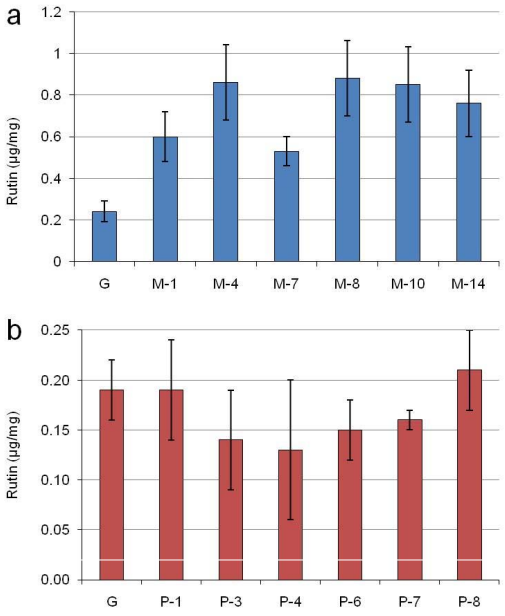

Fig 2. Rutin contents in (a) AtMYB12- and (b) PAP1-overexpressed hairy roots of common buckwheat.