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**Systems biology and "omics" tools: A cooperation for next-generation mycorrhizal studies**

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**Abstract**

Omics tools constitute a powerful means of describing the complexity of plants and soil-borne microorganisms. Next generation sequencing technologies, coupled with emerging systems biology approaches, seem promising to represent a new strategy in the study of plant–microbe interactions. Arbuscular mycorrhizal fungi (AMF) are ubiquitous symbionts of plant roots, that provide their host with many benefits. However, as obligate biotrophs, AMF show a genetic, cellular and physiological complexity that makes the study of their biology as well as their effective agronomical exploitation rather difficult. Here, we speculate that the increasing availability of omics data on mycorrhiza and of computational tools that allow systems biology approaches represents a step forward in the understanding of arbuscular mycorrhizal symbiosis. Furthermore, the application of this study-perspective to agriculturally relevant model plants, such as tomato and rice, will lead to a better in-field exploitation of this beneficial symbiosis in the frame of low-input agriculture.

## 1. An overview of the mycorrhizal world

Beneficial micro-organisms, including soil-borne symbionts, such as N2-fixing bacteria, and arbuscular mycorrhizal fungi (AMF), provide nutrients to plants and are directly involved in crop production. AMF are members of the Glomeromycota phylum [1] and, with a few exceptions, are ubiquitous in all terrestrial soil ecosystems, in which they colonize the roots of 80% of plants, including agricultural crops. They thus form one of the most widespread mutualistic associations in nature: arbuscular mycorrhiza (AM) [2]. As a consequence of a molecular dialogue, the fungus in this symbiosis colonizes the host root cells by developing intercellular hyphae and extensive intracellular branched hyphae called arbuscules [3] (Fig. 1). The fungus in the soil forms massive networks of extraradical hyphae that take up mineral nutrients (mainly phosphate) and water, and transfer them across the symbiotic interface to the root cells of the plant [2,4]. The plant provides the fungus with organic carbon, which has been estimated to represent about 20 percent of plant photoassimilates [5]. The host plants also gain other benefits from AM symbiosis, that is protection from pathogens [6], tolerance to drought [7] and pollutants [8], as well as an improved soil structure [9]. In the context of AMF use in low input farming, recent findings have demonstrated that they influence the uptake of several other nutrients beside phosphorus [10]: N nutrition is found to be significantly improved [11], thanks to mechanisms which include increased mineralization of the soil organic matter, N uptake and translocation to the fungus-plant interface [12], and the induction of mycorrhiza-specific plant N transporters [13]. Furthermore, a growing body of evidence shows that AMF can reduce nutrient loss in soils, and possibly limits the N release in the biosphere caused by chemical fertilization [14]. It is thus not surprising that, over the years, both basic and applied researchers have directed their efforts towards a better understanding of what happens upon mycorrhizal establishment, with the aim of dissecting the response of the plant to fungal colonization and of trying to effectively exploit AMF from an agronomic point of view. The establishment of an AM symbiosis requires the implementation of a specific and fine-tuned cellular reprogramming with the signalling between the two partners playing a fundamental role [3]: the

