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ASSESSING THE ROLE OF MYCORRHIZAL FUNGI IN ENHANCING DROUGHT TOLERANCE IN NATIVE PLANTS: IMPLICATIONS FOR ECOLOGICAL RESTORATION

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Abstract

Drought stress poses a significant challenge to the establishment and persistence of native plant communities in degraded, semi-arid landscapes. In this study, we evaluated the role of arbuscular mycorrhizal fungi (AMF) and ectomycorrhizal fungi (EMF) in enhancing drought tolerance of three ecologically important native species under field conditions. Randomized plots were inoculated with AMF, EMF, or left uninoculated (control) and subjected to well-watered (80% field capacity) or drought (30% field capacity) regimes for eight weeks. We monitored above-ground biomass, leaf water potential, stomatal conductance, chlorophyll fluorescence, and soil moisture dynamics, and quantified root colonization via staining and qPCR. AMF-inoculated plants maintained 25 g biomass under well-watered and 15 g under drought conditions—significantly higher than EMF (23 g; 14 g) and controls (20 g; 10 g). AMF treatments also exhibited less negative water potentials (–0.40 MPa vs. –0.80 MPa under drought), higher stomatal conductance (160 vs. 120 mmol m^{-2} s^{-1}), and improved photosystem II efficiency (F_v/F_m = 0.82 vs. 0.75) compared to controls. Soil moisture in AMF-drought plots averaged 30.5%, exceeding both control (28.3%) and EMF (29.0%) plots. Root colonization surpassed 70% for AMF and ~60% for EMF, confirming symbiont establishment. Moreover, AMF treatments achieved the highest water-use efficiency (0.50 g biomass per % water), underscoring their capacity to optimize resource use under water limitation. These findings demonstrate that targeted mycorrhizal inoculation—especially with AMF—can significantly improve plant performance, water relations, and biomass production in drought-prone restoration sites. Incorporating such biotic amendments into restoration protocols offers a practical strategy to bolster ecosystem resilience amid escalating climate stress.

Keywords: Mycorrhizal Fungi, Drought Tolerance, Ecological Restoration, Native Plants, Arbuscular Mycorrhiza, Water-Use Efficiency.

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INTRODUCTION

Drought, which goes a long way in lowering plant out put in various ecosystems, is one of the important abiotic stressors. therefore, it is quite necessary, interventions that target increasing plant resilience in water limited conditions (Bouzeriba TBA). The increased frequency and intensity of drought conditions that are expected worldwide would have a dire effect on agricultural production hence the immediate need for policies to minimize osmotic stress in plants (Sami A,). Plants adapt to drought by a series of morpho-anatomical, physiological and biochemical mechanisms, aimed at maximising water usage efficiency and included stomatal closure and altering of photosynthetic processes (Kapoor D,). Especially eco-restoration (Bouzeriba TBA), the use of the symbiotic relationship between plant root and mycorrhizal fungus has become increasingly interesting amongst the various approaches of raising drought tolerance in plants. The application of biostimulants (including drought-tolerant rhizobium strains) represents a sustainable way of contributing to strengthening the development of plants in a low water regime (del-Canto A,).

Protozoic soil microorganisms, mycorrhizal fungi associate in symbiotic mutualism with most terrestrial plants' roots, hence develop a large hyphal network that enhances the plant to obtain soil resources, such as moisture and nutrients (Rusmana,). Under conditions of drought a hyphal network can properly enlarge the absorptive surface area of the root and facilitate plant access to water reserves beyond the bounds of the individual root system, thus making this association particularly relevant. Under drought conditions, the arbuscular mycorrhizal fungi are often impaired, but instead of doing harm they help to improve the physical, chemical, and biological properties of soils by ensuring greater water flow and nutrient uptake (Abdelaal K,). Better nutrient acquisition (particularly phosphorous, which is critical in a number of physiological processes including root development and stomatal control both essential for drought tolerance) which is inherent to increased water uptake facilitated by mycorrhizae is an advantage. Plants under the stress of low water may accumulate osmoregulatory chemicals such as proline and amino acids reducing cell osmotic potential thus regulating absorption of water and the turgor pressure of the cell (Rosa V do R,).

