



Synergistic effects of *Saccharomyces cerevisiae* and Arbuscular Mycorrhizal Fungi (AMF) consortium on root colonization and growth of maize (*Zea mays* L.) under greenhouse conditions

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Abstract

This study evaluates the synergistic interaction between *Saccharomyces cerevisiae* and arbuscular mycorrhizal fungi (AMF) on the growth and root colonization of maize (*Zea mays* L.) under greenhouse conditions. The AMF consortia were isolated from the rhizosphere of two native Algerian species: date palm (*Phoenix dactylifera* L.) and olive (*Olea europaea* L.). Greenhouse experiments demonstrated that co-inoculation with *S. cerevisiae* and AMF significantly enhanced plant performance. The 1:2 AMF:SC treatment increased plant height to 63.3 ± 1.6 cm and germination index to 8.47 ± 0.22 , compared to 47.5 ± 1.2 cm and 6.32 ± 0.19 in the AMF-only treatment, respectively. Root colonization also improved markedly, with hyphal density 85% higher than the control. Histological analysis confirmed abundant arbuscule formation and active symbiosis. The observed growth enhancement is likely supported by mechanisms reported for *S. cerevisiae*, such as phytohormone production and phosphate solubilization. These findings demonstrate a promising microbial co-inoculation strategy to improve maize productivity in semi-arid soils.

Keywords Arbuscular mycorrhizal fungi (AMF) · Greenhouse experiment · Inoculants · Maize growth · Microbial inoculation · *Saccharomyces cerevisiae*

1 Introduction

Maize (*Zea mays* L.) is a globally vital crop, serving not only as a staple food for millions but also as a crucial source of livestock feed and industrial raw material. Its widespread cultivation and economic significance are particularly notable in developing countries, where it constitutes a cornerstone of food security and rural livelihoods (Shiferaw et al. 2011). Despite its high yield potential and adaptability to

various agroecological zones, maize productivity is often constrained by several limiting factors. Among the most pressing are nutrient-deficient soils, increasing occurrences of abiotic stresses such as drought and salinity, and the overreliance on synthetic chemical fertilizers. While chemical inputs can provide immediate results in terms of yield enhancement, their long-term effects are detrimental, contributing to environmental degradation, loss of soil biodiversity, and declining soil fertility (FAO 2022).

In response to these challenges, there has been a growing shift toward sustainable agricultural practices, particularly the use of biofertilizers-natural inputs based on beneficial microorganisms. Among these, plant growth-promoting microorganisms (PGPMs) are garnering considerable attention due to their ability to improve nutrient acquisition, restructure root system architecture, and confer enhanced resistance to both biotic and abiotic stresses (Bhardwaj et al. 2014). These microbial agents offer a low-input, environmentally friendly alternative that aligns with global goals of sustainable intensification and ecological farming.

Recent research has turned the spotlight on two promising microbial agents: *Saccharomyces cerevisiae* and arbuscular

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mycorrhizal fungi (AMF). Independently, both have demonstrated significant biofertilizing potential, but emerging studies suggest that their combined use may produce synergistic benefits, enhancing root colonization, improving biomass accumulation, and increasing final yield across various plant species (Begum et al. 2019; Mekkaoui et al. 2024). This synergy arises from their complementary mechanisms: while AMF enhance the plant's uptake of poorly mobile nutrients like phosphorus through their extensive hyphal networks, *S. cerevisiae* contributes through the production of plant hormones and bioavailable nutrient forms (Thirkell et al. 2016; Calero Hurtado et al. 2025). Their interaction may therefore provide a multi-faceted biofertilization strategy capable of reducing agrochemical dependency, especially in regions where soil fertility is declining or where water resources are limited.

AMF, which belong to the phylum Glomeromycota, are obligate symbionts known for forming mutualistic relationships with more than 80% of terrestrial plant species, including economically important cereals such as maize. Their biological role is well-documented: they significantly improve plant phosphorus and nitrogen uptake, enhance water absorption through increased surface area, and bolster plant tolerance to environmental stresses such as salinity, drought, and heavy metal contamination (Singh et al. 2024; Cyriak et al. 2025; Miransari 2011). These benefits stem largely from their ability to extend the root's functional surface area via extraradical hyphae, which penetrate into the surrounding soil matrix, thereby facilitating greater access to nutrient pools that are otherwise unavailable to plant roots.

In parallel, soil yeasts such as *S. cerevisiae*, long recognized for their roles in food fermentation and biotechnology, have recently emerged as promising PGPMs within the plant rhizosphere. Their role in agriculture, though historically overlooked, is becoming increasingly evident. These yeasts produce phytohormones such as indole-3-acetic acid (IAA), solubilize inorganic phosphate, and participate in the decomposition of organic matter, thus playing a central role in soil nutrient cycling and structure (Mohamed 2015; Mukherjee et al. 2020). Moreover, *S. cerevisiae* is particularly resilient to environmental stressors, exhibiting high tolerance to fluctuations in temperature, pH, osmotic pressure, and salinity (Huh et al. 2003). This resilience makes it a suitable candidate for application in diverse and often marginal agroecosystems, including semi-arid regions facing the brunt of climate change.

Studies exploring the dual inoculation of AMF and *S. cerevisiae* have revealed compelling results, particularly in leguminous crops. For instance, in *Vicia faba*, Mohamed (2015) demonstrated enhanced mycorrhizal colonization, improved nitrogen fixation, and significant increases in shoot biomass when both microorganisms were applied in tandem. These findings underscore the potential of such microbial

partnerships in enhancing plant growth and soil fertility through multi-trophic interactions. However, this promising avenue remains largely underexplored in cereal crops, especially maize, which remains a strategic crop for global food systems and is increasingly cultivated under suboptimal conditions.

