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Improving Phosphate Efficiency by Phosphate Solubilizing Bacteria and Organic Matter Estimated by Radio Isotop (^{32}P) Technique in Some Soils

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ABSTRACT

Phosphorous (P) contents in many soils are varies highly along with their ability to provide available P to plant growth. Soils may contain very high in total P, but low in available P due to high P adsorbed by soil matrix and all their adsorbing agents. This research which using natural materials was aimed to increase P availability in some high-P absorption soils. The natural materials utilized for extracting P were organic matter, P extracting bacterial, and rock phosphate. Those materials were interacted to high P absorption soils which were: Oxisol, Inceptisol, and Andisol. The detail objectives of this research were: (1) to study the potential of P-extracting agents (organic matter, and P-extracting bacteria) in releasing P of three high P- absorption soils; (2) to quantify the -age of P coming from the treatments; (3) to calculate the efficiency of P utilization by plant; and (4) to quantify Residual P in soils. The P mobility was analyzed by radioisotope technique using $KH_2{}^{32}PO_4$ carrier free solution. The results showed that adding soil organic matter increased the available P by 15.24% in Oxisol, 40.18 in Inceptisol, and by 7.34% in Andisol. Plant sorption toward P from % P used efficiency(%-PUE) up to 60 days was still very low, 0.65 to 9.34%. This was in accordance with the residual P in soils which were still quite high ranging from 94% to 96% in Andisol, 91%97% in Inceptisol, and 96%-98% in Oxisol. The implication of the results of this research, however, is that the application of natural materials in improving soil P availability provides a longtimeresidual effect which could give benefit to the following crops.

Keywords: Andisols, Inceptisol, Oxisols, *P. diminuta*, PUE, rock phosphate

INTRODUCTION

The phosphorus content in many soils varies highly along with the soil ability in providing P to plants. P availability in the soil is greatly influenced by the soil reaction (Blair 1994), within the pH value ranges between 5.5-7.0. P availability decreases if soil pH is lower or higher than those pH range. P deficiency often becomes a serious problem on the acid soil as the result of P- fixation of specific adsorption which bind tightly phosphate ion (Kasno *et al.* 2006). In acidic soils, P can be dominantly adsorbed by Al/Fe oxides and hydroxides, such as gibbsite, hematite, and goethite (Parfitt 1989). P can initially be adsorbed on the surface of clay minerals and Fe/Al oxides by forming various complexes. The non- protonated and protonated bidentate surface complexes may coexist at pH 4 to 9, while protonated bidentate innersphere complex is predominant under acidic soil conditions (Luengo *et al.* 2006; Arai and Sparks 2007). Clay minerals and Fe/Al oxides have large speciûc surface areas, which provide large number of adsorption sites. The adsorption of soil P can be enhanced with increasing ionic strength. With further reactions, P may be occluded in nanopores that frequently occur in Fe/Al oxides, and thereby become unavailable to plants (Arai and Sparks 2007).

The rhizosphere is the critical zone of interactions among plants, soils, and microorganisms. Plant roots can greatly modify the rhizosphere environment through their various physiological activities, particularly the exudation of organic compounds such as mucilage, organic acids, phosphatases, and some speciûc signaling substances, which are key drivers of various rhizosphere processes. The chemical and biological processes in the rhizosphere are not only determine mobilization and acquisition of soil nutrients as well as microbial dynamics, but also control nutrient use efûciency of crops, and thus profoundly inûuence crop productivity (Richardson *et al*. 2009; Wissuwa *et al*. 2009).

On high adsorption soil, plants use approximately less than 10-20% P from fertilizer, consequently 80-90% of P fertilizer is still stay in the soil (Hammond and Diamond 1987; Moersidi *et al.* 1990). On Andisol, phosphate anion is absorbed by allophane mineral, immogolite, and humus-complex of Fe and Al compund. Andisol has a very high adsorption capacity of P which is approximately 8,000 to 15,000 mg kg^{-1}. On acid soils such as Oxisols and Ultisols, phosphate anion reacts with several fraction of Al and Fe in soil solution to form sediment of $Al(PO_4)_3$ and $Fe(PO_4)_3$.

