Trends in Plant Science

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Opinion

Non-Mycorrhizal Plants: The Exceptions that Prove the Rule

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The widespread symbiotic interaction between plants and arbuscular mycorrhizal (AM) fungi relies on a complex molecular dialog with reciprocal benefits in terms of nutrition, growth, and protection. Approximately 29% of all vascular plant species do not host AM symbiosis, including major crops. Under certain conditions, however, presumed non-host plants can become colonized by AM fungi and develop rudimentary AM (RAM) phenotypes. Here we zoom in on the mustard family (Brassicaceae), which harbors AM hosts, non-hosts, and presumed non-host species such as *Arabidopsis thaliana*, for which conditional RAM colonization has been described. We advocate that RAM phenotypes and redundant genomic elements of the symbiotic 'toolkit' are missing links that can help to unravel genetic constraints that drive the evolution of symbiotic incompatibility.

Highlights

The interaction between arbuscular mycorrhizal (AM) fungi and plant roots is one of the most widespread symbioses on Earth.

Approximately 29% of all vascular plant species, including the plant model *Arabidopsis thaliana* and major crops such as sugar beet and rapeseed, are considered to be non-hosts. However, under certain conditions some non-host species do develop rudimentary AM (RAM) phenotypes.

The Brassicaceae family harbors non-host, AM, and RAM species. With the increasing genomic information on the presence or absence of symbiotic toolkit genes, this plant family has an important potential to shed new light on the genetic constraints that drive the evolution of symbiotic incompatibility.

Non-mycorrhizal Plants Shed Light on Symbiosis

Approximately 71% of all vascular plant species, including many important agricultural crops, harbor in their roots a multifunctional symbiosis with AM fungi (subphylum Glomeromycotina) [1–3]. The remaining 29% of vascular plant species apparently lost or suppressed during evolution their abilities to host AM symbiosis, although some may host the less frequent ecto-, ericoid, or orchid mycorrhizas [2]. Among plants considered to be non-hosts for the widespread AM fungi are various members of the families Proteaceae, Chenopodiaceae, Caryophyllaceae, and Brassicaceae, including several major agricultural crops and weeds, the plant model *A. thaliana*, and many Brassicaceae species of scientific interest whose genomes were recently sequenced [4–6]. Recent phylogenomic studies [7–9] revived an earlier approach that employs non-host plants as a tool to shed light on symbiotic processes [10]. By postulating that non-host plants have lost orthologs of putative symbiotic genes, these genome-wide comparison studies between AM host and non-host plant species identified numerous candidate genes with potential roles in AM symbiosis [7–9]. The functions of the symbiotic 'toolkit' genes whose orthologs are absent in specific non-host plant genomes (Table 1) have been characterized in detail in the model AM plants *Medicago truncatula*, *Lotus japonicus*, and *Oryza sativa*. They include well-known regulators of key steps of the symbiotic interaction such as presymbiotic dialog, fungal entry into the root, intraradical hyphal proliferation, and arbuscule development and functioning [11]. These key steps, however, are not always entirely absent in presumed non-host plants [5,12–20], opening a new avenue for research and debate with the potential to unravel the genetic constraints that drive the evolution of symbiotic incompatibility and the discovery of parallel molecular mechanisms important for symbiosis in terrestrial plants.

AM Host: To Be or Not to Be?

By definition, the roots of a non-host plant are never colonized by AM fungi [5,21]. However, this definition depends on the evidence of absence, which can lead to misclassification because of insufficient investigation. An example of this is *Buddleja davidii*, a species that was first

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Table 1. Symbiotic 'Toolkit' Genes That Are Absent in Non-host Plant Species According to Delaux *et al*. [8][a]

Symbiotic gene	Affected colonization step	Gene absence phenotype
NFP	Presymbiotic	Reduced number of arbuscules
DMI2	Presymbiotic	Reduced colonization
DMI3	Presymbiotic	No colonization
CASTOR	Presymbiotic	Reduced colonization
IPD3	Presymbiotic	Reduced colonization and no arbuscules
RAM1	Fungal entry into the root	No hyphopodia
RAM2	Fungal entry into the root	No hyphopodia
VAPYRIN	Intraradical hyphal colonization and arbuscule formation	Reduced colonization and no arbuscules
STR	Intraradical hyphal colonization and arbuscule formation	Reduced colonization and stunted arbuscules
STR2	Intraradical hyphal colonization and arbuscule formation	Reduced colonization and stunted arbuscules
PT4	Arbuscule formation	Reduced colonization, reduced phosphate uptake and increased arbuscule degeneration

[a]The colonization steps affected by the symbiotic toolkit genes and the colonization phenotype in transformed model plants are described according to Delaux *et al*. [91].

considered a non-host plant but later appeared to be a true AM host [22]. To strengthen non-host classifications, some authors use taxonomic extrapolation by assuming that if a plant species belongs to a predominantly non-host family it is likely to be a non-host species [23]. However, the non-host status of a species may still be attributed in cases where AM colonization occurs but does not conform to a recognizable functional type [5,23,24]. The criteria used to define functional AM colonization evolved with time [5]. In earlier years, some authors accepted a plant as a mycorrhizal symbiont when either fungal vesicles or arbuscules were observed in roots [25], while the presence of both was required by others [26]. As the arbuscule became widely recognized as the main site for the symbiotic transfer of phosphate (Pi), a range of authors use the presence of arbuscules as a mainstream criterion for a functional AM phenotype [13,15,23,24]. Hence, fungal colonization without arbuscules has been considered symptomatic of a non-host condition without direct empirical evidence for the lack of physiological functionality [13,15,23,24]. However, as we discuss in more detail below, the 'typical' arbuscule is not the only functional symbiotic structure in nature [27] and is also not always absolutely absent from the roots of presumed non-host plants [12,16,18,20,28–30]. Thus, it is difficult to draw a clear line between host and non-host plants, and the definition of the host status deserves further consideration.