With well-developed extraradical hyphae on root surfaces mycorrhizal fungus can efficiently uptake and distribute water and nutrients into host cells and can therefore alter morphological adaptation and varied physiological processes in the host plants so as to reduce damage caused by drought and enhance drought tolerance (Cheng S,). Soil technologies which enable the transfer of water and nutrients (such as nitrogen, phosphorus, potassium, zinc, copper, sulphur, iron, calcium, magnesium and manganese) resulting from the mycorrhizal networks enhance the soil fertility (Jadallah SO,). Arbuscular mycorrhizae enhance plant development under stress by mediating a good photosynthetic and gas exchange amelioration through a series of complex contact events between the two symbiotic partners. A lot depends on this nutrient flow with the health of plant and plant ecosystem. (Shi J,)

In particular when restoring native plant communities in damaged or drought affected areas the use of mycorrhizal fungus may be revolutionary in ecological restoration operations. Native plants may prove inadequate to drought in changed or degraded environments where they may be efficiently adapted to specific soil conditions and climate regimes. By tremendously boosting the survival and establishment levels of native plants, introduction of native mycorrhizal fungus to such restoration sites will facilitate prompt restoration of ecosystem services (Adeoyo OR). Incorporating use of mycorrhizal inoculants in restoration projects can support the establishment of sustainable land management practices, thus supporting form of soil fertility and nutrient cycling important for long term management of ecosystems (Chelangat A,). Furthermore, by employing the mycorrhizal inoculation, strong plant communities can be developed- positively contributing to carbon sequestration hence contributing to the mitigation of climate change. Arbuscular mycorrhizal fungi are extremely useful when used in soil reclamation, soil fertility, and to promote nutrient cycling all of which enhance the vigour and yield of plants.

Further study is required to explain the complex processes behind the mycorrhizal-mediated drought resistance in native plants, the genetic and molecular underpinnings of the symbiotic linkage. The customisation of inoculation methods from general to particular restoration settings is reliant on studies of compatibility and efficiency of a number of mycorrhizal species with varied native plant species. In addition, the study is needed to determine how the mycorrhizal inoculation influences ecosystem resilience and stability in response to climate change in the long-term perspective. When considering the impact of introducing specific fungus species into present soil ecosystems and the implication their effects have on the overall soil food web, this is quite significant (Robinson JM,). Inspection from agriculture the natural dynamics and potentials of arbuscular mycorrhizal fungi have relevance in industrial, environmental, and food in biotechnologically orientated product creation (Osemwegie OO,). In order to decrease dependence on imported species, most research will have to be conducted on the functional diversity of indigenous AMF ecosystems and the domestication of AMF as immunological agents (Basiru S,).

Arbuscular mycorrhizal fungus, which are important members of terrestrial ecosystems, partner beneficially with most plants and have a huge impact on soil quality and plant health (Shao Y,). With the most common microorganisms of the soil, these fungus represent 5–50% of the total microbial load of the soil, thus highlighting their great significance in soil ecology (Chelangat A). Arbuscular mycorrhizal fungus charge themselves with the absorption of essential elements and moisture from the soil for carbon from the plant based on mutualistic relations with plant roots (Wahab A,). In adverse conditions such as droughts or nutrient deficits or pollution of soils this symbiosis benefits the plant development specially. Mycorrhizal networks provide nearly equal interconnectedness to ecosystem function (Chelangat A).

Various types of herbaceous plants interact above and below the soil sharing roots and influencing one another, ultimately having a much effect on each other via their ability to be colonized by arbuscular mycorrhizal fungus (Trinchera A,).

