Measurement of P Contribution From Several P Sources by Using ^{32}P Method

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ABSTRACT

P-fertilizer other than chemical fertilizers has been used extensively in agriculture. However, the extent to which P-fertilizer contributes to the growth of plants has only been discussed a few, meanwhile the information will be very helpful to the use of P-fertilizer efficiently. The ^{32}P method was used to distinguish P contribution from several sources, i.e soil, chemical fertilizer (Sp) and manure/organic fertilizer (Pk). The isotope carrier free solution of $KH_2{}^{32}PO_4$, which is contained of 98% ^{32}P, was applied to the soil and thus making it as the only source of labeled-P. Radioactivity counting of soil samples will lead to the measurement of P-contribution from several sources of P given. The experiment result showed that most of the P taken up by the plants was from soil. Thus, the P from Sp (P-Sp) and Pk (P-Pk) became un-significantly support the plant growth expressed in lesser dry weight of straw, grain and plants compared to those who taken its P from soil. Although soil contributed most of its available P to straw and grain of lowland rice, but Sp and Pk still contributed P to both plant parts. It was obtained that in straw 64 – 82% P was derived from soil; 12 – 21% P was derived from Pk; and 18 – 29% P was derived from Sp. For grain 49 – 89% P was derived from soil; 11 – 15% P was derived from Pk; and19 – 45% P was derived from Sp.

INTRODUCTION

In the last two decades, a very high rate of phosphorus fertilization has been given continuously to lowland rice. The leveling off of lowland rice production, especially in the Island of Java, is blamed on this practice. This long-term P-fertilization resulted in high P-content of the soil. Although high rates of P-fertilization might be of great beneficial of food plant production, it also causes an imbalance in the soil-nutrient status. The high P-soil content is able to suppress the Zn and Cu availability to the plants. As it is well known, Zn and Cu play important roles in enzymes needed to form growth regulators in plants. Low activity of growth regulators in plant may decrease plant production.

In addition to the imbalance of soil nutrients, low organic-C soil content (<2%) also occurs in lowland rice soils in Java [1]. These two factors, P-soil imbalance with other soil nutrients and low organic-C soil, need to be addressed seriously. Further P-fertilization will be absorbed by soil-colloids, making it more unavailable for plants. This is the cause of P-fertilizer inefficiency, which had raised fertilizer cost but decreased plant production. As late as 1986, Sisworo and Rasjid [2], and later Idawati and Haryanto [3] have found that P-fertilizer efficiency is less than 10% in lowland, as well as in upland soils. The high P-deposit due to the residual effect of P-fertilizer is a potential P-source for plant growth and could increase P-efficiency. Some methods are needed to release this P, which has been fixed by soils colloids. Several methods could be used to improve its availability, as mentioned by Aisyah [4], such as applying organic matter, liming, fertilizer application, and bio-technology (P-solubilizing microorganism).

The findings of Setyorini, *et al.* [5] showed that there is closer correlation between organic-C soil and lowland soil productivity. They found that soil productivity decreases as the organic-C in the soil decreases. As mentioned previously, soil productivity could be improved by adding organic matter whether in the form of green manure, animal manure, etc. It is expected that the addition of organic matter could increase the P-soil availability to the plants. It is also needed to be considered that

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soil with different P-content has to be treated with different P-fertilizers rates so as to prevent the occurrence of soil nutrient imbalance, i.e. soil with high P-contents needs only low P-fertilizer, while soil with low P-contents need higher P-fertilizer rates.

EXPERIMENTAL METHODS

Plant material

The experiment was conducted in the greenhouse, at the Centre for Application of Isotope and Radiation – National Nuclear Energy Agency, Jakarta. The tested plant was lowland rice var. IR-64. The seeds were planted until they were three weeks old and thereafter the seedlings were transferred to the polyethylene pots where the soil have been submerged before and the water level was maintained at 5 cm above soil surface. The plants were harvested at 125 days after transplanting (DAT) and separated between straw and grain.