In *A. thaliana* the occurrence of arbuscules has not been documented, but its host status is not entirely consensual. While some authors reported *A. thaliana* as a non-host plant [8,15], others reported intraradical hyphal proliferation and vesicle development without arbuscule formation [14] or stated that *A. thaliana* is clearly mycorrhizal [31]. The latter statement has been used to classify *A. thaliana* as a weak or facultative AM plant [2,5,21,32,33], in contrast with recent bioinformatic studies that opted instead for the non-host classification [7–9]. It is unclear whether this inconsistency results from different criteria [23] or from different conditions tested in different studies [8,14,15,31]. What is clear is that this model plant species is not a true AM host [8,14,15,31], making it an interesting candidate for genomic comparisons [7–9]. However, for accurate biological interpretation of *in silico* analyses, the morphological and functional features of

plant–AM fungus interactions in *A. thaliana* require detailed investigation. The same applies to other Brassicaceae species whose genomes were recently sequenced [4], some of which do not have a described host status, or when they have it, it is not always consensual (Figure 1).

Symbiotic Functions and Morphologies

Although between 8% and 33% of species of Brassicaceae were estimated to host AM fungi [15,33,34], it is a matter of debate whether such fungal colonization is functionally relevant

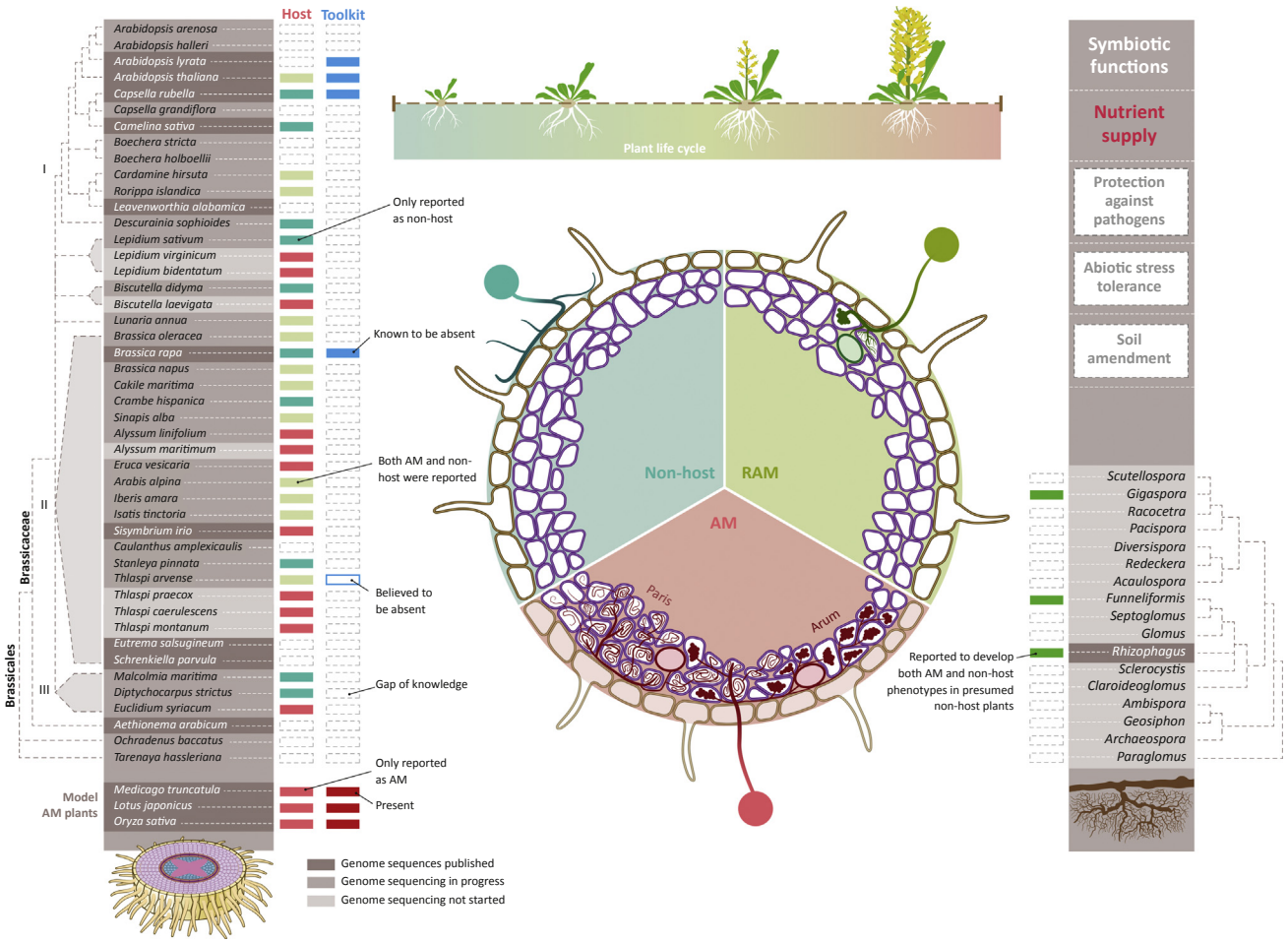

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Figure 1. Interactions between Members of the Brassicaceae Family and Arbuscular Mycorrhizal (AM) Fungi. On the left side, a phylogenetic tree depicts the members of the Brassicaceae family of which genome sequences have been published or are being processed according to Koenig and Weigel [4]. The phylogenetic tree was complemented by a few additional Brassicaceae members for which information on AM host status was available in the literature. The model AM plant species used to describe the required symbiotic toolkit are listed at the bottom. The column 'Host' summarizes information on plant host status compiled from literature cited in this Opinion. The column 'Toolkit' summarizes information on the symbiotic toolkit as documented by Delaux *et al*. [8]. For a detailed list of the toolkit genes that are absent in non-host plant species, see Table 1. In the center, a root transversal cross-section illustrates different host phenotypes: the (AM) host phenotype that accommodates the Paris type of colonization, the Arum type of colonization, or intermediate types of both as described by Dickson *et al*. [27]; the non-host, in which endogenous AM fungal colonization never occurs; and the rudimentary AM (RAM) host as coined in this Opinion to characterize those plant species that do not form prominent AM phenotypes but can harbor a few symbiotic fungal structures. The illustrated plant growth stages presented at the top reflect the potential variation in host ability to form AM symbiosis throughout plant development. On the right, various symbiotic functions of AM fungi are summarized, along with a phylogenetic tree of AM fungal genera according to Krüger *et al*. [90]. The color-coded column associated with this tree summarizes the AM fungal genera for which at least one species was shown to interact with at least one presumed non-host plant species.

