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RESEARCH ARTICLE

ARBUSCULAR MYCHORRHIZAL FUNGI INDUCED MOLECULAR RESPONSES IN *CITRUS*

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ABSTRACT

Citrus is amongst the world's most frequently farmed commercial fruit crops, and it is constantly exposed to a variety of environmental constraints including abiotic and biotic stresses. Since citrus plants have a very few and short root hairs. So, in order to get sufficient nutrient and water they need mycorrhizal colonization. Numerous soil microbes, particularly arbuscular mycorrhizal fungi, dwell into the citrus rhizosphere and form a mutualistic relationship with citrus plant roots. AMF has been considered as a valuable biofertilizer for sustainable agriculture since it provides resistance to host plants against environmental challenges. Moreover, mycorrhizal hyphae contribute towards soil aggregation wich ultimately increases the soil fertility. Although, AMF possess a broad array of applications in citrus plant performance; however, the molecular regulatory mechanisms underlying the AMF response have not yet been fully characterized in citrus plants. In this review, we aimed to dissect the intricate molecular and metabolic pathways induced within citrus following AMF colonization using a variety of approaches such as transcriptomics, proteomics and metabolomics. And further scrutinized the relative contributions of diverse processes to the modulation of host defense response. The insights provided from a more comprehensive understanding of this peculiar symbiotic relationship will contribute in the agricultural biotechnology advancements.

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INTRODUCTION

A varied microbial population colonizes the roots of plants at the root-soil interfaces, giving rise to the rhizosphere. These microbes regulate the growth of plants by forming an interactive relationship with the roots of plants and the microorganisms present in adjacent soil[1]. A lot of research have been conducted in recent years with the explicit intent to determine the impact of AMF (Arbuscular Mycorrhizal Fungus) in a variety of plant species including the citrus, strawberry, apple, pepper and lettuce[2,3,4,5,6]. The phrase "mycorrhiza" is derived from two distinct Greek words: "myco" signifying "fungus" and "rhiza" indicating "roots[1]. Mycorrhizal fungi come in a variety of forms, but the two most frequent are endo and ectomycorrhizae.

Arbuscular mycorrhizae, a form of endo-mycorrhizae is the most widely distributed plant root symbiosis, found in 80 percent of all terrestrial botanical species, including the citrus plant[7]. Endo-mycorrhiza especially, AM have a wide host range and more than 150 species of AM fungi can colonize 2,25,000 species of plant host[8]. Ectomycorrhizal fungi infiltrate the root cortex without invading plant cells and induce plant cell responses that makes nutrient exchanges available. As Citrus are soil and environment selective plants. Mainly grows in light as well as dried soil conditions where plants get more benefit from mycorrhizae. Read and Fremont (1935) was the first to realize the importance of mycorrhizae for citrus plants. Srivastava et al., (2002) noticed that mycorrhizae are highly effective in low fertility and coarse textured soils.

Citrus is known for highly dependency on Arbuscular Mycorrhizal Fungi (AMF), due to its superficial root systems and less developed root hairs[9, 10]. It helps the host plant to absorb nutrients and water from soils, enhance the tolerance against stresses, improves soil structure, and induce higher level root development[11, 12, 13]. Studies have shown about forty-five species of AMF within citrus rhizosphere, belongs to as many as seven genera[14, 15].

CITRUS PRODUCTION: The citrus fruit is grown with an extreme passion throughout the world's tropical and subtropical climates. The recent figures depict that the area utilized for the growth of the citrus has shifted from 876.73hm2 to 1343.27hm2 in a very short period that comprises 15 years. The increase in the production of citrus has rose from 11517.8 million tonnes to 17848.2 million tonnes globally. At present, Asia is the world's largest citrus producer, accounting for almost 52.90% of total citrus production area, followed by America, which accounts for 24.50%, Africa with 16.60%, and in Europe and Oceania, it accounts for 6% of the total land allocated for the citrus production. Recently the production of China, India, and Morocco has witnessed its peak. China and India's growing output has increased significantly in comparison to other major producers, from 9.2358 million tonnes and 4.41 million tonnes in 2000 to a peak of 35.4693 million tonnes and 11.466 million tonnes in 2014. This increasing rate is followed by other countries such as Egypt, Turkey, and South Africa. Between 2000 and 2014, their rates of increase climbed by 85.69%, 70.26%, and 56.93%, respectively[16].

