



The Art of Self-Control – Autoregulation of Plant–Microbe Symbioses

Chenglei Wang, James B. Reid and Eloise Foo*

School of Natural Sciences, University of Tasmania, Hobart, TAS, Australia

OPEN ACCESS

Edited by:

Katharina Pawlowski,
Stockholm University, Sweden

Reviewed by:

Bettina Hause,
Leibniz-Institut für Pflanzenbiochemie
(IPB), Germany
Michael Djordjevic,
Australian National University,
Australia

*Correspondence:

Eloise Foo
Eloise.foo@utas.edu.au

Specialty section:

This article was submitted to
Plant Evolution and Development,
a section of the journal
Frontiers in Plant Science

Received: 27 March 2018

Accepted: 19 June 2018

Published: 10 July 2018

Citation:

Wang C, Reid JB and Foo E (2018)
The Art of Self-Control –
Autoregulation of Plant–Microbe
Symbioses. *Front. Plant Sci.* 9:988.
doi: 10.3389/fpls.2018.00988

Plants interact with diverse microbes including those that result in nutrient-acquiring symbioses. In order to balance the energy cost with the benefit gained, plants employ a systemic negative feedback loop to control the formation of these symbioses. This is particularly well-understood in nodulation, the symbiosis between legumes and nitrogen-fixing rhizobia, and is known as autoregulation of nodulation (AON). However, much less is understood about the autoregulation of the ancient arbuscular mycorrhizal symbioses that form between Glomeromycota fungi and the majority of land plants. Elegant physiological studies in legumes have indicated there is at least some overlap in the genes and signals that regulate these two symbioses but there are major gaps in our understanding. In this paper we examine the hypothesis that the autoregulation of mycorrhizae (AOM) pathway shares some elements with AON but that there are also some important differences. By reviewing the current knowledge of the AON pathway, we have identified important directions for future AOM studies. We also provide the first genetic evidence that *CLV2* (an important element of the AON pathway) influences mycorrhizal development in a non-legume, tomato and review the interaction of the autoregulation pathway with plant hormones and nutrient status. Finally, we discuss whether autoregulation may play a role in the relationships plants form with other microbes.

Keywords: autoregulation, nodulation, arbuscular mycorrhizae, *CLAVATA*, *CLE* peptide, tomato

INTRODUCTION

Mycorrhizal symbioses between plants and fungi are widespread and ancient, with evidence from fossils and extant basal plants indicating that such interactions evolved during the colonization of land by plants between 450 and 475 mya (Field et al., 2015; Martin et al., 2017). The mycorrhizal fungi invade and extend the host plant's root system, enabling enhanced nutrient uptake in exchange for fixed carbon. The most widespread are the arbuscular mycorrhizal (AM) associations that are characterized by the presence of a unique nutrient exchange unit, the fungal arbuscule. A significant amount of plant-derived carbon is invested, with estimates of between 4 and 20% of the carbon fixed by the plant transferred to the AM fungi (Bago et al., 2000; Douds et al., 2000). To prevent the energy cost to the plant outweighing the benefits of the interaction, plants might be expected to regulate AM colonization. Indeed, elegant physiological studies in flowering

plants have revealed powerful systemic regulation of AM colonization. In split root studies, pre-colonization of one side of the root system can suppress subsequent colonization of the other side of the root system, providing evidence for a root–shoot feedback system termed autoregulation of mycorrhiza (AOM) (Vierheilig et al., 2000a, 2008; Meixner et al., 2005). Although there is no direct evidence that such control mechanisms also occur in more basal plant lineages, it is fair to assume that autoregulation would be an important element in the evolution of mutually beneficial plant–mycorrhizal interactions to prevent a potentially parasitic relationship developing.

Significant progress has been made in the past few decades in our understanding of the plant genes and signals that regulate AM symbioses, assisted greatly by the cross-over with the more recently evolved nodulation symbioses that occur between nitrogen-fixing rhizobial bacteria and (almost exclusively) legumes. In particular, the identification of a common symbiotic signaling pathway, required for the formation of both nodules and AM, has revealed that elements of the ancient AM pathway were co-opted into the more recently evolved nodulation pathway (Delaux et al., 2013; Martin et al., 2017). In particular, the common symbiotic pathway includes genes essential for initial communication between the plant host and rhizobia or AM fungi. However, a missing element in these comparisons has been the autoregulation of nodulation (AON) pathway. Like AM, nodulation is under powerful systemic control and the identity of AON signals and transduction pathways are now becoming clear (for review see Reid et al., 2011b; Soyano and Kawaguchi, 2014). Studies in legumes suggest some key cross-overs in the autoregulation pathways, as plant mutants disrupted in the AON pathway display not only hypernodulation but also hypermycorrhizal colonization (Morandi et al., 2000; Shrihari et al., 2000) and split root studies indicate nodulation can systemically suppress AM and vice versa (Catford et al., 2003). In this article, we examine our current understanding of AOM and begin to extend this beyond legumes by providing evidence of a role for the key AON gene in legumes, *CLAVATA2* (*CLV2*), in the AOM pathway of tomato. We also consider the role of plant hormones in autoregulation and examine the potential for the autoregulation of other beneficial plant–microbe interactions.

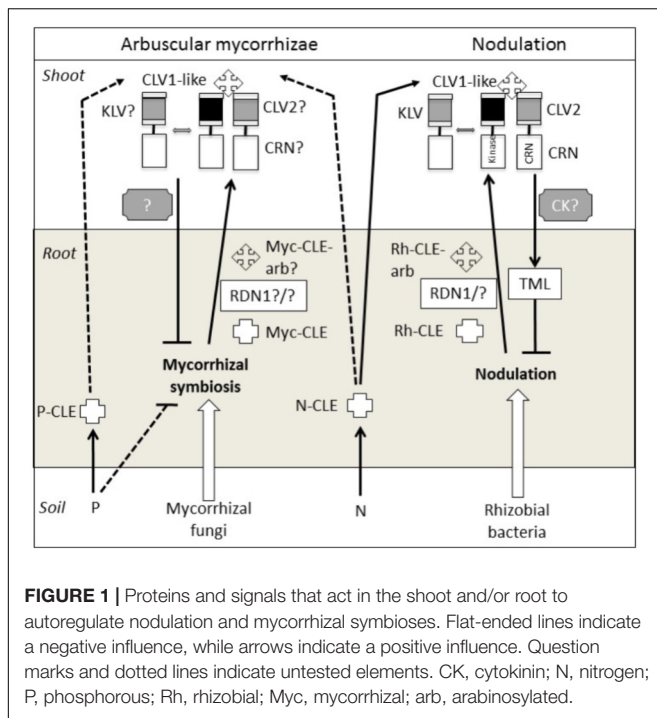
GENES AND SIGNALS OF THE AUTOREGULATION PATHWAY(S)

