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In tomato plants, arbuscular mycorrhizal fungus regulate the growth-defense trade-off and mitigate the detrimental effects of drought.

Indire Adnamia¹, Rhdi Febriamasah²

Faculty of Animal Husbandry, Universitas Andalas, Kampus UNAND Limau Manis Padang, West Sumatra 25163 Indonesia

Research Article

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Abstract

A variety of arbuscular mycorrhizal fungi (AMF) may be used as biofertilizers in agricultural soils to boost crop productivity and growth. AMF may boost plant defences against a variety of herbivores and diseases, according to recent study. However, our current incapacity to forecast the performance of various AMF-plant complexes in changing settings has significantly hindered the effective application of AMF in agriculture. For example, AMFs may reduce allocation constraints related to growth vs defences by boosting plant foraging capacity. However, the in situ variables may determine whether this impact takes place. Investigating the context-dependency of AMF's capacity to regulate plant development and resistance against herbivores under varying soil water availability was the primary objective of this work. In order to achieve our objective, we conducted a greenhouse experiment in which we measured the effects of various AMF inocula (*Funneliformis mosseae*, *Rhizophagus irregularis*, or both) on the growth and defences of tomato plants (*Solanum lycopersicum*) against an insect herbivore under two conditions: normal watering regimes and drought conditions. We assessed the plants' physiological, chemical, and functional characteristics. Findings: We discovered that the presence of AMF often reduced plant development while boosting chemical defences and resistance to generalist caterpillars. Nevertheless, the mycorrhizal inoculum's identity and the water content of the soil determined this growth-defense trade-off. Inoculated tomato plants reduced their defensive investment under drought, but noninoculated plants reduced their growth. In conclusion, our work emphasises how fungal identity and abiotic variables affect plant-AMF-herbivore interactions. More broadly, our findings highlight the need to identify AMF species with less context-dependency on climatic parameters in order to increase their usage in organic

KEYWORDS

Glycoalkaloids, hydric stress, climatic change, chemical defence, biological control, and plant-microbe-insect relationships

1 | INTRODUCTION

Plants are continuously attacked by herbivores since they are at the base of the food chain. As a result, plants have developed a variety of defence mechanisms, such as the development of physical features like trichomes or spines, the synthesis of poisonous compounds (often referred to as plant secondary metabolites), or the reallocation of resources within their tissues (Walters, 2010). Both wild and farmed plants always struggle with how to divide their energy between growth, reproduction, and defences due to the energy-intensive nature of defence

manufacturing (Cipollini & Redman, 1999; Gershenzon & Dudareva, 2007) (Herms & Mattson, 2015). In particular, plants must manage their resources along many axes of trade-offs throughout their lives, such as deciding whether to use energy to create chemical defences or new tissues (plant development) (Herms & Mattson, 2015). The availability of resources primarily determines how strong these trade-offs are (Coley et al., 1985; Defosse et al., 2018; Rasmann et al., 2011). For instance, when resources are few, plants often develop more slowly in order to focus their energy on defences since even minor tissue losses may be lethal to the plant (Karban, 2011). Plants may connect with a variety of rhizosphere- or root-associated microorganisms (fungi and bacteria) to reduce resource restrictions. Soil bacteria improve plants' intake of water and minerals in return for photosynthetic products or root exudates (Schnitzer et al., 2011). Particularly in situations when nutrients are scarce or when dealing with other environmental stressors like drought, heat, salt, flooding, or heavy metals, association with soil microorganisms may promote plant development (Grover et al., 2011; Kumar & Verma, 2018; Singh et al., 2018; Vimal et al., 2017). Furthermore, soil microbes have the ability to induce defence responses and promote constitutive resistance against both aboveground and belowground plant consumers, such as pathogens and herbivores (Formenti & Rasmann, 2019; Guerrieri et al., 2004; Lugtenberg & Kamilova, 2009; Martinez-Medina et al., 2016; Van Wees et al., 2008). As a result, the plants' deficiencies in water and nutrients are lessened by the interaction with beneficial bacteria, which reduces the growth-defense trade-off (Dias et al., 2017; Weyens et al., 2009; Yang et al., 2009). Arbuscular mycorrhizal fungi (AMF; Glomeromycota) are a diverse collection of soil-dwelling microorganisms that may form associations with several plants in almost every terrestrial habitat (Smith & Read, 2010). By promoting plant growth and reproduction (Bennett & Bever, 2007; Koide, 1991, 2010; Wilson & Hartnett, 1997) and modulating plant-herbivore interactions (Gange, 2001; Hartley & Gange, 2009; Papantoniou et al., 2021; Pozo et al., 2009), AMF play a number of important functions in plant health. AMF-mediated stress tolerance in plants is ubiquitous and prevalent, although it greatly depends on the kind of contact. This encompasses the genetic makeup of the interacting partners as well as the environment or soil quality in which the interaction is occurring (Davitt et al., 2011; Zytynska, 2021). Although AMF treatment alone is a very promising substitute for synthetic fertilisers and pesticides (Gosling et al., 2006), its context-dependency necessitates more study to determine how it influences the results of AMF-plant interactions (Hoeksema et al., 2010; Simard & Durall, 2004). Understanding the efficacy of microbe-based products in a variety of environmental conditions is crucial because they exhibit varying degrees of efficacy (Hart et al., 2018; Holland et al., 2018; Kokkoris et al., 2019) (Hartman & Tringe, 2019; Lenoir et al., 2016; Teotia et al., 2017; Yadav et al., 2017). The considerable variability of microbial-based fertiliser effects may be better understood and their commercial use encouraged by experimentally evaluating the context-dependency of plant-microbe interactions in terms of plant growth-defense trade-offs (Bulgari et al., 2019; Lee Díaz et al., 2021). The rise in drought occurrences brought on by climate change is one of the biggest issues facing agricultural growers today (Li et al., 2009; Olesen et al., 2011; Porter & Semenov, 2005). In a number of situations, soil microorganisms have been shown to reduce drought stress (Kim et al., 2012). For example, it has been shown that the AMF species *Rhizophagus irregularis* and *Funneliformis mosseae* reduce drought stress in potato (*Solanum tuberosum*) and tomato (*Solanum lycopersicum*) plants (Latef et al., 2016; Yooyongwech et al., 2016). Thus far, the majority of research has focused on how individual AMF strains affect plants. However, more complex mixes of AMF are seen in both commercial goods and natural soils (Brígido et al., 2017). Studies on the impact of AMF on plant health and development across habitats have shown conflicting results, most likely due to the biological complexity of the soil (Bennett & Bever, 2007; Bennett et al., 2018; Maherali & Klironomos, 2007). Fungal-plant-insect interactions may also be studied using the microbial consortium strategy, which involves inoculating many species simultaneously (Aguilera et al., 2022). Studying how soil microbial combinations respond to abiotic stressors like drought is crucial for crop productivity and product development. We investigated how

combinations of several AMF species can support plant development and defences under drought circumstances in order to close these gaps. As a model system for tomato crop production, we concentrated on the domesticated tomato *Solanum lycopersicum* "MoneyMaker." In particular, we posed the following question: (1) Do AMFs typically help tomato plants under abiotic stress? We hypothesised that mycorrhized plants would outperform nonmycorrhized plants in terms of biomass production, chemical defence, and resistance to insect herbivores under drought. (2) When it comes to defending tomato plants against herbivory under drought stress, is the combination of two AMF strains superior than single-strain inoculations? We anticipated that compared to single-strain inoculations, the double-strain inoculation would boost plant growth defences and resistance. Lastly, we posed question (3): What impact does AMF inoculation have on potential growth-defense trade-offs for plants? We hypothesised that because AMF might boost plant resource acquisition, it should mitigate certain growth-defense trade-offs. In order to clarify the impact of abiotic elements while utilising AMF-based fertilisers and biostimulants for tomato production, our research attempts to better understand the context-dependency in plant-microbe-herbivore interactions.

