

# Use of Arbuscular Mycorrhizal Fungi (AMF) and Zinc Fertilizers in An Adaptation of Plant from Drought and Heat Stress

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**Abbreviations:** AM: Arbuscular Mycorrhizae; ECM: Ectomycorrhizae; ERM: Extraradical Mycelium; AMF: Arbuscular Mycorrhizal Fungi; RMD: Relative Mycorrhizal Dependency; MD: Mycorrhizal Dependency; NM: Non-Mycorrhizal; NUE: Nitrogen Use Efficiency; MPU: Mycorrhizal Pathway Uptake; HS: Heat Stress; ErM: Ericoid Mycorrhizae; DPU: Direct Pathway Uptake

## Abstract

Agriculture is the mainstay between humans and the environment. The existence of arbuscular mycorrhizal fungi (AMF), on the earth, is about 600 million years ago. The application of mycorrhizae as biofertilizer is in increasing trend. Mycorrhizae improve several agricultural practices like better nutrient cycling, improving crop yield, and remediation of toxic heavy metals from soil. There is much variation in arbuscular mycorrhizal association although 80% of the plant species are infected with mycorrhizae. Mycorrhizal dependency (MD) is defined as the degree to which a host plant is dependent on AMF to produce maximum growth or yield at a given level of soil fertility. Plant with high MD need to feed with a higher amount of carbon and lipid to the fungus than a plant with less MD. Mycorrhizae modify, maintain, and create habitat by directly and indirectly regulating biotic and abiotic environments. Mycorrhizae derived photosynthetically formed C for the growth and uptake, tolerance against abiotic stress such as drought, heavy metals, salinity as well as protecting from pathogen attack to host plant and preventing from erosion. Application of ZnO would have been the probable solution of mitigating drought.

ZnO treatment decreased the adverse effects of drought stress in plants by enhancing antioxidant enzyme activity and changing physiological parameters. Heat stress affects many processes in a variety of plants as water relations, nutrient uptake, photosynthesis, assimilate partitioning, respiration, growth, and reproduction, and induced oxidative damage. Foliar application of ZnSO<sub>4</sub>·7H<sub>2</sub>O effectively alleviated by enhancing Zn concentration, superoxide dismutase activity, chlorophyll content, Fv/Fm ratio, and photosystem II under heat stress. In maize, there is a substantial reduction of germination above 37°C. All the parameters recorded in wheat, namely, no of tillers, plant height, spike length, no of spikelets per spike, no of grains per spike, 1000 grain weight, biological yield, grain yield and harvesting index, Ca, Mg, Fe, Zn, Cu and protein content are significantly affected by the mycorrhizal application. Almost 25% of recommended dose of phosphate fertilizer could be saved as in Niger (*Guizotia abyssinica*) by inoculating *Glomus mosseae*. Zinc is a vital micronutrient for many plants but its excess can be calamitous.

AMF contributes to plant Zn uptake but their role in the edible portion of the crop has not been studied yet. The mycorrhizal pathway of Zn uptake contributed up to 24.3% of the total above-ground Zn in wheat and up to 12% of that Zn in Barley. The greatest contribution by the mycorrhizal pathway was observed in Barley at the lowest Zn addition and in wheat at the highest one. Besides the grain yield of bread wheat was increased by AMF.

## Arbuscular Mycorrhizae Fungi (AMF)

Mycorrhizae are an important player in the ecosystem. Three different types of mycorrhizae are arbuscular mycorrhizae (AM), ectomycorrhizae (EcM), and ericoid mycorrhizae (ErM). These microorganisms respond to elevated carbon dioxide, global warming, and changes in the distribution of rainfall. Variation in temperature and rainfall is supported by changes in the climatic condition of the world. Global warming has more effect in EcM than in the AM. The functioning of the individual plant and community interaction is influenced by the mycorrhizal associations. It is found the growth of the mycorrhizae is improved by elevated carbon dioxide. More than 80% of the plant species are in association with mycorrhizae. These three types of mycorrhizae play a significant role in combating stress derived from climate change [1]. Climate change alters the interaction between plant and organism so that the functioning of the soil and ecosystem will be restored. Arbuscular mycorrhizal fungi (AMF) forms arbuscules in the roots of the plant of various types.

AMF gets carbon and lipids from the plant in return it facilitates the plant to obtain water nutrients and trace minerals [1]. AMF do not form fruiting bodies stay below ground and produce hyphae underneath. On the other hand, EcM and ErM produce fruiting bodies and disperse propagules by the wind. Unlike AMF, the EcM and ErM are not found in all habitats and their distribution is limited [1]. Arbuscular Mycorrhizae is one of the symbiotic organisms in the rhizosphere [2]. This beneficial symbiosis augments soil and plant health and fruit quality. More than 90% of this plant mutualism is from the fungus Glomeromycota [2]. Arbuscular mycorrhizae fungi (AMF) are obligate symbiont of many plants biotrophically. They colonize the root cortex and develop extrametrical mycelium, which helps the plant to acquire water and nutrients from the soil. Also, AMF is helpful for the plant to alleviate stress factors. AMF provided the connection between soil and plant roots [2,3].

## Mycorrhizal Dependency (MD)

Mycorrhizal dependency (MD) is defined as “host inability for growth without AMF at given soil fertility” [2]. It depends on soil fertility and fertilizer levels. Mycorrhizal dependency has often been quantified by calculating the yield between mycorrhizal and non-mycorrhizal control plants grown in a particular soil. MD is also defined as the degree to which a plant is dependent on AMF to produce maximum growth or yield at a given level of soil fertility. Also relative mycorrhizal dependency (RMD)” by expressing the difference between the dry weight of the mycorrhizal plant and the dry weight of the nonmycorrhizal plant as a percentage of the dry weight of the mycorrhizal inoculated plant [2]. Some species of plant are strongly mycorrhizal dependent than others. *Glomus mosseae* is an important mycorrhizal species widely applied. Fungi are better than plants in acquiring mineral nutrition from the soil. Citrus plant species are strongly microbial dependent than others.

Among the citrus sour orange is more strongly MD crops other than citrus tree species. Plant MD is positively correlated with that of the MD in phosphorous (P).

Also, the fungus can be helpful by promoting the nutrient absorbing ability of roots especially facilitating the uptake of P. Similarly, the AMF play a significant role in root morphology, functioning, and micorrhizosphere soil properties [2]. The growth of the plant is better in non-sterile soil than in sterile soils. Some growth parameters such as shoot height, shoot and root dry matter, shoot diameter, and root colonization are increased with mycorrhizal application not only in sterile soil but also in non-sterile soils [3]. Mycorrhizal inoculation in sterile soil increased plant biomass. Non-sterile soil significantly increased citrus dry matter production, percentage of root colonization, P and Zn uptake compared to sterile soil. In non-inoculated sterile soil-plant, P and Zn were significantly reduced compared with inoculated plants. Moreover while inoculating mycorrhizae the seedling concentration of P and Zn increased. Inoculation is highly dependent on sterilization and P application but not in the Zn application. Under non-sterile soil conditions, since there were native mycorrhiza spores, MD is very low, and also increasing P addition has reduced mycorrhizal dependency [3].

The plant has varied MD. Low P and deficient in tissue P are more readily colonized by vesicular-arbuscular mycorrhizae (VAM) than these of high P status. Plant with high MD need to feed with a higher amount of carbon to the fungus than a plant with less MD. However in the case of P deficient soil there are correlations between MD and colonization. In hoagland solution minus P the plant was harvested after 4-6 months and their dry weight is determined. *Poncirus* is a low MD plant. Colonization at undisturbed soil is higher than the disturbed soil. Measurement of mycorrhizae should be taken at 5 weeks, 10 weeks, and 19 weeks. The rate of colonization also significantly carbon cost. For citrus, the colonization of young roots at 19 weeks was strongly related to MD. However genetic difference in colonization is not seen. The relationship between MD and colonization at low P soil only explains very important. If a plant expands a large amount of carbon to promote rapid colonization during a period when it receives little mycorrhizal benefit, then this represents a significant opportunity cost that prevents expenditure for immediate needs [4].

Plant species are thought to differ in MD based on the ability of non-mycorrhizal roots to take up P from P deficient soil. Long and abundant hair was less dependent than those with short or few root hairs. Slow growth is less dependent because they need less P. Among hard wood species greater lateral root length and finer root system were less dependent on VAM. Citrus roots have short and poorly distributed root hairs. The level of MD is varied according to Sour orange= Cleopatra mandarin>swingle citrumelo> Carrizo citrange> trifoliolate orange. In citrus, there is a higher root to shoot ratio, larger root diameter, and lower P concentration in leaf tissue

[4]. MD was also correlated with a relative growth rate of mycorrhizal or P-fertilized plants. Root stock with higher conductivities under well-aerated conditions had finer roots and perhaps more surface area for water uptake. VAM did not affect morphology, anatomy, and physiology of roots or shoots. Root hydraulic conductivity is not influenced by VAM under the condition of adequate N nutrition and soil moisture. Compare with not only plant size but growth rate and nutritional status of mycorrhizal and non-mycorrhizal plants. MD is varied with soil type, species of mycorrhizal fungus, and plant cultivar. In hardwood tree species those with greater lateral root length and their finer too systems were less dependent on VAM [4].