The systemic regulation of AM colonization in split root studies has been observed in both legumes and non-legumes (Vierheilig et al., 2000a; Meixner et al., 2007). This negative regulation appears to require a certain threshold of the amount of root colonized by AM (Vierheilig, 2004). AOM does not appear to be due simply to the strength of the carbon sink (Vierheilig et al., 2000b, 2008), but is rather regulated by a more specific pathway. Nodulation can systemically suppress AM and vice versa and even the application of Nod factors, the rhizobial-derived signaling molecules, can suppress AM (Catford et al., 2003), suggesting a clear overlap between the AON and AOM

pathways. Similar conclusions were drawn from studies using a dual inoculation system in which both rhizobia and AM fungi were applied to the one root system (Sakamoto et al., 2013). This has also been explored in the non-legume barley, where it was found that inoculation with *Rhizobium* sp. could systemically inhibit AM formation. However, this inhibitory effect did not rely on Nod-factor production but was linked instead to the type 3 effector proteins of this rhizobial strain (Khaosaad et al., 2010).

A member of the Leucine-rich repeat (LRR) receptor kinase family that shares similarities with the *CLAVATA1* (*CLV1*) protein (outlined below) has a central role in the AON in legumes (**Figure 1**). This *CLV1*-like gene is known as *NODULE AUTOREGULATION RECEPTOR KINASE* (*NARK*) in soybean, *HYPERNODULATION ABERRANT ROOT FORMATION 1* (*HAR1*) in *Lotus japonicus*, *SUPER NUMERIC NODULES* (*SUNN*) in *Medicago truncatula* and *SYM29* in pea, and mutations in these genes result in hypernodulation (reviewed by Reid et al., 2011b). The *CLV1*-like protein appears to function as a shoot receptor for root-derived *CLAVATA*/*ESR*-related (*CLE*) peptides. In the root, events associated with nodulation generate specific rhizobial induced *CLE* peptides (Rh-*CLE* **Figure 1**) which in some cases appear to be arabinosylated via action of the enzyme *ROOT DETERMINED NODULATION1* (*RDN1*) (e.g., *MtCLE12*, *GmRIC1a*, and *GmRIC1b*) (Schnabel et al., 2011; Okamoto et al., 2013; Kassaw et al., 2017; Hastwell et al., 2018; Imin et al., 2018). The *CLE* peptides are then translocated to the shoot where they are perceived by a receptor complex that includes the *CLV1*-like protein (e.g., Okamoto et al., 2013). The perception of the root-derived signal(s) in the plant shoot generates a shoot to root signal that inhibits further nodulation. The shoot to root inhibitor is predicted to be a small molecular weight heat-stable molecule (Lin et al., 2010) that has been suggested to be cytokinin (Sasaki et al., 2014). Other elements of the AON pathway include the shoot acting *CLV2* (Krusell et al., 2011), *CORYNE* (*CRN*) (Crook et al., 2016), and *KLAVIER* (*KLV*) (Miyazawa et al., 2010), all three of which encode LRR receptors that may also play a role in *CLE* perception, and *TOO MUCH LOVE* (*TML*), a root-acting F-Box protein that appears to act downstream of the shoot to root signal (Takahara et al., 2013).

In addition to its role in nodulation, the *CLV1*-like protein is also essential in the AOM pathway since *clv1-like* mutants across legume species (*sym29*, *sunnn*, *nark*, and *har1*) also display hypermycorrhizal colonization (Morandi et al., 2000; Solaiman et al., 2000; Sakamoto and Nohara, 2009). It is important to note that the relatively small increase in AM in these mutant plants compared to wild type (WT) (between 20 and 50%) contrasts with the large (many fold) increase in nodule numbers of these lines compared with WT lines. Some have speculated that this relatively small increase may be due to the ability of the AM fungus to spread laterally in the root, meaning it is sometimes difficult to distinguish the effect of *clv1-like* mutations on subsequent AM infection (Schaarschmidt et al., 2013). One interpretation is that the AOM pathway may inhibit new AM infections at the epidermis but may not limit the spread of AM between cortical cells, although this has not been tested



empirically. Such a hypothesis is consistent with the activation of AON during early stages of rhizobial infection (Li et al., 2009). Another way to examine the role of *CLV1-like* genes in AOM has been through the use of split root studies with mutant plants, which have shown that the *CLV1-like* gene in soybean (*NARK*) influences the suppression of subsequent mycorrhizal colonization (Meixner et al., 2005, 2007; Schaarschmidt et al., 2013). A role for *NARK* in the shoot control of AM was also observed in reciprocal grafts between the soybean *nark* mutant En6500 and WT plants (Sakamoto and Nohara, 2009) but not in a split root system with the same En6500 mutant (Meixner et al., 2007). Studies of orthologous mutants in other legumes species (e.g., *har1*, *sym29*) may be useful to clarify this inconsistency.

Apart from the requirement for the *CLV1-like* protein in AOM it is not yet clear if other AON genes encoding proteins that act in the root (*RDN1*, *TML*), shoot (*CLV2*, *KLV*, *CRN*) or as mobile signals (CLE) are also employed by the AOM pathway (Figure 1). One study examining the effect of a pea *clv2* mutant (*sym28*) found only a small but not significant increase in AM colonization compared with WT, although grafting and split root studies that would reveal if *CLV2* plays a role in the systemic regulation of AM were not attempted (Morandi et al., 2000). This and other mutants disrupted in root and shoot acting elements of the AON pathway outlined above are available in a range of legumes, but to date their AM phenotypes and possible role in systemic AOM regulation has not been examined. Recent work in the legume *Lotus japonicas* indicated that mycorrhizal colonization and phosphate starvation generates CLE peptides distinct from those induced by nodulation or nitrogen (Funayama-Noguchi et al., 2011; Handa

et al., 2015), but the function of these CLE peptides has yet to be tested. In an attempt to understand downstream elements of the AOM pathway, Schaarschmidt et al. (2013) analyzed the soybean transcriptome in split root mycorrhizal studies using WT and *nark* lines. Two putative CCAAT-binding transcription factor genes were identified, *GmNF-YA1a* and *GmNF-YA1b*, that are down-regulated by AM in a *NARK*-dependent manner. Hairy root RNAi lines with reduced expression of *GmNF-YA1a/b* displayed reduced AM colonization and this occurred in both WT and *nark* backgrounds, consistent with *GmNF-YA1a/b* acting downstream of *NARK* to suppress AM.

