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不同猕猴桃品种对溃疡病的抗性差异及其机制研究

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摘 要:【目的】由*Pseudomonas syringae* pv. *actinidiae*(Psa)引起的溃疡病是猕猴桃生产中威胁最大的病害,揭示抗病机制,为利用抗病品种防治病害提供理论依据。**【方法】**通过离体枝条定量接种试验,比对分析了9个常见猕猴桃栽培品种的抗病性,测定了接种前后不同时间点6个抗病相关基因的表达量及防御酶中过氧化物酶(POD)、苯丙氨酸解氨酶(PAL)、超氧化物歧化酶(SOD)和过氧化氢酶(CAT)活性的变化。**【结果】**发现9个品种抗病性差异显著,'徐香'(*Actinidia deliciosa*'Xuxiang')抗病性最强、病斑最小、显症晚(15 d),显著优于其他测试品种,'红阳'(*A. chinensis*'Hongyang')抗病性最弱、显症早(10 d)。抗性相关基因*PR1*和*PR5*基因在抗性品种'徐香'中自接种Psa后至显症前显著上调表达,相对表达量最高上调了92.52倍和61.54倍;而感病品种'红阳'接种后显著上调表达,上调表达量为'徐香'的50%左右;*POD*和*PAL*基因在'徐香'中显症前期显著上调表达,上调了5.02倍和10.21倍;在'红阳'中显症后才显著上调表达,表达量2~3倍远低于'徐香'。2品种的4个防御酶活性在接种前均没有显著差异,接种后'徐香'中POD和PAL活性显著高于'红阳',显症前期达到峰值分别是接种前的1.66倍和2.31倍,与基因表达趋势一致;而在'红阳'中峰值出现时间晚(20 d),分别是接种前的1.46倍和1.8倍。CAT和SOD活性及相关基因表达在抗、感病品种没有显著性差异。**【结论】***PR1*、*PR5*和POD、PAL在'徐香'抵御溃疡病侵染时发挥重要作用。

关键词:猕猴桃;溃疡病;防御酶;基因表达

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Differences in resistance to *Pseudomonas syringae* pv. *actinidiae* and acting mechanism of different kiwifruit varieties

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Abstract:【Objective】The bacterial canker of kiwifruit caused by *Pseudomonas syringae* pv. *actinidiae* is a severe threat to kiwifruit production. The disease, with a truly high pandemic proportion, has caused huge economic loss in many countries. A lot of researchers found that resistance breeding was a safe and effective method to control kiwifruit bacterial canker. However, only a few studies have been focused on the mechanism of kiwifruit resistance to bacterial canker so far. According to the differences in physiological changes between resistant and susceptible varieties after inoculation, we can reveal the mechanism of kiwifruit against *Pseudomonas syringae* pv. *actinidia,* and provide a theoretical basis for controlling kiwifruit bacterial canker. 【Methods】We evaluated nine kiwifruit varieties using quantitative inoculation *in vitro*. The resistant variety (*Actinidia deliciosa* 'Xuxiang') and susceptible variety (*A. chinensis* 'Hongyang') were used to investigate the relative expression levels of resistance genes *PR1*, *PR5*, *POD* and *PAL*, and investigate the dynamic changes of the activity of defense enzymes including catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) and phenylalanine ammonia lyase (PAL). 【Results】Of the 9 kiwifruit varieties that were tested in this study, 25 days later, the results

