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甜樱桃砧木*PcMPK3*基因启动子的克隆及对病原菌感染的响应

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摘 要:【目的】探明*PcMPK3*基因的表达调控规律。【方法】利用染色体步移技术从甜樱桃矮化砧木Gisela 6中克隆*PcMPK3*基因的启动子序列PcMPK3pro。利用Neural Network Promotor Prediction、softberry、PLACE和PlantCARE网站在线预测*PcMPK3*基因的基础启动子、转录起始位点和顺式作用元件。将PcMPK3pro定向替换植物表达载体pBI121-SN1的CaMV35S组成型启动子,构建重组表达载体pBI-PcMPK3pro:*GUS*,瞬时转化烟草叶片。【结果】结果表明,PcMPK3pro含有启动子核心元件TATA-box和CAAT-box等多种响应胁迫的顺式作用元件。受病原菌丁香假单胞菌番茄致病变种(*Pseudomonas syringae* pv. *tomato* DC3000,*Pst* DC3000)侵染,PcMPK3pro能驱动*GUS*报告基因表达且GUS酶活性显著提高。【结论】推测*PcMPK3*基因参与植物响应病原菌感染的胁迫过程。

关键词:甜樱桃;砧木;*PcMPK3*;启动子;*GUS*基因

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Identification of the *PcMPK3* gene promoter from sweet cherry rootstock and its response to plant pathogen infection

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Abstract:【Objective】*PcMPK3* encoding a mitogen-activated protein kinase (MAPK) in sweet cherry dwarf rootstock Gisela 6 (*P. cerasus* × *P. canescens*) is highly identical to *Arabidopsis AtMPK3* gene, which is involved in plant growth, development and responses to biotic and abiotic stresses. Our previous work showed *PcMPK3* responded to osmotic, salt stresses and bacterium pathogen infection significantly on the transcriptional level. However, the upstream regulation sequence of *PcMPK3* has not been reported to date. The purpose of this study is to identify the promoter sequence of *PcMPK3* and investigate its transcriptional regulation characteristics in sweet cherry rootstock.【Methods】The 5'-flanking upstream region of *PcMPK3* gene was cloned by thermal asymmetric interlaced PCR (TAIL-PCR) using a degenerate primer AP1 and three gene-specific primers SP1, SP2 and SP3. Sequence analysis was carried out by online bioinformatics resources, such as Neural Network Promotor Prediction, softberry, PLACE and PlantCARE to reveal its basic core promoter, transcription start site and *cis*-regulatory elements (CREs). The *PcMPK3* promoter sequence (designated as PcMPK3pro) was cloned into the plant expression vector pBI121-SN1 to replace CaMV35S promoter and produce a new recombinant expression vector named pBI121-PcMPK3:*GUS*. The binary plasmid was then transformed into *Agrobacterium tumefaciens* strain LBA4404. Tobacco leaves (*Nicotiana tobacum* cv. Samsun) were incubated at 25℃