The evolutionary origin of the AON/AOM genes is still emerging but is informed by the *CLAVATA-WUSCHEL* (*CLV-WUS*) shoot meristem pathway. This pathway is best understood in Arabidopsis and involves several LRR receptors that act locally in the shoot, including *CLV1*, *CLV2* and *CRN*, to perceive a CLE peptide, *CLV3*, which in turn activates a feedback loop to maintain a defined stem cell population in the shoot apical meristem (Hazak and Hardtke, 2016). Arabidopsis lines disrupted in these genes displayed altered shoot meristem formation. In pea and *Lotus japonicas*, mutant studies have revealed *CLV2* plays a dual role, acting in both shoot development and AON, as *clv2* mutants display hypernodulation and shoot fasciation (Krusell et al., 2011). In contrast, there appear to be specific CLE genes that act in the AON pathway (Mortier et al., 2010). Similarly, disruption of *CLV1-like* genes closely related to *CLV1* in pea, *Lotus*, *Medicago* and soybean (*SYM29*, *HAR1*, *SUNN*, and *NARK*, respectively) result in hypernodulation but not shoot fasciation (reviewed by Reid et al., 2011b). A possible explanation in soybean for divergence between *NARK* and *CLV1*, is that *CLV1* appears to have undergone a duplication, resulting in *NARK* and *CLV1A* (Yamamoto et al., 2000) but this does not appear to be the case in the other legumes examined (see Schnabel et al., 2005). *CLV1A* is more closely related to *AtCLV1* and recent studies have shown it influences shoot architecture but not nodulation (Mirzaei et al., 2017). Although many phylogenetic studies have examined the *CLV1*, *CLV2* and *CLE* gene families, these studies have been almost exclusively limited to angiosperms (e.g., Sun and Wang, 2011; Zan et al., 2013; Wei et al., 2015; Xu et al., 2015; Hastwell et al., 2017), preventing a more comprehensive understanding of the evolutionary history and possible functional diversification of these genes. One recent transcriptomic study failed to find evidence for the *CLAVATA-WUSCHEL* (*CLV-WUS*) pathway in the moss *Physcomitrella* and liverwort *Marchantia* (Frank and Scanlon, 2015). However, a more comprehensive examination of these gene families in basal plant lineages and in mycorrhizal vs. non-mycorrhizal species (see approach of Favre et al., 2014; Delaux et al., 2015) might provide an insight into their possible role in the AM program.

ROLE OF *CLV2* IN AOM OF THE NON-LEGUME TOMATO

To fully understand the genes, signals and evolutionary history of AOM, we must go beyond legumes. Indeed, a non-legume system removes the possible complication of cross-talk between

the AON and AOM pathways to allow us to identify AOM components. Therefore, we employed the tomato *clv2-2* line, a CRISPR-Cas9 knock out line that targets the *CLV2* gene (Xu et al., 2015). As outlined above, this gene is essential for AON in legumes and also acts to control shoot apical meristem formation in legumes (Krusell et al., 2011) and non-legumes, including *clv2* tomato lines that display a weakly fasciated shoot and an increase in the number of floral organs (Xu et al., 2015).

We tested if the *CLV2* gene plays a role in AM development by examining the AM phenotype of the tomato *clv2-2* line. Compared with WT, *clv2* plants displayed a significant increase in AM colonization, including arbuscule frequency, compared with WT plants (Figure 2A). Although more frequent, the mycorrhizal structures observed in *clv2* mutants including arbuscules were similar in appearance to WT (Figure 2B). Therefore, we provide the first evidence that *CLV2* in tomato, known to be important for AON in legumes, also acts as a negative regulator of AM. This provides the first genetic evidence for the AOM pathway in non-legumes.

ROLE OF PLANT HORMONES IN AOM

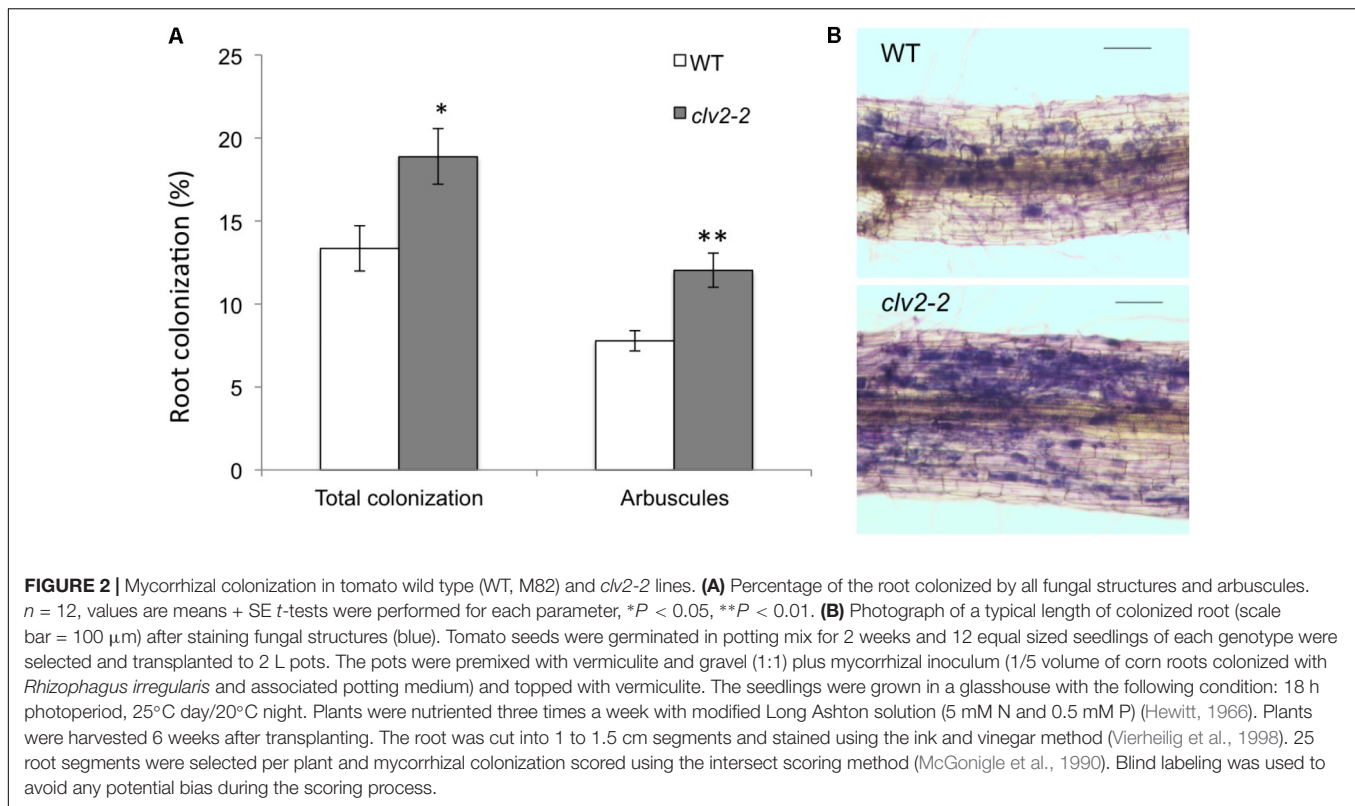
Autoregulation of mycorrhizae and AON are regulated by systemic signals and in addition to mobile CLE peptides, a range of studies have examined the role of plant hormones in AON and in some cases AOM. Double mutant studies in pea indicate gibberellins, brassinosteroids, and strigolactones are not required for the supernodulation phenotype and thus do not appear to act downstream of AON elements, *CLV1*-like, *CLV2* or *RDN1* (Ferguson et al., 2011; Foo et al., 2014b). In contrast, transcriptional studies showed that either jasmonic acid (JA) biosynthesis genes or JA regulated genes were systemically regulated by rhizobial colonization, and this was mediated by GmNARK in soybean. These results suggest the AON pathway influences JA signaling (Kinkema and Gresshoff, 2008). Several studies have also examined a role for auxin in autoregulation. In split root studies in soybean, significant auxin accumulation was observed in AM colonized roots but not uncolonized roots. However, this increase was not as great in the roots of a *nark* mutant (Meixner et al., 2005). This contrasts with nodulation studies with the orthologous mutant in *Medicago*, *sun*, that suggest *SUNN* may be required to suppress auxin accumulation in the root following rhizobial challenge (van Noorden et al., 2006). In WT *Medicago*, challenge with rhizobia leads to the downregulation of auxin transport from the shoot to the root. In contrast, *sun* mutants displayed elevated auxin levels in the infection zone of the root following inoculation with rhizobia. These studies suggest the AON pathway may modulate shoot to root auxin transport but this has yet to be investigated directly in AOM.

