

PpWRKY4通过影响PpCCD4表达调控桃果实类胡萝卜素积累

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摘要:【目的】克隆桃PpWRKY4基因, 探究其在桃果实类胡萝卜素代谢过程中的调控机制。【方法】以白肉桃中油桃14号果实为材料, 分析了其果实中类胡萝卜素含量变化趋势, 克隆桃中PpWRKY4基因, 对其进行生物信息学和表达分析。通过亚细胞定位、酵母单杂交技术和双荧光素酶试验确定PpWRKY4对PpCCD4的调控机制。【结果】随着中油桃14号果实成熟, PpCCD4基因表达量逐渐升高, 类胡萝卜素含量(w, 后同)由1.97 μg·g⁻¹降至0.68 μg·g⁻¹。PpWRKY4基因CDS区共1764 bp, 编码587个氨基酸, 蛋白分子质量为64.06 ku, 等电点为6.00; 通过氨基酸序列比对, 其包含两个WRKY保守基序, 为I类WRKY蛋白。PpWRKY4基因在桃果实发育前期高表达, 后期表达量较低, 与PpCCD4表达趋势相反。亚细胞定位结果表明PpWRKY4定位在细胞核。酵母单杂交技术和LUC试验表明PpWRKY4通过结合PpCCD4启动子抑制其表达。【结论】PpWRKY4通过负调控PpCCD4表达进而调控桃果实类胡萝卜素降解, 研究结果为进一步解析桃果实类胡萝卜素积累分子机制提供理论基础。

关键词:桃; WRKY转录因子; 果肉颜色; 类胡萝卜素

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PpWRKY4 regulates carotenoids accumulation in peach fruit by affecting the expression of PpCCD4

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Abstract:【Objective】Peach (*Prunus persica*) is one of the most important economic fruit tree in the world. The color of fruit flesh is a notable feature for consumers and one of the important breeding objectives. The flesh color of white/yellow is a typical Mendelian trait controlled by a single locus (*Y*), and white phenotype (*YY* or *Yy*) is fully dominant over the yellow one (*yy*). The yellow-flesh peach mainly depends on the accumulation of carotenoids in chromoplasts. The carotenoids is considered as an indispensable part for human diet health and nutrition. The previous studies indicated that three types of the *PpCCD4* mutation caused no expression or extremely lower expression of the *PpCCD4* in yellow-flesh peach, suggesting that the carotenoids would be negatively correlated with the expression of the

