

草莓生长素合成限速酶 *FaYUC11* 基因的克隆和功能分析

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摘要:【目的】克隆草莓 YUC 家族新基因, 探究其在草莓中的具体调控作用。【方法】以‘久香’草莓为材料, 利用同源克隆技术克隆草莓果实大小调控相关的新基因, 利用病毒诱导基因沉默(virus-induced gene silencing, VIGS)结合定量 RT-PCR 技术, 分析新基因在草莓果实膨大调控中的作用。【结果】从‘久香’草莓果实中克隆到 1 个草莓果实大小调控相关的新基因, 命名为 *FaYUC11* (GenBank 登录号: JX417083.1), 用草莓幼果微注射法建立了病毒诱导 *FaYUC11* 基因沉默的转基因草莓体系, 结果发现, *FaYUC11* 基因沉默后果实的膨大和正常生长均受到影响, 并且导致果实瘦果(种子)中游离生长素含量下降、果实纵横径增长率降低。【结论】*FaYUC11* 基因是草莓生长素合成途径中的关键基因, 该基因通过调控生长素的合成继而调控果实的膨大。

关键词: 草莓; *FaYUC11* 基因; 生长素合成; 基因沉默

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Cloning and functional verification of rate-limiting enzyme *FaYUC11* in strawberry auxin biosynthesis

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Abstract: 【Objective】Cloning and functional verification of a new crucial regulator gene in strawberry auxin biosynthesis. 【Methods】The *FaYUC11* for fruit enlargement was isolated from the ‘Jiuxiang’ cultivated strawberry (*Fragaria×ananassa* ‘Jiuxiang’) by using RT-PCR (reverse transcription PCR), and then the function of *FaYUC11* in strawberry fruit was analyzed by VIGS (virus-induced gene silencing) technology to complete our investigation. The total RNA of the strawberry fruit was extracted by CTAB (Hexadecyl trimethyl ammonium Bromide). Specific primers were designed according to the homologous gene sequences of *F. vesca* for PCR amplification. After the PCR products were verified by sequencing, they were then compared with the YUC gene family members, and according to the comparison results, specific areas were selected close to the 5’ end of *FaYUC11* to design the interference primers, using the plasmid with *FaYUC11* full-sequence as the template for amplification. Then the products were integrated into the virus RNA plasmid pTRV2, in order to obtain a pTRV2-d*YUC11* virus induced gene silencing carrier. The infection vector pTRV2-d*YUC11* was constructed and transformed into the *Agrobacterium* strain GV3101. The resultant *Agrobacterium* was microinjected into the strawberry fruit to induce gene silencing. 34 days after infection, an analysis was done of each index of the virus induced gene silencing strawberries. The expression of *FaYUC11* was analyzed using qRT-PCR (Quantita-

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tive Real-time PCR), the content of the free IAA in the strawberry achenes (seeds) was detected by GC-MS, and the phenotypic changes of the strawberry fruits infected by pTRV2-*YUC11* were analyzed, and then compared with the percentage increase of the longitudinal and traverse diameters of the strawberry fruits. 【Results】Compared with wild type strawberry fruits, the expression of fruits with the treatment of the pTRV2 empty vector was 1.13, while the expression of fruits with the pTRV2-*YUC11* infection were 0.18 and 0.44. The results of qRT-PCR showed that, the *FaYUC11* decreased in transcript accumulation levels with different degrees in the strawberry fruits infected with pTRV2-*YUC11*. The content of the free IAA in the strawberry achenes (seeds) was detected by GC-MS, the results showed that, compared with the group that were treated with the pTRV2 empty vector ($515.2 \text{ ng} \cdot \text{g}^{-1}$), it significantly decreased in the fruits infected by pTRV2-*YUC11* ($64.86 \text{ ng} \cdot \text{g}^{-1}$, $307.19 \text{ ng} \cdot \text{g}^{-1}$), which indicated that *FaYUC11* is a significant regulator for IAA accumulation. Phenotypic change analysis of the strawberry fruits at 34 days after infection showed that there were mildly sunken at the top of the fruits that were treated with pTRV2 empty vector, but it did not affect fruit enlargement and its morphology, while the fruits infected by pTRV2-*YUC11* were significantly suppressed, with some of the fruits enlargement of inhibition being minor (RiYUC11-3), but part of the fruits were severely inhibited (RiYUC11-2), with little change showing after 34 days growth, with some still being in the small green fruit stage. Furthermore, by analyzing the percentage increase of longitudinal and traverse diameters of the strawberry fruits found, compared with the wild type fruits, there was a significant decrease in the fruits infected by pTRV2-*YUC11*. This indicates that *FaYUC11* regulates the increase of longitudinal and traverse diameters of strawberry fruits. 【Conclusion】The lack of *FaYUC11* led to free IAA content in strawberry achenes (seeds) and the percentage increase of the longitudinal and traverse diameters of strawberry fruits were reduced, and at the same time, this influenced the fruit enlargement and normal growth. It indicated that *FaYUC11* plays a significantly important role in the regulation of strawberry fruit size. This provides good application prospects in the regulatory functions of strawberry fruit size and has great significance in the development of molecular markers for functional SNP and large strawberry fruit breeding.