[12,15,23,35]. The most-studied function of AM fungi is the supply of soil-derived nutrients to their host plants, in particular Pi. The ability to protect plants against stress caused by drought, high salinity, and pathogens and the ability to improve soil structure around the roots and to reduce nutrient losses due to leaching have also been documented as important functions of AM fungi [36–40]. The classification of the host status, however, is often based on the morphological identification of fungal structures invading the roots, which provides only partial information regarding the various functions of AM fungi.

Currently, two morphological classes of AM colonization are recognized: the Paris type and the Arum type [27]. The Paris type forms intracellular hyphae, coils or arbusculate coils, and vesicles within roots (Figure 1). The Arum type forms intercellular hyphae, the 'typical' arbuscules, and vesicles (Figure 1). Both morphological types depend on the plant and fungus species combination [27]. With the exception of AM-responsive Pi transporter (*PT*) genes [41,42], most symbiotic plant genes have been characterized in the Arum-type colonization [43–52], although the Paris type is thought to be more frequent in nature [27]. This means that one of the two types of AM colonization has not been thoroughly investigated at the molecular level. The localized expression of plant *PT* genes in root cells colonized by arbuscules, coils, or arbusculate coils [41,46,53] provides undisputable evidence that these fungal structures play a pivotal role in the symbiotic function of fungal delivery of Pi to the roots. Likewise, the localized expression of ammonium transporter genes suggests that the arbuscules play a key role in fungal supply of ammonium to the plant [52,54]. Such detailed investigations, however, have not been made for other mineral nutrients, although the external AM mycelium has the capacity to take up and deliver potassium, calcium, sulfur, cupper, and zinc to the host plant [36,55]. It remains to be determined whether the endogenous translocation of these mineral nutrients requires the formation of arbuscules, coils, or arbusculate coils or whether intercellular and intracellular hyphae can also provide a portion of mineral nutrients to the plants. For instance, a *Glomus* sp. isolate (BEG21) did not form arbuscules or vesicles but could still have beneficial effects on a host plant [56]. Moreover, Pi delivery and the extent of AM fungal colonization do not correlate with the AM function of plant protection against pathogen infection [57,58]. Although the absence of arbuscules may imply an absence of Pi or ammonium delivery, the same link has not been clearly established for other fungal symbiotic functions that are also important for ecosystem functioning [1,36,59]. Thus, the occurrence of AM fungal colonization in presumed non-host plants, as observed in various species of Brassicaceae [12–16,18,20,31,60–63], should ideally be characterized in the light of the diversity of functions of AM fungi to value the significance of these plant–AM fungus interactions.

RAM Phenotypes in Non-host Plants

If the presence of arbuscules, coils, or arbusculate coils is the criterion for a symbiotic phenotype [5,23], several species of Chenopodiaceae, Carophyllaceae, and Brassicaceae could still be classified as AM hosts [12,16,18,20,28,60,62–64]. In *Brassica napus*, for instance, a low frequency of arbuscule formation was detected and the invaded root cells had apparently functional nuclei and cytoplasm [12]. As the presence of arbuscules were also reported to occur in *Brassica oleracea* [60], it was proposed that this genus can host AM fungi [12]. However, the rate of fungal development in *B. napus* was threefold lower than that of the positive control AM plant *Trifolium subterraneum*, and it was suggested that future experiments should be run over a long time period to understand whether the maximum proliferation of AM fungi is also lower [12].

An advantage of controlled experiments that use positive controls is that when the tested plants are found uncolonized, the controls allow rejection of the hypothesis that the plants were not in

contact with a viable inoculum [5]. However, most controlled experiments start with young seedlings that grow for 5 to a maximum of 10 weeks [8,12–15,31,60] and have the drawback of not allowing falsification of the hypothesis that plants are susceptible to colonization but were examined at wrong time of the year or stage of plant development [5]. In Brassicaceae this hypothesis was verified in the cases of *Biscutella laevigata* and *Thlapsi praecox* [18,29]. Orłowska *et al*. [18] documented that roots of *B. laevigata* collected from two different sites at the time of flowering harbored AM fungal hyphae, vesicles, and well-developed arbuscules. However, no arbuscules were detected in other periods of the year [18]. In *T. praecox*, symbiosis was observed under controlled conditions in 7-month-old plants during the reproductive stage after 4 months of vernalization but not before vernalization [16,29]. This growth period was longer than the periods used in studies proposing that species related to *T. praecox* cannot develop an AM phenotype [8,13,15,60], which emphasizes the importance of examining colonization at the right time of the year and stage of plant development [5]. The lifespan of the plant might also be an important factor, as became apparent by the examination of field-collected samples of several species of the *Thlapsi* genus of which only the perennial species were distinctly colonized [16].

Also, the context in which an experiment is performed is important. For instance, Veiga *et al*. [14] showed that the hyphae of an AM fungus can colonize the roots of *A. thaliana* and form vesicles when the AM fungus is nursed by neighboring AM host plants. This system simulates the natural process in which early plant growth occurs in the presence of a pre-established AM mycelium [65]. When exposed to an AM host-supported mycorrhizal network, the formation of arbuscule-like structures can occasionally be observed inside the *A. thaliana* roots, albeit at a very low frequency (Figure 2). The roots of the non-host plant *Dianthus deltoides* (Caryophyllaceae) collected in the field were also colonized by AM hyphae and vesicles, and more rarely arbuscules were detected in the colonized roots [64]. Although the arbuscules in specific non-host plants are rarely observed [12,16,28,64], their detection indicates that development is not fully blocked. In the non-host plant *Salsola kali* (Chenopodiaceae), the arbuscule lifespan was relatively shorter than that of the positive control AM plant *Agropyron dasystachyum* [28]. Therefore, a factor that potentially contributes to the rare detection of arbuscules in non-host plants could be rapid arbuscule degeneration. This raises a fundamental question: is it correct to classify these plants as non-hosts? An alternative classification would be RAM for plant species that suppress or have lost their ability to form prominent AM phenotypes but under specific circumstances can harbor symbiotic structures in their roots and might therefore have sufficient genetic tools to activate components of the symbiotic behavior of AM fungi. Considering that current non-host species evolved from ancestral AM host plants [2,66], the proposed RAM phenotype would be analogous to a rudimentary organ.

