Growth Enhancement and Improve Rice Productivity by Mycotrophy and Zinc Solubilizing Bacteria in Coastal Region of Tamil Nadu

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Abstract

Rice (*Oryza sativa* L.) is the most broadly consumed staple crop for almost half of the world's population. Bioinoculants are the optimistic remedy for replacing chemical fertilizers and insecticides in crop production. Arbuscular mycorrhizal fungi (AMF) represent a vital group of root symbionts of higher plants, as they provide a range of soil nutrients (e.g. N and P) to plants in exchange for plant carbohydrates. A pot culture experiment was conducted to assess the effect of *Glomus fasciculatum* and zinc solubilizing bacteria on the growth and yield of rice var.CO-55 during the Kharif season in the pot culture yard of the Department of Agricultural Microbiology, Faculty of Agriculture, Annamalai Nagar, Tamil Nadu. The soil samples were collected from 15 different locations of the Coastal area, Cuddalore district. The results showed a significant improvement in soil biological indicators such as microbial population and enzyme activity due to application of (AM fungi) *G. fasciculatum* and zinc solubilizing bacteria along with graded levels of inorganic fertilizers. Further, AMF plants increase the allocation of N and P to rice panicles compared to non-AMF plants during the grain filling stage. The plant yield parameters as well as quality parameters were highest with this combination. The treatment, T10 (75 % NPK + *Glomus fasciculatum* + zinc Solubilizing Bacteria) was the most successful inoculation of rice among all the thirteen treatments and it significantly increased the growth and yield of rice variety CO-55 by enhancing the nutritional status, especially P and K status of the plants. Hence, this study recommends farmers to reduce the use of inorganic fertilizers by 25 %, when AM fungi and zinc Solubilizing Bacteria are used.

Keywords: AM fungi, *Glomus fasciculatum,* Zinc solubilizing bacteria, Inorganic fertilizers Rice

Introduction

Rice (*Oryza sativa* L.) is the most widely consumed staple crop for approximately half of the world's population (Bao *et al*., 2022). Being a cereal, it is extensively consumed as a main source of nutrition, predominantly in Asia, where 60% of the earth's population lives. China and India itself accounts for 50% of rice cultivation and consumption. Rice has two cultivated species viz., *Oryza sativa* L. and *Oryza glaberrima* Steud and about 20 wild species. *Oryza sativa* L. is grown all over the world while *O. glaberrima* is cultivated only in Africa. Rice is one of the most important crops in the world and is considered as a strategic crop due to its wide distribution in soils and climates worldwide, as well as in a scenario of climate change for food security (Fahad *et al*., 2019; Ríos-Ruiz *et al*., 2020; Redondo-Gómez *et al*., 2023).

Synthetic fertilizers boost growth with negative consequences for human health and the environment. In India, the farmers regularly using more amount of chemical fertilizers for crop production which leads to soil pollution and ground water contamination, ultimately causing health hazards (Giannakoula *et al.*, 2010). In order to avoid the environmental pollution especially soil pollution, most of the scientists are recommending the use of biofertilizers along with inorganic fertilizers in a sustainable manner to maintain the soil health and also the productivity (Sivasakthi *et al*., 2014; Ding *et al*., 2019).

Micronutrients, although necessary in little amount, their deficiency leads to low productivity in several areas of the world. Among micronutrients, Zn is considered to be the most vital as its insufficiency causes

everywhere abiotic stress in Asian countries like Pakistan, India, and Bangladesh and also other countries like Turkey, Syria, Iraq, and also in USA and Africa (Iqbal *et al.*, 2010).

Zinc require for plant in micro quantity but in critical concentration and if the amount available is not enough, plants will suffer from physiological stress brought about by the dysfunction of some enzyme systems and other metabolic functions (Alloway, 2008; Yu *et al*., 2017). Phosphorus is the key element that interferes on zinc uptake, as zinc uptake by plants reduces by increasing phosphorus in soil (Marschner *et al*., 1990; Sharma *et al*., 1986; Chen *et al.*, 2019; Alwahibi *et al*., 2021).

AM fungi and zinc solubilizing bacteria inoculation enhances the growth and development by producing growth promoting hormones and by solubilizing, mobilizing nitrogen, phosphorus, potash and micronutrients like zinc and protecting plants from stresses and pathogens.