Key words: Strawberry; *FaYUC11* gene; Auxin biosynthesis; Virus-induced gene silencing

草莓果实由花托发育而来,属“假果”,而植物学意义上的果实是点缀其表面的种子,后者又称为瘦果。生长素是植物中最早发现的一类激素^[1],目前已知生长素几乎参与了植物生命每一个方面的调控,包括多种组织器官的发育和形态建成以及对环境的响应等。伍涛^[2]研究发现,梨果实早期大果内源 IAA 含量均高于小果,说明果实发育早期内源 IAA 水平有利于光合产物向果实内运转和积累。Nitsch^[3]研究发现,授粉后若去掉草莓花托上的全部瘦果(种子),花托就会停止生长;而去掉花托上的部分种子,则仅含有瘦果的那部分花托发育,最后会长成形状畸形的果实。但是,如果去掉花托上所有的种子,在花托上涂 NAA,最后却能发育成正常的果实。说明草莓花托的生长发育受激素调控,主要是瘦果中合成的生长素起着重要作用。Liu 等^[4]在草

莓中的研究也表明,生长素的合成对草莓生长发育至关重要。

前人对植物内源生长素合成机制已有报道^[5-7]。研究表明,植物体内存在多条生长素合成途径^[8-9],主要分为依赖于色氨酸的途径以及非依赖于色氨酸的途径 2 类^[10]。其中依赖于色氨酸的吲哚丙酮酸途径 (IPyA pathway of IAA biosynthesis)被认为是植物体内生长素合成的主要途径^[11-12]。仅包括 2 步反应的 IPyA 途径又称为 TAA/YUC 途径,首先,色氨酸氨基转移酶 TAA/TAR 催化色氨酸产生吲哚丙酮酸(indole-3-pyruvic acid, IPyA),之后 YUC 家族黄素单加氧酶将 IPyA 转变为 IAA^[13-15]。YUC 基因最早在拟南芥中鉴定^[16],为依赖色氨酸的生长素合成限速步骤的关键酶。在水稻中,过表达 *OsYUCCA1* 基因的水稻植株表现出高生长素表型,而抑制表达 *OsYUC-*

CAI 基因的水稻则与生长素不敏感突变体有相似的缺陷表型^[17]。目前在西红柿、水稻、杨树、玉米中都分离鉴定到 *YUCCA* 基因^[17-20],表明 *YUCCA* 基因功能保守,在高等植物中广泛分布,并对生长素合成起着重要的作用。

VIGS 技术是一种操作简单、快速便捷的基因功能鉴定方法^[21-22],已在多种植物中成功应用,特别是针对性地研究植物繁殖器官中的特异基因,从而揭示植物果实的发育与品质形成的机制^[23-24]。VIGS 广为运用的载体系统是双链 RNA 病毒-烟草脆裂病毒 (TRV)^[25],草莓中有应用成功的报道^[26]。

目前,我国的草莓产业在总面积和总产量上虽称得上“草莓大国”,但与“世界草莓强国”的美国、西班牙等国相比仍存在一定差距,在抗病性、品质优良等方面仍有较大的提升空间。因此,积极发掘鉴定与重要农艺性状相关的基因并解析其功能,对于揭示草莓果实大小调控的分子机制及开发与果实大小、质量等相关的标记和辅助选育适合我国栽培环境和消费习惯的大果草莓新种质具有重要意义。有关草莓果实大小发育调控相关基因的研究仍鲜有报道,笔者利用同源克隆从八倍体草莓‘久香’中克隆了1个新的、完整的草莓果实大小调控基因 *FaYUC11*,并利用 VIGS 技术对其功能进行验证。

