Arbuscular Mycorrhizal Fungi Inoculation as a Climate Adaptation Strategy for Establishment of Swietenia macrophylla King. Seedlings

Lakshmy J. Rajan 1,*, Santhoshkumar A. V. 2, Surendra Gopal K. 3 and Kunhamu T. K. 4

1 Institute for Tropical Forestry & Forest Products, Technische Universität Dresden, 01737 Dresden, Germany
2 Department of Forest Biology & Tree Improvement, College of Forestry, Kerala Agricultural University, Thrissur 680656, India; santhoshkumar.av@kau.in
3 Department of Agricultural Microbiology, College of Horticulture, Kerala Agricultural University, Thrissur 680656, India; gopsurendra@gmail.com
4 Department of Silviculture and Agroforestry, College of Forestry, Kerala Agricultural University, Thrissur 680656, India; kunhamu.tk@kau.in
* Correspondence: lakshmy.jalaja_rajan@mailbox.tu-dresden.de

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Abstract: Research Highlights: Drought stress significantly decreased the performance of seedlings in the nursery. Seedlings inoculated with *Claroideoglomus etunicatum* is recommended to produce superior planting stock of mahogany seedlings with better drought resistance in the nursery. Background and Objectives: With numerous intense droughts across tropical regions due to climate change, it is crucial to understand effects of drought stress on tree seedlings to improve crop management practices and avoid failures on large scale planting. *Swietenia macrophylla*, a commercial timber species in India, is poorly studied in relation to its management including physiological responses to various environmental stresses. Arbuscular mycorrhizal fungi (AMF) is known to improve performance of tree seedlings under drought conditions and produce quality planting stock in nursery. This study aims to understand the responses of mahogany seedlings under different levels of drought stress when inoculated with three types of AMF, namely *Funneliformis mosseae*, *Claroideoglomus etunicatum*, and *Rhizophagus intraradices*. Materials and Methods: The experiment is conducted in pot culture using a factorial completely randomized design. Different irrigation regimes were applied at 100, 80, 60, and 40 percent of weekly cumulative evapotranspiration. The seedlings were tested for biometric, physiological, and mycorrhizal parameters periodically. Results: Physiological attributes such as rate of photosynthesis, stomatal conductance, transpiration rate, chlorophyll content, and water potential were found to be higher in the daily irrigated (control) seedlings. Performance of the seedlings were poorest in the least irrigated treatment. It was apparent that inoculated seedlings performed better than the non-inoculated ones. Conclusions: Among the three different AMF species used, *C. etunicatum* was found to be the most beneficial and suitable for the young mahogany seedlings. These seedlings also recorded higher root colonization percentage and total spore count in the rhizosphere soils. Seedlings inoculated with *C. etunicatum* showed positive influence on rate of photosynthesis, stomatal conductance, transpiration rate, chlorophyll content, relative growth rate (RGR) and water potential of seedlings.

Keywords: drought stress; arbuscular mycorrhizal fungi; *Swietenia macrophylla*; rate of photosynthesis; stomatal conductance; transpiration rate; leaf temperature; chlorophyll content; plant water status
1. Introduction

Changing environmental conditions induced by climate change create abiotic stress in tree seedlings which has detrimental effect on its growth and productivity. Global climate change models have predicted frequent and intense drought across the tropical regions [1]. Drought induced stress is a major limiting factor that hinders the successful establishment of tree seedlings [2,3] and reduce their chance of survival into the next growing season. Generally, tree seedlings in their first year of planting lack extensive and deep root systems. This root system is not adequate to access the receding soil water levels created by drought conditions [4]. Various genetic, silvicultural, and management strategies could be employed for improving the adaptive capacity of these seedlings to drought stress [5]. One such strategy that could increase the rate of seedling survival would be planting nursery grown seedlings inoculated with suitable arbuscular mycorrhizal fungi (AMF) [6]. It is widely reported that AMF can adjust to different soil water regimes and endure extreme habitats [7]. Also, there is considerable evidences to suggest that AMF symbiosis can enhance the tolerance of associated host plants to water deficit [8].

Drought hinders growth and induces mortality in seedlings thereby selecting plants with improved tolerance [2,9]. Modifications at biochemical, physiological and morphological levels allow plants to avoid or increase their tolerance towards the stress [10,11]. Such modifications can be done through the symbiosis of plant roots with AMF. The mutualistic relationship of AMF is seen in around 80% of the terrestrial plants [12]. The AMF hyphae multiply and grow beyond the root [13] and helps to increase nutrient acquisition. It enters root cortical cells and forms a structure called arbuscule that acts as a mediator for the exchange of metabolites between fungus and host cytoplasm. Hence root colonization by AMF is directly associated to better nutrient and water uptake, increased chlorophyll content, increased rate of photosynthesis, and transpiration [14–17] which improves plant’s resilience towards drought conditions. Moreover, AMF plays an important role in maintaining soil structure, which is vital to all ecosystems [18]. The AMF increases tangling and meshing of soil particles by plant roots and root hair [19]. They modify soil structure by (1) growing their external hyphae that extends into the soil to create a skeletal structure that holds soil particles together; (2) creating conditions that are beneficial for the development of micro-aggregates (3) forming macro aggregates by enmeshment of micro aggregates and (4) directly acquiring the carbon resources of the plant [20]. Inoculation experiments have proved that AMF inoculated plants are better immune to crop pests and diseases, especially soil-borne fungal diseases [21]. Rather than total inhibition, a reduction in the severity of diseases is observed due to AMF colonization. This increased resistance may be correlated to enhanced nutrition, along with many other mechanisms operating simultaneously [22].

Big leaf mahogany (Swietenia macrophylla) with its high economic value as an industrial timber species and high adaptability to various environmental conditions [23], is highly preferred for plantation establishment and restoration in India. Being an exotic, it is less studied in relation to its management including AMF symbiosis and physiological responses to various abiotic stresses in Indian conditions. About twenty-three AMF species belonging to genera Glomus, Acaulospora, Gigaspora, and Rhizophagus were found to form symbiosis with mahogany trees growing in its natural habitat [24]. However, no such documentation is available for the species in its introduced habitat of tropical India. A better understanding of suitable native AMF strains and their effect on the host seedlings can help the foresters to effectively utilize them in rehabilitation, restoration, and afforestation activities in a sustainable manner. This study aims to understand the effect of various levels of water stress on the physiology of S. macrophylla seedlings and investigate the efficiency of three native AMF strains in alleviating the drought stress.
2. Materials and Methods

2.1. Experimental Layout

The experiment was conducted in a polyhouse from March to August 2018 at Kerala Agricultural University (10°31’54” N latitude and 76°16’42” E longitude), Thrissur located in southern part of India at an altitude of 22.5 m above mean sea level with warm humid tropical climate. The area receives average annual precipitation of 3025 mm, with a bimodal pattern with major share of the rains received during June to August. Mean monthly maximum temperature ranged from 28 to 36.7 °C and mean monthly minimum temperature from 22.2 to 25.1 °C (Agrometeorological observatory, KAU) during the study period.

The experiment was laid out in a completely randomized design with two factors. Four different levels of irrigation set at 100, 80, 60, and 40 percentage of weekly cumulative evapotranspiration were used as first factor with daily irrigation as a control. Treatments under second factor consists of three different strains of AMF viz; *Funneliformis mosseae*, *Claroideoglomus etunicatum*, and *Rhizophagus intraradices*; and non-inoculum as a control. The three AMF strains were selected after a preliminary screening experiment to select most adapted species to the local edaphic conditions. Thus, the experiment had 20 treatment combinations, each of which was replicated four times with 30 seedlings per replication. Observations were taken at two intervals viz., 90 days corresponding to normal time of selling of seedlings by Indian nurseries and 180 days corresponding to normal planting age in field.

