

**DIVERSITY OF ARBUSCULAR MYCORRHIZAL FUNGI
ASSOCIATED WITH RICE (*Oryza sativa* L.) AND THEIR ROLE
ON RICE TOLERANCE TO DROUGHT AND SALT STRESS**

**A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS OF THE DEGREE OF DOCTOR OF
PHILOSOPHY**

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MZU REG. NO.: 1627 of 2009-10

Ph.D. REG. NO.: MZU/Ph.D./1272 of 08.09.2018



DEPARTMENT OF BOTANY

SCHOOL OF LIFE SCIENCES

SEPTEMBER, 2024

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TO DROUGHT AND SALT STRESS**

BY

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Submitted

**In partial fulfillment of the requirement of the Degree of Doctor of Philosophy
in Botany of Mizoram University, Aizawl**

MIZORAM UNIVERSITY

(A Central University Established by an Act of Parliament of India)

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CERTIFICATE

This is to certify that the thesis work entitled, “Diversity of Arbuscular Mycorrhizal Fungi Associated with Rice (*Oryza sativa* L.) and their Role on Rice Tolerance to Drought and Salt Stress”, submitted by MC Freddy Vanlalmuana (MZU/Ph.D./1272 of 08.09.2018) in partial fulfilment of the requirement of the degree of Doctor of Philosophy in Botany is a record of bonafide work carried out by him under my supervision and guidance.

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DECLARATION

MIZORAM UNIVERSITY

SEPTEMBER, 2024

I, **MC Freddy Vanlalmuana**, hereby declare that the subject matter of this thesis entitled “**Diversity of Arbuscular Mycorrhizal Fungi Associated with Rice (*Oryza sativa* L.) and Their Role on Rice Tolerance to Drought and Salt Stress**” is the record of work done by me, that the contents of this thesis did not form basis of the award of any previous degree to me or to do the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other University/Institute.

This is being submitted to the Mizoram University for the degree of ***Doctor of Philosophy*** in Botany.

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ACKNOWLEDGEMENTS

The completion of this PhD thesis has been a long and challenging journey, and it would not have been possible without the support and guidance of many individuals and institutions, to whom I am deeply indebted.

First and foremost, I give thanks to God Almighty. I wish to express my profound gratitude to my supervisor, Prof. R. Lalfakzuala. Your support, advice, and encouragement have guided me through this research process. You allowed me to explore my ideas while being there with your guidance when necessary. Your guidance has been essential, and I couldn't have asked for a better supervisor on this journey.

I am also immensely grateful to the Head of the Department of Botany, Prof. F. Lalnunmawia. My sincere thanks go to Prof. G. Gurusubramanian, the Dean of the School of Life Sciences. The academic opportunities provided by the Department of Botany; School of Life Sciences have greatly contributed to my growth as a researcher. I would also like to express my heartfelt appreciation to all the faculties of the Department of Botany. I would also like to express my deepest gratitude to Dr. Kripamoy Chakraborty and Dr. Panna Das, Assistant professor, Tripura University for their invaluable guidance and support for my research. I would like to extend my gratitude for the financial support provided by UGC, CSIR, DBT NECAB, DBT-BUILDER, SERB and MoTA. I would like to extend my heartfelt appreciation to my research colleagues from the Laboratory of Microbiology and the Department of Botany, Mizoram university.

To my wife, Gospel Lalawmpuii, and my daughter, Camellia Lalnunmawii, words cannot express my gratitude for your love, patience, and understanding throughout this process. I am equally grateful to my father, M. Sangkunga, and all my family for their unconditional love and support. Lastly, I dedicate this work to the loving memory of my mother, K. Lalzarmawii.

MC Freddy Vanlalmuana

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Chapter 1

Introduction

Mycorrhizae can be referred to as a mutual relationship that occurs between a fungus and a plant root. Mycorrhizae is well known to serve important functions within ecosystem functioning, plant nutrition status, and soil conditions. This type of association is known as mutualism where both the partners involved benefit and has been an important trait as it has altered the evolution of plants and agriculture for the better. All the major lineages of land plants and around 90% of the existing land plant species till this day still develop a mycorrhizal relationship with fungi (Feijen, 2018). Various types of mycorrhizae have been identified, with orchid mycorrhizae, ectomycorrhizae (EM), ericoid mycorrhizae, and arbuscular mycorrhizae (AM) being the most prominent. Each kind includes various fungal species and how they interact with host plants in different ways.

Arbuscular mycorrhiza (AM) is probably the most established terrestrial symbiotic association (Fitter, 2005), being present in the roots of approximately 70-90 % of all the plants (Smith and Read, 2008). In arbuscular mycorrhiza, the fungal hyphae penetrate the cortical cells of the roots, forming structures called arbuscules and, at times, vesicles, improving the uptake of water or soluble nutrients mostly phosphorus in reciprocation of carbohydrates supplied by the plants (Finlay, 2008). AM fungi can also develop a different form of intraradical structures (Dickson, 2004), such as hyphal coils that can form in the absence of arbuscules.

This form of mutualism often involves members of the fungal phylum Glomeromycota, which is thought to be monophyletic (Schüßler *et al*, 2001; Hibbett, D. S *et al.*, 2008). With further refinement of molecular tools, the arbuscular mycorrhizal fungi (AMF) were further resolved at the taxonomic level which led to the better understanding of their biodiversity (Türrner, 2012). Some correlating species of plants are associated with the respective AM-fungal communities which might reflect a certain degree of host preference or specialization. Such host-preference indicates that even when other factors remain constant, plant diversity in an ecosystem

can be a determinant to AM fungal diversity. Johnson *et al.* (2003) pointed out that plant species composition and diversity are usually robust explanations for what types of AMF communities will develop. Conditions such as temperature, precipitation, and humidity are climatic factors affecting AMF diversity and community composition. Studies by Dumbrell *et al.* (2010) showed that climatic factors affect the distribution and amount of AMF taxa in different geographical regions.

First traces of AM fungi have been found as far back as the early Paleozoic era, four hundred million years ago. This kind of symbiosis is thought to have been crucial for the establishment and radiation of early terrestrial plants (Parniske, 2008). Fossil evidence suggests that AM fungi were also associated with the earliest land plants, thus contributing to the earlier success of plants on land. Such associations, in all likelihood, helped in the absorbance of water and minerals from the soil which was quite imperative in the struggle to live in the rough terrestrial world (Strullu-Derrien *et al.*, 2018). Furthermore, molecular phylogenetic studies indicate that the AM symbiosis is a highly ancient and prevalent kind of plant-fungal interaction. The persistence and worldwide occurrence of this relationship across plant evolution is evidence of its basic functional role in plant biology (Wang and Qiu, 2006). The evolution of arbuscular mycorrhizal fungi, in the sense of co-evolution with plants can be understood from their extreme distribution within many different plant families (Corradi and Bonfante, 2012).

The process of AM symbiosis establishment begins with the signalling between plant roots and AMF. The first steps include the mutual exchange of signalling substances from both partners. The plants produce compounds known as strigolactones that induce the spore of the fungi to break dormancy and form hyphae toward the roots (Akiyama *et al.*, 2005). In response to that stimulation, AMF themselves produce several molecules called *Myc* factors, which are received by plant receptors and stimulate a signalling cascade within the plant body (Maillet *et al.*, 2011). During the intracellular stage of symbiosis, arbuscule is formed, erratic frondlike structures that reside in the cells of the cortex that are the contact sites for nutrient exchanges (Harrison, 1999). The arbuscules provide an increase in the area of nutrient exchange thereby facilitating the direct translocation of phosphorus from the fungal partner to the host plant (Smith and Read, 2008). The plant reciprocates by providing the fungus

with carbon molecules obtained through photosynthesis. Nutrient transfer occurs with the help of specific transporter proteins that are existent at the membrane of plants and fungi, facilitating the two – way communication for the needed components (Javot *et al.*, 2007). Hence in plants, some of the phosphate transport, lipid biosynthetic, and defense-related genes are upregulated in the presence of the fungus partner (Guether *et al.*, 2009). Further, AMF alter their biochemical pathways in order to enhance the consumption of the carbon source given by the plant and to enable their growth and reproduction in the host root (Bago *et al.*, 2000).

Arbuscular mycorrhizae play an important role in the function of almost all terrestrial ecosystems by enhancing the growth of a plant, soil health and all forms of productivity. The AM fungus create large networks of hyphae, which enhance the surface area for nutrient absorption. This enables plants to obtain soil nutrients that are located outside the range of their roots. AM fungi promote plant growth because they are efficient in increasing the level of often deficient in soils phosphorus that is essential for plants. Such fungi also assist in the uptake of other nutrients such as nitrogen, zinc and copper (Smith and Read, 2008). The fungal hyphal systems associated with arbuscular mycorrhizal fungi are implicated in soil aggregation thus enhancing soil structure. This influences the capacity of the soil to retain moisture and minimize soil erosion leading to encouraged land management (Rillig, 2004). Arbuscular mycorrhizal (AM) fungi influence the dynamics of plant communities by offering distinct advantages to different species, therefore fostering plant variety. This symbiosis also increases the plants' adaptability to various environmental stresses, such as droughts and salinity, as well as providing protection from soil borne diseases (van der Heijden *et al.*, 1998; Begum *et al.*, 2019).

Rice (*Oryza sativa* L.) is one of the most important crops within any agricultural system in the world. More than half of the world's population relies on it as a staple food (Rahman, 2023). Its nutritional value, economic significance and religious and cultural prominence are reasonable in Asian countries where it is part of food and economic stability. There are many challenges for rice cultivation including water availability as it is necessary to bear heavy inundation as in deep water rice cultivation or non-availability of water as in upland cultivation (Khush, 2005). Together with maize and wheat, rice contributes nearly 44% of the overall global

dietary caloric intake. Rice provides over 70% of the caloric supply in several Asian countries (GRiSP, 2013; Maclean *et al.*, 2002). Rice is considered a highly strategic commodity on a worldwide scale due to its significant impact on global food security, as well as its tight association with economic growth, employment, social stability, and regional peace (Yadev and Kumar, 2018). For years, in several of the world's largest rice growing countries such as India, Bangladesh, and China, there has been little change in yield and total production (Shetty *et al.*, 2013) while the demand from impoverished populations continues to rise.

As far as global climate change is concerned it is the change in the climate that persist for a longer time on a larger scale. The Intergovernmental Panel on Climate Change (IPCC) reports that as a result of human-induced activities, the global temperature has increased by approximately 1.2°C above the pre-industrial average (IPCC report, 2021). Global warming is here to stay and it is going to have large impacts on ecosystems, weather patterns and people's well-being. The weather changes fostered by global warming are also changing the traditional distribution and intensity of rainfall, so that some places experience more severe or frequent storms while others, more prolonged periods of drought. These phenomena have the potential to impact water sources, food production systems and biodiversity (Trenberth *et al.*, 2007). In Mizoram alone, the total amount of rainfall decreases at the rate of 5.22 mm per year. During the period of 32 years, total annual rainfall is highest in 2007 (3121.86 mm) and lowest in 2014 (1936.83 mm). The average annual rainfall received every year is 2551.88. (Climate profile of Mizoram, Mizoram State Climate Change Cell). Due to the melting of polar ice sheets, glaciers, and thermal expansion of water in oceans, sea levels are increasingly on the rise. Studies reveal that since 1880 global sea levels have risen by around 20 centimetres and in particular the last few decades have seen dramatic changes in the rate of this increase (Church and White, 2011).

The potential impact of climate change is that rice productivity is forecasted to decline (IFPRI, 2009). Rice cultivation is heavily reliant on the presence of water, and alterations in precipitation patterns might disturb the essential water supply necessary for rice fields. The sensitivity of rice production to temperature and water availability makes it particularly vulnerable to these changes (Wassmann *et al.*, 2009). Rice growth and development are negatively impacted by drought stress, resulting in decreased leaf

size, stunted plant height, and reduced biomass accumulation. Physiological functions such as photosynthesis, respiration, and nutrient absorption are impaired. Water deficit affects the yield of grains extensively, in particular during the flowering and grain filling period of the crop. Studies have shown about 30–50% yield loss in severe drought conditions (Bouman *et al.*, 2007; Lafitte *et al.*, 2004). Lower or suboptimal moisture conditions too affect the quality of the rice grains because poor grain filling reduced grain size and lower milling quality (Kamoshita *et al.*, 2008).

As soon as salinity stress occurs, growth of the rice during its vegetative and reproductive stages is invariably retarded. Other effects of salinity include the reduction of the germination percentages, the development of seedlings and even the ultimate height of the entire plant. Almost all aspects of the yield including number of tillers, panicle length and grain weight got diminished considerably when subjected to salinity (Zeng *et al.*, 2003). Salinity also brings ionic stress when high concentrations of toxic ion such as Na⁺ or Cl⁻ are present and affect the absorption and balance of nutrients (Munns *et al.*, 2008). Several mechanisms allow rice plants to adjust to salinity stress such as the development of thicker cuticle on leaves, modification of root structures or osmotic adjustment via the synthesis of proline and glycine betaine among others (Horie *et al.*, 2012).

The effects of the relationship between rice growing and AM fungi have been shown to enhance plant growth and yield. AM colonization can improve P uptake and biomass production of rice plants (Zhang *et al.*, 2018; Chen *et al.*, 2017). Additionally, the AM fungi may enhance the tolerance of rice to drought and salt by regulating certain physiological and biochemical activities (Li *et al.*, 2020; Evelin *et al.*, 2019). AMF colonization results in an increased osmolytes concentration of which, proline and soluble sugars are osmoprotectants and assist in osmotic adjustment under drought stress (Ruiz-Lozano, 2001). On the other hand, AMF-associated plants also exhibit enhanced antioxidant enzyme activity for the removal of reactive oxygen species produced under salt stress (Evelin *et al.*, 2009). Besides nutrient uptake and the ability to withstand stress, they also play other very vital roles involving the regulation of plant hormonal levels and gene expression. These interactions foster increased quality and resistance in plants, thus promoting the sustainable use of agriculture (Pozo and Azcón-Aguilar, 2007).

The role of arbuscular mycorrhiza (AM) in salt and drought tolerance of rice is essential in the scope of global agricultural problems. With climate change aggravating the occurrence and intensity of several abiotic stresses such as salinity and drought, there is a need for more sustainable and eco-friendly agricultural practices. The present study focused on investigating the diversity of arbuscular mycorrhizal fungi (AMF) associated with rice plants in Mizoram, a region in North East India. Additionally, it aimed to evaluate how these locally isolated AMF strains influence the biochemical and physiological responses of rice plants under drought and salt stress conditions. The research specifically examined two rice genotypes to understand how AMF inoculation modulates their tolerance to these abiotic stresses.

The objectives of the present research were:

1. To isolate and identify AM fungal community associated with rice plant
2. To study the role of AMF on rice tolerance to drought and salt stresses
3. To analyse the soil physio-chemical and biochemical properties of the study site.

Chapter 2

Review of Literature

2.1 Overview of Rice Cultivation

2.1.1 Historical Context of Rice Cultivation

The cultivation of rice has a long and extensive history that dates back several millennia. It is believed that rice have been domesticated over 9,000 years ago in the Yangtze River basin in China (Fuller *et al.*, 2009). The process of domesticating rice had a crucial role in human history by facilitating the formation of stable agricultural communities throughout Asia. The spread of rice cultivation from Asia to other regions of the world took place through trade routes and colonization, resulting in its establishment as a widely grown crop worldwide (Huke and Huke, 1997).

2.1.2 Global Importance of Rice

One most important staple food crop in the world is Rice (*Oryza sativa* L.) in which more than half of the world's population depend. Rice plays a vital role in ensuring global food security, since it contributes 20% of the world's dietary energy supply. This makes it an essential crop in the battle against hunger and malnutrition (FAO, 2021). In many countries, the production of rice is deeply rooted in both the culture and the economy respectively.

Rice cultivation holds great importance in many Asian countries, not only as a means of sustenance but also a deeply ingrained cultural and religious practice. 78% of the world's rice is grown under irrigated or rainfed lowland conditions (Itao *et al.*, 1999). Rice production in 2003 reached over 600 million metric tons, covering a land area of 149 million hectares. This accounts for 23% of the global caloric intake (Khush, 2005). The global population growth is driving an upward trend in the demand for rice. As per the International Rice Research Institute (IRRI, 2022), in order to satisfy the increasing demand, rice production needs to be augmented by 25% from its present levels by 2050.

2.1.3 Rice Cultivation in India

India is the world's second-largest rice producer, after China, accounting for around 20% of worldwide rice production. Rice cultivation in India is widespread, spanning several agro-climatic regions. The eastern, southern, and northeastern parts of the country are the primary places where rice is produced. Rice cultivation occurs in three primary seasons: Kharif (monsoon), Rabi (winter), and summer. Kharif is the most important season, contributing approximately 85% of the overall rice production in India (Government of India, 2023). According to Ghosh *et al.* (2020), rice is cultivated across multiple agro-climatic zones in India, including the Indo-Gangetic Plains, Eastern and Western Coastal Plains, and the hilly regions of the Northeast and Western Ghats.

In India, rice production is mostly defined by traditional methods, however there has been a gradual transition towards the adoption of modern techniques and high-yielding varieties (HYVs). The introduction of high-yielding varieties (HYVs), coupled with the use of chemical fertilizers, pesticides, and irrigation, led to a substantial increase in rice production (Pingali, 2015). The Green Revolution had a substantial impact on the transformation of rice farming in India, namely in states such as Punjab and Haryana, where there was a widespread adoption of intensive rice-wheat cropping systems (Swaminathan, 2020). Nevertheless, this escalation has also resulted in several ecological problems, such as soil deterioration and limited water supply. Climate change poses additional threats, including increased frequency of droughts, floods, and temperature fluctuations, which can adversely affect rice yields (Aggarwal *et al.*, 2019). Additionally, the overuse of chemical fertilizers and pesticides has led to soil degradation, reduced soil fertility, and the contamination of water resources (Basu and Paroda, 2018).

2.1.4 Challenges in Rice Cultivation

Traditional rice cultivation practices, such as puddling and continuous flooding, require substantial amounts of water as rice is a water intensive crop. Efforts are being made to investigate sustainable methods of rice farming, such as the System of Rice Intensification (SRI) and direct-seeded rice (DSR), in order to decrease water use. This is particularly important as groundwater levels decline and competition for

water resources becomes more intense (Bouman *et al.*, 2007). The effects of climate change on rice agriculture are becoming more apparent. Climate change, characterized by escalating temperatures, unpredictable rainfall patterns, and a rise in the occurrence of severe weather phenomena such as floods and droughts, has a negative impact on rice production. Studies have shown that a rise in temperature of 1°C could lead to a decrease in rice yields of 10% (Lobell *et al.*, 2011). This requires the creation of rice varieties that are resilient to climate change and the implementation of measures to adapt to these changes.

Rice is vulnerable to a variety of pests and diseases, which can result in substantial reductions in crop productivity. The Brown Planthopper (*Nilaparvata lugens*) and Bacterial Blight (*Xanthomonas oryzae*) are highly destructive pests. Implementing Integrated Pest Management (IPM) measures, such as utilizing resistant cultivars, employing biological control methods, and employing pesticides in a careful and controlled manner, is crucial for effectively managing these risks (Heong and Hardy, 2009). Persistent rice cultivation, particularly in intensive agricultural systems, results in the depletion of soil nutrients and a deterioration of soil health. Unbalanced fertilizer application, namely excessive use of nitrogen-based fertilizers, has resulted in problems such as soil acidity and decreased fertility. To tackle this issue, it is necessary to advocate for the implementation of balanced fertilization and integrated nutrient management methods (Fageria *et al.*, 2011).

Socio-economic factors have a significant role in rice farming, with smallholder farmers being the primary participants, especially in places like India. These farmers frequently have difficulties with their ability to obtain loans, access markets, and utilize technology. Moreover, variations in rice prices and the exorbitant expenses associated with inputs might impact the profitability of rice cultivation. Policy interventions targeting the enhancement of the economic sustainability of rice cultivation and providing assistance to small-scale farmers are of utmost importance (Pingali *et al.*, 2019).

2.2 Abiotic Stress in Rice

Rice production frequently faces many challenges from abiotic stresses, including drought, salt, temperature fluctuations, and flooding. The effect of these

stresses can have a substantial impact on rice production, thereby impacting the livelihoods of millions of people. Gaining insight into the mechanisms of stress tolerance and cultivating stress-resistant rice varieties is crucial for maintaining rice production in the face of shifting climatic conditions.

Drought is a major abiotic stress factor that has a considerable impact on rice production, especially in rainfed regions. Drought stress causes a decline in leaf water potential, closure of stomata, and a decrease in photosynthetic activity, ultimately resulting in a loss in grain yield. The research conducted by Serraj *et al.* (2009) emphasizes the significance of root characteristics in drought tolerance. Specifically, the presence of deeper and thicker roots enhances the ability to absorb water more effectively during drought situations. Genes linked to drought tolerance in rice have been found through molecular investigations. For instance, the *DREB1A* gene, responsible for encoding a transcription factor that plays a role in responding to drought, has been demonstrated to enhance the ability of transgenic rice to withstand drought conditions when it is overexpressed (Ito *et al.*, 2006). In addition, QTL mapping has been used to discover specific areas of the genome that are associated with drought tolerance. One example is the *qDTY* loci, which have been found to be linked to crop yield under drought conditions (Venuprasad *et al.*, 2009). Flooding, especially submergence, is a prevalent kind of stress in low-lying regions where rice is cultivated. Rice plants have the ability to endure brief periods of being submerged, but extended inundation can result in hypoxia, which in turn leads to diminished growth and output. The *Sub1* QTL, located on chromosome 9, has played a crucial role in the development of rice cultivars that can tolerate submergence. The *Sub1A* gene, which is a significant constituent of this QTL, improves survival under submergence by inhibiting elongation and preserving carbohydrate reserves (Xu *et al.*, 2006).

Salinity stress is a significant abiotic factor that has a negative impact on the growth of rice, especially in coastal and irrigated regions. Elevated salinity levels result in osmotic stress, ion toxicity, and nutritional imbalance, all of which hinder the growth and development of plants. Munns and Tester (2008) found that salinity stress causes a disturbance in the balance of ions within plant cells, resulting in the excessive buildup of sodium (Na^+) and chloride (Cl^-) ions in plant tissues. One of the primary focuses of developing salt-tolerant rice varieties has been on the identification of genes

and QTLs that are associated with salt tolerance. The *Saltol* QTL, located on chromosome 1, has been demonstrated to provide resistance to salinity during the early growth stage by controlling the balance of sodium and potassium ions (Thomson *et al.*, 2010). Another crucial gene, *OsHKT1;5*, plays a role in excluding Na⁺ from the shoot, which in turn improves salt tolerance (Ren *et al.*, 2005).

Rice exhibits a high degree of sensitivity to both low and high temperatures, which can have an impact on its ability to germinate, grow vegetatively, and fill its grains. Exposure to high temperatures during the flowering stage might cause the spikelets to become sterile, resulting in a considerable decrease in crop yield (Jagadish *et al.*, 2010). Conversely, exposure to low temperatures, especially in the early stages of plant growth, might hinder growth and decrease the formation of tillers. The breeding efforts to enhance temperature tolerance have primarily concentrated on the identification of tolerant cultivars and the elucidation of the genetic mechanisms underlying the response to temperature. An example of this is the *qHTSF* QTL, which has been discovered and mapped in different rice populations, and is linked to the ability to tolerate high temperatures during the flowering stage (Ye *et al.*, 2012). In addition, rice varieties that are able to withstand cold temperatures, such as those containing the *Ctb* gene, have been created specifically for growing in temperate areas (Saito *et al.*, 2010).

Rice frequently experiences the simultaneous occurrence of various abiotic stressors in its natural habitats. Gaining a comprehensive understanding of the cumulative impacts of these stressors is essential for the development of robust rice cultivars. For instance, Venuprasad *et al.* (2011) found that rice varieties exhibiting simultaneous tolerance to drought and salinity shown superior performance in areas prone to stress. Progress in genomics, such as genome-wide association studies (GWAS) and transcriptomics, have facilitated the discovery of crucial genes and regulatory networks implicated in various stress responses (Zhang *et al.*, 2016).

2.3 Arbuscular Mycorrhiza Fungi (AMF)

2.3.1 Overview

Arbuscular mycorrhizas (AM) are the most common mycorrhizal type and are a relatively small group of fungi that form endomycorrhizal symbioses. They are

formed in an enormously wide variety of host plants by obligately symbiotic fungi which have recently been reclassified on the basis of DNA sequences into a separate fungal phylum, the Glomeromycota (Schüßler, *et al.*, 2001). They are obligate biotrophs that develop a close mutualistic symbiotic association with the roots of a living host plant to complete their life cycle. They can symbiotically interact with almost all the plants that live on the earth, i.e., almost 90% of plant families (Smith and Read, 2008).

Based on the occurrence of AM fungal structures within plant roots, AM morphology has been classified as Arum, Paris or intermediate-types (Dickson, 2004). In response to particular environmental stimuli, asexual spores of variable size (up to 400µm), depending on the species, are normally produced in the soil and sometimes within the roots. The AMF mycelium that is connected to the root system can indeed acquire nutrients from distant soil areas or small soil particles that are inaccessible to roots (Allen, 2011; Smith *et al.*, 2000). In particular, they are commonly recognized to boost the uptake of phosphorus (Smith *et al.*, 2011) which is rather difficult for the plant to absorb since it is mostly available in insoluble forms. Most of nutrient trading preferentially happens inside root cortical cells, where AMF form the so-called arbuscules (Balestrini *et al.*, 2015). These structures, whose shape recalls that of a small shrub, form from intercellular hyphae branching into multiple very thin hyphae that have the purpose of maximizing the surface area on which nutrients are exchanged. As aforementioned, AMF profusely grow outside the plant roots, and generate a hyphal network that can extend over long distances beyond the nutrient depletion zone around the roots.

2.3.2 Role of Arbuscular Mycorrhizal Fungi (AMF)

The mutualistic association between arbuscular mycorrhizal fungi (AMF) and plants has existed for more than 400 million years. Throughout this time, they have undergone co-evolution to improve plant adaption to diverse environmental circumstances (Smith and Read, 2008). Arbuscular mycorrhizal fungi (AMF) establish colonization in the root cortex of plants, giving rise to specialized structures known as arbuscules and vesicles. Arbuscules are dendritic formations that facilitate nutrition exchange, whereas vesicles function as storage organs. These structures enable a

symbiotic connection, where AMF help plants obtain vital nutrients from the soil, particularly phosphorus, in return for carbon from the plant (Parniske, 2008). The colonization of crop plants by arbuscular mycorrhizal (AM) fungi can improve their nutrients uptake predominantly phosphorus and are linked with crop yields (Tinker, 1975; Gianinazzi and Scheuepp, 1994). Arbuscular mycorrhizal fungi absorb nitrogen, phosphorus, potassium, calcium, sulfur, ferric, manganese, copper and zinc from the soil and then translocate these nutrients to the plants with whose roots they are associated (Gerdemann, 1975).

The functioning of AMF is supported by multiple processes that enhance plant health and growth. An important process is the improvement of nutrient acquisition, specifically phosphorus, which is frequently scarce in numerous soils. Arbuscular mycorrhizal fungi (AMF) expand the root system by forming hyphal networks, which enhance the absorptive surface area and enable the plant to obtain soil nutrients that would otherwise be unattainable (Smith and Smith, 2011). This enhanced nutrient acquisition not only supports better plant growth but also improves yield in crops like rice (*Oryza sativa*) (Jansa *et al.*, 2011). Mycorrhizal plants take up Zn over longer distances crossing the depletion zone. Lehman *et al.* (2014) confirmed by a meta-analysis that AMF positively affects Zn concentrations in various crop plant tissues including rice. Hajiboland *et al.* (2009) studied the influence of AMF on the uptake of Zn and P by two contrasting rice genotypes in a pot experiment. After inoculation with mycorrhiza, they observed up to two-fold higher Zn uptake by rice genotypes under Zn deficiency. An enhanced AMF inoculation under aerobic condition has been shown to increase rice Zn uptake as observed by Gao *et al.* (2007). They observed that AMF inoculated plants produced more biomass and took up more Zn than the non-mycorrhizal control.

AMF not only help plants obtain nutrients, but also play a vital role in improving plant resilience against both living and non-living challenges. They have the ability to trigger systemic resistance in plants, which aids in defending against infections. In addition, arbuscular mycorrhizal fungi (AMF) can enhance plant resistance to non-living environmental factors like lack of water and high salt levels by increasing water absorption, strengthening antioxidant defense systems, and altering root structure (Ruiz-Lozano *et al.*, 2012).

Arbuscular mycorrhizal fungi (AMF) play a key role in obtaining nutrients, specifically phosphorus, nitrogen, and micronutrients like zinc and copper. Phosphorus is an essential nutrient that is frequently found in forms that plants cannot readily use. Arbuscular mycorrhizal fungi (AMF) produce enzymes, such as phosphatases, which facilitate the release of phosphorus from organic molecules. This process makes phosphorus accessible for plant absorption (Tisserant *et al.*, 2012). Furthermore, arbuscular mycorrhizal fungi (AMF) aid in the uptake of nitrogen by raising the efficiency of nitrogen utilization and by forming associations with nitrogen-fixing bacteria, so increasing the accessibility of this essential nutrient. Studies have demonstrated that plants that have a symbiotic relationship with arbuscular mycorrhizal fungi (AMF) experience enhanced growth and productivity, particularly in soils that lack sufficient nutrients (Smith and Smith, 2011).

In addition to acquiring nutrients, arbuscular mycorrhizal fungi (AMF) also have a crucial function in controlling plant growth and development. They exert an impact on the hormonal equilibrium in the plant, namely by regulating the concentrations of growth-stimulating hormones such as auxins, cytokinins, and gibberellins. The hormonal regulation results in enhanced root structure, elevated root mass, and improved plant establishment (López-Ráez *et al.*, 2010). Research conducted by Foo *et al.* (2013) has shown that colonization of AMF (Arbuscular Mycorrhizal Fungi) can result in elevated levels of auxins in the root system, which in turn stimulate the elongation and branching of roots. This alteration in the root architecture improves the plant's capacity to assimilate water and nutrients, hence promoting development. The alterations in the proportion of these hormones induced by AMF can also enhance plant growth in different environmental situations, hence increasing plants' ability to withstand stresses such as drought and salinity (Talaat and Shawky, 2013).

Soil enzymatic activities regulate the various indices of soil fertility, soil productivity and soil quality (Busto and Perez-Mateos, 1997). AM fungi can increase soil enzyme activities, such as phosphatase (Mar Vazquez *et al.*, 2000), dehydrogenase, urease, protease and β -glucosidase (Caravaca *et al.*, 2004). Mar Vazquez *et al.* (2000) reported mycorrhizal colonization induced qualitative changes in the microbial population and enzyme activities in the rhizosphere of maize plants.

2.4 AMF and Abiotic Stress Tolerance in Plants

AMF play a vital role in regulating plant stress responses and are well known to improve plant growth and production under abiotic stress conditions (Abdel Latef 2011, 2013; Abdel Latef and Chaoxing, 2011a, b, 2014). AM fungi can also act as biofertilizers, bioprotectors and biocontrol agents and significantly benefit plants (Jeffries and Barea, 2012). Mechanisms underlying the protective roles of AM are credited to AMF-assisted alleviation of oxidative stress in plants (Abdel Latef and Chaoxing, 2011, 2014), rapid water uptake and nutrient absorption (Hajiboland, 2013) and change of transcript levels of genes involved in signalling pathway or stress response (López-Ráez *et al.*, 2010).

AMF enhance the plant's tolerance to environmental stresses by altering the expression of stress-related genes and boosting the plant's antioxidant capacity. The regulating role of AMF is essential for promoting sustainable agriculture, especially in agricultural systems that experience different biotic and abiotic stresses (Ruiz-Lozano *et al.*, 2012). AMF (arbuscular mycorrhizal fungi) can mitigate salt stress in saline settings by promoting the preferential absorption of necessary ions such as K^+ while limiting the uptake of harmful ions like Na^+ . This helps to maintain ion homeostasis within the plant (Giri *et al.*, 2007). In addition, plants that are connected with arbuscular mycorrhizal fungi (AMF) frequently display reduced levels of oxidative stress as a result of increased activities of antioxidant enzymes. These enzymes help safeguard plant cells against harm produced by reactive oxygen species (ROS) during stressful situations (Evelin *et al.*, 2009).

The growth of plants is severely hindered by drought stress, which restricts the availability of water and triggers oxidative stress. AMF improve plants' ability to withstand drought by employing multiple ways. According to Augé (2001), plants that have been colonized by AMF exhibit enhanced water relations, such as increased water intake and higher leaf water potential. AMF also facilitate the buildup of osmolytes such proline, which aids in preserving cell turgor during periods of dryness (Aroca *et al.*, 2008). In addition, plants connected with arbuscular mycorrhizal fungi (AMF) show increased levels of antioxidant enzymes, which help to reduce oxidative damage caused by drought stress (Wu *et al.*, 2013).

Salinity is a significant abiotic stress that impacts plant growth by disturbing the balance of ions and causing osmotic stress. Arbuscular mycorrhizal fungi (AMF) alleviate the negative effects of salt stress by enhancing the uptake of nutrients, namely phosphorus, which is frequently scarce in saline environments (Evelin *et al.*, 2009). In addition, arbuscular mycorrhizal fungi (AMF) decrease the absorption of sodium and increase the absorption of potassium, hence regulating ion balance in plants (Talaat and Shawky, 2011). The accumulation of osmoprotectants such as glycine betaine and the improvement of antioxidant defense mechanisms caused by AMF also contribute to increased tolerance to salinity (Evelin *et al.*, 2009).

The presence of high levels of heavy metals in soils poses a significant risk to the health of plants due to the induction of oxidative stress and disruption of metabolic processes. Arbuscular mycorrhizal fungi (AMF) play a crucial role in reducing heavy metal toxicity by limiting the movement of metals to the aboveground portions of plants, therefore decreasing their harmful effects (Gamalero *et al.*, 2009). AMF also stimulate the synthesis of metal-chelating molecules such as phytochelatins and glutathione, which aid in the sequestration and detoxification of heavy metals within plant cells (Miransari, 2011).

Heat stress can result in significant harm to plant tissues, mostly by disturbing cellular balance and triggering oxidative stress. Studies have demonstrated that arbuscular mycorrhizal fungi (AMF) can alleviate the negative impacts of heat stress on plants by bolstering their antioxidant defense mechanisms and strengthening their ability to absorb water and nutrients. AM fungi significantly enhance the resilience of lettuce to high-temperature stress by improving chloroplast structure, increasing photosynthetic efficiency, and protecting PSII from damage (Yan *et al.* 2021). AMF can mitigate the detrimental effects of high temperature on PSII by enhancing electron transport efficiency and maintaining a higher proportion of active reaction centers, thereby improving the overall photosynthetic performance and stress tolerance in maize plants (Mathur and Jajoo, 2020).

The study conducted by Chen *et al.*, (2013) found that AMF-inoculated seedlings had lower levels of hydrogen peroxide under chilling conditions, suggesting that AMF helps reduce oxidative stress in cucumber seedlings. The beneficial effects of AMF on citrus seedling growth, photosynthesis, and nutrient uptake are largely

dependent on temperature. While AMF provides considerable advantages at moderate temperatures, these benefits are almost entirely lost under low-temperature stress (Wu and Zou, 2010).

The synergistic effects of AMF on stress tolerance are influenced by a complex interplay between plant genetics, environmental factors, and AMF species diversity. Chitarra *et al.* (2016) discovered that the combination of certain tomato genotypes with AMF species resulted in different levels of salt stress tolerance. This highlights the significance of plant genetics in determining how efficient AMF-mediated stress responses are. Bunn *et al.* (2009) state that soil pH and nutrient levels play a crucial role in determining the variety and functionality of AMF (arbuscular mycorrhizal fungi). Van der Heijden *et al.* (1998) demonstrated that a higher diversity of arbuscular mycorrhizal fungi (AMF) improved plant development and ability to withstand stress, especially in environments with limited nutrients.

2.5 AMF Diversity and distribution in Agroecosystems

AMF diversity pertains to the assortment of species or genotypes of AMF that exist inside a specific soil or plant root system. It was reported that rice plants readily form mycorrhizal associations under upland conditions, but under submerged conditions infection is rare due to the anoxic environment (Ilag *et al.*, 1987). However, Barea (1991) reported that AMF can survive in water-logged conditions, and this is supported by the fact that *Glomus etunicatum*, showed fairly high colonization in rice roots and best survival under submerged conditions (Purakayastha and Chhonkar, 2001). Research has demonstrated that AMF communities exhibit a significant level of diversity, with certain estimations indicating the presence of numerous species on a global scale. Nevertheless, the presence of arbuscular mycorrhizal fungi (AMF) in agricultural environments is generally less varied than in natural ecosystems. This is mostly due to the influence of agricultural activities like as tillage, crop rotation, and the application of chemical fertilizers and pesticides (Oehl *et al.*, 2003). Tillage disturbs the arrangement of soil particles and decreases the number and diversity of AMF spores, resulting in less varied AMF communities in tilled soils compared to no-till or reduced-till systems (Jansa *et al.*, 2002). Crop rotation can increase the diversity of arbuscular mycorrhizal fungi (AMF) by introducing diverse host plants that can

support a wide range of AMF species. Monoculture cropping systems, in contrast, have a tendency to decrease the variety of arbuscular mycorrhizal fungi (AMF) by promoting a small number of AMF species (Johnson *et al.*, 1992). Organic farming practices, which abstain from the utilization of artificial fertilizers and pesticides, have been linked to greater arbuscular mycorrhizal fungi (AMF) variety in comparison to conventional agricultural systems. The reason for this is most likely the presence of a wider variety of plant communities and the decreased disruption of soil in organic systems (Mäder *et al.*, 2000).

The spread of arbuscular mycorrhizal fungi (AMF) is mostly influenced by soil pH, as different species of AMF have distinct preferences for certain pH ranges. For example, certain species exhibit robust growth in acidic soils, whilst others are more prevalent in soils with a neutral to alkaline pH (van der Heijden *et al.*, 1998). The spread of arbuscular mycorrhizal fungi (AMF) can be greatly affected by the presence of nutrients, especially phosphorus. Elevated soil phosphorus levels can diminish the necessity for AMF symbiosis, resulting in a decrease in both the quantity and variety of AMF (Smith and Read, 2008). Plant root exudates, which include a range of organic compounds, can attract specific AMF species, thereby influencing their distribution in the soil. Different plant species and even different cultivars within a species can have distinct AMF communities due to variations in root exudate composition (Bever *et al.*, 1996). Plant root exudates, consisting of various organic substances, have the ability to attract particular species of arbuscular mycorrhizal fungi (AMF), therefore affecting their spatial distribution in the soil. Plant species and cultivars can have unique arbuscular mycorrhizal fungi (AMF) communities because of differences in the composition of substances released by their roots (Bever *et al.*, 1996).

2.6 AMF and Rice

It is crucial to comprehend the relationship between arbuscular mycorrhizal fungi (AMF) and rice in India, where rice is a primary crop grown in various agroecological regions. This understanding is vital for enhancing crop productivity and sustainability. It is a fact that mycorrhizal fungus is able to increase growth in a number of agricultural crops (Mosse, 1973; Gerdemann, 1975; Tinker, 1975). Sanni (1976) demonstrated the increase in the growth of rice plants after inoculation with *Giaspora*

gigantia. Certain studies revealed that AM fungi root colonization has seasonal dynamics (Allen, 1996; De Mars and Boerner, 1995). Application of mycorrhizal inoculum increased the soil nutrients and root colonization in rice plants (Yeasmin *et al.*, 2019). The growth and yield of rice plants under field condition is greatly influenced by the native AM fungal composition (Chakraborty *et al.*, 2019).

There are several reports of AMF inoculations in wetland rice culture under greenhouse conditions, but very little information on responses under field conditions. Some studies under pot culture conditions revealed that AMF increased grain and straw yields of wetland rice (Sivaprasad *et al.*, 1990) and increased the grain yield and P and Zn content in plants (Secilia and Bagyaraj, 1994a). Gupta and Ali (1993) reported a significant increase in the grain yield by AMF colonization in wetland rice under both pot and field conditions. Secilia and Bagyaraj (1994b) also reported a positive response of wetland rice to AMF on under field conditions. These reports did not include information for colonization, growth and nutrient acquisition of the seedlings at nursery stages. Solaiman and Hirata (1995) reported that inoculation of AMF directly into the flooded soil was not effective for wetland rice. However, inoculation of seedlings under dry nursery conditions was effective for promotion of wetland rice growth and nutrient acquisition (Solaiman and Hirata, 1996). AMF inoculation at the nursery stage under both dry and wet conditions increased growth, grain yield and nutrient acquisition of wetland rice under field conditions (Solaiman and Hirata, 1997).

The abundance and variety of arbuscular mycorrhizal fungi (AMF) in rice fields in India are affected by multiple factors, such as soil composition, climatic conditions, and agricultural techniques. Research has demonstrated that rice fields in India have a diverse range of species of arbuscular mycorrhizal fungi (AMF), with the genera *Glomus*, *Acaulospora*, and *Scutellospora* being often observed (Verma *et al.*, 2010). The presence of diverse organisms in the soil is crucial not only for the preservation of soil quality, but also for enhancing the ability of rice crops to withstand abiotic stresses. A study conducted by Singh *et al.* (2012) showed that the introduction of arbuscular mycorrhizal fungi (AMF) into rice plants resulted in a substantial increase in phosphorus absorption, resulting in improved plant growth and crop yield. This is especially pertinent in areas with low-input agricultural systems, where

chemical fertilizers are either inaccessible or prohibitively expensive for a significant number of farmers. A study conducted by Sharma *et al.* (2014) found that rice plants that were inoculated with arbuscular mycorrhizal fungi (AMF) had enhanced growth and increased yield when subjected to drought conditions, in comparison to plants that were not inoculated. The study proposed that arbuscular mycorrhizal fungi (AMF) could play a crucial role in the development of rice types that are resistant to drought.

The significance of arbuscular mycorrhizal fungi (AMF) in preserving soil health is crucial for ensuring the long-term viability of rice farming in India. Arbuscular mycorrhizal fungi (AMF) play a crucial role in enhancing soil structure, facilitating nutrient cycling, and inhibiting the growth of soil-borne diseases. Pandey *et al.* (2015) emphasized the significance of arbuscular mycorrhizal fungi (AMF) in decreasing reliance on chemical inputs in rice farming, thereby encouraging sustainable agricultural methods. Organic farming approaches, increasingly popular in India, mainly depend on natural processes to sustain soil fertility and promote plant health. Arbuscular mycorrhizal fungi (AMF) play a crucial role in organic rice production by increasing the availability of nutrients and raising the plant's ability to withstand diseases and pests. Bhattacharjee and Sharma (2016) conducted research that demonstrated the use of AMF in organic rice systems led to increased yields and improved grain quality when compared to conventional techniques.

2.7 AMF and Rice in Mizoram

There is a very little reports on the study of mycorrhiza in Mizoram. Zothanzama and Thari (2018) studied on how soil properties, human activities, and environmental factors such as rainfall and temperature influence AMF colonization in maize roots. The study found that lower AMF colonization in Jhum land is attributed to continued human exploitation, including logging and the use of weedicides, which negatively affected soil properties and AMF colonization. Fallow periods in jhum farming and minimal human disturbance have a critical role in promoting AMF colonization and improving soil fertility in jhum cultivation systems (Thari and Zothanzama, 2018). In another study, it was found that *Melocanna baccifera* from undisturbed site exhibited a higher percentage of AMF colonization compared to the disturbed site (Thari and Zothanzama, 2019). Among the AMF, *Acaulospora* is the

dominant genus followed by *Glomus* in the rhizosphere soil of Mizo Bird's Eye Chilli (*Capsicum frutescens* L.) and it was reported that *Pacispora scintillans* in association with the chili plant for the first time, marking a new record in the literature (Vanlalmalsawmi and Zothanzama, 2022). There is also very little research on AMF in relation to medicinal plants and invasive plants in Mizoram. One study provides important insights into the relationship between AMF colonization and the success of invasive plant species, highlighting the potential role of AMF in facilitating plant invasions (Vanlalmuana *et al.*, 2021). There is a strong symbiotic relationship between AMF and medicinal plants, with significant variation in colonization levels across species (Vanlalmuana *et al.*, 2024). There are no reports related to AM fungal in association with rice, in relation to structural features within the roots and diversity, in Mizoram. This study will provide a valuable insight on the diversity of AMF associated with rice and the function of AMF in enhancing drought and salt tolerance to the rice variety grown in Mizoram.

Chapter 3

Colonization and Diversity of Arbuscular Mycorrhizal Fungi Associated with Rice Plant

3.1 Introduction

Rice is the primary agricultural crop and a significant component of the food grain supply in Mizoram. In the 2022-23 period, a total of 58,836 Metric Tonnes of rice was produced from an area spanning 34,071 Hectares. The state's rice output is now sufficient to cover only 20% of its rice demand, suggesting that further efforts are needed to meet the state's consumption needs. Rice dominates the agricultural landscape in Mizoram, accounting for 60.88% of the total cultivated land. According to the Economic Survey of Mizoram for the year 2023-2024, a total of 22,053 Metric Tonnes of rice was produced from the Jhum area, while the rest 35,788 Metric Tonnes was generated from the Wet Rice Cultivation (WRC) area. The entire arable land in Mizoram is 51,033 hectares, which accounts for 33% of the overall geographical area. However, the WRC area, which is a subset of the arable land, is just 16,866 hectares, making up less than 0.8% of the whole geographical area. Serchhip district has over 2389 hectares of Wet Rice Cultivation Area, and the district production is only just 15.29% of the total state production from wetland cultivation. The potential sites suitable for Wet Rice Cultivation (WRC) in Serchhip district cover 2.61% of the district's total area, which is equivalent to 37.10 km² (Lallianthanga *et al.*, 2013).

There is some debate on whether the presence or absence of AM in rice grown in wetlands. Some studies show little or no colonization was observed in the roots of rice grown in flooded area (Ilag *et al.*, 1987; Lumini *et al.*, 2011). But others have demonstrated that AMF are found in the roots of rice plant grown in wetland condition (Wang *et al.*, 2015; Chen *et al.*, 2017; Bernaola *et al.*, 2018; Vallino *et al.* 2009). Among the AM fungal species, *Rhizophagus irregularis* and *Funnelformis mosseae* are very common and found associated with rice plants (Tisarum *et al.*, 2019; Vallino *et al.*, 2014). Currently, there is a very little report on the studies of arbuscular mycorrhiza in Mizoram, especially in relations to rice plant.

Apart from the biotic community which can influence the growth of rice in a field, soil edaphic factor is an important measure which can influence the biotic community of the ecosystem. The development of plants is determined by soil fertility, which is a crucial element. The composition of an environment is determined by the levels of Carbon, Nitrogen, Phosphorus, Potassium, organic and inorganic substances, micronutrients, and water present. Soil chemical fertility, and specifically the absence of nutrient inputs, is a significant contributor to soil degradation (Hartemink *et al.*, 2010).

Soil enzymes are a collection of enzymes that consistently contribute to the preservation of soil ecology, physical and chemical characteristics, fertility, and overall soil well health. The soil enzymes (Sinsabaugh *et al.*, 1991) regulate the entire process of organic matter decomposition in the soil system. Microbes in the soil rely on enzymes to facilitate essential reactions for their survival and the maintenance of soil structure. These reactions include the breakdown of organic waste, the production of organic matter, and the cycling of nutrients. Enzymes play a vital role in agriculture by catalyzing these processes, contributing to the overall health and productivity of the soil (Dick *et al.*, 1994). The enzymes secreted from plants and microbes may include amylase, arylsulphatases, β -glucosidase, cellulase, chitinase, dehydrogenase, phosphatase, protease, and urease (Miwa *et al.*, 1937; James *et al.*, 1991).

It is very important to look into how rice, AMF, and different soil properties affect each other and the different types of AMF that are linked to rice. This is because different soil properties are known to affect plant growth and yield, and even influence the diversity and colonization of local AM fungi which can greatly benefit the host plant in many ways. This study will also provide an initial report on AMF species found in the rhizosphere soil of rice grown in Mizoram.

3.2 Materials and Methods

3.2.1 Study Sites

Mizoram is situated in Northeastern India, with coordinates ranging from 21°58' to 24°35' N latitude and 92°48' E longitude. The state is characterized by its hilly terrain, rivers, and valleys, and it covers an area of approximately 21,081 square kilometres. The mean annual temperature is 24.75°C with a yearly rainfall of 2323.7

mm. The rice plants in this study were collected from the paddy fields in North Vanlaiphai (23°07'25.0"N 93°02'50.8"E) and Thenzawl (23°19'41.2"N, 92°46'48.6"E) which are located in Serchhip district with 24 hectares and 110 hectares of WRC area respectively (Plate I).

3.2.2 Sample Collection

Root samples were collected from ten randomly selected healthy individuals of each rice plant species. Three random soil cores, taken from a depth of 0–30 cm, were collected from each of the ten duplicate plants and then combined. Root samples were fixed in FAA (Formaldehyde, alcohol, acetic acid; 1:1:1) and transferred to the laboratory for further processing along with soil samples which are kept in Ziplock bags at 4°C.

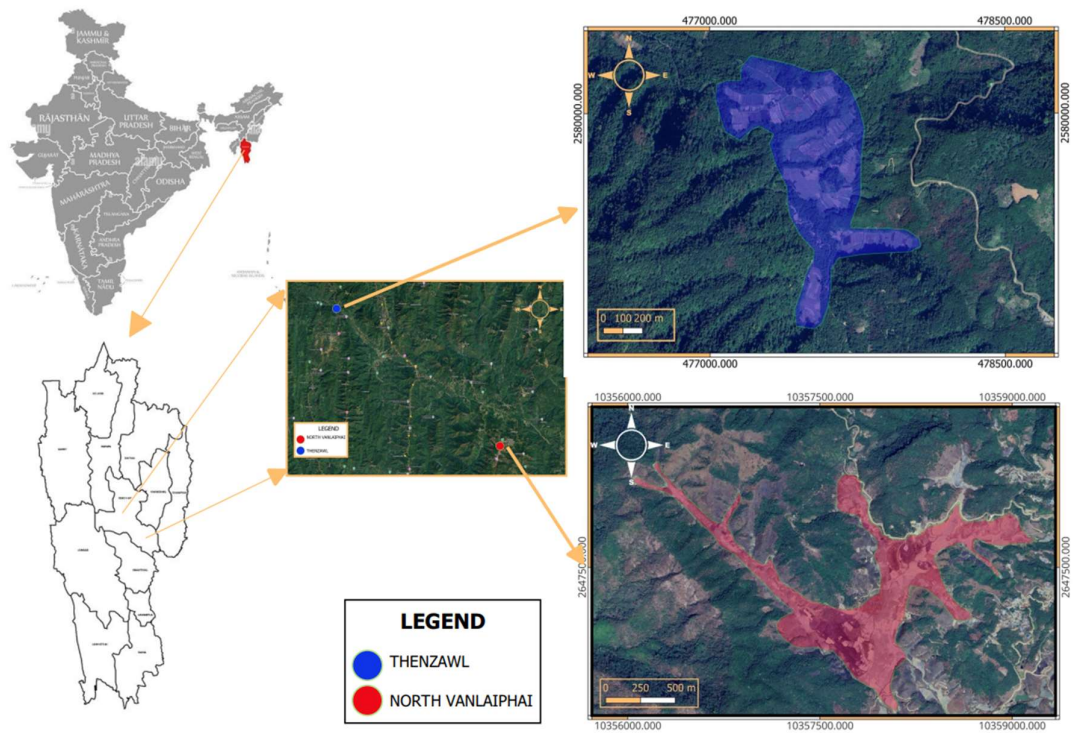
3.2.3 Estimation of Arbuscular Mycorrhizal Colonization in Rice Roots

The root samples were cleaned with tap water to remove dirt particles. Subsequently, the roots were first sectioned into segments (1-2 cm) and were heated with a 10% solution of KOH at a temperature of 90°C for approximately 30 minutes. The root segments are acidified with 2% HCl for 15-20 minutes after rinsing. Subsequently, the roots were treated with a solution of 0.05% trypan blue in lactophenol for a duration of 5 minutes. Excess stain was then removed using clear lactophenol, as described by Phillips and Hayman (1970). Each plant sample was prepared by mounting thirty root segments on a glass slide using lactophenol. The root segments were then viewed under a light microscope (Olympus BX 53) at a magnification of 100X. The extent of arbuscular mycorrhizal (AM) colonization was assessed using the magnified intersection method (Mc Gonigle, 1990). The percentage of arbuscular colonization (AC), vesicular colonization (VC), and hyphal colonization (HC) was calculated using the following formula:

$$AC = \frac{\text{Count of Arbuscules}}{\text{Total number of intersections examined}} \times 100:$$

$$VC = \frac{\text{Count of vesicles}}{\text{Total number of intersections examined}} \times 100$$

Plate I



Geospatial Location and Extent of the Study Sites

Thenzawl and North Vanlaiphai in Mizoram, India

$$HC = \frac{\text{Total number of intersections examined} - \text{Negative intersection}}{\text{Total number of intersections examined}} \times 100$$

3.2.4 Identification of AM fungal spore morphotypes.

The isolation of AM spores from soil was done using the wet sieving and decanting method (Gerdeemann and Nicolson, 1963), followed by sucrose density gradient (20% and 60%) centrifugation (Sieverding, 1983). Spores were examined under a microscope (100X; Olympus BX 53) and separated according to their morpho-anatomical features and mounted on glass slides using polyvinyl lactoglycerol (PVLG) and Melzer's reagent (Brundrett et al., 1996). The spores were morphologically identified to the species level based on the size, color, wall layer thickness, surface ornamentation, and subtending hyphae, following the keys provided on the websites of INVAM (<https://invam.ku.edu/>). The names of AMF species and the corresponding authorities were confirmed and revised in accordance with the AMF Phylogeny website of A. Schüßler (www.amf-phylogeny.com).

3.2.5 Diversity Studies

The composition of arbuscular mycorrhizal (AM) fungus communities was assessed based on spore density (SD), species richness (SR), Relative abundance (%), Shannon-Wiener index (H) and Simpson's Index of Diversity (1-D) (Simpson, 1969; Franke-Snyder *et al.*, 2001). Species richness was quantified by determining the number of arbuscular mycorrhizal (AM) fungi species found in each soil sample. The assessment of species diversity was conducted using the Shannon-Weiner index, and Simpson's Index of Diversity.

$$\text{Shannon-Weiner index } (H) = -\sum_{i=1}^k (P_i \ln P_i)$$

$$\text{Simpson's Index of Diversity } (D) = \sum_{i=1}^s p_i^2.$$

The relative abundance (%) of each species in each area was estimated using the formula:

$$\text{Relative Abundance} = \frac{\text{Number of particular AMF spores}}{\text{Total number of all AMF spores}} \times 100$$

3.2.6 Soil Physico-chemical Analysis

3.2.6.1 Soil Moisture Content (SMC)

10 grams of air-dried soil was taken and then placed in a hot air oven at a temperature of 105°Celsius. Once the soil had fully dried, it was reweighed and documented. The moisture content was expressed in percentage and determined using the following formula:

$$\text{Moisture Content (\%)} = \frac{a-b}{a} \times 100$$

Where, a = weight of air-dried soil

 b = weight of oven dried soil

3.2.6.2 Soil pH

The soil's pH was obtained by measuring it in a soil water suspension with a weight-to-volume ratio of 1:5 using a pH meter (pH 700, EUTECH Instruments). A recently obtained soil sample was measured to weigh 10 grams and placed in a glass beaker with 50 ml of distilled water. The suspension was agitated initially using a glass rod. Following agitation, the pH of the soil solution was measured using a pH meter.

3.2.6.3 Bulk Density of Soil (Bashour and Sayegh, 2007)

The soil's bulk density was determined using a cylindrical soil core sampler. Soil samples were obtained from the top layer, approximately 6 inches thick, from randomly selected plots within the study site. The soil sample was subjected to oven drying at a temperature of 105°C for a duration of 12 hours, after which its weight was measured. The precise volume of the dirt was obtained by measuring the volume of the cylinder.

$$\text{Bulk Density (BD gm}^{-3}\text{)} = \frac{\text{Weight of oven dried soil (g)}}{\text{Volume of soil core (cm}^3\text{)}}$$

3.2.6.4 Available Nitrogen (Subbiah and Asija, 1956)

5 grams of dehydrated and filtered soil was placed into the digesting tube. The tube was inserted into the distillation unit, and on the opposite side of the hose, 20ml of a 2% solution of boric acid was added and mixed with an indicator in a 250ml

conical flask. The distillation unit program automatically added 25ml of potassium permanganate solution (0.32%) and 25ml of sodium hydroxide solution (2.5%). The sample block was heated to a temperature of 410°C by continuously pumping steam through it. The ammonia released during this process was absorbed in a 20ml solution of 2% boric acid, which also contained a mixed indicator solution. The pink hue transformed into a green shade as a result of ammonia absorption.

Approximately 150 millilitres of distillate were collected within a duration of approximately 10 minutes. Next, the green distillate was titrated with a solution of 0.02N H₂SO₄, resulting in a colour change back to the original tint, which is pink. Concurrently, a blank sample without soil was run alongside. The blank and titre reading was recorded and the concentration of available nitrogen in the soil were quantified. The available nitrogen was expressed in kg/acre and calculated according to the formula:

$$\text{Available N (kg acre}^{-1}\text{)} = \frac{R \times \text{Normality of acid} \times \text{atomic mass of Nitrogen} \times \text{weight of soil in one acre}}{\text{sample weight} \times 1000}$$

3.2.6.5 Available Phosphorous (Olsen *et al.*, 1954)

A 100 ml conical flask was utilized to combine 2.5g of desiccated soil and 50ml of sodium bicarbonate solution. The mixture was then vigorously agitated using Tarson's horizontal orbital plate shaker at a speed of 6000 rpm for a duration of 30 minutes. The suspension underwent filtration using Whatman filter paper No.42, then activated carbon was used to provide a transparent filtrate.

A 5ml portion of the extract was placed in a 25ml volumetric flask. Then, 5ml of Dickman and Bray's reagent was added slowly and continuously mixed until the bubbling caused by the release of CO₂ stops. Finally, 1ml of diluted SnCl₂ was added. The volume was thereafter adjusted to the specified level by adding additional liquid until it reached the 25ml mark on the volumetric flask. The colour remains consistent for a duration of 24 hours, with the highest level of intensity achieved within a span of 10 minutes. The absorbance was measured using a UV-VIS spectrophotometer (Shimadzu UV-1900I) at a wavelength of 660nm. The concentration of Olsen P was calculated from a standard curve and expressed as kg acre⁻¹.

3.2.6.6 Exchangeable Potassium (Metson, 1980)

A quantity of 5 grams of dried and sieved soil was placed into a conical flask with a capacity of 150 ml. Then, 25 ml of ammonium acetate solution was added to the flask. The mixture was vigorously shaken for a duration of 5 minutes using a shaker. The contents were filtered using a Whatman No. 1 filter paper. The initial drops of the filtrate were discarded. The filtered solutions were introduced into the flame photometer following appropriate adjustments and calibration of the device. The same approach was used to run a soilless blank as well. The available potassium concentration was determined using the standard curve.

3.2.6.7 Soil Organic Carbon (Walkey and Black, 1934)

A little quantity of the collected soil was dried by exposure to the sun and then crushed thoroughly. Afterwards, it was filtered using a Lab Standard Sieve with a 0.2mm opening and a diameter of 20cm. A dry conical flask was filled with 0.5g of sieved fine soil. Then, 10ml of a 1N potassium dichromate solution was added to the flask. Next, a 20ml portion of concentrated sulphuric acid was cautiously poured into the flask at an inclined angle. The mixture was then gently stirred multiple times and left undisturbed for approximately 30 minutes. To get a more distinct endpoint during titration, 200ml of distilled water was combined with 10ml of ortho-phosphoric acid. Once 1ml of diphenylamine indicator was added, the solution was titrated with 0.5N ferrous ammonium sulphate solutions until the colour changed from blue-violet to green. The final measurement was taken for each replicate after the green colour development. A soilless blank was conducted concurrently. The SOC is determined using the following formula and is expressed as a percentage.

$$\text{Soil organic carbon (\%)} = \frac{10(A-B) \times 0.003 \times 100}{B \times W}$$

where,

A = Volume of ferrous ammonium sulphate required for blank titration in ml

B = Volume of ferrous ammonium sulphate needed for soil sample in ml

W = Weight of soil in gram

3.2.7 Soil Biochemical Analysis

3.2.7.1 Dehydrogenase (Casida *et al.*, 1964)

Three replicates of fresh soil weighing 1 gram each were mixed with 0.1 grams of CaCO_3 in a test tube. Next, 1 ml of a 1% solution of 2,3,5-triphenyl tetrazolium chloride (TTC) was added to the tubes. The tubes were then placed in an incubator set at a temperature of 30°C for a duration of 24 hours. The resultant mixture was placed onto Whatman filter paper No.1, and triphenyl formazan (TPF) was extracted using multiple portions of concentrated methanol in a 50 ml volumetric flask. The extinction of the pink colour was measured using a Spectrophotometer (Shimadzu UV-1900I) at a wavelength of 485nm, using methanol as the blank solution. The enzyme activity was quantified as $\mu\text{g TPF mg}^{-1} \text{ hr}^{-1}$.

3.2.7.2 Acid Phosphatase (Tabatabai and Bremner, 1969)

A total of 0.1 grams of soil that had been air dried was placed in a 50ml conical flask. This soil was then combined with 4ml of modified universal buffer (MUB) with a pH of 6.5, 0.25ml of toluene, and 1ml of a solution containing 0.115M p-nitrophenyl phosphate (p-NPP). The flask was manually swirled for a brief period and snugly sealed with a cotton stopper. It was then incubated for one hour at a temperature of 37°C in an incubator. Subsequently, a total of 1ml of 0.5M CaCl_2 and 4ml of 0.5M NaOH solutions were concurrently introduced into the mixture, followed by putting the resulting mixture onto Whatman No.1 filter paper. The filtrate of p-nitrophenol phosphate (phosphoric acid), which had a yellow color, was analyzed using a spectrophotometer (Shimadzu UV-1900I) at a wavelength of 410nm. In the case of blank, 1ml of p-NPP was introduced into the mixture, following the addition of CaCl_2 and NaOH, and just prior to filtration, without the presence of soil. The enzyme activity is expressed as $\mu\text{g p-NP per milligram per hour}$.

3.2.7.3 Urease (McGarity and Myers, 1967)

A 100 ml volumetric flask was used to hold 1g of fresh soil, with three replicates for each sample. Each sample was treated with 1ml of toluene, which was let to stand for 15 minutes to ensure full penetration of the toluene into the soil. Next, 10ml of buffer with a pH of 7 and 5ml of a freshly made 10% urea solution were added.

Following a complete agitation, the flask was placed in an incubator and kept at a temperature of 37°C for a duration of 3 hours. In the case of blank, a 5ml portion of a 10% urea solution was substituted with 5ml of sterile distilled water. Following incubation, the flask's volume was adjusted to the mark (100ml) using distilled water. The mixture was vigorously agitated and then filtered using Whatman No.5 filter paper. The ammonia produced by urease activity was quantified using the Indophenol Blue method. Specifically, 0.5ml of the filtrate was transferred into a 25ml volumetric flask, and then 5ml of distilled water was added. Next, 2ml of phenolate solution was introduced, followed by the addition of 1.5ml of sodium hypochlorite solution. The flask's final content was raised to 25ml using distilled water, and the spectrophotometer (Shimadzu UV-1900I) was used to measure the blue colour at a wavelength of 630nm. The enzyme activity was quantified as milligrams of NH_4^+ or NH_3 produced per hour per gram of soil ($\mu\text{mol/hr/g}$ soil).