Some bacteria produces organic acids, protein, antibiotics, growth regulator, and enzyme in their metabolism activities. In the activities of phosphorous solubilizing bacteria which are isolated from soil produce some acids such as citric acid, glutamate, lactic, oxalic, glicoxalic, fumarate, tartric and a-cetoglutiric (Alexander 1978). *Pseudomonas putida* 27.4B and *P. diminuta* produce some organic acid such as citric acid (11.2 and 16.1 mg kg^{-1}), formic (9.1 and 12.4 mg kg^{-1}), succinate (13.4 and 6.9 mg kg^{-1}), acetic (14.1 and 8.9 mg kg^{-1}), propionate (9.4 and 4.2 mg kg^{-1}), butyrate (1.4 and 1.0 mg kg^{-1}), and oxalic (11.7 and 12.4 mg kg^{-1}) (Setiawati and Mihardja 2008). There are some isotopic techniques that can be applied to determine the relative immediate and residual effectiveness of P fertilizers. The advantage of using isotopes as tracers, however, the percentage of utilization by plants of P derived from a fertilizer can be determined. Labeling of plant residues with the radioisotopes ^{32}P or ^{33}P has been used to investigate the recovery of P from plant residues in soil P pools and in growing plants. The radioisotopes ^{32}P could also be added to soil without simultaneous application of ^{31}P (*i.e.* carrier free). In this case, the amount of P introduced with the radioisotope is negligible in relation to native P in the to soil solution and other soil P pools, but the the radioisotope can easily be detected because of the sensitivity of a counting. Carrier-free addition of ^{32}P or P is commonly used to determine isotopically exchangeable P in soil-solution mixtures and in pot experiments (Fardeau *et al*. 1996).

Considering that the role of phosphate solubilizing bacteria (organic material and phosphate solubilizing bacteria) in releasing adsorbed P by soil matrix, it is important to conduct a research to study the role of organic material, along with phosphate solubilizing bacteria (PSB), *P. diminuta*, and rock phosphate. The purpose of this research was to study the influence of those materials towards P dynamics using radio-isotope technique, with free solution carrier $KH_2{}^{32}PO_4$. The organic material and PSB were phosphate solubilizing agents which take part in the P dynamics, mainly on the soils with high P adsorption. Rock phosphate was added as the P source. The application of radioisotope technique using radioisotope materials (^{32}P) obtained from Badan Tenaga Atom Nasional (BATAN), was to quantify: the transformation of P nutrient in plants, the value of *P-use efficiency*, and residual P in soil. The expected result had significant role to determine recommended P fertilization.

The aim of this research were: (1) to study the organic material and phosphate solubilizing bacteria (PSB) capability in releasing P on three soils with high P adsorption; (2) to quantify the amount of P which come from the fertilizer; (3) to calculate the efficiency of P used by the plants; and (4) to quantify the residual P in the soil.

MATERIALS AND METHODS

Research Design

This research was performed as a factorial experiment utilizing a Completely Randomized Designs (3 × 3 × 2), having three factors and three replicates. The first factor was the soil types, consisted of three soils: Oxisol, Inceptisol and Andisol. The second factor was the phosphate solubilizing agents comprises of control (C), plant biomass/organic matter (OM), (50 g pot^{-1}) and phosphate solubilizing bacteria (PSB), *P. diminuta* (10^8 CFU g^{-1} soil). The third factor was the rate of rock phosphate (RP) applied: 0 kg ha^{-1} and 400 kg ha^{-1}.

The P-radioisotope (^{32}P) technique was employed to obtain the P-use efficiency data and the residual P data. The research was installed as a two sets parallel pot experiment. Each consisted of 54 pots. The first set was applied with ^{32}P radio-isotope; and the second set was not applied with ^{32}P radio-isotope. In addition, as a comparison, there was another set of experiment applied with SP36, P fertilizer, with three replications.

Soil and Biomass Preparation

Soil used for the pot experiment purposes was taken from horizon A each soil type. Each pot was filled with 4 kg air-dried soil, approximately 70-80% field capacity. The plant biomass was collected from decomposed legume (soybean) residues. Each pot was applied as much as 50 g plant biomass.

