

of systemic control of mycorrhization, as interaction between the two root sides implicates long-distance transport of signals via aerial parts of the host plant. Using such a split-root system approach, autoregulation of mycorrhization has been first identified in the non-legume barley and thereafter studied in alfalfa and soybean.^{10-13,49,54-56}

In alfalfa, inoculation of one half of a split-root system with the fungus *Glomus mosseae* significantly reduced later AM colonization on the other half. A similar suppressive effect on mycorrhization was observed after inoculation with *Sinorhizobium meliloti*.¹² Furthermore, prior addition of purified rhizobial Nod factors on one half significantly reduced mycorrhization on the other half of the split-root system. Reciprocally, prior mycorrhization on one side suppressed nodule formation on the other side of the split-root system.¹² Taken these data together, they point to a common autoregulation circuit for both symbioses (Fig. 1). We suggest that Nod factor signaling as well as mycorrhizal signaling in response to unknown mycorrhizal signals (“Myc factors”) induces expression or post-translation processing of CLE peptides, which likely function as ascending long-distance signals to the shoot. Moreover, the descending SDI transported to the root seems to inhibit both, nodule formation as well as mycorrhizal root colonization. Indeed, inoculation experiments with the supernodulation mutant *nts1007* provided genetic evidence for autoregulation of mycorrhization in soybean (cv. Bragg). The *nts1007* mutant carries a nonsense mutation that truncates the autoregulation LRR receptor kinase NARK.³¹ In contrast to wild-type soybean plants, prior AM colonization of *nts1007* plants on one half did not suppress later AM colonization by the AM fungus *G. mosseae* on the other half of the split-root system, indicating that NARK is essential for shoot-controlled autoregulation of mycorrhization.¹³ *En6500*, an allelic mutant with a similar nonsense mutation in *NARK* derived from cv. Enrei, retained the ability to systemically regulate AM fungi under these conditions, suggesting that the genetic background (varietal differences) influences autoregulation of mycorrhization under certain experimental conditions.⁵⁶ Using reciprocal grafting experiments however, shoot-controlled autoregulation of mycorrhization has been recently demonstrated for the *En6500* mutant. In this study, abundance of arbuscules was determined in roots colonized by the AM fungus *Gigaspora rosea*.⁵⁷

Similar to autoregulation of nodulation, the SDI signal seems to induce physiological changes in the root that limit AM root colonization. Analysis of phytohormonal changes in soybean points to a possible role of auxin in autoregulation of mycorrhization.¹³ Levels of isoflavonoids such as formononetin and ononin were systemically reduced in non-infected parts, when alfalfa split-roots were infected with AM fungi on one half. On the other hand, exogenous application of ononin to autoregulated root parts stimulated AM colonization, indicating that certain flavonoids thwart SDI-induced inhibition effects on mycorrhization.⁴⁹ Proteomic analysis of the mycorrhized autoregulation-defective *sunm* mutant (mutated in the autoregulation LRR receptor kinase gene *SUNN* of *M. truncatula*) revealed increased accumulation of proteins involved in plant defense reactions, cytoskeleton rearrangements and auxin signaling, which perhaps

reflect the higher number of formed arbuscules in this mutant as compared to wild-type plants.⁵⁸

Data from classical split-root experiments indicate that autoregulation of mycorrhization also exists in non-legume plants. In barley, mycorrhization was significantly reduced when other parts of the root system were already colonized by AM fungi.^{10,11} The feedback inhibition effect depended on the degree of AM colonization in the first half of the split-root system, suggesting a dose-dependent effect.⁵⁴ We suggest that autoregulation processes of mycorrhization in legumes and non-legumes are controlled by similar molecules (Fig. 1). It would be interesting to find out whether CLE peptides¹⁷⁻²³ or other plant bio-active peptides^{28,59} represent the ascending signals in non-legumes.