3.2.8 Statistical Analysis

All statistical analyses were conducted using Python 3.13 within a Jupyter Notebook environment. Descriptive statistics were calculated to summarize the central tendency, variability, and range of each soil parameter, colonization and diversity index for both sites. To assess pairwise differences between selected groups, independent samples t-tests were performed where appropriate. For factorial analysis involving two independent variables, two-way analysis of variance (ANOVA) was employed to evaluate the main effects and interaction effects of the factors on the dependent variables. Correlation analyses were also conducted using Pearson's correlation coefficient to explore the relationships between soil properties, arbuscular colonization, spore density. Assumptions of normality and homogeneity of variance were verified prior to inferential testing. All statistical tests were two-tailed, and a significance threshold of $p < 0.05$ was applied.

3.3 Results

3.3.1 Soil physico-chemical properties of the study sites

The soil physico-chemical properties for the two sites were analysed to understand the basic characteristics and differences. The mean values and standard deviations (SD) for soil physico-chemical properties are presented in Table 3.1. The mean available nitrogen content was found to be 19.33 kg/acre at North Vanlaiphai. In contrast, Thenzawl had a higher mean nitrogen content of 25.67 kg/acre. Available phosphorus levels were relatively consistent between the two sites, with North Vanlaiphai having a mean of 23.00 kg/acre and Thenzawl having a mean of 23.33 kg/acre. There was a notable difference in exchangeable potassium content between the two sites. North Vanlaiphai had a mean potassium content of 145.33 kg/acre, while Thenzawl had a significantly lower mean potassium content of 111.33 kg/acre.

Table 3.1: Soil physico-chemical properties of the study sites

SN	Parameter	North Vanlaiphai	Thenzawl
1.	Available N (kg/acre)	19.33 ± 0.58	25.67 ± 0.58
2.	Available P (kg/acre)	23.00 ± 1.00	23.33 ± 0.58
3.	Exchangeable K (kg/acre)	145.33 ± 0.58	111.33 ± 1.15
4.	Bulk Density (g/cm ³)	7.09 ± 1.05	8.74 ± 0.49
5.	pH	6.94 ± 0.05	6.72 ± 0.04
6.	EC (μS/cm)	88.61 ± 6.43	84.03 ± 4.67
7.	SOC (%)	2.88 ± 0.21	2.95 ± 0.13
8.	SMC (%)	31.97 ± 0.31	30.13 ± 0.30

EC: Electrical Conductivity; SOC: Soil Organic Carbon; SMC: Soil Moisture Content; Values are presented as mean ± SD.

Bulk density, which measures soil compaction and porosity, showed some variation between the sites. North Vanlaiphai had a mean bulk density of 7.09 g/cm³, while Thenzawl exhibited a higher mean bulk density of 8.74 g/cm³. Soil pH was slightly higher at North Vanlaiphai, with a mean of 6.94, compared to a mean of 6.72 at Thenzawl. The mean electrical conductivity (EC) values, which reflect the soil's ability to conduct electricity and often indicate the level of soluble salts, were 88.61 μS/cm at North Vanlaiphai and 84.03 μS/cm at Thenzawl. The soil organic carbon content was relatively similar between the two sites, with North Vanlaiphai showing a

mean SOC of 2.88% and Thenzawl showing a mean SOC of 2.95%. Soil moisture content was slightly higher at North Vanlaiphai, with a mean of 31.97%, compared to 30.13% at Thenzawl.

3.3.2 Soil biochemical properties of the study sites

The biochemical parameters of the soil at the two sites were analyzed to comprehend their fundamental qualities and distinctions. Table 3.2 presents the mean values and standard deviations (SD) for soil physico-chemical characteristics.

Table 3.2: Soil biochemical properties of the study sites

SN	Parameter	North Vanlaiphai	Thenzawl
1.	Dehydrogenase ($\mu\text{g TPF/g /hr}$)	2.89 ± 0.16	3.34 ± 0.13
2.	Phosphatase ($\mu\text{g p-nitrophenol/g /hr}$)	172.49 ± 2.58	202.66 ± 5.99
3.	Urease ($\mu\text{g NH}_3/\text{g /hour}$)	11.42 ± 0.39	14.74 ± 0.30

Values are presented as mean $\pm$ SD.

Dehydrogenase activity, an indicator of microbial activity in the soil, was higher at Thenzawl, with a mean of $3.34 \mu\text{g TPF/g /hour}$, compared to $2.89 \mu\text{g TPF/g /hour}$ at North Vanlaiphai. Phosphatase activity, which is involved in the mineralization of organic phosphorus, was higher at Thenzawl, with a mean of $202.66 \mu\text{g p-nitrophenol/g/hour}$, compared to $172.49 \mu\text{g p-nitrophenol/g/hour}$ at North Vanlaiphai. Urease activity, which is responsible for the hydrolysis of urea into ammonia and carbon dioxide, was also higher at Thenzawl, with a mean of $14.74 \mu\text{g NH}_3/\text{g /hour}$, compared to $11.42 \mu\text{g NH}_3/\text{g /hour}$ at North Vanlaiphai.

3.3.3 Root AM colonization

3.3.3.1 Arbuscular colonization

The mean values and standard deviations (SD) for arbuscular colonization (Plate II, A&B) are presented in Table 3.3. The arbuscular colonization percentage at North Vanlaiphai showed a gradual increase over the three years. In 2019, the mean colonization was 2.32%, which increases to a mean of 2.68% in 2020 and by 2021, the mean colonization further increased to 3.24%.

At Thenzawl, the arbuscular colonization percentage was consistently higher compared to North Vanlaiphai. In 2019, Thenzawl had a mean colonization of 3.06%,

and continued to increase in 2020, reaching 3.52% and by 2021, the mean colonization at Thenzawl was 3.74%.

Table 3.3: Arbuscular colonization percentage from two sites across three different years

SN	Site	AC %		
		2019	2020	2021
1.	North Vanlaiphai	2.32 ± 0.04 ^c	2.68 ± 0.02 ^b	3.24 ± 0.01 ^a
2.	Thenzawl	3.06 ± 0.02 ^b	3.52 ± 0.11 ^a	3.74 ± 0.05 ^a

Different letters indicate significant differences among each site according to Duncan's test at $p < 0.05$.

The assumptions of ANOVA were checked, and both normality and homogeneity of variance were satisfied, ensuring the robustness of the ANOVA results. A two-way ANOVA showed that arbuscular colonization varied significantly due to individual factor (site and year) and their interaction (Table 3.4).

Table 3.4: Two-way ANOVA results for the effects of site and year on arbuscular colonization percentage.

Source	Sum of Squares	df	F-value	p-value
Site	2.1356	1	255.59	<0.0001
Year	1.9204	2	114.92	<0.0001
Site x Year	0.0926	2	5.54	0.0198
Residual	0.1003	12		

3.3.3.2 Vesicular colonization

The mean values and standard deviations (SD) for vesicular colonization (Plate II, C&D) are presented in Table 3.5. At North Vanlaiphai, the mean vesicular colonization percentage was highest in 2019 (4.92%), and there was a notable decline

Table 3.5: Vesicular colonization percentage from two sites across three different years

Site	VC %		
	2019	2020	2021
North Vanlaiphai	4.92 ± 0.012 ^a	3.19 ± 0.15 ^b	2.55 ± 0.23 ^c
Thenzawl	3.25 ± 0.06 ^b	3.83 ± 0.12 ^a	3.87 ± 0.13 ^a

Different letters indicate significant differences among each site according to Duncan's test at $p < 0.05$.

in colonization percentages in the subsequent years, with means of 3.19% in 2020 and 2.55% in 2021, suggesting a possible negative trend over time. In contrast, the Thenzawl site exhibited a steady increase in vesicular colonization percentages over the same period. The mean colonization in 2019 was 3.25%, with a slight increase to 3.83% in 2020, and further to 3.87% in 2021.

Table 3.6: Two-way ANOVA results for the effects of site and year on vesicular colonization percentage.

Source	Sum of Squares	df	F-value	p-value
Site	0.040139	1	1.935441	0.1894
Year	2.3671	2	57.06911	<0.0001
Site x Year	7.374744	2	177.7999	<0.0001
Residual	0.248867	12		

A two-way ANOVA results showed that vesicular colonization varied significantly due to year and site $\times$ year. Site has no significant main effects on vesicular colonization (Table 3.6).

3.3.3.3 Hyphal colonization

In 2019, the average hyphal colonization (Plate III) was 25.34% and increased to 26.60% in 2020 and decreased to 24.14% in 2021 at North Vanlaiphai. At Thenzawl, the average hyphal colonization was 28.00% in 2019, 27.77% in 2020 and 26.71% in 2021. The hyphal colonization varies across both sites and years, with Thenzawl consistently showing higher colonization rates than North Vanlaiphai (Table 3.7).

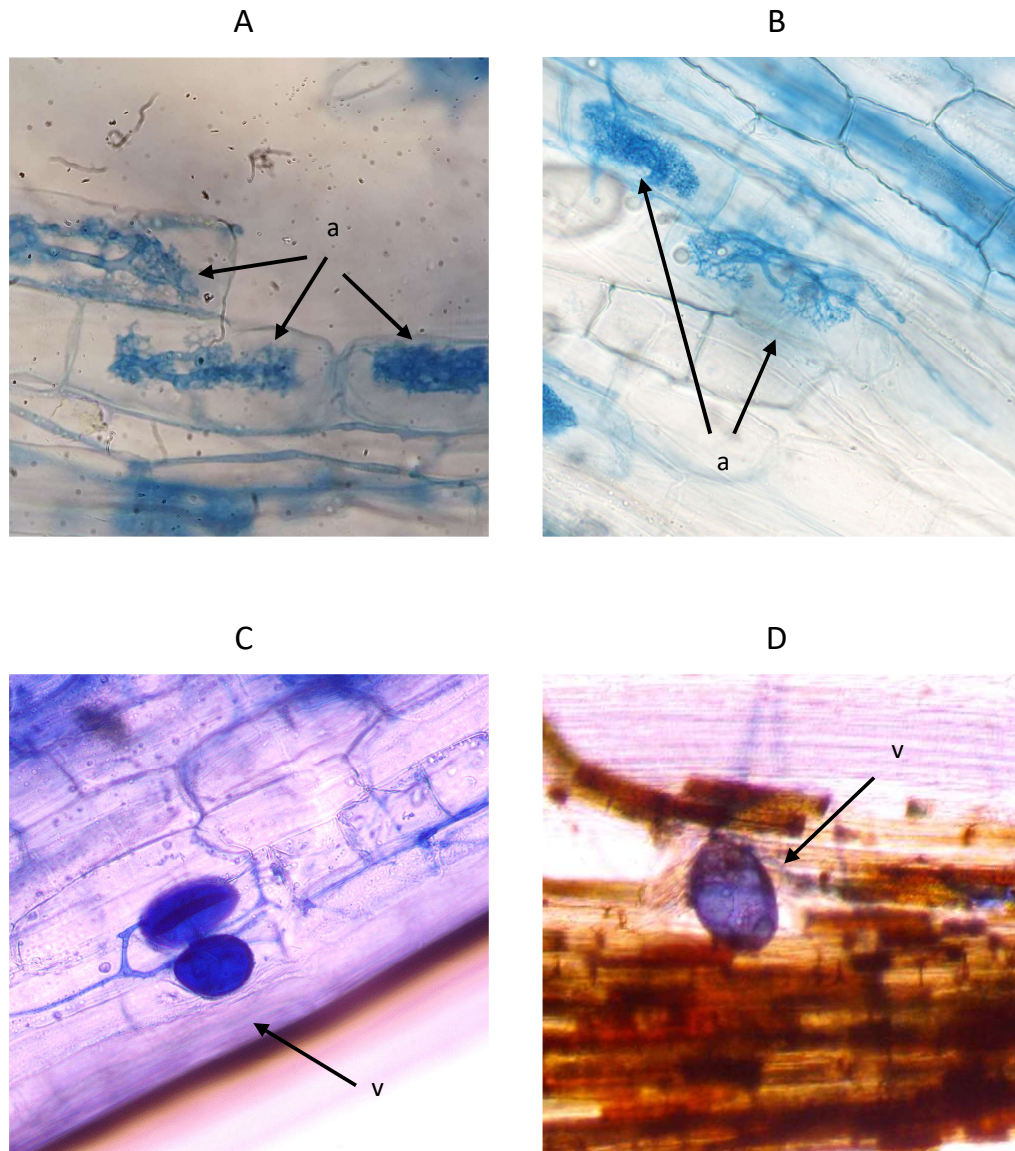
Table 3.7: Hyphal colonization percentage from two sites across three different years

Site	HC		
	2019	2020	2021
North Vanlaiphai	25.35 $\pm$ 0.72 ^{ab}	26.60 $\pm$ 0.21 ^a	23.14 $\pm$ 0.60 ^b
Thenzawl	28.00 $\pm$ 0.24 ^a	28.77 $\pm$ 0.28 ^{ab}	26.04 $\pm$ 0.33 ^b

Different letters indicate significant differences among each site according to Duncan's test at $p < 0.05$.

Results from Two-way ANOVA shows that hyphal colonization varied significantly due to site and year factors (Table 3.8). The interaction between site and

Plate II



AM Fungal structures in roots of *Oryza sativa* L. cv. Changkawi

Arbuscules formed intracellularly in the root cells of rice plants (A & B); Vesicles formed in the intercellular spaces of the roots (C & D); a: arbuscules, b: vesicles.

year was not statistically significant. This implies that the effect of the year on hyphal colonization does not differ significantly between the two sites.

Table 3.8: Two-way ANOVA results for the effects of site and year on hyphal colonization percentage.

Source	Sum of Squares	df	F-value	p-value
Site	20.43736	1	35.05617	<0.0001
Year	9.855544	2	8.452601	0.0051
Site x Year	2.098478	2	1.799758	0.2072
Residual	6.995867	12		

3.3.4 Spore Density

North Vanlaiphai has a mean spore density of 396.83/50g of soil, 401/50g of soil and 363.51/50g of soil in 2019, 2020 and 2021 respectively. At Thenzawl, the mean spore density is 443.83/50g of soil, 496.17/50g of soil and 548.5/50g of soil in the year 2019, 2020 and 2021 respectively. These results indicate that Thenzawl generally had higher spore densities compared to North Vanlaiphai over the three years (Table 3.9).

Table 3.9: Spore Density (per 50g of soil) from two sites across three different years

Site	Spore Density/50g soil		
	2019	2020	2021
North Vanlaiphai	396.83 ± 6.49 ^a	401.00 ± 11.72 ^a	363.50 ± 5.51 ^b
Thenzawl	443.83 ± 14.77 ^b	496.17 ± 14.44 ^{ab}	548.50 ± 66.12 ^a

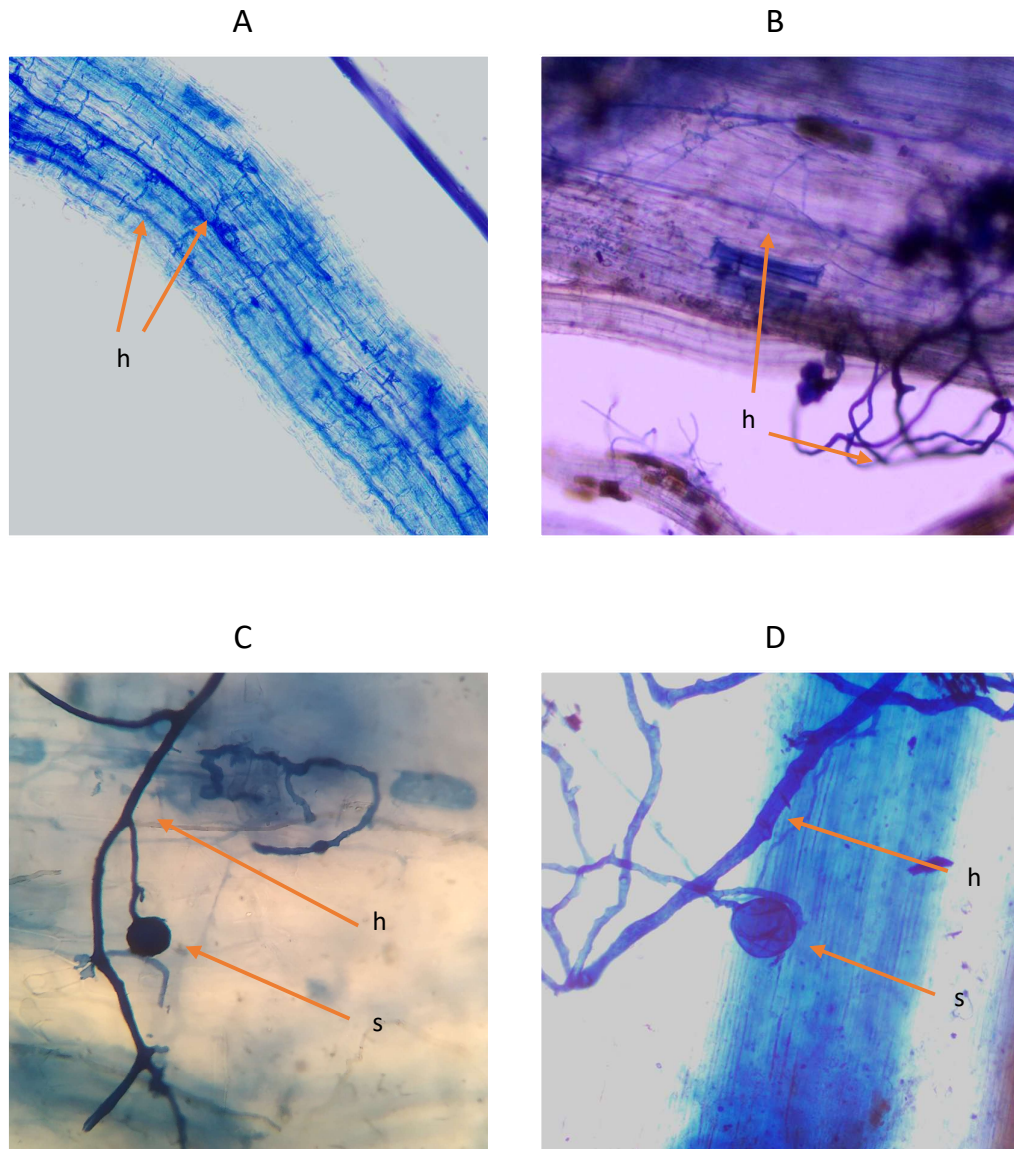
Different letters indicate significant differences among each site according to Duncan's test at $p < 0.05$.

Two-Way ANOVA results (Table 3.10) shows that there was a significant main effect of the site on spore density, year does not have significant main effect and the interaction effect between site and year was significant.

Table 3.10: Two-way ANOVA results for the effects of site and year on spore density

Source	Sum of Squares	df	F-value	p-value
Site	53519.01	1	85.48225	<0.0001
Year	4250.361	2	3.394405	0.06787
Site × Year	14717.03	2	11.75325	0.00149
Residual	7513	12		

Plate III



Hyphal structures associated with the roots of *Oryza sativa* L. cv. Changkawi.

A) a segment of root with intraradical hyphae. B) Intraradical and extraradical hyphae of rice roots. C & D) Extraradical hyphae with a young terminal spore; h: hyphae, s: spore.

3.3.5 AM Fungal diversity

3.3.5.1 Species Richness

The species richness (SR) of Arbuscular Mycorrhizal Fungi (AMF) was assessed at two different sites: North Vanlaiphai and Thenzawl. The results indicate that the Thenzawl site exhibited a higher species richness (SR = 8) compared to the North Vanlaiphai site (SR = 5) (Table 3.11). This suggests that the Thenzawl site may harbor a more diverse AMF community, which could be influenced by various environmental factors or site-specific conditions. In total, 11 AM fungal species were recorded from the two study sites. *Acaulospora* (Plate IV A&B) and *Glomus* (Plate V C&D) represented by three species each were the dominant genera. One species each from the genera *Entrophosphora* (Plate IV C), *Funneliformis* (Plate V A), *Gigaspora*, *Rhizophagus* (Plate IV D) and *Septoglomus* (Plate V B) were recorded.

Table 3.11: Species Richness, Diversity Index, and Relative abundance of AM morphotypes

AM Fungal Species	Relative Abundance %	
	North Vanlaiphai	Thenzawl
<i>Acaulospora longula</i> Spain & N.C.Schenck	18.82	6.85
<i>Acaulospora spinosa</i> C.Walker & Trappe	20.59	5.94
<i>Acaulospora foveata</i> Trappe & Janos	-	19.18
<i>Entrophosphora infrequens</i> I.R. Hall	8.82	-
<i>Funneliformis geosporum</i> C. Walker & A. Schüßler	-	14.61
<i>Gigaspora margarita</i> W.N. Becker & C. Hall	21.76	-
<i>Glomus multicaule</i> Gerd. & Trappe	-	14.16
<i>Glomus</i> sp. 1	26.47	-
<i>Glomus</i> sp. 2	-	17.81
<i>Rhizophagus sinuosus</i> Sieverd., F.A. Souza & Oehl	-	6.39
<i>Septoglomus constrictum</i> Sieverd., F.A. Souza & Oehl	-	12.33
Simpson's Index of Diversity (1-D)	0.76	0.84
Shannon Diversity Index (H)	1.52	1.92
Species Richness	5	8

3.3.5.2 Diversity Index

The mean Shannon Diversity Index is higher for the Thenzawl site (1.92) compared to the North Vanlaiphai site (1.52) (Table 3.11). The mean Simpson's

Diversity Index (1 - D) is also higher for the Thenzawl site (0.84) compared to the North Vanlaiphai site (0.76).

3.3.5.3 Relative Abundance

North Vanlaiphai showed a dominance of *Glomus* sp (26.47%) and *Gigaspora margarita* (21.76%), indicating these species are the most prevalent at this location. Thenzawl had a more even distribution of AMF species, with *Acaulospora foveata* (19.18%), *Glomus* sp (17.81%), and *Funneliformis geosporum* (14.61%) being notably abundant (Table 3.11).

3.3.6 Correlation between soil factors and root AM colonization

The correlation matrix of variables at North Vanlaiphai is shown at Figure 3.2. Arbuscular colonization has a significant strong positive correlation with phosphatase activity and bulk density of the soil, and a significant negative correlation with soil organic carbon. Vesicular colonization has a significant positive correlation with urease activity and a significant negative correlation with soil organic carbon. Hyphal colonization shows a significant strong positive correlation with soil organic carbon, potassium levels and a significant strong negative correlation with phosphorus levels.

At Thenzawl, arbuscular colonization has a significant strong positive correlation with phosphorus levels and bulk density (Figure 3.3). Very strong significant positive correlation is observed between vesicular colonization and phosphorous levels, and pH values. Almost perfect positive correlation is observed between hyphal colonization and pH, and a strong positive correlation between phosphorous and hyphal colonization. Urease shows moderate to weak negative correlations with AC, VC, and HC. Soil Moisture Content shows moderately negative correlation with VC and HC.

3.3.7 Correlation between edaphic factors and spore density

At North Vanlaiphai, there is a very strong negative correlation between Spore Density (SD) and Bulk Density (-0.99), Phosphatase (-0.99), Urease (-0.98). There is a strong positive correlation with pH (0.67), Dehydrogenase activity (0.96) and a moderate positive correlation (0.48) with SMC. Spore density is most strongly

influenced by bulk density, dehydrogenase, and urease activities, with higher bulk density and urease activity associated with less variability (Figure 3.1).

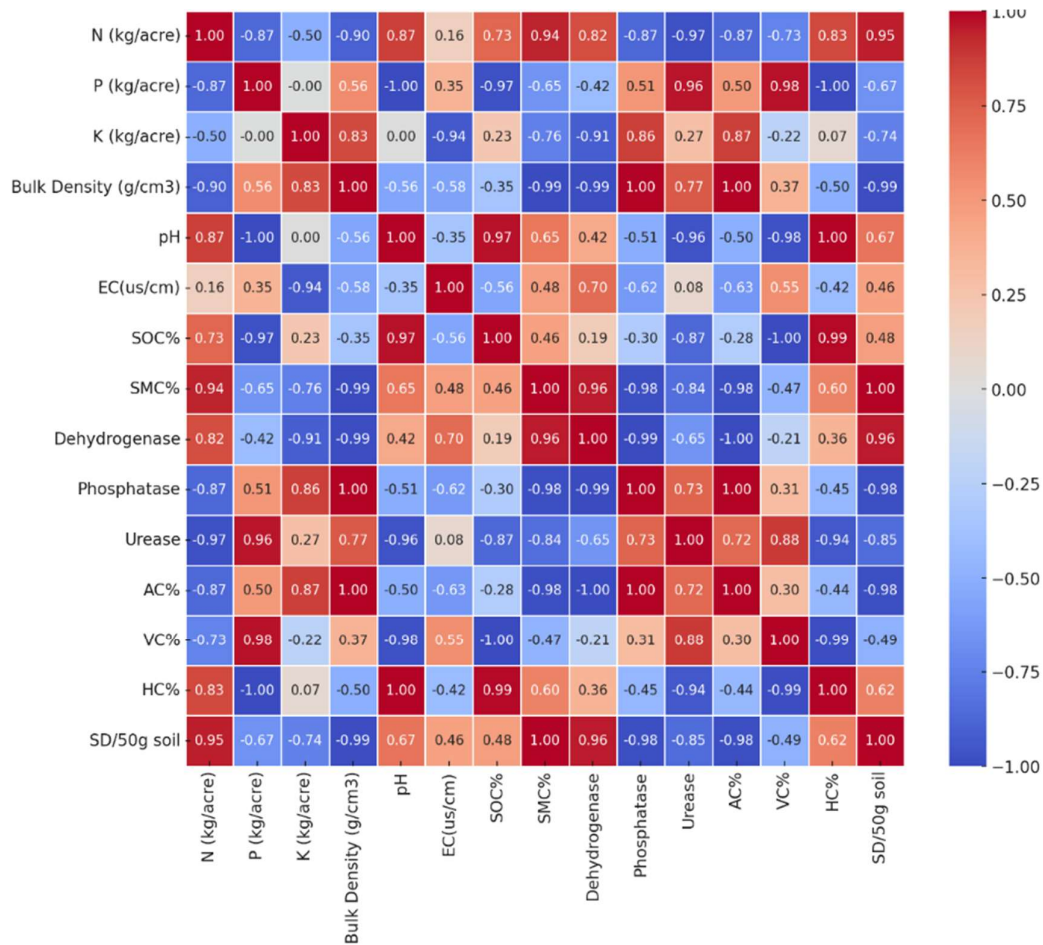


Figure 3.1: Heatmap of Person correlation between edaphic factors, AM colonization and spore density at North Vanlaiphai.

At Thenzawl, the correlation between pH and spore density is extremely high and positive (Figure 3.2). There is also strong positive correlation between Urease activity and spore density. The potassium content in the soil also shows a strong positive correlation with spore density. There is a very strong negative correlation between Phosphatase activity and spore density. Soil organic carbon percentage has a weak positive correlation with spore density. Soil moisture content is strongly negatively correlated with spore density, indicating that higher moisture content is associated with lower spore density. In summary, spore density at Thenzawl is most positively influenced by pH, Urease activity, and potassium content, while

Phosphatase activity and soil moisture content have strong negative effects. Soil organic carbon has a minor positive influence.

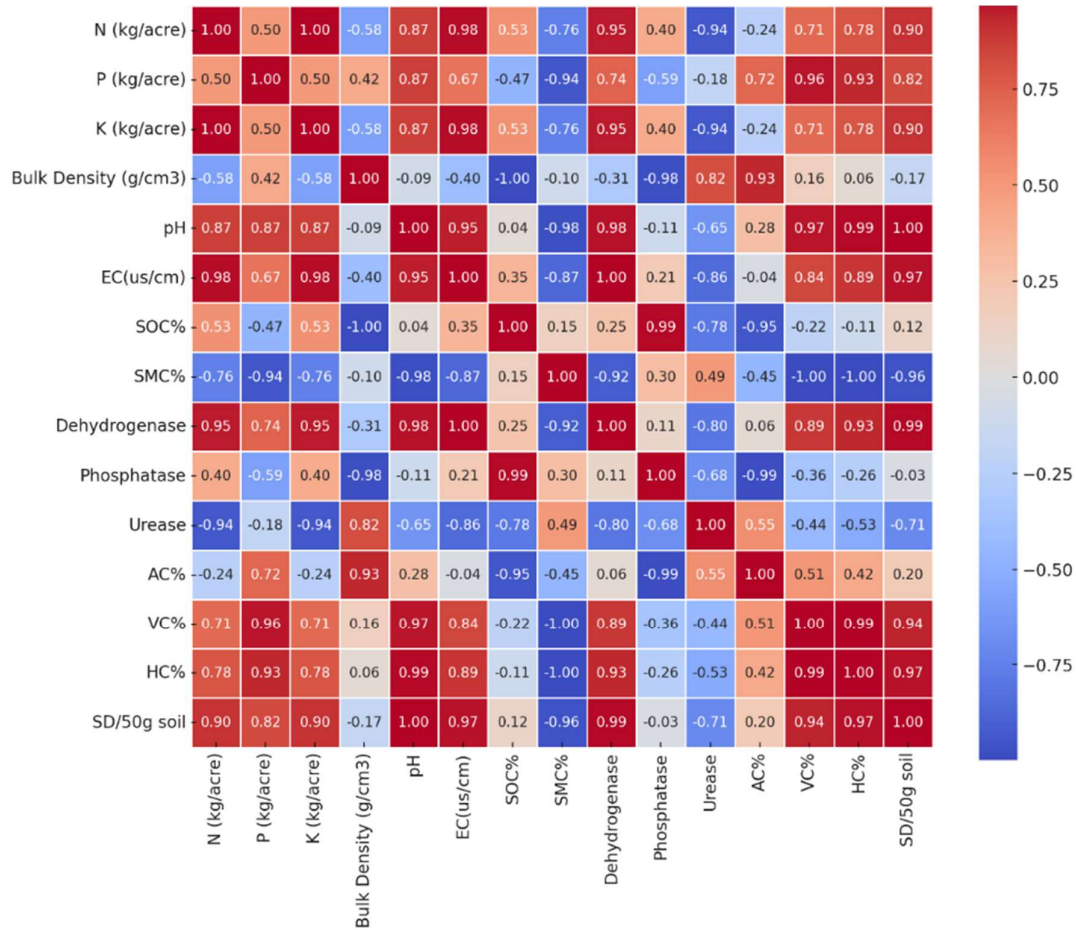


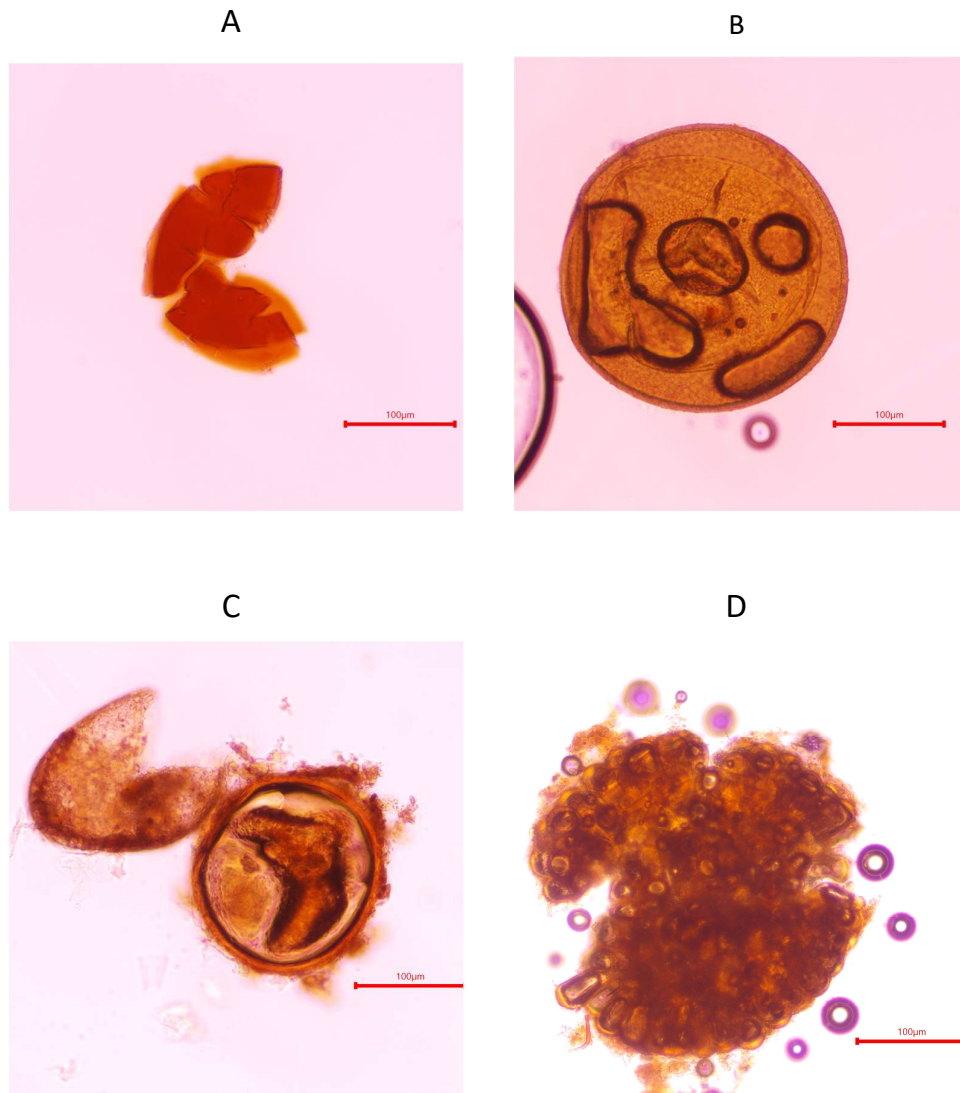
Figure 3.2: Heatmap of Person correlation between edaphic factors, AM colonization and spore density at Thenzawl.

3.4 Discussion

The present study investigated the colonization and diversity of Arbuscular Mycorrhizal Fungi (AMF) associated with rice plants in two distinct sites in Mizoram—North Vanlaiphai and Thenzawl—over a three-year period. The findings reveal significant site-specific differences in AMF colonization, spore density, and diversity, providing valuable insights into the factors that influence AMF communities in rice cultivation.

Arbuscular colonization percentages were consistently higher at Thenzawl compared to North Vanlaiphai throughout the study period. Both sites showed an inc-

Plate IV

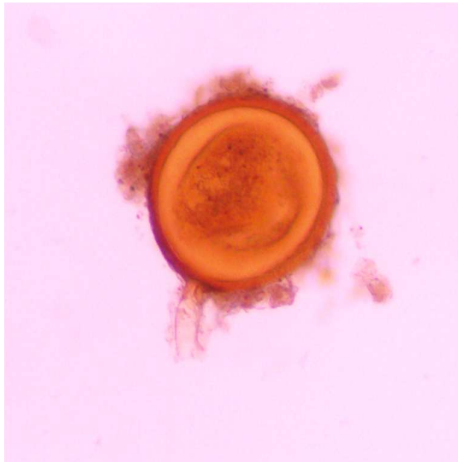


Arbuscular mycorrhizal fungal spores isolated from the study sites

A) *Acaulospora longula* Spain & N.C.Schenck; B) *Acaulospora spinosa* C.Walker & Trappe; C) *Entrophosphora infrequens* I.R. Hall; D) *Rhizophagus sinuosus* Sieverd., F.A. Souza & Oehl

Plate V

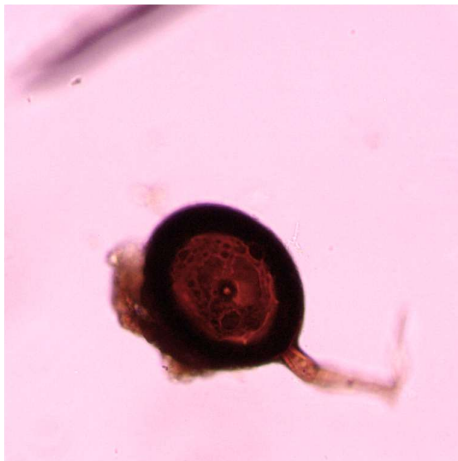
A



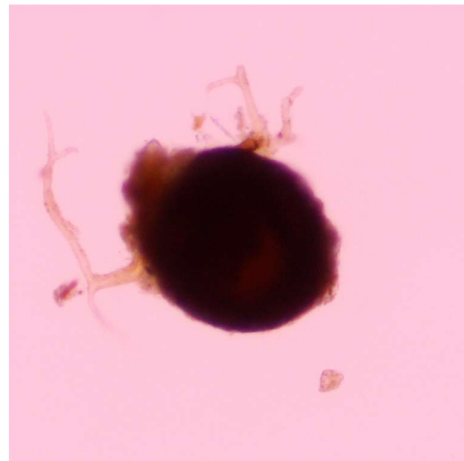
B



C



D



Arbuscular mycorrhizal fungal spores isolated from the study sites

A) *Funneliformis geosporum* C. Walker & A. Schüßler; B) *Septoglomus constrictum* Sieverd., F.A. Souza & Oehl; C) *Glomus* sp. D) *Glomus multicaule* Gerd. & Trappe

rease in arbuscular colonization over the years, but the increase was more pronounced at Thenzawl. This is consistent with previous studies that have found higher AMF colonization in soils with favorable physico-chemical properties, such as optimal pH and nutrient availability (Wang *et al.*, 2015; Chen *et al.*, 2018). The significant interaction between site and year observed in this study suggests that local environmental conditions, possibly influenced by agricultural practices or natural soil variations, play a crucial role in determining AMF colonization levels. These findings align with those of Bernaola *et al.* (2018), who also reported significant site-specific variations in AMF colonization in rice fields.

The contrasting trends in vesicular colonization between the two sites are also noteworthy. At Thenzawl, vesicular colonization increased steadily over the three years, whereas it declined at North Vanlaiphai. This divergence may reflect differences in soil management practices or environmental stressors at the two sites, which have been shown in other studies to impact AMF colonization (Lumini *et al.*, 2011; Tisarum *et al.*, 2020). Hyphal colonization was generally higher at Thenzawl, suggesting that this site offers a more conducive environment for AMF growth. Previous studies have also indicated that soil nutrient content, particularly potassium levels, positively influences hyphal colonization (Vallino *et al.*, 2009), which is corroborated by the strong positive correlation between hyphal colonization and potassium levels observed in this study. The overall colonization percentage in both the study sites is not high as compared to other plants. This may be due to the anaerobic condition when rice roots are under submergence which reduce the colonization rate (Vallino *et al.*, 2014).

The spore density at Thenzawl was consistently higher than at North Vanlaiphai across all years, with significant effects of site and the interaction between site and year. This pattern is consistent with previous research showing that soil pH, nutrient content, and enzyme activities can significantly influence spore production and persistence (Tian *et al.*, 2013). The positive correlation between spore density and soil pH, urease activity, and potassium content at Thenzawl suggests that these factors contribute to a more favorable environment for AMF spore formation.

In terms of AMF diversity, Thenzawl exhibited significantly higher species richness and diversity indices compared to North Vanlaiphai. The higher Shannon Diversity Index and Simpson's Diversity Index at Thenzawl suggest a more diverse

and evenly distributed AMF community. The physico-chemical parameters of the soil in North Vanlaiphai and Thenzawl significantly affect the variety of the AMF community, which is consistent with the results of another research (Ma *et al.*, 2021; Maitra *et al.*, 2021). In addition, pH, P, SOC, and K were identified as the primary factors that influenced the diversity of the AMF community. In addition, the pH and P variables exhibited a strong and statistically significant negative correlation with community diversity. The influence of pH on AMF spore formation and development has been observed (Zhang *et al.*, 2022). Additionally, pH can indirectly impact plant community composition and the uptake and utilization of soil nutrients by plants (Xu *et al.*, 2017).

The relative abundance data, which highlighted the dominance of certain species at each site, further support the notion that site-specific conditions significantly shape AMF community structure. *Glomus* and *Acaulospora*, being the predominant genus, were found to be significantly influenced by soil physicochemical properties. Additionally, most genera were observed to be affected by various soil factors, which is consistent with findings from previous studies (Xu *et al.*, 2017; Kim *et al.*, 2022). Our findings provide additional evidence that soil factors play a significant role in shaping the structure and diversity of AMF communities.

The findings of this study underscore the importance of site-specific soil properties in shaping AMF colonization, spore density, and diversity in rice fields in Mizoram. Thenzawl consistently exhibited higher AMF colonization and diversity, likely due to more favourable soil conditions, including higher pH, urease activity, and potassium content. These results are consistent with those of previous studies, which have emphasized the role of local environmental conditions in determining AMF community structure and function. The study highlights the need for tailored soil management practices that consider local soil characteristics to optimize AMF benefits in rice cultivation. Further research could explore the underlying mechanisms driving these site-specific differences and evaluate the potential for manipulating soil properties to enhance AMF-mediated benefits in agricultural systems.

Chapter 4

Propagation and Mass Production of Arbuscular Mycorrhizal Fungi

4.1 Introduction

Arbuscular mycorrhizal (AM) fungus improves the plant's ability to obtain soil resources, specifically phosphorus, by forming a mutually beneficial relationship. In return, the plant supplies the fungi with carbon, mainly in the form of sugars and lipids (Luginbuehl *et al.*, 2017). AM fungi also have a significant impact on enhancing plant tolerance to many environmental conditions, including drought (Ruiz-Lozano and Aroca, 2010), salinity (Porcel *et al.*, 2012), and heavy metal contamination (Díaz *et al.*, 1996). In addition, they have been found to promote photosynthesis in plants (Boldt *et al.*, 2011) and improve their resilience to diseases (Pozo and Azcón-Aguilar, 2007; Jung *et al.*, 2012). Due to the advantages, they offer, there has been a considerable focus on the production and examination of AM fungus, specifically for their use in agriculture, landscaping, and ecological restoration (Sawers *et al.*, 2018; Berruti *et al.*, 2016). There are three main methods often used for the mass production of AM fungi: substrate-based production, substrate-free production, and in vitro production systems.

The conventional mode of production, known as the substrate-based production system, entails growing plants and their symbiotic AM fungus in soil or sand-based substrates (Gaur and Adholeya, 2002). The methods of wet sieving and decanting are used to separate individual spores (Klironomos and Hart, 2002). There are different types of inoculum formulations available, usually consisting of spores, colonized roots, hyphae, and sterilized soil from pot cultures (Gemma *et al.*, 2002). Typically, the inoculum is positioned below the seed or seedling during the planting process to ensure that the roots pass through the inoculum, which helps with colonization (Jackson *et al.*, 1972).

Generally, while in vitro techniques offer a high degree of control and allow for production without contamination, the substrate-based system is the traditional and often used way for cultivating AM fungus, making them highly suitable for

experimental use. In this experiment also, substrate-based system is used for the mass propagation of single species AM fungal spores.

4.2 Materials and Methods

4.2.1 Experimental Site

Trap culture, single-species culture, and its mass production were conducted in the greenhouse facility at the Department of Botany, Mizoram University.

4.2.2 Selection of Host Plant

In the process of cultivating Arbuscular Mycorrhizal Fungi (AMF) for research and practical applications, the choice of host plants plays a crucial role in the success of both trap culture and single species culture techniques. After careful consideration of various plant species, maize (*Zea mays* L.) and the sorghum-sudangrass hybrid (*Sorghum bicolor* L. Moench-*Sorghum bicolor* var. *sudanense*) were identified as the preferred host plants for these purposes.

Maize was selected as the preferred host for trap culture due to its well-documented efficiency in promoting AMF colonization. Maize is known for its extensive root system and high compatibility with a wide range of AMF species, making it an ideal candidate for capturing and propagating diverse AMF communities from soil samples. On the other hand, the sorghum-sudangrass was selected for single species culture of AMF. This hybrid combines the beneficial traits of both sorghum and sudangrass, including vigorous growth, a dense root system, and high biomass production. The strategic selection of these host plants not only enhances the efficiency of AMF propagation but also ensures the availability of healthy and viable AMF inocula for subsequent experimental and field applications.

4.2.3 Establishment of Trap Culture (INVAM)

The collected rhizosphere soil from the field was chopped, along with the roots, into small fragments. The soil-root mixture was then mixed with double autoclaved soil in 1:1 ratio and transferred to 15 cm plastic pot (Plate VI A). After this, previously surface sterilised maize seeds were sown to the pot. Then the cultures were grown in greenhouse for four months during which the plant cease to grow and sporulation occur

Plate VI



Experimental set-up for trap culture

A) Soil and sand mixture for trap culture; B) Four weeks old trap culture; C) Three months old trap culture.

(Plate VI B&C). Fertilisers are applied in minimum quantity during the growing period. After four months, culture pots are left undisturbed to dehydrate in a shaded room. 1-2 weeks after watering was stopped, spores were collected before the soil was too dry and can be used for starter inoculum.

4.2.4 Extraction of Spores (Gerdemann and Nicolson, 1963)

The isolation of AM spores from trap culture soil was done using the wet sieving and decanting process. A 100g sample of soil was mixed with 1000mL of tap water in a beaker. The soil suspension was agitated with a glass rod and then the sediments were allowed to settle for a duration of 30 seconds. The aliquot was filtered using sieves arranged in series of sizes (0.25-0.037mm). The contents from each sieve were rinsed into individual beakers. The aliquot was subsequently filtered using Whatman filter paper No. 1 and then transferred to Petri plates. The filter paper was observed with a bright-field microscope (Olympus BX53) to detect the existence of spores and sporocarps.

4.2.5 Taxonomic Identification of AM Fungi

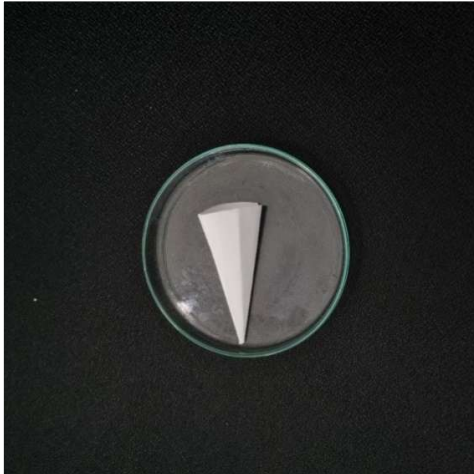
The AMF were morphologically identified by analysing spore size, spore colour, wall layer thickness, spore surface ornamentation, and subtending hyphae. This identification process followed the guidelines provided in the identification manual found on the International Collection of Vesicular and AM Fungi website (<http://invam.wvu.edu/>) and amf-phylogeny.com.

4.2.6 Preparation of Starter Inoculum

Using sorghum-sudangrass as host plant, starter culture or pure culture of AM fungi is initiated using Single-spore inoculation tray method (Selvakumar *et al.*, 2018). A single viable spore is carefully picked under microscope and dispensed to the roots of pregerminated seedling. Then the seedlings are put into filter paper made into funnel shape (Plate VII A) and filled with two times autoclaved soil and sand mixture (1:1). Then, the filter papers were transferred to a seedling tray and watered regularly (Plate VII B). After 30 days, the roots of seedlings were examined for the AM colonization (Phillips and Hayman, 1970). After this, seedling with successful colonization was transferred to bigger pots containing two-time autoclaved sand: soil mixture (1:1) for

Plate VII

A



B



C



D



Preparation of starter inoculum

A) UV sterilised Whatman filter paper for single spore culture; B) AMF single spore inoculated sorghum-sudangrass seedlings in a seedling tray; C) Seven days old sorghum-sudangrass seedlings for substrate-based mass propagation of AM fungal spores; D) Fifteen days old sorghum-sudangrass seedlings for substrate-based mass propagation of AM fungal spores.

Plate VIII

A



B



C



D



Mass production of single species culture

A) Thirty days old sorghum-sudangrass seedlings for substrate-based mass propagation of AM fungal spores; B) 90 days old sorghum-sudangrass seedlings for substrate-based mass propagation of AM fungal spores; C) 120 days old sorghum-sudangrass seedlings for substrate-based mass propagation of AM fungal spores; D) Pots left to dry and sporulate after four months of cultivation.

mass production of spores (Plate VII C&D).

4.2.7 Mass Production of Single Species Culture

The substrate-based production system was utilized for the mass production of a single species. Sorghum-sudangrass hybrid (*Sorghum bicolor* L.) Moench/*Sorghum bicolor* var. *sudanense*) was used as the host plant, and the specific needed arbuscular mycorrhizal (AM) species (starting culture) were grown in sterilized sand: soil mixture (1:1). The pots were overseeded with sorghum-sudangrass seeds in order to reduce the presence of contaminants on exposed surfaces, limit the movement of particles on the surface, and ensure uniform plant growth at a manageable height (Plate VIII A&B). After a period of 4 months, the plants are permitted to undergo desiccation, following which the shoot portion is removed (Plate VIII C&D). The combination of sand and severed roots is thereafter placed into zip-loc bags, properly labelled, and stored at a temperature of 4°C. These bags serve as the inoculant for subsequent research.

4.3 Results

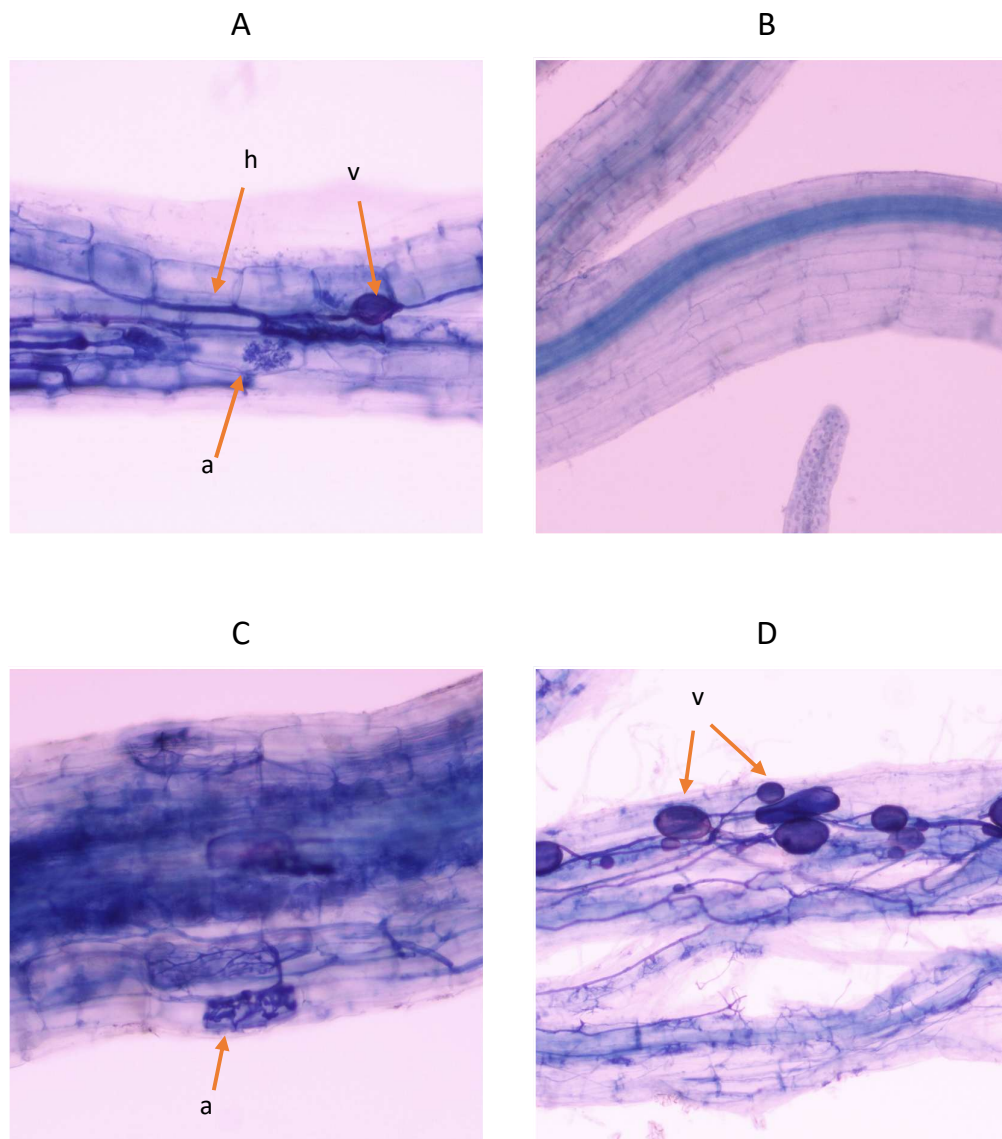
4.3.1 Trap Culture

In the present study, a total of seven species of arbuscular mycorrhizal (AM) fungi can be isolated and refined using trap cultures. Of these, two species each belong to the genera *Glomus* and *Acaulospora*. Additionally, one species from each of the genera *Gigaspora*, *Funneliformis*, and *Rhizophagus* can be obtained. These fungal species can then be utilized for single-species inoculation studies, which allow for a focused investigation into the individual effects of these AM fungi on plant growth and stress tolerance in rice, under controlled conditions. The presence or absence of AM fungal structures were confirmed by analysing the roots under microscope (Plate XI).

4.3.2 Mass Production of Single Species Culture

Out of the seven AM fungal species refined using trap cultures, three AM species *Acaulospora foveata* (Plate X), *Gigaspora margarita* (Plate XI A&B), and *Glomus* sp. (Plate XI C&D), was selected for starter inoculum since *Acaulospora* and *Glomus* was found to be dominant and abundant genera in the study sites. The three

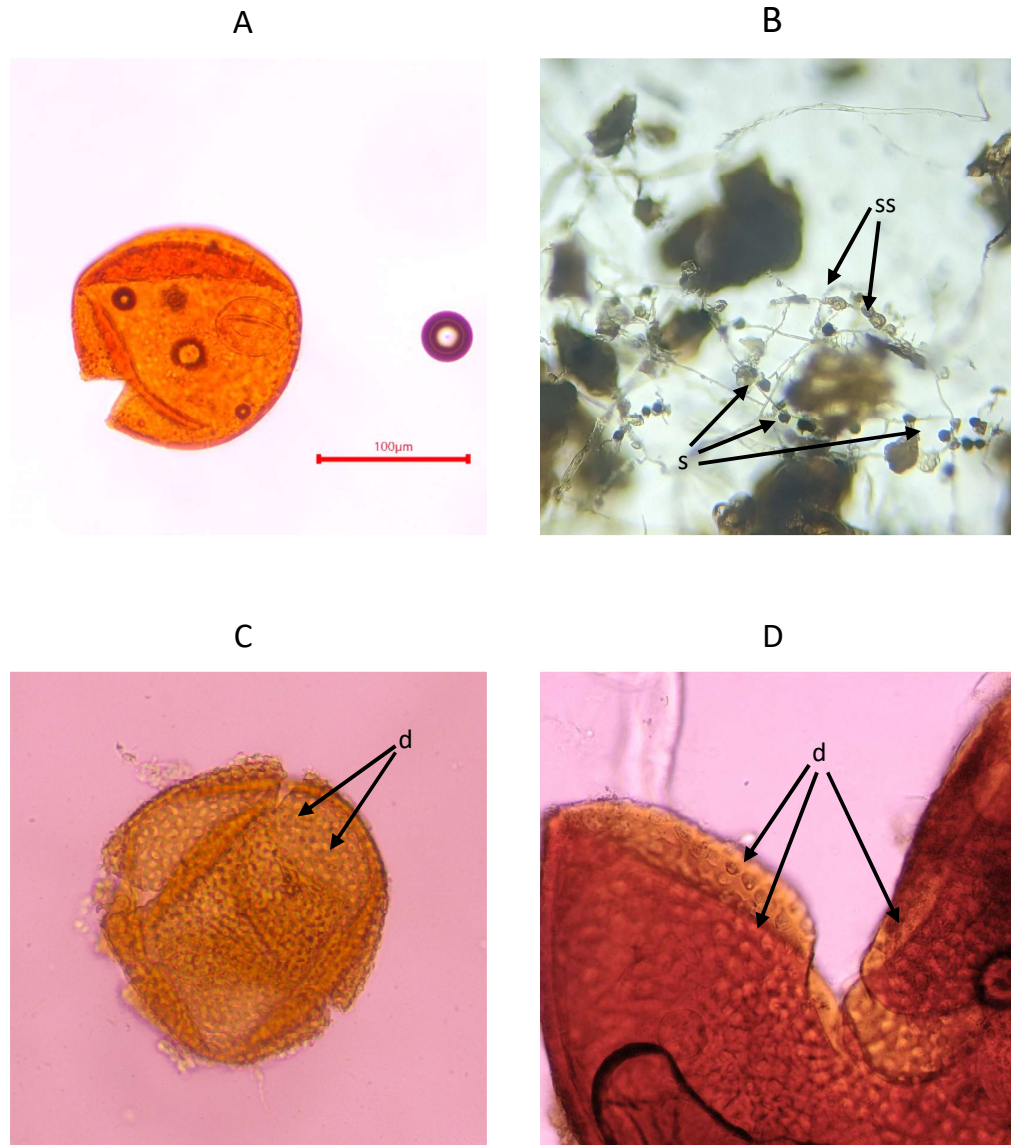
Plate IX



AM fungal structures in the roots of *Zea mays* L.

A) Vesicle and Arbuscule along with hyphae found in the roots of AMF infected roots, B) no fungal structures in controlled roots, C) Arbuscule structure in roots, D) Clusters of vesicles in the roots of AMF infected roots; a: arbuscule, b: vesicle, h: hyphae.

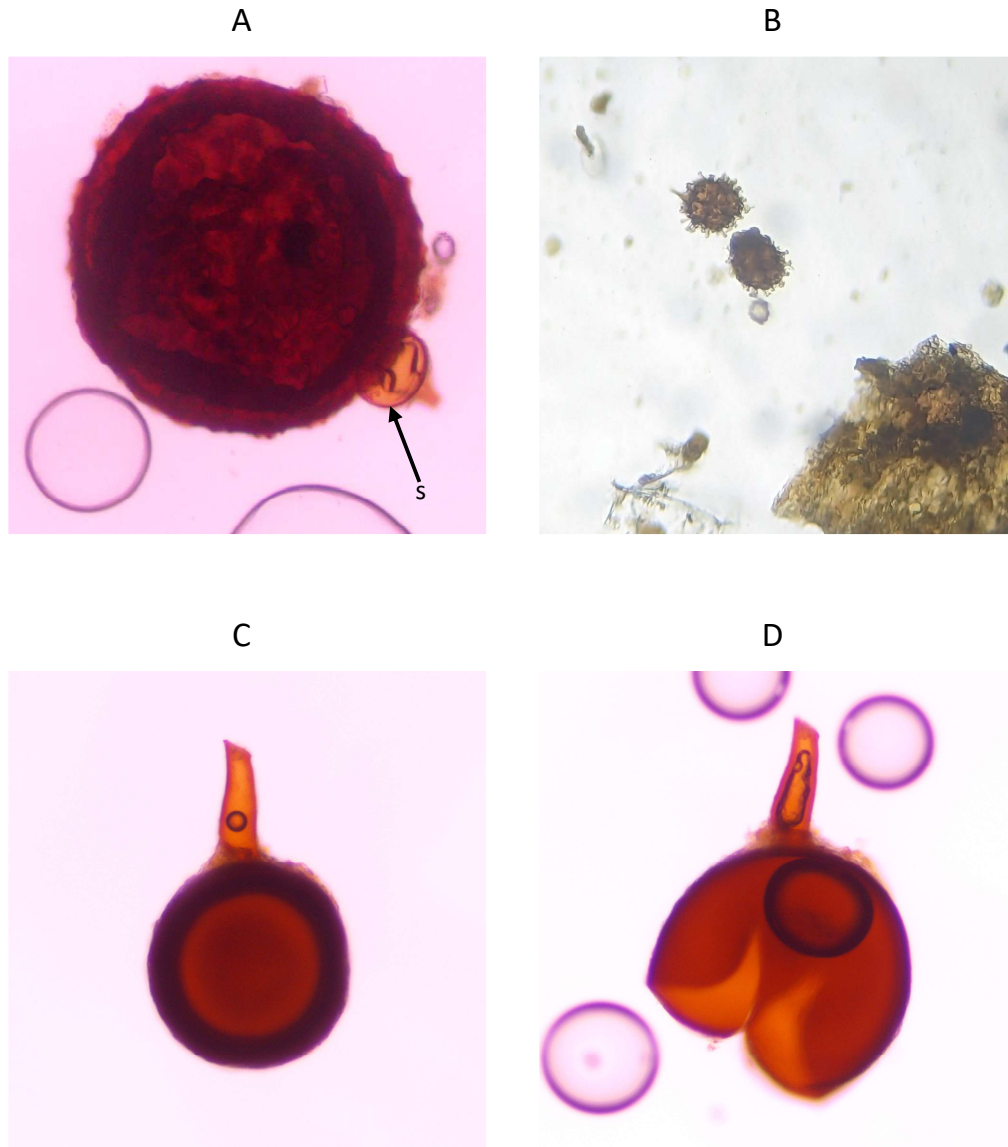
Plate X



Acaulospora foveata spores recovered from single spore culture.

A) Spore mounted in PVLG, B) Spores, hyphae and empty sporiferous saccule in soil suspension, C) Juvenile spore crushed for spore wall study showing depressions on wall surface, D) Depression in surface of spore wall; d: depressions, s: spores, ss: sporiferous saccule.

Plate XI



***Gigaspora margarita* and *Glomus* sp. recovered from single spore culture.**

A) *Gigaspora margarita* spore with swollen subtending hypha, B) Auxiliary cells of *Gigaspora margarita*, C & D) Unidentified *Glomus* spore used for subsequent research. s: swollen subtending hyphae, characteristic of *Gigaspora*.

AM fungal species were then cultured and their spores were successfully multiplied in a large number of pots using sorghum-sudangrass as substrate.

4.4 Discussion

Acaulospora foveata is known for its high efficiency in colonizing plant roots, making it a suitable candidate for studies focusing on mycorrhizal inoculation. This species is particularly effective in phosphorus-poor soils, where its presence can significantly enhance phosphorus uptake by the host plant. Moreover, *A. foveata* has been observed to improve plant growth under stressful conditions, including drought and salinity, which are common in many agricultural settings (Jeffries *et al.*, 2003; Smith and Read, 2008). Studies using *A. foveata* have demonstrated its role in enhancing the resilience of crops like rice (*Oryza sativa*) and maize (*Zea mays*) under adverse conditions, making it a valuable tool in sustainable agriculture research (Gosling *et al.*, 2006).

The biology and genetics of *Glomus* are well-studied, providing a solid foundation for experimental design and data interpretation. Their life cycle, spore morphology, and colonization patterns are well-documented, facilitating reproducibility in experiments (Redecker *et al.*, 2013). *Glomus* are particularly effective in enhancing the uptake of phosphorus, an essential nutrient often limiting in soils. This efficiency is crucial in studying the role of AMF in plant nutrition and soil fertility management (Smith and Smith, 2011).

