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Significance of zinc fertilization and microbial inoculation on phosphorus nutrition of rice (*Oryza sativa*)

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ABSTRACT

The present experiment was conducted during rainy season of 2013-14 at ICAR–Indian Agricultural Research Institute, New Delhi, to compare and calculate variations in phosphorus (P) concentration and uptake in rice (*Oryza sativa* L.) plant as well as soil available P (Olsen's reagent 0.5 M $NaHCO_3$-extractable) as influenced by three different crop establishment methods (CEMs), rates of nitrogen (N), phosphorus (P) and zinc (Zn) fertilization and microbial inoculations in spilt plot design with three replications. The concentration and uptake of P in puddled transplanted rice (PTR) and system of rice intensification (SRI) was significantly higher than aerobic rice system (ARS) and total uptake was increased by 480 and 540 g/ha in PTR and 580 and 660 g/ha in SRI over ARS in first and second year, respectively. The treatment with 100% recommended dose of nutrients (RDN) (25.8 kg P/ha and 120 kg N/ha) had significantly higher P concentration and uptake than 75% RDN and absolute control. The correlation between milled rice yield and P concentration was found positive (R^2= 0.95 and 0.94). Application of microbial inoculation significantly increased P concentration and uptake over fertilizer control (75% RDN) and absolute control which increased in total P uptake by 640 and 680 g/ha due to application of *Anabaena–Pseudomonas* (An-Ps) biofilmed formulations (MI2) and *Anabaena* sp. (CR1) + *Providencia* sp. (PR3) consortia (MI1) over fertilizer control (75% RDN).

Key words: Aerobic rice system (ARS), *Anabaena–Pseudomonas* (An-Ps) biofilmed formulations, Phosphorus, Rice

Phosphorus (P) is second most important nutrient after nitrogen (N) and 42% of the 3.65 million soil samples analysed was found low in P status in India (Tandon 2013). Out of total fertilizer consumed in India, 29.2% N, 27.6% P_2O_5 and 35% K_2O was consumed by rice (*Oryza sativa* L.) and share of P in total fertilizer consumed in rice was 26.8%. Along with this status of P deficiency and fertility addition through fertilizer, the use of high yielding varieties over larger area and intensification of cropping system again aggravate the problem of P deficiency in soil. The rice is grown under different crop establishment methods (CEMs) varying their water regimes such as puddled transplanted rice (PTR), system of rice intensification (SRI) and aerobic rice system (ARS). These conditions create variation in soil P availability and response to applied P. The responses to P fertilization in these various CEMs were studied in relation to concentration and uptake of P in final crop produce and soil P status at initial and at harvest of crop. To know the P dynamics across different CEMs, variation in concentration and uptake of P in rice and soil available P status at frequent interval need to be studied. At the same time, high P fixation in soil, low use efficiency of added P fertilizer (Roberts and Johnston 2015) and high price per unit of P in fertilizer (₹ 60.8 per kg P_2O_5) also need to be addressed. The use of microbial inoculations make fixed P available for plant growth, substitute part of P fertilizer addition and bring economy and sustainability in P nutrition of agricultural crop (Sharma *et al.* 2010, Sharma *et al.* 2011, Alori *et al*. 2017). Taking these research backgrounds into consideration, it was needed to quantify the effect of microbial inoculation and rate of N and P application on P nutrition of rice and their influence on available soil P status under varying CEMs and therefore the present study was conducted.

MATERIALS AND METHODS

A field experiment was conducted for two years (2013, 2014) during *kharif* (June-October). Experimental field was at research farm of ICAR–Indian Agricultural Research institute, New Delhi, India with latitude of 28°38' N, longitude of 77°10' E and altitude of 228.6 m amsl. The mean annual normal rainfall and evaporation was 650 mm and 850 mm, respectively. The soil of the experimental field was sandy clay loam in texture with 0.54% organic C, 257 kg/ha alkaline permanganate oxidizable N, 17 kg/ha available P, 327 kg/ha 1 N ammonium acetate exchangeable K and 0.85 mg/kg of DTPA–extractable Zn with *p*H of 7.6

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(1: 2.5 soil and water ratio).

