



ORSZÁGOS TUDOMÁNYOS DIÁKKÖRI KONFERENCIA

**GOMBA ÉS VÍRUS FERTŐZÉSKOR *FUNNELIFORMIS MOSSEAE* JELENLÉTÉBEN BEKÖVETKEZŐ
VÁLASZREAKCIÓ MEGFIGYELÉSE A FENILALANIN
AMMÓNIA-LIÁZ GÉNEXPRESSZIÓJÁN KERESZTÜL**

***FUNNELIFORMIS MOSSEAE* MEDIATED PHENYLALANINE AMMONIA
LYASE GENE EXPRESSION ALTERATION IN RESPONSE TO FUNGAL
AND VIRAL PATHOGENS**

Készítette:

Arup Ratan Basak

JLE4W9

Mezőgazdasági biotechnológia, MSc

Nappali tagozat, angol nyelven

Szent István Campus

Témavezető: Dr. Posta Katalin, egyetemi tanár, MATE SZIC GBI
Mikrobiológia és Alkalmazott Biotechnológia Tanszék

László Livia, tudományos segédmunkatárs, MATE SZIC GBI
Mikrobiológia és Alkalmazott Biotechnológia Tanszék

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1. INTRODUCTION

Plants, being sessile organisms, experience a wide range of biotic and environmental stressors throughout their life cycle. These stressors fall into two general categories: biotic (like pathogen infections) and abiotic (such drought, salinity, and heavy metal toxicity etc). In recent years, the growing effects of climate change has led to significant shifts in the distribution and prevalence of plant pathogens (Raza & Bebbber, 2022). Changes in temperature, humidity, and precipitation patterns are the main ways that climate change alters the dynamics of plant pathogens, which can increase the virulence and spread of different plant diseases. For instance, the severity of head blight and blotch on wheat caused by *Fusarium* spp. and *Septoria tritici*, respectively, as well as powdery mildew on cucurbits produced by *Sphaerotheca fuliginea*, increased with elevated CO₂ levels (B. K. Singh et al., 2023). Adaptive methods in agricultural practices are required since climate change impacts not only the frequency and severity of plant diseases but also the efficacy of disease management efforts. To enhance plant resilience, researchers have looked into a number of methods such as using beneficial microbes like arbuscular mycorrhizal fungi (AMF), which coexist symbiotically with plant roots. AMF-mediated modulation of plant defense mechanisms involves complex interactions that impact the synthesis of defense-related compounds, signaling molecules, and the expression of specific stress-responsive genes. The phenylpropanoid pathway is a key aspect of plant defense against biotic stress. Phenylalanine ammonia-lyase (PAL), one of the key enzymes in this pathway, catalyzes the initial stage of salicylic acid (SA) synthesis. However, more research is required to figure out whether AMF during pathogen infections modifies the expression of the *PAL* gene. In this study, the effect of the common AMF species *Funneliformis mosseae* was examined on the expression of the *PAL2* gene in tomato plants under biotic stress conditions caused by Cucumber Mosaic Virus (CMV) and powdery mildew.

2. AIMS

The study aims are to examine how AMF (*Funneliformis mosseae*) inoculation influences the separate infection of CMV-Trk and powdery mildew fungus. Additionally, it will investigate how AM fungi affect the expression of the phenylalanine ammonia-lyase gene in response to fungal and viral pathogens. Furthermore, the research will provide insights into how AMF help plants during biotic stress conditions.

3. LITERATURE REVIEW

3.1. OVERVIEW OF TOMATO

Tomatoes are one of the most popular and extensively consumed fruits globally (Motamedzadegan & Tabarestani, 2018a), valued for their distinct flavour, versatility (Waheed et al., 2020), and significant nutritional benefits (Bhowmik et al., 2012). Taxonomically, tomatoes belong to the *Solanaceae* family, with the cultivated variety belonging to the species *Solanum lycopersicum*. Within the *Solanum* genus, numerous tomato species exist, each possessing unique characteristics and adaptations (Gatahi, 2020). Other important food crops from the solanaceous family are Potato, eggplant and pepper. The tomato is native to South America and was later domesticated in Central and South Asia. In 1753, Linnaeus classified the tomato as part of the *Solanum* genus, using the name *S. lycopersicum* L. This crop, which thrives in warm seasons and requires a cooler, drier climate for high-quality yield, is sensitive to frost but can tolerate a range of climatic conditions from temperate to hot and humid tropical climates, with an optimal temperature range of 20–24 °C for growth, yield, and fruit quality. (Padmanabhan et al., 2015). In the year 2022, it is estimated that over 4.9 million hectares of land were dedicated to the production of more than 186 million tons of tomatoes (FAOSTAT, 2020). Tomatoes are in fact the second-largest vegetable crop, both in terms of production and consumption, moreover, Tomatoes are a major cash crop in many countries, contributing significantly to agricultural economics (Gatahi, 2020).

Tomatoes are a rich source of vitamins (Beecher, 1998). They are rich in A, B complex (folate is higher than other B complex vitamins), E, K, and C vitamins, with vitamin C having the highest concentration (Ali et al., 2021) as well as carotenoid contents like β -carotene, lycopene (absent in raw tomatoes) and lutein (absent in raw tomatoes) (Ali et al., 2021; Chaudhary et al., 2018; Kim et al., 2014). They also contain some major mineral elements like Potassium, Calcium, Magnesium and Phosphorus (Ali et al., 2021). Other secondary metabolites include phenolic compounds-flavonoids, phenolic acids and polyphenols which contribute to the antioxidant properties and health benefits of tomatoes (Chaudhary et al., 2018).

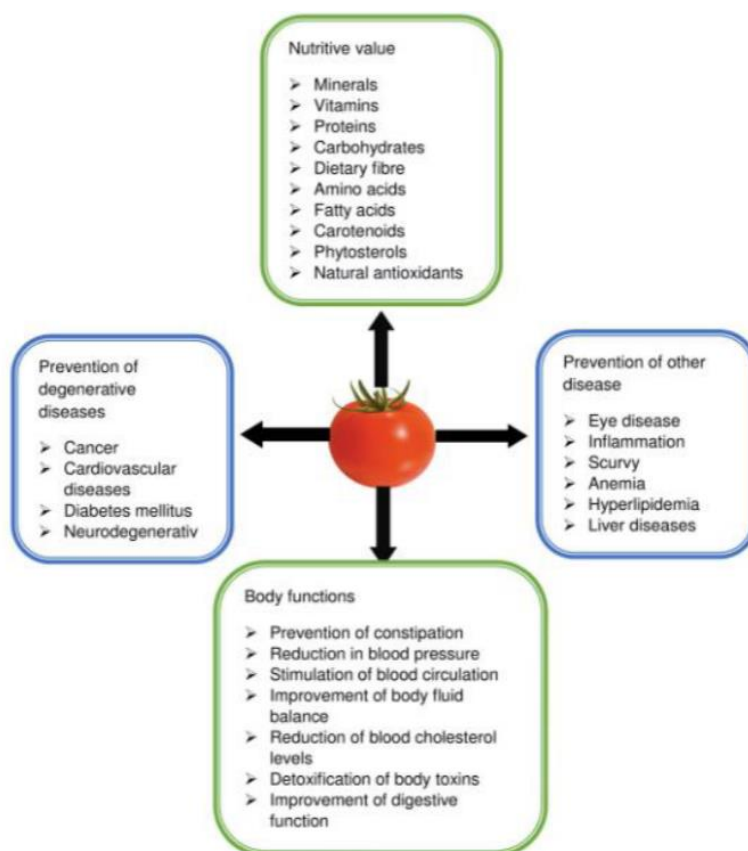


Figure 1: Summary of health benefits of tomato (Ali et al., 2021)

As it is shown in Figure 1, tomatoes are known to help alleviate a variety of diseases, especially chronic ones, since they contain high amounts of antioxidant molecules such lycopene, ascorbic acid (vitamin C), tocopherol (vitamin E), and carotenoids (β -carotenoids and lycopene). Due to the antioxidant properties of vitamins C and E, tomatoes can be used as a medicinal agent to help prevent a number of diseases such as cancer and cardiovascular disease (CVDs) (Ali et al., 2021). Non-nutritive phytochemicals like carotenoids and polyphenols are the other bioactive compounds present in ripened tomatoes and they are known to reduce the oxidative stress and thereby address a number of health issues (Chaudhary et al., 2018). Another carotenoid that lacks branches and has a non-cyclic structure is lycopene. This feature makes its incorporation into the liver, prostate, and adrenal glands, where it prevents the kinds of oxidative alterations associated with the outbreak of various diseases (Borguini & da Silva Torres, 2009). Although this compound is mainly found in tomato skins, due to the antioxidant properties it is linked to reduced risk of heart disease and cancer (Miller et al., 2002). Carotenoids lower the chance of developing several diseases, including

diabetes, gastrointestinal, and CVDs, by lowering the level of oxidized low-density lipoproteins and exhibiting anticancer properties for stomach, lung, and prostate cancer (Giovannucci, 1999).

Moreover, they are a staple ingredient in various food industries, including fresh produce markets, processed food manufacturing, and restaurant businesses. Tomatoes are a versatile ingredient in culinary traditions worldwide, used in salads, sauces, soups, sandwiches, and countless other dishes (Motamedzadegan & Tabarestani, 2018b). So, their culinary versatility makes them not only a flavourful addition to meals but also a significant source of health benefits.

