毛白杨真菌胁迫下miRNA靶基因预测和生物信息分析

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摘要 目前, 关于杨树在真菌胁迫下其miRNAs对基因的调控作用研究较少。准确快速地预测并鉴定miRNA靶基因, 对揭示真菌胁迫下miRNAs在基因调控中的作用至关重要。该文根据miRNA的进化保守性, 通过靶基因预测软件psRNATarget, 以已知的毛果杨miRNAs为探针, 与毛白杨真菌胁迫下转录组的基因序列比对, 找到其中347个miRNAs的772个靶基因, 分别编码与植物激素信号传导、植物病原互作、谷胱甘肽代谢等与植物抗病密切相关的蛋白。miR393通过转录后水平作用于靶基因, 调节生长素信号以响应多种外界刺激。该研究发现了miR393的11个靶基因, 主要参与生长素介导的信号通路; 其中6个靶基因(*SD1-13*、*CYP83B1*、*AFB2*、*TIR1*、*AFB3*和*PSBR*)存在差异表达, 这些基因是研究miR393的生物学功能的关键候选基因。

关键词 毛白杨; 真菌胁迫; miR393; 靶基因

Prediction and Bioinformatics Analysis of miRNA Target Genes in *Populus tomentosa* under Fungus Stress

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Abstract There are few reports profiling the regulatory mechanisms of miRNA in poplar defense responses during fungus stress. It is very important to predict miRNA target genes accurately and rapidly for revealing the regulation effect of miRNA in gene expression during plant-fungus interactions. According to the conservative property of miRNAs, prediction of miRNA target genes was applied with a miRNA target analysis server psRNATarget using previously deposited miRNA sequences from *Populus trichocarpa*. A total of 772 target genes from 347 miRNAs were identified from *Populus tomentosa* under the infection to stem blister canker. Those genes encode proteins involved in disease-resistant categories, like plant hormone signal transduction, plant-pathogen interaction, glutathione metabolism, etc. miR393 regulates auxin signal in response to a variety of external stimulus through the post-transcriptional regulation of target genes. 11 target genes of miR393 were identified, mainly involving in auxin mediated signaling pathway. Among these target genes, 6 genes were differentially expressed in response to fungus stress, including *SD1-13*, *CYP83B1*, *TIR1*, *AFB2*, *AFB3* and *PSBR*. These genes are key candidates for studying the biological function of miR393.

Key words *Populus tomentosa*; fungus stress; miR393; target genes

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microRNAs(miRNAs)是一类在真核生物中发现的长度为21 nt左右、非编码、内源性的单链小分子RNA, 通过序列互补使得靶mRNA降解、翻译抑制或者染色质修饰调节基因表达, 主要发挥转录后水平的负调控作用[1-3]。大多数植物来源的miRNAs能够与靶mRNA近乎完全互补而将其裂解[4]。作为重要的调控分子, miRNAs参与植物生命过程中一系列重要进程, 主要包括植物发育、信号传导、蛋白降解、胁迫相应、病毒入侵, 同时它们也调节自身的生物过程[5-6]。在过去几年里, 有很多miRNAs被发现, 但是目前对于它们的具体作用机制认识还很有限。在高等真核生物中, miRNAs能够调节基因组30%基因的表达[7]。因此, 准确快速地预测并鉴定miRNAs靶基因, 对研究miRNAs的生物学功能具有十分重要的意义。

现有的miRNA靶基因预测软件大多数针对于动物来源的miRNA靶基因预测, 而植物miRNA靶基因预测要求miRNAs更严格地、近乎完全地与靶基因互补。相较于动物miRNAs倾向于在转录水平抑制靶基因表达, 植物miRNAs通过直接降解靶mRNA抑制靶基因表达, 因此需要开发专门的植物miRNA靶基因预测软件[8]。研究者们根据植物miRNA与其靶基因作用的特点开发了多种植物miRNA靶基因预测软件, 如miRU、miRNAassist、PatScan和psRNATarget等。psRNATarget相比于其他预测软件, 能够在Linux系统后台执行高效分布式算法, 满足了针对二代测序数据的高通量分析[9]。

miR393是一个典型的跨植物物种保守miRNA家族[10], 在毛果杨中共有5个成员, 分别为ptc-miR393a-5p、ptc-miR393b-5p、ptc-miR393c、ptc-miR393a-3p和ptc-miR393b-3p。miR393在植物发育(如根系构型)、硝酸盐胁迫、抵抗病原细菌等方面发挥着重要功能。拟南芥(*Arabidopsis thaliana*)经过硝酸盐处理, miR393可被诱导表达, 并且作用于具有基本螺旋–环–螺旋(basic helix-loop-helix, bHLH)结构的转录因子和生长素受体如TIR1(transport inhibitor response protein 1)、AFB1(auxin signaling F-box protein 1)、AFB2和AFB3[11]。miR393/AFB3作为唯一的氮响应模块能够随着内外源氮的可利用量改变根系构型[12-13]。miR393-TIR1之间的精密互作, 是植物发育过程中响应生长素所必需的。在拟南芥中过表达具有miR393抗性的TIR1, 能够提高植物对于生长素的敏感性, 导致植物主根生长抑制、侧根生长过度、叶片表型改变和延迟开花等多种结果[14]。动植物通过编码模式识别受体(pattern recognition receptors, PRRs), 识别病原体相关分子(pathogen-associated molecular pattern, PAMP), 然后调节一系列miRNAs的表达[15]。miR393是最早被发现的能够参与植物体抵御细菌病原的miRNA, 能够响应PAMP。在细菌鞭毛蛋白flg22刺激下, 植物体能够大量生成miR393, 从而抑制生长素信号传导, 增强拟南芥对于假单胞菌(*Pseudomonas syringae*)的抗性, 抑制细菌生长[10]。在杨树中, 有研究者发现欧洲山杨(*Populus tremula*)受到紫外辐射(UV-B)时, 内部miR393表达上调, 并且提出一个响应UV-B的miRNA网络调控模型[16]。