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PpCCD4. Although the *PpCCD4* is considered as the key gene controlling the white/yellow flesh trait of peach, the regulatory mechanism of carotenoids degradation remains unclear. The *PpWRKY4*, a WRKY transcription factors associated with carotenoids degradation, was isolated from a white-flesh peach exhibiting lower carotenoids content in this study and the regulatory mechanism of the *PpWRKY4* was explored in carotenoid metabolism of peach fruit. **【Methods】** The carotenoids content and the relative expression of the *PpCCD4* at S1-S4 stages of the fruits of Zhongyoutao 14 (CN14) were analyzed, respectively. The *PpWRKY4* (the homology of OfWRKY3) was cloned by PCR. *PpWRKY4* and other three homologous proteins were analyzed for domains using MEGA 11. The expression pattern of the *PpWRKY4* and *PpCCD4* at S1-S4 periods of the fruits of CN14 were obtained by qRT-PCR. The *cis*-elements of the *PpCCD4* promoter were analyzed using PlantRegMap and a binding site map was drawn by GSDS. The CDS of the *PpWRKY4* was inserted into pSAK277-GFP vector, and then transformed into GV3101 and Marker mixed annotated *Nicotiana benthamiana*. The fluorescence in tobacco leaf cells was observed using laser scanning confocal microscopy after 36–48 h. The *PpWRKY4* was cloned and inserted into pGADT7 and transfected into Y1HGold with PpAbAi-*PpCCD4-promoter* for Y1H assay. The CDS of the *PpWRKY4* was cloned into the pSAK277 vector to create the effector. The promoter sequence of the *PpCCD4* (-2 Kb upstream of ATG) was cloned into the dual reporter vector pGreenII-0800. The *Agrobacterium* cells containing pSAK277:PpWRKY4 and pGreenII-0800:PpCCD4 were co-infiltrated into the tobacco leaves, and the leaves infiltrated with the empty vector pSAK277 and pGreenII-0800:PpCCD4 were used as controls. 48 h after infiltration, the luciferase (LUC) and Renilla luciferase (REN) activities were measured using a dual-luciferase assay kit. **【Results】** The expression pattern of the *PpCCD4* and the carotenoids content during the S1-S4 periods of the fruits of CN14 showed opposite tendency. The relative expression of the *PpCCD4* increased gradually, while the carotenoids content of CN14 fruits significantly decreased, ranged from 1.97 $\mu\text{g} \cdot \text{g}^{-1}$ at S1 stage to 0.68 $\mu\text{g} \cdot \text{g}^{-1}$ at S4 stage. Several *cis*-elements of the *PpCCD4* promoter were observed, mainly including Dof, MADS, MYB, bHLH, WRKY etc. The *PpWRKY4* contained 1764 bp of ORF (open reading frame) and encoded 587 amino acids, which contained two WRKY conserved domains, as the character of a group I of WRKY gene subfamily. The sequence alignment results indicated that PpWRKY4 showed the highest expected value with OfWRKY3 which is a key transcription activator of the *OfCCD4* gene participating in biosynthesis of carotenoids. qRT-PCR analysis of S1-S4 periods of the fruits of CN14 showed that the *PpWRKY4* showed the opposite expression pattern of the *PpCCD4*. The expression level of the *PpWRKY4* showed higher at S1 stage and then sharply decreased at S2-S4 stages. Based on the expression patterns of the *PpWRKY4* and *PpCCD4*, the *PpWRKY4* might negatively regulate the *PpCCD4*. The subcellular localization analysis demonstrated that the *PpWRKY4* would be a nucleus-localized transcription factor. The Y1H results showed that the yeast cells containing the *PpWRKY4* and *PpCCD4* were able to grow well in SD-Leu/AbA²⁰⁰, but the yeast containing the empty AD vector and the *PpCCD4* did not, indicating that the *PpWRKY4* was bound to the *PpCCD4*. The results of dual-luciferase assays using the transiently transformed tobacco leaves indicated that the activity of the *PpCCD4* promoter greatly decreased after co-infiltration of the promoter reporter construct with a construct expressing *PpWRKY4*. These results showed that the *PpWRKY4* would be bound to the integral *PpCCD4* and repressed its transcription. **【Conclusion】** A homology of OfWRKY3 denoted as the *PpWRKY4* was cloned in CN14 peach. The *PpWRKY4* is a typical group I WRKY transcription factor with two WRKY domains. The *PpWRKY4* was expressed higher at S1 stage of CN14 fruits than that at S2-S4 stages, showing opposite expression pattern with the *PpCCD4*. The *PpWRKY4* could di-

rectly bind to the *PpCCD4* and inhibited its expression. The *PpWRKY4* was likely a transcription inhibitor of the *PpCCD4* gene, involved in regulating carotenoids accumulation.

Key words: Peach (*Prunus persica*); WRKY transcription factor; Flesh color; Carotenoids

桃是我国重要的栽培果树之一,其味甜多汁、香气浓郁、营养物质丰富,深受广大消费者喜爱。类胡萝卜素是植物体次生代谢产物中的一类重要物质,是人体维生素A生物合成的前体,并且具有抗氧化作用^[1]。随着人民生活水平不断提高,人们更加注重健康,黄肉桃因富含类胡萝卜素而受到追捧。类胡萝卜素裂解加氧酶基因家族(carotenoid cleavage dioxygenases, CCDs)是类胡萝卜素降解的关键基因^[2]。白肉桃中由于 *PpCCD4* 高表达而引起类胡萝卜素降解,导致果实中不能积累类胡萝卜素;而黄肉桃中由于 *PpCCD4* 基因突变,类胡萝卜素降解受阻而大量积累,果肉呈现黄色^[3-5]。深入研究类胡萝卜素生物合成和积累的代谢调控网络,对富含类胡萝卜素桃品种改良具有重要意义。

WRKY 转录因子是植物中特有的一类转录因子超家族^[6]。*WRKY* 基因以高度保守的 WRKY 结构域而命名,其包含一个七肽序列(WRKYGQK),并紧邻一个 C_{x4}-C_{x22}-H_xH 或 C_{x7}C_{x23}H_xC 的锌指蛋白基序^[7]。WRKY 转录因子根据其 DNA 结合结构域的数量和锌指蛋白基序的特征可分为三类。第 I 类具有两个 WRKY 结构域。第 II 类只有一个 WRKY 结构域,其锌指蛋白基序与第 I 类相同,为 Cys(2)-His(2),其包含的 WRKY 基因最多;基于氨基酸序列差异,II 类可进一步划分为 IIa、IIb、IIc、IId 和 IIe 5 个亚群。第 III 类 WRKY 转录因子锌指蛋白基序为 C_{x7}C_{x23}H_xC^[8]。WRKY 家族成员在植物中被相继发现和鉴定,其在不同植物中数量各不相同,如在水稻(*Oryza sativa*)中存在 109 个^[9]、玉米中(*Zea mays*)119 个^[10]、拟南芥中(*Arabidopsis thaliana*)74 个^[11]、苹果中(*Malus domestica*)56 个^[12]、番茄中(*Solanum lycopersicum*)81 个^[13]和桃中(*Prunus persica*)58 个^[14]。

