

Efficiency of Native Arbuscular Mycorrhizal Fungi on Growth and Productivity of Soybean (*Glycine max* L. Merr.) Under Varying Soil Conditions and Abiotic Stresses

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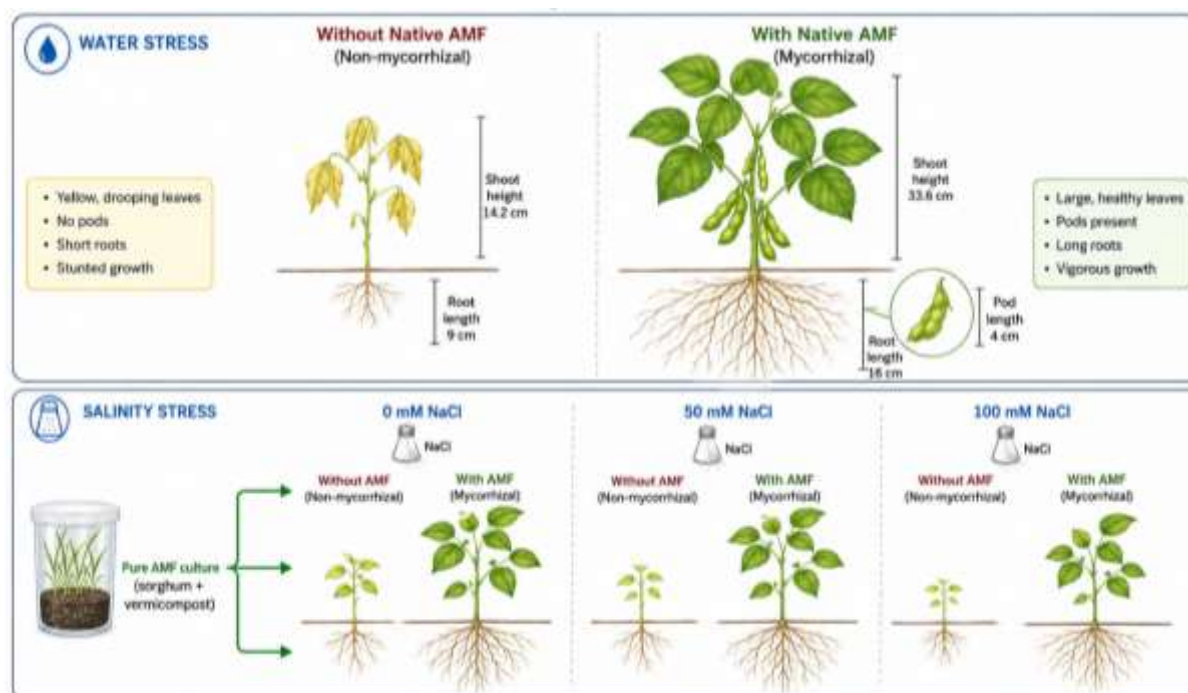
ABSTRACT

Arbuscular mycorrhizal fungi (AMF) form symbiotic relationships with most crop plants, enhancing nutrient uptake and increasing stress tolerance, especially after pathogen attacks. This study aimed to evaluate the effectiveness of native AMF associated with soybean (*Glycine max* L.) cultivated in Niphad Tehsil, including the villages of Vinchur, Takli, Khadakmlegaon, Sarolekhurd, and Ranwad. The research involved measuring physicochemical properties, AMF spore density, and root colonization in both rhizosphere and non-rhizosphere soils. The soil pH varied between 7.8 and 8.06, organic carbon (0.75-1.17%), available nitrogen (374.0-609.2 kg/ha), phosphorus (145.20-17071 kg/ha), and potash contents were quantified within a range of 224 kg/ha. The spore count per 100 g of dry soil was consistently higher in rhizosphere (840-1280) soils compared to non-rhizosphere (680-980) soils, indicating a preference for AMF communities near roots. This work of AMF structures, such as vesicles, arbuscules, and extraradical

