

杧果 *MiOFP1* 基因的表达与功能分析

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摘要:【目的】卵形家族蛋白(ovate family proteins, OFPs)在植物生长发育及逆境响应过程中扮演重要角色。前期通过杧果成花基因酵母文库筛选, 获得了一个 *MiOFP1* 基因, 为明确其功能, 对 *MiOFP1* 的表达模式和转基因功能开展了研究。【方法】在本研究中分析了杧果 *MiOFP1* 的启动子序列; 通过实时荧光定量PCR技术分析 *MiOFP1* 在杧果不同组织器官和不同生长发育期叶片中的表达模式; 转化构建好的超量表达载体并侵染拟南芥研究 *MiOFP1* 的功能。【结果】四季蜜杧 *MiOFP1* 启动子包含激素响应元件: ABA 响应元件、GA 响应元件、SA 响应元件和乙烯响应元件, 逆境响应元件: 盐响应元件、脱水响应元件、MYC 转录因子和 MYB 转录因子结合位点。组织特异性表达分析显示, *MiOFP1* 在各组织器官中均有表达, 且在童期实生树和成年期嫁接树的茎中表达量最高, 在成熟果实中表达量最低; 嫁接树不同成花发育时期表达分析结果显示, *MiOFP1* 在营养生长期的叶中表达量最高, 在成花诱导期和花发育期表达水平较低。转基因功能研究显示, 超量表达 *MiOFP1* 的拟南芥出现晚花表型, 抽薹期叶片中成花抑制基因 *FLOWERING LOCUS C(FLC)* 的表达水平显著上调, 而成花促进基因 *FLOWERING LOCUS T(FT)* 的表达水平显著下调。逆境胁迫处理显示, ABA 处理显著抑制拟南芥种子的萌发与根的伸长, 但通过转基因显著提高了拟南芥种子的萌发率, 降低了拟南芥根长对 ABA 的敏感性。进一步分析显示, *MiOFP1* 显著提高了拟南芥在 ABA 处理后的脯氨酸含量和过氧化物酶活性, 上调了 ABA 代谢相关基因的表达水平。【结论】明确了杧果 *MiOFP1* 抑制成花, 且降低了转基因植株对 ABA 的敏感性, 为进一步探索杧果 *MiOFP1* 参与杧果成花和逆境胁迫应答的分子机制奠定基础。

关键词: 杧果; *MiOFP1*; 表达模式; 功能分析; ABA 处理

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Expression and functional analysis of *MiOFP1* gene in mango

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Abstract: 【Objective】Ovate Family Proteins (OFPs) play roles in the growth and development, hormone response and stress response of plants. In our previous study, the *MiOFP1* gene was obtained from a screen mate and plate library of mango flowering genes. However, the information and functional characteristics of the *MiOFP1* gene in mango were limited. To clarify the function of the *MiOFP1* gene, its expression pattern and transgenic function were studied. The exploration of the *MiOFP1* gene would provide a reference for further exploring the molecular mechanism of the *MiOFP1* gene in mango flowering and resistance to adversity. 【Methods】To learn more about the expression and regulation characteristics of the *MiOFP1* gene, the promoter sequences in the 2000 bp region upstream of the coding region were analysed, and *cis*-acting elements of the promoter were analysed by NEW PLACE and illustrated via TBtools. The expression of the *MiOFP1* in different tissues and different growth stages was verified and analysed by quantitative real-time PCR (qRT-PCR). The transgenic function of the

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MiOFPI was investigated by overexpression of it in *Arabidopsis thaliana*. The phenotypes of homozygous plants of the T3 generation were observed, and the expression of the *FLOWERING LOCUS T (FT)* and *FLOWERING LOUS C (FLC)* in *Arabidopsis thaliana* was determined. The seeds of the T3 generation and seedlings of the T4 generation were treated with ABA, the germination rate and root length were observed and photographed, the physiological indexes, such as proline (Pro) content and peroxidase (POD) activity, and the expression of ABA-related genes, such as *ABA-INSENSITIVE1 (ABII)*, *MOTHER OF FT AND TFL (MFT)*, *DESICCATION-RESPONSIVE PROTEIN 29B (RD29B)* and *Nine-cis-epoxycarotenoid dioxygenase 3 (NCED3)*, were determined. All data were analysed with IBM SPSS Statistics Version 22 (SPSS, Inc., Chicago, IL, USA), and the statistical validity was analysed by one-way ANOVA. Duncan's multiple range test was applied. **【Results】** The promoter sequences of the *MiOFPI* contain many hormone response elements, such as abscisic acid (ABA), gibberellin (GA), salicylic acid (SA) and ethylene response elements, and stress response elements, such as Slat, Dehydration, MYB and MYC binding sites. The results of tissue-specific expression analysis showed that the *MiOFPI* gene was expressed in all tissues and organs in different development stages of mango, with the highest expression in the juvenile and adult stems and the lowest expression in the ripe fruit. The temporal expression pattern analysis showed that the *MiOFPI* was highest in the vegetative growth stage but lower in the flower induction stage and flower development stage and gradually increased in the leaves during the fruit developmental stage. The transgenic *Arabidopsis* overexpression of the *MiOFPI* gene showed that the bolting time and flowering time of three overexpressing lines were 2–4 days later than those of WT. The detection of the expression level of endogenous flowering genes in *Arabidopsis* showed that the *MiOFPI* significantly increased the expression level of the flowering inhibiting gene *AtFLC* in transgenic *Arabidopsis* but significantly decreased the expression level of the flowering promoting gene *AtFT*. The ectopic expression of the *MiOFPI* in transgenic plants resulted in higher tolerance to ABA. More specifically, the transgenic plants showed high ABA level tolerance manifested as increased seed germination and seedling growth. In the medium without ABA, the transgenic seeds germinated slightly faster than WT seeds; on the medium supplemented with ABA, not only containing $2 \mu\text{mol} \cdot \text{L}^{-1}$ ABA but also containing $5 \mu\text{mol} \cdot \text{L}^{-1}$ ABA, the germination of all seeds was inhibited, and the germination of transgenic seeds was significantly faster than that of WT seeds. For root length, no significant difference was observed in growth phenotype or root length between the transgenic *Arabidopsis* overexpression of the *MiOFPI* gene and the WT plants under normal growth; however, the transgenic plants showed significantly greater root elongation than WT plants under $5 \mu\text{mol} \cdot \text{L}^{-1}$ ABA and $10 \mu\text{mol} \cdot \text{L}^{-1}$ ABA conditions. These results suggested that heterologous overexpression of the *MiOFPI* could promote root elongation in *Arabidopsis*. Furthermore, under $20 \mu\text{mol} \cdot \text{L}^{-1}$ ABA stress, the Pro content and POD activity were increased, and the transcript levels of the several stress-responsive genes (*AtABII*, *AtMFT*, *AtRD29B* and *NCED3*) in the transgenic lines were significantly upregulated compared with those in wild-type plants. In addition, the ectopic expression of the *MiOFPI* in *Arabidopsis* resulted in insensitivity to ABA. **【Conclusion】** Promoter sequence analysis showed that the *MiOFPI* promoters contained multiple hormone and stress *cis*-regulatory elements. Tissue expression analysis showed that the *MiOFPI* was mainly expressed in juvenile and adult stems. The temporal expression analysis showed that the *MiOFPI* was highly expressed in the vegetative growth stage. Furthermore, the overexpression of the *MiOFPI* had a significant impact on the bolting time and flowering time. On the other hand, compared with those in WT plants, the overexpression of the *MiOFPI* would improve root length, survival rate, Pro content, POD activity, and the expression of the ABA-related