MATERIAL AND METHODS

1.1 | Biological material

We grew tomato seedlings (*Solanum lycopersicum*, cultivar "MoneyMaker," Sativa Rheinau, Switzerland) that were infected with several AMF species under various watering schedules in order to answer our enquiries. Single inoculations of AMF *Rizophagus irregularis* (previously *Glomus intraradices*) and *Funneliformis mosseae* (Glomeraceae), as well as their mixtures, comprised the AMF therapy. The two AMF species were grown in sandy soil using *Plantago lanceolata* as a nursery plant species, and they came from the Swiss collection of AMF (Agroscope) (Wagg et al., 2011).

1.2 | Experimental design

Tomato seeds were surface-sterilized for ten minutes in a water-sodium hypochlorite (5% NaClO) solution, properly washed with deionised water, and then allowed to germinate on autoclaved potting soil (Ricoter). Before being transplanted into inoculated soil five weeks after germination, the seedlings were kept in the greenhouse with natural sunshine at 24° C/18° C day/night. Eleven individual seedlings (n = 11) were placed in 3 L plastic pots with a sterile combination of potting soil, wood fibre, and sand (2:2:1) for each treatment group. The mycorrhizal treatment, which involves adding 5% (v:v) of the AMF substrate inoculum to a sterile soil mixture, was applied to the inoculated plants upon transplanting. The identical quantity of autoclaved AMF substrate was given to the control plants. To further homogenise the microbial population across all treatments, a 5 ml aliquot of a filtrate (<20 µm) of the inoculum substrate was given to each plant. No additional fertiliser was applied to the plants. The watering treatment began immediately upon transplanting and inoculation, and the insect bioassay experiment was carried out nine weeks later and one week before to plant harvesting. We used a full-factorial randomised design with two main factors to test for the interaction between drought and various AMF inoculations: (1) AMF treatments with four levels (*R. irregularis* inoculation (R), *F. mossae* inoculation (F), both AMF species together (RF), and a control (C) without AMF); and (2) watering treatments with two levels (normal watering regime and drought; 11 replicates per treatment, totalling 88 plants). For the regular watering regime, the watering treatments included either watering to maintain field capacity or, for the drought regime, not watering until the field reached the wilting threshold.

1.3 | Measurements of functional traits and samples collection

The following functional characteristics of plant growth and biomass accumulation were assessed on each duplicate at the conclusion of the experiment: (1) Plant height (mm), expressed as the length of the main stem from the terminal apex to the glycoalkaloids and tomatine cotyledon insertion point. (2) A chlorophyllometer (SPAD, Konica Minolta, Inc.) was used to monitor the leaf's photosynthetic activity six weeks after inoculation. Each plant's terminal leaflet of the final five completely grown leaves was measured, and statistical analyses were conducted using

the mean value per plant. (3) Specific leaf area (SLA $\text{cm}^2\cdot\text{mg}^{-1}$) was computed by dividing the dry weight by the area of a fully extended leaf from the plant's center. The public domain program ImageJ was used to calculate leaf area (Schneider et al., 2012). (4) The ratio of carbon to nitrogen (CN), as determined by an organic elemental analyser (Flash2000, Thermo Scientific). Lastly, we assessed the plants' fresh biomass (5) aboveground (stem and leaves) and (6) belowground (roots). In order to determine the AMF colonisation rate, fresh roots were gathered, frozen, and preserved. For metabolomic investigations, leaf samples were gathered and quickly frozen in liquid nitrogen.

1.4 | Insect resistance bioassay

One fully developed terminal leaf was taken from each plant nine weeks following AMF inoculation and the beginning of the watering schedules. It was then put in a 250 cc plastic box with the petiole kept damp and covered with moist tissue. Five caterpillars (2nd instar) of the extremely polyphagous Egyptian cotton leafworm (*Spodoptera littoralis*, Lepidoptera, Noctuidae) weighing 55 mg ($\pm 10\%$) were placed in each box ($n = 11$ per treatment; 88 total). The eggs of caterpillars supplied by the University of Neuchâtel's Farce laboratory were previously fed tomato leaves and developed to the second instar. For seven days, the larvae were kept in the leaf-filled enclosures at 22°C with a 12-hour photoperiod. Lastly, a larval weight increase value for each plant duplicate for all treatments (a total of 440 caterpillars) was calculated by averaging the fresh biomasses of the five caterpillars.

1.5 | Determination of mycorrhizal inoculation

After thoroughly cleansing the roots under tap water, mycorrhizal inoculation was confirmed. After 30 minutes at 90°C in KOH (10%), the roots were rinsed with water that had been acidified with 1% acetic acid. The technique outlined in Vierheilig et al. (2005) was used to colour the fungal structures found in the roots using a staining solution (5% blue ink diluted in 5% acetic acid). The gridline intersection approach (Giovannetti & Mosse, 1980) was used to determine the mycorrhizal colonisation rate under a 100X stereo microscope (Leica MGD33). Taking into account hyphae, arbuscules, and vesicles, we computed the colonisation rate as the proportion of root length colonised by the AMF.

1.6 | Plant secondary metabolites

The impact of AMF, drought, and herbivory on key defence metabolites in tomato plants was evaluated using a targeted metabolomics method. Among these were the two dehydrotomatines and the flavonoid quercetin-3-O-rutinoside, or rutin. Each plant replicate's leaf samples were extracted using a method based on De Vos et al. (2012). In short, freeze-dried leaf material was ground into a powder, and 20 mg of each sample was double extracted using an extraction solution, MeOH HPLC grade/Acetate buffer pH 4.8 (75:25, v/v%), in 2 ml reaction tubes containing two glass beads. The tubes were shaken in a TissueLyser (Qiagen) at 30 Hz for five minutes, and then centrifuged at 15,000 rpm for ten minutes at 4°C . Before being processed further, the clear supernatants were mixed and kept at -20°C . The diluted crude extracts (1:50) were then analysed using an UltiMate™ 3000 Standard Ultrahigh-Pressure Liquid Chromatography system (UHPLC, Thermo Scientific) fitted with an Acclaim® Rapid Separation Liquid Chromatography (RSLC) 120 column (150×2.1 mm, particle size $2.2\ \mu\text{m}$, ThermoFisher Scientific) using the following gradient at a flow rate of 0.4 ml/min. Using an Apollo II electrospray ion source and an ESI-UHR-QToF-MS (maXis impact quadrupole time-of-flight mass spectrometer, Bruker Daltonics) with the following instrument parameters in positive ion mode, the eluted chemicals were found between m/z 90 and 1600 at a spectra rate of 3 Hz: scan range: 90–1600 m/z ; 3 Hz acquisition rate; 500 V end plate offset; 3500 V capillary voltage; 2 bar nebuliser pressure; 10 L min⁻¹ dry gas; and 220°C dry temperature. Sodium formate clusters (10 mM NaOH solution in 50/50 (v/v) isopropanol water containing 0.2% formic acid) were used for mass calibration. Lastly, *mzMine* 2.53 was used to baseline-correct, deconvolute, and align the chromatograms from the raw QToF-MS Bruker data files. *R* 4.0.5 and *mzMine* 2.53 (Pluskal et al., 2010) were used to assign m/z groups to features. *R* Core

Team (2021) using the packages "xcms" (Smith et al., 2006) and "CAMERA" (Kuhl et al., 2012). Based on their fragmentation spectra at low energy under electrospray ionisation (ESI) in a positive mode, we were able to identify the substances rutin, alpha-tomatine, and dehydrotomatine using the unique observed molecular characteristic (Supporting Information: Figure S1). Based on the peak area of the total fragmented ions, we utilised the relative quantities of the molecules for the analysis.