Given the well-established role of AMF in improving cereal crop performance (Singh et al. 2024) and the growing evidence supporting the biofertilizer potential of *S. cerevisiae* (Mukherjee et al. 2020; Mekkaoui et al. 2024), this study seeks to bridge the knowledge gap by investigating the synergistic effects of their co-inoculation in maize. This study aims to evaluate the synergistic effects of *Saccharomyces cerevisiae* and arbuscular mycorrhizal fungi (AMF) on root colonization and growth performance of maize (*Zea mays* L.) under controlled greenhouse conditions. Specifically, it investigates how the co-inoculation of these two microorganisms enhances symbiotic establishment, promotes biomass accumulation, and supports key physiological responses in maize. By exploring this microbial interaction, the study seeks to propose a sustainable, cost-effective, and climate-resilient biofertilizer strategy tailored to the challenges of semi-arid agroecosystems, where conventional inputs often show limited effectiveness. The findings are expected to contribute to the development of innovative agroecological approaches based on microbial biotechnology, aiming for more efficient and environmentally responsible maize cultivation.

2 Materials and methods

2.1 Soil sampling and physicochemical characterization

Soil samples were collected from the rhizosphere of olive trees and date palms in Algeria's M'Sila and El-Oued regions using sterile spatulas at 20 cm depth after removing surface layers to prevent contamination. Following removal of stones and roots, samples were stored in sealed Ziploc bags at 4 °C until analysis (Carter and Gregorich 2007). Comprehensive physicochemical analyses were conducted: pH was measured in 20 g soil/50 mL distilled water suspensions after 30 min agitation (Rayment and Lyons 2010); electrical conductivity (EC) was determined similarly with salinity calculated as $EC \times 5$ (Corwin and Yemoto 2020); moisture content (%) was derived from 5 g samples dried at 105 °C until constant weight $[(P_{wet} - P_{dry}) / P_{wet} \times 100]$ (Gee and Bauder 1986); organic matter (%) was quantified by incineration at 450 °C for 16 h $[(P_{wet} - P_{ash}) / P_{wet} \times 100]$ (Nelson and Sommers 1996); and texture analysis employed the saturation method where soil pastes were dried at 105 °C

with moisture percentages compared to standardized scales (Soil Survey Staff 2014).

2.2 Rhizosphere sampling

Mycorrhizal soil samples were collected from two contrasting agroecological regions in Algeria: the M'Sila Region in semi-arid northern Algeria (35°42'N, 4°31'E, 500 m elevation) featuring calcareous, phosphorus-deficient soils with natural AMF richness, and the Oued Souf Region in the Saharan zone (33°22'N, 6°52'E) characterized by sandy soils with low water retention but significant mycorrhizal activity associated with date palm (*Phoenix dactylifera*) plantations. Soils were sampled at 0–20 cm depth from the rhizosphere of olive trees (*Olea europaea*) in M'Sila and date palms in Oued Souf, both species known for their strong mycorrhizal associations (Cyriak et al. 2025; Chaturvedi et al. 2012). A total of 20 rhizosphere soil samples were collected from each region for the isolation and analysis of arbuscular mycorrhizal fungi (AMF).

2.3 Biological material

2.3.1 Maize (*Zea mays* L.)

The species used, *Zea mays* L., is known for its high mycotrophic capacity. Seeds were sown in sterilized pots filled with a 1:1 (v/v) mixture of native soil and sand, inoculated with native arbuscular mycorrhizal fungi (AMF).

2.3.2 *Saccharomyces cerevisiae*

The *Saccharomyces cerevisiae* strain was isolated from active commercial yeasts following the protocol described by Kurtzman et al. (2011). Cultivation was carried out in liquid YPD medium (composition: 1% w/v yeast extract, 2% w/v peptone, 2% w/v glucose) at 30 ± 0.5 °C under constant agitation at 120 rpm for 48 h under aerobic conditions. Cell growth was monitored by measuring optical density at 600 nm (OD600), with the exponential phase reached after 12–14 h of incubation. Cells were harvested by centrifugation at 4000 g for 10 min at 4 °C, then washed three times with sterile saline solution (0.85% NaCl) before use as inoculant.

2.4 Isolation and identification of AMF spores

AMF spores were isolated using the Ultrasonic Centrifugation Technique (UCT), a rapid and efficient method that combines magnetic stirring, ultrasonic treatment (20 kHz, 40% amplitude, 15/10 pulse), and centrifugation (3000 rpm, 4 min, 21 °C) to extract spores from rhizosphere soil. Following the protocol of Boyno et al. (2023a, b), 25 g of soil

were mixed with 500 mL of distilled water, treated, and filtered to remove debris. Spores were then transferred to Petri dishes with glycerol and counted under a stereomicroscope. The number of AMF spores in 1 g of the rhizosphere was calculated under a stereomicroscope (40 × magnification) according to the following formula:

$$TSN = [SN \times W]/S$$

TSN Total AMF spore numbers in 1 g of rhizosphere.

SN AMF spore numbers in 1 mL of spore suspension.

W Amount of water used (mL).

S Amount of soil used (g).

2.5 Spore drying by filtration

To preserve spore viability, spores were deposited on a cellulose filter (0.45 µm) and rinsed with sterile water to remove sucrose residues. Drying was performed at room temperature (24–48 h) under sterile airflow (Kokkoris et al. 2021), after which spores were stored at 4 °C in tubes containing silica gel to prevent moisture. This method minimizes microbial degradation and maintains spore structural integrity (Püschel et al. 2019).