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showed that there was a significant difference in the size of lesions among the varieties after they were inoculated with Psa, and no any species was immune to kiwifruit bacterial canker. According to the size of the lesions, the degree of disease showed, 'Hongyang' > 'Xixua' > 'Huayou', 'Cuixiang', 'Jinyan' > 'Yate', 'Qinmei' > 'Hayward' > 'Xuxiang', the lesion of 'Hongyang' was 15.99 cm, and the lesion of 'Xixuan' was 1.76 cm. 'Xuxiang' was proved to be a resistance genotype with a low incidence of infection, however, 'Hongyang' was highly susceptible. After 'Xuxiang' and 'Hongyang' varieties were inoculated with Psa, we compared the process of disease spot spreading. The results showed that, the symptoms of 'Hongyang' appeared at the earliest time, and water-stained lesions were visible under the epidermis 10 days after inoculation, while Xu Xiang's lesions appeared at the latest time, and after 15 days of inoculation, slight lesions could be seen. After 25 days, the lesions of two varieties were stable, no longer expanded, and became dry. Then, we measured the expression level of genes that related to defense enzymes, and the Real-time quantitative PCR results displayed, the relative expression level of these genes were up-regulated in resistant variety 'Xuxiang', and higher than the susceptible variety 'Hongyang'. The expression level of *PR1* gene increased gradually during the whole infection in both varieties. It was up-regulated by 92.52 times on 12th day with 'Xuxiang' and 50.76 times on 14th day with 'Hongyang'. The expression trend of *PR5* gene in two varieties was the same as that of *PR1* gene, and the difference was obvious. The relative expression level in 'Xuxiang' was particularly pronounced, with the highest increase on the 12th day, up-regulated by 61.54 times, and the highest on the 14th day in 'Hongyang', up-regulated 14.78 times. As the genes that could regulate the activity of the corresponding enzymes, the *PAL* and *POD* were up-regulated at 2th day and 4th day, respectively in 'Xuxiang', but in 'Hongyang' they were up-regulated at 10th day after inoculation, and the relative expression level was low. The relative expression levels of *CAT* and *SOD* genes were not significantly different between the two varieties after they were inoculated with Psa, and there was no obvious correlation with the corresponding enzyme activities. We also found that the activities of four defense enzymes were significantly enhanced in both resistant and susceptible varieties compared with uninfected groups. Except for SOD, the CAT, POD and PAL showed a trend of "first increase and then decrease". The change of enzyme activities of 'Xuxiang' was particularly prominent, and the enzyme activities reached peaks before symptoms occurred, which significantly inhibited the expansion of lesions. However, the activities of the enzymes at the early stage of infection were low in 'Hongyang', and the expansion rate of the lesion was very fast, and the enzyme activities reached the maximum at the late stages of the disease. Among them, the peak activity of PAL and POD enzymes was significantly different, 'Xuxiang' was significantly higher than 'Hongyang', and the peak appeared earlier than 'Hongyang'. The activities of PAL enzyme increased rapidly in 'Xuxiang', reaching the peak at 5th day after inoculation, and the enzyme activity was 2.31 times compared with uninfected group. However, the enzyme activity reached a peak at 20th day with 'Hongyang', and the enzyme activity was 1.8 times more than the uninfected group. The POD activity of 'Xuxiang' first reached the peak at 5th day after inoculation, which was 1.66 times more than the uninfected Psa group, and at 20th day it reached the peak again and was 1.81 times higher than the uninfected. But POD showed a slow growth trend in 'Hongyang' variety, reached peak at 20 d after inoculation, and the enzyme activities were very lower than that in 'Xuxiang'. Although the SOD activity was significantly enhanced, there was no significant difference between the two varieties. For CAT, the enzyme activity of 'Xuxiang' only showed a small peak in the early stage of the disease and was higher than that of 'Hongyang', and subsequently, the changes in CAT activity were small. The activity of CAT in 'Hongyang' showed an increasing trend and maintained higher activities during the whole infection period, the en-

zyme activity was much higher than that of ‘Xuxiang’, which reached the peak at 20 d after inoculation, which was 7.34 times more than that of the uninfected group.【Conclusion】After evaluation of disease-resistant varieties, it was indicated that ‘Xuxiang’ can be used as an ideal material for disease-resistant breeding, *PR1* and *PR5* genes can be used as effective markers for kiwifruit resistant variety. PAL and POD activities can be used as one of the biochemical markers for screening resistant plants.

Key words: Kiwifruit; *Pseudomonas syringae* pv. *actinidiae;* Defense enzymes; Gene expression

猕猴桃是重要的果树作物，风味独特、维生素C含量很高、营养价值丰富，产业蓬勃发展，已成为许多地区的经济支柱产业[1-2]。然而，由丁香假单胞菌猕猴桃致病变种（*Pseudomonas syringae* pv. *actinidiae*，Psa）引起的细菌性溃疡病已成为猕猴桃生产上毁灭性病害，造成了严重的经济损失[3-4]。该病害发展快、危害重、防治难，使得猕猴桃产业的发展面临巨大的威胁。