Soil preparation

The soil used was an Ultisols soil with its main characteristics are total N = 0.09%; P_2O_5 = 24 mg/100 g; K_2O = 5 mg/100 g; Ca = 6.75 cmol(+)/kg; organic matter = 1.78%; and has a soil-pH of 5.3. The soil was air-dried and ground roughly just to break its aggregate. Each pot received 10 kg air-dried soil. The soils were submerged prior to seedlings transfer. Before the seedlings were transferred, the soils in all pots were mixed as homogenously as possible with 300 µCi/10 ml in the form of $KH_2{}^{32}PO_4$ carrier free solution. This isotopic solution was produced by Batan Technology. The determination of P-soil content shown that the soil having a medium P content (24 mg 100/kg), which is shown its low ability to support P to the plants.

Experimental procedures for measuring plant responses

After harvesting, the plants were separated into straw and grain. Thereafter the plant materials were oven-dried at 65°C for 72 hours. After the straw and grain were finely ground, a 1 g of sample was taken. The samples were ashed at 550°C and dissolved in HNO_3. Total P was determined using the vanadomolybdat yellow method and the ^{32}P activity was measured in an aliquot for all the samples by Cerenkov Counting using a liquid scintillation analyzer. P-uptake was calculated as mg P/pot.

Experimental treatments and design

The experimental treatment consisted of a 3 × 3 factorial arrangement (3 rates of chemical fertilizer / SP-36) and 3 rates of organic matter in the form of animal manure, with a randomized block design and 3 replicates. The rates of SP-36 and animal manure are as listed in Table 1.

Table 1. The rates of experimental treatment consist of SP-36 and animal manure.

SP-36	Equal to	Code
0 SP-36 kg/ha	0 mg SP-36/pot	Sp_0
50 kg SP-36/ha	902.7 mg SP-36/pot	Sp_1
80 kg SP-36/ha	1111 mg SP-36/pot	Sp_2
Animal Manure		
0 t manure/ha	0 mg manure/pot	Pk_0
10 t manure/ha	50000 mg manure/pot	Pk_1
15 t manure/ha	75000 mg manure/pot	Pk_2

The parameters observed were:

- Dry weight of straw, grain and plants which is straw + grain
- Percentage of total-P (%P-to) of straw and grain, and P-total uptake (P-to) of straw, grain and plants
- Percentage P-derived from ; soil (%P-soil); SP-36 (%P-Sp); and manure (%P-Pk) of straw and grain
- P-uptake derived from soil (P-soil), SP-36 (P-Sp) and manure (P-Pk) of straw, grain and plants

The ANOVA (analysis of variance) was carried out using a factorial arrangement with randomized block design and three replicates. The F-calculated was used to determine the difference of the treatments, to test whether there was any difference among the treatments. The factorial arrangement was set up as combination from Sp_0, Sp_1, Sp_2 against Pk_0, Pk_1, Pk_2.

Isotopic Methods

The indirect isotope method A-value was applied, where ^{32}P in the form of carrier-free $KH_2{}^{32}PO_4$ solution was used. Each pot received 300 µCi/10 ml to evaluate the radioactivity data. This data was expressed in cpm (count per minute) and thereafter transferred to dpm (disintegration per minute). For detailed explanation of the A-value method, see Sisworo, *et al* [6].

RESULTS AND DISCUSSION

Dry weight of straw, grain and plant

The perusal data from Table 2 showed that there was no response of the lowland rice growth expressed in dry weight to P-chemical fertilizer (Sp) and organic matter / manure (Pk). The ANOVA application where F-calculated was derived from showed no difference among treatments and their interaction (Pk, Sp, Pk & Sp). Apparently this could be due to the fact that the soil used in this experiment has a medium P content. This soil P-content could be speculated to be enough for plant growth from planting to harvest. Since the plants obtaining most of their P from the soil, it might be the reason why P applied (P-Pk and P-Sp) become un-significantly support the plant growth expressed in dry weight of the plant and its several parts (straw and grain).

As far as in 1985, Jenkinson *et al*. [7] have introduced the term "primary effect". According to them [7] when Nitrogen (N) is added to plants, this added N will stimulate root growth in abundance. The abundance root growth would be able to contact more soil particles compare to when no N is added. The more the roots contact the soil particles, the more they could take up N-soil and this further could stimulate plant growth.