FACTORS AFFECTING CITRUS PLANTS: Different abiotic and biotic factors have currently affected citrus plants. Among these factors, we can categorize, all kinds of radiations, temperature fluctuations, water content, minerals, and different botanical and zoological factors along with microorganisms. These factors alter the biosynthesis and development process due to the oxidative burst[17]. The effect of climate change in most cases is evident because the abiotic factors of the environment are persistently making the citrus plants move through the hardships. The temperature fluctuations such as high temperature and cold[18,1], the challenging stress of the decreased soil water level[20, 21], the challenges of the salt stress[22, 23], the quality as well as quantity of the nutrients such as phosphorous and iron stress[24, 25, 26] heavy metal stress and waterlogging challenges[27, 28] are continuously obstructing the citrus culture. Following the findings of numerous studies on abiotic stresses, it is clear that AMF can increase the host's tolerance level by regulating plant water content, the efficiency of a plant's uptake of nutrients, the rate of photosynthesis, the capacity for osmotic regulation, reactive oxygen metabolism in the plant, synthesis of plant hormone and induction of molecular responses[29, 30].

The ability of mycorrhiza as a biological entity increases the efficiency of nutrient uptake of the responsive plants from the native sources in the nutrient depleted soils (Marschner, 1995). Once the plant is infected with mycorrhizae for the long period of time, then the plants might be carrying out the mycorrhizal infection. Additionally, the AM fungi are also the allies of the roots whose purpose is to support the plant in coping up with all kinds of biotic stresses. They help the plant to resist pathogenic microorganisms such microbial infections, nematode, necrotrophic pests and ectoparasites[31]. This particular bio protective process has been coined as MIR (Mycorrhiza Induced Resistance)[32]. The mechanism operating behind the process of the biotic stress management is mostly comprised of increased nutrient status, compensation of all kinds of harms and damages to the plant, providing strength to the plant, increased the production of secondary metabolites, the alteration of soil microbial communities and the induction of the defensive genes in plants along with the stimulation of systemic resistance mechanism[33].

EFFECT OF AM SYMBIOSIS ON CITRUS GROWTH: Many soil microorganisms inhabit the citrus rhizosphere that mostly comprises upon bacteria, fungal bodies, nematodes, protozoa, algal structures and certain microarthrops. These soil microorganisms are extremely affected by the exudates of the roots, different plant species, the developmental stages of the plants and diverse properties of the soil.[34, 35] AM species having different responses to different citrus cultivators nutrient uptake particularly less mobile phosphorus (P), zinc (Zn) and copper (Cu). By examining the factor of importance for the roots of the citrus plant, the microorganisms with a symbiotic relationship that are ranked at the top are the AMF[36]. After establishing a symbiotic relationship with plants, AMs extract a considerable amounts of minerals and water from the soil and transfer them to host plants. In exchange, host plants produce photosynthesis for the proliferation of AMF[36, 37]. Levels of H2O2 and melondialdehyde (MDA) contents in leaves was decreased due to AMF symbiosis. Colonization rate, plant growth as well as antioxidant enzymes activity was observed to be incredibly correlated. AMF symbiosis is a potential tool to increase the detriment created by drought stress on young seedling by elevating plant growth, reducing membrane lipid peroxidation, raising cell wall stability and increasing the activity of antioxidant enzymes. Hence, AMF symbiosis helps the plant by elevating plant growth, increasing the activity of antioxidant enzyme, raising cell wall stability and reducing membrane lipid peroxidation. The AM symbiosis enhances plant performance, in addition to taking nutrients, by increasing protection against environmental stresses, whether they are biotic or abiotic. it also improves the soil structure by forming the hydro-stable aggregates essential for optimum soil inclination[38]. It was the common perception that the citrus crop is dependent on the mycorrhiza fungi at a very higher rate since Peyronel (1922) examined the different samples of mycorrhizal structures in Italy[39]. Later on, in the future, Rayner (1935) observed mycorrhizal bodies in the rhizosphere specified for the citrus plants including C. sinensis and C. aurantium. However, these studies were unsuccessful in providing a meaningful perception towards the utility of the presence of the AM associations within citrus[40]. The morphological structures of AM, such as entrance sites, arbuscules, inter and intracellular hyphae, spores and extraradical hyphae are recently described in detail in the Poncirus trifoliata L. Raf grafted with Citrus unshiu Marcovitch. A novel insight was provided that intraradical AM hyphae might infect root maturation areas, root caps, and meristematic regions in roots of citrus plants[41].

