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Gibberellin regulates infection and colonization of host roots by arbuscular mycorrhizal fungi

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Arbuscular mycorrhiza (AM) is established by the entry of AM fungi into the host plant roots and the formation of symbiotic structures called arbuscules. The host plant supplies photosynthetic products to the AM fungi, which in return provide phosphate and other minerals to the host through the arbuscules. Both partners gain great advantages from this symbiotic interaction, and both regulate AM development. Our recent work revealed that gibberellic acids (GAs) are required for AM development in the legume *Lotus japonicus*. GA signaling interact with symbiosis signaling pathways, directing AM fungal colonization in host roots. Expression analysis showed that genes for GA biosynthesis and metabolism were induced in host roots around AM fungal hyphae, suggesting that the GA signaling changes with both location and time during AM development. The fluctuating GA concentrations sometimes positively and sometimes negatively affect the expression of AM-induced genes that regulate AM fungal infection and colonization.

Keywords: arbuscular mycorrhiza, gibberellin, *Lotus japonicus*, *Rhizophagus irregularis*, symbiosis

Abbreviations: AM, arbuscular mycorrhiza; GA, gibberellic acid; GUS, β-glucuronidase; CCaMK, calcium/calmodulin-dependent protein kinase.

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Gibberellin distribution in the host root during AM development

Gibberellins are homeostatically controlled in plants, and excess up- or down-regulation of their biosynthesis or signaling causes abnormal physiological responses: for example, enhanced GA signaling causes shoot elongation, whereas inhibition induces dwarf phenotypes.[1,2] Excess amounts of GAs or overloading of GA signaling inhibits AM fungal colonization in the host root, indicating that GA functions as a negative regulator in AM.[3] Conversely, suppression of GA biosynthesis or signaling during AM development causes abnormal morphology of AM colonization in roots of *L. japonicus*.[4] These results indicate that proper regulation of GA signaling is essential for AM development.

Active and inactive forms of GA accumulate in roots of *L. japonicus* infected with the AM fungus *Rhizophagus irregularis*.[4] Although we could not measure actual GA amounts in tissues or cells, histochemical analyses using fusions of β-*glucuronidase* (*GUS*) with promoters of genes for GA biosynthesis (*GA20ox1, GA20ox2*) and metabolism (*GA2ox1*) indicates that GA biosynthesis/metabolism is upregulated around AM hyphae in the host root.[4] During the early stage of infection, when AM hyphae of *R. irregularis* attached to or entered the host root, GUS staining was detected in epidermal and cortical cells near the sites of fungal attachment or entrance. Following hyphal elongation, it extended to the cortical cells, and strong staining in the arbuscule-containing cells indicated active controls of GA concentrations in these cells. This histochemical expression analysis indicates that GA levels change during AM development, causing different effects on the symbiotic responses and AM fungal colonization.

Hyphal branching in host tissues and pre-penetration apparatus

The regulation of AM fungal colonization by GA signaling is associated with interaction with the symbiosis signaling pathway and AM-induced gene expression (**Fig. 1**).[4] We think that the negative effects of GA on hyphal entry into the host root are caused by interference with AM-induced genes such as *RAM1* and

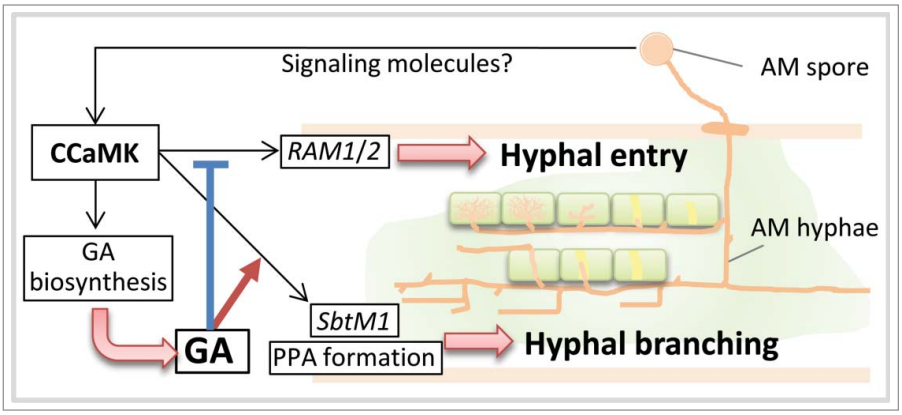

Figure 1. A model of the interference of GA signaling with AM-induced gene expression and AM fungal colonization. The expression of AM-induced genes *SbtM1*, *RAM1*, and *RAM2* and genes for GA biosynthesis is induced by an AM signaling factor via symbiotic signaling kinase CCaMK. The same signal also induces the formation of the pre-penetration apparatus (PPA). The induction of GA biosynthesis/metabolism enzymes changes the GA concentration, which alters GA signaling in the host root. Elevated GA signaling inhibits the expression of AM-induced genes (such as *RAM1* and *RAM2*), resulting in the inhibition of hyphal entry into the host root. On the other hand, GA signaling promotes or maintains the expression of other AM-induced genes (such as *SbtM1*) and PPA formation, which would promote hyphal branching in the host cortex.