Non-host Plants and the Obligate Biotrophic Behavior of AM Fungi

AM fungi have no significant saprotrophic abilities and their capacity to produce a mycelium without a compatible symbiosis depends on limited reserves of energy, mostly from spores and vesicles [55]. The completion of their obligate biotrophic life cycle relies on photosynthates (sugars and fatty acids) supplied by a nurturing autotrophic host [55,67–69]. Therefore, from a fungal perspective, choosing the right partner is crucial for their survival in nature [70]. During the pre-contact stage (i.e., during spore germination and extraradical hyphal growth), the interaction with the plant starts with reciprocal exchange of diffusible signals before the symbiotic partners engage in physical contact [71]. Host roots release strigolactones, which are signal molecules that are perceived by the fungal partner and subsequently induce extensive hyphal branching in the AM fungus [72]. However, there are no obvious indications that AM fungi can recognize unambiguously the roots of non-host plants

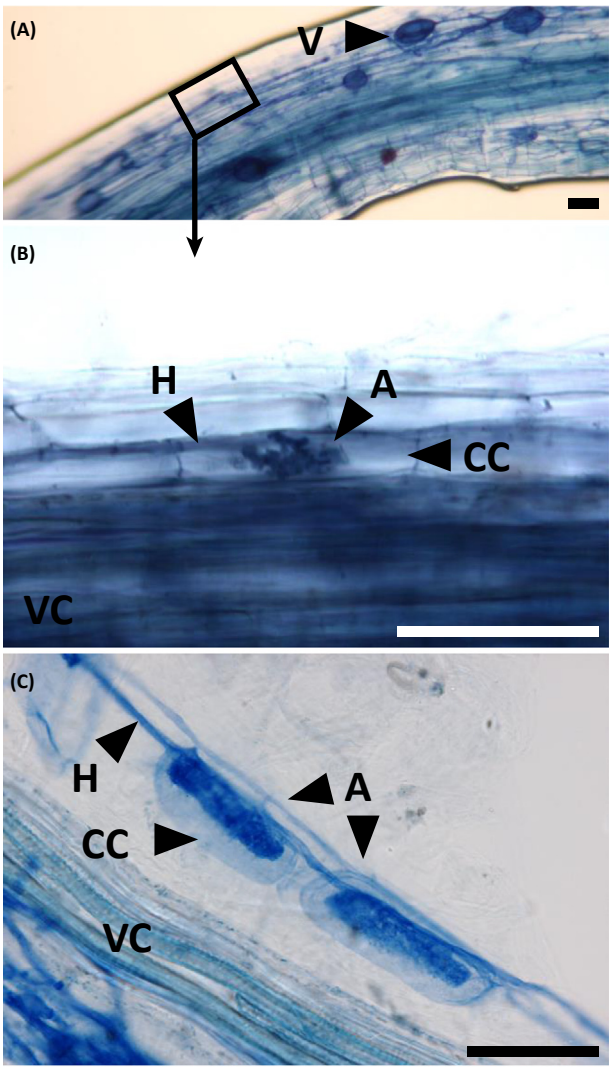

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Figure 2. Arbuscules of *Rhizophagus irregularis* in Roots of *Arabidopsis thaliana*. The arbuscular mycorrhizal (AM) fungus *R. irregularis* colonizes the roots of *A. thaliana* when the mycorrhizal network is nursed by an AM host plant [14]. In colonized *A. thaliana* roots, AM fungal vesicles (V), intercellular hyphae (H), and, on very rare occasions, arbuscule-like structures (A) can be observed. (B) is a magnified region of the root fragment shown in (A) and shows arbuscule-like structures filling a cortex cell (CC) above the vascular cylinder (VC). (C) shows a root fragment whose structure was squeezed to facilitate the observation of two arbuscule-like structures located in separate cortex cells above the vascular cylinder. Bars, 50 μm.

[5,19]. AM fungal discrimination between AM host and non-host roots becomes more obvious with the initial attempts of fungal entry into the roots [19]. When interacting with non-host plants, the AM fungi display an active response comprising the abortion of fungal penetration pegs, the redevelopment of vegetative tips in hyphopodia already attached to the roots [12], septation of hyphae on and in the roots indicating retraction of fungal cytoplasm and senescence [13], and the development of runner hyphae heading away from lignified roots to reach out for other root sections [28]. How AM fungi evaluate the AM host/non-host status of roots is largely unknown.

Despite the apparent incompatibility, some AM fungi may colonize the roots of non-host plants, particularly when they are part of a mycorrhizal network that is supported by neighboring AM host plants. This interaction with host-supported AM fungi often reduces growth of the colonized non-host plant [14,60,64,73,74]. It is unclear whether and how AM fungi benefit from this interaction and which mechanisms are employed. In some cases, the intraradical AM fungal hyphae might absorb photosynthates from non-host plants, and even enough to complete the fungal life cycle [64,75]. In other situations, the AM fungi may benefit indirectly by increasing the competitive advantage of more compatible host plants growing in the same community [65,74]. Whether this fungal manipulation of plant communities results from nutrient reallocation between rhizospheres or from fungal allelochemicals liberated into the soil remains unproven [65,74,76]. Alternatively, it has been speculated that AM fungi switch their beneficial behavior to pathogenic during the infection of non-host plants [6].

