

猕猴桃病程相关蛋白 PR-1 基因的克隆和功能分析

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摘要:【目的】探究猕猴桃病程相关蛋白(pathogenesis-related proteins, PRs)PR-1基因在响应丁香假单胞杆菌中的功能。【方法】以毛花猕猴桃(*Actinidia eriantha*)为材料, 克隆得到PR-1同源基因AePR-1全长序列, 并对其序列进行生物信息学分析。采用实时荧光定量方法检测AePR-1基因在不同组织、花器官以及接种细菌性溃疡病菌(Psa)和不同激素(SA、ABA、GA₃)处理条件下的表达情况。利用亚细胞定位技术分析AePR-1基因在细胞中的表达位置。通过在本氏烟草中过表达AePR-1基因, 验证其在溃疡病菌响应过程中的功能。【结果】猕猴桃AePR-1基因序列全长522 bp, 编码173个氨基酸, 序列中含有6个保守的半胱氨酸结构基序和4个allergen V5/Tpx-1 related保守结构域。亚细胞定位发现AePR-1定位在细胞膜和细胞质中。AePR-1在猕猴桃根和雌蕊中高表达, 且能够响应溃疡病菌及激素处理。过表达AePR-1的烟草在接种溃疡病菌后, 叶片病斑数明显少于对照组。【结论】AePR-1基因在溃疡病菌和激素诱导下显著表达且过表达能够增强烟草对溃疡病的抗性, 说明猕猴桃PR-1基因在响应生物和非生物胁迫中具有重要作用。

关键词:猕猴桃; PR-1基因; 细菌性溃疡病菌(Psa); 抗病

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Cloning and function analysis of PR-1 gene in *Actinidia*

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Abstract:【Objective】China is the origin of the kiwifruit (*Actinidia* spp.), with rich germplasm resources and wide geographical distribution. It is one of the most recently domesticated fruit plants and has become an important horticultural crop. There are 54 species and 21 varieties of the *A.* Lindl. in the world. Kiwifruit bacterial canker is a devastating disease in kiwifruit industry globally and caused by pathogen *Pseudomonas syringae* pv. *actinidiae* (Psa). Psa is highly virulent, and once systemic invade plant may quickly lead plant to death. It has been documented that the Pathogenesis-related 1 protein (PR-1) could resist the spread of viruses, limit the invasion of pathogens and protect plants from adversity stress. In many plant species such as *Arabidopsis* and *tobacco*, the overexpression of the PR-1 gene could enhance plant resistance to *P. syringae*. However, the PR-1 gene in kiwifruit and its role in responses to abiotic stress remain largely unknown. The objective of this study was to explore the function of kiwifruit Pathogenesis-related 1 gene (PR-1) in response to biological stress. This analysis could contribute

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to in-depth understanding the function of the *PR-1* gene in kiwifruit disease resistance. 【Methods】 Annual grafted trees of kiwifruit species *A. eriantha* were used as experimental materials. The full-length sequence of the *PR-1* homologous gene *AePR-1* of *A. eriantha* was cloned and analyzed by multiple bioinformatic tools. The DNAMAN software was used to compare and analyze the sequence of the *AePR-1* gene. The conserved domain of AePR-1 protein sequence was analyzed by NCBI website. The ExPasy ProtParam tool was used to predict the molecular weight, theoretical pI, instability index and grand average of hydropathicity (GRAVY) of AePR-1 protein. The Cell-PLoc 2.0 software was used to predict the subcellular localization of PR-1 protein. The phylogenetic relationship between the AePR-1 protein and PR-1 of other plants was analyzed by the MEGA 11.013 software using neighbor-joining method. The qRT-PCR was performed to analyze the expression level of the *AePR-1* in different tissues and flower organ. The expression of the *AePR-1* gene in response to *P. syringae* pv. *Actinidiae* (Psa) bacterial solution and Jasmonic acid (JA), Salicylic acid (SA), Abscisic acid (ABA), Gibberellin A3 (GA₃) treatments was detected by real-time fluorescence quantitative PCR method. The samples were taken at 0 h, 6 h, 12 h, 24 h, 36 h, 48 h, 72 h and 96 h after treatment and immediately frozen in liquid nitrogen and stored at -80 °C for RNA isolation. The subcellular localization technology was used to analyze the expression position of the *AePR-1* gene in cells. The homologous recombination was used to construct the *AePR-1* overexpressed vector and heterologous expression was carried out in the tobacco to validate the function of the gene *PR-1* under Psa infection. All the experiments and data in this study involved at least three repeats. The Excel, SPSS and Origin2023 software were used to statistics and analysis of test measurement data. LSD test (*p*<0.05) was used to assess significant differences in means. 【Results】 The full length of the cloned *AePR-1* gene sequence was 522 bp, encoded 173 amino acids and contained CAP_PR-1 conserved domain. The relative molecular weight (MW) of protein was 19.28 ku and the isoelectric points (PI) was 9.28. The protein instability index was 41.54 and the protein belonged to unstable protein and the grand average of hydropathicity (GRAVY) was -0.261. The subcellular localization of PR-1 showed that the *AePR-1* gene was mainly localized in the cytoplasm and cell membrane. The phylogenetic tree results showed that the protein AePR-1 was highly homologous to the AthPR-1 from *Arabidopsis* and CsaPR-1 from *Cucumis*. The qRT-PCR results showed that *AePR-1* gene was highly expressed in the roots and pistils. And after inoculation with Psa bacterial solution, the expression level of the *AePR-1* gene decreased at the early stage, increased rapidly after 6 hours, and reached its peak at 24 hours. With the prolongation of the treatment time of different hormones, the expression of the *AePR-1* gene generally showed two peaks. The *AePR-1* expressed the highest on the second day after overexpression of tobacco. Therefore, Psa was used to infect injected tobacco on the second day of tobacco transient expression. The results showed that on the 14th day after infection, the leaves of the empty control group showed large areas of yellow spots, while a small number of yellow spots appeared on the surface of the leaves overexpressing the *AePR-1* gene. 【Conclusion】 This study explored the anti-disease effect of kiwifruit *AePR-1* gene in kiwifruit bacterial canker. The results showed that the *AePR-1* gene of kiwifruit was expressed in large quantities under the induction of Psa bacteria and exogenous hormones, participated in the immune response of kiwifruit and enhanced the disease resistance of kiwifruit. This study shows that the *AePR-1* gene plays an important role in the disease resistance of kiwifruit and can be valuable for resistance breeding of kiwifruit.