WRKY 蛋白通常作为抑制因子或激活因子,参与植物多种发育过程,如生物胁迫、非生物胁迫、发育过程以及生物合成等^[7-8, 15]。过表达小麦 TaWRKY2 转录因子调节抗旱相关基因表达,进而增强小麦植株抗旱性^[16]。甘蔗 ScWRKY3 负调控烟草病镰刀菌蓝色变种的抗性^[17]。*OsWRKY45* 的过

表达除了增强抗病能力外,还增强了耐盐性和耐旱性^[18]。WRKY 转录因子调控植物对温度胁迫的抗性,包括对高温^[19]和冷胁迫^[20-21]。越来越多的研究证明,WRKY 转录因子参与植物次生物质代谢过程。桂花中 OfWRKY3 通过调控 *OfCCD4* 表达进而可能影响挥发性物质 β-ionone 合成^[22]。番茄 WRKY32 基因调控 YELLOW FRUITED-TOMATO 1 基因表达,调控类胡萝卜素积累,进而影响果实颜色^[23]。Sl-WRKY35 通过激活番茄果实 MEP(2-C-methyl-D-erythritol 4-phosphate)通路正向调控类胡萝卜素生物合成^[24]。虽然 WRKY 参与植物代谢调控逐渐增多,但是,WRKY 基因是否参与调控桃果实类胡萝卜素降解尚不清楚。基于此,笔者在本研究中测定了中油桃 14 号果实四个时期(S1、S2、S3、S4)类胡萝卜素含量变化和 *PpCCD4* 基因表达趋势,*PpCCD4* 基因启动子元件分析发现其包含 WRKY 转录因子结合元件,进一步克隆了 *PpWRKY4*,并初步验证了 *PpWRKY4* 能够调控 *PpCCD4* 基因表达。研究结果将有助于对桃类胡萝卜素积累分子机制的理解,同时为桃果肉颜色改良提供理论基础。

1 材料和方法

1.1 试验材料

中油桃 14 号果实取自河南农业大学科教园区,分别采集果实的 S1、S2、S3 和 S4 四个时期的果肉组织进行类胡萝卜素提取和检测,提取 DNA 用于 *PpCCD4* 基因启动子克隆,提取 RNA 用于 *PpWRKY4* 基因克隆和 qRT-PCR 分析。

1.2 类胡萝卜素提取和检测

称取 5 g 研磨成粉的桃果实样品进行类胡萝卜素提取。在样品中加入色素提取液(正己烷、丙酮、无水酒精体积比 2:1:1,含 0.01% BHT)。采用 UV755B 型紫外可见分光光度计在波长 450 nm 下进行扫描,测定桃果实总类胡萝卜素含量。类胡萝卜素的提取与测定参考朱运钦等^[25]的方法。

1.3 *PpCCD4* 基因启动子克隆和转录因子结合位点预测

以中油桃 14 号的 DNA 为模板,使用引物

(PpCCD4- p- F: TGGTAGTTACTAGGGTGTGTT-GCC, PpCCD4- p- R: AAAAAGGTAGTGAGGTGT-GGGA)进行启动子序列扩增。将克隆得到的PpCCD4启动子序列利用PlantRegMap(<https://plantregmap.gao-lab.org/>)网站预测转录因子结合位点,并利用GSDS(<https://gsds.gao-lab.org/>)绘制结合位点图谱。

1.4 PpWRKY4基因克隆及蛋白序列分析

以中油桃14号的cDNA为模板,使用高保真酶2×Phanta Flash Master Mix(P520,诺唯赞生物科技)和引物(PpWRKY4- G- F: ATGGACGCAACCA-CACTC, PpWRKY4- G- R: CTATGGACCTGTTAG-TACCCTT)进行编码区段(coding sequence, CDS)扩增;使用MEGA11软件,将拟南芥AtWRKY20、葡萄VvWRKY20和桂花OfWRKY3蛋白氨基酸序列进行比对分析。PCR扩增和载体构建参照谭彬等^[26]的方法进行。

