

血红鸡爪槭叶片 WD₄₀ 转录因子的克隆及序列分析

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摘要:以血红鸡爪槭叶片总 RNA 为模板, 采用 RT-PCR 和 RACE-PCR 的方法, 研究血红鸡爪槭叶片花色素苷的合成机理, 成功获得了调控花色素苷表达的转录因子 WD₄₀ 基因。结果表明: WD₄₀ 基因全长 1 104 bp, 编码 337 个氨基酸。通过 NCBI 中的 Blast 软件对其进行核苷酸序列和氨基酸序列的同源性进行分析, 表明所获得的核苷酸序列和推导的氨基酸序列与其它物种的转录因子 WD₄₀ 有较高的同源性, 并且 C 端含有 4 个 WD 重复基序, 推断该基因为血红鸡爪槭的 WD₄₀ 转录因子。

关键词:血红鸡爪槭; WD₄₀ 转录因子; 基因克隆; 序列分析

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花色素苷是植物体内的类黄酮次生代谢物质, 是一类水溶性色素, 产生颜色的范围为红色到紫色^[1]。目前研究表明影响花色素苷合成的基因为 2 类: 一类是结构基因, 如 CHS、CHI、ANS、F3H、DFR 等^[2]; 另一类为转录因子调节基因, 如 MYB、MYC、WD₄₀、锌指蛋白 (Zinc finger)、同源域蛋白 (Homeodomain) 等^[3]。转录因子能够通过结构与结构基因启动子中含有的能被其识别的顺式作用元件结合, 从而激活花色素苷生物合成途径中多个基因的表达, 有效地启动花色素苷生物合成途径^[4]。

WD₄₀ 转录因子一般为组成型表达, 在含有花色素苷组织中, 它与 BHLH 和 MYB 型转录因子相互作用, 形成一个转录复合体调节基因表达^[4]。WD₄₀ 具有 β-螺旋桨结构, 它包含 4~8 个短的 WD 重复基序 (Trp-Asp repeats motif), 其每个重复基序由大约 40 个氨基酸残基组成, 包含保守的氨基和羧基终端的核心结构, 同时这些核心结构被短的氨基酸序列分隔开来^[5]。

血红鸡爪槭为园林应用中极为重要的彩色叶树种, 其花色素苷对叶色色彩形成起到主要作用, 所以该试验克隆了与花色素苷合成相关的转录因子 WD₄₀, 为进一步在分子水平上研究彩叶植物提供了依据, 同时也为进一步阐明整个 WD₄₀ 大家族的进化历史提供更多的分析材料。

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1 材料与方法

1.1 试验材料

血红鸡爪槭叶片采自河北农业大学苗圃; Prime Script Reverse Transcriptase, DNA 回收试剂盒, 质粒提取试剂盒, pMD-18T 载体, rTaq DNA 聚合酶, 大肠杆菌 DH5α, 5'-Full Race Kit 等购于 TaKaRa。

1.2 试验方法

1.2.1 总 RNA 的提取 血红鸡爪槭总 RNA 提取方法参照文献[6]。

1.2.2 引物设计 搜索 GenBank 数据库中已报道的 WD₄₀ 转录因子基因序列, Blast 比对, 然后用 DNAMAN 软件设计兼并引物 WD1/WD2 以扩增中间片段, Pw1/Pa 扩增 3' 末端序列, 根据测序结果设计 5' 末端序列特异引物 Pw2 进行 RACE-PCR 扩增, 5' 外侧引物 UP1 和 UP2 由试剂盒提供。

表 1 试验所用引物

Table 1	Primers used in the experiments
引物名称	引物序列 (5'~3')
Name of primer	Sequence of primers (5'~3')
The primer WD1	AAATAGAGARYTCAASCCAAG
The primer WD2	AGGGCTTTCGTAGATGATTGTG
The primer (Pw1)	AGATCTGAAAACCTVCGTCACATA
The primer (Pa)	TCAAACCTYTGAAGCTGCA
The primer (Pw2)	CAGAACTCACTGCTCTTGCTA

1.2.3 反转录合成 cDNA 第 1 条链 参照 Prime Script Reverse Transcriptase 说明书。

1.2.4 血红鸡爪槭叶片 WD₄₀ 转录因子基因的克隆 中间片段的合成: 以经过反转录合成的 cDNA 第 1 条链为模板, WD1 和 WD2 为引物进行 PCR 扩增, 其反应程序为: 94℃ 预变性 5 min, 94℃ 变性 1 min, 52℃ 退火 1 min,

72℃延伸 1 min, 30 个循环后 72℃继续延伸 10 min。3'RACE-PCR:以反转录合成的 cDNA 第 1 条链为模板, Pw1 和 Pa 为引物进行 PCR 反应。反应程序为:94℃预变性 5 min, 94℃变性 1 min, 51℃退火 1 min, 72℃延伸 1 min, 30 个循环后 72℃继续延伸 10 min。5'RACE-PCR:参照试剂盒说明进行, 根据已知序列设计特异引物 Pw2, 用 WD1 和 5'UP1 进行 PCR, 以此产物为模板, 用 Pw2 和 5'UP2 进行巢式 PCR。反应条件:94℃ 3 min, 94℃ 1 min, 58℃ 1 min, 72℃ 1 min, 共进行 30 个循环。