Key words: *Actinidia*; *PR-1* gene; *Pseudomonas syringae* pv. *actinidiae* (Psa); Disease-resistant

植物会产生一类病程相关蛋白(pathogenesis-related proteins, PRs)来抵御病原微生物的侵染或非生物因子的刺激^[1]。自从在烟草花叶病毒(TMV)侵染烟草叶片试验中首次检测到该蛋白至今^[2], PR蛋白的研究得到了广泛关注。超敏反应(HR)、系统获得性反应(SAR)、信号通路、生物及非生物胁迫都能引起植物体内PR蛋白的响应和积累^[3-5]。PR蛋白相对分子质量较小,可分为酸性蛋白和碱性蛋白,稳定性较强,能有效地在细胞内和细胞间积累^[6],在植物适应不良环境方面具有重要作用。

PR蛋白可划分为17个家族,大多数可参与防御信号传导,从而提高植物对病原菌的抗性^[7]。如PR-3、PR-4、PR-8和PR-11等蛋白具有几丁质酶活性^[6];PR-5蛋白具有抵御真菌侵染、激活防御反应的作用^[8];PR-6具有蛋白酶抑制剂活性,能够抵抗线虫和昆虫的入侵^[9];PR-10具有核糖核酸酶活性^[10];PR-15和PR-16具有细胞重塑、类萌发素等特性^[11]。PR-1具有甾醇结合活性和防御信号肽,可参与植物超敏反应和系统获得性反应,在植物抗病过程中发挥重要作用^[12-13]。欧洲花椒*SaPRI-like*基因在生物胁迫下显著表达,增强了植株的抗逆性^[14];水稻*PR-1*基因家族成员*PR-1-3*和*PR-1-13*在接种水稻纹枯病菌(*Thanatephorus cucumeris*)^[15]和白叶枯病菌(*Xanthomonas oryzae*)后^[16]表达量均显著提高;辣椒*CAB-PRI*基因在烟草中过表达后,能够增强转基因烟草对重金属胁迫的耐受性,以及对烟草疫霉菌(*Phytophthora nicotianae*)、青枯菌(*Ralstonia solanacearum*)和丁香假单胞杆菌(*Pseudomonas syringae* pv. *tabaci*)的抗性^[17]。拟南芥*PR-1*基因的积累增强了其对丁香假单胞杆菌(*P. syringae* pv. *tomato* DC3000)的抗性;将葡萄*VvPR1b1*基因过表达于烟草中,能增强其对烟草野火病病原细菌(*P. syringae* pv. *tabaci*)的抗性^[18-19]。从上述可见,不同物种的PR-1蛋白受诱导条件不同,其作用机制尚不一致,但其在防御反应中都具有相似的功能。

丁香假单胞杆菌(*P. syringae* pv. *actinidiae*, Psa)是引起猕猴桃(*Actinidia Lindl.*)细菌性溃疡病的致病菌^[20-21],该病害具有传染性强、蔓延快、致病性强、根除难度大等特点,目前尚无有效的根治方法^[22]。从1984年首次在日本发现至今,细菌性溃疡病已成为制约我国乃至世界猕猴桃产业发展的重要病害^[23-24]。研究表明,茉莉酸、水杨酸和脱落酸等植物

激素能诱导*PR-1*基因表达而对病害具有防御作用^[25]。迄今为止,PR-1蛋白在防御反应中的功能在番茄^[26]、葡萄^[27]、柑橘^[28]、核桃^[29]、小麦^[30]、玉米^[31]等多种植物上已有相关研究,但在猕猴桃中未见报道。笔者在前期系统评价鉴定猕猴桃不同品种(系)溃疡病抗性的基础上^[32],以筛选的抗病种质为试材,分析了*PR-1*基因的结构、组织表达及响应溃疡病和外源激素的表达情况,并进一步明确其在植物病原响应过程中的功能,以期为寻找猕猴桃细菌性溃疡病抗性基因及解析抗病机制提供理论基础。

1 材料和方法

1.1 材料

毛花猕猴桃(*A. eriantha*)品种华特嫁接苗,砧木为对萼猕猴桃(*A. valvata*)品种中猕抗砧1号,保存于国家园艺种质资源库猕猴桃分库(郑州);本氏烟草(*N. benthamiana*)由中国农业科学院郑州果树研究所栽培生理实验室保存;Psa病菌由浙江省农业科学院园艺研究所提供。猕猴桃苗木嫁接后在营养钵中培养3个月,再移至人工气候箱中培养,条件设置为温度25 °C、相对湿度80%、12 h光照/12 h黑暗。Psa菌液采用金氏培养基培养,在20 °C、200 r·min⁻¹摇床振荡24 h,浓度稀释至1×10⁸ cfu·mL⁻¹后进行侵染。使用5 mmol·L⁻¹水杨酸(SA)、250 mg·L⁻¹赤霉素(GA₃)、50 μmol·L⁻¹脱落酸(ABA)对嫁接苗进行叶面喷施处理,并置于人工气候箱中培养至96 h。试验过程中采集的叶片样本经液氮处理后均置于-80 °C冰箱保存备用。

1.2 RNA提取、反转录

利用多糖多酚RNA提取试剂盒(天根生化科技公司)提取样品总RNA,于-80 °C冰箱保存备用。使用TOYOBO ReverTra Ace qPCR RT Kit(FSQ-101)将RNA反转录合成cDNA,反应体系为4 μL 5×RT Buffer、1 μL Enzyme Mix、1 μL Primer Mix、4 μL RNA、10 μL ddH₂O。先将RNA和ddH₂O加入PCR管中,采用PCR仪65 °C变性5 min后置于冰上,再加入其他组分,反应程序:37 °C、15 min,98 °C、5 min。反转录后的cDNA保存于-20 °C冰箱备用。

1.3 *PR-1*基因克隆及系统发育分析

在前期组学研究的基础上^[33],通过在猕猴桃基因组数据库^[34]中进行目的序列比对,提取目的基因*AePR-1*的CDS序列,使用Primer Premier 6.0软件设

引物(上游引物:5'-ATGGGGTGGTTGTGTA-3',下游引物:5'-CTAAATATTTCTACATAGGTC-3')扩增全长序列;使用2×TransStart® Fast Pfu Master Mix(北京全式金生物技术有限公司)进行PCR扩增,PCR产物回收后连接T载体(pClone007 Blunt Simple Vector Kit,北京擎科生物科技有限公司),并转化至大肠杆菌感受态细胞,挑取单克隆菌落检测后送测序。引物合成和测序在上海生工生物工程有限公司进行。使用Expasy ProtParam工具对AePR-1序列进行相对分子质量、等电点及稳定性等理化特性分析;使用DNAMAN软件对AePR-1基因序列进行比对分析;在NCBI网站分析AePR-1蛋白序列的保守结构域;用猕猴桃PR-1蛋白序列在phytozome数据库中Blast检索其他同源序列,并使用MEGA-X软件的邻接法(Neighbor-Joining)构建系统发育树,进行系统发育分析。