Plant Genetics of Symbiotic Incompatibility

Until recently, mechanisms of AM incompatibility have been debated mostly at the plant physiological level [5,19]. Delaux *et al*. [8] provided the pioneer step towards an evolutionary genetics explanation. The current hypothesis proposes that independent non-host plant lineages lost their symbiotic capacity due to convergent deletions of the orthologs of important symbiotic genes (Table 1) [7–9]. It remains a matter of debate which symbiotic genes are deleted in specific non-host plants [7]. Moreover, it remains to be determined whether the complementation of non-host plants with symbiotic genes can restore AM symbiosis to these plants, which would provide decisive evidence to further test the current hypothesis. Such complementation will contribute not only to testing a theoretical principle but also to the development of biotechnological solutions to implement microbial symbioses in major crop plants.

The Brassicaceae family is a remarkable model in this context due to the large genetic toolbox of its member *A. thaliana* and its fast growing number of species with sequenced genomes [4]. It was proposed that the entire family lost its symbiotic capacity due to deletions of symbiotic orthologs that occurred in the order Brassicales before the divergence of Limnanthaceae [8]. Because this hypothesis is based on transcriptome data, whole-genome data from additional Brassicaceae genomes (Figure 1) should generate additional evidence for this notion. Furthermore, although the Brassicaceae family is generally considered as non-host [23], various species of Brassicaceae were reported to host AM fungi [12–16,18,20,31,63]. There are at least two possible explanations for this inconsistency: (i) the deletions of symbiotic genes did not occur at the phylogenetic position proposed [8], but instead there were multiple independent deletions along the phylogeny of Brassicales; or (ii) there could be other, partially redundant mechanisms supporting levels of AM fungal colonization. The later hypothesis seems consistent with the evolutionary theory of AM abandonment [77], which proposes that the evolution of non-host states in plants occurred most often via transitory states; that is, via the weakening of AM symbiosis as a necessary precursor for abandonment of mutualism by plants. The Brassicaceae family offers excellent opportunities to unravel the genetic constraints leading to symbiotic weakening. Non-host plant species specialized in severely impoverished soil fertilities (e.g., the Proteaceae type as defined by Lambers *et al*. [6]) may have evolved under different evolutionary pressures and model genomes of this non-host type are needed to perform comparative genomics. Moreover, it has been recently estimated that 30–50 different separate evolutionary origins of non-host plants have occurred during plant evolution [24]. The genetic basis underlying all of these separate origins has not been thoroughly investigated so far due to the lack of genomic information.

It should be noted that the importance of specific symbiotic genes in AM development might depend on growth conditions and functional redundancy. For instance, the Pi transporter gene *PT4* was once considered indispensable for AM symbiosis based on evidence showing that, under reduced Pi availability, the *M. truncatula* mutant *pt4* accelerated arbuscule degeneration and discouraged fungal colonization [78]. However, this gene was later shown to become redundant for AM development in *pt4* under nitrogen deficiency due to an alternative symbiotic recruitment by the ammonium transporter *AMT2;3*, which arrests arbuscule degeneration and stimulates fungal colonization [79]. Additionally, the systemic expression of the *PT4* ortholog in fine lateral roots of *O. sativa*, which do not host AM fungi, suggested that this gene might not be specific for the arbuscule interface [80]. Moreover, the plant species *Moringa oleifera* (Moringaceae) apparently lacks *PT4* [8] but can host AM symbiosis and benefit from Pi uptake [81,82]. The fungal endophyte Helotiales sp. F229 (Ascomycota) can also increase Pi uptake in *Arabis alpina* [83], although this Brassicaceae species presumably lacks *PT4* [8]. Together this implies the existence of alternative pathways for fungal contribution to plant Pi nutrition. For instance, *Solanum tuberosum* PT3 was suggested to regulate Pi acquisition in root cells harboring thick-coiled AM fungal hyphae [41]. Furthermore, non-orthologs of symbiotic genes may potentially replace the function of symbiotic genes. For instance, *A. thaliana* lacks an ortholog of the glycerol 3-phosphate acyl transferase (GPAT) gene *REDUCED ARBUSCULAR MYCORRHIZATION2* (*RAM2*) [8]. However, the complementation of *M. truncatula* mutant *ram2* with *GPAT* genes of *A. thaliana* promoted AM colonization of *ram2* [45], indicating that the non-orthologous *GPAT* genes can take over the function of *RAM2*. Moreover, plant mutants defective in the gene *NFP*, which encodes a receptor of a presymbiotic signal released by an AM fungus, can host AM symbiosis [84]. Therefore, it is assumed that additional receptors are likely to exist [84]. How many plant receptors are involved in the presymbiotic signaling recognition of all AM fungi is unknown [84].

Other conserved molecular components in plants could operate in parallel with the symbiotic toolkit to affect the degree of compatibility or incompatibility, which might help to explain the occurrence of RAM phenotypes. Such components could be involved in phytohormone pathways [85,86], encode elements of MAMP signal perception and transduction [87], or represent targets for one of the more than 200 AM fungal effectors that have been recently identified [88,89]. While much research has been done in recent years to characterize the mechanisms of AM compatibility, almost no attention was given to unraveling the molecular constrains causing incompatibility. However, an encouraging number of powerful research tools are now available to generate new lines of evidence, test complementary and alternative hypotheses, and substantiate the debate on the causes of symbiotic incompatibility.

Concluding Remarks and Future Directions

Although relatively less frequent in nature, many non-host plant species are major agricultural crops and important model organisms. Their whole-genome sequences have emerged as valuable tools to shed light on the molecular mechanisms governing AM symbiosis. A recurring theme in non-host plant species is that they may become colonized by the AM fungi and develop RAM phenotypes. Comprehensive knowledge on the conditions governing non-host status across the full plant life cycle is paramount for accurate biological and functional interpretation of concomitant genomic information. The function of weaker AM fungal colonization should be characterized by direct physiological assessment considering not only the function of Pi delivery but also other well-established functions of AM fungi (i.e., uptake of other mineral nutrients, protection against pathogens, enhanced abiotic stress tolerance, soil amendment). Although often perceived as generalists, AM fungi develop strong genotype-specific interactions with plants, and the fungal side during incompatible interactions offers

exciting directions for future research. The Brassicaceae arises as a resourceful model family to unravel the genetic constraints driving the evolution of symbiotic incompatibility as well as to uncover partially redundant mechanisms controlling symbiosis. Detailed information on molecular mechanisms modulating AM symbiosis will contribute not only to the design of future crops that produce more with less agrochemical input but also to advancing our knowledge on the biological factors that delimit symbiosis in terrestrial plants (see Outstanding Questions).

