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基于microRNA测序分析miRNA在刺葡萄抗白腐病中的作用

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摘 要:【目的】在前期的种质资源评价过程中,发掘一株中国野生刺葡萄0943,该株系高抗葡萄白腐病。基于microRNA(miRNA)在植物抗病中的重要作用,拟从miRNA水平探讨刺葡萄在受到病原菌侵染后的表达调控机制。**【方法】**以抗病的中国野生刺葡萄为试材,对比感病的欧亚种'美人指',分别以病原菌诱导,在0 hpi(hours post inoculation)和病菌诱导后的12 hpi、36 hpi处理后采样,进行二代测序,并对数据进行KEGG及表达量的差异分析。**【结果】**对比感病葡萄'美人指',分析了抗病刺葡萄在基础代谢和抗病途径中的差异,结合miRNA的表达量,获得了150个表达量发生变化的miRNA,其中44个miRNA的表达在刺葡萄和'美人指'之间存在差异。5个miRNA在刺葡萄中特异表达,但是在'美人指'中不表达,实施定量验证了这一结果。靶基因预测显示,其靶基因包括与抗病的紧密相关的WRKY、SPL、EFR等转录因子,还包括与抗病直接相关的LRR类的抗病基因。**【结论】**筛选出5个在刺葡萄上特异表达候选miRNA(miR172a、miR172b、miR845a、novel_81和miR159a),可作为刺葡萄抗白腐病研究的目标。

关键词: 葡萄白腐病;microRNA;抗病;测序

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Analysis of the function of miRNA on the resisitance to white-rot disease in *Vitis davidii* based on microRNA sequencing

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Abstract:【Objective】Grape white- rot [*Coniella diplodiwlla* (speg.) Petrak & Sydow] is one of the most destructive diseases in grape. The traditional fungicide control not only could increases the production cost, but also would affect the environment. Therefor, it is necessary to use the resistance genes of wild grape in China and improve the resistance of new varieties. An wild grape strain 0943 (*Vitis davidii*) was found to be resistant to the grape white rot in our previous study. It has been known that microRNAs (MiRNAs) play important role in plant disease resistance. This study intended to explore the miRNA mechanism of expression regulation in *V. davidii* after infection by pathogenic bacteria.【Methods】For providing plant tissues for sRNA sequencing analysis, 2-year-old plant of *Vitis davidii* and *Vitis vinifera* 'Manicure Finger' were grown in a greenhouse at 28 ℃ with a 16 h photoperiod. These plants were inoculated with *C. diplodiella* by fixing four mycelium gelose discs (diameter of 2 mm) on each leaf with small pins and covering the leaf. Leaf samples were collected at 0, 12 and 36 hours post inoculation (hpi). 3 μg of total RNA for each sample was taken to create a small RNA library. Gene Ontology's study of the distribution of candidate target genes in Gene Ontology would elucidate how the sample differences in experiments were reflected in gene function. KEGG analysis, and significant enrichment through pathway were used to identify the most important biochemical and signal transduction

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pathways for candidate target genes involved. KEGG enrichment analysis was made using KOBAS. 【Results】We sequenced three small RNA libraries from *Vitis davidii*. In this study, we obtained 3.282 G data, each library data was over 0.5 G. 106 miRNA mature bodies were obtained through sequencing, of which 91 were able to match the miRNA precursor, and the types of miRNA that could match were 6 567. After statistics, the miRNA number of *V. davidii* was more thanthat of the ‘Manicure Finger’ at 0 hpi, but the induced expression of miRNA was lower than the ‘Manicure Finger’. The number of miRNA species increased at 12 hpi and 36 hpi points in both *V. davidii* and ‘Manicure Finger’, and the miRNA species of *V. davidii* increased from 862 to 1 300 and 1 115, while ‘Manicure Finger’ miRNA species increased from 828 to 1 384 and 1 078. In the differential expression analysis of miRNAs, the miRNAs with significantly different expressions at 12 hpi and 36 hpi were used as references for the thorny grape at the 0 hpi treatment point, and their target genes were analyzed by KEGG enrichment. The analysis showed that miRNAs played a role in 11 life pathways through 847 target genes, of which the target genes involved in the protein processing in endoplasmic reticulum pathway were the most, and the target genes involved in the Pantothenate and CoA biosynthesis pathway were the least, accounting for 23. Through the prediction of miRNA target genes, the basal metabolic pathways with sugar metabolism as the core were analyzed. The results showed that in spiny grapes, all basal metabolic pathways including the Citrate cycle and the oxidative phosphorylation of the NADH energy synthesis pathway, were in a down regulation mode; CoA-mediated energy release Coumaroyl-CoA pathway was in an up regulation mode. In this study, the differences between *V. davidii and* ‘Manicure Finger’ were analyzed. The different expression of 150 was obtained. And 44 miRNA was different between *V. davidii* and ‘Manicure Finger’. At the same time, 5 miRNA were specifically expressed in *V. davidii,* but were not expressed in ‘Manicure Finger’. Target gene prediction showed that the target gene included WRKY, SPL, EFR and other transcription factors related to disease resistance, and also included LRR disease- resistant genes. 【Conclusion】In this study, 5 specific expressions of candidate miRNA, mir172a, miR172b, miR845a, novel_81 and miR159a in *V. davidii* were screened, which could be used for the study of resistance to grape white rot in *V. davidii.*