Cytokinin is an interesting case as it has been suggested to be a candidate for the shoot-derived inhibitor in AON, based on several lines of evidence from plants with altered cytokinin or CLE peptide biosynthesis, and measurement of cytokinin levels and transport (Sasaki et al., 2014). However, a key finding of this paper, that the LORE *ipt3-2* mutant allele, which is disrupted

in a key cytokinin biosynthesis gene, has increased nodulation could not be repeated in an independent study (Reid et al., 2017). In addition, another study indicated cytokinin may promote nodulation via the AON pathway. In *Medicago*, application of cytokinin directly to roots could induce the expression of the *MtCLE13* gene (Mortier et al., 2012) believed to encode the root to shoot AON signal. Given the ability for nodulation to suppress AM and vice versa outlined above, it is likely that the shoot-derived inhibitor is a common signal between the AON and AOM pathways. Studies with grafts between legume species certainly suggest that the shoot-derived inhibitor of nodulation is conserved across species (e.g., Lohar and VandenBosch, 2005; Ferguson et al., 2014; Foo et al., 2014a). However, unlike its clear role in nodulation there is less evidence to suggest cytokinin has an influence on AM. For example, the cytokinin receptor mutant in *M. truncatula*, *cre1*, did not display any alteration in AM development (Laffont et al., 2015). However, pharmacological studies suggest that cytokinin may promote AM development in pea (F. Guinel, personal communication). Clearly, questions remain around the role of CK in AON, in particular as the shoot-derived inhibitor, and studies directly testing its endogenous role in AOM are required.

DO NUTRIENT STATUS AND OTHER BENEFICIAL PLANT–MICROBE SYMBIOSES INTERACT WITH THE AUTOREGULATION PATHWAY(S)?

Forming symbioses with rhizobial or AM partners may only be beneficial to the plant under conditions of low mineral nutrient availability. In particular, legumes severely reduce nodulation when roots are exposed to elevated nitrogen levels and there are important roles for elements of the AON pathway in this nitrate-response (see Reid et al., 2011b). For example, the *clv1-like* mutants across legumes display a reduced ability to suppress nodulation in response to nitrate (Schnabel et al., 2005; Lim et al., 2011; Okamoto and Kawaguchi, 2015). This reduced response to nitrogen is also seen in *klv* and *rdn1* mutants (e.g., Jacobsen and Feenstra, 1984; Oka-Kira et al., 2005). However, this has not been comprehensively examined for all AON mutants across species. In addition, nitrate treatment induces the expression of specific CLE peptides, which in some cases are the same as those that are induced by rhizobia (Okamoto et al., 2009; Reid et al., 2011a). Whether the AOM pathway plays any role in nitrogen-regulation of AM symbioses has not been explored and, unlike the clearly suppressive effects on nodulation, it is not even clear if nitrogen is a promoter or inhibitor of AM (Correa et al., 2015). In contrast, phosphate has a strong suppressive influence on AM and this influence is systemic and does not require strigolactones (Breuillin et al., 2010; Foo et al., 2013b). Although phosphate induces expression of specific CLE peptides (Funayama-Noguchi et al., 2011), there is no direct evidence that the AOM pathway mediates the phosphate response of AM. Indeed phosphate regulation of AM is maintained in soybean and pea mutants disrupted in the *clv1-like* mutants, *nark* and *sym29* (Wyss et al.,



1990; Foo et al., 2013a). However, phosphate positively regulates nodule number and studies in pea have found this is disrupted in the *sym29* mutant (Foo et al., 2013a), suggesting a cross-over in the AON and phosphate response pathways. MicroRNAs of the 399 family have also been shown to play a role in the phosphate response and some were shown to be induced by phosphate-starvation in AM colonized *Medicago* (Branscheid et al., 2010). However, as overexpression of these miR399 genes in tobacco did not influence AM colonization, no clear role for these mobile microRNAs were established in the AM phosphate response (Branscheid et al., 2010).

In addition to nodulation and AM, plants form a range of other beneficial interactions with soil microbes. These include actinorhizal symbioses between members of the fabid clade and *Frankia* bacteria, ectomycorrhizal symbioses and interactions with fungal and bacterial endophytes. Systemic regulation of colonization has been demonstrated for actinorhizal associations (Wall and Huss-Danell, 1997), and for the interaction between *Arabidopsis* and the fungal endophyte *Piriformospora indica* (Pedrotti et al., 2013). Indeed, it has been shown in some split root studies that plants infected with endophytes have a decreased level of AM colonization, although this was not found in all studies (Müller, 2003; Omacini et al., 2006; Mack and Rudgers, 2008). Phylogenetic studies have suggested that the common symbiotic pathway is conserved in AM, rhizobial and actinorhizal associations, although the role of these genes in ectomycorrhizae and endophyte relationships is not known (Martin et al., 2017). *Arabidopsis* is a particularly interesting case as it appears to have lost the majority of the common symbiotic pathway (Delaux et al.,

2014), consistent with the lack of AM colonization and suggesting that this pathway is not important for hosting fungal endophytes. However, as these studies did not include AON/AOM genes the evolutionary origin of these pathways and their potential role across species is still not clear.