RAM2, whereas the positive effect of GA on hyphal branching is closely related to the expression of AM-induced genes such as *SbtM1* and the formation of the pre-penetration apparatus (PPA).[5] Transgenic roots carrying gain-of-function calcium/calmodulin-dependent protein kinase (CCaMK) showed the induction of *SbtM1* expression without AM fungal infection and the formation of PPA-like structures was observed in cortical cells in which *SbtM1* expression was highly induced.[6] The expression of *SbtM1* induced by gain-of-function CCaMK was clearly reduced in the presence of a GA biosynthesis inhibitor, which decreased the cells containing a PPA-like structure.[4] The decrease in the formation of PPA-like structures would be directly linked to the reduction in hyphal branching under low-GA conditions. The branched hyphae of *R. irregularis* elongate mainly between the cells, or sometimes penetrate the cells accompanied by PPA formation in *L. japonicus*.[6] Therefore, the decrease in the formation of PPA-like structures induced by gain-of-function CCaMK under low-GA conditions is directly linked to the reduction in hyphal branching in the host root.

Intercellular hyphal branching between the cells should also be inhibited in low-GA conditions.[4] The mechanism of hyphal elongation in the intercellular spaces is largely unknown. However, a recent genomic sequence analysis of *R. irregularis* revealed that the fungus lacks cell-wall–degrading enzymes, which are required for invasion into the host plant and are usually abundant in pathogenic and ectomycorrhizal fungi.[7] This fact suggests that the host plant should support hyphal elongation by loosening the intercellular spaces. Putative cell-wall–degrading enzymes, such as pectinesterases, galactosidases, xyloglucan endoglucanases, and proteases, were upregulated during AM formation in the host plant.[4,8] GAs induce cell extension in company with reconstruction of cell walls by inducing cell-wall–degrading enzymes.[9] The accumulation of GAs during AM formation might induce such enzymes, reducing cell wall stiffness around inner AM hyphae. In addition, SbtM1, whose induction is also promoted by GA signaling, is a serine protease with a secretion signal peptide and digests apoplastic proteins during AM fungal infection.[10] Although substrates of SbtM1 have not been isolated, SbtM1 or other AM-induced proteases might digest structural proteins of the cell wall, facilitating the intercellular hyphal elongation of AM fungi.

Does only the host plant make and respond to GA?

In our study, we assumed that only the host plant produces GA, and considered the effects only on the host plant. However, strigolactones influence AM hyphal development inside and outside the host roots,[11] indicating that plant hormones can act as symbiosis signaling factors also for the microbial symbiont. Moreover, mycorrhizal fungi produce molecules with GA-like activity.[12,13] The pathogenic fungus *Gibberella fujikuroi* secretes a GA which reduces host resistance and enhances colonization of the host plant.[14] Thus, it might be important to consider GA biosynthesis and signaling in both the host plant and AM fungi to fully understand the interrelationship between GA signaling and AM development.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Ikeda A, Ueguchi-Tanaka M, Sonoda Y, Kitano H, Koshioka M, Futsuhara Y, Matsuoka M, Yamaguchi J. *slender rice*, a constitutive gibberellin response mutant, is caused by a null mutation of the *SLR1* gene, an ortholog of the height-regulating gene *GAI/RGA/RHT/D8*. Plant Cell 2001; 13:999-1010; PMID:11340177; http://dx.doi.org/10.1105/tpc.13.5.999
2. Ueguchi-Tanaka M, Ashikari M, Nakajima M, Itoh H, Katoh E, Kobayashi M, Chow TY, Hsing YI, Kitano H, Yamaguchi I, Matsuoka M. *GIBBERELLIN INSENSITIVE DWARF1* encodes a soluble receptor for gibberellin. Nature 2005; 437:693-8; http://www.nature.com/nature/journal/v437/n7059/full/nature04028.html; PMID:16193045; http://dx.doi.org/10.1038/nature04028