1.2.5 目的基因的回收、克隆及分析 对 PCR 产物进行 1%琼脂糖凝胶电泳, 然后用 TaKaRa 公司 DNA 快速纯化回收试剂盒进行胶回收, 得到目的基因片段, 与 pMD-18T 载体 16℃连接过夜, 将连接产物转化大肠杆菌 DH5 α 感受态细胞, 在 Amp-LB 平板上筛选, 挑取阳性单克隆于含 Amp-LB 的培养基中进行培养, 以菌液为模板进行菌液 PCR 验证。然后对菌液进行提质粒及双酶切验证并测序。

1.2.6 序列分析及系统进化树的构建 利用 NCBI 中的 VecScreen 程序去除所测序列当中的载体序列, 并且在 DNAMAN 中进行 2 段基因的拼接, 并用 Blastn 进行同源性分析。利用 DNAMAN 软件将核酸序列翻译成氨基酸序列, 用 Blastp 进行氨基酸序列比对, 找到相似性高的蛋白质序列, 然后用 ClustalX 软件进行多序列比对, 用 MEGA 4.0 软件中 Alignment 和 Phylogeny 程序进行构建系统进化树。蛋白质结构预测利用 ExPASy 系统 (<http://swissmodel.expasy.org>) 的 Swiss-Model 软件。

2 结果与分析

2.1 总 RNA 提取

总 RNA 通过用 ND-1000 核酸蛋白检测仪测定结果为:OD₂₆₀/OD₂₈₀ 为 1.98, OD₂₆₀/OD₂₃₀ 为 2.11; 电泳结果见图 1, 表明总 RNA 纯度较好, 无降解现象, 符合试验的要求。

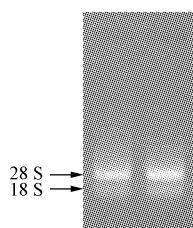


图 1 总 RNA 凝胶电泳图

Fig. 1 Gel electrophoresis of total RNA

2.2 血红鸡爪槭叶片 WD₄₀ 基因的分离

以 WD1 和 WD2 为引物扩增反转录 cDNA 模板, 得到大约 600 bp 的特异性条带(图 2-a 的泳道 1), 以 Pw1 和 Pa 为引物扩增得到大约 1 000 bp 的片段(图 2-b 的泳

道 1), 条带大小符合预期片段大小, 酶切结果均出现载体片段和目的片段, 且片段大小一致。以 WD2/UPM 扩增得到大约 650 bp 条带(图 2-c 的泳道 1), 以此 PCR 产物为模板, 以 Pw2/UPM 为引物进行巢式 PCR, 扩增得到近 400 bp 的特异片段(图 2-c 的泳道 2)。

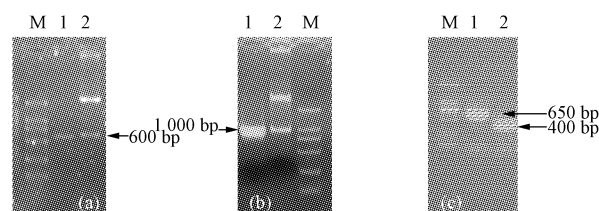


图 2 PCR 产物凝胶电泳图

注:(a)中间片段;(b)3'RACE-PCR;(c)5'RACE-PCR。Lane M, Marker DL 2000 (从上到下: 2.0 kb, 1.0 kb, 0.75 kb, 0.5 kb, 0.25 kb, 0.1 kb)。

Fig. 2 Gel electrophoresis of PCR amplified product

Note: (a) between segment; (b) 3'RACE-PCR; (c) 5'RACE-PCR. Lane M, Marker DL 2000 (from top to bottom: 2.0 kb, 1.0 kb, 0.75 kb, 0.5 kb, 0.25 kb, 0.1 kb).

2.3 测序结果及序列分析

将中间片段、3'末端和 5'末端序列的测序结果进行序列拼接, 得到总长为 1 104 bp 的核苷酸序列, 通过 ORF finder 寻找该基因的开放阅读框, 结果显示在 3~1 016 bp 处包含 1 个 1 014 bp 的 ORF 框, 推断其编码 337 个氨基酸, 利用 DNAMAN 软件对血红鸡爪槭 WD₄₀ cDNA 序列进行推导, 翻译成氨基酸序列(图 3)。

将核苷酸序列和推导的氨基酸序列在 NCBI 中进行 Blast 比对, 结果发现, 血红鸡爪槭 WD₄₀ 编码的氨基酸序列与百脉根(*Lotus japonicus*)、锦鸡儿(*Caragana jubata*)、陆地棉(*Gossypium hirsutum*)、紫花牵牛(*Ipomoea purpurea*)、苹果(*Malus domestica*)、紫叶李(*Prunus cerasifera*)、葡萄(*Vitis vinifera*)、拟南芥(*Arabidopsis thaliana*)的 WD₄₀ 编码的氨基酸同源性分别为 80.35%、78.72%、83.33%、84.50%、83.09%、79.47%、84.50%(图 4)。而核苷酸序列的同源性也均在 71% 以上。