2.2. Mass Multiplication of AMF

Vermipaste based pure cultures of *Funneliformis mosseae*, *Claroideoglomus etunicatum*, and *Rhizophagus intraradices* having a spore count of 1000 spores in 100 g were obtained from TERI (The Energy Research Institute, New Delhi). Mass multiplication was done in grow bags filled with autoclaved sterile vermiculite as a medium and *Zea mays* as host. The plants were irrigated daily with sterile water and each plant was provided with 50 mL of Hoagland’s solution every 10 days [25]. After 45 days, the maize roots were tested for colonization using clearing and staining method using Tryphan blue stain [26]. When colonization percentage reached 80% or above, the shoots of maize plants were removed. The root portion along with vermiculite was mixed up thoroughly to obtain inoculum and stored in 4 °C for inoculating mahogany seedlings [27].

2.3. Preparation of Seedlings and Inoculation of AMF

Mahogany seeds obtained locally were sown in nursery beds containing solarized soil treated with 5% formaldehyde solution for 20 days [27]. Solarization was done by covering with white translucent polyethylene sheet for 8 days [28]. The germinated seedlings were then transplanted into polybags of 30 cm × 15 cm dimension, containing solarized and 5% formaldehyde treated potting mixture of soil, sand, and farmyard manure at 1:1:1 proportion. The potting mixture in each polybag was then inoculated with 50 grams of vermiculite mixture containing mass multiplicated AMF. The poly bags were maintained in polyhouse.

2.4. Irrigation Scheduling

The evaporation inside the polyhouse was estimated using a Piche evaporimeter [29] which was calibrated against the evaporation data obtained from the local Agrometeorological observatory situated 700 m away. The crop evapotranspiration (ETcrop) was estimated as a product of reference evapotranspiration (ETo) estimated from Piche evaporimeter and crop coefficient (Kc) as per the FAO’s modified Penman Monteith procedure [30]. Weekly cumulative evapotranspiration (ET) was then calculated using the available crop evapotranspiration data. Various irrigation regimes (Table 1) were then fixed as in relation to weekly cumulative evaporation and irrigated at weekly intervals. The plants received irrigation water (IW) as per the different treatments (IW/ET = 1, IW/ET = 0.8, IW/ET = 0.6 and IW/ET = 0.4) on a weekly basis and was not provided with any irrigation in the intervening days.
The plants were thus exposed to the increased drought stress till the next scheduled weekly irrigation. A treatment with daily irrigation was maintained as control.

### Table 1. Different levels of irrigation applied as treatments. IW: irrigation water; ET: evapotranspiration.

| Treatment Code | Treatment Description |
|----------------|-----------------------|
| Control        | Treatment with daily irrigation ad abundantium |
| IW/ET = 1      | Treatment with irrigation at 100% weekly cumulative ET applied once weekly |
| IW/ET = 0.8    | Treatment with irrigation at 80% weekly cumulative ET applied once weekly |
| IW/ET = 0.6    | Treatment with irrigation at 60% weekly cumulative ET applied once weekly |
| IW/ET = 0.4    | Treatment with irrigation at 40% weekly cumulative ET applied once weekly |

### 2.5. Parameters Measured

All parameters were measured at 90 and 180 days after inoculation. Rate of photosynthesis, stomatal conductance, rate of transpiration and leaf temperature were measured using infra-red gas analyzer (LI 6400 Portable photosynthesis system, LI-COR, Lincoln, NE, USA). The chlorophyll content of the seedlings was recorded using a chlorophyll meter (SPAD-502, Minolta, Japan) from selected three mature leaves from the second whorl [31]. The plant water status console [32] was used for measuring the water potential. Relative growth rate [33] was estimated at 90 and 180 days through destructive sampling.

- **Root colonization percentage**

  Mycorrhizal colonization in roots was determined [26] and percentage of root colonization was estimated using gridline intersect method [34]. Fine roots were randomly selected, washed with distilled water and cut into bits of one-centimeter length. The root bits were fixed with FAA (formaldehyde:acetic acid:alcohol) solution for 24 h. These were then treated with 10 percentage KOH solution and cleared by boiling at 90 °C for 60 min. The KOH solution was decanted, and excessive KOH neutralized with two percentage hydrochloric acid for 10 min. The root segments were then stained with 0.05 percent trypan blue in lactophenol. This mixture was gently heated at 80 °C for 10 min. Ten such bits of stained roots amounting to a total length of 10 cm were then arranged on a clean slide and covered with cover slips. The root bits are then examined under a compound microscope for the presence of mycelium, vesicles and arbuscules. The AMF colonization percentage was calculated from the formula (1).

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  \text{AMF colonization percentage} = \frac{\text{(Number of root bits of positive AMF colonization)}}{\text{(Total number of root bits observed)}} \times 100 \tag{1}
  \]

- **Total spore count**

  The extramatrical chlamydospores produced by the AMF were estimated using wet sieving and decanting technique at 90 and 180 days [35]. Twenty-five grams of the freshly collected soil samples from each poly bag was suspended with about 250 mL of tap water in a plastic beaker. Soil macro-aggregates were crushed with hands and thoroughly stirred. The suspension after settling down was then passed through a series of sieves ranging from 600 µm, 300 µm, 212 µm, 150 µm, 105 µm, and 45 µm kept one below the other in the order. The contents of sieve were washed down to lower sieves using a jet of water and top sieve removed. The contents of the 105 µm and 45 µm sieves were transferred to a Whatman No.1 filter paper placed in Petri dish and observed under stereo microscope to estimate the spore count.
2.6. Analysis of Data

Data were analyzed using the factorial ANOVA with four levels of irrigation and a control as first factor. Three strains of AMF and non-inoculated seedlings as control were used as the second factor. The data was tested for normality and transformed wherever necessary. The means were separated using Duncan Multiple Range Test. The statistical software SPSS 20.0 was used for analysis including transformation.

3. Results

3.1. Physiological Parameters

3.1.1. Rate of Photosynthesis

Different AMF species and irrigation regimes significantly influenced photosynthetic rate of mahogany seedlings at 90 days (Table 2). The seedlings treated with *R. intraradices* (1.44 μ mol CO₂ m⁻² s⁻¹) and *C. etunicatum* (1.29 μ mol CO₂ m⁻² s⁻¹) showed higher rate of photosynthesis compared to non-inoculated seedlings. Lowest rates were recorded for seedlings inoculated with *F. mosseae* (1.16 μ mol CO₂ m⁻² s⁻¹) and non-inoculated control (1.04 μ mol CO₂ m⁻² s⁻¹). However, these differences in photosynthetic rate disappeared at 180 days (Table 3). Among the irrigation treatments, IW/ET = 1 and IW/ET = 0.8 had higher photosynthetic rates (1.59 μ mol CO₂ m⁻² s⁻¹ and 1.58 μ mol CO₂ m⁻² s⁻¹ respectively) at 90 days; while, IW/ET = 0.4 had lowest value (0.78 μ mol CO₂ m⁻² s⁻¹). At 180 days, highest photosynthesis (2.98 μ mol CO₂ m⁻² s⁻¹) was recorded for treatment IW/ET = 0.8 and the lowest for IW/ET = 0.6 (1.40 μ mol CO₂ m⁻² s⁻¹) and IW/ET = 0.4 (1.12 μ mol CO₂ m⁻² s⁻¹).