Key words: Grape white-rot; microRNA; Resistance; Sequence

葡萄是世界上重要的水果之一，2016年我国葡萄栽培面积为79.93万hm^2，位居世界第二位；产量1 367.6万t，位居世界第一位；鲜食葡萄的面积和产量已连续多年位居世界第一位（国家统计局数据）。葡萄白腐病[*Coniella diplodiwlla*（Speg）Petrk & Sydow]成为目前生产上的最重要的病害之一，该病主要危害果粒、叶片和枝条，在葡萄生长期引起果实腐烂，导致严重减产[1]，传统的药剂防治不但增加生产成本，还会破坏生境，而利用中国野生葡萄抗资源中的抗性基因，提高现有品种的抗性，是实现葡萄产业的可持续发展的必要途径。

我国是葡萄起源中心之一，已知的葡萄野生种约38个种，种类约占世界的60%。遗传资源丰富且大多含有抗性基因，本研究团队已经完成收集保存了其中的25个种。如何利用抗性资源挖掘抗性基因，聚合欧亚种优良品质和野生种的抗性性状，改良现有栽培品种是本研究团队长期从事的一项研究工作。本课题组在中国野生刺葡萄株系中发现高抗白腐病的优异种质刺葡萄0943（*Vitis davidii* Foex），该种质在病原菌接种鉴定中稳定表现高抗，同时能够抑制菌丝的生长和侵染。

葡萄作为重要的经济树种，其分子生物学的研究方面处于世界前沿，并于2007年完成了全基因组测序[2]。高通量测序的广泛应用，使植物性状在基因表达水平的研究逐步深入，不同类型的RNA和功能不断被发现[3-5]。植物受到生物胁迫时，会发生相应的应激反应，在防御病害过程中引起一系列的基因激活和抑制，研究发现内源miRNAs参与了植物免疫反应。

目前已经鉴定的葡萄miRNA有138种，主要集中在酿酒葡萄和欧亚种的成花结实方面[4-6]，与葡萄

抗病相关的miRNA研究较少，而针对葡萄抗白腐病的miRNA未见报道[7]。深度测序（deep sequencing）技术的出现对miRNA的研究起到了巨大的推动作用[8]，miRNA测序（RNA-seq）已成为miRNA发现和分析的重要手段。miRNA通常通过在转录水平剪切靶基因实现功能其调控，确定靶基因成为研究miRNA的重要内容。探讨特定miRNA的靶基因及其在抗病过程中的作用，可以全面揭示抗病种质刺葡萄的抗病机制。

笔者以抗病刺葡萄为研究对象，对比感病的欧亚种‘美人指’，利用深度测序技术研究其在白腐病侵染过程中miRNA的表达种类，结合转录组分析miRNA受诱导种类及靶基因表达差异，从而筛选葡萄受到病原菌侵染后反应中的关键miRNA及其靶基因，为提高葡萄的抗病性提供理论基础。

1　材料和方法

1.1　材料与方法

供试葡萄株系为2 a（年）树龄的成年中国野生刺葡萄和欧亚种‘美人指’，叶片采自当年生枝条上第3~5枚成龄叶片，每个植株选取3枚叶片。葡萄白腐病病原菌[*Coniella diplodiwlla*（Speg）WR01]来自中国农业科学院植物保护研究所，病原菌采用PDA培养基，28 ℃黑暗培养3~5 d，菌丝块接菌到葡萄叶片并保湿，每个叶片设2~4个接种点，分别在接种后0 hpi（hours post inoculation）、12 hpi、，36 hpi后采样，设3株葡萄为独立重复，−80 ℃保存。为数据处理方便，刺葡萄标记为VD，‘美人指’标记为MF，对应0 hpi、12 hpi、36 hpi分别为（Vd1、Vd2、Vd3和MF1、MF2、MF3）。

1.2　Small RNA文库制备与miRNA测序

每个样品取3 μg总RNA作为起始原料来建small RNA文库。所有总RNA的RIN值都在8.0以上。根据Illumina TruSeq™ RNA Sample Preparation Kit（Illumia，San Diego，USA）的操作说明分别选取不同的index标签建库。使用TruSeq PE Cluster Kit v3-cBot-HS（Illumia）试剂在cBot上生成簇。之后在Hiseq 2000测序平台上运行单端测序程序（SE50），得到50 bp的单端测序reads。

1.3　miRNA测序分析比对

通过bowtie[9]软件（完全匹配）将sRNA比对到参考序列，来分析其在参考序列上的分布以及表达情况。miRNA预测软件miREvo[10]和mirdeep2[11]进行新miRNA的分析。

1.4　miRNA差异表达分析

对各样本中miRNA进行表达量的统计，并用TPM[12]进行表达量归一化处理，公式：归一化表达量=readCount×1 000 000）/total_ readCount，之后用DEGseq[13]进行差异分析，差异miRNA筛选条件默认设置为：q value<0.01，$|\log_2$（fold change）$|>1$。