AMF enhance rice plant growth and development by improving photosynthesis and other metabolic activities (Ruiz-Sánchez *et al*., 2010), such as the uptake of several macro- and micronutrients, including nitrogen (N), phosphorus (P), potassium (K), copper (Cu), and zinc (Zn) (Panneerselvam *et al*., 2017; Pandey *et al*., 2020). In addition, they modify soil properties and increase the ability of rice plants to withstand various abiotic stresses, while improving their productivity (Madhushan *et al*., 2023).

Materials And Methods

The present study was carried out to screen and identify the efficient zinc solubilizing bacteria and AM fungi up to the species level from the rhizosphere soils of rice The roots and rhizosphere soil samples (5-15 cm depth) were collected from 15 different locations of the Coastal area, Cuddalore district, Tamil Nadu. The samples were transferred to polythene bags for further analysis. The physico-chemical properties viz., soil texture, pH, EC, organic carbon content, available N, P, and K content were estimated by adopting the standard methods. The survey for the occurrence of AM fungi was done to ascertain the colonization ability of AM fungi on rice.

Isolation and characterization of AM fungal spores

All soil samples collected were examined for the presence of AM fungal spore by wet sieving (1000 - 45 µm) and decanting method of Gerdemann and Nicolson (1963). These spores were cleaned from soil particles by sucrose density gradient centrifugation method and washed with distilled water (Mertz *et al*., 1979) and the numbers of spores present in each soil were counted with a Stereo zoom microscope (45x). During counting, identical spores were separated into groups, mounted, and identified. Later based on the soil types of 15 locations the average number of AM fungal spores was worked out. Based on the taxonomic key of Gerdemann and Trappe (1974) the spores of *G. fasciculatum, G. mosseae, G. versiforme, A. laevis*, and *G. Margarita* was identified. Further, the characterization and screening of selected AM fungal species for percent root colonization, spore population, acid and alkaline phosphatase enzyme activities, relative mycorrhizal dependency, and mycorrhizal inoculation effect were elucidated. Among the screened AM fungal isolates, *Glomus fasciculatum* was found to be the most efficient culture for rice in terms of root colonization, spore number, acid and alkaline phosphatase activity, relative mycorrhizal dependency, mycorrhizal inoculation effect, etc. Hence, it was selected for further study.

Mass production of *Glomus fasciculatum*

For the multiplication of *G. fasciculatum* soil inoculum, the pots of 40 cm diameter were filled with sterilized sand: soil (1:1) fumigated by using 2 percent formaldehyde solution. About 50 g of *G. fasciculatum* inoculum was added to each pot containing sterilized soil. The sorghum seeds at 10 seeds pot-1 were sown. After 30 days of growth, the plants were pulled out and the roots were tested for AM fungal colonization. AM fungal spores were counted from the soil by wet sieving and decanting method. After examining the roots for AM fungal colonization, the roots were cut into small pieces and the top soils from the inoculated pots were incorporated into the pots containing the sterilized soil mixture. The sorghum seeds were sown on AM fungi treated pot for multiplication. After 40 days, roots and soil were tested for the colonization of AM fungi and spore number. This stage was known as “Mother culture - I”. The root pieces from the mother culture - I as well as the top soil were incorporated again into a cement tray containing a similar soil mixture and sown with the seeds of sorghum. After 40 days the root and soil were tested for mycorrhizal colonization and the number of spores. The top soils (10 - 15 cm) along with root pieces were taken. This soil-based root inoculum

containing more than 15 spores per gram of the soil was used as inoculum in the pot culture and field studies. The soil-based root inoculum was stored at 5°C.

Effect of *G. fasciculatum* and zinc solubilizing bacteria on the growth and yield of rice under pot culture condition: A pot culture experiment was conducted to assess the effect of *G. fasciculatum* and zinc solubilizing bacteria inoculated with graded levels of inorganic NPK fertilizers on the growth and yield of rice during the Kharif (July October, 2024) season in the pot culture yard of the Department of Agricultural Microbiology, Faculty of Agriculture, Annamalai University, Annamalai Nagar.