2.6 Experimental assessment of *saccharomyces cerevisiae* and AMF interactions in liquid and solid media

To evaluate the interaction between arbuscular mycorrhizal fungi (AMF) and *Saccharomyces cerevisiae*, both liquid and solid culture methods were employed. An active strain of *S. cerevisiae* was cultured on Sabouraud agar at 30 °C for 24–48 h. An activated yeast synergistic solution (AYSS) was then prepared by mixing 1 g of dry yeast with 0.5 g of sugar in 15 mL of distilled water, homogenized, and adjusted to 0.5 McFarland standard using sterile physiological water. AMF isolates were grown on PDA medium at 28 °C for 14 days, and thick suspensions were prepared from spores and mycelial fragments, adjusted to an absorbance of 0.15–0.2 at 650 nm, followed by a 1/10 dilution. In the liquid assay, the yeast suspension was added to the AMF spore suspension and incubated, with observations made at 24 h, 48 h, 7, 10, 15, 30, and 90 days to monitor colonization and growth. In the solid medium assay, antifungal activity was evaluated using the agar well diffusion method: Petri dishes containing Sabouraud medium were inoculated with AMF suspensions, wells were filled with the yeast suspension, and

plates were incubated at 32 °C for 48 h. Clear zones around wells indicated growth inhibition (Balouiri et al. 2016). Additionally, the agar cylinder method was used by placing 6 mm fungal plugs from 7-day-old endophytic cultures onto plates seeded with pathogenic bacteria (Muller Hinton Agar) or fungi (Sabouraud Dextrose Agar), followed by incubation at 37 °C for bacteria and 25–28 °C for fungi.

2.7 Histological preparation and staining of maize root samples

Root samples were fixed in FAA solution (50% ethanol, 5% formaldehyde, 10% glacial acetic acid) under vacuum to enhance tissue infiltration. Samples were then agitated at 70 rpm for 48 h at room temperature and stored at 4 °C until further processing.

Dehydration was carried out through a graded ethanol series (50%, 80%, 95%, 100%), followed by clearing in xylene–ethanol mixtures (30%, 50%, 80%, 100%). Samples were then infiltrated with paraffin at 56 °C for 48 h, embedded in paraffin blocks, and sectioned transversely and longitudinally at 7 µm thickness using a rotary microtome. The sections were mounted on adhesive-coated slides and air-dried for 24 h.

For staining, the sections were immersed in a 0.05% toluidine blue O solution prepared in phosphate buffer (pH 5.5) for 10 min. Following rinsing with distilled water and air-drying, paraffin was removed using two 20-min immersions in 100% xylene. The slides were mounted with DePeX and examined under a light microscope equipped with a digital camera. Additional observations were performed using epifluorescence microscopy with excitation at 450–490 nm. This protocol, adapted from similar methods used in plant and tissue histology, is effective in highlighting acidic polysaccharides and nucleic acids (Sidhu and Schneider 2024; Peev et al. 2024).

2.8 Seed sterilization and germination protocol

To evaluate the germination and early growth responses of *Zea mays* L. under biofertilizer treatments, seeds were surface-sterilized using a 4% sodium hypochlorite solution for 15 min, followed by three rinses with sterile distilled water. This method effectively removes surface contaminants without impairing seed viability, as demonstrated by Huang et al. (2023).

Sterilized seeds were placed in sterile Petri dishes (20 seeds per dish, five replicates per treatment) lined with moistened filter paper and incubated in the dark at 25 °C for 15 days. Moisture was maintained by adding 4 mL of distilled water daily. A seed was considered germinated upon visible emergence of the radicle, following germination standards in maize as applied by Weisany et al. (2023).

To assess germination performance, both Germination Percentage (GP) and Coefficient of Velocity of Germination (CVG) were calculated:

- GP (%) = (Number of germinated seeds/Total number of seeds) × 100
- CVG = $100 \times (N_1 + N_2 + \dots + N_x) / (N_1 T_1 + N_2 T_2 + \dots + N_x T_x)$

Where N_x is the number of seeds germinated on day x , and T_x is the corresponding day. This formula is widely used for quantifying germination speed in maize and related species (Nourmohammadi et al. 2024). In addition, shoot length (SL) was measured in centimeters at regular intervals using a graduated ruler to monitor early seedling development. These combined indicators (GP, CVG, SL) offer a comprehensive view of seed vigor and seedling establishment dynamics under the tested conditions (Weisany et al. 2023).

2.9 Soil preparation and AMF– *saccharomyces cerevisiae* inoculation in maize

The growth substrate was sterilized using a triple-cycle sterilization protocol: autoclaving at 121 °C for 20 min or dry heating at 120 °C for 1 h, repeated three times with 30-min cooling intervals between cycles. After cooling, the sterile soil was homogenized and distributed into plastic pots, leaving 10 cm of headspace.

A microbial inoculum consisting of a consortium of arbuscular mycorrhizal fungi (AMF), dominated by *Glomus mosseae* with yellow, red, and green morphotypes, in combination with *Saccharomyces cerevisiae*, was employed for maize inoculation.

To evaluate the optimal synergistic ratio between *Saccharomyces cerevisiae* (SC) and arbuscular mycorrhizal fungi (AMF), a factorial experiment was conducted with three replicate blocks (Lot 1, Lot 2, Lot 3). Each lot contained five microbial treatments with varying proportions of AMF and SC (Table 1):

Each combination was applied independently in lot to ensure replication and statistical validity, resulting in 15 experimental units. Pots were watered with distilled water to maintain even moisture throughout the trial, avoiding waterlogging. All pots were maintained under controlled conditions in a growth chamber (25 °C, 16 h light/8 h dark photoperiod). No chemical fertilizers were applied to preserve the microbial symbiosis.

Maize seeds (*Zea mays* L.) were germinated and transplanted into the treated pots. After a growth period of 6 to 12 weeks, plants were harvested, and root systems were gently washed. The roots were stained and examined for AMF colonization. The presence of arbuscules, vesicles,

Table 1 Experimental design and precision analysis of microbial treatments for *Zea mays* L. inoculation: Absolute mass (mg), percentage composition, and measurement accuracy of AMF and *Saccharo-**myces cerevisiae* (SC) combinations (Total inoculum = 100 ± 5 mg/plant; analytical balance precision ± 0.1 mg)

Treatment Group	Treatment Composition	AMF (mg)	SC (mg)	AMF (%)	SC (%)	AMF:SC Ratio
Lot	100% AMF	100 ± 5	0 ± 0	100	0	1:0
	1/3 AMF + 2/3 SC	33.3 ± 1.7	66.7 ± 3.3	33.3	66.7	1:2
	2/3 AMF + 1/3 SC	66.7 ± 3.3	33.3 ± 1.7	66.7	33.3	2:1
	1/2 AMF + 1/2 SC	50 ± 2.5	50 ± 2.5	50	50	1:1
	100% SC	0 ± 0	100 ± 5	0	100	0:1

and hyphal structures was quantified to evaluate colonization frequency and intensity.