3.2. STRESS RESPONSE OF PLANTS

Plant stress refers to any detrimental condition experienced by plants that disrupt their metabolism, normal growth and development processes (Lichtenthaler, 1996). Such stresses can have a deleterious impact on plant physiology, limiting critical functions such as photosynthesis, respiration, and nutrient uptake. Plant stress can be broadly categorized into two types: biotic stress, which is caused by living organisms such as fungi, bacteria, nematode etc. and abiotic stress, which is induced by environmental variables such as drought, cold, salt etc. (Umar et al., 2021)

3.2.1. ABIOTIC STRESS

Detrimental effects of abiotic stress on plant growth and development are one of the most obvious ecological effects of climate change that are becoming apparent (Bellard et al., 2012). Abiotic stresses are non-living environmental factors that can have deleterious effects on plant growth and development. Drought stress, for instance, may significantly impact plant's physiological, metabolic and biochemical activities such as reduced photosynthesis, delayed growth and development, substantial production loss, in the worst situations, the total death of adjacent cells (Bijalwan et al., 2022). Similarly, salinity stress caused by high salt concentrations in the soil can disrupt ionic balance and overall homeostasis in plant cell which affects photosynthesis, interferes nutrient absorption by root and translocation of nutrients paired with disruption of cellular membrane integrity (Mushtaq et al., 2020). Temperature extremes, both heats, and cold, lowers the germination rate, hampers growth and reduces photosynthesis (Kai & Iba, 2014). The presence of toxic heavy metals, such as cadmium, chromium, lead, mercury etc. damages plant cell with various mechanisms including peroxidation of lipids, oxidation of protein, inactivation of enzymes,

DNA damage and/or interact with other vital constituents of plant cells (M. Gill, 2014). Furthermore, excessive exposure to UV radiation can damage DNA, proteins, and lipids within plant cells (Hollósy, 2002)

3.2.2. BIOTIC STRESS

Biotic stress encompasses living organisms that can negatively affect plant growth, productivity, and survival (Schulze et al., 2019). Bacteria, fungi, viruses, and nematodes can cause various diseases in tomato plants by disrupting their physiological processes. Filamentous organism fungi are the most destructive pathogens in plants (Keane, 2012). Phytopathogenic fungi possess specialized hyphal structures that facilitate the invasion of plant tissues. Key initial events include adhesion to the plant cuticle and directed growth of the germ tube across the surface. At the site of plant penetration, appressoria often develop, which may feature melanized walls and high turgor pressure to support the penetration process. The penetration hypha concentrates cytoskeletal components at its tip and secretes a range of cell wall-degrading enzymes in a highly regulated manner, enabling it to breach the cuticle and plant cell wall (Mendgen et al., 1996). For instance, *Magnaporthe oryzae* causes devastating blast diseases in rice and wheat (Doehlemann et al., 2017). In cool temperate regions, tomatoes grown in large greenhouses are vulnerable to powdery mildew infection caused by *Erysiphe neolycopersici* which is spread by airborne fungal spores from outside the greenhouse (Sokolidi et al., 2023).

Free-living bacterial pathogens need to penetrate the plant's surface defences and enter the apoplast to start an infection. One obvious entry point for bacteria is the stomata. Phytopathogens may enter the apoplast through wounds on the plant's epidermis in addition to through the stomata. For this reason, many pathogens produce proteins and enzymes that break down the cell wall, allowing them to enter the plant tissue (Pfeilmeier et al., 2016). Most bacteria are incapable of actively penetrating plant surfaces. However, they can break down plant cell walls by secreting a variety of enzymes, including cellulases and pectinases, once they have entered the apoplast through wounds or stomata (Schulze et al., 2019). The bacterial infection process in plants involves several steps. First, bacteria survive on plant surfaces and form biofilms. Then, they use flagella or pili to migrate across plant surfaces and enter the apoplastic space. Once inside, they secrete enzymes to degrade plant cell walls and tissues, and release toxins to manipulate plant physiology, metabolism, and

immune responses, further facilitating infection. Some common plant pathogenic bacterial species are *Xanthomonas* spp. *Pseudomonas* spp. etc. (Pfeilmeier et al., 2016).

Viruses are made up of a protein coat that encloses nucleic acids (DNA or RNA) that contain the instructions needed to synthesize a small number of proteins. These proteins must be produced by the host cell in order for them to replicate; these proteins then assemble to form new virus particles (Schulze et al., 2019). Viruses need host machinery to reproduce since they are obligatory biotrophs. Through physical wounds, environmental influences, or vectors – which could include insects, nematodes, fungus, or even mites – they passively enter plant cells (Yadav & Chhibbar, 2018). Viruses like the Tomato mosaic virus and Tomato yellow leaf curl virus can stunt growth, inhibit fruit development, and reduce yields (Hanssen et al., 2010). One of the most common plant viruses in the world, it is a single-stranded RNA virus belonging to the *Bromoviridae* family. Aphids are the main vector of CMV's non-persistent transmission, which allows the virus to be swiftly acquired and spread by insects in a brief period (Mochizuki & Ohki, 2012).

Plant parasitic nematodes possess specialized structures, including stylets and esophageal secretory glands, which are essential for their parasitism. The stylet is used to pierce plant cell walls, while the esophageal glands produce secretions that trigger the development of feeding structures within the host plant. During feeding, the nematode inserts the stylet through the cell wall without piercing the plasma membrane and withdraws nutrients from the cytosol through a small opening (Williamson & Husseyb, 1996).

Herbivorous insects, like aphids, beetles, and caterpillars, can physically damage tomato plant tissues by feeding on leaves, stems, and fruits. This can lead to reduced photosynthetic area, increased susceptibility to pathogens, and diminished fruit quality and quantity (Walling, 2000). Additionally, weeds can outcompete crops for vital resources like light, water, and nutrients, which can slow down the growth and output of the crops. Tomato plants may be outcompeted by rival plants like grasses and broadleaf weeds (Zimdahl & Basinger, 2024). This competition can limit the tomato plant's growth, development, and overall productivity.

3.2.3. STRESS RESPONSE

Reactive oxygen species (ROS) are generated as unavoidable by-products of normal plant metabolic processes like photosynthesis and respiration, but their production is greatly increased under various environmental stresses such as drought, high salinity, extreme temperatures, and pathogen attacks, leading to progressive oxidative damage and ultimately cell death (Sharma et al., 2012). ROS are a group of free radicals, reactive molecules, and ions that are derived from O_2 such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical ($\bullet OH$). It has been estimated that about 1-2 % of O_2 consumed by plants is diverted to produce ROS in various subcellular loci such as chloroplasts, mitochondria, peroxisomes (Bhattacharjee, 2019). ROS are widely known to have two distinct roles, depending on the quantity in plants: they can be harmful or beneficial (Sharma et al., 2012). At high concentrations, reactive oxygen species can cause significant oxidative damage to cellular components. This includes lipid peroxidation, where ROS attack lipids in cell membranes, leading to a loss of membrane integrity and function. Additionally, ROS can modify proteins, resulting in a loss of enzyme activity and structural integrity, which can disrupt various cellular processes. High levels of ROS can also cause mutations and damage to nucleic acids, potentially leading to cell death, with the cumulative effect of oxidative damage triggering programmed cell death and ultimately causing the death of the affected cells. In contrast, at low to moderate concentrations, ROS acts as important signalling molecules in various physiological processes. ROS are involved in intracellular signalling cascades that mediate plant responses to environmental stimuli, such as stomatal closure and the activation of defence mechanisms against pathogens. They also play a role in the acquisition of tolerance to both biotic and abiotic stresses by modulating gene expression and activating defence pathways. Furthermore, ROS can interact with plant hormones, such as jasmonic acid, to regulate processes like lignin biosynthesis in response to stress. The balance between ROS production and scavenging is crucial for maintaining cellular homeostasis and determining whether ROS will exert harmful or beneficial effects in plants. Environmental stresses such as drought, salinity, chilling, metal toxicity, and UV-B radiation as well as pathogens attack led to enhanced generation of ROS in plants due to disruption of cellular homeostasis (Banerjee & Roychoudhury, 2017). At large quantities, all ROS are extremely hazardous to organisms. A cell is said to be in a state of "oxidative stress" when the amount of ROS surpasses the capacity of its defence mechanisms. Increased oxidative stress can be harmful to cells because it can lead to lipid peroxidation, protein oxidation, nucleic acid damage, enzyme inhibition, activation of the programmed cell death (PCD) pathway, and eventually cell

death. ROS are well-known second messengers in a number of cellular activities, including tolerance to external stressors, despite their destructive nature.

3.2.4. MECHANISMS FOR ROS ELIMINATION

Enzymatic antioxidants play a crucial role in detoxifying reactive oxygen species in plants. Superoxide dismutase (SOD) catalyses the conversion of the superoxide anion (O_2^-) into hydrogen peroxide (H_2O_2), which is less toxic (Mittler, 2002). Hydrogen peroxide is then broken down by catalase (CAT) into water and oxygen, preventing its accumulation and potential cellular damage (Mittler, 2002). Another important enzyme, ascorbate peroxidase (APX), utilizes ascorbate to reduce H_2O_2 into water as part of the ascorbate-glutathione cycle, ensuring efficient ROS detoxification (Foyer & Kunert, 2024). Additionally, glutathione peroxidase (GPX) reduces both H_2O_2 and organic peroxides by using reduced glutathione as a substrate, further contributing to the plant's antioxidant defence system (Foyer & Noctor, 2005).