3.1.2. Stomatal Conductance

No significant difference in stomatal conductance was observed between different AMF treatments after 90 days. However, at 180 days, seedlings treated with *C. etunicatum* showed the highest (0.033 mol H₂O m⁻² s⁻¹) stomatal conductance. The treatments IW/ET = 1 (0.11 mol H₂O m⁻² s⁻¹) and IW/ET = 0.8 (0.13 mol H₂O m⁻² s⁻¹) had higher conductance at 90 days. At 180 days IW/ET = 1 continued showing highest stomatal conductance (0.04 mol H₂O m⁻² s⁻¹) value.

3.1.3. Rate of Transpiration

At 90 days, seedlings inoculated with *C. etunicatum* (2.61 mmol H₂O m⁻² s⁻¹) recorded lowest transpiration rate. Non-inoculated control and seedlings inoculated with *F. mosseae* had higher transpiration loss. However, at 180 days, *C. etunicatum* recorded the highest transpiration (1.06 mmol H₂O m⁻² s⁻¹) respectively while the non-inoculated seedlings had lowest values (0.87 mmol H₂O m⁻² s⁻¹) (Table 3). At 90 days, IW/ET = 1 (3.10 mmol H₂O m⁻² s⁻¹), IW/ET = 0.8 (3.21 mmol H₂O m⁻² s⁻¹) and control treatments (3.22 mmol H₂O m⁻² s⁻¹) had higher values while, IW/ET = 0.6 (2.64 mmol H₂O m⁻² s⁻¹) and IW/ET = 0.4 (2.43 mmol H₂O m⁻² s⁻¹) recorded lower values. The treatment IW/ET = 0.8 had highest transpiration (1.11 mmol H₂O m⁻² s⁻¹) after 180 days while IW/ET = 0.6 and IW/ET = 0.4 recorded the least (0.90 mmol H₂O m⁻² s⁻¹ and 0.86 mmol H₂O m⁻² s⁻¹). Generally, lower rates of transpiration were observed at 180 days when compared to 90 days after inoculation. This might be pointing to improved drought tolerance of seedlings.
Table 2. Performance of mahogany seedlings as influenced by arbuscular mycorrhizal fungi (AMF) and irrigation at 90 days after the application of treatments.

| Factors | Treatments | Rate of Photosynthesis (µmol CO₂ m⁻² s⁻¹) | Stomatal Conductance (µmol H₂O m⁻² s⁻¹) | Rate of Transpiration (mmol H₂O m⁻² s⁻¹) | Leaf Temperature (°C) | Chlorophyll Content (SPAD Units) | Water Potential (MPa) | Relative Growth Rate (g g⁻¹ day⁻¹) |
|---------|------------|---------------------------------------------|----------------------------------------|----------------------------------------|------------------------|-------------------------------|----------------------|-------------------------------|
| AMF     | Non inoculated (±SE) | 1.04 c (±87 × 10⁻²) | 0.07 (±82 × 10⁻⁴) | 3.16 a (±90 × 10⁻³) | 39.05 a (±76 × 10⁻²) | 31.36 c (±13 × 10⁻¹) | −1.87 c (±15 × 10⁻¹) | 0.003 c (±80 × 10⁻⁵) |
|         | R. intraradices (±SE) | 1.44 a (±10 × 10⁻²) | 0.08 (±10 × 10⁻³) | 2.88 b (±14 × 10⁻²) | 37.62 ab (±90 × 10⁻²) | 35.38 b (±14 × 10⁻¹) | −1.76 b (±13 × 10⁻¹) | 0.006 b (±82 × 10⁻⁵) |
|         | C. etunicatum (±SE) | 1.29 ab (±93 × 10⁻³) | 0.08 (±87 × 10⁻⁴) | 2.61 c (±14 × 10⁻²) | 35.19 c (±10 × 10⁻¹) | 38.57 a (±15 × 10⁻¹) | −1.65 a (±15 × 10⁻¹) | 0.006 b (±62 × 10⁻⁵) |
|         | E. mossae (±SE) | 1.16 bc (±98 × 10⁻³) | 0.09 (±84 × 10⁻⁴) | 3.02 ab (±64 × 10⁻³) | 36.56 bc (±99 × 10⁻²) | 33.82 b (±14 × 10⁻¹) | −1.85 c (±15 × 10⁻¹) | 0.004 ab (±50 × 10⁻⁵) |
|         | Control (±SE) | 1.12 b (±11 × 10⁻²) | 0.06 b (±82 × 10⁻⁴) | 3.22 a (±10 × 10⁻²) | 32.61 c (±11 × 10⁻¹) | 45.06 a (±13 × 10⁻¹) | −0.91 a (±37 × 10⁻²) | 0.005 b (±59 × 10⁻⁵) |
|         | IW/ET = 1 (±SE) | 1.59 a (±11 × 10⁻²) | 0.11 a (±12 × 10⁻³) | 3.10 a (±12 × 10⁻²) | 34.82 b (±11 × 10⁻¹) | 41.04 b (±14 × 10⁻¹) | −1.31 b (±25 × 10⁻²) | 0.002 c (±38 × 10⁻⁵) |
|         | IW/ET = 0.8 (±SE) | 1.58 a (±10 × 10⁻²) | 0.13 a (±11 × 10⁻³) | 3.21 a (±94 × 10⁻³) | 40.30 a (±69 × 10⁻²) | 37.31 c (±11 × 10⁻¹) | −1.83 c (±19 × 10⁻²) | 0.008 a (±10 × 10⁻⁴) |
|         | IW/ET = 0.6 (±SE) | 1.09 b (±87 × 10⁻³) | 0.06 b (±68 × 10⁻⁴) | 2.64 b (±11 × 10⁻²) | 36.66 b (±10 × 10⁻¹) | 27.31 d (±73 × 10⁻²) | −2.27 d (±50 × 10⁻²) | 0.005 b (±91 × 10⁻⁵) |
|         | IW/ET = 0.4 (±SE) | 0.78 c (±83 × 10⁻³) | 0.04 c (±22 × 10⁻⁴) | 2.43 b (±16 × 10⁻²) | 41.17 a (±65 × 10⁻²) | 23.19 e (±35 × 10⁻²) | −2.62 e (±32 × 10⁻²) | 0.003 bc (±69 × 10⁻⁵) |
| Irrigation | F value | 17.19 * | 23.38 * | 17.19 * | 21.24 * | 106.86 * | 9.44 * | 9.74 * |
|         | SEM (Irrigation) | ± 84 × 10⁻³ | ± 8 × 10⁻³ | ± 88 × 10⁻³ | ± 78 × 10⁻² | ± 89 × 10⁻² | ± 22 × 10⁻² | ± 1 × 10⁻³ |
| AMF × Irrigation | F value | 8.22 * | 7.75 * | 17.34 * | 8.86 * | 5.37 * | 4.11 * | 4.82 * |
|         | SEM (Interaction) | ± 17 × 10⁻² | ± 15 × 10⁻³ | ± 17 × 10⁻² | ± 16 × 10⁻¹ | ± 18 × 10⁻¹ | ± 45 × 10⁻² | ± 1 × 10⁻³ |

* Significant at 0.05 levels; ns—nonsignificant at 0.05 levels. Values with similar superscript along the column do not differ significantly.
Table 3. Performance of mahogany seedlings as influenced by AMF and irrigation at 180 days after the application of treatments.