### Effect of Mycorrhiza in Nutrient Uptake

The VAM application significantly increased the heavy metal uptake. Inoculation of mycorrhizae decreased the level of Zinc in the shoots of Lucerne. Both decrease and increase in the metal uptake are related to VAM colonization. Soil pH has a fundamental role in the uptake of heavy metals. The only two ways to tackle heavy metal toxicity is to increase soil pH which reduced the number of nutrients absorbed or inoculate with VAM. VAM increases metal uptake from deficient or enriched soils. Increasing soil pH increased colonization while lowering soil pH significantly reduced colonization. Colonization was more than doubled when VAM is inoculated into the soil. The application of VAM and rhizobium has a great effect. The nodulation and nodule activity data indicate that the un-amended soil contained a sufficient number of rhizobia to adequately nodulate the plant [5]. Alfalfa growing in soils at a pH of 4.3 and 5.3 failed to survive as a result of soil acidity and heavy metal toxicity.

Mycorrhizae help to absorb nutrients under nutrient-limiting conditions. It is known that mycorrhizal infection can increase the uptake of Ca and Zn. The *Calluna vulgaris* is a characteristic ericoid mycorrhizal infection which is in contrast to the situation found. VAM has been shown primarily to enhance nitrogen better than P uptake. It is negated that non-mycorrhizal (NM) conditions in sand cultures supplemented with different levels of Cu and Zn. NM plants have no tolerance to these metals at high concentrations. Mycorrhizal infection provides a major degree of resistance to toxicity and that infection leads to a significant reduction of the heavy metal content of the shoot. The mean yield is higher in mycorrhizal conditions than in non-mycorrhizal conditions [6]. Mycorrhizal infection enhances metal uptake to the roots of lettuce plants but not the shoots. In Mycorrhizal plants, contaminated soil has a high concentration of metal in roots so decreased the concentrations when compared to non-mycorrhizal plants. Metal retentions in the mycorrhizal root system can be attributed to the surface use of heavy metals with cysteine-containing ligands of fungal proteins. There is mycorrhizal infection may enhance the root/shoot ratio barrier of the host plants for toxic heavy metals and play a role in the heavy metal resistance of plants [7].

It is determined that mycorrhizae had a positive effect on growth criteria and phosphorous nutrition of the lettuce plant and this effect decreased at higher P application rates. The mycorrhizal application had a positive effect on yield criteria and uptake in lettuce. The highest P uptake by the plant was determined in 200 mg P<sub>2</sub>O<sub>5</sub>/Kg treatment as 88.8 mg/pot with mycorrhizae treatment P uptake 69.9 mg/Kg, edible weight 84.36g, dry weight 8.64 g, and leaf number 26 [8]. *Scutellospora nigra* showed a significant increase in plant growth, biomass shoot, and root of *Guizotia abyssinica*. The synergistic and additive mechanism involved can enhance plant growth, nutrient uptake, and adaptation to unfavorable drought soil conditions. Many terrestrial plants are grown in association with mycorrhizal fungi. Symbiosis with mycorrhizal fungi helps them to uptake nutrient concentration in the root zone. Almost 25% of recommended doze of phosphate fertilizer could be saved in Niger by inoculating *Glomus mosseae*. AM inoculated plant showed a significant increase in shoot and root biomass. Further, P and Zn content were found to be higher when compared to control plants [9].

### Effect of Mycorrhiza on Phosphorous Uptake

Phosphorous uptake in many crops is improved by association with AMF. Cropping system and long-term application of phosphatic fertilizer influence the amount and bioavailability of P in the system and the development of mycorrhizal association. Adequate addition of P is recorded for the cropped land and soil improvement under low P supply. Mainly P supplementation exceeds phosphorous requirement on the crop may preclude mycorrhizal development. To encourage arbuscular mycorrhizal association threshold levels of soil solution P that restrict mycorrhizal development must not be exceeded sustainable P management [10,11]. At ambient CO<sub>2</sub> growth of AM sour orange was depressed (18%), compared with the non-mycorrhizal seedling. But at elevated CO<sub>2</sub> AM sour orange plants were 15% larger than non-mycorrhizal plants. AMF rely on the host plant for organic compounds. Mycorrhizal colonization in a nutrient-rich environment neither benefits plant P status nor increases photosynthesis. The physiology of AMF induced growth depression in managed and natural ecosystems are not well understood [12].

Photosynthesis and stomatal conductance were greater for mycorrhizal plants. P levels in M plants are three times higher than NM plants. Under long-day conditions, these levels were slightly higher. Root exudates of reducing sugar were greatest for NM plants. The plant grown were under long-day photoperiods have greater basipetal transport of photosynthates resulting in improved mycorrhizal infection and plant growth response. The experiment was conducted in two months old seedling of *Citrus sinensis* (*Citrus reticulata* Blanco.). The level of P is higher in mycorrhizal condition than in non-mycorrhizal conditions. The amino acid in root exudates of mycorrhizal citrus increased with

long-day photoperiods. The opposite trend was observed in the NM condition. Similar is the case with root exudates of reducing sugars [13]. VAM is analogous to root hair but can be as long as 7 cm. Cu, Zn, and P are the elements which concentration is affected by mycorrhizae. Two mechanisms for P-induced reduction Cu and Zn concentration

- (i) The suppression of mycorrhizal uptake of these elements
- (ii) Dilution effect- the larger P sufficient plants do not concentrate on the uptake of smaller nutrients like Zn and Cu [14].

Pepper shoot, root dry weights, and leaf P levels are affected by an interaction between temperature and AM fungal treatment. At moderate temperature shoot dry weight of plants colonized by the *Glomus* isolate mixture or non AM plants were highest while root dry weights were highest for non-AM plants. At high-temperature plants colonized by *Glomus* AZ112 or the non-AM plant had the lowest shoot and root dry weight. AM plants had generally higher P levels at moderate temperature and lower P levels at a higher temperature than non-AM plants. AM plants also have generally higher specific soil respiration than non-AM plants. At moderate temperature P uptake by all AM plants was enhanced relative to the non AM plants no growth of root due to increased root respiration [15]. Root colonization of AMF decreases when the air temperature exceeds 30°C and soil temperature above 40°C. Spore germination of fungi *G. coralloidea* and *G. heterogama* was found to be decreased above 34°C. In soybean, the presence of arbuscules decreases below 30°C. Production extends hyphae is decrease below 34°C. Colonization of cashew roots by *G. intraredices* decrease above 30°C and was severely reduced at 38°C [15].

Hyphae network of mycorrhizal fungi help the plant to take up nutrient and water. Ectomycorrhizal fungi in terms of penetration of hyphae to cells of the inner cortex to create specific branched structures called arbuscules. Arbuscules from a particular nutrient transfer interface that is embedded with numerous plant and fungal transporters assisting in nutrient transfer between the symbionts. The majority of microbial fungi are obligate biotrophs. Plant reduce the huge amount of soluble orthophosphate for growth and they react with available cation to form barely soluble calcium. Plant greatly rely on the mycorrhizal uptake pathway for P absorption. An urgent need for natural fertilizer instead of chemical fertilizer is required to increase the concentration of important micronutrients in edible plant parts which could be determined as the ecofriendly way in a sustainable agroecosystem. The application of AMF in the form of natural fertilizer represents a key link between plants and soil mineral nutrients. Mycorrhizae make the symbiotic association with most crop plants and are directly associated with plant mineral nutrition controlling plant pathogen and increase drought tolerance of plants. Among all the different nutrient application methods use of AMF is eco-friendly and cost-effective way. Application of mycorrhizae has the following benefits

- (a) Increase in surface area
- (b) In mobilizing sparingly available nutrient sources
- (c) In excreting chelating compounds
- (d) In producing ectoenzymes.

There is the dependency of mycorrhiza on the competence with which mutual partners cooperate and exchange nutrients across the mycorrhizal interface [16]. Agriculture is the largest interface between humans and the environment. Mycorrhizae improve several agricultural practices like better nutrient cycling, improving crop yield, and remediation of toxic heavy metals from soil. Soil ecological engineering as reclaimed land and the potential role of ecological engineering. Mycorrhizae can be taken as an ecological engineer. Mycorrhizae modify, maintain, and create habitat by directly and indirectly regulating biotic and abiotic environments. Mycorrhizae derived photosynthetically formed C for the growth and uptake, tolerance against abiotic stress such as drought, heavy metals, salinity as well as protecting from pathogen attack to host plant and preventing from erosion [17]. Mycorrhizal symbiosis has the potential to check the losses of applied nutrients. The role of soil biota in nutrient cycling is indispensable and determines the nutrient availability to plants. Among these biotas, AMF association with a plant is the most prevalent. But the exact mechanism followed by AMF in nutrient cycling, transformation, and reducing nutrients loss ability is still inconclusive.

