

EFFECT OF INOCULATION WITH THREE DIFFERENT PADDY SOILS SUSPENSIONS ON GROWTH OF RICE PLANTS

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Abstract

Inoculation with plant growth promoting rhizobacteria (PGPR) are considered as a solution to the environmental and economic problems of using chemical fertilizers and pesticides. Inoculation with consortia of several bacterial strains is suggested as an alternative to inoculation with single species and mixed species introduced strains. Rice variety BG 250 grown in modified Yoshida rice nutrient solution was inoculated with suspensions of 3 different paddy soils separately with the aim of selecting a soil with effective microbial consortia. Inoculations did not affect any vegetative or reproductive parameter 3 or 14 weeks after transplanting despite the non-competitive conducive environment prevailing in the pots. The major reason could be presence of nutrients particularly nitrogen in sufficient quantities since this suppresses the effect of microbes. Further studies are needed with inoculation under low levels of nitrogen preferably with different rice varieties and different soils for development of biofertilizer with effective PGPR consortia.

Key words: Rice, biofertilizer, PGPR consortia

Introduction

It is unanimously admitted that the chemical fertilizers and pesticides used in modern agriculture create a real environmental and public health problem and use of bio-resources, including PGPRs becoming a promising solution (Noumavo, 2016). It is envisioned that PGPRs will begin to replace the use of chemicals in agriculture, horticulture, silviculture, and environmental cleanup strategies (Glick 2012). Plant growth promoting bacteria enhance plant growth and protect plants from disease and abiotic stresses through a wide variety of mechanisms: fixation of atmospheric nitrogen transferred to the plant, production of siderophore, that chelate iron and make it available to the plant roots, solubilizing of minerals such as phosphorus and synthesis of phytohormones (Souza *et al.*, 2015).

A number of PGPRs are used as biofertilizers in commercial scale. Biofertilizers are formulated as single species of bacteria (Kloepper, 1993; Vessey, 2003), mixture of bacteria obtained from different soils (Hadacek and Kraus, 2002) and biofilms (Kim *et al.* 2008; Brenner and Arnold, 2011). Single species and mixed species inoculations though show positive effects the response is sometimes not reflected at greenhouse and field levels (Buddhika *et al.*, 2013) and the introduced microbes suppress the better adapted soil native microbes due to the competition (Van Veen *et al.*, 1997). Inoculation with a consortium of several bacterial strains could be an alternative to inoculation with individual strains and likely to reflect the different mechanisms used by each strain in the consortium. (Souza *et al.*, 2013). An important research need is selection of effective and competitive multi-functional bio-fertilizers for a variety of crops (Aggani 2013). Native microorganisms include microbes that are living together in harmony with the rest of nature and thus may play a multifunctional and effective role as biofertilizers.

Soil type has a key role in determining the specific dominant bacteria colonizing the rhizosphere (Marschner et al., 2001). The number and kinds of microorganisms present in soil depend on many environmental factors: amount and type of nutrients available, available moisture, degree of aeration, pH, temperature etc. (Pre-scott et al., 1993). Thus, the types of microbes present in the different soils are different and therefore the effect of the microbes on the plant of different soils could be different. The objective of the present study was to investigate the effect of microbes of three different paddy soils on growth of rice plants with the aim of using microbial consortia as biofertilizer.

Materials and Methods

Preparation of inoculum suspensions

Paddy soils from Sammanthurai in Ampara District, Anamaduwa in Puttalam District and Navaly, Manippai in Jaffna District were used. The soils were collected from the surface (15 cm depth) from three random locations in each field, pooled, dried, ground, mixed and sieved using a 2 mm sieve. The pH of the soil samples was measured. The soil suspensions prepared by thoroughly shaking 10 g each of the above 3 soils in 100 milliliters of distilled water separately and leaving few minutes for settling were used as inoculum of the respective soils. The mixed inoculum suspension was prepared using a mixture of above 3 soils in equal quantities.