AM SPECIES IN CITRUS RHIZOSHPERE: AMF is part of a class *Glomeromycetes* of phylum *Glomeromycota* [42]. Around 45 AMF species belonging to seven genera were found in the citrus rhizosphere, including *Acaulospora, Entrophospora, Gigaspora, Glomus, Pacispora, Sclerocyste and Scutellospora*. However, the citrus rhizosphere is primarily dominated by *Acaulospora, Gigaspora* and *Glomus*, particularly *G. fasciculatus* and *G. constrictus,* as given in

Table.1 [43]. Although AMF species with citrus roots can develop a favorable symbiotic arrangement, the development of AM also depends on some internal and external environmental variables, including arbuscular mychorrhizae, genotypes of host plant, moisture, nutrients and pH value of soil[44, 45, 46, 47, 48, 49, 50].

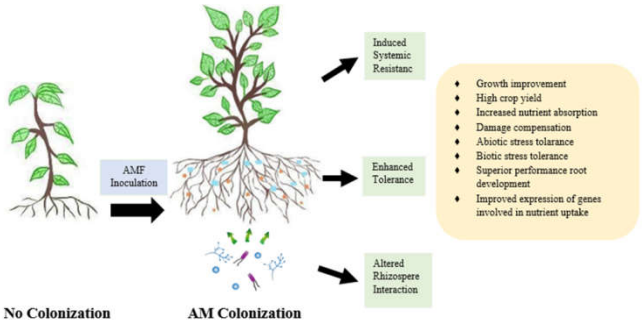

Figure 1. Overview of the possible mechanisms by which arbuscular mycorrhizal fungi contribute to sustainable host plant production

Table 1. The diversity of AMFs identified in the rhizosphere of citrus

AMF Species	Host Plant	Reference
Glomus tortuosum, G. geosporum, G. claroideum, G. aggregatum, G. tenebrosum, G. diaphanum, G. chimonobambusa, G. etunicatum, G. claroideum	*Poncirus trifoliata* L. Raf.	[51]
Scutellospora nigra		
Entrophospora baltica		
Pacispora robigina		
Acaulospora bireticulata, A. spinosa, A. scrobiculata, A. laevis	*Citrus reticulata*	[52]
Acaulospora brieticulata, A. spinosa, A. paulinae	*Citrus unshiu* Marc.	[51]
Gigaspora rosea, G. decipiens, , G. margarita	Blanco	[52]
Glomus mosseae, G. aggregatum, G. fasciculatum, G. monosporum, G. clarum, G. intraradices	Nine citrus Rootstocks	[53]
Sclerocystis sinuosa		
Glomus etunicatum, G. caledonium, G. geosporum	Newhall navel	[54]
Acaulospora koskei		
Gigaspora albida		

MOLECULAR MECHANISMS UNDERLYING AMF RESPONSE IN CITRUS: Studies of the molecular mechanism of AM fungal developmental and growth processes taking place at various phases of mycorrhizal colonization are very important. It helps to identify the subsets of the responsible genes for fungal sequential reprogramming necessary for the symbiosis. Understanding the function of such ecological symbionts in improving crop nutrition and protection of the ecosystems is crucial to the knowledge of AM's genetic underpinnings[55, 56, 57, 58, 59, 38]. Like other biotrophs, AM fungus in the earliest stages can activate plant defensive mechanisms through a process known as priming[60, 32]. The plant is placed in a 'active' state by the priming where defense mechanism not only precisely triggered but where the reaction to an assault happens more quickly and strongly than those plants which that have not previously been subjected to the priming stimulus. As a result, it increases plant resistance and provides significant benefits to plant health[61, 62]. The defensive priming of AM, therefore, possess a vast ecological importance[31].