1.5 基因表达分析

将中油桃14号发育期果实的RNA进行反转录合成cDNA,利用实时荧光定量PCR仪检测PpCCD4和PpWRKY4基因相对表达量。定量引物序列:PpCCD4-F: GGCTAGAGAGCCCGAGA-ATC, PpCCD4-R: GAGGAGACTTGGCATCCATC; PpWRKY4-F: GATCGGCCGTGATGACAAGC, Pp-WRKY4-R: CCAACATCCATCCTCCTCCTT, 内参基因PpEF2(Elongation factor 2), PpEF2-F: GGTGT-GACGATGAAGAGTGATG, PpEF2-R: TGAAGG-AGAGGAAAGGTGAAAG。利用 $2^{-\Delta\Delta CT}$ 法计算基因相对表达量^[26],每个样品3次重复。

1.6 亚细胞定位

将PpWRKY4编码区序列(不含终止密码子)克隆到含有绿色荧光蛋白的载体pSAK277-GFP上,构建PpWRKY4-GFP融合表达载体,将融合表达载体转入GV3101农杆菌菌株中。将阳性菌液注射烟草叶片,48 h后取样,使用激光共聚焦显微镜(尼康A1R HD25)观察荧光信号。

1.7 酵母单杂交验证PpWRKY4结合PpCCD4启动子

将PpCCD4基因起始密码子上游2000 bp片段(PpCCD4-promoter)连接到pAbAi载体上,之后转化到Y1H Gold酵母菌株中,在SD/-Ura琼脂板上培养2~3 d,筛选阳性菌株。随后,筛选PpAbAi- PpCCD4-

*promoter*背景抑制质量浓度200 ng·mL⁻¹的AbA。

将PpWRKY4的编码区序列连接到pGADT7载体上,将pGADT7- PpWRKY4质粒转化进含PpAbAi-PpCCD4-*promoter*质粒的酵母菌株中。将阳性对照(pAbAi-p53+pGADT7-rec-53)、阴性对照(pAbAi-PpCCD4-*promoter*+pGADT7-EMPTY)和试验组(pAbAi- PpCCD4- *promoter* + pGADT7- PpWRKY4),分别在SD^{-leu}和SD^{-leu}+200 ng·mL⁻¹ AbA培养基中观察酵母生长情况。

1.8 双荧光素酶报告系统检测PpWRKY4对PpCCD4的调控作用

将PpCCD4-*promoter*连接到pGreenII 0800-LUC载体中作为报告质粒,将PpWRKY4编码序列连接到pSAK277载体上作为效应质粒。将两种质粒分别转化农杆菌,并进行扩繁(OD₆₀₀=0.9~1.1)。离心后(10 min, 4000 r·min⁻¹),使用重悬液(0.5 mol·L⁻¹ MES, 10 mmol·L⁻¹ MgCl₂, 100 mmol·L⁻¹ AS)重悬菌体并调整菌液OD₆₀₀=0.8左右。注射烟草叶片,培养48 h后,按照Promega双荧光素酶报告基因检测试剂盒实验方法,测定酶活性。