1 材料和方法

1.1 材料

‘久香’草莓果实采自上海市农业科学院林木果树研究所草莓种质资源圃;大肠杆菌 DH5 α 保存于上海市农科院林果所草莓生物技术实验室;高保真 DNA 聚合酶购自北京全式金生物技术有限公司;基因克隆载体 pUCm-T 购自上海生工生物工程有限公司;限制性内切酶购自大连宝生物公司;农杆菌 GV3101、烟草脆裂病毒载体 pTRV1 和 pTRV2 由清华大学刘玉乐老师惠赠。PCR 引物由上海生工生物工程有限公司合成(表1)。

1.2 方法

1.2.1 草莓 RNA 的提取及 cDNA 第 1 链的合成 采用改良的 CTAB 法提取‘久香’草莓果实中的 RNA,1%(ω)琼脂糖凝胶电泳结合 Nanodrop 2000(Thermo Scientific, Wilmington, USA)检测 RNA 的质量和浓度。反转录前先对 RNA 中的 DNA 进行消化,消化体系包含:RNA 6 μ L, 10 \times buffer 1 μ L, DNase I 1 μ L,

表 1 试验所用引物

Table 1 Primers used in the test

引物名称 Primer name	引物序列(5'-3') Primer sequences(5'-3')
18S-F	ACCGTTGATTCGCACAATTGGTCATCG
18S-R	TACTGCGGGTCGGCAATCGGACG
<i>FaYUC11</i> -F	AAAATGGAGAACAATGTGTTGGGA
<i>FaYUC11</i> -R	GGACTAGACCTCTCTGCAGCAT
<i>FaYUC11</i> -Ri-F	TTCTGCTCTCTGCCGATGAT
<i>FaYUC11</i> -Ri-R	CTCCAGTCGCAATTACCAAG
<i>FaYUC11</i> -RT-F	GGAAAGGTGAAAAGGGCGT
<i>FaYUC11</i> -RT-R	GACCTCTCTGCAGCATTAC
<i>FaYUC3</i> -RT-F	AGATGTGTTGGTTGTCGGATCAG
<i>FaYUC3</i> -RT-R	GGGCTTGACCATTGTGTTCTCT
<i>FaYUC4</i> -RT-F	CCACGCTCTGAGTACAAGTCTG
<i>FaYUC4</i> -RT-R	CAATGCTGCCGAGTATCAACCA
<i>FaYUC5</i> -RT-F	AGAGTTGCCTGGAATAGAGACCT
<i>FaYUC5</i> -RT-R	TACCATCCCAAGCCGAACAAGT
<i>FaYUC6</i> -RT-F	CCGTCTCCACCTTCACCTCA
<i>FaYUC6</i> -RT-R	TCATTGTTTCATCGCCTTCACCAC
<i>FaYUC7</i> -RT-F	CTGAACCGCCTCAATATCTCCAA
<i>FaYUC7</i> -RT-R	TGACAAGGACACACCATTATACC
<i>FaYUC10</i> -RT-F	TGGCTTGCTGACAAGATATTGCT
<i>FaYUC10</i> -RT-R	TGAAGGAACATTGCTGCGATACC
TRV2-F	TTACGACGAACCAAGGGAGTACTAC
TRV2-R	AGTCACAATTAGCCCTATTTAGATGT

RNase Inhibitor 0.5 μ L, RNase free water 2.5 μ L。37 $^{\circ}$ C 反应 30 min, 65 $^{\circ}$ C 反应 10 min。反应完成后在上述体系中加入 dNTP 和 Oligo-dT 各 2 μ L, M-MLV 1 μ L, 5 \times buffer 8 μ L, RNase free water 补足体系至 40 μ L, 混匀, 42 $^{\circ}$ C 反应 60 min, 65 $^{\circ}$ C 反应 10 min。-20 $^{\circ}$ C 保存备用。

1.2.2 *FaYUC11* 全长基因的克隆与鉴定 运用生物学软件,从森林草莓基因组序列信息中获得同源基因的序列信息,设计 PCR 扩增引物:正向引物 *FaYUC11*-F 和反向引物 *FaYUC11*-R(表1)。PCR 扩增体系为: cDNA 2 μ L, 10 \times buffer 5 μ L, dNTP (2.5 mmol L⁻¹) 2 μ L, 高保真 *Taq* 酶 0.5 μ L, 上、下游引物 (10 μ mol L⁻¹) 各 1 μ L, ddH₂O 补足体积至 50 μ L, 充分混匀。PCR 反应程序: 94 $^{\circ}$ C 2 min, 94 $^{\circ}$ C 30 s, 58 $^{\circ}$ C 30 s, 72 $^{\circ}$ C 2 min, 30 次循环, 72 $^{\circ}$ C 终延伸 10 min, 4 $^{\circ}$ C 保存。PCR 产物回收纯化后连接克隆到 pUCm-T 载体上,连接产物转化大肠杆菌 DH5 α , 挑取单克隆测序验证。