1.4 PR-1基因的表达分析

采用实时荧光定量技术,分析AePR-1基因在不同组织(根、茎、叶、花、果)和花器官(雌蕊、雄蕊、子房、花瓣、萼片、花托),以及病原菌Psa和激素(SA、GA₃、ABA)处理后0、6、12、24、36、48、72、96 h的表达情况。荧光定量引物为AePR-1F:5'-AAGACTACCTAACGCCACAAAC-3',AePR-1R:5'-TTCTTCTCGTCCACCCACATT-3';荧光定量试剂为NovoStart SYBR qPCR SuperMix Plus;反应体系为10 μL 2×NovoStart SYBR qPCR SuperMix、2 μL cDNA、1 μL PR1 Primer Forward、1 μL PR1 Primer Reverse、6 μL RNase Free Water;反应程序为95 °C预变性5 min,95 °C变性20 s,60°C退火20 s,72 °C延伸20 s,变性至延伸步骤为40个循环。采用2^{-ΔΔCT}法计算每个样品相对猕猴桃内参基因β-actin的表达量^[35]。所有反应均包含3个独立的生物学重复和技术重复。

1.5 亚细胞定位

使用一步定向克隆试剂盒(上海近岸科技有限公司),将目的片段克隆到pCAM35s-GFP载体位点中(上游引物:5'-GGGGACGAGCTCGGTACCAT-GGGGTGGTTGTGTTAGGATG-3',下游引物:5'-CATGGTGTGCACTCTAGAAATATTTCTACATA-GGTCTTAAGCTTAATACATAGG-3'),形成融合表达载体后转化大肠杆菌感受态细胞,单克隆菌落PCR阳性鉴定后送上海生工生物工程有限公司测

序。将测序正确的阳性质粒转化农杆菌GV3101。以空载体pCAM35s-GFP为对照,采用注射方法侵染烟草下表皮^[36],置于人工气候箱中培养(条件设置同1.1)。培养2~3 d后,使用激光共聚显微镜对侵染区域进行亚细胞定位观察。

1.6 过表达载体构建及烟草瞬时转化

采用同源重组的方法将猕猴桃PR-1基因插入到表达载体PBI121中(PR-1-121-F:5'-AACACGGGGACTCTAGAATGGGGTGGTTGTGTA-3',PR-1-121-R:5'-CTGACCACCCGGGGATCCCTAAAT-ATTTCTACATAGGTC-3'),转化农杆菌GV3101后,以空载体PBI121作对照,采用烟草下表皮注射的方法进行瞬时表达^[36],将处理和对照均放置人工培养箱中培养(条件设置同1.1)。在接种1、2和3 d时分别检测PR-1的表达量,筛选Psa菌液侵染瞬时表达烟草的最佳时间。然后将Psa菌液注射到瞬时表达的阳性烟草叶片中,处理后放于上述条件的人工气候箱中继续培养,连续观察叶片的表型变化^[37]。

1.7 数据分析

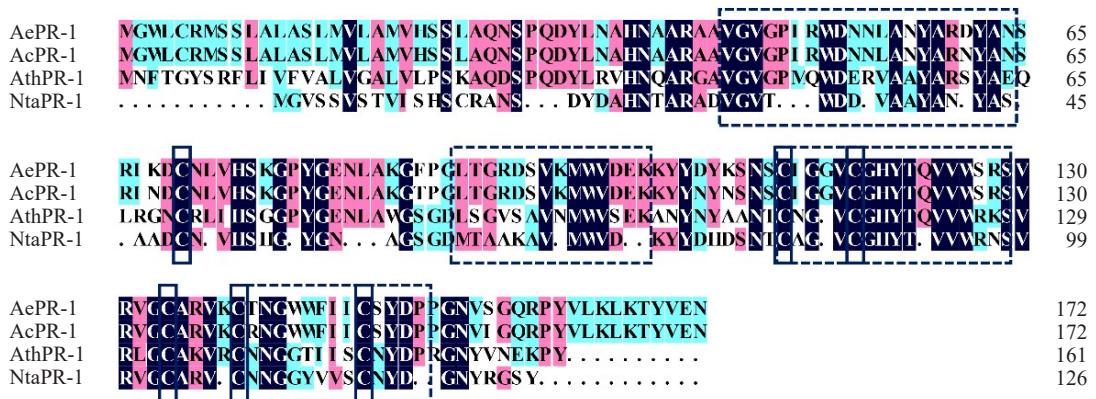
采用Excel和Origin 2023软件对试验数据进行统计分析及相关图表绘制,利用Dunn-Sidak多重比较方法进行差异显著性检验,显著水平设定为0.05。

2 结果与分析

2.1 PR-1基因的克隆及序列分析

在华特猕猴桃中克隆获得1条PR-1基因,命名为AePR-1。克隆得到cDNA全长为522 bp,共编码173个氨基酸。通过在线网站Expasy对其理化特性分析,表明AePR-1蛋白相对分子质量为19.28 ku、等电点为9.28、不稳定系数为41.54。将AePR-1的序列与中华猕猴桃(*A. chinensis* Planch. var. *chinensis*)AcPR-1序列进行同源比对,显示基因CDS序列中共有12个碱基位点不同、蛋白序列中有7个氨基酸不同,但二者皆编码了173个氨基酸。通过对AePR-1与AcPR-1、AthPR-1、NtaPR-1同源蛋白序列分析,发现这些序列都具有6个保守的半胱氨酸结构基序(实线框表示)和4个allergen V5/Tpx-1 related保守的结构域(虚线框表示)(图1)。

构建了猕猴桃AePR-1与其他植物PR-1蛋白的系统发育树,发现发育树分为包含了大桉EgrPR-1、大豆GmaPR-1等分支和可可TcaPR-1、葡萄VviPR-1等分支的两个明显大分支(图2)。毛花猕猴桃