The initial plant residue analysis was carried out to acquire the value of C/N and C/P ratios. C/N ratio was 31 and C/P ratio was 108.

Bacteria Revival

Phosphate Solubilizing Bacteria (PSB) was revived using an NB media. Then it was retested at *Pikovskaya* solid media. They population was counted using the standard curve attained from the previous research (Setyawati and Mudjiharjati 2005). The organic acids produced by the bacterias were recognized based on the previous research by Setyawati and Paniman (2004), and Setyawati and Mudjiharjati (2007), (Unpublished).

Radio-Isotope Application

The application of the radio-isotope was conducted utilizing of $_{32}$P material obtained from *Badan Tenaga Atom Nasional* (BATAN). Initial activity of $_{32}$P was 1,415.2 µCi for each pot. The application of plant biomass application and the inoculation Phosphorous Solubilizing Bacteria (PSB) were applied one week before planting. While the rock phosphate (RP) as P source was applied at planting time. Each pot was planted 4 corn plants, then was left 2 plants after one week.

Soil and Plant Tissue Analyses

Soil chemical analysis was carried out for all soil samples which were taken from rhizosphere zone. These analyses were conducted periodically in every ten days. The P-plant tissue analysis was conducted using a dry-destruction method. The analysis for plant tissue containing isotopic element was cried out by Liquid Sciltilation Counter (LSC). The plant tissue analyses were done in soil laboratory of *Tanah Pasir Batan*. Furthermore, based upon P-shoot tissue, data could be determined the P-use efficiency (PUE), as well as quantifying the residual P.

RESULTS AND DISCUSSION

Change in P Availability in Soil

The soil P availability changed with respect to time, and was measured periodically every 10 days. There was no interaction among factors observed on the 10th day after planting (DAP). On the 20th day, however, there was a significant interaction among the factors (soil types, phosphate solubilizing and, and rock phosphate). Furthermore, on the 30th and 40th days, there were some significant interactions of second degree interactions which were: between soil type and phosphate solubilizing agent; between soil type and rock phosphate, between rock phosphate and phosphate solubilizing agent. This could explain that time was very important in determining the process of organic material decomposition, as well as the role of phosphate solubilizing bacteria and the rate of dissolving rock phosphate.

The addition of phosphate solubilizing agents which comprised of organic material and phosphate solubilizing bacteria *(Pseudomonas diminuta)* showed a significant interactions with soil types (Oxisol, Inceptisol and Andisol) (Table 1). The dominant influence on P availability was found on organic material addition. The significant interaction between soil type and OM was discovered on Oxisol and Inceptisol, while the interaction between soil type and PSB was found on Inceptisol and Andisol. The highest discharge of P-available for all treatment could be seen in the organic material addition in Inceptisol. In the interaction between OM and soil type showed that the performance of OM increased P availability by

Table 1. The interaction between soil type and phosphate solubilizing agents on the P availability.

Soil type	Phosphate solubilizing agents		
	Control	Organic matter	Phosphate solubilizing bacteria
	…………………………..… mg kg-1 ………….………..…….		
Oxisol	1.83 b	2.11 b	1.47 b
	(A)	(A)	(A)
Inceptisol	6.67 a	9.36 a	6.64 a
	(B)	(A)	(B)
Andisol	1.79 b	1.92 b	1.58 b
	(A)	(A)	(A)

Means value with the same letter in each column or row are not significantly different in confidential to 95% by DMRT. Small letters are read horintal and capital letter in paranthese are read verical.

