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蔬菜

芜菁二氢黄酮醇4－还原酶基因的克隆与功能鉴定

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Cloning and Function Identification of Dihydroflavonol 4-reductase Genes in Turnip

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摘要 二氢黄酮醇4－还原酶（dihydroflavonol 4-reductase，DFR）是花青素生物合成晚期阶段的关键酶，属于NAD/NADP依赖型还原酶家族，催化从二氢黄酮醇转变成无色花色素苷的反应。利用UV-A处理‘津田’芜菁（‘Tsuda’turnip）和‘赤丸’芜菁（‘Yurugi Akamaru’turnip）块根24 h后提取总RNA，通过RT-PCR方法分别克隆了‘津田’芜菁*BrDFR1*和‘赤丸’芜菁*BrDFR2*基因。*BrDFR1*和*BrDFR2*的开放读码框分别为1 158 bp和999 bp，分别编码385和332个氨基酸。氨基酸序列分析显示，BrDFR1和BrDFR2与大白菜DFR具有高度同源性，从第8到第302位氨基酸的肽段具有FR_SDR_e结构域。BrDFR1和BrDFR2基因组全长序列均含有5个位置与序列完全相同的内含子。Southern杂交结果显示，‘津田’芜菁*BrDFR1*和‘赤丸’芜菁*BrDFR2*基因均为单一拷贝。UV-A可以诱导*BrDFR1*基因表达，对*BrDFR2*基因表达的诱导效果不明显。*BrDFR1*和*BrDFR2*基因在大肠杆菌细胞中可以表达并纯化出分子量约为42.8 kD的BrDFR1蛋白和37.5 kD的BrDFR2蛋白。过量表达*BrDFR1*和*BrDFR2*基因的烟草花色加深。芜菁*DFR*基因的RNAi载体遗传转化烟草后，转基因植株的花色变浅。这些工作将为阐明依光型和非依光型花青素生物合成机理奠定研究基础。

关键词： 芜菁 二氢黄酮醇4－还原酶基因 基因克隆 遗传转化 功能鉴定

Abstract： Dihydroflavonol 4-reductase（DFR）is the critical catalyze enzyme in the later stage of anthocyanins biosynthesis，which belongs to a large redox enzyme superfamily that shares a Rossmann-fold NAD(P)H/NAD(P)(+) binding（NADB）domain. DFR catalyzes the reaction from dihydroflavonol to unstable leucoanthocyanidin. The roots of ‘Tsuda’ turnip and ‘Yurugi Akamaru’ turnip were irradiated with UV-A light for 24 h. Total RNA was isolated，and then *BrDFR1* and *BrDFR2* genes were cloned by RT-PCR method. The open reading frame（ORF）of *BrDFR1* and *BrDFR2* genes contained 1 158 bp and 999 bp encoding proteins of 385 and 332 amino acids respectively. Amino acid sequence analysis showed that BrDFR1 and BrDFR2 have high homology with DFR of *Brassica rapa* subsp. *pekinensis*. The FR_SDR_e domain was in the amino acid sequence from 8 to 302 of BrDFR1 and BrDFR2. The whole genome sequences of *BrDFR1* and *BrDFR2* had five introns with the same location and sequence. Southern blotting result showed that the copy of *BrDFR1* and *BrDFR2 in ‘Tsuda’turnip and ‘Yurugi Akamaru’turnip genome is only one. Northern blotting result indicated that the expression of BrDFR1 could be induced by irradiation of UV-A，and the expression of the gene was correlated with light-exposure time. The induction of UV-A irradiation on the expression of BrDFR2 gene was indistinctively. The 42.8 kD and 37.5 kD proteins of BrDFR1 and BrDFR2 were successfully purified after BrDFR1 and BrDFR2 genes expressed in E.coli cell，respectively. The color of the flowers from BrDFR1 and BrDFR2 over-expressed tobacco plants was darker than that of wild type. The transgenic plants with light-colored flowers were obtained after the RNAi vector containing the DFR gene fragment of turnip was introduced. The present study will establish the experiment foundation for preliminarily clarifying the mechanism of light-dependent and light-independent anthocyanin biosynthesis.*

Keywords： turnip， dihydroflavonol 4-reductase gene， gene clone， genetic transformation， function identification

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