氨基酸序列分析还发现, 在转录因子 WD₄₀ 的 C 端有 4 个功能保守域, 为 4 个 WD 重复基序, 123E-156D、164T-198D、216T-245D、258R-289E(图 4 中下划线部分), 符合 WD₄₀ 转录因子的结构特征, 因此可推断所获得的 cDNA 序列为转录因子 WD₄₀ 基因。

2.4 血红鸡爪槭 WD₄₀ 转录因子结构预测

将转录因子 WD₄₀ 全长基因所推导出的氨基酸序列提交给 ExPASy(<http://swissmodel.expasy.org>) 的 Swiss-Model 服务器。根据该序列与数据库中的序列相似度, 拟合出几个保守结构域的空间结构, 4 个 WD 重复基序(motif 1, motif 2)均为 3 个 β -折叠组成的片层结构, 基序 1 如图 5-a, 基序 2 如图 5-b, 基序 3 和基序 4 如图 5-c。

AAATGGAGAGCTCAAGAACCGAATCCCACCTGAGATCTGAAACTACGTACATATGAGTCCCCATACCGCTCTACGCCATGGCCCTCTCTCTCCCGCCCTCCCGCCACCAACGCATC
M E S S R T E S H L R S E H Y V T Y E S P Y Q L Y A M A L S S S S A S R H Q R I
GCTCTCGGACGTTCTCGAAGATTTCAACACCGGGTCGATCTGCTCTTTACCCGGAACTCTCAATCAAACCCACCCTTCTCTCTTTGACCACCCCTACCCACCAC
A I G S F I E D F H H B V D I V S F D P E T L S I K T H P S L S F D H P Y P P T
AAGTCATGTTCCAAACCCCTCCCTCCGCAAGTCTCCGACCTCTCGCTCCGCTGTCCTCGGAGGTCCTGACTCTCCGTCGAGCCCTCTACCGTCTCAAT
E I M F Q P T S L B E S S D L L A S S G D L L B L W E V B D S S V E P L T V L H
AATAGCAAGAGCAGTGAGTTCTCGCTCCGTTGACCTCTCGACTGGAACGAAATCGAACCAAGAGACTCGGTACCTCCAGCATCGACACCCTGCACCATCTGGGATATCGAAGA
H S E S S E F C A P I T S F D W H E I E P K B L G T S S I D T T C T I W D I E B
GGCGTCGTCGAAACCAATGATCGCTACGACAAAGGAGGCTACGACATTTGCTGGGGCAGGCCAGGGTTTCGCTTCAAGTTCCGCTGATGGGTCGTGAGAATTTTCGATTGAGA
G V V E T Q L I A H D E E V Y D I C W G E A B V F A S V S A D G S V B I F D L B
GACAAGGAACATCCCAATATCTACGAAAGCCCTCAACCCGACACGCCCTTGCTTCGATGGCTGGAACAAGCAGGATTTGAGGTACATGGCGACAGCTCTGATGGACAGTAACAA
D E H S T I I Y E S P Q P D T P L B L A W H K Q D L B Y M A T D L S H K
GTCGTGATTTGGATATAAGATCGCAACATGCTGTTCGGAGCTAGAGAGACATAAAGGCGGTGTCAATGCCATGCTTGGGCTCTCAGAGTTGTAGGCACATATGCTCTGTCGGG
V V I L D I B S P T M P V A E L E B H E A G V H A I A W A P Q S C B H I C S V G
GACGACACCAAGCTCTCAATTTGGGAGTTCGCCGAGTGGACCAATGGGATTTGATCCATGCTTATGTACTCTGGGCTGAGATTAAACAGCTTCAAGTGGCTGCTGCTGCTAG
D D T Q A L I W E L P P V A G P H G I D P M S M Y S A G S E I H Q L Q W S A A Q
CCTGATTGGATGCCATCGCATTTTCGAACCAATGCGACGCTCTCTTAAAGTTGA
P D W I A I A F S H E C S L L H V *

图3 血鸡爪槭 WD₄₀ cDNA 的核苷酸序列及其推导的氨基酸序列

Fig. 3 Nucleotide sequence and deduced amino-acid sequence of the gene of WD₄₀ from *Acer palmatum*