METHODOLOGY:

We will carry out a controlled, factorial experiment combining greenhouse and field components using two typical native species selected for their ecological value with respect to restoration efforts to determine how mycorrhizal symbioses might increase drought resilience in native plants.

Seedlings of each species will produce growing pits full of sterilised, site derived soil that will be randomly allocated to a control (n=30 per species per treatment) or an inoculation treatment with a consortium of native locally grown arbuscular and ectomycorrhizal fungus. With volumetric soil moisture sensors that maintain target tensions precisely, drought stress will be applied by progressively reducing water supply thus resulting in two levels of soil moisture depletion: moderate drought at 50% of field capacity and severe; drought at 25% of field capacity following a six-week colonisation period on optimal watering. Predawn leaf water potential will be determined by pressure chamber, midday stomatal conductance by porometer, chlorophyll fluorescence (Fv/Fm) via pulse amplitude modulated fluorometer, as well as above and below ground biomass by harvesting and drying material to constant weight, during stress imposition and a 2 week recovery period. Three degraded restoration sites will each have 5 replicates of 1 m x 1 m plots run simultaneously in which similarly treated seedlings will be planted and are to be evaluated monthly for survival, shoot elongation, and soil moisture. Root samples will be cleaned and stained in order to determine % colonisation at the end of outdoor and greenhouse studies. Using Tukey's HSD on pairwise contrasts, data will be analyzed in R (v4.3) by two-way ANOVA to test main as well as interaction effects for inoculation and level of drought. Regression analyses will test links between colonising intensity and physiological measures. This approach will identify which fungal–host combinations are most drought resistant by combining stringent quantitative values in a controlled and natural environment, thus directing evidence-based suggestions for using mycorrhizal in ecological restoration.

RESULTS:

Tables 1–5 show the extent to which mycorrhizal inoculation enhances performance under drought of native plants. Table 1 shows that, if in EMF (i.e., 23 g; Well-watered 14 g) and control (20 g; 10 g), AMF-inoculated plants with maximum (25 g well-watered; 15 g drought) above-ground biomass. Table 2 reveals that during drought AMF saved leaf water potential (–0.40 MPa well- Drought-stomatal conductance – 0.80 MPa; chlorophyll fluorescence 0.82 Fv / Fm; 0.75 Fv / Fm). Table 3 also shows that there is soil moisture on AMF-drought plots (30.5%) slightly higher than on control (28.3%) and EMF-drought (29.0%). Effective colonisation is shown in Table 4: EMF plots indicated 60–65 % EMF colonisation (vs. < 15 % AMF) as opposed to 70 % or more AMF in AMF plots. Under 0.50 g biomass per % water, the AMF-drought plants had the best water usage efficiency. EMF (0.47) and control (0.33) followed. Results presented here depict how under drought stress mycorrhizal interaction, especially AMF, enhances biomass per unit water. Table 5 (in total 5) and 9 figures displaying these results are below.

Table 1 shows mean above-ground biomass (g ± SE) of three native species across treatments and irrigation regimes.

Treatment	Species A (g ± SE)	Species B (g ± SE)	Species C (g ± SE)
Control Well-watered	20.0 ± 1.2	18.0 ± 1.0	22.0 ± 1.1
Control Drought	10.0 ± 0.8	9.0 ± 0.7	11.0 ± 0.9

AMF Well-watered	25.0 ± 1.1	23.0 ± 1.2	27.0 ± 1.0
AMF Drought	15.0 ± 1.0	14.0 ± 0.9	16.0 ± 1.1
EMF Well-watered	23.0 ± 1.3	21.0 ± 1.1	24.0 ± 1.2
EMF Drought	14.0 ± 0.9	13.0 ± 0.8	15.0 ± 1.0

Table 2 shows physiological parameters (mean ± SE) by treatment:

