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Original Research

Role of Arbuscular Mycorrhizal Fungi in Enhancing Thermotolerance and Growth Performance of Onion (*Allium cepa* L.)

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Abstract

Global warming and increasing heat stress adversely affect crop development. This study assessed the potential of Arbuscular Mycorrhizal Fungi (AMF) to mitigate high temperature (>35°C) stress in thermosensitive onion (*Allium cepa*). A completely randomized design with four treatments—Control (CT), AMF, high temperature (HT), and HT+AMF—each with three replicates, revealed significant differences ($p < 0.05$). HT-stressed plants showed the lowest shoot height (9.33 cm), root length (474.58 cm), and biomass (2.28 g), whereas HT+AMF improved these traits by >200%, >140%, and 75%, respectively, demonstrating AMF's mitigating effect. Plants with AMF alone produced the highest growth (shoot 35.50 cm, root 1613.43 cm). Enzyme activity (acid and alkaline phosphatase) that peaked under HT, was reduced by AMF and was intermediate in plants with HT+AMF treatment, indicating moderation of stress responses. Nutrient acquisition (N, P, K) increased by 188%, 49%, and 24% in plants with HT+AMF treatments relative to HT-stressed plants, with mycorrhizal dependency highest (47.17%). Mechanistically, AMF improved nutrient uptake and phosphorus-related enzymatic functions, enhancing physiological stability. PCA and correlation analyses highlighted biomass, shoot, and root length as primary contributors to treatment differences and strong associations among nutrient uptake, enzyme activity, and growth. In conclusion, AMF inoculation enhances thermotolerance in onion, offering a sustainable strategy for maintaining productivity under rising temperatures.

Keywords: Arbuscular Mycorrhizal Fungus (AMF), high temperatures (HT), phenotypic, nutrient availability.

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1. Introduction

The current rate of greenhouse gas emissions is driving a progressive increase in Earth's temperature, leading to global warming. It is likely that by the end of 2024, the average global temperature will exceed pre-industrial values by more than 1.5°C (Morgan, 2024). Future forecasts indicate that the average global temperature is expected to increase by 2.0 to 6.4 °C (Alotaibi, 2023). Agricultural productivity is dependent on climatic factors which encompass variations in precipitation, atmospheric temperature, solar radiation intensity, wind speed, and the concentration of atmospheric gases such as CO_2, O_2, CH_4, N_2, and SO_2 with fluctuations in atmospheric temperature and precipitation leading to significant reductions in agricultural yield (Goud et al., 2022). The abiotic stress of increasing temperature imposed on agricultural land results in alterations in the morphological, biochemical, and physiological processes of plants. This leads to decreased rates of germination, growth, photosynthesis, and respiration; disruption of hormone and enzyme activity; increased oxidative stress; impaired reproduction; and ultimately, a decline in crop productivity (Oyebamiji et al., 2024).

Temperatures above 35°C affect both vegetative and reproductive growth of plants, ranging from germination to grain filling (Hussain et al., 2019), negatively impacting plant height, tiller number, and total biomass (Mitra & Bhatia 2008). Seedling stages are particularly vulnerable in rice, mungbean, and wheat, as are reproductive stages in cereals (Parthasarathi et al., 2022). Plant development and yield are negatively affected by heat waves, heat stress (both short- and long-term), and other forms of extreme heat (Jagadish et al., 2021). Heat waves and stress can cause leaf abscission, burning, senescence, and restricted root and shoot growth, resulting in lower yields (Parthasarathi et al., 2022). Even while heat stress can affect any part of a plant at any point in its life cycle, the reproductive tissues are particularly sensitive. Hence, it is crucial to counteract the impact of elevated temperatures on agricultural products and enhance their productivity by mitigating the adverse effects of high temperature. One potential solution to address this heat stress in plants is developing heat-tolerant crop cultivars, but this has challenges such as genetic complexity, adaptation periods, and environmental impacts (Moreno & Orellana, 2011). Consequently, it is imperative to explore diverse approaches that leverage natural resources. Rhizosphere microorganisms can play a crucial role in protecting plants from environmental stresses (Koza et al., 2022). Among these microorganisms, arbuscular mycorrhizal fungi (AMF) have a particularly important role in altering the physiological parameters of plants under stress situations (Mathur et al., 2021).

AMF, which form mutualistic relationships with the roots of over 80% of terrestrial plants (Robinson et al., 1997), have been shown to protect plants under various stressors, including high temperature, salt, drought, and heavy metals (Mathur et al., 2021). Considering the global impact of high temperatures, it is essential to study the role of AMF-enriched soil in mitigating this issue. Mycorrhizal fungi establish a direct connection between plant roots and soil, extending their exploration beyond the rhizosphere by expanding the absorptive root surface through hyphae plants (Robinson et al., 1997). This symbiotic relationship aids the host plant in obtaining water and vital nutrients, particularly phosphorus (P). Studies on maize have highlighted the protective role of AMF under stress, particularly regarding physiological parameters (Mathur et al., 2021). The significant impact of AMF on plant growth and development in heat stress is mostly attributed to the fungal capacity to enhance morphological and physiological processes, resulting in increased plant biomass and improved uptake of crucial immovable nutrients such as phosphorus (P) (Begum et al., 2019).

The release of phosphatase by plants, coupled with the enhanced phosphatase activity of the intraradical hyphae of AMF, aids in the hydrolysis of a larger quantity of organic phosphorus (Peng et al., 2020). Phosphorus is a critical macronutrient for plant growth, essential in macromolecules like proteins, nucleic acids, and phospholipids. The colonization of roots by AMF resulted in the mycorrhizal pathway being the primary means of acquiring phosphorus (P). Phosphatases play a crucial role in phosphate cycling, with their hydrolysis of phosphate esters being vital for energy metabolism, regulation, and cellular signalling (Sambuk et al., 2011). Phosphorus deficiencies severely impact plant growth and development (Tabaldi et al., 2006). Plants often release more acid phosphatases under low phosphate conditions (Lim et al., 2003). Higher acid phosphatase levels have been observed in stressed and nutrient-deficient plants like tomatoes (Sharma et al., 2023).

Phosphatase activity in soil is temperature dependent. Global warming and other factors that increase soil temperature significantly affect soil enzymes and, consequently, agricultural productivity. Each enzyme has an optimal temperature range; below this range, enzyme activity decreases due to inactivation, and above it, activity decreases due to denaturation. Higher temperatures denature enzymes, reducing nutrient availability and indirectly affecting productivity (Kumari et al., 2017).

Research on the impact of elevated temperatures on onions, particularly regarding nutrient absorption and enzyme secretion, has been limited compared to studies on other stresses like drought and salinity in crops such as rice and maize. Our study aims to address this gap by investigating the effects of elevated temperatures on onion growth, enzyme secretion, and nutrient absorption from soil. Additionally, we propose using AMF to mitigate the adverse effects of elevated temperatures, offering a sustainable and environmentally friendly strategy to address the challenges in onion cultivation posed by rising temperatures.

2. Materials and Methods

2.1 Plant material

Onion (*Allium cepa*) (Rashidpura Onion) was chosen as the experimental plant. The onions were obtained from the local market, and the plants were germinated from the bulbs. The experiment was conducted in 8 kg plastic pots (with a 7 kg capacity and drainage holes) filled with sand and soil in a 1:1 v/v ratio, autoclaved twice at 24-hour intervals. Sterile inoculum ensured the absence the any microbes that might impact the growth of the plants. Ten days after the emergence of plantlets in all the pots, the pots were randomly assigned to the treatments. Each pot initially received 5 onion bulbs, which were later thinned to three plants per pot.