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under a light intensity of 300 μmol·m^{-2}·s^{-1} and 16 hours/8 hours light/dark cycles for 4 weeks and then used for *Agrobacterium*-mediated transient transformation. The tobaccos were classified as control and treatment groups. For the control group, the leaves were inoculated by the suspension of the *Agrobacterium* strain LBA4404 (OD_{600}=0.1) containing pBI121-PcMPK3:*GUS* vector. For the treatment groups, the tobacco leaves were inoculated by the suspension mixture of *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst* DC3000) (OD_{600}=0.01) and *Agrobacterium* LBA4404 (OD_{600}=0.1) harboring pBI121-PcMPK3:*GUS* vector. Both suspensions for the two groups were prepared with the infiltration buffer containing 10 mmol·L^{-1} $MgCl_2$, 10 mmol·L^{-1} 2-(N-morpholino) ethanesulfonic acid (MES) and 150 μmol·L^{-1} Acetosyingon and injected into the tobacco leaves by 1 mL syringe, respectively. The infiltrated leaves were harvested for GUS activity assay at 12 hours and 24 hours after infiltration. For the GUS activity assay, fresh leaves were subjected to X-Gluc solution for histochemical staining and were quantitatively measured using the fluorometric determination method.【Results】The first and second round TAIL-PCR products showed dispersive results during electrophoresis detection. For the third round PCR, two distinct fragments, more than 2 000 bp, were amplified and purified from gel for sequencing respectively. A sequence of 2 027 bp, located in the 5'-flanking upstream of *PcMPK3* gene, was finally cloned and designated as 'PcMPK3pro'. It was registered at GenBank as KY434098. Sequence analysis showed basic promoter may exist at the position of −123 bp to −172 bp, −1 652 bp to −1 702 bp and −1 795 bp to −1 845 bp in the upstream of ATG initiation codon. Typical TATA box and CAAT box elements and numbers of *cis*-regulatory elements relating to plant defense responses were also contained within PcMPK3pro, including the bacterium and fungal elicitor, abscisic acid, ethylene, salicylic acid (SA), methyl jasmonate (MeJA) and gibberellin responsive elements. This means PcMPK3pro may be controlled by phytohormones and involved in physiological growth and development during pathogen infection. Two MYB transcription factors binding sites were also contained within the promoter sequence, which meant MYB transcription factors family may take responsibility to regulate *MAPK* genes expression. In order to uncover further evidence of PcMPK3pro characteristics, the recombinant plasmid pBI121-PcMPK3pro:*GUS* was digested by *Hind*III and *BamH*I for vector construction determination. Different infiltration suspensions, either agrobacterium LBA4404 harboring pBI121-PcMPK3pro:*GUS* vector or bacterium mixture with *Pst* DC3000 and LBA4404 (pBI121-PcMPK3pro:*GUS* contained) were injected into tobacco leaves, respectively. For the histochemical assay, blue staining can be observed in both groups 12 hours after inoculation. However, the intensity of the treatment group was much higher than the control group, about 4.42 times higher than that in control groups according to the fluorometric quantitative assay. More obvious differences were observed 24 hours after inoculation. This result indicated that the transcriptional expression of *GUS* gene can be driven by the promoter PcMPK3pro and induced quickly in response to the challenge of plant pathogen.【Conclusion】Previous research showed most of the plant MAPK members can be regulated at transcriptional level during biotic or abiotic stresses challenging. However, more attentions were paid to phosphorylation of the kinase and their interaction elements in the downstream. Few study on the transcriptional regulation of plant *MAPK* genes was reported. Revealing the characteristics of *MAPK* genes' promoters and their CREs will provide valuable information for the cultivar improvement on sweet cherry rootstocks by genetic engineering methods. In this study, the *PcMPK3* promoter was isolated and characterized. It contained typical TATA box and CAAT box elements and numbers of stress-related CREs and can drive the reporter genes quickly during *Pst* DC3000 infection. Therefore, *PcMPK3* gene may be involved in plant physiological responses to pathogen challenging.

Key words: Sweet cherry; Rootstock; *PcMPK3*; Promoter; *GUS* gene

促分裂原活化蛋白激酶级联途径（Mitogen-activated protein kinase cascades，MAPK cascades）是植物识别病原相关分子模式（Pathogen/microbe-associated molecular patterns，PAMPs/MAMPs）和病原菌效应子的早期信号转导事件，参与调控多个防御反应的信号传递过程，包括防卫基因的激活、植保素合成、细胞壁加固、过敏反应（Hypersensitive responses，HR）和诱导植物产生系统获得性抗性（Systemic acquired resistance，SAR）[1-3]。研究表明，高等植物的A组MAPK（如拟南芥AtMPK3/AtMPK6、烟草SIPK/WIPK）对植物病原相关分子模式所触发的免疫反应（PAMP-triggered immunity，PTI）和效应子触发的免疫反应（Effector-triggered immunity，ETI）均具有重要的调控作用。例如，鞭毛蛋白Flg22能够激活拟南芥MEKK1-MKK4/5-MPK3/6通路，通过该信号通路激活下游WRKY22/WRKY29转录因子，最终诱导相关防卫基因的表达[1]。Flg22调控MPK3的靶向底物是一种bZIP转录因子VIP1（VirE1-interacting protein 1），其活化后可激活下游防卫基因的转录[4]。烟草WIPK和SIPK被烟草花叶病毒（*Tobacco mosaic virus*，TMV）激活后与其上游的NtMEK2共同调控N基因介导的TMV抗性过程[5-6]。因此对该信号系统进行精细调控，在植物抗病育种中具有潜在的应用价值[7-8]。