1.5　KEGG富集分析

针对差异表达miRNA的靶基因（以下称“候选靶基因”）进行KEGG（Kyoto Encyclopedia of Genes and Genomes）分析[14]，通过Pathway显著性富集能确定候选靶基因参与的最主要生化代谢途径和信号转导途径，用KOBAS[15]来进行的KEGG富集分析。

2　结果与分析

2.1　基本数据

供试样品分别0 hpi、12 hpi、36 hpi时间点采样，测序后分别获得3.282 G数据，reads最少为10 939 616条，错误率都在0.01%以下，Q2都在98%以上，Q3的96%以上，GC含量在51.29%以上，满足后续转录组数据分析的数据质量要求（表1）。

表1　数据产出质量情况（平均）

Table 1　List of data quality （mean）

样品 Sample	读数 Reads	碱基数 Bases/G	错误率 Error rate/%	百分比 Q20/%	Q30百分比 Q30 Percentage/%	GC含量 GC content/%
MF1	13 491 979	0.675	0.01	99.01	96.42	52.38
MF2	13 544 859	0.677	0.01	98.95	96.09	51.77
MF3	10 939 616	0.547	0.01	99.06	96.66	51.77
Vd1	12 091 958	0.605	0.01	99.27	97.30	52.35
Vd2	14 243 221	0.712	0.01	99.05	96.39	52.33
Vd3	14 817 029	0.741	0.01	98.99	96.36	51.29

测序获得106个miRNA的成熟体，其中91个能够匹配到的miRNA前体，能匹配的miRNA的种类为6 567个。在数据统计中，刺葡萄的本地miRNA数量多于‘美人指’，但是受诱导表达的sRNA却少于‘美人指’。无论在刺葡萄和‘美人指’中，12 hpi和36 hpi时间点上，miRNA的种类数量都有所增加，刺葡萄sRNA种类从862增加到了1 300和1 115，而‘美人指’中miRNA种类从828增加到了1 384和1 078个，而且数量的高点都出现在12 hpi上，在36 hpi处理点出现了下降（表2）。

表 2 各样本已知 miRNA 比对情况统计

Table 2 Statistical of known miRNA for each sample

种类 Type	合计 Total	MF1	MF2	MF3	Vd1	Vd2	Vd3
miRNA成熟体 Mapped mature	106	81	99	93	82	88	89
miRNA前体 Mapped hairpin	91	81	85	84	72	80	84
miRNA的种类 Mapped uniq miRNA	6 567	828	1 384	1 078	862	1 300	1 115
miRNA的个数 Mapped total miRNA	1 173 945	191 075	347 171	231 201	103 578	164 755	136 165

注：(1)Mapped 成熟体：比对上的 miRNA 成熟体。第 2 列为所有的样本总共匹配上的 miRNA 成熟体个数；第 3 到第 n+2 列分别为样本 1 到 n 配上的 miRNA 成熟体个数。(2)Mapped 前体：比对上的 miRNA 前体。第 2 列为所有的样本总共匹配上的 miRNA 前体个数；第 3 到第 n+2 列分别为样本 1 到 n 匹配上的 miRNA 前体个数。(3) miRNA 的种类：比对上的 sRNA 的种类。第 2 列为所有的样本总共匹配到已知 miRNA 前体的 miRNA 的种类；第 3 到第 n+2 列分别为样本 1 到 n 匹配到已知 miRNA 前体的 mRNA 的种类。(4) miRNA 的个数：比对上的 sRNA 的个数。第 2 列为所有的样本总共匹配到已知 miRNA 前体的 sRNA 的个数；第 3 到第 n+2 列分别为样本 1 到 n 匹配到已知 miRNA 前体的 miRNA 的个数。

Note: (1) Mapped mature: The miRNA mature body on the match. 2nd List of all samples in total matching the number of miRNA mature bodies; The number of miRNA mature bodies with samples 1 to n on the 3rd to n+2 columns，respectively. (2) Mapped hairpin: The miRNA precursor on the match. 2nd List of all samples in total matching the number of miRNA precursors. The number of miRNA precursors on the matching of samples 1 to n for the 3rd to n+2 columns，respectively. (3) Mapped uniq miRNA: The type of sRNA on the match. The 2nd column lists all samples that match the type of miRNA known to the miRNA precursor in total. The 3rd to n+2 columns are samples 1 to n that match the types of mRNA known as the miRNA precursor. (4) Mapped total miRNA: The number of sRNA on the match. 2nd List of the total number of Srna that all samples match to known miRNA precursors. The 3rd to N+2 lists the number of miRNA that match samples 1 to N to known miRNA precursors.