| Factors   | Treatments          | Rate of Photosynthesis (μmol CO₂ m⁻² s⁻¹) | Stomatal Conductance (mol H₂O m⁻² s⁻¹) | Rate of Transpiration (mmol H₂O m⁻² s⁻¹) | Leaf Temperature (°C) | Chlorophyll Content (SPAD Units) | Water Potential (MPa) | Relative Growth Rate (g g⁻¹ day⁻¹) |
|-----------|---------------------|------------------------------------------|---------------------------------------|------------------------------------------|-----------------------|----------------------------------|------------------------|-------------------------------|
| AMF       | Non inoculated (±SE) | 1.60 (±11 × 10⁻²)                        | 0.02 b (±11 × 10⁻⁴)                   | 0.87 d (±23 × 10⁻³)                      | 42.67 a (±32 × 10⁻²)  | 35.44 b (±16 × 10⁻¹)             | -2.15 c (±14 × 10⁻¹)     | 0.001 c (±48 × 10⁻⁵)             |
|           | R. intraradices (±SE) | 1.68 (±16 × 10⁻²)                        | 0.02 b (±98 × 10⁻⁵)                   | 0.94 c (±26 × 10⁻³)                      | 42.89 a (±38 × 10⁻²)  | 38.45 ab (±15 × 10⁻¹)             | -1.94 b (±14 × 10⁻¹)     | 0.004 b (±42 × 10⁻⁵)             |
|           | C. etunicatum (±SE)  | 2.00 (±21 × 10⁻²)                        | 0.03 a (±73 × 10⁻⁴)                   | 1.06 a (±38 × 10⁻³)                      | 41.07 b (±82 × 10⁻²)  | 40.21 a (±14 × 10⁻¹)             | -1.75 a (±15 × 10⁻¹)     | 0.006 a (±54 × 10⁻⁵)             |
|           | F. mossae (±SE)      | 1.84 (±11 × 10⁻²)                        | 0.02 b (±77 × 10⁻⁵)                   | 0.99 b (±22 × 10⁻³)                      | 43.11 a (±31 × 10⁻²)  | 36.46 b (±18 × 10⁻¹)             | -1.99 b (±14 × 10⁻¹)     | 0.001 c (±62 × 10⁻⁵)             |
|           | F value              | 2.41 ns                                  | 9.16 *                                | 19.93 *                                  | 9.04 *                | 4.13 *                          | 43.61 *                 | 41.31 *                        |
| Irrigation | Control (±SE)        | 1.50 c (±95 × 10⁻⁷)                      | 0.02 b (±95 × 10⁻⁷)                   | 1.03 b (±32 × 10⁻³)                      | 41.79 b (±35 × 10⁻²)  | 48.6 a (±13 × 10⁻¹)              | -1.16 a (±39 × 10⁻²)     | 0.003 b (±38 × 10⁻⁵)             |
|           | IW/ET = 1 (±SE)      | 1.92 b (±18 × 10⁻²)                      | 0.04 a (±90 × 10⁻⁷)                   | 0.90 cd (±25 × 10⁻³)                     | 39.39 c (±97 × 10⁻²)  | 45.16 b (±15 × 10⁻¹)             | -1.45 b (±41 × 10⁻²)     | 0.003 b (±56 × 10⁻⁵)             |
|           | IW/ET = 0.8 (±SE)    | 2.98 a (±21 × 10⁻²)                      | 0.01 b (±36 × 10⁻⁷)                   | 1.11 a (±43 × 10⁻³)                      | 43.21 a (±11 × 10⁻²)  | 39.38 c (±16 × 10⁻²)             | -2.00 c (±70 × 10⁻²)     | 0.004 ab (±77 × 10⁻⁵)            |
|           | IW/ET = 0.6 (±SE)    | 1.40 cd (±11 × 10⁻²)                     | 0.01 b (±78 × 10⁻⁴)                   | 0.94 c (±25 × 10⁻³)                      | 43.96 a (±31 × 10⁻²)  | 30.07 d (±12 × 10⁻¹)             | -2.35 d (±51 × 10⁻²)     | 0.005 a (±77 × 10⁻⁵)             |
|           | IW/ET = 0.4 (±SE)    | 1.12 d (±82 × 10⁻³)                      | 0.02 b (±14 × 10⁻⁴)                   | 0.86 d (±28 × 10⁻³)                      | 43.82 ab (±42 × 10⁻⁵) | 24.98 e (±36 × 10⁻²)             | -2.81 e (±28 × 10⁻²)     | 0.001 c (±73 × 10⁻⁵)             |
|           | F value              | 32.21 *                                  | 13.04 *                               | 24.79 *                                  | 30.64 *               | 72.65 *                         | 614.59 *                | 11.63 *                        |
|           | SEM (Irrigation)     | ± 13 × 10⁻²                              | ± 3 × 10⁻³                            | ± 20 × 10⁻³                              | ± 34 × 10⁻²           | ± 12 × 10⁻¹                     | ± 27 × 10⁻²             | ± 1 × 10⁻³                   |
| AMF ×     | F value              | 7.26 *                                   | 13.73 *                               | 21.14 *                                  | 23.96 *               | 4.15 *                          | 3.61 *                 | 3.11 *                        |
| Irrigation| SEM (Interaction)    | ± 26 × 10⁻²                              | ± 6 × 10⁻²                            | ± 40 × 10⁻²                              | ± 69 × 10⁻²           | ± 23 × 10⁻¹                     | ± 56 × 10⁻²             | ± 1 × 10⁻³                   |

* Significant at 0.05 levels; ns—nonsignificant at 0.05 levels. Values with similar superscript along the column do not differ significantly.
3.1.4. Leaf Temperature

At 90 and 180 days of treatment, seedlings inoculated with *C. etunicatum* had lowest leaf temperatures (35.19 °C and 41.07 °C). Between different irrigation treatments, lowest leaf temperatures were recorded by control seedlings (32.61 °C) at 90 days and treatment IW/ET = 1 at 180 days (39.39 °C).

3.1.5. Chlorophyll Content

Highest chlorophyll content was recorded from seedlings inoculated with *C. etunicatum* both at 90 (38.57 SPAD units) and 180 (40.21 SPAD units) days. Non inoculated seedlings recorded the lowest chlorophyll content (31.36 SPAD units) at 90 days after inoculation. With respect to irrigation regimes, control seedlings displayed higher chlorophyll content; and treatment IW/ET = 0.4 maintained lower chlorophyll content.

3.1.6. Plant Water Status

Both 90 and 180 days recorded highest water potential in seedlings inoculated with *C. etunicatum* had highest value (−1.65 MPa and −1.75 MPa). Similarly, the lowest values were recorded for non-inoculated seedlings (−1.87 MPa and −2.15 MPa). Seedlings inoculated with *F. mosseae* (−1.77 MPa) also showed lower water potential at 90 days. Water potential showed a steady decline with increasing drought stress in seedlings.

3.1.7. Relative Growth Rate (RGR)

Highest relative growth rate was recorded by seedlings inoculated with *R. intraradices* (0.006 g·g⁻¹·day⁻¹) 90 days after inoculation. At 90 and 180 days, seedlings inoculated with *C. etunicatum* had higher RGR (0.006 g·g⁻¹·day⁻¹ and 0.006 g·g⁻¹·day⁻¹ respectively) when compared to seedlings inoculated with other AMF strains. Non inoculated seedlings maintained lower RGR consistently at 90 and 180 days. On par with this, seedlings inoculated with *F. mosseae* (0.001 g·g⁻¹·day⁻¹) also had lower RGR at 180 days. At 90 days, IW/ET = 0.8 had highest RGR (0.008 g·g⁻¹·day⁻¹); whereas, lowest values were recorded by the treatment IW/ET = 1 (0.002). The treatment IW/ET = 0.8 and IW/ET = 0.6 had higher RGR at 180 days (0.004 g·g⁻¹·day⁻¹ and 0.005 g·g⁻¹·day⁻¹ respectively) while IW/ET = 0.4 recorded the lowest (0.001 g·g⁻¹·day⁻¹). The non-inoculated seedlings most frequently exhibited lower RGR values except at 90 days.