compounds present in root exudates are the first signals perceived by the fungus in the soil. Identified as strigolactones [15,16], these molecules play multiple roles. They not only elicit the AMF hyphal branching, but also act as plant hormones, influencing shoot and root architecture, and triggering germination of parasitic weeds [17,18]. On the other hand, AMF release diffusible signals known as Myc factors, that have been identified as lipochitoligosaccharide molecules [19] which trigger root colonization and root branching. The perception of the Myc factor by the plant activates responses that are under the control of a characterized signalling pathway, partly overlapping with Rhizobium-legume symbiosis: the so-called common symbiosis (SYM) pathway [20]. Molecular biology studies have been instrumental in elucidating the crosstalk events that lead to mycorrhiza establishment [21–23], as well as in the identification of the molecular basis of the nutrient exchange taking place in mycorrhizal roots [24–26]. High throughput analyses now allow a more detailed examination to be made of the complex molecular modifications that are induced by mycorrhization in plant roots. A consistent set of genes is differentially expressed upon mycorrhiza establishment, and this shows that not only nutrient transport, but also processes such as responses to biotic and abiotic stimuli and plant developmental processes are regulated, suggesting that a profound molecular reprogramming occurs in the mycorrhizal roots (see Table 1 for a summary of the related literature). When these analyses have been extended from the root to the whole plant (Table 1), novel findings have been unravelled, such as the systemic induction of defence-related genes in the shoot and the existence of a core set of genes specifically induced by mycorrhiza formation, regardless of which organ is considered [27]. Taken on the whole, transcriptomic data support the idea that, upon colonization, plants activate an organism-wide reprogramming of their major regulatory networks [3]. Fungal lipochitoligosaccharides in fact elicit such a reprogramming before the colonization process begins [28]. At the moment, it is not known whether these “Myc factors” are also involved in the systemic metabolic changes that have been seen in the host plants. This leads to questions on whether AM-induced long-distance signals exist, and if so, about their nature (are they released by the fungus or produced by the plant, and mediated by the AMF intraradical network?). The longer AMF research continues, the more complicated the picture becomes. The genetic, cellular and metabolic complexity of AMF which are obligate and multinucleated microbes, is still hampering the study of their interplay with plants and other soil microbes. In addition, a stable transformation protocol is not yet available for such microbes, and this hampers a general strategy for their genetic improvement. Thanks to the availability of new technologies and computational approaches, the mycorrhiza research community is currently making major efforts in the molecular analysis of both the genetic and functional diversity of the AMF world. Investigating genetic diversity answers to fundamental questions concerning the dynamics of AMF communities in natural versus agricultural systems through the use of metagenetics approaches [29–31] and is fundamental for the prediction of ecosystem services. In this approach, the high-throughput pyrosequencing of a DNA amplicon (e.g., a fragment of the AMF 18s rRNA gene) from an environmental sample allows the description of the AMF community composition. Another approach is represented by the so-called DNA barcoding: here, small species-specific DNA motifs present in the amplicons obtained from the environmental samples are compared with a DNA barcode reference dataset of known fungal species [32]. The description of AM functions, through omics studies, has instead been mostly developed under laboratory conditions to date. The aim of this commentary is to focus on high-throughput molecular methodologies and systems biology approaches which allow the benefits provided by AMF to be

investigated more in depth. This knowledge could contribute to lay the foundations of a more focused field application.

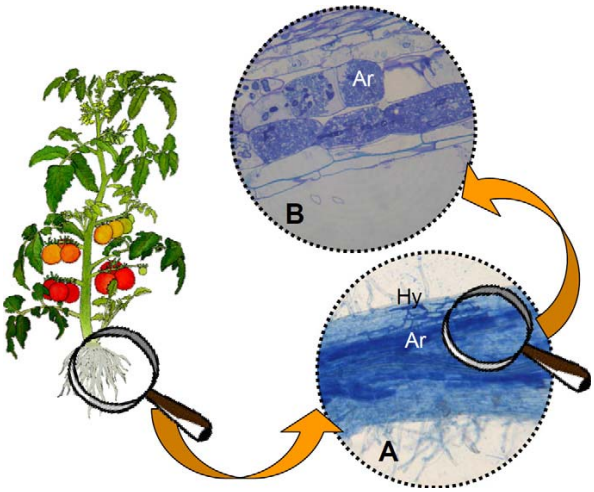


**Fig. 1. Morphology of an AMF-colonized root.** The Figure shows that the fungus enters the host root by means of a structure known as the hyphopodium, and then colonizes the host root cells by developing intracellular branched hyphae called arbuscules. A: Colonized root fragment stained with cotton blue; B: Semithin section (1 mm) from a resin-embedded mycorrhizal root stained with toluidine blue. Ar: arbuscule; Hy: hyphopodium.