目前，生产上此病害的防治并没有理想的办法，主要依赖农业措施和化学措施（铜制剂和链霉素）进行防治，低效且不稳定[5-6]，还会造成猕猴桃产品和生态环境污染。而栽培抗性品种被认为是有效的防治措施[7]。例如，新西兰黄肉猕猴桃产业因Psa损失惨重，但将高感品种‘Hort16A’替换为相对抗病品种‘Zesy002’后产业基本恢复[4]。中国猕猴桃野生资源丰富，育种工作发展迅速，品种种类繁多，品种间抗性存在很大差异。对品种抗病性的田间调查研究发现，中华猕猴桃较美味猕猴桃更易感病[8-11]。本实验室前期对6个猕猴桃品种连续3 a的田间调查结果发现：‘红阳’发病最重，‘徐香’发病最轻，平均病株率分别为37.8%和8.8%[12]。

植物受到病原物侵染会诱发一系列的防卫反应，可使植物体内POD、PAL、SOD、CAT等防御酶发生变化。石志军等[10]和易盼盼等[13]研究表明，猕猴桃体内防御酶的活性与植株的抗病性密切相关，抗病品种PAL、CAT、POD等酶的活性显著高于感病品种。

笔者以鉴定的高抗品种‘徐香’和高感品种‘红阳’为材料，检测与抗病性相关的防御酶活性和相应基因的表达量，探讨其在抗病中的作用，为猕猴桃抗病品种的选育和溃疡病的防治提供理论依据。

1　材料和方法

1.1　供试材料及病原菌

供接种材料为1 a生枝条，美味猕猴桃品种‘徐香’‘海沃德’‘哑特’‘秦美’‘翠香’；中华猕猴桃品种‘西选’‘红阳’‘金艳’以及中华-美味杂交品种‘华优’（表现中华猕猴桃性状）。材料来源于陕西杨凌周边农户。供试菌株为Psa强致病力菌株M228，由西北农林科技大学果树病害病原生物学及综合防治研究团队实验室保存[14]。

1.2　方法

1.2.1　溃疡病菌菌悬液的制备　取超低温保存的M228菌液，于LB平板上划线，25 ℃活化培养48 h，挑取单菌落，于液体LB培养基中25 ℃培养14 h。5 000 *g*离心收集菌体，无菌水洗3次，最后用无菌水悬浮菌体，用分光光度计将浓度调至OD_{600}=0.1（1×10^8 CFU·mL^{-1}）。

1.2.2　离体枝条有伤接种　将采集枝条用0.6%的次氯酸钠溶液消毒20 min，用无菌水冲洗3次；截成15 cm短枝条，并将枝条两端用石蜡密封；人为制造伤口（单面刀片切割，1 mm宽，切至韧皮部），滴加10 μL菌液，以加无菌水作为CK；于人工气候培养箱（光周期L/D：16 h/8 h；昼夜温度：18 ℃/14 ℃；相对湿度95%）培养[15]。

1.2.3　猕猴桃枝条发病情况　接种25 d后，测量9个不同供试品种枝条病斑的大小，以此作为猕猴桃品种的抗性评价标准。并比较两者在整个感病周期内的病斑扩展进程。

1.2.4　不同抗感品种抗病相关基因的表达　取样：接种后的‘红阳’和‘徐香’猕猴桃枝条每隔5 d定期取样，对照组同样处理；用无菌单面刀片切取接种点上下2 cm范围内的枝条组织，用锡箔纸包裹后迅速置于液氮中，并于−80 ℃保存备用。

（1）RNA提取及cDNA的合成。取−80 ℃保存的材料0.5 g于液氮中进行研磨，后续步骤按照华越洋植物组织RNA提取试剂盒（华越洋）说明书进行操作。RNA样品反转录参照RevertAid First Strand cDNA Synthesis Kit（Thermo Scientific）使用说明书进行。

（2）实时荧光定量PCR检测。以Actin作为内参基因[16]，对猕猴桃抗性相关基因进行定量PCR检

测。在定量引物设计网站https://www.idtdna.com/Scitools/Applications/RealTimePCR/)设计引物(表1)及参考其他文献[17-18]。引物合成由擎科生物公司完成。以cDNA为模板进行qRT-PCR检测。反应程序为:95 ℃预变性10 min;95 ℃变性15 s,55 ℃退火20 s,72 ℃延伸45 s,40个循环。