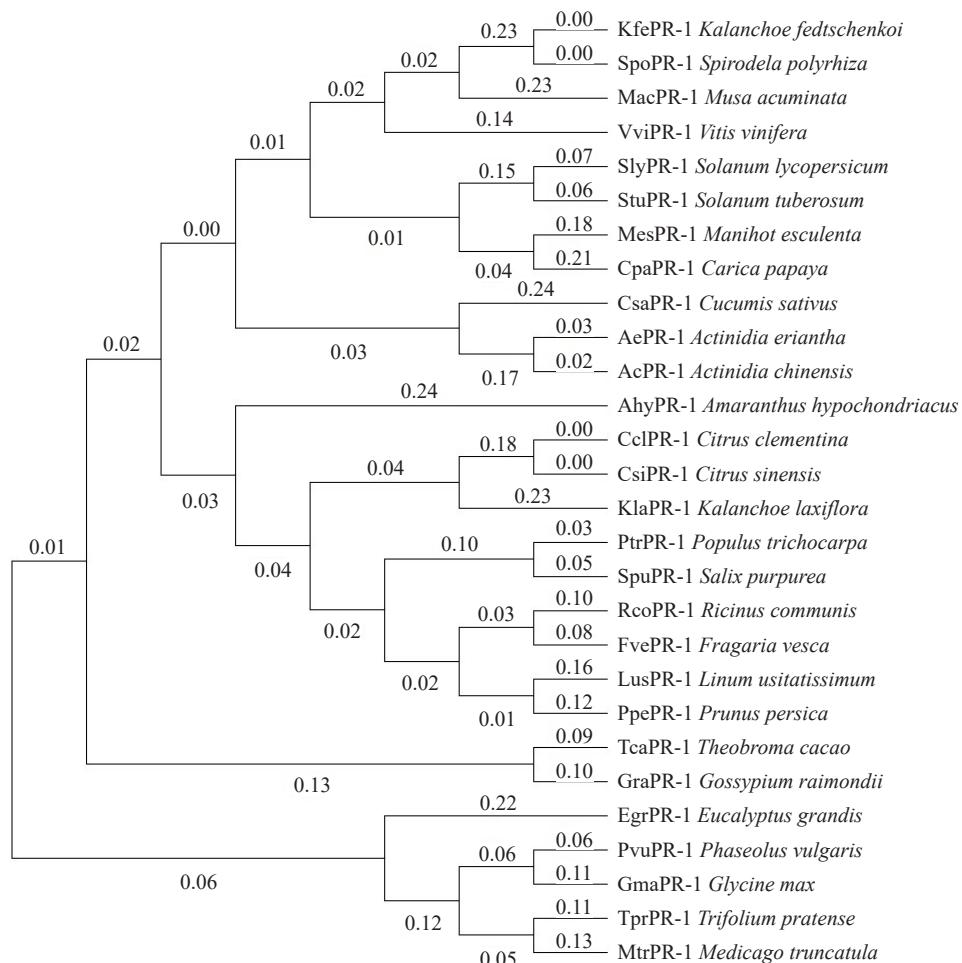


毛花:AePR-1 (DTZ79_19g02670); 中华:AcPR-1 (Ach26g253311); 拟南芥:AthPR-1 (AT2G14610); 烟草:NtaPR-1 (P08299)。实线框表示保守的半胱氨酸结构基序,虚线框表示 allergen V5/Tpx-1 related 保守的结构域。

A. eriantha: AePR-1 (DTZ79_19g02670); *A. chinensis*: AcPR-1 (Ach26g253311); *Arabidopsis thaliana*: AthPR-1 (AT2G14610); *Nicotiana tabacum*: NtaPR-1 (P08299). Solid border indicates conserved cysteine, dotted box indicates conserved allergen V5/Tpx-1 related domain.

图 1 AePR-1 与其他 PR-1 蛋白序列同源性分析

Fig. 1 Alignment of the predicted amino acid sequences of AePR-1 and other PR-1



*AePR-1*与中华猕猴桃*AcPR-1*及黄瓜*CsaPR-1*具有很高的同源性。

2.2 *PR-1*基因的亚细胞定位分析

为揭示*AePR-1*蛋白的定位情况,构建了pCAM35s-*PR-1*-GFP融合表达载体并转化烟草表皮细胞,并以pCAM35s-GFP空载体为对照,培养48 h后在激光共聚焦显微镜下观察。如图3所示,对照组的空载载体在细胞膜、细胞质和细胞核中均能发

出绿色荧光,而试验组的烟草叶片荧光信号主要集中在细胞膜上和细胞质中,表明*PR-1*主要在细胞膜和细胞质中表达,是功能基因。

2.3 *PR-1*基因的表达分析

为了解*AePR-1*基因的时空表达特征,首先对*AePR-1*基因在猕猴桃不同组织和花器官中的表达量进行了分析,发现该基因在根和雌蕊中高表达,而在叶、花瓣、雄蕊和子房中低表达(图4-A~B)。

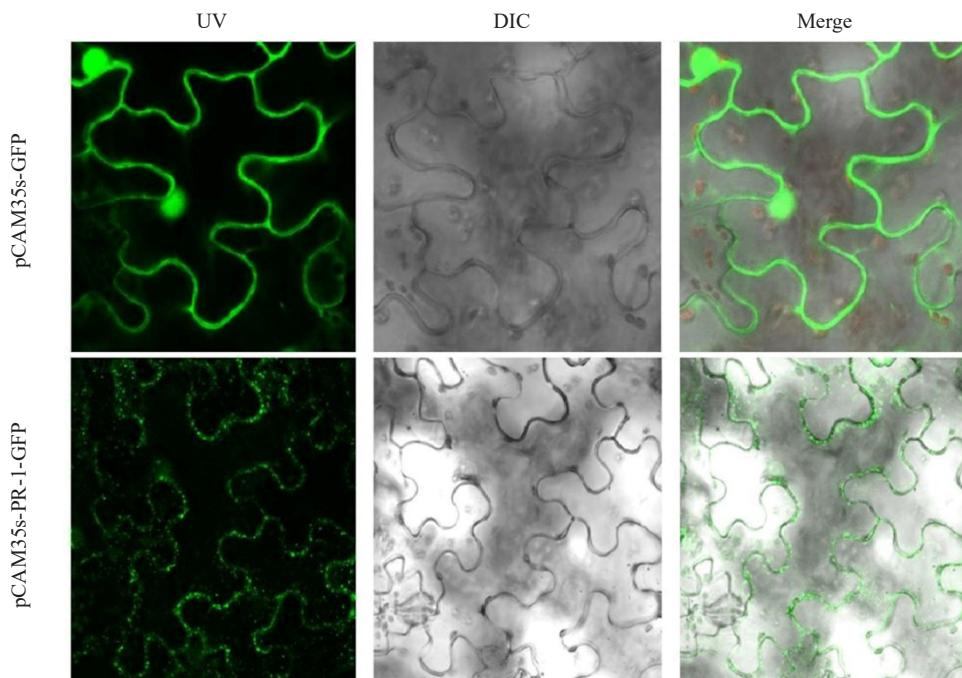
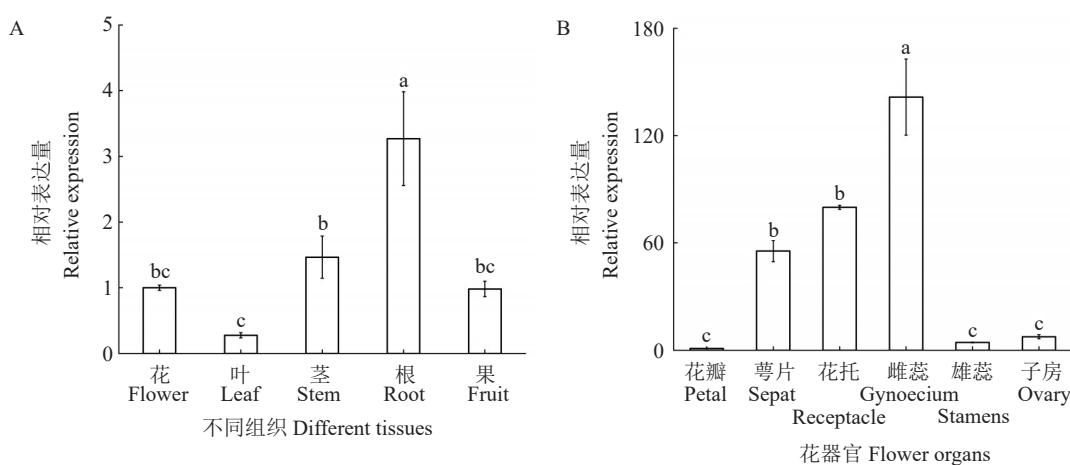


图3 *PR-1*基因在烟草表皮细胞中的亚细胞定位
Fig. 3 Subcellular localization of *PR-1* in tobacco epidermal cells



A. 不同组织相对表达量;B. 花器官相对表达量。不同小写字母代表差异显著 $p<0.05$ 。下同。
A. The relative expression of different tissues; B. The relative expression of flower organs. Different small letters represent significant difference at $p<0.05$. The same below.