2 | STATISTICAL ANALYSIS

R4.0.5 was the software used for the analyses (R Core Team, 2021). To answer the questions in the introduction, we conducted the following analyses. The nine plant traits (plant size, leaf chlorophyll index (SPAD), SLA, CN), aboveground biomass, belowground biomass, insect weight gain, alpha-tomatine, dehydrotomatine, and rutin) were compared across all treatment combinations using a two-way multivariate analysis of variance (MANOVA, implemented with Wilks' lambda test). Univariate analyses of variance (ANOVAs) were then used to test for individual main effects. Similarly, two-way ANOVAs were used to evaluate the impact of AMF and dryness on larval weight increase. To meet the homoscedasticity of variance criteria, chemical characteristics and larval weight increase were log-transformed prior to analysis. (1) We determined the effect size within each AMF treatment to evaluate the impact of various inoculations on plant growth, defence, and drought responses. Cohen's d measure (Cohen, 2013) was used to determine effect sizes, which were estimated using the "effsize" package (Torchiano, 2020). In this instance, a positive impact size means that each treatment's plants fared worse during the drought than they did on the regular watering schedule. A principal component analysis (PCA) computed using the "ade4" package (Dray & Dufour, 2007) was used to ordinate all plant functional attributes assessed (as previously stated) in order to summarise plant growth responses inside a single variable. The effect sizes were obtained using the PCA's first axis. Then, as proxies for plant chemical defences and plant insect resistance, respectively, we computed effect sizes on alpha-tomatine (known for defensive qualities) and larval biomass weight increase. (2) Using the first axis of the coinertia analysis between the three chemical defence features and the seven growth-related variables, we created an interaction plot to evaluate the impact of AMFs and drought on the plant growth-defence trade-off. A strong co-structuration (i.e., correlation) between the plant growth features and the chemical defence matrix is shown by the coinertia analysis (coin function in the "vegan" package [Oksanen et al., 2020]). The "vegan" package's nonmetric multidimensional scaling (NMDS) was used to depict this multi-trait trade-off. Permutational multivariate ANOVA (PERMANOVA, utilising the adonis function in the "vegan" package) was used to investigate the AMF $\times$ drought treatment impact. For both the NMDS and PERMANOVA, the euclidean metric was used to determine sample dissimilarity; nevertheless, the results held up well to other distance measures. Lastly, the vegan package's envfit function was used to fit larval weight growth on the NMDS ordination.

3 | RESULTS

3.1 | Are AMFs beneficial for tomato plants under drought stress?

3.1.1 | Effects of drought on AMF colonization

The species of AMF and how they interacted with drought (D) determined AMF colonisation (see significant AMF and D $\times$ AMF interaction effects in Table 1). All AMF species showed uniform colonisation rates of 3.7%, 4.8%, and 4.2% for F (*F. mossae*), R (*R. irregularis*), and RF under drought circumstances. *R. irregularis* had 8.3% root colonisation under the typical watering schedule, but *F. mossae* only had 2.6% of the root length (Table 1, Supporting Information: Figure S2). The lowest colonisation rate, around 0.6% of the root length, occurred when both species were present (Table 1, Supporting Information: Figure S2).

3.1.2 | Effects of AMF and drought on plant functional traits

Drought and AMF treatments had significant main and interacting impacts on every plant attribute (MANOVA findings for drought treatment: $F_{8,72} = 1479$, $p < 0.001$, AMF treatment effect: $F_{24,222} = 9.14$, $p < 0.001$, and drought $\times$ AMF interaction: $F_{24,222} = 4.06$, $p < 0.001$). AMF-treated plants were generally 23% smaller and 47% lighter than control (nonmycorrhized) plants for both well-

watered and drought-stressed plants (Table 1, Figure 1). Additionally, we discovered an interaction between AMF and drought treatments for the majority of the features under investigation (Figure 1, Table 1). Drought reduced aboveground biomass by 34% (Figure 1b) and plant size by 26% (Figure 1a) in nonmycorrhized plants. Additionally, drought raised belowground biomass by 76% (Figure 1c), SLA by 28% (Figure 1e), and C/N by 30% (Figure 1f). The drought therapy had less of an impact on mycorrhized plants. Following *R. irregularis* inoculation, drought reduced plant size by 23%, aboveground biomass output only by 6%, SLA rose by 8%, and belowground biomass and C/N did not vary from well-watered plants (Figure 1). Following *F. mossae* inoculation, drought decreased plant size by 11%, aboveground biomass output by 7.6%, belowground biomass by 9.4%, SLA by 21%, and C/N by 12% as compared to regular watering (Figure 1). Drought reduced plant growth by 19.6%, aboveground biomass output by 22%, belowground biomass by 31.6%, SLA by 2.5%, and C/N by 39% after inoculation with the two AMF species mix (Figure 1). Under drought, the SPAD measurement of chlorophyll content rose for both the control and *R. irregularis* treatments, however it was not as much influenced for *F. mossae* and the double inoculation treatment. Positive and negative trait correlations were found when all characteristics were analysed collectively. In particular, plants with higher C/N and SLA values—which mostly included mycorrhized plants—achieved reduced biomass and plant height (Figure 2a). Nonmycorrhized plants, on the other hand, were heavier and larger than AMF plants (Figure 2a). As a result, we saw a change in how resources were used. Moving along the first axis of the PCA, which is mediated by the presence or absence of AMF on tomato roots, reveals a shift from a syndrome of plants investing more energy in biomass allocation and chlorophyll activity (the negative values) to a syndrome of high carbon storage and thicker leaves (the positive values) (Figure 2a). Watering schedules also influenced this change in carbon allocation tactics. In particular, we found that nonmycorrhized drought-stressed plants have the lowest C/N in the leaves (Figure 1f) and invest most in root development (Figure 1c). The closeness of *R. irregularis* and noninoculated control plants to the drought impact size (Figure 2c) may be explained by similar patterns in the chlorophyll index (SPAD: Figure 1d).

3.1.3 | Effects of AMF and drought on tomato chemical defences

The constitutive concentration of the three main secondary metabolites observed in tomato leaves was shown to be generally positively impacted by AMFs (Figure 1g,h, Table 1). Alpha-tomatine and dehydrotomatine, two glycoalkaloids, as well as the flavonoid complex rutin, were found in considerably higher amounts in leaves after AMF treatments (Table 1; AMF effects, $p < 0.001$). For both watering schedules, the concentrations of rutin, dehydrotomatine, and alpha-tomatine were greater when AMF was present. Alpha-tomatine concentrations in AMF treatments rose by 37% on average for both watering regimes combined as compared to control plants. Tomatine concentrations in the leaves of infected plants were 51.8% (F), 70.7% (R), and 86.8% (RF) higher than in control plants in well-watered plants (Figure 1g). When subjected to a drought environment, AMF-inoculated plants' leaves had 18.9% (R), 17.8% (F), and 24.7% (RF) more rutin than those of nonmycorrhized plants (Figure 1i). Mycorrhiza's interaction with the watering schedule had an impact on tomatine levels in the leaves (Table 1; $D \times \text{AMF}$ interaction effect, $p = 0.02$). Tomatine levels in the leaves of drought-stressed plants were 25.6% higher in control plants, whereas well-watered plants in mycorrhized plants had greater tomatine levels (Figure 1g). There was no interaction between rutin and dehydrotomatine in response to mycorrhization and dryness. Drought did not substantially alter the quantities of these three molecules in the leaves (Table 1; D effects $p > 0.05$), therefore the concentrations exhibit comparable patterns in the two hydric regimes.

3.2 | How do AMF species and their combined inoculation differentially affect plants under drought stress?

