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Mycorrhiza-induced resistance: more than the sum of its parts?

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Plants can develop an enhanced defensive capacity in response to infection by arbuscular mycorrhizal fungi (AMF). This 'mycorrhiza-induced resistance' (MIR) provides systemic protection against a wide range of attackers and shares characteristics with systemic acquired resistance (SAR) after pathogen infection and induced systemic resistance (ISR) following root colonisation by non-pathogenic rhizobacteria. It is commonly assumed that fungal stimulation of the plant immune system is solely responsible for MIR. In this opinion article, we present a novel model of MIR that integrates different aspects of the induced resistance phenomenon. We propose that MIR is a cumulative effect of direct plant responses to mycorrhizal infection and indirect immune responses to ISR-eliciting rhizobacteria in the mycorrhizosphere.

Mycorrhiza-induced resistance (MIR)

Mycorrhizal symbiosis is a mutualism between plants and mycorrhizal fungi during which photosynthetic products are exchanged for soil-derived mineral nutrients [1]. The true age of this relationship and the extent of host–mycorrhiza coevolution has been revealed by fossil evidence and phylogenetic analyses [2,3], dating the emergence of this symbiosis to 450 million years ago. It has been estimated that 80% of plant species retain these ancient arbuscular mycorrhizal associations [1], illustrating the importance of this mutualism to both partners.

Research on plant–mycorrhiza interactions has mostly focussed on the physiology of nutrient-for-carbon exchange and plant signal-transduction pathways controlling the interaction. Comparatively little is known about the mechanisms conferring non-nutritional benefits by mycorrhiza, such as suppression of soil-borne diseases and enhancing plant resistance to pests and diseases [4]. Plants routinely signal to conspecific organisms in the rhizosphere by releasing primary and secondary metabolites from their roots. Some of these metabolites recruit beneficial microbes, including AMF. Furthermore, AMF infection is known to stimulate biological activity in the rhizosphere, a phenomenon commonly referred to as the 'mycorrhizosphere effect' [5] (Box 1). This effect includes the attraction and selection of specific bacterial strains, such as plant growth-promoting rhizobacteria (PGPR) that possess the capacity to enhance plant growth and suppress pests and diseases. Some of these mycorrhizosphere-inhabiting bacteria can act as 'mycorrhiza-helper bacteria' and promote the efficiency of mycorrhizal symbiosis [6] (Box 1). As a consequence of these interactions, it has been suggested that the benefits of AMF on whole-plant physiology are at least partially determined by biological activities in the mycorrhizosphere [6–8].

AMF can suppress plant pests and diseases through induction of systemic resistance [9–11]. Nutrient supply experiments have revealed that MIR cannot be attributed to improved nutritional status [12]. The induced resistance shares characteristics with both pathogen-induced SAR and rhizobacterial ISR; MIR has been associated with SAR-like priming of salicylic acid (SA)-dependent genes, but more often coincides with priming of jasmonic acid (JA)-dependent defences and cell wall defences (Table 1). Accordingly, MIR confers protection against a wide range of attackers, including biotrophic pathogens, necrotrophic pathogens, nematodes, and herbivorous arthropods (Table 1). It has been proposed that MIR is the result of active suppression of components in the SA-dependent defence pathway, causing systemic priming of JA-dependent defences [10]. However, the exact contribution of jasmonates in MIR remains unclear [13] and the long-distance signals controlling MIR remain to be resolved. Most instances of MIR have been reported for non-sterile systems. It is thus possible that MIR is not solely determined by the fungus, but that bacteria in the mycorrhizosphere have a complementary contribution to the full MIR response. Here, we present a four-phase spatiotemporal model explaining MIR as a cumulative outcome of direct plant–AMF interactions and responses to ISR-eliciting bacteria in the mycorrhizosphere (Figure 1).

