草莓蔗糖非发酵-1-相关蛋白激酶1 (SnRK1) α亚基编码基因的克隆及表达分析

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摘要: 植物的蔗糖非发酵-1-相关蛋白激酶1 (SnRK1)与酵母蔗糖非发酵蛋白激酶1 (SNF1)以及哺乳动物AMP激活的蛋白激酶(AMPK)同源, 都是异源三聚复合体结构, 含有α催化亚基和β、γ两个调节亚基来维持蛋白结构的稳定和激酶活性。本试验以'妙香7号'草莓(*Fragaria* × *ananassa*)为材料, 通过反转录PCR克隆得到一个SnRK1的α催化亚基编码基因, 命名为*FaSnRK1α*。序列分析显示该基因全长1 557 bp, 共编码518个氨基酸, 预测FaSnRK1α蛋白分子质量为59.159 kDa, 理论等电点为8.54, 定位于细胞质和细胞核。生物信息学分析发现FaSnRK1α的氨基酸序列与其他植物SnRK1α蛋白具有较高同源性, 含有KD (kinase domain)、UBA (ubiquitin associated domain)和β-SID (β-submit interaction domain)三个保守结构域。组织特异性分析表明*FaSnRK1α*在草莓根、茎、叶、花和果实中均有表达, 在果实发育进程中*FaSnRK1α*的表达水平呈上升趋势。荧光定量PCR分析表明, 果实中*FaSnRK1α*受脱落酸(ABA)诱导, 表明该基因可能与ABA诱导的果实发育和成熟有关。

关键词: 草莓; SnRK1; 脱落酸; 基因克隆; 表达模式

植物的蔗糖非发酵-1-相关蛋白激酶1 (sucrose non-fermenting-1-related protein kinase 1, SnRK1)与酵母的蔗糖非发酵蛋白激酶1 (SNF1)以及哺乳动物的AMP激活的蛋白激酶(AMPK)同源, 是一个能量感受器, 可被能量缺乏所激活(Polge和Thomas 2007; Baena-González和Sheen 2008; Emanuelle等2015)。植物中, SnRK1是由α催化亚基和β、γ两个调节亚基组成的异源三聚体结构(Ramon等2013; Emanuelle等2016), 其中α亚基有两个功能结构域: N末端的激酶结构域(kinase domain, KD)和C末端的调节域(regulatory domain, RD) (Ghillebert等2011; Crozet等2014; Emanuelle等2016)。KD结构域内存在保守的磷酸化位点, 可被上游激酶(如拟南芥GRIK1和GRIK2)磷酸化, 使其具有催化活性(Polge和Thomas 2007; Halford 和Hey 2009; Shen等2009)。

植物中, 脱落酸(abscisic acid, ABA)可以影响SnRK1的活性: 超表达*SnRK1α1*的拟南芥(*Arabidopsis thaliana*)在种子萌发和幼苗生长过程中对ABA表现出高度敏感性(Jossier等2009; Tsai和Gazzarrini 2012); ABI1和PP2CA两种PP2C类磷酸酶都可以使SnRK1α1发生去磷酸化, 研究证明在成熟的光合组织中ABA可通过抑制ABI1和PP2CA来激活SnRK1 (Rodrigues等2013); 在种子萌发和幼苗阶段, ABA通过与SnRK1A的负调节因子互作抑制SnRK1 (Lin等2014); 在小麦(*Triticum aestivum*)根系中ABA可诱导SnRK1降解(Coello等2012)。不同组织中ABA对SnRK1的调控也存在差异。