2.2 AMF inoculum

For the AMF-enriched pots, a mixed starter culture consortium of 4 AMF species of *Rhizophagus* spp. (*R. irregularis, R. intraradices, R. clarus, R. aggregatus*) was acquired from Symbiotic Sciences Pvt Ltd, India. The sterile inoculum consisted of a mixture of spores, root fragments, and hyphae. AMF were enriched at a rate of 2000 propagules per plant (approximately 1000 spores), which sums up to 6000 propagules per pot (approx. 3000 spores). The mycorrhizal inoculum was administered to the rhizosphere area as soon as the plantlets emerged, and the pots were designated for respective treatments.

2.3 Growing conditions

The plants were watered daily with approximately 250 mL per pot to prevent any water stress and maintain uniform conditions across treatments, ensuring observed effects were due to temperature and AMF. The high temperature (HT) of 35°C and control temperature (CT) of 23°C were maintained in the greenhouse with a variation of ±2°C, following a cycle of 16 hours of daylight and 8 hours of night. No fertigation was done during the entire growth period.

2.4 *Experimental design*

The experiment was laid out in a Completely Randomized Design (CRD) with four treatments and three replicates per treatment, using one pot per replicate. Each pot contained three onion (*Allium cepa*) plants, and pots were repositioned randomly each week to minimize environmental variation (Fig. 1). The treatments included:

- HT = onion plants cultivated at 35°C ± 2°C
- AMF + HT = onion plants grown in AMF-enriched substrate at 35°C ± 2°C
- AMF = onion plants grown in AMF-enriched substrate at 23°C ± 2°C
- CT= onion plants cultivated at 23°C ± 2°C

The plants were harvested at a maturity of 110 days. The entire plant biomass and rhizosphere soil samples were collected from both high temperature exposure and control plants by uprooting the plants and gently shaking off the soil adhering to the roots. The soil was then air-dried, sieved, and processed for estimating AM spore density. The roots were used for assessing AMF colonization and enzyme secretion.

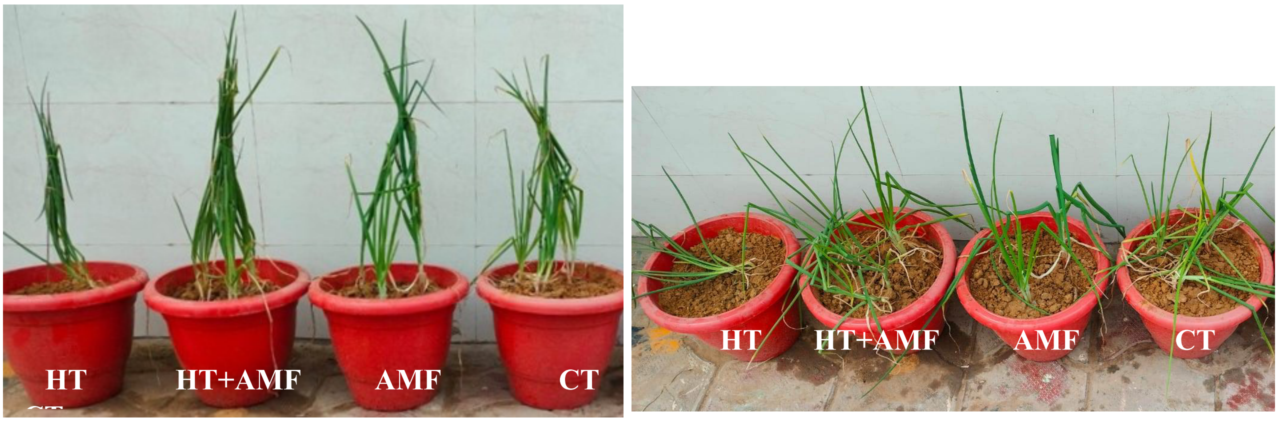


Figure 1. Experimental setup and representative pots of treatment conditions. Description of the four treatments: HT (35°C), HT+AMF (35°C with AMF inoculation), AMF (23°C with AMF inoculation), and CT (23°C).

2.5 *Phenotypical traits of plants*

The growth metrics assessed comprised shoot height (in cm), root length (in cm), dry shoot weight (in g), dry root weight (in g), and the number of flowers per plant after 110 days. The shoot height was determined by measuring the distance from the edge of the container to the top of the main stem of the plant before harvesting using a steel ruler (Buddies & Buddies, 2021). On the other hand, the root length was measured by utilizing the WinRhizo Scanner after removing any unwanted materials (Arsenault et al., 1995). The shoot and root samples were dried in an oven at a temperature of 60°C for 72 hours, and the resulting dry biomass was recorded.

2.6 *Mycorrhizal dependency (MD), mycorrhizal infection, and spore density*

Mycorrhizal dependency (MD) refers to the percentage at which a plant relies on mycorrhizal conditions to achieve optimal production or growth. It refers to the extent to which the plant growth is altered because of colonization by AMF (Graham & Syvertsen, 1985). MD is calculated by:

MD = [(DW inoculated plants - DW non-inoculated plants)/DW inoculated plants] X 100
where DW represents dry weight.

The spore density of AMF per gram of substrate was determined using the wet sieving and decanting technique (Gerdemann & Nicolson, 1963) following thorough mixing of the entire substrate to ensure even distribution of spores throughout. The root samples were subjected to a cleaning process using a 5% (w/v) solution of potassium hydroxide and then stained using a 5% solution of ink vinegar (Vierheilig et al., 1998). The extent of mycorrhization was measured by McGonigle method (McGonigle et al., 1990) through microscopic analysis of intersections of 100 root fragments, each measuring 1 cm in length, that were randomly selected.

2.7 Enzymatic activity: phosphatases

The phosphatase enzyme assay (IU g^{-1} FW) utilized p-nitrophenyl phosphate as the substrate which is hydrolysed to p-nitrophenol by the enzyme (Tabatabai & Bremner, 1969). To measure acid phosphatase activity, 1 g of freshly cleaned roots was mixed with 5 mL of 0.05 M sodium acetate buffer at a pH of 4.8, whereas for alkaline phosphatase activity, the blending process involved using a pre-chilled mortar and pestle with 0.05 M sodium carbonate-bicarbonate buffer at a pH of 10. Subsequently, the homogenate underwent centrifugation at 10,000 revolutions per minute for 15 minutes. This process produced a supernatant known as the crude enzyme extract. The produced pNP was measured using colorimetry at a wavelength of 570 nm.

2.8 Nutrient uptake by plants

For determining the macronutrients in plant material, each plant from every pot was tested. 0.1 g of plant samples was weighed into a 100 mL micro digestion test tube with 2 mL of concentrated H_2SO_4 and left overnight, followed by the addition of 1g of a 10:1 K_2SO_4 and $CuSO_4$ catalyst mixture. The tubes were heated in a KELPLUS digestion unit to 200°C, then 450°C, and turned off after 30 minutes.