genes under ABA conditions. We speculate that it is the key gene that affects mango flowering correlation and participates in the ABA response.

Key words: Mango; *MiOFP1*; Expression pattern; Function research; ABA

卵形家族蛋白(ovate family proteins, OFPs)是具有保守OVATE结构域的转录因子,为植物所特有,因番茄果实形态由卵形变为梨形而发现^[1]。研究发现,*OFPs*参与植物次生细胞壁形成,水稻维管束发育,拟南芥胚珠发育,辣椒和番茄果实的形状、香蕉果实的成熟及品质形成等过程^[2];在拟南芥中过表达*AtOFP1*发现花粉活力受到影响,植物发育迟缓^[3];此外,*OFPs*参与植物逆境抵御和赤霉素(gibberellin, GA)、脱落酸(abscisic acid, ABA)、乙烯(ethylene)及油菜素内酯(brassinolide, BR)等激素的信号传递^[4]; *OsOFP22*通过抑制GA和BR信号传导,调节水稻形态发育^[5];外源施加GA₃抑制*SIOFP20*的表达,低温、干旱、盐胁迫和外源施加吲哚-3-乙酸(indole-3-acetic acid, IAA)和ABA能促进*SIOFP20*的表达^[6]。ABA是一种在植物生长发育和各种非生物胁迫耐受过程中起重要调节作用的激素,参与调节种子成熟、种子发芽、幼苗生长和气孔运动等^[7],外源施加ABA可以有效缓解植物受到的冷害、盐胁迫和干旱胁迫等^[8-10]。

杧果(*Mangifera indica* L.)是漆树科杧果属的热带水果,是世界五大热带水果之一,适时开花和对非生物胁迫的抵御能力影响杧果的产量和品质。*OFPs*参与植物的成花调控且与逆境胁迫应答有关^[11],目前杧果尚无*OFP*基因的研究报道,在前期研究中,笔者从杧果成花酵母文库中筛选获得了一个*OFP*基因,命名为*MiOFP1*。笔者在本研究中对杧果*MiOFP1*基因的表达模式和转基因功能进行研究,为揭示*MiOFP1*参与杧果成花和杧果逆境应答的分子机制提供参考。

1 材料和方法

1.1 植物材料及取样

供试杧果品种为四季蜜杧,栽培于广西大学农学院果树标本园,拟南芥(*Arabidopsis thaliana*)为哥伦比亚野生型,种子由广西大学农学院杧果课题组保存。组织表达特性样品采集:2022-02-15采集6株长势一致的童期实生杧果树(3年生)冬梢的成熟叶、芽和茎(韧皮部组织),6株长势一致的成年期嫁

接杧果树(12年生)冬梢的成熟叶、茎(韧皮部组织)、花,以及盛花后20 d和100 d的植物果实胚和果肉。时间表达特性样品为6株长势一致的嫁接杧果树的成熟叶,样品采集时间为2021年9月至2022年5月。采样时间统一定为17:00—18:00,样品采集后立即处理,放入-80 °C冰箱冷冻保存备用。

1.2 试验方法

1.2.1 *MiOFP1* 启动子序列分析 利用NEW PLACE (<https://www.dna.affrc.go.jp/PLACE/?action=newplace>)在线软件预测四季蜜杧*MiOFP1*启动子区域顺式作用元件,利用TBtools绘制启动子顺式作用元件位置信息图。

