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RESEARCH ARTICLE

Optimizing mycorrhizal spore application for effective colonization and early seedling establishment in rice

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Abstract

Arbuscular mycorrhizal (AM) fungi form a symbiotic association with plant roots, promoting plant growth and fostering resilience. However, the efficacy of this symbiotic association highly depends on the nature and abundance of spores, but there is no clear evidence regarding the relationship between the quantity of spores in the inoculum and colonization efficiency. Therefore, the present study was conducted to establish quality standards for AM inoculum and to investigate the effect of varying mycorrhization levels of *Rhizophagus intraradices* on root colonization and early seedling vigor of rice (*Oryza sativa* L., var. CO51) under semi-dry cultivation conditions. The experiment was set up with seven treatments (T1-T7), with spore inoculation ranging from 0 to 6 spores per seed, arranged in a completely randomized design (CRD) with three replications. Results evidenced a clear dose-dependent promotive effect of increasing AM spore density on germination percentage, chlorophyll content, seedling biomass and root colonization. Notably, the study also confirmed that a threshold effect of 2 or more spores per seed is required to achieve optimal germination and effective AM colonization. Furthermore, the treatments T6 and T7 (5 and 6 spores per seed respectively) recorded the highest shoot and root lengths, chlorophyll concentration and biomass accumulation throughout the observation period (30 days of plant growth). T7 also exhibited the fastest and highest level of colonization by mycorrhizal fungi, recording efficient early symbiotic establishment. However, results were not strictly linear among treatments, underscoring the significance of establishing threshold and optimal inoculum dosages rather than depending solely on higher doses. These findings highlight the potential of low-dose AM fungal seed biotization in enhancing early rice vigor and mycorrhizal colonization.

Keywords: percent mycorrhizal colonization; plant growth; seed biotization; spore density; symbiotic efficiency

Abbreviations: ANOVA - Analysis of Variance; CV - Coefficient of Variance; AMF - Arbuscular Mycorrhizal Fungi; TPF - Tri Phenyl Formazan; PVLG - Polyvinyl alcohol lactoglycerol; INVAM - International Vesicular Arbuscular Mycorrhiza culture collection; DMRT - Duncan's Multiple Range Test

Introduction

AM fungi are essential members of the soil ecosystem, forming mutualistic relationships with the roots of about 80 % of all terrestrial plants. These symbiotic interactions provide a wide range of benefits to the host plants, including enhanced plant growth and improved acquisition and absorption of nutrients (especially phosphorus and other micronutrients) through their extensive extraradical hyphae. Furthermore, AM fungi enhance plant resilience against environmental stresses such as salinity, drought and heavy metal accumulation (1). Additionally, AM fungi can also facilitate host plants' coping with various biotic stresses including phytopathogens and herbivores by triggering morphophysiological and biochemical responses such as plant phenomics, nutrition and toxicity (2-3). Moreover, AM fungi colonizing plants can more effectively utilize soil water since their extraradical mycelia enhance soil structure and soil physicochemical properties play a vital role in dry conditions (4-7). AM fungi may also aid in regulating stomatal conductance in the host plant during drought stress (8). Several research studies have demonstrated that inoculating cereals like rice, maize, wheat and barley with AM fungi often boosts plant growth and resilience in adverse environments (2, 9).

O. sativa, one of the world's most important food crops, can be grown widely as a lowland crop across the state. Particularly in drought-prone areas, rice can be cultivated in semi-dry conditions with moderate availability of water. In such methods, direct seeding is recommended with the efficient use of rainwater while minimizing the irrigation by up to 40 % (10). Rice roots are capable of forming symbiotic associations with AM fungi, both native and introduced. These partnerships can help rice plants grow stronger roots, absorb more nutrients, water and better withstand environmental stress. Further, this relationship also tends to reshape the root system, making it more branched and efficient during water deficit conditions (11-12). Despite the potential advantages of AM fungal bio-inoculant in rice production being well established, spore

quantity, viability, delivery mechanism and soil conditions continue to affect the practical use and quality of AM fungal inoculant production for rice cultivation. Furthermore, the efficiency of AM fungi colonization in plant roots is highly influenced by spore viability and quantity (13).

With this information, it is hypothesized that optimizing the threshold level of spore quantity for seed treatment is crucial to ensure effective colonization and to maximize the plant growth-promoting effects of AM fungi. More importantly, determining the optimal spore count and threshold level is a critical step for enhancing the practical application of AM fungi in rice cultivation. Thus, the present study aimed to standardize and evaluate the different spore quantities and to identify the threshold spore level required for effective colonization. Furthermore, the study assesses how varying spore inoculum levels affect root colonization, plant growth and early seedling establishment of rice plants under semi-dry conditions, thereby elucidating the potential benefits of AM fungi in enhancing plant performance under water-limited environments. Rice is used as a model crop to evaluate the impact of optimal spore dosage, aiming to improve the efficiency and consistency of AM fungal inoculants and make them more reliable tools for promoting sustainable rice farming, especially in water-deficit conditions.