To determine total nitrogen (N) in plant material, the digested material after cooling at room temperature was distilled with 20 mL of 4% boric acid and 25 mL of 40% NaOH for 3 minutes. The distillate was titrated with 0.02N H2SO4 until the colour changed from blue to pink. %N was calculated as:

$$\%N = [(\text{Sample titration value} - \text{Blank titration value}) \times 0.00028 \times 100] / 0.1$$

The phosphorus concentration (mg g^{-1}) was determined using the molybdovanadophosphate method, originally described by Olsen et al. (2018), with a modification to use plant samples instead of soil. 5 mL of digested sample was transferred into a 50 mL volumetric flask, followed by the addition of 5 mL of vanadate-molybdate solution, diluted to 50 mL with water, mixed, and allowed to develop colour for 10 minutes. The colour intensity was measured using a spectrophotometer at 420 nm. A blank and various concentrations of stock solution (0.50 to 2.50 mL of 100 mg P L^{-1}) were run simultaneously to prepare a standard curve. The P concentration in the sample was determined using this standard curve.

The flame photometer was used to measure the potassium levels (ppm), following the procedure outlined by Proehl and Nelson (1950). 5 mL of digested plant sample was aspirated into the flame photometer to analyze the potassium concentration.

2.9 Statistical analysis

The experimental data was analyzed using R version 4.3.0 software to investigate and interpret the statistical findings (R: The R Project for Statistical Computing). The analyses consisted of conducting an analysis of variance (ANOVA), performing Tukey's honestly significant difference (HSD) test with a significance level of $p < 0.05$, utilizing a Compact Letter Display (CLD), and examining the correlation matrix. ANOVA evaluated the overall differences between groups in the response variable. This was followed by Tukey's HSD test, which allowed for detailed pairwise comparisons to identify individual groups that had statistically significant variations in their means. The CLD offered a brief description of the findings, utilizing short letters to denote significant variations among the groups. The correlation matrix examined the relationships and interdependencies among various variables in the dataset by generating their correlation coefficients. Principal Component Analysis (PCA) found the proportion of variance extracted from the dataset. The combination of these tests and visualizations offered a thorough method for investigating and comprehending the fluctuations in the data, enabling a detailed comprehension of differences and correlations across groups.

3. Results

3.1 Phenotypical traits of plants

In one of the triplicate pots for the HT treatment, two plants did not survive, leaving only one plant remaining in the pot. The shoot height varied significantly among treatments, with mean values ranging from 9.33 ± 0.25 cm (HT) to 35.50 ± 1.47 cm (AMF). Root length also showed pronounced differences across treatments, ranging from 474.58 ± 11.10 cm (HT) to 1613.43 ± 68.69 cm (AMF). Flowers per plant exhibited variation, with the highest mean of 0.89 ± 0.31 observed in the AMF treatment and the lowest of 0 in HT. Similarly, the number of leaves per plant varied, with the highest mean count of 7.11 ± 0.20 in AMF and the lowest of 5.67 ± 0.17 in HT+AMF. Regarding biomass, the highest mean shoot dry weight was 2.19 ± 0.17 g in AMF, and the lowest was 1.25 ± 0.02 g in HT. For root dry weight, the highest mean was 2.13 ± 0.04 g in HT+AMF, and the lowest was 1.03 ± 0.00 g in HT. Total dry biomass showed the highest mean of 3.99 ± 0.06 g in HT+AMF and the lowest of 2.28 ± 0.02 g in HT. Ratios of root length to shoot height ranged from 46.00 ± 2.53 (AMF) to 56.12 ± 2.49 (HT+AMF), while ratios of root to shoot biomass ranged from 0.55 ± 0.05 (AMF) to 1.16 ± 0.04 (HT+AMF). The ratios of root length to dry shoot biomass ranged from 380.65 ± 12.45 cm g^{-1} (HT) to 789.22 ± 93.43 cm g^{-1} (AMF) (Fig. 2).

3.2 Enzyme activity: phosphatases

Both Alkaline Phosphatase (ALP) activity and Acid Phosphatase (ACP) activity varied among the different treatments. The highest ALP activity was observed in the HT treatment, with a mean value of 1.180 IU g^{-1} FW. This was followed by the CT treatment, with a mean value of 1.007 IU g^{-1} FW, and the HT+AMF treatment, which showed a slightly lower mean value of 1.018 IU g^{-1} FW. The lowest ALP activity was recorded in the AMF treatment, with a mean value of 0.984 IU g^{-1} FW. The highest ACP activity was again observed in the HT treatment, with a mean value of 1.209 IU g^{-1} FW. The HT+AMF treatment showed a mean value of 1.081 IU g^{-1} FW, while the CT treatment had a mean value of 1.067 IU g^{-1} FW. The AMF treatment exhibited the lowest ACP activity with a mean value of 0.995 IU g^{-1} FW (Fig. 3).