1.2.2 *MiOFP1* 组织特性和时间表达特性分析 提取杧果组织表达特性样品和时间表达特性样品的RNA,逆转录为cDNA待用,根据四季蜜杧*MiOFP1*基因序列设计荧光定量PCR引物qOFP1 F(5' CTG-TACCTGCCGTGCTACAA 3')、qOFP1 R(5' CTG-TACCTGCCGTGCTACAA 3')。以杧果*MiActin1*为内参基因,引物为qActin F(5' CCGAGACATGAAGGAGAAGC 3')、qActin R(5' GTGGTCTCATGGATACGAGCA 3')。实时荧光定量PCR仪器为ABI7500。扩增反应体系和程序参照试剂盒SYBR Premix Dimer Eraser(TaKaRa)说明书进行^[12]。

1.2.3 拟南芥转化与成花表型分析 实验室前期已构建好四季蜜杧*MiOFP1*的超量表达载体,命名为pBI121-MiOFP1载体,转化EHA105感受态。通过花序侵染法转化哥伦比亚野生型拟南芥^[13]。利用抗生素筛选和PCR技术鉴定阳性植株。以T3代纯合植株为试验材料进行相关试验。成花表型观察:利用半定量技术鉴定杧果*MiOFP1*在转基因拟南芥中的表达水平,并观察转基因拟南芥的抽薹时间和第一朵花开放的时间。通过荧光定量技术,采集抽薹期拟南芥叶片,检测内源成花基因的表达水平,以拟南芥*AtActin2*为内参基因,引物为AtActin2 F(5'-GCAGAGCGGGAAATTGTAAG-3')、AtActin2 R(5'-GGATATCAGGAAGGATCTGTAC-3'),AtFLC F(5'-ATCATCATGTGGGAGCAGAAG-3')、AtFLC R(5'-TTCAACCGCCGATTTAAGG-3'),AtFT F

(5'-CTTGGCAGGCAAACAGTGTATGCAC-3')、AtFT R (5'-GCCACTCTCCCTCTGACAATTGTA-GA-3'),检测方法参考课题组研究报道^[44]。

1.2.4 转基因拟南芥响应植物激素 ABA 试验 萌发率测定:将转基因拟南芥 T3 代纯合株系种子播种于含 0、2、5 $\mu\text{mol}\cdot\text{L}^{-1}$ ABA 的 1/2 MS 固体培养基上,每个处理 3 次重复,以胚根伸出为萌发依据,每 12 h 记录一次萌发率,10 d 后拍照记录。**根长测定:**将 T3 代纯合株系种子播种于 1/2 MS 固体培养基上培养,5 d 后移至含 0、5、10 $\mu\text{mol}\cdot\text{L}^{-1}$ ABA 的 1/2 MS 固体培养基上继续培养,每个处理 3 次重复,移栽 5 d 后拍照并统计根长。**生理指标测定:**播种 9 d 后移栽幼苗到穴盆中,培养 12 d 后进行 20 $\mu\text{mol}\cdot\text{L}^{-1}$ ABA 喷施处理,以清水处理为对照,处理 1 d 后采集拟南芥叶片,每个处理 3 次重复,采用试剂盒检测脯氨酸含量和过氧化物酶活性(北京索莱宝科技有限公司);提取 ABA 处理组及对照组拟南芥叶片的 RNA,逆转录后通过荧光定量技术检测参与拟南芥 ABA 响应的内源基因表达量,引物为 AtABI1 F (5'-

GTTTTCCCGTCTCACATCTTCGT-3')、AtABI1 R (5'-CTTCATCCGTCA TTACATCCCAA-3'), At-MFT F (5'-CGAGCCGAACATGAGAGAAT-3')、At-MFT R (5'-AAGTATCTCTTTTCTCTTGAGGG-3')、AtDREB2B F (5'-CATCAGAGCCAAGAC-CAAACC-3')、AtDREB2B R (5'-TGTAGGAC-CATTGCCTCAGAAC-3')^[45]。

1.3 数据分析

将所得数据采用 Excel 进行数据处理及图片制作,利用 IBM SPSS 22.0 软件进行方差分析。

2 结果与分析

2.1 *MiOFPI* 基因启动子序列分析

对四季蜜杧 *MiOFPI* 的启动子序列(2000 bp)进行顺式作用元件预测,发现 *MiOFPI* 启动子中存在的激素响应元件有:1 个乙烯响应元件,5 个 ABA 响应元件,7 个 SA 响应元件,9 个 GA 响应元件;逆境响应元件有:1 个盐响应元件,4 个脱水响应元件,9 个 MYC 转录因子和 10 个 MYB 转录因子结合位点(图 1)。

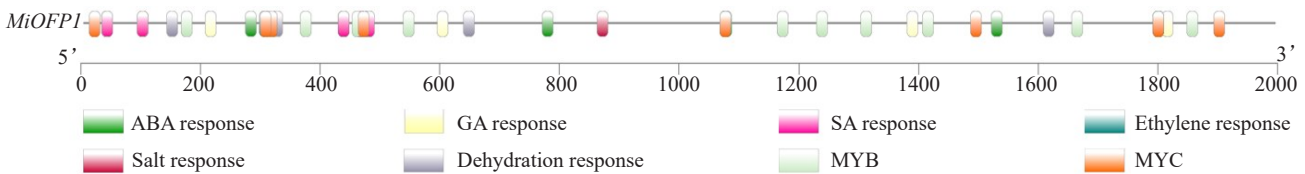


图 1 四季蜜杧 *MiOFPI* 启动子主要顺式作用元件

Fig. 1 Cis-elements analysis of *MiOFPI* promoter in SiJiMi mango

2.2 *MiOFPI* 表达模式分析

对 *MiOFPI* 在杧果不同生长发育周期的不同组织器官中的表达模式进行实时荧光定量分析。结果如图 2-A 所示, *MiOFPI* 存在组织表达差异性。 *MiOFPI* 在童期树组织中的表达水平高于成年期树。 *MiOFPI* 在茎的韧皮部中表达水平较高,在叶、花和幼果中表达水平最低,在成熟果实中表达水平最低。不同成花发育时期叶片中的表达模式分析显示, *MiOFPI* 在营养生长期的叶片中表达水平较高,在成花诱导期和花发育期的叶片中表达水平较低,而在果实发育期的叶片中表达水平逐步上升(图 2-B)。