1.2.3 *FaYUC* 家族基因表达模式分析 选取‘久香’草莓发育进程中各时期的果实,包括绿果、白果、半红半白果(转色期果)、红果(成熟果)以及成熟的花托,分别将果实的种子和果肉分开。通过半定量 RT-PCR 分析 *FaYUC* 家族基因的表达模式(引物见

表1),以明确*FaYUC11*基因的优势表达组织。

1.2.4 病毒诱导基因沉默(VIGS)载体的构建 经测序鉴定克隆成功后,根据‘久香’草莓YUC家族成员序列比对结果,选取靠近5'端的基因特异区域,设计干涉引物*FaYUC11*-R iF和*FaYUC11*-R iR(表1)。以所获得携带全长基因的质粒为模板,利用干涉引物进行PCR扩增,将获得的基因片段首先克隆到pUCm-T载体上,然后将含有*FaYUC11*基因片段的pUCm-T质粒经*Pst*I和*Bam*H I双酶切,通过体外重组将基因片段移入用相同组合酶切开的pBSK-in载体上。之后,再次用*Pst*I和*Sal*I酶切含有*FaYUC11*基因片段的pUCm-T质粒,将切下的基因片段通过体外重组转移进入用*Nsi*I和*Sal*I线性化的已含有1个目标基因片段的pBSK-in载体,最后得到含有2个*FaYUC11*基因片段的重组质粒pBSK-in-dYUC11,其中2个YUC11基因片段方向相反,且中间被1个内含子隔开。经过2次体外重组转移到pBSK-in载体的内含子序列两端,最后经*Kpn*I和*Sac*I双酶切点整合进病毒RNA 2质粒pTRV 2,得到pTRV 2-dYUC11病毒诱导基因沉默载体。

1.2.5 VIGS草莓的获得 选取‘久香’草莓小绿果为试材。将含有目标基因的VIGS表达载体以及空pTRV 1和pTRV 2载体的GV 3101农杆菌分别接种于含有50 mg · L⁻¹ Kan、10 mg · L⁻¹ Rif和50 mg · L⁻¹ Gen的5 mL YEP培养基中,28 °C过夜培养,翌日按照1:50的比例接种于诱导培养基(YEP,含有10 mmol · L⁻¹ MES、20 μmol · L⁻¹ AS、50 mg · L⁻¹ Kan、10 mg · L⁻¹ Rif和50 mg · L⁻¹ Gen)中,28 °C摇菌至对数期(OD值为0.6~0.8),5 000 r · min⁻¹离心10 min,收集菌体。再用侵染缓冲液(10 mmol · L⁻¹ MES、100 μmol · L⁻¹ AS、10 mmol · L⁻¹ MgCl₂)重悬菌体,调节菌液OD值为1.2~1.5,28 °C放置3 h后用于接种草莓果实。然后将含有pTRV 1的GV 3101菌液与含有pTRV 2-dYUC11的GV 3101菌液按体积比为1:1混匀,使用微注射方法将混合的菌液从果实顶部注入,控制环境温度温度为25~28 °C,保证病毒有效侵染。

1.2.6 VIGS草莓的鉴定 分别提取野生型和注射病毒原始空载体以及重组*FaYUC11*病毒诱导沉默载体的草莓果实总RNA,利用随机引物进行反转录合成cDNA。参考Jia等^[21]的烟草脆裂病毒RNA2特异引物TRV2-F和TRV2-R(表1)进行RT-PCR反应,鉴定病毒是否侵染草莓果实。PCR产物进行电泳检

测。

1.2.7 pTRV2-YUC11基因沉默结果分析 选取‘久香’草莓小绿果(横径10~12 mm)为试验材料,将100 μL含有pTRV2-YUC11基因沉默载体的GV3101农杆菌混合菌液通过注射器压迫的方式注入到草莓果实中,处理34 d后,将病毒侵染的草莓果实和野生型同发育时期的草莓果实进行分析比较。进一步通过测定果实纵横径,并与微注射含病毒农杆菌之前的果实大小数据进行比较。草莓果实种子(瘦果)中的IAA含量参考GC-MS法^[27]测定,由中国科学院植物分子生理重点实验室完成测定。分析pTRV2-YUC11基因沉默对草莓果实的影响,从而明确*FaYUC11*基因在草莓果实生长发育过程中的调控作用。