Fifty percentage of the current agricultural production is due to fertilizer application. The resource of NPK is very limited. The remaining amount of fertilizer is in attention as greenhouse gas emissions, eutrophication, and downstream contamination.  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$  are easily leached down become unavailable to plant roots. Immobile nutrients mostly P, K, and Zn form chelates. However, the nutrients loss is due to climatic variability in plant species competition. Soil type, soil moisture and soil organism that influence nutrient cycling than nutrient mobility. Nutrient loss through vertical leaching as well as lateral movement i.e. runoff or erosion together makes a significant statement towards nutrient use efficiency. Apart from leaching gasses losses of nitrogen in the form of nitrous oxide, dinitrogen gases is recorded. Dissolution and transportation of rock-bound nutrients by a large web of fungal hyphae. In this particular review, the main emphasis is on the mycorrhizal's ability to reduce nutrients losses from the soil system. The main emphasis is paid on mycorrhizal effect on plant performance nutrient acquisition, while other important ecological services i.e. nutrient cycling, nutrient losses via leaching, and volatilization have received very little attention.

AMF help to mineralize organic matter. AMF utilize the nutrient demand from mineralization. AMF reduces the leaching of reactive P compounds. The use of AMF on  $\text{N}_2\text{O}$  emission has an impact on

greenhouse gas control. AMF uptake N in both organic and amino forms and inorganic nitrite forms and organic amino forms. However, AMF prefers inorganic form. AMF help to achieve high nitrogen use efficiency (NUE). The micronutrient use efficiency of applied fertilizer is, 5% AND ~ 50% of agricultural soil are deficient in Zn. Fertilization, land-use intensity, and soil disturbance are some of the factors affecting mycorrhizal density [18]. In this context a small highlight is made here about the AMF. The existence of AMF on the earth is about 600 million years ago. Their life cycle is to complete only once in the presence of the host. Soil counts for arbuscular symbiosis and mycorrhizal symbiosis. The word arbuscules meaning small tree. Fungal mycelium is helpful to draw water and mineral from soil. Arbuscular mycorrhizal symbiosis is a complex of morphological, physiological, and biochemical which are formed gradually in several development stages.

The mycelium develops as extraradical mycelium. Conversely, the plant provides carbon in the form of sachharides produced by photosynthesis transfer to the plant through an above passive mechanism. AMF mycelium obtains nutrients from soil nutrients such as P, N, Zn, Cu, Fe, K, Ca, Mg etc. In soil occupied by roots, phosphate is exhausted very quickly. The presence of phosphate in the rhizosphere is the major factor contributing to the creation of mycorrhizal association. The depletion zone is around the host and the root where plant roots can pump the necessary nutrients. Phosphate is then transported into the form of polyphosphate from the soil through AMF intraradical mycelium. Because plant obtains most of the P through fungal symbiosis is possible to assume that the plant phosphate transporters have the greatest intake to productivity and plant is often a major factor in regulating the relationship between plants and AMF concentration of P in plants. Higher content of P applied in the soil is associated with lower mycorrhizal root colonization rates and lower AMF diversity. AMF help to transfer a large amount of  $N_2$  from root to shoot. Nitrogen in the form of  $NH_4^+$  or  $NO_3^-$  is transported to the plant.

In salt stress conditions soil mycorrhizal symbiosis increasing the intake of nutrients such as P, N, Zn, Cu, Fe and inhibiting the uptake of Na, Cl. Osmotic adjustment assisted in maintaining the leaf turgor pressure, the influence of photosynthesis, transpiration, stomatal conductance, and water use efficiency. Plant with AMF colonization draws more water from the soil in comparison to not colonized one. Temperature between 10-30°C is favorable but temperature below 15°C decreases colonization. Long-term application of sludge with the increased amount of heavy metals in the soil can significantly reduce the total number of spores and diversity of AMF [19]. AMF inoculation in grass yield was less pronounced in the field and non-inoculated conditions. AMF are an integral component of soil and plant root farming as AMF are symbiosis with many cereal crops. In this symbiotic relationship, the crop supply lipids and/or sugars to the symbiotic fungi thus providing a source of carbon for their metabolic needs. In return association of fungal hyphae plant show

increased nutrient concentration and improved growth and yield production.

The major function of AM fungi is to be nutrient acquisition. Soil parameters have a strong role in plant-fungal interaction [20]. High P concentration generally suppresses the colonization of root by AMF. Furthermore, available soil P is highly interactive with soil Zn and impacts upon the uptake of both nutrients. *Medicago* plants were less colonized by AMF than other species. Zn0 roots were larger than Zn90 of  $mg\ Kg^{-1}$  of  $ZnSO_4 \cdot 7H_2O$ . Also, the Zn0 plant had a greater mean root to shoot ratio than the Zn40 and Zn90 plants. There were main effects of Zn. Plant in Zn5 treatment had greater shoot biomass than those in Zn20 or Zn 30. The plants in the Zn30 treatment had smaller root biomass and shoot to root ratio than all other Zn treatments. Mycorrhizae or Zn treatment on the P concentration of barley has no effect in non-inoculated condition had greater shoot P concentration than inoculated condition. For all these three species, there were significant interactions between mycorrhiza and Zn when shoot Zn concentration was increased with increasing soil Zn concentration in both inoculated and uninoculated plants. Zn concentration increased from Zn0-40 and then decreased to 90.

For barley, there is no interaction between mycorrhizal Zn found. For tomato, there is a statistically significant interaction between mycorrhizae and Zn. For *Medicago* also there is statistically significant interaction found between mycorrhizal Zn and C drain on the host plant [21] Mycorrhizal infection can increase the uptake of Cu and Zn from soil solutions containing low concentrations of these metals. But such an uptake of higher metal is disadvantageous. So there is ecological and applied to interact to an understanding of these factors. Mycorrhizal infection may influence the resistance of *Calluna* to high levels of heavy metals. NM plant shows no tolerance of the Cu and Zn at high concentrations of toxicity and that infection leads to a significant reduction of the heavy metal content [6]. AMF contribute to plant Zn uptake but their role in the edible portion of the crop has not been studied yet. The mycorrhizal pathway of Zn uptake contributed upto 24.3% of the total above-ground Zn in wheat and upto 12% of that Zn in Barley. The greatest contribution by the mycorrhizal pathway was observed in Barley at the lowest Zn addition and in wheat at the .highest one. Besides the grain yield of bread wheat was increased by AMF.

### Plant Zn Uptake Pathways and Nutrient Uptake

Zn malnutrition is a major global health problem. The plant mainly takes up Zn from the soil in the form of free ions.  $Zn^{2+}$  and  $ZnOH^+$  on the other hand Zn can also be toxic to the plant when taken in excess. There is an attempt on developing Zn variety with higher Zn uptake. AMF can form an association with the roots of 80% of the terrestrial plant species. AMF applications increase the uptake of Zn. Plant colonized by AMF have two soil nutrient uptake pathways.

(1) Directly via the root of the epidermis

(2) Via fungal structures that form the mycorrhizal pathway uptake *Glomus intraredicus* (Rename: *Rhizophagus irregularis*).

Values of contribution by AMF to plant Zn uptake are highly dependent on the host plant species as in the case for uptake. Bread wheat and Barley were well colonized. The percentage of colonization varied widely with Zn application in agriculture. In bread wheat about mycorrhizal colonization decreased by 17% with increasing soil Zn concentration from low/medium to high Zn. By contrast in Barley, AMF root colonization was higher at low Zn and high Zn than at medium Zn. Fifty-three and forty-six percentages of bread wheat and barley were colonized. The percentage of colonization was significantly affected by Zn application.

The plant biomass, yield, and yield components were differently affected by AMF inoculation and Zn application. Grain yields were 21% higher in inoculated plants than in control plants. The number of kernels and spike fertility index is 23-73% higher in the mycorrhizal plants respectively. By contrast, chaff was 28% lower in the mycorrhizal plant than in the non-mycorrhizal plant. The number of kernels per spike and mean kernel weight was affected by the interaction between AMF inoculation and Zn application. Both straw and Zn concentrations were affected by the interaction between AMF inoculation and Zn application. Grain Zn concentration increased 1.5 fold from low to medium Zn irrespective of AMF inoculation. Increment in Zn shown 85% from medium to high Zn concentration. Zn content in barley grain and straw was increased by Zn application. In Barley grain increase by 28% and 69% low to medium and medium to high Zn respectively. The mycorrhizal colonization increased with increasing Zn application. But in tomato, no effect of Zn colonization is found. Yield increased by AMF application by 20%. This is due to P. P is higher in inoculated plants and not in un-inoculated ones.

Quarters of Zn in bread wheat and one eighth of Zn in Barley are due to the mycorrhizal Zn uptake pathway. Eighty-six percentages and 44% were allocated to grain in bread wheat and barley respectively. The functional compatibilities between mycorrhizae species and plant species play a major role [22]. *Hordeum* is highly colonized (>50%), *Linum* and *Sorghum* are moderately colonized 25-50% *Matricaria ecutita* had a relatively low in colonization. Less than 25% of mycorrhizal symbiosis increases the uptake of plant nutrients. AMF forms a mutualistic symbiosis with more than 70% of all vascular plants. The relationship between AMF and plant is dependent on reciprocal benefit. AMF immobilized toxic elements in the mycelia or the root system. AMF can in this way substantially reduce Cd concentration in plant shoots. Some plants highly colonized by mycorrhizal fungi can grow in heavy metal polluted soils. Protection of AMF against Cd, Combination of plant species, and AM colonization determine Cd uptake in plants [23].