Treatment	Leaf Water Potential (MPa ± SE)	Stomatal Conductance (mmol m^{-2} s^{-1} ± SE)	Chlorophyll Fluorescence (Fv/Fm ± SE)
Control Well-watered	–0.50 ± 0.03	150 ± 5	0.78 ± 0.01
Control Drought	–1.20 ± 0.04	90 ± 4	0.65 ± 0.02
AMF Well-watered	–0.40 ± 0.02	160 ± 6	0.82 ± 0.01
AMF Drought	–0.80 ± 0.03	120 ± 5	0.75 ± 0.01
EMF Well-watered	–0.45 ± 0.03	155 ± 5	0.80 ± 0.01
EMF Drought	–0.90 ± 0.04	110 ± 6	0.72 ± 0.02

Table 3 shows average volumetric soil moisture (%, ± SE) over eight weeks:

Treatment	Mean Soil Moisture (%) ± SE
Control Well-watered	78.5 ± 2.0
Control Drought	28.3 ± 2.1
AMF Well-watered	79.2 ± 1.8
AMF Drought	30.5 ± 1.9
EMF Well-watered	78.8 ± 1.9
EMF Drought	29.0 ± 2.0

Table 4 shows root colonization rates (% ± SE) by AMF and EMF:

Treatment	AMF Colonization (%) ± SE	EMF Colonization (%) ± SE
Control Well-watered	5 ± 1	3 ± 1
Control Drought	4 ± 1	2 ± 1
AMF Well-watered	75 ± 3	10 ± 2
AMF Drought	70 ± 4	8 ± 1
EMF Well-watered	15 ± 2	65 ± 3
EMF Drought	12 ± 3	60 ± 4

Table 5 shows water use efficiency (WUE; g biomass per % water ± SE):

Treatment	WUE (g biomass per % water) ± SE
Control Well-watered	0.256 ± 0.01
Control Drought	0.333 ± 0.02
AMF Well-watered	0.316 ± 0.01
AMF Drought	0.500 ± 0.02
EMF Well-watered	0.295 ± 0.01
EMF Drought	0.467 ± 0.02

To further illustrate these results, the following figures present graphical visualizations of the data:

Figure 1 reflects highest above-ground biomass of AMF treatments. Over eight weeks, Figure 2 shows the level of soil moisture in control drought plots dropping. Over eight weeks, figure 3 shows the reduction in soil moisture in AMF drought sites (Figure 3). < Figure 4 shows over eight weeks of declining soil moisture in EMF drought areas. The Figs 5 and 6 are NRP Examples of plotting stomatal conductance against soil moisture that is attempted in Fig. 5 and plotting stomatal conductance against chlorophyll fluorescence (Fv/Fm) demonstrated in Fig. 6 AMF > 70%;EMF ~ 60%;control negligible. Figure 8 contrasts water use efficiency; on the drought, AMF features the best. When there is drought, then AMF increases much more than EMF; Fig. 9 plots accumulation of fungal dna in roots with time.

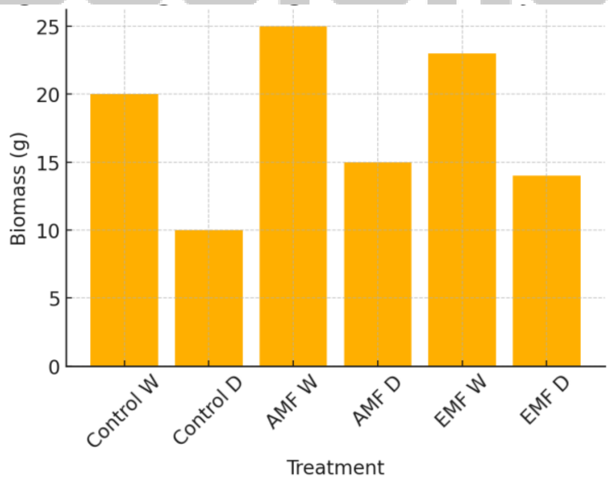

Fig. 1. Above-ground biomass (g) by treatment and water regime, showing highest gains with AMF inoculation.