## 2. What is known and what is novel in the application of mycorrhiza for plant improvement

The increasing demand for safe and healthy food, along with more environmental-friendly agricultural practices, has kindled enthusiasm towards the development of low impact sustainable agriculture, in which the role of AMF as natural fertilizers is predicted to be of great importance [33]. AMFs are in fact particularly promising for improved plant nutrition, protection against pests and crop quality trait promotion. However, the beneficial effect of these microorganisms in conventional agriculture can be hidden by the excessive use of chemical products, and their presence can decrease in intensively exploited fields [30,34,35]. For this reason, their reintroduction into the soil by means of commercially available inocula is considered a valuable agricultural practice. In spite of the nature of obligate biotrophs, methods for the efficient production of large-scale AMF inocula are now available [36]. Furthermore, the advances that have been made in AMF detection and quantification now lead to a better inoculum characterization and standardization, as well as a more reliable estimation of the presence of AMF in both the soil and mycorrhizal roots [37]. The development of beneficial microbial inocula for a large-scale field application is moving forward quickly, and in recent years the market for these products has been growing with an annual rate of approximately 10% [38]. The current market of AMF based inocula comprises applications in horticulture, gardening and organic farming, especially for highly valuable cultures. However, the widespread application of AMF inocula as a routine agricultural practice has been delayed by

their cost-effectiveness, which still has to be improved, and by the unstable performance they sometimes show under field conditions [36]. This latter point very likely depends on the specific plant–fungus–environment combination that actually takes place. In some particular cases, the plant responses to AMF are neutral or even negative in terms of plant growth [26,39]. Conversely, many synergistic interactions between AMF and other soil microbes in promoting plant growth have been reported [40], suggesting that more research is needed to test and define specific consortia that are suitable for commercial use on different crop species and under different field conditions [41,42]. In addition to the acknowledged impact on plant growth and protection, new evidence is emerging on the capabilities of AMF to improve crop production in many other different ways. Recent results suggest that AMF can also negatively influence the growth of some weed species, in the presence of a crop (maize), thus conferring a clear advantage for sustainable field management [43]. Moreover, some evidence suggests that mycorrhiza establishment can improve the nutritional value of different crops, by enhancing the content of nutritionally valuable compounds in the edible part of the plant. AM symbiosis has been shown to increase the lycopene content of tomato fruit, while the production of mutagenic compounds has not been observed as a result of mycorrhiza-induced metabolic changes [44]. On the same line, AMF inoculation has increased the content of antioxidant compounds, such as anthocyanins and carotenoids, in lettuce leaf under greenhouse conditions [45]. Interestingly, AMF biodiversity itself has been shown to promote plant productivity, and also to buffer, in some way, the productivity fluctuations that occur under differing environmental conditions [46]. These latter findings clearly suggest that the multifunctional ability of AMF to improve plant performance is still far from being fully understood, and this makes their use in agriculture much more valuable than as a simple substitute of chemical fertilizers/pesticides. Maximizing the beneficial effects of AMF requires understanding the molecular mechanisms of symbiosis, studying the different responses of plant species and genotypes and developing new formulations of efficient inocula that are suitable for specific applications. As a prerequisite to the achievement of these goals, a closer link between field research and laboratory studies must be achieved, in order to fill the gap that exists between basic and applicative mycorrhizal research. Large and complex field trials are only rarely associated with a deep molecular-scale analysis, while plant response to AM symbiosis may vary extensively between laboratory and field conditions [34,39]. A combination of field and laboratory data (evaluation of biomass, phenotype description, identification of physiological and molecular traits) will be of great importance to elucidate the mechanisms that govern plant–microbe symbioses under field conditions. Adopting this approach, Gao and co-workers have recently assessed that in-field inoculation of rhizobia and AMF decreased red crown rot disease in soybean [47]; they then switched to inpot cultures to verify the reduction of the pathogen growth and found that plant defence-related gene expression was increased by rhizobium and/or AMF inoculation. Such approaches, from the field to the laboratory or even in the opposite direction, will be instrumental in applied mycorrhizal research, and will help close the gap between data obtained at a small-molecular scale and data from field trials. The second level of unpredictability arises from the comparison of results obtained from changing set ups and conditions. The difficulty to collate results from different experimentations often arises because of the functional variation that the symbiosis shows in different contexts, and because of the difficulty in identifying, separating and quantifying the relative importance of the single benefits provided to plants [48]. The mycorrhizal community is reacting to these concerns. A great effort has been made to assess the right control fertilization level for in-pot experiments, so that mycorrhization is not inhibited and non myc controls are not nutrient depleted [49]. Statistical approaches have been