Phase I: root exudation of mycorrhiza-recruiting chemicals

Plant roots exude a diverse array of biologically active compounds [14]. Estimates suggest plants can exude up to 40% of their photosynthates from roots, representing a rich source of energy for soil microbes [15]. Root exudates typically contain sugars, amino acids, carboxylic acids, phenolics, and other secondary metabolites, which all have the capacity to influence the occurrence, physiology, and behaviour of soil organisms. For the interaction between plants and AMF, strigolactones have been identified as

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1360-1385/$ – see front matter.
 http://dx.doi.org/10.1016/j.tplants.2013.06.004

Box 1. The mycorrhizosphere effect

A relatively small volume of soil around plant roots is under the direct influence of root exudates, termed the 'rhizosphere'. This zone is characterised by increased levels of microbial activity. However, 80% of all plant species form symbiotic relationships with AMF [1]. Consequently, the volume of soil influenced by plant-derived carbon via AMF can be extended to encompass the 'mycorrhizosphere'. AMF have a selective influence on microbial communities in the mycorrhizosphere. The enhanced microbial activity surrounding mycorrhizal roots compared with non-mycorrhizal roots is called the 'mycorrhizosphere effect' [5]. Having resolved that extramatrical hyphae from AMF have access to resources from a vast volume of soil, it was discovered that some mycorrhizosphere-inhabiting bacteria, called 'mycorrhiza helper bacteria' (MHB), can stimulate mycorrhizal symbioses [6]. The concept of mutualism between AMF and soil bacteria is not new. In 1962, Mosse first proposed the idea that AMF and bacteria interact directly in the soil, showing that mycorrhizal roots can enhance the survival of *P. fluorescens* bacteria [74]. Since then, multiple studies have demonstrated that MHB can promote mycorrhizal infection and symbiosis through stimulation of mycelial extension and reducing the impact of adverse environmental conditions [6]. Whether increased AMF growth and survival by MHB are due to production of growth factors, detoxification of soil allelochemicals, or antagonism of competitors and/or parasites remains unresolved [6]. With the development of metagenomics technologies and DNA-sequencing methods, the true extent of quantitative and qualitative changes in the microbial community due to AMF is beginning to emerge. The chemical basis driving mycorrhizosphere development is less well resolved, although there are indications that carbon exudation by AMF in the form of the glycoprotein glomalin plays a role [75]. This is an attractive hypothesis, given that up to 5% of active soil organic carbon pools comprise glomalin, which is recalcitrant in the soil and thus represents a 'slow-release' carbon substrate [75]. The consequences of the mycorrhizosphere effect, including recruitment of PGPRs, may not only boost nutrient mobilisation by AMF but could also provide non-nutritional benefits, such as disease suppression via antibiosis and/or competitive exclusion. Crucially, increased densities of selected rhizobacteria in the mycorrhizosphere have the potential to suppress pests and diseases in systemic plant tissues through priming of inducible defences.

important AMF-recruiting signals (Box 2). This class of terpenoid lactones, long known as germination signals for parasitic plants, stimulates hyphal branching in AMF, thereby helping the fungus to localise host roots and so facilitate infection [5,6]. Involvement of root signals in the attraction of both pathogenic and mutualistic soil organisms is not rare. For instance, root-borne isoflavones secreted by soybean can attract the endosymbiotic N-fixing bacterium *Bradyrhizobium japonicum* [16], but can simultaneously attract the pathogenic oomycete *Phytophthora sojae* [17]. Similarly, benzoxazinoid metabolites in root exudates of maize (*Zea mays*) can attract both beneficial *Pseudomonas putida* bacteria [18] and western corn rootworm [19]. From an evolutionary perspective, common attraction of mutualistic and parasitic organisms is unsurprising given the strong selection pressure on parasites to adopt the same plant signals as beneficial mutualists (Box 2).

Phase II: the plant immune system responding to AMF infection

The initial stages of root colonisation by AMF are accompanied by transient induction of selected plant defences, followed by localised suppression at later stages of the interaction [20]. It is plausible that initial induction of plant immunity is based on host recognition of microbe-associated molecular patterns (MAMPs) from the AMF. Recognition of MAMPs by pattern-recognition receptors elicits a series of signalling cascades resulting in enhanced production of the plant defence hormone SA and expression of MAMP-triggered immunity [21]. For instance, infection by *Funneliformis mosseae* (syn. *Glomus mosseae*) induces transient accumulation of SA in pea [22], whereas this response was more pronounced and permanent in symbiosis-resistant P2 pea genotypes. Hence, the initial SA response is suppressed during successive stages of AMF infection. Localised MAMP recognition and SA production can lead to production of long-distance SAR signals and cause systemic priming of SA-dependent defences [23–25]. Because most SAR studies have been conducted with AMF-incompatible *Arabidopsis*, it is difficult to draw direct comparisons between SAR and MIR. However, like SAR, MIR has been associated with systemic priming of SA-dependent defences and protection against (hemi)biotrophic pathogens (Table 1). Furthermore, the primed defence state of SAR is long lasting [26,27] and can act additively on other forms of systemic disease resistance [28]. We therefore propose that SAR-related signals during the early stages of plant–AMF interactions contribute to MIR (Figure 1).

