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RESEARCH ARTICLE

Influence of saline-tolerant arbuscular mycorrhizal fungi on rice growth under varying salinity levels

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Abstract

An investigation was conducted from 2022 to 2024 examined native arbuscular mycorrhizal (AM) fungi in the saline wetland rice ecosystems of the Aghanashini and Kagal regions in the Uttara Kannada district, Karnataka, India. Between the two sites, Aghanashini recorded higher AM fungal diversity across all diversity indices, including the Shannon-Wiener diversity index, Margalef's species richness index and Simpson's dominance index. Three efficient AM fungal isolates–UASDAMFAG8 (*Acaulospora mellea)*, UASDAMFAG10 (*Glomus macrocarpum*) and UASDAMFAG28 (*Glomus aggregatum*)–were selected based on their superior performance in phosphorus uptake, total dry biomass production and peroxidase activity in rice grown under salinity levels of 6 and 8 dS m^{-1} respectively. These isolates were evaluated individually and in combination under controlled microcosm conditions. The AM fungal consortium (*G. macrocarpum* + *A. mellea* + *G. aggregatum*) significantly enhanced plant growth parameters, mycorrhizal root colonization, total glomalin content, relative chlorophyll content, proline accumulation, phosphorus uptake and soil enzyme activities compared to the uninoculated control (UICNotably, the consortium increased proline content in rice to 13.47 and 14.06 µmol g^{-1} fresh weight (FW) under 6 and 8 dS m^{-1} salinity respectively. Among individual inoculants, *G. macrocarpum* showed the most pronounced effects across all measured parameters. The statistical interaction between AM fungi and salinity stress revealed that the AM fungal consortium effectively mitigated the adverse effects of salinity at both levels. These findings suggest that native AM fungal isolates, particularly in consortium form, can be exploited for enhancing salt tolerance and promoting sustainable rice cultivation in saline wetland ecosystems.

Keywords: consortium; diversity; isolates; microcosm

Introduction

Rice (*Oryza sativa* L.) is the most widely consumed staple crop globally, serving as a primary source of calories and protein, particularly in South and Southeast Asia (1). However, the increasing problem of soil salinity, coupled with rapid global population growth, poses a significant challenge to ensuring sufficient rice production in these regions (2). Current estimates suggest that salinity causes approximately 50 % yield loss in saline rice-growing areas (3). Rice is inherently sensitive to salinity, especially during its vegetative and reproductive phases, with elevated salt levels in the soil leading to substantial declines in productivity. Salinity stress primarily induces osmotic and ionic imbalances, which in turn provoke oxidative stress, disrupt cellular homeostasis and impair critical physiological and biochemical processes. These include reductions in photosynthetic efficiency, alterations in enzymatic activities and disturbances in mineral nutrient uptake. Collectively, these stress responses adversely affect plant growth and development, resulting in poor spikelet formation and significantly reduced grain yield (4, 5).

Arbuscular mycorrhizal fungi (AMF) are specialised symbiotic organisms belonging to the phylum Glomeromycota that colonise plant roots and establish mutually beneficial associations with their host plants. The host plant provides the fungi with carbohydrates produced through photosynthesis, while the fungi use their extensive hyphal network in the soil to enhance water and nutrient uptake by the plant (6). The term mycorrhiza was first introduced in 1885 by the German botanist Albert Bernhard Frank and is derived from the Greek words mykēs (fungus) and rhiza (root).

Arbuscular mycorrhizal fungi have emerged as promising biological agents for alleviating the detrimental effects of salinity stress in plants. They are well known for improving plant nutrient acquisition, enhancing water uptake efficiency and enhancing plant tolerance to various abiotic stresses, including salinity (7). Although AMF are obligatory aerobes, they have also been reported in waterlogged and saline ecosystems such as mangroves, marshes and salt-affected wetlands, indicating their remarkable adaptive capacity in challenging environmental conditions (8).

All specific statements in this paragraph should be supported with appropriate references, including the cultivation of Kagga rice in the Aghanashini estuarine region, its reported salinity tolerance (EC = 14 dS m^{-1}) and the described morphological traits. The claim regarding limited research on AMF colonisation in saline lowland rice systems should also be substantiated with relevant citations. If any of these details are based on observations from the present study, this should be clearly indicated in the text.

It is hypothesized that saline wetland ecosystems differ in arbuscular mycorrhizal (AM) fungal diversity and that native AM fungal inoculation enhances rice growth, nutrient acquisition and physiological resilience under salinity stress. Further, it is postulated that AM fungal consortia confer greater benefits than individual isolates by improving mycorrhizal colonisation, osmoprotectant accumulation, antioxidant activity and soil biological functions. By harnessing native AM fungal diversity, this research seeks to develop sustainable and eco-friendly strategies to mitigate salinity stress and enhance rice productivity in salt-affected wetland ecosystems.

Materials and Methods

The present study was conducted from 2022 to 2024 at the Department of Microbiology, University of Agricultural Sciences, Dharwad, Karnataka. Native AM fungal isolates were obtained from rhizosphere soil samples collected from saline wetland rice ecosystems of the Aghanashini (EC 11.46 dS m^{-1}) and Kagal (EC 15.50 dS m^{-1}) regions in the Aghanashini River estuary, Uttara Kannada District. Initial soil physicochemical and biological properties were analysed.