recently used in the attempt to provide a clearer view of the influence of AMF on plants [39,48]. This is the case of statistics assisted metanalyses, and in particular structural equation modelling, that have recently emerged as powerful techniques to assess causal relationships among variables, by calculating global indexes like correlation coefficients. A good example is given by the contradictory reports concerning whether AMF protect or do not protect plants from pathogens. A meta-analysis conducted on more that 100 papers has revealed that the identity of the AM isolate has had a dramatic effect on the level of pathogen protection [50]. These kinds of approaches should be encouraged since they provide useful indications on how to turn research into in-field applications. Obtaining high-quality high-throughput molecular data from field conditions, coupled with innovative analytical strategies to manage them, would help mycorrhizal research to fill the existing gap between field and laboratory studies. We believe that these approaches will lead to a better exploitation of AM symbiosis and, more in general, to the adoption of agricultural practices that produce more predictable and consistent effects.

**Table 1**
A consistent number of plant genes are differentially regulated upon mycorrhiza establishment in different target organs.

| Plant species | AM F species | Target organ(s) | Regulated genes n° | Technology used | Studied genes n°[d] | Reference |
|---|---|---|---|---|---|---|
| *Medicago truncatula* | *Glomus versiforme* | roots | 92 | cDNA Macroarrays | 2268 | [106] |
| *Solanum lycopersicum* | *Glomus mosseae* | leaves | 5 | differential mRNA display | ND | [107] |
| *M. truncatula* | *Glomus intraradices* | roots | 182 | cDNA Microarrays | 5651 | [108] |
| *M. truncatula* | *G. intraradices* | roots | 115 | EST sequencing, SSH, in silico screening | 5646 | [109] |
| *Oryza sativa* | *G. intraradices* | roots | 256 | Affymetrix GeneChip | 51279 | [110] |
| *M. truncatula* | *G. intraradices, G. mosseae* | roots | 201 co-induced | cDNA Microarrays | 16086 | [111] |
| *Lotus japonicus* | *G. mosseae commercial inoculum* | roots | 62 | cDNA Macroarrays | 18144 | [112] |
| *M. truncatula* | *Gigaspora margarita* | roots (early infection) | 107 | SSH | ND | [113] |
| *M. truncatula* | *G. intraradices* | roots | 652 | Affymetrix GeneChip | 61200 | [114] |
| | *G. intraradices, G. versiforme* | arbusculated microdissected root cells | 13 validated | RT-PCR | 32 | |
| *Lotus japonicus* | *G. margarita* | roots | 558 | Affymetrix GeneChip | 50000 | [115] |
| | | arbusculated microdissected root cells | 7 | RT-PCR | 7 | |
| *S. lycopersicum* | *G. intraradices, G. mosseae* | roots | 59 co-regulated | Affymetrix GeneChip | >9200 | [49] |
| *M. truncatula* | *G intraradices, G. mosseae* | roots | 512 | Affymetrix GeneChip | 61200 | [116] |
| | | microdissected root cells | 25 arbuscule-specific regulation | RT-PCR | 71 | |
| *M. truncatula* | *G. intraradices* | roots | 647 | QIAGEN Medicago OligoArray | 16000 | [27] |
| | | shoots | 599 | | | |
| *S. lycopersicum* | *G. mosseae* | roots | 655 | TOM2 microarray | 12000 | [95] |
| | | shoots | 422 | | | |
| *M. truncatula* | *Myc-LCO*[a] | roots | About 500[b] | Affymetrix GeneChip | 61200 | [28] |
| *M. truncatula* | *G. intraradices* | microdissected root cells | 535 arbuscule-specific regulation | Affymetrix GeneChip | 61200 | [117] |
| *O. sativa* | *G. intraradices* | leaves | 144 | Macroarray[c] | 6144 | [118] |
| *M. truncatula* | *G. margarita* | roots (early contact) | 248 | Affymetrix GeneChip | 61200 | [119] |
| *S. lycopersicum* | *G. mosseae* | fruit | 11 | TOM2 microarray | 12000 | [104] |

Notes:
ND = not definable for this study.
[a] Mycorrhizal lipochitooligosaccharides, diffusible AM fungal signals that trigger AM symbiosis establishment [23].
[b] Depending of the considered time and the nature of the used Myc-LCO (sulfated or nonsulfated).
[c] Tags only represented genes whose expression is induced in leaves by *Magnaporthe oryzae* infection.
[d] For the arrays the number of ESTs or oligonucleotide probes is given.