表1 实时荧光定量引物序列

Table 1 Sequences for primers used in real-time fluorescent quantitative PCR

基因 Gene	基因登录号 Genebank accession number	序列(5'-3') Sequences(5'-3')	参考文献 References
Actin	FG520231	F:GCAGGAATCCATGAGACTACC R:GTCTGCGATACCAGGGAACAT	Petriccione M et al.,2015b[16]
PR1	FG499230	F:GCAGGAATCCATGAGACTACC R:GTCTGCGATACCAGGGAACAT	Wurms K V et al., 2017[17]
PAL	AAC18870	F: AAACGACAACCCCTTGATTG R: ACAAGCTCCGAAATTTGTGC	Wurms K V et al., 2017[18]
POD	FJ422811	F:TCTGTCGTCTTCTGTTTGTATGG R:CTCCTCCTTTGAGAGGGTTATTG	This study
PR5	AJ871175.2	F:GAGCACCTTCAAATCTCTCTCC R:GGTGAAAGGGCAGTTGTTTATG	This study
CAT	FG470670	F: GCTTGGACCCAACTATCTGC R: TTGACCTCCTCATCCCTGTG	Petriccione M et al.,2015b[16]
SOD	FG471220	F: CACAAGAAGCACCACCAGAC R: TCTGCAATTTGACGACGGTG	Petriccione M et al.,2015b[16]

1.2.5 *防御酶活性测定* 粗酶液的提取参照殷丽华[19]的方法,略有改动。

过氧化氢酶(catalase,CAT)活性的测定采用紫外吸收法,方法参照李合生[20],略有改进;SOD活性测定采用光化还原(NBT)法[21];POD活性测定采用愈创木酚法[22];PAL活性测定方法参照孙红梅等[23]的报道。

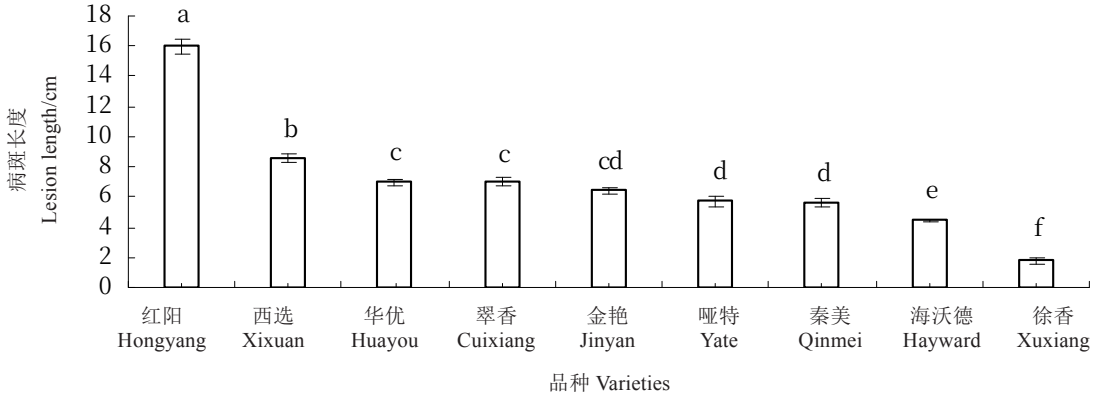
1.3 数据处理

采用SPSS 19.0软件进行数据统计分析,Microsoft Excel 2010作图。

2 结果与分析

2.1 猕猴桃不同品种对溃疡病的抗性评价

如图1所示,接种Psa后,各品种发病程度差异显著,显症时间不同。9个品种中'红阳'和'西选'的发病时间最早,于接种后10 d出现明显病斑,'徐香'和'海沃德'发病时间最晚,于接种后15 d出现可见病斑。25 d后,测量其病斑大小后,其抗病强弱依次为:'徐香'>'海沃德'>'秦美''哑特'>'金艳''翠香''华优'>'西选'>'红阳','红阳'枝条发病最严重,病斑长度为15.99 cm,'徐香'枝条发病最轻,病斑长度为1.76 cm。从图2可知,'红阳'显症时间最早,在接种后10 d刮开表皮可见水渍状病斑,且扩展速度快,并伴随大量菌脓流出;'徐香'出现时间最晚,接种15 d后,可见轻微病斑,扩展速度慢。25 d



图中数据为均值±SD,相同字母表示无显著差异 $p < 0.05$)。试验重复3次,下同。菌体浓度为 1×10^8 CFU·mL^{-1}。

The data in the figure is the mean±SD, the same letter are not statistically different at the 5% confidence level based on Duncan's multiple range test. results obtained from three independent experiments. The same below. Concentration of bacteria:1×10^8 CFU·mL^{-1}.