Collection of samples

Rhizosphere soil samples were collected from two different saline wetland rice ecosystems with distinct salinity levels. Prior to soil sampling, the salinity of the sampling sites was analyzed using EC meter (Compact conductivity meter LAQUAtwin-EC-22). Three healthy plants were selected from three different positions in the field. Soil and root samples were taken from a depth of 0-25 cm, in polyethylene bags and brought to the laboratory. The rhizosphere soil of the three healthy plants at flowering stage from each site was pooled to form composite sample. Samples were then divided into three parts, (i) for isolation and enumeration of initial AM spores, (ii) for preparing trap cultures and (iii) for soil analysis. The samples were stored under refrigerated conditions.

Soil analysis

Soil pH was measured in 1:2.5 soil–water suspension using a pH meter. Electrical conductivity was measured using EC meter (Compact conductivity meter LAQUAtwin-EC-22). Walkley-Black rapid titration method was used to estimate organic carbon content. Total nitrogen (N) was assessed by micro-Kjeldahl method. Available phosphorus (P) was estimated using Bray and Kurtz method. Available potassium (K) was determined by the ammonium acetate method (10).

Soil biological parameters

The initial microbial biomass carbon was estimated by the chloroform fumigation–extraction method (11). Soil total glomalin content was measured by extraction and quantification (12). Root colonization by AMF was determined by washing roots in tap water, cutting into 1-cm fragments, clearing with 10 % KOH and staining with Trypan blue (13).

Isolation and identification of arbuscular mycorrhizal spores

Spores were isolated from rhizosphere soil samples using wet sieving and decanting method (14). The single spore was extracted from the respective funnels in which each morphotypes were multiplied and were mounted on clean glass slides in lactophenol cotton blue and examined under a Motic research compound microscope (B1-Series). Morphological identification of the spores was carried out by using the culture database established by International Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM).

Diversity studies and statistical analysis

Mycorrhizal diversity within each ecosystem was analysed separately using the following ecological indices:

(A) Species richness (S)

Species richness was determined as the total number of AM fungal species recorded in each ecosystem.

(B) Shannon–Wiener diversity index (H') (15)

The Shannon–Wiener diversity index was calculated using the formula:

$$H' = -\sum_{i=1}^{S} \left(\frac{n_i}{N}\right) \ln\left(\frac{n_i}{N}\right)$$

Where:

ni = number of individuals belonging to the i^{th} species

N = total number of individuals of all species

(C) Margalef's species richness index (d) (16)

Margalef's richness index was computed as:

$$d = \frac{S-1}{\ln N}$$

Where:

S = total number of species

N = total number of individuals

ln = natural logarithm ($\log_e$)

(D) Simpson's dominance index (D) (17)

Simpson's dominance index was calculated using the formula:

$$D = \sum \left(\frac{n_i}{N}\right)^2$$

Where:

ni = number of individuals belonging to the i^{th} species

N = total number of individuals in the sample

Rapid screening of native arbuscular mycorrhizal fungal isolates to assess their phosphorus uptake under varied saline conditions

Single pre-colonized paddy seedlings inoculated with native AMF cultures were transplanted individually into plastic cups and maintained under two saline wetland conditions: moderate salinity (6 dS m^{-1} EC) and high salinity (8 dS m^{-1} EC), achieved using saline solutions. The treatment details included the native AMF isolates and a control without AMF. These treatments were laid out in a completely randomized design (CRD) design and replicated three times. Thirty native AMF isolates were screened based on P uptake (18), peroxidase activity (19) and dry biomass accumulation, all of which were assessed through destructive sampling. Top three promising AMF isolates which showed higher P uptake, higher antioxidant activity and biomass were selected for further validation under microcosm studies.

Assessing the plant growth and physiological response of mycorrhized rice plants under varied saline conditions in microcosms

Based on the rapid screening, top three native AM fungal isolates *viz Glomus macrocarpum* (UASDAMFAG10)*, Acaulospora mellea* (UASDAMFAG8) and *Glomus aggregatum* (UASDAMFAG28) were selected for further studies under varied saline conditions in microcosms. Each pot was filled with 4 kg of sterilised soil collected from the paddy fields of the Aghanashini River estuary and pre-colonized paddy seedlings containing native AMF were transplanted into the respective pots. There were 10 treatments with three replications. The crop used was rice of the variety 'Kagga'. Fertilizer was applied at a rate of 100:50:50 kg ha^{-1} for N:P:K. The experiment was conducted under microcosm conditions.

The treatments included five AMF inoculation levels and two salinity levels, making a total of 10 treatment combinations, each replicated three times in a factorial CRD. The AMF treatments were:

- M_1: *G. macrocarpum*
- M_2: *A. mellea*
- M_3: *G. aggregatum*
- M_4: A consortium of *G. macrocarpum*, *A. mellea* and *G. aggregatum*
- M_5: Uninoculated control (UIC)

The salinity levels were:

- S_1: Moderate saline soil (EC 6 dS m^{-1})
- S_2: High saline soil (EC 8 dS m^{-1})

This setup allowed for evaluation of AMF performance under different salinity conditions in rice cultivation.

Plant growth parameters were recorded at the flowering stage. Measurements included plant height, number of tillers, chlorophyll content (measured using a SPAD meter), proline content (20), glomalin content, root colonisation and soil enzyme activities such as dehydrogenase (21) and phosphatase (22). Root traits, including average root diameter, root surface area and root volume, were analysed using a WinRHIZO scanner and plant P content was estimated following standard protocols.

Statistical analysis

The observations were recorded, tabulated and the final data were statistically analysed using a factorial CRD at $p < 0.01$ with OPSTAT online software.

Results and Discussion

Preliminary assessment of the physicochemical and biological properties of soils from saline wetland rice ecosystems was carried out prior to the experiment and the results are presented in Table 1. Variations in soil salinity and fertility parameters created distinct ecological conditions, influencing AM fungal diversity and functionality.