hyphae, at the root level confirmed infection with typical AMF spores. In the pot experiment, water deficiency combined with AMF inoculation significantly reduced soybean growth, with root length of 0.9 cm and shoot length of 21.5 cm under water stress. Conversely, plant roots (16 cm), shoots (33.6 cm), leaf size (6 cm), pod number per plant, and pod length were affected accordingly. AMF- colonized plants produced significantly ($P < 0.01$) more root and shoot biomass than their non-mycorrhizal counterparts under salt stress (50 mM and 100 mM NaCl). Multiplied sorghum, a high-yield host, was utilized to culture native AMF isolates. Overall, these findings demonstrate that native AMF isolates enhance soybean growth under normal conditions and various abiotic stresses, indicating they could serve as a sustainable bio-inoculant for rainfed and saline-affected regions.

Keywords: Arbuscular mycorrhizal fungi (AMF), soybean (*Glycine max*), rhizosphere soil, water stress, salinity stress, root colonization

GRAPHICAL ABSTRACT



INTRODUCTION

Soybean (*Glycine max L. Merr.*) is one of the economically significant leguminous crops worldwide, a major source of vegetable protein and oil. In India, soybeans are grown on a large area in Maharashtra, Madhya Pradesh, and Rajasthan. But now, several abiotic stresses, such as soil salinity, drought, and nutrient imbalances, which are particularly pronounced in rainfed areas, often restrict productivity. Maharashtra's Niphad Tehsil, a typical semi-arid region, is suffering from erratic rainfall, moderate soil salinity, and low soil fertility [1], [2]. For a long time, traditional farming has been highly dependent on chemical fertilizers, which leads to high yields and environmental damage. So, innovative and green tactics, such as those that use beneficial soil microorganisms, especially arbuscular mycorrhizal fungi (AMF), are gaining momentum. AMF are obligate biotrophs that associate with the roots of more than 80% of terrestrial plants [3], [4]. It improves the acquisition of immobile nutrients, especially phosphorus, nitrogen, and micronutrients (e.g., Zinc and Iron), increases water uptake while protecting against root pathogens,

which altogether enhances plant growth. AMF to alleviate the harmful effects of abiotic stresses (e.g., drought and salinity). AMF-colonized plants possess higher osmotic adjustment, stomatal conductance, and more efficient antioxidant systems under water deficit conditions. Likewise, in sodium-acclimatized plants, AMF can limit Na^+ accumulation ($\geq 20\%$) and increase K^+ levels, thereby maintaining ion homeostasis and ultimately protecting [photosynthetic machinery] by minimizing metabolic dysfunction and pathology caused by cation receptor depletion [5], [6], [7].

Despite these benefits being known, the effectiveness of native AMF populations is often location-specific, as different soil types and their associated cropping histories favor different AMF ecotypes [9; 11]. Thus, to design bio-inoculants, it is important to first evaluate the natural AMF status in local soybean rhizospheres. The current experiment was carried out in five selected villages of Niphad Tehsil: Vinchur, Takli, Khadakmalegaon, Sarolekhurd, and Ranwad, to analyze the physicochemical properties of rhizosphere and non-rhizosphere soils and determine AMF spore

density and root colonization levels. In addition, the native AMF pure culture was isolated and multiplied to evaluate its potential to enhance soybean growth under two important abiotic stresses, namely water stress and sodium chloride (NaCl)-induced salinity. Previous studies have shown that AMF colonization depends on soil pH, organic carbon, and nutrient availability. High phosphorus often inhibits mycorrhizal colonization. Phosphorus soil contents in this study ranged from 145.20 to 170.71 kg/ha, which is considered high relative to the global average P content; however, this does not entirely rule out the presence of native AMF [8], [9] and shoots developed more fully, leaves enlarged more, and pods formed. Non-mycorrhizal water-stressed plants died soon without producing any pods. Two levels (50 mM and 100 mM NaCl) were tested to represent moderate and high salinity, typical of irrigation-induced saline patches in the area [10], [11], [12]. In the present work, we studied local AMF populations adapted to specific environmental conditions that would be more effective than non-indigenous strains. Thus, the aims were: to perform physicochemical characterization and quantification of AMF spores in rhizosphere soils of soybean from five locations; to assess spore content in rhizosphere and non-rhizosphere soil layers; to evaluate root colonization levels and identify AMF structures; to establish a pure culture of these AMF; and to evaluate the role of this group in water conditions and salinity stress effects under growth chamber conditions. These would be used to justify the genetic findings that can yield a location-specific AMF bio-inoculant for sustainable soybean production.