图1 不同猕猴桃品种离体枝条有伤接种 Psa 25 d 后抗性评价结果

Fig. 1 Resistance evaluation of nine kiwifruit varieties against to Psa by wound inoculation on detached canes after 25 days

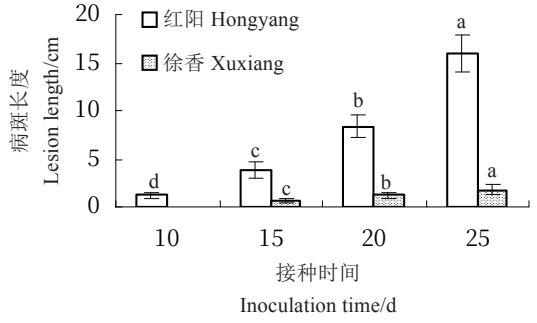


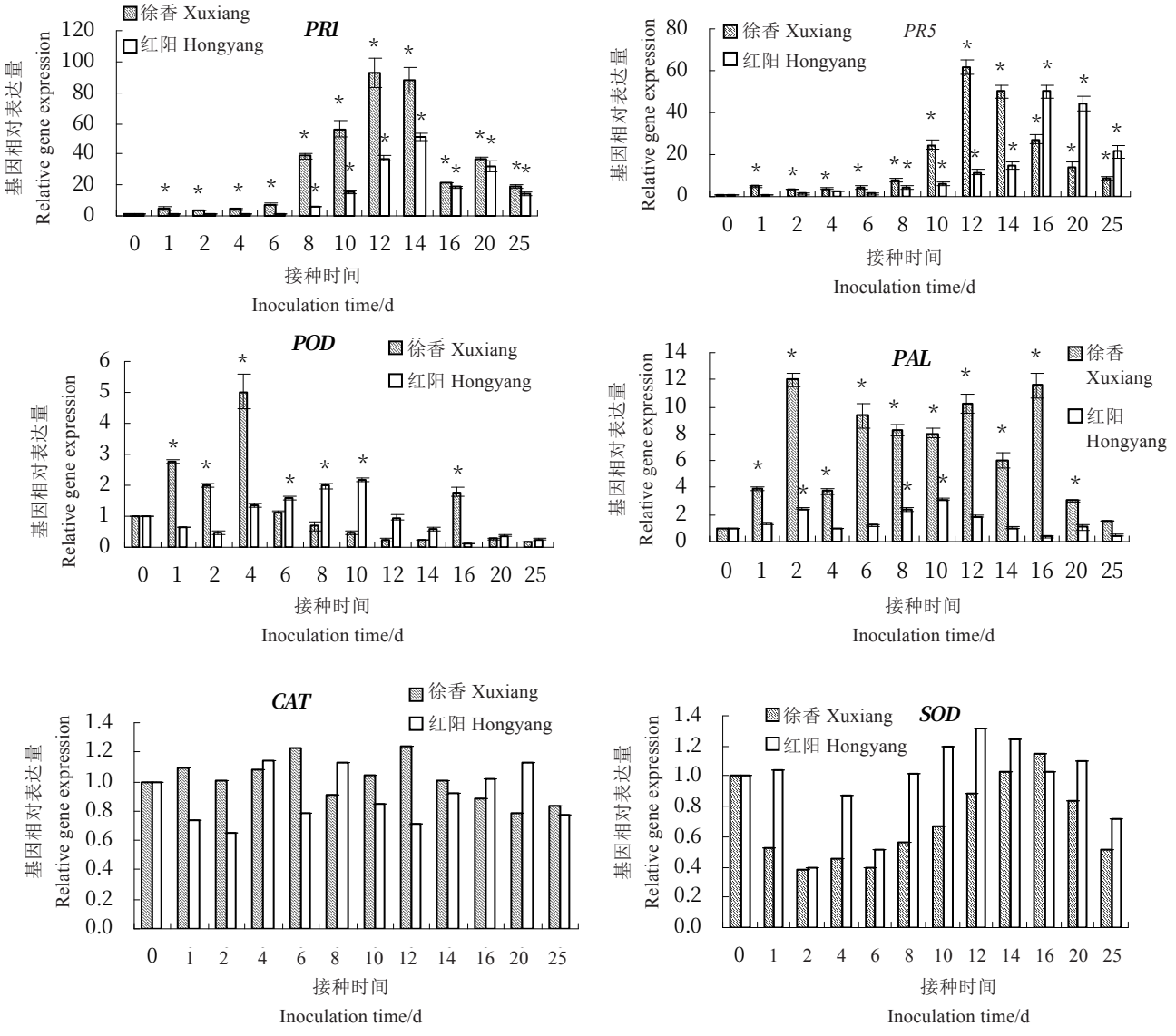
图 2　猕猴桃材料离体枝条接种 Psa 后病斑长度比较

Fig. 2 Comparison of the disease length of vitro branch of kiwifruit materials after inoculation

后，2 品种病斑稳定，不再有扩展，病斑部位干枯。

2.2　溃疡病菌侵染后猕猴桃枝条内抗性相关基因的表达

实时定量 PCR 结果显示（图 3），接种 Psa 后，各基因在‘徐香’中的表达水平较‘红阳’中高。*PR1* 和 *PR5* 基因在‘徐香’中自接种后至显症前上调表达显著，12 d 时表达量最高，分别上调了 92.52 倍和 61.54 倍，在‘红阳’中上调表达显著但远低于其在‘徐香’中的表达量，显症后表达量才达到最高，分别上调了 50.76 倍和 50.39 倍。*POD* 基因在‘徐香’接种后 1 d 显著上调表达，于接种后 4 d 表达量最高，上调 5.02



图中数据为平均值±SD，相同色柱与对照（0 d）相比，*表示有著差异 $p < 0.05$）。试验 3 次重复。

The data in the figure is the mean ±SD，The same color column compared to the control (0 d)，* indicates statistically different at the 5% confidence level based on Duncan's multiple range test. Results obtained from three independent experiments.