3.2. Mycorrhizal Parameters

3.2.1. Root Colonization Percentage

The root colonization percentage was calculated at 90 and 180 days of the experiment. At both periods, highest colonization percentage was observed for *C. etunicatum* (45.48% and 44.89% respectively) and the lowest was observed for non-inoculated seedlings (Table 4). With respect to various irrigation regimes, the root colonization percentage generally decreased with increasing levels of drought stress. The irrigation treatment IW/ET = 1 had highest colonization percentage (40.59%) while, the lower values were recorded by IW/ET = 0.6 (17.22% and 17.49%) and IW/ET = 0.4 (13.34% and 12.95%) respectively, at 90 and 180 days. The interaction effect of different AMF strains and irrigation regimes were not significant at 90 and 180 days. Higher root colonization percentage were observed for well-watered and moderately stressed seedings when treated with AMF.
Table 4. Mycorrhizal parameters of mahogany seedlings under different irrigation treatments.

| Factors      | Treatments                  | Root Colonization Percentage (%) | Total Spore Count |
|--------------|-----------------------------|----------------------------------|-------------------|
|              |                             | 90 Days | 180 Days | 90 Days | 180 Days |
| AMF          | *R. intraradices* (±SE)     | 35.07 b | 32.68 b  | 114.8 a | 64.00 b |
|              | *C. etunicatum* (±SE)       | 45.48 a | 44.89 a  | 82.05 a | 85.55 a |
|              | *F. mosseae* (±SE)          | 27.13 c | 27.38 b  | 50.60 ab| 54.30 b |
| F value      |                             | 147.24 *| 91.64 *  | 3.70 *  | 95.20 * |
| SEM (AMF)    |                             | (±1.60) | (± 1.98) | (± 3.19) (± 3.73) |
| Irrigation   | Control (±SE)               | 29.65 b | 26.04 c  | 52.81 (± 9.61) | 49.81 c |
|              | IW/ET = 1 (±SE)             | 40.59 a | 42.11 a  | 138.12 (± 12.37) | 79.87 a |
|              | IW/ET = 0.8 (±SE)           | 33.80 b | 32.59 b  | 60.75 (± 10.19) | 64.81 b |
|              | IW/ET = 0.6 (±SE)           | 17.22 c | 17.49 d  | 31.44 (± 6.99) | 33.50 d |
|              | IW/ET = 0.4 (±SE)           | 13.34 c | 12.95 d  | 26.19 (± 5.22) | 26.81 d |
| F value      |                             | 40.47 * | 27.78 *  | 2.51 ns  | 27.55 * |
| SEM (Irrigation) |                         | (± 1.79) | (± 2.21) | (± 3.57) | (± 4.17) |
| AMF x Irrigation |                        | F value | 5.86 *   | 4.13 *   | 1.27 *   | 4.54 *   |
| SEM (Interaction) |                    | (± 3.58) | (± 4.43) | (± 7.14) | (± 8.34) |

* Significant at 0.05 levels; ns—non significant at 0.05 levels. Values with similar superscript along the column do not differ significantly.
3.2.2. Total Spore Count

Total spore count under different treatments had significant variations at 90 and 180 days (Table 4). Generally, the spore count decreased with increasing levels of drought stress. At 90 days, high total spore count was observed for both R. intraradices (114.8) and C. etunicatum (82.05). C. etunicatum recorded the highest total spore count at 180 days (85.55). Among the irrigation regimes, no significant variations were observed for spore count at 90 days. At 180 days, however, IW/ET = 1 recorded the highest spore count (79.87), while the control had the lowest spore count (49.81).

4. Discussion

Among the various physiological, biochemical, and molecular processes that controls plant growth [36], photosynthesis is the fundamental process that provides organic blocks that contributes substantially to the plant growth and development. However, stressful environments such as drought can hinder photosynthesis in most seedlings by changing the ultrastructure of organelles and thereby inducing stomatal or non-stomatal limitations [37]. A moderate drought stress is reported to impede stomatal conductance and thereby leaf photosynthesis and stomatal conductance in most tree seedlings [38]. From the observations, it was apparent that increasing levels of drought stress decreased photosynthetic rate; the rate of decrease being slower in seedlings inoculated with AMF. This point to an increased capacity of gas exchange caused due to AMF colonization by reducing the resistance at stomatal level and improving transpiration fluxes [39].

The present investigation indicated that stomatal conductance was higher on the AMF inoculated seedlings. Stomatal closure has been recognized as an immediate reaction of plants towards drought stress [40]. Stomatal control happens either due to a decrease in leaf turgor or plant water status [41]. Inoculation with AMF can regulate the opening and closing of stomata through hormonal signals and thereby control the gaseous exchange, imparting better drought stress tolerance. Mycorrhizal seedlings showing increased photosynthetic rate than non-mycorrhizal seedlings due to decreased stomatal resistance is well documented [42]. Mycorrhizal seedlings showing increased photosynthetic rate than non-mycorrhizal seedlings due to decreased stomatal resistance is well demonstrated [42].

Symbiosis with AMF also benefits the plants through improvement in absorption of soil water and nutrients through the development of external hyphae increasing the rate of photosynthetic assimilation and export, enhancing the conductivity of roots and regulating the concentration of cellular osmolytes [8,43–45]. Under water stress, plants tend to negotiate between CO₂ uptake and transpiration water loss [42]. The results shows that AMF inoculated seedlings were less stressed than non-inoculated ones which could be related to a combination of increased water uptake by mycorrhizal roots, high soil-to-plant hydraulic conductance and improved stomatal conductance in response to high water potential [30,42,46]. The findings are on par with [47], who noted that transpiration rate of black locust seedlings was inhibited by drought stress and colonization with AMF greatly improved it in well-watered and mild drought stress treatment but not for severe drought stress treatment, but not in severe stress treatment.

Leaf temperature can be an early indicator of plants in drought stress [3]. It is widely accepted that temperature influences leaf stomatal conductance [48]. The results show comparatively high rates of transpiration and improved stomatal conductance in seedlings inoculated with AMF. Improved transpiration leads to evaporative cooling of leaf surface, which brings down the surface temperature of leaves. Hence, low leaf temperature can be considered as an indirect measure of transpiration rate. However, the relationship between stomatal conductance, transpiration and leaf temperature can also be conflicting in nature.

Previous studies states that leaf temperature and stomatal conductance can be either directly, inversely or neutrally related [49–51]. In the present study, the nature of the relationship between photosynthesis, stomatal conductance, transpiration, and leaf temperature were confirmed through correlation analysis. The analysis showed positive correlation of photosynthesis with transpiration and stomatal conductance at 90 days. Correlation between stomatal conductance and photosynthetic
capacity has been observed in many studies [52,53]. However, at 180 days, analysis showed no correlation between photosynthesis and stomatal conductance. This is similar to the finding of [54] who reported that stomatal conductivity of seedlings under resource constrained conditions cannot be determined by the photosynthetic capacity of guard cells or the leaf mesophyll directly. This indicates to the possibility that in seedlings with more developed root systems, under resource constraint in the form of limited area to expand as in a poly bag, AMF may not afford any advantage.