Inoculation of seeds

Rice variety BG 250 collected from Sammanthurai Rice Research Station was used for the study. Few paddy grains were separately soaked overnight in the four soil suspensions (inoculations) and distilled water (for non-inoculated treatment). The seeds soaked were transferred to petri dishes containing cotton wool covered with filter paper soaked with respective soil suspensions and distilled water. Petri dishes were covered with black polythene and left at room temperature for seeds to germinate.

Pot experiment

Around 5 mm sized stone chippings were used as the pot medium. The stone chippings obtained from a quarry in Sammanthurai were well washed and pH adjusted to neutral. Thirty-six pots (plastic buckets of 15.7 cm height and 15.5 cm diameter) were filled up to $\frac{3}{4}$ of the pot with stone chippings and submerged with rice nutrient solution (Yoshida et. al., 1976). For treatments with full dose of nitrogen the solution was prepared as per the original recipe and for treatments with half dose of nitrogen, half the amount of prescribed NH_4NO_3 was used.

Five days old seedlings were transplanted in pots (two seedlings per pot) and the remaining soil solution was added to the respective pots. The pots were covered with black polythene to prevent algal growth. Tap water was added in equal volumes as required to keep the medium submerged. The relevant nutritional solutions were applied in equal volumes when required to keep the plants healthy.

The following six treatments were maintained: T1- full nitrogen with no inoculation, T2- half nitrogen with no inoculation, T3- half nitrogen inoculated with Jaffna soil suspension, T4- half nitrogen inoculated with Anamaduwa soil suspension, T5 - half nitrogen inoculated with Sammanthurai soil suspension and T6 - half nitrogen inoculated with mixed soil suspension. Six pots (3 replicates per harvest) per treatment were maintained in completely randomized manner in the plant house.

Data collection and analysis:

Three replicates from each treatment were harvested 3 Weeks After Transplanting (WAT). The shoots of the plants were cut 2 cm above the pot medium level and washed. Roots were separated from the chippings carefully by dipping in tap water. Number of tillers, shoot and root length and leaf area of each replicates were recorded. Leaf area was measured using the leaf area meter (LI-Portable Area Meter, LI-3050C transparent belt conveyer accessory). The total leaf area for a pot was considered as Leaf Area Index (LAI), because the ground area of all pots was same. Dry weight of shoot and root were recorded after drying in an oven for three days at 45°C. Same vegetative data except leaf area, the number of filled seeds, number of unfilled seeds and 100 grain weight were recorded of remaining plants 14 WAT.

Above data were analyzed statistically by one-way ANOVA and the significance of differences between mean values was evaluated by Turkey's model using MINITAB 16.1.

Enumeration of soil bacterial population

The bacterial population of the three soils and a non-agricultural soil were enumerated using plate count method using Nutrient Agar media of which pH was adjusted to 5.5 and 7.0.

Results and Discussion

The results 3 WAT showed no difference in any of the vegetative parameters measured, except leaf area index which was the highest in the treatment with full dose of nitrogen. Although not significant the total dry weight of Jaffna soil inoculated treatment was the highest (Table 1).

This could be due to some growth promoting effect of microbes of Jaffna soil since it had the highest bacterial population. Other than this none of the inoculation treatments showed any positive effect compared to the negative control treatment (half dose of nitrogen).

The positive effects of Jaffna soil found 3 WAT were not reflected at reproductive stage (Table 2). At final harvest all the parameters were highest in the full dose nitrogen treatment. None of the inoculation treatments showed any improvement in any parameter compared to the negative control (Half dose of nitrogen) treatment indicating no effect of the microbes of inoculated soils.

Table 1:Growth parameters of rice plants 3 weeks after transplanting

Treatment	Shoot length (cm)	Shoot dry weight (mg)	Root dry weight (mg)	Root length (cm)	Total dry weight (mg)	LAI	No of tillers
1	61.133 ^a	1470.5 ^a	1113.2 ^a	32.500 ^a	2583.6 ^a	320.77 ^a	7.667 ^a
2	55.167 ^a	1471.6 ^a	912.9 ^a	31.500 ^a	2384.6 ^a	160.48 ^b	8.667 ^a
3	55.833 ^a	1643.8 ^a	1002.1 ^a	29.833 ^{ab}	2645.8 ^a	211.42 ^{ab}	8.667 ^a
4	57.167 ^a	1382.4 ^a	758.3 ^a	26.133 ^{ab}	2140.6 ^a	198.48 ^{ab}	9.667 ^a
5	53.000 ^a	1371.1 ^a	873.8 ^a	23.167 ^b	2244.9 ^a	158.94 ^b	7.000 ^a
6	54.200 ^a	1444.2 ^a	2344.1 ^a	25.667 ^{ab}	899.9 ^a	162.88 ^b	8.000 ^a

