



Inorganic phosphorus fractions and crop productivity in response to different rates of phosphate-solubilising fungi *Penicillium bilaii*

Y V SINGH¹ and SUNITA GAIND²

ICAR-Indian Agricultural Research Institute, New Delhi 110 012

Received: 27 May 2017; Accepted: 31 August 2018

ABSTRACT

Two field experiments were conducted at experimental farm of ICAR-Indian Agricultural Research Institute, New Delhi during 2014-15 to investigate the interactive effect of three rates of seed inoculation with wettable formulation of phosphate solubilising fungi (*Penicillium bilaii*) and two rates (60 kg and 30 kg P₂O₅/ha) of chemical phosphorus (P) fertilization on soil P availability and productivity in wheat (*Triticum aestivum* L.) and mustard (*Brassica campestris* L.) crops. Inorganic P distribution in different fractions was conducted by successive extraction of soil P with water, NaHCO₃ (SB-P), NaOH (SH-P) and HCl. Application of fungal formulation @10.604 g/kg mustard seed and 4.894 g/kg wheat seed + 30 kg P₂O₅/ha as chemical P increased the SB-P fraction of mustard and wheat grown soil by 145.7% and 140.7%, respectively compared to their respective un-inoculated controls at maturity. HCl-P was the major fraction under wheat soil with average contribution of 46% and 64.8% of total extractable P at 60 and 120 d of crop growth whereas SH-P was the major fraction under mustard grown soil. Improved soil P availability due to fungal inoculation could explain the role of phosphate solubilising fungi in soil P mobilization. The crop productivity however, was highest with highest rate of chemical P and fungal based formulation.

Key words: Fertilization, *Penicillium bilaii*, Phosphate solubilising fungi, Phosphorus fractions.

Low availability of phosphorus (P) in about 50% of the Indian soils is a matter of great concern due to its adverse effect on crop productivity. Under prevalent soil conditions and agronomically acceptable fertilization rates (based on soil recommendations), the P precipitation reactions with soil clays and oxides, make the P relatively immobile in the soil. This low P-fertilizer efficiency is often corrected through high fertilizer application, which may not be a viable option for cultivators. Increasing the plant availability of added chemical P fertilizers is of utmost importance for enhancing productivity and food security to the projected population during the next century (United Nations, 2007). The primary approach in agronomic management of P is to scavenge the native or fixed P and also to overcome the fixation of applied P-fertilizer. The former option seems more feasible as microorganisms play a fundamental role in releasing inorganic P (Pi) from both organic and inorganic P sources present in soil or added exogenously (Sharma *et al.* 2013). The phosphate solubilising fungi (PSF) have higher potential to improve the availability of P in soil at a relatively low cost. Recent research has focused on using actively sporulating *Penicillium* fungi as inoculants to

enhance P mobilization in soil. Due to their non-specificity for plant and soil association, *Penicillium* species have a broad agro-ecological range, indicating their potential to be developed as inoculants for a range of plant production systems (Harvey *et al.* 2009).

Novozymes is the largest suppliers of industrial enzymes and microorganisms in India, catering to the detergent, food, feed, textile, leather, oils and fats and beverage alcohol industries. Novozymes developed a fungus-based P fertilizer (Jumpstart) that contains PSF *Penicillium bilaii*. Field trials with Jumpstart in wheat crop showed that seed inoculation with *P. bilaii* increased the soil P availability by 18%, stimulated production of root hairs and enhanced root growth (Gulden and Vessey 2000, Vesse and Heisinger 2001) compared to non-inoculated plants. Inoculant product based on *P. bilaii* (Jumpstart) recorded average yield benefits of ~6% in different crops including canola (Kucey and Leggett 1989) and pasture legumes (Beckie *et al.* 1998) but for wheat up to 66% (Novozymes Biologicals Pvt. Ltd. data from over 400 field trials). Seed inoculation with *P. bilaii* and *Penicillium* sp. +100% recommended dose of chemical P improved the P uptake by maize plants at harvest (Patil *et al.* 2012) besides plant biometric parameters.

The soil P contains different P fractions and the transformations are primarily mediated by microbial activity, which is influenced by a combination of factors

¹Principal Scientist (e mail:yvsingh63@yahoo.co.in), CCUBGA, ²Principal Scientist (e mail: sugaind175@rediffmail.com), Division of Microbiology

that can affect the P dynamics in the soil, including plant species, environmental conditions, soil type and soil management (Wright 2009). The challenge for cultivation of soils with low P availability is to develop strategies of management that can enhance P availability and subsequent acquisition by plant. One of these important strategies is use of phosphate dissolving microorganisms. The effect of tillage and P fertilization on distribution of soil P fractions under corn-soybean rotation has been documented by Shi *et al.* (2015). However, information on distribution of P in different fractions induced by different rates of PSF *Penicillium bilaii* inoculation under different chemical P fertilization regimes is scarce. Thus, the present study was undertaken with the objectives (i) to compare the interactive effects of co-application of single super phosphate and seed inoculation with different rates of *P. bilaii* on changes in soil inorganic P fractions under winter grown wheat and mustard crop (ii) to measure the above ground biomass and grain yield responses in tropically grown wheat and mustard under field conditions with different P availabilities. Distribution of soil P pools can increase our understanding about sinks and sources of P in the soil, and is essential for an efficient P management programme. The study was conducted to generate better information on soil P cycling process, optimize dose of P fertilizer and fungal based formulation to improve the availability of soil P for uptake by crop plants and help overcome the limited information that total P analysis provides.