The correlation analysis also showed negative correlation between leaf temperature and transpiration rate consistently. It was noted that transpirational cooling of leaves decreased leaf temperature by 9 °C in well-watered and by 1 °C in drought-stressed poplar and loblolly pine [41]. However, this mechanism is only possible with enough hydraulic conductivity. In the present study, leaf temperature increased with increasing levels of drought stress following a decreasing trend transpiration rates due to the reduced water availability. Seedlings inoculated with C. etunicatum had significantly lower leaf temperature than the non-inoculated seedlings. This may be due to high rate of transpiration and stomatal conductance for the seedlings inoculated with C. etunicatum, a fact which is substantiated earlier in this study (Table 3).

Chlorophyll content is an important tool for evaluating plant photosynthetic efficiency and effect of environmental stress [55]. Conversely, plastids which contains chloroplast are extremely sensitive water deficit and other stressful conditions. Water deficit can impose severe damage to both photosynthetic pigments and thylakoid membrane; thereby decreasing the photosynthetic efficiency of plants under water stress [56]. Hence, chlorophyll content has a premier role in the modulation of plant responses to various stresses [57,58]. The chlorophyll content is generally found to be decreasing with increased drought stress [59,60] The outcomes of the present study are also in agreement to these finding. Moreover, chlorophyll content in seedlings of Albizia lebbeck and Cassia siamea was found to be less under water stress [61]. Results shows similarity to [62,63] who found increased chlorophyll concentrations in AMF inoculated seedlings subjected to drought stress.

Plant water status provides direct evidence for physiological and biochemical changes in seedlings. Experimental evidence prove that reduction in soil water can considerably lower plant water potential [64]. As substantiated above, AMF colonization can improve water potential of host plant through improved stomatal conductivity, root absorptivity and cellular osmotic adjustments. Higher water potential of AMF inoculated plants would thereby help to increase photosynthetic assimilation and in turn meet the carbon needs of fungal symbiont [65].

This experiment shows reduced growth rate of seedlings with increasing levels of drought stress. Moreover, seedlings inoculated with C. etunicatum and R. intraradices, displayed relatively higher RGR under water stressed conditions. There are reports which correlate difference in RGR to dry matter partitioning. They explain reduction in RGR can be attributed to reduced allocation of biomass to leaves, the site of photosynthesis [66]. Cell division being a turgor driven process is easily affected by drought. Significant reduction in RGR consequent to drought stress was observed in the mahogany [42] and teak seedlings [3].

It is important to select the AMF species that can adapt well to the conditions on which plants are grown. Different AMF isolates vary in their ability to colonize roots, proliferate, and produce hyphae that can help in mineral acquisition [67,68]. Several factors such as host-fungus compatibility, AMF isolate and soil environmental conditions determine the efficiency of AMF—root symbiosis [68]. Therefore it is imperative to have a better understanding about these factors for effectively manipulating AMF strains to optimize the growth of plants. In this study, the total spore count of C. etunicatum was consistently higher across all the irrigation levels indicating its better suitability and adaptedness to its host and local conditions.

Microscopic assessment of the AMF in the polybags suggests that increasing drought stress brought a significant decline in percentage of AMF colonization and spore abundance especially for F. mosseae and R. intraradices. Interestingly, the percentage of AMF colonization was more adversely affected by severe stress than moderate stress. It was also noted that the daily irrigated AMF inoculated seedlings
(least stressed) had lower colonization percentage compared to those in mild stress. This finding agrees with [42] who reported that drought stress in mild form can significantly improve the root colonization of AMF.

Reports from similar studies suggest significantly higher percentage of root colonization by *G. claroideum* in well-irrigated seedlings compared to water stressed ones [67]. While screening different AMF for their symbiotic efficiency with *Tectona grandis*, [68] observed that a significantly higher percentage of teak roots were colonized with *G. margarita*. However, spore numbers were highest in soil samples inoculated with *G. leptotichum*, indicating the better proliferating ability of this AMF with teak as the host. Hence, it can be suggested that root colonization percentage is not always directed proportional to its spore abundance. The improved growth and performance of the seedlings and AMF isolates when inoculated with *C. etunicatum* therefore shows better compatibility with mahogany as its host. It also demonstrates the potential of mycorrhizal inoculation in reducing the adverse effects of drought stress in the seedlings.

5. Conclusions

A significant reduction in the performance of mahogany seedlings was observed with the increasing levels of drought stress. *R. intraradices* and *C. etunicatum* showed better suitability with mahogany seedlings during study. Among these two AMF species, *C. etunicatum* is recommended to produce superior planting stock of mahogany seedlings in the nursery; as parameters such as rate of photosynthesis, stomatal conductance, transpiration rate, chlorophyll content, RGR and water potential were found to be positively influenced in these seedlings. Under the threats of climate change, a better understanding of the effects of abiotic stress on plant responses and environmentally friendly remedies will help nursery managers and foresters to avoid large scale failures on different planting program. The insight on mechanisms involved in plant responses to drought stress and ability of AMF as a biofertilizer to improve their adaptive capacity can lead to effective utilization of AMF in forestry.

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**References**

1. Williams, J.W.; Jackson, S.T.; Kutzbach, J.E. Projected distributions of novel and disappearing climates by 2100 AD. *Proc. Natl. Acad. Sci. USA* 2007, 104, 5738–5742. [CrossRef] [PubMed]
2. Engelbrecht, B.M.J.; Kursar, T.A. Comparative drought-resistance of seedlings of 28 species of co-occurring tropical woody plants. *Oecologia* 2003, 136, 383–393. [CrossRef] [PubMed]
3. Sneha, C.; Santhoshkumar, A.V.; Sunil, K.M. Quantifying water stress using crop water stress index in mahogany (Swietenia macrophylla King) seedlings. *Curr. Sci.* 2013, 104, 348–351.
4. Mahari, A. Factors Affecting Survival of Tree Seedlings in the Drylands of Northern Ethiopia. Available online: https://www.semanticscholar.org/paper/Factors-Affecting-Survival-of-Tree-Seedlings-in-the-Mahari/0c9891093a560cee79c71f9261fe636f499ceea (accessed on 16 April 2020).
5. Lamb, D. Restoration of Degraded Tropical Forest Landscapes. *Science* 2005, 310, 1628–1632. [CrossRef]
6. Dar, M.H.; Reshi, Z.A. Vesicular Arbuscular Mycorrhizal (VAM) fungi- as a major biocontrol agent in modern sustainable agriculture system. *Russ. Agric. Sci.* 2017, 43, 138–143. [CrossRef]
7. Mosse, B.; Stribley, D.P.; LeTacon, F. Ecology of Mycorrhizae and Mycorrhizal Fungi. *Adv. Microb. Ecol.* 1981, 137–210. [CrossRef]

8. Augé, R.M. Arbuscular mycorrhizae and soil/plant water relations. *Can. J. Soil Sci.* 2004, 84, 373–381. [CrossRef]

9. Breneres-Arguedas, T.; Roddy, A.B.; Coley, P.D.; Kursar, T.A. Do differences in understory light contribute to species distributions along a tropical rainfall gradient? *Oecologia* 2010, 166, 443–456. [CrossRef]

10. Bray, E.A. Plant responses to water deficit. *Trends Plant Sci.* 1997, 2, 48–54. [CrossRef]

11. Rahimzadeh, S.; Pirzad, A. Arbuscular mycorrhizal fungi and Pseudomonas in reduce drought stress damage in flax (*Linum usitatissimum* L.): A field study. *Mycorrhiza* 2017, 27, 537–552. [CrossRef]

12. Giovannetti, M.; Avio, L.; Fortuna, P.; Pellegrino, E.; Sbrana, C.; Strani, P. At the Root of the Wood Wide Web. *Plant Soil*. 2006, 1, 1–5. [CrossRef] [PubMed]