Materials and Methods

Seed material and mycorrhizal spores

The rice cultivar CO 51 (an early-maturing variety with a duration of 105-110 days and moderately drought-tolerant) was used for this study. The AM fungal species used for inoculation was *R. intraradices* RMS6, a native species isolated from rice rhizosphere soils of Ramanathapuram district by the wet sieving and decanting method (14). Preliminary screening showed that *R. intraradices* exhibited maximum root colonization (60 %) in maize, outperforming other native AM fungal species in terms of infection or colonization potential. The spores were propagated using maize as a trap crop and stored under sterile conditions until they were used for experimentation.

Seed biotization with mycorrhizal spores

Seeds of the rice variety CO 51 were surface-sterilized using 0.1 % mercuric chloride for 2-3 min, followed by thorough rinsing with sterile distilled water thrice to eliminate surface contaminants. The surface-sterilized seeds were then shade-dried and subsequently coated with the AM fungal formulation, which included mycogel/sticker containing *R. intraradices* spores.

The AM fungal formulation was prepared by incorporating 1 to 6 spores per seed, with the recommended field application dose of 1 lakh spores per acre. To facilitate uniform coating and enhance spore adherence, a mycogel-based sticker was used during seed biotization (15). After coating, the seeds were shade-dried for 45 min to ensure proper adhesion of the inoculum. The presence and uniform distribution of spores on the seed surface were confirmed through microscopic examination (Fig. 1). The treated seeds were then used for direct sowing in pots after 3 days of germination under semi-dry conditions.

Mycorrhizal seed biotization

Assessing inoculum requirements using a scaled pot experiment

The pot experiment was conducted in a net house at the Department of Agricultural Microbiology, Tamil Nadu Agricultural University (TNAU), Coimbatore (11° N latitude, 77° E longitude and altitude: 426.7 m AMSL). Field soil (soil texture: clay loam, pH: 8.2, EC : 0.7 dS/m, Clay: 36.2 %, Silt: 21.7 %, Sand: 40.2 %) from Thondamuthoor was taken for the experiment and autoclaved by intermittent sterilization. The study followed a CRD using the CO 51 rice variety grown in clay loam soil. The treatments were structured based on incremental levels of *R. intraradices* spore density per rice seed, as follows: T_1 - Control (no AM fungal spores), T_2 - 1 spore per seed, T_3 - 2 spores per seed, T_4 - 3 spores per seed, T_5 - 4 spores per seed, T_6 - 5 spores per seed and T_7 - 6 spores per seed, each replicated three times. This experimental design was formulated by the field-level recommendation of 1 lakh spores per acre, aiming to determine the optimal spore density for seed biotization under semi-dry cultivation conditions. To achieve uniform mycorrhizal colonization, AM fungal inoculum was applied at a field-equivalent rate of 1 lakh spores per acre. Based on an assumed average topsoil weight of 1000000 kg per acre, the inoculum requirement was proportionally scaled for 5 kg pot experiments, corresponding to approximately one spore per seed. To ensure successful establishment, a practical inoculum dose of 1 - 6 spores per seed was applied (15).

In vitro germination assay

Two experimental setups were maintained for the *in vitro* germination assay, viz. from experimental setup 1, germination percentage was recorded on the 5th day after sowing (DAS). Germination was recorded when the radicle of the seeds extended a minimum of 2 mm in length. Germination was expressed as %.

$$\text{Germination percentage (\%)} = \frac{\text{Number of germinated seeds}}{\text{Number of seeds kept for germination}} \times 100$$

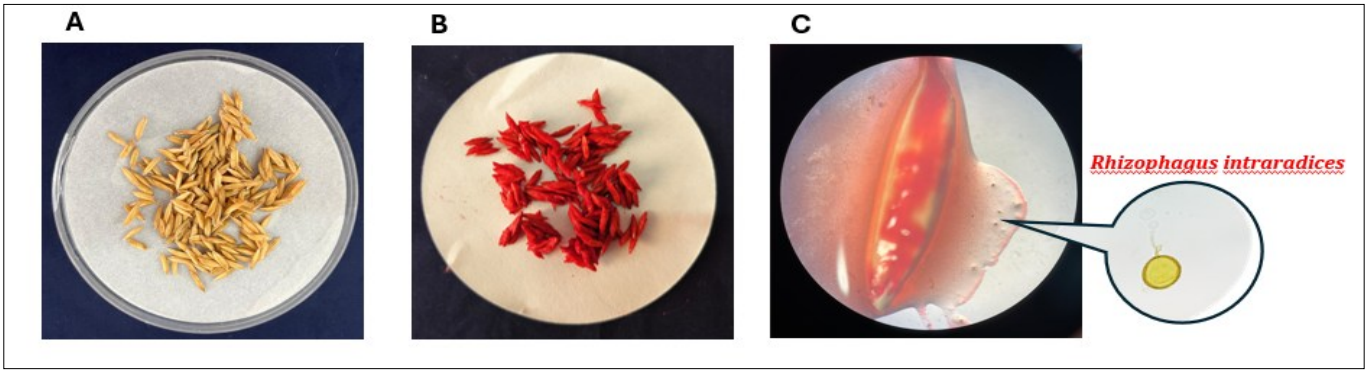

Fig. 1. Seed biotization with AM spores a) Surface sterilized rice seeds; b) Mycogel with AM spore-coated seeds; c) Microscopic image of AM spores adhering to rice seed at 40x magnification.