图 3　猕猴桃抗性相关基因的定量分析

Fig. 3 Quantitative analysis of kiwifruit resistance related genes

倍，而在‘红阳’中第8天才表现明显的上调表达，接种后10 d表达量达到最高，上调了2.16倍；*PAL*基因在2种猕猴桃品种中差异显著，其中在‘徐香’中显著上调表达，第2天时表达量最高，上调了10.21倍；在‘红阳’中上调表达不显著，在第10天时表达量最高，上调了3.13倍；而*CAT*和*SOD*基因的表达量在两者中没有明显的差异，上调表达不显著。

2.3 防御酶活性变化

在接种Psa前，2个品种中酶活性均处于较低水平且没有明显差异，接种Psa后，两个品种间的POD、PAL、CAT和SOD活性变化显著，都呈现出先升高后降低的趋势。‘徐香’的酶活性变化尤为显著，结合图2枝条的病斑扩展进程，显症前酶活性就达到峰值(5 d)，遏制了病斑扩展，病斑出现晚(15 d)，且病斑小；而‘红阳’在感病前期酶活性较低，病斑出现时间早(10 d)，扩展速度快，在感病后期酶活性才达到最大(20 d)。其中PAL、POD酶活性差异显著且与基因表达一致。‘徐香’的POD于接种后5 d达到高峰，是未接种的1.66倍，此后POD酶活性略下降，随着病斑出现，酶活性再次达到峰值为接种前的1.81倍；而‘红阳’在显症前酶活性增速快，而在接种后10 d，即‘红阳’出现病斑后，POD酶活性增长慢，于接种后20 d酶活性达到峰值为接种前的1.46倍。‘徐香’的PAL活性峰值显著高于接种前的活性，5 d时就达到了峰值，是接种前的2.31倍，有效抑制了病原菌扩展，随后酶活性下降。而‘红阳’呈现缓慢增长趋势，直到20 d时酶活性才达到峰值为接种前的1.8倍；两品种的SOD活性较接种前都有显著的升高，但两者之间没有显著差异；而‘徐香’的CAT活性在显症前上升快达到高峰且高于‘红阳’，而‘红阳’在症状出现时酶活性显著升高；表明感病前期‘徐香’较‘红阳’的CAT活性上升快，该阶段CAT活性可能与品种抗性相关。可见Psa侵染显著提高了‘徐香’PAL、POD的活性，且活性远高于感病品种‘红阳’且峰值出现的时间也远早于‘红阳’，表明2种酶与品种抗病性有关(图4)。