CONCLUSION AND FUTURE PERSPECTIVES

The similarities between the AOM and AON pathways and their shared genetic components is consistent with the AON pathway evolving at least in part from a pre-existing AOM pathway in early land plants. However, a lack of phylogenetic, genetic and physiological studies in non-legumes, including basal land plants, has hampered our understanding of the origin and diversification of the autoregulation pathway. In this paper, we show that in the non-legume tomato, the *CLV2* gene suppresses AM development, providing the first genetic evidence for an AOM gene in a non-legume. As found in legumes, this gene also plays a role in shoot apical meristem maintenance. However, the precise delineation in function of other AON/AOM elements such as the *CLV1* and *CLE* genes in shoot apical meristem maintenance is still not clear. Furthermore, it is likely that multiple systemic pathways regulate symbioses (Kassaw et al., 2015). For example, novel peptides and accompanying perception pathways with roles in nodulation and root development are now emerging (e.g., CEP1 and CRA2, Mohd-Radzman et al., 2016). Future studies could more systematically examine the role of AON genes and peptide

signals in AM development and take a phylogenetic approach to examine the evolutionary origin of symbiotic autoregulation.

AUTHOR CONTRIBUTIONS

EF conceived the project. CW carried out experiments. CW, JR, and EF wrote the manuscript.

REFERENCES

- Bago, B., Pfeffer, P. E., and Shachar-Hill, Y. (2000). Carbon metabolism and transport in arbuscular mycorrhizas. *Plant Physiol.* 124, 949. doi: 10.1104/pp.124.3.949
- Branscheid, A., Sieh, D., Pant, B. D., May, P., Devers, E. A., Elkrog, A., et al. (2010). Expression pattern suggests a role of MiR399 in the regulation of the cellular response to local Pi increase during arbuscular mycorrhizal symbiosis. *Mol. Plant Microbe Interact.* 23, 915–926. doi: 10.1094/MPMI-23-7-0915
- Breuillin, F., Schramm, J., Hajirezaei, M., Ahkami, A., Favre, P., Druège, U., et al. (2010). Phosphate systemically inhibits development of arbuscular mycorrhiza in *Petunia hybrida* and represses genes involved in mycorrhizal functioning. *Plant J.* 64, 1002–1017. doi: 10.1111/j.1365-313X.2010.04385.x
- Catford, J. G., Staehelin, C., Lerat, S., Piché, Y., and Vierheilig, H. (2003). Suppression of arbuscular mycorrhizal colonization and nodulation in split-root systems of alfalfa after pre-inoculation and treatment with Nod factors. *J. Exp. Bot.* 54, 1481–1487. doi: 10.1093/jxb/erg156
- Correa, A., Cruz, C., and Ferrol, N. (2015). Nitrogen and carbon/nitrogen dynamics in arbuscular mycorrhiza: the great unknown. *Mycorrhiza* 25, 499–515. doi: 10.1007/s00572-015-0627-6
- Crook, A. D., Schnabel, E. L., and Frugoli, J. A. (2016). The systemic nodule number regulation kinase SUNN in *Medicago truncatula* interacts with MtCLV2 and MtCRN. *Plant J.* 88, 108–119. doi: 10.1111/tj.13234
- Delaux, P.-M., Radhakrishnan, G. V., Jayaraman, D., Cheema, J., Malbreil, M., Volkening, J. D., et al. (2015). Algal ancestor of land plants was preadapted for symbiosis. *Proc. Natl. Acad. Sci. U.S.A.* 112, 13390–13395. doi: 10.1073/pnas.1515426112
- Delaux, P.-M., Séjalon-Delmas, N., Bécard, G., and Ané, J.-M. (2013). Evolution of the plant–microbe symbiotic ‘toolkit’. *Trends Plant Sci.* 18, 298–304. doi: 10.1016/j.tplants.2013.01.008
- Delaux, P. M., Varala, K., Edger, P. P., Coruzzi, G. M., Pires, J. C., and Ane, J. M. (2014). Comparative phylogenomics uncovers the impact of symbiotic associations on host genome evolution. *PLoS Genet.* 10:e1004487. doi: 10.1371/journal.pgen.1004487
- Douds, D. D., Pfeffer, P. E., and Shachar-Hill, Y. (2000). “Carbon partitioning, cost, and metabolism of arbuscular mycorrhizas,” in *Arbuscular Mycorrhizas: Physiology and Function*, eds Y. Kapulnik and D. D. Douds (Dordrecht: Springer), 107–129. doi: 10.1007/978-94-017-0776-3_6
- Favre, P., Bapaume, L., Bossolini, E., Delorenzi, M., Falquet, L., and Reinhardt, D. (2014). A novel bioinformatics pipeline to discover genes related to arbuscular mycorrhizal symbiosis based on their evolutionary conservation pattern among higher plants. *BMC Plant Biol.* 14:333. doi: 10.1186/s12870-014-0333-0
- Ferguson, B. J., Foo, E., Ross, J. J., and Reid, J. B. (2011). Relationship between gibberellin, ethylene and nodulation in *Pisum sativum*. *New Phytol.* 189, 829–842. doi: 10.1111/j.1469-8137.2010.03542.x
- Ferguson, B. J., Li, D., Hastwell, A. H., Reid, D. E., Li, Y., Jackson, S. A., et al. (2014). The soybean (*Glycine max*) nodulation-suppressive CLE peptide, GmRIC1, functions interspecifically in common white bean (*Phaseolus vulgaris*), but not in a supernodulating line mutated in the receptor PvNARK. *Plant Biotechnol. J.* 12, 1085–1097. doi: 10.1111/pbi.12216
- Field, K. J., Pressel, S., Duckett, J. G., Rimington, W. R., and Bidartondo, M. I. (2015). Symbiotic options for the conquest of land. *Trends Ecol. Evol.* 30, 477–486. doi: 10.1016/j.tree.2015.05.007
- Foo, E., Ferguson, B. J., and Reid, J. B. (2014a). Common and divergent roles of plant hormones in nodulation and arbuscular mycorrhizal symbioses. *Plant Signal. Behav.* 9, 1037–1045. doi: 10.4161/psb.29593
- Foo, E., Ferguson, B. J., and Reid, J. B. (2014b). The potential roles of strigolactones and brassinosteroids in the autoregulation of nodulation pathway. *Ann. Bot.* 113, 1037–1045. doi: 10.1093/aob/mcu030
- Foo, E., Yoneyama, K., Hugill, C., Quittenden, L. J., and Reid, J. B. (2013a). Strigolactones: internal and external signals in plant symbioses? *Plant Signal. Behav.* 8:e23168. doi: 10.4161/psb.23168
- Foo, E., Yoneyama, K., Hugill, C. J., Quittenden, L. J., and Reid, J. B. (2013b). Strigolactones and the regulation of pea symbioses in response to nitrate and phosphate deficiency. *Mol. Plant.* 6, 76–87. doi: 10.1093/mp/sss115
- Frank, M. H., and Scanlon, M. J. (2015). Transcriptomic evidence for the evolution of shoot meristem function in sporophyte-dominant land plants through concerted selection of ancestral gametophytic and sporophytic genetic programs. *Mol. Biol. Evol.* 32, 355–367. doi: 10.1093/molbev/msu303
- Funayama-Noguchi, S., Noguchi, K., Yoshida, C., and Kawaguchi, M. (2011). Two CLE genes are induced by phosphate in roots of *Lotus japonicus*. *J. Plant Res.* 124, 155–163. doi: 10.1007/s10265-010-0342-5
- Handa, Y., Nishide, H., Takeda, N., Suzuki, Y., Kawaguchi, M., and Saito, K. (2015). RNA-seq transcriptional profiling of an arbuscular mycorrhiza provides insights into regulated and coordinated gene expression in *Lotus japonicus* and *Rhizophagus irregularis*. *Plant Cell Physiol.* 56, 1490–1511. doi: 10.1093/pcp/pcv071
- Hastwell, A. H., Corcilius, L., Williams, J., Gresshoff, P. M., Payne, R. J., and Ferguson, B. J. (2018). Triarabinsylation is required for nodulation-suppressive CLE peptides to systemically inhibit nodulation in *Pisum sativum*. *Plant Cell Environ.* doi: 10.1111/pce.13325 [Epub ahead of print].
- Hastwell, A. H., De Bang, T. C., Gresshoff, P. M., and Ferguson, B. J. (2017). CLE peptide-encoding gene families in *Medicago truncatula* and *Lotus japonicus*, compared with those of soybean, common bean and Arabidopsis. *Sci. Rep.* 7:9384. doi: 10.1038/s41598-017-09296-w
- Hazak, O., and Hardtke, C. S. (2016). CLAVATA 1-type receptors in plant development. *J. Exp. Bot.* 67, 4827–4833. doi: 10.1093/jxb/erw247
- Hewitt, E. J. (1966). *Sand and Water Culture Methods Used in the Study of Plant Nutrition*. Wallingford: Centre for Agriculture and Bioscience International.
- Imin, N., Patel, N., Corcilius, L., Payne, R. J., and Djordjevic, M. A. (2018). CLE peptide tri-arabinsylation and peptide domain sequence composition are essential for SUNN-dependent autoregulation of nodulation in *Medicago truncatula*. *New Phytol.* 218, 73–80. doi: 10.1111/nph.15019
- Jacobsen, E., and Feenstra, W. J. (1984). A new pea mutant with efficient nodulation in the presence of nitrate. *Plant Sci. Lett.* 33, 337–344. doi: 10.1016/0304-4211(84)90025-7
- Kassaw, T., Bridges, W. Jr., and Frugoli, J. (2015). Multiple autoregulation of nodulation (AON) signals identified through split root analysis of *Medicago truncatula* sunn and rdn1 Mutants. *Plants* 4, 209–224. doi: 10.3390/plants4020209
- Kassaw, T., Nowak, S., Schnabel, E., and Frugoli, J. A. (2017). ROOT DETERMINED NODULATION1 is required for *M. truncatula* CLE12, but not CLE13 peptide signaling through the SUNN receptor kinase. *Plant Physiol.* 174, 2445–2456. doi: 10.1104/pp.17.00278
- Khaosaad, T., Staehelin, C., Steinkellner, S., Hage-Ahmed, K., Ocampo, J. A., Garcia-Garrido, J. M., et al. (2010). The *Rhizobium* sp. strain NGR234 systemically suppresses arbuscular mycorrhizal root colonization in a split-root system of barley (*Hordeum vulgare*). *Physiol. Plant.* 140, 238–245. doi: 10.1111/j.1399-3054.2010.01396.x
- Kinkema, M., and Gresshoff, P. M. (2008). Investigation of downstream signals of the soybean autoregulation of nodulation receptor kinase GmNARK. *Mol. Plant Microbe Interact.* 21, 1337–1348. doi: 10.1094/MPMI-21-10-1337