Preparation of pots: Rectangular cement pots of size 18" x 12" x 12" were filled with 45 kg of paddy field soil, flooded with water for 2 days and brought to fine puddled condition. Seeds of rice variety CO-55 were loosely packed in small gunny bag and soaked in water for 12 hrs.

Then it should be puddled twice and basal application of 0.5 kg DAP is recommended when the seedlings are to be pulled out in 20-25 days after sowing. The pre-germinated seeds of rice (CO-55) were sown uniformly in a well levelled and puddled nursery seed bed of convenient length (4 m) with width of 1.5 m. Then, the rice seedlings of 25 days old were transplanted in rows in pots, separately at the rate of 15 seedlings per pot with 3 hills (5 seedlings $hill^{-1}$) with a spacing of 20 x 15 cm. A control pot without inoculation was also maintained. Gap filling was done between 7^{th} and 10^{th} DAT. The experiment was conducted in Completely Randomized Block Design (CRD) with three replications. The crop was given hand weeding at regular intervals and well protected against pests and diseases.

A fertilizer schedule of 150:50:50 NPK (kg/ha) was followed for rice. The entire dose of nitrogen was applied in two split doses. The remaining dose of P_2O_5 and the entire dose of K_2O was applied basally as DAP (Di-ammonium phosphate) and murate of potash, respectively. The seedlings were raised under wet conditions and the age of the seedlings were counted from the time of transplanting (DAT) and the following treatments were tested. Five representative samples of plant hills in each pot were peg marked for periodical observation.

Experiment details

Crop	Variety	Design	Replication	Recommended dose of fertilizer (Kg/ha)
Rice	CO-55	CRD	3	150:50:50

Treatment details

T_1 : Control
T_2 : 100% NPK alone
T_3 : *Glomus fasciculatum* alone
T_4 : zinc solubilizing bacteria
T_5 : 100% NPK + *G. fasciculatum*
T_6 : 100% NPK +zinc solubilizing bacteria
T_7 : 100% NPK + *G. fasciculatum*+zinc solubilizing bacteria
T_8 : 75% NPK + *G. fasciculatum*
T_9 : 75% NPK + zinc solubilizing bacteria
T_{10} : 75% NPK + *G. fasciculatum* +zinc solubilizing bacteria
T_{11} : 50% NPK + *G. fasciculatum*
T_{12} : 50% NPK + zinc solubilizing bacteria
T_{13} : 50% NPK + *G. fasciculatum* + zinc solubilizing bacteria

Results and Discussion

Inoculation effect of *G. fasciculatum* and zinc solubilizing bacteria on the biometric observations of rice var.CO-55 – Pot culture study: A pot culture study was conducted to evaluate the individual and combined effects of zinc solubilizing bacteria and *G. fasciculatum* with graded levels of inorganic fertilizers on the growth and yield parameters of rice var. CO-55 up to 120 days were presented below.

The treatment T_{10} (75% NPK + *G. fasciculatum* + zinc solubilizing bacteria) recorded maximum plant height of 38.33 cm, 64.12 cm, 93.05 cm and 111.35 cm on 30, 60, 90 and 120 DAT respectively. It was followed by T_7 (100% NPK + *G. fasciculatum* + zinc solubilizing bacteria) as 37.85 cm, 63.86 cm, 90.82 cm and 110.84 cm (Table 1).

The treatments T_3 (*G. fasciculatum* alone) and T_4 (zinc solubilizing bacteria alone) were observed the plant height of 101.76 cm and 99.97 cm on 120 DAT. Whereas, the treatment T_2 (100% NPK alone) recorded 99.53 cm on 120 DAT and found to be better than the individual application of either *G. fasciculatum* or zinc solubilizing bacteria.

