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INTERACTION OF DIAZOTROPHS WITH PHOSPHORUS-SOLUBILIZING BACTERIA: THEIR EFFECT ON SEED GERMINATION, GROWTH AND GRAIN-YIELD OF MAIZE, UNDER RAINFED CONDITIONS

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ABSTRACT

Interaction between two diazotrophs (*Rhizobium* and *Azospirillum*) and one phosphorus -solubilizing bacterium (PSB), *Pseudomonas* was studied in laboratory using soil extract agar plates and carrier models. On soil extract agar medium, the three microbes grew without inhibiting each other. In the carrier, all three microorganisms sustained their population equal or more than 107cfu/ g of carrier (effective enough for successful inoculation) after eight weeks storage at 28°C, irrespective of in what combination they exist. *Rhizobium* maintained highest population at the end of 8 weeks and seems to play a probiotic role for PSB, as population of PSB was higher in the presence of *Rhizobium* as compared to PSB alone after 8 weeks. Maize seed germination was 25% higher than in control after 24 h of inoculation when seeds were inoculated with coinoculant, *Azospirillum* + PSB or with *Azospirillum+Rhizobium* + PSB than in un-inoculated seeds. In field maize crop model was used to study the performance of three microbes in the presence of each other. Increase in grain yield ranged from 27%-50% over the control due to inoculation. Of all the inoculated treatments, highest increase was recorded when inoculant containing all three microbes *Rhizobium, Azospirillum* and *Pseudomonas* were inoculated in the presence of nitrogen @30kg/h and phosphorus @20kg/h. The grain yield in this case was equal to that when 120kg N and 80kg P was applied without bacterial inoculation. Highest seed nitrogen as well as seed phosphorus was recorded when *Pseudomonas* was applied in the presence of 120kg nitrogen and 40 kg phosphorus. Grain yield showed good correlation with straw Phosphorus (r0.0= 0.58) and leaf nitrogen (r0.0=0.63).

Key words: Coinoculant, diazotrophs, PSB, maize, grain-yield, seed-N and P

INTRODUCTION

Co-inoculation with beneficial bacteria has been recommended to ensure crop inoculation response (Hassouna *et al.*, 1991) in field to cope with variability in soil conditions. Mixture of rhizobacteria has been reported to work better for wheat by Freitas *et al.*, (2000). Indigenous isolates of *Azospirillum*, *Rhizobium* and *Pseudomonas* at soil microbiology labs NARC, are being used as single microbe inoculant. *Azospirillum* has previously been reported to increase grain yield by reducing the effect of drought through mitigating the effect on water potential and chlorophyll content of leaves (Casanovas *et al.*, 2003). *Rhizobium* operates through promoting root growth. *Pseudomonas* is a phosphate solubilizing bacteria (PSB). However their metabolic products may affect each other's role. The present work aimed at determining the abilities of different isolates to co-exist *in vitro* and in carrier and to improve seed germination, growth and yield of maize, under rainfed conditions.

MATERIALS AND METHODS

In vitro Interaction

Three test microorganisms viz. *Azospirillum*, *Rhizobium* and *Pseudomonas*, were grown in their respective liquid media to a mid log phase so as to have a cell population of 108ml-1. The three microbes were allowed to interact on soil-extract agar medium (Boothe, 1971) in Petri plate model following Eugene *et al* (2001), using cellophene discs instead of filter paper discs.

Co-culturing in Carrier

Two diazotrophs (*Azospirillum* and *Rhizobium*) and one phosphorus–solubilizing bacterium *Pseudomonas* (all previously proved satisfactory as solo performers) were prepared in NARC carrier in seven combinations (viz. *Rhizobium, Azospirillum, Pseudomonas, Rhizobium+ Azospirillum, Azospirillum+ Pseudomonas, Rhizobium+ Azospirillum+ Pseudomonas*) in equal ratios. The inoculants thus prepared were kept at 28oC for a period of 8 weeks. Samples were retrieved at 2-weeks intervals and enumeration of each of the constituting microbe was made on selective media.