吉塞拉（Gisela）系列砧木（*P. cerasus* × *P. canescens*）是目前甜樱桃生产中应用最广的矮化砧木[9]，但对细菌性溃疡病抗性较差是该系列砧木的的主要缺点之一。研究探明细菌性溃疡病致病原为一种丁香假单胞菌致病变种，感染该致病原可导致树干流胶，树势衰弱，直至死亡[10-11]。利用生物技术对吉塞拉系列砧木进行抗病性遗传改良，能够克服传统育种的局限性，提高育种效率。

笔者前期从甜樱桃砧木Gisela 6中分离了12个*MAPK*基因，并筛选出受丁香假单胞菌丁香致病变种（*Pseudomonas syringae* pv. *syringae*）、水杨酸（salicylic acid，SA）和茉莉酸甲酯（Methyl jasmonate，MeJA）显著诱导的抗病相关基因*PcMPK3*[12]。该基因是拟南芥*AtMPK3*的同源基因，木本植物中关于该基因对植物抗病性调控作用的研究尚无报道。笔者从甜樱桃矮化砧木Gisela 6中克隆了*PcMPK3*基因启动子序列，利用Neural Network Promotor Prediction、softberry、PLACE和PlantCARE网站在线预测*PcMPK3*基因的基础启动子、转录起始位点和顺式作用元件。将PcMPK3pro定向替换植物表达载体pBI121-SN1的CaMV35S组成型启动子，构建重组表达载体pBI-PcMPK3pro-GUS，利用注射法瞬时表达于烟草叶片。通过组织化学染色和GUS活性定量检测揭示该启动子对病原菌感染的响应特性，为深入研究该基因的功能奠定基础，同时为利用该基因进行甜樱桃砧木的抗病性育种提供依据。

1　材料和方法

1.1　材料

甜樱桃砧木Gisela 6（*P. cerasus* × *P. canescens*）组培苗保存于山东省果树生物技术育种重点实验室；三生烟（*Nicotiana tabacum* var. *samsun*）由山东农业大学植物保护学院烟草系提供；试验用植物表达载体pBI121-SN1、根癌农杆菌（*Agrobacterium tumefacieus*）菌株LBA4404及植物病原菌丁香假单胞菌番茄致病变种（*Pseudomonas syringae* pv. *tomato* DC3000）均为本实验室保存；染色体步移试剂盒（Genome Walking kit）购自TaKaRa公司；PCR引物合成和DNA测序均由生工生物工程公司完成。

1.2　甜樱桃砧木叶片总DNA提取及*PcMPK3*基因启动子的克隆

选取MS增殖培养基继代培养三周的甜樱桃砧木Gisela 6组培苗叶片为试材，采用植物基因组DNA提取试剂盒（北京天根）提取叶片总DNA保存于−80 ℃备用。根据*PcMPK3*基因组序列设计3条特异性引物SP1、SP2、SP3，采用染色体步移技术进行热不对称交错PCR（thermal asymmetric interlaced PCR，TAIL-PCR）扩增。具体步骤如下：利用染色体步移试剂盒AP1引物和特异性引物SP1进行第一轮PCR扩增，取适量第一轮反应液做模板，利用AP1和SP2引物进行第二轮PCR扩增，取适量第二轮反应液做模板，利用AP1和SP3引物进行第三轮PCR扩增，将3次PCR进行电泳检测，回收凝胶清晰条带进行测序，测序引物为SP3。测序结果与参考序列进行比对，验证序列的正确性，命名为PcMPK3pro，并提交至GenBank注册（表1）。

1.3　启动子顺式作用元件分析

利用Neural Network Promoter Prediction在线软件预测*PcMPK3*基因基础启动子；利用softberry网站（http://www.softberry.com）预测可能的转录起

表 1 启动子扩增中所用引物序列

Table 1 Sequences of primers used for promoter amplification

引物名称 Primer name	引物序列(5'→3') Sequences (5'→3')
SP1	GATCCAAATGACGAAGCAGCTTAA
SP2	GCTACAAAATCCCAAACAACACTCCAA
SP3	AAAAACGACGTACCAAACGATGCC
PcMPK3pro-F	AGTAGTGTACAAATAGATATAA
PcMPK3pro-R	TATTTGTTGGTGATCTCGAAGAG