One of the key features of *Gigaspora margarita* is its ability to form extensive hyphal networks that significantly enhance nutrient uptake, particularly phosphorus, in host plants. This trait is vital for studying the mechanisms through which AMF enhance plant growth and stress tolerance, particularly under nutrient-deficient conditions. Additionally, the large spore size of *Gigaspora margarita* facilitates easier identification and isolation, making it a preferred choice for researchers working on the taxonomy and phylogeny of AMF species (Berruti *et al.*, 2016).

The findings of this experiment are consistent with earlier studies, especially in terms of the selection of particular host plants and the effectiveness of substrate-based production systems for propagating AMF. The substrate-based method is widely acknowledged for its ability to closely replicate field conditions, making it the most

often used technique for propagating AMF. The trap culture and monosporic culture techniques are the main methods employed in this approach to multiply spores (Douds *et al.*, 2006; Panwar *et al.*, 2008).

Trap plants, which rely heavily on mycorrhizae, are commonly employed as hosts in pot cultures to enhance the production of arbuscular mycorrhizal fungi (AMF) spore. Nevertheless, Gryndler *et al.* (2003) observed a decrease in the frequency of root colonization, the number of arbuscules, and the growth of extraradical mycelium after 11 months of cultivation. Trejo-Aguilar *et al.* (2013) discovered that employing a variety of arbuscular mycorrhizal fungi (AMF) species from diverse families to commence spore production led to superior outcomes. However, it was observed that specific species from the Glomeraceae family were largely prevalent in the resulting AMF cultures. These findings emphasize the intricacy of producing pure AMF cultures, as the inability to completely recreate natural conditions in trap pot cultures may hinder sporulation and AMF growth. In addition, the selection of AMF-host combinations may be influenced by factors such as soil type, substrate type, and processing procedures, which might introduce bias (Bever, 2002).

Chapter 5

Biochemical and Physiological Responses of Rice Plant Inoculated with Arbuscular Mycorrhizal Fungi Under Drought Stress

5.1 Introduction

Climate change and food security are among the most urgent global concerns. (Raza *et al.*, 2019). Drought is becoming a major worldwide environmental issue as a result of the acceleration of climate change (Naumann *et al.*, 2018; Piao *et al.*, 2010; Trenberth *et al.*, 2014; Mathur *et al.*, 2021). Over the last ten years, drought stress has caused a 70% decrease in crop productivity (Raza *et al.*, 2019). The occurrence of repeated dry periods and irregular rainfall patterns has led to a shortage of water and subsequent loss or damage in crop production in areas that typically rely on rainfall for irrigation (Vallino *et al.*, 2009).

The need for rice cultivation is increasing steadily due to the ongoing growth of the global population. According to predictions, the global population is expected to reach over 8 billion by 2030. As a result, there is a requirement to boost rice output by 40% within the next two decades (Bernier *et al.*, 2008). Rice possesses the distinctive evolutionary trait of being semi-aquatic and is traditionally cultivated by keeping it submerged for the most of its growth period. Therefore, it exhibits minimal modifications to situations with restricted water availability and is highly susceptible to the effects of drought stress (Kamoshita *et al.*, 2008). Nevertheless, around 50% of the global rice cultivation region lacks adequate water to sustain flooded conditions, resulting in a diminished output due to drought.

Periodic water scarcity during crucial growth phases can lead to significant decreases in crop productivity and even complete crop failure (Bernier *et al.*, 2008). Undoubtedly, drought poses a significant constraint on rice cultivation in rain-fed habitats. The negative impact of drought stress on rice crop has been widely documented, leading to reduced grain output, inhibited plant growth, grain sterility, and yield loss (Mukamuhirwa *et al.*, 2019; Yu, 2018). Evenson *et al.* (1996) estimated that drought caused an annual loss of 18 million tons of rice globally, which accounted

for 4% of the total rice production. This loss was estimated to be worth US \$3.6 billion at the time. The decrease in yield potential is not solely caused by the absence of water, but also by the specific timing and length of drought stress in relation to phenological processes (Jongdee *et al.*, 2002).

One of the most prevalent methods that is utilized to maintain crop productivity is the application of appropriate irrigation, which is determined by the water requirements of each unique crop (Ewaid *et al.*, 2019; Sharma, 2020; Zhang *et al.*, 2022). Alternatively, the introduction of a consortia of microorganisms into the soil has been documented as a sustainable method to enhance the physical and chemical properties of the soil. This results in a greater ability to retain water and alleviate drought in the specific area being targeted (Ahluwalia *et al.*, 2021; Aslam *et al.*, 2022; Etesami *et al.*, 2022; Thangavel *et al.*, 2022).

An effective approach to enhance rice output in response to population growth and climate change is to enhance drought tolerance in rice through the use of the AM symbiosis. Arbuscular mycorrhizal fungi (AMF) have been found to be highly beneficial for enhancing plant development in the long run, making it a profitable strategy for promoting sustainability (Lanfranco *et al.*, 2018; Tisarum *et al.*, 2022). Previous studies have indicated various mechanisms through which the arbuscular mycorrhizal (AM) symbiosis can help mitigate drought stress in host plants. Studies have shown that the arbuscular mycorrhizal (AM) symbiosis can help protect host plants from the harmful effects of water deficit (Auge', 2001, 2004; Ruiz-Lozano, 2003; Ruiz-Lozano *et al.*, 2008). AMF has been linked to various mechanisms that contribute to a plant species' ability to tolerate drought. These mechanisms include improved water and nutrient absorption, enhanced root structure, increased root mass, better osmotic adjustment, and a strengthened antioxidant defense system (Wu *et al.*, 2013; Pavithra and Yapa, 2018; Petipas *et al.*, 2017; Begum *et al.*, 2019).

The most important factors are the direct uptake and transfer of water through the fungal hyphae to the host plant (Hardie, 1985; Ruiz-Lozano and Azcon, 1995), changes in soil water retention properties (Auge' *et al.*, 2001), better osmotic adjustment of AM plants (Auge' *et al.*, 1992; Kubikova *et al.*, 2001; Ruiz-Lozano *et al.*, 1995a), enhancement of plant gas exchange and water use efficiency (Auge' *et al.*, 1992; Ruiz-Lozano *et al.*, 1995a, 1995b), and protection against the oxidative damage

generated by drought (Porcel and Ruiz-Lozano, 2004; Porcel *et al.*, 2003; Ruiz-Lozano *et al.*, 2001). The importance of this process has been acknowledged (Ruiz-Lozano, 2003), as various environmental challenges, such as water scarcity, lead to the generation of reactive oxygen species (ROS) in plants. This, in turn, exacerbates oxidative stress and contributes to degenerative effects.

The term ROS is inclusive, encompassing not only free radicals like superoxide ($O_2^{\bullet-}$) and hydroxyl radicals, but also H_2O_2 and singlet oxygen. Although it is commonly believed that the hydroxyl radical and singlet oxygen are highly reactive and should be produced in minimal amounts (Jakob and Heber, 1996), $O_2^{\bullet-}$ and H_2O_2 are actually synthesized at very high rates even under ideal conditions (Noctor and Foyer, 1998). The primary toxicity of $O_2^{\bullet-}$ and H_2O_2 is believed to be associated with their capacity to initiate cascade reactions, leading to the generation of hydroxyl radicals. The radicals and their derivatives are considered to be highly reactive in the field of chemistry. They have the ability to react without discrimination, leading to oxidative damage in biomolecules. This damage includes lipid peroxidation, denaturation of proteins, and mutation of DNA (Halliwell and Gutteridge, 1984).

The enhancement of antioxidant compounds in plants is often associated with the improvement of stress tolerance. Considering the toxicity of reactive oxygen species (ROS), it is crucial for plants to possess efficient detoxification mechanisms that enable swift elimination of these compounds. The systems consist of many antioxidant enzymes as well as non-enzymatic substances like ascorbate, glutathione, flavonoids, carotenoids, and tocopherols (Ma *et al.*, 2008). Therefore, the generation of antioxidant molecules is frequently employed as a measure of a plant's ability to withstand environmental conditions that result in oxidative stress (Noctor and Foyer, 1998).

AMF implementation in rice typically faces challenges in lowland paddy fields that are flooded with water. These fields are often not suitable for AMF inoculation, and the diversity of AMF consortium that can thrive in these conditions is determined by the anaerobic root zones (Bao *et al.*, 2019; Sarkodee-Addo *et al.*, 2020; Wang *et al.*, 2021). Rice plants easily establish mycorrhizal connections in upland circumstances, but infection is uncommon in submerged situations due to the lack of oxygen (Ilag *et al.*, 1987). Nevertheless, certain reports have uncovered the

colonization of AMF in flooded settings (Secilia and Bagyaraj, 1994a, 1994b; Solaiman and Hirata, 1996).

In the case of an AMF consortium, researchers often utilize a combination of multiple AMF species to maximize their positive effects. For instance, the combination of *Funneliformis* and *Acaulospora* species has been found to greatly enhance the physiological and biochemical adaptabilities of *Sorghum bicolor* to drought conditions (Neuman and George, 2004). Furthermore, the application of a combination of 25 arbuscular mycorrhizal fungi (AMF) species from four different genera, namely *Funneliformis*, *Acaulospora*, *Gigaspora*, and *Scutellospora*, led to enhanced plant growth and development. (Yooyongwech *et al.*, 2013). *Glomus* is a frequently studied arbuscular mycorrhizal fungus (AMF) genus. It is often utilized in research to investigate its potential to enhance various aspects of plant physiology and growth under challenging environmental conditions. For instance, studies have shown that *Glomus mosseae* can improve chlorophyll content, photosynthetic efficiency, mineral nutrient uptake, and shoot-root traits in host plants subjected to stressors like heavy metal exposure and drought (Zhang *et al.*, 2018; Cheng *et al.*, 2024).

Rice can be cultivated using an alternate irrigation system to minimize the amount of water required for production. This technique induces aerobic conditions in the soil, which promote the colonization of rice roots by arbuscular mycorrhizal (AM) fungi. However, there is a limited number of research that give a comprehensive understanding of the colonization of arbuscular mycorrhizal fungi (AMF) in rice roots. Currently, there is no clear understanding of how this connection might be utilized to improve crop productivity in unfavorable environmental conditions (Vallino *et al.*, 2009). A recent study focused on using non-flooded upland paddy cultivation as a model system to investigate the interaction between AMF and plants in the presence of water deficit stress (Lumini *et al.*, 2011; Bernaola *et al.*, 2018; Parvin *et al.*, 2021; Surendirakumar *et al.*, 2021).

In order to study the control of physiological and biochemical characteristics in upland rice during the reproductive phase under water scarcity, we selected *Acaulospora foveata*, *Gigaspora margarita*, and *Glomus* sp. both separately and in combination. Based on the idea, the AMF consortium has the potential to control the mechanisms of host plant attributes during periods of water shortage stress. The aim

of this study was to examine if the arbuscular mycorrhizal (AM) symbiosis can mitigate the detrimental impacts of drought stress on rice plants. According to the hypothesis, the inoculation of rice plants with different mycorrhizal treatments will enhance drought tolerance compared to non-inoculated plants, with differences observed between rice varieties.

5.2 Materials and Methods

5.2.1 Experimental Site

The pot experiment took place in the greenhouse facility (Plate XII A) at the Department of Botany, Mizoram University, located in Aizawl, Mizoram, India (23°44'11.4"N 92°39'46.6"E).

5.2.2 Preparation of Inoculum

The combination of sand and severed roots which was stored at a temperature of 4°C was taken out from the refrigerator. The roots and soil particles were carefully removed from the zip lock bag and kept separately in a sterile container according to the species. This mixture serves as inoculum for the current experiment which was then mixed with a double steam sterilised soil.

5.2.3 Preparation of Pot Substrate Material

The soil used for rice seedling culture is composed of a mixture of forest soil (sieved through a 2-mesh sieve) and river sand, in a volume ratio of 2:1. Afterwards, it underwent two rounds of sterilization at a pressure of 0.14 MPa and a temperature of 125°C. The pH of the soil is 6.5, the electrical conductivity (EC) is 103.8 µS/cm, the soil organic carbon content is 18.03 g/kg, the available phosphorus content is 23.93 mg/kg, the available nitrogen content is 46.6 mg/kg, and the available potassium content is 189.2 mg/kg. The culture pots were washed with 75% ethanol and thereafter stored for future use.

5.2.4 Plant Materials and Seedling Preparation

A local rice cultivar named Changkawi was obtained from North Vanlaiphai. Simultaneously, DRR Dhan 50, which is drought and submergence tolerant variety, was obtained from ICAR – IIRR seed portal. DRR Dhan 50 is a rice variety developed at ICAR-Indian Institute of Rice Research (ICAR-IIRR), Hyderabad collaboration

with ICAR-National Institute of Plant Biotechnology, New Delhi and IRRI, Philippines, through molecular breeding with introgression of *qsub1* for submergence tolerance, and *qDTY2.1* and *qDTY3.1* for drought tolerance. The seeds of both varieties were pretreated in order to break dormancy and have a high germination rate. Seeds were exposed to direct sunlight for four days and then primed by soaking for 5 hours and dried again prior to sowing. Before germination, the primed seeds were pretreated for 1 min in 1% sodium hypochlorite (NaOCl) and then rinsed three times with sterile water. After surface sterilisation, the seeds were submerged in sterile water and kept inside a plant growth chamber with a temperature of 30°C, humidity 75%, and 12 hr light and 12 hr dark period. The seeds were kept in this condition until a radical and plumule appeared from the embryo. After germination, the seedlings were transferred to the pots in the greenhouse and grown for 90 days.

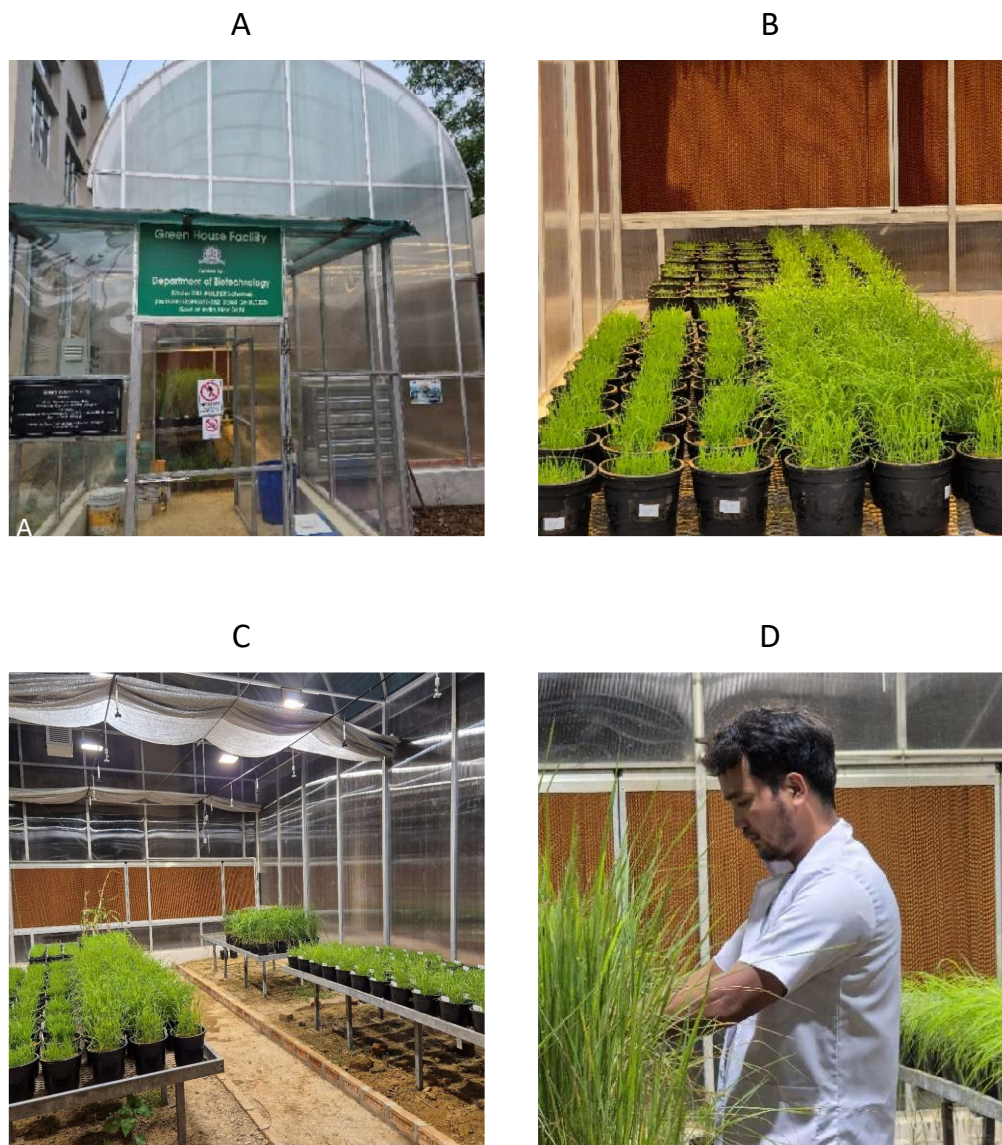
5.2.5 Experimental Design

A full-factorial experiment with completely randomized design (CRD) was used consisting of three factors (*viz.* Rice variety, Water treatment and Mycorrhizal treatment). There are two levels of rice variety - Changkawi (CHA), DRR Dhan 50 (DHA); two levels of water treatment - Well-watered (WW), Moderate Drought (MD) and six levels of mycorrhizal treatment – Non-mycorrhizal (NM), *Acaulospora foveata* (AM1), *Gigaspora margarita* (AM2), *Glomus* sp. (AM3), mixture of AM1, AM2 and AM3 (AM4) and Commercial AMF propagules (AM5). There was a total of 24 treatment combinations each replicated three times (Figure 5.1). The total number of experimental units is 72. Each of the experimental units were randomly assigned to ensure unbiased results.

5.2.6 Greenhouse Study

The pots were filled with a layer of steam sterilised soil and AMF inoculant. Only sterilised soil was used for non-mycorrhizal treatment. Ten seedlings of rice plants were grown in each of the experimental pots and they were cultivated in greenhouse until it reaches the booting stage. The temperature in the greenhouse was set at $32 \pm 2^\circ\text{C}$ for daytime and the relative humidity at $75 \pm 5\%$. Subsequently, each pot, together with the substrate, was watered individually to reach near field capacity, as determined by the mass of the pot. In order to promote optimal early development

Plate XII



Greenhouse experimental setup for rice cultivation under controlled conditions

A) Greenhouse facility where pot experiment was performed; B) Experimental pots 14 days after sowing; C) Rice seedlings after 1 month cultivation; D) Researcher measuring growth parameters in rice plants.

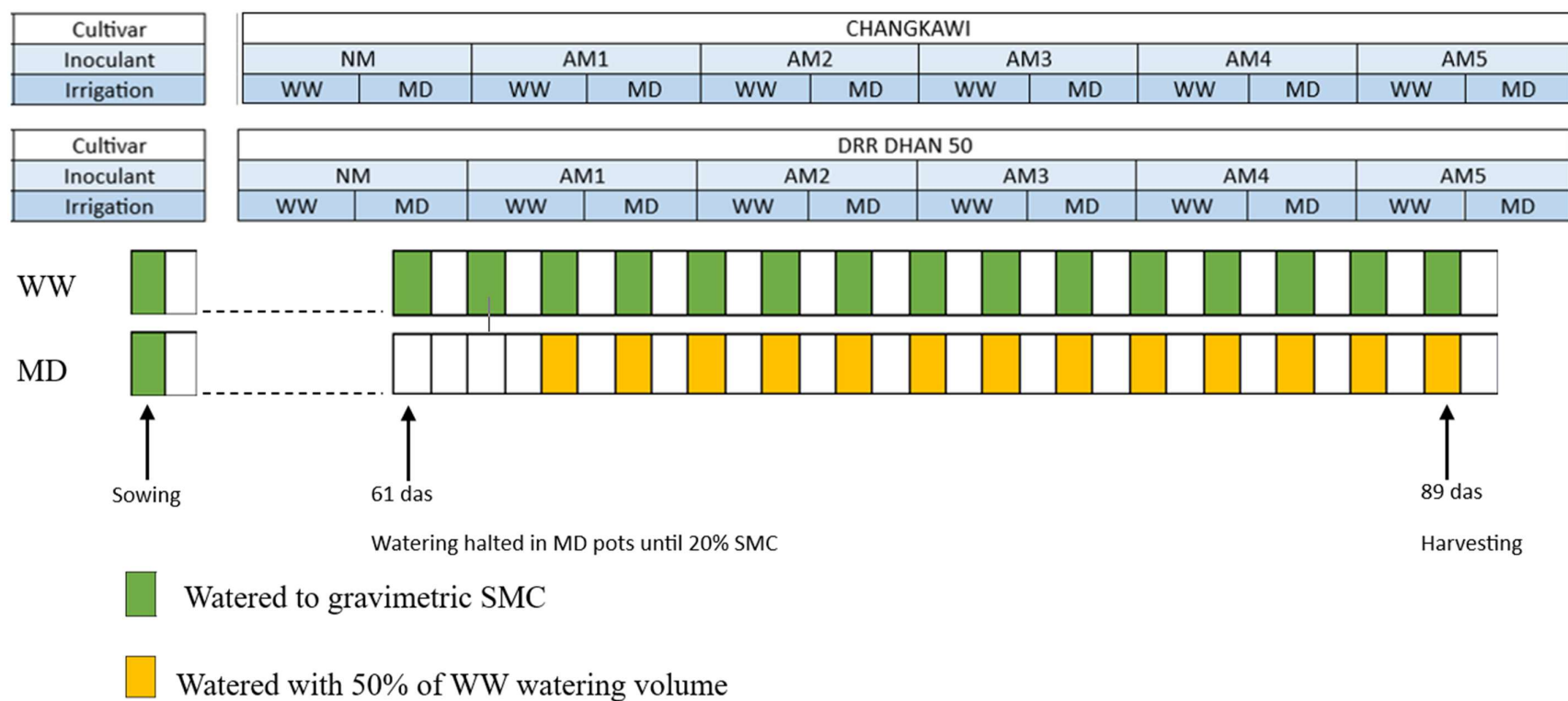


Figure 5.1. Experimental set-up. Two rice cultivars (*Oryza sativa* L.; Changkawi, Dhan 50) were either non-inoculated (NM) or inoculated with single mycorrhizal fungus (AM1-*Acaulospora foveata*, AM2 – *Gigaspora margarita*, AM3 – *Glomus* spp) or with a mixture of the three fungi (AM4) or with commercial propagules (AM5). Following germination, the seeds were directly planted in pots and irrigated every alternate day. This irrigation schedule was maintained until 60 days after sowing (das), at which point the plants were divided into different water regimes: well-watered (WW) and moderate drought (MD), based on the soil moisture content (SMC).

and facilitate the establishment of mycorrhizal symbiosis in rice plants, the pots were irrigated weekly with 60 ml of sterile Hoagland's nutrition solution with a low level of phosphorous and maintained under well-watered circumstances for the initial 60 days.

After 60 days of normal water supply, the plants were subjected to two different water regimes (n=3 pots per rice cultivar, water status and mycorrhizal treatment, respectively). The Well-Watered plants (WW) were additionally watered to almost maximum field capacity every alternate day. In Moderate drought (MD) treatment, the pots were not watered after 61 days after sowing until they achieved 50% field capacity, which occurred at 65 days after sowing. From day 65 after seeding, the MD plants were given only half the quantity of water that the WW plants received every other day. The water treatment continued for 28 days and stops at 88 days after sowing. The rice plants were harvested at 89 days after sowing. Before harvesting, growth parameters like shoot length, leaf length and leaf size were calculated.

5.2.7 Estimation of AM Colonization in Roots (McGonigle, 1990)

The root samples were meticulously cleaned with tap water to eliminate dirt particles. Subsequently, the roots were subjected to thermal treatment with a 10% solution of potassium hydroxide (KOH) at a temperature of 90°C for approximately 30 minutes. Prior to this treatment, the roots were first sectioned into segments measuring 1-2 cm in length. The root segments are acidified with 2% HCl for 15-20 minutes after rinsing. Subsequently, the roots were treated with a solution of 0.05% trypan blue in lactophenol for a duration of 5 minutes. Any surplus stain was then eliminated using clear lactophenol, as described by Phillips and Hayman (1970). Each plant sample was prepared by mounting thirty root segments (1-2 cm) on a glass slide using lactophenol. The root segments were then viewed under a light microscope (Olympus BX 53) at a magnification of 100X. The extent of arbuscular mycorrhizal (AM) colonization was assessed using the magnified intersection approach, as described by Mc Gonigle (1990). The plane of focus was adjusted throughout the entire root to examine each point of intersection, and any arbuscules, vesicles, or hyphae that were crossed by the vertical crosshair were recorded. To estimate arbuscular colonization (AC) and vesicular colonization (VC), the numbers of arbuscules and vesicles were divided by the total number of intersections studied.

Hyphal colonization (HC) was determined by calculating the ratio of crossings where all fungal structures (arbuscles, vesicles, and hyphae) were present. The calculation of arbuscular colonization (AC), vesicular colonization (VC), and hyphal colonization (HC) percentages was performed using the following formula:

$$AC = \frac{\text{Count of Arbuscules}}{\text{Total number of intersections examined}} \times 100$$

$$VC = \frac{\text{Count of vesicles}}{\text{Total number of intersections examined}} \times 100$$

$$HC = \frac{\text{Total number of intersections examined} - \text{Negative intersection}}{\text{Total number of intersections examined}} \times 100$$

5.2.8 Mycorrhizal Growth Responsiveness (Hetrick *et al.* 1992)

The mycorrhizal growth responsiveness index was calculated by expressing the dry weights of the mycorrhizal plants in question as a percentage of the dry weight of the plants without mycorrhizal association (Hetrick *et al.*, 1992). The mycorrhizal growth responsiveness percentage was determined using the following formula:

$$MGR = \frac{\text{shoot dry weight of mycorrhizal plant} - \text{shoot dry weight of non-mycorrhizal plant}}{\text{shoot dry weight of non-mycorrhizal plant}} \times 100$$

5.2.9 Plant Growth Performances (Height, Leaf Area, Biomass)

The height and dry mass per fresh mass of the rice shoot were assessed using the IRRI protocol (IRRI. *Standard evaluation system for rice*). The height of the plant is measured in centimetres from soil surface to tip of the tallest leaf. For biomass measurement, the plant samples are oven dried at 70 °C until constant weight achieved. The shoot dry mass of plant and leaves are taken and are expressed in g plant⁻¹. Leaf area index was measured using Petiole Pro Leaf Area Meter (<https://www.petiolepro.com/leaf-area-meter-petiole-pro/>) (Figure 5.2).

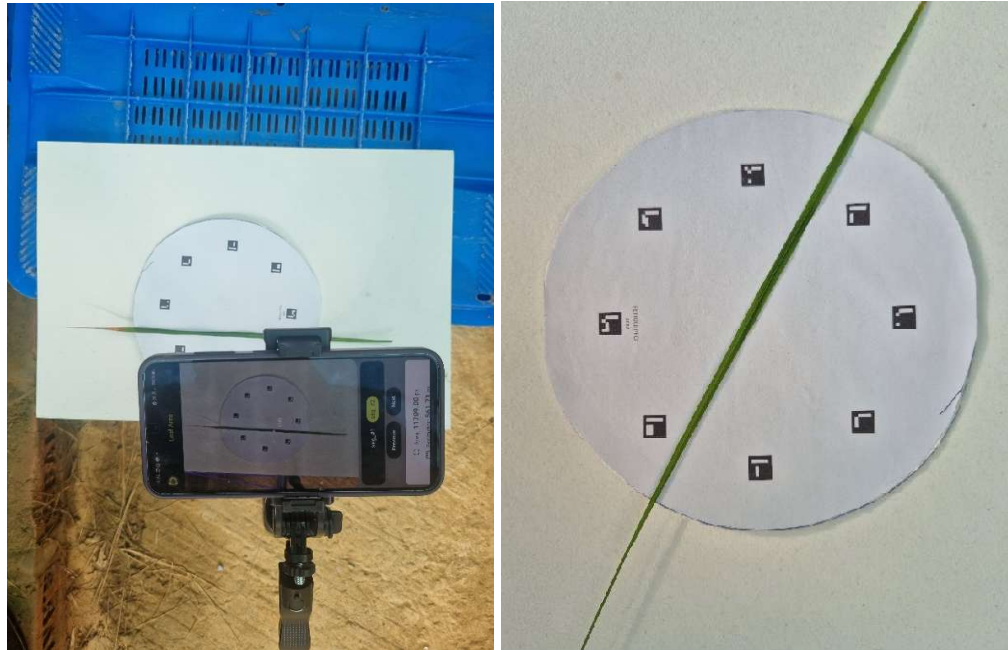


Figure 5.2: Measuring leaf area index using Petiole Pro

5.2.10 Specific Leaf Area and Leaf Dry Matter Content (Wilson, 1999)

Specific leaf area (SLA) is the proportion obtained by dividing the leaf area of a plant by its leaf dry mass. The parameter is crucial for plant growth models as it dictates the allocation of new leaf area for every unit of biomass generated. Five intact leaves from each plant were selected and the surface area of the leaf sample was measured by Petiole Pro. Once the leaves area was measured, immerse the leaf base in water and store them in a cool, dim environment overnight to restore moisture. After rehydrating, the leaf sample was dried by blotting it with tissue paper to eliminate any residual water on the surface. Subsequently, the sample was promptly weighed to obtain the saturated weight measurement. The specimen underwent a drying process at a temperature of 80°C for a duration of 3 days. Subsequently, it was weighed again to determine its dry weight. Leaf dry matter content (LDMC) values were determined by calculating the ratio of dry weight to saturated weight, represented as a percentage. The formulas for calculating SLA and LDMC are as follows:

$$SLA = \frac{\text{Leaf surface area (sq.mm)}}{\text{Leaf dry mass (mg)}}$$

$$\text{LDMC} = \frac{\text{Leaf dry mass (mg)}}{\text{Leaf saturated mass (g)}}$$

5.2.11 Leaf Relative Water Content (Smart and Bingham, 1974)

Relative water content (RWC) was calculated based on the fully expanded leaves of three plants per experimental unit. The leaves were divided into segments of 2 cm in length. The fresh weight (FW) of the leaf segments was determined accordingly. In order to prevent any loss of respiratory activity, leaf segments were weighed and thereafter immersed in distilled water for a duration of 4 hours at a temperature of 20°C, while being exposed to low levels of light. Subsequently, the leaf discs were meticulously dried to eliminate any residual moisture on the surface. The turgid weight (TW) was used to measure the amount of water absorbed. The dry weight (DW) of the leaf discs was obtained by subjecting the tissues to a drying process at a temperature of 70 °C for a duration of 3 days. g. RWC is measured by the following formula:

$$\text{RWC (\%)} = \frac{(\text{Fresh Weight} - \text{Dry Weight})}{(\text{Turgid Weight} - \text{Dry Weight})} \times 100$$

5.2.12 Determination of Photosynthetic Pigments (Hiscox and Israelstam, 1979)

A quantity of 25 mg of finely minced fresh leaf sample was deposited into the test tubes, followed by the addition of 5 ml of DMSO. The tubes were covered in aluminium foil and placed inside an oven set at a temperature of 65°C for a duration of 4 hours. The absorbance of the Chlorophylls was measured at 663 and 645 nm using spectrophotometer (Shimadzu UV-1900I) after a duration of 4 hours, with DMSO serving as the blank. The Chlorophylls were determined using the extinction values, according to the equation proposed by Arnon (1949).

Chlorophyll content is calculated using following formula:

$$\begin{aligned} \text{Chlorophyll } a \text{ (mg/ml)} &= \frac{12.7D_{663} - 2.69D_{645}}{1000 \times W} \times V \\ \text{Chlorophyll } b \text{ (mg/ml)} &= \frac{22.9D_{645} - .68D_{663}}{1000 \times W} \times V \\ \text{Total Chl. (mg/ml)} &= \frac{20.2D_{645} + .02D_{663}}{1000 \times W} \times V \end{aligned}$$

$$\text{Carotenoids (mg/ml)} = \frac{7.60D_{480} - 1.49D}{1000 \times W} \times V$$

where,

D663 = absorbance at 663 nm

D645 = absorbance at 645 nm

D510 = absorbance at 510 nm

D480 = absorbance at 480 nm

V = final volume of Chlorophyll extract (in ml)

W = fresh weight of leaf tissue extracted (in grams).

5.2.13 Determination of Leaf Anthocyanin (Neff and Chory, 1998)

The fresh material weighing 0.2g was pulverized using liquid nitrogen in a mortar and then mixed well with 2ml of a solution containing 1% hydrochloric acid in methanol. The homogenates were placed in a shaker and incubated at a temperature of 4°C for a duration of 24 hours, while being kept in the dark. After the incubation period, the mixtures were spun at a temperature of 4°C at a speed of 7000 rpm for a duration of 15 minutes. The spectrophotometer (Shimadzu UV-1900I) was used to detect the absorbance of anthocyanin at wavelengths of 530 nm and 657 nm. The anthocyanin concentration was expressed as A mg/g of fresh tissue.

5.2.14 Determination of Membrane Permeability (Bajji *et al.*, 2002)

The method described by Bajji (2002) was used to quantify electrolyte leakage (EL) and assess the membrane permeability of plant tissues. From each treatment, six randomly selected plants were chosen, and three fully developed leaves were obtained from each plant. These leaves were then chopped into segments measuring 1 centimeter in length. The leaf samples were rinsed thrice with distilled water to eliminate any surface impurities, and thereafter transferred to separate vials with stoppers, each holding 10 mL of distilled water. The specimens were placed in a controlled environment with a temperature of 25 °C and subjected to agitation at a speed of 100 revolutions per minute for a duration of 24 hours. The electrical conductivity of the bathing solution (EC1) was measured following incubation. Subsequently, the identical samples were subjected to an autoclave at a temperature of 120 °C for a duration of 20 minutes. Following the cooling of the solution to room

temperature, a second measurement of the electrical conductivity (EC2) was taken. The EL was determined by dividing EC1 by EC2 and then expressing the result as a percentage.

$$\text{EL (\%)} = \frac{\text{Electrical conductivity of solution before autoclaving}}{\text{Electrical conductivity of solution after autoclaving}} \times 100$$

5.2.15 Determination of Leaf Malondialdehyde (Hodges *et al.*, 1999)

Leaf malondialdehyde (MDA) was measured using 2-thiobarbituric acid (TBA). 100mg fresh material was ground with liquid nitrogen in a mortar. The plant tissue samples were mixed with 2.5 ml of a solution containing 80% ethanol and 20% water. The resulting mixture was then spun in a centrifuge at a force of 3000xg for a duration of 10 minutes. A 1ml portion of the sample was combined with 1 ml of either a solution without TBA or a solution containing TBA in a test tube. The samples were thereafter agitated, subjected to a temperature of 95°C in a water bath for a duration of 25 minutes, cooled, and then centrifuged at a force of 3000xg for a period of 10 minutes. Measurements of the absorbances were taken at wavelengths of 440 nm, 532 nm, and 600 nm using spectrophotometer (Shimadzu UV-1900I). The calculation of malondialdehyde equivalents was performed using the following method-

$$A = [(A_{532+\text{TBA}} - A_{600+\text{TBA}}) - (A_{532-\text{TBA}} - A_{600-\text{TBA}})]$$

$$B = [(A_{440+\text{TBA}} - A_{600+\text{TBA}}) 0.0571]$$

$$\text{MDA equivalents (nmol.ml}^{-1}\text{)} = (A-B/157000) 10^6$$

5.2.16 Determination of Leaf Hydrogen Peroxide (Velikova *et al.*, 2000)

The concentration of hydrogen peroxide (H₂O₂) in fresh plant material was determined. The frozen powder (500 mg) was homogenized directly with 5 ml of 0.1% trichloroacetic acid (TCA). The mixture was subjected to centrifugation at a force of 12,000xg for a duration of 15 minutes. 0.5 ml of the liquid remaining after centrifugation was combined with 0.5 ml of a solution containing 10 millimoles of potassium phosphate buffer with a pH of 7.0, and 1 ml of a solution containing 1 mole of potassium iodide. The blank probe contained a solution of 0.1% TCA without any leaf extract. The reaction was incubated for 1 hour in the absence of light, and the

absorbance was measured at a wavelength of 390 nm using (Shimadzu UV-1900I). The measurement of hydrogen peroxide was conducted using an extinction coefficient (ϵ) of $0.28 \mu\text{M}^{-1} \text{cm}^{-1}$ and the results were represented as $\mu\text{mol g}^{-1} \text{FW}$.

5.2.17 Determination of Soluble Sugars

The estimation of total soluble sugars (TSS) in fresh leaves was conducted by following Irigoyen *et al.* (1992). The newly picked leaves (0.5 g) were crushed into a fine powder using liquid nitrogen and homogenized with 5 ml of 95% ethanol (v/v). The insoluble portion of the extract was rinsed two times with 5 ml of 70% ethanol. The soluble fractions were subjected to centrifugation with a force of 3,500 xg for a duration of 10 minutes. The liquid portions were combined for sugar estimation. Add 0.1 ml of the alcoholic extracts to 3 ml of freshly made anthrone reagent. Immerse the mixture in a boiling water bath for 10 minutes. The anthrone reagent was prepared by combining 150 mg of anthrone with 100 ml of 72% (w/v) sulphuric acid, 100 mM Tris-HCl (pH 7.4) solution containing 100 mM MgSO_4 , and 0.1 g of PVP-40. The absorbance at 625 nm was measured using a spectrophotometer (Shimadzu UV-1900I) after the sample had cooled. A standard curve was created using various concentrations of glucose. Based on this standard curve, the final total soluble sugars (TSS) were measured and expressed in milligrams per gram of fresh weight ($\text{mg g}^{-1} \text{FW}$).

The concentration of sucrose and fructose was measured using the method of Wu *et al.* (2015). The dehydrated plant materials were pulverized and filtered using a 0.5 mm sieve. A 50 mg sample of the ground material was placed in a 10 ml centrifugal tube along with 4 ml of 80% ethanol. The mixture was then incubated at a temperature of 80°C for a duration of 40 minutes. After the incubation, the tube was subjected to centrifugation at a force of 2,500xg for a period of 5 minutes. The centrifugal residues were extracted once more using the aforementioned technique, and the supernatants from the two extractions were mixed for carbohydrate tests. The test for sucrose involved combining 0.15 mL of supernatant with 0.15 mL of a solution containing 2 mmol/L of NaOH. This mixture was heated to 100°C for 5 minutes. Then, 2.1 mL of 30% HCl and 0.6 mL of 0.1% resorcinol were added to the mixture, which was further heated to 80°C for 10 minutes. Finally, the absorbance of the assay was measured at 480 nm using sucrose as a reference. The fructose content was determined by

analysing a solution containing 0.8 mL of 0.1% resorcinol, 0.4 mL of water, and 0.4 mL of the supernatant. The solution was then heated at a temperature of 80°C for a duration of 10 minutes. The absorbance of the solution was measured at a wavelength of 480 nm using fructose as a reference solution.

5.2.18 Determination of Leaf Proline Content (Bates *et al.*, 1973)

The estimation of Proline (Pro) was conducted using the methodology described by Bates *et al.* (1973). The fresh material was pulverized with liquid nitrogen in a mortar, resulting in 250 mg of ground material. The homogenate powder was combined with 5mL of an aqueous solution of sulfosalicylic acid (3%, w/v) and subsequently centrifuged at 5,000 x g for 5 minutes. The filtrate/supernatant, measuring 2 ml, was combined with 2 ml of glacial acetic acid and 2 ml of acid-ninhydrin reagent. The Acid - Ninhydrin reagent was made by dissolving 1.25 g of ninhydrin in a mixture of 30 ml of Glacial Acetic acid and 20 ml of 6 M orthophosphate. The dissolution process took place at a temperature of 70 °C. The mixture was then incubated at a temperature of 95°C for a duration of 1 hour. Finally, the reaction was stopped by placing the mixture in an ice bath. The reaction mixture was forcefully agitated with 4mL of toluene for 15-20 seconds. The chromophore present in toluene was extracted from the aqueous phase, heated to a temperature of 25°C, and the absorbance was measured at a wavelength of 520 nm using toluene as a blank in spectrophotometer (Shimadzu UV-1900I).

5.2.19 Determination of Total Phenolic Content (Sarker, 2018)

Total phenolic content was measured by Folin-Ciocalteu assay. 0.1 g of leaves was ground in mortar with liquid nitrogen. The powder was dissolved in 4 ml of 90% methanol and thereafter transferred to a tightly closed bottle. The bottle was then subjected to shaking at a temperature of 80 °C for a duration of 1 hour. The solution was subjected to centrifugation at a speed of 6000 rpm for a duration of 10 minutes. A volume of 50 µl of the leaf extract solution was combined with 1 ml of folin-ciocalteu reagent, which had been previously diluted at a ratio of 1:4 with distilled water. The mixture was then carefully mixed. Distilled water was used to prepare a reagent blank. After a duration of 3 minutes, a volume of 1 ml of sodium carbonate solution (10% NaCO₃) was introduced into the combination, which was then left undisturbed for a

period of 1 hour in the absence of light. The spectrophotometer (Shimadzu UV-1900I) was used to measure the absorbance at a wavelength of 760 nm. The concentration of total phenolic compounds in the leaf extracts was quantified using an equation derived from a standard gallic acid graph. The results are reported in micrograms of gallic acid equivalent (GAE) per gram of fresh weight (mg GAE g⁻¹).

5.2.20 Determination of Superoxide Dismutase Activity (Dhindsa *et al.*, 1981)

The measurement of superoxide dismutase (SOD) (EC 1.15.1.1) activity in leaves was conducted using the procedure outlined by Dhindsa *et al.* (1981). The process relies on the creation of a blue complex called formazan, which occurs when nitro-blue tetrazolium reacts with the O₂^{•-} radical. The reduction in absorbance for this blue-colored compound is measured at a wavelength of 560 nm. 0.2 g of fresh plant leaves was ground with liquid nitrogen and homogenized with 2 ml of extraction buffer. The extraction buffer consisted of phosphate buffer (0.1 M at pH 7.5) containing EDTA (0.5 mM). The sample was subjected to centrifugation at a force of 15,000xg for a duration of 10 minutes at a temperature of 4°C. 0.03 ml of enzyme extract was introduced into the reaction mixture, which consisted of phosphate buffer (50 mM at pH 7.8), methionine (13.33 mM), sodium carbonate (50 mM), EDTA (0.1 mM), 75uM NBT, and 0.95 ml water. The reaction was started by adding riboflavin (2 µM) and the test tubes were exposed to a 15 W fluorescent light for 15 minutes. By turning off the light and placing the tubes in darkness, the reaction was halted. A reaction mixture that had not been exposed to light was used as a blank. The absorbance was measured at a wavelength of 560 nm using (Shimadzu UV-1900I). One unit of enzyme activity was defined as the amount of enzyme that caused a 50% decrease in absorbance compared to tubes without enzyme. The percentage inhibition of NBT reduction by SOD is calculated using the following formula:

$$\text{Percentage Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

The measurement of SOD activity is expressed as units per milligram of protein (U/g FW) in the enzyme extract and can be calculated using the following formula:

$$\text{SOD Activity} = \frac{\text{SOD units/ml} \times \text{volume of extract}}{\text{Fresh weight of tissue}}$$

5.2.21 Determination of Catalase Activity (Aebi, 1984)

The activity of the enzyme catalase (CAT, EC 1.11.1.6) was examined using the method described by Aebi (1984) in freshly harvested leaves. 0.2 g of fresh plant leaves was ground with liquid nitrogen and homogenized with 2 ml of extraction buffer. The extraction buffer comprised of a phosphate buffer with a concentration of 0.1 M at a pH of 7.5, supplemented with EDTA at a concentration of 0.5 mM. 10 µl of enzyme was added to a reaction mixture consisting of potassium phosphate buffer (50 mM), hydrogen peroxide (12.5 mM), and demineralized water to achieve a total volume of 3 ml. The reaction was initiated by adding hydrogen peroxide (H₂O₂), and the decrease in absorbance was measured every 15 seconds at a wavelength of 240 nm for a duration of 1 minute. The enzyme activity was determined by quantifying the decomposition of H₂O₂ using the extinction coefficient ($\epsilon = 0.0436 \text{ mM}^{-1} \text{ cm}^{-1}$).

5.2.22 Statistical Analysis

All statistical analyses were conducted using Python 3.13 within a Jupyter Notebook environment. Descriptive statistics, including means, standard errors, and ranges, were computed for each dependent variable across treatment groups. To assess the collective influence of the independent variables on the measured traits, Multivariate Analysis of Variance (MANOVA) was performed on 20 dependent variables related to growth, physiology, and biochemistry. Three-way Univariate ANOVA was used to determine the individual and interactive effects of rice variety, water status, and mycorrhizal treatment on each variable. Principal Component Analysis (PCA) was employed to reduce dimensionality and identify key traits contributing to variation in drought tolerance. Cluster analysis was conducted to classify treatment combinations based on similarities in their overall response patterns. All statistical tests were conducted at a 5% significance level ($\alpha = 0.05$). Results are reported as mean $\pm$ standard error, and differences were considered statistically significant at $p < 0.05$.

5.3 Results

5.3.1 Arbuscular Colonisation

The total arbuscular colonization (AC) percentage varied across different experimental treatments, which included two rice varieties, two water statuses (well-watered and drought), and five mycorrhizal treatments. Mycorrhizal structures were present in all AM1, AM2, AM3, AM4 and AM5 samples, but not in NM plants. The mean AC percentage across all treatments was approximately 3.84%, with a standard deviation of 1.87%. The minimum AC value recorded was 0.29%, and the maximum value reached 6.73%, reflecting the range of colonization responses. The highest mean AC (6.41%) was observed in Changkawi rice treated with well-watered condition and AM3 (*Glomus* sp.). The lowest mean AC (1.48%) was observed in the drought conditions, in Dhan 50 with the AM5 (Commercial propagules) mycorrhizal treatment (Figure 5.3A). The effect of water status, rice varieties, mycorrhizal treatment on AC was not statistically significant as indicated by the results from three-way ANOVA.

5.3.2 Vesicular Colonisation

Vesicular colonization (VC) percentage varied across the experimental treatments. The mean VC percentage across all treatments was approximately 9.67%, with a standard deviation of 2.58%. The minimum recorded VC value was 5.01%, and the maximum value reached 14.20%, indicating substantial variation in colonization levels under different experimental conditions. The highest mean VC (12.78%) was observed in the well-watered condition, Changkawi variety, and the AM4 mycorrhizal treatment (Figure 5.3 B). The lowest mean VC (5.92%) was observed in the drought condition, Dhan 50 variety, and the AM5 mycorrhizal treatment. Results from three-way ANOVA showed that the effect of water status, rice variety, mycorrhizal treatment and their interaction, on VC was not statistically significant.

5.3.3 Hyphal Colonisation

Hyphal colonization (HC) percentage varied across the experimental treatments, which included two rice varieties, two water statuses (well-watered and drought), and five mycorrhizal treatments. The mean HC percentage across all treatments was 34.82%, with a standard deviation of 5.25%. The minimum HC value

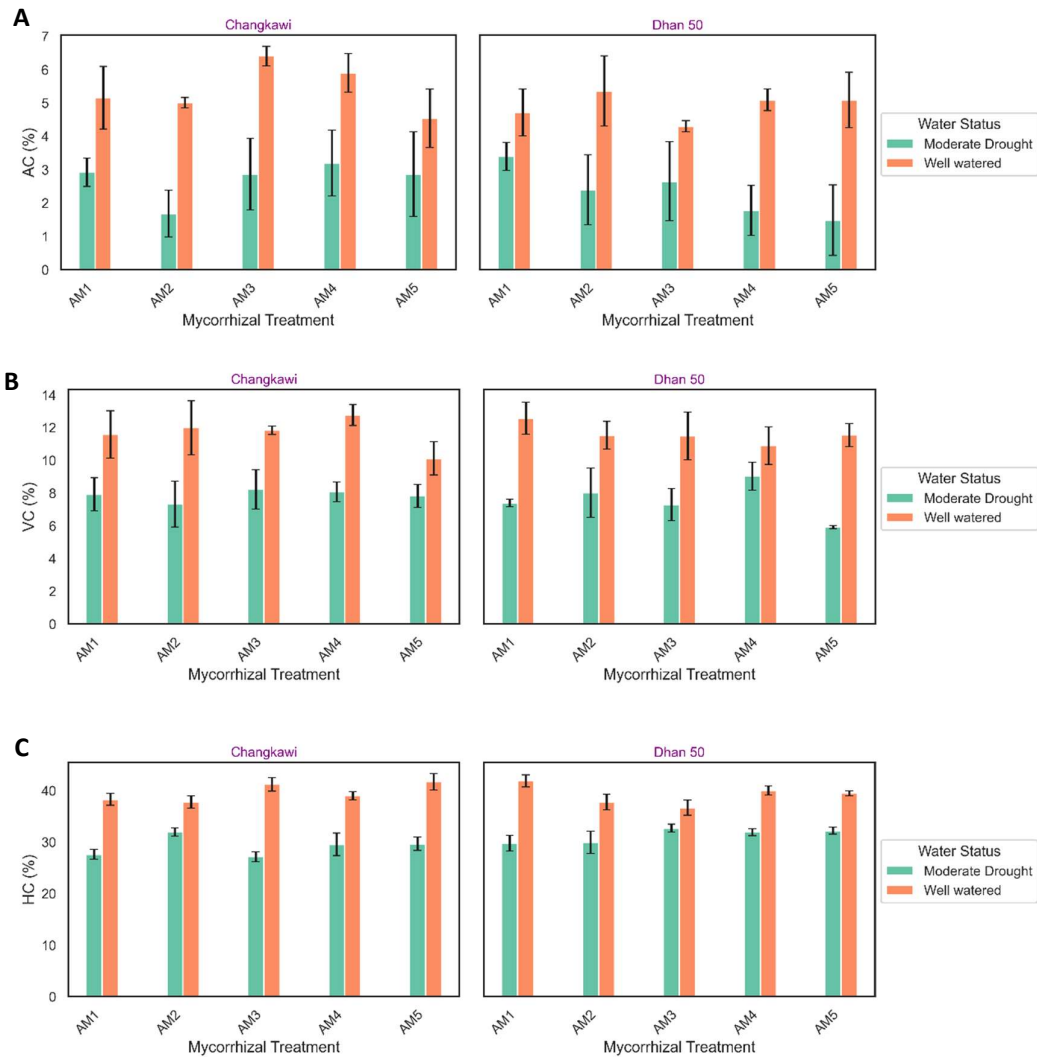
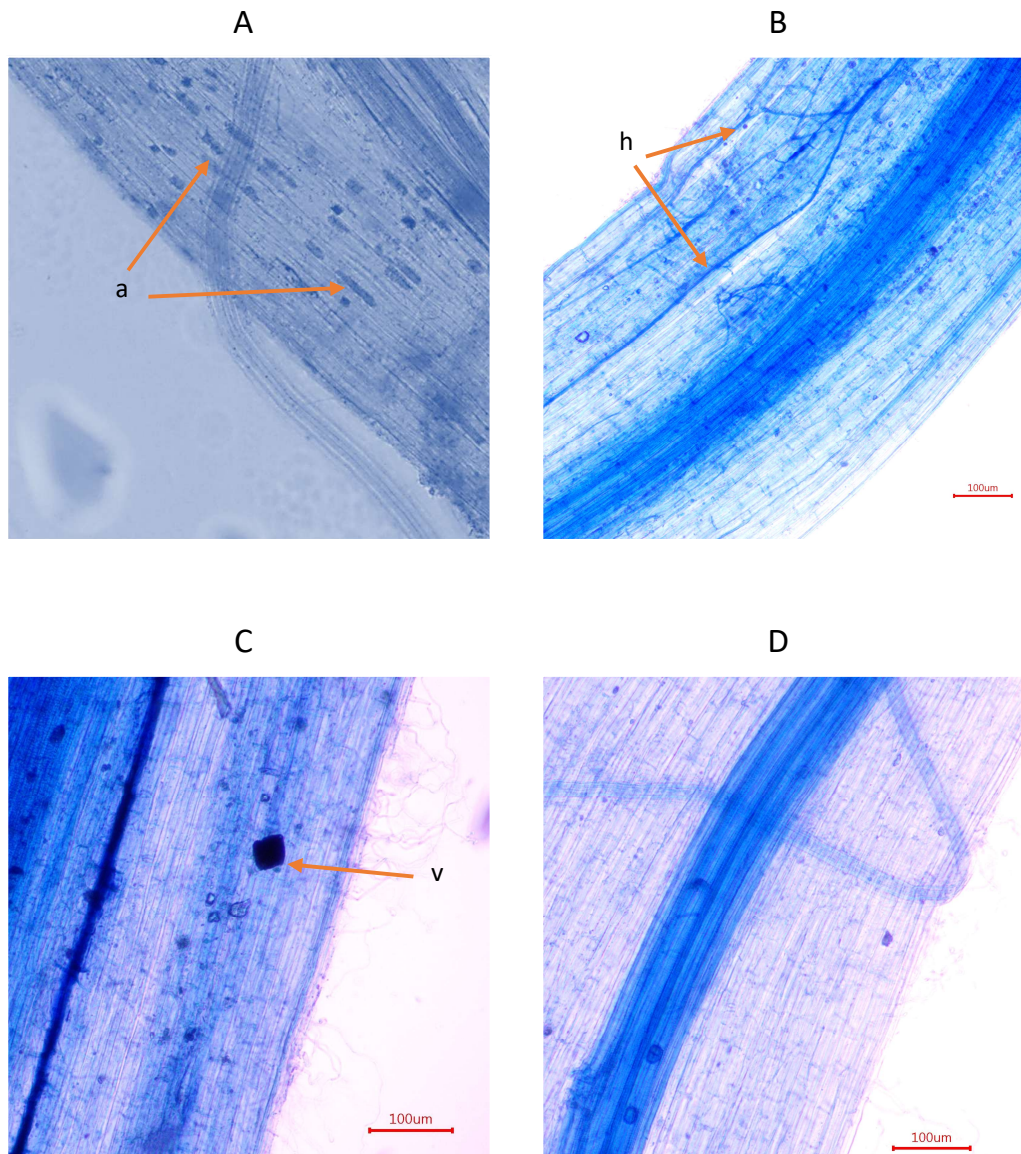


Figure 5.3: Root colonization percentage for – A) Arbuscles; B) Vesicles and C) Hyphae

observed was 27.15%, and the maximum value reached 41.89%, indicating a notable variation in the extent of colonization under different conditions (Figure 5.3 C). The highest mean HC (41.89%) was observed in the well-watered condition, Dhan 50 variety, and the AM1 mycorrhizal treatment. The lowest mean HC (27.15%) was observed in the drought condition, Changkawi variety, and the AM3 mycorrhizal treatment. Results from three-way ANOVA indicate that Rice variety, mycorrhizal treatments and none of the interaction terms, did not significantly affect HC while water status had a significant impact on HC.

Plate XIII



Structures of AMF colonizing the roots of rice plants subjected to drought stress.

A) Root structure showing arbuscules; B) Root structure with intraradical hyphae; C) Root structure with vesicle; D) No AM fungal colonization in NM plants. a: arbuscle, h: hyphae, v: vesicle

5.3.4 Mycorrhizal Growth Responsiveness

The mean MR percentage across all treatments was approximately 25.23%, with a standard deviation of 17.62%. The highest MR (39.73%) was recorded in well-watered Dhan 50 rice treated with AM1, while the lowest MR (12.25%) was observed in well-watered Changkawi rice treated with AM2 (Figure 5.4). The results from three-way ANOVA indicates that neither water status, rice variety, nor mycorrhizal treatment had a statistically significant effect on mycorrhizal responsiveness in rice.

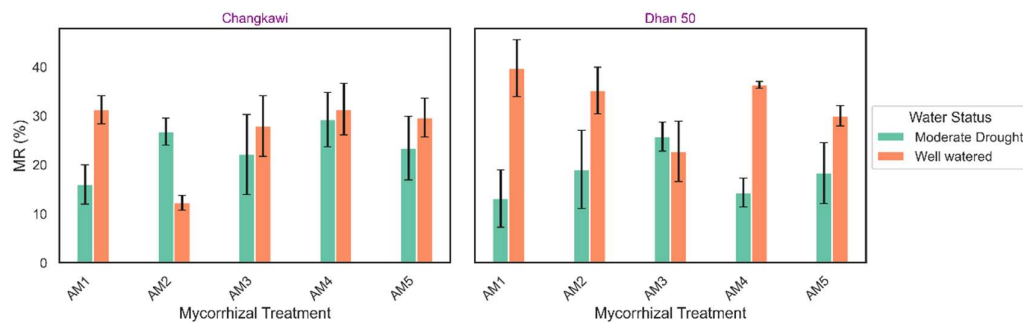


Figure 5.4: Mycorrhizal growth responsiveness of rice plants under different treatment condition.

5.3.5 Plant Growth Performances

5.3.5.1 Plant Height

The average plant height across all treatments was 75.18 cm, with a standard deviation of 7.29 cm (Figure 5.5 A). The minimum recorded height was 63 cm, and the maximum height was 88 cm, showing a wide range influenced by treatment conditions. Plants under well-watered conditions had higher average heights, with an approximate mean of 80 cm. Under drought conditions, the average plant height decreased to around 70 cm, illustrating the negative impact of water stress on plant growth. Changkawi variety showed better growth under well-watered conditions, with an average height of 78 cm, but experienced a more substantial reduction under drought stress, with an average height of 65 cm. Dhan 50 variety exhibited better drought tolerance, with an average height of 72 cm under drought conditions and 82 cm under well-watered conditions.

The multivariate analysis of variance (MANOVA) showed that plant height, as part of the overall physiological and biochemical response, was significantly influenced by the independent variables (Water Status, Rice Variety, and Mycorrhizal Treatment) (Table 5.1). Specifically, water status had a strong effect on the set of dependent variables, including height.

Three-way ANOVA result shows that water status (drought vs. well-watered) and mycorrhizal treatment significantly affect plant height (Table 5.2). The interaction between water status and rice variety is also significant, suggesting that drought affects the two rice varieties differently in terms of height. However, the combination of all three factors does not have a significant combined effect on height.

In the PCA, height was one of the key variables contributing to the variance captured by the first principal component (PC1), which explained 44.5% of the total variance (Figure 5.16). Height was positively correlated with PC1 (Figure 5.17), which was associated with well-watered conditions and favourable growth factors. The second principal component (PC2), which explained 18.2% of the variance, captured variations related to specific mycorrhizal treatments. Plants that were taller tended to have higher loadings along PC1, reflecting the influence of water status and mycorrhizal inoculation on overall growth.

Cluster analysis using K-means identified three distinct clusters based on the physiological and biochemical responses of the rice plants (Figure 5.18). Plant height was a major factor in defining the clusters. The first cluster primarily consisted of plants with greater heights, which were predominantly well-watered and inoculated with AM5. The second cluster included plants with intermediate heights, mostly from the other mycorrhizal treatments under both well-watered and drought conditions. The third cluster contained plants with shorter heights, which were mostly drought-stressed and had lower growth rates.

5.3.5.2 Shoot Dry Biomass

The average shoot dry biomass across all treatments was 4.37 g, with a standard deviation of 1.02g (Figure 5.5 B). Well-watered plants exhibited higher average shoot dry biomass, with values around 4.8 g, while plants under drought conditions had a lower lower under well-watered conditions and 3.6 g under drought conditions. Dhan

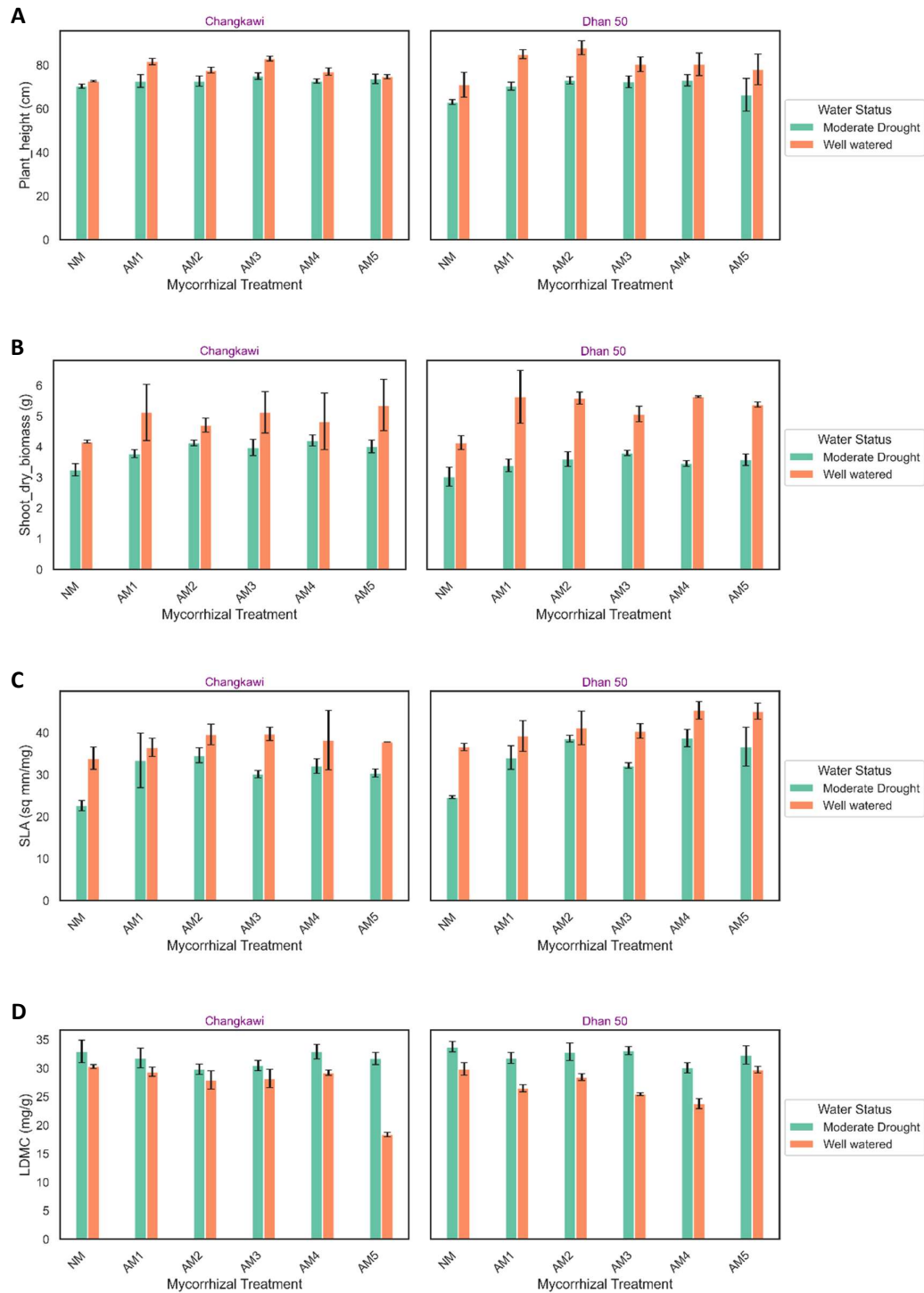


Figure 5.5: Growth responses of rice varieties (Changkawi and Dhan 50) under different water and mycorrhizal treatments. A) Plant height; B) Shoot dry biomass; C) Specific Leaf Area; D) Leaf Dry Matter Content

50 had higher shoot dry biomass, particularly under well-watered conditions, where the average value was 4.9 g. Under drought conditions, the shoot dry matter for Dhan 50 was lower, with an average of 3.7 g. The treatment group with the highest average shoot dry biomass was Dhan 50 inoculated with AM1 mycorrhizal treatment under well-watered conditions, with an average shoot dry matter of 5.63 g. The treatment group with the lowest average shoot dry matter was Dhan 50 without mycorrhizal treatment (NM) under drought conditions, with an average of 3.02 g. Inoculation with AM1 produced the highest shoot dry matter under well-watered conditions. Under drought conditions, the shoot dry matter values were much lower, with AM1 and AM3 treatments maintaining approximately 4.0 g of shoot dry matter, while the non-mycorrhizal (NM) treatment had the lowest value at 3.02 g.