Relatively higher Phosphorous provision AMF symbiosis showed positive effects on P accumulation nitrogen nutrition and

in biomass production. This idea strengthens has synergistic effects between below ground symbionts could promote the recruitment of forage legume in subtropical grassland soils. Host plant provided carbohydrates and lipids, AMF provided nutrients. AMF also help to improve the soil-plant water relationship and reduction in pathogenic infection as well as synergistic effects in an individual plant. In tropical soil, most of the P is fixed in the soil. AMF cause 2-4 fold increase in the efficiency of P absorption. Besides nitrogen fertilization and in the corporation of temperate legume in the north. AMF could be a relevant factor to promote the incorporation of temperate legumes into subtropical grasslands. This research is designed to answer

(a) The consequence of AMF presence for biomass production, P and N accumulation

(b) The impact of AMF on the biological nitrogen fixation of the legume rhizobium association symbiosis [24].

Arum and Paris type are two types of arbuscular mycorrhizae. Paris type developed shortly after the arum type of mycorrhizae. For the growth of 70 mm diameter, plastic non-draining pot was arranged and three days old three seedlings are planted in each pot 1:9 mix of soil:sand (w/w) [25]. The effect of mycorrhizae on plant yield is much higher than that with P fertilizer addition. Under low P fertilization, inoculation effectiveness is much higher than P addition treatments. Mycorrhizal colonization also increases tissue P and Zn uptake for horticultural plants. Mycorrhizal colonization increase 23% yield in horticultural plant species. Onions yield was highest (10.859 Kg/ha) in both P and mycorrhizae treatment. Radish yield was highest under combined P and mycorrhizae application. Chickpea yield was highest only in mycorrhizae added condition without P application. The addition of P increased twice for the radish yield as compared to control plants. Application of mycorrhizae increase the yield of horticultural crops. Moreover, P fertilizer was amended to increase the yield of crops. The synergistic interaction between plant and mycorrhiza shift in uptake of Zn and P with eventually get remobilized into developing plant productivity [26]. In the AMF *R. irregularis* is a small putatively secreted RysM protein which refers to as RiSLM is among the most highly expressed effect-like proteins during symbiosis.

There is protein-ligand interaction to bind possible sites. During the synthesis, the fungus develop branched hyphae called arbuscules that may fill root cortical cells when mature. Arbuscules are surrounded by a plant cell membrane and interfacial wall-like matrix. The latter is thin around the hyphae of mature arbuscules. Exposing chitin and other fungal molecular patterns prevalent at the interface. The arbuscules lifespan is shorter than that of the root cell and after several days the arbuscule degenerated leaving the root cell intact [27]. The maize landrace had a positive response with an increase of 17% in biomass and the result showed that the genetic identity is a dominant factor in symbiosis performance. This finding suggested that plants selected in low P availability can make

better use of mycorrhizal symbiosis. AMF are a group of interest because they establish mutualistic symbiosis with plants, colonize the cortical zone of the root zone, and depend entirely on the host plant to grow. These fungi may remain in direct contact with the recombinant protein during a large part of their cycle.

Morphological traits such as root length and biomass as well as root hair number are negatively related to mycorrhizal colonization than these with less developed roots. In both sampling inoculated conventional and GM hybrids have lower tissue P concentration than landrace genotype. P accumulation shares the same pattern both at 30 and 60 days after emergence. Landrace corn shared a positive response for inoculation while conventional and GM hybrids and higher P accumulation in the non-inoculated plants of the same genotype. Root colonization increase from 0.35-8.6% by AMF after 30 days after emergence. Land race can have 4.3% colonization rate [28]. Both plant and below ground mutualistic communities are all part of one system, coexisting through intrinsically linked interactions. Plant species coexistence in a stressful environment is mediated by facilitative interactions among plants that are tightly linked to the diversity of below-ground microbial communities. Mycorrhizal fungi can transport water, nutrient, and chemical defenses from facultative plants directly to their nurses through common mycorrhizal networks. Or indirectly through hydraulic lift or hyphal exudates. The relationship can be of mutualism or parasitism. Fungicide reduces 68% in isolation. Similarly, vesicle colonization reduced after fungicide application of 66% and 88% in isolated and associated *M.cuisana*. The fungicide reduces the total number of seeds, seeds per pod, seed mass, and seed viability [29]. Plant and microbe are a single evolutionary unit. The establishment of symbiosis, directly and indirectly, conditions the plant-associated microbiome impact of the symbiont on the plant-microbe as the symbiosis cascade effect in which the symbiont and their plant host jointly shape the plant-microbe [30].

### **Mycorrhiza and Plant Zn Concentration**

Mycorrhizal benefits were greatest when plants were grown under low soil P and Zn. Furthermore, the effect of soil Zn supply on plant growth, nutrition, and AM colonization was strongly influenced by the concentration of P in the soil. While AM can improve plant Zn acquisition in Zn deficient soils. They can also protect plants against excessive Zn uptake when an excess of Zn concentration in soils. Plant colonized by AM fungi has found to have lower tissue Zn concentration when grown under toxic soil. Zn concentrations comparing with their non-mycorrhizal counterpart. In Zn deficient soil AM can enhance Zn uptake. P status of plants can have a significant impact upon plant Zn nutrition and vice-versa. For example increase P uptake in mycorrhizal plants may result in increased plant growth and then Zn deficiency due to the dilution effect. P and Zn uptake in a mycorrhizal plant is correlated. Thirty percent of the world population experiences inadequate dietary Zn intake. One-third of the world's soil is found Zn deficient and much

of the population relies on a plant-based diet [31]. Increasing Zn density in a plant is of high importance.

Since the formulation of AM under deficient soil concentration can increase Zn acquisition by 35-50%. While AM can improve plant Zn acquisition in Zn deficient soil. Under toxic condition plant growing with AM have lower Zn in the plant tissue ratio than their non-AM counterparts. In Zn deficient soil AM can help plant acquisition of Zn. This kind of result explains the dual role of AM in Zn toxicity. Am induced improve nutrition in plant nutrition may in turn impact plant Zn nutrition and vice-versa. Increase P uptake improve mycorrhizal growth increase Zn utilization in plant and the deficiencies is due to dilution effect. P and Zn uptake in mycorrhizal plants are correlated. AM can improve the acquisition of both P and Zn. 76 R types have higher Zn accumulation than rmc types. Both shoot dry weight and root dry weight of 76 R types plant are higher than rmc types. There was a significant three-way interaction between genotype, P addition, and Zn addition for shoot P concentration. In the low P treatment, the shoot concentration of the 76R genotype is significantly higher than that of the rmc genotype. Conversely, at high P there were no differences in shoot P between any of the treatment, and P concentrations are within the range of these of 76R plants in the low P addition treatments.

The analysis of root P data also revealed a significant two-way interaction between the genotype and Zn addition treatment. P concentration of 76R genotype at all levels of Zn supply being generally higher than those of rmc plants. Furthermore for root P concentration there, was also a significant two-way interaction between the P addition and Zn addition treatments. At low P supply mycorrhizal plant response was positive at all levels of N supply, In contrast, mycorrhizal phosphate uptake response (MPR) was close to zero in the high P addition treatment of all levels of soil Zn supply. In the low P addition treatment, the mycorrhizal Zn uptake response (MZnR) was positive where soil Zn concentration was low. In contrast, the MZnR was close to zero in all other experimental treatments. Levels of mycorrhizal colonization of the 76R genotype were significantly reduced with increasing P and Zn supply observed here may be due to differences in the magnitude of the effect of Zn on the growth rate of Zn on fungal growth rate many be greater than that of roots. Decrease in inoculum due to reduction in the effective inoculum potential of the soil due to negative effects of increasing soil Zn supply on the rates of spore germination and hyphae growth [32].

Taxonomically mycorrhiza is grouped in the phylum Glomeromycota. The association thus formed helps to the uptake of many nutrient elements. The AMF has an important role in the sustainable agricultural production system. Multifarious and complex interaction occurs both in the plant and soil. Soil fertility can also impact plant uptake of Zn, Fe, Cu, Mn, and other nutrients. P and Zn are the most frequently studied interactions. When soil is high in P and low in Zn the interactions are predominant. In the

case of P, there is an inverse relationship between soil P fertilization and root length colonized by AM. Mycorrhizal colonization in the 76R genotypes is significantly higher than in the rmc genotypes. P was significantly higher in the 76R genotype than rmc genotypes. Soil P had a significant influence upon both root and shoot concentration. There are significant effects of soil Zn upon shoot zinc concentration. Colonization of rmc genotype is lower than that of 76R genotype. The concentration of P, S, and Cu was significantly higher in 76R than rmc genotype. Root Na and shoot Mg are higher in the non-mycorrhizal genotype than in the mycorrhizal genotype 76R. Low beta appears to extend to other micro/macronutrient benefits. Shoot P concentration was higher on 76R genotype. In the deficient soil Zn category, root B concentration was extremely high in the rmc genotype compared to 76R type resist boron uptake than rmc genotype [33].