Seed Germination test

Maize seeds, surface sterilized with 17% Hydrogen Peroxide were inoculated with seven inoculants treatments (as mentioned previously). Two un-inoculated sets of seeds (one non-surface sterilized and other surface sterilized)

were kept as control. The seeds were allowed to germinate in pre-sterilized petri dishes, containing filter paper. Percent germination was recorded daily for a period of three days.

Field Performance with maize

A field experiment was conducted on a low fertility (N=1.5%,P=11.9ppm, organic matter=0.10%) silt loam soil at Tarlai under rainfed conditions. The experiment included seven inoculated treatments, one un-inoculated control (with 25% of recommended dose of N as well as of Phosphorus) and other fertilized (recommended full dose) control. Urea and single superphosphate (SSP) were used as source of nitrogen and phosphorus respectively.

T0: Uninoculated +1/4N,P; T1: *Rhizobium*+ 1/ 2N, P; T2: *Azospirillum*+ 1/ 2N, P; T3: *Pseudomonas*+ N,1/2P
T4: *Rhizobium*+*Azospirillum*+1/ 2N, P; T5: *Rhizobium*+*Pseudomonas* +1/ 2N, 1/2P; T6: *Azospirillum*+ *Pseudomonas*+1/ 2N, 1/2P; T7: *Rhizobium*+*Azospirillum*+*Pseudomonas*+1/ 2N, 1/2P; T8: NPK.

The treatments were arranged in randomized complete block design with four replicates. Seeds were hand sown on ridges. Leaf nitrogen was estimated in flag top leaves at 2 month age. Above ground dry biomass, root dry biomass, thousand grain weight, grain yield,straw –P, seed N and P were recorded at maturity. All N-determinations were made by micro-kjeldahl method and P-determinations by Acid digestion method. The experiment was planted in Kharif 2005. Rainfall recorded during the period was 470mm.

RESULTS AND DISCUSSION

***In vitro* Interactions**

There was no inhibition to either of the partner on soil extract agar medium. Previously liquid synthetic media have been used to study *in vitro* interactions (Tyagi *et al.*, 2002). In the carrier, there was an overall decline from initial population of *Rhizobium, Azospirillum,* as well as *Pseudomonas* from 108 to 107 / g of carrier at the end of eight weeks, with periodic resurgence in population, irrespective of in what combination they exist (Table 1). Previously different legume-rhizobia when given an initial population of 107 / g, were able to maintain this population till 12 weeks (Khalil *et al.*, 1991) in the same carrier. In this context, the decline is not significant. Diluted cultures having initial populations equal to 106 have been reported to have longer shelf life (Somasegaran, 1988). During the present study population of *Pseudomonas* was higher (log10=7.7) in the presence of *Rhizobium* as compared to *Pseudomonas* alone (log10=6.3) at the end of 8 weeks. *Rhizobium* thus seems to play a probiotic effect on *Pseudomonas.* All other combinations maintained the population of their component microbe between 107 and 109 till six weeks. A trend of reduction at the end of eighth week, in all treatments except *Rhizobium* was noticed and can be explained on the basis of intra- or inter- microbe competition when population threshold reaches 108-9 under long storage conditions. However, a shelf life of six weeks is enough for local use of this biofertilizer. Co-culturing of *Rhizobium meliloti* and *Penicillium bilaji* in sterile peat has been reported as successful by Rice *et al.,* (1995). Successful coculturing of five (out of eight) rhizobial strains, with five phosphorus-solubilizing fungi, has been reported (Abd- Allah and Omar, 2001).

Improvement of Maize seed germination by different combinations

Maize seed germination was 25% higher than in control after 24 h of inoculation with *Azospirillum*+ *Psudomonas* or with *Azospirillum*+*Rhizobium*+*Psudomonas*. Effect on seed germination can be related with growth hormone production by the microbe. *Azospirillum* has been reported to produce IAA (Crozier *et al.*, 1988) and promote seed germination in tomato and capsicum (Moya-Rodriguez *et al.,* 2001) The indigenous chickpea-*Rhizobium* has also been reported to produce IAA (Yasmin, 2003). However either of them could not manifest their effect alone in this case. Presence of *Pseudomonas* seems to make them operative. *Pseudomonas* has been reported to play role in biological control of pathogens through eliciting certain chemicals in the rhizosphere (Kumar *et al.*, 2001). If these chemicals are supporting the combination partner here, yet remains to be answered.