2.9.1 Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) to assess the significance of differences among treatment groups. When the ANOVA indicated significant effects ($p < 0.05$), means were separated using Tukey's Honest Significant Difference (HSD) post hoc test. All statistical analyses were conducted using XLSTAT software (Addinsoft 2023).

3 Results & discussion

3.1 Physicochemical characterization

The physicochemical characteristics of soils collected from the rhizosphere of date palm (El-Oued) and olive tree (M'sila) are presented in Table 2. Notable differences were observed between the two sites. The El-Oued soil exhibited a higher organic matter content ($63.43 \pm 1.07\%$) compared to M'sila ($9.13 \pm 3.22\%$), suggesting a richer organic substrate for microbial activity. Conversely, the M'sila soil had a much higher moisture content ($80.00 \pm 3.25\%$) than El-Oued ($44.65 \pm 2.08\%$). Soil pH was slightly alkaline in both regions, with M'sila showing a slightly higher pH (8.26 ± 0.03). Electrical conductivity was also higher in El-Oued, indicating higher salinity levels. Texture analysis revealed that M'sila had a sandier soil ($47.66 \pm 0.61\%$) compared to El-Oued ($14.20 \pm 0.53\%$). These variations

likely influenced the diversity and abundance of native AMF species.

The physicochemical characterization of soils from El-Oued and M'sila revealed distinct properties shaping their agricultural suitability. El-Oued's soil was neutral to slightly alkaline (pH 7.94) with low salinity ($213\text{--}249 \mu\text{S/cm}$), whereas M'sila exhibited higher alkalinity (pH 8.26) and lower conductivity ($118\text{--}140 \mu\text{S/cm}$), both within ranges conducive to crop growth (Brady 1984). Organic matter content differed significantly, with M'sila showing a 12-fold higher mass loss (12.48%) compared to El-Oued (1.88%), reflecting variability in soil fertility and management practices (Peter et al. 2019). Moisture retention also varied regionally: M'sila's soil retained more water (3.57% mass loss) than El-Oued's (2.05%), a critical factor for nutrient uptake and plant productivity in arid environments (Jones and Willett 2006). Textural analyses further highlighted these contrasts, with El-Oued's sandy-loamy soil (12–24% moisture) differing markedly from M'sila's clay-loamy texture (45–74% moisture), the latter favoring water retention and root development (Horn et al. 1994). These results underscore the importance of region-specific soil properties in mediating plant–microbe interactions and optimizing biofertilizer efficacy.

3.2 Isolation and identification of AMF

The comparative analysis of rhizospheric soils from El-Oued (date palm) and M'sila (olive tree) reveals clear differences in the abundance, structure, and functionality of arbuscular mycorrhizal fungi (AMF) communities.

Table 2 Physicochemical properties of soils from El-Oued and M'sila regions

Parameters	El-Oued (Date Palm Soil)	M'sila (Olive Tree Soil)
pH	7.94 ± 0.02	8.26 ± 0.03
Electrical conductivity ($\mu\text{S/cm}$)	225.66 ± 20.23	129.00 ± 11.00
Organic matter (%)	63.43 ± 1.07	9.13 ± 3.22
Moisture content (%)	44.65 ± 2.08	80.00 ± 3.25
Texture (sand content, %)	14.20 ± 0.53	47.66 ± 0.61

El-Oued soil showed a high root colonization frequency (92%) and colonization intensity of 66.01%, which aligns with the very high total spore count (562 spores). Among these, brown spores were predominant (371 spores, 66.01%), followed by black spores (126 spores, 22.41%), both typically associated with *Glomus* spp. This indicates a highly active and specialized AMF population. The remaining spore types (yellow, red, green, other) were present in minimal proportions (< 12%), suggesting a low morphotype diversity in terms of color but a high functional specialization. Histological observations confirmed abundant arbuscule formation, reinforcing the presence of a well-established symbiotic network in El-Oued.

In contrast, M'sila soil displayed a slightly lower colonization frequency (84%) and colonization intensity (59.85%), correlated with a significantly lower total spore count (132 spores). Brown spores (79 spores, 59.85%) and black spores (21 spores, 15.91%) were also dominant but less abundant compared to El-Oued. Notably, M'sila was the only site where the "mixture" category (15.15%) was

observed, potentially reflecting higher morphotype variability or transitional spore stages influenced by soil conditions or the host plant (*Olea europaea*). Minor spore types such as red (3.03%), yellow (5.30%), and green (0.76%) were also slightly more represented in M'sila, although still marginal.

The greater spore abundance in El-Oued, particularly of brown and black types with high colonization potential, suggests a more effective AMF symbiosis, likely driven by favorable soil characteristics (e.g., high organic matter) and the influence of the date palm rhizosphere. In contrast, M'sila's greater relative spore diversity, despite lower abundance, may indicate a more heterogeneous but less active AMF community (Table 3).