ACKNOWLEDGMENTS

We thank Professor Zachary Lippman (Cold Spring Harbor Laboratory) for the kind gift of *clv2* tomato seed, Shelley Urquhart and Michelle Lang for assistance with plant husbandry and the Australian Research Council for financial support to EF and JR (FF140100770 and DP140101709).

- Krusell, L., Sato, N., Fukuhara, I., Koch, B. E. V., Grossmann, C., Okamoto, S., et al. (2011). The *Clavata2* genes of pea and *Lotus japonicus* affect autoregulation of nodulation. *Plant J.* 65, 861–871. doi: 10.1111/j.1365-313X.2010.04474.x
- Laffont, C., Rey, T., André, O., Novero, M., Kazmierczak, T., Debéllé, F., et al. (2015). The CRE1 cytokinin pathway is differentially recruited depending on *Medicago truncatula* root environments and negatively regulates resistance to a pathogen. *PLoS One* 10:e0116819. doi: 10.1371/journal.pone.0116819
- Li, D., Kinkema, M., and Gresshoff, P. M. (2009). Autoregulation of nodulation (AON) in *Pisum sativum* (pea) involves signalling events associated with both nodule primordia development and nitrogen fixation. *J. Plant Physiol.* 166, 955–967. doi: 10.1016/j.jplph.2009.03.004
- Lim, C. W., Lee, Y. W., and Hwang, C. H. (2011). Soybean nodule-enhanced CLE peptides in roots act as signals in GmNARK-mediated nodulation suppression. *Plant Cell Physiol.* 52, 1613–1627. doi: 10.1093/pcp/pcr091
- Lin, Y. H., Ferguson, B. J., Kereszt, A., and Gresshoff, P. M. (2010). Suppression of hypernodulation in soybean by a leaf-extracted, NARK- and Nod factor-dependent, low molecular mass fraction. *New Phytol.* 185, 1074–1086. doi: 10.1111/j.1469-8137.2009.03163.x
- Lohar, D. P., and VandenBosch, K. A. (2005). Grafting between model legumes demonstrates roles for roots and shoots in determining nodule type and host/rhizobia specificity. *J. Exp. Bot.* 56, 1643–1650. doi: 10.1093/jxb/eri160
- Mack, K. M. L., and Rudgers, J. A. (2008). Balancing multiple mutualists: asymmetric interactions among plants, arbuscular mycorrhizal fungi, and fungal endophytes. *OIKOS* 117, 310–320. doi: 10.1111/j.2007.0030-1299.15973.x
- Martin, F. M., Uroz, S., and Barker, D. G. (2017). Ancestral alliances: plant mutualistic symbioses with fungi and bacteria. *Science* 356:eaad4501. doi: 10.1126/science.aad4501
- McGonigle, T. P., Miller, M. H., Evans, D. G., Fairchild, G. L., and Swan, J. A. (1990). A new method which gives an objective measure of colonization of roots by vesicular–arbuscular mycorrhizal fungi. *New Phytol.* 115, 495–501. doi: 10.1111/j.1469-8137.1990.tb00476.x
- Meixner, C., Ludwig-Müller, J., Miersch, O., Gresshoff, P., Staehelin, C., and Vierheilig, H. (2005). Lack of mycorrhizal autoregulation and phytohormonal changes in the supernodulating soybean mutant nts1007. *Planta* 222, 709–715. doi: 10.1007/s00425-005-0003-4
- Meixner, C., Vegvari, G., Ludwig-Müller, J., Gagnon, H., Steinkellner, S., Staehelin, C., et al. (2007). Two defined alleles of the LRR receptor kinase GmNARK in supernodulating soybean govern differing autoregulation of mycorrhization. *Physiol. Plant.* 130, 261–270. doi: 10.1111/j.1399-3054.2007.00903.x
- Mirzaei, S., Batley, J., El-Mellouki, T., Liu, S., Meksem, K., Ferguson, B. J., et al. (2017). Neodiversification of homeologous CLAVATA1-like receptor kinase genes in soybean leads to distinct developmental outcomes. *Sci. Rep.* 7:8878. doi: 10.1038/s41598-017-08252-y
- Miyazawa, H., Oka-Kira, E., Sato, N., Takahashi, H., Wu, G. J., Sato, S., et al. (2010). The receptor-like kinase KLAVIER mediates systemic regulation of nodulation and non-symbiotic shoot development in *Lotus japonicus*. *Development* 137, 4317–4325. doi: 10.1242/dev.058891
- Mohd-Radzman, N. A., Laffont, C., Ivanovici, A., Patel, N., Reid, D. E., Stougaard, J., et al. (2016). Different pathways act downstream of the peptide receptor CRA2 to regulate lateral root and nodule development. *Plant Physiol.* 171, 2536–2548. doi: 10.1104/pp.16.00113
- Morandi, D., Sagan, M., Prado-Vivant, E., and Duc, G. (2000). Influence of genes determining supernodulation on root colonization by the mycorrhizal fungus *Glomus mosseae* in *Pisum sativum* and *Medicago truncatula* mutants. *Mycorrhiza* 10, 37–42. doi: 10.1007/s005720050285
- Mortier, V., De Wever, E., Vuylsteke, M., Holsters, M., and Goormachtig, S. (2012). Nodule numbers are governed by interaction between CLE peptides and cytokinin signaling. *Plant J.* 70, 367–376. doi: 10.1111/j.1365-313X.2011.04881.x
- Mortier, V., Den Herder, G., Whitford, R., Van De Velde, W., Rombauts, S., D'haeseleer, K., et al. (2010). CLE peptides control *Medicago truncatula* nodulation locally and systemically. *Plant Physiol.* 153, 222–237. doi: 10.1104/pp.110.153718