The uniformly germinated seeds obtained from experimental setup 2 were transferred to the pot experiment with appropriate treatments respectively. The pots were maintained under a greenhouse used for further experiments.

Assessment of mycorrhizal colonization in rice roots

Mycorrhizal colonization in rice roots was analyzed on the 7th, 15th and 30th DAS to assess the mycorrhizal infection across treatments. Root samples were collected from treated and non-inoculated plants. The mycorrhizal root colonization was determined using the trypan blue staining technique (16).

Only on the 7th day, root bits from a single plant were collected and checked for mycorrhizal colonization. On the 15th and 30th DAS, approximately 30 root segments, each measuring 1 cm in length were randomly collected from each sample. The root bits were cleared, stained with 0.05 % trypan blue in lactoglycerol and mounted on slides for observation. The stained root samples were examined under a stereo-zoom microscope at 5x magnification to detect the presence of fungal structures including hyphae, arbuscules and vesicles. The percentage of root colonization was calculated based on the number of infected root segments out of the total observed.

$$\% \text{ AM colonization} = \frac{\text{Total number of AM positive segments}}{\text{Total number of root segments observed}} \times 100$$

Evaluation of plant growth parameters

The germinated AM-coated rice seedlings were planted in 5 kg capacity pots filled with sterilized clay soil. Seedlings were thinned to maintain three uniform plants per pot, ensuring equal spacing. All pots were uniformly saturated and maintained under semi-dry conditions in a net house. The experiment was conducted over 30 days to evaluate the responses of AM colonization and plant growth. At 7th, 15th and 30th days after germination, plants from each treatment were carefully uprooted and analyzed for shoot length (cm plant^{-1}) and root length (cm plant^{-1}).

Plant biomass

Fresh weight: On the 7th, 15th and 30th day, the root and shoot portions of each plant were carefully separated. The roots were gently washed under running tap water to remove adhering soil particles without damaging delicate root structures. Both the root and shoot samples were then placed between folded filter papers and gently pressed to remove excess surface moisture. The fresh weight of each plant part was immediately recorded using a precision electronic balance and expressed as g per plant.

Dry weight: The uprooted rice plants were initially sun-dried for one day to remove surface moisture. Subsequently, the samples were transferred to a hot air oven and dried at a temperature of 60 °C-70 °C for three consecutive days until a constant dry weight was achieved. The dry biomass of each plant was then recorded using a precision balance and expressed as g per plant.

Root volume

Root volume was determined using the volume displacement method (17). A graduated measuring cylinder of appropriate size was filled with water to a known volume and the initial water level was recorded. The root sample was carefully dried and then submerged in a measuring cylinder filled with water. The rise in water level was recorded to determine the root volume. The difference between the initial and final water level was calculated as the root volume, expressed in cubic centimeters per plant (cm^3 plant^{-1}).

$$\text{Root Volume}(cm^3) = \text{Final volume} - \text{Initial volume}$$

Estimation of chlorophyll content

Chlorophyll content was assessed in rice leaves at the 7th, 15th and 30th day (18). About 0.1 g of fresh leaf tissue was ground and mixed with 5 mL of 80 % acetone. The mixture was centrifuged at 3000 rpm for 10 min. The extraction process was repeated several times until all pigments were fully extracted. The resulting liquid was pooled and the volume was adjusted to 10 mL using 80 % acetone. Using a digital spectrophotometer (Systronics® Spectrophotometer 166), the absorbance of the solution was measured at 665 nm, with 80 % acetone as the blank. The chlorophyll content was then calculated and expressed as milligrams per gram (mg g^{-1}) of fresh leaf weight.

$$\text{Chlorophyll content (mg g}^{-1}) = \frac{20.2 \times OD_{645} + 8.02 \times OD_{663}}{25}$$

Statistical analysis

The dataset was subjected to a two-way analysis of variance and means were separated using Duncan's multiple range test (DMRT) at a 0.05 probability level. Means were separated by DMRT at a 0.05 level of probability using statistical software analysis of variance. Additionally, means were separated by DMRT at a 0.05 level of probability using SPSS version 20.0. GraphPad Prism 8 was used to construct the graphs. Mean values of the treatments were compared using DMRT at $p \leq 0.05$.

Results

Germination percentage

The germination percentage was evaluated on the 5th DAS for all treatments involving varying concentrations of AM spore inoculation. On the 5th day after germination, treatments T4, T6 and T7 with two or more spores per seed achieved a 100 % germination rate. Treatments T1, T2, T3 and T5 showed slightly lower germination rate ranges from 90 % to 93.3 % (Table 1, Fig. 2).