图4 *PR-1*基因在不同组织和花器官中的表达分析
Fig. 4 Analysis of *PR-1* gene expression in different tissues and flower organs

在接种 Psa 菌液后, *AePR-1* 基因相对表达量呈现出先降低后迅速升高的趋势, 在接种 24 h 时达到最大值, 后又逐渐下降(图 5-A)。

在不同激素处理过程中, 随着处理时间的延长, *AePR-1* 基因相对表达量均呈双峰曲线。在 SA 和

ABA 处理下, *AePR-1* 基因表达量在 24 h 时达到第一次峰值(图 5-B~C); GA₃ 处理可诱导 *AePR-1* 基因迅速表达, 接种 12 h 时达到最大值(图 5-D)。3 种激素处理下首次出现峰值的时间存在差异, 但在 48 h 时均再次达到峰值, 表明 *AePR-1* 基因对不同激素响应

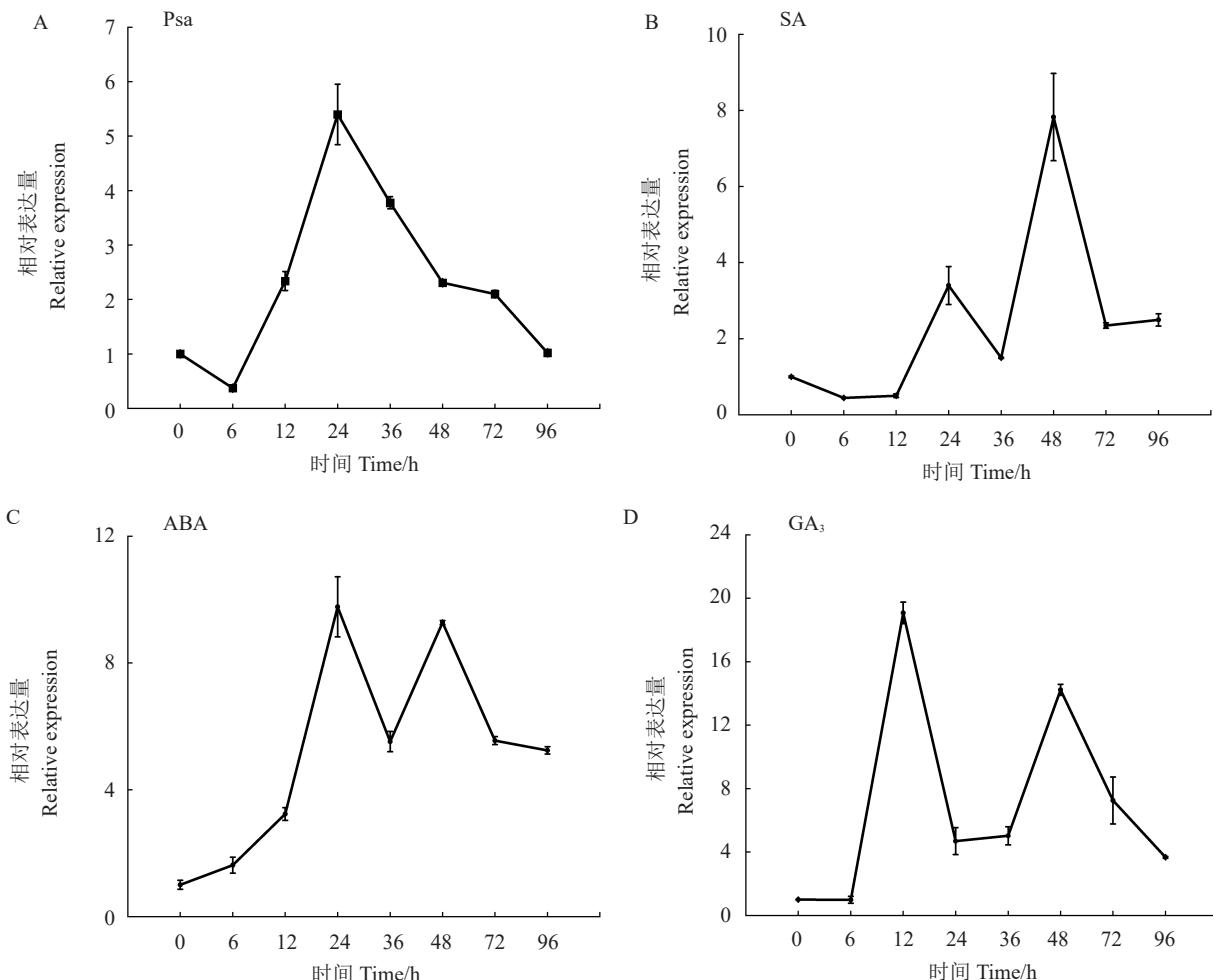


图 5 *PR-1* 基因在 Psa 侵染及不同激素处理下的表达分析

Fig. 5 Analysis of *PR-1* gene expression after Psa infection and hormone treatment

模式存在差异。

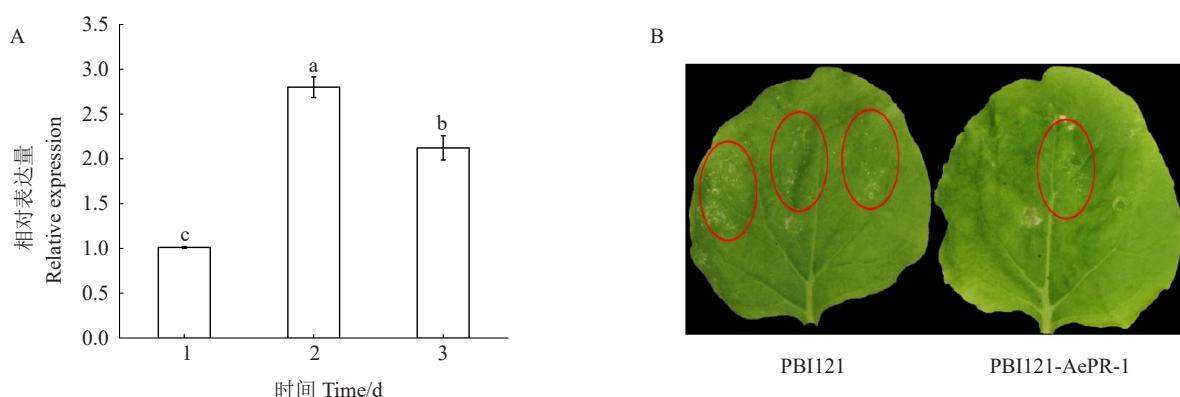
2.4 *PR-1* 基因增强了烟草对 Psa 的抗性

以 pBI121 空载体为对照, 构建过表达载体 pBI121-*AePR-1* 瞬时表达本氏烟草后发现, *AePR-1* 表达量在 2 d 最高(图 6-A)。因此, 在烟草瞬时表达后的 2 d 使用 Psa 菌液侵染, 将接种后的烟草放入人工气候箱中培养, 观察烟草的发病情况。结果表明, 在侵染后第 14 天, 在空载对照组的叶片表面上出现了大量的黄色病斑, 而过表达 *AePR-1* 基因的叶片表面出现少量黄色斑点(图 6-B)。以上结果表明, *AePR-1* 基因能增强本氏烟草对 Psa 的抗性。