SnRK1可以通过抑制与碳氮代谢相关的生物合成酶的活性下调植物的生长发育进程(Sugden等1999; Polge等2008; 罗静静等2018), 也可以影响代谢、信号转导、转录、耐逆性、物质运输、生长发育等多种途径相关的1 000多个基因的表达(Baena-González等2007; Baena-González和Sheen 2008; 赵永飞等2017)。Baena-González等(2007)研究发现在营养缺乏的培养基上, 超表达*KIN10*的拟南芥衰老延缓, 并且在长日照条件下开花延迟。近年来研究发现超表达湖北海棠(*Malus hupehensis*) '平邑甜茶' SnRK1α亚基编码基因*MhSnRK1.1*的番茄(*Solanum lycopersicum*)比野生型番茄果实提前成熟(Wang等2012), 且超表达*MdSnRK1.1*的苹果愈伤在含有蔗糖的培养基上可积累更多的花青苷, 说明*MdSnRK1.1*是蔗糖诱导的花青苷积累所必须的(Liu等2017a)。

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草莓(*Fragaria* × *ananassa*)是非呼吸跃变型果实, 在成熟的过程中没有乙烯释放量的剧烈升高和呼吸作用的增强(Given等1988; Abeles和Takeda 1990)。近年来一系列重要研究进展证实在非呼吸跃变型果实发育过程中ABA水平逐渐增加, 是调控果实成熟的重要激素(Chai等2011; Jia等2011, 2013; Li等2013)。外源ABA可以明显促进果实成熟(Manning 1994; Jiang和Joyce 2003; Terry等2007; Jia等2011; Li等2013), 而ABA抑制剂氟啶酮明显抑制果实成熟(Jia等2011), 但其调控的分子机理目前仍不十分清楚。因此, 本试验以'妙香7号'草莓为材料, 克隆得到了一个*FaSnRK1α*成员, 进行了生物信息学分析, 并分析了其在草莓不同组织和果实不同发育阶段的表达特性, 同时分析了果实中该基因在ABA处理后的表达模式, 为进一步阐述ABA调控下游果实成熟相关的新的信号组分提供理论参考。

1 材料与方法

1.1 材料与处理

试验采用栽培草莓(*Fragaria* × *ananassa* Duch.) '妙香7号', 在山东农业大学南校区试验站进行。选取设施栽培的结果期草莓, 取其根、茎、叶、花和果实用于组织特异性分析。

果实发育时期根据花期后天数和果实发育进程分为小绿果(花后7 d)、中绿果(花后13 d)、大绿果(花后17 d)、褪绿果(花后19 d)、白果(花后22 d)、初红果(花后25 d)、半红果(花后28 d)和红果(花后31 d)。在果实发育的各个时期采集10个相同生长状态下的果实, 去掉种子, 混匀后用液氮冷冻, −80°C保存, 用于果实不同发育阶段基因表达模式分析。

ABA处理试验选择设施栽培大绿果时期的草莓进行。以清水为对照, 不同浓度(30、60、90、120 μmol·L^{-1})的ABA为处理条件, 将全果浸入ABA溶液中5 s, 每处理10个果实, 并于处理后0、1、2、4、8、12、24 h取样, 去种子, 混匀后液氮冷冻, −80°C保存, 用于实时荧光定量PCR分析。

1.2 *FaSnRK1α*克隆与序列分析

以露地栽培的草莓幼嫩叶片为试材, 采用EASYspin Plus植物RNA快速提取试剂盒(北京爱德莱生物科技有限公司)提取样品RNA, 利用反转录试剂盒PrimeScript RT reagent Kit with gDNA Eraser (Perfect Real Time, TaKaRa)获得cDNA模板, 贮存于−20°C备用。根据在草莓基因组数据库中检索到的序列, 设计引物(表1)。反应条件: 95°C预变性5 min; 98°C变性10 s, 55°C退火30 s, 72°C延伸1 min, 35次循环; 72°C延伸10 min。PCR产物用1.5%琼脂糖凝胶进行电泳检测目的条带, 用大量琼脂糖凝胶DNA回收试剂盒(TIANGEN)回收目的条带, 连接到pMD19-T克隆载体进行测序。