鸡爪槭 <i>Acer palmatum</i>	...SSPT...S...V...S...Q...V...I...SSSSASH...C...A...G...S...H...D...N...R...S...D...E...T...I...S...I...K...T...H...P...S...L...S...F...D...H...P...Y...P...P...T...S...L...R...90
百脉根 <i>Lotus japonicus</i>	...SSPT...S...V...S...Q...V...I...SSSSASH...C...A...G...S...H...D...N...R...S...D...E...T...I...S...I...K...T...H...P...S...L...S...F...D...H...P...Y...P...P...T...S...L...R...100
锦鸡儿 <i>Caragana jubata</i>	...SSPT...S...V...S...Q...V...I...SSSSASH...C...A...G...S...H...D...N...R...S...D...E...T...I...S...I...K...T...H...P...S...L...S...F...D...H...P...Y...P...P...T...S...L...R...89
陆地棉 <i>Gossypium hirsutum</i>	...SSPT...S...V...S...Q...V...I...SSSSASH...C...A...G...S...H...D...N...R...S...D...E...T...I...S...I...K...T...H...P...S...L...S...F...D...H...P...Y...P...P...T...S...L...R...96
紫花牵牛 <i>Ipomoea purpurea</i>	...SSPT...S...V...S...Q...V...I...SSSSASH...C...A...G...S...H...D...N...R...S...D...E...T...I...S...I...K...T...H...P...S...L...S...F...D...H...P...Y...P...P...T...S...L...R...96
葡萄 <i>Vitis vinifera</i>	...SSPT...S...V...S...Q...V...I...SSSSASH...C...A...G...S...H...D...N...R...S...D...E...T...I...S...I...K...T...H...P...S...L...S...F...D...H...P...Y...P...P...T...S...L...R...89
苹果 <i>Malus domestica</i>	...SSPT...S...V...S...Q...V...I...SSSSASH...C...A...G...S...H...D...N...R...S...D...E...T...I...S...I...K...T...H...P...S...L...S...F...D...H...P...Y...P...P...T...S...L...R...95
紫叶李 <i>Prunus cerasifera</i>	...SSPT...S...V...S...Q...V...I...SSSSASH...C...A...G...S...H...D...N...R...S...D...E...T...I...S...I...K...T...H...P...S...L...S...F...D...H...P...Y...P...P...T...S...L...R...95
苹果 <i>Malus domestica</i>	...SSPT...S...V...S...Q...V...I...SSSSASH...C...A...G...S...H...D...N...R...S...D...E...T...I...S...I...K...T...H...P...S...L...S...F...D...H...P...Y...P...P...T...S...L...R...95
拟南芥 <i>Arabidopsis thaliana</i>	...SSPT...S...V...S...Q...V...I...SSSSASH...C...A...G...S...H...D...N...R...S...D...E...T...I...S...I...K...T...H...P...S...L...S...F...D...H...P...Y...P...P...T...S...L...R...90
鸡爪槭 <i>Acer palmatum</i>	KSSD...L...A...S...G...L...R...S...R...S...S...V...L...T...V...N...S...S...S...C...A...P...I...T...S...F...L...W...E...E...P...R...G...T...S...I...D...T...C...T...I...W...D...I...E...B...V...I...A...H...D...E...E...V...Y...D...I...C...W...G...E...A...B...V...F...A...S...V...S...A...D...G...S...V...B...I...F...D...L...B...186
百脉根 <i>Lotus japonicus</i>	KSSAV...L...A...S...G...L...R...S...R...S...S...V...L...T...V...N...S...S...S...C...A...P...I...T...S...F...L...W...E...E...P...R...G...T...S...I...D...T...C...T...I...W...D...I...E...B...V...I...A...H...D...E...E...V...Y...D...I...C...W...G...E...A...B...V...F...A...S...V...S...A...D...G...S...V...B...I...F...D...L...B...198
锦鸡儿 <i>Caragana jubata</i>	KTSS...L...A...S...G...L...R...S...R...S...S...V...L...T...V...N...S...S...S...C...A...P...I...T...S...F...L...W...E...E...P...R...G...T...S...I...D...T...C...T...I...W...D...I...E...B...V...I...A...H...D...E...E...V...Y...D...I...C...W...G...E...A...B...V...F...A...S...V...S...A...D...G...S...V...B...I...F...D...L...B...186
陆地棉 <i>Gossypium hirsutum</i>	SSSD...L...A...S...G...L...R...S...R...S...S...V...L...T...V...N...S...S...S...C...A...P...I...T...S...F...L...W...E...E...P...R...G...T...S...I...D...T...C...T...I...W...D...I...E...B...V...I...A...H...D...E...E...V...Y...D...I...C...W...G...E...A...B...V...F...A...S...V...S...A...D...G...S...V...B...I...F...D...L...B...192
紫花牵牛 <i>Ipomoea purpurea</i>	KSAD...L...A...S...G...L...R...S...R...S...S...V...L...T...V...N...S...S...S...C...A...P...I...T...S...F...L...W...E...E...P...R...G...T...S...I...D...T...C...T...I...W...D...I...E...B...V...I...A...H...D...E...E...V...Y...D...I...C...W...G...E...A...B...V...F...A...S...V...S...A...D...G...S...V...B...I...F...D...L...B...192
葡萄 <i>Vitis vinifera</i>	TSFD...L...A...S...G...L...R...S...R...S...S...V...L...T...V...N...S...S...S...C...A...P...I...T...S...F...L...W...E...E...P...R...G...T...S...I...D...T...C...T...I...W...D...I...E...B...V...I...A...H...D...E...E...V...Y...D...I...C...W...G...E...A...B...V...F...A...S...V...S...A...D...G...S...V...B...I...F...D...L...B...185