Before them [7] in 1973 the Letcombe Laboratory in its lab-experiment by Drew and Saker [8] showed pictures of nitrate (N) or phosphate (P), either separated or mixed, could result in tremendous root growth. This is in line with the finding of Jenkinson *et al*. [7]. Based on these findings it could be presumed that in this work the addition of P by Pk or Sp or their interaction was able to stimulate root growth to an extent that the plant roots could contact plenty of soil particles. This further resulted in high P-soil uptake, making the P-Pk and P-Sp uptake un-significantly promote plants growth as shown in Table 2.

Total P-percentage (%P-to) and P-uptake (mgP/pot)

In Table 3 and 4, the %P-to and P-uptake are presented. It illustrates that the response to the treatments and their interaction (Pk, Sp, Pk & Sp) shows no significant difference for %P-to and P-to uptake (mgP/pot). As for the %P, the value found in straw and grain might be the normal values for lowland rice.

For P-to uptake (Table 4) where the data are a result of dry weight x %P-to and P-to, no significances might be proposed as follows. As shown in Table 2, the dry weight of straw, grain, plant and %P-to of straw and grain are not significant for the treatments and their interaction. Hence, it is possible that the P-to uptake become more significant, the increasing rate of SP (Sp_1; Sp_2) showed slightly increasing values of %P-to and P-to uptake. It could be speculated that this P-fertilizer although not too significant could still contribute to plant growth expressed in %P-to and P-to uptake.

Considering the data in Tables 2 and 3, where it was found for parameters observed for plant growth, not a single one showed any response to the P-fertilizers added (Pk and Sp). Whether this is due to the greater role of P-soil it could be shown by calculated for each P-source (soil, Pk and Sp) by using the ^{32}P technique, and this will be discussed here after.

Percentage of P-derived from soil (%P-soil), manure (%P-Pk) and SP-36 (%P-Sp)

Percentage of P-derived from several source including soil for straw and grains are given in Table 5. Remarkable data is for %P-soil found in straw as well as in grain. Here it shown that the %P-soil is several time higher that of % P-Pk and P-Sp. But the role of P-Pk and %P-Sp could not be ignored. These all are shown by both straw as well as grain.

From these data it could be concluded that P-soil has more influenced compare to P-Pk and P-Sp. This could be due to the fact that the soil used has a medium P content, which could mean that it has enough P available to be used by the low rice plant. Further it is shown from Table 3 that when Sp is added, the %P-Pk will decrease significantly, while the reverse is also true, where Pk is added the %P-Sp will decrease. This is valid for straw and grain. It is shown too, that Sp has a greater influence on Pk then Pk on Sp. As shown by these data, that the %P-Sp is much higher than %P-Pk (straw : $\%P\text{-}Sp_1 = 18.23$ and $\%P\text{-}Sp_2 = 28.50$ vs $\%P\text{-}Pk_1 = 11.85$ and $\%P\text{-}Pk_2 = 20.93$; and grain : $\%P\text{-}Sp_1 = 19.06$ and $\%P\text{-}Sp_2 = 44.79$ vs $\%P\text{-}Pk_1 = 10.58$ and $\%P\text{-}Pk_2 = 15.20$). This might be explained by the fact that Pk is an organic fertilizer which need more time to be dissolved making its P available later compared to Sp. But in the long run anorganic fertilizer would be better than an inorganic one, due to its capability to feed the soil with organic matter making the soil in the longer term become more fertile and this is one of the tools to make soil sustainable for agriculture including lowland soil [9].

Table 2. Dry weight of straw, grain and plants (straw + grain) of lowland rice applied with several P-sources.