1.3 生物信息学分析

利用NCBI CDD在线工具(https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi)预测扩增片段推测出的氨基酸序列的结构域, 利用DNAMAN 7.0进行氨基酸序列分析, 利用MEGA 5.0软件绘制系统进化树, 利用Prot Param在线软件(http://web.expasy.org/protparam/)推导蛋白质分子质量、理论等电点等基本理化性质, 利用PSORT II和Soft Berry ProtComp 9.0 (http://www.softberry.com/berry.phtml?group=programs&subgroup=proloc&topic=protcomppl)进行亚细胞定位预测。

1.4 实时荧光定量PCR分析

采用EASYspin Plus植物RNA快速提取试剂盒(北京爱德莱生物科技有限公司)提取样品RNA, 利用反转录试剂盒(Perfect Real Time, TaKaRa)获得用于实时荧光定量PCR的cDNA。实时荧光定量PCR采用SYBR Green PCR *Premix Ex Taq*试剂盒(宝生物公司), 操作参照说明书进行, 引物参见表1。用CFX96 Touch Real-Time PCR Detection System (Bio-Rad, USA)进行实时荧光定量PCR, 反应程序为: 95°C预变性30 s; 95°C变性5 s, 57°C退火30 s, 40次循环。所有实时荧光定量PCR反应均设置3次生物学重复和3次技术重复, 所得数据采用$2^{-\Delta\Delta C_T}$进行计算分析。

2 实验结果

2.1 *FaSnRK1α*基因克隆

根据拟南芥*KIN10*基因序列(登记号: At3g01090)在草莓基因组网站(http://strawberry-garden.kazusa.or.jp/)上进行Blast检索, 得到1个SnRK1α亚基编码基因(FANhyb_rscf00000747.1.g00007.1), 根

表1 本研究所用的引物

Table 1 Primers used in this study

引物名称	引物序列(5′→3′)
FaSnRK1α-F	CTCTAGAGGATCCCCGGGATGGATGGAGCAATTGGCCG
FaSnRK1α-R	GATCGGGGAAATTCGAGCTCTTAAAGAACACGAAGCTGTGCAAGG
FaSnRK1α(RT)-S	GCATCCTCACATTATACGACTCTA
FaSnRK1α(RT)-A	TCCAGACTTCACATACTCCATAAC
FaACTIN(RT)-S	GCCAACCGTGAGAAGATG
FaACTIN(RT)-A	TCCAGAGTCAAGAACAATACCAG

据已知序列设计引物, 通过反转录PCR扩增得到该基因(图1), 测序结果显示该基因全长1 557 bp, 编码518个氨基酸, 与网站上登记的其他栽培品种氨基酸序列相似性达到93.67%, 本试验将其命名为*FaSnRK1α*。

2.2 *FaSnRK1α*基因编码蛋白理化性质及保守结构域分析

利用NCBI CDD在线工具预测扩增片段推测出的氨基酸序列结构域, 该基因含有SnRK1α亚基保守的KD结构域(kinase domain)、UBA结构域(ubiquitin associated domain)和β-SID结构域(β-submit interaction domain) (图2), 因此认为该基因是草莓SnRK1α亚基的编码基因。*FaSnRK1α*基因编码蛋白的理化性质见表2, 利用PSORT II和SoftBerry ProtComp 9.0进行亚细胞定位预测, 显示该蛋白定位于细胞质和细胞核。