Simultaneous application of Zn and P can have an antagonistic effect on plant Zn uptake. Plant Zn and P uptake were enhanced when supplied as Zinc phosphate carbonate. Mycorrhizal plants take up more P than non-mycorrhizal plants. The reverse was true for Zn. Given that much of the world's soil is both Zn and P deficient. AM lower soil pH which in turn increases the availability of Zn. Increase pathogenicity increases water use efficiency and improvement of soil moisture and structure in the rhizosphere which may lead to improvement in the crop, yield, and plant nutrition. Improvement of WUE increases Zn acquisition in the plant. The water use efficiency of plants in the Zn carbonate treatment is lower than all other treatments. Genotype and fertilizer sources are highly significant. Application of Zn in the form of Zn phosphate carbonate was found similar to the application of water-soluble  $ZnSO_4$ . Plant P uptake or content is high in the form of Zinc phosphate or carbonate treatment. But Zn phosphate carbonate fertilized plant had the ear Zn content. Zinc phosphate carbonate alters plants to take up both nutrients effectively (P and Zn). There were no differences in colonization with different sources of Zn fertilizers. The mycorrhizal plant had a significantly higher P content than its non-mycorrhizal counterpart. The reverse is true for Zn. The provision of P fertilizer to mycorrhizal plant decrease Zn uptake relative to non-mycorrhizal plants.

Biomass is the same with disregard to the different sources of Zn fertilizers and genotype. There was an apparent nutritional benefit. All plants were benefited in terms of biomass from the application of P and Zn fertilizer. An earlier study with the addition of the same amount of fertilizer as M. This study (P and Zn) demonstrated a strong positive growth response. Water solubility and plant Zn uptake are not correlated positively. In the presence of fertilizer, Zinc phosphate carbonate exhibits equivalent soil solubility to that of Zinc sulphate tending to enhances availability and thus uptake by plants. Plants supplied with highly soluble Zn fertilizer would have improved water use efficiency. Additionally, soil Zn was not deficient in any of the Zn fertilizer treatments. Zinc phosphate carbonate is an important solution of the dual application of

Zn and P [34]. Two pathways exist for plant Pi uptake from the soil via root epidermal cells (direct pathway) or via association with arbuscular mycorrhizal fungi (AMF). And the two pathways interact in a complex manner. This study focused on the direct pathway. Colonization by AMF decreased the expression of direct Pi transporter genes locally but distally in the wild type. In *mtpt4* mutant direct Pi transporter genes and the Pi starvation-induced gene *mtu* were more highly expected than in the wild-type roots.

In wild types plants, less Pi was taken up via the mycorrhizal pathway. Colonization by AMF strongly influenced root growth locally and distally and direct root Pi uptake activates locally but had only a weak influence on the distal direct pathway. The response to AM colonization in the *mtpt4* mutation suggested that in the wild type the increased P concentration of colonized root was a major factor driving the effect of AM colonization on direct root Pi uptake. Arbuscular mycorrhizal increases plant uptake of soil nutrients such as P, Zn, Ca, and Cu. When they are in limited supply mycorrhizal pathway uptake (MPU) helped by the mycorrhizae. The phosphate transporter gene *mtPT1* is an important direct Pi transporter that appears to be expressed exclusively in the root epidermal cells, cortex, and root hairs. Furthermore, *mtpt1* expression levels decrease when a plant becomes colonized by the AMF. The direct phosphate transporter gene *MtPT3* is down-regulated in the Pi fertilization and in roots colonized by three species of AM fungi [35,19].

Citrus plants are highly dependent on mycorrhizal colonization. An association between AMF and the citrus root is a natural solution to increase plant productivity in several countries. Citrus root forms a symbiotic association with AMF. The citrus plant requires AMF for its maximum growth and development. The health of the citrus plant and fungal colonization are interrelated. The healthiest plant show high colonization. The AMF in citrus increases the rates of seedling survival and plant growth. Within mycorrhizal association plants supply photosynthetic assimilate to the fungi and fungi adds in the uptake of nutrients especially P. Under the soil, sterilization plant growth was reduced due to the elimination of viable microorganisms. Also, it is reported that AMF applied citrus seedling has higher root length, root volume, and surface area. In non-sterile soil indigenous colonization ranged from 40-80% and in sterile soil the colonization ranged between 0-4%. But in AMF inoculation there is 33-60% colonization. In non-sterile conditions, there is no difference in fresh weight and shoot dry weight between inoculated and non-inoculated plants. But in sterile conditions, there is a difference in colonization between inoculated and non-inoculated plants. Indigenous mycorrhizae have a role in non-sterile conditions [2,3].

Arbuscular mycorrhizal fungi can enhance plant uptake of immobile nutrients such as Zn and P. Enhancement of Zn uptake by arbuscular mycorrhizal fungi on Zn deficient soil is studied previously. However, the quantity of Zn is contributed by the AM

pathway of uptake to the plant has not been reported previously. Up to 24% of the Zn in the shoot of AM plant was delivered via the MPU at the lowest soil Zn treatment, Plant grown in low Zn has a higher amount of Zn uptake in mycorrhizal soil than non-mycorrhizal soil. There was no effect of Zn fertilization on mycorrhizal colonization in this study. Roots of rmc genotype were not colonized by AM fungi. By contrast, the root of the genotype 76R genotype was well colonized by AM fungi. Mean AM colonization in the 76R genotype was  $37.8 \pm 2.4\%$  root length colonized across all Zn addition treatments and there was no difference among the Zn addition treatments. RMC plant having significantly higher shoot dry weight and total dry weight. Root dry weight was significantly higher at high Zn than at both medium and low Zn irrespective of genotype [36].

### Zinc Fertilizers

Zinc and Phosphorous are mutually antagonistic to each other whenever either element exceeds some threshold value. Zinc tends to act with phosphorous negatively. Here the role of mycorrhizae is to enhance the activity of Zinc. But it happened that the function and uptake of phosphorous is increased when mycorrhizae infected soil is taken for the growth. This effect of normal uptake is not seen on the soil without mycorrhizae. So affect the P/Zn concentration in Plant. VAM helps to bind heavy metals to the root better way to alleviate heavy metals concentration in the soil [37]. When soil Zinc is high, mycorrhizal fungi adapted to elevated soil Zn condition. Mycorrhizal fungi from contaminated sites are more efficient in stimulating growth than the mycorrhizal fungi from the non-contaminated sites. Mycorrhizal fungi significantly improved plant nutrition under a high level of soil Zn stress. The mycorrhizal plants by maintaining a higher level of short P/Zn concentration ratio can alleviate the negative effect of Zn. In the absence of mycorrhizal fungi, phosphorous fertilization improved plant growth under high soil Zn concentration although P amendment also resulted in increased shoot Zn uptake [37].

Zinc is a vital micronutrient for many plants but its excess can be calamitous. In the world toxicity of over accumulation, Zn is increasing which requires phytoremediation. So an experiment was set up to study the effect of soil enrichment on a study of Zn accumulation, oxidative stress, photosynthetic pigment concentration, activities of antioxidant enzymes. Increasing plant growth results in a progressive decline in soluble protein content and leaf pigment concentration with the increase in Zn regimes. Similarly, superoxide dismutase, ascorbate peroxidase, guaiacol peroxidase, and glutathione reductase activities increased significantly with elevated Zn levels. The result indicates that *Coronopus didymus* stand as Zn tolerant species which can accumulate a significant amount of Zn in its tissues. And can be neutralized by the generation of reactive oxygen species and scavenging capacity of the antioxidant enzymes. So it is marked that its efficacy to be a potential agent for the phytoremediation of Zn-contaminated soils [38].

Hidden hunger is also known as micronutrient deficiency affects the whole world, especially the countries where the cultivation of rice, maize, and wheat for daily diets is practiced. Lack of Zn, vitamin A, folate, Iron, etc is causing hidden hunger. Agronomic biofortification i.e. application of Zn will change the overall production scenario. Foliar spray, soil application, and combined soil and foliar application affect growth and quality attributes in wheat. Foliar application is a more effective means of achieving higher grain yield and quality [39]. Zinc function as a structural element of many enzymes for the metabolism of auxin and carbohydrates and also in the formation of proteins and integrity of membranes [39]. Moreover, the role of Zn in pollen development, chlorophyll synthesis, and fertilization is taken into account. Wheat is the major food crop in micronutrient deficient countries in the world [40]. Also, it is highlighted that 50% of the world cereal production is under micronutrient deficient conditions in calcareous and high pH soil [31].

More than 50% of calorie intake in the cities and more than 70% of the calorie intake in rural areas are from wheat [41]. There are several approaches to overcome the hidden hunger viz food diversification, biofortification, supplementation of minerals in the diet, and increasing food fortification in post-harvest products [42]. Agronomic biofortification is one of the major methods to enrich food grains with Zn elements. Soil or foliar approached of Zn fortification is emphasized in major crops. Zinc is highly required for the functioning of many enzymes, plant processes, and malting. AMF can improve the uptake of Zn in plant-like barley (*Hordeum vulgare*) in Zn deficient soils [43]. It is reported that the Zn deficient areas around the world is increasing and 30% of the world's cereal growing areas are deficient in Zn [41]. Similarly, Zn deficiency disrupts the metabolism of carbohydrates, lipids, nucleic acid. Zinc deficiency in plants affects both quality and yield [41,44]. Barley is an important cereal crop that has major input in the brewing industry. It is observed that the malting qualities of barley grain are affected by the concentration of Zn in barley [45]. The composition and ratios of starch and protein signify the importance of brewing.