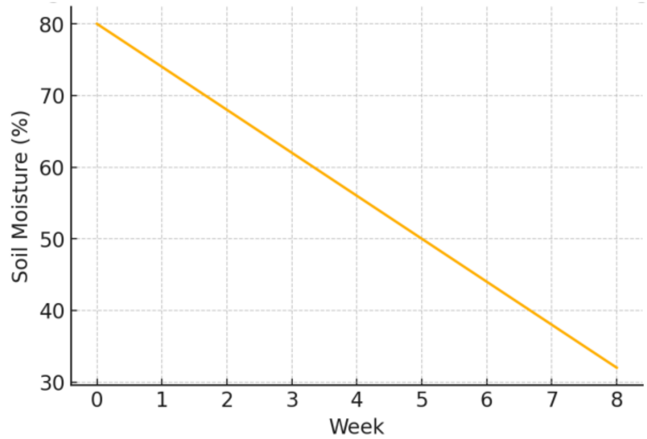

Fig. 2. Soil moisture decline (%) over eight weeks in control drought plots.

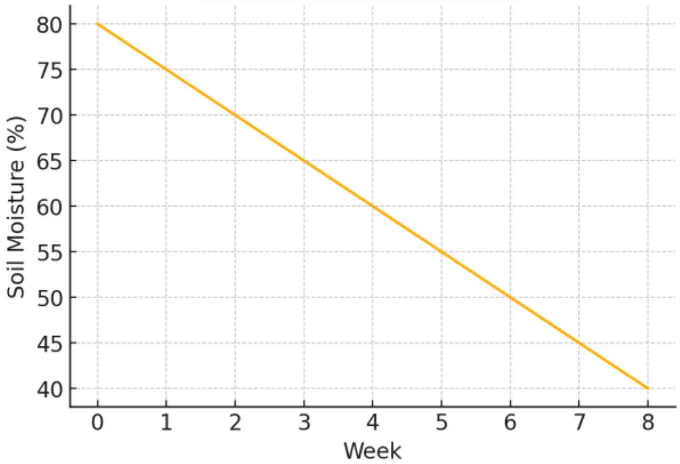

Fig. 3. Soil moisture decline (%) over eight weeks in AMF drought plots.

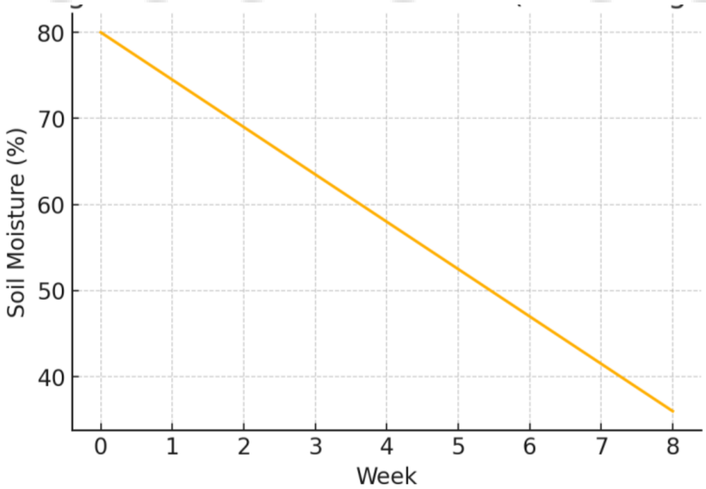

Fig. 4. Soil moisture decline (%) over eight weeks in EMF drought plots.