Phase III: immune suppression by AMF and recruitment of mycorrhizosphere bacteria

The transient nature of MAMP-triggered immune responses during the early stages of mycorrhization suggest that AMF employ strategies similar to those of pathogenic fungi, which secrete specific effector molecules to suppress plant immunity and establish a successful infection [29]. A comparative transcriptome study in rice revealed striking similarities between responses to AMF and pathogenic fungi [30]. Additional evidence for active immune suppression by AMF came from the discovery that the calcium/calmodulin kinase DMI3, a central regulator in the symbiotic pathway, represses early-acting defence genes [31]. Kloppholz *et al.* [32] were the first to identify an effector protein (SP7) from *Rhizophagus irregularis* (syn. *Glomus intraradices*). This secreted protein is expressed during the initial stages of contact between the mycorrhizal fungus and roots and is translocated to the plant nucleus, where it inhibits the transcription factor ERF19 to suppress plant defence and promote infection by biotrophic fungi like *R. irregularis* [32]. AMF induce species-specific changes in defence hormones in their hosts [13,33]. Some of these hormonal changes can restrict AMF colonisation, whereas others function to promote biotrophic AMF infection. For instance, AMF promote production of the plant hormone abscisic acid (ABA) [34]. Experiments with the ABA-deficient tomato (*Solanum lycopersicum*) mutant *sitiens* have revealed that arbuscular development and functionality in tomato are dependent on ABA [35]. Because ABA can suppress SA-dependent defences against biotrophic pathogens [36,37], it is plausible that AMF stimulate ABA production in the roots to promote their

Table 1. Defence mechanisms associated with ISR by AMF

Proposed mode of action of systemic resistance	Resistance-inducing AMF strain	Host plant	Disease or pest	Refs
Priming of JA- and SA-inducible defence genes	*R. irregularis*[a] *G. versiforme* *F. mosseae*[b]	Grapevine (*Vitis vinifera*) Grapevine, tobacco (*Nicotiana tabacum*) Maize	*Xiphinema index* *Meloidogyne incognita* *Rhizoctonia solani*	[76] [77] [50]
SA-independent resistance	*F. mosseae*[b]	Barley	*Gaeumannomyces graminis*	[78]
Priming of SA-inducible *PR* genes	*Glomus* sp. MUCL 41833	Potato	*Phytophthora infestans*	[66]
AMF-specific modulation of herbivore-induced leaf chemicals	*Gigaspora margarita* *Acaulospora longula*	*Lotus japonicus*	*Tetranychus urticae*	[79]
Priming of cell wall defence	*F. mosseae* *Glomus* and *Gigaspora* sp.	Tomato Common bean (*Phaseolus vulgaris*)	*Phytophthora parasitica* *R. solani*	[80–82] [83]
Priming of defence-related protein production	*F. mosseae*	Tomato	*P. parasitica*	[80–82]
Priming of defence-related enzymatic activity	*Glomus* and *Gigaspora* sp.	Common bean	*R. solani*	[83]
Enhanced production of phenolic compounds	*Glomus* and *Gigaspora* sp. *Glomus versiforme*	Common bean Tomato	*R. solani* *Ralstonia solanacearum*	[83] [84]
Enhanced expression of stress-related genes	*R. irregularis*	*Medicago truncatula*	*Xanthomonas campestris*	[85]
Enhanced production of benzoxazinoids	*F. mosseae*[b]	Maize	*R. solani*	[50]

[a]syn. *G. intraradices*.