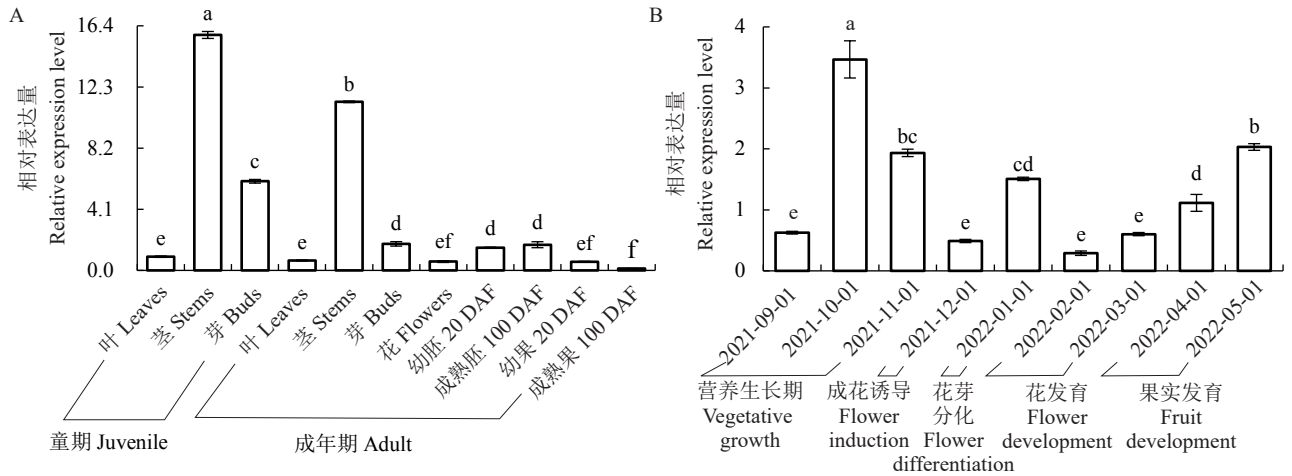
2.3 拟南芥转化及成花表型分析

将包含 pBI121-*MiOFPI* 超量表达载体和空载体的农杆菌通过花序感染法分别转化模式植物拟南芥。半定量 PCR 检测结果显示,转基因株系

MiOFPI#7、*MiOFPI*#8 和 *MiOFPI*#9 中 *MiOFPI* 正常表达,而在对照植株中没有表达(图 3-B)。超量表达 *MiOFPI* 转基因株系表现出晚花表型,其抽薹时间和第一朵花开放时间均比 WT 和转空载体植株(pBI121)晚 2~4 d(图 3-A、C)。转基因拟南芥内源成花基因表达水平检测显示,超量表达 *MiOFPI* 显著提高了拟南芥 *AtFLC* 的表达水平,显著下调了 *AtFT* 的表达水平(图 3-D)。

2.4 外源 ABA 处理对 *MiOFPI* 转基因拟南芥的影响

2.4.1 外源 ABA 处理后 *MiOFPI* 转基因拟南芥种子萌发表型分析 T3 代纯合拟南芥种子在 0、2、5 $\mu\text{mol}\cdot\text{L}^{-1}$ ABA 的 1/2 MS 固体培养基上培养,记录发芽情况。ABA 处理 10 d 后拍照记录如图 4-A 所示,在未添加 ABA 的培养基上,拟南芥幼苗长势基