Non-enzymatic antioxidants play a vital role in scavenging reactive oxygen species and protecting plant cells from oxidative damage. Ascorbate (Vitamin C) directly scavenges ROS, such as hydrogen peroxide (H_2O_2), and aids in the regeneration of other antioxidants, contributing to cellular defence (S. S. Gill & Tuteja, 2010). Glutathione (GSH) works alongside ascorbate, participating in the detoxification of H_2O_2 and playing a crucial role in the ascorbate-glutathione cycle (S. S. Gill & Tuteja, 2010). Carotenoids are responsible for quenching singlet oxygen and protecting chlorophyll from photooxidative damage, thereby safeguarding the photosynthetic apparatus (Mittler, 2002). Tocopherols (Vitamin E), on the other hand, prevent lipid peroxidation by neutralizing lipid radicals, further reducing oxidative stress in plant cells (Štolfa Čamagajevac et al., 2018)

3.2.5. INDUCED DEFENSES IN PLANTS

Induced defences are activated after the plant detects the presence of biotic stress. These involve signal perception and transduction pathways leading to localized and systemic responses (Jones & Dangl, 2006).

Plants utilize a variety of immune responses to defend against pathogens, with two key strategies being the hypersensitive response (HR) and systemic acquired resistance (SAR). The hypersensitive response is an early, localized defence mechanism where plants sacrifice infected cells by inducing programmed cell death (apoptosis) to form necrotic lesions, thereby containing the pathogen and preventing further spread (Jones & Dangl, 2006). This localized cell death traps the invading organism, limiting its ability to proliferate.

Complementing HR, SAR is a long-lasting, systemic immune response that spreads throughout the plant following a local infection. SAR is mediated by salicylic acid and provides broad-spectrum protection against a wide range of pathogens, making the plant more resilient to future attacks (Dangl & Jones, 2001).

In addition to these defence mechanisms, plants recognize conserved molecular patterns from pathogens, known as pathogen-associated molecular patterns (PAMPs), through pattern recognition receptors (PRRs) on their cell surfaces. This process is referred to as PAMP-triggered immunity (PTI). Upon recognizing PAMPs, such as flagellin or chitin, plants trigger various defence responses, including the strengthening of cell walls, the production of antimicrobial compounds, and the closure of stomata to block pathogen entry (Boller & Felix, 2009).

Some pathogens, however, release effectors that suppress PTI, allowing the pathogen to establish infection. In response, plants have evolved a second line of defence called effector-triggered immunity (ETI). ETI involves plant resistance (R) proteins that recognize specific pathogen effectors and activate a stronger immune response. This often leads to the activation of defence-related genes, including those that produce antimicrobial proteins, such as pathogenesis-related (PR) proteins (Dos Santos & Franco, 2023).

3.2.6. CHEMICAL AND HORMONAL SIGNALING

Plant hormones are crucial for controlling the signalling networks and developmental processes that plants use to respond to a variety of biotic and abiotic stressors. It is recognized that three phytohormones – salicylic acid, jasmonic acid, and ethylene – are important in regulating how plants defend themselves against a range of diseases, pests, and abiotic stressors (Glazebrook, 2005). Persistent systemic immunity against secondary infection by a variety of biotrophic, hemi-

biotrophic, and necrotrophic pathogens with different mechanisms of infection is possible with systemic acquired resistance (SAR). SA is essential to plant defence and is typically involved in the development of systemic acquired resistance and the activation of defence responses against biotrophic and hemi-biotrophic diseases (Grant & Lamb, 2006). On the other hand, jasmonic acid and ethylene are usually associated with defence against herbivores and necrotrophic pathogens that kill plant cells to feed (Grant & Lamb, 2006). Gene-for-gene resistance is an important form of resistance against biotrophs. It is associated with activation of SA-dependent signalling and SAR (Glazebrook, 2005).

3.3. MYCORRHIZAL RELATIONSHIP AND THEIR CLASSIFICATION

The term mycorrhiza is derived from the Greek words "mykes" meaning fungus and "rhiza" meaning root, refers to the symbiotic relationship between certain fungi and the roots of plants (Frank, 1885). Unlike the antagonistic relationship between plants and pathogenic fungi, mycorrhizal fungi exhibit a mutualistic symbiosis with plant roots, where the benefits are shared between the two organisms. This cooperative interaction results in a combined potential that exceeds the individual capacities of the fungus and the plant (Smith & Read, 2008). Initially mycorrhizal symbiosis was categorized by Harley in 1961 into two main types: Endomycorrhiza and Ectomycorrhiza (Alizadeh, 2011). Currently the categorization of mycorrhizal associations is based on the nature of the relationship between the fungi and the plant, as well as the state of communication between the root cells and the fungal mycelium. Accordingly, three primary groups are recognized: Endomycorrhiza, Ectomycorrhiza, and Ectomycorrhiza (Smith & Read, 2008). These groups differ in how the fungus penetrates the host cell and establishes diverse fungal states and structures within the host cells.

Ectomycorrhizal fungi belong to the classes including Basidiomycetes, Ascomycetes, and few imperfect fungi like Zygomycetes called Andogone. They do not penetrate the root cells, but instead form a dense network called the Hartig network between the root cells for exchanging metabolites. These fungi also create a thick sheath or pod on the root surface, often changing the root's colour and shape (Ramanankierana et al., 2007).

Endomycorrhizal fungi are classified as Zygomycetes, with two main groups: Glomales and Endogonales. In this form of associations, the fungus penetrates into the root epidermis cells of host plants. They produce specialized structures called arbuscules and vesicles within the host roots to facilitate the exchange of nutrients and metabolites between the fungus and the plant. Arbuscules are the primary site of this metabolic exchange, while vesicles serve as storage and resting organs for the fungi (Duponnois & Plenchette, 2003). Mycorrhizal fungi, which belong to the phylum Glomeromycota, are ubiquitous in the environment and form symbiotic relationships with more than 80% of plant species across a wide range of managed and natural ecosystems (Dhiman et al., 2022).

Ectendomycorrhizal fungi have a reduced or absent fungal sheath, and their Hartig network is not fully expanded, but their roots still penetrate the host plant's root tissue. Conversely, Ectomycorrhizal fungi can form an Ectendomycorrhizal association under suitable conditions when interacting with different host plants. The classification of mycorrhizal fungi is based on the presence or absence of transverse walls in their hyphae. Fungi lacking transverse walls are called Fecomistic Endofits and Fungi having transverse walls are Ascomycetes or Basidiomycetes (Klironomos et al., 2000). These fungi are rarely observed in their free-living state and are instead found dispersed around the host plant's roots, penetrating the intercellular spaces and even entering the host cells, where they form specialized structures such as split suction organs (Arbuscular) inside the cell and vesicles are inside or outside the host tissue, and mostly form spores or Sporocarps with complex structures. This type of mycorrhizal association is termed Vesicular-Arbuscular Mycorrhiza (Alizadeh, 2011).