The multivariate analysis of variance (MANOVA) showed that the combination of Water Status, Rice Variety, and Mycorrhizal Treatment significantly affected the overall growth, physiological and biochemical responses, including shoot dry biomass (Table 5.1).

Three-way ANOVA result shows that water status has a major impact on shoot dry biomass, with drought significantly reducing biomass (Table 5.2). Mycorrhizal treatment also has a significant positive effect on shoot dry biomass, indicating the importance of these fungi in plant biomass accumulation. The interaction between water status and rice variety is significant, suggesting that drought affects shoot dry biomass differently in the two rice varieties. No significant three-way interaction was observed, meaning the combined effects of all three factors did not significantly alter shoot dry biomass beyond their individual contributions.

Shoot dry biomass contributed significantly to the first principal component (PC1), which explained 44.5% of the total variance (Figure 5.16). Biomass was positively correlated with PC1 (Figure 5.17), which captured the variation driven by water status and favorable growing conditions. Plants with higher shoot biomass tended to have higher loadings along PC1, indicating that water availability and AMF inoculation, particularly AM5, were key factors influencing biomass production.

Cluster analysis using K-means shows that shoot dry biomass played a significant role in defining the clusters. The first cluster primarily consisted of plants with high biomass, mainly from well-watered conditions and those inoculated with

AM5 (Figure 5.18). The second cluster included plants with intermediate biomass, mainly from other mycorrhizal treatments under both well-watered and drought conditions. The third cluster contained plants with lower biomass, which were predominantly drought-stressed and had lower growth rates.

5.3.5.3 Specific Leaf Area (SLA)

The average SLA across all treatment groups shows considerable variation, reflecting the impact of water status, rice variety, and mycorrhizal inoculation on leaf morphology. The range of SLA values extends from 22.61 mm²/mg to 45.33 mm²/mg (Figure 5.5 C). Under well-watered conditions, plants exhibited higher SLA values, ranging from 33.90 mm²/mg to 45.33 mm²/mg. Under drought conditions, SLA values were generally lower, ranging from 22.61 mm²/mg to 38.72 mm²/mg, indicating a reduction in leaf area per unit mass due to water stress. Changkawi had lower SLA values compared to Dhan 50. For Changkawi, the highest SLA under well-watered conditions was 39.68 mm²/mg, while under drought conditions, SLA ranged from 24.6 mm²/mg to 38.72 mm²/mg. Dhan 50 demonstrated higher SLA values, with the maximum SLA recorded under well-watered conditions being 45.33 mm²/mg for the AM4 mycorrhizal treatment. Under drought conditions, SLA for Dhan 50 ranged from 22.61 mm²/mg to 34.56 mm²/mg. The treatment group with the highest SLA was Dhan 50 inoculated with AM4 mycorrhizal treatment under well-watered conditions, with an SLA of 45.33 mm²/mg. The treatment group with the lowest SLA was Changkawi without mycorrhizal treatment (NM) under drought conditions, with an SLA of 22.61 mm²/mg. Mycorrhizal inoculation significantly improved SLA, particularly under well-watered conditions. Treatments with AM4 and AM5 resulted in the highest SLA values for both rice varieties. Under drought conditions, AM2 and AM4 helped maintain higher SLA values compared to non-mycorrhizal plants.

The multivariate analysis of variance (MANOVA) demonstrated that SLA, along with other physiological and biochemical traits, was significantly influenced by the independent variables (Table 5.1). Water status had a strong multivariate effect on SLA, indicating that water availability was a major factor driving changes in leaf area per unit dry mass.

Three-way ANOVA result indicate that water Status had a significant effect suggesting that drought conditions significantly affect leaf area (Table 5.2). Mycorrhizal Treatment also had a significant effect indicating that different treatments influence SLA under stress. No significant interaction effects were found.

PCA results showed that SLA was strongly associated with the first principal component (PC1), which explained 44.5% of the total variance (Figure 5.16). SLA positively contributed to PC1, which was primarily driven by traits related to water availability and growth, such as leaf area, biomass, and SLA (Figure 5.17). The second principal component (PC2), explaining 18.2% of the variance, captured variation related to specific mycorrhizal treatments. Plants with higher SLA tended to cluster along PC1, particularly those inoculated with AM5 under well-watered conditions, suggesting that SLA is a key trait in differentiating treatments based on leaf morphology and water relations.

Plants with higher SLA, mostly under well-watered conditions and inoculated with AM5 and AM4, formed the first cluster (Figure 5.18). The second cluster consisted of plants with moderate SLA values, including those under intermediate mycorrhizal treatments across both well-watered and drought conditions. The third cluster represented plants with lower SLA values, primarily drought-stressed plants with smaller, thicker leaves.

5.3.5.4 Leaf Dry Matter Content (LDMC)

Under moderate drought conditions, LDMC values were generally higher, indicating a greater proportion of dry matter in the leaves due to reduced water content. The values ranged from 29.82 mg/g to 33.73 mg/g (Figure 5.5 D). Under well-watered conditions, LDMC values were lower, ranging from 18.34 mg/g to 30.28 mg/g, reflecting higher water content in the leaves due to ample water availability. Changkawi generally exhibited lower LDMC values under well-watered conditions, with the lowest LDMC being 18.34 mg/g (with AM5 treatment). Under moderate drought conditions, the LDMC for Changkawi ranged from 29.82 mg/g to 32.93 mg/g. Dhan 50 showed higher LDMC values, especially under moderate drought conditions. The highest LDMC recorded for Dhan 50 under drought was 33.73 mg/g (without mycorrhizal treatment), indicating a greater accumulation of dry matter in response to

water stress. The treatment group with the highest LDMC was Dhan 50 without mycorrhizal treatment (NM) under moderate drought conditions, with an LDMC of 33.73 mg/g. The treatment group with the lowest LDMC was Changkawi inoculated with AM5 under well-watered conditions, with an LDMC of 18.34 mg/g. Mycorrhizal inoculation generally reduced LDMC under well-watered conditions, indicating improved water retention in the leaves. Under moderate drought, inoculation with AM3 and AM2 helped maintain balanced LDMC values in Dhan 50. However, the non-mycorrhizal (NM) group for both rice varieties consistently exhibited higher LDMC under drought.

The multivariate analysis of variance (MANOVA) indicated that LDMC, as part of the overall physiological and biochemical responses, was significantly affected by the independent variables (Table 5.1). Water status had a significant effect on the combined dependent variables, including LDMC, indicating that water availability plays a critical role in determining leaf dry matter content. Mycorrhizal treatment also had a significant impact, suggesting that AMF inoculation influences leaf density and dry matter accumulation in rice plants.

Three-way ANOVA result indicate that water status significantly affected LDMC with drought stress increasing dry matter content (Table 5.2). Mycorrhizal Treatment also had a significant effect suggesting that different treatments influence leaf dry matter under stress conditions. No significant interactions were observed.

In the PCA, LDMC contributed significantly to the first principal component (PC1), which explained 44.5% of the total variance (Figure 5.16). LDMC was negatively correlated with PC1, indicating that plants with higher LDMC were generally associated with drought stress and other traits related to water conservation (Figure 5.17). The second principal component (PC2), which explained 18.2% of the variance, captured variation related to specific mycorrhizal treatments, with AMF-inoculated plants under well-watered conditions tending to have lower LDMC values.

Cluster analysis using K-means identified three distinct clusters based on physiological and biochemical traits, with LDMC playing a critical role in defining these clusters. The first cluster was characterized by plants with higher LDMC, primarily from drought-stressed conditions, reflecting their adaptation to water scarcity by increasing leaf density (Figure 5.18). The second cluster consisted of plants

with intermediate LDMC values, mostly from well-watered conditions with moderate mycorrhizal inoculation. The third cluster contained plants with lower LDMC values, mostly well-watered and inoculated with AM4 and AM5, suggesting that these AMF treatments helped to maintain lower leaf density and better hydration under favorable conditions.

5.3.6 Plant Physiological Responses

5.3.6.1 Leaf Relative Water Content (RWC)

Relative Water Content (RWC) measures the water content in plant leaves relative to their fully saturated state and provides an indication of the plant's water status and hydration capacity. Under moderate drought conditions, RWC values varied significantly, ranging from 66.56% to 88.80% (Figure 5.6 A). These values indicate how well plants were able to retain water under stress conditions. Under well-watered conditions, RWC values were generally higher, but still varied depending on the treatment. The RWC ranged from 66.80% to 86.78%, reflecting the hydration levels under optimal water availability. Changkawi exhibited higher RWC values under moderate drought, with the highest RWC being 88.80% for the AM5 treatment, indicating its ability to retain water despite water limitations. Under well-watered conditions, Changkawi's RWC ranged from 73.25% to 84.07%. Dhan 50 showed a wider range of RWC values, especially under moderate drought, where the lowest RWC was 66.56% (without mycorrhizal treatment), indicating more water stress in the absence of mycorrhizal symbiosis. Under well-watered conditions, Dhan 50's RWC ranged from 66.80% to 86.78%. The treatment group with the highest RWC was Changkawi inoculated with AM5 under moderate drought conditions, with an RWC of 88.80%, and the treatment group with the lowest RWC was Dhan 50 without mycorrhizal treatment (NM) under moderate drought conditions, with an RWC of 66.56%. Mycorrhizal inoculation generally improved RWC under both drought and well-watered conditions. In particular, AM5 and AM4 treatments consistently resulted in higher RWC values. In contrast, non-mycorrhizal (NM) groups exhibited lower RWC values under both drought and well-watered conditions.

The multivariate analysis of variance (MANOVA) indicated that RWC, along with other physiological and biochemical traits, was significantly affected by the

independent variables (Table 5.1). Plants under drought stress showed significantly lower RWC values compared to well-watered plants.

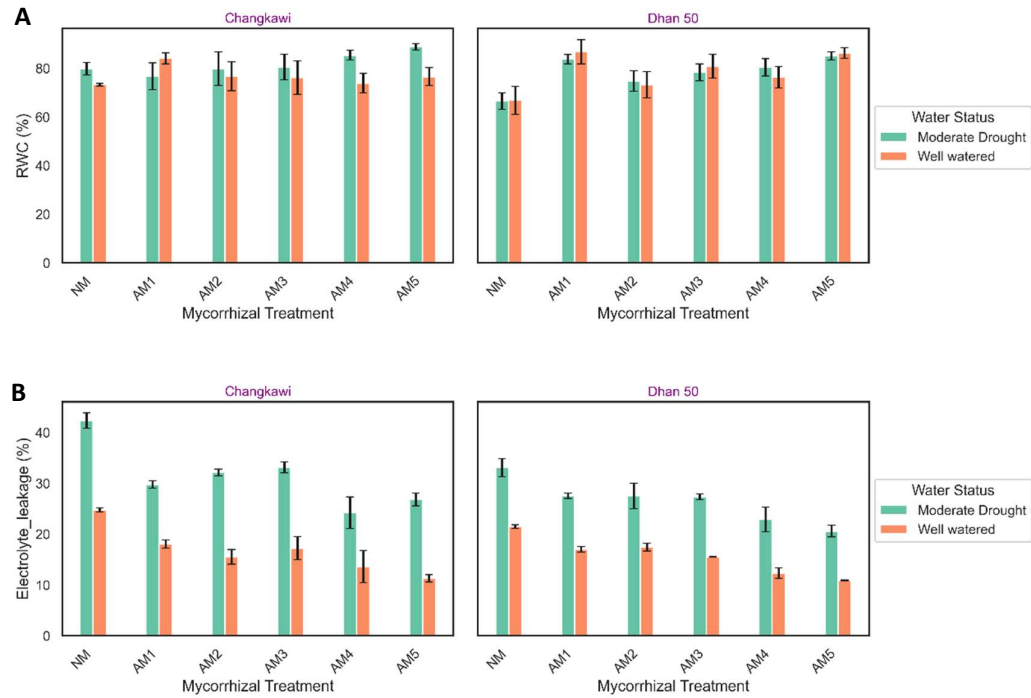


Figure 5.6: Physiological responses (A: Relative water content; B: Electrolyte leakage) of rice Varieties (Changkawi and Dhan 50) under different water and mycorrhizal treatments.

Three-way ANOVA result indicate that mycorrhizal treatment has a significant positive effect on RWC, suggesting its role in helping rice plants maintain water content under stress (Table 5.2). Water status and rice variety alone, or in combination, did not significantly influence RWC. There is no strong evidence of interaction effects among the three factors in influencing RWC.

In the PCA, RWC was strongly associated with the first principal component (PC1), which explained 44.5% of the total variance (Figure 5.16). RWC was positively correlated with PC1 (Figure 5.17), reflecting its strong relationship with well-watered conditions and traits associated with better water retention and overall plant hydration. Plants with higher RWC values, particularly those inoculated with AM5 and AM4, clustered along the positive side of PC1.

Cluster analysis using K-means identified that the first cluster consisted of plants with high RWC values, mostly from well-watered conditions and those

inoculated with AM5 and AM4 (Figure 5.18). The second cluster included plants with intermediate RWC, representing other mycorrhizal treatments across both well-watered and drought conditions. The third cluster contained plants with lower RWC, primarily drought-stressed plants with reduced water retention in their leaves.

5.3.6.2 Electrolyte Leakage (EL)

Electrolyte Leakage is an indicator of membrane stability and damage, typically increasing under stress conditions. Higher EL values indicate greater cell membrane damage and ion leakage. Under moderate drought conditions, EL values were higher, indicating greater membrane damage due to water stress. The values ranged from 20.58% to 42.32%. Under well-watered conditions, EL values were generally lower, ranging from 10.91% to 24.75%, reflecting better membrane stability with adequate water supply (Figure 5.6 B). Changkawi had higher EL values under drought, with the highest EL being 42.32% (without mycorrhizal treatment), indicating significant membrane damage in the absence of mycorrhizal support. Under well-watered conditions, EL values ranged from 11.26% to 24.75%. Dhan 50 showed more resilience under drought, with EL values ranging from 20.58% (with AM5 treatment) to 33.08% (without mycorrhizal treatment). Under well-watered conditions, EL values ranged from 10.91% to 21.49%, reflecting better membrane integrity compared to Changkawi. The treatment group with the highest EL was Changkawi without mycorrhizal treatment (NM) under moderate drought conditions, with an EL of 42.32%, indicating severe membrane damage. The treatment group with the lowest EL was Dhan 50 inoculated with AM5 under well-watered conditions, with an EL of 10.91%. Mycorrhizal inoculation generally reduced EL across all conditions, showing its protective effect on membrane stability. Under drought, AM4 and AM5 treatments resulted in significantly lower EL values for both rice varieties. Under well-watered conditions, EL was consistently lower with AM inoculation, especially with AM5, which had the lowest values for both rice varieties. Non-mycorrhizal plants (NM) exhibited the highest EL values under both drought and well-watered conditions.

The multivariate analysis of variance (MANOVA) indicated that EL, along with other physiological and biochemical traits, was significantly influenced by the independent variables (Table 5.1).

Three-way ANOVA result shows that water status and rice variety significantly affect electrolyte leakage, with drought stress leading to higher leakage and one variety showing greater resilience (Table 5.2). Mycorrhizal treatment significantly reduces electrolyte leakage, suggesting its role in mitigating membrane damage under stress conditions. The significant interaction between water status and rice variety suggests that drought stress affects the two rice varieties differently, with potential implications for selecting drought-tolerant varieties. No significant three-way interaction was found.

PCA revealed that EL was strongly associated with the first principal component (PC1), which explained 44.5% of the total variance (Figure 5.16). EL was positively correlated with PC1, along with other stress-related traits, such as proline content and MDA (Malondialdehyde), indicating its role as a key marker of stress response (Figure 5.17). Plants with higher EL values clustered along the positive side of PC1, primarily corresponding to drought-stressed conditions, while plants with lower EL values, particularly those inoculated with AM5, were positioned along the negative side of PC1.

Cluster analysis using K-means identified three distinct clusters based on physiological and biochemical traits, with EL playing a critical role in defining these clusters. The first cluster included plants with low EL values, mostly from well-watered conditions and those inoculated with AM5 and AM4, indicating better membrane stability (Figure 5.18). The second cluster consisted of plants with intermediate EL values, representing those under moderate drought stress or other mycorrhizal treatments. The third cluster contained plants with high EL values, primarily from drought-stressed conditions with minimal AMF inoculation, highlighting the vulnerability of these plants to membrane damage.

5.3.6.3 Chlorophyll *a*

Chlorophyll *a* is a vital pigment for photosynthesis, and its concentration reflects the plant's ability to capture light energy and perform photosynthesis. Higher chlorophyll *a* values generally indicate better photosynthetic efficiency. Under moderate drought conditions, chlorophyll *a* values ranged from 0.94 mg/g to 3.22 mg/g. Drought stress typically reduced chlorophyll *a* content, particularly in the non-

mycorrhizal group (Figure 5.7 A). Under well-watered conditions, chlorophyll *a* values were generally higher, ranging from 0.64 mg/g to 3.31 mg/g. Changkawi exhibited lower chlorophyll *a* values under both moderate drought and well-watered conditions compared to Dhan 50. The highest chlorophyll *a* value for Changkawi under well-watered conditions was 2.01 mg/g (with AM5 treatment), and under moderate drought, the maximum was 1.74 mg/g. Dhan 50 showed higher chlorophyll *a* values, particularly under well-watered conditions, where the highest value was 3.31 mg/g (with AM4 treatment). Under moderate drought conditions, Dhan 50's chlorophyll *a* ranged from 1.15 mg/g to 3.22 mg/g. The treatment group with the highest chlorophyll *a* was Dhan 50 inoculated with AM4 mycorrhizal treatment under well-watered conditions, with a chlorophyll *a* concentration of 3.31 mg/g. The treatment group with the lowest chlorophyll *a* was Changkawi without mycorrhizal treatment (NM) under well-watered conditions, with a chlorophyll *a* value of 0.64 mg/g. Mycorrhizal inoculation significantly increased Chlorophyll *a* content under both drought and well-watered conditions. Treatments with AM4 and AM3 resulted in the highest chlorophyll *a* concentration, particularly for Dhan 50. In contrast, non-mycorrhizal plants (NM) exhibited the lowest Chlorophyll *a* value under both conditions.

The multivariate analysis of variance (MANOVA) indicated that chlorophyll *a*, along with other physiological and biochemical traits, was significantly influenced by the independent variables (Table 5.1).

Result from three-way ANOVA shows that rice variety and mycorrhizal treatment both have a strong and significant impact on chlorophyll *a* content, with certain rice varieties and mycorrhizal treatments enhancing photosynthetic pigment levels (Table 5.2). The interaction between rice variety and mycorrhizal treatment is highly significant. There is also a significant three-way interaction between water status, rice variety, and mycorrhizal treatment.

PCA revealed that chlorophyll *a* was strongly associated with the first principal component (PC1), which explained 44.5% of the total variance (Figure 5.16). Chlorophyll *a* content was positively correlated with PC1 (Figure 5.17), which was driven by traits related to water availability and photosynthetic performance. Plants

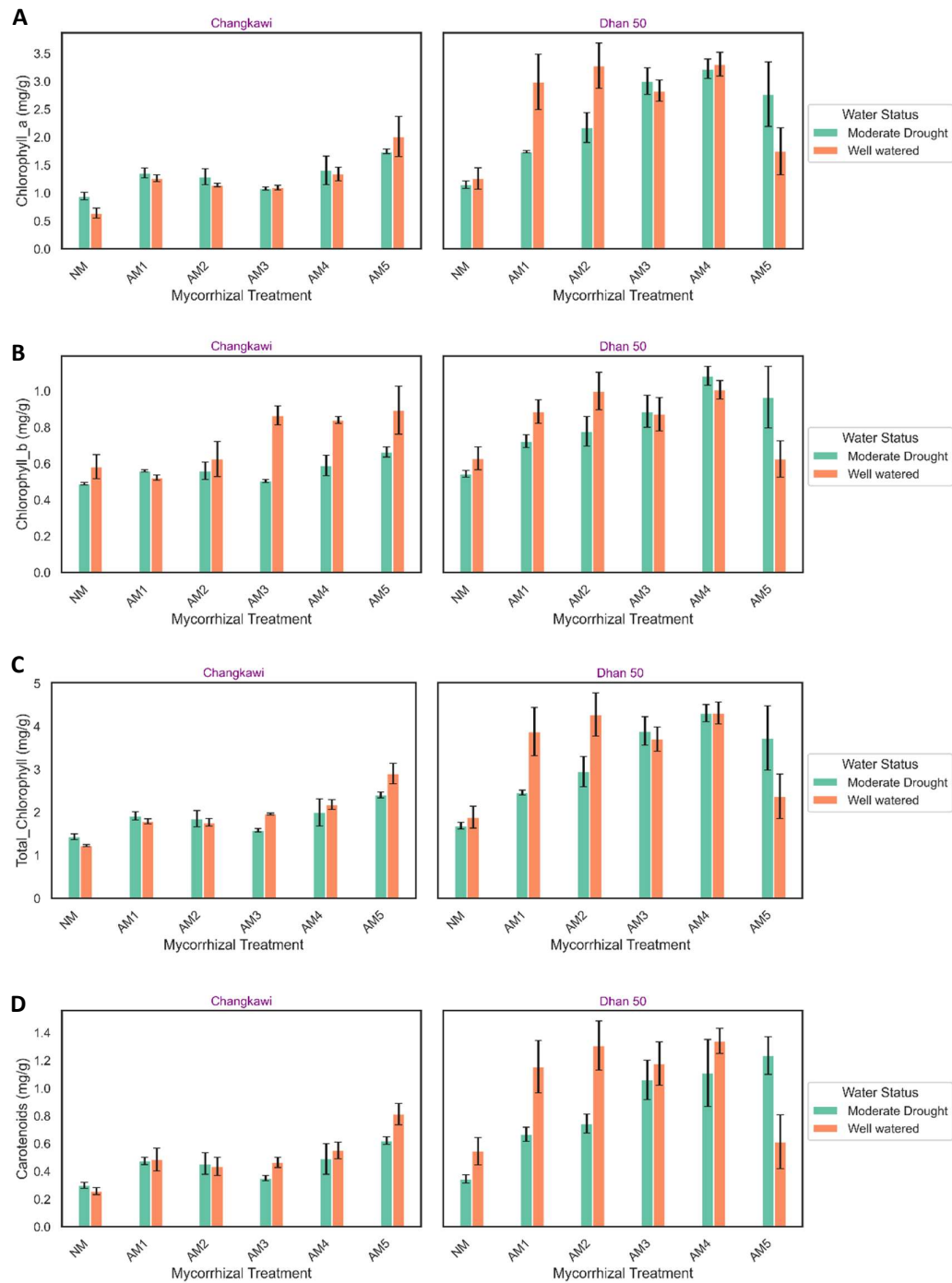


Figure 5.7: Photosynthetic Pigment Content in Rice Varieties (Changkawi and Dhan 50) Under Different Water and Mycorrhizal Treatments. A: Chlorophyll *a*; B: Chlorophyll *b*; C: Total Chlorophyll; D: Carotenoids.

with higher chlorophyll *a* level, particularly those inoculated with AM5 and AM4, clustered along the positive side of PC1, reflecting the role of AMF in maintaining chlorophyll content under both drought and well-watered conditions.

Cluster analysis using K-means identified the first cluster consisted of plants with higher chlorophyll *a* level, mainly well-watered plants and those inoculated with AM5 and AM4 (Figure 5.18). The second cluster included plants with moderate chlorophyll *a* level, primarily under other mycorrhizal treatments. The third cluster contained plants with lower chlorophyll *a* level, mostly from drought-stressed conditions with less effective mycorrhizal treatments, indicating a decline in photosynthetic pigment content under stress.

5.3.6.4 Chlorophyll *b*

Chlorophyll *b* works in conjunction with chlorophyll *a* to capture light energy for photosynthesis. Higher chlorophyll *b* values typically indicate better adaptation to light conditions and an enhanced capacity for photosynthesis. Under moderate drought conditions, chlorophyll *b* values ranged from 0.49 mg/g to 1.08 mg/g, indicating the effect of water stress on chlorophyll content (Figure 5.7 B). Drought typically led to a reduction in chlorophyll *b*, especially in non-mycorrhizal plants. Under well-watered conditions, chlorophyll *b* values were generally higher, ranging from 0.52 mg/g to 1.01 mg/g. Changkawi exhibited lower chlorophyll *b* values under both drought and well-watered conditions compared to Dhan 50. The highest Chlorophyll *b* value for Changkawi under well-watered conditions was 0.89 mg/g (with AM5 treatment), while under moderate drought conditions, the maximum was 0.66 mg/g. Dhan 50 showed higher chlorophyll *b* values, particularly under moderate drought, with the highest value recorded at 1.08 mg/g (with AM4 treatment). Under well-watered conditions, chlorophyll *b* ranged from 0.62 mg/g to 1.01 mg/g for Dhan 50. The treatment group with the highest chlorophyll *b* was Dhan 50 inoculated with AM4 mycorrhizal treatment under moderate drought conditions, with a chlorophyll *b* concentration of 1.08 mg/g. The treatment group with the lowest chlorophyll *b* was Changkawi without mycorrhizal treatment (NM) under moderate drought conditions, with a chlorophyll *b* value of 0.49 mg/g. Mycorrhizal inoculation significantly increased chlorophyll *b* content under both drought and well-watered conditions.

Treatments with AM4 and AM3 resulted in the highest chlorophyll *b* concentrations, particularly for Dhan 50. In contrast, non-mycorrhizal plants (NM) exhibited the lowest chlorophyll *b* values under both conditions.

The multivariate analysis of variance (MANOVA) demonstrated that Water Status and Mycorrhizal Treatment significantly influenced chlorophyll *b* content, along with other physiological traits (Table 5.1).

Result from three-way ANOVA shows that water status, rice variety, and mycorrhizal treatment all have significant impacts on chlorophyll *b* content (Table 5.2). The significant interaction between water status and rice variety indicates that drought stress affects the two rice varieties differently in terms of chlorophyll *b* content. The interaction between rice variety and mycorrhizal treatment suggests that the effect of mycorrhizal inoculation on chlorophyll *b* varies depending on the rice variety. The significant three-way interaction reveals a complex interplay between water status, rice variety, and mycorrhizal treatment, indicating that their combined effects significantly influence chlorophyll *b* levels.

PCA indicated that chlorophyll *b* was strongly associated with the first principal component (PC1), which explained 44.5% of the total variance (Figure 5.16). Chlorophyll *b* was positively correlated with PC1, along with other photosynthetic traits such as chlorophyll *a* (Figure 5.17). Plants with higher chlorophyll *b* content, particularly those inoculated with AM5 and AM4, clustered along the positive side of PC1.

Cluster analysis using K-means revealed three distinct clusters based on physiological and biochemical traits, with chlorophyll *b* playing a significant role in defining these clusters (Figure 5.18). The first cluster consisted of plants with high chlorophyll *b* content, primarily well-watered plants and those inoculated with AM5 and AM4. The second cluster included plants with moderate chlorophyll *b* levels, representing those under other mycorrhizal treatments or mild drought stress. The third cluster comprised plants with lower chlorophyll *b* content, mainly from drought-stressed conditions without effective mycorrhizal inoculation, indicating the negative impact of water stress on chlorophyll *b* levels.

5.3.6.5 Total Chlorophyll

Under moderate drought conditions, total chlorophyll values ranged from 1.43 mg/g to 4.30 mg/g, with drought stress generally reducing total chlorophyll content, especially in the non-mycorrhizal plants (Figure 5.7 C). Under well-watered conditions, total chlorophyll values were higher, ranging from 1.22 mg/g to 4.31 mg/g. Changkawi exhibited lower total chlorophyll content under both drought and well-watered conditions compared to Dhan 50. The highest total chlorophyll value for Changkawi under well-watered conditions was 2.90 mg/g (with AM5 treatment), while under moderate drought, the maximum was 2.41 mg/g. Dhan 50 showed significantly higher total chlorophyll values, with the highest value recorded at 4.31 mg/g (with AM4 treatment) under well-watered conditions. Under moderate drought conditions, Dhan 50's total chlorophyll ranged from 1.69 mg/g to 4.30 mg/g. The treatment group with the highest total chlorophyll was Dhan 50 inoculated with AM4 mycorrhizal treatment under well-watered conditions, with a total chlorophyll concentration of 4.31 mg/g. The treatment group with the lowest total chlorophyll was Changkawi without mycorrhizal treatment (NM) under well-watered conditions, with a total chlorophyll value of 1.22 mg/g, indicating a lower chlorophyll content and photosynthetic efficiency in the absence of mycorrhizal support. Mycorrhizal inoculation significantly increased total chlorophyll content under both drought and well-watered conditions. Treatments with AM4 and AM3 resulted in the highest total chlorophyll concentrations, particularly for Dhan 50. Non-mycorrhizal plants (NM) exhibited the lowest total chlorophyll values under both conditions.

The multivariate analysis of variance (MANOVA) showed that Water Status, Rice Variety, and Mycorrhizal Treatment significantly influenced the combined physiological and biochemical traits, including total chlorophyll (Table 5.1).

Result from three-way ANOVA shows that rice variety and mycorrhizal treatment both have a significant and strong effect on total chlorophyll content, with specific rice varieties and mycorrhizal treatments improving chlorophyll levels (Table 5.2). The interaction between rice variety and mycorrhizal treatment is highly significant, showing that different rice varieties respond uniquely to mycorrhizal inoculation in terms of chlorophyll production. There is a significant three-way interaction between water status, rice variety, and mycorrhizal treatment, indicating

that the combination of these factors has a substantial effect on total chlorophyll content.

PCA indicated that total chlorophyll was strongly associated with the first principal component (PC1), which explained 44.5% of the total variance (Figure 5.16). Total chlorophyll content was positively correlated with PC1, which captured the variation driven by water availability and mycorrhizal inoculation. Plants with higher total chlorophyll levels clustered along the positive side of PC1, particularly those from well-watered conditions and those inoculated with AM5 and AM4.

Cluster analysis using K-means identified three distinct clusters based on physiological and biochemical traits, with total chlorophyll content playing a significant role in defining these clusters (Figure 5.18). The first cluster consisted of plants with high total chlorophyll content, mainly from well-watered conditions and those inoculated with AM5 and AM4. The second cluster included plants with moderate total chlorophyll levels, representing other mycorrhizal treatments under both well-watered and drought conditions. The third cluster contained plants with lower total chlorophyll levels, mostly drought-stressed plants with minimal or no mycorrhizal inoculation, highlighting the negative impact of water stress on chlorophyll content.

5.3.6.6 Carotenoids

Carotenoids are pigments that play a crucial role in photosynthesis by protecting chlorophyll from damage caused by excess light and reactive oxygen species. Higher carotenoid levels are indicative of better photoprotection and stress resistance. Under moderate drought conditions, carotenoid values ranged from 0.30 mg/g to 1.24 mg/g (Figure 5.7 D). Drought stress generally reduced carotenoid levels, particularly in non-mycorrhizal plants, though some mycorrhizal treatments maintained higher values. Under well-watered conditions, carotenoid values were generally higher, ranging from 0.26 mg/g to 1.34 mg/g. Changkawi exhibited lower carotenoid values compared to Dhan 50 under both drought and well-watered conditions. The highest carotenoid value for Changkawi under well-watered conditions was 0.81 mg/g (with AM5 treatment), while under moderate drought, the maximum was 0.62 mg/g. Dhan 50 showed higher carotenoid values, especially under

well-watered conditions, with the highest value recorded at 1.34 mg/g (with AM4 treatment). Under moderate drought conditions, Dhan 50's carotenoid values ranged from 0.35 mg/g to 1.24 mg/g. The treatment group with the highest carotenoid value was Dhan 50 inoculated with AM4 mycorrhizal treatment under well-watered conditions, with a carotenoid concentration of 1.34 mg/g. The treatment group with the lowest carotenoid value was Changkawi without mycorrhizal treatment (NM) under well-watered conditions, with a carotenoid value of 0.26 mg/g. Mycorrhizal inoculation significantly increased carotenoid content under both drought and well-watered conditions. Treatments with AM4 and AM5 consistently resulted in higher carotenoid concentrations, particularly for Dhan 50. Non-mycorrhizal plants (NM) exhibited the lowest carotenoid values under both conditions.

The multivariate analysis of variance (MANOVA) demonstrated that Water Status, Mycorrhizal Treatment, and Rice Variety significantly influenced carotenoids, along with other physiological and biochemical traits (Table 5.1).

Three-way ANOVA result shows that water status, rice variety, and mycorrhizal treatment all significantly affect carotenoid content, with drought stress reducing carotenoids and certain rice varieties and mycorrhizal treatments enhancing carotenoid production (table 5.2). The significant interaction between rice variety and mycorrhizal treatment suggests that different rice varieties respond uniquely to mycorrhizal inoculation in terms of carotenoid production. The three-way interaction shows that the combination of these factors leads to significant changes in carotenoid levels, indicating complex relationships between the factors.

Carotenoids were strongly associated with the first principal component (PC1), which explained 44.5% of the total variance (Figure 5.16). Carotenoid content was positively correlated with PC1 (Figure 5.17), which captured variations driven by water availability and other growth-promoting factors like chlorophyll content. Plants with higher carotenoid levels, particularly those inoculated with AM5 and AM4, were positioned along the positive side of PC1.

In cluster analysis using K-means, the first cluster consisted of plants with high carotenoid content, mainly from well-watered conditions and those inoculated with AM5 and AM4, which exhibited strong carotenoid retention (Figure 5.18). The second cluster included plants with moderate carotenoid levels, representing other

mycorrhizal treatments across both well-watered and drought conditions. The third cluster comprised plants with lower carotenoid levels, mainly from drought-stressed conditions with less effective mycorrhizal inoculation.

5.3.6.7 Total Soluble Sugars

Total soluble sugars reflect the concentration of soluble sugars in plant tissues, which are essential for energy storage and stress responses. Higher total soluble sugars levels are often associated with enhanced stress tolerance. Under moderate drought conditions, total soluble sugars values ranged from 85.38 mg/g to 103.14 mg/g, with non-mycorrhizal plants showing higher sugar accumulation in response to water stress (Figure 5.8 A). Under well-watered conditions, total soluble sugars values were lower, ranging from 62.76 mg/g to 68.97 mg/g. Changkawi exhibited lower total soluble sugars values compared to Dhan 50 under both drought and well-watered conditions. The highest total soluble sugars value for Changkawi under moderate drought was 97.15 mg/g (without mycorrhizal treatment), while under well-watered conditions, the maximum was 66.71 mg/g. Dhan 50 showed higher total soluble sugars values, particularly under moderate drought, with the highest total soluble sugars recorded at 103.14 mg/g (without mycorrhizal treatment). Under well-watered conditions, Dhan 50's total soluble sugars ranged from 63.46 mg/g to 68.97 mg/g. The treatment group with the highest total soluble sugars value was Dhan 50 without mycorrhizal treatment (NM) under moderate drought conditions, with a total soluble sugar's concentration of 103.14 mg/g. The treatment group with the lowest total soluble sugars value was Changkawi inoculated with AM1 under well-watered conditions, with a TSS value of 62.76 mg/g. Mycorrhizal inoculation generally reduced total soluble sugars accumulation, particularly under well-watered conditions. This suggests that mycorrhizal treatments, especially AM4 and AM5, helped improve water and nutrient uptake, reducing the plant's need to accumulate sugars. In contrast, non-mycorrhizal plants (NM) exhibited the highest total soluble sugars values under both conditions.

The multivariate analysis of variance (MANOVA) indicated that Water Status, Mycorrhizal Treatment, and Rice Variety significantly influenced total soluble sugars, along with other physiological and biochemical traits (Table 5.1).

Three-way ANOVA result shows that water status has a major impact on total soluble sugars, with drought conditions leading to a significant increase in sugar accumulation (Table 5.3). Mycorrhizal treatment also plays an important role in modulating total soluble sugars, suggesting that certain mycorrhizal fungi enhance sugar accumulation under stress conditions. The interaction between water status and mycorrhizal treatment is significant, indicating that the response to drought varies depending on the specific mycorrhizal inoculation used. Rice variety does not have a

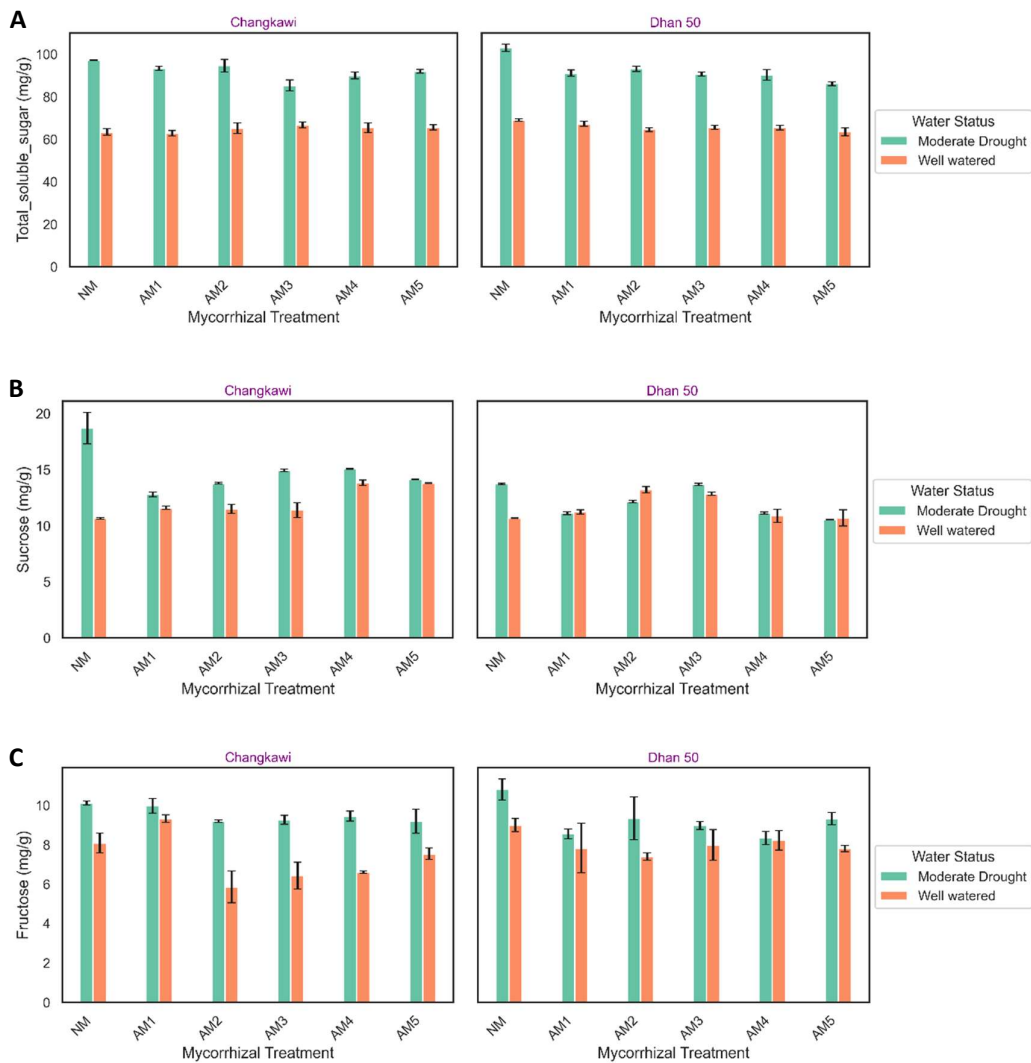


Figure 5.8: Carbohydrates content in rice varieties (Changkawi and Dhan 50) under different water and mycorrhizal treatments. A: Total soluble sugar; B: Sucrose; C: Fructose.

direct significant effect on total soluble sugars, but it does interact with mycorrhizal treatments, suggesting genotype-specific responses.

PCA revealed that total soluble sugars were strongly associated with the first principal component (PC1), which explained 44.5% of the total variance (Figure 5.16). total soluble sugars, along with other stress-related traits like proline and MDA, contributed positively to PC1, indicating that higher total soluble sugars levels were closely associated with drought stress conditions (Figure 5.17). Plants with elevated total soluble sugars levels, particularly those inoculated with AM5, clustered along the positive side of PC1.

In cluster analysis using K-means, the first cluster comprised plants with high total soluble sugars levels, mainly from drought-stressed conditions and those inoculated with AM5 and AM4 (Figure 5.18). The second cluster included plants with moderate total soluble sugars levels, representing other mycorrhizal treatments across both well-watered and drought conditions. The third cluster contained plants with lower total soluble sugars levels, primarily from well-watered conditions with minimal mycorrhizal inoculation, reflecting less reliance on sugar accumulation under non-stressful conditions.

5.3.6.8 Sucrose

Sucrose is an important disaccharide that plays a key role in plant metabolism and energy storage. It also acts as a signal molecule in response to stress, with higher sucrose levels often associated with stress adaptation. Under moderate drought conditions, sucrose levels ranged from 10.54 mg/g to 18.69 mg/g (Figure 5.8 B). Drought stress generally led to increased sucrose accumulation, particularly in non-mycorrhizal plants. Under well-watered conditions, sucrose values were generally lower, ranging from 10.65 mg/g to 13.82 mg/g. Changkawi exhibited higher sucrose values under moderate drought, with the highest sucrose value recorded at 18.69 mg/g (without mycorrhizal treatment). Under well-watered conditions, the highest sucrose value for Changkawi was 13.82 mg/g (with AM4 treatment). Dhan 50 had lower sucrose values under moderate drought, with the highest value recorded at 13.72 mg/g (without mycorrhizal treatment). Under well-watered conditions, Dhan 50's sucrose levels ranged from 10.54 mg/g to 13.21 mg/g. The treatment group with the highest

sucrose value was Changkawi without mycorrhizal treatment (NM) under moderate drought conditions, with a sucrose concentration of 18.69 mg/g. The treatment group with the lowest sucrose value was Dhan 50 inoculated with AM5 under moderate drought conditions, with a value of 10.54 mg/g. Mycorrhizal inoculation generally resulted in lower sucrose accumulation under both drought and well-watered conditions. Treatments like AM4 and AM5 were particularly effective in keeping sucrose levels lower. Non-mycorrhizal plants (NM) had the highest sucrose values under both water conditions.

The multivariate analysis of variance (MANOVA) showed that Water Status, Mycorrhizal Treatment, and Rice Variety significantly influenced sucrose content along with other biochemical and physiological traits (Table 5.1).

Three-way ANOVA result indicates that water status, rice variety, and mycorrhizal treatment all significantly affect sucrose content, with drought leading to increased sucrose accumulation (Table 5.3). The interaction between water status and rice variety is highly significant, indicating that the two rice varieties respond differently to drought in terms of sucrose accumulation. The significant interaction between water status and mycorrhizal treatment suggests that certain mycorrhizal fungi are more effective in enhancing sucrose production under drought conditions. There is a three-way interaction, indicating that the combination of water status, rice variety, and mycorrhizal treatment has a complex effect on sucrose levels.

PCA results indicated that sucrose was strongly associated with the first principal component (PC1), which explained 44.5% of the total variance (Figure 5.16). Sucrose, along with other stress-related traits such as proline and total soluble sugars, contributed positively to PC1 (Figure 5.17). Plants with elevated sucrose content, particularly those inoculated with AM5, clustered along the positive side of PC1.

In cluster analysis, the first cluster consisted of plants with high sucrose levels, primarily drought-stressed plants and those inoculated with AM5 and AM4 (Figure 5.18). The second cluster included plants with moderate sucrose levels, representing other mycorrhizal treatments across both well-watered and drought conditions. The third cluster contained plants with lower sucrose levels, mainly from well-watered conditions with minimal mycorrhizal inoculation.

5.3.6.9 Fructose

Fructose is a simple sugar important in plant metabolism, and it often accumulates under stress conditions, similar to other sugars like sucrose. Higher fructose levels can indicate an adaptive response to stress, especially under drought conditions. Under moderate drought conditions, fructose values ranged from 8.34 mg/g to 10.81 mg/g (Figure 5.8 C). Under well-watered conditions, fructose levels were generally lower, ranging from 5.86 mg/g to 8.99 mg/g, reflecting less need for sugar accumulation when water is plentiful. Changkawi exhibited the highest fructose value under moderate drought at 10.11 mg/g (without mycorrhizal treatment). Under well-watered conditions, the highest fructose value for Changkawi was 8.08 mg/g (without mycorrhizal treatment). Dhan 50 showed higher fructose values under drought, with the maximum being 10.81 mg/g (without mycorrhizal treatment). Under well-watered conditions, Dhan 50's fructose levels ranged from 7.40 mg/g to 8.99 mg/g. The treatment group with the highest fructose value was Dhan 50 without mycorrhizal treatment (NM) under moderate drought conditions, with a fructose concentration of 10.81 mg/g. The treatment group with the lowest fructose value was Changkawi inoculated with AM2 under well-watered conditions, with a fructose value of 5.86 mg/g. Mycorrhizal inoculation generally reduced fructose accumulation under both drought and well-watered conditions. Treatments with AM5 and AM4 helped maintain balanced sugar levels, while non-mycorrhizal plants (NM) showed the highest fructose values in both water conditions.

The multivariate analysis of variance (MANOVA) indicated that Water Status, Mycorrhizal Treatment, and Rice Variety significantly influenced fructose levels, along with other physiological and biochemical traits (Table 5.1).

Three-way ANOVA result indicate that water status has a major impact on fructose content, with drought significantly increasing fructose levels (Table 5.3). Mycorrhizal treatment also plays an important role in regulating fructose levels, suggesting that certain mycorrhizal fungi enhance fructose accumulation under stress. The interaction between water status and rice variety is significant, indicating that the two rice varieties respond differently to drought in terms of fructose content. The interaction between rice variety and mycorrhizal treatment is also significant,

suggesting that different rice varieties respond uniquely to mycorrhizal inoculation in terms of fructose accumulation.

PCA showed that fructose was strongly associated with the first principal component (PC1), which explained 44.5% of the total variance (Figure 5.16). Fructose, along with other osmotic adjustment traits like sucrose and total soluble sugars, contributed positively to PC1 (Figure 5.17). Plants with elevated fructose content, particularly those inoculated with AM5, clustered along the positive side of PC1.

Cluster analysis using K-means identified three distinct clusters, the first cluster comprised plants with high fructose levels, primarily from drought-stressed conditions and those inoculated with AM5 and AM4 (Figure 5.18). The second cluster included plants with moderate fructose levels, representing other mycorrhizal treatments across both well-watered and drought conditions. The third cluster consisted of plants with lower fructose levels, mainly from well-watered conditions with minimal mycorrhizal inoculation

5.3.7 Plant Biochemical Responses

5.3.7.1 Leaf Proline

Proline is an amino acid that accumulates in plants under stress, particularly drought and salinity, functioning as an osmoprotectant and helping the plant maintain cellular functions. Under moderate drought conditions, proline levels ranged from 39.71 $\mu\text{mol/g}$ to 59.36 $\mu\text{mol/g}$. The highest proline accumulation occurred in non-mycorrhizal plants, indicating that plants without mycorrhizal support relied more on proline for stress tolerance (Figure 5.9). Under well-watered conditions, proline levels were significantly lower, ranging from 16.23 $\mu\text{mol/g}$ to 25.73 $\mu\text{mol/g}$. Changkawi exhibited the highest proline accumulation under moderate drought at 55.83 $\mu\text{mol/g}$ (without mycorrhizal treatment). Under well-watered conditions, the highest proline value for Changkawi was 21.85 $\mu\text{mol/g}$ (without mycorrhizal treatment). Dhan 50 showed higher proline accumulation under drought, with the maximum value being 59.36 $\mu\text{mol/g}$ (without mycorrhizal treatment). Under well-watered conditions, Dhan 50's proline values ranged from 16.23 $\mu\text{mol/g}$ to 25.73 $\mu\text{mol/g}$. The treatment group with the highest proline value was Dhan 50 without mycorrhizal treatment (NM) under

moderate drought conditions, with a proline concentration of 59.36 $\mu\text{mol/g}$. The treatment group with the lowest proline value was Dhan 50 inoculated with AM4 under well-watered conditions, with a proline value of 16.23 $\mu\text{mol/g}$. Mycorrhizal inoculation significantly reduced proline accumulation under both drought and well-watered conditions. Treatments with AM4 and AM3 helped maintain balanced proline levels, while non-mycorrhizal plants (NM) exhibited the highest proline values under both drought and well-watered conditions.

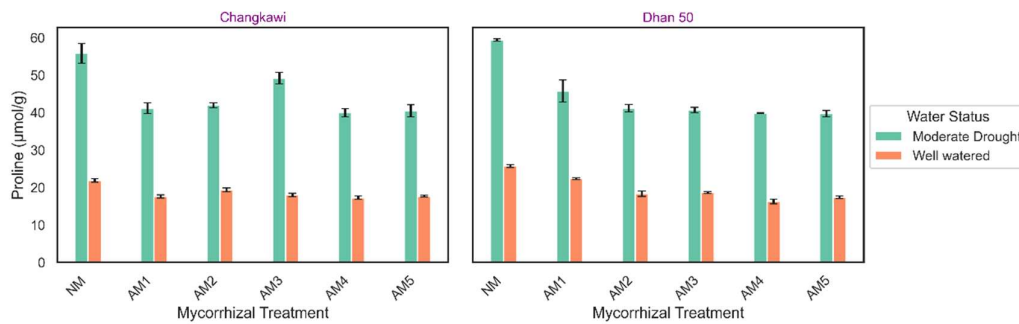


Figure 5.9: Proline content in rice varieties (Changkawi and Dhan 50) under different water and mycorrhizal treatments

Results from MANOVA indicated that Water Status, Mycorrhizal Treatment, and Rice Variety significantly influenced proline levels, along with other physiological and biochemical traits (Table 5.1).

Three-way ANOVA result indicate that water status has a major and highly significant effect on proline content, with drought conditions leading to a sharp increase in proline accumulation. Mycorrhizal treatment also plays a crucial role in enhancing proline levels, suggesting that specific mycorrhizal fungi help plants cope with drought stress by increasing proline production. The interaction between water status and mycorrhizal treatment is significant, indicating that different mycorrhizal treatments vary in their effectiveness at promoting proline accumulation under drought conditions. The three-way interaction is significant, indicating that the combination of water status, rice variety, and mycorrhizal treatment influences proline levels in a complex manner.

PCA results showed that proline was strongly associated with the first principal component (PC1), which explained 44.5% of the total variance (Figure 5.16). Proline

content, along with other stress-related traits such as total soluble sugars and fructose, contributed positively to PC1 (Figure 5.17). Plants with elevated proline levels, especially those inoculated with AM5, clustered along the positive side of PC1, reflecting the association between proline accumulation and improved stress tolerance.

Cluster analysis using K-means identified three distinct clusters based on physiological and biochemical traits, with proline playing a significant role in defining these clusters (Figure 5.18). The first cluster comprised plants with high proline levels, primarily drought-stressed plants and those inoculated with AM5 and AM4. The second cluster included plants with moderate proline levels, representing other mycorrhizal treatments across both well-watered and drought conditions. The third cluster consisted of plants with lower proline levels, mainly from well-watered conditions with minimal mycorrhizal inoculation.

5.3.7.2 Anthocyanin

Anthocyanins are pigments responsible for red, purple, and blue colors in plants depending on the pH level. They play a role in stress tolerance, particularly under conditions like drought, by protecting cells from oxidative stress. Under moderate drought conditions, anthocyanin levels ranged from 0.12 mg/g to 0.65 mg/g. Anthocyanin accumulation generally increased with drought stress, but non-mycorrhizal plants showed the lowest accumulation. Under well-watered conditions, anthocyanin levels were higher in plants with certain mycorrhizal treatments, ranging from 0.15 mg/g to 0.68 mg/g (Figure 5.10). Changkawi showed higher anthocyanin accumulation under both moderate drought and well-watered conditions compared to Dhan 50. The highest anthocyanin value for Changkawi under drought was 0.65 mg/g (with AM5 treatment), while under well-watered conditions, the maximum value was 0.68 mg/g (with AM4 treatment). Dhan 50 exhibited lower anthocyanin accumulation, with the highest value recorded under moderate drought being 0.56 mg/g (with AM2 treatment). Under well-watered conditions, the highest anthocyanin value for Dhan 50 was 0.60 mg/g (with AM2 treatment). The treatment group with the highest anthocyanin value was Changkawi inoculated with AM4 under well-watered conditions, with a value of 0.68 mg/g. The treatment group with the lowest anthocyanin value was Changkawi without mycorrhizal treatment (NM) under

moderate drought conditions, with an anthocyanin concentration of 0.12 mg/g. Mycorrhizal inoculation significantly increased anthocyanin accumulation under both drought and well-watered conditions. Treatments like AM4 and AM5 were particularly effective at enhancing anthocyanin levels, while non-mycorrhizal plants (NM) exhibited the lowest anthocyanin values under both conditions.

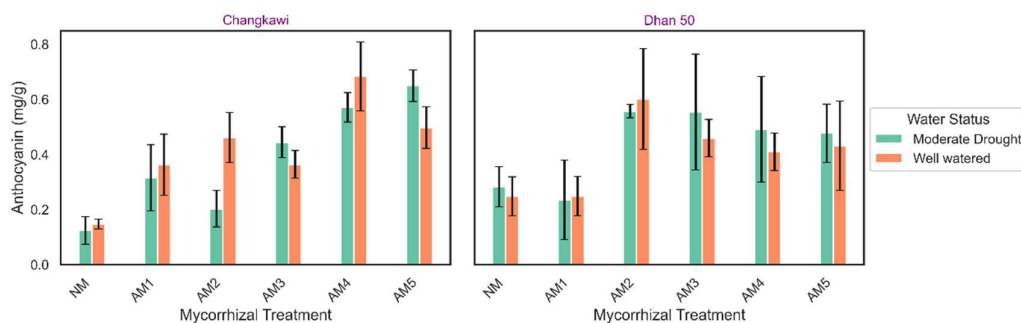


Figure 5.10: Anthocyanin content in rice varieties (Changkawi and Dhan 50) under different water and mycorrhizal treatments

The multivariate analysis of variance (MANOVA) demonstrated that Water Status, Mycorrhizal Treatment, and Rice Variety significantly influenced anthocyanin content, along with other physiological and biochemical traits (Table 5.1).

Result from three-way ANOVA shows that mycorrhizal treatment has a significant positive effect on anthocyanin content (Table 5.3). Neither water status nor rice variety has a significant individual effect on anthocyanin content. The significant interaction between rice variety and mycorrhizal treatment suggests that different rice varieties respond uniquely to mycorrhizal inoculation in terms of anthocyanin production. There is no significant three-way interaction between water status, rice variety, and mycorrhizal treatment.

PCA revealed that anthocyanin content was strongly associated with the first principal component (PC1), which explained 44.5% of the total variance (Figure 5.16). Anthocyanins, along with other stress-related traits like proline and MDA, contributed positively to PC1 (Figure 5.17).

In cluster analysis, the first cluster consisted of plants with high anthocyanin levels, mainly drought-stressed plants and those inoculated with AM5 and AM4. The second cluster included plants with moderate anthocyanin levels, representing other

mycorrhizal treatments under both well-watered and drought conditions. The third cluster comprised plants with lower anthocyanin levels, mainly from well-watered conditions with minimal mycorrhizal inoculation, reflecting less reliance on anthocyanins for photoprotection and stress adaptation.

5.3.7.3 Catalase

Catalase is an important antioxidant enzyme that helps protect cells from oxidative damage by breaking down hydrogen peroxide into water and oxygen. Its activity is often elevated under stress conditions, such as drought, to mitigate reactive oxygen species (ROS) accumulation. Under moderate drought conditions, catalase activity ranged from 1.25 U/min/g to 2.23 U/min/g. Drought stress generally increased catalase activity, particularly in mycorrhizal-treated plants, reflecting the plant's response to oxidative stress (Figure 5.11). Under well-watered conditions, catalase activity was lower, ranging from 0.68 U/min/g to 1.18 U/min/g, as the plants experienced less oxidative stress and required less catalase activity. Changkawi exhibited higher catalase activity under both moderate drought and well-watered conditions compared to Dhan 50. The highest catalase value for Changkawi under moderate drought was 2.23 U/min/g (with AM5 treatment), suggesting strong antioxidant activity in response to stress. Under well-watered conditions, the highest catalase value for Changkawi was 1.18 U/min/g (with AM5 treatment). Dhan 50 showed lower catalase activity, with the highest value under moderate drought being 2.04 U/min/g (with AM3 treatment). Under well-watered conditions, Dhan 50's catalase values ranged from 0.74 U/min/g to 1.12 U/min/g. The treatment group with the highest catalase value was Changkawi inoculated with AM5 under moderate drought conditions, with a catalase activity of 2.23 U/min/g. The treatment group with the lowest catalase value was Changkawi without mycorrhizal treatment (NM) under well-watered conditions, with a catalase activity of 0.68 U/min/g. Mycorrhizal inoculation generally increased catalase activity under both drought and well-watered conditions. Treatments with AM5 and AM3 were particularly effective in boosting catalase activity. Non-mycorrhizal plants (NM) exhibited the lowest catalase values under both water conditions.

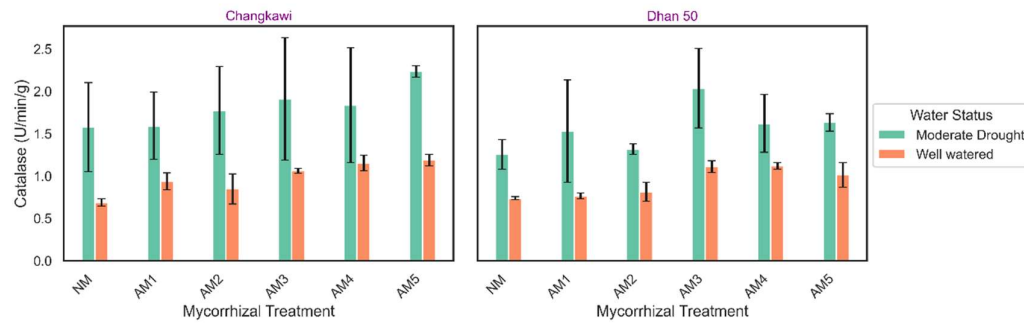


Figure 5.11: Catalase activity in rice varieties (Changkawi and Dhan 50) under different water and mycorrhizal treatments

The multivariate analysis of variance (MANOVA) showed that Water Status, Mycorrhizal Treatment, and Rice Variety significantly influenced catalase activity along with other physiological and biochemical traits (Table 5.1).

Result from three-way ANOVA shows that water status has a highly significant effect on catalase activity, indicating that drought stress significantly increases catalase levels, likely as a response to oxidative stress (Table 5.3). Neither rice variety nor mycorrhizal treatment individually had a significant effect on catalase activity. No significant interaction effects were observed between the independent variables, suggesting that the response of catalase activity is primarily driven by water status.

PCA revealed that catalase activity was strongly associated with the first principal component (PC1), which explained 44.5% of the total variance (Figure 5.16). Catalase, along with other antioxidant traits such as proline and MDA, contributed positively to PC1 (Figure 5.17). Plants with elevated catalase activity, particularly those inoculated with AM5, clustered along the positive side of PC1.

Cluster analysis shows the first cluster consisted of plants with high catalase activity, mainly drought-stressed plants and those inoculated with AM5 and AM4, reflecting the importance of catalase in the antioxidant response to water stress (Figure 5.18). The second cluster included plants with moderate catalase levels, representing other mycorrhizal treatments across both well-watered and drought conditions. The third cluster comprised plants with lower catalase activity, primarily from well-watered conditions with minimal mycorrhizal inoculation, reflecting less reliance on catalase for oxidative stress mitigation under non-stressful conditions.

5.3.7.4 Superoxide Dismutase (SOD)

SOD is a key antioxidant enzyme that plays a crucial role in protecting plant cells from oxidative damage by converting superoxide radicals into hydrogen peroxide and oxygen. SOD activity typically increases in response to oxidative stress, such as drought. Under moderate drought conditions, SOD activity ranged from 9.35 U/g to 12.12 U/g (Figure 5.12). Drought stress significantly increased SOD activity, indicating elevated oxidative stress and the plant's response to reduce the damaging effects of reactive oxygen species. Under well-watered conditions, SOD activity was lower, ranging from 4.56 U/g to 5.66 U/g. Changkawi showed higher SOD activity compared to Dhan 50, particularly under moderate drought conditions. The highest SOD value for Changkawi was 12.12 U/g (with AM3 treatment). Under well-watered conditions, the highest SOD activity for Changkawi was 5.66 U/g (with AM1 treatment). Dhan 50 exhibited similar SOD levels under drought, with the highest SOD value of 12.09 U/g (with AM4 treatment). Under well-watered conditions, Dhan 50's SOD activity ranged from 5.00 U/g to 5.48 U/g. The treatment group with the highest SOD activity was Changkawi inoculated with AM3 under moderate drought conditions, with a value of 12.12 U/g. The treatment group with the lowest SOD activity was Changkawi with AM3 under well-watered conditions, with an SOD value of 4.56 U/g. Mycorrhizal inoculation increased SOD activity in both drought and well-watered conditions. The treatments AM3 and AM4 showed the highest SOD values, while non-mycorrhizal plants (NM) exhibited the lowest SOD values in both conditions.

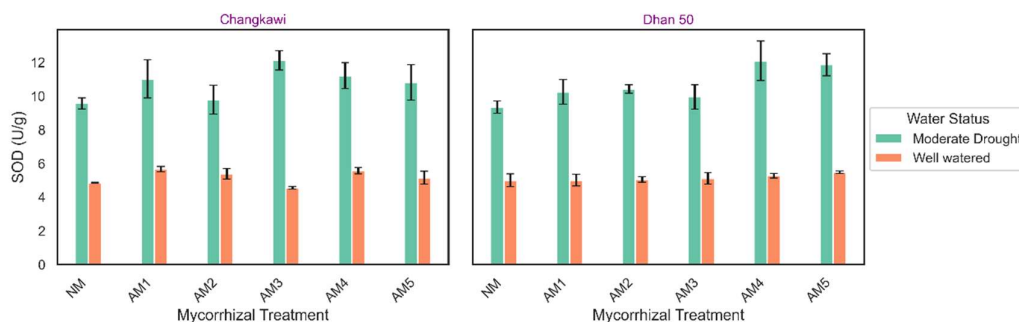


Figure 5.12: Superoxide Dismutase (SOD) activity in rice varieties (Changkawi and Dhan 50) under different water and mycorrhizal treatments

The multivariate analysis of variance (MANOVA) demonstrated that Water Status, Mycorrhizal Treatment, and Rice Variety significantly influenced SOD activity, along with other physiological and biochemical traits (Table 5.1).

Result from three-way ANOVA shows that water status has a highly significant effect on SOD activity (Table 5.3). Mycorrhizal treatment also has a significant effect on SOD activity, suggesting that certain mycorrhizal fungi enhance the plant's antioxidant response under stress conditions. No significant interaction effects were observed between the independent variables, meaning that SOD activity is primarily influenced by water status and mycorrhizal treatment independently.