Studies revealed that AMF exhibited characteristics similar to those associated with compulsory biotrophy, including the activation of a significantly less number of genes involved in plant cell wall breakdown[63, 64, 65], overexpression of signal cascade and transport regulatory genes[66], and the production of numerous tiny proteins[67, 68]. Understanding the mechanism of these agricultural and ecological beneficial organisms in crop nutrition and ecosystem preservation is critical to our understanding of AM's genetic groundwork and the development of new technologies[55]. They aid in the production of various phytochemicals through actions that are beneficial to health and contribute to the formation of nutrient-dense food products[69, 70]. When plants is faced with environmental stresses like drought, in that case AMF manages various mechanisms so that under drought stress there is no oxidative damage. Some of the AMF-mediated mechanisms. These mechanisms include moderations in the content of plant hormone content, for example; stringolactones, jasmonic acid (JA) and abscisic acid (ABA), an hydraulic conductivity is increased by plant water status improvement. AMF helps in enhancement of the plant drought tolerance. It improves the plant water relations by the regulation of the 14-3-3 genes (TFT1- TFT12) in the ABA signaling pathway[71].

AMF AND TRANSCRIPTOMICS: Transcriptomic research focusing in particular on plant protein encoding genes has led to understanding the molecular programming that AMF colonization induced in plants, not just at the root site[72, 73, 74] but systemically in other plant components as well[75, 76]. The AM colonization transcriptomic signature is being used for various purposes. First, for improving the efficiency of AM with the use of chemical and environmental solutions. Second, for successful and unsuccessful AM colonization surveillance, and finally, to identify the upstream regulatory pathways (transcription factors and miRNAs) that govern colonization and symbiosis of AM[77]. Generally, AM symbiosis is accompanied with precise signal reception and transduction, although little is known about the molecular processes of AM fungal signal transduction, owing to the fact that they are obligatory symbiotic organisms and *Arabidopsis thaliana* (a model plant), does not develop AM roots. Research has shown that AMF colonization can cause morphological and functional changes in host roots, which can affect plant development[78, 79, 80]. In particular, transcriptomic study of AMF response in citrus plants was followed by the below-mentioned methods employed.

Subtractive hybridization analysis: Several earlier investigations have found that AMF colonization in citrus[81, 82], barley[83] and *Alnus glutinosa*[84] plants affected the development of root hair, however, AMF's enhanced root hair development regulatory network remains unclear and requires further confirmation. So, for a better understanding, the subtractive hybridization approach[85, 86, 87] cDNA and oligonucleotide array analysis[88, 89, 90, 91, 73] and in silico data analysis approaches[92] have been carried out on a model crop named *Medicago truncatula*[93, 94]. The cDNA library constructed using subtractive hybridization is reported to be one of the most powerful technologies known to detect genes which are differentially expressed across diverse samples[95, 96, 97]. The range of genes discovered in SSH libraries provides a chance to identify functionally significant genes[98].

RNA sequencing: RNA-Sequencing is a revolutionary technique for studying gene pathways and processes that offers