苹果 <i>Malus domestica</i>	KSND...L...A...S...G...L...R...S...R...S...S...V...L...T...V...N...S...S...S...C...A...P...I...T...S...F...L...W...E...E...P...R...G...T...S...I...D...T...C...T...I...W...D...I...E...B...V...I...A...H...D...E...E...V...Y...D...I...C...W...G...E...A...B...V...F...A...S...V...S...A...D...G...S...V...B...I...F...D...L...B...191
紫叶李 <i>Prunus cerasifera</i>	KSND...L...A...S...G...L...R...S...R...S...S...V...L...T...V...N...S...S...S...C...A...P...I...T...S...F...L...W...E...E...P...R...G...T...S...I...D...T...C...T...I...W...D...I...E...B...V...I...A...H...D...E...E...V...Y...D...I...C...W...G...E...A...B...V...F...A...S...V...S...A...D...G...S...V...B...I...F...D...L...B...191
苹果 <i>Malus domestica</i>	KTND...L...A...S...G...L...R...S...R...S...S...V...L...T...V...N...S...S...S...C...A...P...I...T...S...F...L...W...E...E...P...R...G...T...S...I...D...T...C...T...I...W...D...I...E...B...V...I...A...H...D...E...E...V...Y...D...I...C...W...G...E...A...B...V...F...A...S...V...S...A...D...G...S...V...B...I...F...D...L...B...191
拟南芥 <i>Arabidopsis thaliana</i>	RFSS...L...A...S...G...L...R...S...R...S...S...V...L...T...V...N...S...S...S...C...A...P...I...T...S...F...L...W...E...E...P...R...G...T...S...I...D...T...C...T...I...W...D...I...E...B...V...I...A...H...D...E...E...V...Y...D...I...C...W...G...E...A...B...V...F...A...S...V...S...A...D...G...S...V...B...I...F...D...L...B...190
鸡爪槭 <i>Acer palmatum</i>	SVSA...G...V...R...I...F...I...R...K...E...H...S...T...I...Y...E...S...F...O...E...T...F...L...I...A...W...N...K...D...E...R...W...A...T...I...M...N...K...V...I...D...I...R...S...H...P...V...A...E...I...R...H...G...N...A...I...W...A...H...S...S...H...I...S...G...I...T...G...A...I...286
百脉根 <i>Lotus japonicus</i>	SVSA...G...V...R...I...F...I...R...K...E...H...S...T...I...Y...E...S...F...O...E...T...F...L...I...A...W...N...K...D...E...R...W...A...T...I...M...N...K...V...I...D...I...R...S...H...P...V...A...E...I...R...H...G...N...A...I...W...A...H...S...S...H...I...S...G...I...T...G...A...I...298
锦鸡儿 <i>Caragana jubata</i>	SVSA...G...V...R...I...F...I...R...K...E...H...S...T...I...Y...E...S...F...O...E...T...F...L...I...A...W...N...K...D...E...R...W...A...T...I...M...N...K...V...I...D...I...R...S...H...P...V...A...E...I...R...H...G...N...A...I...W...A...H...S...S...H...I...S...G...I...T...G...A...I...286
陆地棉 <i>Gossypium hirsutum</i>	SVSA...G...V...R...I...F...I...R...K...E...H...S...T...I...Y...E...S...F...O...E...T...F...L...I...A...W...N...K...D...E...R...W...A...T...I...M...N...K...V...I...D...I...R...S...H...P...V...A...E...I...R...H...G...N...A...I...W...A...H...S...S...H...I...S...G...I...T...G...A...I...292
紫花牵牛 <i>Ipomoea purpurea</i>	SVSA...G...V...R...I...F...I...R...K...E...H...S...T...I...Y...E...S...F...O...E...T...F...L...I...A...W...N...K...D...E...R...W...A...T...I...M...N...K...V...I...D...I...R...S...H...P...V...A...E...I...R...H...G...N...A...I...W...A...H...S...S...H...I...S...G...I...T...G...A...I...292
葡萄 <i>Vitis vinifera</i>	SVSA...G...V...R...I...F...I...R...K...E...H...S...T...I...Y...E...S...F...O...E...T...F...L...I...A...W...N...K...D...E...R...W...A...T...I...M...N...K...V...I...D...I...R...S...H...P...V...A...E...I...R...H...G...N...A...I...W...A...H...S...S...H...I...S...G...I...T...G...A...I...285
苹果 <i>Malus domestica</i>	SVSA...G...V...R...I...F...I...R...K...E...H...S...T...I...Y...E...S...F...O...E...T...F...L...I...A...W...N...K...D...E...R...W...A...T...I...M...N...K...V...I...D...I...R...S...H...P...V...A...E...I...R...H...G...N...A...I...W...A...H...S...S...H...I...S...G...I...T...G...A...I...291
紫叶李 <i>Prunus cerasifera</i>	SVSA...G...V...R...I...F...I...R...K...E...H...S...T...I...Y...E...S...F...O...E...T...F...L...I...A...W...N...K...D...E...R...W...A...T...I...M...N...K...V...I...D...I...R...S...H...P...V...A...E...I...R...H...G...N...A...I...W...A...H...S...S...H...I...S...G...I...T...G...A...I...291
苹果 <i>Malus domestica</i>	SVSA...G...V...R...I...F...I...R...K...E...H...S...T...I...Y...E...S...F...O...E...T...F...L...I...A...W...N...K...D...E...R...W...A...T...I...M...N...K...V...I...D...I...R...S...H...P...V...A...E...I...R...H...G...N...A...I...W...A...H...S...S...H...I...S...G...I...T...G...A...I...291