## 3. When next generation sequencing meets mycorrhiza

DNA sequencing has evolved to a great extent from Sanger's original "first generation" technology set up, and especially after the launching of the 454 system in 2005, which opened the way to the next generation sequencing era [51,52]. Currently, three sequencing platforms dominate the market: Roche 454 Life Sciences, Illumina Genome Analyzer, and Applied Biosystems' SOLiD [53]. These sequencing technologies have provided powerful new ways of understanding complicated eukaryote transcriptomes [53]. The so-called RNA-Seq (RNA sequencing) offers advantages over existing transcriptomic approaches: it is not limited to detecting transcripts that correspond to a known genomic sequence; it is reliable in quantifying expression levels and has a very low background; it is sensitive for transcripts that exist in very high and very low quantities [54]. After sequencing, the resulting reads are produce a genome-scale transcription map that consists of both the

transcriptional structure and/or the expression level of each gene [54]. In addition, all RNA populations, such as microRNAs and short interfering RNAs, can be sequenced. However, while it is true that RNASeq offers some important advantages over previous transcriptomic approaches, this technique also presents some weaknesses: the statistical analysis is less mature, replication experiments are more expensive, and sequencing errors can occur. De novo transcriptome assembly is still considered challenging, and the gold standard for this procedure is not yet available [55]. Regardless of this, RNAseq has been effective in transcript quantification and in differential expression studies in different research fields, and has shown high sensitivity in the detection of genes with low expression levels as well as an overall consistency with microarray generated data [56–58]. However, further investigations are needed to have a better understanding of the advantages and limitations of RNASeq, since the application of this technique is still rather new. On the other hand, the sequencing manufacturers are making a competitive effort to develop new strategies in order to obtain an increase in the amount of sequence output per run as well as in the read length, an improvement in the sequencing accuracy and a reduction in costs [52]. The academic world is also contributing to these technical advances, by developing new algorithms for the computational analysis [59]. In addition to traditional genomics and transcriptomics, other omics studies can be added to the list, at the edge of sequencing applications. This is the case of the recent development of DNA methylation assays, which can provide a comprehensive picture of genome epigenetic modifications [60], and the 'high-throughput degradome sequencing' approach, which enables the large-scale validation of small RNA targets [61]. However, the field is still experiencing a fast and ongoing evolution, with new strategies being proposed, like single-molecule sequencing [62]. The most promising and innovative application is represented by the use of nanopores, i.e. nanostructures based on bacterial pore proteins which are able to identify the single bases of a DNA molecule passing through them by the way they affect the ion flow across the pore. Single-molecule sequencing is expected to avoid some of the problems related to the current sequencing approach, by simplifying sample preparation, reducing the amount of nucleic acid required, and eliminating the need for template amplification [62]. Although the generation of huge amounts of sequence data from a single experiment has provided a way to deepen the analysis at a molecular level, it also requires the availability of powerful computing technology and specific expertises to store, analyze and manage this bulk of data. Pipelines devoted to the analysis of specific datasets have already been and are constantly being set up, in order to try to fill the gap between the acquisition of such a large amount of data and the ability to understand their biological meaning [63–65]. In the plant biology field, next-generation sequencing has predictably boosted the availability of genomic information, and has allowed the analysis of reference-free transcriptomes [66,67] as well as the development of markers for molecular breeding and mutant mapping [68]. With the important exception of Arabidopsis thaliana, many plants that have been sequenced so far, are hosts for AMF: from rice (see below) to the grapevine, from the apple tree to the poplar and Medicago truncatula, all of these genomes have opened new opportunities to have a better understanding of their interactions with microbes. Another good example is given by Solanum lycopersicum genome sequencing, which has recently been achieved by the Tomato Genome Consortium using a combination of long Sanger and 454/Roche GS FLX reads, assembled with high-coverage, shorter SOLiD and Illumina reads [69]. This result represents a milestone towards improving solanaceous crops, on one hand allowing the identification of polymorphisms linked toeither aligned to a reference genome, or de novo assembled to agronomically relevant traits, and on the other the analysis of relevant gene expression patterns through genotype-to-phenotype

