PLANT SIGNALING & BEHAVIOR
2020, VOL. 15, NO. 4, e1744347 (4 pages)
https://doi.org/10.1080/15592324.2020.1744347

Taylor & Francis
Taylor & Francis Group

SHORT COMMUNICATION

Expression of rice siR109944 in *Arabidopsis* affects plant immunity to multiple fungal pathogens

Lulu Qiao[a,b], Hongwei Zhao [a,b], Hailing Jin [c], and Dongdong Niu[a,b]

[a]College of Plant Protection, Nanjing Agricultural University, Nanjing, China; [b]Key Laboratory of Integrated Management of Crop Diseases and Pests (Ministry of Education), Nanjing, China; [c]Department of Microbiology & Plant Pathology, University of California, Riverside, CA, USA

ABSTRACT

Plant small RNA (sRNA)-mediated gene expression has a conserved role in regulating plant growth, development, and immunity. Heterologous expression of sRNA contributes to determining whether the function of sRNA is conservative or independent. We recently characterized the Tourist-miniature inverted-repeat transposable element (MITE)-derived siR109944 had a conserved function that enhanced susceptibility to *Rhizoctonia solani* infection by affecting auxin homeostasis in rice and *Arabidopsis*. To ascertain whether the function of rice siR109944 has a broad-spectrum immunity in *Arabidopsis*, we infected *Arabidopsis* with a variety of fungal pathogens. Overexpression of siR109944 in *Arabidopsis* increased susceptibility to *Botrytis cinerea, Sclerotinia sclerotium*, and *Verticillium dahliae* infection. Further studies found that *Arabidopsis* auxin-related miRNAs were suppressed in *siR109944 OE*. Our results demonstrated that overexpression of rice siR109944 in *Arabidopsis* affected immune responses to multiple pathogens by inhibiting auxin-related miRNA expression *in planta*.

ARTICLE HISTORY
Received 8 February 2020
Revised 27 February 2020
Accepted 28 February 2020

KEYWORDS
Heterologous expression; siRNA; *Arabidopsis* immunity; multiple fungal pathogens

sRNA-mediated RNA interference (RNAi) is a conserved immune regulatory mechanism in most eukaryotes, which is involved in host immunity and pathogen virulence.[1–3] Many studies have identified the role of sRNAs in rice and *Arabidopsis* in plant immunity; however, the heterologous regulating function of sRNAs has scarcely been studied. Heterologous gene expression utilizes gene resources and can be useful for the screening of disease-resistant breeding and increasing the cognition of immune regulatory responses among different species. For example, heterologous expression of *Vitis amurensis VaERF20* in *Arabidopsis* improves resistance to *Botrytis cinerea* and *Pseudomonas syringae* pv. *tomato* DC3000.[4] Host-induced gene silencing is a combination of heterologous expression and RNAi technology to enhance host resistance by constructing transgenic plants by targeting important pathogenic genes that are necessary for growth, development, and pathogenicity.[5–7]

Based on our present study, we found that rice Tourist-miniature inverted-repeat transposable element (MITE)-derived siR109944 suppressed plant immunity to sheath blight, and overexpression of siR109944 in *Arabidopsis* could enhance susceptibility to *Rhizoctonia solani* by modulating auxin homeostasis.[8] To explore whether overexpression of siR109944 enhanced the broad-spectrum immune response in *Arabidopsis*, the OE line with a higher expression level was selected for this study.[8] We first inoculated *siR109944 OE* and Col-0 WT seedlings with *B. cinerea*, a destructive fungus. *Arabidopsis* plants with siR109944 overexpression were more susceptible to *B. cinerea* infection than Col-0 WT plants (Figure 1(a,b)). In addition to *B. cinerea, Sclerotinia sclerotiorum* is another serious plant pathogenic fungus that can cause white mold disease.[9] *Verticillium dahliae* causes verticillium wilt in many plant species and causes *Arabidopsis* leaves to curl and discolor.[10] Then, *siR109944 OE* and Col-0 WT plant roots and leaves were inoculated with *V. dahliae* spores and *S. sclerotiorum* mycelium plugs, respectively. Compared to Col-0 WT plants, we found that *siR109944 OE* plants displayed more sensitive disease phenotypes caused by *S. sclerotium* and *V. dahliae* (Figure 1(c–f)). It has been shown that auinx is involved in the modulation of various plant defense signals against pathogens, including *B. cinerea, S. sclerotium*, and *V. dahlia*.[11–13] Collectively, our results demonstrated that the expression of rice siR109944 in *Arabidopsis* regulated plant broad-spectrum immunity against fungi.