MATERIALS AND METHODS

The experimental field was situated in ICAR-Indian Agricultural Research Institute (IARI), New Delhi, India at latitude: 28° 38' N, longitude: 77° 90' E and altitude of 229 m above mean sea level. The soil of the experimental fields was Inceptisol, Maharuli series with sandy loam texture, deep percolating and well drained, hypothermic family of the Typic Ustochrept (old alluvium). The total rainfall received during wheat and mustard cultivation was 19.80 mm. The experimental fields had an initial pH 8.3, EC 0.26 dS/m, soil organic carbon 0.38%, alkaline KMnO_4 oxidizable N 66.7 mg/kg soil, 0.5 M NaHCO_3 extractable P 26.2 mg/kg soil and exchangeable K 62.6 mg/kg soil. The mustard and wheat crop were sown on 14th Nov and 8th Dec, 2014, respectively. A row width of 20 cm was maintained for wheat and 30 cm for mustard crop. The seed rates for wheat (cv. HD 2967) and mustard (cv. Pusa M 30) were 80 kg and 4 kg/ha, respectively. For proper seed distribution along the rows, strip paper containing seeds were used. The fertilization rates were $\text{N}_{120}\text{P}_{60}\text{K}_{60}$ for wheat and $\text{N}_{80}\text{P}_{40}\text{K}_{40}$ for mustard. For wheat cultivation, half of chemical nitrogen as urea (60 kg N/ha) was applied as basal and the other half at tillering stage. The fields were irrigated as and when required and weeding was done 25 and 45 days after sowing. Both the crops were harvested in the last week of March. The threshing of harvested above ground crop biomass was done mechanically to get grain yield for each treatment. All the samples were sun dried prior to oven

drying and weighed on electronic balance.

Penicillium bilaii (commercial product, Jumpstart) was obtained from Novozymes Asia Pvt Ltd. It had fungal spores' population of 7.2×10^8 colony-forming units/g (CFU/g). The wheat (*Triticum aestivum*) and mustard (*Brassica campestris*) seeds were treated separately with the wettable powder formulation of test fungus at three different rates as per schedule given in Table 1. The inoculated seeds were spread on a sheet (in to a flat layer) under shade, and allowed to dry for 2-3 h prior to sowing.

Three sub-samples of soil were taken twice (mid season and crop maturity) from different locations of each treatment plot at a depth of 0-15 cm and mixed together to make a composite sample that was pooled in a sterile plastic bag and transported to the laboratory. Field (moist) samples were passed through a 2 mm sieve and divided into

Table 1 Effect of fungal inoculation and P fertilization on soil M_{BP} ($\mu\text{g/g}$ soil) under mustard and wheat crop

Treatment	Crop	P rates (kg P_2O_5 / ha)	Inoculum rate g/kg seed	M_{BP}	
				60 day	120 day
T1	Mustard	30	0	8.85	4.36
T2		30	2.651	18.85	2.91
T3		30	5.302	24.45	5.8
T4		30	10.604	25.80	12.91
T5		60	0	7.27	4.36
T6		60	2.651	15.97	2.91
T7		60	5.302	46.52	29.45
T8		60	10.604	20.27	4.36
P<0.05				5.32	2.15
T1	Wheat	30	0	36.25	62.56
T2		30	1.223	87.36	119.3
T3		30	2.446	54.32	21.82
T4		30	4.894	45.30	14.55
T5		60	0	52.32	24.72
T6		60	1.223	32.65	4.36
T7		60	2.446	54.32	17.27
T8		60	4.894	70.65	26.17
P<0.05				12.54	8.50