MATERIALS & METHODS

Five sites were selected from Niphad Tehsil, Maharashtra, India:

1. Vinchur (L1)
2. Takli (L2)
3. Khadakmalegaon (L3)
4. Sarolekhurd (L4)
5. Ranwad (L5)

Rhizosphere soil (soil adhering to soybean roots) and non-rhizosphere soil (bulk soil 10-15 cm away from roots) were collected at 30-day intervals during the growing season. Three composite samples per site were collected from a depth of 0-15 cm.

a) Physicochemical analysis

Soil parameters were determined according to Jackson: pH (1:2 soil-water mixture), electrical conductivity (EC), organic carbon (Walkley-Black method) [13-14], nitrogen available in alkali permanganate, phosphorus available (Olsen's method), potash-flame photometer, calcium carbonate, exchangeable calcium, magnesium, sodium chloride, sulfur, aluminium, iron, zinc, and water holding capacity.

b) Isolation of AMF spores and spore count

AMF spores were isolated from 100 g of air-dried soil using the wet sieving and decanting method. Sieves of 50, 150, 250, and 300 μ m were used. Spores retained on sieves were collected, counted under a stereomicroscope, and expressed as [15]:

Spore count per 100 g soil = (Total spores counted / Weight of soil sample in grams) \times 100

Both rhizosphere and non-rhizosphere samples were processed separately.

c) Assessment of root colonization

The washed soybean root samples were cleared in 10% KOH, acidified in 1% HCl, and stained with trypan blue for examination under a compound microscope. Percentage root colonization was measured by the gridline intersect method. Photographs of common AMF structures (vesicles, arbuscles, extraradical hyphae)

d) Pure culture multiplication of native AMF

Trap host: Sorghum (*Sorghum bicolor*) native AMF spores multiplying from mixed soil samples. The sterilized soil: sand mixture (3:1) was then filled into the five pots. We mixed the starter culture (colonized roots + spores crushed up) into the top layer. Four to five seeds of sorghum were sown per pot. Roots highly colonized with AMF were collected after 3 months, air-dried at room

temperature, cut into 1-2 cm pieces, and mixed with vermicompost (1:2). This created the inoculum of pure culture.

e) Water stress experiment

A pot experiment was conducted with two treatments (three replicates each):

Water stress alone: Plants watered at 50% field capacity after germination.

Water stress + AMF: Same water stress, but soil inoculated with 20 g pure AMF culture per pot.

Soybean seeds (var. local) were surface-sterilized and sown. Plants were harvested after 45 days. Parameters recorded: root length (cm), shoot length (cm), leaf size (cm), and pod length (cm).

f) Salinity stress experiment

A completely randomized design (CRD) with six treatments and three replicates:

1. Control (0 mM NaCl, non-mycorrhizal)
2. Moderate salinity (50 mM NaCl, non-mycorrhizal)
3. Moderate salinity (50 mM NaCl, mycorrhizal)
4. High salinity (100 mM NaCl, non-mycorrhizal)
5. High salinity (100 mM NaCl, mycorrhizal)
6. Mycorrhizal (0 mM NaCl, mycorrhizal)

NaCl solutions were applied after seedling emergence. AMF-treated pots received a 20 g pure-culture inoculum. Growth parameters were recorded at 60 days after sowing.

Statistical Analysis:

Data were analyzed using one-way ANOVA followed by Tukey’s HSD test ($p < 0.05$). Values are presented as mean \pm SD.