- Müller, J. (2003). Artificial infection by endophytes affects growth and mycorrhizal colonisation of *Lolium perenne*. *Funct. Plant Biol.* 30, 419–424. doi: 10.1071/FP02189
- Oka-Kira, E., Tateno, K., Miura, K. I., Haga, T., Hayashi, M., Harada, K., et al. (2005). klavier (klv), A novel hypernodulation mutant of *Lotus japonicus* affected in vascular tissue organization and floral induction. *Plant J.* 44, 505–515. doi: 10.1111/j.1365-313X.2005.02543.x
- Okamoto, S., and Kawaguchi, M. (2015). Shoot HARA1 mediates nitrate inhibition of nodulation in *Lotus japonicus*. *Plant Signal. Behav.* 10:e1000138. doi: 10.1080/15592324.2014.1000138
- Okamoto, S., Ohnishi, E., Sato, S., Takahashi, H., Nakazono, M., Tabata, S., et al. (2009). Nod factor/nitrate-induced CLE genes that drive HARA1-mediated systemic regulation of nodulation. *Plant Cell Physiol.* 50, 67–77. doi: 10.1093/pcp/pcn194
- Okamoto, S., Shinohara, H., Mori, T., Matsubayashi, Y., and Kawaguchi, M. (2013). Root-derived CLE glycopeptides control nodulation by direct binding to HARA1 receptor kinase. *Nat. Commun.* 4:2191. doi: 10.1038/ncomms3191
- Omacini, M., Eggers, T., Bonkowski, M., Gange, A. C., and Jones, T. H. (2006). Leaf endophytes affect mycorrhizal status and growth of co-infected and neighbouring plants. *Funct. Ecol.* 20, 226–232. doi: 10.1111/j.1365-2435.2006.01099.x
- Pedrotti, L., Mueller, M. J., and Waller, F. (2013). *Piriformospora indica* root colonization triggers local and systemic root responses and inhibits secondary colonization of distal roots. *PLoS One* 8:e69352. doi: 10.1371/journal.pone.0069352
- Reid, D. E., Ferguson, B. J., and Gresshoff, P. M. (2011a). Inoculation-and nitrate-induced CLE peptides of soybean control NARK-dependent nodule formation. *Mol. Plant Microbe Interact.* 24, 606–618. doi: 10.1094/MPMI-09-10-0207
- Reid, D. E., Ferguson, B. J., Hayashi, S., Lin, Y. H., and Gresshoff, P. M. (2011b). Molecular mechanisms controlling legume autoregulation of nodulation. *Ann. Bot.* 108, 789–795. doi: 10.1093/aob/mcr205
- Reid, D. E., Nadzieja, M., Novak, O., Heckmann, A. B., Sandal, N., and Stougaard, J. (2017). Cytokinin biosynthesis promotes cortical cell responses during nodule development. *Plant Physiol.* 175:pp.00832.2017. doi: 10.1104/pp.17.00832
- Sakamoto, K., and Nohara, Y. (2009). Soybean (*Glycine max* [L.] Merr.) shoots systemically control arbuscule formation in mycorrhizal symbiosis. *Soil Sci. Plant Nutr.* 55, 252–257. doi: 10.1111/j.1747-0765.2009.00358.x
- Sakamoto, K., Ogiwara, N., and Kaji, T. (2013). Involvement of autoregulation in the interaction between rhizobial nodulation and AM fungal colonization in soybean roots. *Biol. Fertil. Soils* 49, 1141–1152. doi: 10.1007/s00374-013-0804-8
- Sasaki, T., Suzuki, T., Soyano, T., Kojima, M., Sakakibara, H., and Kawaguchi, M. (2014). Shoot-derived cytokinins systemically regulate root nodulation. *Nat. Commun.* 5:4983. doi: 10.1038/ncomms5983
- Schaarschmidt, S., Gresshoff, P. M., and Hause, B. (2013). Analyzing the soybean transcriptome during autoregulation of mycorrhization identifies the transcription factors GmNF-YA1a/b as positive regulators of arbuscular mycorrhization. *Genome Biol.* 14:R62. doi: 10.1186/gb-2013-14-6-r62
- Schnabel, E., Journet, E. P., De Carvalho-Niebel, F., Duc, G., and Frugoli, J. (2005). The *Medicago truncatula* SUNN gene encodes a CLV1-like leucine-rich repeat receptor kinase that regulates nodule number and root length. *Plant Mol. Biol.* 58, 809–822. doi: 10.1007/s11103-005-8102-y
- Schnabel, E. L., Kassaw, T. K., Smith, L. S., Marsh, J. F., Oldroyd, G. E., Long, S. R., et al. (2011). The *ROOT DETERMINED NODULATION1* gene regulates nodule number in roots of *Medicago truncatula* and defines a highly conserved, uncharacterized plant gene family. *Plant Physiol.* 157, 328–340. doi: 10.1104/pp.111.178756
- Shrihari, P. C., Sakamoto, K., Inubushi, K., and Akao, S. (2000). Interaction between supernodulating or non-nodulating mutants of soybean and two arbuscular mycorrhizal fungi. *Mycorrhiza* 10, 101–106. doi: 10.1007/s005720000064
- Solaiman, Z. M., Senoo, K., Kawaguchi, M., Imaizumi-Anraku, H., Akao, S., Tanaka, A., et al. (2000). Characterization of mycorrhizas formed by glomus sp. on roots of hypernodulating mutants of *Lotus japonicus*. *J. Plant Res.* 113, 443–448. doi: 10.1007/PL00013953
- Soyano, T., and Kawaguchi, M. (2014). “Systemic regulation of root nodule formation,” in *Advances in Biology and Ecology of Nitrogen Fixation*, ed. T. Ohyama (Rijeka: InTech).
- Sun, X., and Wang, G.-L. (2011). Genome-wide identification, characterization and phylogenetic analysis of the rice LRR-Kinases. *PLoS One* 6:e16079. doi: 10.1371/journal.pone.0016079

