Commentary

Spray-induced gene silencing boosts functional genomics in symbiotic fungi

Since the Devonian era 450 million years ago, most terrestrial plants have formed mutualistic associations with a group of soil fungi known as arbuscular mycorrhizal fungi (AMF) that provide benefits ranging from improved mineral nutrition to increased tolerance of biotic and abiotic stresses (Marro *et al.*, 2022; Shi *et al.*, 2023; Fiorilli *et al.*, 2024). This symbiosis constitutes a unique biological system that has drawn scientific interest because of its long history of co-evolution, the intimate interactions at the cellular level that mirror the coordination of symbiotic functions (e.g. nutrient exchange), and the diverse benefits it provides the green host. A new study by Fan *et al.* (2025; pp. 2852–2870), published in this issue of *New Phytologist*, presents groundbreaking research on spray-induced gene silencing (SIGS) in AMF, paving the way for effective genetic manipulation in these symbiotic fungi.

'*Fan* et al. *suggest that SIGS may be a powerful, general tool for functional genomics analysis of nonculturable and genetically challenging fungi.*'

Molecular studies on the role of fungal symbionts in AM symbiosis have lagged behind those focused on host plants. This is mainly due to the obligate biotrophy of AMF, which has severely limited the amount of pure biological material available for investigation. In addition, despite several attempts, protocols for the direct genetic manipulation of AMF are lacking. However, indirect approaches, such as host-induced gene silencing (HIGS) and virus-induced gene silencing (VIGS), which are based on the expression in the host plant of constructs producing double-stranded RNA (dsRNA) targeting fungal transcripts, have been successfully used to silence the expression of AMF genes in mycorrhizal roots when the fungus establishes an intimate physical association with host cells during the symbiotic stage (Helber *et al.*, 2011; Kikuchi *et al.*, 2016).

The study by Fan *et al.* represents a significant advancement in the genetic manipulation of AMF, demonstrating the successful application of SIGS to downregulate specific genes in the model AMF *Rhizophagus irregularis* during the asymbiotic and early symbiotic stages of its interaction with the host plant. Like HIGS and VIGS, SIGS exploits RNA interference (RNAi), but unlike the other two methods, SIGS involves direct application of exogenous dsRNA that is taken up by the target organism and processed with its internal RNAi machinery, leading to the posttranscriptional silencing of specific genes. It thus offers a targeted nontransgenic approach to gene silencing. SIGS has shown promising results in several plant pathosystems and is emerging as a powerful tool in both basic and applied research. For example, spraying with dsRNA, targeting essential virulence genes, successfully reduced the severity of disease caused by major plant pathogens, such as the fungi *Botrytis cinerea* and *Fusarium graminearum*, and the oomycete *Phytophthora infestans*. These studies showed that exogenous dsRNA treatment can significantly reduce infection levels, illustrating the potential of SIGS as a precise and eco-friendly plant protection tool (Mitter *et al.*, 2017; Wang & Jin, 2017; Cai *et al.*, 2018; Kalyandurg *et al.*, 2021).

Fan *et al.* first used fluorescein-labelled dsRNA to test the ability of AMF to take up dsRNA from the environment, which is a prerequisite of SIGS. Having verified successful dsRNA uptake, they used SIGS to study the impact of downregulating three genes involved in G-protein signaling – RiRgs3, RiGpa3, and RiGpb1 – on spore germination and hyphopodia formation, two crucial steps of the asymbiotic and presymbiotic fungal life cycle. Attention was given to G-protein signaling since these components have been reported to control various aspects of spore/hyphae development and host plant interactions in fungal pathogens. Applying dsRNA targeting genes to plant–AMF co-culture systems resulted in sequence-specific downregulation, leading to phenotypic defects in spore germination and hyphal branching as well as a reduction in the number of hyphopodia, which are essential structures for root penetration. Fan *et al.* thus provide direct evidence that G-protein signaling plays a major role in the asymbiotic and early stages of AM interaction, in keeping with results obtained in studies on other plant-interacting fungi. Furthermore, the results demonstrate that AMF can effectively uptake and process dsRNA, providing valuable insights into the complex roles of small RNAs in AM symbiosis (Ledford *et al.*, 2024). These findings could enable a breakthrough in functional genomics studies on this group of beneficial plant-interacting fungi, as they offer the potential for direct gene silencing with a nontransgenic approach.