Outstanding Questions

Do non-host and RAM plant phenotypes depend on environmental conditions and/or plant developmental stage?

What are the ecological functions of RAM phenotypes?

Is fungal genotype an important factor during the interaction between AM fungi and non-host or RAM plants?

Do AM fungi perceive the incompatibility of the roots and how?

How much and which photosynthates (sugars and/or lipids) can AM fungi absorb from RAM plants?

Do RAM phenotypes allow AM fungi to complete their life cycle?

What mechanisms do AM fungi employ to antagonize non-host plants growing in a community with AM plants?

What are the molecular constrains responsible for evolutionary weakening of AM symbiosis in land plants?

What is the role of phytohormone pathways, MAMPs, and the plant targets of AM fungal effectors in modulating the degree of incompatibility with AM fungi?

Do molecular pathways newly acquired by non-host or RAM plants influence the degree of symbiotic incompatibility?

What are the advantages and disadvantages of losing/suppressing AM symbiosis?

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References

1. Van der Heijden, M.G.A. *et al.* (2015) Mycorrhizal ecology and evolution: the past, the present, and the future. *New Phytol.* 205, 1406–1423
2. Wang, B. and Qiu, Y.L. (2006) Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza* 16, 299–363
3. Spatafora, J.W. *et al.* (2016) A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data. *Mycologia* 108, 1028–1046
4. Koenig, D. and Weigel, D. (2015) Beyond the thale: comparative genomics and genetics of *Arabidopsis* relatives. *Nat. Rev. Genet.* 16, 285–298
5. Tester, M. *et al.* (1987) The phenomenon of "nonmycorrhizal" plants. *Can. J. Bot.* 65, 419–431
6. Lambers, H. and Teste, F.P. (2013) Interactions between arbuscular mycorrhizal and non-mycorrhizal plants: do non-mycorrhizal species at both extremes of nutrient availability play the same game? *Plant Cell Environ.* 36, 1911–1915
7. Bravo, A. *et al.* (2016) Genes conserved for arbuscular mycorrhizal symbiosis identified through phylogenomics. *Nat. Plants* 2, 15208
8. Delaux, P.-M. *et al.* (2014) Comparative phylogenomics uncovers the impact of symbiotic associations on host genome evolution. *PLoS Genet.* 10, e1004487
9. Favre, P. *et al.* (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, 1–20
10. Beveged, I. and Bowen, G.D. *et al.* (1975) *Endogone* strain and host plant differences in development of vesicular–arbuscular mycorrhizas. In *Endomycorrhizas* (Sanders, F.E., ed.), pp. 77–86, Academic Press
11. Gutjahr, C. and Parniske, M. (2013) Cell and developmental biology of arbuscular mycorrhiza symbiosis. *Annu. Rev. Cell Dev. Biol.* 29, 593–617
12. Tommerup, I.C. (1984) Development of infection by a vesicular–arbuscular mycorrhizal fungus in *Brassica napus* L. and *Trifolium subterraneum* L. *New Phytol.* 98, 487–495
13. Glenn, M.G. *et al.* (1985) Hyphal penetration of *Brassica* (Cruciferae) roots by a vesicular–arbuscular mycorrhizal fungus. *New Phytol.* 99, 463–472
14. Veiga, R.S.L. *et al.* (2013) Arbuscular mycorrhizal fungi reduce growth and infect roots of the non-host plant *Arabidopsis thaliana*. *Plant Cell Environ.* 36, 1926–1937
15. DeMars, B.G. and Boerner, R.E.J. (1996) Vesicular arbuscular mycorrhizal development in the Brassicaceae in relation to plant life span. *Flora* 191, 179–189
16. Regvar, M. *et al.* (2003) Colonization of pennycresses (*Thlaspi* spp.) of the Brassicaceae by arbuscular mycorrhizal fungi. *J. Plant Physiol.* 160, 615–626
17. Pongrac, P. *et al.* (2007) Changes in elemental uptake and arbuscular mycorrhizal colonisation during the life cycle of *Thlaspi praecox* Wulfen. *Chemosphere* 69, 1602–1609
18. Orłowska, E. *et al.* (2002) Influence of restoration on arbuscular mycorrhiza of *Biscutella laevigata* L. (Brassicaceae) and *Plantago lanceolata* L. (Plantaginaceae) from calamine spoil mounds. *Mycorrhiza* 12, 153–159
19. Giovannetti, M. and Sbrana, C. (1998) Meeting a non-host: the behaviour of AM fungi. *Mycorrhiza* 8, 123–130
20. Peterson, R. *et al.* (1985) Vesicular–arbuscular mycorrhizal associations of vascular plants on Heron Island, a Great Barrier Reef coral cay. *Aust. J. Bot.* 33, 669–676
21. Menzel, A. *et al.* (2017) Mycorrhizal status helps explain invasion success of alien plant species. *Ecology* 98, 92–102
22. Dickie, I.A. *et al.* (2007) On the perils of mycorrhizal status lists: the case of *Buddleja davidii*. *Mycorrhiza* 17, 687
23. Brundrett, M.C. (2009) Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. *Plant Soil* 320, 37–77
24. Brundrett, M.C. and Tedersoo, L. (2018) Evolutionary history of mycorrhizal symbioses and global host plant diversity. *New Phytol.* Published online January 22, 2018. http://dx.doi.org/10.1111/nph.14976
25. Reeves, F.B. *et al.* (1979) The role of endomycorrhizae in revegetation practices in the semi-arid West. I. A comparison of incidence of mycorrhizae in severely disturbed vs. natural environments. *Am. J. Bot.* 66, 6–13
26. Iqbal, S.H. and Qureshi, K.S. (1976) The influence of mixed sowing (cereals and crucifers) and crop rotation on the development of mycorrhiza and subsequent growth of crops under field conditions. *Biologia (Lahore)* 22, 287–298
27. Dickson, S. (2004) The Arum–Paris continuum of mycorrhizal symbioses. *New Phytol.* 163, 187–200
28. Allen, M.F. *et al.* (1989) Responses of the non-mycotrophic plant *Salsola kali* to invasion by vesicular–arbuscular mycorrhizal fungi. *New Phytol.* 111, 45–49
29. Vogel-Mikuš, K. *et al.* (2006) Colonisation of a Zn, Cd and Pb hyperaccumulator *Thlaspi praecox* Wulfen with indigenous arbuscular mycorrhizal fungal mixture induces changes in heavy metal and nutrient uptake. *Environ. Pollut.* 139, 362–371
30. Ross, J.P. and Harper, J.A. (1973) Hosts of a vesicular–arbuscular *Endogone* species. *J. Elisha Mitchell Sci. Soc.* 89, 1–3
31. Kruckelmann, W. *et al.* (1975) Effects of fertilizers, soils, soil tillage, and plant species on the frequency of *Endogone* chlamydospores and mycorrhizal infection in arable soils. In *Endomycorrhizas* (Sanders, F.E., ed.), pp. 511–525, Academic Press
32. Hempel, S. *et al.* (2013) Mycorrhizas in the Central European flora: relationships with plant life history traits and ecology. *Ecology* 94, 1389–1399