拟南芥 <i>Arabidopsis thaliana</i>	SVSA...G...V...R...I...F...I...R...K...E...H...S...T...I...Y...E...S...F...O...E...T...F...L...I...A...W...N...K...D...E...R...W...A...T...I...M...N...K...V...I...D...I...R...S...H...P...V...A...E...I...R...H...G...N...A...I...W...A...H...S...S...H...I...S...G...I...T...G...A...I...290
鸡爪槭 <i>Acer palmatum</i>	IWLE...I...A...G...N...C...I...D...I...E...H...Y...A...S...S...I...N...O...I...C...W...S...S...E...I...M...T...C...S...A...P...I...T...S...F...L...W...E...E...P...R...G...T...S...I...D...T...C...T...I...W...D...I...E...B...V...I...A...H...D...E...E...V...Y...D...I...C...W...G...E...A...B...V...F...A...S...V...S...A...D...G...S...V...B...I...F...D...L...B...336
百脉根 <i>Lotus japonicus</i>	IWLE...I...A...G...N...C...I...D...I...E...H...Y...A...S...S...I...N...O...I...C...W...S...S...E...I...M...T...C...S...A...P...I...T...S...F...L...W...E...E...P...R...G...T...S...I...D...T...C...T...I...W...D...I...E...B...V...I...A...H...D...E...E...V...Y...D...I...C...W...G...E...A...B...V...F...A...S...V...S...A...D...G...S...V...B...I...F...D...L...B...348
锦鸡儿 <i>Caragana jubata</i>	IWLE...I...A...G...N...C...I...D...I...E...H...Y...A...S...S...I...N...O...I...C...W...S...S...E...I...M...T...C...S...A...P...I...T...S...F...L...W...E...E...P...R...G...T...S...I...D...T...C...T...I...W...D...I...E...B...V...I...A...H...D...E...E...V...Y...D...I...C...W...G...E...A...B...V...F...A...S...V...S...A...D...G...S...V...B...I...F...D...L...B...336
陆地棉 <i>Gossypium hirsutum</i>	IWLE...I...A...G...N...C...I...D...I...E...H...Y...A...S...S...I...N...O...I...C...W...S...S...E...I...M...T...C...S...A...P...I...T...S...F...L...W...E...E...P...R...G...T...S...I...D...T...C...T...I...W...D...I...E...B...V...I...A...H...D...E...E...V...Y...D...I...C...W...G...E...A...B...V...F...A...S...V...S...A...D...G...S...V...B...I...F...D...L...B...342
紫花牵牛 <i>Ipomoea purpurea</i>	IWLE...I...A...G...N...C...I...D...I...E...H...Y...A...S...S...I...N...O...I...C...W...S...S...E...I...M...T...C...S...A...P...I...T...S...F...L...W...E...E...P...R...G...T...S...I...D...T...C...T...I...W...D...I...E...B...V...I...A...H...D...E...E...V...Y...D...I...C...W...G...E...A...B...V...F...A...S...V...S...A...D...G...S...V...B...I...F...D...L...B...342
葡萄 <i>Vitis vinifera</i>	IWLE...I...A...G...N...C...I...D...I...E...H...Y...A...S...S...I...N...O...I...C...W...S...S...E...I...M...T...C...S...A...P...I...T...S...F...L...W...E...E...P...R...G...T...S...I...D...T...C...T...I...W...D...I...E...B...V...I...A...H...D...E...E...V...Y...D...I...C...W...G...E...A...B...V...F...A...S...V...S...A...D...G...S...V...B...I...F...D...L...B...342
苹果 <i>Malus domestica</i>	IWLE...I...A...G...N...C...I...D...I...E...H...Y...A...S...S...I...N...O...I...C...W...S...S...E...I...M...T...C...S...A...P...I...T...S...F...L...W...E...E...P...R...G...T...S...I...D...T...C...T...I...W...D...I...E...B...V...I...A...H...D...E...E...V...Y...D...I...C...W...G...E...A...B...V...F...A...S...V...S...A...D...G...S...V...B...I...F...D...L...B...341
紫叶李 <i>Prunus cerasifera</i>	IWLE...I...A...G...N...C...I...D...I...E...H...Y...A...S...S...I...N...O...I...C...W...S...S...E...I...M...T...C...S...A...P...I...T...S...F...L...W...E...E...P...R...G...T...S...I...D...T...C...T...I...W...D...I...E...B...V...I...A...H...D...E...E...V...Y...D...I...C...W...G...E...A...B...V...F...A...S...V...S...A...D...G...S...V...B...I...F...D...L...B...341
苹果 <i>Malus domestica</i>	IWLE...I...A...G...N...C...I...D...I...E...H...Y...A...S...S...I...N...O...I...C...W...S...S...E...I...M...T...C...S...A...P...I...T...S...F...L...W...E...E...P...R...G...T...S...I...D...T...C...T...I...W...D...I...E...B...V...I...A...H...D...E...E...V...Y...D...I...C...W...G...E...A...B...V...F...A...S...V...S...A...D...G...S...V...B...I...F...D...L...B...341
拟南芥 <i>Arabidopsis thaliana</i>	IWLE...I...A...G...N...C...I...D...I...E...H...Y...A...S...S...I...N...O...I...C...W...S...S...E...I...M...T...C...S...A...P...I...T...S...F...L...W...E...E...P...R...G...T...S...I...D...T...C...T...I...W...D...I...E...B...V...I...A...H...D...E...E...V...Y...D...I...C...W...G...E...A...B...V...F...A...S...V...S...A...D...G...S...V...B...I...F...D...L...B...340