3.2.1 | Effects of AMF and drought on plant growth

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We also found that dryness had less of an impact on aboveground and belowground biomass in AMF treatments than it does on noninoculated plants (Figure 1b). We used a PCA to find the underlying trait correlations. Overall, the PCA ordination revealed relationships between both positive and negative qualities. In particular, increased root and leaf biomass, plant height, and chlorophyll content were linked to lower CN and SLA values (Figure 2a). Compared to AMF plants, nonmycorrhized plants were larger and heavier (Figure 2b). Conversely, with AM

TABLE 1 Two-way interaction ANOVA table for measuring the effect of drought (two levels) and AMF treatments (four levels) on plant growth and chemical defence traits

Response variable	Treatment	Df	SumSq	F value	p value	Significance
AMF colonization	Drought (D)	1	4.4	1.17	0.28	
	AMF	3	462.2	40.56	<0.001	***
	D × AMF	3	143.3	12.57	<0.001	***
	Residuals	79	300.1			
Plant height	Drought (D)	1	705,990	37.10	<0.001	***
	AMF	3	874,752	15.32	<0.001	***
	D × AMF	3	123,335	2.16	0.1	
	Residuals	79	1,503,301			
SPAD	Drought (D)	1	54.9	7.50	0.01	**
	AMF	3	652.1	29.71	<0.001	***
	D × AMF	3	152.7	6.96	<0.001	***
	Residuals	79	578.1			
SLA	Drought (D)	1	0.0089	0.61	0.44	
	AMF	3	0.332	7.63	<0.001	***
	D × AMF	3	0.106	2.44	0.07	.
	Residuals	79	1.1461			
CN	Drought (D)	1	92.3	3.63	0.06	
	AMF	3	1700.5	22.33	<0.001	***
	D × AMF	3	1649.6	21.66	<0.001	***
	Residuals	79	2005.8			
AG biomass	Drought (D)	1	0.903	15.81	<0.001	***
	AMF	3	7.814	45.59	<0.001	***
	D × AMF	3	0.537	3.13	0.03	*
	Residuals	79	4.513			
BG biomass	Drought (D)	1	1.02E + 00	21.43	<0.001	***
	AMF	3	5.99E + 00	42.04	<0.001	***
	D × AMF	3	8.11E-01	5.7	0.001	**
	Residuals	79	3.75E + 00			
Alpha-tomatine	Drought (D)	1	7.62E + 11	3.65	0.06	.
	AMF	3	6.13E + 12	9.79	<0.001	***
	D × AMF	3	2.06E + 12	3.29	0.02	*
	Residuals	79	1.65E + 13			
Dehydrotomatine	Drought (D)	1	1.04E + 12	1.24	0.27	
	AMF	3	1.48E + 13	5.89	0.001	**

Rutin	D × AMF	3	4.47E + 12	1.78	0.16	***
	Residuals	79	6.63E + 13			
	Drought (D)	1	1.01E + 11	0.34	0.56	
	AMF	3	6.99E + 12	7.73	<0.001	

TABLE 1 (Continued)

Response variable	Treatment	Df	SumSq	F value	p value	Significance
	D × AMF	3	1.21E + 12	1.34	0.27	
	Residuals	79	2.38E + 13			

Abbreviations: AMF, arbuscular mycorrhizal fungi; CN, carbon to nitrogen ratio; SLA, specific leaf area; SPDA, leaf chlorophyll index. Asterisks indicate significant effect.

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$.

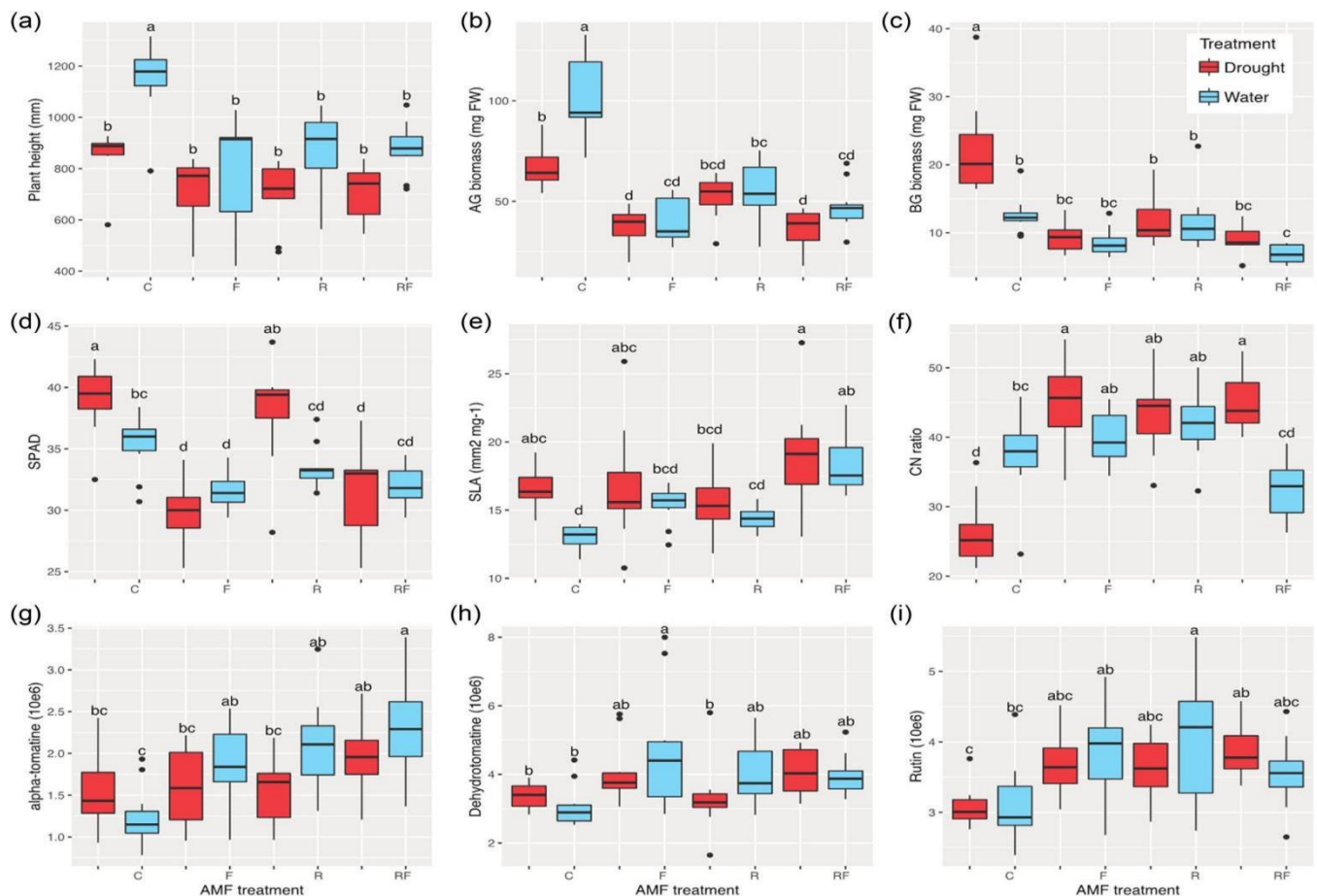


FIGURE 1 impact of drought and AMF on plant defence and growth. Boxplots display the raw data of the recorded variables for each AMF treatment (C: control; F: Funneliformis mossae; R: Rhizophagus irregularis; RF: R. irregularis + F. mossae) and watering regime (blue: normal watering; red: drought condition): plant height (a); AG, aboveground biomass (b); BG, belowground biomass (c); SPAD, chlorophyll index (d); SLA, specific leaf area (e); carbon to nitrogen (C/N) ratio (f); and the relative chemical concentrations of alpha-tomatine (g); dehydrotomatine (h);

and rutin (i) in the leaves prior to herbivory. Significant changes between AMF and drought treatments are shown by letters above boxplots (Tukey HSD test, $p < 0.05$). Boxplots show the minimum, first quartile, median, third quartile, and maximum from bottom to top; outliers are shown by dots. Tomato leaves were thinner and contained 38% less nitrogen, as shown by their higher CN ratios and SLA values. Photosynthetic activity was impacted by drought in both *R. irregularis*-inoculated and noninoculated plants (SPAD, Figure 1d). This might account for the close proximity of these therapies on the PCA1.

(Figure

2c).