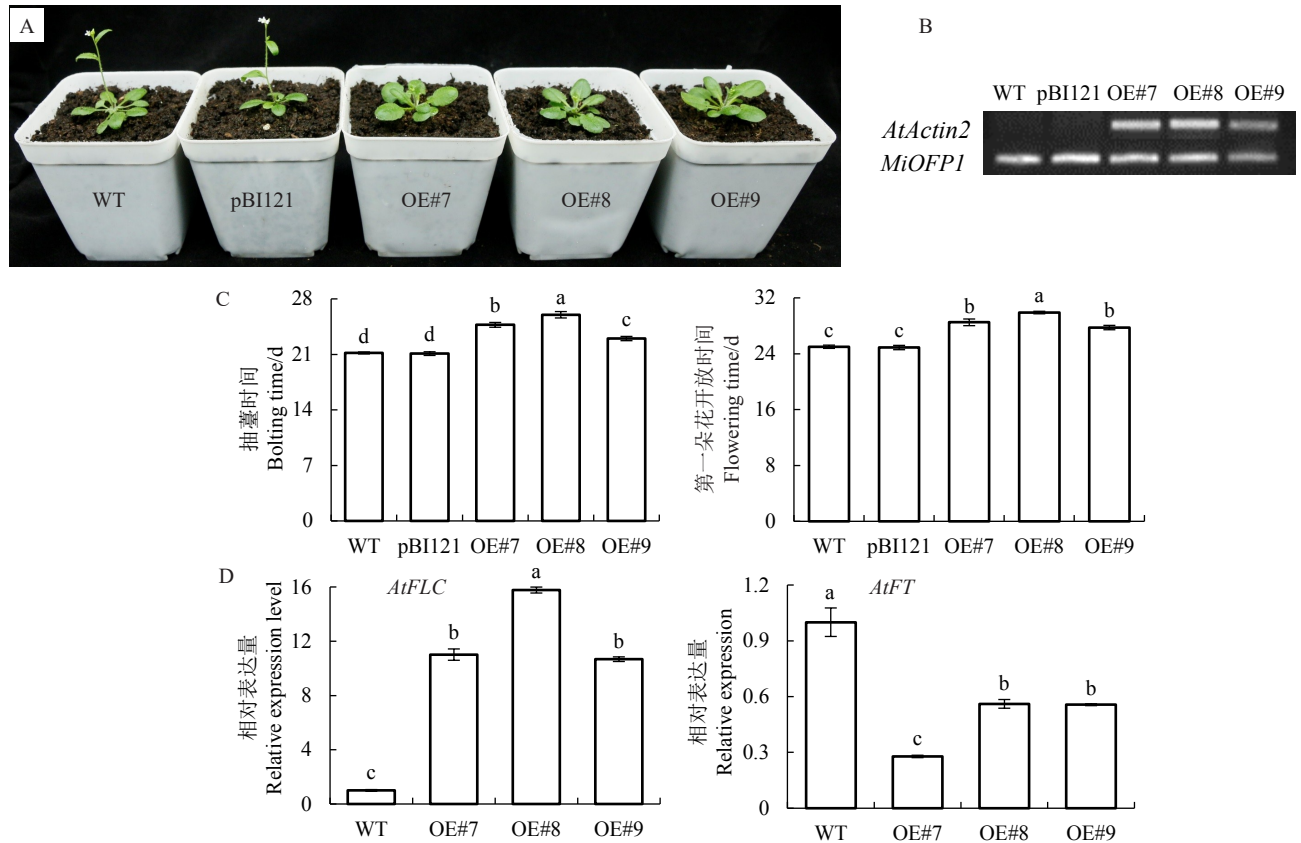


A. 四季蜜杧童期和成年期不同组织中 *MiOFP1* 基因表达模式; B. 四季蜜杧不同发育时期叶片中 *MiOFP1* 基因表达模式。字母表示差异显著水平 ($p < 0.05$)。

A. Expression pattern of *MiOFP1* in different tissues of SiJiMi; B. Expression pattern of *MiOFP1* in leaf of SiJiMi at different developmental stages. Letters indicate the level of significant difference ($p < 0.05$).

图2 四季蜜杧 *MiOFP1* 基因的表达模式分析

Fig. 2 Expression pattern analysis of *MiOFP1* gene of SiJiMi



A. 过量表达 *MiOFP1* 转基因拟南芥植株表型; B. 半定量检测外源基因 *MiOFP1* 在转基因和对照植物叶片中的表达; C. 过量表达 *MiOFP1* 转基因拟南芥抽薹时间和开花时间; D. 拟南芥叶片中内源 *AtFLC* 和 *AtFT* 基因表达水平检测。字母表示差异显著水平 ($p < 0.05$)。

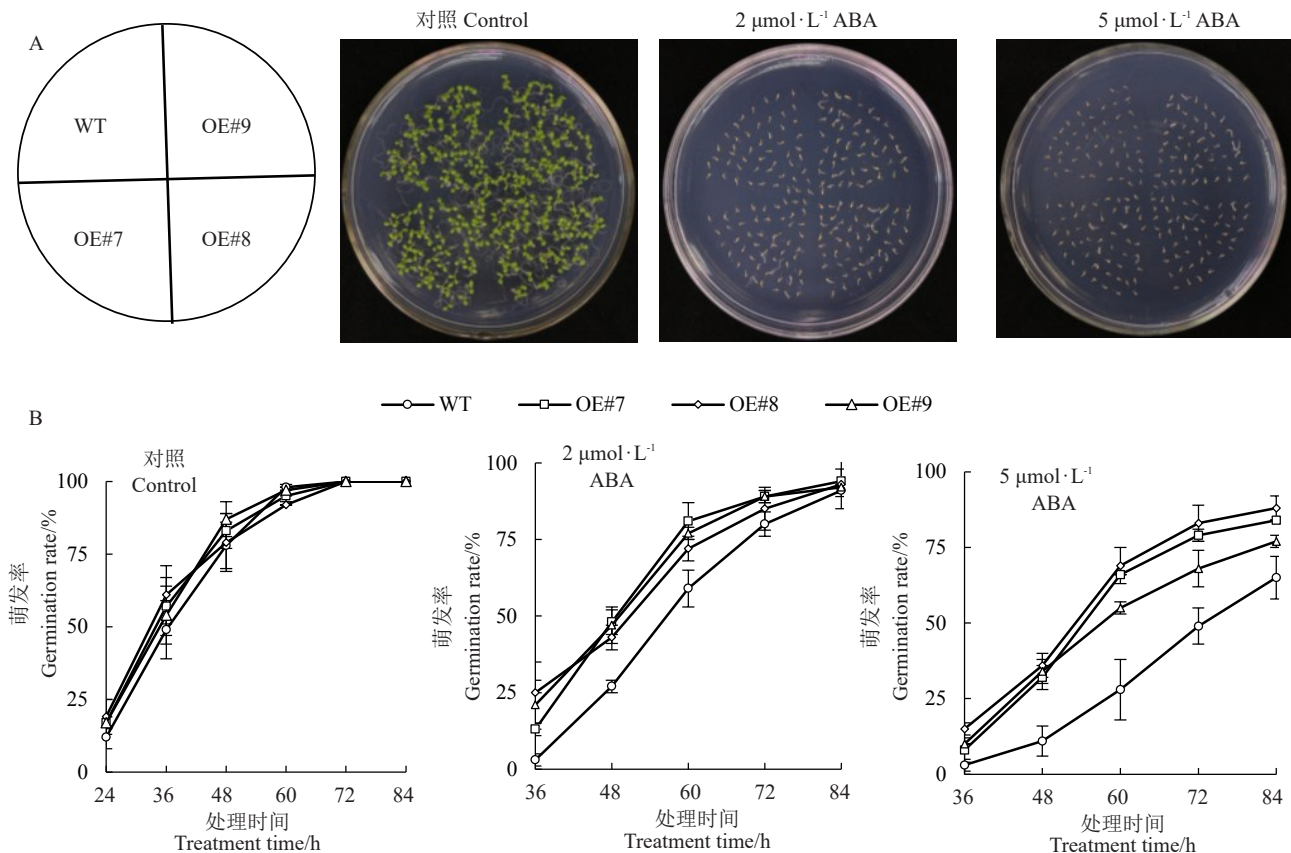
A. Overexpression of *MiOFP1* transgenic *Arabidopsis* plant phenotypes; B. The expression of exogenous genes *MiOFP1* verified by semi-quantitative PCR in the leaf of transgenic plant; C. The bolting time and flowering time of *MiOFP1* transgenic *Arabidopsis*; D. The expression of endogenous genes about *AtFLC* and *AtFT* were verified by quantitative RT-PCR in the leaf of transgenic plant. Letters indicate the level of significant difference ($p < 0.05$).