greater sensitivity as well as the ability to identify splicing isomers and sometimes even somatic mutations[99, 100]. It is widely employed in the exploration of gene expression analyses in a number of organisms[101, 102, 103]. The symbiosis of AMF with a citrus species termed *Poncirus trifoliata* L. Raf was investigated by Chen *et al*., (2017), and it was discovered that it promotes lateral root development (LR) in citrus through nutrient management. The main objective was to discover the channels by which AMF regulates lateral root growth and to test the various regulatory circuits, using RNA-Seq technology[104]. To date, most root hair research has concentrated on the genetic foundation of herbaceous plants, while AMF-associated woody plants have received little attention[105]. However recently, Liu *et al*., (2020) discovered a mutual association between trifoliate orange and AMF by sequencing the RNA. He got valuable insights into the molecular mechanisms of AMF-enhanced root hair growth and observed that the expression of a group of genes activated by AMF was closely correlated with the growth of root hairs and the size of third lateral roots[106]. Transcriptome studies on the detection of differential expression of AMF-induced plant genes have been extensively documented up to this moment[87, 75, 107]. But, no attempt was made to establish a unifying transcriptome signature for AM symbiosis that could be used to assess the computational biological systems, like promoter analysis, identification of prevalent regulators as well as relevant target exploration, pointing towards critical regulators and targets of the AM symbiosis pathway[77].

Integration of machine learning: Recent advancements in the use of autonomous machine learning techniques have offered a whole new arena for data mining strategies to minimize possible the batch effects and integrate disparate investigations[108, 109]. It analyses a wide range of data from multiple research experiments with varying statistical backgrounds and also promotes the integration of AM types as variables and study in the AM transcriptome signature prediction model[110, 111, 112].

Modulation of meta-analysis: More recently, integrated autonomous machine learning techniques alongside meta-analysis were used to identify a bio-signature of mastitis and a preliminary prognosis of its progression. Drawing on the combination of meta-analysis with automated attributes weighting models, AM symbioses meta-genes can be used to efficiently distinguish between both AM-inoculated and non-inoculated samples[113, 114]. In a subsequent RNA-sequence experiment for validation, the generated signature differente the AM-induced roots with high accuracy[77]. According to the latest findings, Luo *et al*., (2020) have identified the sweet orange MRLK gene families and their potential impact on the use of bioinformatics and gene expression during colonization under the drought stress conditions[115]. Additionally, global analyses of expression and co-expression revealed the active participation of CBLs and CIPK genes throughout the drought stress and AMF interrelations in citrus seedlings, as well as their motif characteristics, gene recombination, organization of coding and non-coding RNAs, chromosome dispersion, phylogenetic analysis and cis-elements[116].

Identification of AMF induced non-coding RNAs using transcriptomics: Complex molecular processes, such as reprogramming the stress-related expression of genes through ncRNAs (non-coding RNAs), emerged to address various kinds of biotic and abiotic stresses in plants[117, 118, 119]. Generally, ncRNA is a subclass of functional RNAs that are synthesized with the guidance of RNA polymerase but lack the ability to code[120, 121]. Traditional ncRNAs are categorized into the following kinds depending upon the size and structure: lncRNAs (long non-coding RNAs) and sncRNAs (small non-coding RNAs), which are mostly comprised of miRNAs (micro RNAs) and siRNAs (small interfering RNAs).[122, 123, 124, 125]

Long non coding RNAs (lncRNAs): Long noncoding RNAs (lncRNAs) are a subclass of ncRNAs that are more than 200 nucleotides long and can be found in a variety of organisms[126, 127]. Previous researches have demonstrated that lncRNAs play a crucial role in regulating a variety of biological mechanisms and emerging as a critical modulators of gene expression[128, 129]. Some lncRNAs have been shown to activate nearby genes through a cis-regulatory method[130]. However, certain ncRNAs can exert epigenetic control over gene expression through transcriptional regulation, chromatin-mediated suppression, or histone modification[129, 130]. A significant number of lncRNAs sensitive to cold and drought stress, nitrogen stress, osmotic stress, salt stress and phosphate deficiency, were discovered using high-performance RNA-seq approach[126, 128, 26, 131]. lncRNAs are considered to be involved with induction of defense response in plants against pathogenic microorganisms such as viruses, bacteria, and fungi[23, 132, 133, 134]. It is found that tomato lncRNA33732 operate as a positive regulator and increase the tomato resistance to a fungal pathogen (*Phytophthora infestants*) by transcribing the respiratory-burst oxidase gene and increasing H_2O_2 buildup[135]. Despite their involvement in regulating the interaction between plants and microorganisms, there are fewer studies about the role of the lncRNA in response to mychorhhizal symbiosis. Up to present, 18,165 lncRNAs of high confidence have been identified from 749 RNA-seq studies in various inbred tissues of maize line B73[136]. In a study, Han *et al*., (2020), made a comparison between two libraries of lncRNAs and reported that numerous lncRNAs are transcribed in roots as well. They also discovered that 63 lncRNAs were expressed differently in fungus-infested roots when compared to the control roots. Additional network analysis demonstrates that DELs can alter the expression of AMF symbiosis pathways both in a direct and indirect manner[137]. In another study, which was conducted on the TYLCV-resistant tomato line CLN277A, several differently regulated lncRNAs were revealed in response to the TYLCV, and found that lncRNAs positively impacted the expression of miRNA targeting protein-coding genes via miRNA target imitation[131]. It is believed that lncRNAs play a significant role in the flow of bidirectional nutrient exchange in maize, which both partners must control in order to maintain a stable symbiotic connection. However, the specific method by which bidirectional nutrient exchange is regulated remains unknown[128, 137]. Furthermore, there has been no single study conducted to far on the role of differentially regulated lncRNAs in response to AM symbiosis in citrus plants.