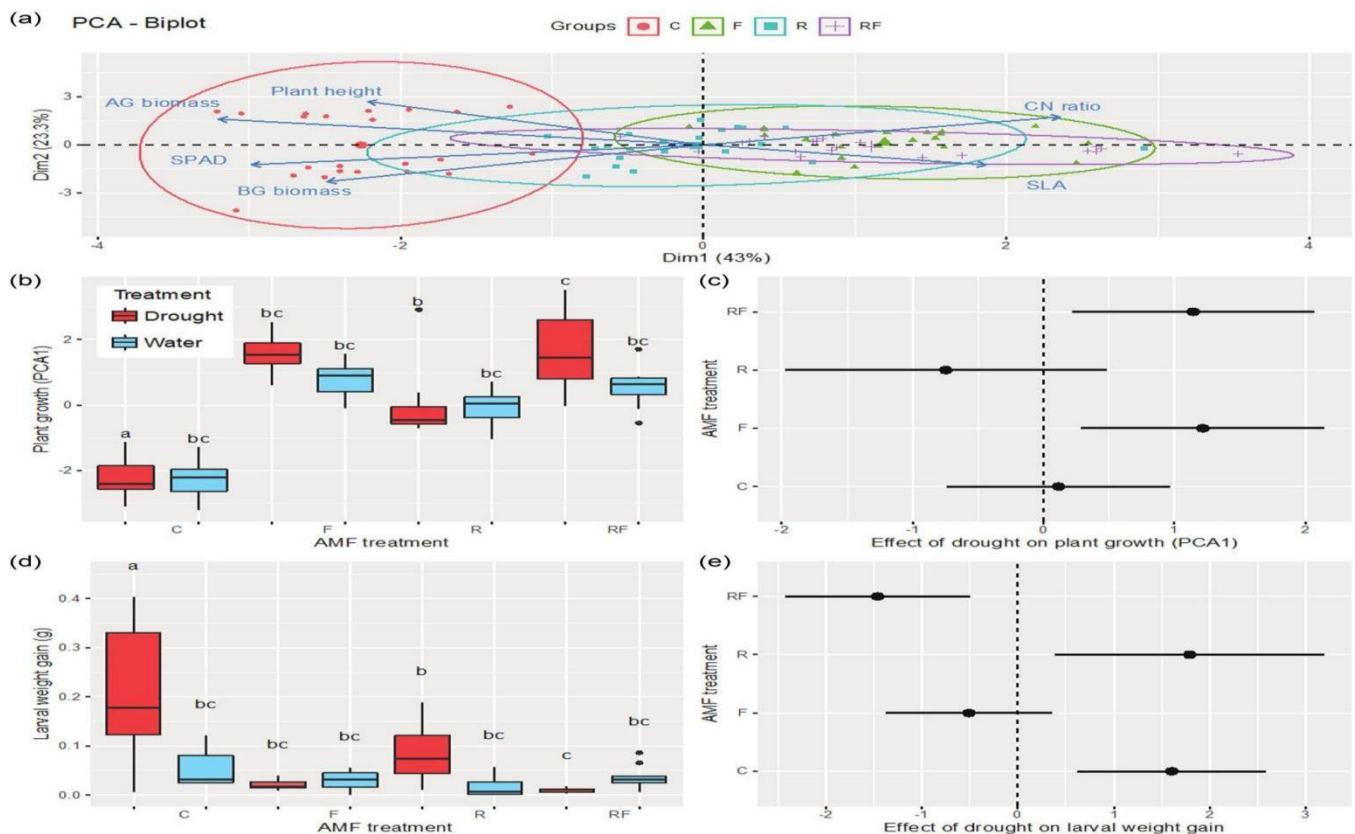


FIGURE 2; Drought's impacts on plant growth responses, whether or not AMF is present. Principal component analysis (PCA) ordination plots for all plant growth-related characteristics (plant height, aboveground [AG] biomass, belowground [BG] biomass, chlorophyll content [SPAD], carbon-to-nitrogen [CN] ratio, and specific leaf area [SLA]) are shown. The various AMF treatments (C: control; F: *Funneliformis mossae*; R: *Rhizophagus irregularis*; RF: *R. irregularis* + *F. mossae*) are shown as ellipses with 95% confidence intervals. (b) The first axis's average loadings of the PCA (PCA1) as previously displayed, grouped by the drought treatment (blue: normal watering; red: drought condition) and by the various AMF treatments as previously mentioned in the (c) Cohens' D effect sizes between the drought condition and normal watering across all AMF treatments using the loading of PCA1 (averages $\pm$ 95% c.i.). (d) Average larvae weight increase across plants under the two watering regimes and AMF treatments, and (e) Cohens' D effect sizes for larval weight gain across all AMF treatments between the drought condition and regular watering (averages $\pm$ 95% c.i.). Significant changes between AMF and drought treatments are shown by letters above boxplots (Tukey HSD test, $p < 0.05$). Boxplots show the minimum, first quartile, median, third quartile, and maximum from bottom to top; outliers are shown by dots.

3.2.2 Effects of AMF and drought on insect performance

The performance of *S. littoralis* caterpillars was significantly impacted by AMF (Figure 2d; AMF effect; $F_{3,79} = 28.83$, $p < 0.001$). Caterpillar biomass dropped to 25% when fed on AMF-inoculated

plant leaves compared to caterpillars raised on nonmycorrhized tomato plant leaves. Drought (Figure 2d; drought effect; $F_{1,72} = 0.05$, $p < 0.001$) and the combination of AMF and drought (Figure 2d,e; $F_{3,72} = 13.45$, $p < 0.001$) had a significant impact on this effect. All AMF treatments produced much smaller caterpillars under drought stress, but there were no appreciable variations in larval weight increase under the regular watering schedule (Figure 2d). Overall, we discovered that *S. littoralis* larvae fed leaves from well-watered plants grew 63% less than those fed leaves from drought-stressed plants (Figure 2d). Drought also significantly affected herbivore performance (Table 1). Larval weight increase was 74% lower on well-watered control plants and 78% lower on well-watered *R. irregularis*-inoculated plants when comparing normal watering to drought stress for each AMF treatment. For both well-watered and drought-stressed plants, caterpillar biomass was typically low when fed on leaves from the mixed AMF inoculum (RF) and *F. mossae*. In both hydric environments, the single *F. mossae* inoculation had no discernible impact on herbivore resistance (Figure 2d,e).

3.3 | How do AMF inoculation and drought affect the growth-defence trade-off in tomato plants?

3.3.1 | Effects of AMF and drought on growth-defence trade-off

We discovered that mycorrhized plants invested more in chemical defences, while control, nonmycorrhized plants invested more in biomass accumulation (Figure 3, Supporting Information: Figure S3). The plant phenotypes in the multivariate space were clearly differentiated by AMF treatments (Figure 4a, PERMANOVA with 999 permutations; $F_{3,42} = 5.71$, $p = 0.001$ for the water treatment, and Figure 4b, $F_{3,43} = 4.40$, $p = 0.002$ for the drought treatment, Supporting Information: Figure S3). In particular, we found that the content of rutin in leaves was negatively correlated with aboveground biomass (Supporting Information: Figure S4, $r = -0.38$, $p < 0.001$). When plants were well-watered, this intraspecific growth-defence trade-off was more noticeable, but when watering circumstances were lowered, it was lessened (i.e., plants were more comparable along the trade-off axis) (Figure 3). Lastly, we found that AMF-treated plants, which made more defensive investments, were also more resilient to *S. littoralis* feeding along this trade-off axis. However, the association between larval weight increase and the growth-defence matrix under normal watering regimes (Figure 4a, $r = 0.3$, $p = 0.167$) was only significant under drought circumstances (Figure 4b, $r = 0.74$, $p = 0.001$).

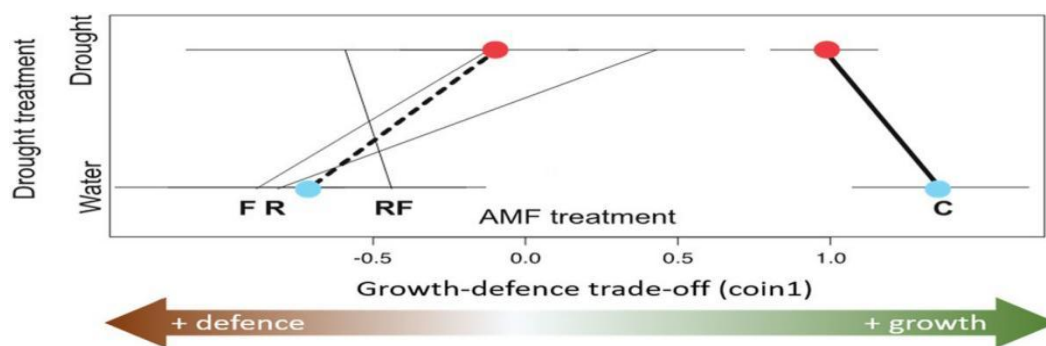


FIGURE 3; Growth-defence trade-off interaction plot. The trade-off is based on the coinertia axis, which is computed between the three chemical defences and the matrix of the seven growth features (coinertia: $r = 0.188$, $p < 0.001$). A strategy of investing in chemical defences is associated with negative values of the coinertia axis, while a strategy of investing in biomass accumulation is associated with positive values. Each AMF treatment (C: control (bold plain line); F: funneliformis mossae; R: *Rhizophagus irregularis*; RF: *R. irregularis* + *F. mossae* (bold dashed line, representing the average of the three AMF treatments)) across the two and each watering