2.2 KEGG分析统计

为了分析抗病和感病葡萄中miRNA的整体变化，从miRNA差异表达入手，以0 hpi处理点为参照，分别比较12 hpi和36 hpi处理后表达差异显著的miRNA，并对其靶基因进行KEGG富集分析。分析显示，miRNA通过90个靶基因在11条生命途径中发挥作用，占总基因的10.6%。参与Protein processing in endoplasmic reticulum途径的靶基因最多，达到了22个，参与Pantothenate and CoA biosynthesis途径的靶基因最少，只有3个。miRNA通过降解目标mRNA来行使功能，因此RNA degradation途径可能是miRNA直接作用的途径，该途径中预测到8个靶基因表达变化显著(表3)。

2.3 基于KEGG途径的基础代谢和抗病途径分析

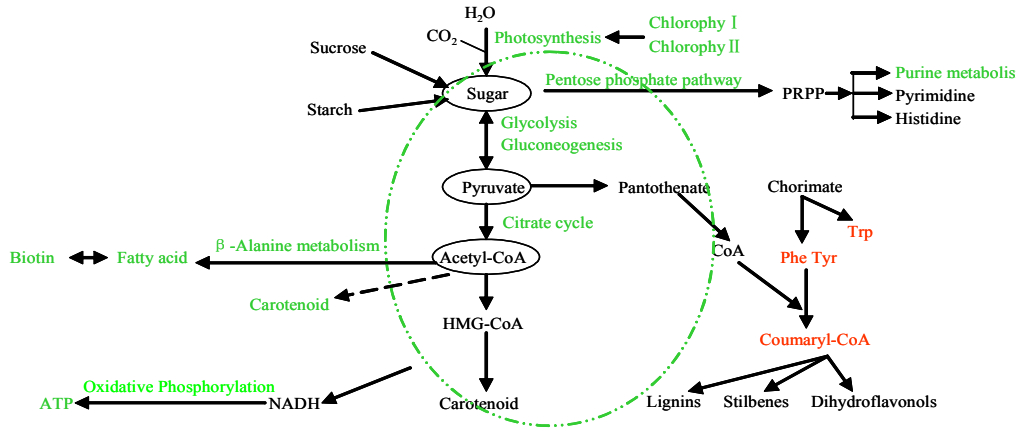
表 3 候选靶基因 KEGG 显著性富集

Table 3 Candidate target genes KEGG significant enrichment

描述 Term	编码 Id	差异基因数 Sample number	背景基因数 Background number	*P*值 *P*-value	矫正*P*值 Corrected *P*-value
Endocytosis	vvi04144	10	71	1.193 1e-03	8.109 8e-02
Protein processing in endoplasmic reticulum	vvi04141	22	186	1.732 5e-03	8.109 8e-02
Nucleotide excision repair	vvi03420	10	52	2.061 8e-03	8.109 8e-02
Ubiquitin mediated proteolysis	vvi04120	10	106	2.773 5e-03	8.181 8e-02
Spliceosome	vvi03040	10	146	3.146 7e-02	6.112 6e-01
Basal transcription factors	vvi03022	5	41	3.561 4e-02	6.112 6e-01
RNA transport	vvi03013	12	135	4.056 6e-02	6.112 6e-01
RNA degradation	vvi03018	8	87	4.144 1e-02	6.112 6e-01
Pantothenate and CoA biosynthesis	vvi00770	3	23	8.233 0e-02	1.000 0e+00
合计 Total		90	847		

整合KEGG中各个代谢途径中基因的表达变化，绘制刺葡萄和‘美人指’代谢图，分别以其糖代谢为核心，围绕光合作用、糖孝解、柠檬酸循环、氧化磷酸化，以及能量代谢中的CoA途径进行比对分析。结果显示，在刺葡萄和‘美人指’中，从光合作用(Photosynthesis)中的Chlorophy Ⅰ和Chlorophy Ⅱ开始，包括基础代谢的糖酵解(Glycolysis)、柠檬酸循环(Citrate cycle)、氧化磷酸化的NADH能量合成途径，所有基础代谢途径均处于下调模式；而Trp和CoA介导的能量释放Coumaroyl-CoA途径在刺葡萄中处于上调模式(图1、图2)，而在‘美人指’中处于下调模式，揭示病菌的侵染全面影响了葡萄的代谢，但是刺葡萄中的能量代谢反而增加。

为了进一步研究刺葡萄的抗病反应，根据KEGG途径的分析，绘制了刺葡萄和‘美人指’中关键的抗病途径。结果显示，刺葡萄和‘美人指’中



红色为正调控，绿色为负调控，黑色为不变。下同。

Red. Positive regulation; Green. Negative regulation; Black. Unchanged. The same below.

图 1　病原菌诱导后刺葡萄基础代谢途径基因表达变化

Fig. 1　Based metabolic pathway gene expression changes after pathogen induced in *V. davidii*

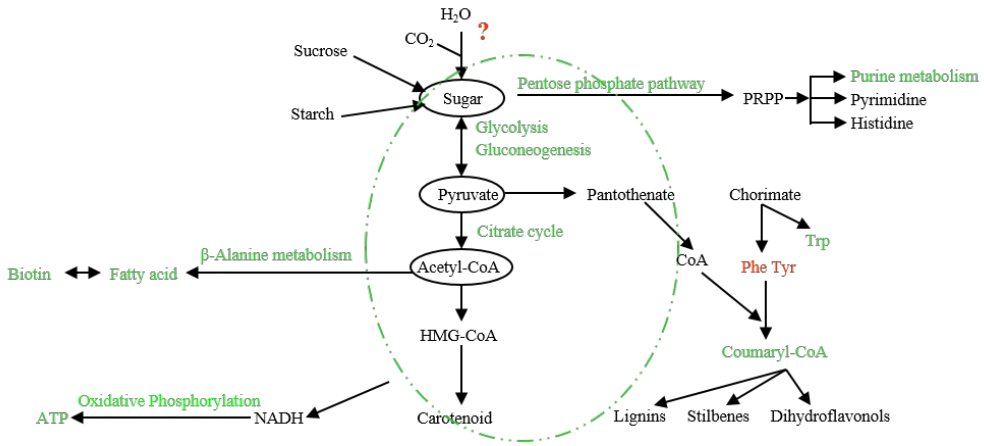


图 2　病原菌诱导后‘美人指’葡萄基础代谢途径基因表达变化

Fig. 2　Based metabolic pathway gene expression changes after pathogen induced in *V. vinifera* ‘Manicure Finger’