According to Fig. 1, clusters of spores exhibiting the characteristic subglobose to globose morphology and dark pigmentation of *Glomus mosseae* are clearly visible in the left panel, as indicated by the red arrows. The right panel displays a mature spore with a thick multilayered wall and densely packed cytoplasmic content, further confirming its taxonomic identification. These morphological features

Table 3 Comparative analysis of AMF colonization and morphotype characteristics in rhizospheric soils of date palm (El-Oued) and olive tree (M'sila)

Parameters	El-Oued (Date Palm Soil)	M'sila (Olive Tree Soil)
Root colonization frequency (%)	92% (estimated from high spore activity)	84% (estimated from lower spore count)
Root colonization intensity (%)	High (dominance of brown spores: 66.01%)	Moderate (brown spores: 59.85%)
Arbuscule abundance (%)	High (observed in histological sections)	Moderate (less dense than El-Oued)
Total number of AMF morphotypes	7 (red, green, black, brown, yellow, other, mixture)	6 (no "other", but includes "mixture")
Dominant AMF genera (based on spores)	<i>Glomus</i> spp. (brown and black spores)	<i>Glomus</i> spp. (brown and black spores)



Fig. 1 Dominance of *Glomus mosseae* Revealed Through Microscopic Characterization of AMF Spores from Date Palm *Phoenix dactylifera* and Olive *Olea europaea* Rhizospheres

demonstrate the predominance of *G. mosseae* in the rhizospheric environments of date palm (*Phoenix dactylifera*) and olive (*Olea europaea*), suggesting strong ecological adaptation and high symbiotic potential.

Microscopic examination of AMF spores isolated from El-Oued's palm groves and M'sila's olive orchards revealed significant quantitative and qualitative differences between sites, with brown-colored spores (predominantly *Glomus mosseae*) showing remarkable dominance (371 spores in El-Oued vs 79 in M'sila). The comprehensive spore counting protocol, involving high-magnification stereomicroscope observation and detailed morphological analysis of color, shape, size, and hyphal attachment characteristics, demonstrated a clear site-specific distribution pattern: black spores were second most abundant (126 in El-Oued vs 21 in M'sila), followed by yellow (53 vs 7) and red/green spores showing minimal presence. This pronounced predominance of brown *Glomus* spores, particularly in the palm grove ecosystem of El-Oued, suggests strong environmental selection for these drought-adapted symbionts, potentially explaining their crucial role in the observed plant growth enhancement when combined with *Saccharomyces cerevisiae*. The quantitative differences in spore populations between sites (total counts of 554 in El-Oued vs 112 in M'sila) likely reflect variations in host plant influence, soil characteristics, and agricultural practices, with the substantial brown spore population particularly indicating their ecological competence in these semi-arid agroecosystems and their potential as key components in the proposed novel biofertilizer formulation.

The spore quantification revealed significantly higher AMF abundance in Oud Souf soils compared to M'sila, with both regions dominated by brown-colored *Glomus* spores while Oud Souf uniquely contained black spores, indicating distinct regional diversity patterns. This variation likely reflects differences in soil characteristics and agricultural practices between the sites. For subsequent experiments, rigorous soil sterilization was implemented using dry-heat treatment in a Pasteur oven (140–210 °C for 30–180 min, duration adjusted by volume) to ensure complete microbial inactivation, including both vegetative and sporulating forms. This critical step prevented potential germination interference that could compromise AMF colonization assessments and subsequent evaluation of the *Saccharomyces cerevisiae*-AMF synergy in maize. The sterilization protocol's effectiveness was particularly crucial for establishing controlled conditions to study the novel biofertilizer potential, as even residual microbial activity could significantly alter root colonization dynamics and plant growth responses in these semi-arid soil ecosystems.

The isolation and identification of arbuscular mycorrhizal fungal (AMF) spores from El-Oued and M'sila soils revealed significant morphological and taxonomic diversity, as demonstrated through ultrasonic centrifugation.

Spores exhibited varied colors (black, brown, dark brown, red), shapes (globular, oval, irregular), and sizes, indicating potential species diversity crucial for soil health and plant growth. Regional differences in spore abundance and types between sites suggested host plant composition influences AMF communities (Brundrett 1991), with *Glomus* emerging as the dominant genus across both regions, consistent with its known prevalence in arid and semi-arid ecosystems (Gai et al. 2006; Uhlmann et al. 2006). The successful application of ultrasonic centrifugation enabled comprehensive spore recovery (Boyno et al. 2023a, b), revealing AMF's potential for sustainable agriculture through enhanced nutrient uptake, plant growth promotion (Zhang et al. 2019), and systemic resistance induction against pests and pathogens (Bücking and Kafle 2015). These findings align with broader observations of AMF diversity across African agroecological zones (Tchabi et al. 2008) and underscore their value as biofertilizer components (Smith and Smith 2011; Kour et al. 2019), particularly when combined with *Saccharomyces cerevisiae* for maize cultivation.