目前, 关于miR393的研究局限于拟南芥[11,14,17]、水稻(*Oryza sativa*)[18-19]等非多年生植物, 在多年生木本植物中研究较少; 对于miR393的研究更多地集中于其在盐碱、干旱、低温等非生物胁迫下的调控作用, 对于病理条件下miR393的功能研究较少, 仅局限在细菌病原的研究, 对于miR393是否响应真菌胁迫迄今未见报道。依据毛白杨与毛果杨具有高度同源性[20], 本文利用毛果杨的miRNA数据, 通过miRNA靶基因预测软件psRNATarget寻找毛白杨在真菌胁迫下转录组中miR393的靶基因, 并通过生物信息学手段对靶基因进行生物信息分析, 以期为进一步了解杨树miR393家族的功能奠定基础。

1 材料与方法

1.1 靶基因预测流程

从miRNA数据库miRBase(www.mirbase.org, release 20)[21-24]下载毛果杨成熟miRNA序列。毛白杨受溃疡病[致病菌: 葡萄座腔菌(*Botryosphaeria dothidea*)]感染14 d后获得的树皮组织构建cDNA库(BD)和对照组(CK, 未接种致病菌)cDNA库, 作为寻找靶基因的mRNA来源(具体实验方法详见参考文献[20]; 转录组数据收录号为SRP033626, BD库和CK库的实验收录号分别为SRX389421和SRX389392)。本研究利用在线靶基因预测软件psRNATarget(http://plantgrn.noble.org/psRNATarget/)在毛白杨真菌胁迫下转录组数据库中预测miRNA的靶基因。按照软件方法所述设置严格的筛选标准, 设置最大期望值为3, 其他为默认参数; 得分小于3

且UPE(maximum energy to unpair the target site)小于25.0 kcal/mol的序列为miRNA的潜在靶基因。

1.2 靶基因功能注释

经过psRNATarget预测得到的靶基因进行Gene Ontology(GO; http://www.geneontology.org/)注释和Kyoto Encyclopedia of Genes and Genomes(KEGG; http://www.genome.jp/kegg/)代谢通路功能分析。通过Blast2GO[25]比对得到基因的功能注释信息, 通过对应数据库中的映射关系, 得到对应基因的GO信息。并以此为基础, 对得到的注释信息利用在线GO作图软件BGI WEGO(http://wego.genomics.org.cn/cgi-bin/wego/index.pl)进行GO的功能分类, 分别统计基因在细胞组成、生物过程以及分子功能三个类别中的注释情况。通过KAAS server[26]进行KEGG代谢通路分析, 利用Blast信息得到对应的KO号, 分析不同代谢通路的响应情况, 同时找出关键的代谢通路。

1.3 靶基因的差异表达分析

为了鉴别获得的miRNA靶基因在BD库和CK库是否存在差异表达, 采用RPKM统计法来计算靶基因的表达丰度[27]。根据各个靶基因的表达丰度值做差异分析, 满足2倍上调/下调($|\log_2(BD/CK)|\geq 1$)的条件, 则视为差异基因。对以上方法得到的差异基因, 进行GO和KEGG富集分析, 使用超几何分布算法(Phyper)计算前景转录本同GO/Pathway分类中某个特定分支的*P*值, 结果用FDR(false discovery rate)法进行校正, $P<0.05$代表具有统计学意义的GO分类和KEGG通路。

2 结果

2.1 psRNATarget预测靶基因的获得

通过psRNATarget算法, 得到1 711个靶基因预测结果, 包括来自于99个miRNA家族的347个的miRNA对应上772个靶基因。其中, 预测靶基因总数最多有30个miRNA家族(图1)。ptc-miR169(31, 4.0%)、ptc-miR475(28, 3.6%)、ptc-miR156(26, 3.4%)、ptc-miR396(26, 3.4%)和ptc-miR172(22, 2.8%)是毛白杨受溃疡病感染条件下, 寻找到的靶基因最多的5个miRNA家族(图1)。ptc-miR169基因家族有31个miRNA成员, 是杨树中规模最大的miRNA基因家族[22], 故其预测到的靶基因数目也最多。