始位点；利用 PLACE（http://www.dna.affre.go.jp/PLACE/signalscan.html）和 PlantCARE（http://bioinformatics.psb.ugent.be/webtools/plantcare/html/）网站在线分析*PcMPK3*基因启动子内部顺式作用元件。

1.4 pBI121-PcMPK3pro-*GUS*表达载体的构建及瞬时转化烟草

将连有PcMPK3pro序列的pMD18-T重组质粒和植物表达载体pBI121-SN1分别用*Hin*d III和*Bam*H I进行双酶切，凝胶回收PcMPK3pro和pBI121-SN1载体片段利用T4连接酶将启动子PcMPK3pro重组至pBI121-SN1载体，构建pBI121-PcMPK3pro:*GUS*融合表达载体，冻融法转入农杆菌LBA4404中，采用注射法进行烟草瞬时表达，步骤如下：用10 mmol·L^{-1} $MgCl_2$溶液重悬农杆菌（LBA4404::PcMPK3pro-*GUS*），配置成终浓度为OD_{600}=0.1的侵染液，用不加针头的注射器将侵染液从下表皮注射至烟草叶片，接种方式为全叶接种，即整片叶片出现湿润状。

1.5 植物病原菌*Pst* DC3000接种

为了检测启动子PcMPK3pro对植物病原菌*Pst* DC3000的响应，以病原菌*Pst* DC3000及含有重组表达载体的农杆菌LBA4404的混合菌液注射法接种处理组烟草叶片；以仅含有重组质粒的农杆菌LBA4404侵染液接种对照组烟草叶片。采用组织化学染色法及荧光定量法检测GUS活性。

处理组烟草接种方法如下：以10 mmol·L^{-1} $MgCl_2$溶液悬浮*Pst* DC3000菌体将浓度稀释为OD_{600}=0.02，以10 mmol·L^{-1} $MgCl_2$溶液悬浮含有重组质粒的农杆菌LBA4404，将浓度稀释至OD_{600}=0.2，将两种菌液1∶1等体积混合，配置成终浓度分别为OD_{600}=0.01、OD_{600}=0.1的混合菌液，用注射器将混合侵染液注射至整个叶片。对照组烟草接种方法如下：以仅含有重组质粒的农杆菌LBA4404侵染液（OD_{600}=0.1）采用注射法接种烟草叶片。

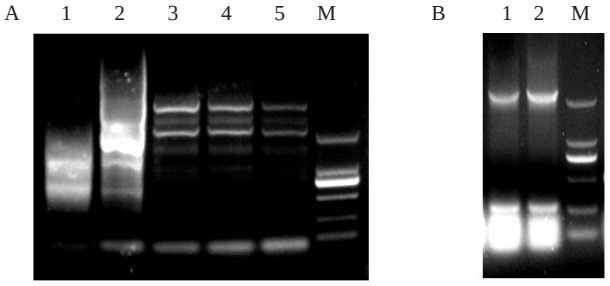
1.6 GUS组织化学染色及荧光定量检测

分别取接种后12 h和24 h的处理组及对照组烟草叶片，浸于含有X-Gluc的GUS染色液中，37 ℃培养箱中温育过夜，95%乙醇中脱色。GUS荧光定量检测参照Blázquez等[13]的方法进行。

2 结果与分析

2.1 *PcMPK3*基因启动子序列的克隆

经1%琼脂糖凝胶电泳检测3轮PCR产物，结果显示，第1、2轮的PCR产物电泳成弥散状，无特异性条带，第3轮PCR产物电泳显示至少扩增得到4个条带，其中2个条带较为清晰，且均大于2 000 bp，分别回收5号泳道中大于2 000 bp的两条特异片段（图1-A），测序后与*PcMPK3*基因进行同源性比对，确认其中1个序列为*PcMPK3*基因5'侧翼序列。根据测序序列设计特异引物PcMPK3pro-F和PcMPK3pro-pro-R，以甜樱桃砧木Gisela 6基因组DNA为模板，PCR扩增*PcMPK3*基因5'侧翼序列，序列长度为2 027 bp（图1-B），命名为PcMPK3pro，GenBank注册号为KY434098。