	Straw-dry weight (g/pot)				Grain-dry weight (g/pot)				Plant-dry weight (g/pot)			
	Pk0	Pk1	Pk2	Ro-Sp	Pk0	Pk1	Pk2	Ro-Sp	Pk0	Pk1	Pk2	Ro-Sp
Sp0	51.76	53.61	53.09	52.82	67.23	69.63	69.44	68.77	118.99	123.24	122.87	121.70
Sp1	52.40	51.03	53.26	52.23	63.63	65.40	67.70	65.58	116.02	116.45	120.96	117.81
Sp2	55.21	49.98	60.00	55.06	67.27	62.54	77.58	69.13	122.48	112.51	137.59	124.19
Ro-Pk	53.12	51.54	55.45		64.04	65.86	71.58		119.16	117.40	127.14	
F-calculated												
Treatments		1.19	ns			1.43	ns			1.37	ns	
Pk		1.62	ns			2.36	ns			2.18	ns	
Sp		0.94	ns			0.86	ns			0.84	ns	
Pk x Sp		1.10				1.25	ns			1.24	ns	
F-table		5%	1%			5%	1%			5%	1%	
Treatments		2.59	3.89			2.59	3.89			2.59	3.89	
Pk		3.63	6.23			3.63	6.23			3.63	6.23	
Sp		3.63	6.23			3.63	6.23			3.63	6.23	
Pk x Sp		3.01	4.77			3.01	4.77			3.01	4.77	
CV (%)		8.68				9.35				8.70		

Remarks : ns = not significant

Table 3. Percentage P-total (%P-to) in straw and grain of lowland rice applied with several P sources.

	Straw - %P-to				Grain - %P-to			
	Pk0	Pk1	Pk2	Ro-Sp	Pk0	Pk1	Pk2	Ro-Sp
Sp0	0.0316	0.032	0.0359	0.0331	0.2169	0.3516	0.2981	0.3039
Sp1	0.0356	0.0281	0.0302	0.0313	0.2699	0.3915	0.3479	0.3364
Sp2	0.0364	0.0346	0.0303	0.0338	0.416	0.3946	0.2914	0.3673
Ro-Pk	0.0346	0.0315	0.0321		0.3159	0.3793	0.3125	
F-calculated								
Treatments		0.7795	ns			2.4455	ns	
Pk		0.6541	ns			3.1138	ns	
Sp		0.4307	ns			2.2248	ns	
Pk x Sp		1.0166	ns			2.2218	ns	
F-table		5%	1%			5%	1%	
Treatments		2.59	3.89			2.59	3.89	
Pk		3.63	6.23			3.63	6.23	
Sp		3.63	6.23			3.63	6.23	
Pk x Sp		3.01	4.77			3.01	4.77	
CV (%)		17.95				19.01		

Remarks : ns = not significant

Table 4. P-total uptake (mgP/pot) in straw, grain, plants (straw+grain) of lowland rice applied with several P-sources.

	P-to uptake straw (mg P/pot)				P-to uptake grain (mg P/pot)				P-to plants (mg P/pot)			
	Pk0	Pk1	Pk2	Ro-Sp	Pk0	Pk1	Pk2	Ro-Sp	Pk0	Pk1	Pk2	Ro-Sp
Sp0	16.39	17.14	17.88	17.14	175.34	239.86	205.55	206.92	191.73	256.99	223.43	224.05
Sp1	19.37	14.04	18.24	17.22	177.75	261.22	234.82	222.93	190.66	275.27	253.07	239.66
Sp2	20.9	17.27	17.66	18.61	277.38	253.05	226.08	249.95	297.4	263.64	210.41	268.26
Ro-Pk	18.89	16.15	17.93		208.49	249.15	222.15		226.6	265.3	240.08	
F-calculated												
Treatments		2.22	ns			1.51	ns			1.47	ns	
Pk		3.56	ns			1.5	ns			1.3	ns	
Sp		0.51	ns			1.65	ns			1.7	ns	
Pk x Sp		2.03	ns			1.45	ns			1.27	ns	
F-table		5%	1%			5%	1%			5%	1%	
Treatments		2.59	3.89			2.53	3.89			2.53	3.89	
Pk		3.63	6.23			3.63	6.23			3.63	6.23	
Sp		3.63	6.23			3.63	6.23			3.63	6.23	
Pk x Sp		3.01	4.77			3.01	4.77			3.01	4.77	
CV (%)		12.52				22.39				21.76		

Remarks : ns = not significant

Table 5. Percentage P-derived from soil (%P-soil), and two unlabelled sources (%P-Pk and %P-Sp) in grain and straw of lowland rice.