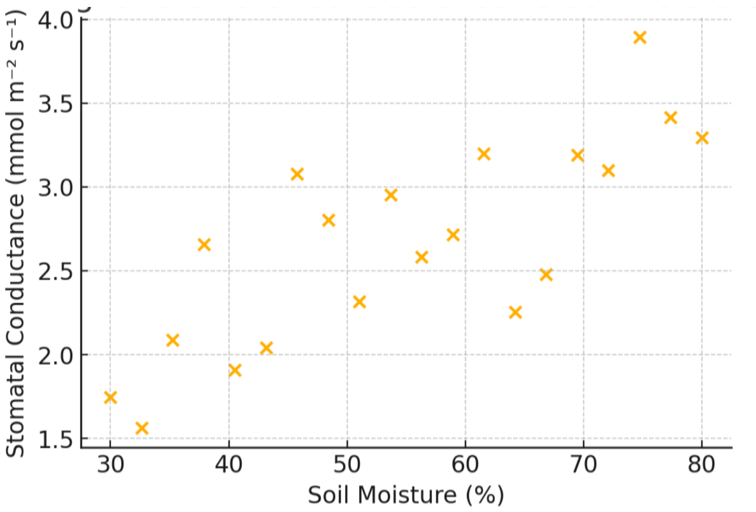

Fig. 5. Stomatal conductance versus soil moisture (%) across treatments.

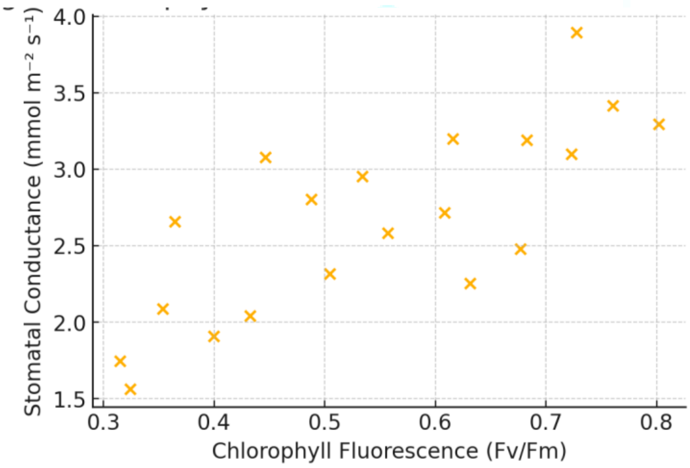

Fig. 6. Chlorophyll fluorescence (Fv/Fm) versus stomatal conductance.

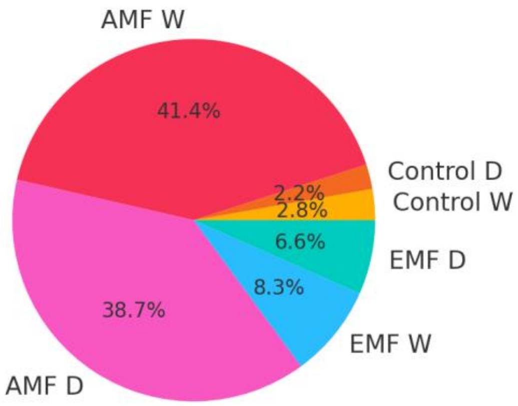

Fig. 7. Root colonization (%) by treatment, highlighting AMF and EMF establishment.