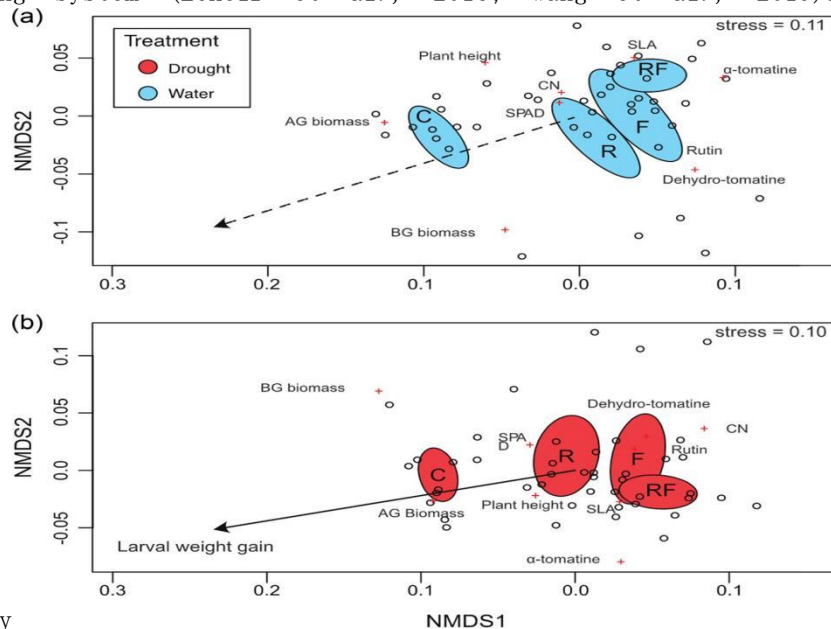
regime (blue: normal watering; red: drought condition) is represented by dots that show the average $\pm$ 1 S.E.

4 DISCUSSION

In domesticated tomato plants under drought stress, we investigated plant-AMF-leaf herbivore interactions. During the course of our investigation, we discovered that dryness typically reduced plant development and resistance against generalist caterpillars of noninoculated plants. The identification of the mycorrhizal inoculum and watering schedules had a context-dependent impact on growth and defence. Interestingly, AMF-inoculated plants maintained a greater amount of glycoalkaloids and rutin than nonmycorrhizal plants, and although being generally smaller, they exhibited a reduced drop in height and biomass owing to dryness. This suggests that the presence of AMFs in the tomato roots typically reduced the growth-defense trade-off.

4.1 | AMF effects on plant growth and defences

Our research produced two main findings: first, we demonstrate that AMF generally reduced plant growth while simultaneously boosting defences and resilience to caterpillars. Second, we demonstrate that the impact of dryness on plant functional characteristics varied and interacted with the AMF inocula's identity or presence. Although it may not be as often documented in the literature, it has already been shown that AMF reduces plant development and biomass accumulation (Koltai & Kapulnik, 2010). This result could be explained by: (i) trade-offs in energy allocation (Cavagnaro et al., 2021; Unger et al., 2016); (ii) mycorrhization costs outweighing the advantages of increased resource acquisition through the mycorrhizal network (Höpfner et al., 2015; Ngo et al., 2021; Sanchez-Bel et al., 2016); or (iii) physical limitations imposed by the potting system (Lenoir et al., 2016; Wang et al., 2019). In this instance, the most



likely

FIGURE 4; The *Solanum lycopersicum* plant's functional and chemical characteristics are shown using nonmetric multidimensional scaling (NMDS) of ([a]: Water, for the typical watering regime; [b]: Drought, for hydric stress). The following variables were used to create distance matrices: aboveground biomass, belowground biomass, plant height, specific leaf area (SLA), SPAD, the carbon to nitrogen ratio of the leaves, and the concentrations of the chemicals alpha-tomatine, dehydro-tomatine, and rutin in the leaves. The four distinct AMF treatments (Control, *F. mossae*, *R. irregularis*, and *R. i. + F. m.*) are used to illustrate the 95% confidence interval ellipses. Stress values: B = 0.10, A = 0.11. Figure 4a shows PERMANOVA with 999 permutations for the water treatment ($F_{3,42} = 5.71$, $p = 0.001$) and Figure 4b shows $F_{3,43} = 4.40$, $p = 0.002$ for the drought treatment. The displayed Bray-distance of the permanova of the larval weight growth in the NMDS statistical space is shown by the arrows. The association between larval weight increase and the

growth-defense matrix under normal watering regimes (Figure 4a, $r = 0.3$, $p = 0.167$) and under drought circumstances (Figure 4b, $r = 0.74$, $p = 0.001$) is significant only for the drought treatment. Allocation trade-offs mediated by the AMFs provide an explanation. In fact, mycorrhized plants were better protected but grew less than nonmycorrhized plants. Second, we discovered that plant resistance to herbivory under drought circumstances peaked when the AMF inocula were combined (Figure 2), and mycorrhization was similar to that of the other AMF treatments (Supporting Information: Figure S2). The two-species inoculum (RF) had the lowest colonisation rate under a typical watering schedule, but its effects on herbivore performance were similar to those of the single inoculation treatments (F and R). These findings support our theory that the way tomato plants react to helpful fungus depends on both species and environment (Barber et al., 2013). Drought had a greater influence on tomato plant development when *R. irregularis* was present, whereas *F. mossae* and the combination of the two AMF species lessened the detrimental impacts of drought. As a result, it is still challenging to forecast three-way interactions between fungi, plants, and their enemies (Bennett et al., 2006). This difficulty increases when an additional factor, like drought, a major abiotic factor affecting the growth of the inoculated plants, is present (Bennett & Bever, 2007). However, regardless of the watering schedule, we showed that tomato plants infected with AMF (*R. irregularis*, *F. mossae*, or a combination of both) raised the constitutive concentration of harmful alkaloids. A variety of insect herbivores, including generalist and specialist caterpillars, are negatively impacted by the steroidal glycoalkaloids (including alpha-tomatine) that *S. lycopersicum* produces, according to earlier research (Friedman, 2002, 2004; Leite-Mondin et al., 2021; Papantoniou et al., 2021). In a similar vein, the rise in the flavonoid compound rutin (quercetin-3-O-rutinoside) in all AMF treatments—during both drought and regular watering schedules—indicates a definite impact of mycorrhizal fungus on the chemical makeup of tomato leaves (Figure 1j). Thus, our results support the hypothesis that AMF affects chemical defences, which is probably mediated via the hormonal system that triggers chemical defence activation (Aseel et al., 2019; Delavaux et al., 2017; Minton et al., 2016). Drought and AMF inoculation's effects on tomato plants' growth-defense trade-off We demonstrated intricate biotic and abiotic impacts on the growth-defense trade-offs of tomato plants. While AMF-treated tomato plants had larger quantities of chemical defence chemicals, noninoculated tomato plants invested more in growth under a regular watering schedule. More precisely, all AMF-inoculated treatments boosted alpha-tomatine production, but non-inoculated plants often had lower levels of rutin and alpha-tomatine in their leaves than AMF-inoculated plants. It's interesting to note that these trade-offs were more noticeable with regular irrigation than during drought. Inoculated tomato plants reduced their defensive investment under drought, but noninoculated plants reduced the development of their shoots. This implies that mycorrhized plants are more adaptable and may explore a larger phenotypical space in terms of growth and defences when resources, in this instance water, are readily available (Gratani, 2014; Toby Kiers et al., 2010). Alternatively put, environmental stress would likely to restrict plant AMF-enhanced resistance to herbivores, indicating that climate change may have a greater influence on plant-herbivores-AMF interactions than is predicted from research on bilateral interactions (Nicotra et al., 2010). The physiological and metabolic underpinnings of how AMFs influence such growth-defense trade-offs should be the focus of future studies (Meena et al., 2017). This would assist in determining which AMF strains are most suitable for application in situations of severe drought (Grover et al., 2011). It's interesting to note that all AMF-inoculated tomato plants showed significant constitutive concentrations of rutin and alpha-tomatine both under 9 weeks of drought treatment and when the plants were well-watered. The impact of AMF in initiating the constitutive chemical defence of tomato plants under hydric physiological restrictions is confirmed by the increase in the amounts of these glycoalkaloid and flavonoid chemicals. When creating tomato varieties that will probably be impacted by severe drought occurrences in the future, further research is needed to determine if this effect is

mediated by physiological limitations or whether it is the outcome of an adaptive response of the plants (Basu et al., 2018).