Using split-root systems, a recent study demonstrated that the broad-host range strain *Rhizobium* sp. NGR234 systemically suppressed mycorrhization of barley roots.⁶⁰ Rhizobia cannot infect barley, but bacterial rhizosphere colonization in one half of the split-root system systemically affected AM root colonization on the other half. The observed suppression effect was independent of Nod factors, as a mutant strain of NGR234 deficient in Nod factor synthesis (strain *NGRΔnodABC*) suppressed mycorrhization in a similar way. These data suggest that barley roots can perceive the rhizobia in the rhizosphere. We postulate that there are rhizobial elicitors (microbe-associated molecular patterns), which are recognized by corresponding pattern recognition receptors and trigger activation of plant defense reactions in barley roots. Indeed, roots challenged with NGR234 showed increased levels of free salicylic acid, a typical defense response against pathogens.⁶⁰

Work on cucumber (*Cucumis sativus*) revealed that application of root exudates from mycorrhizal plants reduced the degree of AM root colonization, whereas root exudates from non-infected plants stimulated mycorrhization. Root exudates from the non-mycorrhizal half of a split-root system (with mycorrhizal roots on the other half) partially inhibited mycorrhization of cucumber plants.⁶¹ These data indicate that the composition of root exudates is systemically regulated and suggest a systemic plant defense response against AM fungi. This is reminiscent to the effects of the “endogenous elicitor” systemin, a peptide hormone of tomato (*Lycopersicon peruvianum*) involved in systemic activation of plant defense reactions.⁶²

Interestingly, prior mycorrhization in barley split-root systems not only suppressed later mycorrhization, but also systemically reduced subsequent infection of the pathogenic fungus *Gaeumannomyces graminis*.⁶³ Hence, autoregulation of mycorrhization and the systemic biocontrol effect of AM fungi (or rhizobia) on pathogenic fungi could be regulated by a similar SDI signal.⁶⁴ Apparently, autoregulation of mycorrhization possesses certain parallels with the phenomenon of “systemic acquired resistance” in plant-pathogen interactions, where prior infection by a pathogen systemically induces plant defense reactions in the host plant.⁶⁵ It is worth mentioning in this context that autoregulation signaling affected root-knot nematode infection in *L. japonicus* roots. The *har1* mutant (mutated in the autoregulation LRR receptor kinase gene *HAR1*) was hyperinfected by *Meloidogyne incognita* and formed significantly more galls than

wild-type plants.⁶⁶ Similarly, as compared to the parent cv. Enrei, the supernodulating soybean line Sakukei4 was more damaged by red crown rot, which is caused by *Calonectria ilicilola*.⁶⁷ These differences could be due to reduced expression of disease resistance genes in loss-of-autoregulation mutants and point to a possible crosstalk between autoregulation and defense signaling pathways.⁴⁶

Concluding Remarks

Whereas knowledge on autoregulation of nodulation considerably increased during the recent years, studies on autoregulation of mycorrhization are still in their infancy. In legumes, autoregulation of nodulation and mycorrhization seem to be regulated by the same signaling pathway in the shoot. Autoregulation of mycorrhization in non-legumes is reminiscent to “systemic acquired resistance” in plant-pathogen interactions. CLE peptides are putative ligands for the autoregulation LRR receptor kinases HAR1/SYM29/NARK/SUNN. It will be of interest to investigate these receptor-ligand interactions biochemically in order to characterize their specificity. Further research on loss-of-autoregulation mutants in legumes and identification of mutants from non-legumes will provide a way to understand autoregulation signaling in more detail.

The overlaps between autoregulation of symbiosis and plant developmental processes might complicate the molecular analysis of symbiotic autoregulation circuits. Redundancy in expression and multiple functions of CLE peptides likely contribute to a high level of complexity. CLE peptides are processed from longer

polypeptide chains and certain CLE peptides undergo post-translational modifications, such as arbinosylation to gain their biological activity.^{20-23,68} Furthermore, CLE peptides seem to affect nodule inhibition not only via shoot-controlled autoregulation, but also directly within roots. Thus, short-distance transport of CLE peptides within roots could interfere with shoot-controlled autoregulation of symbiosis. We hypothesize that expression and short-distance transport of CLE peptides is particularly important for nitrate-mediated inhibition of nodulation as well as for phosphate-mediated inhibition of mycorrhization. Indeed, recent expression studies indicated that *LjCLE-RS2* in *L. japonicus* roots is induced by KNO₃, whereas expression levels of *LjCLE19*/*LjCLE20* were stimulated when plants were grown at high phosphate concentrations.^{18,69} It is tempting to speculate that autoregulation LRR receptor kinases expressed in roots are receptors for CLE peptides induced by high nitrate or phosphate levels. Consequently, an inhibitor identical or related to the SDI signal would be also locally synthesized in roots. In other words, mechanisms of nutrient-mediated inhibition of symbiosis and autoregulation of symbiosis controlled by long-distance signaling would represent variations of a common theme.

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