	Pk0	Pk1	Pk2	Ro-Sp	Pk0	Pk1	Pk2	Ro-Sp	Pk0	Pk1	Pk2	Ro-Sp
Straw												
Sp0	99.99	86.1	75.47	87.19		13.89	24.53	19.21				
Sp1	75.36	73.75	66.35	72.02		11.89	21.56	16.73	24.03	14.35	16.3	18.23
Sp2	71.02	60.72	51.33	61.02		9.78	16.7	13.24	27.97	29.49	28.05	28.5
Ro-Pk	82.32	73.53	64.38			11.85	20.93		26	21.92	22.18	
	F-calc.	F table			F-calc.	F table			F-calc.	F table		
		5%	1%			5%	1%			5%	1%	
Treat.	299.18**	2.59	3.89		84.47	3.33	5.64		41.06**	3.33	5.64	
Pk	365.19**	3.63	6.23		319.78	4.96	10.04		153.25**	4.1	7.56	
Sp	783.00**	3.63	6.23		46.5	4.1	7.56		10.10**	4.96	10.04	
Pk x Sp	24.27**	3.01	4.77		4.83	4.1	7.56		15.93**	4.1	7.56	
CV (%)		1.92				7.08				7.53		
	Pk0	Pk1	Pk2	Ro-Sp	Pk0	Pk1	Pk2	Ro-Sp	Pk0	Pk1	Pk2	Ro-Sp
Grain												
Sp0	99.92	86.83	80.87	89.21		13.1	19.06	16.08				
Sp1	75.27	74.32	67.53	72.37		11.2	15.98	13.59	24.67	14.41	18.09	19.06
Sp2	52.57	49.34	44.85	48.92		7.44	10.56	9	47.05	43.18	44.12	44.79
Ro-Pk	75.92	70.16	71.66			10.58	15.2		35.86	28.8	31.55	
	F-calc.	F table			F-calc.	F table			F-calc.	F table		
		5%	1%			5%	1%			5%	1%	
Treat.	740.70**	2.59	3.89		39.25**	3.33	5.64		277.62**	3.33	5.64	
Pk	216.77**	3.63	6.23		77.32**	4.96	10.04		34.13**	4.1	7.56	
Sp	2863.61**	3.63	6.23		59.13**	4.1	7.56		1306.37**	4.96	10.04	
Pk x Sp	31.21**	3.01	4.77		2.33^{ns}	4.1	7.56		6.74*	4.1	7.56	
CV (%)		1.67				8.88				4.73		

Remarks : * = significant; ** = highly significant

Table 6. P-uptake derived from soil (P-soil uptake), manure (P-Pk uptake), and P-chemical fertilizer (P-SP uptake) in straw, grain and plant of lowland rice.