[b]syn. *G. mosseae*.

own infection. The role of ABA in disease resistance is complex and depends on the stage and nature of the interaction. Although ABA typically suppresses relatively late-acting defence mechanisms during plant–pathogen interactions (e.g., SA-dependent mechanisms), it can promote defence mechanisms that act relatively early in the interaction, such as MAMP-induced stomatal closure, induction of reactive oxygen species, and cell wall reinforcements [36]. The mobility of ABA through both xylem and phloem makes this hormone an attractive candidate to act as a complementary long-distance MIR signal to the shoot, where it could contribute to priming of cell wall defences (Figure 1). Indeed, application of ABA to maize roots induces above-ground resistance to the (hemi)necrotrophic fungi *Setosphaeria turcicia* and *Colletotrichum graminicola* [38,39] and ABA treatment of rice enhances resistance against the necrotrophic fungus *Cochliobolus miyabeanus* [40]. However, shoot profiling of plant hormones in melon did not reveal consistent changes in ABA levels after root infection by either *G. intraradices* or *G. mosseae* [41], suggesting that a potential role for ABA as a systemic MIR signal may be transient.

Mycorrhization increases transport of photosynthates to the roots, influencing sugar-dependent signaling pathways [42]. This, in combination with modulation of defence metabolism and improved phosphate uptake, leads to quantitative and qualitative changes in the composition of root exudates. For instance, AMF-mediated uptake of phosphorus suppresses strigolactone exudation [43], whereas other studies have reported quantitative and qualitative changes in primary metabolites from root exudates [44]. Some of these changes can have negative and positive impacts on other rhizosphere microbes [45–47]. Apart from plant-mediated changes in root exudate chemistry, metabolic activity by the fungus itself can also alter the chemical composition of mycorrhizal root exudates. Pulse–chase labeling experiments with $^{13}CO_2$ revealed that plant-assimilated carbon is transferred within hours to the fungus and can be traced back in specific mycorrhizosphere bacteria a few days later [47]. Exactly which AMF-induced changes in mycorrhizal root exudate chemistry shape the bacterial composition of the (mycor)rhizosphere remains difficult to predict on the basis of correlative studies and *in vitro* chemotaxis assays, but it is likely that a combination of primary and secondary metabolites is involved. In non-mycorrhizal *Arabidopsis*, mutation in the malate transporter gene *ALMT1* affects recruitment of ISR-eliciting *Bacillus subtilis* FB17 after treatment of the leaves with MAMPs [48], indicating that

Box 2. Strigolactones: multipurpose rhizosphere signals

The strigolactones are a group of plant sesquiterpenes that are exuded from roots [86]. They serve as signals to induce hyphal branching of mycorrhizal fungi, leading to enhanced root colonisation by AMF [87,88]. However, 40 years ago, the same strigolactones were first identified as germination stimulants of parasitic plants from the Orobanchaceae family and the obligate root hemiparasitic plant *Striga hermonthica* (giant witchweed) [89], the first of these, strigol, being isolated from non-host cotton (*Gossypium hirsutum*) [89]. These observations generated a conundrum: why would plant roots produce signals to promote parasitic plant infection? Despite this unresolved issue, research continued to focus on understanding the biosynthesis of strigolactones and the diversity of Orobanchaceae species, for which they are able to induce germination. Consequently, until recently little was known about their additional beneficial role in the rhizosphere, despite the fact that non-hosts for parasitic Orobanchaceae produce strigolactones profusely. The breakthrough came from the observation that the synthetic strigolactone GR24 induces hyphal branching in *in vitro* cultures of the AMF *Gigaspora margarita* [87] and *Gigaspora rosea* [88]. Strigolactone-induced hyphal branching is thought to increase the probability of cellular contact between plant and fungus and, consequently, enhance root colonisation by AMF. The discovery of strigolactones as plant stimulants of mycorrhization demonstrates that parasitic plants have 'hijacked' an important host–symbiont signalling mechanism that predates their evolution by at least 200–300 million years [2,3]. To date, it remains unclear whether there are additional signals involved in the recruitment of AMF to plant roots.