2.2 靶基因的功能注释和代谢通路分析

通过GO注释和KEGG代谢通路分析, 772个靶基因中有561(72.67%)个基因获得了GO注释(图2), 映射到40个GO terms(level 2), 分别注释到Cellular component分类的10个GO terms, Biological process分类的19个GO terms以及Molecular function分类的11个GO terms。结果显示, 预测靶基因集合分别聚集在绑定、代谢过程、细胞过程等一系列基本生物学过程。作为基本的生物学过程, 即使在病理条件下, 这些GO terms仍然占据着主导地位。在KEGG通路分析中, 139(18.0%)个靶基因注释到159条KEGG代谢通路, 除了集合于RNA运输(ko03013. RNA transport)、核糖体(ko03010. Ribosome)以及内质网蛋白加工(ko04141. Protein processing in endoplasmic reticulum)等与基本生命过程相关的代谢通路之

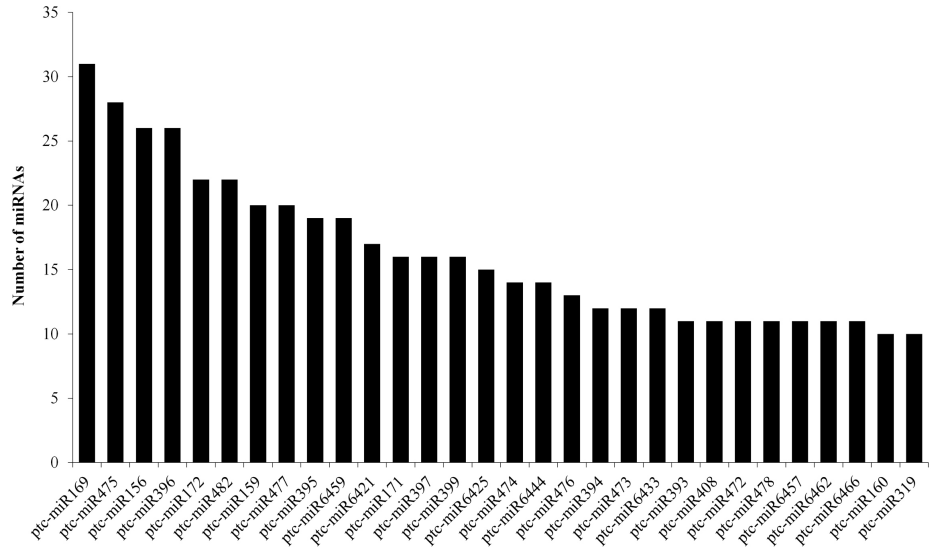


图1 预测靶基因数目分布图

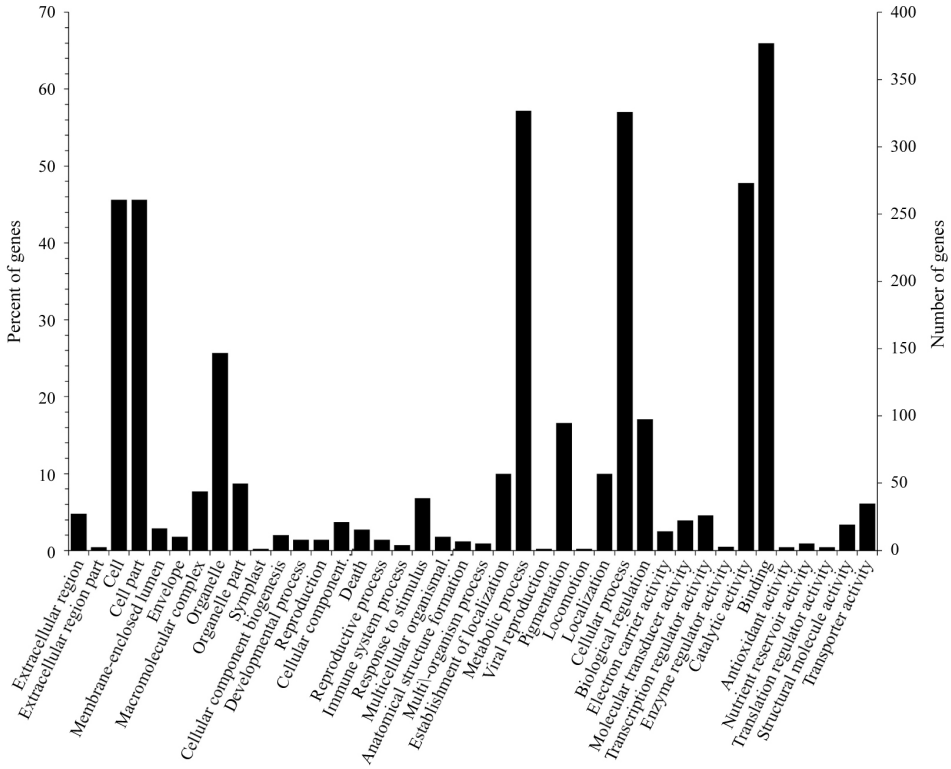
Fig.1 Distribution for the number of predicted target genes

外, 靶基因集合还显著富集于植物激素信号传导(ko04075. Plant hormone signal transduction)、植物病原互作(ko04626. Plant-pathogen interaction)、谷胱甘肽代谢(ko00480. Glutathione metabolism)、抗原加工(ko04612. Antigen processing and presentation)、泛素介导的蛋白水解(ko04120. Ubiquitin mediated proteolysis)等与植物抗病密切相关通路, 如ko04626是直接与病原作用相关的通路, 通路中包括抗病相关蛋白RPS2、钙离子绑定蛋白CML以及蛋白激酶CPK等(表1)。