Effect on growth and yield of Maize

Increase in grain yield ranged from 27-50% over the control due to inoculation. Of all the inoculated treatments, highest increase was recorded when multi-microbe inoculant containing all three microbes *Rhizobium*, *Azospirillum* and *Pseudomonas* was inoculated (Table 3) in the presence of half of recommended dose of both N and P.
The diazotrophs associated with roots of non-legumes are less specific than symbiotic ones and is subject to varying effects of the environment. To cope with this situation, application of multi-microbe inoculant has been

recommended to increase the probability of inoculation success in the field (Dommerguez, 1988) where environmental conditions fluctuate or vary in different ecologies. Growing evidence of favorable effects of combined application of *Azotobacter*, *Azospirillum* and *Pseudomonas* bacteria providing plant nutrients to cereals and vegetables and supplementing inorganic fertilizer has been reported (Belimov *et al*., 1995).Peat or Bran inoculants of *Rhizobium leguminosarum* RCR 1044 +A. *niger* and *Bradyrhizobium japonicum* USDA +A.*niger* significantly increased dry matter yield of faba bean and soybean (Abd-Allah and Omar,2001). Co-inoculation of *Pseudomonas fluorescenes* 2137 and *Bradyrhizobium japonicum* A1017 increased the colonization of *B. japonicum* A1017 on soybean roots and ARA at 10 and 20 days after inoculation (Chebotar *et al.*, 2001). Co-inoculation of maize with *Azospirillum+ Rhizobium+ Pseudomonas* saved the cost of 50% N (urea) as well as 50%P (super phosphate) without compromising on yield. Previously, 40% saving on N-fertilizer has been reported by Santa *et al.*, (2004) by inoculating maize cv Carqil-909 in Brazil while 50% saving due to *Azorhizobium* and *Azospirillum* has been reported by Woodard and Bly (2000).

Table 1. Population Interaction of Diazotrophs with Phosphorus Solubilizing Bacteria in NARC carrier at 28 °C.

Treatment (microorganism)	Colony forming units/gram of carrier (log 10) At				
	Zero-time	2-weeks	4-weeks	6-weeks	8-weeks
(*Rhizobium*)	8.0	7.0	7.6	7.0	8.4
(*Azospirillum*)	8.0	9.0	8.0	7.6	6.7
(PSB)	8.0	7.0	9.0	8.0	6.3
Rhizobium + *Azospirillum*)	8.0	7.0	7.0	6.0	6.7
Rhizobium + PSB	8.0	7.0	7.2	7.0	6.9
Azospirillum + PSB	8.0	7.7	7.0	7.7	7.7
Rhizobium + *Azospirillum* +PSB	8.0	8.0	7.0	7.6	7.7
	8.4	7.0	8.7	7.7	<6
	8.0	7.8	8.6	9.0	6.7
	8.0	7.0	8.0	8.6	6.3
	8.0	7.0	8.0	7.8	<6.0
	8.0	7.0	8.0	8.7	6.5

Table 2. Effect of multi-microbe inoculant on Maize seed (NARC3001) germination.

Inoculant	Percent germination		
	Day1	Day2	Day3
(Unsterilized control)	50	65	75
(surface-sterilized control)	55	75	90
(*Rhizobium*)	60	80	95
(*Azospirillum*)	55	80	95
(PSB)	65	90	100
(*Rhizobium*+*Azospirillum*)	65	90	100
(*Rhizobium*+ PSB)	70	95	100
(*Azospirillum* +PSB)	75	95	100
(*Rhizobium* + *Azospirillum* +PSB)	75	95	100

Effect on Nitrogen and Phosphorus content

Highest percent (2.4%) nitrogen of grain was recorded when *Pseudomonas* was applied in the presence of nitrogen @120kg/h and phosphorus @ 40kg/h, though not significantly different from when inoculated with *Azospirillum* in the presence of nitrogen @60kg/h and phosphorus@ 80kg/h or with *Rhizobium+Azospirillum* in the presence of nitrogen @60kg/h and phosphorus @ 80kg/h or with *Rhizobium +Pseudomonas* in the presence of

nitrogen @60kg/h and phosphorus @40kg/h (Table 4). Lesser percentage of grain nitrogen on application of multi-microbe inoculant may be attributed to larger grain yield produced.