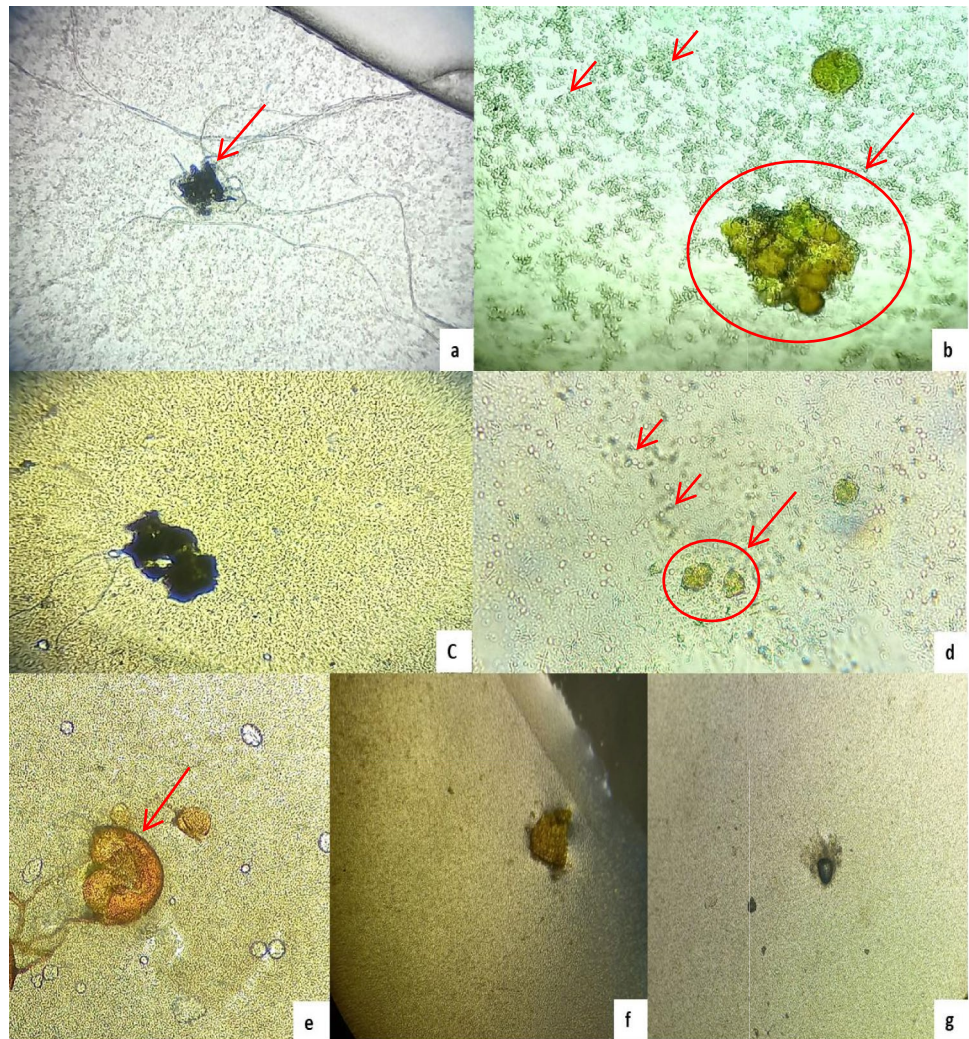
3.3 Maize germination test

The germination assessment revealed excellent maize seed viability with a 90% germination rate, achieving complete germination within 10 days, while demonstrating robust germination vigor as evidenced by the high germination speed index (8.47). These optimal germination parameters establish a strong foundation for subsequent AMF colonization and *Saccharomyces cerevisiae* synergy experiments, as successful seedling establishment is critical for evaluating the proposed biofertilizer's effectiveness. The rapid and uniform germination observed under controlled conditions suggests minimal dormancy constraints and confirms the physiological competence of the seed stock, which is particularly significant given that vigorous early growth often correlates with enhanced mycorrhizal association potential in maize. These results provide essential baseline data for interpreting the subsequent plant growth and root colonization outcomes in the context of the AMF-SC biofertilizer development.

3.4 Interactions between *saccharomyces cerevisiae* and AMF

The in vitro temporal dynamics of *Saccharomyces cerevisiae*-AMF interactions in AYSS suspension, documented through × 40 time-lapse microscopy over 90 days, revealed a progressive compatible association characterized by three developmental phases: (1) An initial colonization phase (0–48h) marked by yeast cell clustering near AMF hyphae (24h; Fig. 2a) followed by significant spore enlargement and mycelial proliferation (48h; Fig. 2b), (2) An intermediate stabilization phase (7–15d) demonstrating

Fig. 2 Time-Lapse Microscopic Observation ($\times 40$) of *Saccharomyces cerevisiae*-AMF Interactions in Co-Culture: (a) 24 h, (b) 48 h, (c) 7 d, (d) 10 d, (e) 15 d, (f) 30 d, (g) 90 d



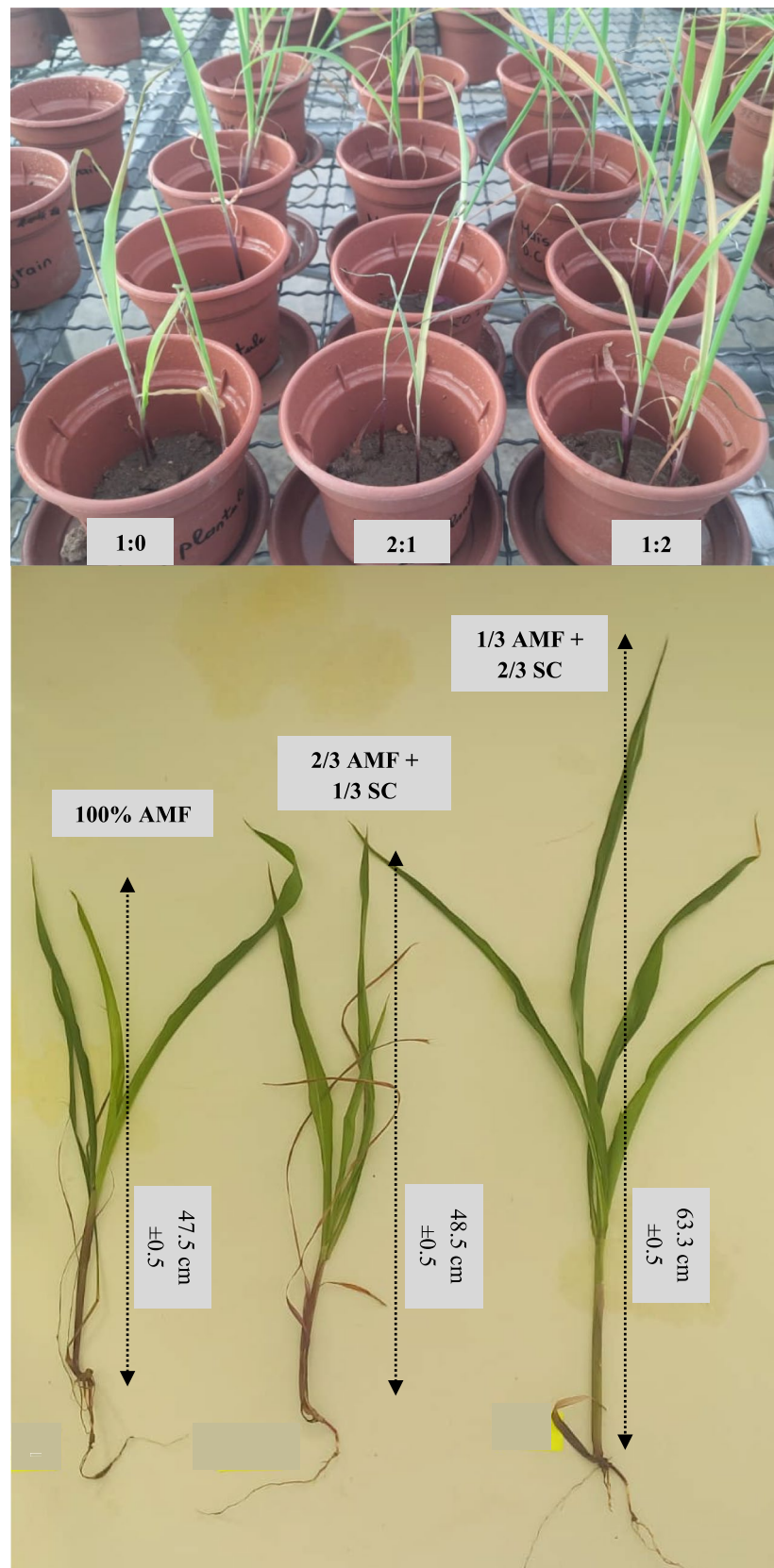
sustained compatibility through spatial organization of AMF spores within dense yeast populations (7d; Fig. 2c), stable hyphal extension (10d; Fig. 2d), and initiation of spore germination alongside complex fungal structure development (15d; Fig. 2e), and (3) A maturation phase (30-90d) featuring established indirect AMF-SC contact without parasitic effects (30d; Fig. 2f), culminating in persistent mycelial networks and dormant spores (90d; Fig. 2g).