PCA results revealed that SOD activity was strongly associated with the first principal component (PC1), which explained 44.5% of the total variance (Figure 5.16). SOD, along with other antioxidant-related traits such as catalase and proline, contributed positively to PC1. Plants with elevated SOD activity, particularly those inoculated with AM5, clustered along the positive side of PC1.

Cluster analysis shows that the first cluster consisted of plants with high SOD activity, primarily drought-stressed plants and those inoculated with AM5 and AM4 (Figure 5.18). The second cluster included plants with moderate SOD levels, representing other mycorrhizal treatments across both well-watered and drought conditions. The third cluster contained plants with lower SOD levels, mainly from well-watered conditions with minimal mycorrhizal inoculation, reflecting lower oxidative stress and reduced reliance on SOD activity.

5.3.7.5 Malondialdehyde (MDA)

MDA is a marker of lipid peroxidation, indicating oxidative damage in plant cells. Higher MDA values suggest greater membrane damage and oxidative stress, particularly under drought conditions. Under moderate drought conditions, MDA values ranged from 64.81 nmol/g to 98.62 nmol/g for Changkawi, and from 65.78 nmol/g to 81.73 nmol/g for Dhan 50 (Figure 5.13). Non-mycorrhizal plants showed the highest MDA values, indicating significant oxidative damage under drought stress. Under well-watered conditions, MDA levels were much lower, ranging from 33.07 nmol/g to 58.54 nmol/g. Changkawi exhibited the highest MDA value under moderate drought at 98.62 nmol/g (without mycorrhizal treatment). Under well-watered

conditions, the highest MDA value for Changkawi was 58.54 nmol/g (without mycorrhizal treatment). Dhan 50 showed slightly lower MDA values compared to Changkawi under drought, with the highest value being 81.73 nmol/g (without mycorrhizal treatment). Under well-watered conditions, the MDA levels for Dhan 50 ranged from 33.07 nmol/g to 56.45 nmol/g. The treatment group with the highest MDA value was Changkawi without mycorrhizal treatment (NM) under moderate drought conditions, with an MDA concentration of 98.62 nmol/g. The treatment group with the lowest MDA value was Dhan 50 inoculated with AM4 under well-watered conditions, with an MDA concentration of 33.07 nmol/g. Mycorrhizal inoculation generally lowered MDA levels, particularly under both drought and well-watered conditions. Treatments with AM4 and AM5 helped reduce oxidative damage, as indicated by lower MDA values. Under drought conditions, AM4 significantly reduced MDA levels for both rice varieties, while non-mycorrhizal plants (NM) consistently showed the highest MDA values, especially under drought conditions.

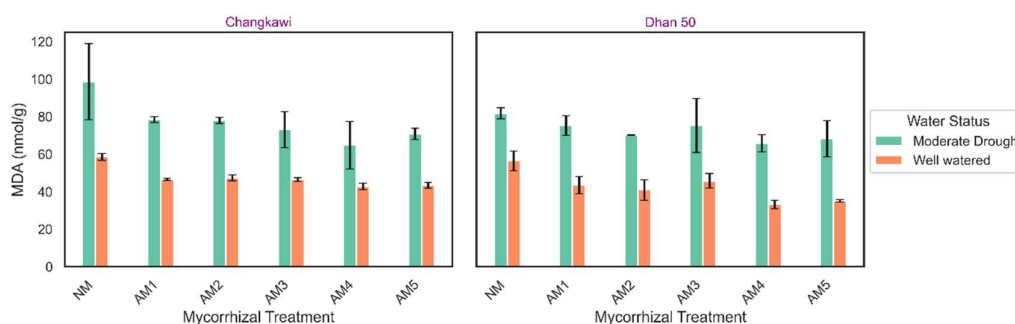


Figure 5.13: Malondialdehyde (MDA) content in rice varieties (Changkawi and Dhan 50) under different water and mycorrhizal treatments

The multivariate analysis of variance (MANOVA) demonstrated that Water Status, Mycorrhizal Treatment, and Rice Variety significantly influenced MDA content along with other physiological and biochemical traits (Table 5.1).

Result from three-way ANOVA shows that water status has a highly significant effect on MDA content (Table 5.3). Mycorrhizal treatment also has a significant impact on MDA levels. No significant interaction effects were observed between water status, rice variety, and mycorrhizal treatment.

PCA revealed that MDA was strongly associated with the first principal component (PC1), which explained 44.5% of the total variance (Figure 5.16). MDA, along with other oxidative stress markers such as proline and SOD, contributed positively to PC1 (Figure 5.17). Plants with higher MDA levels, particularly those without effective mycorrhizal inoculation, clustered along the positive side of PC1, while those with lower MDA levels (inoculated with AM5) clustered along the negative side.

Cluster analysis shows the first cluster consisted of plants with high MDA levels, primarily drought-stressed plants with minimal mycorrhizal inoculation (Figure 5.18). The second cluster included plants with moderate MDA levels, representing other mycorrhizal treatments under both well-watered and drought conditions. The third cluster contained plants with low MDA levels, primarily well-watered plants and those inoculated with AM5 and AM4, indicating reduced lipid peroxidation and improved membrane stability.

5.3.7.6 Hydrogen Peroxide

Hydrogen peroxide (H_2O_2) is a reactive oxygen species (ROS) that accumulates under stress conditions, leading to oxidative damage if not efficiently scavenged by antioxidant systems. Higher H_2O_2 levels indicate more oxidative stress. Under moderate drought conditions, H_2O_2 values for Changkawi ranged from 61.74 nmol/g (AM2) to 90.53 nmol/g (non-mycorrhizal treatment), while for Dhan 50, values ranged from 54.35 nmol/g (AM4) to 74.62 nmol/g (NM) (Figure 5.14). Drought stress increased H_2O_2 accumulation, with non-mycorrhizal plants showing the highest levels of oxidative stress. Under well-watered conditions, H_2O_2 values were lower, ranging from 42.99 nmol/g (AM5 for Changkawi) to 53.22 nmol/g (non-mycorrhizal treatment for Changkawi) and 36.74 nmol/g (AM5 for Dhan 50) to 50.95 nmol/g (non-mycorrhizal treatment for Dhan 50). Mycorrhizal treatments helped reduce oxidative stress even under well-watered conditions. Changkawi exhibited higher H_2O_2 accumulation under both drought and well-watered conditions compared to Dhan 50. The highest H_2O_2 value for Changkawi was 90.53 nmol/g (non-mycorrhizal treatment) under moderate drought. Under well-watered conditions, Changkawi's H_2O_2 levels ranged from 42.99 nmol/g to 53.22 nmol/g. Dhan 50 had slightly lower H_2O_2

accumulation, with the highest value under drought being 74.62 nmol/g (non-mycorrhizal treatment). Under well-watered conditions, Dhan 50's H₂O₂ values ranged from 36.74 nmol/g to 50.95 nmol/g. The treatment group with the highest H₂O₂ value was Changkawi without mycorrhizal treatment (NM) under moderate drought conditions, with a value of 90.53 nmol/g. The treatment group with the lowest H₂O₂ value was Dhan 50 inoculated with AM5 under well-watered conditions, with a value of 36.74 nmol/g. Mycorrhizal inoculation generally reduced H₂O₂ accumulation under both drought and well-watered conditions. Treatments with AM5 and AM4 were particularly effective at reducing oxidative stress, as indicated by lower H₂O₂ levels. Under drought conditions, these treatments helped mitigate the buildup of H₂O₂, while non-mycorrhizal plants (NM) exhibited the highest H₂O₂ levels, particularly under drought.

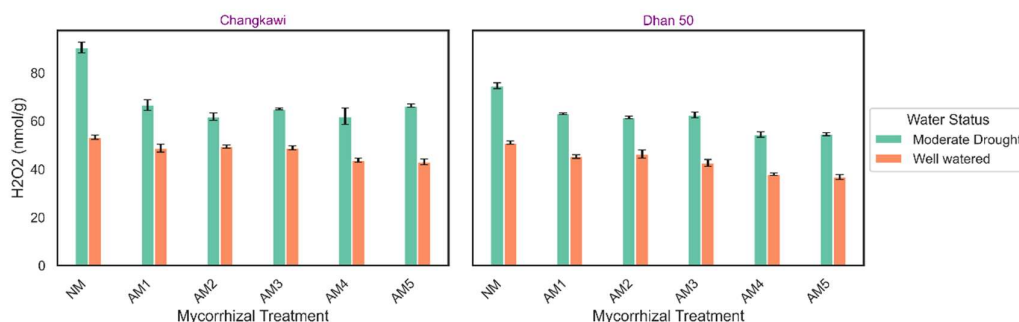


Figure 5.14: Hydrogen Peroxide content in rice varieties (Changkawi and Dhan 50) under different water and mycorrhizal treatments

The multivariate analysis of variance (MANOVA) demonstrated that Water Status, Mycorrhizal Treatment, and Rice Variety significantly influenced H₂O₂ levels, along with other physiological and biochemical traits (Table 5.1).

Three-way ANOVA result shows that water status, rice variety, and mycorrhizal treatment all significantly affect H₂O₂ content, with drought conditions greatly increasing oxidative stress, as indicated by higher H₂O₂ accumulation (Table 5.3). There is a significant interaction between water status and rice variety, water status and mycorrhizal treatment, and a three-way interaction between water status, rice variety, and mycorrhizal treatment.

PCA results revealed that H₂O₂ was strongly associated with the first principal component (PC1), which explained 44.5% of the total variance (Figure 5.16). H₂O₂,

along with other oxidative stress markers such as MDA and SOD, contributed positively to PC1 (Figure 5.17).

Cluster analysis shows that the first cluster consisted of plants with high H₂O₂ levels, primarily drought-stressed plants with minimal mycorrhizal inoculation. The second cluster included plants with moderate H₂O₂ levels, representing other mycorrhizal treatments across both well-watered and drought conditions. The third cluster contained plants with low H₂O₂ levels, mainly well-watered plants and those inoculated with AM5 and AM4.

5.3.7.7 Total Phenolic Content

Total phenolic content (TPC) reflects the concentration of phenolic compounds in plant tissues, which play an essential role in defense against oxidative stress, particularly under drought conditions. Phenolic compounds have antioxidant properties and help protect cells from damage caused by reactive oxygen species (ROS). Under moderate drought conditions, TPC values for Changkawi ranged from 0.557 mg GAE/g (non-mycorrhizal treatment) to 0.800 mg GAE/g (with AM5 treatment). For Dhan 50, TPC values ranged from 0.498 mg GAE/g (non-mycorrhizal treatment) to 0.653 mg GAE/g (with AM5 treatment). Under well-watered conditions, TPC values were generally lower, with Changkawi ranging from 0.437 mg GAE/g (non-mycorrhizal treatment) to 0.526 mg GAE/g (with AM5 treatment), and Dhan 50 ranging from 0.448 mg GAE/g (non-mycorrhizal treatment) to 0.539 mg GAE/g (with AM4 treatment). Changkawi exhibited the highest TPC under moderate drought, with the maximum value recorded at 0.800 mg GAE/g (with AM5 treatment). Dhan 50 showed lower TPC under drought, with the highest value being 0.653 mg GAE/g (with AM5 treatment). Under well-watered conditions, Dhan 50's TPC ranged from 0.448 mg GAE/g to 0.539 mg GAE/g. The treatment group with the highest TPC value was Changkawi inoculated with AM5 under moderate drought conditions, with a value of 0.800 mg GAE/g. The treatment group with the lowest TPC value was Changkawi without mycorrhizal treatment (NM) under well-watered conditions, with a value of 0.437 mg GAE/g. Mycorrhizal inoculation generally increased TPC under both drought and well-watered conditions. The treatments AM5 and AM4 consistently

resulted in higher TPC levels, particularly under drought conditions, while non-mycorrhizal plants (NM) exhibited the lowest TPC values under both conditions.

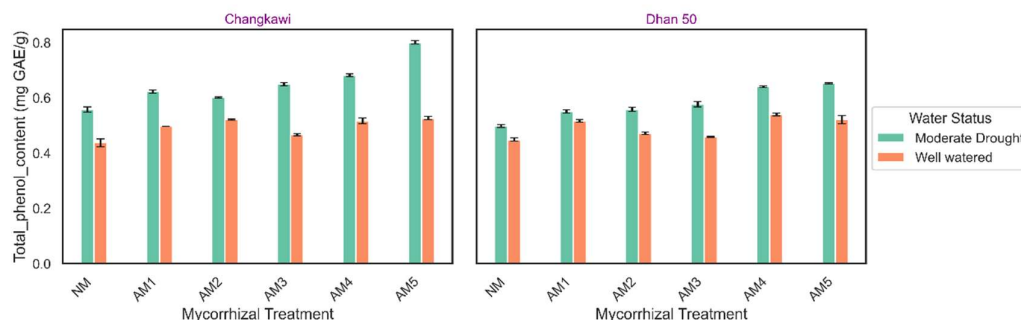


Figure 5.15: Total phenolic content in rice varieties (Changkawi and Dhan 50) under different water and mycorrhizal treatments

MANOVA demonstrated that Water Status, Mycorrhizal Treatment, and Rice Variety significantly influenced TPC along with other physiological and biochemical traits (Table 5.1).

Three-way ANOVA result shows that water status, rice variety, and mycorrhizal treatment all have highly significant effects on total phenolic content (TPC), with drought conditions significantly increasing TPC (Table 5.3). There is a significant interaction between water status and rice variety, water status and mycorrhizal treatment, and a three-way interaction between all factors.

Table 5.1: Summary of MANOVA Results for the Effects of Water Status, Rice Variety, and Mycorrhizal Treatment on Rice Physiological, Biochemical, and Growth Responses

Effect	Wilks' Lambda	F Value	P value
Water Status	0.002	738.88	<0.0001
Rice Variety	0.044	31.67	<0.0001
Mycorrhizal Treatment	0.000	6.32	<0.0001
Water Status × Rice Variety	0.116	11.00	<0.0001
Water Status × Mycorrhizal Treatment	0.001	4.25	<0.0001
Rice Variety × Mycorrhizal Treatment	0.002	3.542	<0.0001
Water Status × Rice Variety × Mycorrhizal Treatment	0.007	2.59	<0.0001

PCA results showed that TPC was strongly associated with the first principal component (PC1), which explained 44.5% of the total variance (Figure 5.16). TPC, along with other antioxidant-related traits such as catalase and proline, contributed

Table 5.2: Three-way ANOVA results showing F-ratios and level of significance for growth and physiological parameters of rice

Effect	Height	Biomass	SLA	LDMC	RWC	EL	Chl_a	Chl_b	Total Chl	Carotenoids
Water Status	36.54****	63.94****	36.83****	111.36****	1.95ns	429.00****	0.74ns	7.73**	1.96ns	5.60*
Rice Variety	0.04ns	0.03ns	9.61**	0.73ns	0.37ns	22.83****	137.30****	41.05****	127.60****	105.64****
Mycorrhizal Treatment	4.55**	2.87*	5.60***	5.05***	4.63**	38.40****	13.57****	8.88****	14.13****	10.58****
Water Status × Rice Variety	5.03*	4.99*	0.00ns	0.51ns	2.36ns	10.43**	1.97ns	6.65*	0.28ns	1.50ns
Water Status × Mycorrhizal Treatment	0.67ns	0.42ns	1.08ns	2.70*	1.10ns	1.10ns	2.23ns	1.15ns	1.88ns	2.48*
Rice Variety × Mycorrhizal Treatment	1.24ns	0.12ns	0.78ns	9.65****	1.60ns	1.66ns	7.52****	3.19*	7.06****	4.64**
Water Status × Rice Variety × Mycorrhizal Treatment	0.41ns	0.53ns	0.08ns	6.90****	0.51ns	0.83ns	3.96**	4.61**	4.68**	5.03***

SLA: Specific Leaf Area; LDMC: Leaf Dry Matter Content; RWC: Relative Water Content; EL: Electrolyte Leakage; TC: Total Chlorophyll

* Level of significance, $p < 0.05$.

** Level of significance, $p < 0.01$.

*** Level of significance, $p < 0.001$.

ns Level of significance, not significant.

Table 5.3: Three-way ANOVA results showing F-ratios and level of significance for biochemical parameters of rice

Effect	Proline	Anthocyanin	Catalase	SOD	MDA	H ₂ O ₂	TPC	TSS	Sucrose	Fructose
Water_Status	3194.12****	0.00ns	31.14****	548.48****	113.24****	1266.29****	1931.12****	1697.61****	100.76****	64.05****
Rice Variety	0.80ns	0.11ns	1.35ns	0.10ns	2.88ns	107.30****	179.13****	1.08ns	109.43****	1.00ns
Mycorrhizal Treatment	68.03****	6.33***	1.45ns	2.74*	4.97***	89.87****	206.16****	8.57****	10.14****	5.02***
Water Status × Rice Variety	2.69ns	0.61ns	0.58ns	0.01ns	0.01ns	4.58*	160.51****	0.28ns	50.91****	5.98*
Water_Status × Mycorrhizal Treatment	15.86****	0.73ns	0.11ns	1.58ns	0.09ns	17.82****	52.20****	7.04****	26.82****	1.52ns
Rice Variety × Mycorrhizal Treatment	8.46****	2.49*	0.24ns	1.02ns	0.25ns	5.01***	11.41****	4.15**	16.71****	2.75*
Water Status × Rice Variety × Mycorrhizal Treatment	2.93*	0.32ns	0.14ns	1.56ns	0.39ns	5.69***	12.66****	1.94ns	4.56**	1.17ns

SOD: Superoxide Dismutase; MDA: Malondialdehyde; TPC: Total Phenolic Content; TSS: Total Soluble Sugars

* Level of significance, $p < 0.05$.

** Level of significance, $p < 0.01$.

*** Level of significance, $p < 0.001$.

ns Level of significance, not significant.

positively to PC1 (Figure 5.17). Plants with elevated TPC levels, particularly those inoculated with AM5 and AM4, clustered along the positive side of PC1.

Cluster analysis shows the first cluster consisted of plants with high TPC levels (Figure 5.18), primarily drought-stressed plants and those inoculated with AM5 and AM4, reflecting a strong association between mycorrhizal inoculation and enhanced phenolic content under stress conditions. The second cluster included plants with moderate TPC levels, representing other mycorrhizal treatments across both well-watered and drought conditions. The third cluster contained plants with lower TPC levels, primarily from well-watered conditions with minimal mycorrhizal inoculation indicating that phenolic accumulation was less critical under favourable conditions.

5.3.8 Multivariate Correlation Analysis

The correlation matrix (Figure 5.19) revealed several important relationships between the dependent variables. The correlations between the traits were examined, and significant correlations ($p < 0.05$) were marked with an asterisk (*) in the heatmap. The correlation analysis suggests that Proline, SOD, and TSS are strongly correlated with one another and serve as primary indicators of stress responses in rice plants. These markers likely represent a coordinated defense mechanism activated under drought conditions. Higher levels of these compounds are associated with better stress mitigation but may also result in trade-offs, such as reduced growth (e.g., lower height and SLA). Conversely, traits related to growth and photosynthesis, such as chlorophyll a, height, and shoot dry biomass, showed weaker or negative correlations with stress markers. This suggests that the activation of defense responses under drought may come at the cost of growth and photosynthetic efficiency.

5.4 Discussion

This study underscores the significant impact of arbuscular mycorrhizal fungi (AMF) on mitigating the detrimental effects of drought stress in rice plants. The current study involved subjecting two rice cultivars, Changkawi and Dhan 50, inoculated with various arbuscular mycorrhizal fungi (AMF), to water-deficit stress. The AMF inoculations are AM1 (*Acaulospora foveata*), AM2 (*Gigaspora margarita*), AM3 (*Glomus* sp), AM4 (*Acaulospora foveate*, *Gigaspora margarita*, *Glomus* sp), and

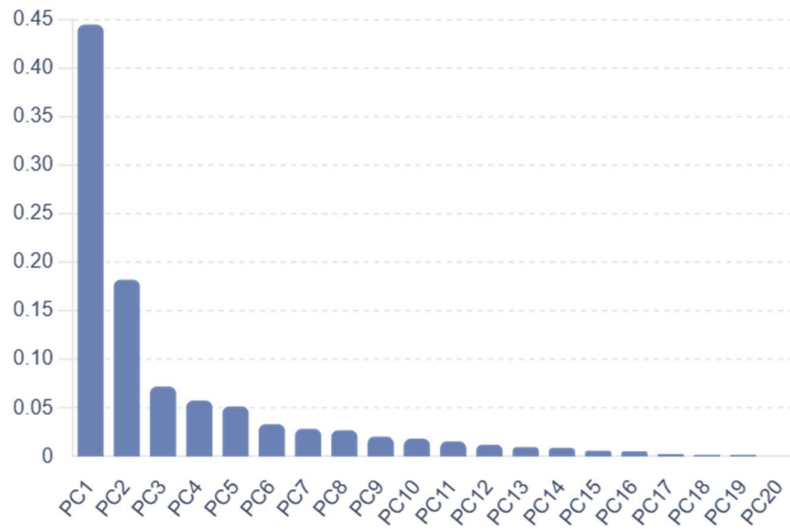


Figure 5.16: Bar plot showing how much variance each principal component explains. The first two principal components (PC1 and PC2) explained a significant portion of the total variance in the dataset: PC1 explained 44.5% of the variance; PC2 explained 18.2% of the variance. Together, these two components captured 62.7% of the total variation, indicating that they effectively summarize the overall patterns in the data.

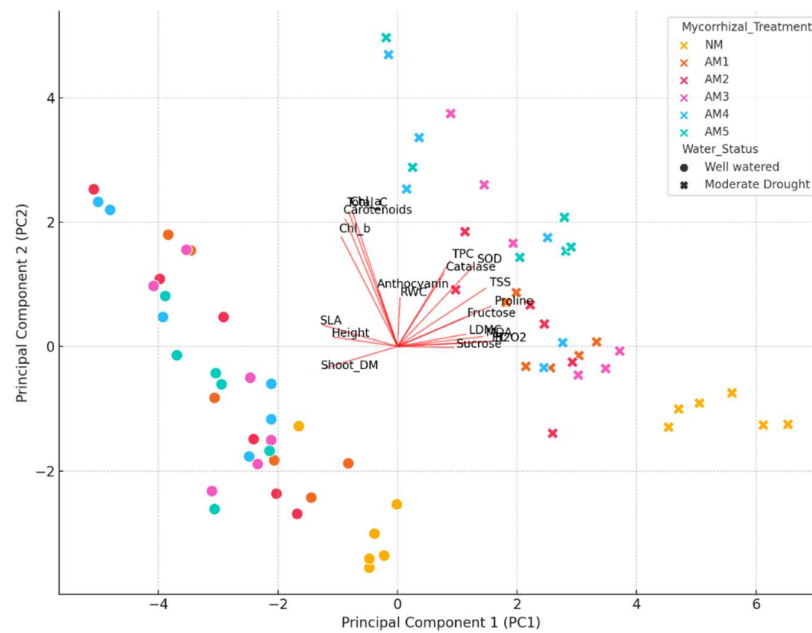


Figure 5.17: PCA biplot (PC1 vs PC2), showing the relationship between the different Mycorrhizal Treatments and Water Status. PC1 was predominantly influenced by stress-related biochemical markers such as Proline, H₂O₂, Electrolyte Leakage, Total Soluble Sugars, and MDA. PC2 was primarily driven by photosynthesis-related variables such as Chlorophyll a, Chlorophyll b, Carotenoids, and Total Chlorophyll.

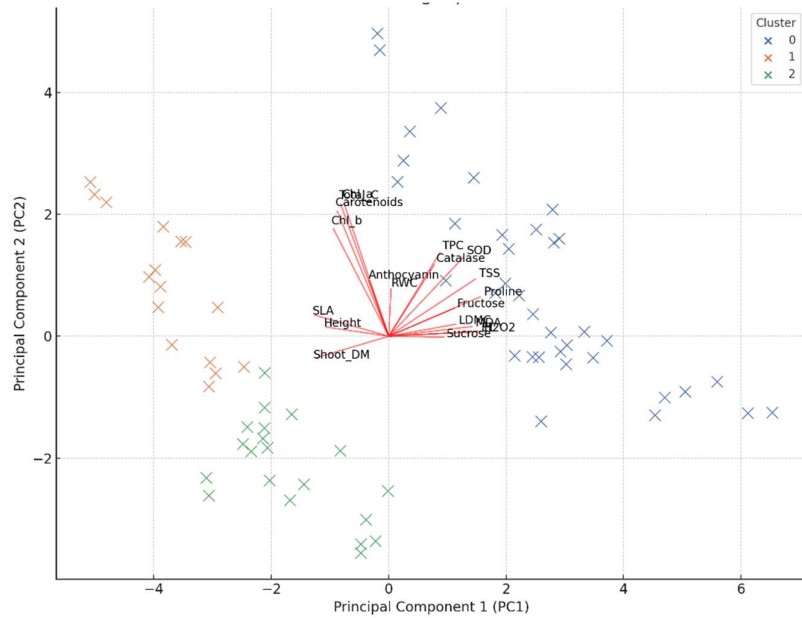


Figure 5.18: Cluster Visualization Based on Principal Components 1 and 2. The arrows represent the loading vectors of the variables, showing how each variable contributes to the principal components. The clusters are indicated by the different colors.

AM4 (Commercial AMF propagules). Both individual and combined applications of arbuscular mycorrhizal fungi (AMF) have been utilized to enhance plant resilience in the face of drought conditions. (Begum, 2019; Boutasknit *et al.*, 2020). The present findings demonstrate that AMF colonization percentage varied across treatments, with the highest AC%, VC% and HC% observed in the Changkawi (AM3), Changkawi (AM4) and Dhan 50(AM1) rice variety under well-watered conditions, particularly with the AM3 treatment. Conversely, drought conditions reduced colonization, which is consistent with the well-documented decline in AMF colonization under water-limited environments.

Previous studies have also indicated that drought stress can significantly reduce the germination of AMF spores and restrict the growth of extra-radical hyphae, thereby limiting colonization potential (Zhang *et al.*, 2018; Tang *et al.*, 2022). However, certain species such as *Glomus* are known for their resilience in arid environments, as they maintain colonization even under severe drought conditions (Verma *et al.*, 2008; Tang *et al.*, 2022).

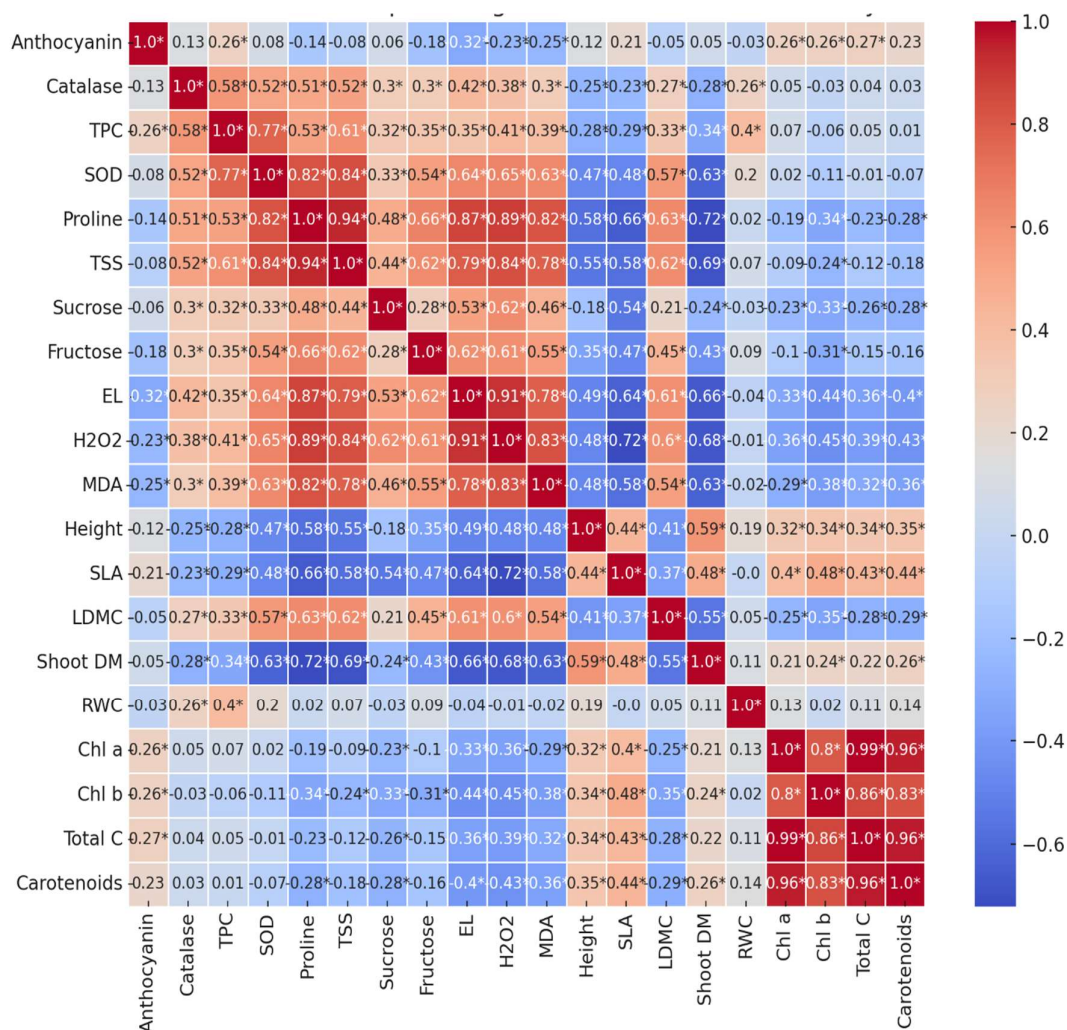


Figure 5.19: Correlation heatmap of biochemical, physiological, and growth parameters in rice under different mycorrhizal and water treatments. * indicates significant correlations ($p < 0.05$).

Rice seedlings that were inoculated with AMF exhibited superior growth compared to non-inoculated seedlings in both rice cultivars. This phenomenon, referred to as positive Mycorrhizal Growth responsiveness (MGR), has been well-documented (Johnson and Graham, 2013). Interestingly, the mycorrhizal growth responsiveness (MR) showed substantial variation across treatments, with the highest responsiveness observed in Dhan 50 under well-watered conditions and treated with AM4. This highlights the beneficial effect of AMF in enhancing plant growth under optimal conditions. It is commonly believed that the enhanced plant growth resulting from mycorrhiza is due to improved nutrient uptake, particularly phosphorus. This is

because the hyphal network of AMF provides access to a significant soil surface area in the mycorrhizal root. However, under drought, the growth-promoting effect of AM was diminished, indicating that the benefits of mycorrhizal inoculation are more pronounced under non-stress conditions (Pons and Müller, 2022).

Water status had a profound effect on plant height and biomass accumulation, with well-watered conditions resulting in significantly taller plants and higher shoot dry biomass. Dhan 50 displayed superior drought tolerance compared to Changkawi, maintaining higher plant height and biomass under drought conditions. This suggests that Dhan 50 possesses a more robust physiological mechanism to withstand water stress, which could be attributed to its inherent genetic traits or its interaction with mycorrhizal fungi.

The positive effect of mycorrhizal inoculation on growth was particularly evident in plants treated with AM1 and AM3, which exhibited greater resilience under drought conditions, reflected in their ability to maintain height and biomass. These results are consistent with previous findings that AMF can enhance plant growth by improving water uptake, nutrient acquisition, and drought tolerance mechanisms (Mirshad and Puthur, 2016). The specific leaf area (SLA) and leaf dry matter content (LDMC) further supported these observations, as SLA was reduced under drought stress, particularly in non-mycorrhizal plants, while LDMC increased, indicating greater dry matter accumulation as a survival strategy.

The increase in LDMC under drought conditions suggests that rice plants, particularly those without mycorrhizal inoculation, allocate more resources to structural biomass in response to water stress (Chen *et al.*, 2017; Mitra *et al.*, 2019). However, mycorrhizal inoculation, especially with AM3 and AM4, helped maintain more favorable growth parameters, even under drought conditions, highlighting the significant role of AMF in promoting drought resilience.

Drought stress caused a significant reduction in relative water content (RWC) and an increase in electrolyte leakage (EL), both of which are indicative of compromised water status and membrane integrity in rice plants. Mycorrhizal treatments, particularly AM4 and AM5, substantially improved RWC, especially under drought conditions. This improvement suggests that mycorrhizal inoculation enhances the plant's ability to retain water under water-limited conditions, likely

through improved root water uptake or altered stomatal conductance (He *et al.*, 2017; Chandrasekaran *et al.*, 2019). In contrast, non-mycorrhizal plants experienced the most severe water loss, as reflected by the lowest RWC values, further reinforcing the critical role of AMF in enhancing water-use efficiency under drought stress. This is consistent with previous studies demonstrating that AMF inoculation improves water relations in host plants by enhancing root hydraulic conductivity and osmotic adjustment (Kubikova *et al.*, 2001; Morte *et al.*, 2000; Mena-Violante *et al.*, 2006; Zhu *et al.*, 2012).

Electrolyte leakage, a key indicator of cell membrane stability and oxidative stress, increased significantly under drought conditions, particularly in non-mycorrhizal plants. The higher EL values in drought-stressed plants indicate greater membrane damage due to dehydration and oxidative stress. However, mycorrhizal inoculation, particularly with AM4 and AM5, significantly reduced EL, suggesting that AM symbiosis helps maintain membrane integrity and reduces oxidative damage. These findings are consistent with previous studies demonstrating the role of AMF in protecting plants from oxidative stress through enhanced antioxidant enzyme activity and improved water relations (Chandrasekaran, 2022).

Biochemical markers such as proline, total soluble sugars (TSS), and antioxidant enzymes (SOD and catalase) exhibited substantial variability depending on water status and mycorrhizal treatment. Proline, an osmoprotectant, plays a critical role in maintaining cell turgor during drought. The results showed that proline accumulation was significantly higher in non-mycorrhizal plants, particularly in Changkawi under drought conditions. This suggests that plants without mycorrhizal symbiosis rely more on proline accumulation to cope with osmotic stress. Mycorrhizal plants, especially those treated with AM3 and AM4, exhibited lower proline levels, indicating that these treatments helped alleviate the need for excessive proline accumulation by improving water uptake. This is in line with findings that mycorrhizal symbiosis enhances the plant's ability to cope with osmotic stress, reducing the reliance on osmoprotectants like proline (Wu *et al.*, 2017; Azmat and Moin, 2019; Ruiz-Sánchez *et al.*, 2010; Yooyongwech *et al.*, 2013).

Total soluble sugars (TSS) play a critical role in plant metabolism, especially under stress conditions like drought, where they act as osmoprotectants, helping

maintain cell turgor and stabilize cellular structures. In this study, drought stress significantly increased TSS levels in both rice varieties, particularly in non-mycorrhizal plants. The highest TSS accumulation was observed in the non-mycorrhizal Dhan 50 under drought conditions. This suggests that without the aid of mycorrhizal symbiosis, plants rely heavily on sugar accumulation to cope with osmotic stress. Interestingly, mycorrhizal inoculation, especially with AM4 and AM5, resulted in lower TSS levels under both drought and well-watered conditions. This reduction in sugar accumulation suggests that mycorrhizal-treated plants experienced less osmotic stress and therefore had a reduced need for sugar buildup. The lower TSS levels in mycorrhizal plants imply improved water and nutrient uptake, reducing the necessity for osmotic adjustment via sugar accumulation. The ability of AMF to alleviate stress without excessive TSS accumulation aligns with previous research, suggesting that AMF improve the plant's physiological balance by facilitating more efficient resource utilization under stress conditions (Yooyongwech *et al.*, 2016; Tsoata *et al.*, 2015; Hajiboland *et al.*, 2015).

Sucrose, a primary disaccharide in plants, plays a pivotal role in carbon partitioning, energy storage, and signaling, especially under stress conditions. In this study, drought stress led to a significant increase in sucrose accumulation, particularly in non-mycorrhizal Changkawi. This suggests that under drought conditions, non-mycorrhizal plants may depend on increased sucrose accumulation as part of their defense mechanism to cope with water stress. The elevated sucrose levels are likely involved in osmotic adjustment, serving as a compatible solute to stabilize cellular structures under dehydration. Mycorrhizal inoculation, especially with AM4 and AM5, significantly reduced sucrose accumulation under drought conditions, which indicates a more efficient stress response and less reliance on osmotic adjustment through sugar accumulation (Zhang *et al.*, 2018). AM4 and AM5 helped plants manage stress more effectively, likely by enhancing water and nutrient uptake, thus diminishing the need for excessive sucrose buildup. In well-watered conditions, sucrose levels were naturally lower across all treatments, but mycorrhizal plants consistently showed reduced sucrose levels compared to non-mycorrhizal ones. This highlights the role of AMF in improving plant energy balance and reducing the metabolic cost associated with drought stress. The significant interaction between rice

variety and mycorrhizal treatment suggests that different rice varieties respond uniquely to mycorrhizal inoculation in terms of sucrose accumulation. Changkawi, in particular, benefited from AM4 and AM5 treatments, which helped reduce the need for sucrose accumulation as a stress response.

Fructose, a simple monosaccharide, plays an essential role in osmotic regulation, carbohydrate metabolism, and stress adaptation in plants. The study revealed that drought stress led to higher fructose accumulation, particularly in non-mycorrhizal Dhan 50 plants. This accumulation is likely part of the plant's adaptive response to maintain osmotic balance under drought conditions. The highest fructose levels were recorded in non-mycorrhizal plants under drought, reflecting their increased reliance on simple sugars to mitigate water stress. Conversely, mycorrhizal inoculation, especially with AM5 and AM4, significantly reduced fructose levels under both drought and well-watered conditions. The lower fructose levels in mycorrhizal-treated plants suggest that these plants were better equipped to manage drought stress without excessive sugar accumulation. This can be attributed to the improved water and nutrient acquisition provided by the mycorrhizal symbiosis, which reduced the need for osmotic adjustment through fructose accumulation. The ability of AMF to modulate fructose levels under stress aligns with their known role in enhancing nutrient uptake and promoting more balanced physiological responses in plants under drought conditions (Wu *et al.*, 2017).

The role of antioxidant enzymes, including superoxide dismutase (SOD) and catalase, was also evident in the study. These enzymes are critical for scavenging reactive oxygen species (ROS) that accumulate under stress conditions. Drought significantly increased SOD and catalase activity, especially in plants inoculated with AMF. The AM3 and AM4 treatments were particularly effective in boosting SOD activity, suggesting that these mycorrhizal treatments enhanced the plant's ability to cope with oxidative stress by reducing ROS accumulation (Li *et al.*, 2019).

Drought stress significantly reduced the levels of chlorophyll a, chlorophyll b, and total chlorophyll, particularly in non-mycorrhizal plants. The reduction in chlorophyll content under drought stress is indicative of impaired photosynthesis, as water limitation often leads to chlorophyll degradation and reduced carbon assimilation (Pal and Pandey, 2016). However, mycorrhizal inoculation, particularly

with AM4 and AM5, helped maintain higher levels of chlorophyll under drought conditions, suggesting that AMF can mitigate the negative effects of drought on photosynthetic efficiency. The increase in carotenoids, which play a protective role by quenching excess light energy and reducing oxidative damage, further highlights the photoprotective effects of AMF, especially under stress conditions (Hart *et al.*, 2015).

Oxidative stress, as indicated by malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) levels, was significantly higher in non-mycorrhizal plants under drought conditions. MDA, a marker of lipid peroxidation, was most elevated in Changkawi under drought conditions, particularly in the absence of mycorrhizal inoculation. The high MDA levels suggest severe oxidative damage to cell membranes due to drought-induced stress. In contrast, mycorrhizal treatments, especially AM4 and AM5, significantly reduced MDA and H_2O_2 levels, indicating enhanced protection against oxidative stress. This aligns with research showing that AMF enhance the plant's antioxidant defense mechanisms, reducing oxidative damage under stress (Zou *et al.*, 2021; Zhang *et al.* 2018b; Ma *et al.*, 2022).

Total phenolic content (TPC), which reflects the plant's antioxidant capacity, increased under drought conditions, particularly in mycorrhizal-treated plants. Phenolic compounds are known to play a critical role in protecting plants from oxidative stress by neutralizing ROS. The highest TPC was observed in plants inoculated with AM5 under drought conditions, suggesting that mycorrhizal inoculation enhances the plant's antioxidant defenses by promoting the biosynthesis of phenolic compounds. This finding supports previous research indicating that AMF can stimulate the production of secondary metabolites, such as phenolics, which contribute to enhanced drought tolerance (Baslam *et al.*, 2011; Mirshad and Puthur, 2016).

The multivariate analysis confirmed that water status, rice variety and mycorrhizal treatment had significant effect on the combined physiological, biochemical, and growth responses, with drought stress substantially altering these parameters. Mycorrhizal treatment, particularly in combination with rice variety, also had a significant effect, indicating that specific combinations of AMF and rice varieties result in improved drought tolerance. Principal component analysis (PCA) revealed that stress-related biochemical markers (proline, MDA, H_2O_2) and photosynthetic

pigments (chlorophyll and carotenoids) were the most influential variables determining the plant's response to drought (Wahab *et al.*, 2022). Notably, AM3 and AM5 emerged as the most effective treatments, enhancing both stress mitigation and photosynthetic performance. NM (non-mycorrhizal) consistently had the lowest values across most variables, showing the detrimental effect of not using mycorrhizal inoculation. The other AM treatments (AM1, AM2, and AM4) showed moderate effects, with significant differences depending on the variable in question. AM3 consistently outperformed NM (non-mycorrhizal) and some other mycorrhizal treatments (particularly AM1 and AM2) across nearly all dependent variables. It played a crucial role in improving stress-related biochemical markers (e.g., catalase, SOD, proline, phenolics) and enhancing growth and physiological traits (e.g., height, shoot DM, RWC, chlorophyll). AM3 was particularly effective in mitigating the negative effects of drought stress, indicating its strong potential for improving plant resilience and performance under stress conditions.

Chapter 6

Biochemical and Physiological Responses of Rice Plant Inoculated with Arbuscular Mycorrhizal Fungi Under Salt Stress

6.1 Introduction

Salinization of soil is a significant issue of land degradation that is progressively worsening in various regions across the globe, especially in arid and semiarid areas (Al-Karaki 2006; Giri *et al.*, 2003). Approximately 5% or 3.85 million hectares of the global cultivated area, which is 77 million hectares in total, is believed to be impacted by salt stress (Sheng *et al.*, 2008). This accounts for over 50% of arable land (Wang *et al.*, 2003). It is estimated that by 2050, the area of soil contaminated by salt is expected to expand to 16.2 million hectares. This might potentially lead to food insecurity for the global population (Yadav *et al.*, 2017). Within the Southeast Asian region, there has been an identification of approximately 5.8 million hectares of arable land that is affected by salinity (Shrestha, 2006). The issue of saline soil is prevalent in the Northeastern region of Thailand, affecting agricultural areas to varying degrees of salinity (Arunin and Pongwichian, 2015).

The expanse of salt-affected soils in India is estimated to be around 6.73 million hectares. Gujarat and Uttar Pradesh have the largest areas of saline tracts, with 2.23 million hectares and 1.37 million hectares respectively. Maharashtra and West Bengal have salt-affected areas measuring 0.61 million hectares and 0.44 million hectares respectively, according to (Mandal *et al.*, 2018). According to Sharma *et al.*, (2014), the area under Saline condition in India is projected to triple to 20 million hectares by 2050. Plants that grow in soils with high salt content are exposed to the harmful impact of certain ions, such as sodium and chloride. These ions interfere with the functioning of enzymes and other large molecules, harm cell organelles, and disturb the processes of photosynthesis and respiration. Soil salinity causes a physiological drought in plants and disrupts nutrient balance by reducing nutrient absorption and/or transport to the aboveground parts of the plant (Munns and Tester, 2008; Ruiz-Lozano *et al.*, 2012).

As per the FAO (2005) report, it is noted that the global population is steadily increasing and, as a result, global agriculture will need to produce 70% more food by the middle of the twenty-first century. Rice is a significant carbohydrate crop that serves as a staple food for over half of the global population (Khush, 2005). Nevertheless, salinity poses a significant challenge to rice cultivation globally, leading to a substantial decrease in productivity (Pareek et al., 2010; Mantri et al., 2012). The crop is extremely vulnerable to salt stress, leading to a decrease in productivity even at minimal salt concentrations (Zeng and Shannon, 2000; Grattan *et al.*, 2002). Hence, the exploration of various approaches to enhance the tolerance and productivity of rice plants in saline conditions poses a significant challenge for researchers in addressing the decline in food production caused by soil salinization (Ruiz-Lozano *et al.*, 2012; Augé *et al.*, 2014).

Arbuscular mycorrhizal fungi (AMF) play a crucial role in regulating the phosphorus (P) content, growth, and yield of the host plant. These symbiotic microorganisms have a significant impact on the overall health and productivity of the plant (Gosling *et al.*, 2006). AMF establishes a symbiotic relationship with the host plant's root organs, influencing its photosynthetic abilities, growth characteristics, and ability to withstand abiotic stress (Panneerselvam *et al.*, 2017; Basu *et al.*, 2018; Mbodj *et al.*, 2018). Several species of arbuscular mycorrhizal fungi (AMF), including *Glomus mosseae*, *Glomus geosporum*, *Glomus intraradices*, *Acaulospora* sp., and *Scutellospora* sp., have been found to commonly colonize rice plants (Gosling *et al.*, 2006; Maiti *et al.*, 2013; Zhang *et al.*, 2015; Tisarum *et al.*, 2019).

In addition to their inherent protective mechanisms, plants can counteract the negative effects of salinity by engaging with various beneficial soil microbes, such as arbuscular mycorrhizal fungus. Multiple investigations have demonstrated that the AM symbiosis has the ability to mitigate the effects of salt stress (Evelin *et al.*, 2009; Ruiz-Lozano *et al.*, 2012; Porcel *et al.*, 2012; Augé *et al.*, 2014; Ruiz-Lozano and Azcón, 2000). The enhanced salt tolerance of arbuscular mycorrhizal plants has been ascribed to a more effective assimilation of nutrients (Talaat and Shawky 2011; Beltrano *et al.*, 2013), preservation of enzyme functions (Rabie and Almadini 2005; Talaat and Shawky 2011), augmentation of photosynthetic capacity (Sheng *et al.*, 2008; Hajiboland *et al.*, 2010; Estrada *et al.*, 2013), facilitation of water absorption by

plants (Aroca *et al.*, 2007; Sheng *et al.*, 2008), and alleviation of ionic imbalance (Giri *et al.*, 2007; Evelin *et al.*, 2012).

AMF-inoculated plants have been found to exhibit improved defense responses against salt stress. This includes higher production of free proline, glycine betaine, and soluble sugars (Campanelli *et al.*, 2013; Evelin *et al.*, 2013; Garg and Baher, 2013; Talaat and Shawky, 2014). AMF inoculation has been found to regulate ion homeostasis, compartmentalization, and Na⁺ translocation from root to shoot. These processes involve influx/efflux, vacuolar storage, and can occur *via* apoplastic and/or symplastic routes (Evelin *et al.*, 2009; Porcel *et al.*, 2012; He and Huang, 2013; Porcel *et al.*, 2016; Yadav *et al.*, 2017; Evelin *et al.*, 2019). Salt stress induces the upregulation of various antioxidant enzymes in plants inoculated with arbuscular mycorrhizal fungi (AMF). These enzymes include superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) (Borde *et al.*, 2011; Ruiz-Lozano *et al.*, 2012; Evelin and Kapoor, 2014; Chang *et al.*, 2018).

The aim of this study was to evaluate the effectiveness of three different species of arbuscular mycorrhizal fungi—*Acaulospora foveata*, *Gigaspora margarita*, and *Glomus* sp.—in mitigating the negative effects of salt stress in two rice cultivars, Changkawi and Dhan 50. The study focused on analyzing a range of physiological, biochemical, and growth-related parameters to determine the potential benefits of AMF inoculation under saline conditions. Through this comprehensive approach, the study aimed to determine which AMF species provided the most effective protection against salt stress, to compare the tolerance levels between the two rice cultivars, and to identify the physiological and biochemical mechanisms by which AMF contributes to stress alleviation. The findings of this research are expected to provide insights into the potential use of AMF as a sustainable biological tool for improving rice resilience in saline soils, which is increasingly important due to the growing threat of soil salinity in agricultural regions worldwide.

6.2 Materials and Methods

6.2.1 Experimental Site

The pot experiment took place in the greenhouse facility at the Department of Botany, Mizoram University, located in Aizawl, Mizoram, India (23°44'11.4"N 92°39'46.6"E).

6.2.2 Preparation of Inoculum

The combination of sand and severed roots which was stored at a temperature of 4°C was taken out from the refrigerator. The roots and soil particles were carefully removed from the zip lock bag and kept separately in a sterile container according to the species. This mixture serves as inoculum for the current experiment which was then mixed with a double steam sterilised soil.

6.2.3 Pot Substrate Material

The soil used for rice seedling culture is composed of a mixture of forest soil (sieved through a 2-mesh sieve) and river sand, in a volume ratio of 2:1. Afterwards, it underwent two rounds of sterilization at a pressure of 0.14 MPa and a temperature of 125°C. The pH of the soil is 6.5, the electrical conductivity (EC) is 103.8 µS/cm, the soil organic carbon content is 18.03 g/kg, the available phosphorus content is 23.93 mg/kg, the available nitrogen content is 46.6 mg/kg, and the available potassium content is 189.2 mg/kg. The culture pots were washed with 75% ethanol and thereafter stored for future use.

6.2.4 Plant Materials and Seedling preparation

A local rice cultivar named Changkawi was obtained from North Vanlaiphai. Simultaneously, DRR Dhan 50, which is drought and submergence tolerant variety, was obtained from ICAR – IIRR seed portal. DRR Dhan 50 is a rice variety developed at ICAR-Indian Institute of Rice Research (ICAR-IIRR), Hyderabad collaboration with ICAR-National Institute of Plant Biotechnology, New Delhi and IRRI, Philippines, through molecular breeding with introgression of *qsub1* for submergence tolerance, and *qDTY2.1* and *qDTY3.1* for drought tolerance. The seeds of both varieties were pretreated in order to break dormancy and have a high germination rate. Seeds were exposed to direct sunlight for four days and then primed by soaking for 5 hours

and dried again prior to sowing. Before germination, the primed seeds were pretreated for 1 min in a 1% sodium hypochlorite (NaOCl) and then rinsed three times with sterile water. After surface sterilisation, the seeds were submerged in sterile water and kept inside a plant growth chamber with a temperature of 30°C, humidity 75%, and 12 hr light and 12 hr dark period. The seeds were kept in this condition until a radical and plumule appeared from the embryo. After germination, the seedlings were transferred to the pots in the greenhouse and grown for 90 days.

6.2.5 Experimental Design

A full-factorial completely randomized design (CRD) was used. The experiment consists of three factors: Rice variety, Salt treatment, and Mycorrhizal treatment. There are two rice cultivars: Changkawi (CHA) and DRR Dhan 50 (DHA). The salt treatment includes three levels: 0 mM NaCl (0 mM), 75 mM NaCl (75 mM), and 150 mM NaCl (150 mM). The mycorrhizal treatment comprises six levels: Non-mycorrhizal (NM), *Acaulospora foveata* (AM1), *Gigaspora margarita* (AM2), *Glomus* sp. (AM3), mixture of AM1, AM2 and AM3 (AM4) and Commercial AMF propagules (AM5). There was a total of 36 treatment combinations, with each combination repeated 3 times and a total of 108 experimental units (Figure 6.1).

6.2.6 Greenhouse Study

The pots were filled with a layer of soil that had been sterilized with steam, and an inoculant of arbuscular mycorrhizal fungi (AMF) was added. The non-mycorrhizal treatment exclusively utilized sterilized soil. A total of twelve rice plant seedlings were planted in each experimental pot and nurtured in a greenhouse until they reached the booting stage (Figure 6.2). The temperature in the greenhouse was maintained at a constant level of $32 \pm 2^\circ\text{C}$ during the daytime, while the relative humidity was kept at $75 \pm 5\%$. Afterwards, each pot, together with the substrate, was watered separately until it reached a moisture level close to its maximum capacity, as indicated by the weight of the pot. To enhance early development and boost the formation of mycorrhizal symbiosis in rice plants, the pots were watered once a week with 60 ml of sterile Hoagland's nutrition solution containing a low amount of phosphorous. The plants were watered regularly for the first 60 days.

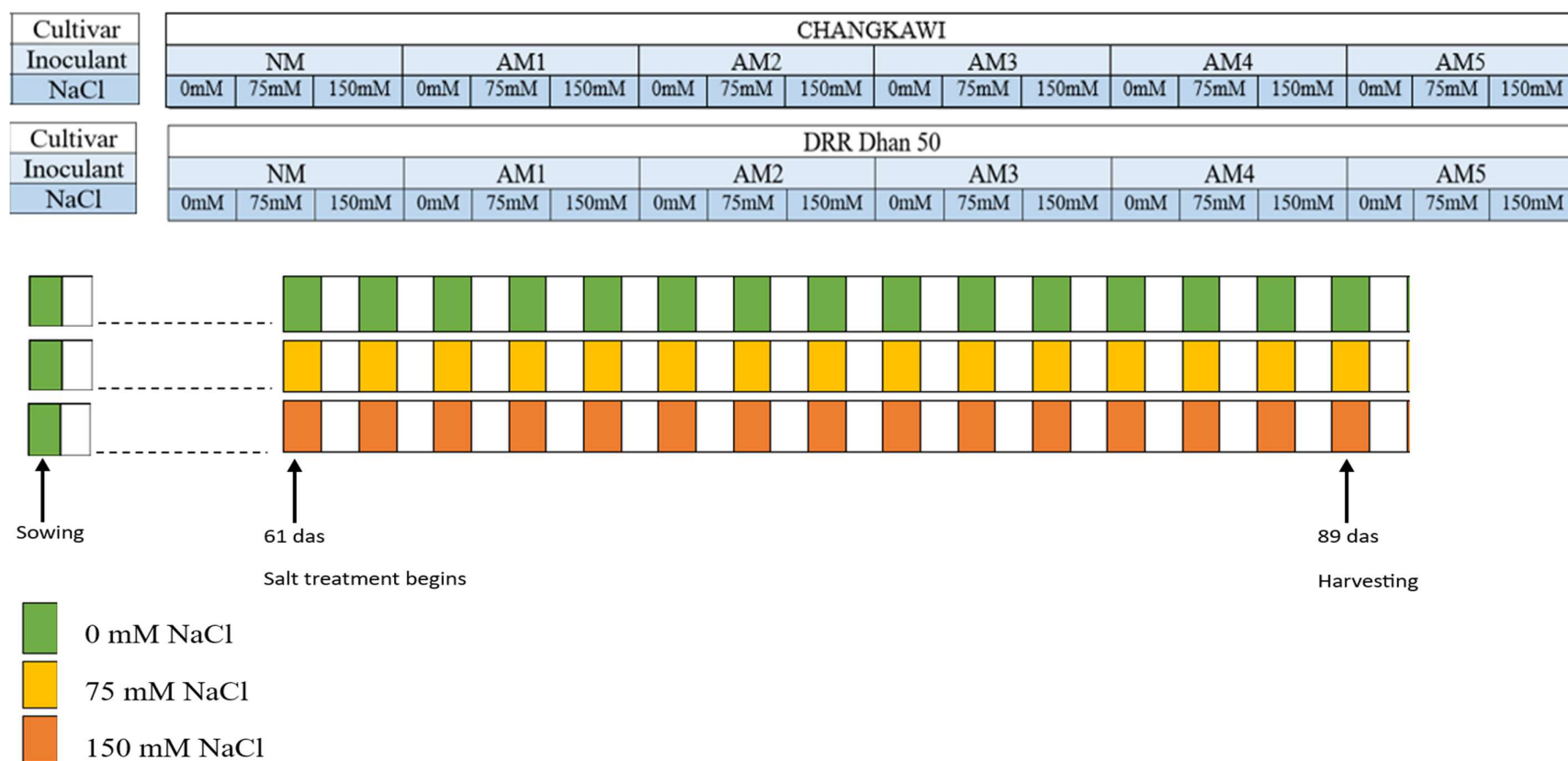


Figure 6.1. Experimental set-up. Two rice cultivars (*Oryza sativa* L.; cv Changkawi and Dhan 50) were either left non-inoculated (NM) or inoculated with a single species of mycorrhizal fungi (AM1 – *Acaulospora foveata*, AM2 – *Gigaspora margarita*, AM3 – *Glomus spp.*). Additionally, plants were inoculated either with a mixture of the three fungi (AM4) or with commercial mycorrhizal propagules (AM5). After germination, the seedlings were transferred into pots and irrigated every other day. This irrigation regimen was maintained up to 60 days after sowing (DAS). At this point, the plants were subjected to different NaCl treatments: 0 mM, 75 mM, and 150 mM, to evaluate the impact of salt stress under varying mycorrhizal treatments.



Figure 6.2: Greenhouse experimental setup for rice cultivation under controlled conditions

Following a 60-day period of regular water supply, the plants were exposed to three distinct salt treatments. Each treatment consisted of three pots per rice cultivar, salt treatment, and substrate regime, respectively. The 0mM group was kept under normal water conditions, without any NaCl, every other day. Thirty-six pots (75mM) were regularly filled with a 75 mM NaCl solution four times per week and the rest thirty-six pots (150 mM) were regularly filled with 150 mM NaCl solution for four days in a week. The application of salt was maintained for a duration of 28 days and ceased 88 days after the seeds were planted. The rice seedlings were harvested 89 days after being sown. Prior to the harvest, growth characteristics such as shoot length, leaf length, and leaf size were measured.

6.2.7 Plant Growth Performances (Height, Leaf Area, Biomass)

The height and dry mass per fresh mass of the rice shoot were assessed using the IRRI protocol (IRRI. *Standard evaluation system for rice*). The height of the plant is measured in centimetres from soil surface to tip of the tallest leaf. For biomass measurement, the plant samples are oven dried at 70 °C until constant weight achieved. The shoot dry mass of plant and leaves are taken and are expressed in g plant⁻¹. Leaf area index was measured using Petiole Pro Leaf Area Meter (<https://www.petiolepro.com/leaf-area-meter-petiole-pro/>) (Figure 6.3).

6.2.8 Specific Leaf Area and Leaf Dry Matter Content (Wilson, 1999)

Specific leaf area (SLA) is the proportion obtained by dividing the leaf area of a plant by its leaf dry mass. The parameter is crucial for plant growth models as it dictates the allocation of new leaf area for every unit of biomass generated. Five intact leaves from each plant were selected and the surface area of the leaf sample was measured by Petiole Pro. Once the leaves area was measured, immerse the leaf base in water and store them in a cool, dim environment overnight to restore moisture. After rehydrating, the leaf sample was dried by blotting it with tissue paper to eliminate any residual water on the surface. Subsequently, the sample was promptly weighed to obtain the saturated weight measurement. The specimen underwent a drying process at a temperature of 80°C for a duration of 3 days. Subsequently, it was weighed again to determine its dry weight. Leaf dry matter content (LDMC) values were determined by calculating the ratio of dry weight to saturated weight, represented as a percentage. The formulas for calculating SLA and LDMC are as follows:

$$SLA = \frac{\text{Leaf surface area (mm}^2\text{)}}{\text{Leaf dry mass (mg)}}$$

$$LDMC = \frac{\text{Leaf dry mass (mg)}}{\text{Leaf saturated mass (g)}}$$

6.2.9 Leaf Relative Water Content (Smart and Bingham, 1974)

Relative water content (RWC) was calculated based on the fully expanded leaves of three plants per experimental unit. The leaves were divided into segments of 2 cm in length. The fresh weight (FW) of the leaf segments was determined accordingly. In order to prevent any loss of respiratory activity, leaf segments were weighed and thereafter immersed in distilled water for a duration of 4 hours at a temperature of 20°C, while being exposed to low levels of light. Subsequently, the leaf discs were meticulously dried to eliminate any residual moisture on the surface. The turgid weight (TW) was used to measure the amount of water absorbed. The dry weight (DW) of the leaf discs was obtained by subjecting the tissues to a drying process at a temperature of 70 °C for a duration of 3 days. g. RWC is measured by the following formula:

$$\text{RWC (\%)} = \frac{(\text{Fresh Weight} - \text{Dry Weight})}{(\text{Turgid Weight} - \text{Dry Weight})} \times 100$$

6.2.10 Determination of Photosynthetic Pigments (Hiscox and Israelstam, 1979)

A quantity of 25 mg of finely minced fresh leaf sample was deposited into the test tubes, followed by the addition of 5 ml of DMSO. The tubes were covered in aluminium foil and placed inside an oven set at a temperature of 65°C for a duration of 4 hours. The absorbance of the Chlorophylls was measured at 663 and 645 nm using spectrophotometer (Shimadzu UV-1900I) after a duration of 4 hours, with DMSO serving as the blank. The Chlorophylls were determined using the extinction values, according to the equation proposed by Arnon (1949).

Chlorophyll content is calculated using following formula:

$$\begin{aligned} \text{Chlorophyll } a \text{ (mg/ml)} &= \frac{12.7D_{663} - 2.69D_{645}}{1000 \times W} \times V \\ \text{Chlorophyll } b \text{ (mg/ml)} &= \frac{22.9D_{645} - 4.68D_{663}}{1000 \times W} \times V \\ \text{Total Chl. (mg/ml)} &= \frac{20.2D_{645} + .02D_{663}}{1000 \times W} \times V \\ \text{Carotenoids (mg/ml)} &= \frac{7.60D_{480} - 1.49D_{510}}{1000 \times W} \times V \end{aligned}$$

where,

D663 = absorbance at 663 nm

D645 = absorbance at 645 nm

D510 = absorbance at 510 nm

D480 = absorbance at 480 nm

V = final volume of Chlorophyll extract (in ml)

W = fresh weight of leaf tissue extracted (in grams).

6.2.11 Determination of Leaf Anthocyanin (Neff and Chory, 1998)

The fresh material weighing 0.2g was pulverized using liquid nitrogen in a mortar and then mixed well with 2ml of a solution containing 1% hydrochloric acid in methanol. The homogenates were placed in a shaker and incubated at a temperature of 4°C for a duration of 24 hours, while being kept in the dark. After the incubation period, the mixtures were spun at a temperature of 4°C at a speed of 7000 rpm for a

duration of 15 minutes. The spectrophotometer (Shimadzu UV-1900I) was used to detect the absorbance of anthocyanin at wavelengths of 530 nm and 657 nm. The anthocyanin concentration was expressed as A mg/g of fresh tissue.