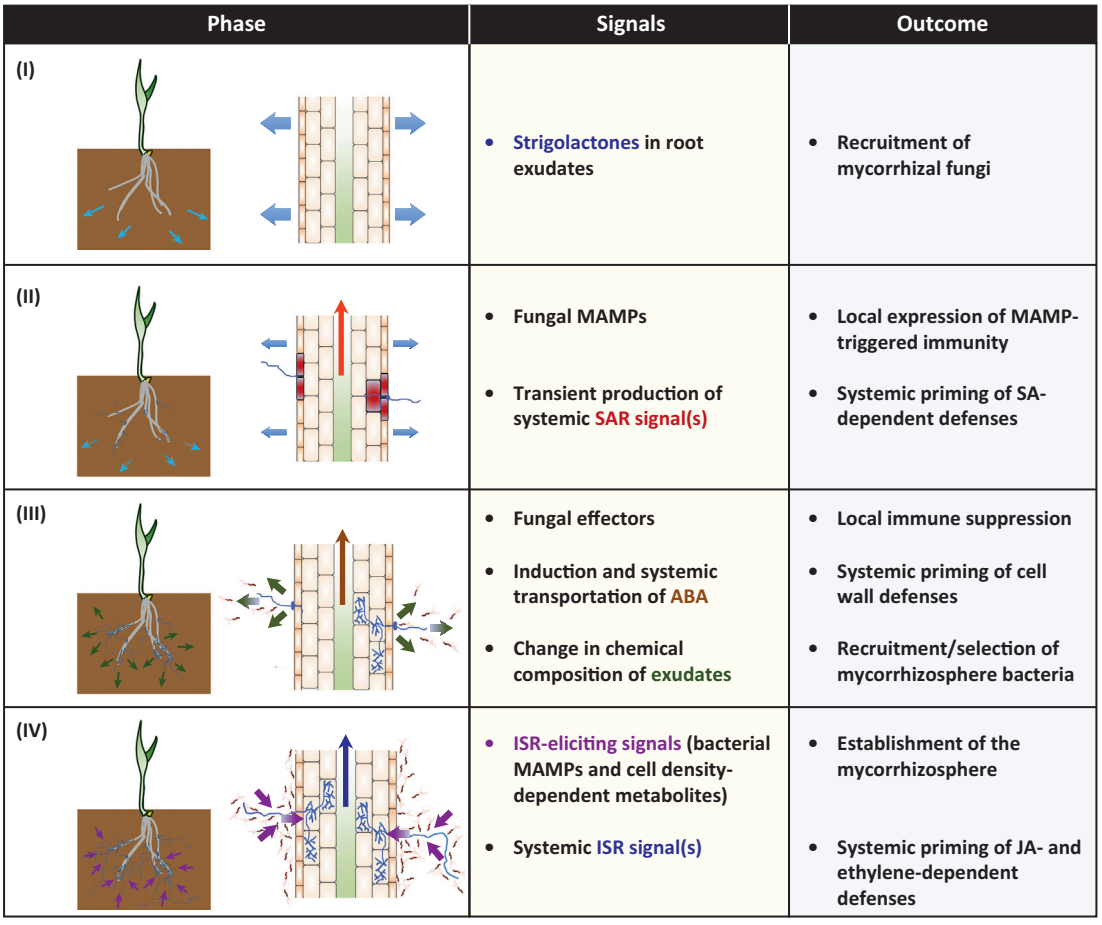

Figure 1. Spatiotemporal model of mycorrhiza-induced resistance (MIR). Phase I: Root exudation of strigolactones (blue arrows) induces hyphal branching in arbuscular mycorrhizal fungi (AMF) and stimulates infection. Phase II: AMF initiate infection of the root cortex. Microbe-associated molecular patterns (MAMPs) from the fungus are recognised by the plant innate immune system. This leads to transient expression of MAMP-triggered immunity (red cells) and generation of long-distance signals in the vascular tissues (red arrow), which induce long-lasting priming of salicylic acid (SA)-dependent defences and systemic acquired resistance (SAR). Phase III: AMF employ specific effector molecules and stimulate production of abscisic acid (ABA) to suppress MAMP-triggered immunity locally. ABA can be transported through the xylem to the shoot (brown arrow), where it can prime cell wall defences. Formation of intracellular arbuscules increases sugar import from the shoot and delivers phosphorous and other nutrients from the soil, thereby altering root metabolism and exudates. Moreover, metabolically active hyphae can alter the chemical composition of root exudates. The combined impact of plant immune modulation, enhanced sugar allocation, increased nutrient uptake, and fungal modification of root exudates leads to changes in root exudation chemistry (green arrows) and recruitment/selection of specific mycorrhizosphere bacteria. Phase IV: Establishment of the mycorrhizosphere is associated with dense colonisation by selected bacteria that metabolise mycorrhizal root exudates and deliver ISR-eliciting signals at the root surface and/or fungal hyphae (purple arrows). After perception of these signals by the host plant, long-distance signals (blue arrow) are generated that prime jasmonate- and ethylene-dependent plant defences and cause induced systemic resistance (ISR).