Mycorrhizal colonization in rice roots

Table 1. Effect of AM spore abundance in rice seed germination after 5 DAS

Treatments		Germination (%) recorded on 5th day
T1	Control	90±(5.6)^b
T2	1 spore (50000 spores/acre)	90±(5.7)^b
T3	2 spore (100000 spores/acre)	93.3±(6.6)^b
T4	3 spore (150000 spores/acre)	100±(0.0)^a
T5	4 spore (200000 spores/acre)	90±(5.7)^b
T6	5 spore (250000 spores/acre)	100±(0.0)^a
T7	6 spore (300000 spores/acre)	100±(0.0)^a

T1 - T7 represents varied levels of spores from 0 to 6 spores per seed respectively. Values represent mean ± standard error. Different letters within the same column indicate statistically significant differences at $p < 0.05$ (ANOVA).

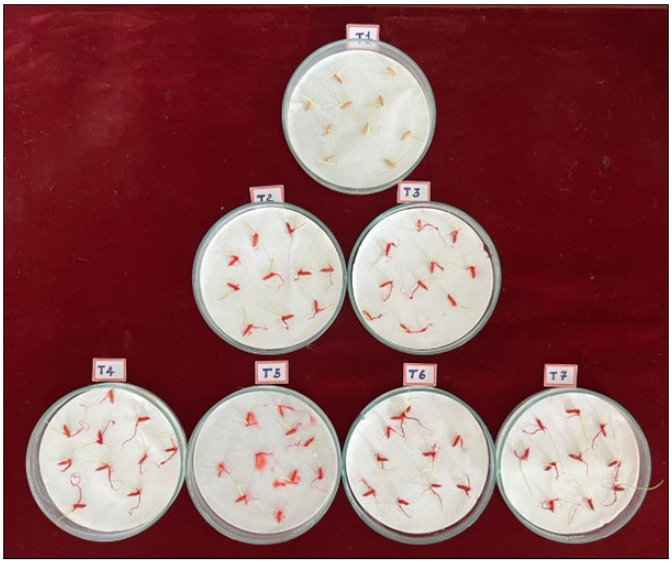

Fig. 2. Effect of AM spore abundance on rice seed germination on the 7th day. T1-T7, where T1-T7 represent varied levels of spores, ranging from 0 to 6 spores per seed respectively.

According to the root clearing and staining method, the percentage of mycorrhizal colonization was assessed. The results in Table 2 indicated a significant increase in rice root colonization when inoculated with varying levels of mycorrhiza spores. The availability of AM fungal spore richness positively influenced the percentage of mycorrhizal colonization in rice roots. The initiation of mycorrhizal infection was observed within 7 days after biotized seeds were planted. The plant was uprooted and only mycorrhizal hyphae were observed on the roots of rice. Mycorrhizal infection showed statistically significant differences among treatments at all observation intervals. On 7th day, colonization varying from 10 % (T2) to 27 % (T7), with T7 representing 2.7-fold higher colonization than T2.

By the 15th day, colonization had intensified, ranging from 23 % (T2) to 56.6 % (T7), exhibiting a 2.46-fold increase. A similar trend was observed on the 30th day, with the highest infection rate in T7 (92.2 %), which is significantly higher than T4 (70.0 %), T3 (52.0 %) and T2 (46.6 %).

T3 showed optimum growth. T4, T5, T6 and T7 recorded satisfactory growth, root volume and chlorophyll content. T4 can be taken for further development because it demonstrated better colonization and plant growth in rice (Fig. 3).

Total plant height

Seeds treated with AM fungi exhibited significantly enhanced early-stage morphological growth compared to the uninoculated control, particularly in shoot and root development (Fig. 4). The AM fungal application markedly improved plant height and leaf number. Seedling growth performance was evaluated through measurements of shoot and root lengths at 7, 15 and 30 days after sowing across seven treatment groups (T1-T7).

Shoot length

Shoot length increased progressively with time in all treatments. On the 7th day, shoot length ranged from 3.1 cm (T1) to 4.1 cm (T6 and T7). By the 15th day, T7 recorded the maximum shoot length (24.6 cm) followed by T6 (22.4 cm), while the lowest shoot length was again observed in T1 (12.4 cm). At the 30th day, T7 continued to show superior growth reaching 36.2 cm, while T1 and T2 had the shortest shoots (20.1 cm and 20.3 cm respectively) (Fig. 5a).

Root length

Similar to shoot length, root length also increased over time in all treatments. On the 7th day, T1 showed the shortest root (0.7 cm), while T6 and T7 recorded the highest (1.5 cm). By the 15th day, root lengths increased significantly, with T7 reaching 5.2 cm and T6 at 4.5 cm. On the 30th day, T7 exhibited the longest root at 10.4 cm, followed by T6 (9.5 cm), whereas T1 had the shortest root length (3.8 cm) (Fig. 5b).