A. TAIL-PCR 扩增 *PcMPK3* 基因启动子序列。1. TAIL-PCR 第一轮扩增产物；2. TAIL-PCR 第二轮扩增产物；3~5. TAIL-PCR 第三轮扩增产物；M. DNA 分子量标准 DM2000。B. 启动子全长序列的扩增。

A. Amplification of *PcMPK3* promoter by TAIL-PCR. 1. The first round TAIL-PCR products; 2.The second round TAIL-PCR products; 3-5. The third round TAIL-PCR products; M.DNA marker. B. Amplification of full length *PcMPK3* promoter sequence by specific primers.

图 1 *PcMPK3*基因启动子序列的扩增

Fig. 1 Amplification of *PcMPK3* promoter

2.2 *PcMPK3*基因启动子顺式作用元件分析

利用Neural Network Promoter Prediction在线预测*PcMPK3*基因基础启动子，结果显示在基因起始密码子ATG上游−123~−172 bp、−1 652~−1 702 bp、−1 795~−1 845 bp存在基础启动子序列的可能性

分别为0.98、0.97、0.97。PLANTCARE软件分析显示，该启动子序列含有33个TATA-box、29个CAAT-box以及多个响应病原刺激及相关信号分子的顺式作用元件（表2、图2），其中包括脱落酸响应元件、病

表2　启动子区域顺式作用元件分析

Table 2　Cis-acting regulatory elements analysis of promoter sequences

基序名称 Motif name	位置 Position	信号序列 Signal sequences	功能注释 Function annotation
ABRE	-1 085	TACGTG	ABA响应元件 Cis-acting element involved in the abscisic acid responsiveness
ARE	-1 717，-1 852，-1 943	TGGTTT	厌氧诱导顺式调控元件 Cis-acting regulatory element essential for the anaerobic induction
AT-rich sequence	-386	TAAAATACT	激发子激活响应元件 Element for maximal elicitor-mediated activation
CCAAT-box	-320，-860	CAACGG	MYBHv1结合位点 MYBHv1 binding site
CGTCA-motif	-123，-1 774	CGTCA	茉莉酸甲酯响应元件 Cis-acting regulatory element involved in the MeJA-responsiveness
ERE	-585，-1 416	ATTTCAAA	乙烯响应元件 Ethylene-responsive element
GARE-motif	-1 807	AAACAGA	赤霉素响应元件 Gibberellin-responsive element
O2-site	-1 426	GATGACATGA	蛋白代谢调节顺式作用元件 Cis-acting regulatory element involved in zein metabolism regulation
P-box	-338	CCTTTTG	赤霉素响应元件 Gibberellin-responsive element
TCA-element	-139，-696，-1 276	GAGAAGAATA	水杨酸响应元件 Cis-acting element involved in salicylic acid responsiveness
W box	-1 816	TTGACC	真菌触发子响应元件 Fungal elicitor responsive element