Mustard crop treatment: T₁: No seed inoculation + 50% P/ha, T₂: PSF seed inoculation @2.651 g/kg seed+ 50% P/ha, T₃: PSF seed inoculation @5.302 g/kg seed+ 50% P/ha, T₄: PSF seed inoculation @10.604 g/kg seed+ 50% P/ha, T₅: 100% P/ha, T₆: PSF seed inoculation @2.651 g/kg seed+ 100% P/ha, T₇: PSF seed inoculation @5.302 g/kg seed+ 100% P/ha, T₈: PSF seed inoculation @10.604 g/kg seed+ 100% P/ha. *Wheat crop treatment:* T₁: No seed inoculation +50% P/ha, T₂: PSF seed inoculation @1.223g/kg seed + 50% P/ha, T₃: PSF 2.447 g/kg seed + 50% P/ha, T₄: PSF seed inoculation @ 4.894 g/kg seed+ 50% P/ha, T₅: PSF 100% P/ha, T₆: PSF seed inoculation @1.223 g/kg seed + 100% P/ha, T₇: PSF seed inoculation @ 2.447 g/kg seed+ 100% P/ha, T₈: PSF seed inoculation @4.894 g/kg seed + 100% P/ha

two parts. One part was stored in cold room for microbial biomass phosphorus (M_{BP}). Nother part was oven dried and ground finely, prior to estimation of pH and different P fractions. The soil pH was determined in 1:2.5 (soil: water ratio) using digital pH meter. M_{BP} was measured by fumigation- extraction method (Joergensen *et al.* 1995) as per equation given below:

$$\text{Microbial biomass P} = E_p/k_{EP}$$

where, E_p = P extracted from fumigated compost – P extracted from non-fumigated compost, k_{EP} = 0.40 (Brookes *et al.* 1982).

Soil samples were sequentially extracted for various P fractions in soil under wheat and mustard crop by modified Hedley procedure (Hedley *et al.* 1982). The sequential method extracts P of differing degrees of availability and gives an indication of P transformations in the soil. P concentration in above ground biomass and grain samples was measured by phospho–vanademolybdate yellow method. The above ground biomass (AGB) and grain samples of respective crops were ground finely and P uptake was estimated by standard method of Jackson (1972). Standard deviation of means was calculated with Microsoft Excel. The difference in mean values were analyzed by analysis of variance (ANOVA) and least significance difference test. Statistically significant differences and correlation were set at $P < 0.05$.

RESULTS AND DISCUSSION

Microbial biomass P

Microbial biomass P (M_{BP}) is an important source of P for plants. Microbial inoculation can bring changes in its values. In mustard grown with treatments, PSF seed inoculation @5.302 g/kg seed+ 50% P/ha (T_3), PSF seed inoculation @10.604 g/kg seed+ 50% P/ha (T_4) and PSF seed inoculation @5.302 g/kg seed+ 100% P/ha (T_7), recorded higher M_{BP} values at 60 day sampling, compared to other treatments (Table 1). However, at maturity only PSF seed inoculation @ 5.302 g/kg seed+ 100% P/ha (T_7) could maintain its relatively high M_{BP} values till 120 day sampling. The results were in agreement with the findings of Saini *et al.* (2004) who reported higher M_{BP} values in chickpea grown soil in response to *Bacillus megaterium* after 30 days of crop growth. The M_{BP} values for wheat crop were much higher than mustard crop. At 60 day sampling, interactive effect of 30 kg P_2O_5 /ha+ 1.223 g (T_2) fungal based formulation/kg seed application resulted in highest M_{BP} values (87.4 $\mu\text{g/g}$ soil). The trend was maintained till 120 day sampling. All other treatments showed relatively low M_{BP} values. The variation in M_{BP} values of different treatments may be attributed to rate of fungal application on specific crop seeds. Higher the rate of fungal based product application, more were the M_{BP} values. Availability of soluble P during initial stages of crop growth helped in microbial proliferation leading to assimilation of P by microbial biomass.

Soil P fractionation

Water used as an extracting solution does not alter the soil P sharply and its extraction power reflects the P extracting power of plant roots. Water and bicarbonate extractable inorganic P (Pi) considered as the most labile P fraction, showed different trends when soil samples were analyzed at 60 and 120 day of mustard crop growth (Table 2). The former showed a decrease in its content while the latter increased with crop maturity irrespective of the rate of chemical P. However, dose of fungal inoculation showed variability as far as the availability of W-P was concerned. At 60 day of crop growth, treatment with PSF seed inoculation @10.604 g/kg seed+ 50% P/ha (T_4) recorded the highest W-P content of 18.75 mg/kg soil compared to 15.23 mg/kg in un-inoculated control (T_4). The values in PSF seed inoculation @10.604 g/kg seed+ 50% P/ha and no seed inoculation+ 50% P/ha reduced to 12.06 and 13.38 mg/kg soil, respectively at 120 day of crop growth. Contrarily W-P content in wheat grown soil was relatively low compared to mustard grown soil at 60 day and 120 d of crop growth (Table 2). A decrease in water soluble P under wheat and mustard cultivated soil with crop maturity showed the P sorption capacity of soil and P uptake by crop plants.