Morphological characterization and diversity of native arbuscular mycorrhizal fungi

A total of thirty AM fungal species were identified from saline wetland rice rhizosphere soils. Of these, twenty-two species belonged to the genus *Glomus*, six to *Acaulospora* and two to *Septoglomus* (Table 2). The predominance of *Glomus* species under saline conditions has been reported earlier and is attributed to their ecological plasticity and higher tolerance to abiotic stress (23, 24).

The highest number of AM fungal species (ten) was recorded in the rice rhizosphere of Aghanashini at a salinity level of 11.46 dS m^{-1}, whereas only eight species were recorded in Kagal at a higher salinity level of 15.50 dS m^{-1} (Table 3). This reduction in species number with increasing salinity suggests that salt stress exerts a strong selective pressure on AM fungal communities, favouring only stress-tolerant taxa (25).

Diversity indices of arbuscular mycorrhizal fungi

The diversity indices presented in Table 4 reveal clear differences in AM fungal communities between the two study sites. The Shannon–Wiener diversity index was higher in the Aghanashini rice rhizosphere (1.84) than in Kagal (1.52), indicating greater AM fungal diversity under comparatively lower salinity conditions. Margalef's species richness index was also higher in Aghanashini (d = 1.56) compared to Kagal (d = 1.06), reflecting a richer AM fungal community. Simpson's dominance index showed higher values in Aghanashini (0.79) than in Kagal (0.72), suggesting greater dominance of certain well-adapted AM fungal species such as *Glomus multicaule*, *Glomus fasciculatum*, *Glomus etunicatum*, *Glomus* spp., *G. macrocarpum*, *Glomus nicolsonii*, *A. mellea*, *Acaulospora morrowae*, and *G. aggregatum* under lower salinity conditions. Overall, these findings confirm that salinity significantly influences AM fungal diversity, species richness and dominance patterns (25)

Rapid screening of native arbuscular mycorrhizal fungal isolates under saline conditions

Rapid screening of native AM fungal isolates under two salinity levels (6 and 8 dS m^{-1}) revealed significant variation in P uptake, dry biomass accumulation and peroxidase activity among treatments (Table 5At 6 dS m^{-1}, isolate UASDAMFAG8 exhibited the highest peroxidase activity (10.00 U mg^{-1} protein), whereas at 8 dS m^{-1}, UASDAMFAG28 showed the highest activity (12.70 U mg^{-1} protein). The non-mycorrhizal controls (UIC) recorded the lowest peroxidase activity, with values of 3.90 and 5.10 U mg^{-1} protein at 6 and 8 dS m^{-1} respectively. Increased peroxidase activity in AMF-inoculated plants indicates enhanced antioxidant defense mechanisms that help mitigate salinity-induced oxidative stress (26).

Table 1. Geographical location, physico-chemical characteristics and biological properties of the study area

Sl. No.	Name of soil physicochemical properties	Aghanashini (Uttara Kannada District)	Kagal (Uttara Kannada District)
Location	Latitude	14˚52′ 25.09″	14˚ 50′ 27″
	Longitude	74° 37′ 22.57″	74° 37′ 18.4″
1	pH	5.40	4.10
2	EC (dS m^{-1})	11.46	15.50
3	Organic carbon (%)	0.97	0.80
4	Available N (kg ha^{-1})	214.00	175.00
5	Available P (kg ha^{-1})	43.53	13.22
6	Available K (kg ha$^{-1)}$	304.60	276.00
7	Soil microbial biomass carbon (µg g^{-1} soil)	193.00	207.00
8	Glomalin content (mg g^{-1} soil)	0.44	0.51
9	AM fungal spore count (No. of spores 50 g^{-1}soil)	105.00	125.00
10	AM fungal root colonization (%)	11.00	14.00
11	Dehydrogenase activity (µg TPF formed g-^{1} soil d^{-1})	61.66	69.23
12	Phosphatase activity (µg pNP released g^{-1} soil h^{-1})	19.64	44.64

Table 2. Morphological characterisation of native arbuscular mycorrhizal fungal morphotypes from saline wetland rice rhizosphere