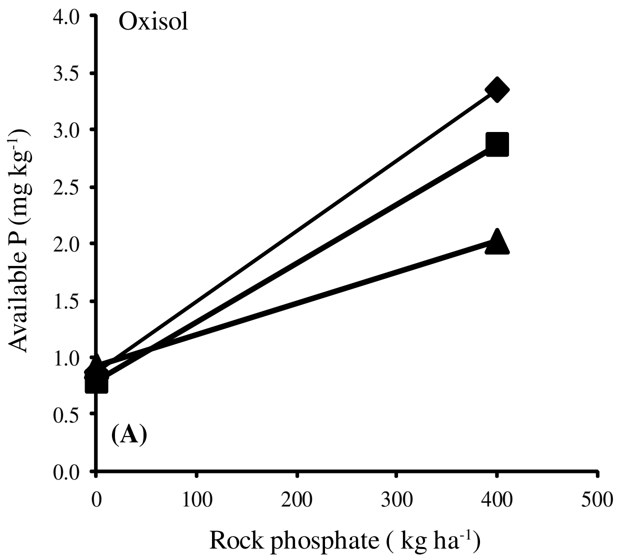
15.24%, 40.18% and 7.34% in Oxisol, Inceptisol, and Andisol, respectively.

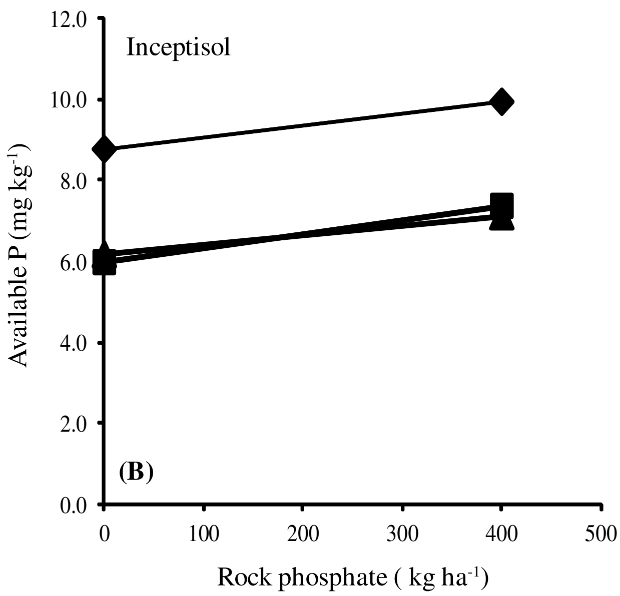
The organic acids produced from the decomposition process of soybean biomass were able to release P from the absorbed condition and Pin the soil become P-available. The roles of organic acids were different in the desorption process of P on the three type of soils (Oxisol, Inceptisol and Andisol) used (Mudjiharjati *et al.* 2007).

The interaction between phosphate solubilizing agents and rock phosphate showed that the role of organic material was better than the control and phosphate solubilizing bacteria (Figures 1). The role of organic material was also found in along with increasing rates of rock phosphate. The organic acids produced from organic material decomposition were able to dissolve rock phosphate into available P for plants. The organic acids produced such as oxalic acid, citric acid, acetic acid, and others, were able to release absorbed P especially for soils rich in Fe-oxide-hydroxides. The oxalic acid would compete with $H_2PO_4^-$ on the absorption sites which caused $H_2PO_4^-$ became more available to the plants. The role of organic acids was different in the P desorption on the three soils (Oxisol, Inceptisol and Andisol) (Mudjiharjati *et al.* 2007). The organic acids including the ligand were capable in forming chellate through coordinating group, which then formed a ring structure which usually has a strong bound with metal ion and anion (Violante and Glanfreda 2000). This condition assisted P desorption in the soil solution. Hu *et al.* (2002) proved that the oxalic acid and citric acid in the solution increased P desorption bound by the surface of Al oxide-hydroxide and Al complex as much as 4.1-4.75%. While, without the presence of organic acids, P desorption was only 2.5%. The desorption rates increased along with the increase of the organic acid concentration in the solution.