3 讨 论

植物受到生物或非生物胁迫时, 会激活其免疫防御反应, 而 *PR-1* 基因是 SAR 反应的标志基因, 在抗病反应中具有积极作用^[38]。牡丹受叶斑病菌(*Cylindrocladium canadense*)侵染后, *PsPR-1* 基因被显著诱导参与抗病反应, 且在 24 h 达到最高峰^[39]。这与本研究结果相似, 猕猴桃在接种 Psa 病菌后, *PR-1* 表达量显著升高, 在 24 h 达峰值, 表明其参与了抗病防御反应。

笔者以华特猕猴桃为材料克隆得到了 *AePR-1* 基因, 通过生物信息学分析发现, 该基因的蛋白序列

图 6 *AePR-1* 瞬时表达烟草后表达分析 (A) 及 Psa 侵染结果 (B)Fig. 6 The expression patterns of *AePR-1* after transient expression of tobacco (A) and infection results of Psa (B)

与其他物种的PR-1具有高度的同源性,其理化特性与葡萄^[25]、向日葵^[40]、核桃^[29]等相似且包含了相同的保守结构域。具有该结构域的蛋白积极参与了先天性免疫反应和适应性免疫反应,从而提高植株的抗逆性^[41]。这表明不同植物中的PR-1基因在防御反应中可能存在相似功能。

进一步对PR-1基因在不同组织和花器官中的表达量检测,发现猕猴桃在根中高表达,而大豆则表现为在叶片中高表达^[42],这可能是由于不同物种中PR-1基因表达模式存在差异,猕猴桃PR-1基因的组织特异性表达差异可能与其在植物生长发育及胁迫反应中的不同作用相关。

植物激素能诱导PR-1基因表达量的升高,在防御反应中发挥至关重要的作用^[25, 43]。在葡萄中,JA、SA和ABA均能诱导叶片*VvPRI*基因的显著表达^[25]。水稻中,*OsPRIa*基因受JA、SA和ET的诱导而显著表达^[44];喷施外源SA可以显著降低褐斑病(*P. oryzicola*)的发病率,同时PR-1和NPRI的相对表达量均显著升高^[45]。笔者采用不同激素诱导处理,发现对猕猴桃PR-1基因表达趋势的整体影响相同,但具体表达量与峰值出现时间存在差异。*AePR-1*基因对不同激素的响应差异可能与其启动子中对不同激素的响应元件的有无及数量有关。其中在GA₃处理条件下,*AePR-1*基因响应表现最早且表达量最高,其次依次为ABA和SA。本研究结果与前人研究结果相似,在外源激素诱导下,PR-1基因表达量升高。还有研究发现,干旱、低温、盐等胁迫及H₂O₂、H₂S等因素也能显著诱导PR-1基因的表达^[46]。

本研究结果表明,猕猴桃PR-1基因在响应生物

侵染过程中发挥重要作用,并揭示了过表达*AePR-1*基因能够增强烟草对Psa病菌的抗性。今后有必要进一步开展以激素诱导为前提提高猕猴桃植物抗细菌性溃疡病的作用机制研究,以期为科学防控该病害提供理论和实践基础。

4 结 论

笔者探讨了*AePR-1*基因在猕猴桃细菌性溃疡病和外源激素侵染中发挥的作用。结果表明,猕猴桃PR-1基因在Psa病菌和外源激素诱导下均可大量表达,参与了免疫应答反应,从而增强了猕猴桃的抗病性。笔者研究证实了PR-1基因在猕猴桃抗病过程中具有重要作用。

参考文献 References:

- [1] VAN LOON L C, PIERPOINT W S, BOLLER T, CONEJERO V. Recommendations for Naming plant pathogenesis-related proteins[J]. Plant Molecular Biology Reporter, 1994, 12(3):245-264.
- [2] VAN LOON L C, VAN KAMMEN A. Polyacrylamide disc electrophoresis of the soluble leaf proteins from *Nicotiana tabacum* var. 'Samsun' and 'Samsun NN' II. Changes in protein constitution after infection with tobacco mosaic virus[J]. Virology, 1970, 40(2):199-211.
- [3] LIU J J, EKRAMODDOULLAH A K M. The family 10 of plant pathogenesis-related proteins: Their structure, regulation, and function in response to biotic and abiotic stresses[J]. Physiological and Molecular Plant Pathology, 2006, 68(1/2/3):3-13.
- [4] VAN LOON L C, VAN STRIEN E A. The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins[J]. Physiological and Molecular Plant Pathology, 1999, 55(2):85-97.
- [5] BALINT-KURTI P. The plant hypersensitive response: Concepts, control and consequences[J]. Molecular Plant Pathology, 2019, 20(8):1163-1178.