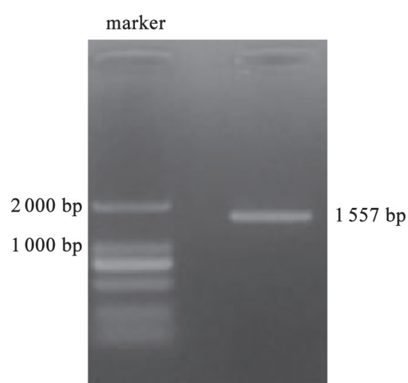

图1 *FaSnRK1α*的PCR扩增

Fig.1 PCR amplification of *FaSnRK1α*

2.3 FaSnRK1α序列比对和进化树分析

利用DNAMAN 7.0分析草莓和拟南芥*SnRK1*家族基因编码的蛋白氨基酸序列, 显示FaSnRK1α与拟南芥AtKIN10和AtKIN11 (登记号: At3g29160)的同源性高达80%以上(图3)。从NCBI网站的BLAST程序检索到与草莓SnRK1α亚基编码基因同源的其他物种的氨基酸序列, 系统进化分析表明栽培草莓SnRK1的氨基酸序列与野草莓(*F. vesca*)、月季(*Rosa chinensis*)、白梨(*Pyrus bretschneideri*)、甜樱桃(*Prunus avium*)、桃(*P. persica*)、苹果(*Malus pumila*)等蔷薇科植物亲缘关系较近(图4)。

2.4 *FaSnRK1α*的组织特异性与果实不同发育阶段的表达模式

为了解草莓*FaSnRK1α*基因的表达特性, 将草莓分为根、茎、叶、花和大绿果, 利用荧光定量PCR的方法, 检测了*FaSnRK1α*在草莓不同部位中的表达水平, 结果显示: *FaSnRK1α*在叶中表达量最高, 在根、茎和花中的表达量相对较低, 在果实中表达量最低(图5)。由于草莓果实发育分不同阶段, 又分析了*FaSnRK1α*在果实不同阶段的表达量, 结

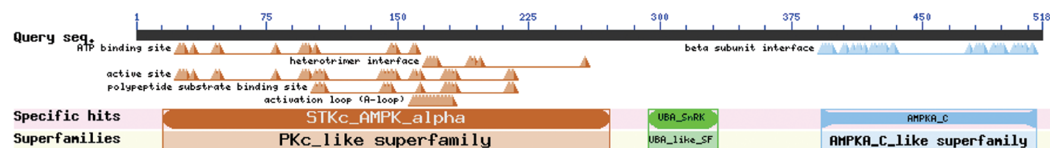

图2 FaSnRK1α保守功能结构域分析

Fig.2 Conserved functional domain analysis of FaSnRK1α

Query seq.: 序列查询; Specific hits: 特异性匹配; Superfamilies: 超家族。橙色部分: KD结构域; 绿色部分: UBA结构域; 蓝色部分: β-SID结构域。

表2 *FaSnRK1α*编码氨基酸序列的理化特性

Table 2 Physicochemical characterization of amino acid sequences coded by *FaSnRK1α*

蛋白	氨基酸数	分子质量/kDa	理论等电点	亚细胞定位
FaSnRK1α	518	59.159	8.54	细胞质、细胞核

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AtKIN10    MDGSGTGS.RSGVESILPNYKLGRTLGIGSFGRVKIAEHALTGHKVAIKILNRRKIKNMEMEEKVRREIKILRLFMHPHIIRLYEVIETPTDIYLVMEYV    99
AtKIN11    MDHSSNRFGNNGVESILPNYKLGKTLGIGSFGKVKIAEHVVTGHKVAIKILNRRKIKNMEMEEKVRREIKILRLFMHPHIIRQYEVIETTSDIYVVMEYV   100
FaSnRK1α   MDGAIGRG.GSSADAYLPNYKLGKTLGIGSFGKVKIAEHALTGHKVAIKILNRRKIKNMEMEEKVRREIKILRLFMHPHIIRLYEVIETPSDIYVVMEYV    99
Consensus  md              lpnyklg tlgigsfg vkiaeh  tghkvaikilnrrkiknmemeekvrreikilrlfmhphiir yeviet  diy vmeyv