Sl. No.	Isolate code	Salinity level	Shape	Colour	Spore surface	Spore size (µm)	Spore wall size (µm)	No of walls	Mean hyphal size (µm)	AM fungal species (Tentative identification)
1	UASDAMAG1	11.46	Round	Honey	Rough	103.4	-	-	39.5	*Glomus multicaule*
2	UASDAMFAG2	11.46	Round	Orange	Smooth	157.0	6.00	2	101.9	*Glomus fasciculatum*
3	UASDAMFAG3	11.46	Round	Honey	Rough	124.4	7.50	2	-	*Glomus etunicatum*
4	UASDAMFAG4	11.46	Oval	Honey	Smooth	141.2	-	-	-	*Glomus* spp.
5	UASDAMFAG5	11.46	Round	Light brown	Smooth	182.0	7.50	3	-	*G. macrocarpum*
6	UASDAMFAG6	11.46	Round	Yellow	Rough	87.6	4.70	1	-	*Acaulospora nicolsonii*
7	UASDAMFAG7	11.46	Round	Dark brown	Rough	50.8	-	-	-	*Glomus* spp.
8	UASDAMFAG8	11.46	Round	Light yellow	Rough	80.4	4.74	1	-	*A. mellea*
9	UASDAMFAG9	11.46	Round	Light yellow	Rough	69.2	5.20	1	8.2	*A. mellea*
10	UASDAMFAG10	11.46	Round	Light brown	Smooth	184.2	5.10	3	49.3	*G. macrocarpum*
11	UASDAMFAG11	11.46	Round	Light yellow	Rough	40.8	2.80	1	-	*Acaulospora morrowae*
12	UASDAMFAG12	11.46	Oval	Yellow	Smooth	61.2	3.30	3	33.9	*A. mellea*
13	UASDAMFAG13	11.46	Round	Light yellow	Rough	49.4	3.10	1	-	*Acaulospora morrowae*
14	UASDAMFAG14	11.46	Round	Light yellow	Rough	44.4	4.00	1	9.6	*G. aggregatum*
15	UASDAMFAG15	11.46	Round	Orange	Smooth	129.8	3.50	2	23.3	*Glomus etunicatum*
16	UASDAMFAG16	15.5	Round	Light brown	Rough	184.4	4.00	2	96.2	*Glomus albidum*
17	UASDAMFAG17	15.5	Round	Honey	Rough	156.0	7.40	2	65.8	*Glomus diaphanum*
18	UASDAMFAG18	15.5	Round	Yellow	Smooth	135.8	-	-	52.8	*Glomus leptotichum*
19	UASDAMFAG19	15.5	Round	Yellow	Smooth	138.4	3.20	3	76.2	*Glomus leptotichum*
20	UASDAMFAG20	15.5	Round	Light brown	Rough	281.0	16.10	1	-	*Glomus* spp.
21	UASDAMFAG21	15.5	Round	Light yellow	Rough	133.0	9.40	2	36.9	*Glomus deserticola*
22	UASDAMFAG22	15.5	Round	Yellow	Rough	71.4	3.90	1	-	*Glomus aggregatum*
23	UASDAMFAG23	15.5	Round	Light brown	Rough	77.8	4.70	2	27.8	*G. aggregatum*
24	UASDAMFAG24	15.5	Round	Light yellow	Smooth	74.2	1.60	2	37.1	*G. aggregatum*
25	UASDAMFAG25	15.5	Round	Brown	Rough	67.0	2.60	1	-	*Septoglomus* spp.
26	UASDAMFAG26	15.5	Round	Light brown	Rough	76.0	-	-	-	*Septoglomus* spp.
27	UASDAMFAG27	15.5	Oval	Yellow	Smooth	46.8	4.40	2	13.2	*Glomus citricolum*
28	UASDAMFAG28	15.5	Round	Yellow	Rough	77.2	1.60	3	36.3	*G. aggregatum*
29	UASDAMFAG29	15.5	Round	Light yellow	Rough	54.4	4.70	2	-	*Glomus citricolum*
30	UASDAMFAG30	15.5	Round	Honey	Rough	39.6	1.00	2	-	*G. aggregatum*

Table 3. Species of arbuscular mycorrhizal fungi present in different salt-affected areas of saline wetland rice ecosystems

Sl. No.	Aghanashini (EC 11.46 dS m^{-1}) (Uttara Kannada District)	Sl. No.	Kagal (EC 15.50 dS m^{-1}) (Uttara Kannada District)
1	*G. multicaule*	1	*Glomus albidum*
2	*G. fasciculatum*	2	*Glomus diaphanum*
3	*G. etunicatum*	3	*Glomus leptotichum*
4	*Glomus* spp.	4	*Glomus* spp.
5	*G. macrocarpum*	5	*Glomus deserticola*
6	*G. nicolsonii*	6	*G. aggregatum*
7	*Glomus* spp.	7	*Septoglomus* spp.
8	*A. mellea*	8	*Glomus citricolum*
9	*A. morrowae*		
10	*G. aggregatum*		
Total	10		8

Table 4. Diversity of arbuscular mycorrhizal fungi in the rhizosphere of rice in varied saline wetland ecosystems

Parameter	**Aghanashini (EC 11.46 dS m^{-1}) (Uttara Kannada District)**	**Kagal (EC 15.50 dS m^{-1}) (Uttara Kannada District)**
Number of species (S)	10.00	8.00
Total number of individuals of all species (N)	324.00	724.00
Shannon's diversity index (H')	1.84	1.52
Species richness index (Margalef's richness index, d)	1.56	1.06
Simpon's dominance index (D)	0.79	0.72

Table 5. Rapid screening and selection of efficient native arbuscular mycorrhizal fungal isolates under 6 and 8 dS m^{-1} salinity based on phosphorus uptake, total dry biomass and peroxidase activity in paddy