2 结果与分析

2.1 草莓*FaYUC11*全长基因序列的获得

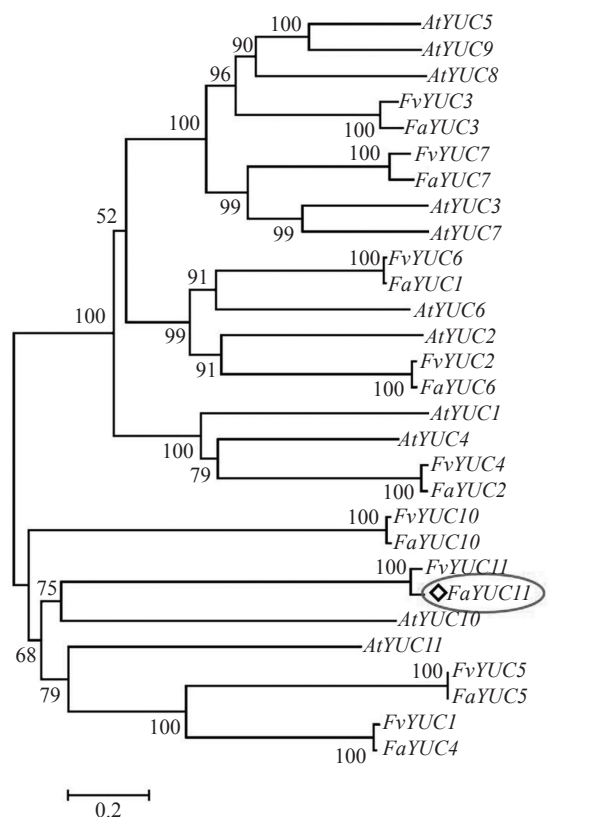
以野生型‘久香’草莓果实cDNA为模板,根据森林草莓同源基因的序列设计引物,进行PCR扩增,将扩增产物克隆到pUCm-T载体上,经过菌落PCR、质粒酶切鉴定后,送上海华大基因测序,利用DNAMAN软件对测序结果进行拼接确认。鉴定结果表明,成功克隆得到*FaYUC11*全长DNA序列,将该序列已提交至GenBank(登录号:JX417083.1)。

2.2 YUCCA基因进化树构建

利用MEGA 5软件将9个栽培草莓YUCCA基因家族、9个野生草莓YUCCA基因家族、11个拟南芥YUCCA基因家族所推导出的氨基酸序列进行分析,构建进化树(图1)。进化树上的数字表示基因分在同一枝上的可信程度,也被认为是它们之间的亲缘关系程度,数字越大表示亲缘关系越近,同时它们的基因功能作用也可能类似。从进化上可看出,这些YUCCA基因主要分为两大枝,而各自里面又分了一些小枝。总体上,草莓的YUCCA基因家族在进化树上的各个小枝上都有分布,其中*FaYUC11*与*FvYUC11*的相似度为100%,且与拟南芥*AtYUC10*有较高的相似度。

2.3 *FaYUC*家族基因表达模式分析

为了明确*FaYUC11*基因在草莓中的优势表达部位,运用半定量RT-PCR分析*FaYUC*家族基因的表达模式特征(图2)。*FaYUC3*在前期果实的种子中有表达,生长到转色期后不再表达;*FaYUC4*在绿果种子和花托中均有表达,而在其他各阶段中均未



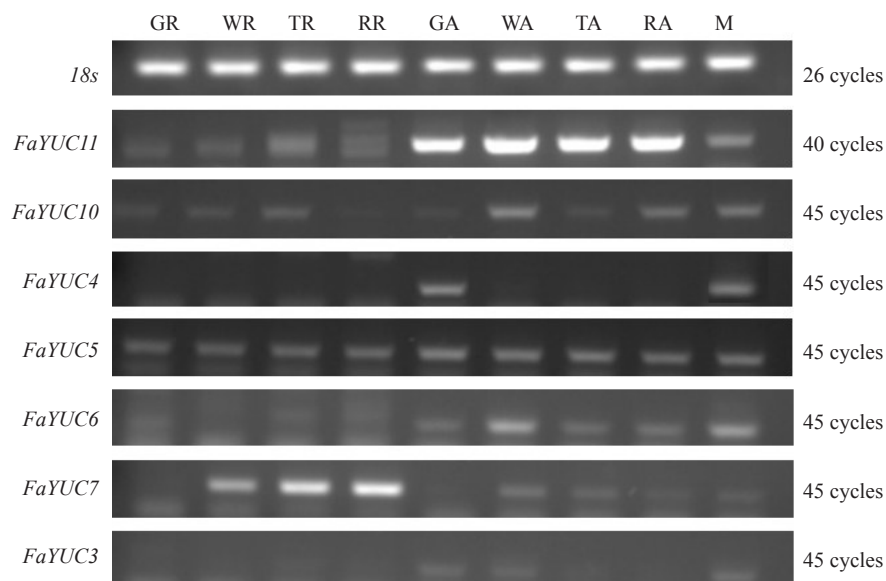
At. 拟南芥; Fa. 栽培草莓; Fv. 森林草莓。
At. *Arabidopsis thaliana*; Fa. *Fragaria x ananassa* Duch.; Fv. *Fragaria vesca* L..