Under mustard grown soil, SB-P values increased consistently from T_1 to T_4 with higher rate of fungal based formulation at 60 day sampling. However, the peak SB-P value of 164.3 mg/kg was registered in 100% P/ha (T_5) but the values in T_6 - T_8 declined with increasing rate of fungal inoculant (Table 2). The SB-P content at 120 day of crop growth declined in 100% P/ha, but the same increased by >2 fold in most of fungal inoculated (T_2 - T_4 and T_6 - T_8) treatments compared to their 60 day counterpart. The trend for SB-P under wheat growth was different from that of mustard crop. Higher SB-P values were recorded at 60 day sampling compared to 120 day of crop growth. Low rate of chemical P and highest rate of fungal based formulation registered SB-P values comparable to recommended dose (60 kg P_2O_5 /ha) of chemical P (Table 2). Improved availability of SB-P in PSF seed inoculation @10.604 g/kg seed+ 100% P/ha (T_8) emphasized the role of phosphate dissolving fungal inoculants in mobilizing chemically fixed soil P. The decline in P, with crop growth reflected the P uptake by plants. The difference in SB-P availability under mustard and wheat soil may be due to variation in their nutritional requirements.

SH-P was the most abundant fraction of P recorded in mustard grown soil at 60 day of crop growth and declined with crop maturity in all the treatments irrespective of the dose of added P (Table 2). The values were high with 30 kg P_2O_5 /ha as chemical P + fungal based formulation compared to 60 kg P_2O_5 /ha as chemical P (100% P/ha) with not much variation among fungal inoculated treatments with exception of seed inoculation with PSF @2.651 g/kg seed+ 100% P/ha. Contrarily, wheat grown soil registered much lower values for SH-P compared to mustard grown soil and T_4 showed the highest SH-P fraction (210.95 mg P/kg soil).

When readily available SB-P in mustard field was low at 60 d interval and SH-P was high, and when SB-P

Table 2 Effect of chemical-P and fungal inoculation on sequentially extracted mustard and wheat grown soil P fractions

DAS	P fraction	Phosphorus (mg/kg soil)								SD
		T1	T2	T3	T4	T5	T6	T7	T8	
<i>Mustard</i>										
60 day	W-P	15.23	13.29	14.06	18.75	15.50	11.42	10.00	12.34	2.74
	SB-P	43.20	58.00	82.51	88.00	164.27	30.30	57.96	63.11	41.16
	SH-P	347.03	351.65	419.40	393.51	302.80	275.21	318.02	300.11	51.40
	HCl-P	103.52	113.10	143.26	169.88	137.56	195.70	159.04	127.14	30.95
	Sum of fractions	508.98	536.04	659.23	670.14	620.13	512.63	545.03	502.70	69.51
120 day	W-P	13.38	5.30	11.32	12.06	12.32	10.24	10.00	6.54	2.87
	SB-P	68.42	97.85	116.20	168.11	140.15	122.40	91.36	183.45	39.01
	SH-P	88.66	91.72	85.62	128.43	76.42	116.17	103.96	76.41	18.77
	HCl-P	239.28	249.53	201.50	208.56	167.51	221.07	201.78	108.20	44.78
	Sum of fractions	409.74	444.40	414.64	517.16	396.40	469.88	407.1	374.60	45.91
<i>Wheat</i>										
60 day	W-P	10.19	6.80	8.36	12.64	11.40	7.95	11.62	11.36	2.06
	SB-P	43.19	73.09	76.42	103.96	110.00	79.50	91.57	105.02	17.87
	SH-P	88.68	116.88	138.66	210.95	110.01	137.58	131.45	129.22	35.68
	HCl-P	211.90	186.07	171.28	168.22	167.29	222.24	220.38	196.93	29.02
	Sum of fractions	353.98	382.84	394.72	495.77	398.70	447.27	455.02	442.53	40.71
120 day	W-P	6.10	6.18	4.89	4.07	9.58	7.13	5.30	13.65	3.12
	SB-P	22.56	33.63	46.40	51.96	64.70	30.54	42.29	64.00	39.52
	SH-P	73.88	97.81	107.83	134.49	103.94	110.08	138.05	110.04	20.61
	HCl-P	195.59	305.78	308.09	306.69	286.66	320.31	322.28	328.97	38.57
	Sum of fractions	298.13	443.40	467.21	497.21	464.88	468.06	507.92	516.66	34.68

W-P, Water soluble P, SB-P: sodium bicarbonate extractable P, SH-P: sodium hydroxide extractable P, HCl-P: hydrochloric acid extractable P, DAS: days after sowing, SD- standard deviation

increased at 120 day interval, SH-P showed a sharp decline (Fig 1). Although, SH-P may not be as available as SB-P but there was no clear cut separation between SB-P and SH-P. SH-P was not a static fraction in either of the crop grown soil, rather it declined under cropping. The decrease of the moderately labile P could be due to its transformation into labile P fractions followed by transportation with surface runoff.