Sl. No.	**Isolate code**	**EC 6 dS m^{-1}**			**EC 8 dS m^{-1}**		
		P uptake (mg $plant^{-1}$)	**Total dry matter (g $plant^{-1}$)**	**Peroxidase activity (U mg^{-1} protein)**	**P uptake (mg $plant^{-1}$)**	**Total dry matter (g $plant^{-1}$)**	**Peroxidase activity (U mg^{-1} protein)**
1	UASDAMFAG1	0.80	0.40	6.00	0.70	0.30	7.90
2	UASDAMFAG2	0.70	0.29	7.00	0.60	0.22	6.80
3	UASDAMFAG3	0.70	0.47	4.50	0.60	0.40	5.40
4	UASDAMFAG4	0.60	0.25	4.70	0.40	0.21	6.90
5	UASDAMFAG5	1.60	0.55	4.20	1.30	0.51	6.80
6	UASDAMFAG6	0.80	0.49	8.30	1.20	0.45	9.00
7	UASDAMFAG7	1.20	0.40	7.10	0.70	0.33	8.60
8	UASDAMFAG8	1.50	0.60	10.00	1.30	0.58	12.40
9	UASDAMFAG9	1.20	0.60	9.00	1.30	0.55	10.40
10	UASDAMFAG10	1.70	0.60	9.80	1.50	0.57	12.60
11	UASDAMFAG11	1.00	0.38	6.60	0.40	0.21	8.50
12	UASDAMFAG12	0.90	0.43	5.70	0.80	0.27	7.90
13	UASDAMFAG13	1.40	0.46	4.40	0.60	0.28	9.90
14	UASDAMFAG14	1.50	0.62	6.10	1.10	0.40	8.00
15	UASDAMFAG15	1.50	0.49	5.40	0.70	0.38	11.10
16	UASDAMFAG16	0.50	0.25	7.70	0.40	0.19	8.30
17	UASDAMFAG17	1.00	0.41	4.40	0.90	0.34	5.80
18	UASDAMFAG18	1.30	0.45	7.40	0.90	0.90	0.44
19	UASDAMFAG19	1.30	0.47	5.40	0.70	0.70	0.27
20	UASDAMFAG20	1.30	0.40	9.30	1.20	1.20	0.41
21	UASDAMFAG21	0.90	0.45	8.10	0.80	0.80	0.38
22	UASDAMFAG22	1.00	0.45	9.20	0.90	0.90	0.40
23	UASDAMFAG23	1.50	0.59	5.90	1.20	1.20	0.48
24	UASDAMFAG24	1.60	0.62	9.00	1.00	1.00	0.45
25	UASDAMFAG25	1.30	0.38	6.50	1.10	1.10	0.53
26	UASDAMFAG26	1.60	0.60	7.00	0.60	0.60	0.36
27	UASDAMFAG27	1.20	0.55	4.30	1.00	1.00	0.41
28	UASDAMFAG28	1.80	0.64	9.10	1.60	1.60	0.59
29	UASDAMFAG29	1.50	0.60	6.50	1.20	1.20	0.57
30	UASDAMFAG30	1.10	0.59	4.40	0.80	0.80	0.34
31	UIC	0.20	0.15	3.90	0.10	0.10	0.13
	S.Em. ±	0.04	0.01	0.10			
	C.D. (p = 0.01)	0.12	0.03	0.29			

A comparable trend was observed for dry biomass accumulation. Isolate UASDAMFAG28 produced the highest biomass (0.64 and 0.59 g $plant^{-1}$ at 6 and 8 dS m^{-1}), followed by UASDAMFAG8 (0.60 and 0.58 g $plant^{-1}$) and UASDAMFAG10 (0.60 and 0.57 g $plant^{-1}$). The non-inoculated plants (UIC) recorded the lowest biomass (0.15 and 0.13 g $plant^{-1}$). Enhanced biomass production in AMF-inoculated plants is associated with improved nutrient uptake and better carbon assimilation under saline stress (7, 26).

Phosphorus uptake was highest in plants inoculated with UASDAMFAG28 (1.80 and 1.60 mg $plant^{1}$ at 6 and 8 dS m^{-1} respectively) followed by UASDAMFAG10 (1.70 and 1.50 mg plant ^{1}) and UASDAMFAG8 (1.50 and 1.30 mg $plant^{-1}$), while the non-inoculated control (UIC) exhibited the lowest uptake. Improved P uptake is a well-documented benefit of AM symbiosis, resulting from increased root absorptive surface and phosphatase activity (26).

Based on consistent superior performance, UASDAMFAG28, UASDAMFAG10 and UASDAMFAG8 were selected for further evaluation under microcosm conditions (Fig. 1).

Table 6. Plant height and number of tillers of rice as influenced by selected efficient arbuscular mycorrhizal fungal isolates at different salinity levels

	Plant height (cm)			**Number of tillers plant^{-1}**		
	Flowering stage			**Flowering stage**		
AMF	**S_1**	**S_2**	**Mean of M**	**S_1**	**S_2**	**Mean of M**
G. macrocarpum	79.45	74.17	76.81	5.37	4.47	4.92
A. mellea	72.23	69.73	70.98	4.91	3.95	4.43
G. aggregatum	73.7	71.6	72.65	5.16	4.18	4.67
G. macrocarpum + A. mellea+ G. aggregatum	88.6	82.83	85.71	6.37	5.07	5.72
UIC	53.17	47.83	50.5	3.02	2	2.51
Mean S	73.43	69.23		4.96	3.93	
	S.Em. ±	C.D. (p =0.01)		S.Em. ±	C.D. (*p* =0.01)	
C.D of M (AMF)	0.5	1.48		0.04	0.13	
C.D of S (salinity)	0.31	0.93		0.03	0.08	
C.D of M*S	0.7	2.09		0.06	0.18	

Note- Salinity level 1 (S_1) = 6 dS m^{-1} EC , Salinity level 2 (S_2) = 8 dS m^{-1} EC , M= Arbuscular mycorrhizal fungal isolates

Table 7. Relative chlorophyll content and proline content of rice as influenced by selected efficient arbuscular mycorrhizal fungal isolates under different salinity levels

Treatment	**Relative chlorophyll content (SPAD values)**			**Proline (µmol g^{-1} FW)**		
	Flowering stage			**Flowering stage**		
AMF	**S_1**	**S_2**	**Mean of M**	**S_1**	**S_2**	**Mean of M**
G. macrocarpum	40.40	38.14	39.27	7.77	12.49	10.13
A. mellea	37.95	36.51	37.23	5.93	10.84	8.39
G. aggregatum	38.51	37.63	38.07	6.77	11.52	9.15
G. macrocarpum + A. mellea + G. aggregatum	42.56	39.32	40.94	13.47	14.06	13.77
UIC	32.16	31.69	31.92	3.12	4.59	3.86
Mean S	38.32	36.66		7.41	10.70	
	S.Em. ±	C.D. (*p* = 0.01)		S.Em. ±	C.D. (*p* =0.01)	
C.D of M (AMF)	0.33	0.97		0.28	0.82	
C.D of S (salinity)	0.21	0.61		0.17	0.52	
C.D of M*S	0.46	1.37		0.39	1.16	