Table 1: Effect of *G. fasciculatum* and zinc solubilizing bacteria with graded levels of inorganic nutrients on the plant height of rice

Treatments	Plant height (cm plant^{-1})			
	30 DAT	60 DAT	90 DAT	120 DAT
T_1	29.27	55.10	80.16	99.48
T_2	37.77	62.24	91.19	99.53
T_3	31.35	57.45	86.58	101.76
T_4	30.86	55.27	85.90	99.97
T_5	34.92	60.09	89.36	105.19
T_6	33.54	60.02	88.51	104.95
T_7	37.85	63.86	92.82	110.84
T_8	36.29	61.33	90.24	107.71
T_9	35.65	60.91	89.73	106.60
T_{10}	38.33	64.12	93.05	111.35
T_{11}	32.12	59.78	87.62	104.26
T_{12}	31.79	57.13	87.47	103.48
T_{13}	37.28	61.91	90.65	108.32

Table 2: Effect of *G. fasciculatum* and zinc solubilizing bacteria on the leaf area index (LAI), number of tillers per hill, number of panicles per plant and panicle length of rice

Treatment	Leaf area index	Number of tillers per hill	Number of panicles per plant	Panicle length
T_1	2.62	7	3.27	21.40
T_2	5.84	15	5.12	24.41
T_3	3.14	9	3.91	21.68
T_4	2.97	8	3.48	21.63
T_5	3.43	12	4.54	22.89
T_6	3.40	11	4.43	22.72
T_7	6.65	16	5.26	24.55
T_8	4.79	14	4.77	23.34
T_9	4.01	13	4.65	23.00
T_{10}	6.98	17	5.89	24.96
T_{11}	3.36	10	4.23	22.17
T_{12}	3.23	9	4.18	22.11
T_{13}	5.02	15	4.80	23.57

Table 3: Effect of *G. fasciculatum* and zinc solubilizing bacteria on the total number of grains per panicle, 1000 grain weight, grain yield and straw yield of rice

Treatment	Total number of grains per panicle	1000 grain weight	Grain yield	Straw yield
	Mean			
T_1	145.50	20.52	11.71	13.28
T_2	156.67	25.80	16.84	17.33
T_3	148.17	21.74	13.98	14.81
T_4	147.62	20.37	12.53	13.99
T_5	153.73	24.00	15.11	16.58
T_6	152.76	23.00	15.06	16.25
T_7	157.04	26.70	17.21	17.97
T_8	154.38	24.50	16.05	17.00
T_9	153.90	24.30	15.47	16.92
T_{10}	157.29	27.30	17.89	18.43
T_{11}	151.81	22.95	14.68	15.94
T_{12}	149.74	22.36	14.24	15.68
T_{13}	5.02	15	4.80	23.57

Table 4: Effect of *G. fasciculatum* and zinc solubilizing bacteria with graded levels of inorganic nutrients on the plant dry matter production of rice

Treatments	Plant dry matter production (g plant^{-1})			
	30 DAT	60 DAT	90 DAT	120 DAT
T_1	1.40	9.32	19.41	29.82
T_2	1.63	12.64	23.45	34.84
T_3	1.42	9.09	21.23	30.58
T_4	1.41	9.85	19.51	30.37
T_5	1.48	11.45	22.06	31.25
T_6	1.47	11.23	21.92	31.22
T_7	1.78	13.60	24.69	35.03
T_8	1.50	12.38	22.88	31.64
T_9	1.49	11.53	22.52	31.32
T_{10}	1.82	14.97	26.07	37.78
T_{11}	1.45	10.12	21.72	31.07
T_{12}	1.44	10.89	21.41	30.71
T_{13}	1.56	12.51	23.34	32.97

Effect of *G. fasciculatum* and zinc solubilizing bacteria on chlorophyll content of rice: The inoculation effect of *G. fasciculatum* and zinc solubilizing bacteria along with graded levels of inorganic fertilizers on chlorophyll content of rice were presented in Table 5. The chlorophyll content was estimated at 60 DAT. The significant effect on the increase in chlorophyll content of rice by the application of (AM fungi) *G. fasciculatum* and zinc solubilizing bacteria along with graded levels of inorganic fertilizers was observed. The maximum total chlorophyll content of 1.87 mg g^{-1} of leaf was recorded in T_{10}, which was followed by T_7 (1.43 mg g^{-1}). The minimum amount of total chlorophyll content was recorded in control T_1 (0.92 mg g^{-1}).