1.9 数据统计与分析

利用SPSS 17.0软件对数据进行显著性分析,采用Excel 2010进行数据统计和作图。

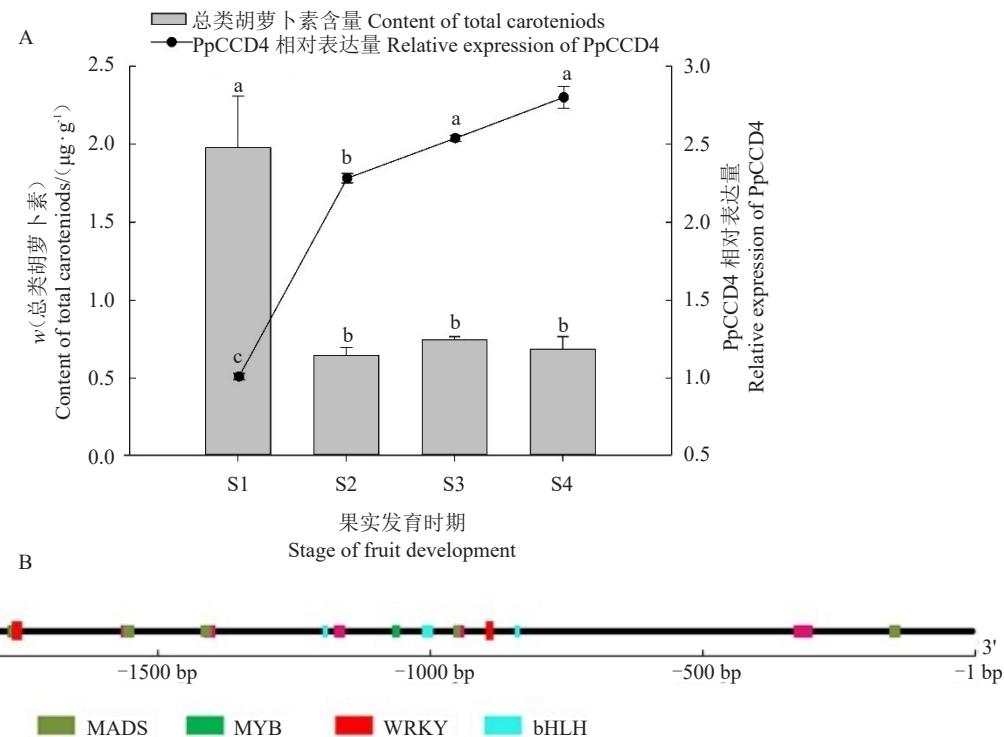
2 结果与分析

2.1 中油桃14号果实类胡萝卜素含量及PpCCD4表达模式分析

对不同发育阶段中油桃14号果实类胡萝卜素含量进行测定,结果显示,在果实发育S1时期类胡萝卜素含量为1.97 μg·g⁻¹,随后类胡萝卜素含量急剧下降,S2时期为0.64 μg·g⁻¹,S2-S4时期果实中类胡萝卜素含量差异不显著(图1-A)。对类胡萝卜素裂解加氧酶PpCCD4基因在中油桃14号果实不同发育时期表达分析显示,其在S1时期低表达,随后急剧上升,说明类胡萝卜素的降解是由PpCCD4的高表达所导致的。对PpCCD4上游2000 bp序列进行转录因子结合元件分析,发现区域内包含多种转录因子结合基序,包括Dof、MADS、MYB、bHLH以及WRKY等(图1-B)。由此推测,PpCCD4可能会受到WRKY、MYB等转录因子的调控。

2.2 PpWRKY4基因克隆与表达分析

以中油桃14号果肉组织cDNA为模板克隆



A. 类胡萝卜素含量与 *PpCCD4* 表达趋势分析, 不同小写字母表示差异显著($p<0.05$, 下同); B. *PpCCD4* 启动子结合元件分析。

A. The content of total carotenoids and expression of *PpCCD4*. Different small letters mean significant differences ($p<0.05$, the same below); B. The motif analysis in the promoter of *PpCCD4*.

图 1 中油桃 14 号不同时期果实类胡萝卜素含量与 *PpCCD4* 表达及启动子结合元件分析

Fig. 1 The content of total carotenoids and expression pattern of *PpCCD4* in the fruit of Zhongyoutao14 at different stages and motif analysis in the promoter of *PpCCD4*

PpWRKY 基因, 测序分析发现, 其 CDS 区共 1764 bp, 编码 587 个氨基酸, 预测 *PpWRKY4* 蛋白分子质量为 64.06 ku, 等电点为 6.00。序列比对结果显示 *PpWRKY4* 与 *AtWRKY20* 和 *OvWRKY3* 高度同源, 含有两个 WRKY 保守结构域, 属于 I 类 WRKY 蛋白(图 2-A)。对 *PpWRKY4* 基因在中油桃 14 号果实不同发育期表达趋势分析发现 *PpWRKY4* 在 S1 时期高表达, 随后急剧下降(图 2-B), 与 *PpCCD4* 表达趋势相反, 且 *PpCCD4* 基因启动子区有 WRKY 结合元件, 据此推测 *PpWRKY4* 可能负调控 *PpCCD4* 表达。