表1 靶基因的10条显著KEGG通路

Table 1 The top 10 significant KEGG pathways in target genes

KEGG通路 KEGG pathway	基因数量 Gene number	预测蛋白 Predicted protein
Ko03013. RNA transport	10(7.2%)	PMRT15, ABH1, NUPL2, NUP93, POP1, ACIN1, NUP210, NUP205, SEH1, THOC1
Ko03010. Ribosome	10(7.2%)	RPS16, RPS3A, ARP2, RPS7, RPL13, RPL40, RPL11, RP10, RPS17
Ko04141. Protein processing in endoplasmic reticulum	9(6.5%)	RIN2, RNF185, DER1, PDIL1-4, BAP31, SKP1B, HR23, PLAA, OST6
Ko04075. Plant hormone signal transduction	8(5.8%)	ARR14, TIR1, SAUR, HAB1, EIN3, TGA1, ERF1B
Ko04626. Plant-pathogen interaction	6(4.3%)	RPS2, CPK4, SK5, CML36
Ko03040. Spliceosome	6(4.3%)	U2AF1, ABH1, LSM4, ACIN1, RBM17, THOC1
Ko00500. Starch and sucrose metabolism	5(3.6%)	BAM3, GAUT6, SPS3, sacA
Ko00480. Glutathione metabolism	5(3.6%)	GOR, PGD, RRM2, GST
Ko04612. Antigen processing and presentation	4(2.9%)	NFYA1, NFYA7, NFYA10
Ko04120. Ubiquitin mediated proteolysis	4(2.9%)	APC5, UBE1, SKP1, UBC27



靶基因的功能在Cellular component分类中主要聚集于Cell和Cell part, 在Biological process分类中主要聚集于Metabolic process和Cellular process, Molecular function分类中主要聚集于Binding和Catalytic activity。

"Cell" and "Cell part" were dominant within the cellular component terms, "Metabolic process" and "Cellular process" in the Biological process terms, and "Binding" and "Catalytic activity" in the Molecular function terms.

图2 靶基因的功能分类

Fig.2 Functional classification of the target genes

2.3 靶基因的差异表达分析

来源于毛白杨感病BD库和对照组CK库的miRNA靶基因在两个库之间存在差异表达。经过差异表达分析, 满足2倍上调/下调的差异靶基因共有372个, 其中89(23.92%)个上调(log_2(BD/CK)≥1), 283(76.08%)个下调(log_2(CK/BD)≥1)。275个差异靶基因获得了GO注释, 映射到38个GO terms(level 2)。对于GO分类进行富集分析, Cellular component、Biological process以及Molecular function分类中各自显著的GO terms与所有靶基因GO分类结果(图2)相同, 例如在Cellular component分类中主要聚集于Cell和Cell part。在KEGG富集分析中, 共有50个差异基因获得了通路分析, 映射到92个代谢通路。其中植物激素信号传导(ko04075. Plant hormone signal transduction)注释到靶基因总数为8个(表2), 其中差异基因数量(5个)最多, 分别为*ERF1*、*AFB2*、*TIR1*、*PP2C*和*SAUR*, 这些基因都是该通路中参与调控植物激素表达和作用的关键因子。植物激素信号传导通路与多个通路相关, 如泛素介导蛋白质水解(ko04120. Ubiquitin mediated proteolysis)、色氨酸代谢(ko00380. Tryptophan metabolism)、玉米素生物合成(ko00908. Zeatin biosynthesis)等。

2.4 miR393靶基因功能预测

miR393作为*TIR1*、*AFB2*和*AFB3*的关键调控因子, 在拟南芥与假单胞菌的鞭毛蛋白作用时, 能够被诱导表达, 同时也在多种非生物胁迫(低温、干旱、高盐等)发挥着重要作用[28]。在毛白杨感染溃疡病的转录组中, 我们总共发现了11个miR393靶基因, 每个miR393家族成员能够对应多个靶基因, 同时每个靶基因也能够与多个miRNA碱基互补(表3)。根据GO富集分析显示, miR393靶基因主要涉及生长素介导的信号通路(GO: 0009734; Auxin mediated signaling pathway)。在KEGG富集分析中发现, 靶基因主要富集到植物激素信号传导通路。经过差异表达分析, 鉴定出6个差异表达靶基因, 分别为*SD1-13*、*CYP83B1*、*AFB2*、*TIR1*、*AFB3*和*PSBR*。根据通路富集分析的结果显示, 在植物激素信号传导通路中, miR393靶基因占3/8(表2), 说明miR393家族成员及其靶基因在该通路发挥着重要作用, 推测其可能在毛白杨溃疡病病理条件下担当重要的内源响应元件。