Table 2. Impact of AM spore abundance on percent root colonization in rice at 7th, 15th and 30th DAS

Treatments		Mycorrhizal infection on 7th day (%)	Mycorrhizal infection on 15thday (%)	Mycorrhizal infection on 30thday (%)
T1	Control	0±(0.00)^{f}	0±(0.00)^{g}	0±(0.00)^{e}
T2	1 spore	10.0±(0.1)^{e}	23.0±(0.1)^{f}	46.6±(1.0)^{d}
T3	2 spores	10.5±(0.1)^{e}	32.5±(0.03)^{e}	52.0±(1.2)^{c}
T4	3 spores	14.0±(0.2)^{d}	39.0±(1.0)^{d}	70.0±(1.5)^{b}
T5	4 spores	15.0±(0.3)^{c}	42.0±(0.7)^{c}	89.6±(1.9)^{a}
T6	5 spores	20.0±(0.3)^{b}	50.0±(0.2)^{b}	91.0±(0.7)^{a}
T7	6 spores	27.0±(0.1)^{a}	56.6±(0.6)^{a}	92.2±(0.05)^{a}

T1 - T7 represent varied levels of spores from 0 to 6 spores per seed respectively. Values represent mean ± standard error. Different letters within the same column indicate statistically significant differences at $p < 0.05$ (ANOVA).

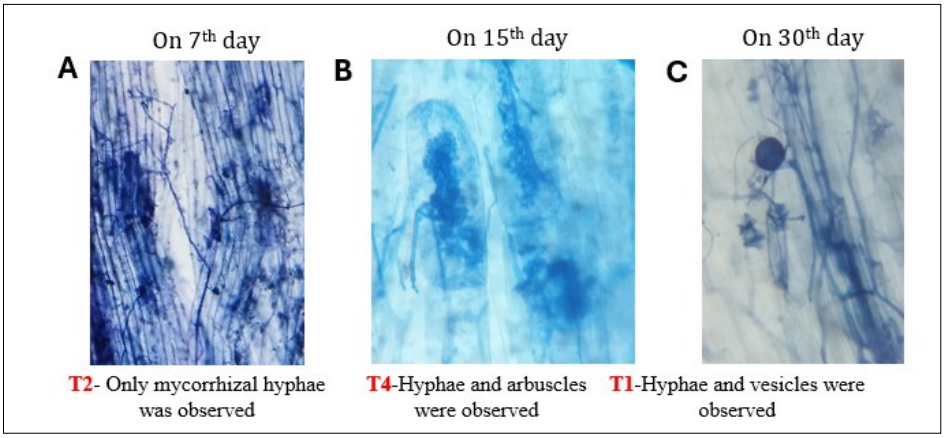

Fig. 3. Effect of AM spore abundance on root colonization of rice seedlings visualized in a compound microscope at 40x magnification. A) 7th, B) 15th and C) 30th DAS.

Fig. 4. Growth enhancement of rice by different AM spore treatments (T1-T7), where T1 - T7 represent varied levels of spores from 0 to 6 spores per seed respectively. (The photograph was taken just before harvesting, therefore, watering was done prior to capturing the image).

Plant biomass

In rice plants, the total fresh weight and total dry weight increased in AM-inoculated plants compared to the control; however, the increase was not significant. Mycorrhizal infection seems to cause a non-significant increase in fresh and dry weight at 30 days of plant growth for T5, T6 and T7.

Fresh weight

At the 7th day, fresh weight ranged from 0.3 g (T1) to 0.8 g (T7). A significant increase in fresh weight was observed by the 15th day, with T7 (2.8 g) and T6 (2.6 g) showing the highest values, while T2 recorded the lowest (0.7 g). By the 30th day, T7 had the highest fresh weight (5.0 g), followed closely by T6 (4.9 g) and T5 (4.8 g). The lowest fresh weight at 30 days was seen in T1 (1.5 g), indicating poor biomass accumulation (Fig. 5c).

Dry weight

On the 7thday, dry weight was lowest in T2 (0.01 g) and highest in T7 (0.3 g). By the 15th day, T7 maintained the highest dry weight (1.5 g), followed by T6 (1.4 g) and T5 (1.2 g). At 30 days, T5, T6 and T7 all recorded the same maximum dry weight (1.8 g), indicating a plateau in biomass accumulation. The lowest dry weight at 30 days was again noted in T1 (0.3 g) (Fig. 5d).

