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Field evaluation of nursery bed inoculated arbuscular mycorrhiza and rootdip inoculated *Azotobacter chroococcum* and *Aspergillus awamori* on aerobic rice

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Abstract: Field evaluation of nursery bed inoculated with *Glomus fasciculatum* and root dip treatement with *Azotobacter chroococcum* and *Aspergillus awamori* was carried out on aerobic rice. All the inoculated treatments with *G. fasciculatum* either singly or incombination showed increased growth and yield of rice compared to control and dual and triple inoculation were performed better than single inoculation treatment. *G. fasciculatum* inoculated seedlings in nursery bed shown better root colonization in field after transplantation compared to un-inoculated plants. The root dip inoculation with *A. chroococcum* and *A. awamori* during transplantation also increased the population of N_2 fixer and Phosphate solubilizers besides increasing the population of general microflora in the rhizosphere. The results revealed the possibility of nursery inoculation of arbuscular mycorrhiza and root dip inoculation of other biofertilizers for aerobic rice.

Keywords: Arbuscular mycorrhiza, Nursery inoculation, Root dipping, *Azotobacter,* Aerobic rice, *Aspergillus awamori*

INTRODUCTION

Aerobic rice also known as upland rice is becoming increasingly popular in recent years as it does not require standing water for growth. It can be cultivated in upland conditions with protective irrigation. Along with manures and fertilizers, biofertilizers application has been proved in improving crop growth and yield in various agricultural and horticultural crops (Muthukumar and Udaiyan, 2000). Nursery inoculation of Arbuscular Mycorrhizal fungi (AMF) has been showed to carry the inocula through the colonized roots in to field (Quoreshi *et al.,* 2008). AMF inoculation at the nursery stage under both dry and wet conditions increased growth, grain yield and nutrient acquisition of wet land rice under field conditions (Solaiman and Hirata, 1997). Amaranthus and Steinfeld (2005) observed that the improved plant growth and survival of seedlings inoculated with mycorrhiza at nursery level. Al-karaki (2006) reported that the pre-inoculation of tomato transplants with AM fungi improved yield and helped in tolerance to salt stress. Similarly root dip inoculation of other biofertilizers found useful in transplantable crops (Earanna and Bagyaraj, 2008).

In the rain fed farming, application of biofertililzers in field condition has become difficult due to lack of suitable techniques. The common practice at present is that the carrier based cultures of *Azotobacter,* P solubilizers and arbuscular mycorrhiza are applied to rhizosphere or to planting pit (for transplantable crops) while transplanting. Broad casting along with manures is also not recommendable due to sensitivity of live organisms in adverse conditions. Moreover, this is inconvenient for larger area application and leads to loss of microbes due to moisture stress. Similarly, soil based arbuscular mycorrhizal culture is difficult to transport and apply in field. Because of all these reasons biofertilizer application often fails to reach the target. Hence, the study was carried out to explore the possibility of Nursery bed inoculation technique for arbuscular mycorrhiza and root dip inoculation of N_2 fixer and P solubilizer during tansplanting.

MATERIALS AND METHODS

Culture collection and preparation: Efficient arbuscular mycorrhiza, the *Glomus fasciculatum* maintained in the glass house of the Department of Agricultural Microbiology was used for inoculation to the nursery beds prepared for aerobic rice. *Azotobacter chroococcum* (N_2 fixer) and *Aspergillus awamori* (P solubilizer) was grown in Waksman No.77 Nitrogen free broth and Potato dextrose broth respectively on rotary shaker for 7 days. Fully grown culture was mixed with sterile lignite carrier material and used for root dipping.

Nursery inoculation of *G. fasciculatum*: Raised nursery beds measuring 1.5M X 9M were prepared in the field. The beds were watered one day prior to sowing. Seeds of aerobic rice (MAS-946) were obtained from the Department of Genetics and Plant Breeding, University of Agricultural Sciences, GKVK, Bangalore. The seeds were soaked and pre-germinated using wet gunny bags.

Table 1. Response of aerobic rice to biofertilizers inoculation in the field.

Treatments	Plant height (cm) at harvest	Average number of tillers	Average straw weight (Kg)	Average grain weight (Kg)
Control	64.33^{d}	9.07^{d}	5.83^{c}	0.97
Glomus fasciculatum (Gf)	67.33^{bc}	14.00^{c}	6.00^{c}	1.15
Azotobacter chroococcum (Ac)	68.67^{ab}	15.73^{b}	6.33^{bc}	1.23
Aspergillus awamori (Aa)	64.17^{d}	15.23^{b}	5.92^{c}	0.97
Gf + Ac	69.67^{a}	18.53^{a}	7.25^{a}	1.33
Gf + Aa	65.83^{cd}	15.23^{b}	7.17^{a}	1.00
Gf + Ac + Aa	66.83^{bc}	15.43^{b}	6.83^{ab}	1.08
LSD @ 0.5%	2.29	0.99	0.71	NS

Note: Means of the same super script do not differ significantly at 5% level of duncan multiple range test.