a single primary metabolite can be critical for recruitment of a specific rhizobacterial strain. There is also evidence for bacterial attraction by more complex secondary metabolites. Recently, it was found that mutation of the benzoxazinoid biosynthesis pathway in maize reduces attraction of ISR-eliciting *P. putida* KT2440 [18,49]. Interestingly, two additional studies have reported that infection of maize by the AMF *F. mosseae* or *R. irregularis* boosts production of root benzoxazinoids [50,51]. It is therefore tempting to speculate that AMF-induced exudation of a blend of benzoxazinoids contributes to cereal mycorrhizosphere development.

Phase IV: establishment of the mycorrhizosphere and induction of systemic resistance by mycorrhizosphere bacteria

Most MIR studies have quantified the level of resistance when the plant–AMF symbiosis and the mycorrhizosphere are fully established [9]. It is therefore possible that MIR involves an ISR component elicited by bacteria in the mycorrhizosphere (Figure 1). Like AMF, rhizobacteria possess MAMPs, which can trigger MAMP-induced immune responses [52]. Well-known examples of defence-eliciting MAMPs from bacteria are rhamnolipids, the elongation factor Tu, flagellin, and cell-wall lipopolysaccharides [53]. The spatially confined structure of the mycorrhizosphere allows rhizobacterial strains to reach exceptionally high cell densities [5]. Under these conditions, bacterial gene expression can be controlled by small diffusible signal molecules from members of the population themselves. This autoinduction process, known as quorum sensing (QS), allows bacteria to adjust community gene expression in accordance with their environment [54]. Many rhizosphere-colonizing bacteria, including *Pseudomonas* and *Burkholderia* strains, employ QS to control gene expression [55]. Some QS autoinducer molecules, like *N*-3-oxo-tetradecanoyl-*L*-homoserine lactone, can elicit

resistance in *Arabidopsis* to *Pseudomonas syringae* and *Golovinomyces orontii* and in barley (*Hordeum vulgare*) to *Blumeria graminis* f. sp. *hordei* [56]. In addition to direct effects by autoinducer molecules, cell density-controlled processes in bacteria can also contribute to disease suppression. The PhzI/PhzR QS system in *Pseudomonas chlororaphis* mediates synthesis of the heterocyclic fungicide phenazine [57], whereas 2,4-diacetylphloroglucinol (2,4-DAPG) produced by *Pseudomonas fluorescens* has autoinducer activity that can be counteracted by metabolites from other soil microbes [58]. Interestingly, both phenazine and 2,4-DAPG have been associated with ISR; expression of *P. chlororaphis*-mediated ISR in tobacco against necrotrophic *Erwinia carotovora* requires production of phenazine [59], whereas production of 2,4-DAPG by *P. fluorescens* is critical for JA-dependent ISR in *Arabidopsis* [60]. Considering that some bacterial strains reach sufficiently high cell densities in the mycorrhizosphere for autoinduction processes, it is plausible that cell density-dependent bacterial metabolites, like phenazine and 2,4-DAPG, contribute to MIR (Figure 1). Delivery of these ISR-eliciting determinants can be directly on the root surface, but can also be facilitated by efficient transportation into the root cortex through mycorrhizal hyphae [61]. Hence, the potential for mycorrhizosphere bacteria to elicit ISR is not only determined by their presence, but also depends on their metabolic activity in relation to chemical signals from mycorrhizal root exudates, their cell density, and the presence of competing microbes.