2.3 P_pWRKY4 亚细胞定位

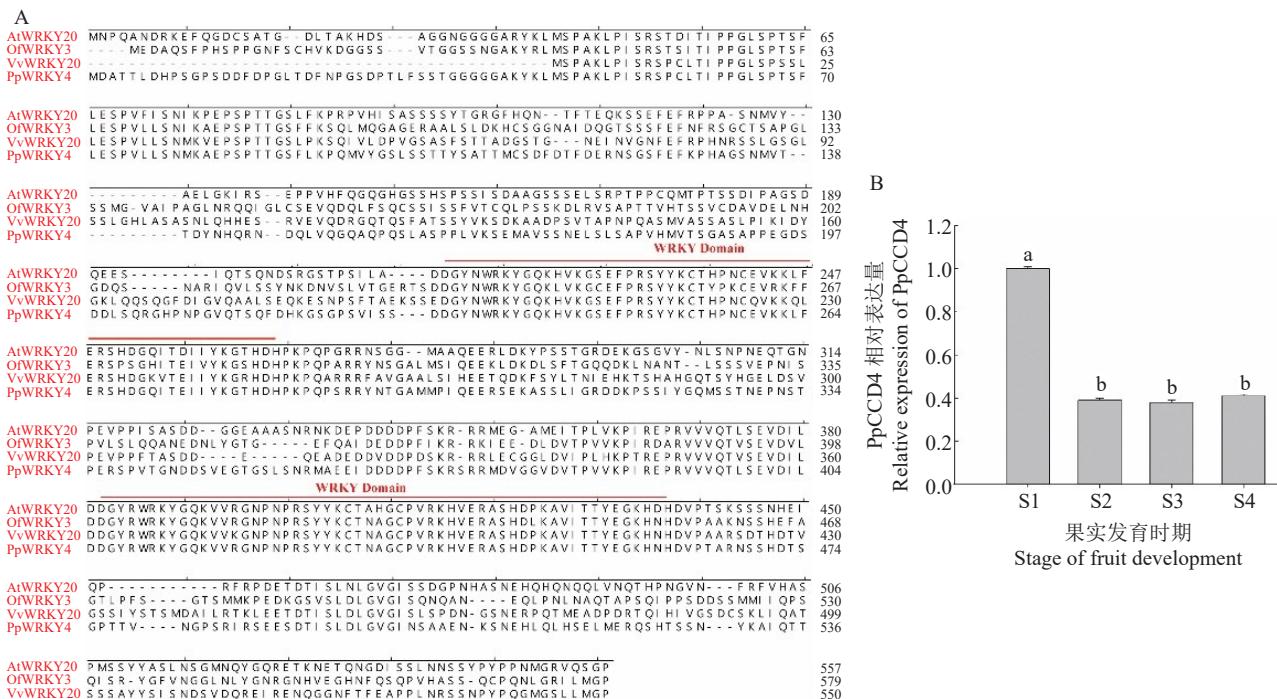
为了确认 *PpWRKY4* 蛋白在细胞内可能发挥作用的部位, 构建了 pSAK277-P_pWRKY4-GFP 融合表达载体和 pSAK277-GFP 空载, 通过农杆菌侵染烟草。亚细胞定位结果显示 P_pWRKY4-GFP 融合蛋白仅在烟草表皮细胞的细胞核中观察到绿色荧光(图 3), 表明 P_pWRKY4 定位于细胞核。

2.4 P_pWRKY4 与 *PpCCD4* 启动子的互作分析

为了验证候选的 P_pWRKY4 是否可以直接结合在 *PpCCD4* 启动子上, 将 *PpCCD4* 启动子片段(*PpCCD4P*)连接到 PAbAi 载体上, 并转化到酵母中, PAbAi-P_pCCD4-promoter 质粒在 AbA 为 200 ng·mL⁻¹ 下被抑制生长。将 P_pWRKY4 的 CDS 全长连接到 pGADT7 载体上, 与 PAbAi-P_pCCD4-promoter 载体共转, 验证 P_pWRKY4 是否可以结合 *PpCCD4* 启动子。结果显示, 当 SD-leu 培养基中 AbA 质量浓度为 0 时, 阳性对照 p53-promoter+AD-Rec-p53、阴性对照 P_pCCD4-promoter+AD-empty、P_pCCD4-promoter+AD-P_pWRKY4 均可以正常生长; 当培养基中 AbA 质量浓度为 200 ng·mL⁻¹ 时, 仅有阳性对照和 P_pCCD4-promoter+AD-P_pWRKY4 可以正常生长, 阴性对照酵母的生长受到严重的抑制, 表明 P_pWRKY4 可以结合 *PpCCD4* 启动子(图 4)。