- Takahara, M., Magori, S., Soyano, T., Okamoto, S., Yoshida, C., Yano, K., et al. (2013). Too much love, a novel Kelch repeat-containing F-box protein, functions in the long-distance regulation of the legume-Rhizobium symbiosis. *Plant Cell Physiol.* 54, 433–447. doi: 10.1093/pcp/pct022
- van Noorden, G. E., Ross, J. J., Reid, J. B., Rolfe, B. G., and Mathesius, U. (2006). Defective long-distance auxin transport regulation in the *Medicago truncatula* super numeric nodules mutant. *Plant Physiol.* 140, 1494–1506. doi: 10.1104/pp.105.075879
- Vierheilig, H. (2004). Further root colonization by arbuscular mycorrhizal fungi in already mycorrhizal plants is suppressed after a critical level of root colonization. *J. Plant Physiol.* 161, 339–341. doi: 10.1078/0176-1617-01097
- Vierheilig, H., Coughlan, A. P., Wyss, U., and Piché, Y. (1998). Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. *Appl. Environ. Microbiol.* 64, 5004–5007.
- Vierheilig, H., Garcia-Garrido, J., Wyss, U., and Piché, Y. (2000a). Systemic suppression of mycorrhizal colonization of barley roots already colonized by AM fungi. *Soil Biol. Biochem.* 32, 589–595. doi: 10.1016/S0038-0717(99)00155-8
- Vierheilig, H., Maier, W., Wyss, U., Samson, J., Strack, D., and Piché, Y. (2000b). Cyclohexenone derivative- and phosphate-levels in split-root systems and their role in the systemic suppression of mycorrhization in precolonized barley plants. *J. Plant Physiol.* 157, 593–599. doi: 10.1016/S0176-1617(00)80001-2
- Vierheilig, H., Steinkellner, S., Khaosaad, T., and Garcia-Garrido, J. M. (2008). “The biocontrol effect of mycorrhization on soilborne fungal pathogens and the autoregulation of the AM symbiosis: one mechanism, two effects?” in *Mycorrhiza: State of the Art, Genetics and Molecular Biology, Eco-Function, Biotechnology, Eco-Physiology, Structure and Systematics*, ed. A. Varma (Berlin: Springer), 307–320.
- Wall, L. G., and Huss-Danell, K. (1997). Regulation of nodulation in *Alnus incana*-Frankia symbiosis. *Physiol. Plant.* 99, 594–600. doi: 10.1111/j.1399-3054.1997.tb05362.x
- Wei, Z., Wang, J., Yang, S., and Song, Y. (2015). Identification and expression analysis of the LRR-RLK gene family in tomato (*Solanum lycopersicum*) Heinz 1706. *Genome* 58, 121–134. doi: 10.1139/gen-2015-0035
- Wyss, P., Mellor, R. B., and Wiemken, A. (1990). Mutants of soybean (*Glycine max*) unable to suppress nodulation in the presence of nitrate retain the ability to suppress mycorrhization in the presence of phosphate. *J. Plant Physiol.* 136, 507–509. doi: 10.1016/S0176-1617(11)80045-3
- Xu, C., Liberatore, K. L., Macalister, C. A., Huang, Z., Chu, Y.-H., Jiang, K., et al. (2015). A cascade of arabinosyltransferases controls shoot meristem size in tomato. *Nat. Genet.* 47, 784–792. doi: 10.1038/ng.3309
- Yamamoto, E., Karakaya, H. C., and Knap, H. T. (2000). Molecular characterization of two soybean homologs of *Arabidopsis thaliana* CLAVATA1 from the wild type and fasciation mutant. The sequence data reported in this paper have been submitted to the GenBank data libraries under the accession numbers AF197946 for GmCLV1A and AF197947 for GmCLV1B. *Biochim. Biophys. Acta* 1491, 333–340. doi: 10.1016/S0167-4781(00)00061-0
- Zan, Y., Ji, Y., Zhang, Y., Yang, S., Song, Y., and Wang, J. (2013). Genome-wide identification, characterization and expression analysis of populus leucine-rich repeat receptor-like protein kinase genes. *BMC Genomics* 14:318. doi: 10.1186/1471-2164-14-318

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2018 Wang, Reid and Foo. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.