表2 参与植物激素信号传导通路(ko04075)的靶基因

Table 2 Target genes involved in plant hormone signal transduction (ko04075)

miRNA miRNA	基因编号 Gene ID	编码蛋白 Predicted protein	靶基因 Target gene	功能 Function
Ptc-miR169t, ptc-miR169z	Comp92404	ERF1(ethylene-responsive transcription factor 1)	Potri.002G039000.1	Sequence-specific DNA binding transcription factor
Ptc-miR393a-3p, ptc-miR393b-3p	Comp103443	AFB2(auxin signaling F-box protein 2)	AT3G26810.1	Ubiquitin-protein ligase activity
Ptc-miR393a-3p, ptc-miR393b-3p	Comp110720	EIN3(ethylene-insensitive protein 3)	AT1G73730.1	Cellular response to sulfate starvation
Ptc-miR393a-5p, ptc-miR393b-5p, ptc-miR393c	Comp106164	TIR1(transport inhibitor response 1)	AT3G62980.1	Response to molecule of bacterial origin
Ptc-miR482c-3p	Comp106396	PP2C(protein phosphatase 2C)	AT1G72770.1	Protein serine/threonine phosphatase activity
Ptc-miR530a, ptc-miR530b	Comp111939	ARR-B(two-component response regulator ARR-B family)	AT2G01760.1	Cytokinin mediated signaling pathway
Ptc-miR6433-5p	Comp72671	SAUR(SAUR family protein)	Potri.012G102700.1	Auxin mediated signaling pathway
Ptc-miR6466-5p	Comp113355	TGA(transcription factor TGA)	AT5G65210.1	bZIP transcription factor

基因编号: 实验中靶基因的编号; 靶基因: 靶基因序列在Phytozome V9.1(http://www.phytozome.net/)Blast获得的同源基因(毛果杨或者拟南芥)编号。

Gene ID: gene number in the present study; Target gene: gene sequence was blasted against the Phytozome V9.1(http://www.phytozome.net/)to get the accession number of homologous gene from *Populus trichocarpa* or *Arabidopsis thaliana*.

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Ptc-miR393a-5p    21   CUA GUU ACG CUA GGG AAA CCU   1     (3′→5′)
                       |  | * | | ||   ||| *| | ||| | ||
Comp70101         165  GUU UAA UGG GAU UCC UUU GGA   185   (5′→3′)

Ptc-miR393b-5p    20   UAG UUA CGC UAG GGA AAC CU    1     (3′→5′)
                       || | |  |* | ||* ||| | ||| *
Comp113948        728  AUC AUU GUG AUU CCU UUG GG    747   (5′→3′)

Ptc-miR393a-3p    20   UAG GUU UCC CUA UCG UAC UA    1     (3′→5′)
                       ||| ||| ||| ||| || ||  *|
Comp103443        535  AUC CAA AGG GAU CGC AUU GU    554   (5′→3′)

Ptc-miR393c       20   UAG UUA CGC UAG GGA AAC CU    1     (3′→5′)
                       *  | ||| || | ||| || | ||| ||
Comp106164        1765 GAC AAU GCG AUC CCU UUG GA    1784  (5′→3′)

Ptc-miR393b-3p    20   UAG GUU UCC CUA UCG UAC UA    1     (3′→5′)
                       ||| ||| || | ||| || ||  * |
Comp113305        745  AUC CAA AGG GAU CGC AUU GU    764   (5′→3′)