Auxin plays an important role in regulating many aspects that affect *Arabidopsis* growth and development,[14,15] and some miRNAs are involved in the regulation of auxin. Since the expression of siR109944 could affect *Arabidopsis* disease immunity by affecting the auxin-regulated gene expression,[8] we explored whether overexpression of siR109944 could alter auxin-related miRNA expression in *Arabidopsis*. Overexpression of miR165 alters the expression of genes involved in auxin signaling and vascular development of *Arabidopsis*.[16] Repression of *AUXIN RESPONSE FACTOR10* (*ARF10*) and *ARF17* by miR160 is critical for seed germination and post-germination stages.[17,18] Auxin-related miRNAs, including miR160, miR167, and miR165/166 in Col-0 WT and *siR109944 OE*, were detected in *Arabidopsis* by northern blot. The results showed that the expression levels of these miRNAs were more down-regulated in *siR109944 OE* than in Col-0 WT plants (Figure 2(a)). Moreover, the target genes of these miRNAs had the opposite expression levels as the related miRNAs, including *ARF10* (miR160), *ARF6*

CONTACT Dongdong Niu ddniu@njau.edu.cn College of Plant Protection, Nanjing Agricultural University, No 1 Weigang, Nanjing, 210095, China

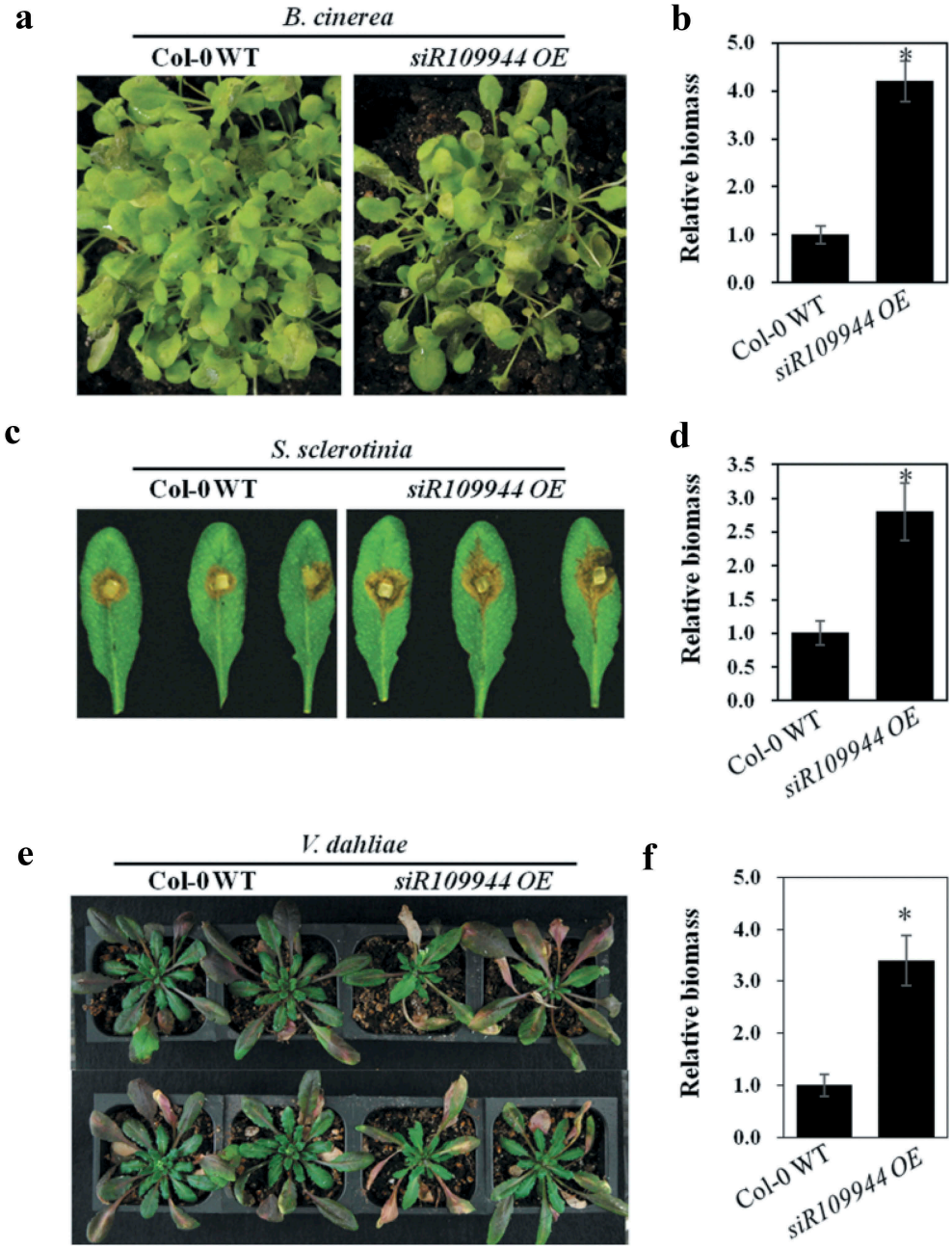

Figure 1. Heterologous expression siR109944 suppresses *Arabidopsis* immunity against fungal infection (a) The phenotypes of Col-0 WT and *siR109944 OE Arabidopsis* seedlings were inoculated by foliar spraying with *Botrytis cinerea* spore suspension, and photos were taken 2 days post-inoculation (dpi). (b) The *B. cinerea* relative DNA content (relative biomass) was measured using quantitative PCR, and error bars represent the standard deviation (s.d.) of three technical replicates. Asterisks indicate significant differences. (c) The phenotypes of *Sclerotinia sclerotiorum* infect Col-0 WT and *siR109944 OE* transgenic *Arabidopsis*, and photos were taken at 2 dpi. (d) The relative biomass of *S. sclerotiorum* was measured using quantitative PCR, and error bars represent the s.d. of three technical replicates. Asterisks indicate significant differences. (e) *Arabidopsis* Col-0 WT and *siR109944 OE* were infected by *Verticillium dahliae* spores using the root dipping method, and photos were taken at 10 dpi. (f) Relative biomass of *V. dahliae* was detected by quantitative PCR, and error bars represent the s.d. of three technical replicates. The experiments were repeated three times with similar results. Asterisks indicate significant differences (*P < .05).

(miR167), *PHB*, and *PHN* (miR165/166) in Col-0 WT and *siR109944 OE* plants (Figure 2(b)). It was speculated that overexpression of siR109944 enhanced disease susceptibility against multiple fungi might be due to the inhibition of auxin-related miRNA expression in *Arabidopsis*.

RNA interference (RNAi) is a regulatory mechanism for gene silencing by which siRNAs guide RNA-induced silencing complexes (RISC) to cleave homologous transcripts.[19] Therefore, the RNAi mechanism is conserved among different species. We found that the target genes of siR109944 in rice had highly homologous genes in *Arabidopsis*, but these genes were not the target of siR109944 in *Arabidopsis*.[8] Though the regulatory networks of small RNAs vary among plant species and are highly diverse, siRNA pathways have adopted new functions to create novel plant morphologies and immune response by heterologous expression.