图3 *MiOFP1* 转基因对拟南芥成花的影响

Fig. 3 Effect of *MiOFP1* transgene on flowering of *Arabidopsis thaliana*

本一致;在添加ABA的培养基中,所有种子发芽均受抑制,种子在含 $5\ \mu\text{mol}\cdot\text{L}^{-1}$ ABA的1/2 MS固体培养基上的长势较在含 $2\ \mu\text{mol}\cdot\text{L}^{-1}$ ABA的1/2 MS固体培养基上更弱。萌发率统计分析显示(图4-B),未添加ABA时,前60 h转基因拟南芥种子的萌发率略高于WT种子,但差异不显著;在 $2\ \mu\text{mol}\cdot\text{L}^{-1}$ ABA处

理时,所有种子萌发均受到抑制,转基因拟南芥种子萌发速度明显快于WT种子;在 $5\ \mu\text{mol}\cdot\text{L}^{-1}$ ABA处理时,种子萌发受到的抑制更强,转基因拟南芥种子的萌发率极显著高于对照植株,最终的转基因拟南芥种子发芽率分别为84%、88%、77%,WT种子发芽率为65%。



A. 超量表达 *MiOFPI* 的转基因拟南芥种子在 0、2、5 $\mu\text{mol}\cdot\text{L}^{-1}$ ABA 处理下的萌发类型;B. 超量表达 *MiOFPI* 的转基因拟南芥种子在 0、2、5 $\mu\text{mol}\cdot\text{L}^{-1}$ ABA 处理下的萌发率统计数据。

A. The germination phenotype of transgenic *Arabidopsis* seeds overexpressing *MiOFPI* under ABA treatment; B. The germination rate of transgenic *Arabidopsis* seeds overexpressing *MiOFPI* under ABA treatment.