Table 5: Effect of *G. fasciculatum* and zinc solubilizing bacteria on chlorophyll content of rice

Treatments	Chlorophyll content (mg g^{-1} of leaf)		
	A	B	Total
T_1	0.70	0.62	0.92
T_2	1.25	1.27	1.39
T_3	0.92	0.95	1.07
T_4	0.85	0.84	0.95
T_5	1.17	1.14	1.31
T_6	1.14	1.10	1.26
T_7	1.33	1.28	1.43
T_8	1.22	1.21	1.34
T_9	1.21	1.16	1.32
T_{10}	1.37	1.36	1.87
T_{11}	1.12	1.05	1.20
T_{12}	1.03	1.03	1.13
T_{13}	1.24	1.22	1.38

Effect of *G. fasciculatum* and zinc solubilizing bacteria with graded levels of inorganic nutrients on the N, P, K uptake of rice: The nitrogen, phosphorus and potassium uptake of rice were noticed and presented in Table 6.The nitrogen uptake ranged from 40.53 to 48.47 kg ha^{-1} among the various treatments studied. Maximum N uptake of 48.47 kg ha^{-1} was recorded in the treatment T_{10} (75% NPK + *G. fasciculatum* + zinc solubilizing bacteria). This was followed by T_7 (47.96 kg ha^{-1}), T_2 (46.90 kg ha^{-1}).

Regarding the phosphorus uptake, (T_{10}) 75% NPK + *G. fasciculatum* + zinc solubilizing bacteria showed highest uptake of 15.89 kg ha^{-1}. It was followed by T_7 (100% NPK + *G. fasciculatum* + zinc solubilizing bacteria) as 14.64 kg ha^{-1}, T_2 (100% NPK alone) as 14.36 kg ha^{-1}. The minimum P uptake of 10.03 kg ha^{-1} was observed in the control treatment T_1.The potassium uptake of rice plant ranged between 45.65 and 57.59 kg ha^{-1}. The 100% recommended dose of fertilizers i.e., 100% NPK alone (T_2) recorded 55.80 kg ha^{-1}. The maximum K uptake of 57.59 kg ha^{-1} was recorded in the treatment T_{10} (75% NPK + *G. fasciculatum* + zinc solubilizing bacteria) followed by (T_7) 100% NPK + *G. fasciculatum* + zinc solubilizing bacteria (56.51 kg ha^{-1}) and the lowest value of 45.65 kg ha^{-1} was found in control T_1.

In the present study, plant uptake of N, P, and K was maximum in the treatment (T10). These types of improvements were mainly due to the transport of NPK nutrients along with water by AM fungal mycelium and improved root development by the inoculation of AM fungi and zinc solubilizing bacteria. Similar studies were made by Yeasmin *et al*., (2007), Xiao *et al*., (2010) Suzuki *et al*., (2015) and Panneerselvam *et al*., (2019), Vaid *et al*., (2014).

Table 6: Effect of *G. fasciculatum* and zinc solubilizing bacteria with graded levels of inorganic nutrients on the N, P, K uptake of rice

Treatments	NPK (kg ha$^{-1)}$		
	N	P	K
T_1	40.53	10.03	45.65
T_2	47.90	14.36	55.80
T_3	43.61	10.49	50.63
T_4	42.24	10.27	48.29
T_5	45.40	12.81	53.28
T_6	45.35	12.05	53.07
T_7	47.96	14.64	56.51
T_8	46.00	13.99	54.72
T_9	45.64	12.70	54.43
T_{10}	48.47	15.89	57.59
T_{11}	44.98	11.66	52.66
T_{12}	44.62	11.12	51.91
T_{13}	46.79	14.28	54.87

Figure 1: An experimental Pot culture view

Summary and Conclusion

On the basis of results obtained in present investigation, it may be concluded that there was a significant improvement in the growth and yield of rice with the combined inoculation of *G. fasciculatum* and zinc solubilizing bacteria as compared to control and single inoculation. There was a significant improvement in soil biological indicators such as microbial population and enzyme activity due to inoculation of zinc solubilizing bacteria. Further, AMF plants increase the allocation of N and P to rice panicles compared to non-AMF plants during the grain filling stage, and the grain yield of rice was significantly increased than non-mycorrhizal plants. The plant yield parameters as well as quality parameters were highest with this combined inoculation. So, the selective application and proper management of microbial inoculants to field crops is essential in order to allow reduction of chemical fertilizers and pesticides input and high economic output.

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