2.5 P_pWRKY4 抑制 *PpCCD4* 表达

为了进一步明确 P_pWRKY4 对 *PpCCD4* 基因的



A. PpWRKY4、AtWRKY20、OfWRKY3 和 VvWRKY20 氨基酸序列比对; B. PpWRKY4 表达趋势分析。

A. Alignment of WRKY domain of PpWRKY4, AtWRKY20, OfWRKY3 and VvWRKY20; B. Expression pattern of PpWRKY4.

图 2 PpWRKY4 结构域及表达趋势分析

Fig. 2 Characterization and expression of PpWRKY4

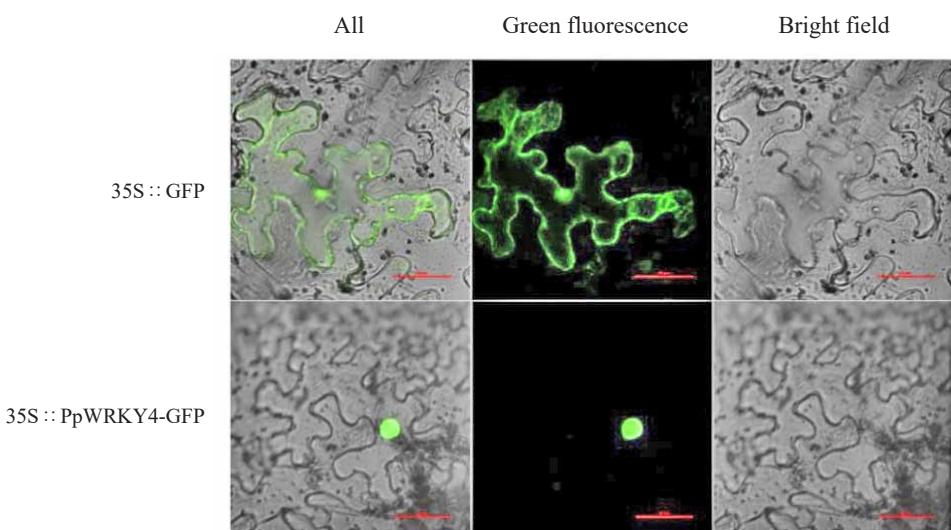


图 3 PpWRKY4 蛋白亚细胞定位

Fig. 3 Subcellular localization of PpWRKY4

转录调控活性,利用双荧光素酶系统,构建 pSAK277-PpWRKY4 过表达载体,以及将 PpCCD4 启动子片段连接到 pGreenII0800-LUC 载体上。利用农杆菌介导方法,将 pSAK277-PpWRKY4 和

pGreenII0800-LUC-PpCCD4-promoter 共转化烟草叶片。结果显示,阴性对照的 LUC/REN 值是试验组 PpWRKY4 的 4 倍左右,表明 PpWRKY4 可显著抑制 PpCCD4 启动子活性(图 5)。

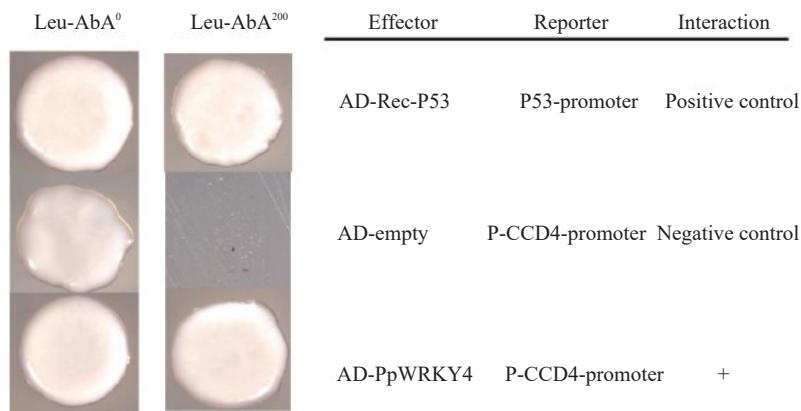


图 4 酵母单杂交揭示 PpWRKY4 可以结合 PpCCD4 启动子

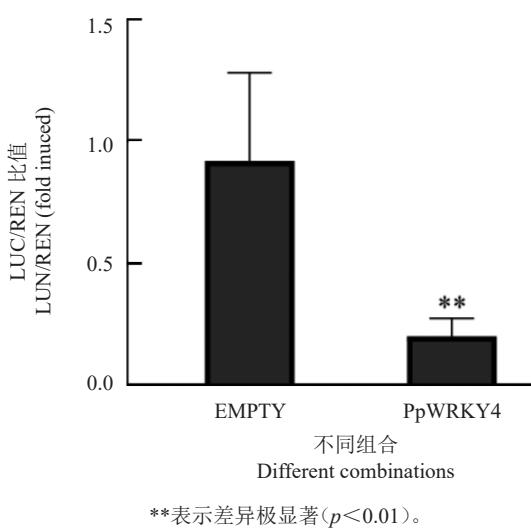
Fig. 4 PpWRKY4 binds to the promoter of *PpCCD4* by yeast one-hybrid

图 5 双荧光素酶试验证明 PpWRKY4 抑制 PpCCD4 基因转录

Fig. 5 PpWRKY4 repress transcription of *PpCCD4* by Dual-Luciferase reporter assay