Table 8. Mycorrhizal parameters as influenced by selected efficient arbuscular mycorrhizal fungal isolates at different salinity levels in rice

Treatment	**Per cent root colonization (%)**			**Total glomalin content (mg g^{-1} soil)**		
	Flowering stage			**Flowering stage**		
AMF	**S_1**	**S_2**	**Mean of M**	**S_1**	**S_2**	**Mean of M**
G. macrocarpum	54.67	50.33	52.50	0.161	0.123	0.142
A. mellea	50.50	41.17	45.83	0.147	0.102	0.124
G. aggregatum	52.00	44.00	48.00	0.155	0.110	0.132
G. macrocarpum + A. mellea + G. aggregatum	62.33	59.00	60.67	0.177	0.170	0.174
UIC	22.00	18.00	20.00	0.092	0.054	0.073
Mean S	48.30	42.50		0.146	0.112	
	S.Em. ±	C.D. (*p* = 0.01)		S.Em. ±	C.D. (*p* = 0.01)	
C.D of M (AMF)	0.56	1.66		0.004	0.011	
C.D of S (salinity)	0.35	1.05		0.002	0.007	
C.D of M*S	0.79	2.35		0.005	0.015	

Table 9. Soil enzyme activity as influenced by selected efficient arbuscular mycorrhizal fungal isolates at different salinity levels in rice

Treatment	**Dehydrogenase activity (µg TPF formed g^{-1} soil d^{-1})**			**Phosphatase activity (µg pNP released g^{-1} soil h^{-1})**		
	Flowering stage			**Flowering stage**		
AMF	**S_1**	**S_2**	**Mean of M**	**S_1**	**S_2**	**Mean of M**
G. macrocarpum	87.48	80.10	83.79	75.94	67.89	71.91
A. mellea	83.91	75.70	79.80	67.02	55.94	61.48
G. aggregatum	85.14	77.24	81.19	68.67	57.09	62.88
G. macrocarpum + A. mellea + G. aggregatum	93.94	92.57	93.26	81.61	74.54	78.07
UIC	53.13	45.27	49.20	52.08	48.89	50.48
Mean S	80.72	74.18		69.06	60.87	
	S.Em. ±	C.D. (*p* = 0.01)		S.Em. ±	C.D. (*p* = 0.01)	
C.D of M (AMF)	0.48	1.42		0.59	1.75	
C.D of S (salinity)	0.30	0.90		0.37	1.11	
C.D of M*S	0.67	2.00		0.84	2.48	

Table 10. Root surface area and root volume of rice as influenced by selected efficient arbuscular mycorrhizal fungal isolates at different salinity levels

Treatment	**Root surface area (cm^2)**			**Root volume (cm^3)**		
	Flowering stage			**Flowering stage**		
AMF	**S_1**	**S_2**	**Mean of M**	**S_1**	**S_2**	**Mean of M**
G. macrocarpum	190.90	183.30	187.10	2.30	1.87	2.08
A. mellea	182.23	163.00	172.62	1.81	1.67	1.74
G. aggregatum	186.63	167.90	177.27	1.97	1.68	1.82
G. macrocarpum + *A. mellea* + *G. aggregatum*	229.40	214.20	221.80	2.77	2.27	2.52
UIC	108.03	100.30	104.17	1.37	1.33	1.35
Mean S	179.44	165.74		2.04	1.76	
	S.Em. ±	C.D. ($p = 0.01$)		S.Em. ±	C.D. ($p = 0.01$)	
C.D of M (AMF)	1.58	4.69		0.05	0.15	
C.D of S (salinity)	1.00	2.97		0.03	0.10	
C.D of M*S	2.23	6.64		0.07	0.22	

Table 11. Phosphorus content influenced by selected efficient arbuscular mycorrhizal fungal isolates at different salinity levels in rice

Treatment	**P concentration in plants (%)**		
	Flowering stage		
AMF	**S_1**	**S_2**	**Mean of M**
G. macrocarpum	0.51	0.447	0.478
A. mellea	0.45	0.393	0.422
G. aggregatum	0.465	0.41	0.438
G. macrocarpum + *A. mellea* + *G. aggregatum*	0.72	0.617	0.668
UIC	0.343	0.293	0.318
Mean S	0.498	0.432	
	S.Em. ±	C.D. (p=0.01)	
C.D of M (AMF)	0.006	0.018	
C.D of S (salinity)	0.004	0.011	
C.D of M*S	0.008	0.025	

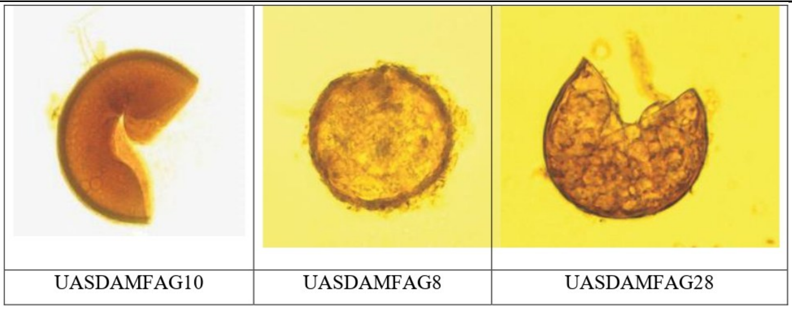

Fig. 1. Selected native mycorrhizal isolates based on their phosphorus uptake, total dry biomass and peroxidise activity in paddy (Microscopic image captured using a Motic microscope at 40× magnification).