5 | CONCLUSION

The goal of this research was to learn more about how AMF affects tomato growth and defences under drought by integrating functional, physiological, and chemical characteristics. We demonstrated that AMF's interactions with tomato plants are significantly influenced by the watering schedule throughout the culture cycle. In particular, even if there are now a number of microbe-based solutions on the market, it is crucial to comprehend how effective they are under various environmental pressures. Our method suggests that in order to really boost plant productivity, complex interactions between plants, microbes, insects, and the environment must be properly taken into account. Our results confirm the need for further information to completely comprehend how AMF impacts rely on eco-climatic conditions in order to accurately estimate production systems with substantial economic stakes. Therefore, in order to better understand the molecular basis of the abiotic and biotic stress attenuation caused by AMF colonisation, we recommend assessing the impact of AMF under realistic climatic settings and enhancing these studies with a thorough metabolomic research.

REFERENCES

- Arbuscular mycorrhizal fungi from acidic soils promote tomato production and lycopene concentration (Aguilera P, Becerra N, Alvear M, Ortiz N, Turrini A, Azcón-Aguilar C, et al.). *J. Sci. Food Agric.* 102(6):2352–8, 2022.
- Rashad YM, Hammad SM, and Aseel DG. In order to protect tomatoes against tomato mosaic virus, arbuscular mycorrhizal fungus stimulate the transcriptional expression of genes involved in the flavonoid and chlorogenic acid biosynthesis pathways. *Sci Rep.* 9(1):9692, 2019.
- Context-dependency of arbuscular mycorrhizal fungi on plant-insect interactions in an agroecosystem, Barber N, Kiers ET, Hazzard R, Adler L. *Front Plant Sci.* 4:338 (2013).
- AMF: the prospects for sustainable agriculture in the future, Basu S, Rabara RC, Negi S. *Physiol Mol Plant Path.* 102:36–45. 2018.
- Bever JD, Bennett AE. Different mycorrhizal species change how plants develop and react to herbivory. *Ecology.* 88(1):210–8, 2007.
- Bever JD, Alers-Garcia J, Bennett AE. Mutualistic mycorrhizal fungi, plants, and plant enemies interact in three ways: theories and synthesis. *Am Nat.* 167(2):141–52, 2006.
- Bennett AE, Orrell P, Malacrino A, Pozo MJ. The community approach, stability, evolution, processes, and uses of fungal-mediated above-belowground interactions. In: Johnson S, Wurst S, Ohgushi T, editors. *Community ecology aboveground and belowground.* Ecological Research. Springer, Cham, 2018, vol. 234. https://doi.org/10.1007/978-3-319-91614-9_5
- Brígido C, van Tuinen D, Brito I, Alho L, Goss MJ, Carvalho M. Managing the biological diversity of AM fungi by combining extraradical mycelium integrity and host plant succession. 2017; 112:237–47; *Soil Biol Biochem.*
- Application of biostimulants in horticultural crops under abiotic stress conditions, Bulgari R, Franzoni G, Ferrante A. *Agronomy.* 9(6):306, 2019.
- Grimoldi AA, Oosterheld M, Cavagnaro RA, and Oyarzabal M. Trade-offs between mycorrhizas and regrowth in response to phosphate addition and defoliation are species-specific. *Fungal Ecol.* 51:101058, 2021.
- Redman AM, Cipollini DF. effects of wind exposure and jasmonic acid therapy on tomato foliar oxidase activity and insect resistance that vary with age. *J. Chem. Ecol.* 25(2):271–81, 1999.
- Cohen J. *Analysis of statistical power in the behavioural sciences.* Academic Press, 2013.
- Plant antiherbivore defence and resource availability (Coley PD, Bryant JP, Chapin FS). *science.* 1985; 230(4728):895–9.
- Rudgers JA, Chen C, and Davitt AJ. Recognising the impact of biotic and abiotic settings on host fitness and the rate of symbiont transmission in plant-microbe symbiosis. *The Environ Exper Bot.* 2011; 71(2):137–45.
- Hall RD, Schipper B, and DeVos RCH. Analysis of plant metabolites in Brassicaceae using high-performance liquid chromatography and mass spectrometry. Hardy NW, Hall RD, editors. *Plant*

- metabolomics: techniques and procedures. Humana Press, Totowa, NJ, 2012 [cited May 3, 2021], pp. 111 – 28.
- Rasmann S, Pellissier L, and Defosse E. plant growth form-defense syndrome development along elevation gradients. 2018 Ecol Lett. 21(5): 609 – 18.
- Kuebbing SE, Smith-Ramesh LM, and Delavaux CS. Beyond nutrients: a meta-analysis of the many ways that arbuscular mycorrhizal fungus affect soils and plants. ecology. 2017; 98(8):2111 – 9.
- Dias MP, Santarém ER, Xavier VB, Cassel E, Bastos MS, and Astarita LV. In tomatoes, *Streptomyces* spp. increase plant development and resistance. Plant Physiol Biochem. 118:479 – 93 (2017).
- Dufour AB, Dray S. The duality diagram for ecologists is implemented by the ade4 package. J Stat Softw. 22(1):1 – 20, 2007.
- Formenti L, Rasmann S. In tomato plants that produce less jasmonic acid, mycorrhizal fungus increase tolerance to herbivores. Agronomy. 9(3):131, 2019.
- Friedman M. Tomato Glycoalkaloids: function in the plant and in the diet. J. Agric Food Chem. 50(21):5751 – 80, 2002.
- Friedman M. Examination of physiologically active substances in the seeds of tomatoes (*Lycopersicon esculentum*), potatoes (*Solanum tuberosum*), and jimson weed (*Datura stramonium*). J Chromatogr A. 1054(1):143 – 55. 2004.
- Gange AC. reactions of a species-specific insect that feeds on roots and shoots to arbuscular mycorrhizal colonisation of its host plant. New Phytol. 150(3):611–8, 2001.
- Terpene natural products' role in the natural environment, Gershenzon J, Dudareva N. 2007; Nat Chem Biol. 3(7):408 – 14.
- An assessment of methods for quantifying vesicular arbuscular mycorrhizal infection in roots by Giovannetti M, Mosse B. New Phytol. 84(3):489 – 500, 1980.
- R Core Group. R is a statistical computing language and environment. R Foundation for Statistical Computing, Vienna, Austria, 2021. Arbuscular mycorrhizal fungi and organic farming is accessible at <https://www.R-project.org/Gosling>
- P, Hodge A, Goodlass G, Bending GD. Agric Ecosyst Environ. 113(1): 17 – 35 (2006).
- Gratani L. Plants' ability to adapt their phenotypes to their surroundings. 2014:e208747 in Adv Botany.
- Microorganisms in agricultural crops' adaptability to abiotic stressors, Grover M, Ali SkZ, Sandhya V, et al. World Journal of Microbiology and Biotechnology. 2011; 27(5):1231 – 40.
- Do interactions between plant roots and the rhizosphere influence parasitoid behaviour? Guerrieri E, Lingua G, Digilio MC, Massa N, Berta G. Entomol Ecol. Hart MM, Antunes PM, Chaudhary VB, Abbott LK, 2004; 29(6):753 – 6.
- Is the benefit of using fungal inoculants in the field outweighed by the risk? Funct Ecol. 2018; 32(1): 126 – 35.
- Gange AC, Hartley SE. Impacts of plant symbiotic fungus on insect herbivores: mutualism in a multitrophic setting. Annu Rev Entomol. 54(1):323 – 42, 2009.
- Plant-soil interactions that shape the root microbiota under abiotic stress, Hartman K, Tringe SG. Biochem J. 476(19): 2705 – 24, 2019.
- Herms DA, Mattson WJ. Plants' dilemma: to protect or expand. Q Rev Biol. 22:283 – 308, 2015.
- A meta-analysis of context-dependency in plant response to mycorrhizal fungal inoculation was conducted by Hoeksema JD, Chaudhary VB, Gehring CA, Johnson NC, Karst J, Koide RT, et al. 2010; 13(3):394 – 407 Ecol Lett.
- Holland T, Vukicevich E, Thomsen C, Pogiatis A, Hart M, Bowen P. Should we use biofertilizers for arbuscular mycorrhizal fungi in viticulture? Catalyst: Putting Knowledge into Action. Nov. 152(2):59 – 63, 2018.
- pfner I, Bartelheimer M, Werner C, Unger S, Beyschlag W. The competitive interactions between the grassland species *Plantago lanceolata* and *Ngo HTT*, Watts-Williams SJ, and Cavagnaro TR depend on mycorrhization and nutrient availability.