The experiment was conducted in split plot design involving three crop establishment methods (CEMs), viz. conventional puddled transplanted rice (PTR), system of rice intensification (SRI) and aerobic rice system (ARS) as main plot. In sub–plot treatments, two microbial inoculation, viz. consortia of *Anabaena* sp. (CR1) + *Providencia* sp. (PR3) (MI1) and *Anabaena–Pseudomonas* biofilmed bio-fertilizer (MI2) were applied with 75% recommended dose of nutrients (RDN) and compared with 100% RDN (120 kg/ha N and 25.8 kg P/ha) and 75% RDN making total four treatments. These four treatments were applied with and without Zn fertilization along with one absolute control making total nine sub–plot treatments and were replicated thrice. Rice variety Pusa Sugandh 5 was used.

The sowing of rice in main field for ARS and sowing rice in nursery for transplanting in both PTR and SRI was done at the same date. For ARS, direct sowing of seed (seed rate 60 kg/ha) was done with spacing of 20 cm between two rows using seed-drill. In SRI, single healthy seedling of 14–days old was transplanted/hill at a spacing of 20 × 20 cm, whereas in PTR, two healthy seedlings of 25–days old were transplanted/hill at a spacing of 20 × 15 cm. For application of microbial inoculants, a thick paste of respective culture was made and applied to rice seedling in PTR and SRI method of rice cultivation by dipping roots in paste of respective culture for half an hour before transplanting. In direct seeded ARS pre-soaked seeds were treated with thick paste of culture made in carboxyl methyl cellulose. The whole dose of phosphorus as per the treatment involved was applied at the time of sowing. Nitrogen was applied in split in all treatments irrespective of dose at sowing, 30 days after sowing (DAS) and 60 DAS in ARS and 5 days after transplanting (DAT), 25 DAT and 55 DAT in both PTR and SRI. Potassium was applied uniformly (49.8 kg K/ha) in all plots at the time of sowing. Zinc was soil applied at the rate of 5 kg Zn/ha through zinc sulphate heptahydrate as basal dose at the time of sowing/transplanting.

P in rice plant at different growth stages as well in white rice kernel, bran and hull parts was determined by wet digestion method following vanadomolybdophosphoric acid yellow colour method. Available soil P was determined by Olsen's procedure. Alkaline phosphatase activity was assayed in soil suspended in modified universal buffer (*p*H 11), along with 1 ml p-nitro phenyl phosphate (Tabatabai and Bremner 1969).

RESULTS AND DISCUSSION

Phosphorus concentration: Phosphorus concentration showed decreasing trend from 40 DAS toward maturity in straw with highest concentration recorded in bran followed by 40 DAS and the lowest in hull (Table 1). Major reasons for decreasing concentration from 40 DAS towards crop maturity in straw are dilution effect due to increase in dry matter accumulation, decrease in P absorption toward maturity and translocation of most of P from straw to rice grain. The P concentration in milled rice grain was 1.9 to 2.0 times higher than straw which might be due to translocation of P from straw to rice grain and dilution effect due to higher straw yield. Among CEMs, the highest concentration was recorded in SRI and remained at par with PTR; while ARS stood inferior to both SRI and PTR. This superiority was explained as better crop growth leading to higher P absorption, increased P availability under puddled condition, and higher contribution by microbial inoculation and lower weeds competition.

The concentration was not affected significantly due to nutrient management options at 40 DAS; while at 70 and 100 DAS, concentration of P in T2 was significantly higher than T4 and T1 remained inferior to both of them (Table 1). The superiority of T2 over T4 was indication of positive effect of optimal P dose than sub–optimal dose and superiority of both over control signifies the importance of P fertilization. The T6 and T8 stand statistically superior to T4 and on par with T2 indicating impact of inoculation on P concentration (Lavakush *et al*. 2014). The interaction between CEMs and nutrient management options on P concentration was found significant at 100 DAS and in straw, milled rice and hull.