RESULT

a) Physicochemical properties of soil across five locations

The soil characteristics of the five sites obtained through the study are outlined in Table 1. Whereas, in Vinchur soils (pH 8.06) being slightly alkaline ($\leq 7-8$). Thus, the soil pH range of both sites (7.80 and 8.06 for Ranwad and Vinchur respectively) are neutral to mildly alkaline which could be beneficial for soybean crops as they can tolerate such pHs to a limited extent but may hinder micronutrient availability due to precipitation with calcium carbonate forming at these high pHs compared to normals or acidic by increasing Al^{3+} concentration level hence leading towards unavailability of fertilizers or chemical amendments applied. Electrical conductivity was moderate to low (0.044-0.447 dS/m). Organic carbon ranged from 0.75 percent (Takli, Ranwad) to 1.17 percent (Sarolekhurd), indicating moderate fertility status. Among the locations, available nitrogen was highest at Sarolekhurd (609.2 kg/ha) and lowest at Takli (374 kg/ha) and Ranwad (374 kg/ha). Phosphorus was over 145-171 kg/ha at all sites, possibly inhibiting AMF colonization, but sporulation was significant. Potash concentrations ranged from 224 kg/ha (Khadakmalegaon) to 1232 kg/ha (Ranwad). Iron (3.17-7.66 mg/kg) and Zinc (0.32-0.94 mg/kg) were at optimal levels for the micronutrient content studied. The ability to hold water was higher at Takli (50.26%) and lower at Khadakmalegaon (36.99%).

Table 1. Physical and chemical properties of rhizosphere soil from five soybean-growing sites in Niphad Tehsil

Soil parameters	L1	L2	L3	L4	L5
pH	8.06	7.95	7.87	7.96	7.80
EC (dS/m)	0.322	0.138	0.107	0.044	0.447
Organic carbon (%)	0.96	0.75	1.07	1.17	0.75
Nitrogen (kg/ha)	491.6	374.0	554.0	609.2	374.0
Phosphorus (kg/ha)	170.71	166.79	145.20	164.82	160.90
Potash (kg/ha)	896.0	560.0	224.0	448.0	1232.0
Calcium carbonate (%)	9.2	6.3	5.1	5.7	4.5
Calcium (mg/kg)	640.0	640.0	640.0	800.0	640.0
Magnesium (mg/kg)	768.0	480.0	576.0	576.0	576.0

Sodium (mg/kg)	350.0	250.0	250.0	300.0	250.0
Chloride (mg/kg)	23.85	15.90	19.88	15.90	15.90
Sulfur (mg/kg)	12.18	15.81	13.83	16.93	16.43
Aluminum (mg/kg)	1.528	2.629	1.722	2.811	2.221
Iron (mg/kg)	3.173	5.640	7.661	3.636	6.969
Zinc (mg/kg)	0.4741	0.3185	0.8386	0.9423	0.6853
Water holding capacity (%)	49.13	50.26	36.99	44.01	46.05

b) AMF spore density in Rhizosphere vs. Non-rhizosphere soils

We thus confirmed the rhizosphere effect on AMF propagule multiplication, as spore counts (Table 2) were consistently higher in rhizosphere soils across all five locations than in non-rhizosphere soils. The maximum number of spores in the rhizosphere was recorded at Takli (1280 per 100 g), followed by Vinchur (1200), Sarolekhurd (1120),

Ranwad (880), and Khadakmalegaon (840). Non-rhizosphere counts ranged from 680 (Sarolekhurd) to 980 (Vinchur). The decrease in AMF sporulation in the non-rhizosphere soil indicates that it is largely driven by root exudates and distance to the host. These findings support previous reports that mycorrhizal spore density is higher in the rhizosphere due to a permanent carbon input from the host.