Small non-coding RNAs (sncRNAs): In response to AMF, the regulation of gene expression is dependent on a wide range of variables that are involved in transcriptional, post-transcriptional, and translational processes. The next level of regulation is based on the process of RNA interference (RNAi) using small RNAs (sRNAs), which are short, 20–30 nucleotides long, non-coding RNA molecules, involved in the control of numerous endogenous mechanisms in eukaryotes[138]. It is worth noting that a current study has discovered that sRNAs have a role in cross-kingdom communication[139, 140]. In

the case of contacts with plant fungal diseases, they can migrate through the contact surface between both donor and receiver organisms. Once inside the host cell, sRNAs can specifically target certain host mRNAs and occasionally stimulate secondary sRNA synthesis, and therefore modulating the host metabolic processes and defensive reactions[141, 142, 143]. When it comes to pathogens, this is of considerable relevance as a novel crop defense method evolved[128, 142]. At the moment, very little is known about the AMF RNAi machinery as well as potential sRNA transit and reciprocity of sRNA-mediated interactions among both AMF and host plants[143]. However, gene silencing mechanisms such as VIGS and HIGS have been shown to be efficient strategies in AMF and indicates that RNA transfer from the host to the fungus occurs and that RNAi-related pathways are active in AMF[66, 144, 145, 146]. According to a few recent research, several plant microRNAs have been shown to be expressed in a differential manner during the mychorrhizal symbiosis[147, 148]. Although their functional capabilities are still mostly unknown, several of them may constitute prospective mobile sRNAs molecules. Plant miRNAs play a critical role in a variety of physiological and biochemical pathways essential for plant growth and tolerance to abiotic and biotic stress factors[149, 150]. Researchers used the high-throughput sequencing to discover miRNAs in *Medicago truncatula* and discovered that they have key regulatory activities in the AMS system[151]. During mycorrhizal symbiosis, the precursor of miR393 was down-regulated in some plants including *Solanum lycopersicum*, *Oryza sativa* and *Medicago truncatula*. And a substantial drop in the expression of auxin receptor genes decreased arbuscular development was observed after miR393 was overexpressed[152]. In general, studies of AMS-related miRNAs are much more restricted. As a result, it is very important to investigate the AMF-miRNA regulatory network in depth. But, the majority of research on the molecular processes behind AMS use herbaceous model plants, and the mechanism underlying AMS in woody plants remains mostly unclear. However, in order to better understand the mechanism of AMS and the accompanying sRNA-mediated pathway in citrus, extensive illumina sequencing was recently undertaken on a widely used citrus species named *Poncirus trifoliata* L. Raf.[153].