Phosphorus uptake: The uptake increased toward crop maturity with highest uptake in straw and least in hull (Table 1). This might be due to increasing dry matter toward crop maturity. Out of total uptake, 61–63% was accumulated in rough rice and 37–39% remained in straw. Among CEMs, SRI and PTR had statistically identical uptake and both stood superior to ARS at all growth stages and in straw and milled rice. The superiority of PTR and SRI over ARS in P uptake was due to higher dry matter and concentration. The variation in P uptake among methods of cultivation was also reported (Parameswari Y and Srinivas R 2014).

The dry matter and concentration was higher in T2 than T4 which was translated to higher P uptake in T2. The positive effect of P application on dry matter (Amanullah and Inamullah 2016) and P uptake (Prasad *et al*. 2018) was also reported. The zinc fertilization significantly influenced P uptake at 70 and 100 DAS and in straw and milled rice. The positive effect of Zn fertilization on dry matter was translated to increasing P uptake. The application of microbial inoculation of MI1 (0.56 kg/ha) and MI2 (0.62 kg/ha) also showed improvement in total P uptake as corroborated with Sharma *et al*. (2011), Sharma *et al*. (2010) and Madar *et al*. (2011). The interaction between CEMs and nutrient management options on P uptake was found significant at all growth stages and in all components of rough rice.

Soil available phosphorus content: The initial soil available phosphorus (Olsen's reagent 0.5 M $NaHCO_3$–extractable) was doubled at 40 DAS in all treatments having P application (RDN and 75% RDN) except control (Table 2) which was because all P fertilizer was applied as basal and P uptake rate was also very low during initial growth stages. The soil P at harvest was higher by 5–6 kg/ha over initial soil P. The improvement in soil P status due to P fertilization was also reported by Jat R and Ahlawat I

Table 1 Effect of crop establishment methods and nutrient management options on phosphorus concentration and uptake in rice in 2013 and 2014

Treatment	Phosphorus concentration (mg/kg of dry matter)							Phosphorus uptake (kg/ha)							Total uptake (kg/ha)	
	40 DAS	70 DAS	100 DAS	Straw	Milled rice	Bran	Hull	40 DAS	70 DAS	100 DAS	Straw	Milled rice	Bran	Hull	2013	2014
Crop establishment methods																
Puddled transplanted rice (PTR)	1339	1200	524	503	1005	6746	204	1.41	3.83	3.41	3.62	2.68	3.01	0.18	9.55	9.43
System of rice intensification (SRI)	1341	1202	525	504	1007	6748	204	1.40	3.81	3.44	3.62	2.70	3.09	0.18	9.65	9.55
Aerobic rice system (ARS)	1330	1191	514	492	990	6736	199	1.37	3.71	3.27	3.40	2.48	2.91	0.19	9.07	8.89
SEm±	1.3	1.2	1.1	0.9	1.4	2.1	0.7	0.003	0.010	0.010	0.004	0.012	0.069	0.001	0.066	0.058
CD (P=0.05)	5.1	4.8	4.1	3.6	5.6	8.3	2.7	0.011	0.037	0.039	0.016	0.047	0.270	0.005	0.258	0.226
Nutrient management options																
Control ($N_0P_0Zn_0$) (T1)	1322	1176	502	481	981	6718	190	1.24	3.05	2.68	2.96	1.97	2.42	0.15	7.68	7.34
RDN* (T2)	1341	1204	527	505	1007	6751	206	1.42	3.89	3.49	3.64	2.73	3.07	0.19	9.73	9.54
RDN + Zn** (T3)	1342	1205	527	505	1008	6753	207	1.43	4.04	3.60	3.80	2.88	3.16	0.19	9.98	10.08
75% RDN (T4)	1335	1191	516	495	994	6734	200	1.40	3.65	3.25	3.37	2.43	2.96	0.18	9.00	8.86
75% RDN + Zn (T5)	1335	1191	517	496	995	6734	200	1.41	3.71	3.31	3.45	2.52	3.04	0.19	9.32	9.07
75% RDN + MI1 (T6)	1338	1201	524	502	1004	6748	205	1.41	3.83	3.46	3.59	2.69	3.04	0.19	9.59	9.4
75% RDN + MI1 + Zn (T7)	1340	1204	527	505	1006	6751	205	1.42	4.01	3.57	3.76	2.84	3.13	0.19	9.92	9.91
75% RDN + MI2 (T8)	1339	1201	525	502	1005	6749	205	1.41	3.85	3.47	3.60	2.70	3.07	0.19	9.62	9.48
75% RDN + MI2 + Zn (T9)	1341	1205	527	505	1006	6751	206	1.42	4.02	3.58	3.76	2.84	3.15	0.19	9.97	9.93
SEm±	2.7	2.5	2.1	1.7	2.9	4.2	1.2	0.005	0.021	0.020	0.014	0.021	0.102	0.002	0.108	0.088
CD (P=0.05)	7.6	7.0	5.9	4.9	8.3	12.0	3.3	0.02	0.06	0.06	0.04	0.06	0.29	0.01	0.306	0.251
Interaction	NS	NS	Sig.	Sig	Sig.	NS	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.