AtKIN10    NSGELFDYIVEKGRLQEDEARNFFQQIISGVEYCHRNMVVHRDLKPENLLLDSKCNVKIADFGLSNIMRDGHFLKTSCGSPNYAAPEVISGKLYAGPEVD   199
AtKIN11    KSGELFDYIVEKGRLQEDEARNFFQQIISGVEYCHRNMVVHRDLKPENLLLDSRCNIKIADFGLSNVMRDGHFLKTSCGSPNYAAPEVISGKLYAGPEVD   200
FaSnRK1α   KSGELFDYIVEKGRLQEDEARNFFQQIISGVEYCHRNMVVHRDLKPENLLLDSKCNVKIADFGLSNIMRDGHFLKTSCGSPNYAAPEVISGKLYAGPEVD   199
Consensus   sgelfdyivekgrlqedearnffqqiisgveychrnmvvhrdlkpenlllds cn kiadfglsn mrdghflktscgspnyaapevisgklyagpevd

AtKIN10    VWSCGVILYALLCGTLPFDDENIPNLFKKIKGGIYTLPSHLSPGARDLIPRMLVVDPMKRVTIPEIRQHPWFQAHLPRYLAVPPPDTVQQAKKIDEEILQ   299
AtKIN11    VWSCGVILYALLCGTLPFDDENIPNLFKKIKGGIYTLPSHLSSEARDLIPRMLIVDPVKRITIPEIRQHRWFQTHLPRYLAVSPPDTVEQAKKINEEIVQ   300
FaSnRK1α   VWSCGVILYALLCGTLPFDDENIPNLFKKIKGGIYTLPSHLSPGARDLIPRMLVVDPMKRMTIPEIRQHKWFQAHLPRYLAVPPPDTMQQAKKIDEEILQ   299
Consensus  vwscgvilyallcgtlpfddenipnlfkkikggiytlpshls  ardliprml vdp kr tipeirqh wfq hlprylav ppdt  qakki eei q

AtKIN10    EVINMGFDRNHLIESLRNRTQNDGTVTYYLILDNRFRASSGYLGAEFQETMEG.TPRMHPAESVASPVSHRLPGLMEYQGVG...LRSQYPVERKWALGL   395
AtKIN11    EVVNMGFDRNQVLESLRNRTQNDATVTYYLLLDNRFRVPSGYLESEFQETTDSGSNPMRTPEAGASPVGHWIPAHVDHYGLG...ARSQVPVDRKWALGL   397
FaSnRK1α   EVVKMGFDRNHLVESLCGRLQNEGTVAYYLLLDNRFRVSSGYLGAEFQEIVESGFNRMHQAEPASSPIGHRLPGYMEFQGMGSSPFRQQFPVERKWALGL   399
Consensus  ev  mgfdrn   esl  r qn  tv yyl ldnrfr  sgyl  efqe          m   e   sp  h  p      g g    r q pv rkwalgl

AtKIN10    QSRAHPREIMTEVLKALQDLNVCWKKIGHYNMKCRWVPNS..SADGMLSNSMHDNNYFGDESSIIENEAAVKSPNVVKFEIQLYKTRDDKYLLDLQRVQG   493
AtKIN11    QSHAHPREIMNEVLKALQELNVCWKKIGHYNMKCRWVPG...LADGQNT.MVNNQLHFRDESSIIEDDCAMTSPTVIKFELQLYKAREEKYLLDIQRVNG   493
FaSnRK1α   QSRAHPREIMTEVLKALQELRVCWKKIGHYNMKCRWVPGAPGHHEGMVDNPVNNNHYFGDESSIIENDGIMKTPNVVKFEVQLFKTREEKYLLDLQRVQG   499
Consensus  qs ahpreim evlkalq l vcwkkighynmkcrwvp       g            f dessiie        p v kfe ql k r  kylld qrv g

AtKIN10    PQFLFLDLCAAFLAQLRV                                                                                      511
AtKIN11    PQFLFLDLCAAFLTELRV                                                                                      511
FaSnRK1α   LQFLFLDLCAAFLAQLRV                                                                                      517
Consensus   qflfldlcaafl  lrv
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图3 草莓FaSnRK1α与拟南芥AtKIN10和AtKIN11氨基酸序列比对