mapping [70]. Thanks to the increasing availability of highthroughput data on agriculturally relevant plant species, the study of their interaction with soil symbiotic microbes will also benefit: multiple experimental designs targeting different dynamic levels (epigenetics, transcriptomics, proteomics), and different plant organs can be drawn to assess the effects of such symbioses on the whole plant phenotype, and to set up specific breeding strategies. Genomic information on mycorrhizal fungi is still limited to a few species, even though a huge sequencing project is currently under way: the US Department of Energy-Joint Genome Institute has in fact launched a Fungal Genomics Program with the aim of scaling up sequencing and analysis of fungal genomes to explore their diversity and applications for energy and environmental science among which more than 25 symbiotic fungi are included [71]. The genome sequence of the ectomycorrhizal basidiomycete Laccaria bicolor was obtained in 2008 [72], and this was followed by the black truffle (Tuber melanosporum) genome two years later [73]. AMF instead mostly remain a great black box, although substantial steps forward have recently been made: the Glomus intraradices transcriptome has been analyzed [74], and two assemblies of its genome have just been made by two independent research groups (F. Martin and T. Bisseling, personal communication). Mitochondrial genomes from diverse AMF are now available [75,76], as well as sequence information on the obligate endobacterium that lives inside the AMF Gigaspora margarita [77]. The availability of innovative single-molecule sequencing technologies will predictably boost the sequencing of AMF in the near future, facilitating both the reading and the assembly of their large and highly polymorphic genomes. As more mycorrhizal genomes become available, comparative genomics is expected to provide useful information for the identification of important symbiosisrelated genes and to reply to fundamental questions, (such as does a common symbiosis molecular toolbox exist?), as well as to provide novel molecular markers for ecological investigations. Taken on the whole, a brief review of some current NGS projects has revealed that an increasing number of plants that are excellent hosts for AMF have been sequenced while, on the other hand, only a few mycorrhizal genomes have been deciphered, even though their number is rapidly increasing. However, the application of high-throughput technologies to analyze the transcriptomes, proteomes and metabolomes of mycorrhizal plants under various environmental conditions are rapidly developing but still quite limited. There are only a few examples of metatranscriptomics analysis by means of intensive RNA sequencing, which has emerged as a powerful approach to study gene transcription in bacteria [78] and fungal communities from forest soils [79]. The main result from these pioneering studies is the identification of specific functions in the “real” world, like the presence in the soil of diverse enzymes for degrading the organic matter [79]. It would be thus crucial to identify the presence and the efficacy of AM-inducible functional markers in crop-fields by using similar approaches. In conclusion, it seems that Next Generation Sequencing has met host plants and mycorrhizal fungi, but so far only rarely has the methodology been applied to AMs outside the laboratory.

## 4. Next-generation plant-microbe biology: are we still far from it?

In an attempt to describe the complex interactions that govern biological systems, and to take into account all the variables (from environmental to genetics) that affect their responses, a perspective called systems biology has been proposed. This trend is characterized by a holistic rather than reductionist approach to life science, which involves the integration of experimental data and computational research, and relies on the possibility of building a model that allows one to discover