3 讨 论

通过对9个不同品种的猕猴桃的抗性评价研究发现，接种Psa后，‘徐香’的病斑长度最小平均为1.76 cm，而‘红阳’病斑长度最大平均为15.99 cm。这说明‘徐香’比‘红阳’表现出更强的抗性。这与

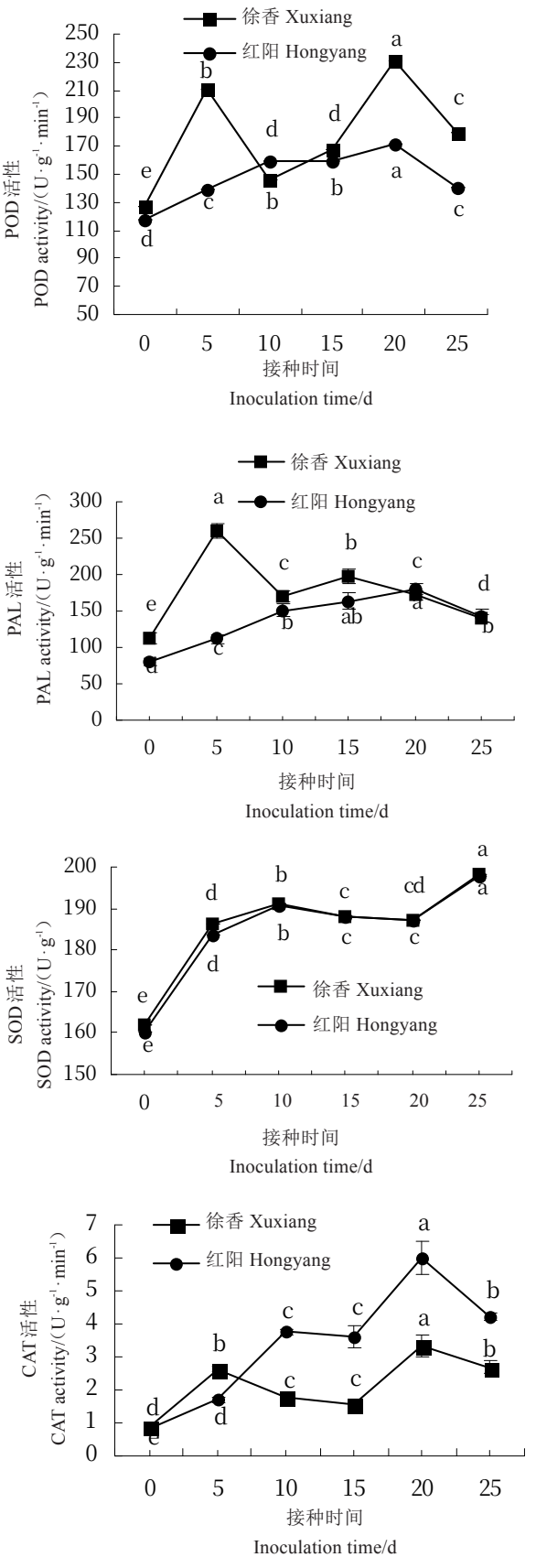


图4 病原菌处理对猕猴桃枝条CAT、POD、SOD和PAL活性的影响

Fig. 4 Effect of pathogen treatment on CAT, POD, SOD and PAL activities in kiwifruit canes