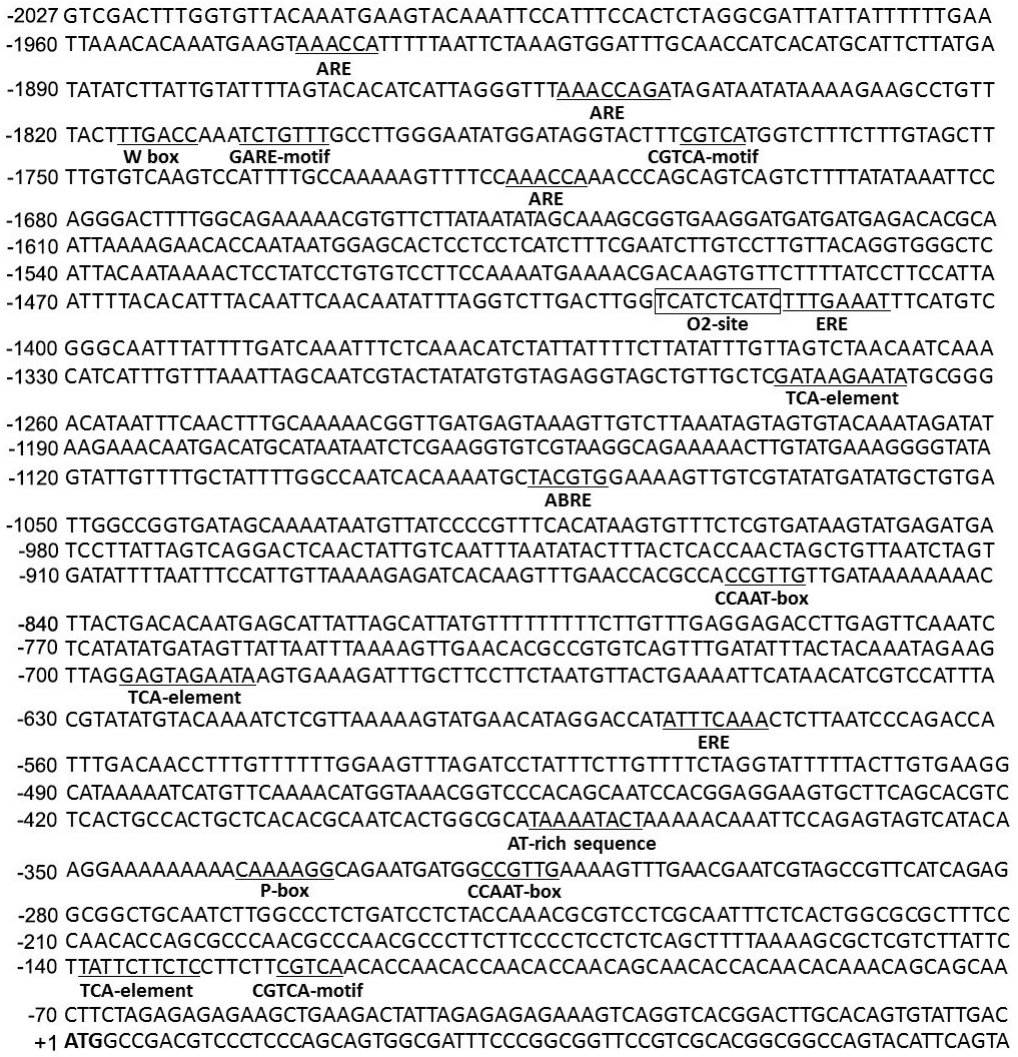


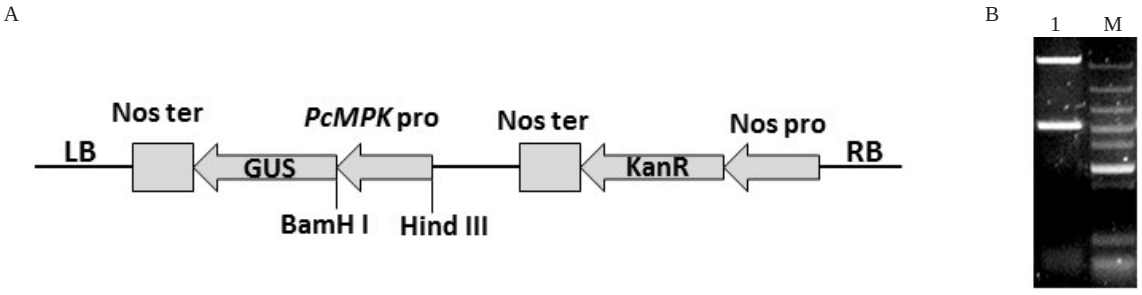
图2　启动子区段顺式作用元件的序列分析

Fig. 2　Sequence analysis of Cis-acting regulatory elements in the promoter

原激发子介导激活元件、茉莉酸甲酯信号响应元件、水杨酸信号响应元件和乙烯响应元件和赤霉素响应元件等。

2.3 重组表达载体pBI121-PcMPK3pro-GUS的构建

利用*Hind* III和*Bam*H I酶切将PcMPK3pro片段引入pBI121-SN1质粒，替换原载体中*GUS*基因的启动子CaMV35S（图3-A），双酶切检测显示约2 000 bp的预期片段已正确插入pBI121-SN1载体中（图3-B）。



