

(O4-01)

AtMYB60, an R2R3-MYB transcription factor encodes a transcriptional repressor of flavonoid metabolism in lettuce (*Lactuca sativa*)

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Transcription factors are regulatory proteins that regulate the repression of specific genes through sequence-specific DNA binding and protein-protein interactions, and act as activators or repressors of gene expression, mediating an increase or decrease of messenger RNA. Since MYB-type transcription factors known to control multiple pathway steps have emerged as powerful tools for the modulation of metabolic pathways in plants.

In this study, we describe the cloning and functional characterization of different R2R3-MYB transcription factors from *Arabidopsis thaliana*. Analysis of *Arabidopsis* genome sequence reveals 125 MYB genes. To examine the regulatory function of these genes, we isolated R2R3 MYB cDNA clones (AtMYB4, 29, 30, 34, 51, 60) which are induced by UV-B irradiation. Because of the absence of a suitable T-DNA tagging mutant, the knowledge about the function is not yet characterized. Transgenic lettuce plants overexpressing AtMYB60 under the control of the cauliflower mosaic virus 35S promoter, showed inhibition of anthocyanin biosynthesis. HPLC analysis and quantification indicate an inhibition of cyanidin pigment in transgenic lines. RT-PCR analysis revealed that the expression of the *dihydroflavonol 4-reductase* (DFR) which is a key structural gene for anthocyanin biosynthesis was inhibited in transgenic lettuce. Together with these data, a possible mechanism showing expression of DFR repressed by AtMYB60 will be described in Fig.

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Increase of recombinant protein by suppression of storage protein gene using RNAi in transgenic rice seeds

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Glutelin is a major seed storage protein, accounting for 60% of total endosperm protein in rice. The low-glutelin cultivar has potential usages as low protein rice not only in diet therapy for patients with kidney disease, but also in molecular farming for accumulation of foreign protein. We have tried to augment recombinant protein by suppression of storage protein gene using RNAi and obtained transgenic glutelin RNAi rice seeds. We have investigated if the expression of a transgene (RFP) in rice seeds could be enhanced by RNAi of an endogenous seed storage protein (glutelin). The seeds of transformants having RFP gene and glutelin that was suppressed by RNAi showed brighter fluorescence than the seeds transformed with RFP gene only. DNA gel blot confirmed integration of RNAi

vector for glutelin into the genome of transgenic rice plants. RNA gel blot analyses indicated that endogenous glutelin gene was severely suppressed in transgenic rice, whereas it was fully expressed in non-transgenic rice. Reverse transcriptase-mediated PCR revealed that glutelin multigene family was suppressed. Western blot analysis showed that the relative accumulation level of RFP in glutelin RNAi seeds was two times of it in the only RFP transgenic seeds. These results suggest that RNAi technology for endogenous storage protein could be of great utility to favor high expression of transgene.

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**Functional analysis of sweet potato peroxidase gene, *swpa4*,
in transgenic tobacco under various stress**

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Secretory class III peroxidases (POD, EC 1.11.1.7) catalyze the reduction of H₂O₂ by taking electrons from various donor molecules. It has been implicated diverse responses under various stress conditions, such as abiotic stress and biotic stress. In the previous studies, ten POD cDNAs were isolated from cell cultures of sweetpotato and their expression levels were characterized under environmental stresses. The expression of *swpa4* gene was strongly induced by treatment of oxidative stress, heavy metal and pathogenic bacteria. In this study, functional characterization of *swpa4* gene was done in transgenic tobacco plants. Transgenic tobacco plant expressing *swpa4* gene under the control of CaMV 35S promoter developed, and it showed 40-fold higher POD activity than non-transgenic (NT) plants. *swpa4* plant had enhanced protection against oxidative stress treatment such as methyl viologen (MV) and hydrogen peroxide (H₂O₂). In 2 μM MV treatment, *swpa4* plant showed about 20% less ion leakage and 16% higher amount of chlorophyll than NT plants. In addition, *swpa4* plants showed higher protection against 200 mM H₂O₂ treatment than NT plants. Interestingly, expression of some pathogenesis-related (PR) gene, such as PR-2 (β-1, 3-glucanase), were increased in *swpa4* plants, suggesting that *swpa4* plants have tolerance against pathogenic bacteria. These results suggest that *swpa4* gene have important roles in adaptation of plants to various environmental stresses including abiotic and biotic stresses.

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