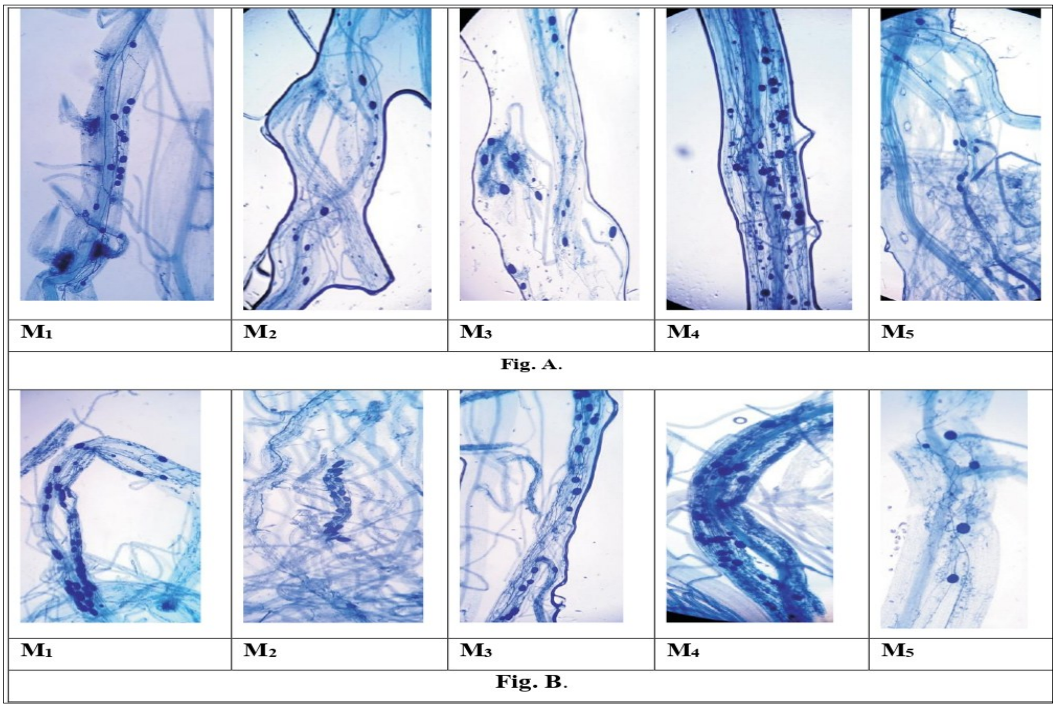

Fig.2. Effect of arbuscular mycorrhizal fungi on root colonization of rice under different salinity (Microscopic image captured using a Motic microscope at 10 × magnification).Levels: (A) 6 dS m^{-1} and (B) 8 dS m^{-1}.(M$_1$- *G. macrocarpum*, M$_2$- *A. mellea* ,M$_3$- *G. aggregatum*, M$_4$- Consortium of M$_1$+M$_2$+M$_3$, M$_5$-Un inoculated control).