Ptc-miR393a-3p    20   UAG GUU UCC CUA UCG UAC UA    1     (3′→5′)
                       || *  | | ||  ||| ||| | || ||
Comp92615         619  AUU GAA AGU GAU AGC AUG AU    638   (5′→3′)
```

miR393家族成员与6个差异靶基因之间碱基互补配对情况; 竖线代表碱基完全互补, “*”代表G:U摆动。

Members of miR393 family and their complementary sites within their 6 target genes; Vertical lines represent Watson-Crick pairing and “*” represents G:U wobble pairing.

图3 miR393与靶基因互补位点图

Fig.3 The complementary site of miR393 and target genes

3 讨论

miRNAs作为一种非编码的基因表达调控因子, 分布范围广泛, 参与复杂的生物学过程。miRNAs调控的靶基因众多, 寻找并鉴定miRNA的靶基因是研究miRNA功能的前提。利用miRNAs靶基因预测软件psRNATarget, 通过设置严格的筛选条件, 在毛白杨真菌胁迫下转录组中共获得了1 711个靶基因预测结果, 包括来自于99个miRNA家族的347个的miRNA比对上772个靶基因。相对于利用植物体的EST(expressed sequence tag)序列寻找靶基因, 本研究利用毛白杨真菌胁迫下, 感病树皮组织构建的cDNA库寻找靶基因, 更容易找出与真菌防御相关的miRNA靶基因, 针对性更强。经过注释和富集分析, miRNAs靶基因集合显著富集于植物激素信号传导、植物病原互作、谷胱甘肽代谢、抗原加工、泛素介导的蛋白水解等与植物抗病密切相关通路。

miR393作为第一个被发现与抗病相关的miRNA家族[10], 通过抑制生长素信号增强植物体对

表3 miR393靶基因预测结果

Table 3 Prediction of miR393 target genes

基因编号 Gene ID	名称 Name	差异倍数 Fold change	AGI登录号 AGI accession No.	miRNA miRNA	抑制作用 Inhibition	期望值 Expectation	能量 UPE
Differentially expressed gene							
Comp70101	SD1-13	1.33	AT1G11350.1	Ptc-miR393a-5p, ptc-miR393b-5p, ptc-miR393c	Cleavage	3	21.10
Comp113948	CYP705A5	1.76	AT5G47990.1	Ptc-miR393a-5p, ptc-miR393b-5p, ptc-miR393c	Cleavage	2.5	18.57
Comp103443	AFB2	–1.50	AT3G26810.1	Ptc-miR393a-3p, ptc-miR393b-3p	Cleavage	3	22.08
Comp106164	TIR1	–1.51	AT3G62980.1	Ptc-miR393a-5p, ptc-miR393b-5p ptc-miR393c	Cleavage	2	21.64
Comp113305	AFB3	–1.21	AT1G12820.1	Ptc-miR393a-3p, ptc-miR393b-3p	Cleavage	3	15.30
Comp92615	PSBR	–2.76	AT1G79040.1	Ptc-miR393a-3p, ptc-miR393b-3p	Cleavage	2.5	11.22
Non-differentially expressed gene							
Comp90123	ABCB26	–0.13	AT1G70610.1	Ptc-miR393a-3p, ptc-miR393b-3p	Cleavage	3	24.82
Comp109624	NDB3	–0.43	AT4G21490.1	Ptc-miR393a-5p, ptc-miR393b-5p, ptc-miR393c	Cleavage	1.5	9.88
Comp110720	EIL3	–0.47	AT1G73730.1	Ptc-miR393a-3p, ptc-miR393b-3p	Translation	3	8.91
Comp110461	–	–0.67	AT5G20610.1	Ptc-miR393a-3p, ptc-miR393b-3p	Translation	3	14.59
Comp97303	OGG1	–0.34	AT1G21710.1	Ptc-miR393a-3p, ptc-miR393b-3p	Cleavage	2.5	20.09

基因编号: 实验中靶基因的编号; 差异倍数: 差异倍数$\log_2$(BD/CK); AGI登录号: 靶基因序列在拟南芥数据库TAIR(http://www.*Arabidopsis*.org/index.jsp, E value<1.0E-5)Blast获得的拟南芥同源基因编号; 抑制作用: psRNATarget预测miRNA抑制靶基因表达的方式, 直接降解(cleavage)或者抑制翻译(translation); 期望值: 靶基因预测期望值; 能量: 解开mRNA靶位点次级结构需要的能量; “–”: 数据库中未给定基因名称。

Gene ID: gene number in the present study; Fold change: the ratio of $\log_2$(BD/CK); AGI accession No.: gene sequence was blasted against the the TAIR database (http://www.*Arabidopsis*.org/index.jsp, with an E value<1.0E-5) to get the accession number of homologous gene from *Arabidopsis thaliana*; Inhibition: the predicted way of miRNA inhibiting mRNA expression, cleavage or translational inhibition; Expectation: the complementary score between miRNA and their target transcript; UPE: allowed maximum energy to unpair the target site; “–”: AGI accession No. without a gene name.

于细菌的抗性。在植物激素信号传导通路中, 属于miR393靶基因占主导地位, 说明miR393与植物激素信号传导涉及的生物学过程密切相关。关于miR393的11个靶基因的分析中, miR393靶基因主要涉及生长素介导的信号通路(GO: 0009734; auxin mediated signaling pathway)。根据靶基因在BD库和CK库的表达情况, 鉴定出6个差异表达靶基因, 分别为*SD1-13*、*CYP83B1*、*AFB2*、*TIR1*、*AFB3*和*PSBR*, 这些差异表达基因通过改变自身表达或者调控其他基因表达, 使得植物体对于真菌感染做出积极的防御。

植物生长素调节植物发育的各个方面, 包括抵御病原入侵的过程。生长素通过与TIR1类似的F-box蛋白直接的物理作用诱导基因的表达, 进而参与植物体的病原抵御过程。植物病原自身能够合成生长素, 或者通过操纵寄主的生长素合成途径干扰植物体的正常生命过程[29]。反之, 植物受到病原感染时, 进化出了抑制生长素信号的机制, 以此抵御病原入侵。TIR1、AFB2、AFB3属于F-box蛋白, 作为植物生长素受体能够被miR393通过DCL1依赖的模式特异地降解[10], 其在真菌感染过后表达量下调, 我们推测使其受到了miR393的作用。PSBR是光系统II(photosystem II, PS II)复合体的一种蛋白, 大小为10 kDa, *PSBR*基因只存在与绿藻和高等植物的细胞核中。目前关于PSBR的研究主要集中于其在蛋白复合体参与光合作用时能够起到稳定作用[30]。有研究者报道, 从小盐芥(*Thellungiella halophila*)分离出了具有抗盐功能的*PSBR*(AT1G79040)基因[31]。在本研究中, *PSBR*作为ptc-miR393a-3p和ptc-miR393b-3p的靶基因, 在毛白杨感染溃疡病条件下, 表达量明显下调, 我们推测其可能参与响应真菌入侵过程。