图1 植物 YUCCA 基因进化树
Fig. 1 Phylogenetic tree of plant YUCCA

检测到。*FaYUC5*在果实的各个阶段,无论是果肉还是种子中的表达都非常稳定;*FaYUC6*在花托和种子中有一定的表达,其中在花托和白果种子中表达稍强,在果肉中基本不表达;*FaYUC7*主要是在果肉中表达,且随着果实的生长发育表达逐渐增强,在成熟果果肉中达到高峰,而在各阶段的种子中表达均较弱;*FaYUC10*在果肉和种子中都有一定的表达,但在白果种子中相对较强;*FaYUC11*主要集中在种子中表达,而且在种子发育的各个阶段,相对于YUC家族其他基因,其表达量都很高,推测其可能与生长素的合成及果实生长发育调控有关。

2.4 VIGS载体的构建

以含有全长目的基因的质粒为模版,利用干涉引物进行PCR扩增,获得了253 bp的基因片段,利用体外重组法分2步将扩增获得的*FaYUC11*基因的253 bp片段克隆进pBSK-in载体的内含子两端,形成了带有2个*FaYUC11*基因片段的重组质粒pBSK-in-d*YUC11*,其中2个YUC11基因片段方向相反,且中间被1个内含子结构隔开。进一步用*Kpn* I和*Sac* I双酶切pBSK-in-d*YUC11*重组质粒,将*FaYUC11*基因片段的双向发夹结构克隆至烟草脆裂病毒pTRV2的2×35S启动子后病毒CP蛋白的下游,获得pTRV2-d*YUC11*重组基因沉默载体(图3)。



GR. 绿果果肉; WR. 白果果肉; TR. 转色期果果肉; RR. 红果果肉; GA. 绿果种子; WA. 白果种子; TA. 转色期果种子; RA. 红果种子; M. 成熟花花托(不含种子)。

GR. Green fruit pulp; WR. White fruit pulp; TR. White and red fruit pulp; RR. Red fruit pulp; GA. Green fruit seeds; WA. White fruit seeds; TA. White and red fruit seeds; RA. Red fruit seeds; M. Receptacle of mature flower (without seeds).

图2 YUCCA基因在草莓各时期果实中的半定量RT-PCR表达分析
Fig. 2 Semi-quantitative RT-PCR analysis of YUCCA transcript level in different fruit stages from strawberry

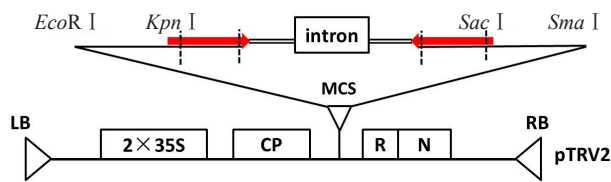
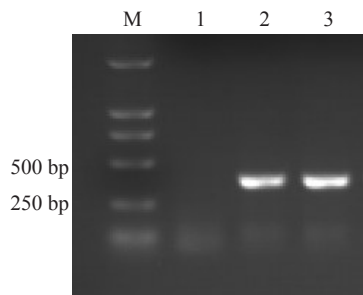


图3 pTRV2-dYUC11重组载体的构建

Fig. 3 The construction of pTRV2-dYUC11 recombinant

2.5 VIGS草莓的鉴定

为了检测烟草脆裂病毒是否成功侵染草莓,分别提取野生型和注射病毒原始载体以及重组 *FaYUC11* 的 VIGS 载体的草莓果实总 RNA, 利用特异引物进行 RT-PCR 扩增, 对扩增产物进行电泳检测。结果发现, 病毒原始载体和 *FaYUC11* 的重组病毒载体侵染的草莓果实均能扩增出病毒特异条带, 而野生型草莓中无此目的条带(图4)。从而确定, 通过微注射法, 烟草脆裂病毒成功侵染了草莓果实。



M. DL2000 Marker; 1. 野生型对照久香果实; 2. 不含目标基因片段的 TRV 农杆菌注射的久香果实; 3. *RiYUC11* 目标基因沉默载体农杆菌注射的久香果实。

M. DL2000 Marker; 1. Wild type fruit; 2. The fruit with treatment of pTRV2 empty vector; 3. The fruit with treatment of *RiYUC11* infection.