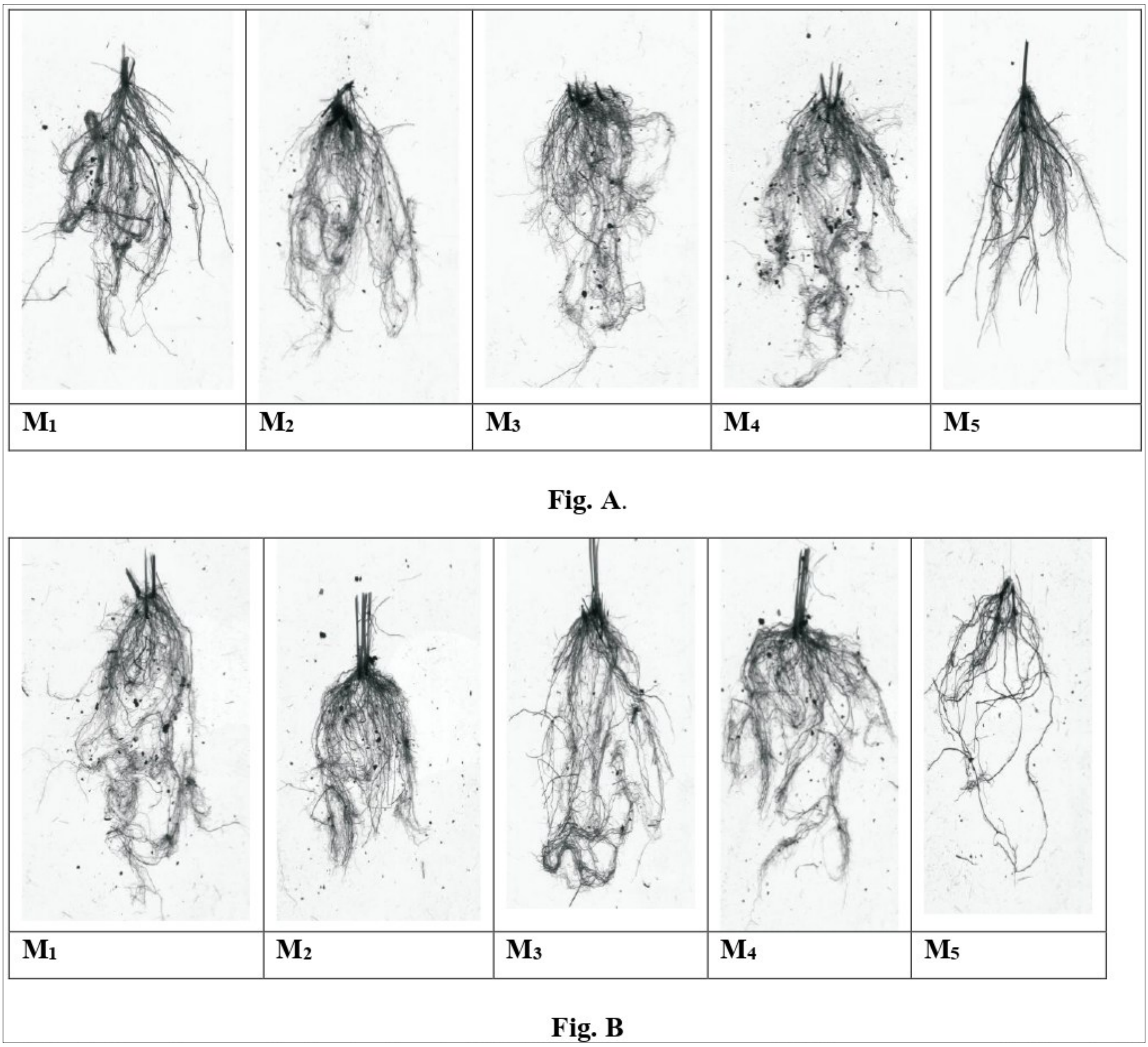

Fig. 3. Root images obtained using the WinRHIZO root scanner. (A) Plants grown at 6 dS m^{-1}; (B) plants grown at 8 dS m^{-1}.

Effect of arbuscular mycorrhizal fungal inoculation under microcosm conditions

At the flowering stage, AM fungal inoculation significantly improved rice growth and physiological traits compared to the UIC (Tables 6–11; Figs. 2 and 3). The AM fungal consortium (*G. macrocarpum* + *A. mellea* + *G. aggregatum*) recorded the highest plant height (85.71 cm), number of tillers (5.72 plant^{-1}), relative chlorophyll content (40.94) and proline content (13.77 µmol g^{-1} FW). These suggest enhanced photosynthetic efficiency and osmotic adjustment under salinity stress (27, 28).

The consortium treatment also recorded the highest root colonization (60.67 %) and glomalin content (0.174 mg g^{-1} soil). Glomalin plays a crucial role in soil aggregation and carbon stabilization, contributing to improved soil structure under stress conditions (29). Soil enzyme activities were significantly enhanced, with dehydrogenase activity of 93.26 µg Triphenyl formazan g^{-1} soil d^{-1} and phosphatase activity of 78.07 µg p-nitrophenol g^{-1} soil h^{-1}, reflecting increased microbial activity and phosphorus mobilization in the rhizosphere (21, 22).

Root surface area (221.80 cm^2) and root volume (2.52 cm^3) were also highest under consortium treatment, indicating improved root architecture that facilitates nutrient and water uptake under saline conditions. Plant P content reached 0.67 %, confirming enhanced nutrient acquisition through AM symbiosis (26). Among single isolates, *G. macrocarpum* showed improved growth and salinity tolerance compared to the UIC, though it was less effective than the consortium.

Influence of salinity levels and interaction effects

Rice plants grown under 6 dS m^{-1} salinity consistently exhibited superior growth and physiological performance compared to those grown under 8 dS m^{-1}, confirming the inhibitory effects of increasing salinity on plant growth and AM fungal efficiency (25). The interaction effects further revealed that consortium application at 6 and 8 dS m^{-1} resulted in the highest plant height (88.60 and 82.83 cm), number of tillers (6.37 and 5.07 plant^{-1}), relative chlorophyll content (42.56 and 39.32) and proline content (13.47 and 14.06 µmol g^{-1} FW) respectively.

Root colonisation (62.33 % and 59.00 %), glomalin content (0.177 and 0.170 mg g^{-1} soil), dehydrogenase activity, expressed as triphenyl formazan (TPF) (93.94 and 92.57 µg g^{-1} soil

d^{-1}) and phosphatase activity, expressed as p-nitrophenol (pNP) (81.61 and 74.54 µg g^{-1} soil h^{-1}), were highest under the consortium treatment at both salinity levels. Root surface area (229.40 and 214.20 cm^2), root volume (1.97 and 1.68 cm^3) and plant phosphorus content (0.72 % and 0.617 %) followed similar trends. These results demonstrate that AM fungal consortia effectively mitigate salinity stress by enhancing nutrient uptake, antioxidant defense, osmotic regulation and soil biological activity (7, 26).

Conclusion

The study confirms that native AM fungal isolates, particularly *G. macrocarpum*, *A. mellea*, *G. aggregatum* and their consortium- significantly mitigate the adverse effects of salinity on rice. These fungi enhance nutrient acquisition, antioxidant defense and root development, thereby promoting plant resilience. The use of native AM fungal consortia offers a promising, eco-friendly strategy for improving crop performance in salt-affected soils.

Authors' contributions

MNN conducted the research, processed, analysed the data and drafted the manuscript. PJN contributed to the design and implementation of the research programme, provided technical guidance and assisted in manuscript preparation. VN and JAH helped finalize the research topic, offered technical guidance and revised the manuscript drafts. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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