Root volume

The root volume of plants measured on the 30th DAS showed significant variation across the different treatments. Among all the treatments, T7 recorded the highest root volume of 2.83 cm^3, followed by T6 (2.65 cm^3) and T4 (2.43 cm^3). Treatments T5 and

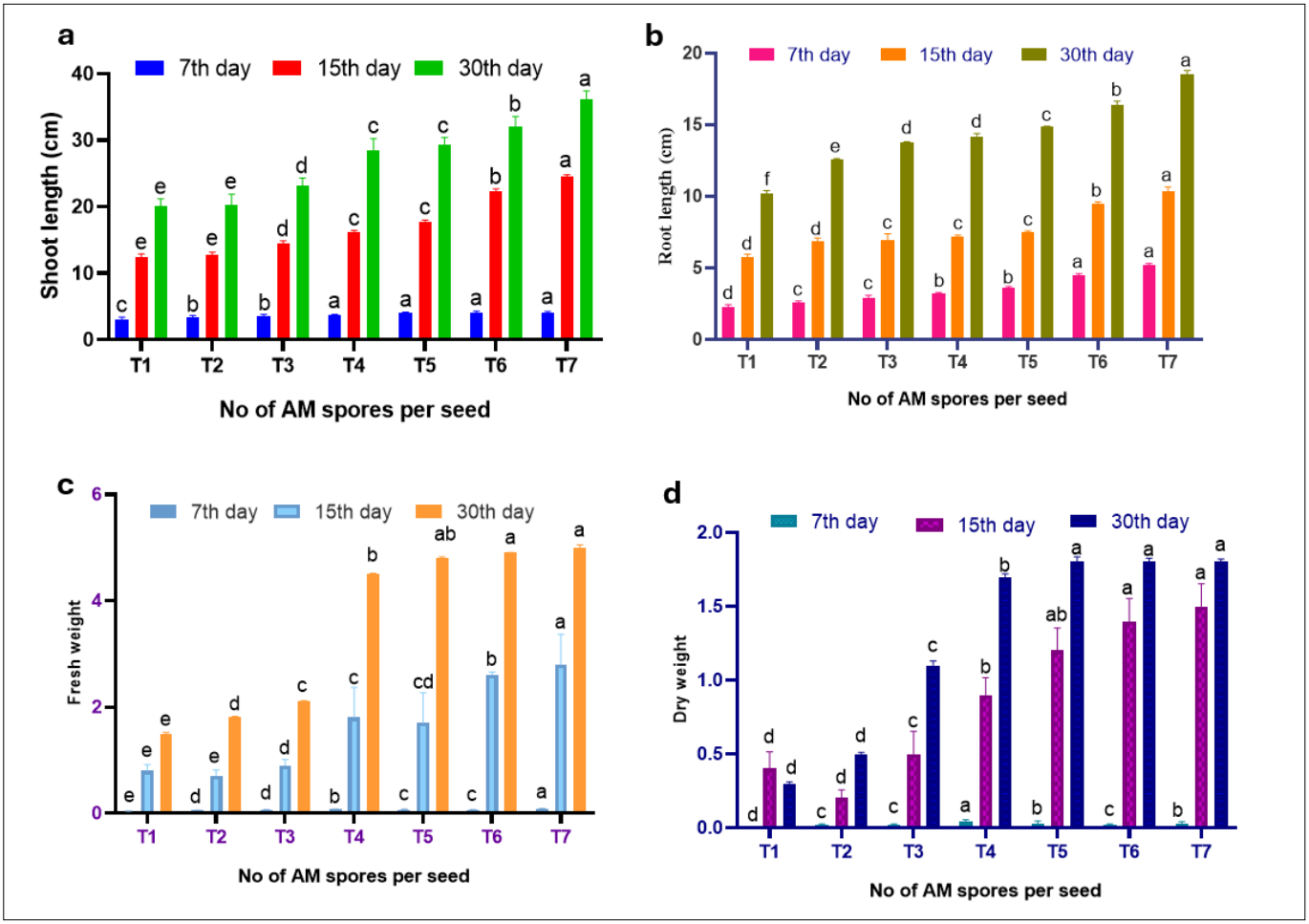

Fig. 5. Effect of AM spore abundance on shoot length, root length, fresh weight and dry weight of rice seedlings at 7, 15 and 30 DAS under different treatment conditions (T1-T7), where T1 - T7 represents varied level of spores from 0 to 6 spores per seed respectively. Error bars represent standard error (n =3) and the different letters on the same observation day are significantly different among treatments ($p \leq 0.05$).

T4 exhibited comparable root volumes (2.4 cm^3 and 2.43 cm^3 respectively), indicating moderate growth. In contrast, T1, T2 and T3 showed relatively lower root development, with volumes of 0.5 cm^3, 0.65 cm^3 and 0.7 cm^3 respectively. These results suggest that the treatments T4 to T7 were more effective in promoting root growth at the early stage of plant development compared with T1 to T3 (Table 3).

Table 3. Impact of AM spore abundance on rice root volume at 30th DAS

Treatments		Root volume (cm^3)
T1	Control	0.5±(0.01)^f
T2	1 spore	0.65±(0.03)^e
T3	2 spores	0.7±(0.01)^e
T4	3 spores	2.43±(0.03)^c
T5	4 spores	2.4±(0.04)^d
T6	5 spores	2.65±(0.03)^b
T7	6 spores	2.83±(0.01)^a

T1 - T7 represent varied levels of spores from 0 to 6 spores per seed respectively. Values represent mean ± standard error. Different letters within the same column indicate statistically significant differences at $p < 0.05$ (ANOVA).