图 4 ABA 处理对 *MiOFPI* 转基因拟南芥种子萌发率的影响

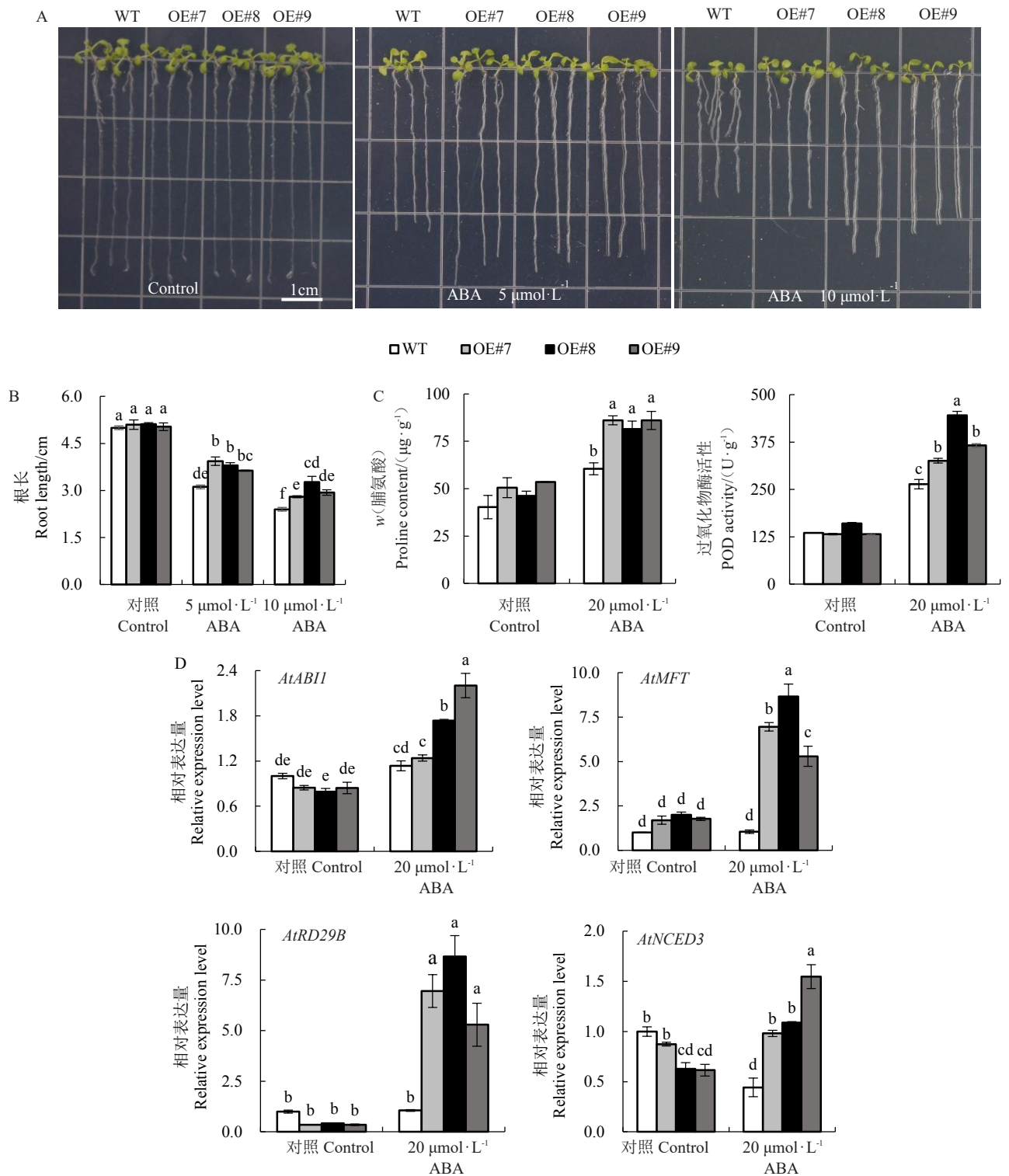
Fig. 4 Effect of ABA treatment on seed germination rate of *MiOFPI* transgenic *Arabidopsis thaliana*

2.4.2 外源 ABA 处理对超量表达 *MiOFPI* 拟南芥根长、生理指标与内源基因表达的影响 不同浓度的 ABA 处理对 T3 代纯合拟南芥幼苗根长的影响如图 5-A,在未施加 ABA 时,转基因拟南芥与对照拟南芥的根长无显著差异,随着 ABA 浓度的增加,转基因拟南芥与对照拟南芥幼苗的根长均表现为受抑制,但在相同处理下,对照植株根长显著短于转基因植株的根长(图 5-B)。

对转基因植株与对照 WT 植株进行 $20\ \mu\text{mol}\cdot\text{L}^{-1}$ ABA 喷施处理,以清水喷施处理为对照,处理后 1 d

采集叶片为样品,对处理组与对照组植株进行生理指标测定和拟南芥内源基因表达水平检测。如图 5-C 所示,清水对照处理下,转基因植株与 WT 植株的脯氨酸含量和过氧化物酶活性无明显差异,但在 ABA 处理后,转基因植株及 WT 植株的脯氨酸含量均有增加,且转基因植株脯氨酸含量显著高于 WT 植株,是 WT 植株的 1.4 倍;在 ABA 处理后,转基因植株的过氧化物酶活性显著高于对照 WT 植株。

内源基因检测显示,清水处理时,*MiOFPI* 转基因株系中 *AtABI1*、*AtRD29B* 和 *AtNCED3* 的表达水平



A. WT 和 OE 植株在 0、5、10 $\mu\text{mol}\cdot\text{L}^{-1}$ ABA 处理下的根长表型; B. WT 和 OE 植株在 0、5、10 $\mu\text{mol}\cdot\text{L}^{-1}$ ABA 处理下的根长统计数据; C. WT 和 OE 植株经 0、20 $\mu\text{mol}\cdot\text{L}^{-1}$ ABA 喷施处理后的脯氨酸含量及过氧化物酶活性; D. WT 和 OE 植株经 0、20 $\mu\text{mol}\cdot\text{L}^{-1}$ ABA 喷施处理后的内源基因 *AtABI1*、*AtMFT*、*AtRD29B* 和 *AtNCED3* 表达水平。字母表示差异显著水平 ($p < 0.05$)。

A. The root length phenotype of transgenic *Arabidopsis* overexpressing *MiOFP1* under ABA treatment; B. The root length of transgenic *Arabidopsis* seeds overexpressing *MiOFP1* under ABA treatment; C. The proline content and POD activity of *MiOFP1* overexpression *Arabidopsis*; D. Expression levels of the ABA stress-related genes *AtABI1*, *AtMFT*, *AtRD29B* and *AtNCED3* in 30 d old WT and transgenic plants. Letters indicate the level of significant difference ($p < 0.05$).