33. Harley, J.L. and Harley, E.L. (1987) A check-list of mycorrhiza in the British flora. *New Phytol.* 105, 1–102
34. Newman, E.I. and Reddell, P. (1987) The distribution of mycorrhizas among families of vascular plants. *New Phytol.* 106, 745–751
35. Pongrac, P. *et al.* (2013) Arbuscular mycorrhiza in glucosinolate-containing plants: the story of the metal hyperaccumulator *Noccaea* (*Thlaspi*) *praecox* (Brassicaceae). In *Molecular Microbial Ecology of the Rhizosphere*, pp. 1023–1032, John Wiley & Sons
36. Marschner, H. and Dell, B. (1994) Nutrient uptake in mycorrhizal symbiosis. *Plant Soil* 159, 89–102
37. Augé, R.M. (2001) Water relations, drought and vesicular–arbuscular mycorrhizal symbiosis. *Mycorrhiza* 11, 3–42
38. Evelin, H. *et al.* (2009) Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. *Ann. Bot.* 104, 1263–1280
39. Rillig, M.C. and Mummey, D.L. (2006) Mycorrhizas and soil structure. *New Phytol.* 171, 41–53
40. Pozo, M.J. and Azcon-Aguilar, C. (2007) Unraveling mycorrhiza-induced resistance. *Curr. Opin. Plant Biol.* 10, 393–398
41. Karandashov, V. *et al.* (2004) Evolutionary conservation of a phosphate transporter in the arbuscular mycorrhizal symbiosis. *Proc. Natl. Acad. Sci. U. S. A.* 101, 6285–6290
42. Glassop, D. *et al.* (2005) Cereal phosphate transporters associated with the mycorrhizal pathway of phosphate uptake into roots. *Planta* 222, 688–698
43. Zhang, Q. *et al.* (2010) Two *Medicago truncatula* half-ABC transporters are essential for arbuscule development in arbuscular mycorrhizal symbiosis. *Plant Cell* 22, 1483–1497
44. Gobbato, E. *et al.* (2012) A GRAS-type transcription factor with a specific function in mycorrhizal signaling. *Curr. Biol.* 22, 2236–2241
45. Wang, E. *et al.* (2012) A common signaling process that promotes mycorrhizal and oomycete colonization of plants. *Curr. Biol.* 22, 2242–2246
46. Harrison, M.J. *et al.* (2002) A phosphate transporter from *Medicago truncatula* involved in the acquisition of phosphate released by arbuscular mycorrhizal fungi. *Plant Cell* 14, 2413–2429
47. Pumplin, N. *et al.* (2010) *Medicago truncatula* Vapyrin is a novel protein required for arbuscular mycorrhizal symbiosis. *Plant J.* 61, 482–494
48. Catoira, R. *et al.* (2000) Four genes of *Medicago truncatula* controlling components of a Nod factor transduction pathway. *Plant Cell* 12, 1647–1665
49. Bonfante, P. *et al.* (2000) The *Lotus japonicus LjSym4* gene is required for the successful symbiotic infection of root epidermal cells. *Mol. Plant Microbe Interact.* 13, 1109–1120
50. Gutjahr, C. *et al.* (2015) Rice perception of symbiotic arbuscular mycorrhizal fungi requires the karrikin receptor complex. *Science* 350, 1521–1524
51. Guether, M. *et al.* (2009) A mycorrhizal-specific ammonium transporter from *Lotus japonicus* acquires nitrogen released by arbuscular mycorrhizal fungi. *Plant Physiol.* 150, 73–83
52. Koegel, S. *et al.* (2013) The family of ammonium transporters (AMT) in *Sorghum bicolor*: two AMT members are induced locally, but not systemically in roots colonized by arbuscular mycorrhizal fungi. *New Phytol.* 198, 853–865
53. Rausch, C. *et al.* (2001) A phosphate transporter expressed in arbuscule-containing cells in potato. *Nature* 414, 462–470
54. Gaude, N. *et al.* (2012) Arbuscule-containing and non-colonized cortical cells of mycorrhizal roots undergo extensive and specific reprogramming during arbuscular mycorrhizal development. *Plant J.* 69, 510–528
55. Smith, S.E. and Read, D.J. (2008) *Mycorrhizal Symbiosis*. (3rd edn), Academic Press
56. Van der Heijden, M.G.A. *et al.* (1998) Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. *Ecology* 79, 2082–2091
57. Powell, J.R. *et al.* (2009) Phylogenetic trait conservatism and the evolution of functional trade-offs in arbuscular mycorrhizal fungi. *Proc. Biol. Sci.* 276, 4237–4245
58. Fernández, I. *et al.* (2014) Defense related phytohormones regulation in arbuscular mycorrhizal symbioses depends on the partner genotypes. *J. Chem. Ecol.* 40, 791–803
59. Maherali, H. and Klironomos, J.N. (2007) Influence of phylogeny on fungal community assembly and ecosystem functioning. *Science* 316, 1746–1748
60. Hirrel, M.C. *et al.* (1978) Vesicular–arbuscular mycorrhizae in the Chenopodiaceae and Cruciferae: do they occur? *Can. J. Bot.* 56, 2813–2817
61. DeMars, B. and Boerner, R.J. (1995) Arbuscular mycorrhizal development in three crucifers. *Mycorrhiza* 5, 405–408
62. Koske, R.E. *et al.* (1992) Mycorrhizae in Hawaiian angiosperms: a survey with implications for the origin of the native flora. *Am. J. Bot.* 79, 853–862
63. Medve, R.J. (1983) The mycorrhizal status of the Cruciferae. *Am. Midl. Nat.* 109, 406–408
64. Lekberg, Y. *et al.* (2015) The fungal perspective of arbuscular mycorrhizal colonization in 'nonmycorrhizal' plants. *New Phytol.* 205, 1399–1403
65. Francis, R. and Read, D.J. (1994) The contributions of mycorrhizal fungi to the determination of plant community structure. *Plant Soil* 159, 11–25
66. Redecker, D. *et al.* (2000) Glomalean fungi from the Ordovician. *Science* 289, 1920–1921
67. Keymer, A. *et al.* (2017) Lipid transfer from plants to arbuscular mycorrhiza fungi. *Elife* 6, e29107
68. Luginbuehl, L.H. *et al.* (2017) Fatty acids in arbuscular mycorrhizal fungi are synthesized by the host plant. *Science* 356, 1175–1178
69. Bravo, A. *et al.* (2017) Arbuscular mycorrhiza-specific enzymes FatM and RAM2 fine-tune lipid biosynthesis to promote development of arbuscular mycorrhiza. *New Phytol.* 214, 1631–1645
70. Kiers, E.T. *et al.* (2011) Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science* 333, 880–882
71. Bonfante, P. and Genre, A. (2015) Arbuscular mycorrhizal dialogues: do you speak 'plantish' or 'fungish'? *Trends Plant Sci.* 20, 150–154
72. Lopez-Raez, J.A. *et al.* (2011) Strigolactones: a cry for help in the rhizosphere. *Botany* 89, 513–522
73. Ocampo, J.A. *et al.* (1980) Influence of plant interactions on vesicular–arbuscular mycorrhizal infection. I. Host and non-host plants grown together. *New Phytol.* 84, 27–35
74. Ocampo, J.A. (1986) Vesicular–arbuscular mycorrhizal infection of "host" and "non-host" plants: effect on the growth responses of the plants and competition between them. *Soil Biol. Biochem.* 18, 607–610
75. Allen, M.F. and Allen, E.B. (1990) Carbon source of VA mycorrhizal fungi associated with Chenopodiaceae from a semiarid shrub-steppe. *Ecology* 71, 2019–2021
76. Veiga, R.L. *et al.* (2012) No evidence for allelopathic effects of arbuscular mycorrhizal fungi on the non-host plant *Stellaria media*. *Plant Soil* 360, 319–331
77. Maherali, H. *et al.* (2016) Mutualism persistence and abandonment during the evolution of the mycorrhizal symbiosis. *Am. Nat.* 188, E113–E125
78. Javot, H. *et al.* (2007) A *Medicago truncatula* phosphate transporter indispensable for the arbuscular mycorrhizal symbiosis. *Proc. Natl. Acad. Sci. U. S. A.* 104, 1720–1725
79. Breuillin-Sessoms, F. *et al.* (2015) Suppression of arbuscule degeneration in *Medicago truncatula phosphate transporter4* mutants is dependent on the ammonium transporter 2 family protein AMT2;3. *Plant Cell* 27, 1352–1366
80. Fiorilli, V. *et al.* (2015) Host and non-host roots in rice: cellular and molecular approaches reveal differential responses to arbuscular mycorrhizal fungi. *Front. Plant Sci.* 6, 636