6.2.12 Determination of Membrane Permeability (Bajji *et al.*, 2002)

The method described by Bajji *et al.* (2002) was used to quantify electrolyte leakage (EL) and assess the membrane permeability of plant tissues. From each treatment, six randomly selected plants were chosen, and three fully developed leaves were obtained from each plant. These leaves were then chopped into segments measuring 1 centimeter in length. The leaf samples were rinsed thrice with distilled water to eliminate any surface impurities, and thereafter transferred to separate vials with stoppers, each holding 10 mL of distilled water. The specimens were placed in a controlled environment with a temperature of 25 °C and subjected to agitation at a speed of 100 revolutions per minute for a duration of 24 hours. The electrical conductivity of the bathing solution (EC1) was measured following incubation. Subsequently, the identical samples were subjected to an autoclave at a temperature of 120 °C for a duration of 20 minutes. Following the cooling of the solution to room temperature, a second measurement of the electrical conductivity (EC2) was taken. The EL was determined by dividing EC1 by EC2 and then expressing the result as a percentage.

$$EL (\%) = \frac{\text{Electrical conductivity of solution before autoclaving}}{\text{Electrical conductivity of solution after autoclaving}} \times 100$$

6.2.13 Determination of Leaf Malondialdehyde (Hodges *et al.*, 1999)

Leaf malondialdehyde (MDA) was measured using 2-thiobarbituric acid (TBA). 100mg fresh material was ground with liquid nitrogen in a mortar. The plant tissue samples were mixed with 2.5 ml of a solution containing 80% ethanol and 20% water. The resulting mixture was then spun in a centrifuge at a force of 3000xg for a duration of 10 minutes. A 1ml portion of the sample was combined with 1 ml of either a solution without TBA or a solution containing TBA in a test tube. The samples were thereafter agitated, subjected to a temperature of 95°C in a water bath for a duration of 25 minutes, cooled, and then centrifuged at a force of 3000xg for a period of 10 minutes. Measurements of the absorbances were taken at wavelengths of 440 nm, 532

nm, and 600 nm using spectrophotometer (Shimadzu UV-1900I). The calculation of malondialdehyde equivalents was performed using the following method-

$$A = [(A_{532+TBA} - A_{600+TBA}) - (A_{532-TBA} - A_{600-TBA})]$$

$$B = [(A_{440+TBA} - A_{600+TBA}) 0.0571]$$

$$\text{MDA equivalents (nmol.ml}^{-1}\text{)} = (A-B/157000) 10^6$$

6.2.14 Determination of Leaf Hydrogen Peroxide (Velikova *et al.*, 2000)

The concentration of hydrogen peroxide (H₂O₂) in fresh plant material was determined. The frozen powder (500 mg) was homogenized directly with 5 ml of 0.1% trichloroacetic acid (TCA). The mixture was subjected to centrifugation at a force of 12,000xg for a duration of 15 minutes. 0.5 ml of the liquid remaining after centrifugation was combined with 0.5 ml of a solution containing 10 millimoles of potassium phosphate buffer with a pH of 7.0, and 1 ml of a solution containing 1 mole of potassium iodide. The blank probe contained a solution of 0.1% TCA without any leaf extract. The reaction was incubated for 1 hour in the absence of light, and the absorbance was measured at a wavelength of 390 nm using (Shimadzu UV-1900I). The measurement of hydrogen peroxide was conducted using an extinction coefficient (ϵ) of 0.28 $\mu\text{M}^{-1} \text{ cm}^{-1}$ and the results were represented as $\mu\text{mol g}^{-1} \text{ FW}$.

6.2.15 Determination of Soluble Sugars

The estimation of total soluble sugars (TSS) in fresh leaves was conducted by following Irigoyen *et al.* (1992). The newly picked leaves (0.5 g) were crushed into a fine powder using liquid nitrogen and homogenized with 5 ml of 95% ethanol (v/v). The insoluble portion of the extract was rinsed two times with 5 ml of 70% ethanol. The soluble fractions were subjected to centrifugation with a force of 3,500 xg for a duration of 10 minutes. The liquid portions were combined for sugar estimation. Add 0.1 ml of the alcoholic extracts to 3 ml of freshly made anthrone reagent. Immerse the mixture in a boiling water bath for 10 minutes. The anthrone reagent was prepared by combining 150 mg of anthrone with 100 ml of 72% (w/v) sulphuric acid, 100 mM Tris-HCl (pH 7.4) solution containing 100 mM MgSO₄, and 0.1 g of PVP-40. The absorbance at 625 nm was measured using a spectrophotometer (Shimadzu UV-1900I)

after the sample had cooled. A standard curve was created using various concentrations of glucose. Based on this standard curve, the final total soluble sugars (TSS) were measured and expressed in milligrams per gram of fresh weight (mg g^{-1} FW).

The concentration of sucrose and fructose was measured using the method of Wu *et al.*, (2015). The dehydrated plant materials were pulverized and filtered using a 0.5 mm sieve. A 50 mg sample of the ground material was placed in a 10 ml centrifugal tube along with 4 ml of 80% ethanol. The mixture was then incubated at a temperature of 80°C for a duration of 40 minutes. After the incubation, the tube was subjected to centrifugation at a force of 2,500xg for a period of 5 minutes. The centrifugal residues were extracted once more using the aforementioned technique, and the supernatants from the two extractions were mixed for carbohydrate tests. The test for sucrose involved combining 0.15 mL of supernatant with 0.15 mL of a solution containing 2 mmol/L of NaOH. This mixture was heated to 100°C for 5 minutes. Then, 2.1 mL of 30% HCl and 0.6 mL of 0.1% resorcinol were added to the mixture, which was further heated to 80°C for 10 minutes. Finally, the absorbance of the assay was measured at 480 nm using sucrose as a reference. The fructose content was determined by analysing a solution containing 0.8 mL of 0.1% resorcinol, 0.4 mL of water, and 0.4 mL of the supernatant. The solution was then heated at a temperature of 80°C for a duration of 10 minutes. The absorbance of the solution was measured at a wavelength of 480 nm using fructose as a reference solution.

6.2.16 Determination of Leaf Proline Content (Bates *et al.*, 1973)

The estimation of Proline (Pro) was conducted using the methodology described by Bates *et al.* (1973). The fresh material was pulverized with liquid nitrogen in a mortar, resulting in 250 mg of ground material. The homogenate powder was combined with 5mL of an aqueous solution of sulfosalicylic acid (3%, w/v) and subsequently centrifuged at 5,000 x g for 5 minutes. The filtrate/supernatant, measuring 2 ml, was combined with 2 ml of glacial acetic acid and 2 ml of acid-ninhydrin reagent. The Acid - Ninhydrin reagent was made by dissolving 1.25 g of ninhydrin in a mixture of 30 ml of Glacial Acetic acid and 20 ml of 6 M orthophosphate. The dissolution process took place at a temperature of 70 °C. The mixture was then incubated at a temperature of 95°C for a duration of 1 hour. Finally,

the reaction was stopped by placing the mixture in an ice bath. The reaction mixture was forcefully agitated with 4mL of toluene for 15-20 seconds. The chromophore present in toluene was extracted from the aqueous phase, heated to a temperature of 25°C, and the absorbance was measured at a wavelength of 520 nm using toluene as a blank in spectrophotometer (Shimadzu UV-1900I).

6.2.17 Determination of Total Phenolic Content (Sarker, 2018)

Total phenolic content was measured by Folin-Ciocalteu assay (Sarker, 2018). 0.1 g of leaves was ground in mortar with liquid nitrogen. The powder was dissolved in 4 ml of 90% methanol and thereafter transferred to a tightly closed bottle. The bottle was then subjected to shaking at a temperature of 80 °C for a duration of 1 hour. The solution was subjected to centrifugation at a speed of 6000 rpm for a duration of 10 minutes. A volume of 50 µl of the leaf extract solution was combined with 1 ml of folin-ciocalteu reagent, which had been previously diluted at a ratio of 1:4 with distilled water. The mixture was then carefully mixed. Distilled water was used to prepare a reagent blank. After a duration of 3 minutes, a volume of 1 ml of sodium carbonate solution (10% NaCO₃) was introduced into the combination, which was then left undisturbed for a period of 1 hour in the absence of light. The spectrophotometer (Shimadzu UV-1900I) was used to measure the absorbance at a wavelength of 760 nm. The concentration of total phenolic compounds in the leaf extracts was quantified using an equation derived from a standard gallic acid graph. The results are reported in mg of gallic acid equivalent (GAE) per gram of fresh weight (mg GAE g⁻¹).

6.2.18 Determination of Superoxide Dismutase (Dhindsa *et al.*, 1981)

The measurement of superoxide dismutase (SOD) (EC 1.15.1.1) activity in leaves was conducted using the procedure outlined by Dhindsa *et al.* (1981). The process relies on the creation of a blue complex called formazan, which occurs when nitro-blue tetrazolium reacts with the O₂^{•-} radical. The reduction in absorbance for this blue-colored compound is measured at a wavelength of 560 nm. 0.2 g of fresh plant leaves was ground with liquid nitrogen and homogenized with 2 ml of extraction buffer. The extraction buffer consisted of phosphate buffer (0.1 M at pH 7.5) containing EDTA (0.5 mM). The sample was subjected to centrifugation at a force of 15,000xg for a duration of 10 minutes at a temperature of 4°C. 0.03 ml of enzyme

extract was introduced into the reaction mixture, which consisted of phosphate buffer (50 mM at pH 7.8), methionine (13.33 mM), sodium carbonate (50 mM), EDTA (0.1 mM), 75uM NBT, and 0.95 ml water. The reaction was started by adding riboflavin (2 µM) and the test tubes were exposed to a 15 W fluorescent light for 15 minutes. By turning off the light and placing the tubes in darkness, the reaction was halted. A reaction mixture that had not been exposed to light was used as a blank. The absorbance was measured at a wavelength of 560 nm using (Shimadzu UV-1900I). One unit of enzyme activity was defined as the amount of enzyme that caused a 50% decrease in absorbance compared to tubes without enzyme. The percentage inhibition of NBT reduction by SOD is calculated using the following formula:

$$\text{Percentage Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

The measurement of SOD activity is expressed as units per milligram of protein (U/g FW) in the enzyme extract and can be calculated using the following formula:

$$\text{SOD Activity} = \frac{\text{SOD units/ml} \times \text{volume of extract}}{\text{Fres weigh of tissue}}$$

6.2.19 Determination of Catalase (Aebi, 1984)

The activity of the enzyme catalase (CAT, EC 1.11.1.6) was examined using the method described by Aebi (1984) in freshly harvested leaves. 0.2 g of fresh plant leaves was ground with liquid nitrogen and homogenized with 2 ml of extraction buffer. The extraction buffer comprised of a phosphate buffer with a concentration of 0.1 M at a pH of 7.5, supplemented with EDTA at a concentration of 0.5 mM. 10 µl of enzyme was added to a reaction mixture consisting of potassium phosphate buffer (50 mM), hydrogen peroxide (12.5 mM), and demineralized water to achieve a total volume of 3 ml. The reaction was initiated by adding hydrogen peroxide (H₂O₂), and the decrease in absorbance was measured every 15 seconds at a wavelength of 240 nm for a duration of 1 minute. The enzyme activity was determined by quantifying the decomposition of H₂O₂ using the extinction coefficient ($\epsilon = 0.0436 \text{ mM}^{-1} \text{ cm}^{-1}$).

6.2.20 Statistical Analysis

All statistical analyses were performed using Python 3.13 within a Jupyter Notebook environment to evaluate the effects of rice variety, NaCl concentrations, and mycorrhizal treatment on growth, physiological, and biochemical parameters. Descriptive statistics, including means, standard errors, and ranges, were calculated for each dependent variable across treatment groups. Multivariate Analysis of Variance (MANOVA) was conducted to assess the combined effects of the independent variables on a set of 20 dependent variables related to growth, physiological, and biochemical responses. In addition, three-way Univariate ANOVA was used to examine the individual and interactive effects of rice variety, NaCl concentration, and mycorrhizal treatment on each dependent variable. Principal Component Analysis (PCA) was applied to reduce the dimensionality of the dataset and identify key variables contributing to variation in salt stress tolerance. Cluster analysis was used to group treatment combinations based on similarities in their overall response profiles. Multivariate correlation analysis was also performed to explore relationships among the dependent variables. All statistical tests were conducted at a 5% significance level ($\alpha = 0.05$). Results are expressed as mean $\pm$ standard error, and statistical significance was considered at $p < 0.05$.

6.3 Results

6.3.1 Plant Growth Performances

6.3.1.1 Plant Height

The average height of rice plants in the experiment was 68.04 cm, with a standard deviation of 14.82 cm (Figure 6.3). The highest mean plant height (97cm) was observed in the Changkawi variety with 0 mM salt treatment and AM5 mycorrhizal inoculation. In contrast, the lowest mean height (41cm) was recorded in the Dhan 50 variety with 150 mM salt treatment and no mycorrhizal inoculation (NM).

Multivariate analysis of variance (MANOVA) indicated that the combined effect of salt treatment, rice variety, and mycorrhizal treatment was statistically significant (Table 6.1).

The three-way ANOVA results indicate that salt treatment, rice variety, and mycorrhizal treatment independently have significant effects on the height of rice plants (Table 6.2). The combination of salt treatment and rice variety has a near-significant effect on height. However, no significant interactions were observed between the other combinations of factors.

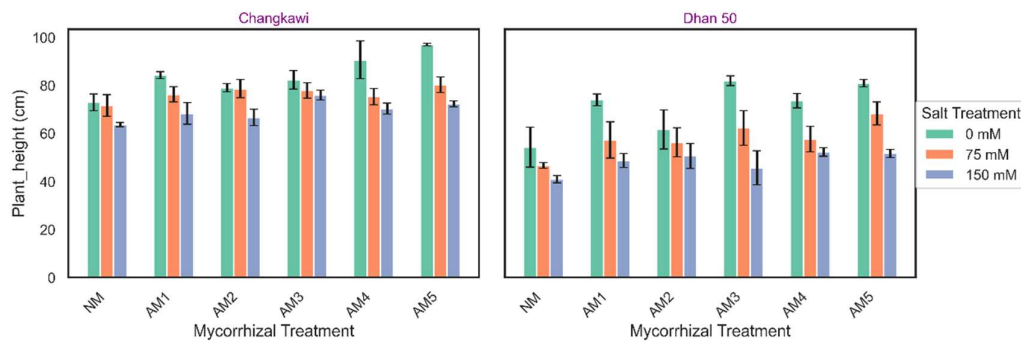


Figure 6.3: Plant height of two rice varieties (Changkawi and Dhan 50) under different salt and mycorrhizal treatments.

PCA results showed that height was closely associated with the first principal component (PC1), which explained 45.40% of the total variance (Figure 6.23). Height contributed negatively to PC1, along with other stress-related traits such as proline and MDA (Figure 6.24). Plants with greater height, particularly those inoculated with AM5 mycorrhiza and subjected to 0 mM salt, clustered on the negative side of PC1. The second principal component (PC2), explaining 14.39% of the variance, captured variations related to mycorrhizal treatments and rice variety, showing that these factors had a more subtle effect on plant height under different environmental conditions.

Cluster analysis using K-means identified three distinct clusters based on growth, physiological, and biochemical traits, with height playing a significant role in defining these clusters (Figure 6.25). The first cluster included plants with high heights, primarily in the 0 mM salt treatment, inoculated with AM5 and AM4 mycorrhiza. The second cluster comprised plants with moderate heights, representing a mix of treatments across different salt and mycorrhizal conditions. The third cluster contained plants with low heights, primarily those exposed to higher salt concentrations and minimal mycorrhizal inoculation.

6.3.1.2 Shoot dry biomass

The descriptive analysis of shoot biomass reveals an average biomass of 3.87 g, with a standard deviation of 1.69 g (Figure 6.4). The highest mean shoot biomass was observed in the Dhan 50 variety with 0 mM salt treatment and AM3 mycorrhizal inoculation, where the mean biomass reached 6.36 g. On the other hand, the lowest biomass was recorded in the Changkawi variety with 150 mM salt treatment and no mycorrhizal inoculation (NM), with a mean biomass of 1.38g.

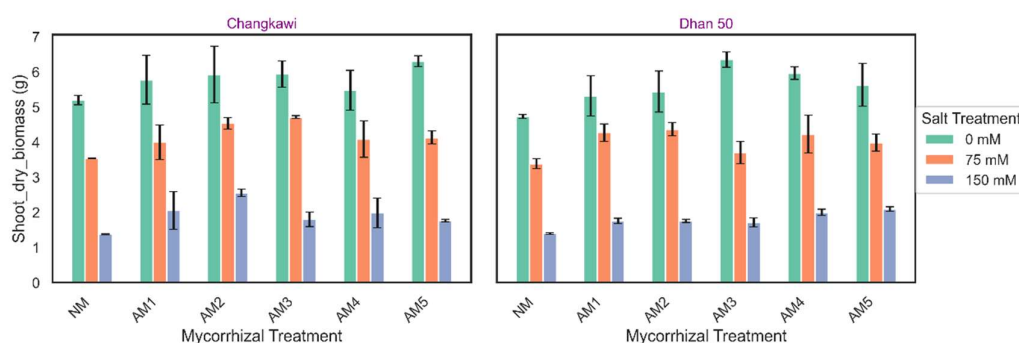


Figure 6.4: Shoot biomass of two rice varieties (Changkawi and Dhan 50) under different salt and mycorrhizal treatments.

Multivariate analysis of variance (MANOVA) showed that Salt Treatment, Rice Variety, and Mycorrhizal Treatment had significant effects on Shoot Dry Matter (DM) along with other biochemical and physiological traits (Table 6.1).

The three-way ANOVA results indicate that salt treatment and mycorrhizal inoculation significantly influence shoot biomass. However, rice variety did not have a significant effect, nor did the interactions between the factors. These findings suggest that biomass production is more strongly influenced by the presence of mycorrhiza and the level of salt stress, with mycorrhizal inoculation helping mitigate the negative effects of salt.

PCA results revealed that shoot dry biomass was closely associated with the first principal component (PC1), which explained 45.40% of the total variance. Shoot DM contributed positively to PC1, along with other growth-related traits such as height and relative water content. Plants inoculated with AM5 and AM4, particularly under well-watered conditions, clustered on the positive side of PC1. The second

principal component (PC2), which explained 14.39% of the variance, captured variations related to mycorrhizal treatments and salt stress.

Cluster analysis shows that the first cluster included plants with high shoot biomass, primarily from well-watered conditions and those inoculated with AM5 and AM4 mycorrhiza. The second cluster consisted of plants with moderate shoot DM, representing a mix of treatments across both salt and mycorrhizal conditions. The third cluster contained plants with low shoot DM, primarily from high salt stress treatments with limited mycorrhizal inoculation

6.3.1.3 Specific Leaf Area (SLA)

Among the various treatment groups, the highest mean SLA was observed in the treatment group with 0 mM salt, Dhan 50 variety, and AM2 mycorrhizal inoculation, with a mean SLA of 48.35 mm²/mg (Figure 6.5). On the other hand, the lowest mean SLA was recorded in the 150 mM salt, Changkawi variety, and NM (no mycorrhizal) treatment group, with a mean SLA of 20.84 mm²/mg. This indicates that low salt conditions (0 mM) combined with Dhan 50 variety and AM2 inoculation enhance SLA, whereas high salt conditions (150 mM) significantly reduce SLA, especially in the Changkawi variety without mycorrhizal inoculation.

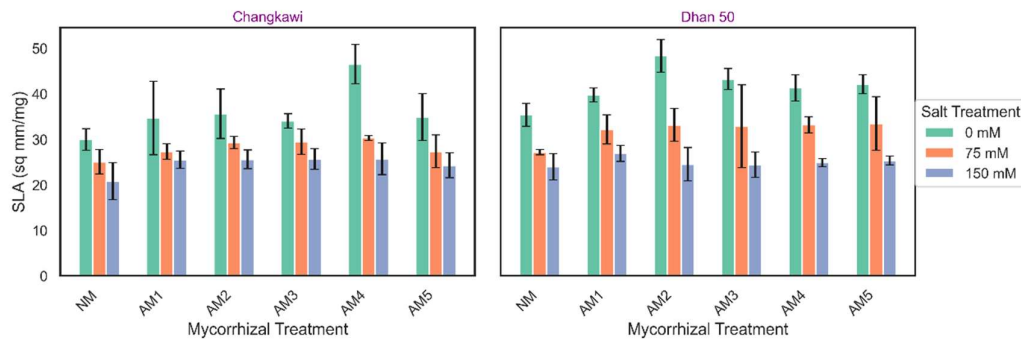


Figure 6.5: Specific Leaf Area of two rice varieties (Changkawi and Dhan 50) under different salt and mycorrhizal treatments.

Multivariate analysis of variance (MANOVA) showed that Salt Treatment, Rice Variety, and Mycorrhizal Treatment significantly affected SLA along with other growth, physiological and biochemical traits (Table 6.1).

The three-way ANOVA results demonstrate that while salt treatment, rice variety, and mycorrhizal treatment independently impacted SLA, the interactions between these factors were not significant (Table 6.2).

PCA results revealed that SLA was closely associated with the second principal component (PC2), which explained 14.39% of the total variance. SLA contributed positively to PC2, along with other traits related to plant morphology and leaf characteristics (Figure 6.24).

In cluster analysis, the first cluster included plants with high SLA (Figure 6.25), primarily from 0 mM salt treatments inoculated with AM5 and AM4 mycorrhiza, and exhibited larger, thinner leaves. The second cluster consisted of plants with moderate SLA, representing a mix of treatments with moderate salt stress and various mycorrhizal inoculations. The third cluster contained plants with low SLA, primarily from 50 mM and 100 mM salt treatments with limited mycorrhizal inoculation.

6.3.1.4 Leaf Dry Matter Content (LDMC)

Among the various treatment combinations, the highest mean LDMC was observed in the treatment group with 150 mM salt, Dhan 50 variety, and no mycorrhizal inoculation (NM), with a mean LDMC of 41.68 mg/g (Figure 6.6). On the other hand, the lowest mean LDMC was recorded in the treatment group with 0 mM salt, Dhan 50 variety, and AM4 mycorrhizal inoculation, with a mean LDMC of 17.35mg/g. These results suggest that high salt concentrations, particularly in the absence of mycorrhizal inoculation, can increase the dry matter content of leaves, while AM4 inoculation under no salt stress resulted in lower LDMC values.

MANOVA demonstrated that Salt Treatment, Rice Variety, and Mycorrhizal Treatment had significant multivariate effects on LDMC alongside other growth, physiological and biochemical traits (Table 6.1).

The three-way ANOVA results indicate that salt treatment and mycorrhizal inoculation independently affected LDMC (Table 6.2). The interactions between Salt

Treatment and Rice Variety, Salt Treatment and Mycorrhizal Treatment, and Rice Variety and Mycorrhizal Treatment were not statistically significant. The three-way interaction among salt treatment, rice variety, and mycorrhizal inoculation was also not significant.

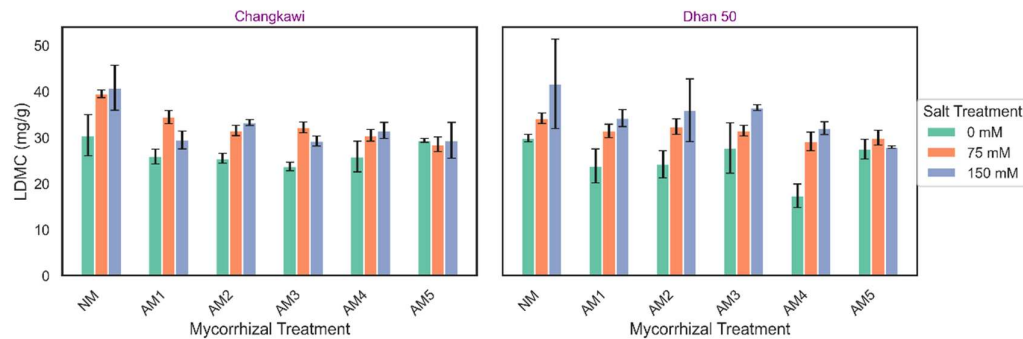


Figure 6.6: Leaf dry matter content of two rice varieties (Changkawi and Dhan 50) under different salt and mycorrhizal treatments.

PCA results revealed that LDMC was strongly associated with the second principal component (PC2), which explained 14.39% of the total variance (Figure 6.24). LDMC contributed positively to PC2, along with traits related to leaf structural integrity and stress tolerance. Plants with moderate LDMC values, particularly those inoculated with AM5 mycorrhiza, clustered along the positive side of PC2.

In cluster analysis, the first cluster contained plants with high LDMC, primarily from the 100 mM salt treatment and those with limited mycorrhizal inoculation (Figure 6.25). These plants exhibited thicker, more rigid leaves. The second cluster consisted of plants with moderate LDMC, which included those inoculated with AM5 and subjected to moderate salt stress. The third cluster consisted of plants with low LDMC, primarily from well-watered conditions with optimal mycorrhizal inoculation, and had more flexible leaf structures.

6.3.2 Plant Physiological Responses

6.3.2.1 Leaf Relative Water Content

The descriptive statistics for Relative Water Content (RWC) across different treatment groups revealed variability (Figure 6.7). The overall mean RWC was

76.48%, with a standard deviation of 12.73%. Among the treatment groups, the highest mean RWC was observed in plants subjected to 0 mM salt, Dhan 50 rice variety, and AM5 mycorrhizal inoculation, with a mean RWC of 95.40%. In contrast, the lowest mean RWC was recorded in plants subjected to 150 mM salt, Changkawi rice variety, and no mycorrhizal inoculation (NM), with a mean RWC of 52.24%.

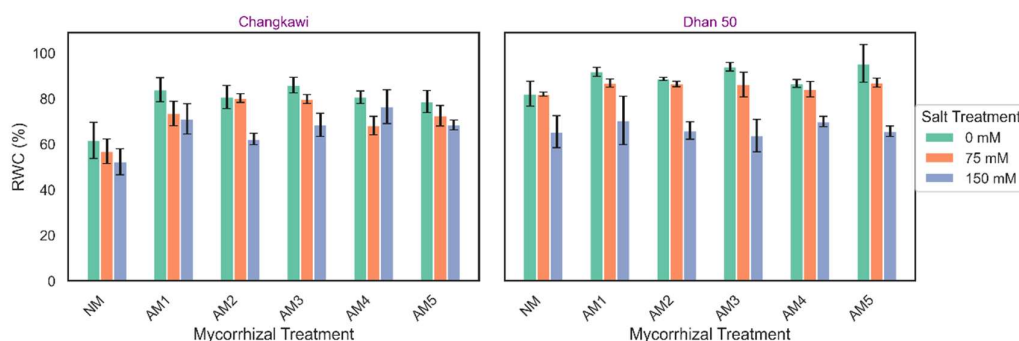


Figure 6.7: Leaf relative water content of two rice varieties (Changkawi and Dhan 50) under different salt and mycorrhizal treatments.

MANOVA results showed that Salt Treatment, Rice Variety, and Mycorrhizal Treatment significantly affected RWC along with other biochemical and physiological traits (Table 6.1).

The three-way ANOVA results indicate that salt treatment, rice variety and mycorrhizal inoculation independently affected RWC (Table 6.2). The interactions between Salt Treatment and Rice Variety was statistically significant. The three-way interaction among salt treatment, rice variety, and mycorrhizal inoculation was not significant.

PCA results showed that RWC was closely associated with the first principal component (PC1), which explained 45.40% of the total variance (Figure 6.24). RWC contributed positively to PC1, along with other growth-related traits such as height and shoot biomass. Plants inoculated with AM5 and AM4 under well-watered conditions clustered positively along PC1. The second principal component (PC2), explaining 14.96% of the variance, was more associated with leaf morphology traits.

Cluster analysis showed that the first cluster consisted of plants with high RWC, primarily from well-watered conditions inoculated with AM5 and AM4 mycorrhiza and these plants had the highest water retention (Figure 6.25). The second

cluster contained plants with moderate RWC, representing plants subjected to moderate salt stress or less effective mycorrhizal inoculation. These plants managed to retain some water, although not as much as those in the first cluster. The third cluster included plants with low RWC, mainly from high salt stress treatments with minimal mycorrhizal inoculation.

6.3.2.2 Electrolyte Leakage (EL)

Among the treatment combinations, the highest mean EL was recorded in plants subjected to 150 mM salt, Changkawi rice variety, and no mycorrhizal inoculation (NM), with a mean EL of 36.61% (Figure 6.8). Conversely, the lowest mean EL was observed in the group treated with 0 mM salt, Changkawi variety, and AM5 mycorrhizal inoculation, where the mean EL was 18.76%.

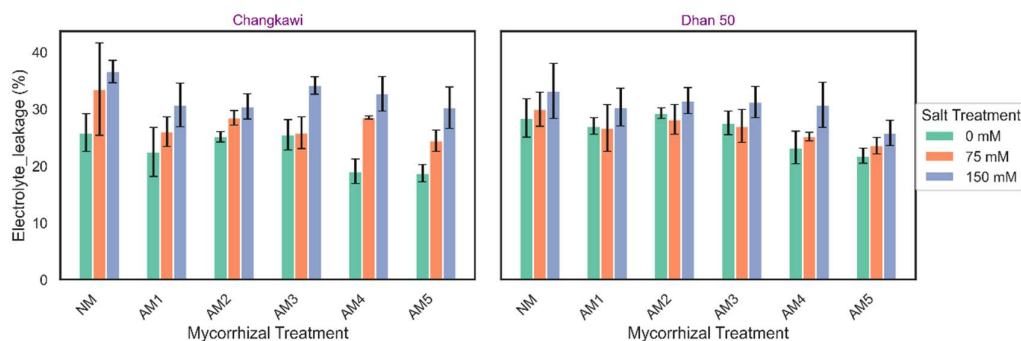


Figure 6.8: Electrolyte leakage from the leaves of two rice varieties (Changkawi and Dhan 50) under different salt and mycorrhizal treatments.

MANOVA results indicated that Salt Treatment, Rice Variety, and Mycorrhizal Treatment significantly affected EL and other physiological and biochemical traits (Table 6.1).

The three-way ANOVA revealed that salt treatment and mycorrhizal inoculation had significant independent effects on Electrolyte Leakage. However, the rice variety did not significantly influence EL. The lack of significant interactions indicates that the effects of salt stress and mycorrhizal inoculation on EL were largely independent of the rice variety.

PCA results revealed that EL was strongly associated with the first principal component (PC1), which explained 45.40% of the total variance (Figure 6.23). EL contributed positively to PC1 (Figure 6.24), along with other stress-related traits such

as proline and malondialdehyde (MDA). Plants inoculated with AM5 and subjected to lower salt concentrations clustered toward the negative side of PC1.

Cluster analysis showed that the first cluster contained plants with high EL (Figure 6.25), primarily from the 100 mM salt treatment and plants with minimal mycorrhizal inoculation, and these plants exhibited severe membrane damage. The second cluster included plants with moderate EL, representing those subjected to 50 mM salt or inoculated with moderate levels of mycorrhiza. The third cluster consisted of plants with low EL, mainly from 0 mM salt treatments inoculated with AM5 and AM4.

6.3.2.3 Chlorophyll *a*

The overall mean chlorophyll *a* was 1.72 mg/g, with a standard deviation of 0.62 mg/g (Figure 6.9). The highest mean chlorophyll *a* was observed in plants treated with 0 mM salt, Changkawi rice variety, and AM5 mycorrhizal inoculation, where the mean chlorophyll *a* was 3.08 mg/g. The lowest mean chlorophyll *a* was recorded in plants subjected to 150 mM salt, Changkawi rice variety, and no mycorrhizal inoculation (NM), with a mean chlorophyll *a* of 0.71 mg/g.

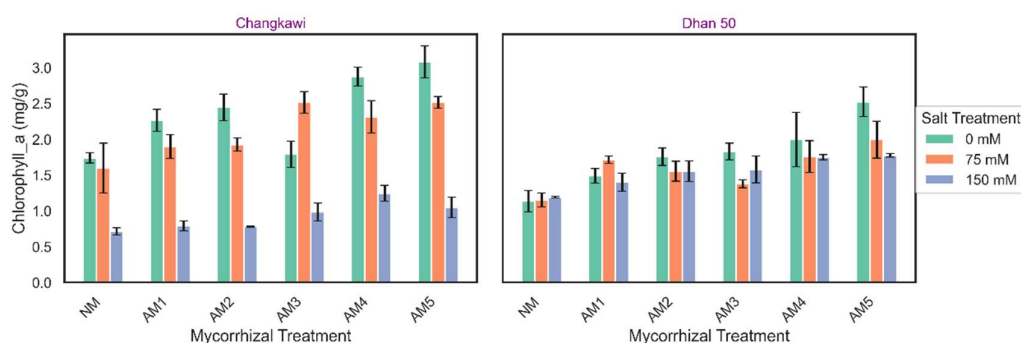


Figure 6.9: Chlorophyll *a* content of two rice varieties (Changkawi and Dhan 50) under different salt and mycorrhizal treatments.

MANOVA indicated that Salt Treatment, Rice Variety, and Mycorrhizal Treatment significantly influenced chlorophyll *a* along with other physiological and biochemical traits (Table 6.1).

The three-way ANOVA results show that salt treatment, rice variety and mycorrhizal inoculation had independent significant effect on chlorophyll content

(Table 6.2). Significant interactions were observed between Salt Treatment $\times$ Rice Variety and Salt Treatment $\times$ Mycorrhizal Inoculation. The three-way interaction was close to significance.

PCA results showed that chlorophyll *a* was closely associated with the first principal component (PC1), which explained 45.40% of the total variance (Figure 6.23). Chlorophyll *a* contributed positively to PC1 (Figure 6.24), along with other growth-related traits such as height and shoot biomass. Plants inoculated with AM5 and subjected to lower salt concentrations clustered positively along PC1. The second principal component (PC2), explaining 14.39% of the variance, captured variations in physiological responses related to leaf structure and pigment concentration.

Cluster analysis showed that the first cluster contained plants with high chlorophyll *a* (Figure 6.25), primarily from 0 mM salt treatments inoculated with AM5 and AM4. These plants had higher photosynthetic capacity and healthier overall growth. The second cluster included plants with moderate chlorophyll *a*, representing a mix of treatments with moderate salt stress and various mycorrhizal inoculations. The third cluster consisted of plants with low chlorophyll *a*, mainly from high salt treatments with minimal mycorrhizal inoculation.

6.3.2.4 Chlorophyll *b*

The overall mean chlorophyll *b* was 0.53 mg/g, with a standard deviation of 0.17 mg/g (Figure 6.10). The highest mean chlorophyll *b* was recorded in plants subjected to 150 mM salt, Dhan 50 rice variety, and AM5 mycorrhizal inoculation, with a mean chlorophyll *b* of 0.84 mg/g. On the other hand, the lowest mean chlorophyll *b* was observed in plants subjected to 150 mM salt, Changkawi rice variety, and no mycorrhizal inoculation (NM), with a mean chlorophyll *b* of 0.22 mg/g.

MANOVA revealed that Salt Treatment, Rice Variety, and Mycorrhizal Treatment significantly influenced chlorophyll *b* along with other physiological and biochemical traits (Table 6.1).

The three-way ANOVA results demonstrate that Salt treatment, rice variety and mycorrhizal treatment significantly influenced chlorophyll *b* content (Table 6.2). The interaction between salt treatment and rice variety was highly significant. The three-

way interaction between salt treatment, rice variety, and mycorrhizal inoculation was also significant.

PCA results showed that chlorophyll *b* was closely associated with the first principal component (PC1), which explained 45.40% of the total variance (Figure 6.23). Chlorophyll *b* contributed positively to PC1 (Figure 6.24), along with other growth-related traits such as height and shoot biomass. Plants inoculated with AM5 and subjected to lower salt concentrations clustered positively along PC1.

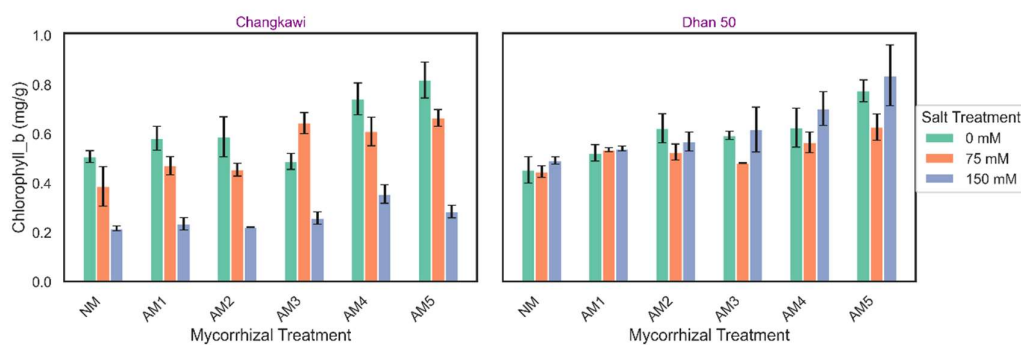


Figure 6.10: Chlorophyll *b* content of two rice varieties (Changkawi and Dhan 50) under different salt and mycorrhizal treatments.

Cluster analysis showed that the first cluster consisted of plants with high chlorophyll *b* (Figure 6.25), primarily from 0 mM salt treatments inoculated with AM5 and AM4 mycorrhiza, and these plants exhibited higher photosynthetic efficiency. The second cluster included plants with moderate chlorophyll *b*, representing plants subjected to moderate salt stress or lower levels of mycorrhizal inoculation. The third cluster contained plants with low chlorophyll *b*, mainly from high salt treatments with minimal mycorrhizal inoculation.

6.3.2.5 Total Chlorophyll

The overall mean total chlorophyll content was 2.25 mg/g, with a standard deviation of 0.77 mg/g (Figure 6.11). The highest mean total chlorophyll was observed in plants subjected to 0 mM salt, Changkawi rice variety, and AM5 mycorrhizal inoculation, with a mean total chlorophyll of 3.90 mg/g. The lowest mean total chlorophyll was recorded in plants treated with 150 mM salt, Changkawi rice variety, and no mycorrhizal inoculation (NM), with a mean of 0.93 mg/g.

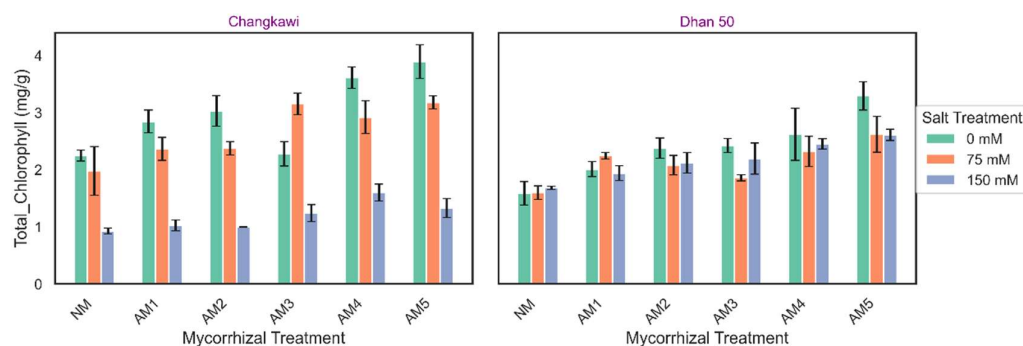


Figure 6.11: Total chlorophyll content of two rice varieties (Changkawi and Dhan 50) under different salt and mycorrhizal treatments.

MANOVA) revealed that Salt Treatment, Rice Variety, and Mycorrhizal Treatment significantly influenced total chlorophyll alongside other physiological and biochemical traits (Table 6.1).

The three-way ANOVA results indicate that salt treatment and mycorrhizal inoculation had a highly significant effect on total chlorophyll content (Table 6.2). The interaction between salt treatment and rice variety, salt treatment $\times$ mycorrhizal inoculation was significant. The three-way interaction approached significance.

PCA results revealed that total chlorophyll was strongly associated with the first principal component (PC1), which explained 45.40% of the total variance (Figure 6.23). Total chlorophyll content contributed positively to PC1 (Figure 6.24), along with other growth-related traits such as height, shoot biomass. Plants inoculated with AM4 and AM5 and under lower salt concentrations clustered along the positive side of PC1.

The first cluster contained plants with high total chlorophyll content, primarily from 0 mM salt treatments inoculated with AM5 and AM4 (Figure 6.25). These plants had better photosynthetic capacity and overall health. The second cluster consisted of plants with moderate total chlorophyll content, representing plants subjected to moderate salt stress or plants with less effective mycorrhizal inoculation. The third cluster included plants with low total chlorophyll content, mainly from high salt treatments and plants with minimal mycorrhizal inoculation.

6.3.2.6 Carotenoids

The descriptive statistics for carotenoid content across different treatment groups revealed significant variation. The overall mean carotenoid content was 0.65 mg/g, with a standard deviation of 0.29 mg/g (Figure 6.12). The highest mean carotenoid content was observed in plants treated with 0 mM salt, Changkawi rice variety, and AM5 mycorrhizal inoculation, with a mean carotenoid content of 1.30 mg/g. In contrast, the lowest mean carotenoid content was recorded in plants subjected to 150 mM salt, Changkawi rice variety, and no mycorrhizal inoculation (NM), with a mean of 0.24 mg/g.

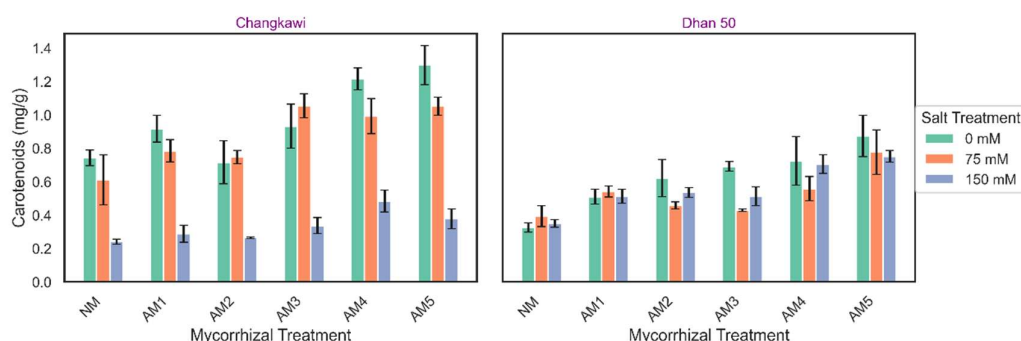


Figure 6.12: Carotenoids content of two rice varieties (Changkawi and Dhan 50) under different salt and mycorrhizal treatments.

MANOVA revealed that Salt Treatment, Rice Variety, and Mycorrhizal Treatment significantly influenced carotenoid levels along with other physiological and biochemical traits (Table 6.1).

The three-way ANOVA results show that salt treatment, rice variety and mycorrhizal inoculation had significant independent effects on carotenoid content (Table 6.2). The significant interaction between salt treatment and rice variety suggests that the two rice varieties responded differently to salt stress in terms of carotenoid content.

PCA results revealed that carotenoid content was closely associated with the first principal component (PC1), which explained 45.40% of the total variance (Figure 6.23). Carotenoid content contributed positively to PC1, along with other growth and

stress-related traits such as shoot biomass and relative water content. The second principal component (PC2), which explained 14.39% of the variance, was more associated with traits related to leaf structure and pigment retention.

Cluster analysis revealed that the first cluster consisted of plants with high carotenoid levels, primarily from 0 mM salt treatments inoculated with AM5 and AM4 mycorrhiza (Figure 6.25). These plants showed better photosynthetic protection and oxidative stress management. The second cluster included plants with moderate carotenoid levels, representing plants subjected to moderate salt stress or plants with less effective mycorrhizal inoculation. The third cluster contained plants with low carotenoid levels, primarily from high salt treatments with minimal mycorrhizal inoculation.

6.3.2.7 Total Soluble Sugars

The highest mean Total Soluble Sugars (TSS) content was recorded in plants subjected to 75 mM salt, Dhan 50 rice variety, and no mycorrhizal inoculation (NM), with a mean TSS of 98.94 mg/g (Figure 6.13). In contrast, the lowest mean TSS was observed in plants treated with 150 mM salt, Dhan 50 variety, and AM4 mycorrhizal inoculation, with a mean of 44.65 mg/g. These results highlight that intermediate salt stress (75 mM) in Dhan 50 without mycorrhiza increases soluble sugar levels, while high salt concentrations reduce sugar levels, even in combination with AM4.

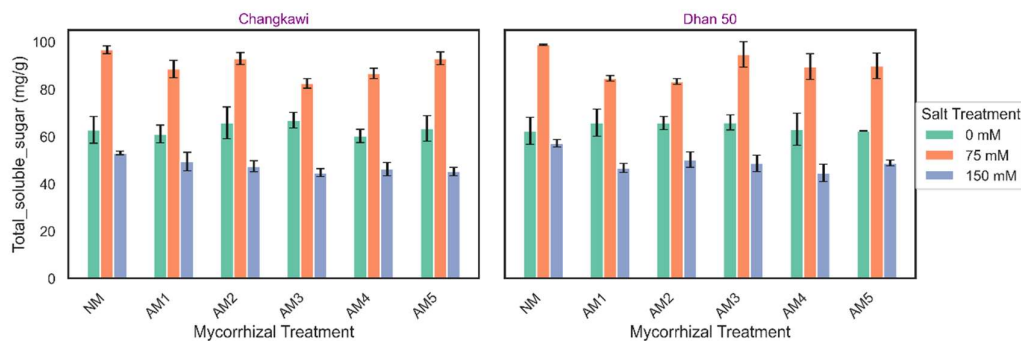


Figure 6.13: Total soluble sugar content of two rice varieties (Changkawi and Dhan 50) under different salt and mycorrhizal treatments.

MANOVA revealed that Salt Treatment, Rice Variety, and Mycorrhizal Treatment significantly influenced TSS along with other physiological and biochemical traits (Table 6.1).

The three-way ANOVA results demonstrate that salt treatment and mycorrhizal inoculation significantly affect total soluble sugar content (Table 6.3). No significant interactions between salt treatment, rice variety, and mycorrhizal inoculation were observed.

PCA results revealed that TSS was strongly associated with the first principal component (PC1), which explained 45.40% of the total variance (Figure 6.23). TSS contributed positively to PC1 (Figure 6.24), along with other stress-related traits such as proline and malondialdehyde (MDA).

Cluster analysis revealed that the first cluster included plants with high TSS levels, primarily from 100 mM salt treatments inoculated with AM5 and AM4. These plants exhibited enhanced osmotic adjustment and stress tolerance. The second cluster contained plants with moderate TSS levels, representing plants subjected to 50 mM salt or plants with moderate mycorrhizal inoculation. The third cluster consisted of plants with low TSS levels, primarily from 0 mM salt treatments with minimal mycorrhizal inoculation.

6.3.2.8 Sucrose

The overall mean sucrose content was 3.21 mg/g, with a standard deviation of 0.96 mg/g (Figure 6.14). The highest mean sucrose content was recorded in plants subjected to 75 mM salt, Changkawi rice variety, and AM3 mycorrhizal inoculation, with a mean of 5.81 mg/g. Conversely, the lowest mean sucrose content was observed in plants treated with 150 mM salt, Changkawi rice variety, and no mycorrhizal inoculation (NM), with a mean of 1.35 mg/g. These findings indicate that moderate salt stress combined with AM3 inoculation enhances sucrose accumulation, while high salt stress in the absence of mycorrhiza drastically reduces sucrose levels.

MANOVA revealed that Salt Treatment, Rice Variety, and Mycorrhizal Treatment significantly influenced sucrose levels along with other physiological and biochemical traits (Table 6.2).

The three-way ANOVA results show that salt treatment significantly affects sucrose content (Table 6.3). There is a significant interaction between salt treatment and rice, salt treatment and mycorrhizal inoculation. The three-way interaction between salt treatment, rice variety, and mycorrhizal inoculation was not significant.

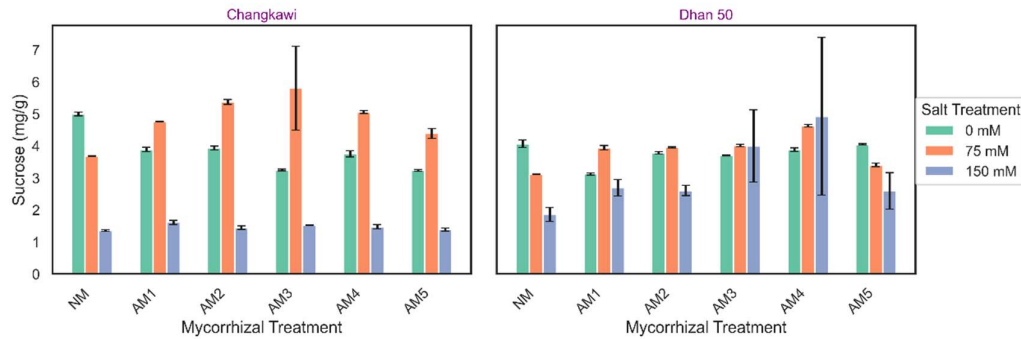


Figure 6.14: Sucrose content of two rice varieties (Changkawi and Dhan 50) under different salt and mycorrhizal treatments.

PCA results revealed that sucrose was closely associated with the first principal component (PC1), which explained 45.40% of the total variance (Figure 6.23). Sucrose contributed positively to PC1 (Figure 6.24), along with other stress-related traits such as proline and malondialdehyde (MDA).

The first cluster consisted of plants with high sucrose levels, primarily from 100 mM salt treatments inoculated with AM5 and AM4 (Figure 6.25). The second cluster contained plants with moderate sucrose levels, representing plants subjected to 50 mM salt or those with less effective mycorrhizal inoculation. The third cluster consisted of plants with low sucrose levels, mainly from 0 mM salt treatments with minimal mycorrhizal inoculation.

6.3.2.9 Fructose

The highest mean fructose content was recorded in plants subjected to 75 mM salt, Dhan 50 rice variety, and AM3 mycorrhizal inoculation, with a mean of 7.36 mg/g (Figure 6.15). The lowest mean fructose content was observed in plants treated with 0 mM salt, Dhan 50 rice variety, and no mycorrhizal inoculation (NM), with a mean of 2.60 mg/g. These findings suggest that moderate salt stress, especially when combined

with AM3 inoculation, promotes fructose accumulation, whereas the absence of salt stress and mycorrhizal inoculation results in lower fructose levels.

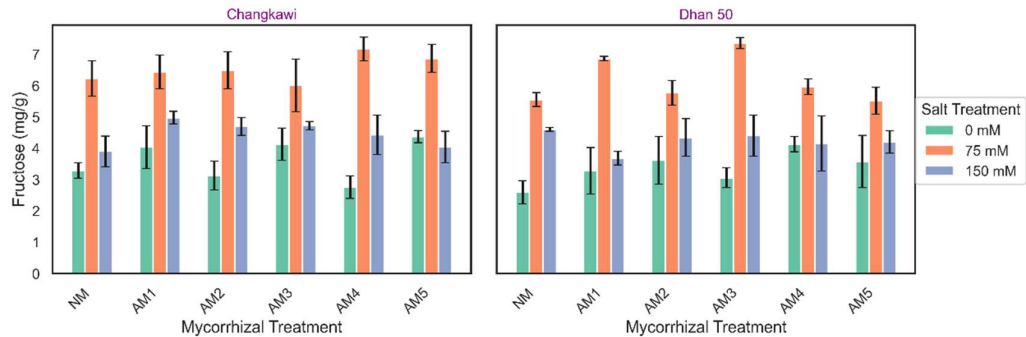


Figure 6.15: Fructose content of two rice varieties (Changkawi and Dhan 50) under different salt and mycorrhizal treatments.

MANOVA revealed that Salt Treatment, Rice Variety, and Mycorrhizal Treatment significantly influenced fructose levels along with other physiological and biochemical traits (Table 6.1).

The three-way ANOVA results demonstrate that salt treatment significantly affects fructose content (Table 6.3). The significant three-way interaction suggests that the combined effects of salt, rice variety, and mycorrhizal inoculation are complex and merit further exploration.

PCA results revealed that fructose was closely associated with the first principal component (PC1), which explained 45.40% of the total variance (Figure 6.23). Fructose contributed positively to PC1 (Figure 6.24), along with other stress-related traits such as proline and MDA. The second principal component (PC2), which explained 14.39% of the variance, captured variations related to growth and physiological traits, showing that fructose accumulation is integrated with overall plant health and metabolic function.

Cluster analysis revealed that the first cluster included plants with high fructose levels, primarily from 100 mM salt treatments inoculated with AM5 and AM4 (Figure 6.25). These plants exhibited strong osmotic adjustment and stress tolerance. The second cluster contained plants with moderate fructose levels, representing plants subjected to 50 mM salt or plants with moderate mycorrhizal inoculation. The third

cluster consisted of plants with low fructose levels, mainly from 0 mM salt treatments with minimal mycorrhizal inoculation.

6.3.3 Plant Biochemical Responses

6.3.3.1 Leaf Proline

The overall mean proline content was 28.45 $\mu\text{mol/g}$, with a standard deviation of 8.19 $\mu\text{mol/g}$ (Figure 6.16). The highest mean proline content was recorded in plants subjected to 150 mM salt, Dhan 50 rice variety, and no mycorrhizal inoculation (NM), with a mean of 47.77 $\mu\text{mol/g}$. Conversely, the lowest mean proline content was observed in plants treated with 0 mM salt, Changkawi rice variety, and AM2 mycorrhizal inoculation, with a mean of 13.52 $\mu\text{mol/g}$.

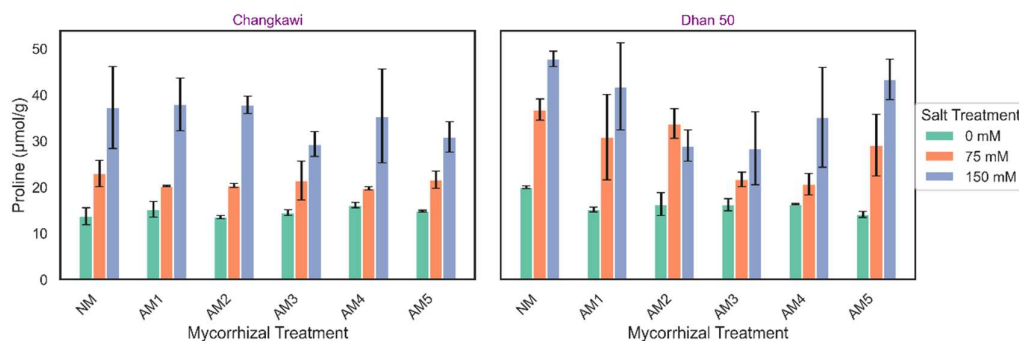


Figure 6.16: Proline content of two rice varieties (Changkawi and Dhan 50) under different salt and mycorrhizal treatments.

MANOVA revealed that Salt Treatment, Rice Variety, and Mycorrhizal Treatment significantly influenced proline levels along with other physiological and biochemical traits (Table 6.1).

The three-way ANOVA results demonstrate that salt treatment and rice variety had a significant independent effect on proline content (Table 6.3). Although mycorrhizal inoculation approached significance, its interaction with salt treatment and rice variety did not significantly impact proline levels. The three-way interaction between salt treatment, rice variety, and mycorrhizal inoculation was also not significant.

PCA results revealed that proline was strongly associated with the first principal component (PC1), which explained 45.40% of the total variance (Figure 6.23). Proline contributed positively to PC1 (Figure 6.24), along with other stress-related traits such as MDA and TSS.

The first cluster consisted of plants with high proline levels, primarily from 100 mM salt treatments inoculated with AM5 and AM4 (Figure 6.25). These plants exhibited enhanced osmotic adjustment and stress tolerance. The second cluster included plants with moderate proline levels, representing plants subjected to 50 mM salt or plants with less effective mycorrhizal inoculation. The third cluster consisted of plants with low proline levels, mainly from 0 mM salt treatments with minimal mycorrhizal inoculation.

6.3.3.2 Anthocyanin

The descriptive statistics for anthocyanin content across different treatment groups showed considerable variation (Figure 6.17). The overall mean anthocyanin content was 1.08 mg/g, with a standard deviation of 0.67 mg/g. The highest mean anthocyanin content was recorded in plants subjected to 0 mM salt, Changkawi rice variety, and AM4 mycorrhizal inoculation, with a mean of 2.34 mg/g. In contrast, the lowest mean anthocyanin content was observed in plants treated with 75 mM salt, Dhan 50 rice variety, and no mycorrhizal inoculation (NM), with a mean of 0.025 mg/g.

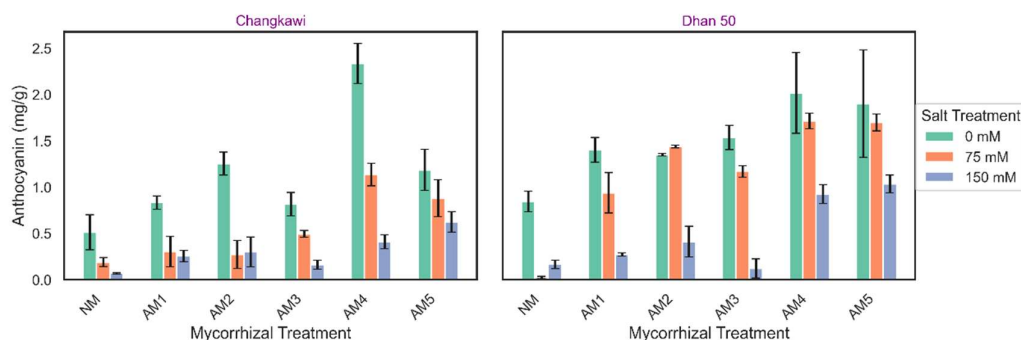


Figure 6.17: Anthocyanin content of two rice varieties (Changkawi and Dhan 50) under different salt and mycorrhizal treatments.

MANOVA revealed that Salt Treatment, Rice Variety, and Mycorrhizal Treatment significantly influenced anthocyanin levels along with other physiological and biochemical traits (Table 6.1).

The three-way ANOVA results show that salt treatment, rice variety and mycorrhizal inoculation had significant independent effect on anthocyanin content (Table 6.3). The interaction between salt treatment and rice variety, salt treatment and mycorrhizal was significant. The three-way interaction between salt treatment, rice variety, and mycorrhizal inoculation was also significant.

PCA results revealed that anthocyanin was strongly associated with the first principal component (PC1), which explained 45.40% of the total variance (Figure 6.23). Anthocyanin contributed positively to PC1 (Figure 6.24), along with other stress-related traits such as proline and MDA. Plants inoculated with AM5 and subjected to higher salt concentrations clustered positively along PC1.

Cluster analysis showed the first cluster consisted of plants with high anthocyanin levels, primarily from 100 mM salt treatments inoculated with AM5 and AM4 (Figure 6.25). These plants exhibited enhanced antioxidant defenses. The second cluster contained plants with moderate anthocyanin levels, representing plants subjected to 50 mM salt or those with moderate mycorrhizal inoculation. The third cluster consisted of plants with low anthocyanin levels, mainly from 0 mM salt treatments with minimal mycorrhizal inoculation.

6.3.3.3 Catalase

The overall mean catalase activity was 1.01 U/min/mg fresh weight, with a standard deviation of 0.22 U/min/g fresh weight (Figure 6.18). The highest mean catalase activity was recorded in plants subjected to 150 mM salt, Dhan 50 rice variety, and AM3 mycorrhizal inoculation, with a mean of U/min/g fresh weight. Conversely, the lowest mean catalase activity was observed in plants treated with 0 mM salt, Changkawi rice variety, and no mycorrhizal inoculation (NM), with a mean of 0.64 U/min/g fresh weight.

MANOVA revealed that Salt Treatment, Rice Variety, and Mycorrhizal Treatment significantly influenced catalase activity alongside other physiological and biochemical traits (Table 6.1).

The three-way ANOVA results show that salt treatment and mycorrhizal inoculation significantly influenced catalase activity (Table 6.3). However, the interactions between salt treatment, rice variety, and mycorrhizal inoculation were not significant, suggesting that these factors largely operate independently in their effects on catalase activity.

PCA results revealed that catalase was strongly associated with the first principal component (PC1), which explained 45.40% of the total variance (Figure 6.23). Catalase activity contributed positively to PC1, along with other stress-related traits. Plants inoculated with AM5 and subjected to higher salt concentrations clustered positively along PC1.

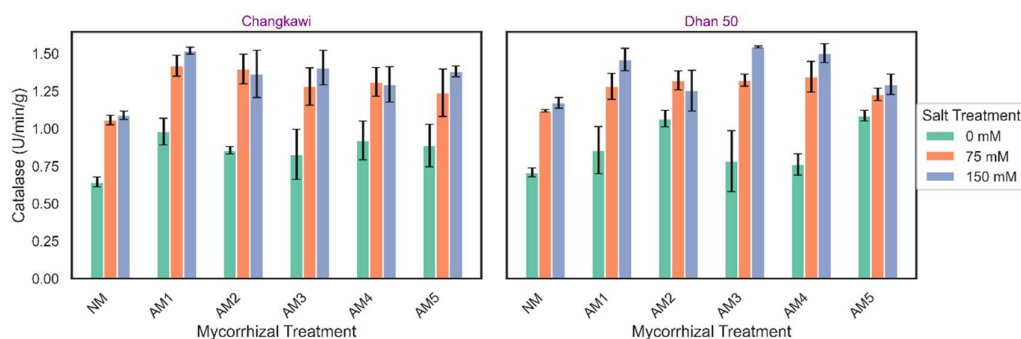


Figure 6.18: Catalase activity of two rice varieties (Changkawi and Dhan 50) under different salt and mycorrhizal treatments.

Cluster analysis showed the first cluster consisted of plants with high catalase activity, primarily from 100 mM salt treatments inoculated with AM5 and AM4 (Figure 6.25), and these plants exhibited strong antioxidant defenses. The second cluster contained plants with moderate catalase activity, representing plants subjected to 50 mM salt or those with moderate mycorrhizal inoculation. The third cluster consisted of plants with low catalase activity, mainly from 0 mM salt treatments with minimal mycorrhizal inoculation.

6.3.3.4 Superoxide Dismutase (SOD)

The highest mean SOD activity was recorded in plants subjected to 150 mM salt, Changkawi rice variety, and AM5 mycorrhizal inoculation, with a mean of 14.70 U/g. Conversely, the lowest mean SOD activity was observed in plants treated with 0

mM salt, Changkawi rice variety, and AM4 mycorrhizal inoculation, with a mean of 4.35 U/g (Figure 6.19). High salt stress induces higher SOD activity, particularly in Changkawi with AM5 inoculation, while non-saline conditions with AM4 inoculation result in lower SOD activity.

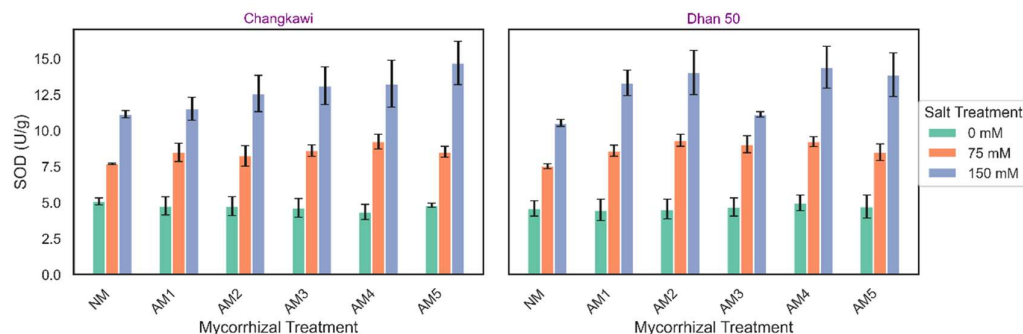


Figure 6.19: SOD activity of two rice varieties (Changkawi and Dhan 50) under different salt and mycorrhizal treatments.

MANOVA revealed that Salt Treatment, Rice Variety, and Mycorrhizal Treatment significantly influenced SOD activity along with other physiological and biochemical traits (Table 6.1).

The three-way ANOVA results show that salt treatment and mycorrhizal inoculation significantly influenced SOD activity (Table 6.3). However, the interactions between salt treatment, rice variety, and mycorrhizal inoculation were not significant.