- [6] 焦文晓,王晓梅,范新光,姜微波.病程相关蛋白在采后果蔬诱导抗病性中的研究进展[J].保鲜与加工,2020,20(1):206-211.
JIAO Wenxiao, WANG Xiaomei, FAN Xinguang, JIANG Weibo. Advances of research on pathogenesis-related protein in induction of disease resistance of postharvest fruits and vegetables[J]. Storage and Process, 2020, 20(1):206-211.
- [7] VAN LOON L C, REP M, PIETERSE C M J. Significance of inducible defense-related proteins in infected plants[J]. Annual Review of Phytopathology, 2006, 44:135-162.
- [8] EL-KEREAMY A, EL-SHARKAWY I, RAMAMOORTHY R, TAHERI A, ERRAMPALLI D, KUMAR P, JAYASANKAR S. *Prunus domestica* pathogenesis-related protein-5 activates the defense response pathway and enhances the resistance to fungal infection[J]. PLoS One, 2011, 6(3):e17973.
- [9] SELITRENNIKOFF C P. Antifungal proteins[J]. Applied and Environmental Microbiology, 2001, 67(7):2883-2894.
- [10] 赵琦,崔梦杰,韩锁义,郭腾达,杜培,刘华,徐静,黄冰艳,董文召,张新友.植物病程相关蛋白 PR10 及其在花生中的研究进展[J].四川农业大学学报,2024,42(1):1-10.
ZHAO Qi, CUI Mengjie, HAN Suoyi, GUO Tengda, DU Pei, LIU Hua, XU Jing, HUANG Bingyan, DONG Wenzhao, ZHANG Xinyou. Plant pathogenesis-related protein PR10 and their research progress in peanut[J]. Journal of Sichuan Agricultural University, 2024, 42(1):1-10.
- [11] PARK C J, AN J M, SHIN Y C, KIM K J, LEE B J, PAEK K H. Molecular characterization of pepper germin-like protein as the novel PR-16 family of pathogenesis-related proteins isolated during the resistance response to viral and bacterial infection[J]. Planta, 2004, 219(5):797-806.
- [12] 李爽,熊樱,müllerxing r,邢倩.转录因子 WRKY6 和 PR1 在拟南芥胁迫记忆中的表达模式[J].植物研究,2019,39(5):752-759.
LI Shuang, XIONG Ying, MÜLLERXING R, XING Qian. Distinct expression patterns of WRKY6 and PR1 in *Arabidopsis* stress memory assays[J]. Bulletin of Botanical Research, 2019, 39(5):752-759.
- [13] BREEN S, WILLIAMS S J, OUTRAM M, KOBE B, SOLOMON P S. Emerging insights into the functions of pathogenesis-related protein 1[J]. Trends in Plant Science, 2017, 22(10):871-879.
- [14] 刘亚辉,李佳兴,王升,王铁霖,袁杰,郭兰萍.欧洲花楸病程相关蛋白基因 SaPR1-like 的克隆及序列分析[J].中国实验方剂学杂志,2019,25(21):118-123.
LIU Yahui, LI Jiaxing, WANG Sheng, WANG Tielin, YUAN Jie, GUO Lanping. Cloning and sequence analysis of pathogenesis related protein 1-like gene from *Sorbus aucuparia*[J]. Chinese Journal of Experimental Traditional Medical Formulae, 2019, 25(21):118-123.
- [15] LIU Z Y, LI X J, SUN F, ZHOU T, ZHOU Y J. Overexpression of *OsCIPK30* enhances plant tolerance to *Rice stripe virus*[J]. Frontiers in Microbiology, 2017, 8:2322.
- [16] 程雨果,魏炳峰,李春霞,陶均.水稻 PR1 基因响应生物胁迫的表达模式[J/OL].分子植物育种,2023:1-24. (2023-06-20). <https://kns.cnki.net/kcms/detail/46.1068.s.20230619.1458.002.html>.
- [17] CHENG Yuguo, WEI Bingzheng, LI Chunxia, TAO Jun. Expression patterns of rice PR1 family genes in response to biotic stresses[J/OL]. Molecular Plant Breeding, 2023: 1-24. (2023-06-20). <https://kns.cnki.net/kcms/detail/46.1068.s.20230619.1458.002.html>.
- [18] SAROWAR S, KIM Y J, KIM E N, KIM K D, HWANG B K, ISLAM R, SHIN J S. Overexpression of a pepper basic pathogenesis-related protein 1 gene in tobacco plants enhances resistance to heavy metal and pathogen stresses[J]. Plant Cell Reports, 2005, 24(4):216-224.
- [19] LI Z T, DHEKNEY S A, GRAY D J. PR-1 gene family of grapevine: A uniquely duplicated PR-1 gene from a *Vitis interspecific* hybrid confers high level resistance to bacterial disease in transgenic tobacco[J]. Plant Cell Reports, 2011, 30(1):1-11.
- [20] SEO J S, DILOKNAWARIT P, PARK B S, CHUA N H. *elf18*-induced long noncoding RNA 1 evicts fibrillarin from mediator subunit to enhance pathogenesis-related gene 1 (Pr1) expression[J]. New Phytologist, 2019, 221(4):2067-2079.
- [21] DONATI I, CELLINI A, SANGIORGIO D, VANNESTE J L, SCORTICHINI M, BALESTRA G M, SPINELLI F. *Pseudomonas syringae* pv. *actinidiae*: Ecology, infection dynamics and disease epidemiology[J]. Microbial Ecology, 2020, 80(1):81-102.
- [22] 裴艳刚,马利,岁立云,崔永亮,刘晓敏,龚国淑.不同猕猴桃品种对溃疡病菌的抗性评价及其利用[J].果树学报,2021,38(7):1153-1162.
PEI Yangang, MA Li, SUI Liyun, CUI Yongliang, LIU Xiaomin, GONG Guoshu. Resistance evaluation and utilization of different kiwifruit cultivars to *Pseudomonas syringae* pv. *actinidiae*[J]. Journal of Fruit Science, 2021, 38(7):1153-1162.
- [23] 钟彩虹,李黎,潘慧,邓蕾,陈美艳.猕猴桃细菌性溃疡病的发生规律及综合防治技术[J].中国果树,2020(1):9-13.
ZHONG Caihong, LI Li, PAN Hui, DENG Lei, CHEN Meiyuan. Occurrence rule and comprehensive control of kiwifruit bacterial canker disease[J]. China Fruits, 2020(1):9-13.
- [24] VANNESTE J L. The scientific, economic, and social impacts of the new zealand outbreak of bacterial canker of kiwifruit (*Pseudomonas syringae* pv. *actinidiae*)[J]. Annual Review of Phytopathology, 2017, 55:377-399.
- [25] SAWADA H, FUJIKAWA T. Genetic diversity of *Pseudomonas syringae* pv. *actinidiae*, pathogen of kiwifruit bacterial canker[J]. Plant Pathology, 2019, 68(7):1235-1248.
- [26] 侯丽霞,高超,车永梅,赵方贵,刘新.葡萄病程相关蛋白 1 基因的克隆和表达分析[J].植物生理学报,2012,48(1):57-62.
HOU Lixia, GAO Chao, CHE Yongmei, ZHAO Fanggui, LIU Xin. Gene cloning and expression analysis of pathogenesis-related protein 1 in *Vitis vinifera* L[J]. Plant Physiology Journal, 2012, 48(1):57-62.
- [27] KARABULUT D, CALIS O. The role of *PR1* gene in resistance mechanism to bacterial canker and wilting disease in promising tomato mutant plants[J]. Journal of Tekirdag Agriculture Faculty, 2022, 19(1):120-131.
- [28] LI P, TAN X B, LIU R T, RAHMAN F U, JIANG J F, SUN L,