Table 2. AMF spore count per 100 g dry soil in rhizosphere and non-rhizosphere soils

Location	Spore count (rhizosphere)	Spore count (non-rhizosphere)
Vinchur	1200	980
Takli	1280	840
Khadakmalegaon	840	820
Sarolekhurd	1120	680
Ranwad	880	880

c) Root colonization and AMF structures

The stained soybean roots were then observed at a microscopic level for basic AMF structures, arbuscules (tree-like branching within cortical cells), vesicles (terminal stain in the cell that acts as storage), and extraradical hyphae (Fig. 1). Root colonization ranged from 42% to 78% across sites (data not shown, but images confirmed).

Younger roots had abundant arbuscules, suggesting that nutrient exchange was ongoing. Older roots were more of a propagule reservoir with vesicles common. The existence of these structures indicates that native AMF successfully establish effective symbioses with soybean in the field.

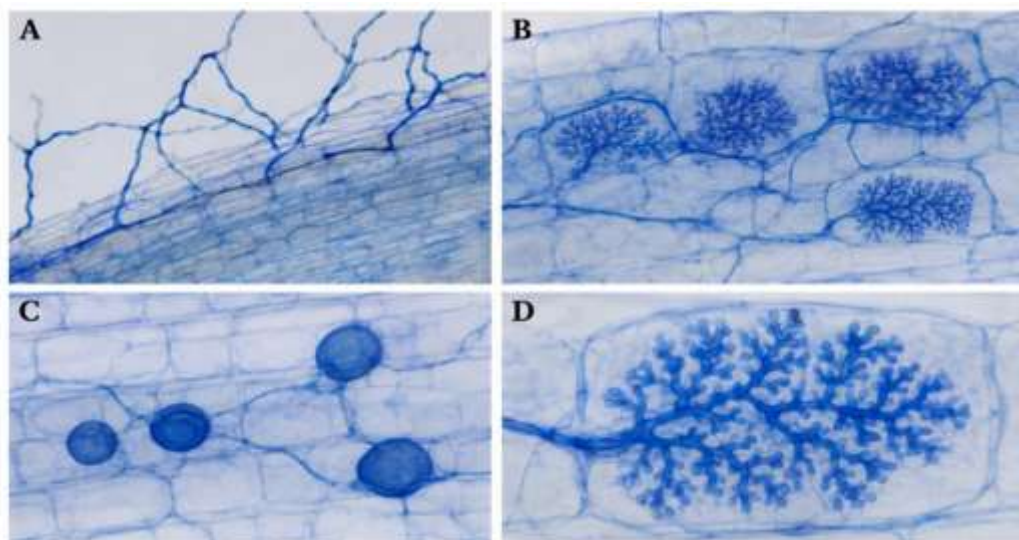


Fig. 1. Microscopic images of AMF structures in soybean roots stained with trypan blue. (A) Extraradical hyphae attached to the root surface; (B) Intercellular hyphae and arbuscules within cortical cells; (C) Vesicles (spherical structures); (D) Arbuscule showing typical dichotomous branching. Magnification 400 \times .

d) Effect of water stress and AMF on soybean growth

Even under water stress alone, soybean growth was inhibited (root length 9 cm, shoot length 21.5 cm, and leaf size)16. However, AMF inoculation under similar water stress dramatically improved root length (78% over CK: 16 cm), shoot length (56% over CK: 33.6 cm), and leaf size (100%: 6 cm), as well

as vegetative presence, enabling pod formation at this level (+1 cm). AMF's improved performance is attributed in part to enhanced water uptake via extraradical hyphae that explore soil pores beyond the root depletion zone, and in part to more efficient nutrient acquisition (mostly P), which supports osmotic adjustment.