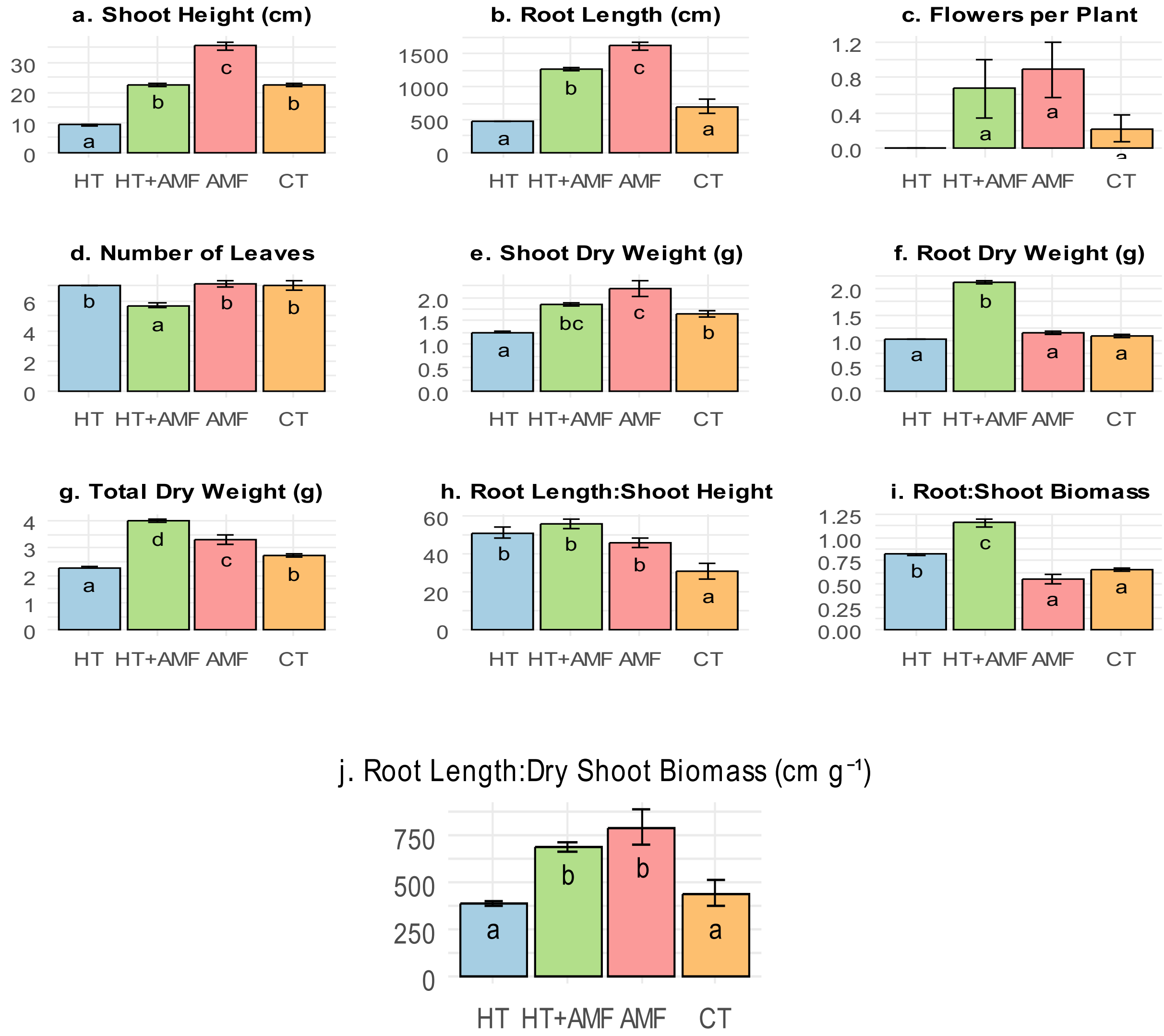


Figure 2. Phenotypical traits of onion plants and their corresponding ratios in different treatments. Bars represent the standard error of the difference (SED) of density. Values with different letters are significantly different at P<0.05 (LSD).

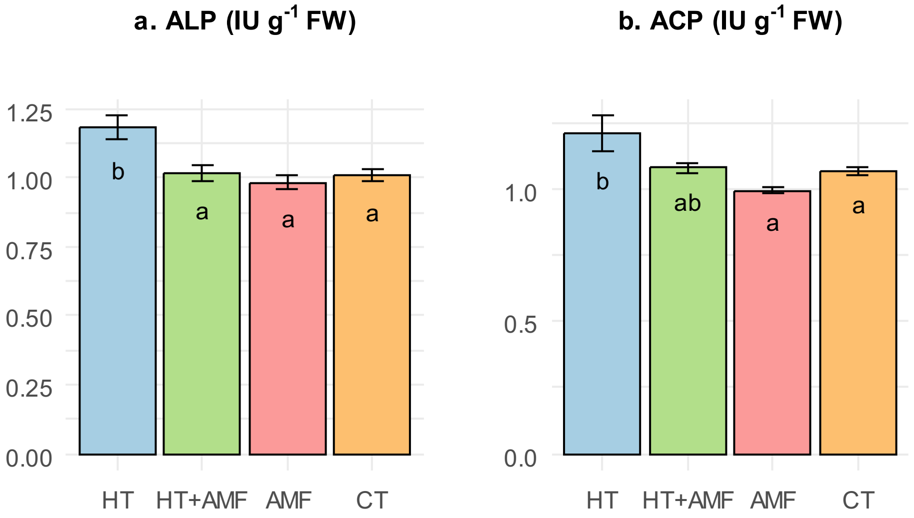


Figure 3. Phosphatase activity in different treatments. Bars represent the standard error of the difference (SED) of density. Values with different letters are significantly different at P<0.05 (LSD).

HT treatment exhibited the highest levels of both ALP and ACP activities, indicating that this treatment may significantly enhance these enzyme activities in the plant tissue. HT+AMF treatment showed intermediate levels of ALP and ACP activities, suggesting that the combined effect of high temperature and AMF might moderate the enzyme activities compared to the HT treatment alone. AMF treatment had the lowest levels of both ALP and ACP activities, indicating that AMF alone might not be as effective in promoting these enzyme activities. CT treatment displayed moderate enzyme activities, falling between the HT and AMF treatments. This suggests that the stress induced by higher temperatures prompted the plants to secrete more enzymes.

3.3 Nutrient acquisition by plants

Total nitrogen content in plant tissues ranged from 0.18% in the HT treatment to 0.52% in the HT+AMF treatment, with intermediate values of 0.48% in the AMF treatment and 0.35% in the CT treatment. Total phosphorus levels followed a similar trend, with concentrations ranging from 7.59 mg g^{-1} in the HT treatment to 13.42 mg g^{-1} in the AMF treatment, and intermediate values of 11.30 mg g^{-1} in the HT+AMF treatment and 9.03 mg g^{-1} in the CT treatment. Total potassium concentrations varied from 147.29 ppm in the HT treatment to 204.44 ppm in the AMF treatment, with values of 182.78 ppm in the HT+AMF treatment and 179.78 ppm in the CT treatment (Fig. 4).

3.4 Mycorrhizal dependency (MD), sporulation, and colonization in inoculated treatments

The assessment of mycorrhizal parameters showed varying degrees of colonization and dependency across treatments. Mycorrhizal dependency was significantly higher in the HT+AMF treatment at 47.17%, compared to 16.17% in the AMF treatment. AMF colonization was higher in the AMF treatment, reaching 50.67%, while the HT+AMF treatment had a colonization rate of 27.00%. Hyphal colonization was similar between treatments, with 15.43% in the HT+AMF treatment and 14.40% in the AMF treatment. Arbuscular colonization was higher in the AMF treatment at 13.03%, compared to 9.10% in the HT+AMF treatment.

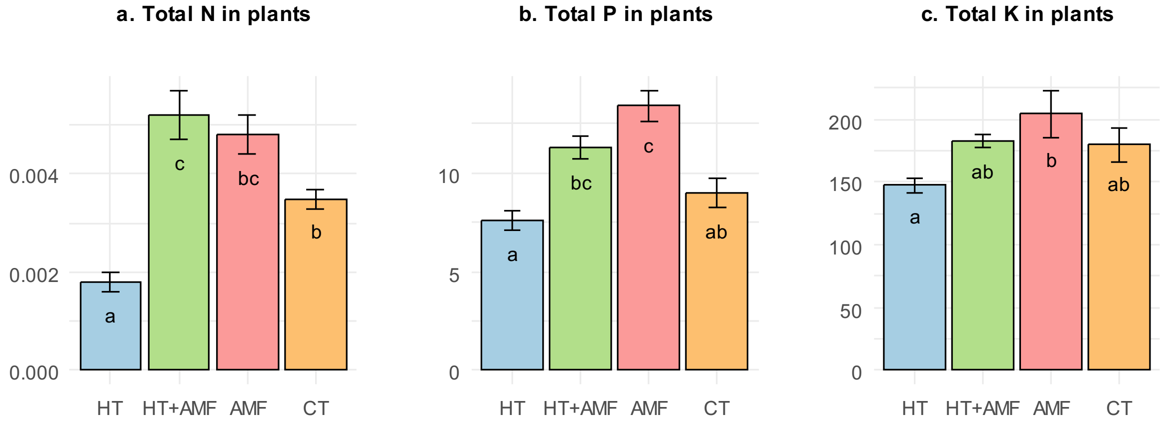


Figure 4. Macronutrients uptake in different treatments. Bars represent the standard error of the difference (SED) of density. Values with different letters are significantly different at P<0.05 (LSD).

Vesicle colonization was notably higher in the AMF treatment at 23.23%, whereas the HT+AMF treatment exhibited only 2.47% vesicle colonization. The spore density per gram of substrate was higher in the AMF treatment, averaging 1.89 spores per gram, compared to 1.00 spores per gram in the HT+AMF treatment (Fig. 5).