3.3.1. ARBUSCULAR MYCORRHIZA FUNGI

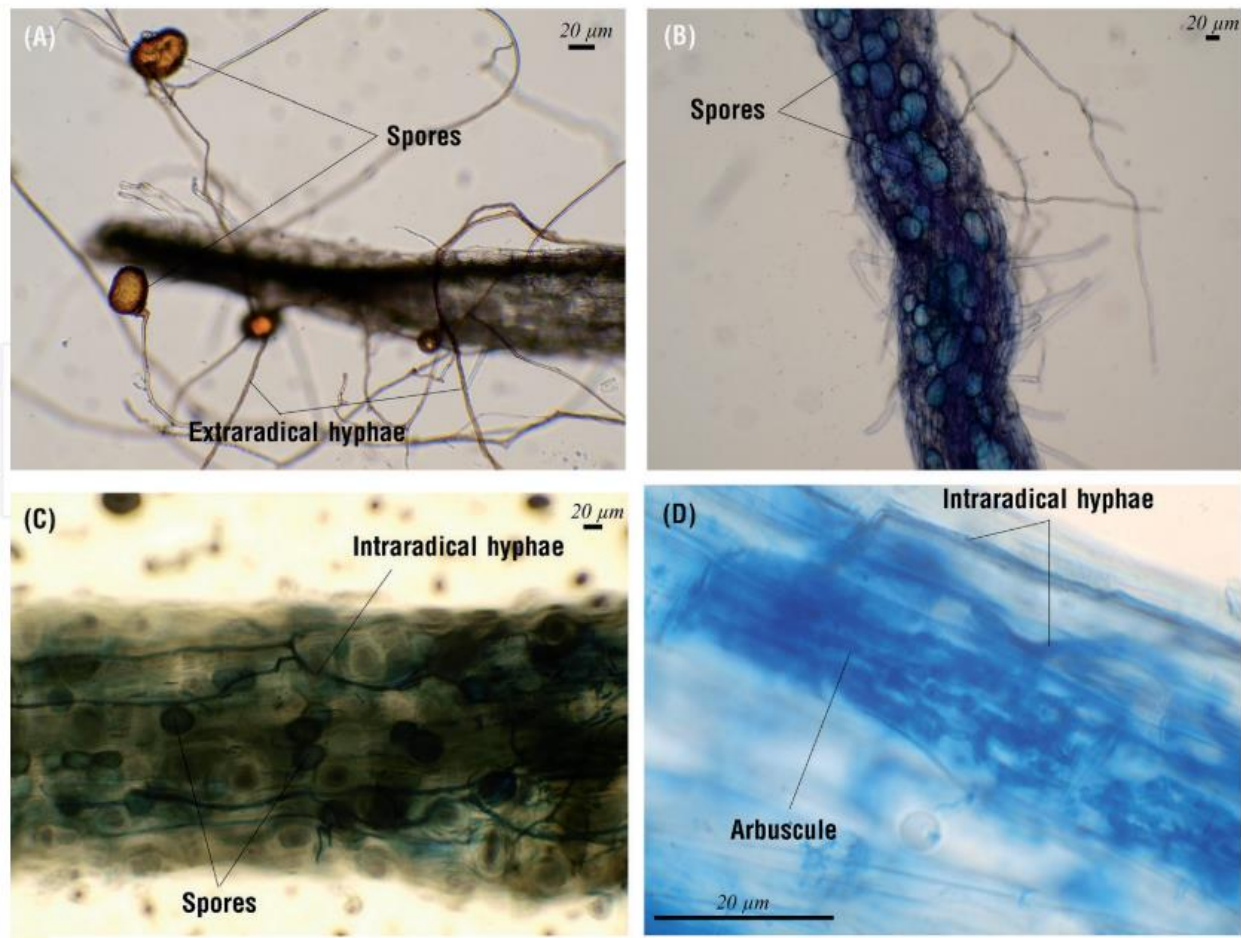


Figure 2: Tomato roots without (A) and with (B-D) staining showing fungal structures- arbuscules, vesicles, intraradical hyphae, and spore. (Posta et al., 2019)

Arbuscular mycorrhizal fungi belong to the endomycorrhizal group, where the fungal hyphae penetrate inside the plant root cells. AMF are classified within the Glomeromycota phylum and are distinguished by their capacity to develop highly specialized structures known as arbuscules inside the cortical cells of the host plant's roots (shown in Figure 2).

AMF are obligate biotroph root symbionts that inhabit most terrestrial environments. They can form a symbiotic relationship with approximately 80% of vascular plants and 90% of agricultural species (Smith & Read, 2008). AMF establish themselves in the cortical cells of roots where they aid in the uptake of macro and micronutrients, especially phosphorus and water and protects the host plant from various biotic and abiotic stresses. (Dowarah et al., 2021). This mutualistic association also involves the fungus receiving 10-20% of the host plant's total photosynthates and

lipids, while the plant benefits from enhanced absorption of water and mineral nutrients facilitated by the mycorrhizal partner (Posta et al., 2019). Plants in symbiosis with AMF can have a root surface area that is more than 100 times larger than plants without AMF association (Smith & Read, 2008) that merely expands the root's absorbing area.

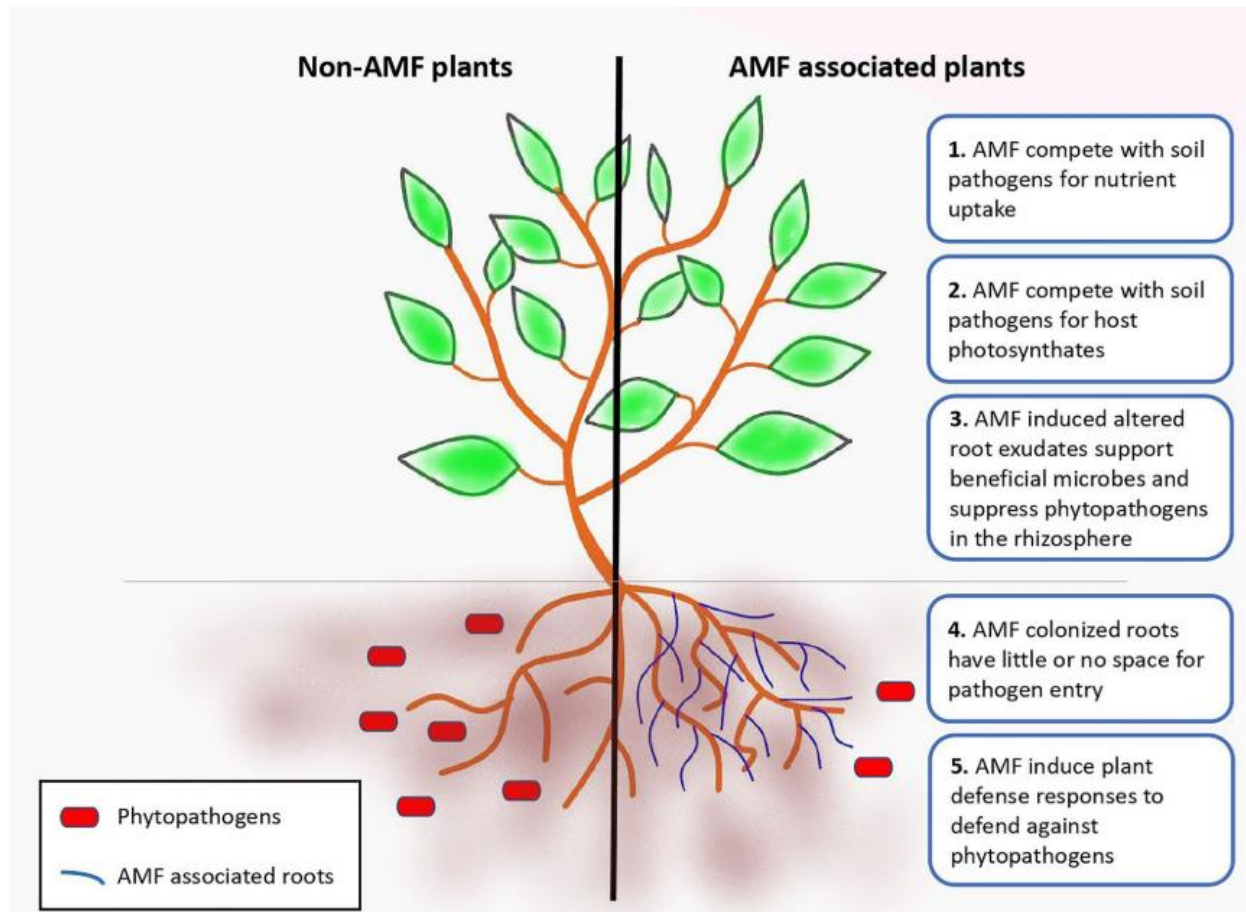


Figure 3: Different ways of AMF induced biotic stress tolerance in plants presented diagrammatically (Dowarah et al., 2021)

Before establishing the symbiotic relationship, during pre-symbiosis, both organisms communicate via oligosaccharides and butenolides. To attract their symbiotic partner and start cellular reprogramming for infection, plants and AMF emit a bouquet of diffusible molecules into the soil before coming into physical contact. In order to initiate the proper symbiotic responses, signalling cascades must be activated by plant and fungal receptor proteins upon perception of the signalling

molecules and cellular transmission of this information (Choi et al., 2018). The symbiotic reactions are induced by the release of Myc (mycorrhization) factors by fungi and strigolactones by plant root exudates. When these two elements work together, it turns on the signalling cascades downstream, which reinforce the link even more (Basu et al., 2018). Myc factors which are fungus-derived soluble compounds induce the expression of mycorrhizal-responsive genes, ultimately triggering phenotypic changes in the roots. SYM genes common to fungal endosymbioses are upregulated upon colonization by arbuscular mycorrhizal fungi and perception of the Myc factors by the roots, suggesting a conserved and coordinated gene signalling cascade in response to fungal colonization (Basu et al., 2018).

In the early symbiotic phase of arbuscular mycorrhizal symbiosis, the process begins with the development of appressoria, which are specialized structures formed by the fungus at the site of contact with the plant root. This morphological change signifies successful presymbiotic recognition between the fungal and plant partners, indicating their commitment to the interaction (Paszkowski, 2006). Changes in fungal gene transcription reflect this morphological shift (Breuninger & Requena, 2004). After that apoplastic fungal spread takes place. It refers to the movement of the AM fungus through the apoplast, which is the space outside the plant cell membranes, allowing for rapid colonization along the axes of root (Paszkowski, 2006).

In the mature symbiotic phase, the formation of haustoria is a key feature. Since arbuscules are an extreme form of intimacy and compatibility, they are the defining characteristic of the AM symbiosis. Arbuscules, which are dichotomous branched tree-like structures formed by the fungus, represent the hallmark of this mature phase (Paszkowski, 2006). They develop within the inner cortex cells of the plant and are crucial for nutrient exchange between the two partners. The formation of arbuscules involves extensive branching of the fungal hyphae, which can fill the living cortical cell. Overall, the mature symbiotic phase is characterized by a highly coordinated exchange of nutrients (Paszkowski, 2006).