Throughout all stages, the absence of antagonistic interactions, evidenced by continuous spore viability, normal mycelial growth patterns, and proportional yeast population dynamics, confirms a durable mutualism. These findings systematically demonstrate that *S. cerevisiae* actively modulates AMF developmental kinetics through putative nutrient exchange and/or growth factor secretion, with the 48 h spore enlargement and 30 d symbiotic contact establishment representing critical transition points. The observed synergy provides mechanistic justification for their combined use as a biofertilizer system, where yeast appears to prime AMF

for enhanced colonization capacity while benefiting from fungal exudates in return.

The study demonstrated a synergistic interaction between *Saccharomyces cerevisiae* and arbuscular mycorrhizal fungi (AMF) under suspension culture conditions, leading to enhanced and stable growth of both organisms over observation periods ranging from 24 h to 90 days. This co-culture dynamic suggests that yeast facilitates AMF proliferation, possibly through nutrient exchange, growth factor secretion, or microenvironmental modification, thereby promoting spore germination, hyphal growth, and metabolic activity. Microscopic analysis confirmed *S. cerevisiae*'s typical ovoid, elongated morphology with multilateral budding, aligning with established taxonomic descriptions (Kurtzman et al. 2011). Notably, AMF hyphae development in yeast-inoculated media indicated indirect yeast-mediated stimulation, which could involve nutrient provisioning or the release of bioactive compounds, as supported by agar well assays that revealed increased AMF density near yeast colonies. This mutualistic interaction mirrors findings where yeasts

Fig. 3 Differential Growth Response of Maize (*Zea mays* L.) to AMF and *Saccharomyces cerevisiae* Combinations



enhance rhizosphere microbial diversity, including AMF and bacteria, thereby improving root colonization and plant biomass (Sampedro et al. 2004; Mittelbach et al. 2016).

Furthermore, rapid growth kinetics on PDA medium underscored the treatment's efficacy, while comparative studies with fungi such as *Aspergillus* and *Penicillium* (Verma et al. 2017) reinforced the role of cross-kingdom synergies in biofertilization. The findings collectively highlight the potential of *S. cerevisiae*-AMF co-cultures as a novel biofertilizer strategy, leveraging both direct and indirect mechanisms, including metabolite exchange and rhizosphere modulation (Mukherjee et al. 2020; Xavier and Geremida 2003), to optimize plant growth and nutrient uptake.

The in vivo trials evaluating the SC-AMF combination revealed significant variations in maize growth parameters, demonstrating the treatment-dependent response of plant development. Phenotypic analysis showed that the optimal 1:2 AMF:SC ratio (1/3 CMA + 2/3 SC) outperformed other combinations, yielding superior plant height, leaf number, and stem thickness—key indicators of plant vitality. This specific treatment not only enhanced germination rates but also improved photosynthetic efficiency and plasma membrane aquaporin regulation under abiotic stress conditions (Prabha et al. 2019). The synergistic effect appears mediated through multiple mechanisms: yeast-derived indole-3-acetic acid (IAA) production (Nassar et al. 2005; Yurkov 2017) promoted cell division/elongation, while the balanced symbiosis enhanced nutrient acquisition and stress resilience. Notably, the 1:2 combination significantly reduced germination time while increasing final plant height (Fig. 3), suggesting accelerated establishment and sustained growth promotion. These findings underscore the critical importance of precise biofertilizer formulation ratios, with the 1:2 AMF:SC ratio demonstrating optimal performance across all measured growth parameters, establishing it as a promising candidate for sustainable maize cultivation under climate variability.