图4 血鸡爪槭 WD₄₀ 氨基酸的多序列比较

Fig. 4 Multiple sequence alignment of the WD₄₀ protein from *Acer palmatum*

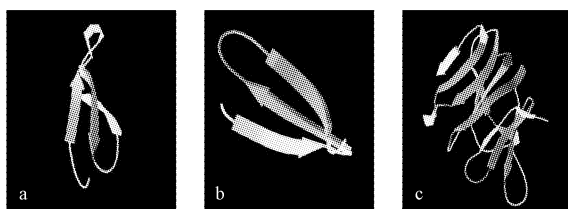


图5 血鸡爪槭 WD₄₀ 重复基序的结构模型

注: a. motif 1; b. motif 2; c. motif 3~4。

Fig. 5 The structure model of WD₄₀ repeat motif of

Acer palmatum

Note: a. motif 1; b. motif 2; c. motif 3~4.

2.5 血鸡爪槭 WD₄₀ 氨基酸序列的系统进化树分析

利用 MEG A4.0 软件中的 Alignment 和 Phylogeny

程序,采用邻结法构建出 WD₄₀ 系统进化树(图 6)。选择毛果杨 *Populus trichocarpa* (XP002318500)、桃树 *Prunus persica* (ACQ65867)、苹果 *Malus x domestica* (AAF27919)、沙梨 *Pyrus pyrifolia* (ADU25044)、葡萄 *Vitis vinifera* (XP002270777)、蓖麻 *Ricinus communis* (XP002530884)、覆盆子 *Rubus idaeus* (AEI55401)、陆地棉 *Gossypium hirsutum* (AAM95645)、豌豆 *Pisum sativum* (ADQ27310)、蒺藜苜蓿 *Medicago truncatula* (ABW08112)、烟草 *Nicotiana tabacum* (ACN87316)、百脉根 *Lotus japonicus* (BAH28880)、马铃薯 *Solanum tuberosum* (AEF01097)、石榴 *Punica granatum* (AAM95645) 和锦鸡儿 *Caragana jubata* (EF543141) 的 WD₄₀ 氨基酸序列以及该试验中获得的 WD₄₀ 进行进化分析。

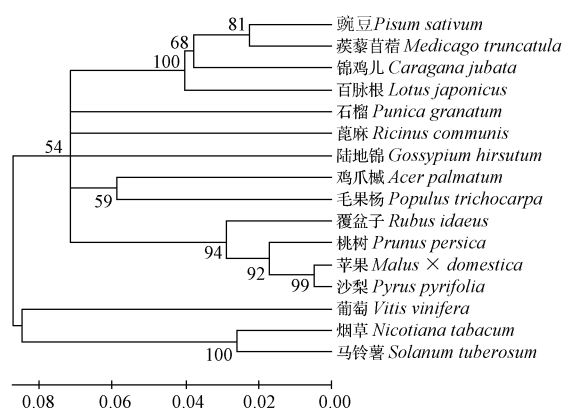
图6 血红鸡爪槭 WD₄₀ 氨基酸序列系统进化树

Fig. 6 Phylogenetic tree of the amino acid of the gene of WD₄₀ from *Acer palmatum*

血红鸡爪槭 WD₄₀ 氨基酸序列与其它植物的聚类分析表明,血红鸡爪槭与毛果杨为 1 个小分支,表明二者之间的距离较近,其同源性较高;与拟南芥、蔓菁等植物亲缘关系较远,这与传统分类相一致。这些结果显示了血红鸡爪槭 WD₄₀ 蛋白的分子进化过程,同时也表明血红鸡爪槭的 WD₄₀ 蛋白是从同一个祖先进化而来,其功能也必然相似。

3 结论与讨论

植物叶色形成过程的调控很复杂,不同的转录因子调控生物合成途径的不同部位,而不同转录因子间又存在相互作用^[7]。目前,几个重要的调节花色苷合成的转录因子的研究已有一些报道,而对调节机制的了解还只是初级阶段^[8],WD₄₀ 就是这些转录因子中极为重要的一种。WD₄₀ 如何构成转录复合体,如何调控结构基因的时空表达等还需要进一步的深入阐明。

近几年来,已经在拟南芥、矮牵牛、玉米等植物中鉴定了花色苷合成相关的 WD₄₀ 类转录因子^[9],但要全面认识 WD₄₀ 类转录因子,还需要大量试验进行验证。该研究克隆了血红鸡爪槭调节花青苷合成的转录因子 WD₄₀ 基因,并对该基因序列进行了深入分析。该基因编码的氨基酸序列的 C 端含有 4 个保守序列区,其同源性较高;而 N 端在很多植物的 WD₄₀ 中相似性较低,因此为基因的 5' 末端扩增带来难度,试验采用 RACE 的方法成