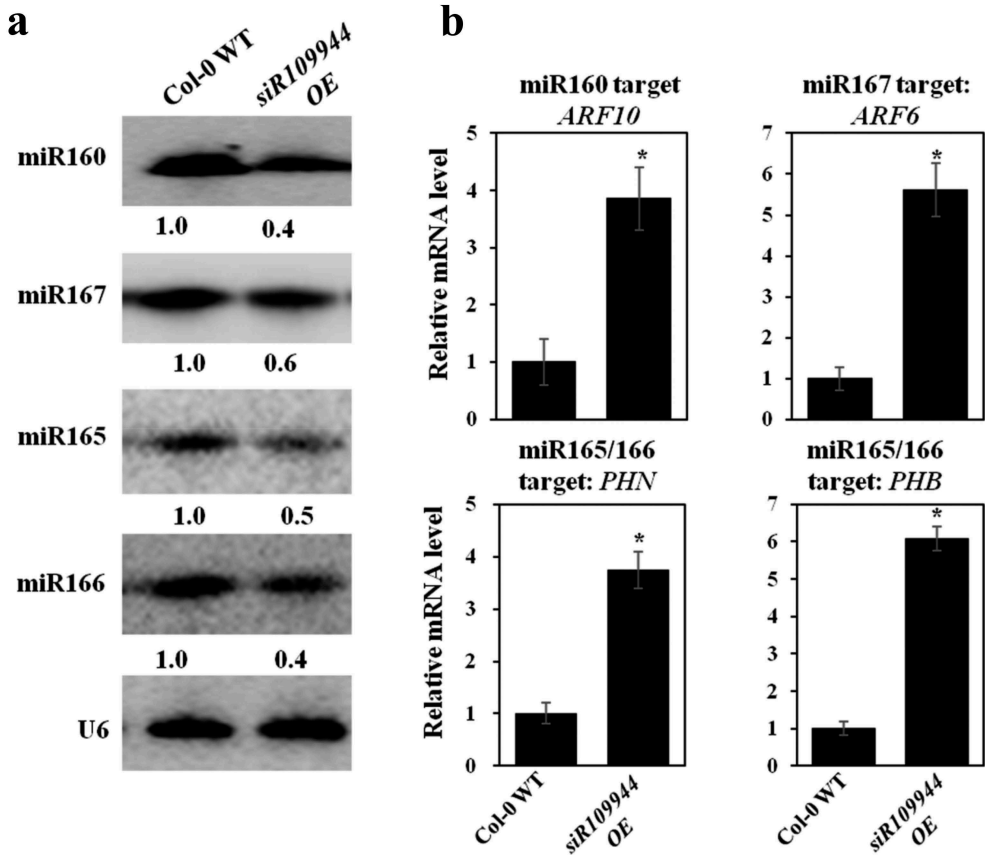

Figure 2. Heterologous expression siR109944 affects *Arabidopsis* auxin-related miRNA expression. (a) The expression levels of auxin-related miRNAs (miR160, miR167, and miR165/166) in Col-0 WT and *siR109944 OE* plants by northern blot. Values below each section represent the relative abundance (RA) of miRNA normalized to U6. (b) Quantitative PCR analysis of the relative expression of the miR160 target gene *ARF10*, miR167 target gene *ARF6*, and miR165/166 target genes *PHN* and *PHB* in Col-0 WT and *siR109944 OE* plants. The internal reference was *AtActin*. The experiments were repeated three times with similar results. Asterisks indicate significant differences (*P < .05).

Disclosure of Potential Conflicts of Interest

There are no potential conflicts of interest to disclose.

Funding

This work was supported by the Jiangsu Agricultural Science and Technology Innovation Fund [CX(19)3103]; National Natural Science Foundation of China [31501621]; Natural Science Foundation of Jiangsu Province of China [BK20171382].

ORCID

Hongwei Zhao http://orcid.org/0000-0001-9107-765X
Hailing Jin http://orcid.org/0000-0001-5778-5193

References

1. Baulcombe D. RNA silencing in plants. Nature. 2004;431 (7006):356–363. PMID: 15372043. doi:10.1038/nature02874.
2. Vaucheret H. Post-transcriptional small RNA pathways in plants: mechanisms and regulations. Genes Dev. 2006;20:759–771. PMID: 16600909 http://www.genesdev.org/cgi/doi/10.1101/gad.1410506
3. Baldrich P, San Segundo B. MicroRNAs in rice innate immunity. Rice. 2016;9(1):6. PMID: 26897721. doi:10.1186/s12284-016-0078-5.
4. Wang M, Zhu Y, Han R, Yin W, Guo C, Li Z, Wang X. Expression of *Vitis amurensis VaERF20* in *Arabidopsis thaliana* improves resistance to *Botrytis cinerea* and *Pseudomonas syringae* pv. Tomato DC3000. Int J Mol Sci. 2018;19(3):696. PMID: 29494485. doi:10.3390/ijms19030696.
5. Qi T, Guo J, Peng H, Liu P, Kang Z, Guo J. Host-induced gene silencing: a powerful strategy to control diseases of wheat and barley. Int J Mol Sci. 2019;20(1):206. PMID: 30626050. doi:10.3390/ijms20010206.
6. Shivakumara TN, Chaudhary S, Kamaraju D, Dutta TK, Papolu PK, Banakar P, Sreevathsa R, Singh B, Manjaiah KM, Rao U, et al. Host-induced silencing of two pharyngeal gland genes conferred transcriptional alteration of cell wall-modifying enzymes of meloidogyne incognita vis-a-vis perturbed nematode infectivity in eggplant. Front Plant Sci. 2017;8:473. PMID: 28424727. doi:10.3389/fpls.2017.00473.
7. Govindarajulu M, Epstein L, Wroblewski T, Michelmore RW. Host-induced gene silencing inhibits the biotrophic pathogen causing downy mildew of lettuce. Plant Biotechnol J. 2015;13 (7):875–883. PMID: 25487781. doi:10.1111/pbi.12307.
8. Qiao L, Zheng L, Sheng C, Zhao H, Jin H, Niu D. Rice siR109944 suppresses plant immunity to sheath blight and impacts multiple agronomic traits by affecting auxin homeostasis. Plant J. 2020. PMID: 31923320. doi:10.1111/tpj.14677.
9. Bolton MD, Thomma BP, Nelson BD. *Sclerotinia sclerotiorum* (Lib.) de Bary: biology and molecular traits of a cosmopolitan pathogen. Mol Plant Pathol. 2006;7(1):1–16. PMID: 20507424. doi:10.1111/j.1364-3703.2005.00316.x.
10. Veronese P, Narasimhan ML, Stevenson RA, Zhu JK, Weller SC, Subbarao KV, Bressan RA.. Identification of a locus controlling Verticillium disease symptom response in *Arabidopsis thaliana*. Plant J. 2003;35(5):574–587. PMID: 12940951. doi:10.1046/j.1365-313X.2003.01830.x.