HCl-P represents the mineral P (Tiessen and Moir 1993), since the Fe or Al-P that remains unextracted with NaOH is soluble in acid. This P is least available to plants. There are conflicting reports that HCl-P is increased by application of fertilizer-P (McKenzie *et al.* 1992) and P application had no effect on HCl-P in Brazilian soil (O'Halloran *et al.* 1987). Changes in HCl-P indicated a similar trend in its values under both the crops and at both the intervals (Table 2). The most stable fraction of HCl-P was low in mustard grown soil compared to HCl-P values of wheat grown soil and values increased with crop maturity irrespective of the test crop. The high values of HCl-P indicated the presence of Ca-containing minerals. HCl-P may act as a buffer for available P in presence of fungal inoculants. The decline in HCl-P content of T8 under mustard soil might be due to-conversion of HCl-P to an available P by highest rate of

inoculated fungi. During crop growth, release of H⁺ through root activity might also be responsible for solubilizing the relatively less soluble HCl-P and residual P forms.

The distribution (%) of different soil P fractions with respect to the total extractable P in wheat and mustard soil at different stages of crop growth is presented in Fig1 (a, b and c). These fractions were influenced both by rate of chemical P and fungal inoculum. Few significant changes were depicted in inorganic P fractions as a result of fungal inoculation.

Since W-P and SB-P are considered as the most labile fraction, these pools though analyzed separately were combined together as W-P+SB-P to determine the available P fraction. Under mustard grown soil, though 60 kg P₂O₅/ha recorded the highest available P fraction of 28.99% and 38.46% at 60 day and 120 day of soil sampling, compared to 15.93 and 34.84% in T4 (30 kg P₂O₅/ha + highest rate fungal based formulation) but the latter indicated the soil P availability at par with 60 kg P₂O₅/ha (Fig 1a). The relatively less increase in available P fraction of wheat grown soil towards maturity indicated the high P nutritional requirement of crop. A part of the available P must have been assimilated by crop plants to biomass and for grain production and some P might have been assimilated by the

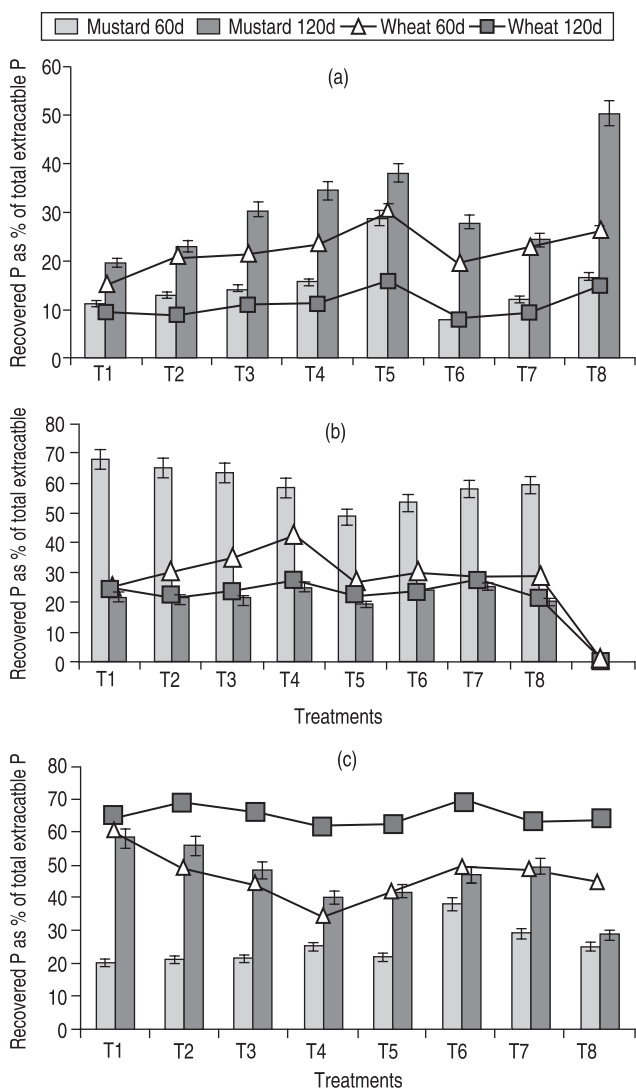


Fig 1 (a) Available P (water-P+ SB-P), (b) Sodium hydroxide extractable P (c) HCl extractable P as % of total extractable P of wheat and mustard grown soil fertilized with chemical P and different rates of *P. bilaii*.

fungal population introduced exogenously.

SH-P was the most abundant Pi fraction accounting an average of 59.5% of total extractable mustard field at 60 day of crop growth (Fig 1b). Contrarily, wheat grown soil recorded an average distribution of SH-P fraction of 31.2% of total extractable P at 60 day sampling and the same reduced to 23.9% at 120 day sampling. This is a moderately labile P fraction and is considered to be associated with Fe and Al ions through chemisorptions to surface of Fe and Al component (Wager *et al.* 1986). However, some Ca-P may also get extracted with 0.1 M NaOH.