13. Marschner, H.; Dell, B. Nutrient uptake in mycorrhizal symbiosis. *Plant Soil* 1994, 159, 89–102. [CrossRef]

14. Peng, S.; Esselstyn, D.M.; Graham, J.H.; Williams, K.; Hodge, N.C. Growth Depression in Mycorrhizal Citrus at High-Phosphorus Supply (Analysis of Carbon Costs). *Plant Physiol.* 1993, 101, 1063–1071. [CrossRef] [PubMed]

15. Mathur, N.; Vyas, A. Influence of arbuscular mycorrhizal on biomass production, nutrient uptake and physiological changes in *Ziziphus mauritiana* Lam. under water stress. *J. Arid Environ.* 2000, 45, 191–195. [CrossRef]

16. Begum, N.; Ahanger, M.A.; Su, Y.; Lei, Y.; Mustafa, N.S.A.; Ahmad, P.; Zhang, L. Improved Drought Tolerance by AMF Inoculation in Maize (*Zea mays*) Involves Physiological and Biochemical Implications. *Plants* 2019, 8, 579. [CrossRef]

17. Begum, N.; Chuan, C.; Ahanger, M.A.; Raza, S.; Khan, M.I.; Ashraf, M.; Ahmed, N.; Zhang, L. Role of Arbuscular Mycorrhizal Fungi in Plant Growth Regulation: Implications in Abiotic Stress Tolerance? *Front. Plant Sci.* 2019, 10. [CrossRef]

18. Ryan, M.H.; Graham, J.H. Is there a role for arbuscular mycorrhizal fungi in production agriculture. In *Diversity and Integration in Mycorrhizas*; Smith, S.E., Smith, F.A., Eds.; Springer: Dordrecht, The Netherlands, 2002; pp. 263–271. ISBN 978-90-481-5933-8.

19. Rillig, M.C.; Mummey, D.L. Mycorrhizas and soil structure. *New Phytol.* 2006, 171, 41–53. [CrossRef]

20. Miller, R.M.; Jastrow, J.D. Mycorrhizal Fungi Influence Soil Structure. In *Arbuscular Mycorrhizas: Physiology and Function*; Kapulnik, Y., Douds, D.D., Eds.; Springer: Dordrecht, The Netherlands, 2000; pp. 3–18. ISBN 978-94-017-0776-3.

21. Borowicz, V.A. Do Arbuscular Mycorrhizal Fungi Alter Plant–Pathogen Relations? *Ecology* 2001, 82, 3057–3068. [CrossRef]

22. Karagiannidis, N.; Bletsos, F.; Stavropoulos, N. Effect of Verticillium wilt (*Verticillium dahliae* Kleb.) and mycorrhiza (*Glomus mosseae*) on root colonization, growth and nutrient uptake in tomato and eggplant seedlings. *Sci. Hortic.* 2002, 94, 145–156. [CrossRef]

23. Larekeng, S.H.; Restu, M.; Arsyad, M.A. Mutia Observation of morphological and physiological characteristics on Abangares Mahogany (*Swietenia macrophylla* King) In South Sulawesi. *IOP Conf. Ser. Earth Environ. Sci.* 2019, 270, 012022. [CrossRef]

24. Rodríguez-Morelos, V.H.; Soto-Estrada, A.; Pérez-Moreno, J.; Franco-Ramírez, A.; Díaz-Rivera, P. Arbuscular mycorrhizal fungi associated with the rhizosphere of seedlings and mature trees of *Swietenia macrophylla* (Magnoliophyta: Meliaceae) in Los Tuxtlas, Veracruz, Mexico. *Rev. Chil. Hist. Nat.* 2014, 87. [CrossRef]

25. Hoagland, D.R.; Arnon, D.I. The water-culture method for growing plants without soil. *Circ. Calif. Agric. Exp. Stn.* 1950, 347, 32.

26. Phillips, J.; Hayman, D.S. Improved procedure for declaring and staining parasitic and VAM fungi for rapid assessment of infection. *Trans. Br. Mycol Soc.* 1970, 55, 158–161. [CrossRef]

27. Ajeesh, R.; Santhoshkumar, A.; Gopal, S.; Binu, N. Screening of selected native arbuscular mycorrhizal fungi at different levels for their symbiotic efficiency with *tectona grandis* seedlings. *J. Trop. For. Sci.* 2017, 29, 395–403.

28. Sharma, M.P.; Gaur, A.; Bhatia, N.P.; Adholeya, A. Growth responses and dependence of Acacia nilotica var. cupriciformis on the indigenous arbuscular mycorrhizal consortium of a marginal wasteland soil. *Mycorrhiza* 1996, 6, 441–446. [CrossRef]
29. Domuță, C.; Cărbunar, M.; Sandor, M.; Borza, I.; Brejea, R.; Domuță, C.; Gîtea, M.; Vușcan, A.; Oneț, C. Researches regarding the use of the piche evaporimeter in the irrigation scheduling of the tomatoes’ solarium crops. *Analele Univ. Din Oradea Fasc. Protectia Mediu.* 2011, 16, 229–234.

30. Allen, M.F. Influence of vesicular-arbuscular mycorrhizae on water movement through bouteloua gracilis (h.b.k.) lag ex steud*. *New Phytol.* 1982, 91, 191–196. [CrossRef]

31. Takebe, M.; Yoneyama, T. Measurement of leaf color scores and its implication to nitrogen nutrition of rice plants. *Ipn. Agric. Res. Q.* 1989, 23, 86–93.

32. Scholander, P.F.; Bradstreet, E.D.; Hemmingsen, E.A.; Hammel, H.T. Sap Pressure in Vascular Plants: Negative hydrostatic pressure can be measured in plants. *Science* 1965, 148, 339–346. [CrossRef]

33. Williams, R.F. The Physiology of Plant Growth with Special Reference to the Concept of Net Assimilation Rate. *Ann. Bot.* 1946, 10, 41–72. [CrossRef]

34. Giovannetti, M.; Mosse, B. An Evaluation of Techniques for Measuring Vesicular Arbuscular Mycorrhizal Infection in Roots. *New Phytol.* 1980, 84, 489–500. [CrossRef]

35. Gerdemann, J.W.; Nicolson, T.H. Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. *Trans. Br. Mycol. Soc.* 1963, 46, 235–244. [CrossRef]

36. Rapparini, F.; Peñuelas, J. Mycorrhizal Fungi to Alleviate Drought Stress on Plant Growth. In *Use of Microbes for the Alleviation of Soil Stresses*, Volume 1; Miransari, M., Ed.; Springer: New York, NY, USA, 2014; pp. 21–42. ISBN 978-1-4614-9466-9.

37. Rahnama, A.; Poustini, K.; Tavakkol-Afshari, R.; Tavakoli, A. Growth and Stomatal Responses of Bread Wheat Genotypes in Tolerance to Salt stress. *Int. J. Biol. Life Sci.* 2010, 7, 216–221.

38. Medrano, H. Regulation of Photosynthesis of C3 Plants in Response to Progressive Drought: Stomatal Conductance as a Reference Parameter. *Ann. Bot.* 2002, 89, 895–905. [CrossRef] [PubMed]

39. Zhu, X.-C.; Song, F.-B.; Liu, S.-Q.; Liu, T.-D. Effects of arbuscular mycorrhizal fungus on photosynthesis and water status of maize under high temperature stress. *Plant Soil* 2011, 346, 189–199. [CrossRef]

40. Cornic, G.; Massacci, A. Leaf Photosynthesis under Drought Stress. In *Photosynthesis and the Environment*; Springer: Dordrecht, The Netherlands, 1996; pp. 347–366. [CrossRef]

41. Ludlow, M.M. Adaptive significance of stomatal responses to water stress. *Adapt. Signif. Stomatal Responses to Water Stress* 1980, 123–138.