我们田间调查的结果及申哲等[8]和石志军等[10]的研究结果相一致。因此可将‘徐香’作为抗性研究的理想材料，以此研究猕猴桃抗溃疡病的抗性机理。

病原菌侵染会使植物体内产生一系列的信号转导及诱导防御基因的表达，抵御病原菌入侵。植物在受到生物或非生物胁迫时会诱发病原相关蛋白（PRs）的表达，参与防御信号的传递，对提高植物抗性起重要作用[24]。*PR1*在很多植物中作为系统获得抗性的分子标记[25]，在植物抵御病原菌的过程中参与很多生物进程包括MAPK信号通路，植物激素转导等，在猕猴桃中受到Psa侵染前后的转录组测序中基因表达量存在明显差异，也证明了其具有病害抗性的功能[26]；*PR5*只要在伤口或病原菌侵染时才会显著表达[27]。有研究发现，猕猴桃在受Psa侵染后会诱导*PR1*和*PR5*的上调表达[7]。本研究中，在‘徐香’中显症前分别上调了92.52倍和61.54倍，上调幅度显著，表现出品种抗性有较强的相关性，而在‘红阳’中显症后才显著上调表达。*PAL*和*POD*作为关键酶的调节基因在‘徐香’中的表达量远高于‘红阳’，峰值在‘徐香’中均在显症前出现较‘红阳’的显症后期，时间上早且基因的表达量显著高于‘红阳’。而*CAT*和*SOD*的基因表达量没有明显的上调甚至下调表达，且与酶活性的变化趋势没有相关性，推测可能是由多种酶活性基因调节引起的。

病原菌侵染会引起植物产生一系列的生理生化反应，植物体内活性氧的积累与其抗病性密切相关[28]。POD、SOD和CAT是植物机体内源活性氧清除剂，清除植物体内的自由基，维持活性氧平衡[29]。此外，POD在木质素、酚类和植保素等抗菌物质的合成中起重要作用[30]。PAL是植物苯丙烷代谢途径的关键酶和限速酶，参与植物抗病次生物质的合成与积累，活性升高有利于细胞壁木质化而阻止病原菌的侵入和扩展，该酶可作为衡量植物抗病性的一个生化指标[31]。

本研究中发现抗、感品种的病斑扩展及其酶活性峰值出现的时间存在差异。抗病品种‘徐香’病斑扩展速度慢，显症时间晚，于接种后15 d出现轻微病斑，25 d后达到最大且不再扩展；而‘红阳’显症时间早（10 d），病斑扩展速度快。石志军等[10]和易盼盼等[13]的研究结果中抗性品种在感病前后POD、PAL、SOD的活性有显著提高，且高于感病品种，与抗病性成正相关相。本研究中，POD活性在‘徐香’中远高于‘红阳’；PAL在‘徐香’中的酶活性变化幅度大，在感染病原菌早期上升较快（5 d），且达到峰值；而‘红阳’的PAL酶活性上升缓慢，在感病后期才达到峰值（20 d）且远低于‘徐香’。2种酶活性的变化与上述中相应酶酶调节基因的变化一致。抗病品种‘徐香’在感病初期CAT酶活性上升较快，接种后5 d时酶活性达到第1个峰值，比‘红阳’早。而‘红阳’的CAT活性在后期远高于‘徐香’，可能早期CAT活性低，积累大量H_2O_2，但过量的H_2O_2对植物细胞产生损害，促使中后期CAT活性快速上升；王竹青等[32]对甘蔗感染黑穗病的CAT酶活性测定中发现，抗病基因型的CAT活性在接种初期上升时间快，后期酶活性变化低于感病基因型，表明CAT对病害的早期防御有一定的作用。抗、感品种的SOD酶活性虽较接种前有明显的升高，但两者上升的幅度没有明显的差别。李庚飞等[33]对不同品种的猕猴桃进行酶活性测定发现，SOD活性与抗性之间的相关性不大，结果显示抗性品种的酶活性变化幅度低于感病品种，认为SOD是诱导酶。本研究认为植物内SOD受到任何生物胁迫和非生物胁迫都可导致该酶活性的变化，不能以此酶活性变化作为品种抗性强弱的表现。

4　结　论

通过对不同猕猴桃品种的抗性评价发现，‘徐香’的抗性最强，以此作为抗性机理研究的材料。抗、感2个品种在感染Psa后，*PR1*、*PR5*及POD和PAL酶活性变化及基因表达与‘徐香’品种病斑扩展的进程相吻合，在‘徐香’防御Psa侵染过程中起重要作用。

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