Several studies have demonstrated that plants' ability to withstand environmental challenges may be enhanced through interaction with these beneficial microbes (Ma et al., 2019). Among them AMF is critically important in agricultural systems, as mycorrhizal fungi enhance plant nutrient and water uptake, stimulate growth (Posta et al., 2019; Smith & Read, 2008), enhance seedling

survival and development, promote crop uniformity, and enhance reproductive capacity (Posta & Hong Duc, 2020). Common mycorrhizal networks by AMF interconnect plants, this phenomenon plays a crucial role in redistributing nutrients and draining carbon from the different plant partners at varying rates (Ma et al., 2019). In fact, arbuscular mycorrhizal fungi are a cost-effective and sustainable solution for enhancing plant nutrition and resilience to environmental and biotic stresses (shown in Figure 3). By colonizing plant roots, the fungi not only promote mineral uptake but also trigger molecular and physiological mechanisms that prime the plant's systemic defence responses (Miozzi et al., 2020). For instance, to combat the drought stress, along with the increased water uptake a second mechanism entails an enhancement in the production of enzymatic and non-enzymatic antioxidants. The capacity of the antioxidant machinery governed by these enzymatic and non-enzymatic antioxidants work in plants to control and scavenge ROS. Moreover, the modified root physiology affects root-to-shoot cross-talk and helps to maintain homeostasis in terms of water, hormones, and ions in the plant (Bahadur et al., 2019). The mycorrhiza-induced resistance enhances the plant's tolerance to a range of challenges, such as pathogenic microbes, nematodes, and insect pests (Miozzi et al., 2020).

3.3.2. AMF MEDIATED PLANT PROTECTION

AMF enhances plant tolerance against various biotic stresses such as bacterial, fungal, and viral pathogens, as well as herbivores and the key mechanisms include- competition for nutrients, alteration of the rhizosphere, host defence induction (Dowarah et al., 2021). When AMF colonizes a plant's root, root its cortex becomes occupied with AMF mycelium, making it more difficult for infections to colonize cortex. Therefore, the soil borne pathogen compete for colonization and infection site (A. Singh et al., 2019). So, both AM and pathogen compete for infection site, space and food. When AMF has primary access to photosynthates, it may negatively impact pathogens (Poveda et al., 2020). However, there is no conclusive evidence that competition for photosynthates is a general mechanism for pathogen biocontrol by AMF symbiosis. But when nutrients are scarce, the competition for nutrients between AMF and soil borne pathogen occurs (Dowarah et al., 2021). So, competition for nutrients and food between these two is a possible mechanism of pathogen control by AMF.

The rhizosphere is the soil region directly influenced by plant roots, where various metabolites and signals are exchanged between roots and microorganisms. Arbuscular mycorrhizal fungi can alter the quality and quantity of root exudates, in turn impacting the rhizosphere microbiota which suppresses pathogen populations or recruit plant growth promoting rhizobacteria (PGPR). (Dowarah et al., 2021). With its both direct and indirect approaches, PGPR functions as a biofertilizer and biopesticide, respectively, to improve soil fertility, encourage plant growth, and reduce phytopathogens in order to create environmentally friendly, sustainable agriculture. (Gupta et al., 2015)

Plant metabolites have an impact on the microbial genome, and vice versa; as a result, alterations in the transcriptome, proteome, and ultimately the metabolome of the plant and microbes are brought about by the plant–microbe relationship (Kaur & Suseela, 2020). By increasing nutrient and water uptake, improving photosynthetic capacity, altering the concentration of phytohormones, and generating signalling molecules, the AMF symbiosis association with plant roots serves as an active bridge between the soil and the plant and improves the primary and secondary metabolites of plants (Amani Machiani et al., 2022). The carbon demands of AMF have an impact on the plant's primary metabolism, which in turn influences the quantity of organic acids, sugars, and amino acids (Kaur & Suseela, 2020). Plant primary metabolites undergo a wide spectrum of alterations as a result of AMF interaction. Increased amounts of sugars and amino acids, improved lipid synthesis and increased formation of organic acids are the key characteristics of these alterations (Amani Machiani et al., 2022; Kaur & Suseela, 2020). Different AMF species regulate the sugars, organic acids, and amino acids differently in different plant species. To maximize the plant's benefit and reduce the costs of the resource under biotic and abiotic stressors, they are mostly elevated or decreased. Additionally, depending on the plants' growth condition, AMF showed the capacity to either upregulate or downregulate plant metabolism. AMF-induced modifications to primary metabolites enhance the quality of plant products and offer protection against abiotic stressors like drought and salinity (Kaur & Suseela, 2020).

While primary metabolites are essential for the basic growth and development of plants, secondary metabolites play a key role in the plant's defence against biotic stressors like pathogens (Zaynab et al., 2018). Phenolic compounds are crucial secondary metabolites in defence mechanisms against biotic stressors. A potential reason for mycorrhizae's function in protecting plants against

pathogens is that they increase the phenolic content of plants (Charitha Devi & Reddy, 2002). The AMF colonized roots' upregulation of signalling pathways including hydrogen peroxide, salicylic acid, and nitrogen oxide pathways results in a greater synthesis of these phenolics (Zhang et al., 2013). Beginning with the signalling and carotenoid pathways and progressing to the phenylpropanoid pathway, the plant-AMF symbiotic interaction modifies the plant defence system (Kaur & Suseela, 2020). Moreover, increased resistance to pests and diseases, as well as higher-quality plant products, can be explained by the plant's increased amount of antioxidant chemicals (Kaur & Suseela, 2020).

4. MATERIALS AND METHODS

4.1. BIOLOGICAL MATERIALS, MYCORRHIZAL INOCULATION AND GROWTH CONDITIONS

Using molecular methods and spore morphology, arbuscular mycorrhizal (AM) fungi were identified as *Funneliformis mosseae* (Glomerales, *Glomeraceae*). This strain came from the collection of the Department of Microbiology and Applied Biotechnology of the Hungarian University of Agriculture and Life Sciences, Hungary. For three consecutive multiplication cycles, each lasting five months, *F. mosseae* was cultivated on maize (*Zea mays* L. 'Golda F1') grown on sterilized peat (Klasmann TS3, 100 mg L⁻¹ P₂O₅) and sand 1:3 (v/v) substrate.

In a greenhouse under controlled environmental conditions, tomato (*Solanum lycopersicum* Mill. Moneymaker var.) seeds were sown and cultivated in sterile soil. Half of the tomato plants were inoculated with AMF spores following standard inoculation procedures. Plants without AM fungal treatment were supplemented with 50 g of three-times-sterilized (121 °C for 60 min) inoculum, whereas mycorrhizal inoculation was carried out at the time of planting for each pot with 50 g (30 spores g⁻¹) of the sand containing the previously described inoculum.

Afterwards, selected 42-day-old tomato plants were then exposed to either cucumber mosaic virus subgroup Trk7 infection or powdery mildew (*Erysiphe neolycopersici*), mimicking biotic stress conditions. Infected plant material was used for powdery mildew inoculation, and mechanical

inoculation was performed with infected sap to cause CMV infection. The final treatment groups, which had six-six plants, were thus created:

- AMF- mock: no AMF, no infection;
- AMF+ mock: AMF inoculation, no infection;
- AMF- Trk: no AMF, CMV-Trk infection;
- AMF+ Trk: AMF inoculation, CMV-Trk infection;
- AMF- Pm: no AMF, powdery mildew infection;
- AMF+ Pm: AMF inoculation, powdery mildew infection.

4.2. ASSESSING BIOMASS AND ROOT COLONIZATION LEVEL

Leaf samples were collected 28 days after infection, and the fresh weight of the plant's roots, shoots, and biomass were all measured. and the dry weight of each sample was then measured after two days of drying at 70 °C in a hot-air oven. The roots were placed in a 10% aqueous solution of KOH (w/v). After heating the roots and KOH in a water bath at 90 °C for 60 minutes, the solution was decanted, and the roots were rinsed under running water. They were then rinsed with 5% vinegar (acetic acid) for one to two minutes, placed in 5% ink (Pelikan blue), and boiled for two minutes (Vierheilig & Piche, 1998). Mycorrhizal colonization was determined with the gridline intersection method by observing the presence or absence of mycorrhizal structures at the intersections between the root fragments and the gridlines (Giovannetti & Mosse, 1980).

4.3. RELATIVE GENE EXPRESSION OF *PAL2*

Total RNA was extracted from leaf samples using E.Z.N.A. Plant RNA kit made by the company named Omega Bio-tek Inc. (USA) following the manufacturer's protocol. RNA integrity was evaluated by Implen Nanophotometer (Implen GmbH, München, Germany). cDNA was synthesized from 1.0 µg of RNA by using RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific Inc., USA). Quantitative real-time PCR reactions were performed in a total volume of 25 µl containing 12.5 µl of Absolute SYBR Green Low ROX qPCR Mix, 1-1 µl of forward and reverse oligos (10 mM), and 9.5 µl of Nuclease Free Water, and 1 µl of 1:10 diluted template cDNA using Stratagene Mx3000 P Real-Time PCR System programmed with an initial

step of 15 min at 95 °C followed by 40 rounds of 15 s at 95 °C, 30 s at 59 °C, 20 s at 72 °C. Melting curve cycling consisted of 60 s at 95 °C, 30 s at 59 °C, 30 s at 95 °C. The primer sequences of the selected reference genes – elongation factor 1-alpha and actin –, as well as the gene specific primer of *PAL2* are presented in Table 1. Three biological replicates and technical triplicate qRT-PCR were implemented for each treatment. The results were analysed by $2^{-\Delta\Delta C_t}$ method.