Straw												
Sp0	16.39	14.78	13.49	14.89	-	2.35	4.39	3.37	-	-	-	-
Sp0	14.74	10.35	11.1	12.4	-	1.65	3.94	2.8	4.63	2.04	2.2	2.95
Sp2	14.22	10.45	9.06	11.24	-	1.7	2.95	2.33	5.77	5.12	5.66	5.51
Ro-Pk	15.12	11.86	11.55	-	-	1.9	3.76	-	5.2	3.58	3.93	-
	F-calc.	F-table			F-calc.	F-table			F-calc.	F-table		
		5%	1%			5%	1%			5%	1%	
Treat.	6.07*	2.59	3.89		25.8**	3.33	5.64		15.73**	3.33	5.64	
Pk	10.31**	3.63	6.23		101.64**	4.96	10.04		8.01**	4.1	7.56	
Sp	71.6**	3.63	6.23		10.8**	4.1	7.56		54.13**	4.96	10.04	
Pk x Sp	1.19^{ns}	3.01	4.77		2.89^{ns}	4.1	7.56		4.24*	4.1	7.56	
CV (%)		13.55				13.82				17.43		
Grain												
Sp0	175.19	208.36	166.44	183.33	-	31.33	38.98	35.15	-	-	-	-
Sp0	129.98	193.26	158.73	160.66	-	29.65	37.54	33.6	42.33	38.15	38.41	39.63
Sp2	146.44	121.55	101.63	123.21	-	18.28	24.26	21.27	130.83	106.44	100.1	112.4
Ro-Pk	150.54	174.37	142.27	-	-	26.42	33.6	-	86.98	72.3	69.26	-
	F-calc.	F-table			F-calc.	F-table			F-calc.	F-table		
		5%	1%			5%	1%			5%	1%	
Treat.	2.78*	2.59	3.89		3.06^{ns}	3.33	5.64		18.16**	3.33	5.64	
Pk	2.00^{ns}	3.63	6.23		3.81^{ns}	4.96	10.04		1.83^{ns}	4.1	7.56	
Sp	6.46**	3.63	6.23		5.71*	4.1	7.56		85.06**	4.96	10.04	
Pk x Sp	1.36^{ns}	3.01	4.77		0.03^{ns}	4.1	7.56		1.04^{ns}	4.1	7.56	
CV (%)		23.02				25.99				22.03		
Plant (straw + grain)												
Sp0	191.59	223.24	179.93	198.25	-	33.58	43.37	-	-	-	-	
Sp0	143.63	202.72	170.83	172.73	-	31.17	36.36	46.92	40.18	40.6	42.57	
Sp2	160.7	132.06	110.77	134.51	-	19.93	23.59	136.6	78.79	105.64	107	
Ro-Pk	165.31	183.64	153.84	-	-	28.24	-	91.75	54.49	73.12	-	
	F-calc.	F-table			F-calc.	F-table			F-calc.	F-table		
		5%	1%			5%	1%			5%	1%	
Treat.	2.91*	2.59	3.89		3.76	3.33	5.64		14.49**	3.33	5.64	
Pk	1.85^{ns}	3.63	6.23		6.07*	4.96	10.04		4.79*	4.1	7.56	
Sp	7.01**	3.63	6.23		6.30**	4.1	7.56		56.89**	4.96	10.04	
Pk x Sp	1.3	3.01	4.77		0.06^{ns}	4.1	7.56		3.98^{ns}	4.1	7.56	
CV (%)		21.58				23.96				24.3		

Remarks : ns = not significant; * = significant; ** = highly significant

P-soil, P-Pk and P-Sp Uptake

The P-uptake of soil, Pk and Sp in straw, grain and plant (straw + grain) could be perused in Table 6.

Like the data in Table 5 (%P of soil, Pk and Sp) P-soil uptake expressed in mgP/pot is high above the P-Pk and P-Sp uptake in the two plant parts and the plant. This is to be expected because the P-uptake derived from each sources (soil, Pk, Sp) is a result of %P-soil, Pk and Sp times P-total uptake. Another point to be forwarded is that P-uptake from soil, Pk and Sp in grain is always higher than that of straw. For grain food plants as has been explained before, the terminal sink are the grains. So it is expected that most of the nutrients including P-taken up by the vegetative plant part (straw) will be distributed to the sink that is in the grain. This result as shown by the data in Table 6 where P-uptake in grain is higher compared to straw.

Nearly all the observations found in %P-derived from soil, straw and grain is also valid for P-uptake. One important observations worth to be explained is that of the P-soil uptake. Here like in %P-soil it also shown that P-soil uptake is influence by the P-fertilizer added. It is clearly shown that P-soil uptake decrease when P-Pk and P-Sp rates increases (Table 6, P-soil uptake: straw, grain and plant $Pk_0 > Pk_1 > Pk_2 > Sp_0 > Sp_1 > Sp_2$).

CONCLUSIONS

From all of these data it could be concluded that P-soil is the main P contributor for the plant growth which is expressed finally in dry weight of straw, grain and plant (Table 2). Here all plants with different treatment have the same chance to take up P-soil and indeed the P-soil was the largest portion of P taken up by the plants. Although P-Pk and P-Sp have contributed P for the plant growth (Table 4 and 5) but compared to P-soil it was negligible. And this might be the reason why there is no significant difference for all the treatments when expressed in dry weight.

Without ^{32}P technique it could not be explained why the dry weight of straw, grain and plants give no differences when P fertilizers (Pk and Sp) were added. With this technique it was able to distinguish between the contribution of several sources (soil, Pk and Sp) and it was shown that most of the P was derived from the soil. This resulted in no difference found in plant growth parameters.

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