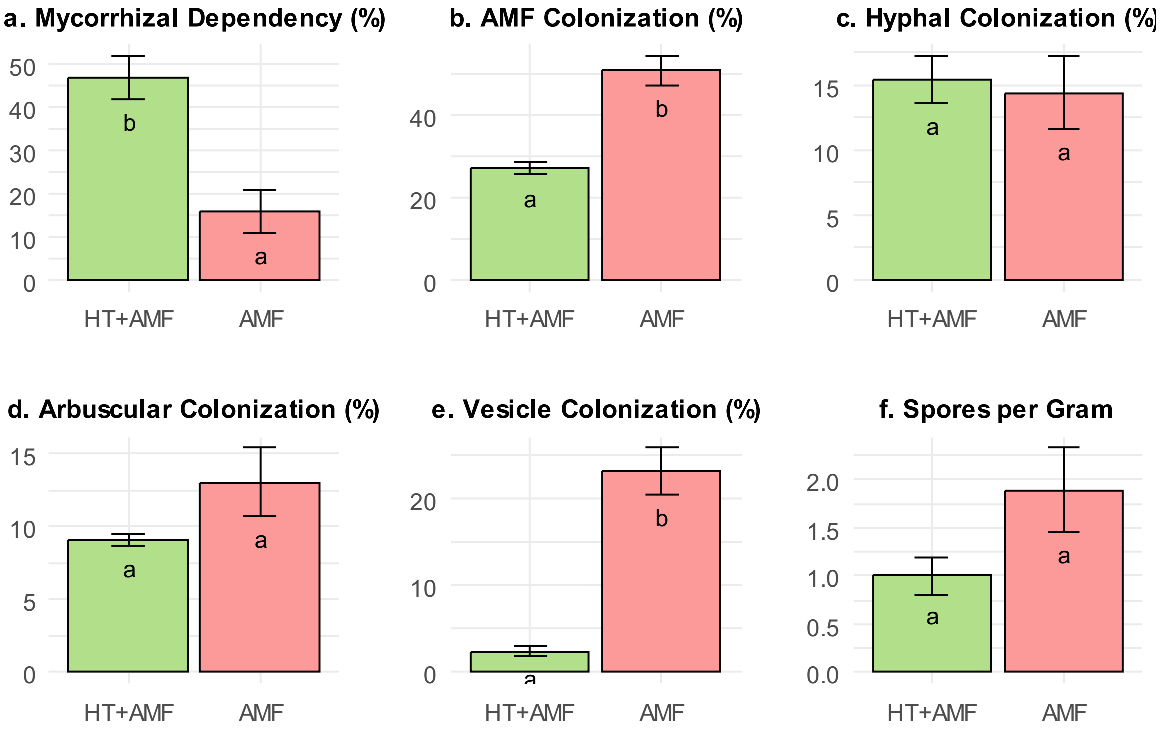
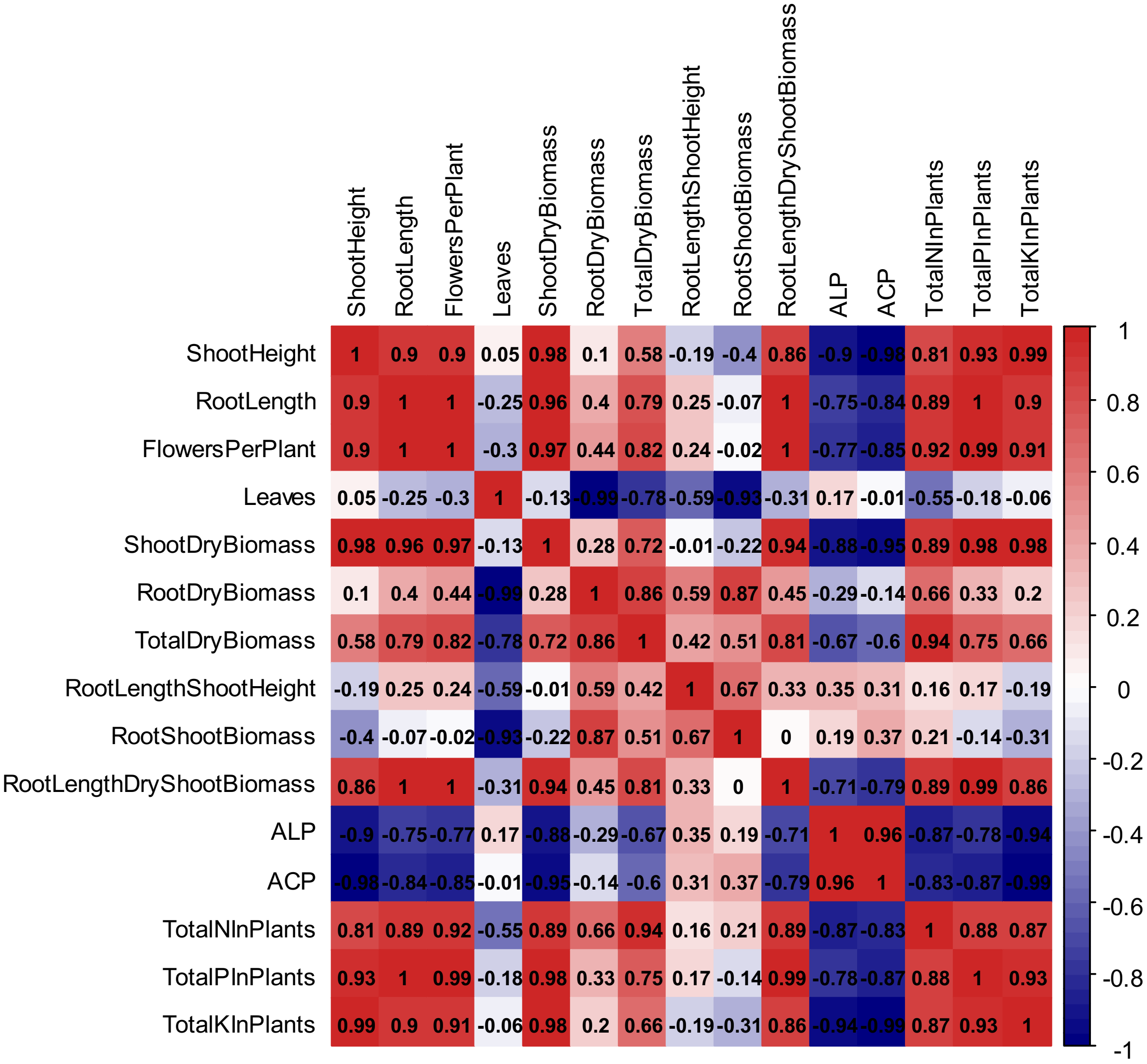


Figure 5. Mycorrhization and spore density in AMF-enriched treatments. Bars represent the standard error of the difference (SED) of density. Values with different letters are significantly different at P<0.05 (LSD).

3.5 *Correlation analysis*

The total nitrogen (N), phosphorus (P), and potassium (K) levels exhibited distinct correlations with key plant growth parameters and nutrient uptake across different treatments. Nitrogen availability showed strong positive associations with shoot height (r = 0.81), root length (r = 0.89), shoot dry biomass (r = 0.89), total dry biomass (r = 0.94), and total phosphorus (r = 0.88), indicating its pivotal role in promoting overall plant growth and biomass accumulation. Phosphorus availability correlated positively with root length (r = 1.00), shoot height (r = 0.93), flowers per plant (r = 0.99), and total potassium (r = 0.93), highlighting its influence on structural and reproductive traits. Potassium availability exhibited strong positive correlations with shoot height (r = 0.99), root length (r = 0.90), flowers per plant (r = 0.91), and shoot dry biomass (r = 0.98), underscoring its importance in enhancing plant stature and biomass production. ALP and ACP show a negative correlation with total phosphorus, with a coefficient of approximately -0.78 and -0.87, respectively. These correlations underscored the intricate relationships between nutrient availability and plant physiological responses, crucial for understanding plant nutrition and optimizing agricultural practices (Fig. 6).