Cf9、CNG C5、FLS2 途径的基因都处于下调模式；刺葡萄的 EFR 途径中 MEKK2、MKK4/5、MPK3/6 和 WRKY33 基因处于上调模式，‘美人指’中只有 MEKK2、MKK4/5 上调（图 3、图 4）。

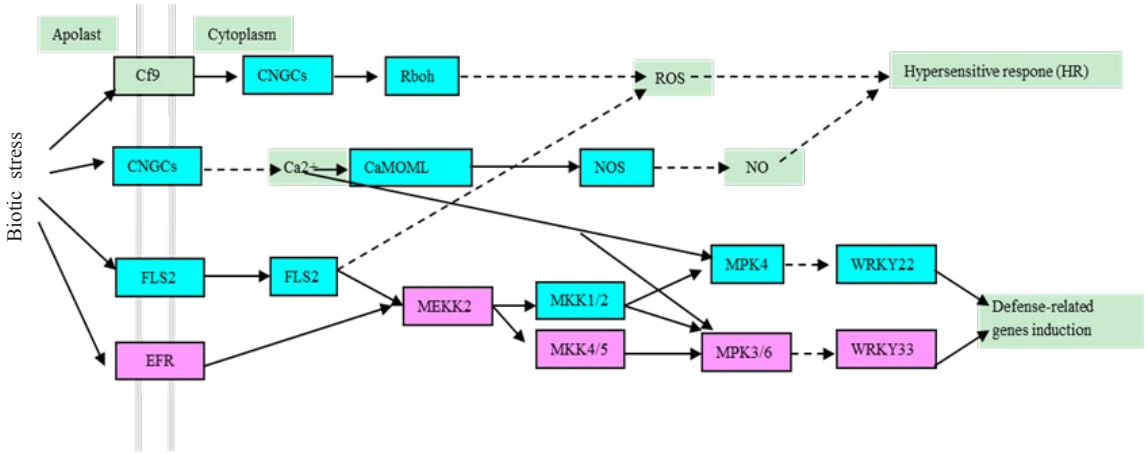


图 3　抗病途径中刺葡萄受病原菌侵染后 miRNA 靶基因的表达变化

Fig. 3　Expression of miRNA target gene in biotic stress pathway after infection of *V. davidii* by pathogenic bacteria

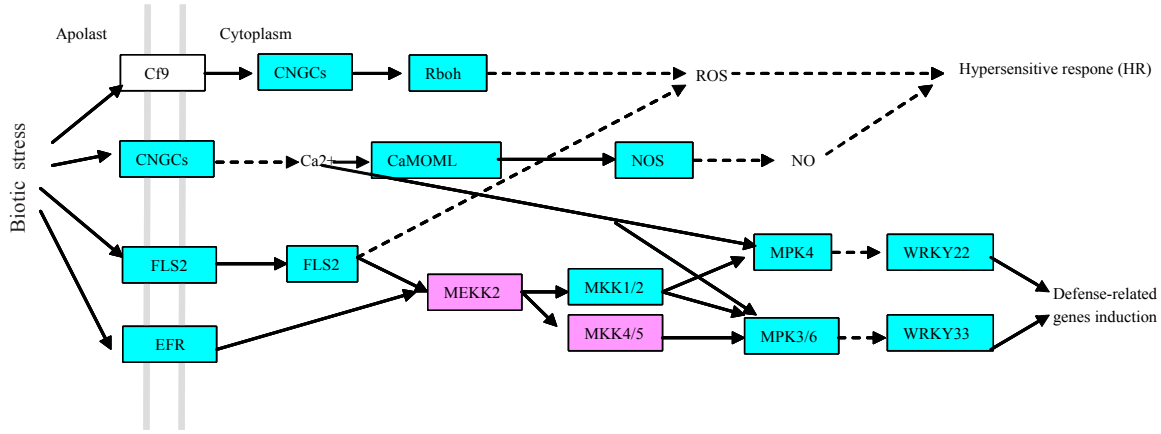


图 4 抗病途径中‘美人指’葡萄受病原菌侵染后 miRNA 靶基因的表达变化

Fig. 4 Expression of miRNA target gene in biotic stress pathway after infection of *V. vinifera* ‘Manicure Finger’ by pathogenic bacteria

2.4 miRNA差异表达分析

为了挖掘刺葡萄抗白腐病中的miRNA，本研究中对150个差异表达变化超过2倍的miRNA表达量进行热图分析（图5-A），抗病刺葡萄中的miRNA存在特异表达种类，这一部分的44个miRNA表达量进行热图分析（图5-B），刺葡萄受病原菌诱导的miRNA数量高于‘美人指’，最高上调$2^{8.9}$，最高下调$2^{5.8}$，且刺葡萄的miRNA本底表达低于‘美人指’（Vd1vsMF1），在病菌诱导后依然低于‘美人指’中的miRNA表达量（Vd2vsMF2、Vd3vsMF3）。