PCA results revealed that SOD was strongly associated with the first principal component (PC1), which explained 45.40% of the total variance (Figure 6.23). SOD activity contributed positively to PC1, along with other stress-related traits (Figure 6.24).

Cluster analysis revealed the first cluster consisted of plants with high SOD activity, primarily from 100 mM salt treatments inoculated with AM5 and AM4 (Figure 6.25). These plants exhibited strong antioxidant defenses. The second cluster contained plants with moderate SOD activity, representing plants subjected to 50 mM salt or those with moderate mycorrhizal inoculation. The third cluster consisted of plants with low SOD activity, mainly from 0 mM salt treatments with minimal mycorrhizal inoculation.

6.3.3.4 Malondialdehyde (MDA)

The overall mean MDA content was 125.43 nmol/g, with a standard deviation of 45.87 nmol/g (Figure 6.20). The highest mean MDA content was observed in plants subjected to 150 mM salt, Changkawi rice variety, and no mycorrhizal inoculation (NM), with a mean of 225.35 nmol/g. The lowest mean MDA content was recorded in plants treated with 0 mM salt, Dhan 50 rice variety, and AM4 mycorrhizal inoculation, with a mean of 35.27 nmol/g. High salt stress increases lipid peroxidation, particularly in Changkawi without mycorrhizal inoculation, while Dhan 50 under non-saline conditions with AM4 inoculation shows reduced MDA levels, indicating lower oxidative stress.

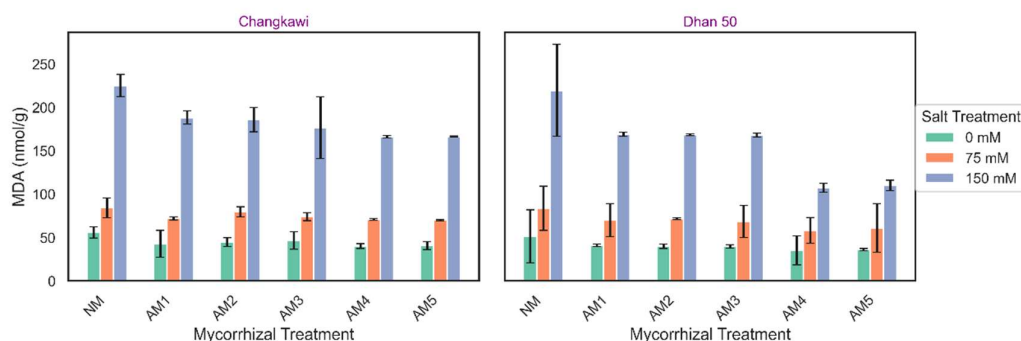


Figure 6.20: Malondialdehyde (MDA) content of two rice varieties (Changkawi and Dhan 50) under different salt and mycorrhizal treatments.

MANOVA revealed that Salt Treatment, Rice Variety, and Mycorrhizal Treatment significantly influenced MDA levels along with other physiological and biochemical traits (Table 6.1).

The three-way ANOVA results indicate that salt treatment, rice variety and mycorrhizal inoculation had significant independent effect on MDA content (Table 6.3). Although the interactions between salt treatment, rice variety, and mycorrhizal inoculation were not significant, the main effects of these factors play crucial roles in determining MDA levels.

PCA results showed that MDA was closely associated with the first principal component (PC1), which explained 45.40% of the total variance (Figure 6.23). MDA

contributed positively to PC1 (Figure 6.24), along with other stress-related traits such as proline and catalase.

Cluster analysis showed the first cluster consisted of plants with high MDA levels (Figure 6.25), primarily from 100 mM salt treatments with minimal mycorrhizal inoculation. These plants exhibited significant oxidative stress and membrane damage. The second cluster contained plants with moderate MDA levels, representing plants subjected to 50 mM salt or plants with moderate mycorrhizal inoculation. The third cluster consisted of plants with low MDA levels, primarily from 0 mM salt treatments or plants inoculated with AM5 and AM4.

6.3.3.5 Hydrogen Peroxide

The overall mean H_2O_2 content was $62.34 \mu\text{mol/g}$, with a standard deviation of 12.98 nmol/g (Figure 6.21). The highest mean H_2O_2 content was recorded in plants subjected to 150 mM salt, Changkawi rice variety, and no mycorrhizal inoculation (NM), with a mean of 79.79 nmol/g . Conversely, the lowest mean H_2O_2 content was observed in plants treated with 0 mM salt, Dhan 50 rice variety, and AM4 mycorrhizal inoculation, with a mean of 44.18 nmol/g . These results indicate that high salt stress leads to elevated oxidative stress, as indicated by increased H_2O_2 levels, particularly in Changkawi without mycorrhizal inoculation. In contrast, Dhan 50 under non-saline conditions with AM4 inoculation shows reduced oxidative stress.

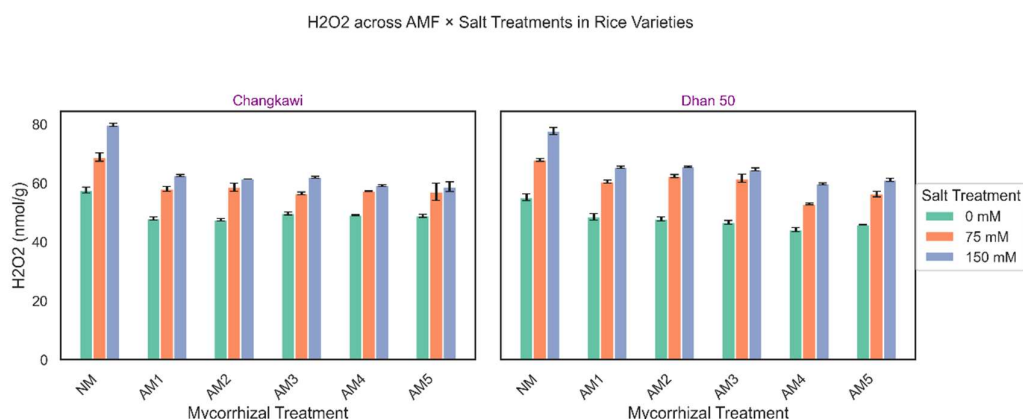


Figure 6.21: Hydrogen peroxide content of two rice varieties (Changkawi and Dhan 50) under different salt and mycorrhizal treatments.

MANOVA showed that Salt Treatment, Rice Variety, and Mycorrhizal Treatment significantly influenced H₂O₂ levels along with other physiological and biochemical traits (Table 6.1).

The three-way ANOVA results demonstrate that only rice variety doesn't have significant main effect (Table 6.3). The two-way and three-way interaction between factors were all significant.

PCA results showed that H₂O₂ was strongly associated with the first principal component (PC1), which explained 45.40% of the total variance (Figure 6.23). H₂O₂ contributed positively to PC1, along with other stress-related traits such as MDA and catalase.

The first cluster consisted of plants with high H₂O₂ levels, primarily from 100 mM salt treatments with minimal mycorrhizal inoculation, and these plants exhibited severe oxidative stress. The second cluster contained plants with moderate H₂O₂ levels, representing plants subjected to 50 mM salt or plants with moderate mycorrhizal inoculation. The third cluster consisted of plants with low H₂O₂ levels, mainly from 0 mM salt treatments or plants inoculated with AM5 and AM4.

6.3.3.6 Total Phenolic Content

The descriptive statistics for total phenolic content (TPC) across different treatment groups revealed significant variation (Figure 6.22). The overall mean TPC was 0.029 mg GAE/g, with a standard deviation of 0.008 mg GAE/g. The highest mean TPC was recorded in plants subjected to 75 mM salt, Dhan 50 rice variety, and AM2 mycorrhizal inoculation, with a mean of 0.040 mg GAE/g. Conversely, the lowest mean TPC was observed in plants treated with 150 mM salt, Dhan 50 rice variety, and no mycorrhizal inoculation (NM), with a mean of 0.019 mg GAE/g. Moderate salt stress (75 mM) and AM2 inoculation promoted higher phenolic content, while high salt stress (150 mM) in Dhan 50 without mycorrhiza resulted in the lowest phenolic levels, likely due to oxidative damage.

MANOVA demonstrated that Water Status, Mycorrhizal Treatment, and Rice Variety significantly influenced TPC along with other physiological and biochemical traits (Table 6.1).

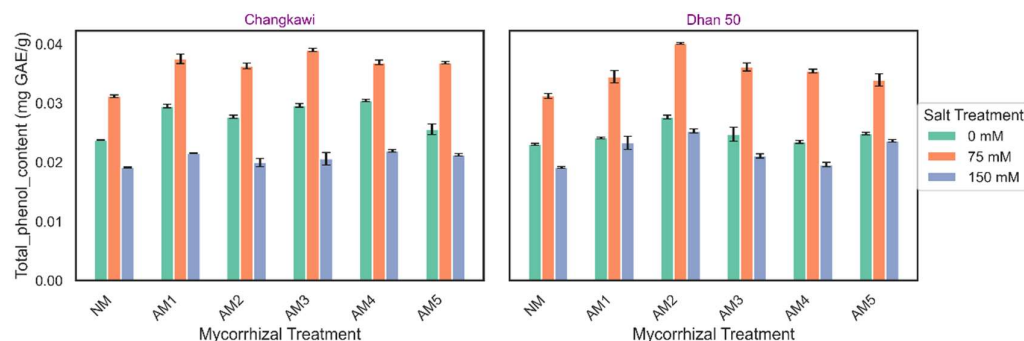


Figure 6.22: Total phenolic content content of two rice varieties (Changkawi and Dhan 50) under different salt and mycorrhizal treatments.

The three-way ANOVA results show that salt stress, rice variety, and mycorrhizal inoculation independently and interactively influence total phenolic content (Table 6.3).

PCA results showed that TPC was strongly associated with the first principal component (PC1), which explained 40.45% of the total variance (Figure 6.23). TPC, along with other antioxidant-related traits such as proline and catalase, contributed positively to PC1 (Figure 6.24).

Cluster analysis showed the first cluster contained plants with high TPC levels, primarily from drought-stressed and salt-stressed plants inoculated with AM5 and AM4 (Figure 6.25). The second cluster consisted of plants with moderate TPC levels, representing a mix of well-watered and moderately stressed plants inoculated with other mycorrhizal treatments. The third cluster included plants with low TPC levels, mainly from well-watered plants with no or minimal mycorrhizal inoculation, indicating that phenolic accumulation was less critical under non-stress conditions.

Table 6.1: Summary of MANOVA Results for the Effects of Salt Treatment, Rice Variety, and Mycorrhizal Treatment on Rice Physiological, Biochemical, and Growth Responses

Effect	Wilks' Lambda	F Value	p-value
Salt Treatment	0.013	22.08	<0.0001
Rice Variety	0.4699	3.07	<0.0001
Mycorrhizal Treatment	0.0122	1.65	0.001
Salt Treatment $\times$ Rice Variety	0.4083	2.5	<0.0001
Salt Treatment $\times$ Mycorrhizal Treatment	0.0363	2.33	<0.0001
Rice Variety $\times$ Mycorrhizal Treatment	0.1058	1.65	0.001
Salt Treatment $\times$ Rice Variety $\times$ Mycorrhizal Treatment	0.03	1.28	0.0187

Table 6.2: Three-way ANOVA results showing F-ratios and level of significance for growth and physiological parameters of rice

Effect	Height	Biomass	SLA	LDMC	RWC	EL	Chl_a	Chl_b	Total Chl	Carotenoids
Salt Treatment	57.51***	355.63***	48.19***	22.01***	41.89***	16.66***	88.84***	32.79***	78.79***	66.46***
Rice Variety	152.40***	2.12ns	8.01**	0.05ns	27.56***	0.02ns	9.60**	44.36***	0.66ns	36.45***
Mycorrhizal Treatment	10.54***	4.43**	2.47*	5.76***	6.24***	3.94**	23.18***	19.25***	24.00***	23.01***
Salt Treatment × Rice Variety	2.66ns	0.02ns	1.76ns	1.81ns	6.48**	2.84ns	53.16***	57.29***	58.77***	56.00***
Salt Treatment × Mycorrhizal Treatment	1.30ns	0.56ns	0.46ns	1.04ns	0.97ns	0.45ns	2.54*	0.90ns	2.18*	1.11ns
Rice Variety × Mycorrhizal Treatment	0.48ns	0.61ns	0.61ns	0.87ns	2.25ns	0.27ns	0.36ns	0.79ns	0.46ns	1.43ns
Salt Treatment × Rice Variety × Mycorrhizal Treatment	1.05ns	0.92ns	0.54ns	0.48ns	0.36ns	0.12ns	1.97*	2.01*	1.95ns	1.57ns

SLA: Specific Leaf Area; LDMC: Leaf Dry Matter Content; RWC: Relative Water Content; EL: Electrolyte Leakage; TC: Total Chlorophyll

* Level of significance, $p < 0.05$.

** Level of significance, $p < 0.01$.

*** Level of significance, $p < 0.001$.

ns Level of significance, not significant.

Table 6.3: Three-way ANOVA results showing F-ratios and level of significance for biochemical parameters of rice

Effect	Proline	Anthocyanin	Catalase	SOD	MDA	H ₂ O ₂	TPC	TSS	Sucrose	Fructose
Salt Treatment	62.74***	92.03***	91.41***	302.50***	221.02***	958.98***	2243.36***	399.74***	50.90***	108.50***
Rice Variety	7.32**	46.66***	0.16ns	0.17ns	6.07*	0.32ns	29.75***	0.55ns	1.23ns	2.95ns
Mycorrhizal Treatment	2.07ns	34.28***	6.79***	2.79*	5.31***	191.27***	59.25***	2.43*	2.17ns	1.06ns
Salt Treatment × Rice Variety	1.52ns	5.00**	0.19ns	0.12ns	2.04ns	15.21***	50.35***	0.15ns	20.26***	0.06ns
Salt Treatment × Mycorrhizal Treatment	0.76ns	2.48*	1.14ns	1.54ns	1.77ns	9.40***	7.10***	1.19ns	2.08*	0.53ns
Rice Variety × Mycorrhizal Treatment	1.09ns	1.94ns	0.62ns	0.78ns	0.52ns	9.50***	28.59***	0.73ns	1.46ns	0.44ns
Salt Treatment × Rice Variety × Mycorrhizal Treatment	0.74ns	2.61**	1.02ns	0.63ns	0.34ns	1.98*	5.33***	0.98ns	1.07ns	2.35*

SOD: Superoxide Dismutase; MDA: Malondialdehyde; TPC: Total Phenolic Content; TSS: Total Soluble Sugars

* Level of significance, $p < 0.05$.

** Level of significance, $p < 0.01$.

*** Level of significance, $p < 0.001$.

ns Level of significance, not significant.

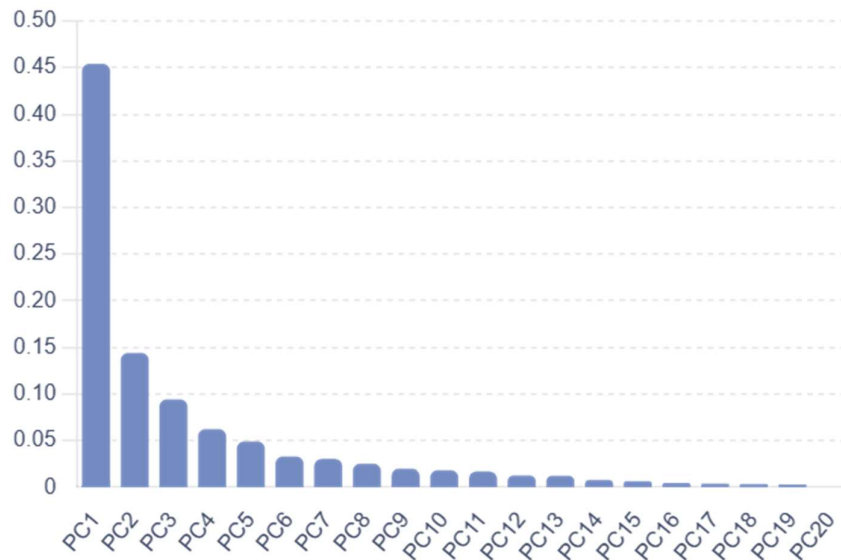


Figure 6.23: Bar plot showing how much variance each principal component explains. The first two principal components (PC1 and PC2) explained 59.79% of the total variance. PC1 explained the largest proportion of the variance (45.4%), PC2 explained an additional 14.39% of the variance.

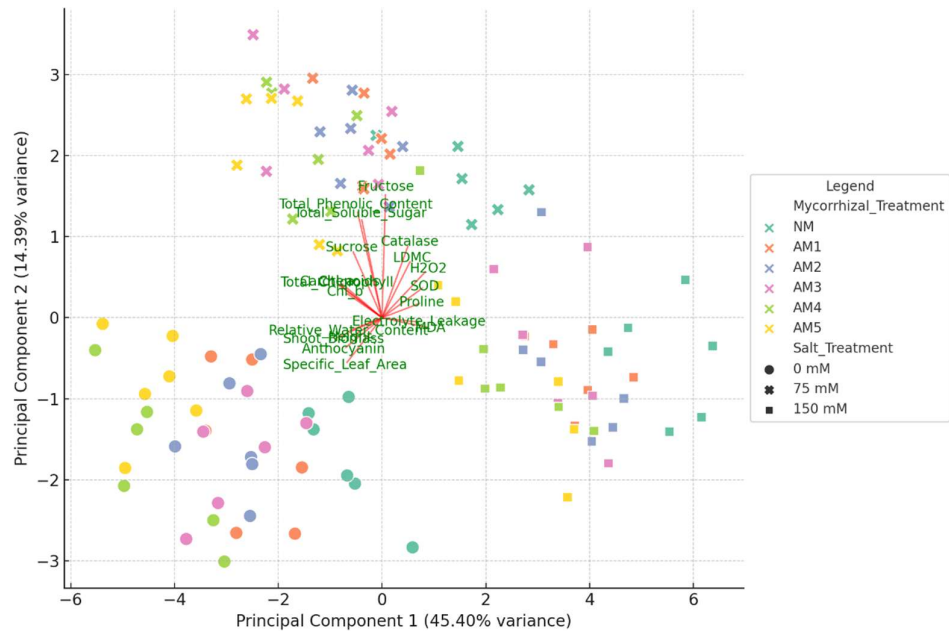


Figure 6.24: PCA biplot (PC1 vs PC2), showing the relationship between the different Mycorrhizal Treatments and NaCl concentrations. PC1 was predominantly influenced by variables related to oxidative stress, such as Catalase, SOD, MDA, and H₂O₂. was influenced by variables related to photosynthetic pigments (such as Chlorophyll a, Chlorophyll b, Total Chlorophyll) and osmoprotectants like Proline.

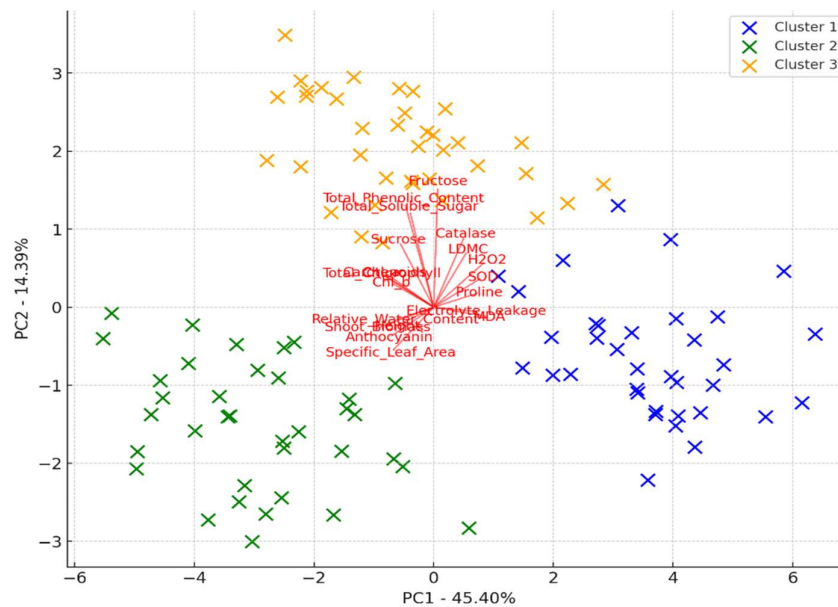


Figure 6.25: K-means clustering biplot, with each cluster represented by a different color (blue, green, and orange). The arrows indicate the variable loadings, showing how the original variables contribute to the first two principal components. The clusters are indicated by the different colors.

6.3.4.4 Multivariate Correlation Analysis

The correlation matrix revealed several significant associations between the measured variables. Significant correlations ($p < 0.05$) are denoted by an asterisk (*) and visualized in the heatmap (Figure 6.26). Proline content, a known stress indicator, was significantly positively correlated with superoxide dismutase (SOD) activity ($r = 0.63^*$) and catalase activity ($r = 0.47^*$). This suggests that proline accumulation is accompanied by increased antioxidant enzyme activity, indicating an enhanced defense mechanism in rice plants under salt stress. Total chlorophyll content showed significant positive correlations with anthocyanin content ($r = 0.50^*$) and height ($r = 0.50^*$). These relationships suggest that healthier plants, with higher chlorophyll content, tend to exhibit increased anthocyanin production and greater shoot height, highlighting their better adaptability to stress conditions. A strong positive correlation was observed between SOD and malondialdehyde (MDA) levels ($r = 0.73^*$), indicating that higher oxidative stress, as reflected by increased lipid peroxidation, is mitigated by elevated antioxidant enzyme activity.

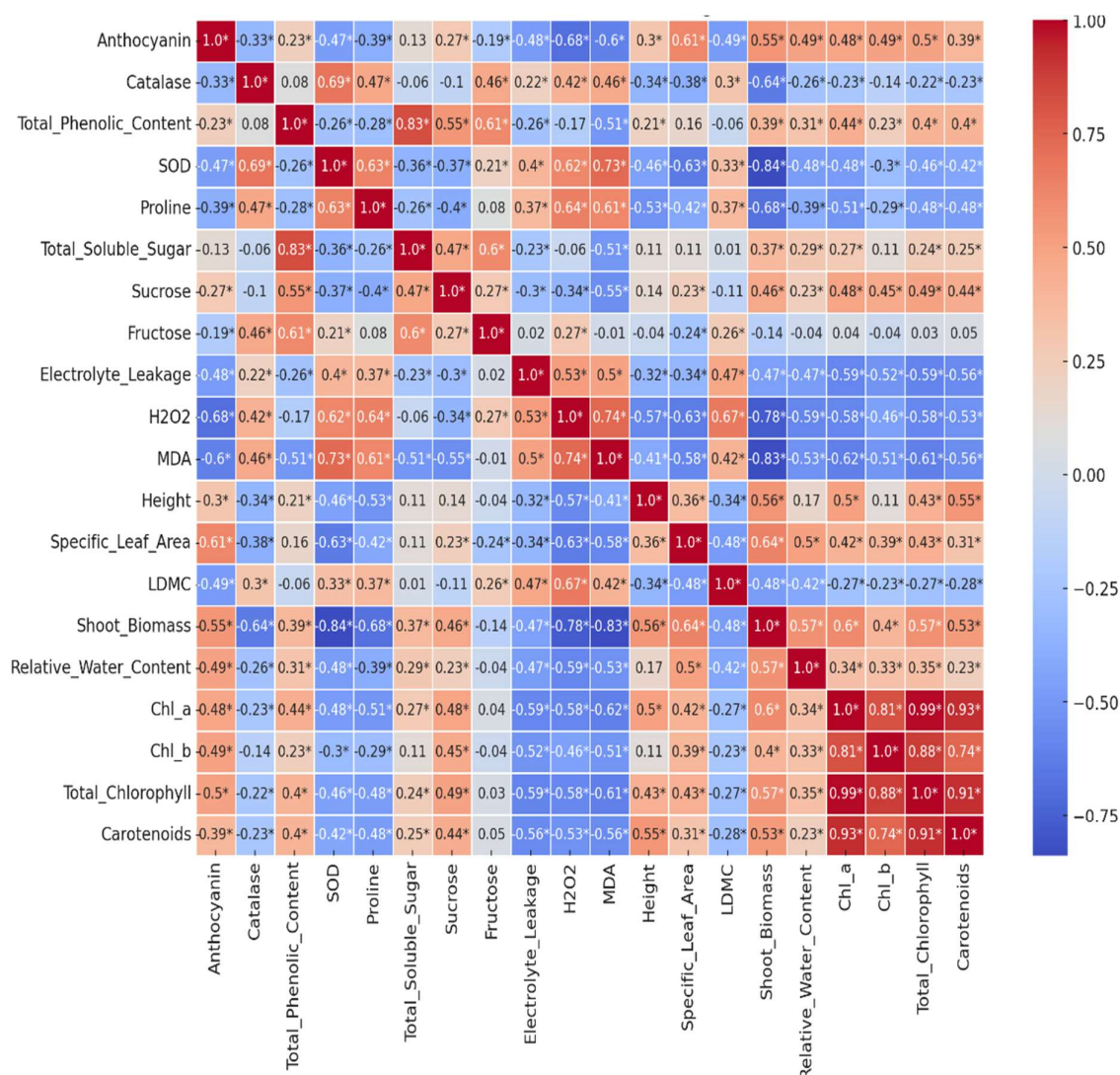


Figure 6.26: Correlation heatmap of biochemical, physiological, and growth parameters in rice under different mycorrhizal and salt treatments. * indicates significant correlations ($p < 0.05$).

The significant correlations observed in this study underline the interdependence of various physiological and biochemical responses in rice plants subjected to salt stress. For instance, the strong correlations between proline, antioxidant enzymes (SOD, catalase), and lipid peroxidation (MDA) suggest that rice plants inoculated with arbuscular mycorrhiza exhibit coordinated biochemical responses to mitigate oxidative damage under salt stress. Additionally, the correlations between chlorophyll, growth parameters, and anthocyanin levels highlight the crucial role of pigmentation and photosynthetic efficiency in enhancing plant growth and resilience.

6.4 Discussion

This study aimed to explore the combined effects of salt stress, rice variety, and mycorrhizal inoculation on the physiological, biochemical, and growth responses of rice plants. The results demonstrate a clear interaction between these factors, with salt stress being the dominant factor influencing plant responses, while mycorrhizal inoculation and rice variety contributed significantly to enhancing stress tolerance.

The data on plant height and biomass revealed that salt stress negatively impacted plant growth, with the most severe reductions observed at 150 mM NaCl treatment. Mycorrhizal inoculation, particularly AM5, mitigated the effects of salt stress on plant height, especially in the Changkawi variety. These results indicate that mycorrhizal inoculation, particularly AM5, significantly enhances growth under saline conditions, which aligns with previous findings that highlight the role of AMF in improving plant stress tolerance through enhanced nutrient acquisition and growth promotion (Talaat and Shawky, 2014; Abdel Latef and Chaoxing, 2014).

Similarly, the shoot dry biomass data showed that Dhan 50 plants responded better to AM3 inoculation under non-saline conditions, with the highest biomass recorded at 6.36 g. However, salt stress reduced biomass, particularly in Changkawi at 150 mM NaCl without mycorrhizal inoculation, which further emphasizes the negative impact of salt stress on plant growth. The lack of significant interactions between salt treatment and rice variety for biomass suggests that while both rice varieties experience growth reductions under stress, the effects of salt stress are more strongly mediated by mycorrhizal inoculation than by rice variety alone. This result is consistent with studies that show mycorrhiza can enhance plant growth under salt stress by improving nutrient acquisition and reducing the effects of salt toxicity (Hashem *et al.*, 2018; Khalloufi *et al.*, 2017; Evelin *et al.*, 2009).

The highest mean SLA was observed in the Dhan 50 variety with 0 mM salt and AM2 inoculation, while the lowest SLA was recorded in Changkawi with 150 mM salt and no inoculation. This suggests that mycorrhizal inoculation helps retain leaf area, especially in less stressful environments. The three-way ANOVA showed that while salt, variety, and mycorrhizal treatment affected SLA independently, their interactions were not significant. This suggests that Dhan 50, especially with AM2 inoculation, retains more leaf area under stress.

High salt concentrations, particularly in the absence of mycorrhizal inoculation, led to increased leaf dry matter content, which could indicate a stress response where plants accumulate more dry matter to cope with osmotic stress. Conversely, AM4 inoculation under no salt stress resulted in the lowest leaf dry matter content, indicating healthier plant growth. This points to mycorrhizal fungi's role in optimizing water retention and nutrient uptake, enhancing plant performance under varying stress conditions (Hajiboland *et al.*, 2010; Hajiboland *et al.*, 2015).

The highest RWC was observed in Dhan 50 with AM5 inoculation under 0 mM salt, while the lowest was in Changkawi without mycorrhizal inoculation under 150 mM salt. These findings highlight that salt stress drastically reduces water content in leaves, especially in Changkawi, while AM5 inoculation helps plants retain water under both stress and non-stress conditions. This demonstrates the role of AMF in improving water relations under saline conditions, likely through enhanced water uptake (Jixiang *et al.*, 2017; Mokhtar *et al.*, 2019). Salt stress increased membrane damage, as indicated by higher EL values, especially in Changkawi under 150 mM salt without mycorrhizal inoculation. In contrast, AM5 inoculation significantly reduced EL, indicating better membrane stability. This aligns with previous studies showing that AMF helps plants maintain membrane integrity by reducing oxidative damage under stress (Abdel Latef and Chaoping 2011, Evelin and Kapoor 2014).

Proline accumulation increased under salt stress (Moukhtari *et al.*, 2020), with the highest proline content recorded in Dhan 50 under 150 mM salt without mycorrhizal inoculation. Proline acts as an osmoprotectant (Yoshida *et al.*, 1997; Verslues and Sharma, 2010) helping plants cope with osmotic stress caused by high salinity. Interestingly, plants inoculated with AM2 under non-saline conditions showed the lowest proline levels, suggesting that AMF reduces the need for osmoprotectants downregulating the Glutamic acid pathway (Zou *et al.*, 2013).

Chlorophyll content decreased with increasing salt stress, particularly in Changkawi without mycorrhizal inoculation. The highest chlorophyll a and total chlorophyll levels were observed in Changkawi with AM5 under 0 mM salt, highlighting the role of AMF in maintaining chlorophyll content and photosynthetic activity under stress. Carotenoid content followed a similar pattern, with the highest levels recorded in Changkawi with AM5 inoculation under 0 mM salt and the lowest

in Changkawi under 150 mM salt without inoculation. Carotenoids are essential for protecting chlorophyll and photosynthetic machinery from oxidative stress. The higher carotenoid content in AM5-inoculated plants suggests that mycorrhizal inoculation enhances the plant's ability to protect its photosynthetic apparatus under stress. The results indicate that mycorrhizal inoculation helps plants sustain photosynthesis by protecting photosynthetic pigments from degradation under saline conditions (Elhindi *et al.*, 2017; Cekic *et al.*, 2012; Hajiboland *et al.*, 2010).

MDA and H₂O₂, markers of oxidative stress (Morales and Munné-Bosch, 2019), were highest in Changkawi under 150 mM salt without mycorrhizal inoculation, indicating severe lipid peroxidation and oxidative damage. Mycorrhizal inoculation, particularly AM4, significantly reduced MDA and H₂O₂ levels, suggesting that AMF mitigates oxidative stress by enhancing the plant's antioxidant defenses. These findings align with studies showing that AMF reduces oxidative damage by suppressing lipid membrane peroxidation under salinity stress (Abdel Latef and Chaoxing, 2014; Talaat and Shawky, 2014). Catalase and superoxide dismutase (SOD) activities increased under salt stress, indicating an upregulation of the plant's antioxidant defense system to combat oxidative damage. The highest catalase activity was recorded in Dhan 50 with AM3 inoculation under 150 mM salt, while the highest SOD activity was observed in Changkawi with AM5 under 150 mM salt. These results suggest that different mycorrhizal inoculants enhance antioxidant enzyme activity to varying extents, with AM5 being particularly effective in Changkawi. This further supports the role of AMF in reducing oxidative stress under saline conditions (Hashem *et al.*, 2018; Evelin *et al.*, 2019; Huang *et al.*, 2023).

The accumulation of soluble sugars, particularly sucrose and fructose, increased under moderate salt stress (75 mM), but high salt stress (150 mM) reduced sugar levels, especially in Dhan 50 without mycorrhizal inoculation. Mycorrhizal inoculation, particularly AM3, helped maintain sugar levels, indicating that AMF supports osmotic adjustment under stress by promoting the accumulation of compatible solutes like sugars (Wu and Xia, 2006; Begum, 2019; Wu *et al.*, 2017).

Anthocyanin content increased under non-saline conditions with AM4 inoculation, while salt stress reduced anthocyanin levels, particularly in Dhan 50 without inoculation. Phenolic content followed a similar trend, with the highest levels

recorded under 75 mM salt and AM2 inoculation. Phenolic compounds play a critical role in antioxidant defense (Zeb, 2020; Negro *et al.*, 2003), and the results suggest that moderate salt stress and mycorrhizal inoculation promote phenolic accumulation (Bencherif *et al.*, 2019), enhancing the plant's ability to scavenge ROS.

The MANOVA results showed that salt stress had the most significant effect on the collective physiological and biochemical responses of rice plants, followed by rice variety and mycorrhizal inoculation. Significant interactions between salt treatment and rice variety, as well as salt treatment and mycorrhizal inoculation, suggest that the response to salt stress varies across rice varieties and mycorrhizal treatments. PCA revealed that oxidative stress markers (SOD, catalase, MDA, and H₂O₂) were the major contributors to the variance explained by the first principal component (PC1), indicating that these variables are key indicators of the plant's response to salt stress. Photosynthetic pigments and osmoprotectants, such as chlorophyll and proline, were aligned with the second principal component (PC2), highlighting the role of mycorrhizal inoculation in modulating these variables.

The K-means clustering revealed distinct groups of rice plants based on their physiological and biochemical responses to the treatments. Cluster 1 represented plants with moderate responses, Cluster 2 included plants with higher tolerance to salt stress (mostly inoculated with AMF), and Cluster 3 consisted of plants under severe stress (uninoculated or poorly performing under high salt stress). The clustering patterns emphasize the importance of mycorrhizal inoculation in enhancing stress tolerance and suggest that specific mycorrhizal treatments, such as AM5, are more effective in mitigating salt stress.

The multivariate correlation analysis demonstrated several significant associations among key physiological and biochemical parameters in rice plants under salt stress. These correlations reveal important relationships that are likely to contribute to the enhanced stress tolerance observed in mycorrhizal-inoculated rice plants. The results support the hypothesis that AMF inoculation improves stress resilience through coordinated modulation of antioxidant defense systems, osmolyte accumulation, and growth regulation.

Chapter 7

Summary and Conclusions

This study examined the colonization frequency and diversity of Arbuscular Mycorrhizal Fungi (AMF) associated with low land rice plants grown in North Vanlaiphai and Thenzawl wet land rice paddy fields. The study aimed to determine the effects of soil properties on AMF colonization, spore density, and community diversity. Key soil properties such as pH, bulk density, potassium, soil organic carbon, and enzyme activities (dehydrogenase, phosphatase, urease) were analyzed at both sites. AMF colonization (arbuscular, vesicular, and hyphal), spore density, and species diversity were assessed through standardized procedures.

The results showed that rice sample collected from Thenzawl paddy field consistently had higher AMF colonization, spore density, and species richness compared to North Vanlaiphai. Soil factors like higher pH, potassium content, and enzyme activities in Thenzawl created a more conducive environment for AMF colonization. Over the three years assessment, arbuscular colonization increased at both sites, though more significantly at Thenzawl. Vesicular and hyphal colonization showed divergent trends, with a steady increase at Thenzawl and a decline at North Vanlaiphai. Spore density was significantly higher at Thenzawl, with strong positive correlations to pH and enzyme activities. The AMF community in Thenzawl also exhibited greater species richness, diversity indices, and a more even distribution of species, particularly favouring genera like *Acaulospora* and *Glomus*.

Propagation and mass production of AMF was performed by using substrate-based systems. The study utilized two main host plants: maize (*Zea mays* L.) for trap culture and the Sorghum-Sudangrass hybrid for single-species culture. Trap culture was established using rhizosphere soil collected from the field, mixed with sterilized soil, and used to propagate AMF spores. After four months of growth, spores were collected and identified. Seven AMF species were isolated, including two species each from the genera *Glomus* and *Acaulospora*, and one species each from *Gigaspora*, *Funneliformis*, and *Rhizophagus*. Of these, three species—*Acaulospora foveata*, *Gigaspora margarita*, and *Glomus* sp.—were selected for mass production. Sorghum-

Sudangrass was used as the host plant for the substrate-based system to propagate single-species spores, which were successfully cultured over four months. The study demonstrated the effectiveness of substrate-based systems for producing high-quality AMF inocula under controlled conditions. It highlighted the importance of selecting appropriate host plants and substrate mixtures to maximize AMF colonization and spore production.

The biochemical and physiological responses of rice plants inoculated with AMF under drought and salt stress was investigated. The study aimed to explore whether AMF inoculation could alleviate the negative impacts of drought and salt stress on rice growth and physiology and identify the most effective AMF species in promoting stress tolerance. Two rice cultivars such as Changkawi and DRR Dhan 50, were chosen for the experiment. Changkawi is a local cultivar, while DRR Dhan 50 is a drought and submergence-tolerant variety developed through molecular breeding. The rice plants were subjected to two different water and salt treatments. Additionally, the plants were either left non-mycorrhizal (NM) or inoculated with five different mycorrhizal treatments: *Acaulospora foveata* (AM1), *Gigaspora margarita* (AM2), *Glomus sp.* (AM3), a combination of the three species (AM4), and a commercial AMF product (AM5). The study assessed a wide range of plant responses, including AMF root colonization, growth parameters (plant height, biomass, and leaf area), water relations (relative water content), photosynthetic pigments (chlorophyll and carotenoids), and stress-related biochemical markers (proline, antioxidant enzyme activities such as superoxide dismutase, catalase, and membrane permeability).

AMF-treated rice plants exhibited improved physiological and biochemical responses under drought conditions compared to non-mycorrhizal plants. AMF inoculation enhanced water retention, chlorophyll content, and photosynthetic efficiency, contributing to better growth under stress. The DRR Dhan 50 variety performed better than Changkawi under drought conditions, with higher shoot biomass, chlorophyll content, and antioxidant activity, indicating it is more suited to drought-prone environments. The mixed inoculum (AM4) and commercial AMF (AM5) treatments provided the greatest benefits in terms of plant growth and stress resilience, demonstrating their potential as effective inoculants for improving rice drought tolerance. AMF inoculation significantly enhanced the activity of antioxidant

enzymes such as superoxide dismutase (SOD) and catalase, which play crucial roles in mitigating oxidative stress caused by drought. This suggests that AMF can protect plants from oxidative damage under water-limited conditions. The results also suggest that incorporating AMF inoculation into rice cultivation practices could enhance drought tolerance and improve yield stability in water-scarce regions. This approach may contribute to more sustainable agricultural practices in the face of climate change-induced water shortages.

AMF significantly enhanced rice growth, water retention, photosynthetic pigment levels, and antioxidant enzyme activity under salt stress. The study demonstrates that AMF can be a sustainable solution to improve salt tolerance in rice cultivation. DRR Dhan 50 exhibited greater salt tolerance compared to Changkawi, performing better in terms of plant height, biomass, water retention, and biochemical responses under all salt treatments. This suggests that Dhan 50 is more suitable for saline environments. Both AM4 and AM5 treatments provided the most consistent and significant improvements across various parameters, suggesting that mixed or commercial AMF inocula may be highly effective in mitigating salt stress in rice cultivation. Proline, MDA, and H_2O_2 levels were important markers of salt-induced stress. AMF inoculation helped reduce MDA and H_2O_2 levels, indicating lower oxidative stress and membrane damage in inoculated plants. Additionally, AMF treatments improved antioxidant enzyme activities, contributing to enhanced plant defense mechanisms. This study underscores the potential of AMF as a biological tool to enhance rice resilience to salt stress, offering a promising approach for sustainable agriculture in regions affected by soil salinization. The findings suggest that integrating AMF into rice cultivation practices could improve productivity and stability in saline soils, providing an eco-friendly solution to an increasingly pressing agricultural challenge.

In conclusion, this study highlights the significant role of Arbuscular Mycorrhizal Fungi in improving the resilience of rice plants to both drought and salt stress. AMF inoculation, particularly with mixed species (AM4) and commercial products (AM5), enhanced plant growth, water retention, chlorophyll content, and antioxidant enzyme activity, resulting in better stress tolerance in both drought and saline conditions. The rice variety DRR Dhan 50 exhibited greater overall resilience

compared to Changkawi, as DRR Dhan 50 is a drought- and submergence-tolerant rice variety that was developed using molecular breeding techniques, making it more suitable for cultivation in challenging environments. The study underscores the importance of AMF as a sustainable, biological solution for enhancing stress tolerance and ensuring stable rice yields in the face of climate change-induced water scarcity and soil salinization. Integrating AMF into rice cultivation practices can provide a promising strategy for improving productivity and agricultural sustainability.

The findings of this study have several important future implications for sustainable agriculture and food security. First, integrating AMF into rice cultivation practices could significantly improve crop resilience to abiotic stresses, such as drought and salinity, which are expected to become more prevalent due to climate change. The enhanced water retention, nutrient absorption, and oxidative stress mitigation provided by AMF inoculation suggest that these fungi could be a crucial component in developing more robust agricultural systems, particularly in regions with limited water resources or saline soils. Moreover, the superior performance of certain AMF inocula, such as the mixed AMF (AM4) and commercial AMF (AM5), points to the potential for developing specific AMF products tailored to different environmental conditions and crop varieties. Future research could explore the optimization of AMF inocula for large-scale agricultural use, as well as the long-term impacts of AMF on soil health and crop yield stability. The application of AMF in rice cultivation offers a promising, eco-friendly approach to addressing food security challenges in the face of growing environmental pressures.

Appendix I

Detailed Experimental Protocols

1. Proline determination (Bates *et al.* 1973)

- a. Materials
 - i. Sulphosalicylic acid
 - ii. Proline
 - iii. Ninhydrin
 - iv. Acetic acid
 - v. Orthophosphate
 - vi. Toluene
 - vii. Whatman#1 filter paper
- b. Equipment
 - i. Centrifuge
 - ii. Spectrophotometer
 - iii. Water bath
- c. Reagent
 - i. Acid - Ninhydrin - 1.25 g ninhydrin is successively added to 30 ml Glacial Acetic acid and 20 ml 6 M orthophosphate, and then dissolved at 70 °C. After cool down, the reagent can be stored in brown bottle at 4 °C for less than 24 h.
- d. Procedure
 - i. 250 mg of fresh material was ground with liquid nitrogen in a mortar.
 - ii. The homogenate powder was mixed with 5mL of aqueous sulfosalicylic acid (3%, w/v) and filtered through filter paper or centrifuge homogenized samples at 5,000 x g for 5 min, and then collect the supernatant.
 - iii. 2 ml of filtrate/supernatant was reacted with an 2ml of glacial acetic acid and 2ml acid-ninhydrin reagent and incubated at 95°C for 1h and the reaction terminated in an ice bath.
 - iv. The reaction mixture was mixed vigorously with 4mL of toluene for 15-20 sec.
 - v. The chromophore containing toluene was aspirated from the aqueous phase, warmed to room temperature (25°C) and the absorbance read at 520 nm using toluene for a blank.
 - vi. The proline concentration was determined from a standard curve and calculated on a fresh weight basis as follows:
$$[(\mu\text{g proline/ml} \times \text{ml toluene}) / 115.5 \mu\text{g}/\mu\text{mole}] / [(\text{g sample})/5] = \mu\text{moles proline/g of fresh weight material.}$$
 - vii. Preparation of standard curve

- viii. A serial concentration of Proline is made in 3% sulphosalicylic acid: 1 μ M, 10 μ M, 50 μ M, 100 μ M, 150 μ M, 200 μ M, 300 μ M, 1 ml for each dilution.
- ix. Each 0.5ml standard solution is added with 0.5ml acetic acid and 0.5ml acid-ninhydrin reagent in 5 ml tube and boil for 45 min, and then cooled in ice for 30 min.
- x. Add equal volume of toluene (1ml) to each sample and shake vigorously for 1 minute.
- xi. Measure the optical density of toluene solution at 520 nm by spectrophotometer and make the standard curve.

2. Soluble sugars determination (sucrose, glucose and fructose), Wu *et al.*, (2015)

a. Materials

- i. 80% Ethanol
- ii. 2mol/L NaOH
- iii. 30% HCl
- iv. 0.1% Resorcinol in HCL
- v. 1mg/mL o-dianisidine dihydrochloride
- vi. 1mg/mL horseradish peroxidase
- vii. 1Unit/mL glucose oxidase
- viii. 10mol/L H₂SO₄
- ix. Distilled water

b. Equipment

- i. Mortar & Pestle
- ii. Centrifuge
- iii. Spectrophotometer
- iv. Water bath

c. Procedure

- i. The oven-dried plant matters were ground and sieved through a 0.5 mm sieve. The ground material (50 mg) was placed in a 10 ml centrifugal tube containing 4 ml 80% ethanol, incubated for 40 min at 80°C, and centrifuged at 2,500 g for 5 min.
- ii. The centrifugal residues were extracted again using the above procedure, and the two-time supernatants was combined for carbohydrate assays.
- iii. Sucrose was assayed with a mixture of 0.15 mL supernatant and 0.15 mL 2 mmol/L NaOH at 100°C for 5 min, which were then mixed together with 2.1 mL 30 % HCl and 0.6 mL 0.1 % resorcinol at 80°C for 10 min and followed by measurement of absorbance of assay at 480 nm using sucrose as one standard.
- iv. Glucose concentration was determined by mixing 0.5 mL of supernatant with 1.0 mL of prepared solution (1 mg/mL o-dianisidine dihydrochloride, 1 mg/mL horseradish peroxidase, and 1 U/mL glucose oxidase) at 30°C for 5 min, then added 2

mL 10 mol/L H₂SO₄ solution to terminate the reaction, and followed by measurement of absorbance at 460 nm using one glucose standard.

- v. Fructose was assayed using a mixture of 0.8 mL 0.1 % resorcinol, 0.4 mL H₂O, and 0.4 mL supernatant at 80°C for 10 min. The absorbance of the mixed solution was determined at 480 nm using fructose as one standard solution.

3. TBARS assay for estimating lipid peroxidation (Hodges *et al.*, 1999)

- a. Materials
 - i. Thiobarbituric acid
 - ii. Trichloroacetic acid
 - iii. Malondialdehyde
 - iv. Ethanol
 - v. Hydroxytoluene
- b. Equipment
 - i. Centrifuge
 - ii. Spectrophotometer
- c. Reagent
 - i. -TBA solution comprised of 20.0% (w/v) trichloroacetic acid and 0.01% butylated hydroxytoluene
 - ii. +TBA solution containing the above plus 0.65% TBA
- d. Procedure
 - i. 100mg fresh material was ground with liquid nitrogen in a mortar.
 - ii. Plant tissue samples were homogenized in 1:25 (g FW:ml) 80:20 (v:v) ethanol:water, followed by centrifugation at 3000 g for 10 min.
 - iii. A 1ml aliquot of appropriately diluted sample was added to a test tube with 1 ml of either (i) -TBA solution or (ii) +TBA solution.
 - iv. Samples were then mixed vigorously, heated at 95°C in water bath for 25 min, cooled, and centrifuged at 3000 g for 10 min.
 - v. Absorbances were read at 440 nm, 532 nm, and 600 nm.
 - vi. Malondialdehyde equivalents were calculated in the following manner:
 - 1) $[(\text{Abs } 532_{+TBA}) - (\text{Abs } 600_{+TBA}) - (\text{Abs } 532_{-TBA} - \text{Abs } 600_{-TBA})] = A$
 - 2) $[(\text{Abs } 440_{+TBA} - \text{Abs } 600_{+TBA}) 0.0571] = B$
 - 3) MDA equivalents (nmol · ml⁻¹) = $(A - B / 157\ 000) 10^6$

4. Determination of H_2O_2 (Velikova *et al.*, 2000)
 - a. Materials
 - i. H_2O_2
 - ii. Potassium Iodide
 - iii. TCA
 - iv. Potassium-phosphate buffer
 - b. Equipment
 - i. Centrifuge
 - ii. Spectrophotometer
 - c. Reagent
 - i. 10 mM K-phosphate buffer
 - ii. 0.1% TCA
 - iii. 1 M KI (w/v in dd H_2O)
 - d. Procedure
 - i. Frozen powder (500 mg) was directly homogenized with 5 ml of 0.1% TCA.
 - ii. The homogenate was centrifuged at $12\,000\times g$ for 15 min.
 - iii. 0.5 ml of the supernatant was added to 0.5 ml 10 mM potassium phosphate buffer (pH 7.0) and 1 ml 1 M KI.
 - iv. The blank probe consisted of 0.1% TCA in the absence of leaf extract.
 - v. The reaction was developed for 1 h in darkness and absorbance measured at 390 nm.
 - vi. Hydrogen peroxide was measured using a standard curve of H_2O_2 and expressed as $\text{nmol g}^{-1} \text{FW}$.

5. Determination of electrolyte leakage (Bajji *et al.*, 2002)
 - a. Procedure
 - i. Three mature leaves per plant from six randomly chosen plants per treatment were taken and cut into 1-cm segments.
 - ii. Leaf samples were washed three times with distilled water to remove surface contamination and then placed in individual stoppered vials containing 10 mL of distilled water.
 - iii. The samples were incubated at room temperature (25 °C) on a shaker (100 rpm) for 24 h.
 - iv. Electrical conductivity of the bathing solution (EC1) was read after incubation.
 - v. The same samples were then placed in an autoclave at 120 °C for 20 min and a second reading of the EC (EC2) was made after cooling the solution to room temperature.
 - vi. The EL was calculated as EC1/EC2 and expressed as percentage.

6. Determination of chlorophyll content (JD Hiscox, 1979)

- a. Materials
 - i. DMSO
 - ii. Test tubes
 - iii. Aluminium foil
- b. Equipment
 - i. Oven
 - ii. Spectrophotometer
- c. Procedure
 - i. 25 mg of finely chopped fresh leaf sample is placed in the test tubes to which 5 ml DMSO is added.
 - ii. The tubes are covered with aluminium foil and kept in an oven at 65°C for 4 hrs.
 - iii. After 4 hrs, the absorbance of the chlorophyll solution is read at 663 and 645 nm.
 - iv. Chlorophyll content (chl 'a' and 'b') is calculated using formula given by Arnon (1949) and finally expressed in terms of mg/g fw.
 - v. DMSO is used as blank.
- d. Calculation:

Chlorophyll content is calculated using following formula:

$$\begin{aligned}\text{Chlorophyll } a &= \frac{12.7D_{663} - 2.69D_{645}}{1000 \times W} \times V \\ \text{Chlorophyll } b &= \frac{22.9D_{645} - 4.68D_{663}}{1000 \times W} \times V \\ \text{Total Chlorophyll } (a+b) &= \frac{20.2D_{645} + .02D_{663}}{1000 \times W} \times V \\ \text{Carotenoids} &= \frac{7.60D_{480} - 1.49D_{510}}{1000 \times W} \times V\end{aligned}$$

Where, D663 = absorbance at 663 nm

D645 = absorbance at 645 nm

D510 = absorbance at 510 nm

D480 = absorbance at 480 nm

V = final volume of chlorophyll extract (in ml)

W = fresh weight of leaf tissue extracted (in grams).

7. Anthocyanin (Neff & Chory, 1998)

- a. Materials
 - i. Methanol
 - ii. HCl
- b. Equipment
 - i. Centrifuge
 - ii. Spectrophotometer
- c. Reagent
 - i. Methanol:water:HCl (80:20:1)
 - ii. Sample-to-buffer ratio 1:10

d. Procedure

- i. 0.2g fresh material was ground with liquid nitrogen in a mortar and homogenized with 2ml of 1%HCl in methanol.
- ii. The homogenates were incubated at 4°C in the dark in the shaker for 24 h.
- iii. At the end of the incubation period, the homogenates were centrifuged at 4°C and 7000 rpm for 15 min.
- iv. Dilute the anthocyanin extract with acidified methanol to ensure the absorbance readings fall within the linear range of the spectrophotometer. (DF=100ul sample+100ulmethanol in my case)
- v. The absorbance of anthocyanin was measured by the spectrophotometer at 530 and 657 nm.
- vi. Anthocyanin concentration was calculated by the following formula and was given as A/g fresh fruit tissue, where TA = total anthocyanin, A = absorbance at 530 and 657 nm, V = volume of extract (mL) and M = fresh mass of the sample (g).

$$TA = \frac{A_{530} - 0.3 A_{657} \times V}{M}$$

8. Phenols (Folin-Ciocalteu assay) (Sarker, 2018)

a. Materials

- i. Gallic acid
- ii. Methanol 90%
- iii. Folin Ciocalteu phenol reagent (diluted 1:4 in distilled water)
- iv. 10% Na₂CO₃

b. Equipment

- i. Spectrophotometer
- ii. Centrifuge

c. Procedure

- i. Take 0.1 g of plant material and ground in mortar with liquid nitrogen.
- ii. Dissolve the powder in 4 ml of 90% methanol and the tightly capped bottle was then placed in an 80 °C shaking water bath for 1 hr.
- iii. Centrifuge at 6000rpm for 10 minutes.
- iv. Fifty microliters of the leaf extract solution were placed in a test tube along with 1 ml of folin-ciocalteu reagent (previously diluted 1:4, reagent: distilled water) and then mixed thoroughly.
- v. An aliquot (1 ml) of extracts or standard solution of gallic acid (20, 40, 40, 60, 80 and 100 µg/ml) was added to a 25 ml volumetric flask, containing 9 ml of distilled water.

- vi. A reagent blank was prepared using distilled water.
- vii. After 3 min, 1 ml of NaCO₃ (10%) was added, and the mixture allowed to stand for 1 h in the dark.
- viii. The absorbance was measured at 760 nm using a spectrophotometer.
- ix. The concentration of total phenolic compounds in the leaf extracts was determined using an equation obtained from a standard gallic acid graph. The results are expressed as microgram gallic acid equivalent (GAE)g⁻¹ DW.

9. Superoxide dismutase activity

- a. Materials
 - i. Potassium Phosphate Buffer
 - ii. L-Methionine
 - iii. NBT
 - iv. EDTA
 - v. Riboflavin
- b. Equipment
 - i. Spectrophotometer
 - ii. Centrifuge
 - iii. Mortar and Pestle
- c. Procedure
 - i. Take 200 mg of plant material and ground in mortar with liquid nitrogen.
 - ii. Dissolve the powder in 2 ml of extraction buffer (0.1M Potassium phosphate buffer, pH7.5 with 0.5mM EDTA).
 - iii. Centrifuge the samples at 15,000xg for 10 minutes at 4°C.
 - iv. Pipette out 1ml of the supernatant and store in a refrigerator for further use.
 - v. The 3 ml reaction mixture consist of- 50mM phosphate buffer, pH 7.8; 13mM Methionine; 75uM NBT, 0.1mM EDTA, 2uM riboflavin and 300ul enzyme extract.
 - vi. Duplicate tubes with the same reaction mixture were kept in the dark and used as blanks.
 - vii. The absorbance was recorded at 560 nm and one unit of enzyme activity was taken as that amount of enzyme, which reduced the absorbance by 50 % in comparison with tubes lacking enzyme.

10. Catalase activity

- a. Materials
 - i. 50mM Phosphate buffer (pH – 7.0)
 - ii. 30mM H₂O₂ in phosphate buffer
 - iii. Reaction mixture (3ml)

- iv. 0.01ml enzyme extract
 - v. 1.99 ml 50mM phosphate buffer
 - vi. 10mM H₂O₂ (1ml of 30mM H₂O₂)
- b. Measurement:
- i. Immediately mix and measure the decrease in absorbance at 240 nm every 15 seconds for 1-2 minutes.
 - ii. The decrease in absorbance is due to the decomposition of hydrogen peroxide by catalase.
 - iii. Enzyme activity was computed by calculating the amount of H₂ O₂ decomposed using extinction coefficient ($\epsilon = 0.0436 \text{ mM}^{-1} \text{ cm}^{-1}$).