	ShootHeight	RootLength	FlowersPerPlant	Leaves	ShootDryBiomass	RootDryBiomass	TotalDryBiomass	RootLengthShootHeight	RootShootBiomass	RootLengthDryShootBiomass	ALP	ACP	TotalNInPlants	TotalPInPlants	TotalKInPlants
ShootHeight	1	0.9	0.9	0.05	0.98	0.1	0.58	-0.19	-0.4	0.86	-0.9	-0.98	0.81	0.93	0.99
RootLength	0.9	1	1	-0.25	0.96	0.4	0.79	0.25	-0.07	1	-0.75	-0.84	0.89	1	0.9
FlowersPerPlant	0.9	1	1	-0.3	0.97	0.44	0.82	0.24	-0.02	1	-0.77	-0.85	0.92	0.99	0.91
Leaves	0.05	-0.25	-0.3	1	-0.13	-0.99	-0.78	-0.59	-0.93	-0.31	0.17	-0.01	-0.55	-0.18	-0.06
ShootDryBiomass	0.98	0.96	0.97	-0.13	1	0.28	0.72	-0.01	-0.22	0.94	-0.88	-0.95	0.89	0.98	0.98
RootDryBiomass	0.1	0.4	0.44	-0.99	0.28	1	0.86	0.59	0.87	0.45	-0.29	-0.14	0.66	0.33	0.2
TotalDryBiomass	0.58	0.79	0.82	-0.78	0.72	0.86	1	0.42	0.51	0.81	-0.67	-0.6	0.94	0.75	0.66
RootLengthShootHeight	-0.19	0.25	0.24	-0.59	-0.01	0.59	0.42	1	0.67	0.33	0.35	0.31	0.16	0.17	-0.19
RootShootBiomass	-0.4	-0.07	-0.02	-0.93	-0.22	0.87	0.51	0.67	1	0	0.19	0.37	0.21	-0.14	-0.31
RootLengthDryShootBiomass	0.86	1	1	-0.31	0.94	0.45	0.81	0.33	0	1	-0.71	-0.79	0.89	0.99	0.86
ALP	-0.9	-0.75	-0.77	0.17	-0.88	-0.29	-0.67	0.35	0.19	-0.71	1	0.96	-0.87	-0.78	-0.94
ACP	-0.98	-0.84	-0.85	-0.01	-0.95	-0.14	-0.6	0.31	0.37	-0.79	0.96	1	-0.83	-0.87	-0.99
TotalNInPlants	0.81	0.89	0.92	-0.55	0.89	0.66	0.94	0.16	0.21	0.89	-0.87	-0.83	1	0.88	0.87
TotalPInPlants	0.93	1	0.99	-0.18	0.98	0.33	0.75	0.17	-0.14	0.99	-0.78	-0.87	0.88	1	0.93
TotalKInPlants	0.99	0.9	0.91	-0.06	0.98	0.2	0.66	-0.19	-0.31	0.86	-0.94	-0.99	0.87	0.93	1

Figure 6. Heatmap showing correlation among various variables.

The principal component analysis (PCA) revealed that the variables studied exhibited distinct patterns across the treatments. The first principal component (PC1) accounted for approximately 67% of the total variance, highlighting strong correlations primarily with variables such as shoot height, root length, flowers per plant, shoot dry biomass, and total dry biomass. These variables contributed significantly to the separation of treatments along PC1, indicating that they varied the most across the treatments of HT, HT+AMF, AMF, and CT. PC2 captured an additional 26% of the variance, where variables like leaf and root dry biomass showed notable correlations, albeit less pronounced than those on PC1. PC3 contributed minimally to the variance (approximately 7%), mainly influenced by variables such as root length shoot height ratio and root length shoot biomass, suggesting their lesser but still discernible impact on treatment differentiation (Fig. 7).

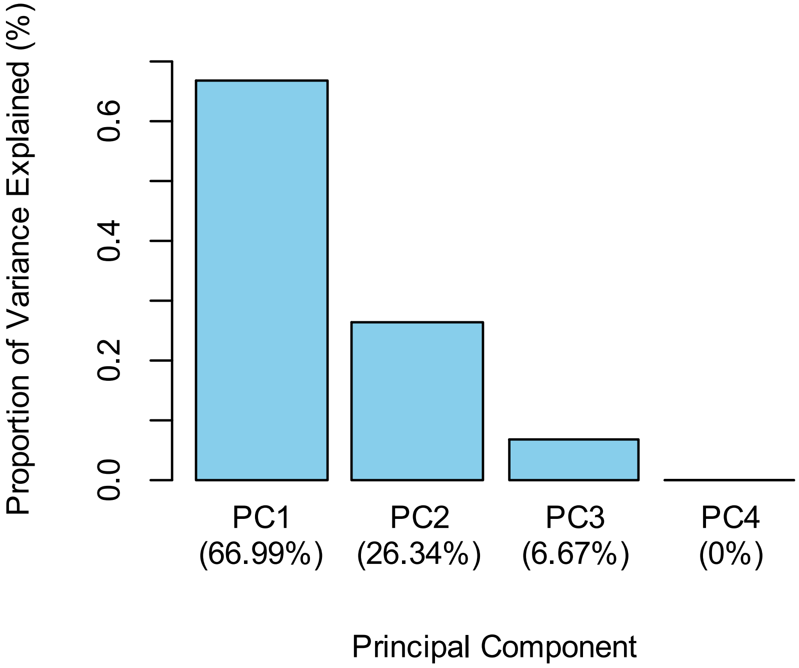
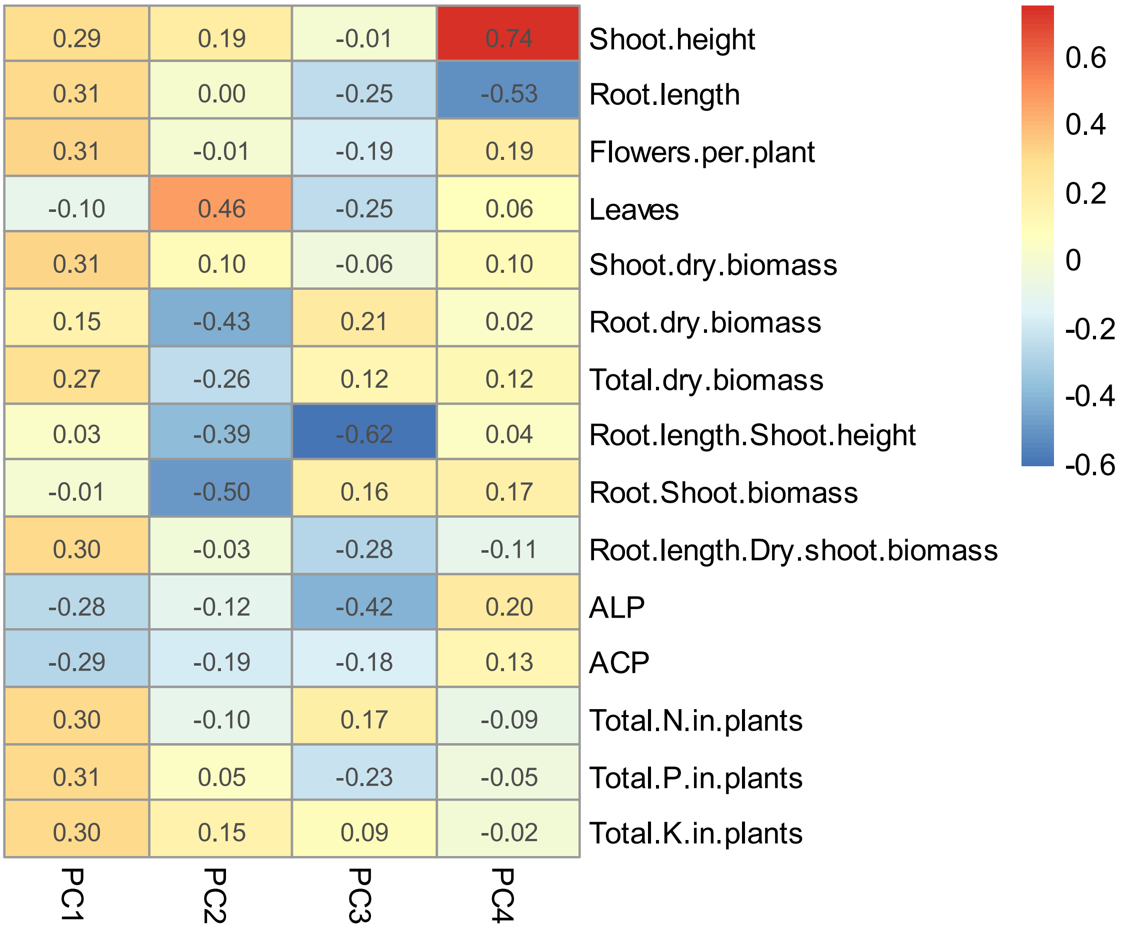