RDN*, Recommended dose of nutrients 120 kg N/ha and 25.8 kg P/ha; Zn**, Soil applied 5 kg Zn/ha through zinc sulphate heptahydrate; MI1, (*Anabaena* sp. (CR1) + *Providencia* sp. (PR3) consortia; MI2, *Anabaena–Pseudomonas* biofilmed formulations; DAS, Days after sowing; NS, Non-significant; Sig., Significant.

Table 2 Effect of crop establishment methods and nutrient management options on soil available phosphorus (kg/ha) and alkaline phosphatase activity in rice at different growth stages during 2013 and 2014

Treatment	2013					2014					Total phosphorus present in soil (kg/ha)		Calculated balance (kg/ha) (Total soil P – total P uptake)		Alkaline phosphatase activity (APA) (µg PNP/g of soil/hr)			
	Initial	40 DAS	70 DAS	100 DAS	At harvest	Initial	40 DAS	70 DAS	100 DAS	At harvest	2013	2014	2013	2014	2013 70 DAS	2014 100 DAS	2013 70 DAS	2014 100 DAS
Crop establishment methods																		
Puddled transplanted rice (PTR)	17	33.2	29.8	29.3	23.6	22.5	35.8	29.4	25.8	19.4	35.6	41.13	26.1	31.7	68.1	117.0	47.2	84.9
System of rice intensification (SRI)	17	33.2	29.8	29.3	23.5	22.4	35.6	29.3	25.5	19.1	35.6	41.03	26.0	31.5	71.3	121.6	48.0	86.0
Aerobic rice system (ARS)	17	32.9	29.0	28.2	22.9	20.7	33.8	27.5	23.7	17.7	35.6	39.33	26.6	30.4	63.0	111.3	43.5	79.0
SEm±	-	0.005	0.01	0.01	0.06	0.13	0.16	0.19	0.22	0.25	–	0.13	0.07	0.17	0.34	0.61	0.29	0.56
CD (P=0.05)	-	0.020	0.04	0.04	0.23	0.50	0.62	0.74	0.86	0.99	–	0.50	0.26	0.66	1.33	2.41	1.13	2.19
Nutrient management options																		
Control ($N_0P_0Zn_0$) (T1)	17	18.1	18.5	21.3	17.0	10.7	12.6	13.8	17.1	12.1	17	10.7	9.3	3.4	30.3	56.1	11.1	28.2
RDN* (T2)	17	38.2	32.4	29.7	24.0	23.0	40.2	30.6	23.7	17.3	42.8	48.8	33.1	39.2	67.6	117.8	48.9	87.6
RDN + Zn** (T3)	17	38.2	32.3	29.6	23.4	21.6	38.8	29.0	22.2	15.4	42.8	47.4	32.8	37.3	68.8	118.3	49.4	87.9
75% RDN (T4)	17	32.5	27.8	25.8	20.5	16.7	29.3	21.7	16.6	10.7	36.4	36.05	27.3	27.2	68.5	116.8	48.5	86.7
75% RDN + Zn (T5)	17	32.5	27.7	25.8	20.3	16.2	28.8	21.2	16.1	10.1	36.4	35.55	27.0	26.5	68.7	117.5	48.6	85.8
75% RDN + MI1 (T6)	17	34.6	31.9	32.1	26.4	28.0	42.4	36.5	33.2	27.0	36.4	47.35	26.8	38.0	74.3	128.2	51.6	92.6
75% RDN + MI1 + Zn (T7)	17	34.6	31.7	31.9	25.9	26.4	40.8	34.7	31.6	24.9	36.4	45.75	26.4	35.9	74.6	128.8	52.1	93.2
75% RDN + MI2 (T8)	17	34.6	31.9	32.1	26.4	27.8	42.2	36.2	33.0	26.7	36.4	47.15	26.7	37.6	77.0	132.4	52.7	93.2
75% RDN + MI2 + Zn (T9)	17	34.6	31.7	31.9	25.9	26.3	40.7	34.6	31.5	24.8	36.4	45.65	26.4	35.7	77.3	133.6	52.8	94.7
SEm±	-	0.007	0.02	0.02	0.09	0.19	0.24	0.28	0.33	0.38	–	0.19	0.11	0.26	0.59	1.04	0.50	1.11
CD (P=0.05)	-	0.020	0.06	0.06	0.25	0.55	0.68	0.80	0.93	1.07	–	0.55	0.31	0.73	1.68	2.95	1.42	3.17
Interaction		Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	–	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.