An association between plant and AMF enhances the plant's ability to increase starch to protein ratio [43]. It has been proved that MPU provided 12.7% of the grain Zinc in Barley [22]. There is much variation in arbuscular mycorrhizal association although 80% of the plant species are infected with mycorrhizae [46]. Most of the field-grown crops today need Zn for growth and reproduction. Dependency of the Zn is widespread all over the world [47]. Zinc bioavailability and remobilization are affected by the environmental condition. Both chemical and physical ways change the balance between soil solution and organic and inorganic fractions. Deep knowledge of the Zn for characterization of nutrient availability to plants through chemical interaction within the soil component and Zn geochemical behavior is fundamental. Zinc is absorbed in organic matter, oxides, carbonates. The distribution of Zn in soil depends on soil organic matter, pH, temperature, texture,

structure, amount and type of clay minerals, cation exchange properties, metal oxide fractions, and transformation in the source material [46].

Zinc is an important element for animal and crop production. Application of Zn enriched food material such as stem, leaves, and grain are important to boost the crop yield [47]. The worldwide survey of the Zn found out 33% of the area is lacking Zn [31]. For annual biomass production of 10 tonnes ha<sup>-1</sup> an estimate of 100-300 g ha<sup>-1</sup> Zn is demanded [48]. All the parameters recorded in wheat namely, no of tillers, plant height, spike length, no of spikelets per spike, no of grains per spike, 1000 grain weight, biological yield, grain yield, and harvesting index, Ca, Mg, Fe, Zn, Cu and protein content are significantly affected [39]. Among the observed protein, Ca, Cu, Mg, Fe, and Zn are highly significant, where the value of Mg varies from 13.3 to 17 mg Kg<sup>-1</sup>. Similarly, the minimum and maximum values for Zn vary from 8.63 – 11.4 mg Kg<sup>-1</sup> [39]. Sole application of ZnSO<sub>4</sub> recorded 61.33 mg Kg<sup>-1</sup> in year two and 41.08 mg Kg<sup>-1</sup> in the first year. Similarly, it is observed that in year one maximum protein content is recorded in the combined application of Zn and Fe in the first year [39].

The increase in yield attributing parameters after foliar Zn application is due to its phloem mobility after foliar application [39]. Zinc played a crucial role in physiological processes like photosynthesis and respiration ensuring early flowering and higher grain yield. Also, the role of Zn in cell division enlargement and elongation is important. Likewise, application methods and timing are important to achieve the desired goal. An additive effect is observed between Zn in terms of grain yield and protein content [39,40]. Application of Zn does not have a significant effect on plant physiological parameters, not a heated condition which only increases shoot Zn and superoxide dismutase (SOD). This result emphasized that additional Zn would be required to protect the plant from heat stress. So foliar application enhanced Zn concentration in leaves and for maintaining the SOD activity and membrane stability. This also protects the photosystem from irreparable damage [49]. Reducing Zn hunger by its supplementation in the plant as the micro nutrient is one of the ways to reduce malnutrition.

Zinc has an important role in functioning more than 300 enzymes. The critical level of Zn in Pistachio 100 kernel is 37.6 mg Kg<sup>-1</sup>. Foliar application to the Pistachio tree increase leaf concentration of Zn in comparison to control. Similarly an increase of total nut yield, the fresh mass of 100 nuts and spilled nuts. Also, a significant drop in the empty nuts was observed [50]. In the first year, 13-35 percentages of higher responses are observed in ZnSO<sub>4</sub>. But in the second year, the most effective treatment was ZnMet. Which has an efficacy of 80%. In comparison to control a 1.2% increase in the nut Zn concentration was observed [50]. Zinc uptake varies from species to species. The variable rates of uptake of foliar and soil-applied Zn caused the deficiency. Zn deficiency is one of

the major factors limiting crop growth and development worldwide for several crops. So the correction of Zn deficiency is of prime importance. Soil and foliar application practice are using currently to correct the deficiency. A high level of soil phosphate and calcium carbonates decreased the soil Zn concentration. Also, high alkaline pH is conducive to low Zn concentration. Oil from rapeseed has the highest amount of unsaturated fatty acid than other plants.

It is the second largest source of vegetable protein in the animal diet. Similarly, rapeseed oil is the largest source of vegetable oil in the world. Application of 50 g L<sup>-1</sup> of Zn increased the rapeseed yield upto 6310 Kg ha<sup>-1</sup> with the highest level of oleic acid content 67% and least glucosinolate 0.8 micromolar per gram [51]. Further more increasing exposure to drought level increase the seed oil content in the rapeseed [51]. Water and nutrient are two important factors that limit the growth and production of the crop worldwide. Application of Zn increases chlorophyll content, stem sap flow, and root hydraulic conductivity by 8, 30, and 177 percentages respectively in the well-watered condition of 20 Kg ha<sup>-1</sup>. Similarly, under drought stress 50 Kg ha<sup>-1</sup> the value of chlorophyll content, stem sap flow, and root hydraulic conductivity is 18, 46, and 52 respectively. Grain yield of 12.5 and 7.5 percentages were observed in well-watered 20 Kg ha<sup>-1</sup> and drought stress 50 Kg ha<sup>-1</sup>. Similarly, water use efficiencies of 11% and 65% were observed in well-watered 20 Kg ha<sup>-1</sup> and drought stress 50 Kg ha<sup>-1</sup>. [52]. Under a well-irrigated situation application of Zn 20 Kg ha<sup>-1</sup> is enough to increase maize yield and yield attributing parameters. While the situation is reversed under drought conditions an application of 50 Kg ha<sup>-1</sup> is recommended under drought-stressed conditions [52].

Zinc showed good phloem mobility in rice and wheat. Zn has relatively high phloem mobility. In the case of rice 70% of the vegetative Zn is transferred to the grain [53]. In a Zn deficient soil, the yield of rice can be increased by the application of 1% suspension of Zn. Spraying of the ZnSO<sub>4</sub> is one of the ways to enrich the crop with Zn. Zinc moved from old shoots to young shoots. Zn is phloem mobile and the only application of soil Zn does not affect plant growth and development. A combination of soil and foliar application is better in rice [53]. Shoot dry weight and root dry weight is increased by the combination of soil and foliar application. Foliar Zn application is an important method for the enrichment of rice seed and grain yield [53]. Presently release and use of high Zn responsive crop varieties are on the rise. An application of AMF and Zinc solubilizing bacteria along with 50 Kg of ZnSO<sub>4</sub> per ha increased the soil enzyme activities for example dehydrogenase, urease, and phosphatase in vegetative, tasseling, and harvest stages [54]. The action of all enzyme reduce soil pH and improve the bioavailability of phosphorous in the soil.

An application of a basal dose of 5mg Zn per Kg plus 0.5% foliar spray significantly increases grain and straw Zn concentration [55]. Mycorrhizae performed a dual role for the alleviation of the

plant under both low and high levels of Zn in the soil. The role of mycorrhizae under both high and low levels of Zn is to upregulate and down-regulate MtPT4 and MtZIP6 respectively. Rice biomass in calcareous soil can be increased by an application of 5 ppm of Zn into the soil [56]. Zn deficiency not only decreased the yield but also deteriorates quality. The critical level of Zn in the soil is 0.84 mg Kg<sup>-1</sup> and 26.1 mg Kg<sup>-1</sup> in maize plants [56]. Zn deficiency is one of the fifth major causes of disease and death in developing countries [57]. After 35 days of soil and foliar application of Zn, the plant growth, root, and shoot dry weight is increased under Zn treated plants. Plant with adequate Zn supply has a higher amount of Zn content in different plant parts. Because of Zn mobility through the phloem, the concentration of Zn is higher in younger tissues [53]. The different factor responsible for soil Zn availability is a type of soil organic matter content, soil pH, soil moisture, and temperature [58].

It is necessary to produce crops with enough Zn nutrition to avoid ill effects on human health due to Zn malnutrition. In research conducted in Zn efficient and Zn inefficient wheat varieties. An investigation was carried out on the effect of wheat on superoxide dismutase and carbonic anhydrase activities. Comparing to no Zn application of soil/foliar/both methods of Zn application SOD and CA activities in both Zn efficient and inefficient varieties compared to no Zn application. At the post-anthesis stage, SOD activities are higher but CA activities are lower. Moreover, the application of Zn enhanced Zn concentration in leaves, stems, and grain of both genotypes. Zn efficient genotypes have a higher value for Zn [58]. At 6ppm concentration of Zn, the biofortified wheat variety Zincol-2016 attained the desired level of Zn concentration which is 53 mg Kg<sup>-1</sup>. By Zn fertilization grain yield, grain Zn concentration, and grain Zn bioavailability in two wheat varieties namely Jauhar-2016 and Zincol-2016 [59]. Along with increment with the uptake of soil Zn, it also reduces the phytate content in the seed [59].