在miR393预测的6个差异靶基因中, 有2个靶基因*SD1-13*和*CYP83B1*表达上调。SD1-13, 又称RKS2, 是一种S-domain受体蛋白激酶(receptor-like kinase, RLK), 能够与AtPUB-ARM E3泛素连接酶作用, 参与保守的信号通路; SD1-13作为积极的调节蛋白, 能够介导植物本身的自交不亲和(self-incompatibility, SI)[32], 也能够被水杨酸诱导[33]。有研究也发现, 该类蛋白激酶也能一定程度上响应ABA(abscisic acid)信号, 参与植物的胁迫相应。近年来发现, 很多S-domain *RLK*基因在受到病原感染、机械损伤或者防御相关物质(如水杨酸)作用时, 表达上调, 参与植物体防御过程[34-37]。CYP83B1是一种细胞色素P450蛋白, 能够调节生长素的合成[38]。细胞色素P450蛋白广泛参与植物体的次级代谢, 包括合成大分子物质如木质素、植物生长调节剂(如茉莉酸、赤霉素)等[39]。*CYP83B1*可能通过调节植物体的次级代谢, 对病原入侵做出积极响应。拟南芥受到黑斑病菌(*Alternaria brassicicola*)感染时, *CYP83B1*基因表达可被激活[40]。

本研究应用psRNATarget进行毛白杨在真菌胁迫下转录组中miRNA靶基因的预测, 并采用生物信息学方法对相关的靶基因功能注释和富集。共预测得到772个靶基因, 靶基因集合显著富集于植物激素信号传导、植物病原互作、谷胱甘肽代谢等与植物抗病密切相关通路。由此可见, miRNAs在杨树溃疡病条件下活跃地调节基因表达。miR393靶基因与生长素介导的信号通路具有密切关系, 其靶基因包括*SD1-13*、*CYP83B1*、*AFB2*、*TIR1*、*AFB3*和*PSBR*等。本文对于miR393靶基因的预测能够为后期靶基因的实验鉴定及其生物学功能研究提供理论基础。进一步研究目标为*TIR1*和*AFB2*等关键靶基因的功能, 需要克隆靶基因, 并构建植物表达载体转化毛白杨, 获得稳定表达的转基因植株, 研究其在真菌胁迫下的功能, 相关实验目前正在进行中。

参考文献 (References)

1 Jones-Rhoades MW, Bartel DP, Bartel B. MicroRNAs and their regulatory roles in plants. Annu Rev Plant Biol 2006; 57: 19-53.

2 Jung JH, Seo PJ, Park CM. MicroRNA biogenesis and function in higher plants. Plant Biotechnology Rep 2009; 3(2): 111-26.

3 Jin H. Endogenous small RNAs and antibacterial immunity in plants. Febs Lett 2008; 582(18): 2679-84.

4 Bartel DP. MicroRNAs: Genomics, biogenesis, mechanism, and function. Cell 2004; 116(2): 281-97.

5 Chen X. MicroRNA biogenesis and function in plants. Febs Lett 2005; 579(26): 5923-31.

6 Dugas DV, Bartel B. MicroRNA regulation of gene expression in plants. Curr Opin Plant Biol 2004; 7(5): 512-20.

7 Yu Z, Jian Z, Shen SH, Purisima E, Wang E. Global analysis of microRNA target gene expression reveals that miRNA targets are lower expressed in mature mouse and *Drosophila* tissues than in the embryos. Nucleic Acids Res 2007; 35(1): 152-64.

8 Dai X, Zhao PX. psRNATarget: A plant small RNA target analysis server. Nucleic Acids Res 2011; 39(suppl 2): W155-W59.

9 Dai X, Zhuang Z, Zhao PX. Computational analysis of miRNA targets in plants: Current status and challenges. Brief in Bioinform 2011; 12(2): 115-21.

10 Navarro L, Dunoyer P, Jay F, Arnold B, Dharmasiri N, Estelle M, *et al*. A plant miRNA contributes to antibacterial resistance by repressing auxin signaling. Sci Signal 2006; 312(5772): 436.

11 Si-Ammour A, Windels D, Arn-Bouldoires E, Kutter C, Ailhas J, Meins F, *et al*. miR393 and secondary siRNAs regulate expression of the TIR1/AFB2 auxin receptor clade and auxin-related development of *Arabidopsis* leaves. Plant Physiol 2011; 157(2): 683-91.

12 Vidal EA, Araus V, Lu C, Parry G, Green PJ, Coruzzi GM, *et al*. Nitrate-responsive miR393/AFB3 regulatory module controls root system architecture in *Arabidopsis thaliana*. Proc Natl Acad Sci USA 2010; 107(9): 4477-82.

13 Vidal EA, Moyano TC, Riveras E, Contreras-López O, Gutiérrez RA. Systems approaches map regulatory networks downstream of the auxin receptor AFB3 in the nitrate response of *Arabidopsis thaliana* roots. Proc Natl Acad Sci USA 2013; 110(31): 12840-5.

14 Chen ZH, Bao ML, Sun YZ, Yang YJ, Xu XH, Wang JH, *et al*. Regulation of auxin response by miR393-targeted transport inhibitor response protein 1 is involved in normal development in *Arabidopsis*. Plant Mol Biol 2011; 77(6): 619-29.

15 Navarro L, Jay F, Nomura K, He SY, Voinnet O. Suppression of the microRNA pathway by bacterial effector proteins. Science 2008; 321(5891): 964-67.

16 Jia X, Ren L, Chen QJ, Li R, Tang G. UV-B-responsive microRNAs in Populus tremula. J Plant Physiol 2009; 16(18): 2046-57.

17 Kepinski S, Leyser O. The *Arabidopsis* F-box protein TIR1 is an auxin receptor. Nature 2005; 435(7041): 446-51.

18 Gao P, Bai X, Yang L, Lü D, Pan X, Li Y, *et al*. Osa-MIR393: A salinity-and alkaline stress-related microRNA gene. Mol Biol Rep 2011; 38(1): 237-42.

19 Jian X, Zhang L, Li G, Zhang L, Wang X, Cao X, *et al*. Identification of novel stress-regulated microRNAs from *Oryza sativa* L. Genomics 2010; 95(1): 47-55.