Prior to sowing, shallow furrows were made at a distance of 20cm on the nursery bed. In to the furrows, 1.5 kg of *G. fasciculatum* inoculums having 1400 IP (infective propagules) was added. Then the pre-germinated rice seeds were sown in the furrows and covered with top soil. The beds were watered. The seedlings in the bed were maintained for 30 days with protective irrigation. The control plants were maintained in un-inoculated nursery beds.

Field experiment: Field experiment was carried out at Regional Research station, University of Agricultural Sciences, GKVK, Bangalore. Soil of the site was alfisol of the type kaolinite, isothermic typic kanhalplustalf (pH 6.4) with 168.33 kg/ha available nitrogen, 24.68 kg/ha phosphorus and 305.60 kg/ha potassium. Experimental layout was made with plots measuring 2X3M size with proper bunding and channels for irrigation.

Slurry of carrier based cultures of *A.chroococcum* and *A. awamori* was made in a sterilize buckets using good quality water. The population was determined in the slurry by dilution plate method. The seedlings from the nursery bed were uprooted and dipped in the inoculum slurry of *A. chroococcum* (3.2X 10^{8} colony forming unit (CFU/ml) and *A. awamori* (5.6 X10^{7} CFU/ml) for 10 minutes. Thus treated seedlings either singly or in combination of both were transplanted in the main field as per the treatment allocation given below with a spacing of 20cm X 20cm. The control was maintained with un-inoculated seedlings. Experiment was carried out in the field using RCBD. There were three replications. The crop was maintained till harvest with protective irrigation.

Treatment (T) allocation: $T_{1:}$ Control (No Inoculation), T_{2}: *Glomus fasciculatum* (Gf), $T_{3:}$ *Azotobacter chroococcum* (Ac), T_{4}: *Aspergillus awamori* (Aa), T_{5}: Gf + Ac, T_{6}: Gf + Aa, T_{7}: Gf + Ac + Aa

Five plants in a plot were labeled for recording the growth parameters. The growth parameters viz., plant height (cm) and number of tillers per plant were recorded. The crop was harvested on 90th days after transplanting and yield (straw and grain) was recorded. Nitrogen content of the plant was estimated by Microkjeldahl method (AOAC, 1980). Phosphorus content of the plant was estimated by Vanodomolybdate yellow colour method (Jackson, 1973). Total microbial population (fungi, Bacteria and Actinomycetes) in the root zone soil was estimated by dilution plate method using appropriate media and population of *Azotobcter* and P solubilizers in the root zone soil were estimated by dilution plate method using Waksman No.77 N-free agar and Pikovasky agar respectively. The data obtained was analysed using m-

Table 2. Effect of inoculation of biofertilizers on nitrogen and phosphorous content of aerobic rice.

Treatments	Nitrogen content (%)		Phosphorus content (%)	
	Shoot	Root	Shoot	Root
Control	1.00	0.45^{d}	0.12	0.11
Glomus fasciculatum (Gf)	1.13	0.66^{bcd}	0.13	0.18
Azotobacter chroococcum (Ac)	1.21	0.70^{abc}	0.14	0.21
Aspergillus awamori (Aa)	1.15	0.76^{abc}	0.13	0.16
Gf + Ac	1.27	0.94^{a}	0.13	0.26
Gf + Aa	1.21	0.90^{ab}	0.14	0.18
Gf + Ac + Aa	1.25	0.65^{cd}	0.13	0.10
LSD @ 0.5%	NS	0.22	NS	NS

Note: Means of the same super script do not differ significantly at 5% level of duncan multiple range test.

Table 3. Effect of inoculation of biofertilizers on microbial population in the root zone soils of aerobic rice.

Treatments	Microbial population				
	Actinomycetes (x 10^2/g)	**Fungi (x10^4/g)**	**Bacteria (x10^6/g)**	***A.Chroococcum* (x10^2/g)**	**PSM (x10^2/g)**
Control	9.33^{d}	12.33^{e}	23.67^{g}	13.67^{e}	3.33^{c}
Glomus fasciculatum (Gf)	16.00^{b}	14.00^{d}	26.67^{f}	16.00^{d}	4.00^{bc}
Azotobacter chroococcum (Ac)	15.67^{b}	15.67^{c}	31.00^{d}	26.33^{a}	5.00^{bc}
Aspergillus awamori (Aa)	9.67^{cd}	18.33^{b}	30.00^{e}	15.33^{d}	17.00a
Gf + Ac	11.33^{c}	12.67^{de}	31.67^{c}	27.33^{a}	5.67^{b}
Gf + Aa	16.67^{b}	17.00^{bc}	35.00^{b}	17.67^{c}	4.67^{bc}
Gf + Ac + Aa	19.67^{a}	22.67^{a}	42.67^{a}	24.33^{b}	16.33^{a}
LSD @ 0.5%	1.75	1.56	0.56	1.39	1.96

Note: Means of the same super script do not differ significantly at 5% level of duncan multiple range test.