3. El Ghachtouli N, Martin-Tanguy J, Paynot M, Gianinazzi S. First report of the inhibition of arbuscular mycorrhizal infection of *Pisum sativum* by specific and irreversible inhibition of polyamine biosynthesis or by gibberellic acid treatment. FEBS Letts 1996; 385:189-92; http://www.sciencedirect.com/science/article/pii/0014579396003791; PMID:8647248; http://dx.doi.org/10.1016/0014-5793(96)00379-1
4. Takeda N, Handa Y, Tsuzuki S, Kojima M, Sakakibara H, Kawaguchi M. Gibberellins interfere with symbiosis signaling and gene expression, and alter colonization by arbuscular mycorrhizal fungi in *Lotus japonicus*. Plant Physiol 2015; 167:545-57; http://www.plantphysiol.org/content/167/2/545.full; PMID:25527715; http://dx.doi.org/10.1104/pp.114.247700
5. Genre A, Chabaud M, Timmers T, Bonfante P, Barker DG. Arbuscular mycorrhizal fungi elicit a novel intracellular apparatus in *Medicago truncatula* root epidermal cells before infection. Plant Cell 2005; 17:3489-99; http://www.plantcell.org/content/17/12/3489.long; PMID:16284314; http://dx.doi.org/10.1105/tpc.105.035410
6. Takeda N, Maekawa T, Hayashi M. Nuclear-localized and deregulated calcium- and calmodulin-dependent protein kinase activates rhizobial and mycorrhizal responses in *Lotus japonicus*. Plant Cell 2012; 24:810-22; http://www.plantcell.org/content/24/2/810.long; PMID:22337918; http://dx.doi.org/10.1105/tpc.111.091827
7. Tisserant E, Malbrei M, Kuo A, Kohler A, Symeonidi A, Balestrini R, Charron P, Duensing N, Frei dit Frey N, Gianinazzi-Pearson V, et al. Genome of an arbuscular mycorrhizal fungus provides insight into the oldest plant symbiosis. Proc Natl Acad Sci USA 2013; 110:20117-22; http://www.pnas.org/content/110/50/20117.long; PMID:24277808; http://dx.doi.org/10.1073/pnas.1313452110
8. Maldonado-Mendoza IE, Dewbre GR, Blaylock L, Harrison MJ. Expression of a xyloglucan endotransglucosylase/hydrolase gene, *Mt-XTH1*, from *Medicago truncatula* is induced systemically in mycorrhizal roots. Gene 2005; 345:191-7; http://www.sciencedirect.com/science/article/pii/S0378111904006523; PMID:15716119; http://dx.doi.org/10.1016/j.gene.2004.10.028
9. Jan A, Yang G, Nakamura H, Ichikawa H, Kitano H, Matsuoka M, Matsumoto H, Komatsu S. Characterization of a xyloglucan endotransglucosylase gene that is up-regulated by gibberellin in rice. Plant Physiol 2004; 136:3670-81; http://www.plantphysiol.org/content/136/3/3670.long; PMID:15516498; http://dx.doi.org/10.1104/pp.104.052274
10. Takeda N, Sato S, Asamizu E, Tabata S, Parniske M. Apoplastic plant subtilases support arbuscular mycorrhiza development in *Lotus japonicus*. Plant J. 2009; 58:766-77; http://onlinelibrary.wiley.com/doi/10.1111/j.1365–313X.2009.03824.x/full; PMID:19220794; http://dx.doi.org/10.1111/j.1365-313X.2009.03824.x
11. Akiyama K, Matsuzaki K, Hayashi H. Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. Nature 2005; 435:824-7; http://www.nature.com/nature/journal/v435/n7043/full/nature03608.html; PMID:15944706; http://dx.doi.org/10.1038/nature03608
12. Strzelczyk E, Sitek J, Kowalski S. Production of gibberellin-like substances by fungi isolated from mycorrhizae of pine (*Pinus silvestris*/L.). Acta microbiologica Polonica. Series B: Microbiologia applicate 1975; 7:145-53; PMID:117264; http://link.springer.com/article/10.1007%2FBF02197150
13. Barea JM, Azcon-Aguilar C. Production of plant growth-regulating substances by the vesicular-arbuscular mycorrhizal fungus *Glomus mosseae*. Appl Env Microbiol 1982; 43:810-3; PMID:16345991; http://aem.asm.org/content/43/4/810.long
14. Wiemann P, Sieber CMK, Von Bargen KW, Studt L, Niehaus EM, Espino JJ, Huß K, Michielse CB, Albermann S, Wagner D, et al. Deciphering the cryptic genome: genome-wide analyses of the rice pathogen *Fusarium fujikuroi* reveal complex regulation of secondary metabolism and novel metabolites. PLoS Pathog 2013: 9:e1003475; http://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1003475; PMID:23825955; http://dx.doi.org/10.1371/journal.ppat.1003475
15. Takeda N, Handa Y, Tsuzuki S, Kojima M, Sakakibara H, Kawaguchi M. Gibberellins interfere with symbiosis signaling and gene expression, and alter colonization by arbuscular mycorrhizal fungi in *Lotus japonicus*. Plant Physiology 2015; 167:545-57; http://www.plantphysiol.org/content/167/2/545.full; PMID:25527715; http://dx.doi.org/10.1104/pp.114.247700