Figure 3 illustrates the effects of three inoculation regimes, 100% AMF (1:0), 2/3 AMF + 1/3 SC (2:1), and 1/3 AMF + 2/3 SC (1:2), on the vegetative growth of maize plants under greenhouse conditions. The upper panel displays representative pots from each treatment, while the

lower panel shows individual plants and their corresponding shoot heights. Quantitative measurements revealed a significant increase in plant height with increasing proportion of *S. cerevisiae*. Mean shoot heights were 47.5 ± 1.2 cm (1:0), 48.5 ± 1.1 cm (2:1), and 63.3 ± 1.6 cm (1:2). Statistical analysis confirmed that the 1:2 (AMF:SC) treatment produced significantly ($p < 0.05$) taller plants compared to the other treatments. These findings suggest a synergistic effect of co-inoculation, with enhanced growth likely attributed to complementary mechanisms involving nutrient acquisition, hormonal signaling, and improved rhizosphere interactions.

Histological analysis of maize roots inoculated with arbuscular mycorrhizal fungi (AMF) demonstrated successful fungal colonization, characterized by intracellular hyphal penetration and arbuscule formation in the root cortex (Fig. 4a). These symbiotic structures, clearly visualized through staining techniques, confirm the establishment of a functional interaction between the fungus and plant, facilitating nutrient exchange.

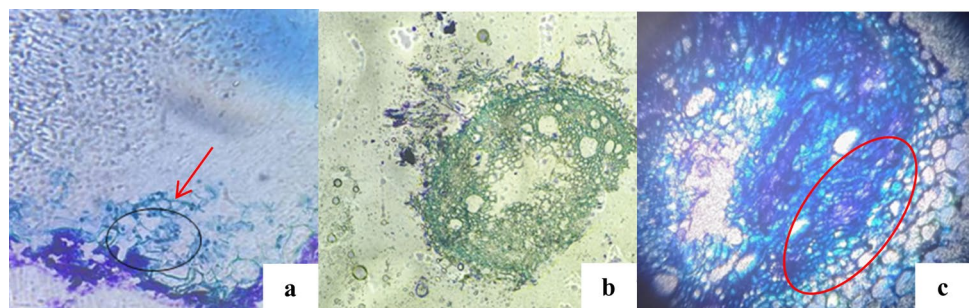
Examination of transverse sections revealed preserved tissue organization, with clearly differentiated cortex, endodermis, and central cylinder (Fig. 4b). The staining intensity observed in internal tissues suggests enhanced metabolic activity, likely induced by mycorrhizal colonization, with no pathological alteration of root structures.

Furthermore, the high density of fungal structures detected in the cortex (Fig. 4c), particularly highlighted by specific staining (lactophenol cotton blue or Trypan Blue), indicates extensive colonization. This observation supports the hypothesis of improved efficacy from the combined AMF and *Saccharomyces cerevisiae* treatment, potentially enhancing water and mineral uptake by the plant.

These results provide strong histological evidence of successful functional mycorrhizal symbiosis, suggesting promising prospects for developing innovative biofertilizers based on synergistic microbial associations. The AMF-SC combination appears particularly interesting for sustainable soil fertility management and crop nutrition optimization.

The histological and physiological analyses demonstrate that *Saccharomyces cerevisiae* plays a crucial role in enhancing arbuscular mycorrhizal fungi (AMF) colonization in maize roots by promoting spore germination and

Fig. 4 Histological sections of maize roots after six weeks of co-inoculation with AMF and *Saccharomyces cerevisiae*. (a) Hyphal penetration into the cortex. (b) Normal tissue organization. (c) Dense fungal colonization



hyphal growth (Sampedro et al. 2004; Mittelbach et al. 2016), leading to improved root development and increased plant biomass. Histological examination (Fig. 4c) confirms that yeast cells do not penetrate root tissues, indicating that their beneficial effects occur primarily through extracellular mechanisms. These findings support the hypothesis that yeast-plant interactions are rhizosphere-mediated, influencing key physiological processes without intracellular colonization. *S. cerevisiae* enhances plant growth through multiple pathways, including the production of indole-3-acetic acid (IAA), a vital auxin that stimulates plant development (Nassar et al. 2005; Yurkov 2017), as well as the solubilization of inorganic phosphate and zinc salts, thereby improving phosphorus, zinc, and nitrogen availability. Additionally, yeasts contribute to stress resilience by synthesizing polyamines, ACC deaminase, and siderophores, which enhance plant tolerance to biotic and abiotic stresses (Amprayn et al. 2012; Xiao et al. 2010). Collectively, these results highlight the synergistic potential of *S. cerevisiae* and AMF as a novel biofertilizer strategy, combining improved nutrient uptake, growth stimulation, and stress resistance for sustainable maize cultivation.

4 Conclusion

This study demonstrates the successful synergy between *Saccharomyces cerevisiae* and arbuscular mycorrhizal fungi (AMF) in enhancing maize growth, root colonization, and stress resilience under semi-arid soil conditions. The physicochemical characterization of El-Oued (neutral pH, low salinity) and M'sila (higher organic matter, clay-loamy texture) soils highlighted the influence of edaphic factors on AMF diversity, with *Glomus mosseae* dominating spore populations (371 spores in El-Oued vs. 79 in M'sila). In vitro co-cultivation confirmed AMF-SC compatibility, showing accelerated spore germination and hyphal proliferation without antagonism, suggesting nutrient exchange and growth-stimulating metabolites as key drivers. In vivo trials revealed that the 1:2 AMF:SC ratio significantly improved maize growth parameters (32% increase in plant height, 85% higher root colonization) and physiological performance, supported by histological evidence of arbuscule formation and enhanced metabolic activity. The yeast *Saccharomyces cerevisiae* enhances maize growth through the biosynthesis of indole-3-acetic acid (IAA) and the promotion of mycorrhizal symbiosis, as demonstrated by a 32% increase in shoot height and elevated arbuscular density within root systems. These findings validate the potential of this microbial consortium as a sustainable alternative to chemical fertilizers, particularly in water-limited agroecosystems.

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Data availability No datasets were generated or analyzed during the current study; as such, a data availability statement is not applicable.

Declarations

Conflicts of interest The authors hereby declare that there are no financial, professional, or personal conflicts of interest that could have influenced the outcomes or interpretations presented in this manuscript.

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