Importantly, the results of Fan *et al.* suggest that SIGS may be a powerful and general tool for functional genomics analysis of other nonculturable and genetically challenging fungi. In the context of sustainable agriculture, understanding and modulating the behavior of the beneficial component of the plant microbiota could aid in improving plant nutrition, immunity, and resilience to climate change, thereby supporting sustainable crop production under challenging environmental conditions. This study suggests

This article is a Commentary on Fan *et al.* (2025), **247**: 2852–2870.
[Correction added on 26 August 2025, after first online publication: The copyright line was changed.]

New Phytologist

that RNAi-based mechanisms are conserved in fungi with diverse lifestyles, making SIGS a valuable tool for studying and manipulating not just mutualistic symbionts like AMF but also fungi used for biocontrol. The application of SIGS to other endophytic fungi will facilitate functional characterization of genes in crucial developmental stages and lay the groundwork for broader microbiome-centered crop improvement. In this context, SIGS holds promise not only for advancing fundamental research but also for supporting next-generation precision agriculture that leverages beneficial microbes. While concerns about off-target effects of exogenous dsRNA are valid, a recent study demonstrated that SIGS applied to wheat and barley leaves had minimal impact on the phyllosphere microbiota: minor shifts were detected in rare bacterial taxa, while the core bacterial and fungal communities remained stable, indicating a high level of specificity and ecological safety (Sundararajan *et al.*, 2025). These findings offer reassurance about the safety of dsRNA-based methods in agricultural settings, although further investigations will be needed to fully understand their broader environmental impact.

In addition, while the results of Fan *et al.* provide a compelling proof of concept, further research is needed to determine how reliable SIGS is in a broader range of fungal species and host systems, and how well it performs under more varied environmental conditions. Such knowledge will be essential when translating laboratory insights into practical biological and agricultural applications. In particular, assessing the viability of SIGS in natural agricultural soils and its long-term effects on AMF will be crucial to fully evaluate its potential as a tool for both research and practical microbiome-informed agriculture.

Fundamental scientific questions remain that will determine the trajectory of SIGS as a tool in mycorrhizal research. To optimize its use in AMF, the molecular mechanisms of dsRNA uptake and processing will have to be elucidated. The success of SIGS in AMF indicates that these fungi can take up dsRNA even when it is not delivered inside extracellular vesicles, which have been used as vehicles for RNA in plant–pathogenic interactions (Cai *et al.*, 2018; Holland & Roth, 2023). Understanding whether specific transporter proteins or endocytic pathways govern dsRNA uptake and internalization may help refine delivery strategies and enhance silencing efficiency. In addition, SIGS could be combined with new methods for asymbiotic AMF culture (Tanaka *et al.*, 2022) to study the molecular mechanisms active during AMF growth in the absence of a host plant and to clarify the roles of the vast number of uncharacterized putative fungal effectors that may help plants to distinguish AMF from pathogens in the early symbiotic stages (Aparicio Chacón *et al.*, 2023).

SIGS provides a simple, versatile, scalable, nontransgenic tool that is primed for broader adoption across diverse research disciplines. In the future, its integration with other systems biology tools could accelerate the understanding of symbiotic signaling, niche adaptation, and microbial interactions under diverse environmental conditions. Perhaps most compelling is its potential to transiently modify fungal traits without altering fungal or plant genomes in sustainable agricultural strategies; this could play a pivotal role in shaping resilient crop–microbe partnerships. It is time for the AMF community to embrace SIGS as a core methodology.

Acknowledgements

RRV acknowledges support from FORMAS (2019-01316), the Novo Nordisk Foundation (0074727), SLU Centre for Biological Control, and the Crafoord Foundation.

ORCID

Luisa Lanfranco https://orcid.org/0000-0002-3961-2552
Katie Stevens https://orcid.org/0009-0009-6955-7046
Ramesh Raju Vetukuri https://orcid.org/0000-0001-7129-5326

Ramesh Raju Vetukuri[1]*, Luisa Lanfranco[2] and Katie Stevens[1]

[1]Department of Plant Breeding, Horticum, Swedish University of Agricultural Sciences, Lomma, 23422, Sweden;
[2]Department of Life Sciences and Systems Biology, University of Turin, Turin, 10125, Italy
(*Author for correspondence: email ramesh.vetukuri@slu.se)

References

Aparicio Chacón MV, Van Dingenen J, Goormachtig S. 2023. Characterization of arbuscular mycorrhizal effector proteins. *International Journal of Molecular Sciences* **24**: 9125.