The distribution of P fraction extractable by HCl averaged 25% of the total extractable P after 60 day of mustard crop growth whereas the same increased to 44% at harvest (Fig 1c). This showed that more P entered into HCl-P pool and more SH-P pool was available for plant growth at 120 day interval. Contrarily, under wheat grown soil, 42% and 59.8% of the total extractable P was in HCl

fraction (at 60 and 120 day growth respectively) making it the largest individual pool. The results were in agreement with Farrell *et al.* (2014) who reported 46% of the total P as HCl-P under wheat soil. The decrease in SB-P, SH-P and W-P with crop growth indicated the available P uptake by plant at maturity.

The orders of different fractions as percentage of total extractable P in mustard and wheat soil varied. In mustard field at 60 day of crop growth, it followed a pattern SH-P>HCl-P>W-P+SB-P. However at 120 day, the % P fraction availability order was HCl-P> SB-P> SH-P. Under wheat grown soil, the fraction distribution % order at 60 day and 120 day sampling was HCl-P> SH-P>SB+W-P. Seed inoculation @4.894 g/kg seed+50% P/ha treatment receiving 30 kg P₂O₅/ha + highest dose of fungal inoculant resulted in SB-P and SH-P availability equivalent to 100% P/ha receiving 60 kg P₂O₅/ha. This indicated that using highest dose of *P. bilaii*, half the chemical P fertilizer can be saved without reduction in yield. Effect of inoculation was more prominent under mustard soil with low dose of chemical P compared to wheat soil.

Correlation among different P fractions

Data in Table 3 gives an idea of the interdependence of two random variables that range in value from -1 to +1, indicating a perfect negative correlation at -1. At harvest, most of the mustard soil phosphorus fractions were weakly related to each other. However, a positive correlation between SH-P and HCl-P was observed with correlation value of 0.448 and 0.7457 for mustard and wheat grown soil, respectively. W-P was negatively correlated with SB-P under mustard soil and SH-P under wheat grown soil.

Above ground biomass and grain yield

P uptake by biomass and grain for both the test crops are given in Table 4. Higher crop yield and above ground biomass was recorded at higher chemical P rates (Table 4). The climatic conditions and crop management practices

Table 3 Correlation co-efficient among different phosphorous fractions of soil under mustard and wheat crop at maturity

P fraction	W-P	SB-P	SH-P	HCl-P
<i>Mustard soil</i>				
W-P	1			
SB-P	-0.1961	1		
SH-P	0.2022	0.0503	1	
HCl-P	0.1876	-0.7527	0.448	1
<i>Wheat soil</i>				
W-P	1			
SB-P	0.5565	1		
SH-P	-0.1969	0.4219	1	
HCl-P	0.1903	0.4897	0.7457	1

W-P: Water soluble P, SB-P: sodium bicarbonate extractable P, SH-P: sodium hydroxide extractable P, HCl-P: hydrochloric acid extractable P

Table 4 Effect of PSF based product on wheat and mustard grown above ground biomass, grain yield and phosphorus uptake

Treatment	Crop	P rates (kg P ₂ O ₅ /ha)	Inoculum rate (g/kg seed)	Biomass yield (kg/ha)	Grain yield (kg/ha)	Biomass P uptake (kg/ha)	Grain P uptake (kg/ha)	HI
T1	Mustard	30	0	4064	1645	5.26	9.32	0.404
T2		30	2.651	4567	1704	7.48	11.06	0.373
T3		30	5.302	4632	1803	7.83	11.89	0.389
T4		30	10.604	4687	1874	7.95	12.28	0.400
T5		60	0	4356	1885	6.85	12.42	0.432
T6		60	2.651	4735	1954	8.32	13.36	0.412
T7		60	5.302	4863	1998	8.47	13.85	0.410
T8		60	10.604	4945	2043	8.86	14.29	0.413
P<0.05				254	175	0.64	1.08	0.006
T1	Wheat	30	0	7482	4023	5.83	13.43	0.537
T2		30	1.223	7223	4134	4.97	13.81	0.572
T3		30	2.446	7487	4265	6.69	15.03	0.569
T4		30	4.894	7585	4357	7.34	15.08	0.574
T5		60	0	7664	4345	7.47	14.40	0.567
T6		60	1.223	7783	4473	9.00	15.10	0.574
T7		60	2.446	7804	4571	9.12	15.52	0.585
T8		60	4.894	7796	4575	9.07	15.70	0.586
P<0.05				183	124	0.69	1.17	0.008

HI: Harvest index

allowed the yield potential of both wheat and mustard to be expressed in the highest P rate. This is in agreement with earlier reports on wheat and other cereals. Wheat's grain yield responses to P supply were related to the total AGB. Fungal inoculation improved the AGB with both the P rates compared to their respective un-inoculated controls. Mustard AGP registered an increase of 15.3% and 6.6% and grain yield increased by 13.9 and 8.4% with 30 and 60 kg P₂O₅/ha + highest rate of fungal based formulation, respectively. An increase of 1.37% and 1.72% in wheat AGP and 8.30% and 5.29% in wheat grain yield was recorded with 30 and 60 kg P₂O₅/ha and highest rate of fungal inoculants, respectively. A strong correlation between AGB and grain yield and grain yield and harvest index of wheat was recorded (Fig 2a,c). The same correlation was relatively weak for mustard crop (Fig. 2b,d). P uptake by biomass and grain was also related with their respective yield (Table 4). These results suggest that the above ground biomass production and grain yield of both wheat and mustard crop respond well to improved P availability (due to fungal inoculation) and its uptake by crop plants.