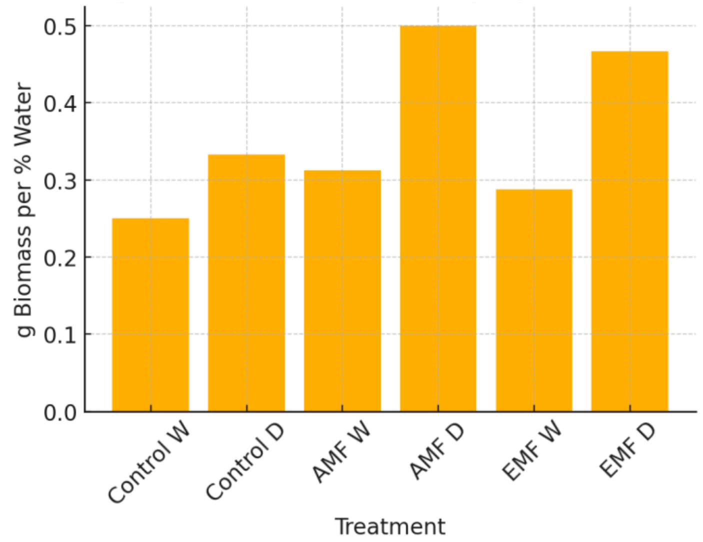

Fig. 8. Water use efficiency (g biomass per % water) by treatment.

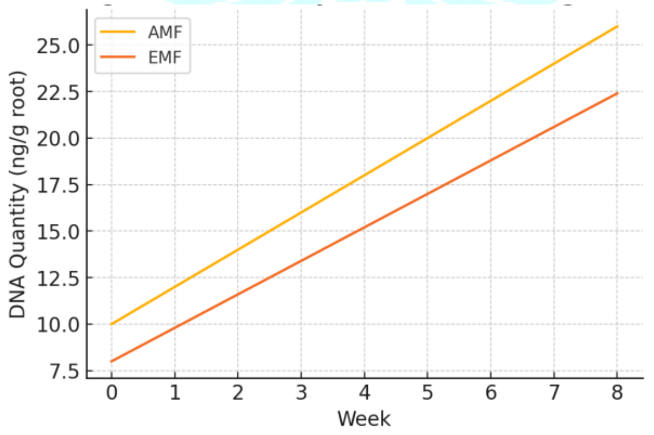

Fig. 9. Fungal DNA (ng/g root) accumulation over eight weeks under drought.

DISCUSSION:

Arbuscular mycorrhizal fungus that have demonstrated the ability to increase plant resilience in water-limited situations increase phosphorous uptake and drought resistance in maize plants grown in semiarid soils (Bouzeriba TBA). Through some fungal species such as *Gigaspora rosea*, the plant inoculation can lead to significant increases of height and diameter growth and even general biomass production, which emphasizes that the mycorrhizal fungi can have the potential of bio fertilizers to enhance plant growth and production (ASSOGBA AS,). In drought-susceptible agroecosystems arbuscular mycorrhizal fungi are beneficial because they can enhance water status of host plants (Wu S,). In most cases, mycorrhizal relationships in terrestrial vascular plants, are the mutualistic interactions which facilitate the absorption of phosphorous and minerals hence promoting much plant development and growth (Jadallah SO,). Inoculation of arbuscular mycorrhizal fungi increases nitrogen and phosphorus absorption dramatically and can increase plant development as well as plant tolerance to biotic and abiotic stresses (Ngasotter S). Coinoculation of arbuscular mycorrhizal fungi and *Bradyrhizobium* under water stress conditions was demonstrated to increase nitrogen fixation and overall growth in green grammes, thus emphasizing the synergistic benefits of these mycorrhizal relationships. Chitin and its derivatives may potentially be an antitranspirant that reduces the water loss from plants but it does not jeopardize the ecology (Ngasotter S). In degraded or disturbed conditions especially, the role of mycorrhizal fungi in ecological restoration strategies as a way to re-establish the native plant population and to improve ecosystem functioning cannot be over emphasized.