Chlorophyll content

After seedling emergence, chlorophyll content remained relatively unchanged or showed a slight increase by day 15 across all treatments. However, a noticeable increase in total chlorophyll content was observed in AM-inoculated plants compared to the uninoculated control over the subsequent 15 days. By 35 DAS, plants inoculated with 6 AM fungal spores exhibited the highest chlorophyll content (2.35 mg/g). Inoculation with 3 to 6 spores resulted in comparable chlorophyll levels after 30 days of sowing , while the uninoculated control recorded the lowest content (0.57 mg/g). These results indicate that mycorrhizal colonization significantly enhanced chlorophyll accumulation in rice leaves.

Chlorophyll content consistently increased over time across all treatments, with varying magnitudes of increase. On 7 DAS, chlorophyll content ranged from 0.21 mg/g (T1) to 0.34 mg/g (T7), with T6 and T7 showing the highest early chlorophyll levels, suggesting enhanced physiological activity. On 15th DAS, all treatments had higher chlorophyll content, with T7 recording the highest (0.74 mg/g), followed by T6 (0.72 mg/g) and T4 (0.66 mg/g). T2 had the lowest content (0.41 mg/g). At 30 DAS, chlorophyll content peaked in all treatments, with T7 again having the highest value (2.35 mg/g), followed closely by T6 (2.34 mg/g), T5 (2.34 mg/g) and T4 (2.33 mg/g). T1 showed the lowest chlorophyll content (0.57 mg/g) throughout the study (Fig. 6).

Discussion

To integrate AM fungal inoculum into agricultural applications, it is necessary to assess the potential of the introduced AM species to thrive and compete in a new environment, as well as compete with the native strains. Further, they must be able to form a mutualistic relationship with the host plants in the natural environment. The right inoculum dose is critical for improving plant growth of several crop species (19-20). Mycorrhizal inoculum is typically developed from infected root bits and propagules obtained from pot cultures of mycorrhizal host plants and spores for commercial purposes (21). Among them, AM spores are considered a vital component of inoculum and are ideal for large-scale applications, facilitating the understanding of the biochemical and molecular interactions of AM fungi with their host.

A preliminary study was carried out to standardize the density of spores in AM fungal inoculum for effective root colonization by seed coating with mycogel using the rice variety CO 51 as the host. Six spore doses ranging from one to six spores were tested in rice in order to standardize AM fungal inoculum levels for root colonization. The current study evaluated the impact of varying AM fungal spore concentration on seedling germination in rice. The beneficial effect of AM fungi was demonstrated by 100 % germination rates in the treatments T4, T5, T6 and T7 with more than two AM spores on seeds on the 7th DAS. The germination rate was slightly lower in treatments T2 (93.3 %) and T3 (96.6 %); these findings are consistent with earlier studies by, who showed the positive impact of *Glomus intraradices* inoculation in papaya (22). Similarly increased seedling growth of cucumbers as a result of AM fungal inoculation, reinforcing the role

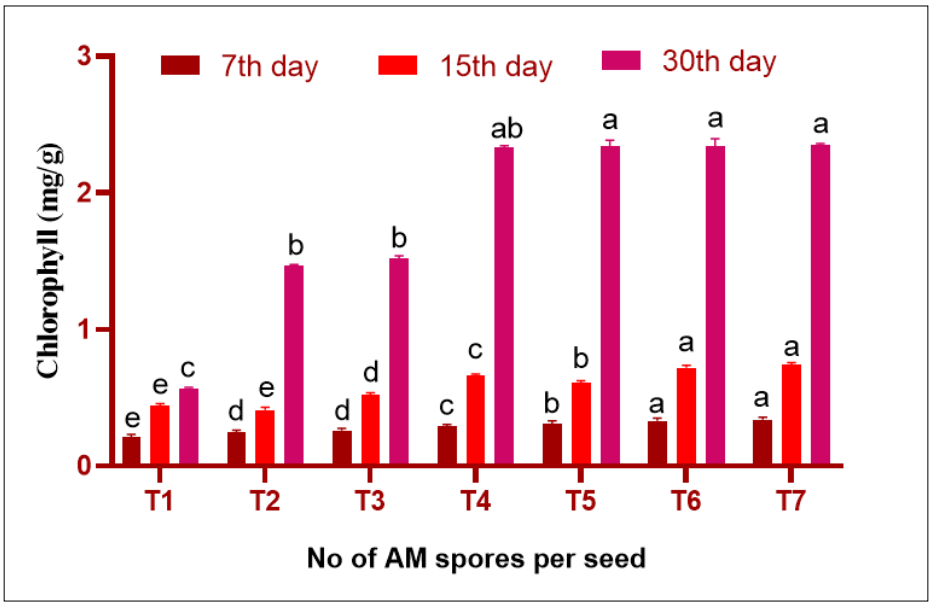

Fig. 6. Effect of AM spore abundance on chlorophyll content of rice seedlings at 7, 15 and 30 DAS under different treatment conditions (T1-T7), where T1-T7 represent varied levels of spores from 0 to 6 spores per seed respectively. Error bars represent standard error (n =3) and the different letters on the same observation day are significantly different among treatments (p ≤0.05).

of AM fungi play in early plant growth and development reported in earlier studies (23). Furthermore, earlier studies have also shown a positive correlation between the mycorrhizal colonies and increased germination. For instance, the inoculation of AM fungi increased germination in three temperate forest species (24). The inoculation of *Funneliformis mosseae* and *R. intraradices* led to increased root colonization, as well as enhanced seedling growth in rice (25).