It was concluded that re-distribution of soil P resulted either due to microbial solubilisation of inorganic P or mineralization of organic P present in soil and its uptake by the crop plants. It might be due to conversion of soil P to microbial biomass P which is subsequently released to soil on microbial cell lyses. In our soil, use of half of the recommended dose of chemical P in conjunction with highest dose of *P. bilaii* inoculums could improve the available P status of mustard grown soil and at par with recommended

dose of P, thereby saving 50% of chemical P fertilizer. Changes in soil labile P fractions including NaOH-P, and HCl-P were influenced by P fertilization.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the Novozymes South Asia Pvt. Ltd., Bangalore, India for financial support to conduct the present investigation.

REFERENCES

- Beckie H J, Schlechte D, Moulin A P, Gleddie S C and Pulkinen D.A. 1998. Response of alfalfa to inoculation with *Penicillium bilaii*. *Canadian Journal of Plant Sciences* **78**: 91–102.
- Brookes PC, Powelson, D S and Jenkinson D S. 1982. Measurement of microbial biomass phosphorus in soil. *Soil Biology and Biochemistry* **14**: 319–29.
- Farrell M, Macdonald L M, Butler G, Chirino-Valle Ivan and Condron L M. 2014. Biochar and fertilizer applications influence phosphorus fractionation and wheat yield. *Biology and Fertility of Soils* **50**:169–78.
- Gulden Robert, H., Vessey and J Kevin. 2000. *Penicillium bilaii* inoculation increases root-hair production in pea. *Canadian Journal of Plant Sciences* **80**(4): 801–4.
- Harvey P R, Warren R A and Wakelin S. 2009. Potential to improve root access to phosphorus: the role of non-symbiotic microbial inoculants in the rhizosphere. *Crop and Pasture Science* **60**: 144–51.
- Hedley M J, Stewart J W B and Chauhan B S. 1982. Changes in inorganic and organic soil phosphorus fractions by cultivation practice and by laboratory incubation. *Soil Science Society of American Journal* **46**: 970–6.
- Jackson M L. 1972. *Soil Chemical Analysis*, p 660. Prentice Hall