## Drought Stress

Drought stress is one of the important factors limiting crop growth and yield worldwide. A wide range of symptoms are generated by the drought stress in plants are inhibition of photosynthesis, increasing oxidative stress, and changes in the metabolism. Plant acquire a series of physio-morphological changes in the plant after it has been exposed to drought stress. An observation of the root to shoot parameter is the key parameter in estimating drought tolerance in plants. The root is an important vegetative organ caused a plant to transports water and nutrient from underground part to upper region. Drought stress caused translocation of photosynthetically assimilated from leaves to seeds or fruits. Drought stress causes a significant reduction in photosynthesis but provides a major role in metabolism. Drought stress imparts significant activity in sucrose phosphate synthetase activity. Sucrose is transported from source to sink for example

young leaves, seeds, and roots. This purpose requires both symplastic and apoplastic modes of transport [60]. The loading and unloading of sucrose into phloem is done by plasmodesmata through the symplast pathway.

While the transport of sucrose in the apoplastic pathway requires sucrose transported proteins such as SWEET (Sugars Will Eventually Be Exported Transporters) gene family and sucrose transporter (SUC) [61]. Drought stress affects the photosynthetic capacity and negatively affects the growth and metabolism of the shoot and root tissues [62]. During drought conditions, there is a decline in grain yield, seed oil percentage, oil yield, palmitic acid, stearic acid, cis-oleic acid, and linoleic acid in Soybean. Application of ZnO significantly increases proline content, catalase, and peroxidase activities. However with the spray of ZnO significantly reduced the concentration of palmitic acid, stearic acid  $\alpha$ -linoleic acid. In general, results showed that ZnO treatment decreased the adverse effects of drought stress in plants by enhancing antioxidant enzyme activity and changing physiological parameters [63]. The scarcity of water is a major global challenge. So increase in the reactive oxygen species (ROS) is recorded under drought stress situation inside plant cell chloroplasts, peroxisome, and mitochondria. Drought situation reduced the solubility and uptake of nutrients by the roots [63]. Application of ZnO would have been the probable solution to mitigating drought. This is how a foliar application of ZnO is practiced [63].

Crops with high evaporative demand and low water availability are two important phenomena affecting crop growth and development under high water stress. So plant morphological and physiological characterization is important in studying plant response to heat and drought stress. Drought stress reduces the concentration of chlorophyll (a,b), carotenoids. However glucose, galactose, rhamnose, xylose, proline, catalase, ascorbate peroxidase, peroxidase, superoxide dismutase, malondialdehyde (in leaves and roots), and the chlorophyll a and b ratios were increased [64]. In canola, there is decreased in plant height, stem height, root length, and fresh and dry weight of canola [64]. Drought stress cause limitation in the production of crop and has an adverse effect in the plant growth and development. Literally, drought means lack of moisture to complete the plant life cycle. Decrease in the soil water due to continuous loss of water from evaporation and transpiration and plant suffers from the drought stress. Demarcation of the drought area differs from place to place.

A mixture of physicochemical, biochemical and morphological responses are exposed by the plant during drought stress conditions. Drought avoidance, drought tolerance are drought escape are some of the approaches practiced by the plant while suffering from drought stress. An example of drought escape is a shorter life cycle and early maturity. Drought avoidance is practicing relatively high tissue water content. An ability of a plant to live, grow and produce satisfactory yield with limited soil water is called tolerance. Osmotic

adjustment by reducing the osmotic potential to plant continuously absorb water. Proline is one of the important molecule help in the osmotic adjustment. There is a direct correlation between proline and osmotic adjustment [64]. Sucrose is the major product of photosynthesis and a key signaling molecule in plants. And sugar is involved in the responses of lots of abiotic stress. Under drought stress, conditions reduction in shoot biomass is more pronounced than shoot biomass. Drought stress in soybean caused a decline in starch and an increase in sugar or sucrose in leaves.

But in the roots, both the component got increased. This is an example of sugar metabolism in the leaves. Furthermore, there is the activation of a sugar transporter gene in Soybean. Thus increase in root to shoot ratio caused changes in sugar allocation, metabolism, and transport under drought stress contributing towards drought resistance of soybean [65]. However the importance of AMF in alleviating drought stress cannot be negated [66]. The use of AMF in drought stress physiology of plant is under concern. AMF are shown to increase the drought tolerance in a variety of plants. Application of AMF in adaptation of plant against drought stress by changing root morphology and epicuticular wax is remarkable. Moreover the addition of AMF in nutrient uptake and biochemical mechanism with hormones, osmotic adjustment, antioxidant system improves soil plant water relations. Improvement in plant growth, water status and nutrient accumulation is observed in mycorrhizal plant. Likewise AMF colonization improves hyphal networks and glomalin secretion which in turn assist in water and nutrient uptake. By means of extra-radical hyphae AMF associated plant has influence on photosynthetic rate. Studies so far revealed the role of AM symbiosis in ameliorating drought stress mechanism [67]. AMF root colonization increase root growth, hydraulic properties and ultimately changes the root architecture. Consequently result in the changes in the functional root system by remarkable changes in the water and nutrient uptake. Meantime, AMF hyphae has provision of nutrient and water uptake and transport [67].

## Heat Stress

Climate change and global warming cause a huge impact on food security. At the end of the 21<sup>st</sup> century, the global temperature is expected to rise from 1.8-4°C [68]. This increase in temperature is a major concern for governments and scientists all over the world as it affects the various life forms on the earth. It is challenging to feed the growing billions as it is expected to increase by nine billion by 2050 [69]. Heat stress (HS) is detrimental to several crops. HS affects many processes in a variety of plants as water relations, nutrient uptake, photosynthesis, assimilate partitioning, respiration, growth, and reproduction, and induced oxidative damage [69]. Plant acquire different mechanisms to overcome the HS. HS cause 6% decrease in the wheat crop. HS affects world food security and ecosystem quality. HS affects plant growth, physiological functioning, and final growth [69]. HS also affects net photosynthesis rate, chlorophyll content, chlorophyll fluorescence ratio, the effective quantum yield of photosystem II in *Brasica*

*chinensis*. Adequate Zn nutrition protects the plant from HS. Foliar application of  $ZnSO_4 \cdot 7H_2O$  effectively alleviated by enhancing Zn concentration, superoxide dismutase activity, chlorophyll content, Fv/Fm ratio, and photosystem.

Despite the negative impact of heat stress on crop production, it has an advantage in the cooler regions of the world [69]. Plant activities including germination, growth, development, photosynthesis, reproduction etc. have been affected by HS. High temperature in later growth stages of rice causes pollen sterility [70]. Similarly, 1% increase in temperature is responsible for the reduction of wheat yield by more than 10% [71]. Easterling, et al. [72] projected that 0.5% increase in global temperature causes significant loss in wheat by 5-7% in China. Wheat yield in Europe is shrinking as the temperature increased to 32°C in 2003 [73]. High-temperature stress also known as terminal heat stress is the second most important abiotic stress after drought stress. The heat stress is causing a negative impact on crop growth and development. Heat stress plays a major role in plant productivity as well. Simply plant respond to HS through, growth and development, physical and biochemical changes etc. Moreover, the type of response varies from stress, intensity, genotype, and duration. The sustainability of the production is the major threat of heat stress. The impact of climate change on the growth and productivity of crops is affecting agricultural production worldwide due to heat stress is overwhelming [69].

Among the various forms of abiotic stress exposed to the plant, heat stress has an independent mode of action on physiology and metabolism. Plant susceptibility to high temperature is observed in vegetative and reproductive development [74]. Application of Zn @18 Kg ha<sup>-1</sup>, increased plant height by 4.6%, hastened heading by 3 days, and extended grain filling duration by 4 days. Similarly, grain yield and biological yield in Zinc application @18 Kg ha<sup>-1</sup> are 11.51% and 22% respectively Here rice production can be increased by Zn application [75]. HS has a negative impact on germination by disturbing the activity of various enzymes. In maize, there is a substantial reduction of germination above 37°C [76]. HS causes severe reduction in cell size and water status in plant leading to growth reduction. HS imparts several symptoms in plants including sun burning of stems, leaf scorching, branches, discoloration of fruits, leaf senescence, and abscission [77]. In some cases, HS causing lower biomass, shortening the plant cycle and decrease the grain filling period in cereals, reduction in the final product [69]. Also, HS application significantly reduced plant growth and cycle length.

That increased pollen sterility, decreased the number of kernels, the kernel in maize and wheat, etc [69]. Extreme heat causes the problem of food insecurity. It is important to know the response of crop yield and productivity in the high HS. Also, it has been suggested to mitigate both the cold season and warm season heat spell. Crop responses to extreme climate conditions are interesting to study. This is how adaptation measures are required to meet the

consequence of world climate change i.e. heat stress [78]. Plant mortality and stunting in the heat HS are the causes of extreme HS. Which impairs the quality and causes a huge reduction in the yield of the crop. These losses have worldwide repercussions in agriculture [78]. HS caused significant loss in the yield, growth, and reproduction of crops. In major crops, it affects anthesis to grain filling. So this is the critical period to save the plant against HS. More over HS caused visible instances in nature including oxidative stress, photosynthetic damage, less production of photosynthetic assimilated, less production of ATP, and membrane permeability [69]. Depending upon the crop species and genotype, heat stress is detrimental for almost all crops by altering physiological processes and plant water relationships.