AMF and Proteomics: Previous investigations have shown that colonization under the control of particular loci is a multi-stage, genetically controlled process. During the colonization phase of pathogenic or symbiotic micro-organisms, this mechanism is expressed and regulated by functional proteins, which eventually form stable mutual symbionts[154]. Moreover, in the analysis of pathogenic mechanisms, these methods are extremely crucial[155]. Proteomics has attested to be a strong technique in the analysis of plant stress response[156, 157, 158] and has been utilized as a powerful method for comparing complicated protein combinations[126]. Presently, just a few proteomic experiments have targeted the adaptive mechanisms of plant symbiotic organisms to the stress factors, which are extremely beneficial for crop development[159, 160]. Protein synthesis and accumulation serve as a key strategy for plants in terms of stress resistance, in addition, changes in protein quantity and quality are a more direct representation of regulatory processes[161]. However, it is still unknown how the AMF's interaction with the host in response to stress affects the global proteome's alteration[162]. While many differential proteome studies were conducted in the roots and leaves of Citrus plants using 2D-DIGE, MS, and iTRAQ approaches in response to different environmental stresses such as aluminium toxicity[163], drought[164], boron deficiency[165], Candidatus Liberibacter asiaticus infection[166] and Citrus tristeza virus[167]. To date, no proteomic work in citrus plants has been documented to identify AMF-induced differential expressed proteins against these challenges. Following proteomics techniques have been used so far for identification of AMF induced proteome in plants.

DE: Recently, Recorbet and colleagues studied Medicago truncatula root proteome responses to two AM fungi colonization. Specifically, they used two-dimensional electrophoresis (2-DE) to separate Glomus mosseae and G. intraradices and found 42 proteins involved in symbiosis, of which 32 could be accurately identified[168]. Aloui et al., (2011) then performed a comparative 2-DE/MALDI-TOF proteome analysis of M. truncatula shooting responses following mycorrhizal colonization and cadmium exposure to determine the processes involved in preventing metal toxicity from Cd-treated mycorrhizal plants[169]. After that, Wang et al., (2013) looked at dynamic fluctuations in AMF colonisation patterns for maize leaves proteins. In that research, Soluble proteins were phenol-extracted and separated by 2-DE, was used to isolate variably expressed proteins from maize leaflets, resulting in 21 distinct gel spots were shown in maize leaves[170] 2-DE

iTRAQ: Along with 2-DE, iTRAQ (Isobaric tags for relative and absolute quantification) is one of the strongest new method for the direct measurement of multiple samples which offers a comparative and quantitative measurement of thousands of proteins[159]. It is a gel-free method for the evaluation of quantitative proteins that utilizes a number of isobaric radioisotope tags to mark enzymatic peptides derived from peptide sources. It is a preferable alternative to the extremely sensitive 2-DE technique and enables more precise measurement, particularly for low-abundance proteins[171, 172, 173]. However, iTRAQ based examinations of the symbiotic activities between AMF and host plants have hardly been documented[155]. Gui et al., (2020) recently employed iTRAQ-based proteomics to distinguish the major proteins differently regulated in AMF-inoculated and non-inoculated southern blueberries under drought stress, represented in Table.2. They found that the photosynthetic capability of AMF plants was greater than those of non-AMF plants when subjected to drought conditions. This transition is mostly due to a high concentration of chlorophyll, which has allowed plants to maintain a significantly higher amount of carbon-fixing proteins, particularly "glyceraldehyde-3-phosphate dehydrogenase" and "sedoheptulose-bisphosphatase" under drought stress[162]. Furthermore, iTRAQ proteomic study revealed that AMF colonization influenced Puccinellia tenuiflora's physiological strategies and molecular regulatory network in AS (alkali-degraded soil). It was discovered that after AMF inoculation, a total of 598 proteins were substantially regulated in AS compared to AS-alone[174].