20 Liao WH, Ji LX, Wang J, Chen Z, Ye MX, Ma HD, *et al*. Identification of glutathione S-transferase genes responding to pathogen infestation in *Populus tomentosa*. Funct Integr Genomic 2014; 14(3): 517-29.

21 Kozomara A, Griffiths-Jones S. miRBase: Annotating high confidence microRNAs using deep sequencing data. Nucleic Acids Res 2014; 42(D1): D68-D73.

22 Kozomara A, Griffiths-Jones S. miRBase: Integrating microRNA annotation and deep-sequencing data. Nucleic Acids Res 2011; 39(Database issue): D152-7.

23 Griffiths-Jones S, Saini HK, van Dongen S, Enright AJ. miRBase: Tools for microRNA genomics. Nucleic Acids Res 2008; 36(Database issue): D154-8.

24 Griffiths-Jones S, Grocock RJ, van Dongen S, Bateman A, Enright AJ. miRBase: microRNA sequences, targets and gene nomenclature. Nucleic Acids Res 2006; 34(Database issue): D140-4.

25 Götz S, García-Gómez JM, Terol J, Williams TD, Nagaraj SH, Nueda MJ, *et al*. High-throughput functional annotation and data mining with the Blast2GO suite. Nucleic Acids Res 2008; 36(10): 3420-35.

26 Moriya Y, Itoh M, Okuda S, Yoshizawa AC, Kanehisa M. KAAS: An automatic genome annotation and pathway reconstruction server. Nucleic Acids Res 2007; 35(Web Server issue): W182-5.

27 Mortazavi A, Williams BA, McCue K, Schaeffer L, Wold B. Mapping and quantifying mammalian transcriptomes by RNA-Seq. Nat Methods 2008; 5(7): 621-8.

28 Sunkar R, Chinnusamy V, Zhu J, Zhu JK. Small RNAs as big players in plant abiotic stress responses and nutrient deprivation. Trends Plant Sci 2007; 12(7): 301-9.

29 Wang D, Pajerowska-Mukhtar K, Culler AH, Dong X. Salicylic acid inhibits pathogen growth in plants through repression of the auxin signaling pathway. Curr Biol 2007; 17(20): 1784-90.

30 Allahverdiyeva Y, Mamedov F, Suorsa M, Styring S, Vass I, Aro EM. Insights into the function of PsbR protein in *Arabidopsis thaliana*. Biochim Biophys Acta 2007; 1767(6): 677-85.

31 Du J, Huang YP, Xi J, Cao MJ, Ni WS, Chen X, *et al*. Functional gene-mining for salt-tolerance genes with the power of *Arabidopsis*. Plant J 2008; 56(4): 653-64.

32 Samuel MA, Mudgil Y, Salt JN, Delmas F, Ramachandran S, Chilelli A, *et al*. Interactions between the S-domain receptor kinases and AtPUB-ARM E3 ubiquitin ligases suggest a conserved signaling pathway in *Arabidopsis*. Plant Physiol 2008; 147(4): 2084-95.

33 Ohtake Y, Takahashi T, Komeda Y. Salicylic acid induces the expression of a number of receptor-like kinase genes in *Arabidopsis thaliana*. Plant Cell Physiol 2000; 41(9): 1038-44.

34 Pastuglia M, Swarup R, Rocher A, Saindrenan P, Roby D, Dumas C, *et al*. Comparison of the expression patterns of two small gene families of S gene family receptor kinase genes during the defence response in *Brassica oleracea* and *Arabidopsis thaliana*. Gene 2002; 282(1): 215-25.

35 Gish LA, Clark SE. The RLK/Pelle family of kinases. Plant J 2011; 66(1): 117-27.

36 Cole SJ, Diener AC. Diversity in receptor-like kinase genes is a major determinant of quantitative resistance to *Fusarium oxysporum* f. sp. matthioli. New Phytol 2013; 200(1): 172-84.

37 Sakamoto T, Deguchi M, Brustolini OJ, Santos AA, Silva FF, Fontes EP. The tomato RLK superfamily: Phylogeny and functional predictions about the role of the LRRII-RLK subfamily in antiviral defense. BMC Plant Biol 2012; 12(1): 229.

38 Bak S, Tax FE, Feldmann KA, Galbraith DW, Feyereisen R. CYP83B1, a cytochrome P450 at the metabolic branch point in auxin and indole glucosinolate biosynthesis in *Arabidopsis*. Plant Cell 2001; 13(1): 101-11.

39 Barlier I, Kowalczyk M, Marchant A, Ljung K, Bhalerao R, Bennett M, *et al*. The SUR2 gene of *Arabidopsis thaliana* encodes the cytochrome P450 CYP83B1, a modulator of auxin homeostasis. Proc Natl Acad Sci USA 2000; 97(26): 14819-24.

40 Stintzi A, Weber H, Reymond P, Farmer EE. Plant defense in the absence of jasmonic acid: the role of cyclopentenones. Proc Natl Acad Sci USA 2001; 98(22): 12837-42.