11. Stotz HU, Jikumaru Y, Shimada Y, Sasaki E, Stingl N, Mueller MJ, Kamiya Y. *Jasmonate-dependent and COI1-independent defense responses against Sclerotinia sclerotiorum* in *Arabidopsis thaliana*: auxin is part of COI1-independent defense signaling. Plant Cell Physiol. 2011;52(11):1941–1956. PMID: 21937677. doi:10.1093/pcp/pcr127.
12. Windram O, Madhou P, McHattie S, Hill C, Hickman R, Cooke E, Jenkins DJ, Penfold CA, Baxter L, Breeze E, *et al*. *Arabidopsis* defense against *Botrytis cinerea*: chronology and regulation deciphered by high-resolution temporal transcriptomic analysis. Plant Cell. 2012;24(9):3530–3557. PMID: 23023172. doi:10.1105/tpc.112.102046.
13. Fousia S, Tsafouros A, Roussos PA, Tjamos SE. Increased resistance to *Verticillium dahliae* in *Arabidopsis* plants defective in auxin signalling. Plant Pathol. 2018;67(8):1749–1757. PMID. doi:10.1111/ppa.12881.
14. Teale WD, Paponov IA, Palme K. Auxin in action: signalling, transport and the control of plant growth and development. Nature Rev Mol Cell Biol. 2006;7(11):847–859. PMID: 16990790. doi:10.1038/nrm2020.
15. Zhao Y. Auxin biosynthesis and its role in plant development. Annu Rev Plant Biol. 2010;61(1):49–64. PMID: 20192736. doi:10.1146/annurev-arplant-042809-112308.
16. Zhou GK, Kubo M, Zhong R, Demura T, Ye ZH. Overexpression of miR165 affects apical meristem formation, organ polarity establishment and vascular development in *Arabidopsis*. Plant Cell Physiol. 2007;48(3):391–404. PMID: 17237362. doi:10.1093/pcp/pcm008.
17. Liu PP, Montgomery TA, Fahlgren N, Kasschau KD, Nonogaki H, Carrington JC. Repression of *AUXIN RESPONSE FACTOR10* by microRNA160 is critical for seed germination and post-germination stages. Plant J. 2007;52(1):133–146. PMID: 17672844. doi:10.1111/j.1365-313X.2007.03218.x.
18. Mallory AC, Bartel DP, Bartel B. MicroRNA-directed regulation of *Arabidopsis AUXIN RESPONSE FACTOR17* is essential for proper development and modulates expression of early auxin response genes. Plant Cell. 2005;17(5):1360–1375. PMID:15829600. doi:10.1105/tpc.105.031716.
19. Dunoyer P, Himber C, Voinnet O. DICER-LIKE 4 is required for RNA interference and produces the 21-nucleotide small interfering RNA component of the plant cell-to-cell silencing signal. Nat Genet. 2005;37(12):1356–1360. PMID: 16273107. doi:10.1038/ng1675.