A. pBI121-PcMPK3pro-*GUS* 重组质粒结构图；B. pBI121-PcMPK3pro-*GUS* 重组质粒的酶切验证。

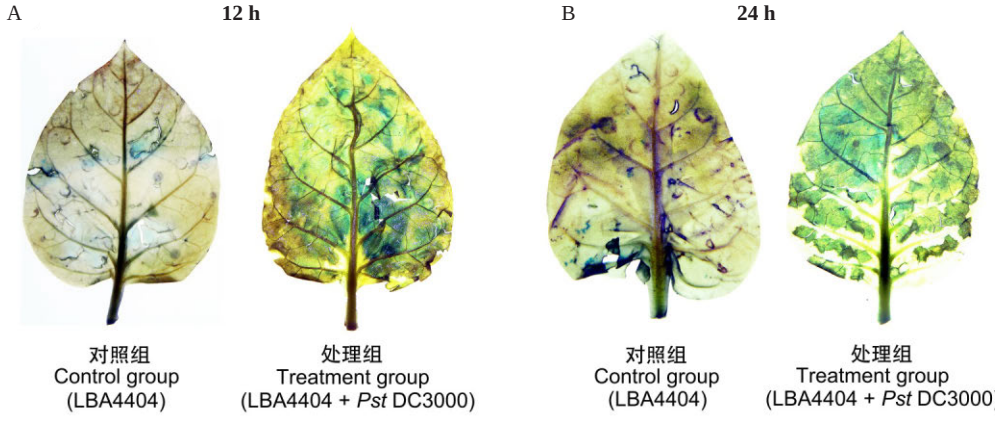
A. Structure of pBI121-PcMPK3pro-*GUS* vector; B. Enzyme digestion of pBI121-PcMPK3pro-*GUS* with *Bam*H I and *Hind* III.

图3 表达载体pBI121-PcMPK3pro-GUS的构建

Fig. 3 Construction of plant expression vector pBI121-PcMPK3pro-GUS

2.4 瞬时表达技术检测PcMPK3pro启动子对病原菌侵染的响应特性

以病原菌*Pst* DC3000与含有重组表达载体的农杆菌LBA4404的混合菌液注射法接种处理组烟草（*Pst* DC3000+LBA4404）叶片；以含有重组质粒的农杆菌LBA4404侵染液接种对照组烟草（LBA4404）叶片。GUS组织化学染色结果显示接种12 h后，处理组与对照组叶片均呈现蓝色区域，表明启动子PcMPK3pro能够驱动*GUS*报告基因的表达。接种12 h后，处理组叶片蓝色区域面积及强度均显著高于对照组叶片。接种24 h后，对照组与处理组GUS染色面积均有增加，但处理组蓝色面积及强度依然显著高于对照组叶片（图4）。GUS荧光定量活性分析显示，接种12 h后，处理组烟草叶片的GUS活性为对照组的4.42倍；接种24 h后，处理组烟草叶片的GUS活性为对照组的2.50倍（图5）。以上结果表明，启动子PcMPK3pro可响应病原菌*Pst* DC3000的侵染。



A. 接种 12 h 后，瞬时表达 *GUS* 基因的烟草叶片的组织化学染色；B. 接种 24 h 后，瞬时表达 *GUS* 基因的烟草叶片的组织化学染色。

A. Histochemical staining analysis of tobacco leaves 12 hours after inoculation; B. Histochemical staining analysis of tobaccos 24 hours after inoculation.

图4 不同处理下瞬时表达*GUS*基因的烟草叶片的组织化学染色

Fig. 4 Histochemical staining analysis of the tobacco leaves with *GUS* gene transient expression under different treatments

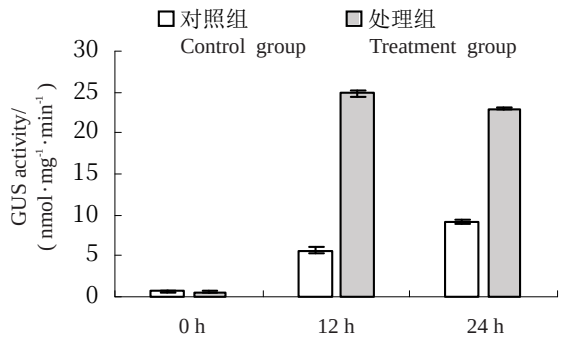


图5　不同处理下瞬时表达*GUS*基因的烟草叶片的GUS酶活性分析

Fig. 5　Quantitative analysis of GUS activity of the tobaccos with *GUS* transient expression under different treatments