- Tomato mycorrhizal growth and phosphorus responses vary according on the source, but not the pace at which phosphorus fertilisers are applied. *Appl Soil Ecol.* 166:104089, 2021.
- Plant phenotypic plasticity in a changing environment Nicotra AB, Atkin OK, Bonser SP, Davidson AM, Finnegan EJ, Mathesius U, et al. 2010; *Trends Plant Sci.* 15(12):684–92.
- Vegan: Community Ecology Package [Internet] Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, et al. 2020 [cited Sep. 7, 2021].
- Accessible at <https://CRAN.R-project.org/package=vegan> Olesen JE, Trnka M, Kersebaum KC, Skjelvåg AO, Seguin B, Peltonen-Sainio P, et al. effects of climate change and how European agricultural production systems have adapted to it. 2011; 34(2):96–112; *Eur J Agron*
- . Cascading consequences of root microbial symbiosis on the development and metabolome of the insect herbivore *Manduca sexta* L. *Metabolites.* 2021;11(11):731. Papantoniou D, Vergara F, Weinhold A, Quijano T, Khakimov B, Pattison DI, et al.
- MZmine 2: a modular framework for processing, displaying, and evaluating chemical profile data derived from mass spectrometry (Pluskal T, Castillo S, Villar-Briones A, Orešič M). *Bioinformatics, BMC.* 2010; 11(1):395.
- Semenov MA, Porter JR. crop reactions to changes in climate. 2005; 360(1463):2021–35. *Philos Trans R Soc, B.*
- Pozo MJ, Verhage A, García-Andrade J, García JM, and Azcón-Aguilar C. Arbuscular mycorrhizal fungi prime plants against diseases. Azcón-Aguilar C, Barea JM, Gianinazzi S, Gianinazzi-Pearson V, editors. *Mycorrhizas: ecological effect and functional mechanisms.* Springer, Berlin, Heidelberg, 2009, pp. 123–35.
- Agrawal AA, Halitschke R, Rasmann S, and Erwin AC. Milkweed (*Asclepias syriaca*) direct and indirect root defences: trophic cascades, trade-offs, and new approaches to researching underground herbivory. Sanchez-Bel P, Troncho P, Gamir J, Pozo MJ, Camañes G, Cerezo M, et al. *J Ecol.* 2011; 99(1):16–25. In tomatoes, mycorrhiza-induced resistance to *Botrytis cinerea* is hampered by the availability of nitrogen.
- Microbiol Front.* Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. 2016;7:1598. 2012; *Nat Methods.* 9(7):671–5.
- Reich PB, Xiao K, Schnitzer SA, Klironomos JN, HilleRisLambers J, Kinkel LL, et al. The traditional pattern of plant variety and production is driven by soil microorganisms. *ecology.* 2011; 92(2):296–303.
- Durall DM, Simard SW. The scope, purpose, and significance of mycorrhizal networks are reviewed. *Can J Bot.* 82(8):1140–65, 2004.
- Singh VK, Singh AK, Singh PP, Kumar A. A review of how microorganisms that promote plant development interact with tomatoes under abiotic stress. *Agric Ecosyst Environ.* 267:129–40 (2018).
- XCMS: processing mass spectrometry data for metabolite profiling utilising nonlinear peak alignment, matching, and identification Smith CA, Want EJ, O’Maille G, Abagyan R, Siuzdak G. *Anal Chem.* 78(3):779–87, 2006. Mycorrhizal symbiosis, Smith SE, Read DJ.
- Teotia P, Kumar M, Prasad R, Sharma S, Kumar V. Endophytic probiotics and plant health: toward a balanced accost, Academic Press, 2010, p. 815. Asad R, Sharma S, Kumar V, Kumar M, editors.
- Plant health and probiotics. 383–99. Singapore: Springer, 2017. Bruno JF, Bronstein JL, Palmer TM, Ives AR, and Toby Kiers E. An evolutionary view of mutualisms in a changing environment. Orchiano M. effsize: Efficient Effect Size Computation [Internet]. 2020. *Ecol Lett.* 2010; 13(12):1459–74.
- Accessible at <https://CRAN.R-project.org/package=effsize> Unger S, Friede M, Hundacker J, Volkmar K, Beyschlag W. Allocation trade-off between root and mycorrhizal surface specifies nitrogen and phosphorus relations in 13 grassland species. *Plant soil.* 2016; 407(1):279–92.
- Pieterse CM, Van Wees SC, and Van der Ent S. Beneficial microorganisms induce plant immune responses. Vierheilig H, Schweiger P, Brundrett M. *Curr Opin Plant Biol.* 2008; 11(4): 443–8. A summary of techniques for identifying and monitoring arbuscular mycorrhizal fungi in roots. *Physiol Plant.* 2005;

125(4):393 – 404. Soil-plant-microbe interactions in stressed agricultural management: a review by Vimal SR, Singh JS, Arora NK, and Singh S. *pedosphere*. 2017; 27(2): 177 – 92. Van der Heijden MGA, Wagg C, Jansa J, and Schmid B. Plant symbionts' influence on belowground biodiversity boost aboveground production. *Ecol Lett*. 2011;14(10):1001 – 9. Walters DR. Plant defence: preventing pathogen, herbivore, and parasitic plant attacks. Wiley-Blackwell, Hoboken, New Jersey, 2010, pp. 1 – 67. Wang X, Yun J, Shi P, Li Z, Li P, Xing Y. Root growth, fruit production, and water usage efficiency of tomatoes grown in greenhouses under various nitrogen levels and watering schedules. Weyens N, van der Lelie D, Taghavi S, Newman L, Vangronsveld J. Using plant-microbe partnerships to enhance biomass production and cleanup. *J Plant Growth Regul*. 2019;38(2):400 – 15. *Biotechnology Trends*. Wilson GWT, Hartnet DC. 2009; 27(10):591-8. Mycorrhizae's effects on plant development and dynamics in tallgrass prairie microcosms used in experiments. *Am J Bot*. 84(4):478 – 82, 1997. Kumar V, Upadhyay N, Kumar N, Sharma S, Yadav G, Vishwakarma K, et al. The growing importance of rhizospheric probiotics and how they affect plant health: the state of sustainable agriculture today. Probiotics and plant health, edited by Kumar V, Kumar M, Sharma S, and Prasad R. Springer, Singapore, 2017, pp. 233 – 51. Rhizosphere microorganisms aid plants in withstanding abiotic stress (Yang J, Kloepper JW, Ryu CM). *Plant Science Trends*, 2009, 14(1):1 – 4. Tisarum R, Theerawitaya C, Yooyongwech S, Samphumphuang T, Cha-um Through osmotic modifications using soluble sugar and free proline, S. Arbuscular mycorrhizal fungus (AMF) enhanced water shortage tolerance in two distinct sweet potato genotypes. *Sci Hort*. 26 198:107 – 17 (2016).