Stat-C software and the treatment means were separated by Duncan's Multiple Range Test (Little and Hills, 1978). Arbuscular mycorrhizal root colonization was estimated by gridline intersection method (Giovannetti and Mosse, 1980) after staining the roots with acid fucshin (0.02%). The chlamydospore numbers in the root zone soil was estimated by wet sieving and decantation technique (Gerdemann and Nicolson, 1963).

RESULTS AND DISCUSSION

Results pertaining to the growth and yield parameters are presented in Table 1. Increased mean plant height of aerobic rice was observed in all biofertilizers and AMF inoculated plants compared to control plants. A significantly maximum plant height (69.67) was found in the treatment combination of Gf+Acwhich is followed by *A. chroococcum* alone inoculated plants. This is due to the influence of nitrogen fixer on the vegetative growth. The next best were the plants inoculated with *G. fasciculatum* alone and its combination with *A.chroococcum* and *A. awamori*. However, *A.awamori* alone treated plants did not differ significantly with the un-inoculated plants. Harwig *et al.* (2002) reported significantly improved biomass of *Lolium perenne* inoculated with *G. intraradices* with high N fertilization. In the present study, interaction effect was showed maximum benefit compared to single inoculation. Further, the biofertilizer treated plants significantly influenced in increasing the number of tillers compared to un-inoculated control plants. The dual inoculation of Gf+Ac produced significantly maximum number of tillers. This may be due to supplement of the two major elements (N and P) by the organisms inoculated to rice plants. These results are in agreement with the findings of Earanna and Bagyaraj (2008) in *Withania somnifera* plant inoculated with VAMF in nursery and PGPRs through root dipping. The straw yield of aerobic rice was significantly higher in co-inoculated treatments compared to single inoculations. This indicated the synergistic effects of the consortia in the root zone of rice plants. The grain yield was though increased in all most all the inoculated plants, did not differ significantly from the control (Table 2). It was observed that after harvest lots of choppy grains in the panicles the reason for which is not known. Further, nitrogen and phosphorus content of shoot and root of aerobic rice was increased in the inoculated plants but did not differ significantly compared to control. AMF inoculation at the nursery stage under both dry and wet conditions increased the growth, grain yield of rice under field conditions (Solaiman and Hirata, 1997). Al-Kanaki (2006) observed increase in nutrient uptake of Tomato plants preinoculated with AMF in the nursery.

The aim of the experiment was to understand the establishment of organism inoculated by root dipping in the rhizosphere soil. It was observed that the microbial population (Bacteria, Fungi and Actinomycetes) was increased in root zone soil of all most all inoculated plants compared to uninoculated plants (Table 3). However, inoculation of all the three consortia significantly increased the microbial population compared to others. Further, root dip inoculation of *A. chroocuccum* significantly increased the free living N_2 fixer population in single as well as in combined treatments. Similarly, the P solubilizers also increased in the rhizosphere of *A. awamori* root dipped seedlings. This indicated that the possibility of root dip inoculation of biofertilizers as more useful compared to other available methods. These results are in agreement with observation made for *Withania somnifera* plants by Earanna and Bagyaraj (2008).

The nursery inoculation of arbuscular mycorrhizal fugus (*G. fasciculatum*) was another important objective of this study as AMF is an obligate symbiont and cannot be cultured on artificial media. In the present study, AMF was inoculated in the nursery beds of aerobic rice and the seedlings were grown for 30 days in the nursery bed and then transplanted to main field. The nursery inoculated plants besides showing improved growth and yield, showed significantly higher root colonization in all the mycorrhizal treatments. So also the chlamydospore

Table 4. Effect of nursery bed inoculation of arbuscular mycorrhiza on root colonization and spore numbers in the root zone soil of the aerobic rice.

Treatments	Root colonization (%)	Spore number per 25g soil
Control	34.33[d]	98.66[b]
Glomus fasciculatum (Gf)	66.33[a]	206.33[a]
Azotobacter chroococcum (Ac)	53.33b[c]	103.66[b]
Aspergillus awamori (Aa)	48.13[c]	96.66[c]
Gf + Ac	65.20[a]	121.66[b]
Gf + Aa	61.66[ab]	242.00[a]
Gf + Ac + Aa	64.33[a]	214.00[a]
LSD @ 0.5%	11.22	60.32

Note: Means of the same super script do not differ significantly at 5% level of duncan multiple range test.

numbers in the root zone soil also increased significantly. This indicated that the establishment of nursery inoculated seedlings in the field. The least root colonization and spore numbers were observed in the un-inoculated plants. Mycorrizal colonization of rice plants inoculated with AMF in the nursery remained higher than those of uninoculated plant under both field and pot conditions (Solaiman and Hirata, 1997). The nursery inoculated *Withania somnifera* and *Phyllanthus amarus* plants showed increased growth, biomass, mycorrhizal colonization and established of bacterial populations in the root zone soil (Earanna and Bagyaraj, 2004; Earanna and Bagyaraj, 2008). Thus, the present study suggests that the nursery inoculation of AMF for aerobic rice could carry and establish the mycorrhizal inocula in field; and root dip inoculation of bacterial and fungal biofertilizers is ideal for field crops.

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