图 5 ABA 处理对 *MiOFP1* 转基因拟南芥的影响

Fig. 5 Effect of ABA treatment on *MiOFP1* transgenic *Arabidopsis thaliana*

略低于 WT 株系,而 *AtMFT* 的表达水平略高于 WT 植株。在 ABA 处理后,上述 4 个基因的表达水平均显著高于 WT 植株(图 5-D)。

3 讨 论

启动子顺式作用元件关系着植物对激素、低温、盐和干旱等非生物胁迫作出反应,非生物胁迫与激素均可诱导相关转录因子结合到下游基因启动子顺式作用元件上,从而诱导其表达^[16-17],*MiOFP1* 前 2000 bp 启动子中包含大量激素响应元件和逆境胁迫响应元件,推测 *MiOFP1* 可能受到激素、非生物胁迫以及上游 MYB、MYC 转录因子的调控和诱导。组织表达分析表明,*MiOFP1* 具有组织表达特异性,主要在童期树和成年期树的茎中表达,在花、叶、果实和胚中表达量相对较低。此前的研究发现,水稻 *OsOFP* 在种子发育时期表达量较高^[18],葡萄 *VvOFP* 在开花前表达量较高,开花一段时间后,表达量显著下降^[19],小麦 *TaOFP* 在根和穗中表达量最高^[20]。对比本研究结果,可以推测,*OFP* 参与多个植物生长发育的过程,但是不同物种 *OFP* 主要发挥的功能和表达的位置可能不同。此外,对过表达 *MiOFP1* 拟南芥进行表型观察,发现抽薹时间和开花时间有显著延迟,这与拟南芥 *OFP1* 功能类似,*AtOFP1* 在拟南芥中过表达出现延迟开花现象^[21]。内源基因表达分析结果表明,在过量表达 *MiOFP1* 的拟南芥株系中,成花抑制基因 *FLC* 的表达得到促进,成花促进基因 *FT* 的表达受到抑制,从而使植物开花时间延迟,这证明了 *MiOFP1* 对转基因拟南芥的开花起到抑制作用。研究发现,过量表达 *SIOFP20* 的番茄开花时间明显推迟,*SIOFP20* 转基因植株的 *SIFT* 表达水平显著低于 WT^[22]。目前尚未见有 *OFP* 在枱果中是否同样具有延迟开花的功能报道,但过量表达 *MiOFP1* 的拟南芥植株与过量表达番茄 *SIOFP20* 的植株均出现延迟开花现象,且植株内 *FT* 的表达量均显著降低,表明二者可能通过相同或相似的途径调控 *FT* 的表达,从而实现了对植株开花时间的调控,综上所述,推测 *MiOFP1* 通过影响 *FLC* 和 *FT* 的表达水平来调节枱果开花时间。

已有研究表明,除参与植物生长发育外,OVATE 家族的多数基因还参与植物激素调节及逆境胁迫响应过程。外源施加 GA₃ 可以减缓 *AtOFP1*

超表达株系的矮化现象;水稻 *OFP1* 通过与 GSK2、OsBZR1 和 DLT 的相互作用来正调控 BR 响应;Os-OFP6-RNAi 转基因株系表现出正常生长条件下侧根变短和 IAA 处理后侧根密度增加^[22];过表达 *AtOFP8* 显著增强了拟南芥的抗旱性,其种子的发芽率和绿叶率更高,叶片中脯氨酸含量提高,丙二醇含量降低,可溶性蛋白含量高,叶片抗氧化酶的活性较强^[23];对去除水稻 *OsOFP6* 的植株进行干旱和低温处理,明显发现植株缺水且相对电导率升高的现象,说明 *OsOFP6* 参与水稻对干旱和低温胁迫的防御过程^[24];苹果 *OFP* 家族基因在盐、干旱和高低温等非生物逆境下呈现出不同的响应差异,尤其是在低温下 *MdOFP* 基因家族中多个基因的表达量均有不同程度的上升^[25];同样的,低温条件下,油桐幼苗 *VjOFP3/7/10/12* 等基因的表达量上升,推测 *VjOFP* 可能在油桐低温胁迫响应方面发挥积极的调节作用^[26]。ABA 是种子萌发和大多数常见非生物胁迫反应的主要介质之一,包括对盐、干旱和寒冷的反应^[27-28]。与野生型植物相比,*MiOFP1* 的超量表达减轻了 ABA 对种子萌发的抑制作用和根长敏感性,提高了外源 ABA 情况下植株的脯氨酸含量和过氧化物酶活性,促进了 ABA 响应基因 *ABII*、*MFT* 和 *RD29B* 以及 ABA 合成基因 *NCED3* 的表达。植物在受到胁迫时,细胞的渗透压发生变化,脯氨酸含量会升高以维持细胞渗透压;同时,细胞内产生大量的过氧化氢及酚类、胺类等物质,对细胞造成损害,过氧化物酶可催化过氧化氢及酚类、胺类等物质,达到减轻上述物质对细胞的毒害作用,通过外源喷施 ABA,提高植物体内的脯氨酸含量和过氧化物酶活性,从而增强植物抵御非生物胁迫的能力^[29-30]; *ABII* 在 ABA 的信号转导过程中起负调控作用^[31],*MFT* 直接抑制 *ABI5* 的表达,对 ABA 信号通路起负反馈调节作用^[32],外源 ABA 处理明显诱导 *RD29B* 表达量升高^[33]。这些发现表明,*MiOFP1* 在枱果开花和外源 ABA 响应中可能具有关键的作用,推测枱果 *MiOFP1* 可能是通过介导 ABA 的信号通路参与种子萌发调控过程和逆境防御过程,但是 *MiOFP1* 是如何参与 ABA 信号通路的、*MiOFP1* 是否影响枱果开花、能否通过提高 ABA 含量来增强枱果逆境抵御能力还不清楚,需要进一步研究。

4 结 论

杧果 *MiOFP1* 在童期茎的韧皮部组织中高度表达,而在成熟果实的果肉中表达水平较低;在营养生长期叶片中表达水平较高,而在成花期表达水平较低。超量表达的 *MiOFP1* 抑制拟南芥开花,但提高了种子在 ABA 处理下的萌发率,减弱了拟南芥对 ABA 胁迫的敏感性。说明 *MiOFP1* 可能与杧果的成花和 ABA 胁迫有关。

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