Figure 7. Scree plot explaining the eigenvalue (proportion of variance) of each principal component (PC) extracted from the dataset.

Principal component 1 (PC1) had the highest level of variance among the variables and mostly characterized the overall growth and biomass characteristics of the plants in this study. The factors that exhibited significant positive relationships with PC1 were shoot height, root length, flowers per plant, and total dry biomass. PC1 can be regarded as a composite measure that encompasses both the dimensions of plant size and production. On the other hand, variables like ALP and ACP, which had negative loadings on PC1, indicate characteristics that are less strongly related to the overall size of the plant but may provide information about specific biochemical processes or nutrient dynamics. PC2, which demonstrates substantial loadings on leaves, root dry biomass, and root length height ratio, seems to indicate the balance between shoot and root growth patterns. The dataset reveals a disparity between the growth of roots and the production of leaves, as seen by positive loadings on root dry biomass and root length height ratio and negative loadings on leaves.

Principal component 3 (PC3) and principal component 4 (PC4) have decreasing impacts on the overall explained variance, but they highlight more aspects of the data. PC3 exhibits loadings that emphasize parameters such as the proportion of root length to shoot biomass and the overall phosphorus content in plants. This suggests a possible relationship between the distribution of nutrients and the morphology of plants. Principal Component 4 (PC4), comprising loadings such as shoot height and root length, provides significant insights into secondary growth patterns and physiological characteristics that are less prominently depicted in PC1 to PC3 (Fig. 8).



	PC1	PC2	PC3	PC4
Shoot.height	0.29	0.19	-0.01	0.74
Root.length	0.31	0.00	-0.25	-0.53
Flowers.per.plant	0.31	-0.01	-0.19	0.19
Leaves	-0.10	0.46	-0.25	0.06
Shoot.dry.biomass	0.31	0.10	-0.06	0.10
Root.dry.biomass	0.15	-0.43	0.21	0.02
Total.dry.biomass	0.27	-0.26	0.12	0.12
Root.length.Shoot.height	0.03	-0.39	-0.62	0.04
Root.Shoot.biomass	-0.01	-0.50	0.16	0.17
Root.length.Dry.shoot.biomass	0.30	-0.03	-0.28	-0.11
ALP	-0.28	-0.12	-0.42	0.20
ACP	-0.29	-0.19	-0.18	0.13
Total.N.in.plants	0.30	-0.10	0.17	-0.09
Total.P.in.plants	0.31	0.05	-0.23	-0.05
Total.K.in.plants	0.30	0.15	0.09	-0.02

Figure 8. Heat Map of PCA Loadings

4. Discussions

<u>Phenotypical traits-vegetative and reproductive traits:</u>In our study it was observed that plants cultivated at HT had smaller stem height compared to the ones grown in ambient temperature. This is consistent to the findings where both plant height and total biomass are decreased by exposure to elevated temperatures (Parthasarathi et al., 2022). During different stages of growth, crops are subjected to physiological changes caused by high temperatures (Bita & Geräts, 2013). Heat stress in bean plants limited their morphophysiological traits, including phenology, dry matter partitioning, and shoot growth (Omae et al., 2012). Moderately high temperatures reduce the final stem height in wheat and barley (Dixon et al., 2019). At ambient temperature (23°C), plants grew to their maximum height; higher temperatures caused plants to grow shorter, like the studies by Nordli et al. (2010), which showed that hydrangea internodes grew longer at 24ºC and increased overall shoot length. High temperatures cause soybeans to produce more nodes and leaves overall, but they also shorten individual internode lengths and ultimate plant heights, which decreases the amount of light that the photosynthetic leaves can capture (Allen et al., 2018). Additionally, throughout the vegetative and reproductive stages, high temperatures increase evapotranspiration, which restricts the amount of water that plants can absorb and use. This leads to dehydration, which inhibits growth of the entire plant as well as individual organs (Fahad et al., 2017).

While the effects of high temperatures on plant parts above ground have been extensively researched, the effects on roots are still little understood (Luo et al., 2020). Plant water and nutrient uptake are significantly influenced by root size. For roots to grow and function properly, there is an optimum temperature range; if this range is exceeded, the uptake is altered (Koevoets et al., 2016). The ideal temperature for roots is typically lower than the ideal temperature for shoots (Gavelienė et al., 2022). Studies have indicated that temperature increases can have one of three effects on root growth: stimulatory, inhibitory, or, after the optimal temperature is attained, stimulatory at first and inhibitory later (De Lima et al., 2021). We found in our study that higher temperatures had a suppressive effect on root growth which corroborates with the findings of other studies. Temperate lettuce plants thrive in conditions that expose their roots to midday temperatures of up to 40^{o}C, which significantly restricts the growth of their roots and shoots (Qin et al., 2007). In sunflower, exposure to high temperatures (>29°C) reduces root growth, lateral root density, and primary root length (De Lima et al., 2021). In sorghum, the temperature of the root zone reduces the rate of root elongation and cell development, thus impeding the root growth by slowing down the rate of cell division (Calleja-Cabrera et al., 2020).

Our study found the higher temperatures lead to the reduced tissue biomass. The effects of high temperatures on biomass have not been thoroughly investigated in comparison to other stress factors. According to a study, root growth ceases when whole plants are grown at high temperatures (above optimum), which reduces root functionality and adversely affects shoot biomass (González-García et al., 2022). Likewise, heat and drought decreased the overall root biomass of *Arabidopsis* in an additive way (Vile et al., 2011).