3　讨　论

MAPK级联途径是植物应答逆境信号通路的重要成员，大量研究表明，参与应答逆境胁迫的植物MAPK家族成员多在转录水平开始调控[14-16]。以往较多关注胁迫刺激对植物MAPK的磷酸化激活及下游互作子的研究。例如，拟南芥AtMPK3/6能够被H_2O_2、ABA等植物信号分子激活[17-18]，并作用于ABA受体，从而调控种子发芽过程中ABA的含量[19]。关于*MAPK*基因的转录调控研究相对较少。启动子是植物基因重要的表达调控元件，分离、鉴定植物*MAPK*基因启动子结构及功能，对进一步探讨胁迫刺激对基因转录的强弱和效率的调控作用，利用基因工程技术改造植物抗逆性，具有重要意义。目前，辣椒、甘蓝型油菜等园艺植物已有*MAPK*基因启动子分离的相关研究[20-21]，但木本园艺植物中鲜有报道。

细菌性溃疡病是危害果树植物的主要病害之一，其致病原为一种丁香假单胞菌丁香致病变种*Pseudomonas syringae* pv. *syringae*[10]。一些研究认为，改变某些植物*MAPK*基因表达水平能够提高转基因植物的抗病能力[22-24]。吉塞拉（Gisela）系列砧木（*P. cerasus* × *P. canescens*）是目前甜樱桃生产中应用最广的矮化砧木[9]，由于其遗传背景的复杂性，利用基因工程方法是对该砧木品种进行遗传改良的最高效的策略。但目前在李属植物中尚无关于*MAPK*基因功能的研究。

笔者课题组前期研究表明，*PcMPK3*基因受丁香假单胞菌丁香致病变种*Pseudomonas syringae* pv. *syringae*诱导后表达显著上调，同时受SA、MeJA、ABA和H_2O_2诱导表达[12]。本研究以樱桃砧木Gisela 6为试材，采用Tail-PCR方法扩增*PcMPK3*基因5’UTR上游2 000 bp的序列，并对序列结构、启动子活性及对病原菌感染的响应特性进行分析。结果显示，启动子PcMPK3pro序列含有核心元件TATA-box和CAAT-box及多种响应防御与胁迫的顺式作用元件。例如，含有多个响应ABA的顺式作用元件ABRE，推测该基因可能与ABA信号途径相关；含有多个响应细菌及真菌激发子的顺式作用元件AT-rich sequence和W box，说明该基因可能在响应病原菌侵染刺激时起作用；含有多个响应MeJA、SA的CGTCA-motif和TCA-element，推测该基因可能通过MeJA和SA信号途径对病原菌触发的植物自身免疫反应进行调控；含有两个MYBHv1转录因子结合位点，推测*PcMPK3*基因可能在转录水平受Myb家族转录因子的调控；含有多个响应赤霉素的GARE-motif和P-box元件，说明该基因可能在参与调控植物对病原菌感染刺激反应的同时参与调节植株的生长发育。

综上所述，本研究进一步证明了该启动子的活性及对病原菌侵染刺激的响应特性，暗示*PcMPK3*基因可能通过MeJA、SA和ABA介导的信号通路对樱桃砧木Gisela 6的抗病反应起调控作用，并调节感病过程中植株的生长发育过程，此信号转导过程可能通过Myb家族转录因子进行调控。未来将进一步鉴定该启动子的顺式作用元件及其结合蛋白，为进一步解析*PcMPK3*调控的抗病性反应及转基因遗传改良奠定基础。

4　结　论

克隆得到樱桃砧木*PcMPK3*基因的5’侧翼序列PcMPK3pro，含有启动子核心元件TATA-box和CAAT-box及多种响应胁迫的顺式作用元件。受病原菌丁香假单胞菌番茄致病变种（*Pseudomonas syringae* pv. *tomato* DC3000，*Pst* DC3000）侵染，PcMPK3pro能驱动*GUS*报告基因迅速表达，GUS酶活性显著增加，推测*PcMPK3*基因参与植物对病原菌侵染的响应过程。

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