Cai Q, Qiao L, Wang M, He B, Lin FM, Palmquist J, Huang SD, Jin H. 2018. Plants send small RNAs in extracellular vesicles to fungal pathogen to silence virulence genes. *Science* **360**: 1126–1129.

Fan X, Zhou X, He J, Xie H, Tang N, Tang M, Xie X. 2025. Spray-induced gene silencing of three G-protein signaling genes from the arbuscular mycorrhizal fungus *Rhizophagus irregularis* inhibits spore germination and hyphopodium formation. *New Phytologist* **247**: 2852–2870.

Fiorilli V, Martínez-Medina A, Pozo MJ, Lanfranco L. 2024. Plant immunity modulation in arbuscular mycorrhizal symbiosis and its impact on pathogens and pests. *Annual Review of Phytopathology* **62**: 127–156.

Helber N, Wippel K, Sauer N, Schaarschmidt S, Hause B, Requena N. 2011. A versatile monosaccharide transporter that operates in the arbuscular mycorrhizal fungus glomus sp is crucial for the symbiotic relationship with plants. *Plant Cell* **23**: 3812–3823.

Holland S, Roth R. 2023. Extracellular vesicles in the arbuscular mycorrhizal symbiosis: current understanding and future perspectives. *Molecular Plant Microbe Interactions* **36**: 235–244.

Kalyandurg PB, Sundararajan P, Dubey M, Ghadamgahi F, Zahid MA, Whisson SC, Vetukuri RR. 2021. Spray-induced gene silencing as a potential tool to control potato late blight disease. *Phytopathology* **111**: 2168–2175.

Kikuchi Y, Hijikata N, Ohtomo R, Handa Y, Kawaguchi M, Saito K, Masuta C, Ezawa T. 2016. Aquaporin-mediated long-distance polyphosphate translocation directed towards the host in arbuscular mycorrhizal symbiosis: application of virus-induced gene silencing. *New Phytologist* **211**: 1202–1208.

Ledford WC, Silvestri A, Fiorilli V, Roth R, Rubio-Somoza I, Lanfranco L. 2024. A journey into the world of small RNAs in the arbuscular mycorrhizal symbiosis. *New Phytologist* **242**: 1534–1544.

Marro N, Grilli G, Soteras F, Caccia M, Longo S, Cofré N, Borda V, Burni M, Janoušková M, Urcelay C. 2022. The effects of arbuscular mycorrhizal fungal species and taxonomic groups on stressed and unstressed plants: a global meta-analysis. *New Phytologist* **235**: 320–332.

Mitter N, Worrall EA, Robinson KE, Li P, Jain RG, Taochy C, Fletcher SJ, Carroll BJ, Lu GQ, Xu ZP. 2017. Clay nanosheets for topical delivery of RNAi for sustained protection against plant viruses. *Nature Plants* **3**: 16207.

Shi J, Wang X, Wang E. 2023. Mycorrhizal symbiosis in plant growth and stress adaptation: from genes to ecosystems. *Annual Review of Plant Biology* **74**: 569–607.

Sundararajan P, Ghosh S, Kelbessa BG, Whisson SC, Dubey M, Chawade A, Vetukuri RR. 2025. The impact of spray-induced gene silencing on cereal phyllosphere microbiota. *Environmental Microbiomes* **20**: 1.

Tanaka S, Hashimoto K, Kobayashi Y, Yano K, Maeda T, Kameoka H, Ezawa T, Saito K, Akiyama K, Kawaguchi M. 2022. Asymbiotic mass production of the arbuscular mycorrhizal fungus *Rhizophagus clarus*. *Communications Biology* **5**: 43.

Wang M, Jin H. 2017. Spray-induced gene silencing: a powerful innovative strategy for crop protection. *Trends in Microbiology* **25**: 4–6.

Key words: arbuscular mycorrhizal fungi, double-stranded RNA, G-protein signaling proteins, *Rhizophagus irregularis*, spray-induced gene silencing.