the emergent properties of the system itself [80,81]. Adopting a systems biology approach, molecular biologists have recently turned attention to the network analytic method as an effective way of managing, organizing and integrating the huge amount of multilevel molecular data (from genome to metabolome) that can be obtained from high-throughput technologies in order to generate hypotheses on their biological meaning and find relevant correlations. Biological networks are mathematical abstractions that catch a part of the global complexity of a system and describe and unravel it in a convenient way (see the Box 1) [82,83]. Network construction has been successful in analyzing the multilevel regulation of gene expression, in dissecting the protein–protein interactions and in characterizing metabolic patterns [84–87]. A number of bioinformatics tools, such as analysis software, web applications and data management frameworks are still being created to support research in systems biology (an extensive list can be found on the www.systemsbiology.org portal). The current availability of many plant genomes, together with transcripts, metabolome and proteome data, has induced scientists to start looking at plant science from a systems biology point of view. For example, the networking approach has been used to describe the complex regulation of small RNAs on plant gene expression [84]. Interestingly, many systems biology approaches have already been applied to the tomato, due to the combination of a relatively large amount of molecular data being available and the particular agronomic/economic interest in this crop. The development and metabolism of the fruit have been particularly targeted, and multilevel molecular data have been integrated to reveal novel regulatory interactions [88,89]. An elegant implementation of such an approach to tomato has been offered by Carrera and co-workers [90], who applied reverse engineering computational methods to plant systems biology. Using transcriptomic, metabolomic and phenomic tomato data, they constructed an in silico model that describes the metabolic profile of the fruit from gene expression. The model was able to reveal connections between metabolic profiles and phenomic data, and to predict changes in fruit quality traits possibly produced by a specific gene expression pattern. Similar approaches could allow computational modelling to be turned into a practical field application for future plant breeding and engineering. These latest technical and analytical advances can surely help in the understanding of plant-microbe symbioses, through the scaling up of network analysis to the whole plant and then at an environmental-wide level [91–93]. A similar approach has recently been proposed for the interpretation and modelling of plant–pathogen interactions mediated by effectors [94]. AMF also trigger a systemic host response, potentially regulate plant gene expression via small RNA and finely modulate plant response to other environmental stimuli [27,95–98]. All this evidence would seem to suggest that a holistic systems biology approach might be a suitable way of symbolizing the complex scenario represented by a mycorrhizal plant living in an (agro)ecosystem; accordingly, a networking approach has been attempted for miRNA regulation and plant degradome as a response to AM symbiosis [99]. Using a deep sequencing approach the authors assessed that small RNAs and degradome sequence tags are regulated by mycorrhization in Medicago truncatula. They identified several AM symbiosis-relevant genes as miRNA targets, and among these, a GRAS transcription factor that is essential in the rhizobia–legume association, suggesting that small RNA-based regulation could play an important role in symbiosis establishment. As stated in paragraph 2, maximizing the beneficial effects of AMF requires a better clarification of questions that are still pending. The application of a systems biology approach to mycorrhiza could be a useful strategy to increase knowledge on the molecular determinants that lead to mycorrhiza establishment and functioning. This approach has recently been applied by Rodriguez- Llorente and co-workers [100], who used computational methods