AMF & Metabolomics: The most often utilized methods to determine the ulterior metabolomics of AM symbiosis in plants are mentioned below

LC–MS or GC–MS: LC-MS or GC-MS (Liquid or gas chromatography with mass spectrometry) are commonly utilized analysis instruments for such metabolomic trials[182]. The LC-MS is extremely sensitive to the identification of abiotic or biotic interactions of essential components of the phenotypic processes that underlie organism responses[196, 197]

Table 2. iTRAQ-identified fungal proteins expressed in Arbuscular Mychorrhizal Fungi

AMF species	Protein name	Reference
Glomus diaphanum	alpha-tubulin	[33]
Rhizophagus clarus	beta-tubulin	[33]
Glomus custos	F-ATPase beta subunit, partial (mitochondrion)	[33]
Rhizophagus intraradices	heat shock protein 60	[33]
Rhizophagus intraradices	binding protein	[33]
Funneliformis mosseae	Phosphoglycerate kinase	[33]

Table 3. List of metabolites induced by AMF in response to stress conditions

AMF Species	Plant	Stress Type	Metabolite produced	Reference
Glomus versiforme	*Poncirus trifoliata*	Drought	Glucose	[187]
Glomus mossae	Maize	Salt	Soluble Sugars	[188]
Funneliformis mossae	*Triticum durum*	Water	Pinitol	[189]
Glomus mossae	Maize	Salt	Acetic acid	[190]
AMF mix and natural inoculum	*Triticum durum*	N stress, P rich	Alanine	[191]
Funneliformis mossae, *Rhizophagus irregularis*	*Solanum lycopersicum*	Minimum P	Aspartic acid	[192]
AMF mix and natural AMF inoculum	*Triticum durum*	N stress, P rich	Fatty acids and their esters	[191]
Gigaspora albida, *Acaulospora longula*	*Anadenanthera colubrina*	Minimal P	Proteins	[193]
Funneliformis mossae	*Cucumis sativa*	Chilling stress	Lignin	[194]
Gigaspora albida + *Acaulospora longula*	*Anadenanthera colubrina*	Increasing P concentration	Phenols	[193]
Rhizophagus irregularis (Ri), *Funneliformis mossae* (Fm), *Claroideoglomu etunicatum* (Ce)	*Solanum lycopersicum*	Salt stress	Jasmonic acid; Methyl jasmonate	[195]

In the past, the impact in root metabolic profiles of *Solanum lycopersicum* in response to two AMF species, called *F. mosseae* and *R. Irregularis*, was measured using the LC–MS technique. According to the findings, the AM operational system entails a precise oxylipine cascade activation, which might contribute to improved stress tolerance in AMF inoculated plants[192]. In the next few research findings, seedlings of *Puccinellia tenuiflora, Cucumis sativus,* and *Lycium barbarum* were examined for their alkali-responsive biochemical and metabolomic characteristics either with or without AMF. It was discovered that salicylic acid and abscisic acid levels have both been increased following AMF inoculation under alkaline stress[186, 193, 194]. Additionally, colonization with *F. mosseae* (an AMF species) has a considerable effect on the remodelling of the carbohydrate and lipids metabolism and regulation of phytohormone within wheat roots[195].

UHPLC-Q-TOF/MS: Recently, non-targeted metabolomics using microscopy and UHPLC-Q-TOF/MS (ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry) has been used to investigate the antifungal efficacy of pinocembroside (PiCB) towards *Penicillium digitatum* in citrus fruit[198]. Nevertheless, no research on the effects of AMF on metabolome of citrus species have indeed been documented yet.

CONCLUSION

It is believed that AM-symbiosis possesses a greater degree of tolerance against environmental stresses in citrus, but, the underlying key mechanistic pathways responsible for this effect have not yet been fully explored. The modulation of gene expression, which encompasses transcriptional, post-transcriptional, and translational processes, is thought to be one of the potential mechanisms through which AM-citrus may withstand the stresses. Taking into account, involvement of diverse combinational approaches, such as transcriptomics, proteomics and metabolomics would help us to gain a better knowledge of the regulatory network triggered between AM fungi and citrus crop.

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Author's contribution

HQ, SM, KSM, PB, NK, PK, DK, MPT, RV did intensive research on various 'Arbuscular Mychorrhizal Fungi Induced Molecular Responses In Citrus' topics developed; HQ, SM, RV contributed in writing the manuscript; Scientist MN designed and supervised the present review article and assisted in writing the paper.

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