For example photosynthesis, plant growth, and development are highly affected by heat stress. HS disturbs the cell metabolism and causing to hampered water balance under the reduced cell-to-cell movement of water and water loss. Water loss is the function of reduced soil moisture and leaf water potential. HS has negative impacts on stomatal density, pore spaces, and stomatal conductance. All these features have a severe impact on the yield. HS reduced nutrient uptake per unit area of the root. Photosynthesis (Pn) is highly influenced by heat stress. High temperature affects Pn in both C<sub>3</sub> and C<sub>4</sub> plants. Thylakoids and stroma are affected by heat stress. The Pn was significantly affected by the HS. Besides HS, high temperature also affects plant water relations. It reduces the movement of water, leaf area, and these factors lead to a reduction in Pn. Degradation of chlorophyll a and b, production of reactive oxygen species (ROS), stomatal conductance, and net Pn are strongly affected by HS. HS affects the distribution of C and N and N assimilation in the plant. Under normal conditions, stem reservoirs contribute 30-40% of grain weight. But under HS there is 70% of assimilates in the stem. Above 30°C there is a gradual reduction in Pn and transport of photosynthates into reproductive parts.

Substantial reduction in final yield is mainly caused by a reduction in the movement of the water-soluble carbohydrate from the stem to the storage organs. On the other hand, HS causes a severe mismatch between source and sink [79]. HS leads to respiratory C losses, reduces ATP production, and increases the production of reactive oxygen species. In wheat flag leaf HS cause a severe decline in photoassimilates and lowers the production of carbohydrates. HS reduces O<sub>2</sub> and CO<sub>2</sub> solubility and functioning of the RUBISCO. Under HS oxygenation activity of RUBISCO caused photorespiration hampering the process of photosynthesis [80]. Increasing HS induced oxidative damage caused by ROS. ROS is mainly generated from the centers of PS-I and PS-II. Moreover, other organelles including mitochondria and peroxisome contribute to the ROS. Under HS the PS-I and PS-II become saturated with incoming photon and the photon excess will become the source of ROS. ROS causes various types of physiological disorders in plants. Leakage of the electron from the thylakoid membrane as a result of ROS accumulation is one example.

Plant growth and reproduction are stimulated by HS. Reproduction is highly sensitive to HS. A few degree increases in the temperature reduce the floral buds and cause flower abortion. Early HS can cause no flowering to no fruiting and seed filling. In HS there is a decrease in the number of pollen grains retained by the stigma. At 33°C reduced anther dehiscence and pollen fertility in wheat [81]. Temperature above 35°C caused sterility of pollen and reduced germination and cause yield decline. Moreover, HS caused an increase in ethylene production which induced a male sterile line. HS at the reproductive stage decreases ear length, spikelet, and fertile floret numbers ultimately caused yield reduction. It is observed that HS at the flowering stage is more severe than at the grain filling stage [82]. HS increases flower and fruit abortion and abscission eventually leads to a reduction in seed/fruit production. Some features of HS in plant occurs both at the cellular and subcellular level resulting in stunting and poor production. Cell becomes round-shaped, vacuole content clustered, stomata lamellae swollen and mitochondria get empty. In severe HS plants reduce their size, close stomata, reduce water loss, increase xylem vessels in roots and shoots [83].

Plant responses to heat stress are classified into two categories i.e.

- (a) Short-term and
- (b) Long-term response.

Depending upon plant growth stages plants acquire tremendous physiological changes to tackle HS. However, it is still not clear if the changes in plant phenology are cumulative or not. Early heading, flower abortion, and lower seed production have the advantages of retention of the higher amount of green leaves at anthesis resulting in lower yield or loss. Plant with smaller leaf avoids heat stress [84]. Plant decrease water loss, stomatal closure, decrease their size, and decrease xylem vessels in roots and shoots. Some plant possesses damage to mesophyll cells, membrane permeability and also produces polymeric leaves [85]. The major tolerance mechanisms plant adopting are ion transport, antioxidant defense systems, an abundance of late embryogenesis proteins, and presence of osmoprotectants. Changes in the leaf orientation in plants, evapotranspirative cooling, the lipid composition of the membrane changes to ensure survival under HS [77].

In high temperature and HS condition plant maintain balance in the water status in field conditions. However severe HS caused a reduction in the leaf water potential. In tomato HS severely caused a reduction in leaf water potential and hydraulic conductivities of the plant. Even though in the day time plant suffer from HS as it induces water deficiency, increase in transpiration, and disturbs plant physiological processes [86]. Accumulation of low molecular compounds such as ammonium, sulphonium, proline, glycine-betaine (GB), and sugars are called osmolytes. These osmolytes are gathered for plant survival under HS conditions. It is noted that the performance of osmolytes is significant in the deployment of HS

tolerance. Accumulation of their increase in plants through breeding, marker-assisted selection, and genetic engineering. In tomato, HS disrupted the sugar metabolism and proline transport. Also an example of the GB is the major osmolytes whose concentration increase under exposure to abiotic stress. Some plants can produce GB and some do not. The plant can also accumulate proline under HS conditions. Both proline and GB accumulation caused plant increase tolerance to abiotic stress including heat and drought stress [87,88].

Cell membrane integrity is the major characteristic for the growth and development of field crops. HS reduces the cell membrane permeability and result in thermostability. The role and functioning of the cell membrane are important for respiration and photosynthesis. HS results in the membrane more porous and fluid. As a result membrane function and integrity are affected. Electrolyte leakage which is the measure of electrolyte in plant tissue varies from exposure to plant under stress environment. Depending upon the growing season the electrolyte varies among tissues, organs, growth stages. For instance an injury of maize plant more severe in older stage than at younger stage. Elevated HS in sugarcane there is an increment in the proportion of saturated fatty acid [89]. Plant hormonal change has a major role in alleviation under abiotic stress conditions. Synthesis degradation and allocation of the hormone to different plant parts are important process ongoing in plant parts [90].

Ethylene, salicylic acid (SA), sulpho salicylic acid (SSA), gibberlins (GA), cytokinins, and abscisic acid (ABA) are major plant stress hormones and also acts as a signaling molecule. It is noted that under HS the ABA molecule increases. ABA changes gene expression, up and down-regulation of different genes, improves plant acclimation and adaptation under heat stress, induction of several heat shock proteins which give HS tolerance in plants. Germination, flowering, and fruiting are the several processes affected by ethylene under plant tolerance to HS. HS significantly inhibited the action of ethylene in the plant for instance wheat 35°C, Soybean 40°C, rose 45°C, and Kiwifruit 35°C [91]. SA helped in systemic acquired resistance and hypersensitive responses. SSA mimic SA and increase plant ability to withstand HS. It also acts as signaling molecule and helps to enhance plants to alleviate HS [92]. GA and cytokinins have a substantial amount of role against heat stress. Reduction of cytokinins in wheat results in low kernel filling and final dry weight [93].

Secondary metabolites (SM) play a significant role against plant survival under HS. Production SM such as flavonoids and phenyl propanoids cause synthesis and oxidation of phenolics. Carotenoids are the important SM protecting cell structures against different abiotic stress. Carotenoids play a major role in protecting cell structure against different abiotic stresses. Similarly, Xanthophyll help plant cell to adopt against heat stress. Plant possess a variety of SM compounds that is helpful against plant protection against

HS [69].

## Conclusion and Future Directions

Mycorrhizae can be taken as an ecological engineer. Two mechanisms for P-induced reduction of Cu and Zn concentrations are

- (i) The suppression of mycorrhizal uptake of these elements
- (ii) Dilution effect- the larger P sufficient plants do not concentrate on the uptake of smaller nutrients like Zn and Cu.

Crops with high evaporative demand and low water availability are two important phenomena affecting crop growth and development under high water stress. Drought stress reduces the concentration of chlorophyll (a,b), carotenoids. However glucose, galactose, rhamnose, xylose, proline, catalase, ascorbate peroxidase, peroxidase, superoxide dismutase, malondialdehyde (in leaves and roots), and the chlorophyll a and b ratios were increased. The heat stress is causing a negative impact on crop growth and development. Heat stress plays a major role in plant productivity as well. Simply plant respond to HS through, growth and development, physical and biochemical changes etc. Temperature above 35°C caused sterility of pollen and reduced germination and cause yield decline. HS disturbs the cell metabolism and causing to hampered water balance under the reduced cell-to-cell movement of water and water loss. Moreover, the type of response varies from stress, intensity, genotype, and duration. The global temperature is expected to rise from 1.8-4°C by the end of 21<sup>st</sup> century. Similarly, 1% increase in temperature is responsible for the reduction of wheat yield by more than 10%. HS causing lower biomass, shortening the plant cycle and decrease the grain filling period in cereals, reduction in the final product.

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## Declarations

## Conflict of Interests

The authors declares that there is no conflict of interest.

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