在44个miRNA中，存在刺葡萄中特异表达的miRNA，其只在刺葡萄中有表达，而在‘美人指’中无表达信号。刺葡萄中特异表达的miRNA存在5个，表达下调的miRNA 4个（miR172a、miR172b、miR845a和novel_81）；表达上调的miRNA为miR159a，这些miRNA基因持续在12 hpi和36 hpi被诱导（表4）。对候选的miR172a、miR172b、miR845a、novel_81和miR159a设计引物进行qPCR验证，结果与测序结果一致（图6）。

为了研究刺葡萄特异表达miRNA的功能，对其进行了靶基因预测。miR172a和b能够预测的靶基因7个，miR845a的靶基因12个，miR159a的靶基因13个，主要包括激素和能量代谢相关的基因，以及与抗性直接相关的LRR类基因、MYB、SPL、EFR等转录因子。而novel_81的靶基因未能预测（表5）。

3 讨 论

葡萄白腐病（*Coniella diplodiwlla*）是葡萄栽培种最具破坏性的病害之一。中国野生葡萄资源中蕴含丰富的抗病种质，刺葡萄对葡萄白腐病为高抗[16-17]。miRNA是一类植物抗病反应中发挥重要作用的非编码RNA，研究发现miRNA在多种植物抗病中起作用[18-20]。

本研究在miRNA测序的基础上，对抗病刺葡萄和感病‘美人指’的数据进行了全面的分析。在KEEG分析的结果显示，刺葡萄和受到葡萄白腐病病原菌侵染后，基础代谢都受到了影响，通过对靶基因的调控网路分析，光合作用、糖酵解（glycolysis）、柠檬酸循环、氧化磷酸化的NADH能量合成途径，均处于下调模式，刺葡萄在抵御白腐病病原侵染时降低了基础代谢的消耗和能量的积累，但是能量代谢中Coumaroyl-CoA途径处于上调模式，推测刺葡萄受到病原菌侵染后，通过快速反应减少其他生命活动，集中能量和物质抵抗病菌侵染。

白腐病病原菌侵染后，150个miRNA的表达量发生变化，44个miRNA的表达在刺葡萄和‘美人指’间存在差异，其中5个miRNA在刺葡萄中特异表达。通过靶基因预测进一步明确了候选miRNA的重要性。靶基因功能中包括了糖代谢（VIT_17s0000g07090、VIT_18s0001g05990）、能量代谢（VIT_18s0001g05990、VIT_13s0158g00340），与抗病直接相关的基因（VIT_01s0010g03210、VIT_01s0127g00070、VIT_14s0006g00680、VIT_15s0021g02040）。具体分析各个候选miRNA基因，miR159a在12 hpi表达上调$2^{6.2}$倍，36 hpi持续上调，其靶基因Myb家族基因，已有的报道中该基因通过调控Myb基因参与拟南芥和杨树的抗病反应[21-22]，并诱导细胞的程序性死亡[23]；另一靶基因为SPL