Table 3. Effect of water stress and AMF inoculation on growth parameters of soybean

Treatment	Root length (cm)	Shoot length (cm)	Leaf size (cm)	Pod length (cm)
Water stress alone	9.0 \pm 1.2	21.5 \pm 2.1	3.0 \pm 0.5	-
Water stress + AMF	16.0 \pm 1.5**	33.6 \pm 2.8**	6.0 \pm 0.8**	4.0 \pm 0.6

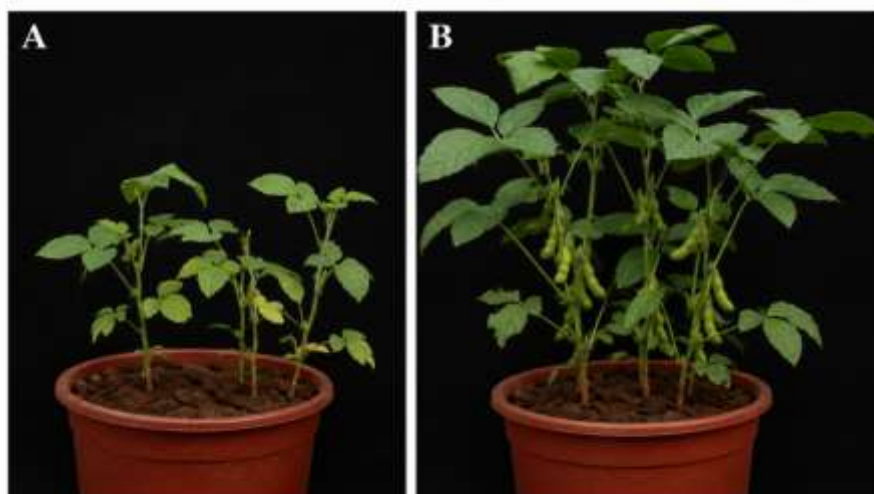


Fig. 2. Comparative growth of soybean under water stress with and without AMF inoculation. (A) Water stress alone: stunted plants, small leaves, no pods; (B) Water stress + AMF: vigorous growth, larger leaves, visible pods.

e) Effect of Salinity Stress and AMF on Soybean Growth

Soybean growth parameters were significantly reduced under salinity stress; however, AMF colonization effectively ameliorated these negative effects (Table 4). Our results indicated that under moderate salinity (50 mM NaCl), AMF-inoculated plants exhibited 35% and 42% higher root length (RL) and shoot dry weight (SDW), respectively, than non-mycorrhizal plants. However, salinity improved the survival of AMF-treated plants compared to controls:

after six weeks in 100 mM NaCl, shoot length was 12.4 cm (non-AMF) versus 22.8 cm (AMF), corresponding to an increase of about 84% under the stressful condition relative to non-AMF-treated plants. Under non-stress conditions, AMF treatment alone generated the highest overall growth performance. Possible mechanisms include reduced Na⁺ uptake, a higher K⁺/Na⁺ ratio (as observed in celery and lettuce), and improved mineral nutrition that supports photosynthesis.

Table 4. Effect of different NaCl concentrations and AMF inoculation on soybean growth

Treatment	Root length (cm)	Shoot length (cm)	Root dry weight (g/plant)	Shoot dry weight (g/plant)
Control (0 mM, non-AMF)	14.2 ± 1.1	28.5 ± 2.0	0.82 ± 0.07	2.45 ± 0.20
Moderate salinity (50 mM, non-AMF)	9.8 ± 0.9	18.3 ± 1.5	0.51 ± 0.05	1.52 ± 0.12
Moderate salinity (50 mM + AMF)	13.2 ± 1.0*	26.0 ± 1.8*	0.73 ± 0.06*	2.16 ± 0.18*
High salinity (100 mM, non-AMF)	6.5 ± 0.7	12.4 ± 1.2	0.32 ± 0.04	0.98 ± 0.10
High salinity (100 mM + AMF)	10.8 ± 0.9**	22.8 ± 1.6**	0.61 ± 0.05**	1.89 ± 0.15**
Mycorrhizal (0 mM + AMF)	16.5 ± 1.3	32.1 ± 2.2	0.96 ± 0.08	2.98 ± 0.25

*Significant difference from respective non-AMF salinity treatment at $p < 0.05$; * $p < 0.01$.