Flowers were absent at higher temperatures in our study. High temperatures are more likely to disrupt crop reproductive activities than vegetative phases (Draeger & Moore, 2017). There have been reports of various plants showing a delay or suppression of flowering at high soil temperatures (Adams et al., 1999). High temperatures have an impact on reproductive organs in both males and females (Wang et al., 2021; Gonzalo et al. 2020; Begcy et al., 2019; Wang et al. 2018). Floral buds and flowers can experience a significant drop in reproduction when exposed to a brief period of heat stress. However, the sensitivity to this stress varies among different plant species and varieties (Sato et al. 2006). Exposure to high temperatures, whether brief or prolonged, has varying effects on all causes of sterility in the reproductive organs (Zhang et al., 2018). During periods of elevated temperatures, the plant may fail to bloom at its reproductive growth phases (Maheswari et al., 2011). Heat waves or temperature extremes are more damaging to reproductive tissues than ambient temperatures which are slightly over normal (Wang et al., 2019). Abiotic stress conditions characterized by high temperatures can increase sterility for several reasons, including but not limited to disrupted fertilization processes, growth obstacles for endosperm and proembryo, reduced viability of ovules, abnormalities in the positions of the stigma and style, a decrease in the number of pollen grains retained by the stigma, and impaired meiosis in both the male and female organs (Cao et al., 2008). According to some research (Su et al., 2001), high temperatures may prevent the synthesis or interconversion of plant hormones like gibberellin or cytokinin, or they may lessen the impact of floral signals.

Our study demonstrated that inoculating the plants with AMF improved the vegetative and reproductive growth characteristics even under stress conditions of elevated temperatures (HT+AMF). The introduction of mycorrhizal fungi into a crop substantially enhances both the accumulation of biomass and the yield (Chen et al., 2014). AMF are regarded as bioremediation agents of stress (Fall et al., 2022). Our findings indicated that AMF-enriched treatments displayed better plant physiological parameters compared to non-inoculated treatments. Moreover, the inoculation with AMF exhibited better physiological traits, showcasing benefits by alleviating stress and enhancing crop productivity (Candido et al., 2013). The accumulation of plant biomass is closely related to the symbiotic influence of AMF and soil nutrients. Ma et al. (2020) showed a direct correlation between the colonization rate of mycorrhizal fungi and overall biomass.

Nutrient acquisition: Typically, soil macronutrients nitrogen (N), phosphorus (P), and potassium (K) have the most significant impact on crop productivity (Elser et al., 2007). Extensive research has examined the effects of long-term global warming on the limiting nutrients, especially for elements other than N, P and K (Litton et al., 2020). The stoichiometry and concentrations of N and P in the soil may be affected, as well as the mechanisms involved in the cycling of nutrients, by changes in the environmental conditions (Peñuelas et al., 2011). Temperature is the most significant abiotic factor influencing the N and P cycles in soil (Bai et al., 2013). According to Conant et al. (2011), a rise in temperature typically speeds up the release of available nutrients from the soil and helps the organic matter in the soil to decompose. On the other hand, several studies revealed that warming had no discernible impact on the rate of net mineralization of N and P (Zhang et al., 2013). The availability of nutrients in soil is even decreased by rising temperatures. A warmer environment led to a reduction in the availability of inorganic N (Jiao et al., 2016). Rising temperatures decreased the levels of available P, supporting our study (Geng et al., 2017).

The growth of plants relies on the availability of essential nutrients. The capacity of plants to absorb these nutrients is influenced by the growth of their root systems and the abundance of nutrients in the rhizosphere and on the form of available nutrient. An effective approach for plants to compensate for nutrient deficiencies is to form symbiotic relationships with AMF, which increase nutrient absorption of the root system by mycelial growth beyond root hair region and improvement of soil structure and nutrient status (Bahadur et al., 2019). The mycelium decreases nutrient and organic matter loss, increases water and mineral retention, and boosts plant productivity (Dierks et al., 2021). AMF effectively mitigates the depletion of non-renewable phosphorus resources (Fall et al. 2023; Trejo et al. 2021; Ghobadi et al. 2020). Plant root secretions stimulate microorganism activity, promoting enzyme production. These enzymes further decompose organic matter in the soil, increasing nutrient levels like N and P, thereby impacting root physical characteristics (Bowles et al., 2016). Microorganisms play vital roles in soil processes like nutrient cycling, absorption, and soil health maintenance, thereby influencing plant growth by altering root structure (Kumar & Verma, 2018). Enhanced N, P, and K tissue contents were found to be positively correlated with mycorrhizal colonization (Abdel-Fattah et al., 2016), as observed in our study, where the concentrations of available NPK were higher in the colonized treatments. In a study by Hussain et al. (Hussain et al., 2019), it was observed that applying AMF to seeds resulted in increased root colonization in maize plants. Another study further demonstrates that AMF not only enhances colonization but also provides protection to plants during periods of high-temperature stress (Alotaibi et al., 2023).

Phosphatase activity: Phosphatases are the enzymes that catalyze the hydrolysis of phosphoric acid esters (Sharma et al., 2023). Acid Phosphatases (ACP) metabolize phosphate in tissues by catalysing transphosphorylation events and breaking down the various orthophosphate esters in acidic conditions (Sharma et al., 2023). In plants, ACP acts as an inorganic phosphate (Pi) scavenger. They mobilize Pi to assist growth when under stress, like in a drought or a Pi scarcity (Plaxton & Carswell, 2018). Some ACP scavenge Pi from extracellular Pi-esters, while others recycle Pi in the vacuoles of Pi-starved plants (Tran et al., 2010). The phosphate scavenging, use, and turnover of phosphate-rich sources are significantly influenced by intracellular and secreted ACP (Gellatly et al., 1994). Research indicates that in low phosphate environments, certain plants release more acid phosphatases (Lim et al., 2003). Higher levels of acid phosphatases have been observed in tomatoes and other plants during stressful and nutrient-deficient periods, primarily in Pi (García & Ascencio, 1992). The studies corroborate our findings that, as compared to plants cultivated at ambient temperature, those subjected to higher temperature stress had higher ACP levels. In plants, ALP is essential for the maintenance of cellular metabolism through the supply and metabolism of inorganic phosphate (Mishra & Dubey, 2008). Increased acid phosphatase levels were observed in the rhizosphere and roots of rape, wheat, and onion infected with AMF compared to uninfected plants (Dodd et al., 1987).

Spore density and mycorrhization: The superior growth observed in AMF-enriched, HT-exposed plants compared to those exposed solely to HT suggests that AMF plays a protective role against temperature stress in maize by enhancing AMF biomass through improved carbon allocation. This enhancement promotes greater colonization and shields the plants from HT-induced damage. Root colonization in both AMF and AMF + HT treatments also contributes to higher photosynthetic rates (Mathur et al., 2021). Our study aligns with these findings, showing that AMF-enriched plants subjected to higher temperatures had significantly higher spore density in the soil and increased root colonization and thus improved morphological traits compared to non-mycorrhizal plants under high-temperature conditions. Thus, the AMF-enriched plants had more active mycelial biomass in the soil, which likely led to better nutrient absorption and more efficient photosynthesis during heat stress.

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