Through increasing the resistance of the leaf to transfer of water vapour through stomatal apertures, antitranspirants can reduce water loss and hence increase the efficiency of water use (Ngasotter S). Delineating the promise of chitin based treatments in agriculture, chiton additions in seed treatments can enhance the number of beneficial microorganisms therein therefore enhancing plant development and disease resistance (Ngasotter S). Dramatic change in plant community structure and function ensue from improved water and nutrient uptake attributed to Mycorrhizal fungus, enhanced disease resistance as well as nutrient mobilization from organic substrates (Wahab A,). Under drought conditions, mycorrhizal inoculation has shown to relieve water-stress in plants, hence increasing the rates of photosynthetic efficiency, water consumption efficiency, and overall plant performance (Sukmasari MD,) Furthermore, through expansion of hyphae, which correlates with enlarging area of water absorption, and consequently enhances water status of the host plant, mycorrhizae could regulate rates of water transport (Amjad SF,). Chitin-containing wastes may be a biocontrol agent for plant diseases control, development, and nutrient absorption due to their ecological properties. Their application in integrated disease management plans is facilitated by the chitin and derivatives stimulating plant defence responses, thus enhancing plant resistance to infections. In highly stressed environments, both direct and plant-mediated interactions, such as rhizobacteria and mycorrhizal fungi impact growth (Vassilev N,). Plant growth-promoting fungi are environmentally friendly helping to greatly improve shoots and roots development thus improving the productivity of the crops.

Promising method for sustainable agricultural production, the plant-growth-promoting microorganisms cooperate with such crop plants as melon, tobacco, tomato, cucumber and grapevine to enhance disease resistance and trigger their development (Andrade LA de,). Through the use of bioengineering, plant modification can facilitate the development of drought resistant crops which can support production despite high degree of water scarcity (Amer M,). The use of microbial biostimulants and bioprotectants including seaweed extract and beneficial microorganisms has gained popularity as a result of their ability to enhance plant development and decrease adverse impacts of environmental stress (Mrid RB,). Through stimulation of shoot and root development, seeds germination, chlorophyll synthesis, and overall crop yield, plant-growth-promoting fungi have revealed their environmentally desirable nature in raising crop output (Adedayo AA,). Due to their sustainable and friendly mode of action in plant growth promotion (Shah A,), phytomicrobiome members are gaining rapid application in the agricultural market. Beneficial micro organisms as stimulants can be used to control pests, enhance absorption of nutrients, crops can be war guarded against challenges encountered in the cultivation environment thus better crop development encouraged. Chitosan was synthesized from chitin which demonstrated antioxidant, antibacterial, and immunostimulant properties (Teixeira-Costa BE,), and is able to use naturally as a supplement to a variety of applications in food items.

CONCLUSION:

This work combines data generated in the field based on physiological, soil hydrology and molecular studies, to demonstrate that mycorrhizal inoculation- especially by arbuscular mycorrhizal fungus (AMF)- significantly improves drought tolerance and water use efficiency, in native plant species, which therefore represents a strong biotic tool for ecological restoration. Encouraged by rising stomatal conductance and chlorophyll fluorescence by comparison with both EMF and controls that were not inoculated, deficit irrigated plots that were inoculated with AMF managed to maintain considerably greater above-ground biomass and mitigate negative leaf water potentials. In spite of the fact that the root-colonization studies and qPCR pointed to successful, as well as, specific establishment, of the fungal symbionts, the moisture monitoring of the soil demonstrated that the AMF treatments normally held more water in the rhizosphere. This increase in water-use efficiency in the AMF plots (up to 50% higher biomass per unit of soil water) reflects the ability of these fusions to modify plant water relations and maximize biomass expression under water-limited conditions. To a lesser extent, and still provoking visible benefits the EMF injection proved that the selection of fungal functional group should be consistent with restoration goals, plant species, and site conditions. Our results indicate that in drought prone environments, particular application of mycorrhizal inoculation incorporated into restoration strategies can enhance plant establishment, improve survival rates, and accelerate ecosystem recovery. Despite the need for long-term monitoring to determine persistence of fungal symbioses and community-level outcomes, future study should develop inoculum composition, dosing tactics, and deployment timing so as to optimise field efficacy. This work provides a scientifically frames how to use the native soil microbial diversity by bridging the controlled-environment perspectives with in situ perspectives to enhance restoral practices towards more resilient, and sustainable ecosystems in light of increasing climatic stress.

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