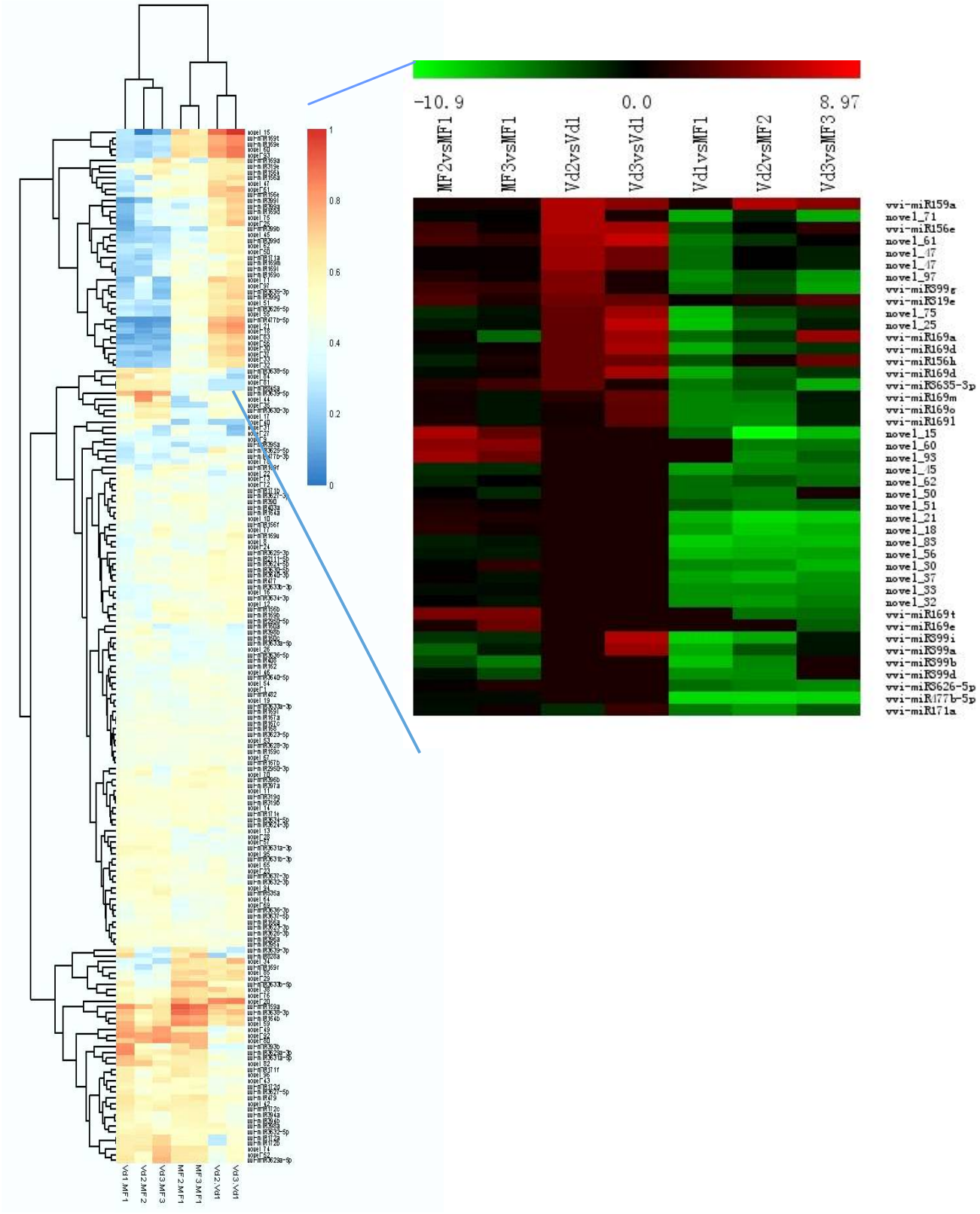


图 5　miRNA 在抗病和感病种质中的表达量热图

Fig. 5　The heatmap of miRNA expression in resistant and susceptible accession

表 4 受白腐病诱导的候选 miRNA

Table 4 Candidate miRNA induced by white rot disease

sRNA	log_2FC						
	MF2 vs MF1	MF3 vs MF1	Vd2 vs Vd1	Vd3 vs Vd1	Vd1 vs MF1	Vd2 vs MF2	Vd3 vs MF3
novel_40	−6.065 2	−3.170 2	−5.820 4	−5.767 7	−0.716 34	NA	NA
miR828a	3.614 4	6.405 5	−5.820 4	−0.964 5	5.369 4	−4.065 1	−1.948 1
novel_81	NA	NA	−4.255 0	−4.182 7	3.905 0	NA	NA
miR172a	NA	NA	−4.530 5	−1.205 4	3.584 5	NA	5.309 1
miR172b	NA	NA	−4.435 9	−1.2054	3.684 5	NA	5.309 1
miR845a	NA	NA	−4.235 5	−4.182 7	3.484 5	NA	NA
novel_75	−1.772 7	−0.737 2	3.409 5	5.388 1	−7.822 7	−3.090 9	−1.832 6
novel_25	−1.063 8	−0.530 7	3.419 5	6.610 5	−8.201 3	−4.178 3	−1.195 2
miR169a	0.549 2	−4.290 2	3.409 5	5.388 1	−4.500 8	−2.090 9	5.309 1
miR169d	−0.450 8	0.572 3	3.408 5	5.803 2	−7.201 3	−3.791 3	−2.105 7
miR156h	−1.450 8	NA	3.459 5	3.803 2	−3.500 8	0.909 12	3.724 1
miR169d	−0.450 8	0.572 3	3.479 5	5.803 2	−7.201 3	−3.791 3	−2.105 7
miR3635-3p	1.212 2	2.151 8	3.469 5	NA	−5.085 8	−3.338 8	−7.293 7
miR319e	2.704 8	1.099 3	3.784 6	3.413 0	0.283 7	1.364 1	2.860 4
novel_97	1.085 3	0.414 8	4.409 5	NA	−5.822 7	−2.948 9	−6.293 7
miR399g	2.093 5	1.736 7	4.458 5	NA	−5.085 8	−3.220 2	−6.878 7
novel_47	0.356 6	0.414 8	5.409 8	3.803 2	−4.500 8	0.101 77	−1.247 7
novel_47	0.356 6	0.414 8	5.449 5	3.803 2	-4.500 8	0.101 77	−1.247 7
novel_61	2.390 5	1.637 2	5.731 5	6.973 1	−5.085 8	−2.195 2	0.114 9
novel_71	−0.293 2	−0.115 7	5.994 5	NA	−7.201 3	−1.363 9	−7.141 7
miR156e	2.008 7	0.414 8	5.994 5	5.803 2	−3.500 8	0.034 653	1.752 3
miR159a	NA	NA	6.216 9	4.803 2	NA	6.196 6	4.724 1

注：FC 表示差异倍数；NA 表示无信号。

Note: FC indicates fold change; NA indicates no signal.