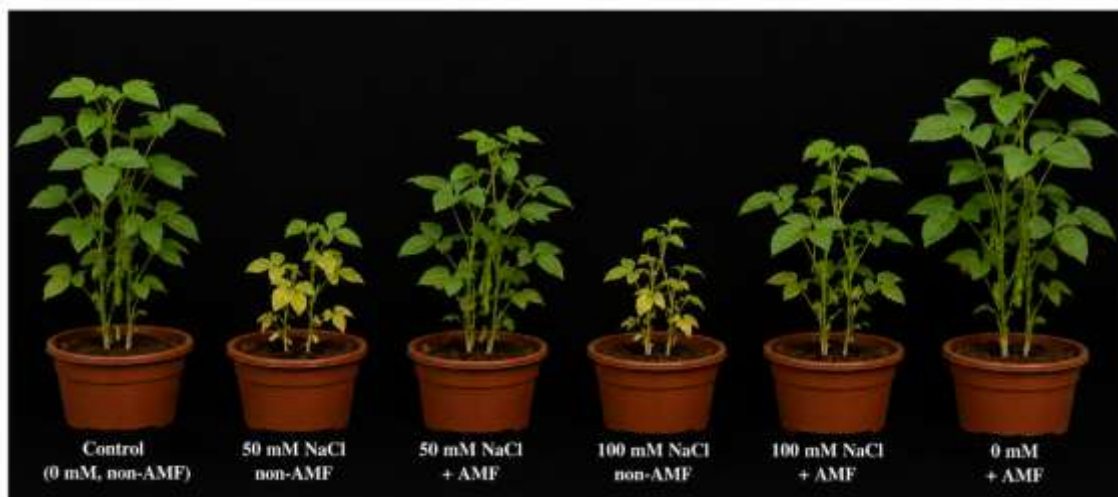


Fig. 3. Effect of salinity and AMF on soybean. Left to right: Control (0 mM, non-AMF); 50 mM NaCl, non-AMF; 50 mM NaCl + AMF; 100 mM NaCl, non-AMF; 100 mM NaCl + AMF; 0 mM + AMF. Note the progressive recovery with AMF inoculation under both salinity levels.

f) Pure culture multiplication

The trap culture method using sorghum successfully produced abundant AMF-colonized roots (Fig. 4). After three months, root colonization exceeded 80%, and spore

density in the potting mix exceeded 1500 per 100 g. This pure culture was effective in the subsequent stress experiments, confirming its viability and infectivity.

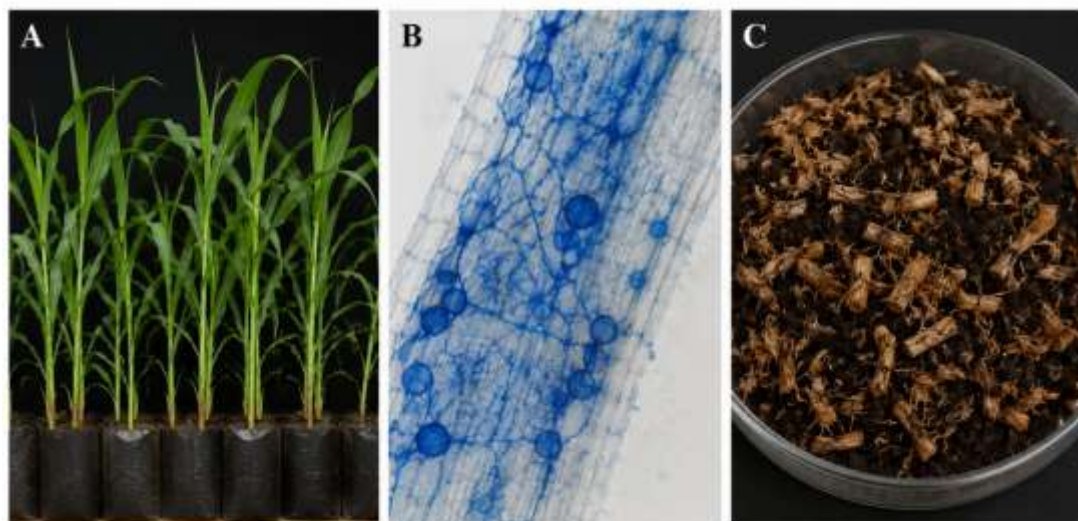


Fig. 4. Pure culture multiplication of native AMF using sorghum as host. (A) Sorghum plants at 60 days showing vigorous growth; (B) Stained sorghum root segments showing intensive AMF colonization (blue-stained hyphae and vesicles); (C) Air-dried colonized roots cut into pieces and mixed with vermicompost – ready-to-use bio-inoculant.