Fig.3 Comparison of FaSnRK1α protein sequence with AtKIN10 and AtKIN11 proteins

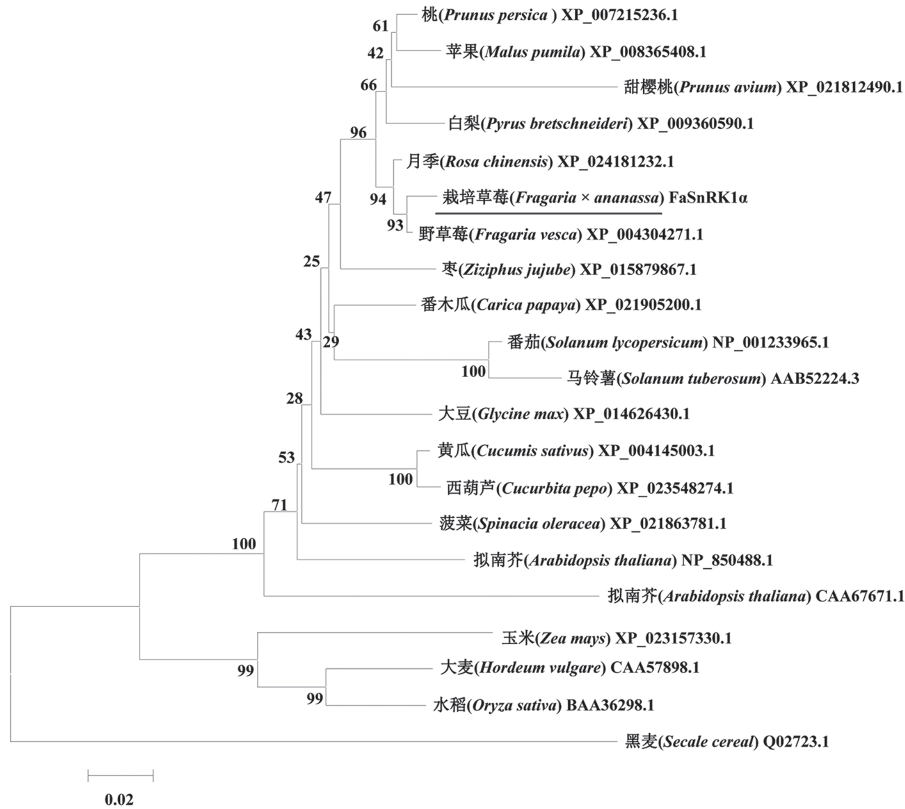

图4 FaSnRK1α与其他物种SnRK1α氨基酸序列的系统进化分析

Fig.4 Phylogenetic analysis of FaSnRK1α and SnRK1α protein sequences from other plants

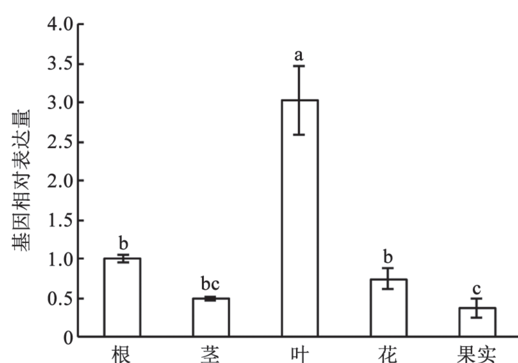

图5 草莓*FaSnRK1α*的组织特异性

Fig.5 Tissue specificity of *FaSnRK1α* in strawberry

各柱形上不同小写字母标识表示数据间差异显著($P<0.05$), 下同。

果表明*FaSnRK1α*在小绿果(花后7 d)中表达量最低, 随着果实发育逐渐升高直至果实半红(花后28 d), 红果时(花后31 d)表达量又略有降低, 暗示*FaSnRK1α*的表达量和果实发育成熟可能存在相关性(图6)。