RDN*, Recommended dose of nutrients 120 kg N/ha and 25.8 kg P/ha; Zn**, Soil applied 5 kg Zn/ha through zinc sulphate heptahydrate; MI1, (*Anabaena* sp. (CR1) + *Providencia* sp. (PR3) consortia; MI2, *Anabaena–Pseudomonas* biofilmed formulations; DAS, Days after sowing; Sig, Significant.

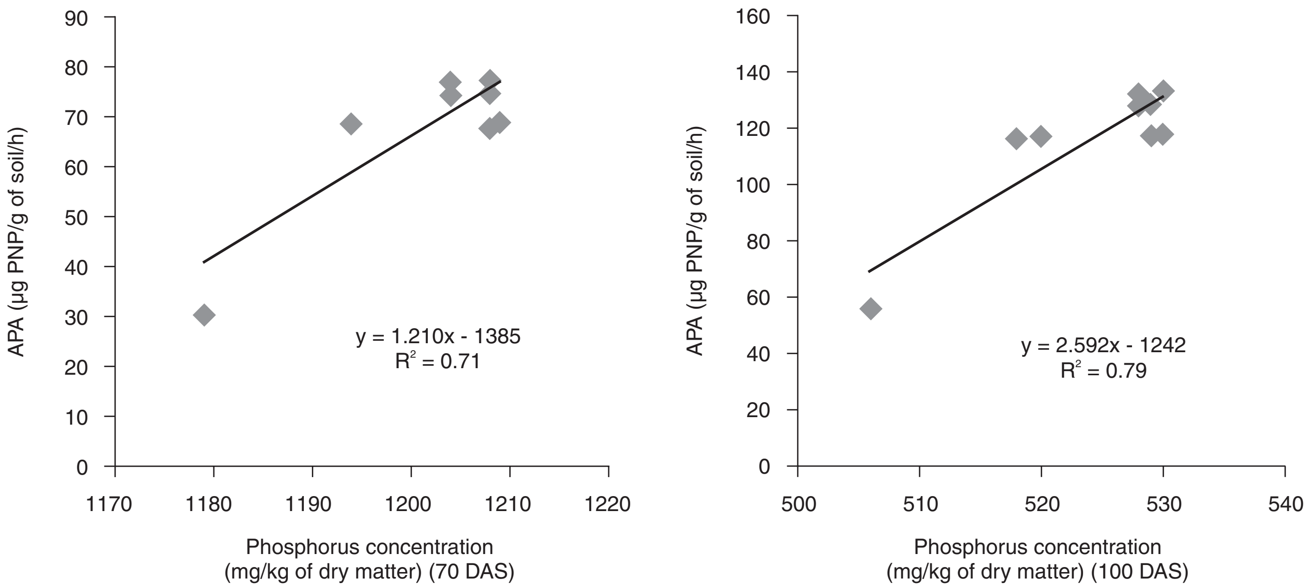

Fig 1 Correlation of phosphorus concentration with alkaline phosphatise activity (APA) 70 days after sowing (DAS) and 100 DAS in 2013.

(2006). In second year, soil P at 40 DAS also increased over soil P at start of second season and after 40 DAS, soil P decreased toward maturity with soil P at harvest lower than initial soil P by 3–4 kg/ha. The soil P in SRI and PTR was consistently and significantly higher than ARS in both years at all observations which might be due to increased soil P in both methods due to puddling and lower P fixation than ARS. The treatment T2 also recorded significantly higher soil P than T4 in both years. The higher application rate (25.8 vs. 19.4 kg/ha) is the major reason for superiority of T2 over T4 in increasing the soil P. The positive effect of Zn fertilization was observed when applied with microbial inoculation (T6 and T8). Hence, even though application rate of Zn and increase in dry matter due to Zn application in T2, T6 and T8 remained same, soil P was significantly higher when Zn was applied with T6 and T8. The treatment T6 and T8 had significantly higher soil P than T4 at all observations in both years which signifies the role of MI1 and MI2 in soil available P (Stephen *et al*. 2015).

Alkaline phosphatase activity (APA): The APA enzyme is responsible for mineralising and dissolving organic and inorganic P in soil, respectively. The APA was higher in first year and 100 DAS than second year and 70 DAS, respectively (Table 2). Among CEMs, SRI and PTR had significantly higher APA than ARS; while SRI stand superior to PTR in first year and remained on par in second year. The application of RDN, 75% RDN and Zn fertilization was not able to influence APA. The inoculated treatment had significantly higher APA than uninoculated one at all observations. This showed that, inoculated microbes played a significant role over inherent soil microbial population and phosphatase secreted by plant roots (Stephen *et al.