Table 3. Effect of multi-microbe inoculant on growth and yield of Maize var. NARC3001 under rainfed conditions.

Treatments	Above ground dry biomass (kg)	Root dry biomass (g)	1000-grain weight (g)	Grain yield (kg/h)
Uninoculated control with ¼ N, ¼ P, K	2.371b	114.075c	54.000c	894.80c
Rhizobium +, ½ N, P	3.076a	127.150b	56.32c	1137.50b
Azospirillum+ 1/ 2N, P	3.072a	127.700b	63.65b	1206.50ab
PSB+N,1/2P	2.941a	128.250b	66.50ab	1234.75ab
Rhizobium +*Azospirillum*+1/2N, P	3.018a	130.92ab	65.75ab	1215.00ab
Rhizobium +PSB+1/2N, 1/2P,K	3.151a	130.47ab	67.25ab	1257.00ab
Azospirillum+PSB+1/2N, 1/2P	3.150a	129.025b	66.75ab	1249.25ab
Rhizobium +*Azospirillum*+PSB+1/2N,1/2P	3.087a	130.975b	67.70ab	1350.00ab
NPK	3.262a	140.425a	69.62a	1395.00a
LSD 0.05	0.516	10.01	5.704	234.3

Table 4. Effect of different inoculants, on nitrogen and phosphorus- concentration (%) of Maize plant.

Treatments	Leaf Nitrogen (%)	Straw Phosphorus (%)	Seed Nitrogen (%)	Seed Phosphorus (%)
Uninoculated control with ¼ N, ¼ P, K	0.44 e	0.04	1.73 c	0.097 d
Rhizobium +, ½ N, P, K	0.56 d	0.05	2.14 b	0.137 cd
Azospirillum+ 1/ 2N, P,K	0.59 cd	0.05	2.19 ab	0.147 c
PSB+N,1/2P,K	0.56 d	0.07	2.41 a	0.195 ab
Rhizobium +*Azospirillum*+1/2N, P,K	0.58 cd	0.06	2.30 ab	0.147 c
Rhizobium +PSB+1/2N, 1/2P,K	0.67 cd	0.06	2.38 ab	0.170 abc
Azospirillum+PSB+1/2N, 1/2P,K	0.61 cd	0.06	2.24 ab	0.152 bc
Rhizobium +*Azospirillum*+PSB	0.79 b	0.07	1.48 cd	0.182 abc
NPK	0.92 a	0.08	1.43 d	0.205 a
LSD0.05	0.09	NS	0.265	0.046

Highest seed Phosphorus due to inoculation of *Pseudomonas* alone, in the presence of nitrogen @ 120kg/h and phosphorus @ 40 kg/h can be attributed to more P-availability. Improved root-uptake of P and hence improved nitrogenase activity and growth of barley has been reported due to inoculation with mixture of nitrogen fixers and phosphate solubilizing bacteria (Belimove *et al.,* 1995). Significant increase in N and P content of faba bean cv.Alborea has also been reported due to inoculation by co-culture of *Bradyrhizobium* and P-solubilizer fungus *Aspergillus niger* (Abdalla and Omar, 2001).

Grain yield showed good correlation with straw Phosphorus (r0.0= 0.58) and leaf nitrogen (r0.0 =0.63).
Best overall performance was shown by tripartite inoculant as it increased seed germination by 25% and grain yield by 50%. It is thus concluded that the three reported microorganisms can be co-cultured in NARC carrier and the multi-microbe inoculant can be used as biofertilizer in low fertility soil in low rainfall area, with low fertilizer input agriculture, so as to cut down the cost of fertilizer without compromising on yield.

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