2.5 果实中*FaSnRK1α*在ABA处理下的表达变化

ABA是调控果实发育和成熟的信号分子(Jia等2011), 本试验设置不同浓度和时间梯度检测了ABA处理后草莓果实中*FaSnRK1α*的表达量, 发现不同浓度ABA处理后*FaSnRK1α*的表达量均上升, 其中60 μmol·L^{-1}处理后效果最显著(图7-A); 又检测了60 μmol·L^{-1}处理后不同时间*FaSnRK1α*的表达量, 表明4 h内表达量呈上升趋势, 4 h后表达量开始下降, 但24 h内表达量仍然是升高的(图7-B)。

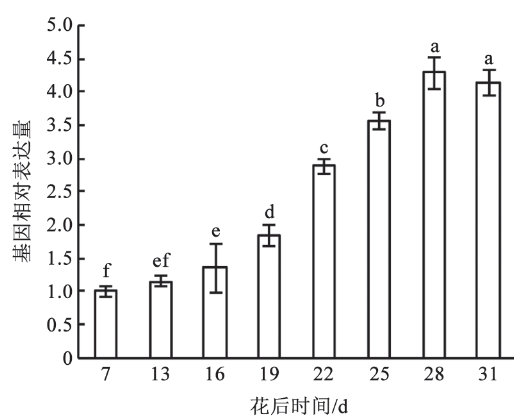

图6 草莓果实不同发育阶段*FaSnRK1α*的表达模式

Fig.6 Expression pattern of *FaSnRK1α* in strawberry fruits during different developmental processes

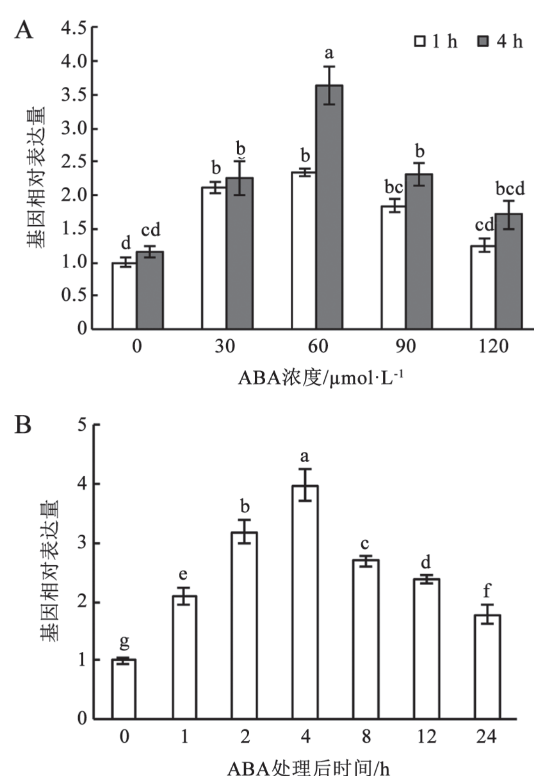

图7 外源ABA处理下草莓果实中*FaSnRK1α*的表达模式

Fig.7 Expression pattern of *FaSnRK1α* in strawberry fruits under exogenous ABA treatments

3 讨论

SnRK1是植物体内复杂信号网络的中心组成部分(Lastdrager等2014), 参与植物碳氮代谢、生长发育、胁迫应答等多种生理过程的调控(Ploge和Thomas 2007; Ghillebert等2011; Emanuelle等2016)。*SnRK1*属于保守的基因家族(Halford和Hardie 1998), 拟南芥中SnRK1α亚基编码基因有两个成员*KIN10*和*KIN11* (Baena-González等2007)。本研究从草莓中克隆得到一个SnRK1α亚基编码基因*FaSnRK1α*, FaSnRK1α氨基酸序列含有保守的KD结构域、UBA结构域和β-SID结构域, 与拟南芥中KIN10和KIN11具有较高的同源性, 与野草莓、月季、白梨、甜樱桃、桃、苹果等亲缘关系较近, 预测其主要定位于细胞质和细胞核中。