Means that share the same letter in a column are not significantly different: Leaf area index(LAI)

Table 2: Growth and yield parameters 14 weeks after transplanting

Treatment	Shoot length (cm)	Shoot dry weight(g)	No of tillers	Total dry weight	No of panicles	100 Grain weight(g)	Weight of filled seeds(g)	Weight of unfilled seeds (g)	Weight of total seeds(g)	Root dry weight(g)
1	57.0 ^a	2.67 ^a	8.00 ^a	6.67 ^a	6.67 ^a	2.23 ^a	3.97 ^a	0.83 ^a	4.8 ^a	4.0 ^a
2	56.7 ^a	1.60 ^a	7.67 ^a	4.00 ^{ab}	5.00 ^{ab}	2.13 ^a	3.27 ^{ab}	0.33 ^b	3.6 ^{ab}	2.4 ^{ab}
3	52.3 ^a	2.43 ^a	8.33 ^a	4.00 ^{ab}	3.67 ^b	2.00 ^a	2.57 ^{ab}	0.30 ^b	2.87 ^b	1.57 ^b
4	60.0 ^a	2.53 ^a	6.33 ^a	4.07 ^{ab}	4.33 ^b	2.20 ^a	2.00 ^b	0.47 ^{ab}	2.47 ^b	1.53 ^b
5	55.0 ^a	1.50 ^a	6.67 ^a	3.53 ^b	4.67 ^{ab}	2.13 ^a	2.73 ^{ab}	0.43 ^{ab}	3.2 ^{ab}	2.0 ^{ab}
6	57.0 ^a	2.33 ^a	6.33 ^a	4.67 ^{ab}	4.00 ^b	2.07 ^a	2.40 ^{ab}	0.33 ^b	2.73 ^b	2.3 ^{ab}

Means that share the same letter in a column are not significantly different. Leaf area index(LAI)

Although not significant, the Jaffna soil had the highest microbial population (Table 3). The microbial population of other two soils didnot differ from the non-paddy soil very much indicating the microbial numbersof the tested paddy soil has not been affected by the agricultural practices. However, it is not known if the microbial composition has been changed by the agricultural practices. The pH values of the soils were ranging between 7.00 – 7.35 and the pH of the rice growth medium in pots were ranging between 5.5 and 7.5 during the experimental period. The bacteria of these soils were found growing at these two pH values and the growth was better at 5.5 pH value (Table 3).Thus the pH of the medium would have not affected the bacterial populations in the rice growth medium.

Also the rice growth medium would have had a conducive environment for the effective microbes of the inoculated soils since there would have been no competitors as in soil. Hence the reason for not observing any effect of the microbes could be due to the amount of nitrogen present in the inoculated treatments Seneviratna et al., (2011) quoting others (Kolb and Marti, 1988; Cruz et al., 2009) reported that nitrogen fertilizers suppress the action of microbes particularly nitrogen fixers.

Table 3: Viable bacterial counts of soils at different pH values

Soil	p ^H	Bacterial population (CFU per gm soil)
Anamadu	7	7.9x10 ⁵
	5.5	1.78x10 ⁶
Sammanthurai	7	9.9x10 ⁵
	5.5	1.49x10 ⁶
Jaffna	7	1.08x10 ⁶
	5.5	2.15x10 ⁶
Non-agricultural soil	7	9.0x10 ⁵
	5.5	1.58x10 ⁶

Conclusion

According to the results of this study, microbes of any of the paddy soils tested did not affect any of the vegetative or reproductive parameter in a considerable manner. Further studies with inoculation under low levels of nitrogen with different rice varieties and different paddy soils may result in development of biofertilizer containing effective microbial consortia.

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