81. Cosme, M. *et al.* (2014) Arbuscular mycorrhizal fungi affect glucosinolate and mineral element composition in leaves of *Moringa oleifera*. *Mycorrhiza* 24, 565–570
82. Radovich, T.J.K. and Habte, M. (2009) Arbuscular mycorrhizal dependency of three *Moringa* genotypes. *Hortscience* 44, 1025–1026
83. Almario, J. *et al.* (2017) Root-associated fungal microbiota of nonmycorrhizal *Arabis alpina* and its contribution to plant phosphorus nutrition. *Proc. Natl. Acad. Sci. U. S. A.* 114, E9403–E9412
84. Bonfante, P. and Requena, N. (2011) Dating in the dark: how roots respond to fungal signals to establish arbuscular mycorrhizal symbiosis. *Curr. Opin. Plant Biol.* 14, 451–457
85. Pozo, M.J. *et al.* (2015) Phytohormones as integrators of environmental signals in the regulation of mycorrhizal symbioses. *New Phytol.* 205, 1431–1436
86. Bucher, M. *et al.* (2014) Through the doors of perception to function in arbuscular mycorrhizal symbioses. *New Phytol.* 204, 833–840
87. Pieterse, C.M.J. *et al.* (2014) Induced systemic resistance by beneficial microbes. *Annu. Rev. Phytopathol.* 52, 347–375
88. Sędzielewska Toro, K. and Brachmann, A. (2016) The effector candidate repertoire of the arbuscular mycorrhizal fungus *Rhizophagus clarus*. *BMC Genomics* 17, 101
89. Kloppholz, S. *et al.* (2011) A secreted fungal effector of *Glomus intraradices* promotes symbiotic biotrophy. *Curr. Biol.* 21, 1204–1209
90. Krüger, M. *et al.* (2012) Phylogenetic reference data for systematics and phylotaxonomy of arbuscular mycorrhizal fungi from phylum to species level. *New Phytol.* 193, 970–984
91. Delaux, P.-M. *et al.* (2013) Evolution of the plant–microbe symbiotic 'toolkit'. *Trends Plant Sci.* 18, 298–304