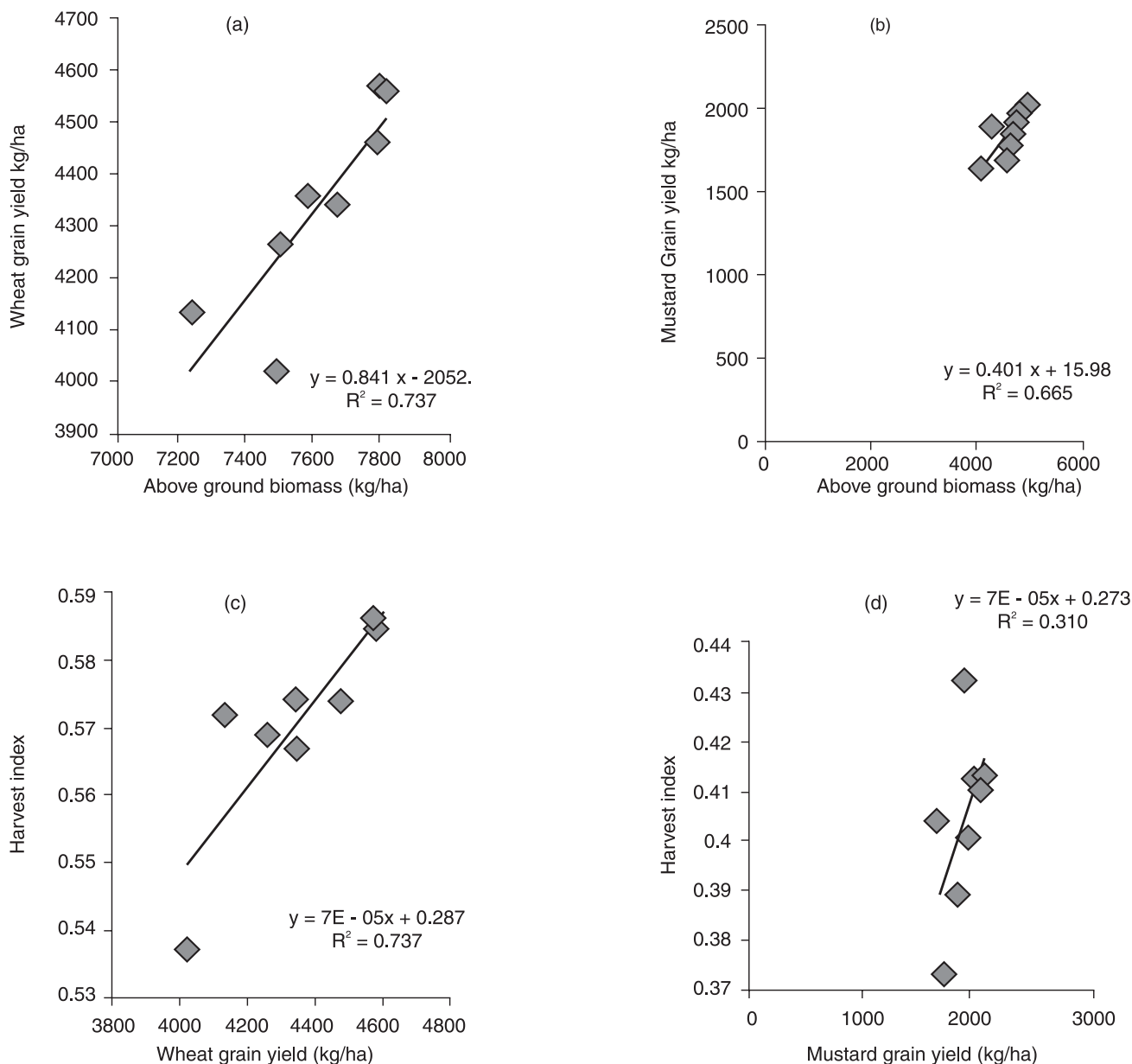


Fig 2 (a) Relationship between grain yield and above ground biomass of wheat (b) grain yield and above ground biomass of mustard crop (c) grain yields and harvest index of wheat (d) grain yield and harvest index of mustard crop.

of India.

Joergensen R G, Kubler H, Meyer B and Wolters V. 1995. Microbial biomass phosphorus in soils of beech (*Fagus sylvatica* L.) forests. *Biology and Fertility of Soils* **19**: 215–9.

Kucey R M N, Janzen H H and Leggett M E. 1989. Microbially mediated increases in plant-available phosphorus. *Advances in Agronomy* **42**: 199–228.

McKenzie R H, Stewart J W B, Dormaar J F and Schaalje G B. 1992. Long-term crop rotation and fertilizer effects on phosphorus transformations. II. In a Luvisolic soil. *Canadian Journal of Soil Science* **72**: 581–9.

O'Halloran I P, Stewart J W and Kachanoski R G. 1987. Influence of texture and management practices on the forms and distribution of soil phosphorus. *Canadian Journal of Soil Science* **67**: 147–63.

Patil M P, Kuligod V B, Hebsur N S, Patil C R and Kulkarni G N. 2012. Effect of phosphate solubilizing fungi and phosphorus

levels on growth, yield and nutrient content in maize (*Zea mays*). *Agricultural Sciences* **25**(1): 58–62.

Saini V K, Bhandan S C and Tarafdar J C. 2004. Comparison of crop yield, soil microbial C, N and P-fixation, nodulation and mycorrhizal infection in inoculated and non-inoculated sorghum and chickpea crops. *Field and Crops Research* **89**: 39–47.

Sharma S B, Sayyed R Z, Trivedi M H and Gobi T A. 2013. Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. *Springer Plus* **2**: 587.

Shi Y C, Ziadi N, Messiga A J, Lalande R and Hu Z Y. 2015. Soil phosphorus fractions change in winter in a corn-soybean rotation with tillage and phosphorus fertilization. *Pedosphere* **25**(1): 1–11.

Tiessen H and Moir J O. 1993. Characterization of available P by sequential extraction. *Soil Sampling and Methods of Analysis*, pp. 75–86. (Ed.) Carter M R. anadian Society of Soil Science,

- Lewis Publication Boca Raton, Florida.
- United Nations Secretariat 2007. World Population Prospects: The 2006 Revision. United Nations, New York.
- Vessey J K and Heisinger K G. 2001. Effect of *Penicillium bilaii* inoculation and phosphorus fertilization on root and shoot parameters of field-grown pea. *Canadian Journal of Plant Science* **81**: 361–6.
- Wager B I, Stewart J W B and Moir J O. 1986. Changes with time in the form and availability of residual fertilizer phosphorus on Chernozemic soils. *Canadian Journal of Soil Science* **66**: 105–19.
- Wright A L. 2009. Phosphorus sequestration in soil aggregates after long term tillage and cropping. *Soil and Tillage Research* **103**: 406–11.