- FAN X C, LIU J H, LIU C H, ZHANG Y. QTL detection and candidate gene analysis of grape white rot resistance by interspecific grape (*Vitis vinifera* L. × *Vitis davidii* Focke) crossing[J]. Horticulture Research, 2023, 10(5):uhad063.
- [28] 郝晨星.响应柑橘溃疡病菌侵染的枸橼C-05抗性基因PR4A的鉴定[D].长沙:湖南农业大学,2019.
- HAO Chenxing. Identification of a defence gene *PR4A* from citron C-05 in response to *Xanthomonas citri* subsp. *citri* infection[D]. Changsha:Hunan Agricultural University,2019.
- [29] 李生萍.核桃*PRI*与*PR4*基因的克隆及表达分析[D].杨凌:西北农林科技大学,2017.
- LI Shengping. Cloning and expression analysis of *PRI* and *PR4* genes in walnut[D]. Yangling: Northwest A & F University, 2017.
- [30] BREEN S, WILLIAMS S J, WINTERBERG B, KOBE B, SOLOMON P S. Wheat PR-1 proteins are targeted by necrotrophic pathogen effector proteins[J]. The Plant Journal, 2016, 88(1): 13-25.
- [31] 石凤梅.病程相关蛋白基因*ZmPR-1*和*ZmPR-4*的克隆及功能研究[D].哈尔滨:东北林业大学,2019.
- SHI Fengmei. Cloning and functional study of pathogenesis-related protein genes *ZmPR-1* and *ZmPR-4*[D]. Harbin: Northeast Forestry University,2019.
- [32] 宋雅林,林苗苗,钟云鹏,陈锦永,齐秀娟,孙雷明,方金豹.猕猴桃品种(系)溃疡病抗性鉴定及不同评价指标的相关性分析[J].果树学报,2020,37(6):900-908.
- SONG Yalin, LIN Miaomiao, ZHONG Yunpeng, CHEN Jinyong, QI Xiujuan, SUN Leiming, FANG Jinbao. Evaluation of resistance of kiwifruit varieties (line) against bacterial canker disease and correlation analysis among evaluation indexes[J]. Journal of Fruit Science, 2020, 37(6):900-908.
- [33] SONG Y L, SUN L M, LIN M M, CHEN J Y, QI X J, HU C G, FANG J B. Comparative transcriptome analysis of resistant and susceptible kiwifruits in response to *Pseudomonas syringae* pv. *actinidiae* during early infection[J]. PLoS One, 2019, 14(2): e0211913.
- [34] YUE J Y, LIU J C, TANG W, WU Y Q, TANG X F, LI W, YANG Y, WANG L H, HUANG S X, FANG C B, ZHAO K, FEI Z J, LIU Y S, ZHENG Y. Kiwifruit Genome Database (KGD): A comprehensive resource for kiwifruit genomics[J]. Horticulture Research, 2020, 7:117.
- [35] AMPOMAH- DWAMENA C, MCGHIE T, WIBISONO R, MONTEFIORI M, HELLENS R P, ALLAN A C. The kiwifruit lycopene beta-cyclase plays a significant role in carotenoid accumulation in fruit[J]. Journal of Experimental Botany, 2009, 60 (13):3765-3779.
- [36] 欧阳梦真,朱磊,孙治强,李胜利,吴帼秀,李阳,何富豪,李严曼.西瓜*CIWRKY54*基因的克隆、亚细胞定位及表达分析[J].中国瓜菜,2019,32(12):8-14.
- OUYANG Mengzhen, ZHU Lei, SUN Zhiqiang, LI Shengli, WU Guoxiu, LI Yang, HE Fuhaao, LI Yanman. Cloning, subcellular localization and expression analysis of *CIWRKY54* in *Citrullus lanatus*[J]. China Cucurbits and Vegetables, 2019, 32(12):8-14.
- [37] 宋雅林.猕猴桃应答溃疡病菌侵染的转录组研究及抗性相关基因挖掘[D].武汉:华中农业大学,2019.
- SONG Yalin. Transcriptome study on kiwifruit response to *Pseudomonas syringae* pv. *actinidiae* infection and mining of resistance related genes[D]. Wuhan: Huazhong Agricultural University,2019.
- [38] KINKEMA M, FAN W, DONG X. Nuclear localization of NPR1 is required for activation of *PR* gene expression[J]. The Plant Cell, 2000, 12(12):2339-2350.
- [39] 杨德翠.*Cylindrocladium canadense*侵染对牡丹(*Paeonia suffruticosa* Andr.)光合特性的影响以及牡丹病程相关蛋白1基因的克隆和遗传转化[D].太谷:山西农业大学,2013.
- YANG Decui. Effects on the photosynthetic characteristics of tree peony (*Paeonia suffruticosa* Andr.) infected by *Cylindrocladium canadense* and gene cloning and genetic transformation of pathogenesis-related Protein 1 of tree peony[D]. Taigu: Shanxi Agricultural University,2013.
- [40] 马立功,张匀华,孟庆林,石凤梅,刘佳,李易初,王志英.向日葵病程相关蛋白*HaPRI*基因的克隆与功能[J].作物学报,2015,41(12):1819-1827.
- MA Ligong, ZHANG Yunhua, MENG Qinglin, SHI Fengmei, LIU Jia, LI Yichu, WANG Zhiying. Cloning and function analysis of pathogenesis related protein gene *HaPRI* from sunflower (*Helianthus annuus*)[J]. Acta Agronomica Sinica, 2015, 41(12): 1819-1827.
- [41] GIBBS G M, ROELANTS K, O'BRYAN M K. The CAP superfamily: Cysteine-rich secretory proteins, antigen 5, and pathogenesis-related 1 proteins: Roles in reproduction, cancer, and immune defense[J]. Endocrine Reviews, 2008, 29(7):865-897.
- [42] CUI H M, QU S, LAMBORO A, JIAO Y L, WANG P W. Bioinformatics analysis of disease resistance gene *PRI* and its genetic transformation in soybeans and cultivation of multi-resistant materials[J]. Phyton, 2022, 91(7):1445-1464.
- [43] PIETERSE C M J, LEON-REYES A, VAN DER ENT S, VAN WEES S C M. Networking by small-molecule hormones in plant immunity[J]. Nature Chemical Biology, 2009, 5(5):308-316.
- [44] AGRAWAL G K, JWA N S, RAKWAL R. A novel rice (*Oryza sativa* L.) acidic *PRI* gene highly responsive to cut, phytohormones, and protein phosphatase inhibitors[J]. Biochemical and Biophysical Research Communications, 2000, 274(1):157-165.
- [45] 尉春雪,苏浩天,张晓宇,何文菡,郑大桂,尹淑霞.外源水杨酸对草地早熟禾抗褐斑病的诱导与抗病基因*PRI*和*NPRI*的表达的影响[J].草业科学,2019,36(5):1249-1254.
- WEI Chunxue, SU Haotian, ZHANG Xiaoyu, HE Wenhan, ZHENG Dacheng, YIN Shuxia. Effects of exogenous salicylic acid on the resistance of Kentucky bluegrass to brown patch and expression of *PRI* and *NPRI* resistance genes[J]. Pratacultural Science, 2019, 36(5):1249-1254.
- [46] 李卓静.*H₂S*通过*CsMYB30*激活黄瓜病程相关蛋白表达[D].太原:山西大学,2021.
- LI Zhuojing. *H₂S* activates the expression of cucumber pathogenesis related protein through *CsMYB30*[D]. Taiyuan: Shanxi University,2021.