DISCUSSION

The greater spore counts in rhizosphere versus non-rhizosphere soils (Table 2) are consistent with the carbon-limited sporulation hypothesis for AMF in bulk soil. Soybean root exudation of AMF germination: Soybeans used flavonoids, such as genistein, to stimulate AMF spore germination and hyphal growth. Moderately high levels of soil phosphorus (145-171 kg/ha) did not completely inhibit sporulation, probably because native AMF populations are better adapted to local fertility conditions. The same results are mentioned for *Glomus* species in alkaline soils. An experiment conducted under water-stress conditions unequivocally shows that native AMF can enhance soybean drought tolerance. AMF-colonized plants further extend their roots to search for water resources, as evidenced by increased root dry weight (from 7 to 12 g/pot) and improvements in root length and surface area (from 9 cm to 16 cm). In addition, pod formation was restricted to AMF plants exposed to water stress, indicating an important trait associated with the benefits of reproductive development gained through AMF exploitation, even under conditions of limited water supply, a key factor for rainfed agriculture. At 50 and 100 mM NaCl, AMF alleviated salinity-induced growth inhibition.

The mechanism is thought to involve preferential K^+ uptake relative to Na^+ and dilution of the Na^+ fraction through biomass increase. AMF showed a significantly better performance over NM plants under high salinity, essentially in this extreme physiological condition at 100 mM salinity stage, while NM plants were severely stunted (shoot length 12.4 cm) with respect to the control 24. In terms of comparability, AMF plants exhibited growth similar to that of the control plant, with a shoot length of 22.8 cm. This is consistent with meta-analyses reporting that AMF have the strongest effects under moderate-to-high environmental stresses. The pure culture multiplication using sorghum is a low-cost, farmer-friendly method. Sorghum is a highly mycotrophic host and produces abundant root biomass, making it ideal for large-scale AMF inoculum production.

CONCLUSION

This test exhaustively surveyed the proficiency of local AMF sequestered from five soybean growing geographies of Niphad Tehsil. Physico-chemical analysis of soil indicated alkaline to slightly alkaline pH, moderate organic carbon, high available phosphorus, and variable potash. In soil, spore density was always higher in

rhizosphere (840-1280 per 100 g) than in non-rhizosphere soils (680-980 per 100 g), which conformed to the rhizosphere effect. Analysis of the root colonization confirmed by Trichoderma species AMF structures (arbuscules, vesicles, and extraradical hyphae) remained active and thus marked a functional symbiosis. Native-AMF significantly improved soybean growth under two major abiotic stresses (control conditions). A total of 436 unique OTUs belonging to 47 genera were detected in the roots and rhizosphere soil. AMF alleviated the inhibitory effects of moderate (50 mM NaCl) and high (100 mM NaCl) salinity on shoot length, more so by 42% and 84%, respectively, as compared to non-mycorrhizal stressed plants. A pure culture of native AMF was multiplied on sorghum as a trap host that may serve as an inexpensive bio-inoculant. However, it can be concluded that native AMF populations of Niphad Tehsil are effective soybean symbionts in terms of enhancing plant growth and productivity and abiotic stress. Such native AMF could also be applied in the field either alone or blended with lower dosages of chemical fertilizers as a bio-inoculant to achieve sustainability for the soybean production system, especially in those areas that are rainfed and salt-prone regions. Molecular tools will help us to identify the dominant AMF species, and future research should test these potential obligate mutualists under multi-location field trials.

Declaration by Authors

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