功获得了其 5' 端序列,获得的 cDNA 序列,全长 1 104 bp,包括起始密码子 ATG 和终止密码子 TGA,编码 337 个氨基酸。蛋白质空间结构的预测,便于探讨 WD₄₀ 与其它转录因子互作的分子机制,研究中发现血红鸡爪槭 WD₄₀ 含有 4 个 WD 重复基序,每个基序形成由 3 个 β -折叠构成的片层结构。Aizza L C B^[10] 在对西番莲的研究中也发现其 WD₄₀ 的 C 端氨基酸序列包含 4 次重复基序。Haar E T 等^[11] 发现,至少 4 个重复的 WD 基元才可以形成 1 个完整的 β -螺旋桨结构,它通过 1 或 2 个片层参与 WD₄₀ 蛋白和其它蛋白的互作。

不同来源的 WD₄₀ 转录因子的进化关系为进一步阐明整个 WD₄₀ 大家族的进化历史提供分析材料,血红鸡爪槭 WD₄₀ 的研究不仅丰富了其基因资源,而且对叶片中花色苷合成相关基因的表达调控机制的研究提供了分子依据,为将来通过基因工程的手段创建新的彩叶植物提供了重要的理论基础。

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Cloning and Sequence Analysis of WD₄₀ Transcriptional Regulator of *Acer palmatum*

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不同 LED 光源对银条组培苗不定芽生长的影响

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摘 要:以银条组培苗为材料,研究了不同 LED 光源对组培苗生长的影响。结果表明:蓝色光下银条组培苗不定芽长势最好,叶片大而展开;绿色光不定芽下增殖倍数最高,达到 317%;白色光 and 对照光源下叶绿素含量最高,蓝光下次之,黄光下叶绿素 a+b 仅为白光的 37%,最少;类胡萝卜素含量趋势和叶绿素含量相近;总酚含量由低到高依次为绿色光、黄色光、白色光、红色光、蓝色光 and 对照。

关键词:LED;银条;不定芽生长;总酚;叶绿素

中图分类号:Q 945.75 **文献标识码:**A **文章编号:**1001-0009(2012)12-0152-03

LED(Light Emitting Diode, LED)光源,具有节能环保、安全可靠、使用寿命长、响应时间短、体积小、重量轻、发热量少、低电压驱动、易于分散或组合控制等一系列传统电光源技术无可比拟的优点,在工业照明、家电诸多领域已得到广泛应用,近些年来在植物栽培方面的应用日益得到重视^[1-3],国内外不少专家学者尝试用 LED 作为组培光源来提高组培效果^[4-5]。

银条(*Stachys floridana* Schuttl. ex Benth)属唇形科水苏属,是原产于我国的一种野生草本薯芋类蔬菜。银条的地下根茎肉质脆嫩、富含蛋白质、多种矿物质、有机酸、酚类、碳水化合物,不但可食用而且具有一定的药用价值^[6-7]。银条无菌条件下离体诱导的种茎,不仅能

解决种性退化和病害蔓延的问题,而且能节约繁种用地^[8]。随着 LED 技术的发展,其在植物组织培养上的应用越来越广泛,但在银条组培上的研究应用尚未见报道。现以银条为材料探讨了 LED 光源对组培苗不定芽发生及生长的影响,为 LED 光源在植物组织培养上应用积累资料。

1 材料与方法

1.1 试验材料

供试材料‘二细一粗’品种的银条组培苗,由河南科技学院园林植物种质资源与生物技术实验室提供。

1.2 试验方法

选择长势一致无污染的组培苗,取长约 1 cm 的带节茎段,接种于 pH 为 5.8 的 MS+1.2 mg/L 6-BA+0.05 mg/L NAA 培养基上,每瓶接种 6 个茎段,每个处理 5 瓶,3 次重复。试验用 LED 光源分别为红色(635~640 nm)、黄色(592~595 nm)、绿色(527~530 nm)、蓝色(455~460 nm)、白色,由湖北匡通电子有限公司生产。将组培苗分别置于上述 5 种 LED 光源和对照光源(日光灯管)下,在温度为(20±1)℃、光照时间 15 h/d 条件下培养 40 d,测定组培苗不定芽数并记录其生长情况。测

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Abstract: With *Acer palmatum* as materials, total RNA was isolated from the leaf of *Acer palmatum* and acted as template for reverse transcription polymerase chain reaction (RT-PCR). The results showed that the full-length cDNA encoding a transcriptional factor was cloned via RACE-PCR method and the cDNA contained an ORF of 1 104 bp encoding 337 amino acids. Through Blast software in NCBI, the nucleotide sequence and the amino acid sequence of the leaf of *Acer palmatum* were analyzed. The results showed that the nucleotide and amino acid sequences of the cDNA in *Acer palmatum* were highly homologous with those of the WD₄₀ transcription factor in other species, and four conserved WD repeat motif were presented in the C-terminal of amino acid sequences, which suggested the sequence was WD₄₀ transcription factor gene.

Key words: *Acer palmatum*; WD₄₀ transcriptional factor; gene cloning; sequence analysis