Gene	Size	Primer sequence (5'-3')
Ef1α	184	F: TGCTGCTGTAACAAGATGGATG R: GCCAGTCAAGGTTGGTTGAT
Actin	200	F: CTTAGCACCTTCCAGCAGAT R: AAAAGAGGCTTGTACTGGAAACA
PAL2	100	F: ATTGCTGGTTTGCTCACTGG R: CACTAATACCAGCCACGCAG

Table 1. Sequences of the applied primers

4.4. STATISTICAL ANALYSIS

For statistical analysis, RStudio 2024.09.0+375 software was used. Data were tested for normality and homogeneity of variance using Shapiro-Wilk and Levene's tests, respectively Multivariate analysis of variance (MANOVA) was used to assess all the data. Means were compared by Tukey post hoc test at $p < 0.05$, except the dry weight of shoots where we used Games-Howell post hoc test. The results of the statistical analysis are presented in Table 2.

5. RESULTS AND DISCUSSION

5.1. COLONIZATION LEVEL

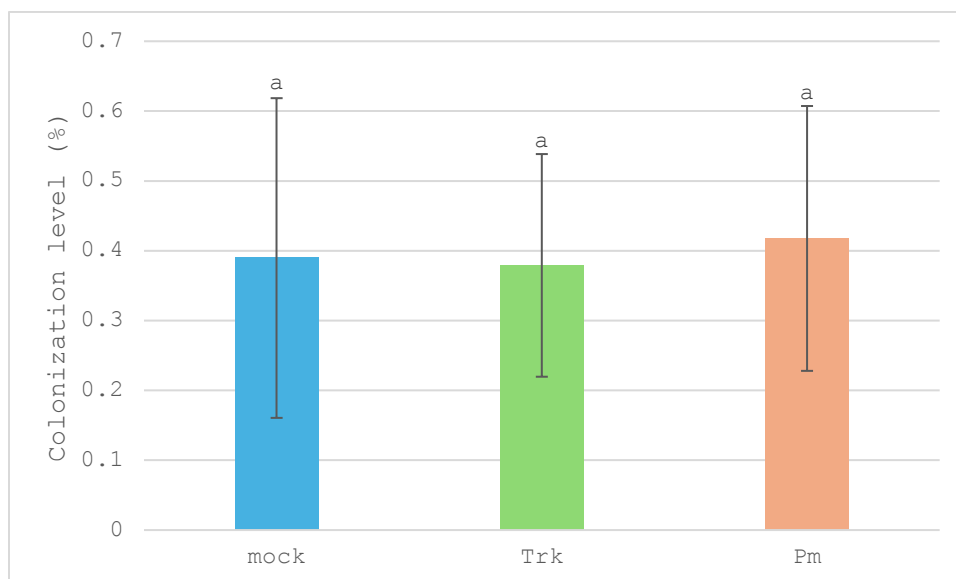


Figure 4. Mycorrhizal colonization level of the AMF inoculated plants. Mock: no infection; Trk: Trk7-CMV infection; Pm: powdery mildew infection. Different regular letters indicate significant differences among treatments according to Tukey's post hoc test at $P < 0.05$.

No statistically significant differences were observed in colonization levels of arbuscular mycorrhizal fungi across the treatments (Figure 4). This suggests that the observed effects are not attributable to variations in colonization levels, but rather to the specific treatment conditions.

5.2. FRESH AND DRY BIOMASS OF SHOOTS AND ROOTS

This section presents the fresh and dry weight measurements of shoot and root under the different treatment conditions described in the Materials and Methods section. Tomato plants were either inoculated with AMF or not inoculated and subjected to infection by CMV-Trk or powdery mildew (PM) pathogen.

Treatment Group	Shoot Fresh Weight (g)	Shoot Dry Weight (g)	Root Fresh Weight (g)	Root Dry Weight (g)	Total Biomass Fresh Weight (g)	Total Biomass Dry Weight (g)

AMF- mock	17.47286	4.695855	5.205603	0.418162	22.67846	5.114018
	SD-	SD-	SD-	SD-	SD-	SD-
	3.026674	0.813424	0.901722	0.072435	3.928397	0.885858
	c	ab	ab	c	ab	ab
AMF+ mock	26.15429	7.930444	5.938747	0.433125	32.09303	8.363568
	SD-	SD-	SD-	SD-	SD-	SD-
	6.433868	1.950863	1.460912	0.106547	7.89478	2.05741
	abc	c	b	bc	b	c
AMF- Trk	16.08333	4.322419	4.791629	0.384908	20.87496	4.707327
	SD-	SD-	SD-	SD-	SD-	SD-
	4.979738	1.338312	1.483589	0.119176	6.463327	1.457487
	ab	ab	ab	abc	ab	ab
AMF+ Trk	19.72857	5.982053	4.479686	0.326712	24.20826	6.308766
	SD-	SD-	SD-	SD-	SD-	SD-
	8.910854	2.701929	2.023351	0.147567	10.9342	2.849496
	bc	bc	ab	abc	ab	bc
AMF- Pm	10.08333	2.70991	3.004078	0.241315	13.08741	2.951226
	SD-	SD-	SD-	SD-	SD-	SD-
	2.655671	0.713715	0.791191	0.063556	3.446862	0.777271
	a	a	a	a	a	a
AMF+ Pm	16.379	4.966404	3.719112	0.271242	20.09811	5.237646
	SD-	SD-	SD-	SD-	SD-	SD-
	5.035788	1.52694	1.143456	0.083395	6.179244	1.610335
	ab	ab	a	ab	a	ab

Table 2: Mean of fresh and dry weights (g) of root and shoot samples of plants from each treatment group. AMF-: no AMF inoculation; AMF+: AMF inoculation; mock: no infection; Trk:

Trk7-CMV infection; Pm: powdery mildew infection. Different regular letters indicate significant differences among treatments according to Games-Howell and Tukey's post hoc test at $P < 0.05$.

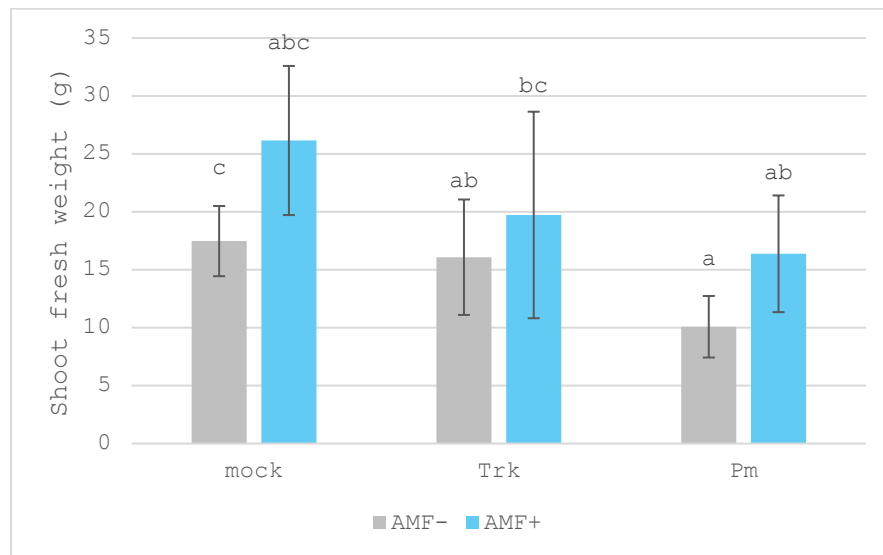


Figure 5. Fresh weight of plant shoots. AMF-: no AMF inoculation; AMF+: AMF inoculation; mock: no infection; Trk: Trk7-CMV infection; Pm: powdery mildew infection. Different regular letters indicate significant differences among treatments according to Tukey's post hoc test at $P < 0.05$.

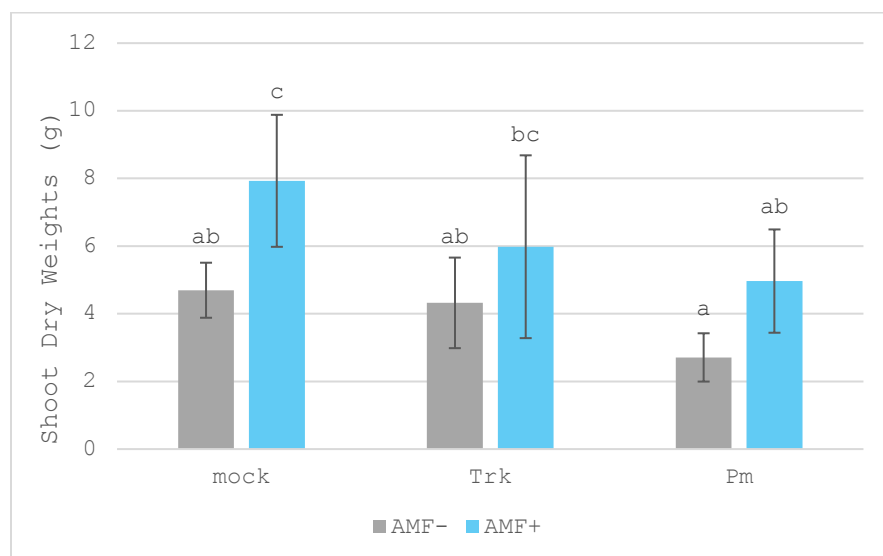


Figure 6. Dry weight of plant shoots. AMF-: no AMF inoculation; AMF+: AMF inoculation; mock: no infection; Trk: Trk7-CMV infection; Pm: powdery mildew infection. Different regular letters indicate significant differences among treatments according to Tukey's post hoc test at $P < 0.05$.

letters indicate significant differences among treatments according to Games-Howell post hoc test at $P < 0.05$.