3 讨 论

桃果肉颜色是一个重要的农艺性状,其类胡萝卜素含量变异丰富^[27-29]。桃果肉颜色(黄肉/白肉)是受一对等位基因控制的质量性状,白肉对黄肉为显性,*PpCCD4*为控制该性状的关键基因^[3,5]。*PpCCD4*的表达与类胡萝卜素含量具有显著相关性,在白肉桃中随着果实成熟*PpCCD4*表达量逐渐升高,而类胡萝卜素含量逐渐降低;黄肉桃果实中,由于*PpCCD4*基因突变,导致其功能丧失或表达量降低,类胡萝卜素积累,果肉呈现黄色^[25,30]。目前,关于桃果实类胡萝卜素积累的研究主要集中在类胡萝卜素

含量的差异以及合成通路相关结构基因表达分析^[3-5,25,31]方面,而对类胡萝卜素合成通路相关结构基因的转录调控因子研究还不清楚。本研究中,在白肉桃中油桃 14 号果实发育过程中,*PpCCD4*表达量随着果实成熟而提高,导致类胡萝卜素降解,果肉呈现白色。对*PpCCD4*启动子元件分析发现,共检测到多种转录因子结合位点,其中包括两个 WRKY 转录因子结合基序。

WRKY 转录因子超家族是植物中最大的转录因子家族之一^[6]。WRKY 蛋白在调节多种形态、发育、生理和生化过程,以及对生物/非生物胁迫的反应中起着重要作用^[32-35]。目前,桃中鉴定到 WRKY 家族基因 58 个,其根据基因保守结构域特性和聚类分析,可将其分为 3 类;通过表达趋势分析,共有 36 个基因在休眠芽中表达,其中 6 个 PpWRKYS 可能参与桃休眠过程^[14]。在桃干旱胁迫响应分子机制研究中,*PpWRKY18*基因表达受干旱胁迫诱导,而复水后,其表达量下降,推测其可能参与干旱胁迫的响应^[36]。本研究中克隆了桃 *PpWRKY4* 基因,对其氨基酸序列分析发现为 I 类 WRKY 蛋白,且与桂花 *Of-WRKY3* 高度同源。桂花中, *OfWRKY3* 调控 *Of-CCD4* 表达,推测其可能通过调控 *PpCCD4* 基因表达参与类胡萝卜素代谢^[22]。

在植物中,许多代谢物的生物合成或降解受到与这些通路相关的结构基因的转录调控,而这种调控作用主要依赖于特定转录因子的调节^[37]。植物中类胡萝卜素合成和积累受到类胡萝卜素生物合成、降解酶及存储相关基因的影响,其受众多转录因子调控^[38]。在柑橘中, *CsERF61* (ethylene response fac-

tor)能够结合至*CsLCYB2*(lycopene β -cyclase)基因的启动子激活其表达,参与类胡萝卜素合成^[39]。在番茄中,通过对类胡萝卜素生物合成途径相关结构基因*S1DXS1*(1-deoxy-D-xylulose 5-phosphate synthase)和*S1PSY1*(phytoene synthase)表达模式与已知调控基因表达模式分析,发现*SIWRKY35*可以控制MEP(2-C-methyl-D-erythritol 4-phosphate)途径的初级代谢,从而诱导下游类胡萝卜素的生物合成^[24]。甜瓜*CmWRKY49*调控*CmPSY1*进而促进 β -胡萝卜素积累^[40]。本研究分析发现,*PpWRKY4*基因在桃果实发育过程中的表达趋势与类胡萝卜素合成关键基因*PpCCD4*的表达趋势相反,且亚细胞定位结果显示*PpWRKY4*蛋白定位在细胞核,与转录因子在细胞核中发挥的作用相符。进一步通过酵母单杂交技术、LUC试验,证明*PpWRKY4*负调控*PpCCD4*。以上研究结果表明,*PpWRKY4*可能具有调控类胡萝卜素积累的功能,在桃果实颜色调控中发挥重要作用,为桃果实颜色形成研究提供更多理论基础。

4 结 论

笔者在研究中克隆了中油桃14号中*PpWRKY4*基因,为I类WRKY转录因子。随着果实成熟,中油桃14号果实的类胡萝卜素含量显著降低,*PpWRKY4*表现出与类胡萝卜素降解基因*PpCCD4*相反的表达模式。进一步研究证明,*PpWRKY4*可直接与*PpCCD4*基因启动子结合并抑制其表达,因此,*PpWRKY4*通过影响*PpCCD4*表达参与调节桃类胡萝卜素的积累,为调节桃的肉色提供了理论依据。

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