The nature of the systemic signals controlling rhizobacterial ISR is unknown. As for pathogen-induced SAR [62], ISR may be controlled by a combination of long-distance signals. Both ISR and MIR have frequently been associated with systemic priming of JA- and ethylene-inducible defences [9,63,64]. Jasmonates also accumulate during mycorrhizal symbiosis [13,33]. It is thus possible that jasmonates function as complementary long-distance signals of MIR, which may be the result of systemic signalling processes similar to autoregulation of nodulation during rhizobia–legume interactions [65]. Although the exact contribution of jasmonates to MIR has yet to be demonstrated, we propose that priming of JA-dependent defences during MIR is partially determined by ISR-eliciting rhizobacteria in the mycorrhizosphere (Figure 1).

Concluding remarks and future research

The concept that MIR is partially determined by resistance-inducing bacteria in the mycorrhizosphere creates a novel impetus to explore the complexity of biotic interactions and chemical signals surrounding mycorrhizal roots. The relative contribution of AMF and mycorrhizosphere-inhabiting bacteria to MIR requires experimental validation. To our knowledge, only one study has demonstrated MIR under strictly axenic conditions [66]. The induced resistance in this study was associated with augmented induction of two SA-inducible *PR* genes following infection by *Phytophthora infestans*. Conversely, no clear transcriptional priming was evident for JA- and ethylene-dependent genes in this study [66], suggesting that axenic conditions prevent JA-dependent MIR. Whether priming of JA-dependent defences is strictly dependent on mycorrhizosphere-inhabiting bacteria would require complementation experiments with bacteria under axenic conditions. It is, however, possible that some ISR-eliciting mycorrhizosphere PGPR are not culturable and thrive only in close proximity to AMF hyphae [67]. It is even possible that endobacteria inside the AMF hyphae contribute to MIR [68]. A global inventory of microbial diversity through 16S RNA gene sequence analysis, coupled to temporal profiling of metabolites in mycorrhizal root exudates would be an alternative strategy to decipher the contribution of mycorrhizosphere bacteria in MIR. Involvement of candidate plant metabolites as regulators of resistance-inducing activities by mycorrhizosphere bacteria can be verified by genetic manipulation of the corresponding biosynthetic pathways in the host plant.

Further research is also required to elucidate the nature of systemic MIR signals. To determine whether selected plant hormones, such as ABA or jasmonates, act as long-distance signals in MIR would require grafting experiments with hormone-deficient plants. Unfortunately, interpretation of such experiments can be challenging, considering that hormone-deficient or -insensitive plant genotypes often develop stress phenotypes that can affect mycorrhizal symbiosis and complicate the interpretation of delicate plant-pathogen assays. In addition to plant hormones, small RNA molecules (sRNAs) are attractive candidates for long-distance defence signals. These 20–25-nucleotide RNAs can act as phloem-mobile long-distance signals [69,70]. Moreover, small interfering (si)RNAs can induce transcriptional gene silencing through RNA-directed DNA methylation (RdDM) [71], a pathway that was recently implicated in transgenerational priming of JA- and SA-dependent plant defence after exposure to herbivory and bacterial speck disease, respectively [72,73]. Global analysis of AMF-induced sRNAs and systemic changes in DNA methylation is needed to provide evidence for a possible contribution of sRNAs in the long-distance regulation of MIR. Finally, more comparative studies on the signalling mechanisms regulating MIR and ISR will be necessary to reveal the exact contribution of rhizobacteria to MIR.

Acknowledgements

J.T.'s research activities are supported by a grant from the European Research Council (ERC; no. 309944; '*PRIME-A-PLANT*') and a Research Leadership Award from the Leverhulme Trust (no. RL-2012-042). D.D.C. is supported by a Royal Society University Research Fellowship. Rothamsted Research receives grant aid from the Biotechnology and Biological Science Research Council of the U.K. S.C.M.W.'s research is supported by the Dutch Technology Foundation STW (VIDI grant no. 11281), which is part of the Netherlands Organisation of Scientific Research. The authors also thank three anonymous reviewers for their helpful suggestions and constructive feedback on earlier versions of this article.

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