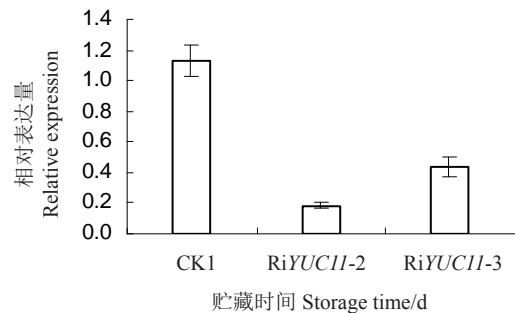
图4 草莓果实中烟草脆裂病毒的检测

Fig. 4 The detection of TRV in strawberry fruits

2.6 qRT-PCR检测VIGS效果

为了进一步检测病毒诱导基因沉默的效果, 设计 *FaYUC11* 特异引物对, 正向引物 *FaYUC11*-RT-F 和反向引物 *FaYUC11*-RT-R(表1), 通过实时荧光定

量 RT-PCR 技术在分子水平上检测果实中 *FaYUC11* 基因的沉默效果。结果表明, 以不作任何处理的果实中 *FaYUC11* 的表达量为 1, 计算得到 pTRV2 空载体处理的果实表达量为 1.13(CK1), 而基因沉默果实中 *FaYUC11* 的表达量分别为 0.18 和 0.44(图5), 说明相比于不做任何处理或者注射含原始病毒质粒农杆菌的果实, 微注射含 *FaYUC11* 重组的病毒诱导沉默质粒农杆菌的果实中 *FaYUC11* 基因转录产物积累水平发生了不同程度的下降。



CK1. pTRV2 空载体处理的对照组; *RiYUC11*-2、*RiYUC11*-3. pTRV2-*YUC11* 侵染处理。下同。

CK1. The control group with treatment of pTRV2 empty vector; *RiYUC11*-2, *RiYUC11*-3. The fruits with pTRV2-*YUC11* infection. The same below.

图5 草莓果实 *FaYUC11* 基因的 qRT-PCR 分析Fig. 5 Quantitative RT-PCR analysis of the *FaYUC11* gene in strawberry fruits

2.7 VIGS表型分析

农杆菌注射 34 d 后, 比较 CK1(不含目标基因插入的病毒空载体转化) 和 2 个病毒诱导基因沉默株系经处理后果实形态的变化。发现含病毒的农杆菌注射后, 果实顶部有轻微凹陷, 但不影响果实膨大和基本形态。而注射重组病毒载体农杆菌的草莓果实膨大明显受到抑制, 其中部分果实膨大所受的抑制比较轻微(*RiYUC11*-3), 而部分果实则受到严重抑制(*RiYUC11*-2), 经过 1 个月的生长几乎没有变化, 仍然处于小绿果阶段(图6)。

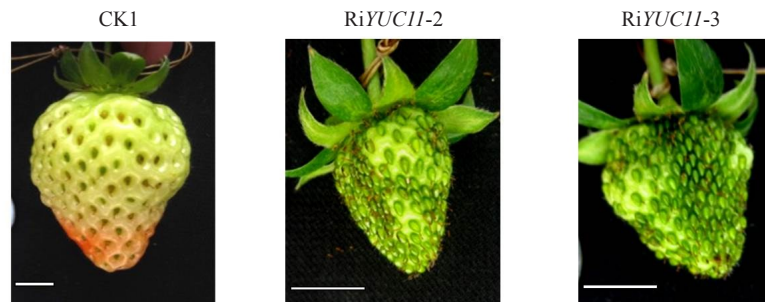


图6 病毒载体 pTRV2-YUC11 侵染后草莓果实 34 d 后表型的变化

Fig. 6 Phenotypic changes of strawberry fruits after 34 days post infection by pTRV2-*YUC11*