42. Augé, R.M. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza* 2001, 11, 3–42. [CrossRef]

43. Subramanian, K.S.; Charest, C. Nutritional, growth, and reproductive responses of maize (*Zea mays* L.) to arbuscular mycorrhizal inoculation during and after drought stress at tasselling. *Mycorrhiza* 1997, 7, 25–32. [CrossRef]

44. Aroca, R.; Vernieri, P.; Ruiz-Lozano, J.M. Mycorrhizal and non-mycorrhizal Lactuca sativa plants exhibit contrasting responses to exogenous ABA during drought stress and recovery. *J. Exp. Bot.* 2008, 59, 2029–2041. [CrossRef]

45. Wu, Q.-S.; Xia, R.-X. Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions. *J. Plant Physiol.* 2006, 163, 417–425. [CrossRef]

46. Augé, R.M.; Schekel, K.A.; Wample, R.L. Osmotic Adjustment in Leaves of VA Mycorrhizal and Nonmycorrhizal Rose Plants in Response to Drought Stress. *Plant Physiol.* 1986, 82, 765–770. [CrossRef] [PubMed]

47. Yang, Y.; Tang, M.; Sulpice, R.; Chen, H.; Tian, S.; Ban, Y. Arbuscular Mycorrhizal Fungi Alter Fractal Dimension Characteristics of Robinia pseudoacacia L. Seedlings Through Regulating Plant Growth, Leaf Water Status, Photosynthesis, and Nutrient Concentration Under Drought Stress. *J. Plant Growth Regul.* 2014, 33, 612–625. [CrossRef]

48. Jones, H.G. *Plants and Microclimate*; Cambridge University Press: Cambridge, UK, 2009; ISBN 978-0-511-84572-7.

49. Mott, K.A.; Peak, D. Stomatal responses to humidity and temperature in darkness. *Plant Cell Environ.* 2010, 33, 1084–1090. [CrossRef] [PubMed]

50. Lahr, E.C.; Schade, G.W.; Crossett, C.C.; Watson, M.R. Photosynthesis and isoprene emission from trees along an urban-rural gradient in Texas. *Glob. Change Biol.* 2015, 21, 4221–4236. [CrossRef] [PubMed]
51. Cerasoli, S.; Wertin, T.; McGuire, M.A.; Rodrigues, A.; Aubrey, D.P.; Pereira, J.S.; Teskey, R.O. Poplar saplings exposed to recurring temperature shifts of different amplitude exhibit differences in leaf gas exchange and growth despite equal mean temperature. *AoB PLANTS* 2014, 6. [CrossRef]

52. Wong, S.-C.; Cowan, I.R.; Farquhar, G.D. Leaf Conductance in Relation to Rate of CO₂ Assimilation: II. Effects of Short-Term Exposures to Different Photon Flux Densities. *Plant Physiol.* 1985, 78, 826–829. [CrossRef]

53. Hetherington, A.M.; Woodward, F.I. The role of stomata in sensing and driving environmental change. *Nature* 2003, 424, 901–908. [CrossRef]

54. von Caemmerer, S.; Lawson, T.; Oxborough, K.; Baker, N.R.; Andrews, T.J.; Raines, C.A. Stomatal conductance does not correlate with photosynthetic capacity in transgenic tobacco with reduced amounts of Rubisco. *J. Exp. Bot.* 2004, 55, 1157–1166. [CrossRef]

55. Zhu, X.C.; Song, F.B.; Liu, S.Q.; Liu, T.D.; Zhou, X. Arbuscular mycorrhizae improves photosynthesis and water status of *Zea mays* L. under drought stress. *Plant Soil Environ.* 2012, 58, 186–191. [CrossRef]

56. Anjum, S.A.; Farooq, M.; Wang, L.C.; Xue, L.L.; Wang, S.G.; Wang, L.; Zhang, S.; Chen, M. Gas exchange and chlorophyll synthesis of maize cultivars are enhanced by exogenously-applied glycinebetaine under drought conditions. *Plant Soil Environ.* 2011, 57, 326–331. [CrossRef]

57. Saravanavel, R.; Ranganathan, R.; Anantharaman, P. Effect of Sodium Chloride on Photosynthetic Pigments and Photosynthetic Characteristics of *Avicennia officinalis* Seedlings. *Recent Res. Sci. Technol.* 2011, 3, 177–180.

58. Biswal, B.; Raval, M.K.; Biswal, U.C.; Joshi, P. Response of Photosynthetic Organelles to Abiotic Stress: Modulation by Sulfur Metabolism. *Sulfur Assim. Abiotic Stress Plants* 2008, 167–191. [CrossRef]

59. Din, J.; Khan, S.U.; Ali, I.; Gurmani, A.R. Physiological and agronomic response of canola varieties to drought stress. *J. Anim. Plant Sci.* 2011, 21, 78–82.

60. Saraswathi, S.G.; Paliwal, K. Drought induced changes in growth, leaf gas exchange and biomass production in *Albizia lebbeck* and *Cassia siamea* seedlings. *J. Environ. Biol.* 2011, 32, 173–178.

61. Morte, A.; Díaz, G.; Rodríguez, P.; Alarcón, J.J.; Sánchez-Blanco, M.J. Growth and Water Relations in Mycorrhizal and Nonmycorrhizal *Pinus Halepensis* Plants in Response to Drought. *Biol. Plant.* 2001, 44, 263–267. [CrossRef]

62. Sánchez-Blanco, M.J.; Ferrándiz, T.; Morales, M.A.; Morte, A.; Alarcón, J.J. Variations in water status, gas exchange, and growth in *Rosmarinus officinalis* plants infected with *Glomus deserticola* under drought conditions. *J. Plant Physiol.* 2004, 161, 675–682. [CrossRef] [PubMed]

63. Ramos, F.R.; Freire, A.L.O. Physiological responses to drought of *Cnidoscolus quercifolius* Pohl in semi-arid conditions. *Adv. For. Sci.* 2019, 6, 493–499. [CrossRef]

64. Augé, R.M.; Toler, H.D.; Sams, C.E.; Nasim, G. Hydraulic conductance and water potential gradients in squash leaves showing mycorrhiza-induced increases in stomatal conductance. *Mycorrhiza* 2008, 18, 115–121. [CrossRef]

65. Poorter, H.; Remkes, C.; Lambers, H. Carbon and Nitrogen Economy of 24 Wild Species Differing in Relative Growth Rate. *Plant Physiol.* 1990, 94, 621–627. [CrossRef]

66. Ananthakrishnan, G.; Ravikumar, R.; Girija, S.; Ganapathi, A. Selection of efficient arbuscular mycorrhizal fungi in the rhizosphere of cashew and their application in the cashew nursery. *Sci. Hortic.* 2004, 100, 369–375. [CrossRef]

67. Rajan, S.K.; Reddy, B.J.D.; Bagyaraj, D.J. Screening of arbuscular mycorrhizal fungi for their symbiotic efficiency with *Tectona grandis*. *For. Ecol. Manag.* 2000, 126, 91–95. [CrossRef]

68. Bagyaraj, D.J. 19 Vesicular-arbuscular *Mycorrhiza*: Application in Agriculture. In *Methods in Microbiology*; Norris, J.R., Read, D.J., Varma, A.K., Eds.; Academic Press: Cambridge, MA, USA, 1992; Volume 24, pp. 359–373.

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