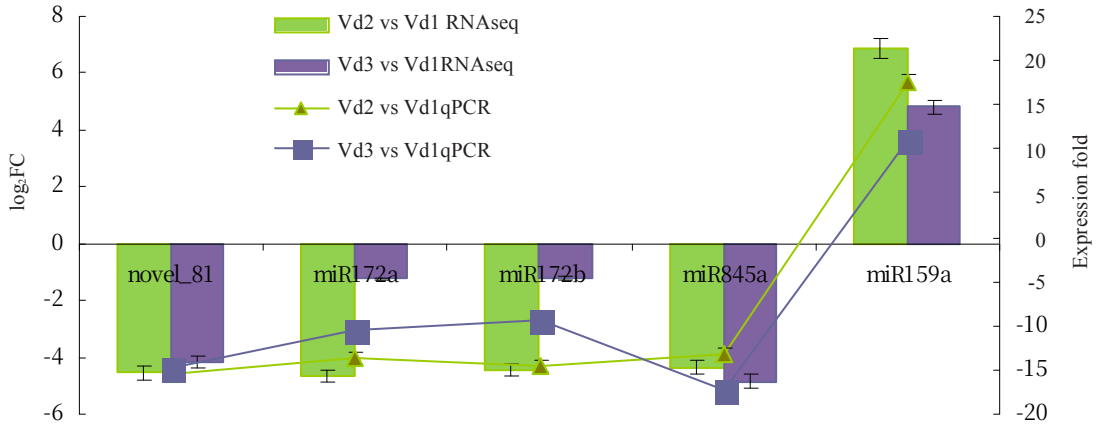


图 6 实施定量与 miRNA 的测序结果验证

Fig. 6 The result of qPCR compare with miRNAseq

（SQUMOSA PROMOTER BINDING PROTEIN LIKE）基因编码重要的转录因子，在已有研究中miR159a作用于拟南芥等多种植物的发育和抗性生命活动中[24-28]，旁证了miR159a在刺葡萄抗白腐病过程中的重要性。miR172a、miR172b、miR845a为显著下调的miRNA，下调倍数都在2^4以上，在拟南芥等多种植物中有参与调控发育和抗性的报道，miR172[29-32]靶基因中有WRKY基因，与本研究中相符，miR845报道协同作用于Myb基因作用于拟南芥和棉花的发育[33]。

基于miRNA表达和靶基因的信息，可以明确刺葡萄在受到白腐病侵染后，基础代谢受到了影响，但是由于能量代谢途径能够高效率运行，保证了刺葡萄抗病过程中的生化反应。该研究结果揭示的5个

表5　靶基因预测

Table 5　microRNA target prediction

miRNA	靶基因 Target gene	靶基因注释 Target gene annotation
miR172a	VIT_00s0516g00030	Dehydrogenase/reductase SDR family member 4
miR172a、miR172b	VIT_01s0127g00070	WRKY trancripter like
	VIT_04s0008g06610	3-hydroxyisobutyryl-coa hydrolase-like protein 2
	VIT_06s0004g03590	Ethylene-responsive transcription factor
	VIT_07s0005g01910	Methyltransferase 1-associated protein
	VIT_07s0031g00220	Floral homeotic protein APETALA
miR172b	VIT_08s0056g01420	Polyribonucleotide nucleotidyltransferase
miR845a	VIT_13s0067g02620	SAC3 family protein
	VIT_13s0158g00340	Glycerol-3-phosphate dehydrogenase [NAD(P)+]
	VIT_15s0021g01560	Leucine carboxyl methyltransferase
	VIT_15s0046g02620	Beta-amylase 2, chloroplastic-like
	VIT_15s0048g01430	Cycloartenol-C-24-methyltransferase 1-like isoform 2
	VIT_16s0098g00540	Golgin candidate 5-like isoform 1
	VIT_17s0000g00600	N-alpha-acetyltransferase 40-like
	VIT_17s0000g07090	UDP-glycosyltransferase 83A1-like
	VIT_18s0001g05990	UDP-glycosyltransferase 85A1
	VIT_18s0001g10980	Patatin group A-3
	VIT_18s0001g11820	Protein DA1
	VIT_18s0001g12210	Cytochrome P450 734A1
miR159a	VIT_00s0226g00070	Protein SHORT-ROOT
	VIT_01s0010g03210	LRR receptor-like serine/threonine-protein kinase GSO2-like
	VIT_03s0017g00940	Cytochrome b6-f complex iron-sulfur subunit, chloroplastic-like
	VIT_03s0038g01150	Auxin-induced protein 10A5
	VIT_04s0043g00760	Probable UDP-N-acetylglucosamine--peptide N-acetylglucosaminyltransferase SEC
	VIT_04s0079g00480	Homeobox-leucine zipper protein ROC8
	VIT_06s0080g00800	Vesicle-associated membrane protein 714
	VIT_07s0104g01230	Auxin response factor 23-like
	VIT_10s0003g02020	Probable LRR receptor-like serine/threonine-protein kinase
	VIT_11s0016g05010	Metallothiol transferase fosb
	VIT_14s0006g00680	Squamosa promoter-binding-like protein 6-like（SPL6）
	VIT_14s0128g00520	Esterase
	VIT_15s0021g02040	Transcription factor MYB48
novel_81	No	No

miRNA刺葡萄的抗病过程中均为特异表达，推测该候选miRNA参与了刺葡萄抗白腐病的免疫反应，可作为后续研究和利用的参考。

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