AMF- mock is the absolute control which is neither inoculated with AMF nor infected with pathogens. Both pathogen treatments resulted in significantly decreased shoot fresh weight (AMF- Trk and AMF- Pm) by 7.953% and 42.291% respectively, meanwhile AMF inoculation (AMF+ mock, AMF+ Trk and AMF+ Pm) compared to the not inoculated one's elevated shoot fresh weight by 49.685%, 22.665% and 62.436% respectively as it is shown on Figure 5. Similarly, the pathogen infections decreased shoot dry weight (Figure 6) by 7.952% and 42.291% respectively compared to the not infected plants. However, in each case when we compare the AMF inoculated and not inoculated plants, the presence of AMF elevated it by 68.881%, 38.396% and 83.268% respectively.

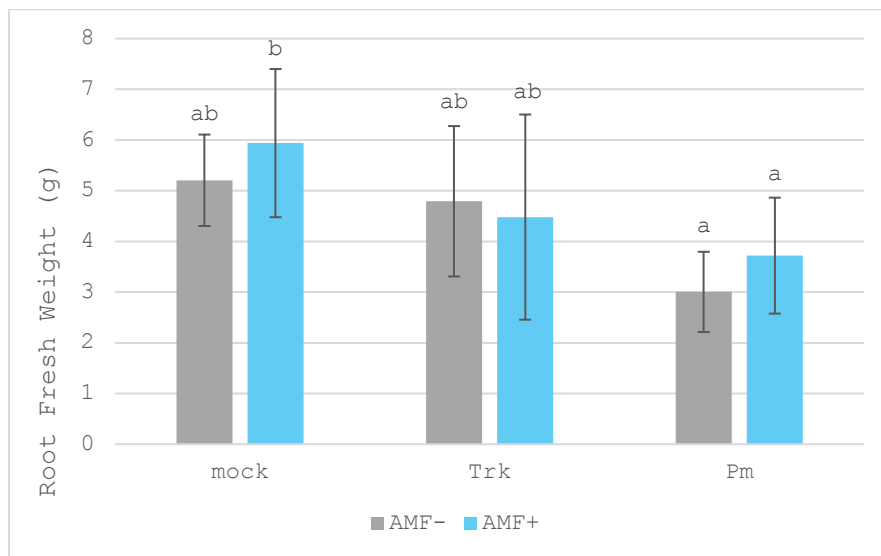


Figure 7. Fresh weight of plant roots. AMF-: no AMF inoculation; AMF+: AMF inoculation; mock: no infection; Trk: Trk7-CMV infection; Pm: powdery mildew infection. Different regular letters indicate significant differences among treatments according to Tukey's post hoc test at $P < 0.05$.

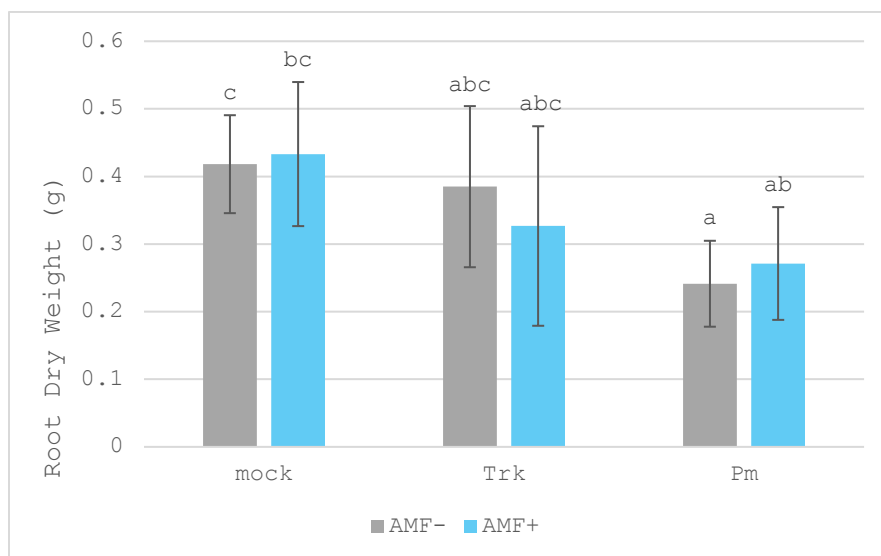


Figure 8. Dry weight of plant roots. AMF-: no AMF inoculation; AMF+: AMF inoculation; mock: no infection; Trk: Trk7-CMV infection; Pm: powdery mildew infection. Different regular letters indicate significant differences among treatments according to Tukey's post hoc test at $P < 0.05$.

Same kind of tendency was found in root fresh and dry weights (Figure 7 and 8) where both pathogens (Trk and Pm) decreased both root fresh and dry weight by 7.952% and 42.291% respectively compared to the not infected plants. The AMF inoculation increased root fresh weight by 14.084% and 23.802% in not infected (AMF+ mock) and Powdery mildew (AMF+ Pm) respectively when we compared them to the not inoculated plants. But in case of CMV-Trk infection (AMF+ Trk), the root fresh weight and root dry weight showed a decrease by 6.510% and 15.119% respectively, but this was not significant.

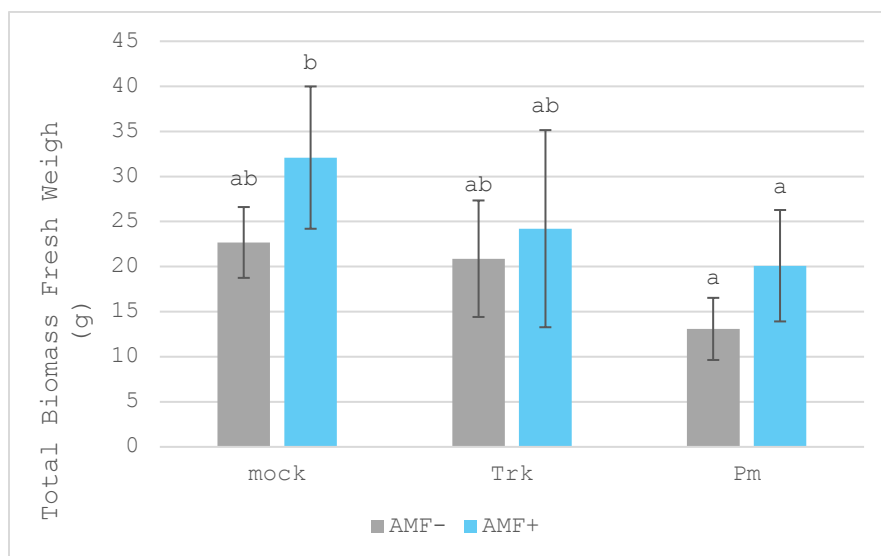


Figure 9. Fresh weight of plant's total biomass. AMF-: no AMF inoculation; AMF+: AMF inoculation; mock: no infection; Trk: Trk7-CMV infection; Pm: powdery mildew infection. Different regular letters indicate significant differences among treatments according to Tukey's post hoc test at $P < 0.05$.

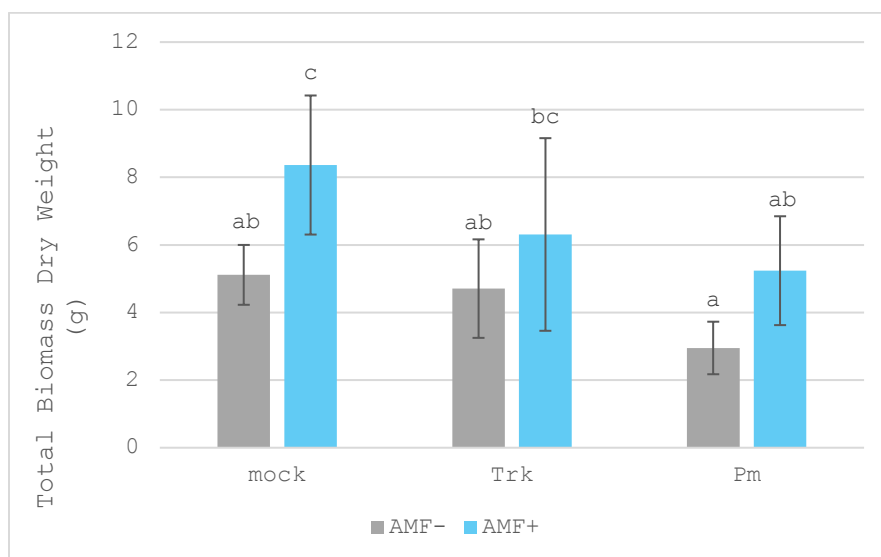


Figure 10. Dry weight of plant's total biomass. AMF-: no AMF inoculation; AMF+: AMF inoculation; mock: no infection; Trk: Trk7-CMV infection; Pm: powdery mildew infection. Different regular letters indicate significant differences among treatments according to Tukey's post hoc test at $P < 0.05$.