组织特异性研究显示, *FaSnRK1α*在草莓根、茎、叶、花和果实中均有表达, 而且在叶片中表达量最高, 果实中表达量最低。结合前人研究发现不同物种中的SnRK1α亚基编码基因表达量存

在差异: *PpSnRK1α*在一年生实生毛桃苗的根、茎和叶中均有表达, 在叶中表达量最高; 在嫁接苗‘鲁星’叶、花和果实中均有表达, 在果实中表达量最高(王贵 2014); *MhSnRK1*在‘平邑甜茶’根、茎和叶中均有表达, 叶中表达量最高(李光杰2009); 番茄中*SlSnRK1*在根、茎、叶和花中均表达, 花中表达量最高(沈庆汤2010)。由于不同物种生长环境及发育特性不同, SnRK1α亚基编码基因在不同组织中的表达也存在差异。

*MdSnRK1.1*在番茄中超表达可以影响碳氮代谢, 促进番茄果实的成熟进程(Wang等2012)。本研究中*FaSnRK1α*的表达量在果实发育过程中呈上升趋势, 尤其在果实褪绿之后表达量上升较快, 至果实成熟后表达量又略有下降, 推测该基因可能在果实发育进程中起重要作用。近来研究表明SnRK1与激素信号(比如水杨酸、茉莉酸和乙烯)尤其是ABA信号之间存在紧密联系(Emanuelle等2016; Hulsmans等2016), MdSnRK1.1可以与MdCAIP1蛋白相互作用, 进而诱导MdCAIP1发生磷酸化修饰, 促进MdCAIP1的降解，从而负调控MdCAIP1的功能，增强对ABA的敏感性(Liu等2017b)。而ABA是调控果实成熟的重要激素, 因此本试验分析了*FaSnRK1α*对ABA处理的响应, 发现果实中*FaSnRK1α*的表达量在ABA处理后表达上调, 说明ABA不仅可以通过抑制ABI1和PP2CA这两个PP2C磷酸酶使SnRK1α1发生去磷酸化而激活SnRK1, 还可以在转录水平调节*FaSnRK1α*表达量, 因此草莓*FaSnRK1α*可能在ABA诱导的草莓果实成熟过程中起重要的调控作用。

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Cloning and expression analysis of sucrose non-fermenting-1-related protein kinase 1 (SnRK1) α-subunit gene in strawberry

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Abstract: SnRK1 (sucrose non-fermenting-1-related protein kinase 1) is the plant ortholog of the budding yeast SNF1 (sucrose-non-fermenting 1) and mammalian AMPK (AMP-activated protein kinase). All three enzymes typically function as heterotrimeric complexes that require a catalytic α-subunit and regulatory β- and γ-subunits for protein stability and kinase activity. In this article a SnRK1 α-subunit gene was cloned from the leaves of strawberry (*Fragaria* × *ananassa* cv. Miaoxiang 7) using reverse transcription PCR, and was designated as *FaSnRK1α*. Sequence analysis showed that the total length of *FaSnRK1α* is 1 557 bp, encoding 518 amino acids. The molecular mass of FaSnRK1α protein is 59.159 kDa and the isoelectric point is 8.54, and FaSnRK1α locates in cytoplasm and nuclear. Bioinformatic analysis showed that FaSnRK1α protein shares high sequence identity with the reported plant SnRK1α proteins, with three conserved domains, annotated as KD (kinase domain), UBA (ubiquitin associated domain) and β-SID (β-submit interaction domain). Tissue specificity analysis showed that *FaSnRK1α* was expressed in root, stem, leaf, flower and fruit and has a rising trend during fruit development. Quantitative real-time PCR analysis demonstrated that the expression of *FaSnRK1α* was highly induced by abscisic acid (ABA), suggesting *FaSnRK1α* might play an important role in strawberry fruit development response to ABA.

Key words: strawberry; SnRK1; abscisic acid; gene cloning; expression pattern

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