进一步分析不同处理果实的纵横径增长率变化发现,与处理前相比,重组*FaYUC11*的病毒诱导沉默载体注射的草莓果实比原始病毒载体注射果实的纵横径增长率低(图7)。说明*FaYUC11*基因可能调控了草莓果实纵横径的增长及果实的正常膨大。

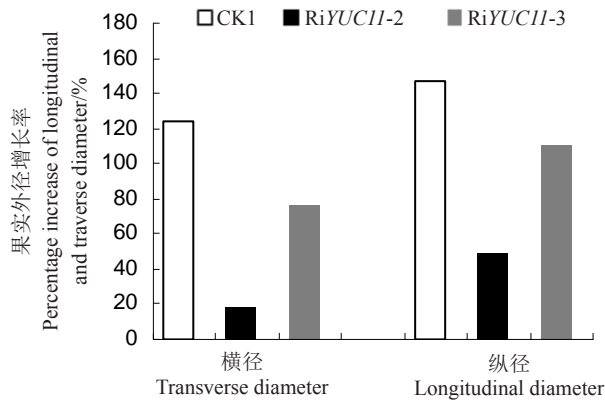


图7 草莓果实纵横径增长

Fig. 7 Increase of longitudinal and transverse diameter of strawberry fruits

为了明确*FaYUC11*基因沉默后是否对草莓果实中生长素含量积累产生影响,运用GC-MS法分别测定对照与病毒诱导基因沉默的草莓果实种子(瘦果)中的IAA含量,结果表明,与只用pTRV2空载体处理的对照组相比,病毒诱导*FaYUC11*沉默的草莓果实不仅膨大受到抑制,生长素含量也明显下降(图8)。说明*FaYUC11*基因对于草莓果实中IAA的积累有重要的作用。

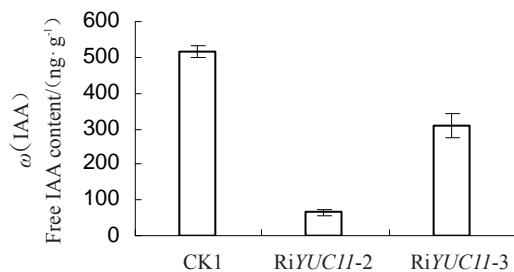


图8 病毒载体 pTRV2-YUC11 侵染后草莓果实中游离 IAA 含量的变化

Fig. 8 Changes of free IAA content in strawberry fruits after infection by pTRV2-YUC11

3 讨论

在多种植物中,YUC作为依赖于色氨酸途径的生长素合成限速步骤关键酶^[13],在TAA/YUC途径的生长素合成过程中发挥着重要作用。Koch等^[28]研

究发现,拟南芥体内YUCCA基因的过表达导致生长素含量的上升并伴随明显的形态学表型。本研究结果表明,与对照相比,*FaYUC11*基因沉默后导致果实中游离IAA含量的下降,且在形态学上与正常果实有明显差异,从相反的方向证实了YUC基因对生长素合成的重要性。

Liu等^[4]对草莓生长素合成基因*FvYUC6*的研究发现,通过RNA干涉抑制*FvYUC6*基因表达,植株的营养生长受到抑制且产量下降。笔者发现,注射基因沉默载体后的草莓果实膨大受到抑制,与对照相比发育明显变缓,这与Liu等^[4]的研究结果相似。Yamamoto等^[17]对水稻的研究发现,通过抑制*OsYUCCA1*基因的表达能够抑制水稻根纵横径的增长,本研究中*FaYUC11*基因的沉默表现为果实纵横径的增长受阻,该基因沉默后果实大小明显比对照小。Cheng等^[29]以拟南芥为试材,发现*YUC1*、*YUC4*、*YUC10*、*YUC11*基因的同时缺失导致子叶下胚轴的消失且影响根的分生,说明YUC家族基因在植物体内分布广泛并且分别影响不同组织的分化和发育。但并不是对YUC家族所有基因的单独抑制都会导致生长素含量的下降^[13],这意味着该家族内其他基因起到了一定的补偿作用。

4 结论

利用VIGS技术对草莓*FaYUC11*基因功能进行验证。通过对病毒诱导基因沉默后果实的表型分析发现,处理34 d后,VIGS株系果实与对照相比,果实纵横径增长显著下降,说明果实的膨大受到抑制。病毒诱导基因沉默后IAA的积累受到抑制。*FaYUC11*基因与草莓果实的生长发育密切相关,是草莓生长素合成途径中的关键基因,该基因通过调控生长素的合成继而调控果实的膨大。

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