Comparing to the not infected plants, the total biomass fresh weight was decreased by 7.95% and 42.291% in Trk infected and Pm infected groups respectively. It showed an increase in AMF inoculated groups (AMF+ mock, AMF+ Trk and AMF+ Pm) by 41.513%, 15.968% and 53.568% respectively. Likewise, the total biomass dry weight in Trk and Pm pathogen infected plants was declined by 7.952% and 42.291% respectively and mycorrhiza inoculation elevated this parameter by 63.542%, 34.02% and 77.474% respectively.

Based on our results, arbuscular mycorrhizal fungal inoculation has a beneficial impact on plant growth parameters such as fresh and dry weight of shoots, roots and total biomass, which matches with the findings of Qin et al., (2020) and Wu et al., (2024). Specifically, for non-infected plants, AMF-inoculated plants exhibited significantly higher dry shoot and total biomass weights compared to non-inoculated plants. However, this significant difference diminished in the presence of pathogens. The biomasses were negatively affected by both the biotic stressors, which is in accordance with the observations of Miozzi et al., (2020) regarding CMV infection and Sunarti et al., (2022) in the case of powdery mildew infection.

5.3. RELATIVE GENE EXPRESSION OF *PAL2*

Plants are constantly subjected to various environmental stressors, among which biotic stress – caused by living organisms such as pathogens – poses significant challenges to their growth and survival. The salicylic acid pathway plays a crucial role in plant defence against biotrophic pathogens, which depend on living host tissue for nourishment. Upon infection, plants activate the SA signalling cascade, leading to the expression of pathogenesis-related genes and the synthesis of defensive compounds. This pathway not only enhances the plant's innate immune response but also mediates systemic acquired resistance, enabling protection against subsequent infections. Additionally, the SA pathway interacts with other hormonal signalling networks, such as those involving jasmonic acid and ethylene, to fine-tune the plant's defensive strategies. Understanding the SA pathway's mechanisms can inform the development of disease-resistant crop varieties and improve agricultural practices. One of the key components of the SA pathway's regulation is the phenylalanine ammonia-lyase, which catalyzes the conversion of L-phenylalanine into trans-

cinnamate- which is the initial committed step of the multi-branched phenylpropanoid pathway in higher plants. Since this biochemical reaction is the first step of phenolic metabolism, PAL is a vital enzyme in both plant development and pathogen defence (Chang et al., 2008).

To assess its function in the defence response of tomato plants subjected to AMF inoculation and biotic stress (CMV or powdery mildew infection), the expression of the *PAL2* gene was evaluated.

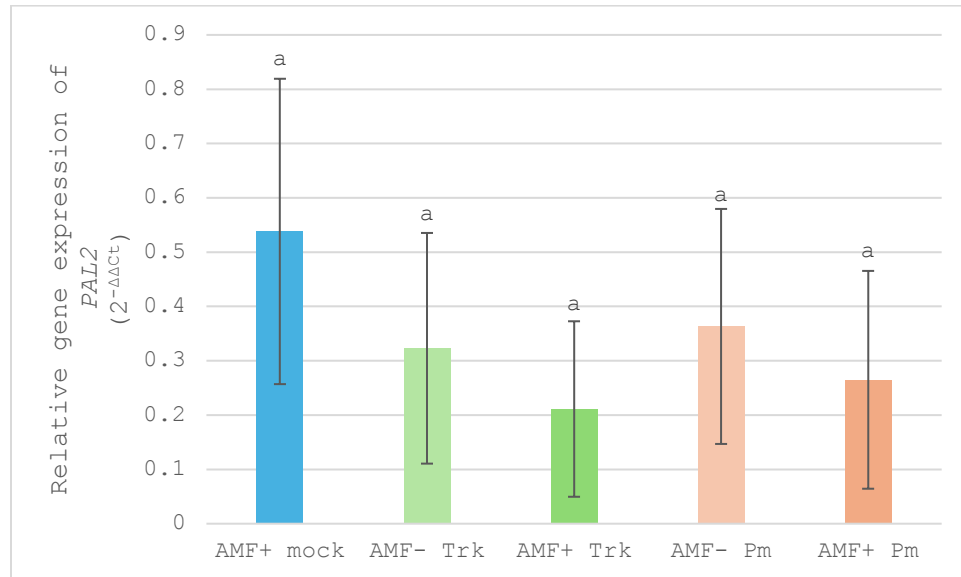


Figure 11. Relative gene expression of *PAL2*. AMF-: no AMF inoculation; AMF+: AMF inoculation; mock: no infection; Trk: Trk7-CMV infection; Pm: powdery mildew infection. Different regular letters indicate significant differences among treatments according to Tukey's post hoc test at $P < 0.05$.

Both AMF inoculation and pathogen infection resulted in an increased *PAL2* gene expression level compared to the absolute control. We observed the highest expression level in the case of mycorrhizal plants without infection. This is an evident result as arbuscular mycorrhizal fungi are obligate biotroph microorganisms. Our results support and complement the study of (Gallou et al., 2012), when they observed the upregulation of *PAL2* in *Solanum tuberosum* due to mycorrhizal inoculation.

The presence of both CMV and powdery mildew induced the expression of the *PAL2* gene, a response consistent with their classification as biotrophic pathogens. This activation can be

attributed to the stimulation of the salicylic acid pathway, a key component of the stress response mechanism in tomato plants triggered by biotrophic pathogen attacks. In the combined treatments involving both *F. mosseae* inoculation and pathogen exposure, we did not observe that elevated gene expression levels that were apparent in the individual treatment groups. The observed increase in the relative gene expression of *PAL2* due to powdery mildew infection supports the findings of (Romero et al., 2008). It is noteworthy that the changes in *PAL2* expression observed in our study were only trends and did not achieve statistical significance. Our findings diverge somewhat from those of (Miozzi et al., 2020), who reported an increase in *PAL2* expression after *F. mosseae* inoculation. In contrast, their study found that viral infection resulted in a decrease in *PAL2* expression, with no significant differences in gene expression observed in plants subjected to both AMF and viral treatments compared to control plants. This divergence could be due to the different sampling time as we examined older plants, these findings indicate that it is important to gather information during the whole process to have a deeper understanding about how does the presence of both AMF and CMV have an impact of stress response genes of the plant.

Our results indicate that the high expression level of *PAL2* due to the *F. mosseae* inoculation result in a more resilient plant to infections, which is supported by the findings of (Maher et al., 1994) and the slightly lower gene expression level in the dual treatments.

Our study aimed to gain a deeper understanding of the influence of *F. mosseae* on plant stress responses under biotic stress, specifically pathogen infection like Trk7-CMV or powdery mildew. While our findings reveal observable trends that support our hypothesis, the lack of statistically significant differences indicates that further investigations are necessary. The beneficial impact of AMF was evident in terms of biomass accumulation; however, additional evidence at the molecular level is required to substantiate these effects.

6. SUMMARY

Arbuscular mycorrhizal fungi assist plants in coping with various abiotic and biotic stresses, including drought, salinity, heavy metal toxicity, and pathogen infections. Through their symbiotic association with plant roots, AMF enhance the absorption of water and nutrients, while also influencing stress-related genes, antioxidants, and hormone levels, thereby boosting plant

resilience. While their role in helping plants withstand abiotic stress is well-established, further research is required to fully comprehend their effects on biotic stress, which could support sustainable agricultural practices and environmental conservation efforts. Our study aims to shed light on how mycorrhizal interaction influences the *PAL2* gene expression of tomato plants (*Solanum lycopersicum* Mill.) during viral or fungal infections. PAL is one of the key enzymes of the salicylic acid pathway, which is induced by biotic stress, especially by biotrophic pathogens.

Tomato seeds were germinated and grown in a sterilized substrate under controlled greenhouse conditions. One group of plants was inoculated with *Funneliformis mosseae* (AMF+) following standard procedures, while the other group remained uninoculated, serving as controls (AMF-). After 6 weeks of growth, both the inoculated and control plants were subjected to either powdery mildew or Cucumber Mosaic Virus infection to mimic biotic stress conditions. Following 4 weeks of growth after infection, the plants were harvested, and their fresh and dry weights were assessed to estimate the physiological condition of them. Additionally, we analysed the changes in the expression of PAL gene by quantitative real time PCR to characterise the stress response of the tomato plants. According to our examinations the combined treatments (AMF and pathogen) resulted in the lowest gene expression level, which could be an indicator of the mitigating impact of AMF inoculation.

Our study sought to explore whether arbuscular mycorrhizal fungi influence plant stress response during pathogen infections. While the positive effect of AMF on biomass was statistically significant, the analysis of relative gene expression showed only trends without significant differences. These findings suggest that AMF may enhance plant resilience to biotrophic pathogens, potentially through the preliminary activation of the salicylic acid pathway. Further research is needed to substantiate these observations in gene expression pattern.

7. ACKNOWLEDGEMENTS

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