Present study examined the effect of different doses of AM spores in semi-dry conditions on the early growth of rice. The results showed that T6 and T7 consistently outperformed other treatments in terms of promoting root and shoot growth. On 30th, DAS showed that T7 had the longest and longest shoot (36.2 cm) and the largest root (10.4 cm), followed by T6 (32.1 cm and 9.5 cm). These results showed that higher spore density can improve seedling health and mycorrhizal association. These results are in line with other evidence that AM fungi promote plant growth primarily by improving nutrient uptake, especially phosphorus. They also have expanded root systems, which facilitate better water absorption (26). In addition, AM fungi alter the phytohormones (auxin and cytokinin) signalling pathways, which aid in root and shoot development (27). The reduced performance in T1 or T2 could be attributed to the low spore concentration used. This reinforces the significance of standardizing the spore dose, as lower concentration levels might not elicit physiological responses in host plants (28).

Mycogel-coated seeds enhanced spore viability and confirmed uniform germination. The efficacy of seed biotization for AM fungal application was confirmed by the positive results recorded in the treatments T6 and T7, especially where direct inoculation procedures are impractical (29). Black-rice development was significantly enhanced by seed treatment with AM fungi, which increased nutrient uptake and metabolic activity (30). The increasing trend of dryweight biomass in treatments T5, T6 and T7 on 30 DAS indicates that these treatments have achieved their maximum biomass accumulation under existing environmental conditions. However, T7 continued to have an early advantage, indicating that high spore levels could result in more effective nutrient absorption and uptake. The AM inoculation enhanced rice growth and yield in varied farming conditions (31).

Chlorophyll is an important indicator of photosynthetic ability and the overall health of a plant. All treatments showed a steady increase in chlorophyll. On the 30th, T5, T6, T7 and T8 recorded the highest levels of chlorophyll at 2.35 mg/g [A1] respectively. This suggests that high spore doses support higher photosynthetic and physiological performance in the host plant. AM fungi are likely to be responsible for the elevated chlorophyll in T6 and T7. This is because AM fungi facilitate nutrient absorption, especially nitrogen and phosphorus. These fungi enhance nutrient uptake, which supports chlorophyll production and photosynthetic efficacy (32). A meta-analysis showed that AM fungal inoculation increases chlorophyll by about 12 % when rain is present, contributing to increased plant growth and productivity. AM fungi also help plants to withstand environmental stress by maintaining chlorophyll and photosynthetic functions even in adverse conditions, as reported in soybean (33). Likewise, AM colonization significantly improved the growth of *Opuntia indica* under limited nutrients and dry conditions (34).

During the present study, the lowest level of chlorophyll was consistently found in T1, indicating poor physiological performance, which might be due to a lack of nutrients and microbial interaction. There is a threshold of spore densities required for functional symbiosis and successful root colonization. A similar pattern has been reported in blackgram seedlings (*Vigna mungo*), where an increase in the number of AM fungus spores led to increased colonization rates and better growth (13).The present study also evidenced an increase in mycorrhizal colonization from 10 %-70 %, which is directly correlated with the plant growth. These results were consistent with the findings in black rice, showing a positive correlation between the spore level and root colonization (30). The increase in colonization was directly associated with the enhanced plant growth and nutrient uptake. These findings emphasize the significance of regulating spore densities for seed biotization to maximize germination and establish an AM symbiosis. Treatments T6 and T7 at higher spore dosages were the most effective at fostering early host-fungus interactions, resulting in enhanced seedling health, vigor and plant growth.

Conclusion

AM fungi inoculation significantly improved seed germination, root colonization and early seedling establishment in rice. A threshold level of spore dosages (>2 spores/seed) facilitated the effective colonization and improved plant growth. In addition, the increase in spore count is further enhanced by root colonization and plant growth parameters, highlighting the importance of AM fungi spore abundance in augmenting early plant establishment under semi-dry conditions. Furthermore, future insights into understanding host-interactions, root colonization and the mechanisms of AM fungi spore inoculation under diverse agro-climatic conditions will be crucial for ensuring ecological adaptability. This paves the way for exploring AM-based inoculants as a sustainable strategy to improve rice productivity and stress resilience.

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Authors' contributions

GM worked on writing the original draft, methodologies, conducting experiments, data curation and analysis. SN contributed to data curation, formal analysis, software, review & amp; editing. AK supervised the workflow along with performing investigation, reviewing, editing and assisted in manuscript preparation. SU conceptualized the workflow and carried out experimental designing, investigation, project supervision, reviewing and editing of the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no competing interests.

Ethical issues: None

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