* 2015). The contribution of inoculants to P concentration was judged from positive correlation between APA with concentration and uptake of P in rice at 100 DAS (Fig 1).

Available phosphorus balance in soil: The available (Olsen's reagent 0.5 M $NaHCO_3$–extractable) and total phosphorus [soil initial P (Olsen's reagent 0.5 M $NaHCO_3$–extractable) + P applied through fertilizer] present in soil at initial stage was higher in second year by 3–5 kg/ha than first year (Table 2). The soil available and total P at initial stage present in soil was not differed among CEMs in first year; while both were found significantly higher in both PTR and SRI than ARS in second year. The balance of P at harvest was significantly higher in ARS than PTR and SRI in first year; while in second year, ARS remained inferior to both PTR and SRI. The superiority of ARS in first year was due to lower uptake in first year and inferiority in second year was aroused because of higher total uptake in first season of rice and wheat planted before second year of rice. The actual P present in soil after harvest in both years was significantly higher in both PTR and SRI than ARS. The available and total P at initial stage was higher in T2 and T3 due to higher rate of application in first year. In second year, T6 and T8 had the highest soil P and found statistically superior to T3, T7 and T9 which showed the role of microbial inoculation on improving soil available P.

Our study concluded that, PTR and SRI performed superior in P nutrition of rice than ARS. The involvement of inoculation of *Anabaena–Pseudomonas* biofilmed formulations and *Anabaena* sp. (CR1) + *Providencia* sp. (PR3) consortia by replacing 25% fertilizer P is useful for increasing contribution of soil fixed P in P nutrition of rice and saving cost on fertilizer.

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