Appendix II

Python Code for Statistical Analysis

```
: import pandas as pd
import numpy as np
import seaborn as sns
import matplotlib.pyplot as plt
import statsmodels.api as sm
from statsmodels.formula.api import ols
from statsmodels.stats.anova import anova_lm
import scikit_posthocs as sp
from itertools import product
from statsmodels.stats.multicomp import pairwise_tukeyhsd

df = pd.read_excel(r'D:\Ph.D\Thesis\Data\1 Drought\Final\Analysis.xlsx')

df.columns = df.columns.str.strip()

print(df.columns.tolist())

['Water_Status', 'Rice_Variety', 'Mycorrhizal_Treatment', 'Anthocyanin', 'Catalase', 'Total_phenol_content', 'SOD', 'Proline', 'Total_soluble_sugar', 'Sucrose', 'Fructose', 'Electrolyte_leakage', 'H2O2', 'MDA', 'Plant_height', 'SLA', 'LDMC', 'Shoot_dry_biomass', 'RWC', 'Chlorophyll_a', 'Chlorophyll_b', 'Total_Chlorophyll', 'Carotenoids']

# Define independent variables
independent_vars = ['Water_Status', 'Mycorrhizal_Treatment', 'Rice_Variety']

# Then extract all columns that are NOT independent
dependent_vars = [col for col in df.columns if col not in independent_vars]

# Ensure strings
df['Water_Status'] = df['Water_Status'].astype(str)
df['Mycorrhizal_Treatment'] = df['Mycorrhizal_Treatment'].astype(str)
df['Rice_Variety'] = df['Rice_Variety'].astype(str)

water_levels = sorted(df['Water_Status'].unique())
print("Water levels:", water_levels)
print("Type of water_levels:", type(water_levels))
water_levels = sorted(df['Water_Status'].unique()) # Correct
Water levels: ['Moderate Drought', 'Well watered']
Type of water_levels: <class 'list'>

# Fix the water_levels declaration (no trailing comma!)
water_levels = sorted(df['Water_Status'].unique())
amf_levels = ['NH', 'AM1', 'AM2', 'AM3', 'AM4', 'AM5']
rice_varieties = sorted(df['Rice_Variety'].unique())
palette = sns.color_palette('Set2', len(water_levels))
bar_width = 0.2
x = np.arange(len(amf_levels))

# Styling
sns.set(style='white')

units = {
    'Anthocyanin': 'mg/g',
    'Chlorophyll_a': 'mg/g',
    'Chlorophyll_b': 'mg/g',
    'Total_chlorophyll': 'mg/g',
    'Carotenoids': 'mg/g',
    'Catalase': 'U/min/g',
    'SOD': 'U/g',
    'H2O2': 'nmol/g',
    'MDA': 'nmol/g',
    'Proline': 'µmoles/g',
    'Total_soluble_sugar': 'mg/g',
    'Sucrose': 'mg/g',
    'Fructose': 'mg/g',
    'Shoot_dry_biomass': 'g',
    'Plant_height': 'cm',
    'SLA': 'sq mm/mg',
    'LDMC': 'mg/g',
    'RWC': '%',
    'Electrolyte_leakage': '%',
    'Total_phenol_content': 'mg GAE/g',
    'Anthocyanin': 'mg/g',
    # Add others as needed
}

sns.set_context("talk", font_scale=1.3)
```

contd.

```
sns.set_context("talk", font_scale=1.3)

plt.rcParams.update({
    'axes.titlesize': 18,
    'axes.labelsize': 16,
    'xtick.labelsize': 14,
    'ytick.labelsize': 14,
    'legend.fontsize': 14,
    'legend.title_fontsize': 15,
    'figure.titlesize': 20
})

for dependent_var in dependent_vars:
    fig, axes = plt.subplots(1, len(rice_varieties), figsize=(14, 6), sharey=True)

    for ax, rice in zip(axes, rice_varieties):
        for i, water in enumerate(water_levels):
            subset = df[(df['Water_Status'] == water) & (df['Rice_Variety'] == rice)]
            means = subset.groupby('Mycorrhizal_Treatment')[dependent_var].mean().reindex(amf_levels)
            sems = subset.groupby('Mycorrhizal_Treatment')[dependent_var].sem().reindex(amf_levels)
            positions = x + i * bar_width

            ax.bar(
                positions, means, yerr=sems,
                width=bar_width,
                label=water, # use water (not salt!)
                capsize=4,
                color=palette[i]
            )

            ax.set_xticks(x + bar_width * (len(water_levels) - 1) / 2)
            ax.set_xticklabels(amf_levels, rotation=45, ha='right')
            ax.set_title(rice, fontsize=14, color='purple')
            ax.set_xlabel('Mycorrhizal Treatment')

        unit = units.get(dependent_var, '')
        ylabel = f'{dependent_var} ({unit})' if unit else f'{dependent_var} (mean ± SEM)'
        axes[0].set_ylabel(ylabel)

    fig.suptitle(f'{dependent_var} across AMF × Water Status in Rice Varieties', fontsize=16)

    handles, labels = axes[0].get_legend_handles_labels()
    fig.legend(
        handles, labels,
        title='Water Status',
        loc='center right',
        bbox_to_anchor=(1.08, 0.5)
    )

    plt.tight_layout(rect=[0, 0, 0.93, 0.95])
    plt.savefig(f'{dependent_var}_barplot.png', dpi=300, bbox_inches='tight')
    plt.close()
```

Appendix III

Appendix Table 1: Descriptive Statistics (Mean $\pm$ SD) of Anthocyanin, Catalase, Total Phenolic Content (TPC), Superoxide Dismutase (SOD), and Proline of Rice Plants Under Different Water Status, Rice Varieties, and Mycorrhizal Treatments

Water Status	Rice Variety	Mycorrhizal Treatment	Anthocyanin (mg/g)	Catalase (U/min/g)	TPC (GAE/g)	SOD (U/g)	Proline (μ moles/g)
Moderate Drought	Changkawi	AM1	0.314 $\pm$ 0.208	1.59 $\pm$ 0.687	0.623 $\pm$ 0.01	11.017 $\pm$ 1.969	41.149 $\pm$ 2.539
Moderate Drought	Changkawi	AM2	0.203 $\pm$ 0.115	1.774 $\pm$ 0.897	0.601 $\pm$ 0.003	9.79 $\pm$ 1.505	41.933 $\pm$ 1.207
Moderate Drought	Changkawi	AM3	0.444 $\pm$ 0.096	1.911 $\pm$ 1.252	0.65 $\pm$ 0.01	12.123 $\pm$ 0.988	49.164 $\pm$ 2.61
Moderate Drought	Changkawi	AM4	0.571 $\pm$ 0.093	1.835 $\pm$ 1.171	0.682 $\pm$ 0.008	11.21 $\pm$ 1.345	39.929 $\pm$ 1.891
Moderate Drought	Changkawi	AM5	0.649 $\pm$ 0.099	2.232 $\pm$ 0.115	0.8 $\pm$ 0.011	10.816 $\pm$ 1.849	40.452 $\pm$ 2.822
Moderate Drought	Changkawi	NM	0.124 $\pm$ 0.087	1.575 $\pm$ 0.911	0.558 $\pm$ 0.016	9.565 $\pm$ 0.565	55.829 $\pm$ 4.596
Moderate Drought	Dhan 50	AM1	0.235 $\pm$ 0.249	1.529 $\pm$ 1.046	0.551 $\pm$ 0.01	10.247 $\pm$ 1.246	45.723 $\pm$ 5.043
Moderate Drought	Dhan 50	AM2	0.557 $\pm$ 0.043	1.315 $\pm$ 0.106	0.559 $\pm$ 0.013	10.417 $\pm$ 0.429	41.149 $\pm$ 1.779
Moderate Drought	Dhan 50	AM3	0.554 $\pm$ 0.365	2.037 $\pm$ 0.81	0.577 $\pm$ 0.017	9.949 $\pm$ 1.255	40.67 $\pm$ 1.247
Moderate Drought	Dhan 50	AM4	0.491 $\pm$ 0.331	1.621 $\pm$ 0.592	0.641 $\pm$ 0.004	12.09 $\pm$ 2.038	39.842 $\pm$ 0.272
Moderate Drought	Dhan 50	AM5	0.477 $\pm$ 0.183	1.633 $\pm$ 0.177	0.653 $\pm$ 0.004	11.862 $\pm$ 1.146	39.711 $\pm$ 1.373
Moderate Drought	Dhan 50	NM	0.282 $\pm$ 0.126	1.254 $\pm$ 0.305	0.498 $\pm$ 0.01	9.347 $\pm$ 0.626	59.358 $\pm$ 0.523
Well-watered	Changkawi	AM1	0.362 $\pm$ 0.192	0.935 $\pm$ 0.175	0.497 $\pm$ 0.002	5.658 $\pm$ 0.287	17.625 $\pm$ 0.755
Well-watered	Changkawi	AM2	0.461 $\pm$ 0.156	0.847 $\pm$ 0.308	0.522 $\pm$ 0.004	5.382 $\pm$ 0.521	19.324 $\pm$ 0.87
Well-watered	Changkawi	AM3	0.363 $\pm$ 0.088	1.06 $\pm$ 0.051	0.467 $\pm$ 0.007	4.559 $\pm$ 0.138	18.017 $\pm$ 0.788
Well-watered	Changkawi	AM4	0.683 $\pm$ 0.218	1.154 $\pm$ 0.157	0.518 $\pm$ 0.018	5.576 $\pm$ 0.336	17.233 $\pm$ 0.788
Well-watered	Changkawi	AM5	0.497 $\pm$ 0.13	1.189 $\pm$ 0.117	0.527 $\pm$ 0.01	5.155 $\pm$ 0.654	17.669 $\pm$ 0.471
Well-watered	Changkawi	NM	0.147 $\pm$ 0.032	0.688 $\pm$ 0.073	0.438 $\pm$ 0.024	4.852 $\pm$ 0.063	21.851 $\pm$ 0.857
Well-watered	Dhan 50	AM1	0.248 $\pm$ 0.123	0.764 $\pm$ 0.062	0.517 $\pm$ 0.01	5.006 $\pm$ 0.605	22.33 $\pm$ 0.528
Well-watered	Dhan 50	AM2	0.6 $\pm$ 0.316	0.814 $\pm$ 0.195	0.472 $\pm$ 0.008	5.051 $\pm$ 0.279	18.322 $\pm$ 1.198
Well-watered	Dhan 50	AM3	0.459 $\pm$ 0.117	1.11 $\pm$ 0.124	0.459 $\pm$ 0.003	5.109 $\pm$ 0.595	18.627 $\pm$ 0.459
Well-watered	Dhan 50	AM4	0.409 $\pm$ 0.118	1.118 $\pm$ 0.069	0.54 $\pm$ 0.008	5.269 $\pm$ 0.233	16.231 $\pm$ 1.07
Well-watered	Dhan 50	AM5	0.431 $\pm$ 0.28	1.012 $\pm$ 0.253	0.521 $\pm$ 0.026	5.48 $\pm$ 0.153	17.32 $\pm$ 0.589
Well-watered	Dhan 50	NM	0.248 $\pm$ 0.122	0.737 $\pm$ 0.031	0.449 $\pm$ 0.01	5.001 $\pm$ 0.668	25.728 $\pm$ 0.671

Appendix Table 2: Descriptive Statistics (Mean $\pm$ SD) of Total Soluble Sugars (TSS), Sucrose, Fructose, Catalase, H₂O₂, and Malondialdehyde (MDA) of Rice Plants Under Different Water Status, Rice Varieties, and Mycorrhizal Treatments

Water Status	Rice Variety	Mycorrhizal Treatment	TSS (mg/g)	Sucrose (mg/g)	Fructose (mg/g)	H ₂ O ₂ (nmol/g)	MDA (nmol/g)
Moderate Drought	Changkawi	AM1	93.374 $\pm$ 1.68	12.791 $\pm$ 0.364	9.977 $\pm$ 0.64	66.667 $\pm$ 3.868	78.387 $\pm$ 2.819
Moderate Drought	Changkawi	AM2	94.607 $\pm$ 5.194	13.779 $\pm$ 0.127	9.201 $\pm$ 0.12	61.742 $\pm$ 2.685	77.935 $\pm$ 2.808
Moderate Drought	Changkawi	AM3	85.375 $\pm$ 4.408	14.932 $\pm$ 0.198	9.261 $\pm$ 0.393	64.962 $\pm$ 0.656	73.063 $\pm$ 16.504
Moderate Drought	Changkawi	AM4	89.96 $\pm$ 2.821	15.062 $\pm$ 0.075	9.449 $\pm$ 0.441	61.932 $\pm$ 5.905	64.808 $\pm$ 22.045
Moderate Drought	Changkawi	AM5	91.993 $\pm$ 1.671	14.154 $\pm$ 0.065	9.183 $\pm$ 1.055	66.288 $\pm$ 1.183	70.75 $\pm$ 5.119
Moderate Drought	Changkawi	NM	97.154 $\pm$ 0.241	18.694 $\pm$ 2.433	10.114 $\pm$ 0.183	90.53 $\pm$ 4.031	98.618 $\pm$ 35.037
Moderate Drought	Dhan 50	AM1	91.086 $\pm$ 2.689	11.112 $\pm$ 0.227	8.55 $\pm$ 0.416	63.068 $\pm$ 0.568	75.207 $\pm$ 9.091
Moderate Drought	Dhan 50	AM2	93.131 $\pm$ 2.278	12.143 $\pm$ 0.192	9.334 $\pm$ 1.875	61.553 $\pm$ 0.868	70.116 $\pm$ 0.419
Moderate Drought	Dhan 50	AM3	90.614 $\pm$ 1.485	13.685 $\pm$ 0.164	8.977 $\pm$ 0.347	62.5 $\pm$ 2.049	75.187 $\pm$ 24.809
Moderate Drought	Dhan 50	AM4	90.31 $\pm$ 4.281	11.126 $\pm$ 0.142	8.343 $\pm$ 0.587	54.356 $\pm$ 1.995	65.776 $\pm$ 7.969
Moderate Drought	Dhan 50	AM5	86.179 $\pm$ 1.599	10.543 $\pm$ 0.022	9.33 $\pm$ 0.537	54.545 $\pm$ 1.136	68.106 $\pm$ 16.849
Moderate Drought	Dhan 50	NM	103.137 $\pm$ 2.977	13.721 $\pm$ 0.099	10.807 $\pm$ 0.918	74.621 $\pm$ 2.151	81.728 $\pm$ 4.975
Well-watered	Changkawi	AM1	62.76 $\pm$ 2.155	11.588 $\pm$ 0.264	9.325 $\pm$ 0.349	48.674 $\pm$ 2.803	46.513 $\pm$ 0.833
Well-watered	Changkawi	AM2	65.202 $\pm$ 4.237	11.487 $\pm$ 0.7	5.861 $\pm$ 1.388	49.432 $\pm$ 1.136	47.311 $\pm$ 2.811
Well-watered	Changkawi	AM3	66.707 $\pm$ 2.58	11.393 $\pm$ 1.161	6.439 $\pm$ 1.17	48.864 $\pm$ 1.503	46.462 $\pm$ 1.632
Well-watered	Changkawi	AM4	65.432 $\pm$ 3.84	13.822 $\pm$ 0.423	6.623 $\pm$ 0.099	43.75 $\pm$ 1.503	42.695 $\pm$ 3.004
Well-watered	Changkawi	AM5	65.56 $\pm$ 2.041	13.8 $\pm$ 0.054	7.536 $\pm$ 0.497	42.992 $\pm$ 1.995	43.254 $\pm$ 2.581
Well-watered	Changkawi	NM	63.342 $\pm$ 2.787	10.651 $\pm$ 0.114	8.082 $\pm$ 0.869	53.22 $\pm$ 1.64	58.539 $\pm$ 3.304
Well-watered	Dhan 50	AM1	67.234 $\pm$ 2.202	11.235 $\pm$ 0.323	7.83 $\pm$ 2.176	45.265 $\pm$ 1.43	43.518 $\pm$ 7.954
Well-watered	Dhan 50	AM2	64.555 $\pm$ 1.655	13.209 $\pm$ 0.47	7.403 $\pm$ 0.325	46.212 $\pm$ 2.86	40.92 $\pm$ 9.513
Well-watered	Dhan 50	AM3	65.498 $\pm$ 1.611	12.835 $\pm$ 0.244	7.99 $\pm$ 1.367	42.614 $\pm$ 2.477	45.694 $\pm$ 6.764
Well-watered	Dhan 50	AM4	65.42 $\pm$ 1.759	10.881 $\pm$ 1.003	8.233 $\pm$ 0.861	37.879 $\pm$ 0.868	33.07 $\pm$ 4.051
Well-watered	Dhan 50	AM5	63.463 $\pm$ 3.055	10.694 $\pm$ 1.247	7.807 $\pm$ 0.275	36.742 $\pm$ 1.736	35.101 $\pm$ 1.305
Well-watered	Dhan 50	NM	68.971 $\pm$ 1.072	10.658 $\pm$ 0.045	8.995 $\pm$ 0.583	50.947 $\pm$ 1.183	56.45 $\pm$ 9.08

Appendix Table 3: Descriptive Statistics (Mean $\pm$ SD) of Height, Specific Leaf Area (SLA), Leaf Dry Matter Content (LDMC), Shoot Biomass, and Relative Water Content (RWC) of Rice Plants Under Different Water Status, Rice Varieties, and Mycorrhizal Treatments

Water Status	Rice Variety	Mycorrhizal Treatment	Height (cm)	SLA (mm ² /mg)	LDMC (mg/g)	Shoot biomass (g/plant)	RWC (%)
Moderate Drought	Changkawi	AM1	72.667 $\pm$ 4.933	33.41 $\pm$ 11.321	31.734 $\pm$ 3.014	3.77 $\pm$ 0.226	76.713 $\pm$ 9.504
Moderate Drought	Changkawi	AM2	72.667 $\pm$ 4.041	34.559 $\pm$ 3.058	29.818 $\pm$ 1.564	4.12 $\pm$ 0.154	79.825 $\pm$ 11.801
Moderate Drought	Changkawi	AM3	75.0 $\pm$ 2.646	30.106 $\pm$ 1.574	30.474 $\pm$ 1.585	3.97 $\pm$ 0.46	80.494 $\pm$ 9.271
Moderate Drought	Changkawi	AM4	72.667 $\pm$ 1.528	32.009 $\pm$ 3.002	32.896 $\pm$ 2.171	4.2 $\pm$ 0.312	85.354 $\pm$ 3.459
Moderate Drought	Changkawi	AM5	73.667 $\pm$ 3.786	30.353 $\pm$ 1.736	31.691 $\pm$ 1.845	4.01 $\pm$ 0.365	88.805 $\pm$ 2.264
Moderate Drought	Changkawi	NM	70.333 $\pm$ 1.528	22.613 $\pm$ 2.195	32.93 $\pm$ 3.39	3.247 $\pm$ 0.342	79.786 $\pm$ 4.553
Moderate Drought	Dhan 50	AM1	70.333 $\pm$ 3.215	34.036 $\pm$ 4.821	31.76 $\pm$ 1.653	3.386 $\pm$ 0.358	83.737 $\pm$ 3.324
Moderate Drought	Dhan 50	AM2	73.0 $\pm$ 3.0	38.553 $\pm$ 1.468	32.839 $\pm$ 2.635	3.596 $\pm$ 0.417	74.727 $\pm$ 7.265
Moderate Drought	Dhan 50	AM3	72.333 $\pm$ 4.509	32.134 $\pm$ 1.147	33.042 $\pm$ 1.236	3.797 $\pm$ 0.156	78.332 $\pm$ 6.112
Moderate Drought	Dhan 50	AM4	73.0 $\pm$ 4.359	38.715 $\pm$ 3.577	30.037 $\pm$ 1.505	3.452 $\pm$ 0.154	80.36 $\pm$ 6.131
Moderate Drought	Dhan 50	AM5	66.333 $\pm$ 13.013	36.606 $\pm$ 8.026	32.32 $\pm$ 2.792	3.573 $\pm$ 0.326	85.011 $\pm$ 2.905
Moderate Drought	Dhan 50	NM	63.0 $\pm$ 2.0	24.604 $\pm$ 0.621	33.733 $\pm$ 1.605	3.022 $\pm$ 0.529	66.562 $\pm$ 5.905
Well-watered	Changkawi	AM1	81.667 $\pm$ 2.517	36.508 $\pm$ 3.732	29.36 $\pm$ 1.352	5.118 $\pm$ 1.588	84.071 $\pm$ 3.854
Well-watered	Changkawi	AM2	77.667 $\pm$ 2.309	39.55 $\pm$ 4.293	27.898 $\pm$ 2.785	4.697 $\pm$ 0.393	76.673 $\pm$ 10.263
Well-watered	Changkawi	AM3	83.0 $\pm$ 2.0	39.684 $\pm$ 2.815	28.191 $\pm$ 2.789	5.122 $\pm$ 1.175	76.19 $\pm$ 11.905
Well-watered	Changkawi	AM4	77.0 $\pm$ 3.0	38.181 $\pm$ 12.284	29.175 $\pm$ 0.785	4.827 $\pm$ 1.604	73.846 $\pm$ 6.96
Well-watered	Changkawi	AM5	74.667 $\pm$ 1.528	37.768 $\pm$ 0.099	18.337 $\pm$ 0.703	5.353 $\pm$ 1.45	76.532 $\pm$ 6.416
Well-watered	Changkawi	NM	72.667 $\pm$ 0.577	33.896 $\pm$ 4.582	30.281 $\pm$ 0.588	4.164 $\pm$ 0.083	73.247 $\pm$ 0.9
Well-watered	Dhan 50	AM1	85.0 $\pm$ 3.606	39.212 $\pm$ 6.328	26.453 $\pm$ 1.061	5.624 $\pm$ 1.503	86.784 $\pm$ 8.544
Well-watered	Dhan 50	AM2	88.0 $\pm$ 5.568	41.128 $\pm$ 6.904	28.407 $\pm$ 1.06	5.583 $\pm$ 0.34	73.189 $\pm$ 9.317
Well-watered	Dhan 50	AM3	80.333 $\pm$ 5.859	40.38 $\pm$ 2.966	25.409 $\pm$ 0.385	5.07 $\pm$ 0.444	80.827 $\pm$ 8.517
Well-watered	Dhan 50	AM4	80.333 $\pm$ 9.018	45.329 $\pm$ 3.684	23.766 $\pm$ 1.535	5.631 $\pm$ 0.052	76.368 $\pm$ 7.586
Well-watered	Dhan 50	AM5	78.0 $\pm$ 12.166	45.091 $\pm$ 3.365	29.705 $\pm$ 1.004	5.369 $\pm$ 0.148	86.225 $\pm$ 3.671
Well-watered	Dhan 50	NM	71.0 $\pm$ 9.849	36.581 $\pm$ 1.483	29.87 $\pm$ 1.922	4.133 $\pm$ 0.381	66.799 $\pm$ 10.073

Appendix Table 4: Descriptive Statistics (Mean $\pm$ SD) of Electrolyte leakage (EL), Chlorophyll a, Chlorophyll b, Total Chlorophyll and Carotenoids of Rice Plants Under Different Water Status, Rice Varieties, and Mycorrhizal Treatments

Water Status	Rice Variety	Mycorrhizal Treatment	EL (%)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total Chlorophyll (mg/g)	Carotenoids (mg/g)
Moderate Drought	Changkawi	AM1	29.8 $\pm$ 1.262	1.359 $\pm$ 0.155	0.559 $\pm$ 0.01	1.918 $\pm$ 0.164	0.476 $\pm$ 0.046
Moderate Drought	Changkawi	AM2	32.122 $\pm$ 1.19	1.293 $\pm$ 0.249	0.56 $\pm$ 0.082	1.852 $\pm$ 0.33	0.456 $\pm$ 0.136
Moderate Drought	Changkawi	AM3	33.129 $\pm$ 1.854	1.078 $\pm$ 0.053	0.504 $\pm$ 0.016	1.582 $\pm$ 0.067	0.351 $\pm$ 0.031
Moderate Drought	Changkawi	AM4	24.184 $\pm$ 5.424	1.407 $\pm$ 0.439	0.589 $\pm$ 0.099	1.995 $\pm$ 0.538	0.49 $\pm$ 0.192
Moderate Drought	Changkawi	AM5	26.838 $\pm$ 2.198	1.744 $\pm$ 0.077	0.663 $\pm$ 0.048	2.406 $\pm$ 0.124	0.621 $\pm$ 0.049
Moderate Drought	Changkawi	NM	42.324 $\pm$ 2.64	0.943 $\pm$ 0.115	0.489 $\pm$ 0.013	1.431 $\pm$ 0.108	0.299 $\pm$ 0.037
Moderate Drought	Dhan 50	AM1	27.552 $\pm$ 0.941	1.738 $\pm$ 0.039	0.723 $\pm$ 0.06	2.46 $\pm$ 0.099	0.667 $\pm$ 0.088
Moderate Drought	Dhan 50	AM2	27.549 $\pm$ 4.355	2.171 $\pm$ 0.461	0.778 $\pm$ 0.142	2.947 $\pm$ 0.602	0.743 $\pm$ 0.12
Moderate Drought	Dhan 50	AM3	27.344 $\pm$ 0.965	3.003 $\pm$ 0.42	0.887 $\pm$ 0.154	3.889 $\pm$ 0.574	1.06 $\pm$ 0.248
Moderate Drought	Dhan 50	AM4	22.866 $\pm$ 4.224	3.222 $\pm$ 0.305	1.083 $\pm$ 0.09	4.304 $\pm$ 0.348	1.109 $\pm$ 0.42
Moderate Drought	Dhan 50	AM5	20.578 $\pm$ 2.012	2.765 $\pm$ 1.001	0.966 $\pm$ 0.295	3.73 $\pm$ 1.294	1.235 $\pm$ 0.235
Moderate Drought	Dhan 50	NM	33.077 $\pm$ 3.109	1.148 $\pm$ 0.111	0.544 $\pm$ 0.032	1.691 $\pm$ 0.135	0.346 $\pm$ 0.049
Well-watered	Changkawi	AM1	18.033 $\pm$ 1.424	1.266 $\pm$ 0.104	0.522 $\pm$ 0.024	1.788 $\pm$ 0.104	0.485 $\pm$ 0.144
Well-watered	Changkawi	AM2	15.536 $\pm$ 2.536	1.141 $\pm$ 0.049	0.625 $\pm$ 0.169	1.765 $\pm$ 0.162	0.435 $\pm$ 0.114
Well-watered	Changkawi	AM3	17.2 $\pm$ 3.937	1.097 $\pm$ 0.073	0.864 $\pm$ 0.091	1.96 $\pm$ 0.044	0.464 $\pm$ 0.064
Well-watered	Changkawi	AM4	13.622 $\pm$ 5.473	1.342 $\pm$ 0.215	0.839 $\pm$ 0.034	2.18 $\pm$ 0.192	0.551 $\pm$ 0.105
Well-watered	Changkawi	AM5	11.258 $\pm$ 1.274	2.01 $\pm$ 0.619	0.894 $\pm$ 0.231	2.904 $\pm$ 0.416	0.812 $\pm$ 0.134
Well-watered	Changkawi	NM	24.751 $\pm$ 0.674	0.641 $\pm$ 0.156	0.583 $\pm$ 0.114	1.223 $\pm$ 0.044	0.256 $\pm$ 0.045
Well-watered	Dhan 50	AM1	17.011 $\pm$ 0.88	2.989 $\pm$ 0.856	0.887 $\pm$ 0.113	3.875 $\pm$ 0.967	1.155 $\pm$ 0.327
Well-watered	Dhan 50	AM2	17.438 $\pm$ 1.335	3.278 $\pm$ 0.7	0.999 $\pm$ 0.18	4.276 $\pm$ 0.875	1.307 $\pm$ 0.308
Well-watered	Dhan 50	AM3	15.541 $\pm$ 0.088	2.83 $\pm$ 0.327	0.871 $\pm$ 0.16	3.701 $\pm$ 0.483	1.178 $\pm$ 0.273
Well-watered	Dhan 50	AM4	12.327 $\pm$ 1.781	3.307 $\pm$ 0.364	1.006 $\pm$ 0.089	4.312 $\pm$ 0.446	1.341 $\pm$ 0.16
Well-watered	Dhan 50	AM5	10.908 $\pm$ 0.139	1.746 $\pm$ 0.724	0.625 $\pm$ 0.173	2.37 $\pm$ 0.897	0.613 $\pm$ 0.335
Well-watered	Dhan 50	NM	21.485 $\pm$ 0.602	1.262 $\pm$ 0.333	0.628 $\pm$ 0.11	1.889 $\pm$ 0.442	0.545 $\pm$ 0.171

Appendix Table 5: Descriptive Statistics (Mean $\pm$ SD) of Anthocyanin, Catalase, Total Phenolic Content (TPC), Superoxide Dismutase (SOD), and Proline of Rice Plants Under Different NaCl Treatments, Rice Varieties, and Mycorrhizal Treatments

Salt Treatment	Rice Variety	Mycorrhizal Treatment	Anthocyanin (mg/g)	Catalase (U/min/g)	TPC (GAE/g)	SOD (U/g)	Proline (μ moles/g)
0 mM	Changkawi	AM1	0.834 $\pm$ 0.125	0.98 $\pm$ 0.155	0.029 $\pm$ 0.0	4.764 $\pm$ 1.081	15.212 $\pm$ 2.958
0 mM	Changkawi	AM2	1.255 $\pm$ 0.216	0.854 $\pm$ 0.042	0.028 $\pm$ 0.0	4.756 $\pm$ 1.129	13.522 $\pm$ 0.579
0 mM	Changkawi	AM3	0.816 $\pm$ 0.218	0.828 $\pm$ 0.289	0.03 $\pm$ 0.0	4.631 $\pm$ 1.117	14.484 $\pm$ 1.118
0 mM	Changkawi	AM4	2.335 $\pm$ 0.373	0.92 $\pm$ 0.224	0.03 $\pm$ 0.0	4.351 $\pm$ 0.897	16.114 $\pm$ 0.943
0 mM	Changkawi	AM5	1.186 $\pm$ 0.385	0.887 $\pm$ 0.244	0.026 $\pm$ 0.002	4.819 $\pm$ 0.273	14.824 $\pm$ 0.307
0 mM	Changkawi	NM	0.513 $\pm$ 0.33	0.644 $\pm$ 0.058	0.024 $\pm$ 0.0	5.09 $\pm$ 0.412	13.705 $\pm$ 3.226
0 mM	Dhan 50	AM1	1.403 $\pm$ 0.232	0.856 $\pm$ 0.273	0.024 $\pm$ 0.0	4.486 $\pm$ 1.279	15.164 $\pm$ 0.929
0 mM	Dhan 50	AM2	1.35 $\pm$ 0.022	1.066 $\pm$ 0.095	0.028 $\pm$ 0.001	4.54 $\pm$ 1.183	16.318 $\pm$ 4.233
0 mM	Dhan 50	AM3	1.537 $\pm$ 0.227	0.783 $\pm$ 0.352	0.025 $\pm$ 0.002	4.69 $\pm$ 1.091	16.188 $\pm$ 2.297
0 mM	Dhan 50	AM4	2.018 $\pm$ 0.754	0.761 $\pm$ 0.123	0.023 $\pm$ 0.0	4.966 $\pm$ 0.928	16.318 $\pm$ 0.2
0 mM	Dhan 50	AM5	1.903 $\pm$ 1.005	1.087 $\pm$ 0.062	0.025 $\pm$ 0.0	4.714 $\pm$ 1.392	14.097 $\pm$ 1.171
0 mM	Dhan 50	NM	0.846 $\pm$ 0.194	0.707 $\pm$ 0.051	0.023 $\pm$ 0.0	4.595 $\pm$ 0.921	19.986 $\pm$ 0.551
75 mM	Changkawi	AM1	0.306 $\pm$ 0.287	1.419 $\pm$ 0.12	0.037 $\pm$ 0.001	8.489 $\pm$ 1.115	20.222 $\pm$ 0.246
75 mM	Changkawi	AM2	0.273 $\pm$ 0.264	1.397 $\pm$ 0.171	0.036 $\pm$ 0.001	8.255 $\pm$ 1.231	20.352 $\pm$ 0.774
75 mM	Changkawi	AM3	0.495 $\pm$ 0.068	1.28 $\pm$ 0.215	0.039 $\pm$ 0.0	8.614 $\pm$ 0.712	21.459 $\pm$ 7.36
75 mM	Changkawi	AM4	1.136 $\pm$ 0.209	1.312 $\pm$ 0.164	0.037 $\pm$ 0.001	9.237 $\pm$ 0.873	19.716 $\pm$ 0.618
75 mM	Changkawi	AM5	0.88 $\pm$ 0.346	1.239 $\pm$ 0.275	0.037 $\pm$ 0.0	8.532 $\pm$ 0.653	21.598 $\pm$ 3.249
75 mM	Changkawi	NM	0.192 $\pm$ 0.085	1.057 $\pm$ 0.055	0.031 $\pm$ 0.0	7.705 $\pm$ 0.074	22.983 $\pm$ 4.934
75 mM	Dhan 50	AM1	0.938 $\pm$ 0.377	1.281 $\pm$ 0.149	0.034 $\pm$ 0.002	8.605 $\pm$ 0.662	30.812 $\pm$ 15.979
75 mM	Dhan 50	AM2	1.439 $\pm$ 0.028	1.32 $\pm$ 0.11	0.04 $\pm$ 0.0	9.328 $\pm$ 0.701	33.77 $\pm$ 5.56
75 mM	Dhan 50	AM3	1.171 $\pm$ 0.11	1.323 $\pm$ 0.07	0.036 $\pm$ 0.001	9.052 $\pm$ 1.036	21.698 $\pm$ 2.711
75 mM	Dhan 50	AM4	1.715 $\pm$ 0.145	1.345 $\pm$ 0.176	0.035 $\pm$ 0.001	9.232 $\pm$ 0.616	20.644 $\pm$ 4.036
75 mM	Dhan 50	AM5	1.7 $\pm$ 0.159	1.226 $\pm$ 0.071	0.034 $\pm$ 0.002	8.504 $\pm$ 0.99	29.104 $\pm$ 11.529
75 mM	Dhan 50	NM	0.025 $\pm$ 0.022	1.117 $\pm$ 0.015	0.031 $\pm$ 0.001	7.532 $\pm$ 0.292	36.784 $\pm$ 4.011
150 mM	Changkawi	AM1	0.257 $\pm$ 0.106	1.518 $\pm$ 0.04	0.022 $\pm$ 0.0	11.519 $\pm$ 1.375	37.925 $\pm$ 9.883
150 mM	Changkawi	AM2	0.301 $\pm$ 0.28	1.364 $\pm$ 0.274	0.02 $\pm$ 0.001	12.574 $\pm$ 2.18	37.838 $\pm$ 3.286
150 mM	Changkawi	AM3	0.162 $\pm$ 0.082	1.406 $\pm$ 0.199	0.021 $\pm$ 0.002	13.117 $\pm$ 2.248	29.344 $\pm$ 4.72
150 mM	Changkawi	AM4	0.409 $\pm$ 0.129	1.295 $\pm$ 0.206	0.022 $\pm$ 0.0	13.254 $\pm$ 2.822	35.399 $\pm$ 17.603
150 mM	Changkawi	AM5	0.627 $\pm$ 0.192	1.381 $\pm$ 0.064	0.021 $\pm$ 0.0	14.705 $\pm$ 2.626	30.868 $\pm$ 5.713

150 mM	Changkawi	NM	0.071 ± 0.012	1.089 ± 0.05	0.019 ± 0.0	11.131 ± 0.431	37.272 ± 15.425
150 mM	Dhan 50	AM1	0.273 ± 0.028	1.461 ± 0.129	0.023 ± 0.002	13.308 ± 1.545	41.802 ± 16.384
150 mM	Dhan 50	AM2	0.412 ± 0.286	1.253 ± 0.234	0.025 ± 0.001	14.04 ± 2.653	28.995 ± 5.836
150 mM	Dhan 50	AM3	0.123 ± 0.178	1.545 ± 0.01	0.021 ± 0.001	11.117 ± 0.337	28.429 ± 13.662
150 mM	Dhan 50	AM4	0.925 ± 0.176	1.501 ± 0.11	0.02 ± 0.001	14.41 ± 2.52	35.137 ± 18.762
150 mM	Dhan 50	AM5	1.035 ± 0.164	1.296 ± 0.117	0.024 ± 0.0	13.872 ± 2.637	43.327 ± 7.563
150 mM	Dhan 50	NM	0.166 ± 0.081	1.171 ± 0.061	0.019 ± 0.0	10.53 ± 0.418	47.77 ± 2.867

Appendix Table 6: Descriptive Statistics (Mean ± SD) of Total Soluble Sugars (TSS), Sucrose, Fructose, Catalase, H₂O₂, and Malondialdehyde (MDA) of Rice Plants Under Different NaCl Treatments, Rice Varieties, and Mycorrhizal Treatments

Salt Treatment	Rice Variety	Mycorrhizal Treatment	TSS (mg/g)	Sucrose (mg/g)	Fructose (mg/g)	H ₂ O ₂ (nmol/g)	MDA (nmol/g)
0 mM	Changkawi	AM1	61.166 ± 6.478	3.89 ± 0.123	4.038 ± 1.174	47.973 ± 0.868	42.701 ± 27.035
0 mM	Changkawi	AM2	65.822 ± 11.649	3.934 ± 0.109	3.133 ± 0.803	47.595 ± 0.656	44.773 ± 8.568
0 mM	Changkawi	AM3	66.945 ± 5.795	3.249 ± 0.045	4.134 ± 0.894	49.678 ± 0.868	46.524 ± 17.661
0 mM	Changkawi	AM4	60.274 ± 4.821	3.754 ± 0.172	2.763 ± 0.627	49.11 ± 0.328	40.137 ± 4.727
0 mM	Changkawi	AM5	63.429 ± 9.301	3.242 ± 0.045	4.375 ± 0.342	48.92 ± 0.984	40.615 ± 7.906
0 mM	Changkawi	NM	62.861 ± 9.868	5.0 ± 0.097	3.292 ± 0.423	57.633 ± 1.826	55.873 ± 11.174
0 mM	Dhan 50	AM1	65.913 ± 9.898	3.126 ± 0.057	3.282 ± 1.283	48.542 ± 1.995	41.273 ± 2.335
0 mM	Dhan 50	AM2	65.837 ± 4.832	3.782 ± 0.062	3.617 ± 1.324	47.784 ± 1.136	39.882 ± 4.271
0 mM	Dhan 50	AM3	66.035 ± 5.594	3.703 ± 0.033	3.057 ± 0.548	46.648 ± 1.136	39.72 ± 3.438
0 mM	Dhan 50	AM4	63.116 ± 11.736	3.883 ± 0.094	4.133 ± 0.425	44.186 ± 1.183	35.271 ± 29.091
0 mM	Dhan 50	AM5	62.516 ± 0.215	4.049 ± 0.033	3.58 ± 1.447	45.89 ± 0.328	36.047 ± 2.198
0 mM	Dhan 50	NM	62.484 ± 9.895	4.071 ± 0.213	2.6 ± 0.641	55.17 ± 2.049	51.366 ± 52.897
75 mM	Changkawi	AM1	88.638 ± 6.429	4.763 ± 0.012	6.439 ± 0.924	58.011 ± 1.503	72.086 ± 3.052
75 mM	Changkawi	AM2	93.029 ± 4.243	5.375 ± 0.135	6.493 ± 1.031	58.58 ± 2.273	79.789 ± 10.173
75 mM	Changkawi	AM3	82.489 ± 3.508	5.808 ± 2.275	6.011 ± 1.458	56.496 ± 0.868	74.068 ± 7.963
75 mM	Changkawi	AM4	86.708 ± 3.711	5.058 ± 0.076	7.168 ± 0.658	57.254 ± 0.328	70.801 ± 1.884
75 mM	Changkawi	AM5	93.137 ± 4.631	4.388 ± 0.275	6.863 ± 0.77	57.064 ± 5.156	70.214 ± 1.289
75 mM	Changkawi	NM	96.723 ± 2.784	3.681 ± 0.033	6.231 ± 0.985	68.807 ± 2.477	84.307 ± 19.983
75 mM	Dhan 50	AM1	84.616 ± 2.088	3.948 ± 0.114	6.868 ± 0.121	60.473 ± 0.868	70.145 ± 33.08
75 mM	Dhan 50	AM2	83.297 ± 2.167	3.963 ± 0.045	5.773 ± 0.673	62.367 ± 0.868	71.692 ± 1.873

75 mM	Dhan 50	AM3	94.709 ± 9.298	4.013 ± 0.057	7.357 ± 0.311	61.61 ± 2.366	68.44 ± 31.988
75 mM	Dhan 50	AM4	89.662 ± 9.512	4.633 ± 0.07	5.961 ± 0.44	52.898 ± 0.568	58.299 ± 25.933
75 mM	Dhan 50	AM5	89.983 ± 9.431	3.408 ± 0.099	5.52 ± 0.742	56.307 ± 1.705	61.062 ± 48.642
75 mM	Dhan 50	NM	98.939 ± 0.526	3.112 ± 0.012	5.558 ± 0.381	67.86 ± 0.868	83.731 ± 44.217
150 mM	Changkawi	AM1	49.426 ± 6.75	1.613 ± 0.112	4.98 ± 0.346	62.557 ± 0.568	188.18 ± 13.106
150 mM	Changkawi	AM2	47.435 ± 4.115	1.447 ± 0.087	4.702 ± 0.493	61.42 ± 0.0	185.983 ± 24.28
150 mM	Changkawi	AM3	44.762 ± 2.804	1.526 ± 0.022	4.724 ± 0.229	61.989 ± 0.568	176.638 ± 61.687
150 mM	Changkawi	AM4	46.29 ± 4.932	1.476 ± 0.119	4.432 ± 1.08	59.148 ± 0.568	166.206 ± 2.599
150 mM	Changkawi	AM5	45.263 ± 3.065	1.39 ± 0.082	4.044 ± 0.868	58.769 ± 2.916	166.497 ± 1.133
150 mM	Changkawi	NM	53.02 ± 1.242	1.354 ± 0.037	3.903 ± 0.844	79.792 ± 0.868	225.345 ± 22.353
150 mM	Dhan 50	AM1	46.786 ± 3.259	2.694 ± 0.449	3.685 ± 0.373	65.398 ± 0.568	169.004 ± 4.032
150 mM	Dhan 50	AM2	50.269 ± 5.544	2.608 ± 0.281	4.347 ± 1.032	65.587 ± 0.328	168.519 ± 1.826
150 mM	Dhan 50	AM3	48.667 ± 6.045	3.999 ± 1.955	4.403 ± 1.128	64.64 ± 0.868	168.137 ± 3.648
150 mM	Dhan 50	AM4	44.652 ± 6.514	4.928 ± 4.263	4.155 ± 1.529	59.716 ± 0.568	107.202 ± 8.89
150 mM	Dhan 50	AM5	48.908 ± 2.221	2.6 ± 0.986	4.198 ± 0.617	61.042 ± 0.868	110.018 ± 10.445
150 mM	Dhan 50	NM	57.186 ± 2.868	1.865 ± 0.38	4.605 ± 0.103	77.708 ± 1.995	219.691 ± 91.807

Appendix Table 7: Descriptive Statistics (Mean ± SD) of Height, Specific Leaf Area (SLA), Leaf Dry Matter Content (LDMC), Shoot Biomass, and Relative Water Content (RWC) of Rice Plants Under Different NaCl Treatments, Rice Varieties, and Mycorrhizal Treatments

Salt Treatment	Rice Variety	Mycorrhizal Treatment	Height (cm)	SLA (mm ² /mg)	LDMC (mg/g)	Shoot biomass (g/plant)	RWC (%)
0 mM	Changkawi	AM1	84.333 ± 2.517	34.709 ± 14.023	25.9 ± 2.762	5.779 ± 1.205	83.903 ± 9.072
0 mM	Changkawi	AM2	79.0 ± 3.0	35.62 ± 9.413	25.467 ± 1.858	5.925 ± 1.393	80.64 ± 8.913
0 mM	Changkawi	AM3	82.333 ± 6.658	34.056 ± 2.706	23.745 ± 1.662	5.94 ± 0.647	85.881 ± 5.945
0 mM	Changkawi	AM4	90.667 ± 13.577	46.545 ± 7.411	25.874 ± 5.737	5.48 ± 0.982	80.635 ± 4.794
0 mM	Changkawi	AM5	97.0 ± 1.0	34.928 ± 8.949	29.39 ± 0.733	6.312 ± 0.269	78.683 ± 8.339
0 mM	Changkawi	NM	73.0 ± 6.083	29.994 ± 4.133	30.492 ± 7.739	5.203 ± 0.235	61.706 ± 13.708
0 mM	Dhan 50	AM1	74.0 ± 4.359	39.748 ± 2.631	23.844 ± 6.422	5.319 ± 0.995	91.809 ± 3.469
0 mM	Dhan 50	AM2	61.667 ± 14.048	48.349 ± 6.201	24.233 ± 5.118	5.439 ± 1.013	88.597 ± 1.171
0 mM	Dhan 50	AM3	82.0 ± 3.606	43.273 ± 4.034	27.739 ± 9.515	6.359 ± 0.378	93.989 ± 3.279
0 mM	Dhan 50	AM4	73.667 ± 5.132	41.305 ± 5.017	17.345 ± 4.404	5.965 ± 0.311	86.602 ± 3.053
0 mM	Dhan 50	AM5	81.0 ± 2.646	42.141 ± 3.632	27.498 ± 3.683	5.632 ± 1.052	95.405 ± 14.29

0 mM	Dhan 50	NM	54.333 ± 14.434	35.406 ± 4.359	29.849 ± 1.414	4.733 ± 0.089	82.121 ± 9.632
75 mM	Changkawi	AM1	76.333 ± 5.508	27.358 ± 3.019	34.453 ± 2.481	4.0 ± 0.855	73.455 ± 9.257
75 mM	Changkawi	AM2	78.667 ± 6.658	29.347 ± 2.304	31.542 ± 1.966	4.54 ± 0.283	80.256 ± 3.363
75 mM	Changkawi	AM3	78.0 ± 5.568	29.495 ± 4.779	32.208 ± 2.07	4.72 ± 0.069	79.845 ± 3.379
75 mM	Changkawi	AM4	75.333 ± 5.859	30.321 ± 0.922	30.462 ± 2.213	4.091 ± 0.901	68.124 ± 7.076
75 mM	Changkawi	AM5	80.333 ± 5.686	27.355 ± 6.253	28.532 ± 2.708	4.132 ± 0.32	72.436 ± 7.811
75 mM	Changkawi	NM	71.667 ± 7.767	25.068 ± 4.618	39.492 ± 1.435	3.546 ± 0.01	56.88 ± 9.343
75 mM	Dhan 50	AM1	57.333 ± 13.051	32.209 ± 5.609	31.445 ± 2.566	4.275 ± 0.425	86.773 ± 3.069
75 mM	Dhan 50	AM2	56.333 ± 10.504	33.177 ± 6.198	32.353 ± 2.923	4.369 ± 0.327	86.45 ± 2.167
75 mM	Dhan 50	AM3	62.333 ± 12.503	32.876 ± 15.791	31.495 ± 1.971	3.707 ± 0.559	86.212 ± 9.412
75 mM	Dhan 50	AM4	57.667 ± 9.292	33.201 ± 3.05	29.172 ± 3.439	4.232 ± 0.931	84.087 ± 5.749
75 mM	Dhan 50	AM5	68.333 ± 8.327	33.499 ± 10.207	29.949 ± 2.807	3.995 ± 0.422	86.989 ± 3.331
75 mM	Dhan 50	NM	46.667 ± 2.082	27.206 ± 1.032	34.204 ± 1.952	3.389 ± 0.248	81.951 ± 1.69
150 mM	Changkawi	AM1	68.333 ± 7.767	25.557 ± 3.341	29.47 ± 3.314	2.058 ± 0.929	71.043 ± 11.57
150 mM	Changkawi	AM2	66.667 ± 5.859	25.613 ± 3.568	33.235 ± 1.094	2.56 ± 0.182	62.208 ± 4.274
150 mM	Changkawi	AM3	76.0 ± 3.606	25.714 ± 3.993	29.231 ± 1.874	1.805 ± 0.358	68.503 ± 8.766
150 mM	Changkawi	AM4	70.333 ± 4.041	25.769 ± 6.041	31.541 ± 3.034	1.989 ± 0.736	76.402 ± 12.788
150 mM	Changkawi	AM5	72.333 ± 2.082	24.287 ± 4.762	29.388 ± 6.76	1.77 ± 0.057	68.477 ± 3.694
150 mM	Changkawi	NM	63.667 ± 1.528	20.838 ± 6.999	40.844 ± 8.493	1.378 ± 0.014	52.244 ± 9.867
150 mM	Dhan 50	AM1	48.667 ± 5.033	26.943 ± 3.061	34.242 ± 3.253	1.762 ± 0.127	70.468 ± 18.288
150 mM	Dhan 50	AM2	50.667 ± 9.074	24.527 ± 6.365	35.952 ± 11.748	1.76 ± 0.077	65.911 ± 6.592
150 mM	Dhan 50	AM3	45.667 ± 12.342	24.442 ± 4.817	36.518 ± 1.158	1.713 ± 0.22	63.743 ± 12.211
150 mM	Dhan 50	AM4	52.333 ± 3.215	24.886 ± 1.506	32.057 ± 2.448	2.0 ± 0.156	69.888 ± 3.979
150 mM	Dhan 50	AM5	51.667 ± 2.887	25.37 ± 1.676	27.91 ± 0.44	2.1 ± 0.111	65.606 ± 3.948
150 mM	Dhan 50	NM	41.0 ± 2.646	23.987 ± 5.016	41.679 ± 16.801	1.403 ± 0.037	65.482 ± 12.168

Appendix Table 8: Descriptive Statistics (Mean ± SD) of Electrolyte leakage (EL), Chlorophyll a, Chlorophyll b, Total Chlorophyll and Carotenoids of Rice Plants Under Different NaCl Treatments, Rice Varieties, and Mycorrhizal Treatments

Salt Treatment	Rice Variety	Mycorrhizal Treatment	EL (%)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total Chlorophyll (mg/g)	Carotenoids (mg/g)
0 mM	Changkawi	AM1	22.467 ± 7.507	2.264 ± 0.267	0.582 ± 0.084	2.846 ± 0.347	0.917 ± 0.138
0 mM	Changkawi	AM2	25.162 ± 1.596	2.447 ± 0.323	0.588 ± 0.141	3.034 ± 0.464	0.717 ± 0.225

0 mM	Changkawi	AM3	25.474 ± 4.615	1.793 ± 0.319	0.489 ± 0.056	2.281 ± 0.369	0.933 ± 0.229
0 mM	Changkawi	AM4	19.047 ± 3.713	2.874 ± 0.224	0.742 ± 0.112	3.615 ± 0.329	1.216 ± 0.114
0 mM	Changkawi	AM5	18.758 ± 2.601	3.08 ± 0.384	0.818 ± 0.126	3.898 ± 0.509	1.299 ± 0.201
0 mM	Changkawi	NM	25.845 ± 5.754	1.741 ± 0.125	0.508 ± 0.043	2.248 ± 0.166	0.743 ± 0.08
0 mM	Dhan 50	AM1	27.04 ± 2.574	1.489 ± 0.174	0.523 ± 0.057	2.012 ± 0.23	0.51 ± 0.077
0 mM	Dhan 50	AM2	29.239 ± 1.64	1.756 ± 0.21	0.624 ± 0.102	2.379 ± 0.31	0.622 ± 0.194
0 mM	Dhan 50	AM3	27.596 ± 3.643	1.829 ± 0.201	0.594 ± 0.029	2.422 ± 0.214	0.692 ± 0.05
0 mM	Dhan 50	AM4	23.236 ± 4.963	1.996 ± 0.655	0.626 ± 0.136	2.621 ± 0.791	0.725 ± 0.254
0 mM	Dhan 50	AM5	21.783 ± 2.259	2.522 ± 0.355	0.775 ± 0.078	3.296 ± 0.433	0.874 ± 0.215
0 mM	Dhan 50	NM	28.454 ± 5.827	1.135 ± 0.26	0.454 ± 0.094	1.588 ± 0.353	0.326 ± 0.05
75 mM	Changkawi	AM1	26.047 ± 4.505	1.896 ± 0.29	0.472 ± 0.065	2.367 ± 0.352	0.786 ± 0.116
75 mM	Changkawi	AM2	28.467 ± 2.193	1.925 ± 0.158	0.455 ± 0.046	2.38 ± 0.204	0.748 ± 0.069
75 mM	Changkawi	AM3	25.859 ± 4.863	2.515 ± 0.264	0.644 ± 0.073	3.158 ± 0.327	1.056 ± 0.124
75 mM	Changkawi	AM4	28.543 ± 0.491	2.312 ± 0.394	0.611 ± 0.101	2.922 ± 0.492	0.993 ± 0.182
75 mM	Changkawi	AM5	24.445 ± 3.197	2.515 ± 0.143	0.665 ± 0.059	3.179 ± 0.195	1.053 ± 0.093
75 mM	Changkawi	NM	33.492 ± 14.081	1.596 ± 0.605	0.387 ± 0.138	1.982 ± 0.74	0.61 ± 0.259
75 mM	Dhan 50	AM1	26.685 ± 7.096	1.713 ± 0.086	0.536 ± 0.013	2.249 ± 0.097	0.541 ± 0.057
75 mM	Dhan 50	AM2	28.186 ± 4.552	1.554 ± 0.242	0.526 ± 0.056	2.08 ± 0.298	0.458 ± 0.036
75 mM	Dhan 50	AM3	27.038 ± 5.066	1.38 ± 0.097	0.482 ± 0.004	1.861 ± 0.093	0.431 ± 0.013
75 mM	Dhan 50	AM4	25.165 ± 1.346	1.757 ± 0.382	0.566 ± 0.074	2.322 ± 0.456	0.558 ± 0.125
75 mM	Dhan 50	AM5	23.573 ± 2.533	1.995 ± 0.449	0.628 ± 0.092	2.622 ± 0.539	0.778 ± 0.23
75 mM	Dhan 50	NM	29.968 ± 5.184	1.153 ± 0.166	0.447 ± 0.042	1.599 ± 0.206	0.393 ± 0.108
150 mM	Changkawi	AM1	30.714 ± 6.63	0.789 ± 0.126	0.236 ± 0.044	1.025 ± 0.167	0.289 ± 0.089
150 mM	Changkawi	AM2	30.455 ± 3.814	0.781 ± 0.012	0.221 ± 0.003	1.001 ± 0.011	0.265 ± 0.006
150 mM	Changkawi	AM3	34.155 ± 2.634	0.985 ± 0.216	0.259 ± 0.043	1.243 ± 0.254	0.337 ± 0.083
150 mM	Changkawi	AM4	32.685 ± 5.291	1.246 ± 0.193	0.356 ± 0.066	1.601 ± 0.257	0.484 ± 0.113
150 mM	Changkawi	AM5	30.259 ± 6.334	1.048 ± 0.246	0.285 ± 0.044	1.332 ± 0.29	0.378 ± 0.104
150 mM	Changkawi	NM	36.608 ± 3.413	0.713 ± 0.085	0.216 ± 0.016	0.929 ± 0.09	0.241 ± 0.027
150 mM	Dhan 50	AM1	30.354 ± 5.745	1.402 ± 0.221	0.54 ± 0.017	1.942 ± 0.226	0.512 ± 0.073
150 mM	Dhan 50	AM2	31.494 ± 3.952	1.555 ± 0.251	0.569 ± 0.068	2.123 ± 0.308	0.536 ± 0.05
150 mM	Dhan 50	AM3	31.253 ± 4.702	1.579 ± 0.325	0.618 ± 0.158	2.196 ± 0.473	0.513 ± 0.097
150 mM	Dhan 50	AM4	30.767 ± 6.883	1.75 ± 0.064	0.704 ± 0.119	2.452 ± 0.154	0.706 ± 0.096
150 mM	Dhan 50	AM5	25.82 ± 3.783	1.775 ± 0.044	0.837 ± 0.214	2.612 ± 0.173	0.752 ± 0.063
150 mM	Dhan 50	NM	33.19 ± 8.407	1.191 ± 0.021	0.492 ± 0.025	1.683 ± 0.045	0.35 ± 0.04

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	M.Sc. 2016	Mizoram University Department of Botany School of Life Sciences	75.10% 8.01 CGPA

Academic Milestones	February 2017	State Eligibility Test (SET/SLET) for Lectureship NE Region Life Sciences; State Level Eligibility Test Commission, ASSAM
	December 2019	National Eligibility Test (NET) for Assistant Professor CSIR-UGC NET (Life Sciences); Qualified for JRF Rank - 139
	February 2025	Graduate Aptitude Test in Engineering Life Sciences
	February 2025	Graduate Aptitude Test in Engineering Biotechnology

Research experience

2020-2022	Junior Research Fellow Supervisor: Prof. R. Lalfakzuala Dept. of Botany, Mizoram University Title: Diversity of arbuscular mycorrhizal fungi associated with rice (<i>Oryza sativa</i> L.) and their role on rice tolerance to drought and salt stress.
2022-2025	Senior Research Fellow Supervisor: Prof. R. Lalfakzuala Dept. of Botany, Mizoram University Title: Diversity of arbuscular mycorrhizal fungi associated with rice (<i>Oryza sativa</i> L.) and their role on rice tolerance to drought and salt stress.

Publications

	Book Chapter
2024	Lalruatpuia, Vanlalmuana M. C. F., Kumar A., and Lalfakzuala R. (2024). Diversity and phytochemical review of arbuscular mycorrhizal fungi associated with selected medicinal plants in Kumar A. (Editor) Advances in Medicinal and Aromatic Plants Research, pp 255-279.
	Journal publications
2025	Lalthazuali, E., Marwein, B., Sailo, H., Lalbiaktluanga, P. S., Vanlalmuana, MC. F., & Ralte, L. (2025). Enhancing rice growth in adverse conditions: the role of dark septate endophytes in salt and water scarcity tolerance. Mycologia, 1–13. https://doi.org/10.1080/00275514.2025.2474900
2024	Vanlalmuana, M.C.F., Martin Lalnunthara, Esther Lalthazuali, Lalruatpuia, Vanlalthiatpui and Lalfakzuala, R. 2024. Exploring the symbiotic relationship between arbuscular mycorrhizal fungi and medicinal plants in Mizoram, North East India. Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 27, No. 3: 2024: 413-418.
2021	Vanlalmuana M.C.F., Chakraborty K. & Lalfakzuala R. (2021). Arbuscular mycorrhizal colonization status in selected alien invasive plants of asteraceae. Science and Technology Journal, 9 (2): 81-84.

Conference papers

- 2018 **Vanlalmuana M. C. F** & Lalfakzuala R. (2018).
Studies on the effects of xenobiotic on radial growth and
dry weight biomass of the soil fungus *Trichoderma* sp.
Proceedings of the Mizoram Science Congress 2018
(MSC 2018) - Perspective and Trends in the
Development of Science Education and Research. DOI
10.2991/msc-18.2018.2.

Presentations and invited lectures

Paper Presentation

- 2024 “Diversity of Arbuscular mycorrhiza associated with
Rice (*Oryza sativa* L.) in Mizoram.”
National Seminar on Biodiversity, Conservation,
Utilization and Commercialization of Medicinal and
Aromatic Plants.
Department of Botany, School of Life Sciences,
Mizoram University
- 2023 “Arbuscular mycorrhizal symbiosis in rice (*Oryza sativa*
L.) plants in flooded conditions in Mizoram.”
National Conference on Recent Advances in Plant
Biology with Special References to North-East India
Department of Botany, School of Life Sciences,
Mizoram University
- 2022 “Arbuscular mycorrhizal colonization enhances drought
tolerance in *Zea mays* under greenhouse condition.”
National Seminar on Emerging Trends in Plant Sciences
(ETPS)
Department of Botany, North-Eastern Hill University,
Shillong, Meghalaya
- 2021 “Arbuscular mycorrhizal colonization alleviates salt
stress in *Zea mays* under greenhouse condition.”
International Conference on Biotechnology for
Environment & Health (ICBEH)
School of Bio Sciences and Technology, Vellore
Institute of Technology

Invited Lecture

- 2023 “Introduction to Mycorrhiza”
Dept. of Botany, Govt. Kolasib College

**Seminar or
Workshop**

- | | |
|------|--|
| 2019 | One Day Workshop on “Invasive Alien Plants in Himalayas: Status, Ecological Impact and Management (Mizoram & Tripura Chapter).”
Botanical Survey of India, Department of Botany, Mizoram University |
| 2019 | One Day National Workshop on “IPR and Plant Protection with special reference to NE India.”
Department of Botany, Mizoram University and Department of Horticulture, Government of Mizoram |
| 2015 | Seminar on Science for Nation Building
Mizo Academy of Sciences in collaboration with Directorate of Science and Technology, Government of Mizoram |

PARTICULARS OF THE CANDIDATE

NAME OF CANDIDATE : MC Freddy Vanlalmuana

DEGREE : Ph.D.

DEPARTMENT : Botany

TITLE OF THESIS : Diversity of arbuscular mycorrhizal fungi associated with rice (*Oryza sativa* L.) and their role on rice tolerance to drought and salt stress

DATE OF ADMISSION : 08.09.2018

APPROVAL OF RESEARCH PROPOSAL

1. DRC : 02.05.2019

2. BOS : 03.05.2019

3. SCHOOL BOARD : 17.05.2019

MZU REGISTRATION NO. : 1627 of 2009-10

Ph.D. REGISTRATION NO. & DATE : MZU/Ph.D./1272 of 08.09.2018

EXTENSION (IF ANY) : NA

(Prof. F. LALNUNMAWIA)
Head
Department of Botany

Abstract

This research aims to demonstrate regarding the colonization and species diversity of some Arbuscular Mycorrhizal Fungi (AMF) with rice plants from two locations in Mizoram India, North Vanlaiphai and Thenzawl, spanning a period of three years (2019-2021). The main aim was to understand the extent to which Arbuscular Mycorrhizal Fungi (AMF) colonization, spore density, and species composition varies in rice fields due to soil properties that are site specific. AMF colonization by the fungal species was evaluated by considering three parameters: arbuscules, vesicles, and hyphae while spore density and diversity were measured relation to soil physicochemical properties.

The findings showed that there were significant differences in terms of the level of AMF colonization at different sites. The Thenzawl site was found to present more arbuscular colonization which increased from 3.06% in 2019 to 3.74% in 2021 whereas North Vanlaiphai rose from 2.32% to 3.24% over the evaluation period. However, the pattern of vesicular colonization was reversed in which it fell in North Vanlaiphai and progressively rose in Thenzawl. The hyphal colonization pattern was generally higher in Thenzawl as well, indicating that soil conditions in Thenzawl were more supportive to AMF development. Spore density followed the same trends with Thenzawl demonstrating more spore density in all the three years with the most spore density obtained in the year 2021 with 548.5 spores per 50g soil.

These factors mainly seem to be controlled by the soil physicochemical properties. In Thenzawl, the higher pH, potassium content, and urease activity were positively correlated with AMF colonization and the number of spores. However, the greater soil moisture content in North Vanlaiphai affected negatively vesicular and hyphal colonization. Correlation studies showed that arbuscular colonization and bulk density and phosphatase activity were strongly positively correlated in North Vanlaiphai while nutrient P levels were important for AMF colonization in Thenzawl.

In terms of AMF diversity, the study identified 11 species across both sites, with Thenzawl showing a higher species richness (8 species) compared to North

Vanlaiphai (5 species). The dominant genera were *Glomus* and *Acaulospora*, with species like *Gigaspora margarita* and *Rhizophagus sinuosus* also being prevalent. Shannon and Simpson diversity indices further confirmed that AMF communities were more diverse and evenly distributed in Thenzawl. These findings suggest that the diversity and colonization of AMF in rice fields are significantly influenced by soil properties, including pH, phosphorous content, and enzyme activities.

Propagation and mass production of AMF was performed by using substrate-based systems. Maize (*Zea mays* L.) was used for trap culture and Sorghum-Sudangrass hybrid was used for single-species culture. Seven AMF species were recovered from trap culture, including two species each from the genera *Glomus* and *Acaulospora*, and one species each from *Gigaspora*, *Funneliformis*, and *Rhizophagus*. Of these, three species—*Acaulospora foveata*, *Gigaspora margarita*, and *Glomus sp.*—were selected for mass production. Sorghum-Sudangrass was used as the host plant for the substrate-based system to propagate single-species spores, which were successfully cultured over four months.

This study also investigates the growth, biochemical and physiological responses of rice plants inoculated with Arbuscular Mycorrhizal Fungi (AMF) under conditions of drought and salt stresses. The experiment was performed in a greenhouse environment at Mizoram University, focusing on two rice varieties: Changkawi (a local cultivar) and DRR Dhan 50 (a drought and submergence-tolerant variety). In one experiment, the rice plants were subjected to two water regimes—well-watered (WW) and moderate drought (MD)—and inoculated with six different mycorrhizal treatments, including *Acaulospora foveata* (AM1), *Gigaspora margarita* (AM2), *Glomus sp.* (AM3), a mixture of these AMF species (AM4), a commercial AMF treatment (AM5), and a non-mycorrhizal control (NM). In another experiment, the rice plants were exposed to three NaCl treatment (0mM, 75mM, 150mM) and inoculated with six different mycorrhizal treatments, including *Acaulospora foveata* (AM1), *Gigaspora margarita* (AM2), *Glomus sp.* (AM3), a mixture of these AMF species (AM4), a commercial AMF treatment (AM5), and a non-mycorrhizal control (NM).

The findings highlighted the advantages of applying AMF inoculation to rice plants during periods of drought. While AMF colonization increased with treatments, the highest levels were found in AM4 (mixture) and AM5 (commercial AMF) treatments in well-watered conditions. In the case of drought, rice plants inoculated with AMF had better drought tolerance as compared with non-inoculated plants. In particular, Dhan 50 was noted to have better drought adaptability than Changkawi in terms of maximum plant height, shoot biomass and leaf area retention. The other set of well irrigated conditions showed more plant height and biomass while moderate water deficit showed a decrease in these traits. The AMF inoculants, on the other hand, proved very much effective in reversing this adverse effect on growth and development of Dhan 50 variety.

On the aspect of physiological responses, it was visible that relative water content was significantly improved through AMF inoculation, particularly in leaves of water stressed plants treated with AM5 and AM4 both in well-watered and drought circumstances. In the AM plants, and particularly in the AM5 subgroup, electrolyte leakage was less, and this parameter assesses the stability of membranes, and the level of their damage, pointing out to the protective effect of AMF on cell membrane of drought-stressed plants. Furthermore, chlorophyll a and b levels, critical for photosynthesis, were higher in AMF-treated plants, with the highest levels observed in Dhan 50 inoculated with AM4 under well-watered conditions.

Proline is an amino acid that accumulates in plants under stress, particularly drought and salinity, functioning as an osmoprotectant and helping the plant maintain cellular functions. The highest proline accumulation occurred in nonmycorrhizal plants, indicating that plants without mycorrhizal support relied more on proline for stress tolerance. The treatment group with the lowest proline value was Dhan 50 inoculated with AM4 under well-watered conditions. Mycorrhizal inoculation significantly reduced proline accumulation under both drought and well-watered conditions. Treatments with AM4 and AM3 helped maintain balanced proline levels, while non-mycorrhizal plants (NM) exhibited the highest proline values under both drought and well-watered conditions.

Additionally, AMF-treated plants showed lower MDA levels which shows lower lipid peroxidation as well as oxidative stress. Antioxidative enzyme activities such as SOD and catalase were seen to be significantly more in the AMF plants thus showing the function of these enzymes in scavenging reactive oxygen species (ROS) and protecting the plants from oxidative stress. The research concludes that AMF inoculation is an effective method for improving drought resistance in rice. Arbuscular mycorrhizal fungi (AMF) can alleviate the adverse impacts of drought on rice plants by strengthening water retention, preserving membrane integrity, increasing photosynthetic pigment concentrations, and augmenting antioxidant defenses. Dhan 50, exhibiting intrinsic drought tolerance, demonstrated the most significant advantage from AMF inoculation, especially with AM4 and AM5 treatments.

The experiment evaluated several growths, physiological, and biochemical responses to ascertain the impact of AMF inoculation under saline stress. Plants inoculated with arbuscular mycorrhizal fungi (AMF), particularly those treated with *Acaulospora foveata* (AM1), *Glomus* sp. (AM3), and the mixed AMF treatment (AM4), demonstrated increased plant height, shoot biomass, and leaf area in saline conditions. The AMF-inoculated plants exhibited elevated relative water content and reduced electrolyte leakage, signifying improved membrane integrity under saline stress. The chlorophyll content, carotenoids, and osmoprotectant levels (proline, TSS, sucrose, and fructose) were elevated in plants treated with AMF, particularly under mild salt stress (75 mM NaCl).

Biochemical responses indicated that AMF inoculation enhanced the activity of antioxidant enzymes, including catalase and superoxide dismutase, thereby alleviating oxidative damage induced by salt stress. The levels of proline and anthocyanins, crucial for osmotic regulation and stress resistance, were markedly elevated in AMF-inoculated plants. Furthermore, AMF inoculation diminished the concentrations of malondialdehyde (MDA), a biomarker of lipid peroxidation, hence suggesting decreased oxidative damage in the inoculated plants. Among the rice cultivars, DRR Dhan 50 exhibited superior salt tolerance compared to Changkawi, as demonstrated by increased shoot biomass, chlorophyll content, and antioxidant enzyme activity under saline conditions. Both cultivars exhibited enhancements from

AMF inoculation, with the most significant gains noted in plants inoculated with *Glomus* sp., the combined AMF treatment and the commercial propagules.

In conclusion, this study highlights the importance of tailoring soil management practices to site-specific conditions to enhance the benefits of AMF in rice cultivation. More favorable soil properties at Thenzawl provided a better environment for AMF colonization and diversity. This study also highlights the significant role of Arbuscular Mycorrhizal Fungi in improving the resilience of rice plants to both drought and salt stress. AMF inoculation, particularly with mixed species (AM4) and commercial products (AM5), enhanced plant growth, water retention, chlorophyll content, and antioxidant enzyme activity, resulting in better stress tolerance in both drought and saline conditions. The rice variety DRR Dhan 50 exhibited greater overall resilience compared to Changkawi, as DRR Dhan 50 is a drought- and submergence-tolerant rice variety that was developed using molecular breeding techniques, making it more suitable for cultivation in challenging environments. The study underscores the importance of AMF as a sustainable, biological solution for enhancing stress tolerance and ensuring stable rice yields in the face of climate change-induced water scarcity and soil salinization. Integrating AMF into rice cultivation practices can provide a promising strategy for improving productivity and agricultural sustainability.