to reconstruct a “symbiosis interactome” in order to illustrate the interaction between the N2 fixing bacterium Sinorhizobium meliloti and its plant hosts. Using functional genomics data from public databases, they constructed an integrated network that represented the functional coupling between gene products in S. meliloti. They then further processed this model to find functional modules (i.e. subnetworks) and to predict functions for proteins on the basis of their association with proteins with a well known role in the symbiosis. This analysis led to the identification of potential novel symbiosis components, and the participation of some of them in the symbiotic process was demonstrated experimentally. In addition, the systems biology approach is suitable for a more widespread application, and could be used in an attempt to fill the gap between field and lab researches that was mentioned in the previous sections. By applying the networking strategy to infield experimental designs, multilevel molecular data could be used to build regulatory networks that represent the plant molecular responses activated in different tested conditions (e.g. AMF inoculated vs. non inoculated). The analysis of these networks is expected to lead to the identification of the key elements (small RNAs, transcripts, proteins...) that are mainly involved in plant response. Furthermore, the integration of molecular data with those from phenological observations will allow specific molecular determinants to be linked to given plant phenotypes. Such an analysis could contribute to the development of molecular markers which are useful to test new inocula formulations based on the response they trigger in the plant, and to assist breeders in the selection of more mycorrhiza-responsive cultivars. Finally, such an approach could be applied to some of the still pending questions about the impact of AMF on plants, such as the impact of mycorrhization on nutritionally valuable traits. However, some of the major experimental complications highlighted in the previous sections are also shared by systems biology; large-scale analyses used in an attempt to reconstruct complex biological systems are not easy to perform, the sampling strategy is crucial as well as the metrics chosen to quantify the plant response; obtaining multilevel data is difficult and expensive; constructing networks with different computational approaches is not an easy task. On the positive side, public databases that integrate the available data and offer a view on the genomic, proteomic, transcriptomic, genetic and functional information are now being created for specific fields of interest (see for example http://integromedb.org/) to enable systems-biology studies. In this context, the development of gene expression atlases, like the new transcriptomic atlas of grapevine [101] and the MtGEA on Medicago truncatula (http://mtgea.noble.org/v2/), offers the exciting opportunity of maximizing the use of publicly-available high-throughput data and of aiding the interpretation of the so far sequenced genomes through functional genomics. MtGEA contains the majority of M. truncatula genes and covers all its major organ systems, with detailed developmental time-series for nodules and seeds, providing excellent views of all the genes that respond to rhizobia and AMF. Such a data set will surely aid gene function determination, biological discovery, and molecular breeding efforts [102]. Among the emerging models of agricultural interest for mycorrhizal studies, rice is surely one of the most interesting and challenging. Starting from the first genome assembly, it has been clear that more detailed knowledge of this crop, given its central role in the diet of hundreds of millions of people, is essential. New data have conclusively demonstrated that rice depends on the AM symbiosis to satisfy its Pi need, which is mediated by a single functional Pi transporter, PT11 [103]. However, rice suffers from an additional problem: AM symbiosis is affected to a great extent by fertilization and wetland conditions, which have a great impact on the whole rice associated microbiome, as shown in detailed barcoding analysis that was conducted on upland versus wetland rice rhizosphere (Lumini et al., in preparation). Another model system of agricultural interest is represented by the

tomato. The recent unravelling of its genome [69] has opened new perspectives in the study of its association with AMF, through a systems biology approach. As previously mentioned, evidence has shown that tomato fruit quality might also be affected by mycorrhization [44]; furthermore, our group recently demonstrated that changes in fruit gene expression and aminoacid composition are induced following Glomus mosseae tomato root colonization [104]. As a second step, we carried out experiments to assess whether the features observed in tomato fruit at a transcriptional and metabolic level were a simple nutritional effect. To test this hypothesis, an experiment was designed in which a detailed RNA-seq analysis was performed on tomato fruit produced by plants treated at different fertilization levels (Zouari et al., in preparation). The preliminary results indicate that the effects of AMF on the fruit transcriptome are not exclusively related to an improved fertilization, thus suggesting a broader impact. These new findings will enhance the interest of applied researchers, who can envisage new opportunities for an evidence-based field application of AMF to tomato. In conclusion, given the increasing availability of omics data on mycorrhiza, and of other informatics interfaces that can serve as a model, we believe that the time is ripe for the development of a mycorrhizal bioinformatics hub that could link molecular-scale data with phenological observations: this will allow cutting-edge working hypotheses to be verified and researchers to be assisted in the design of novel experimental set ups. Such hubs could be focused not only on traditional AM-forming model plants, like Lotus and Medicago, but also, and more ambitiously, on a relevant agronomical species such as tomato or rice, in this way blazing a more direct trail for field applications.

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**Box 1**

What language do networks speak? According to the terminology of the graph theory, a network consists of nodes (vertices) that can be connected by links (edges) [105]. Generally speaking, nodes can be defined as the interacting elements of the network, while edges represent the interactions that occur among such elements. In a given data set (i.e. high-throughput molecular data), each element (=a node) represents a single entity that potentially shares a relationship (=an edge) with the other elements under study [82]. What the nodes and edges are, depends on the nature of the data used for the network construction. The arrangement of the various elements (i.e. nodes and edges) represents the topology of the network, which can be analyzed to find the emergent properties and the causal relationships among the network components.