Interaction of Organic Material and Phosphate Solubilizing Bacteria towards Plant P sorption

There were a significant interaction between phosphate solubilizing agents and rock phosphate in the three soil types on P sorption by corn plants. The P concentration on corn crown tissue on the three soils have different range, which were Oxisol





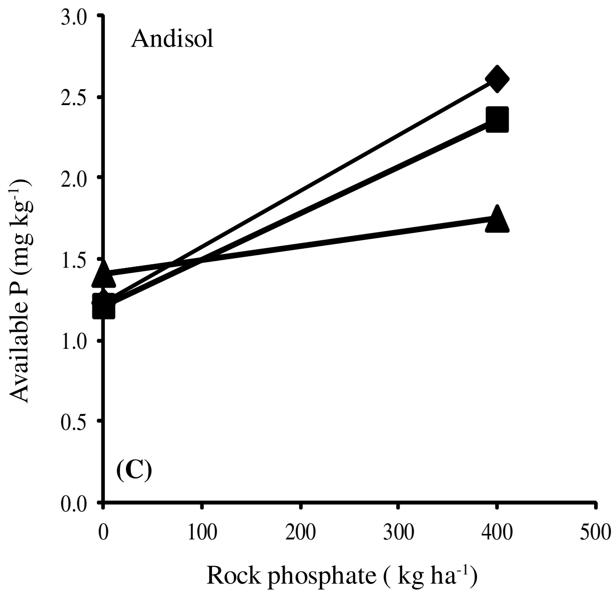


Figure 1. The Interaction between phosphate solubilizing agents and rock phosphate on P-available in Oxisol (A), Inceptisol (B), and Andisol (C). ―◆― = organic matter, ―■― = control, and ―▲― = phosphate solubilizing bacteria.

1.32-1.92 mg P g^{-1}, Inceptisol and Andisol 1.18-1.80 mg P g^{-1}. All of those soils were classified as a low P category (Jones *et al.* 1991).

The interaction between soil types and phosphate solubilizing agents showed that the organic material addition increased P adsorption for all type of soil. Generally, there were several mechanisms in the sorption-desorption of P that influenced the change of P availability, such as: *ligand exchange,* the competition of exchange sites, the change of pH solution, and the soil characteristics. Ion ortho-phosphate in Inceptisol generally bound with ion Ca^{2+} forming CaH_2PO4 compound which has lower dissociation constant (pK = 0.71). While in oxisol which was dominantly bound with ion Fe, Al, or Mn, ortho-phosphate formed the compound of Fe-P, Al-P or Mn-P with high dissociation constants (pK = 2.4 – 5.43) (Olsen and Khasawaneh 1980), so that P availability in soil solution of Inceptisol was higher. However, the performance of organic material in the increasing plant P sorption in the Oxisol was higher than two other soils; in Oxisol it increased 536.36%, while for Inceptisol 32.59% and for Andisol 79.51%. The interaction of phosphate solubilizing bacteria was found on Inceptisol, but not on Andisol and Oxisol. The interaction of OM and phosphate solubilizing bacteria with Inceptisol increased the plant P sorption significantly (Table 2).

Interaction of Organic Material and Phosphate Solubilizing Bacteria towards the Phosphate Use Efficiency

The addition of OM and PSB was significantly increased P availability in soil and plant P absorption as well. There was also a significant interaction between soil types and phosphate solubilizing agents. Table 2 is for phosphate solubilizing agents and Table 4 is for rock phosphate.

The percentage of P and P plants sorption which came from the added materials can be seen in Table 3. The result of this observation could be obtained by using $_{32}P$ tracer. The substance used was $KH_2(_{32}PO_4)$ solution *(carrier free solution),* with an ignorable P concentration.

The %-tage of *P-dff* which was defined as the percentage of plant-P coming from the added materials or source of P, (for example: OM, RP, and PSB). While, the efficiency use of phosphate by plant was calculated as Phosphate Use Efficiency (PUE). The plant P sorption which came from added P source was to show that from the total plant P-sorption, how much P (concentration) was taken not from soil but from added P source.

Referring to Table 3, the result of the analysis using radio isotope, revealed that for all type of soils, there was significant interaction between PSB and rock phosphate on %P-dff value. That meant the interaction of PSB and RP was able to release the highest P in the soil. While, the highest use efficiency value of P by plants (PUE) could be found in the OM addition for Andisol and Inceptisol. Those were because P was in slow release organic P form. Consequently, plant could utilize it more efficiently. The fertilizer use efficiency (PUE) was a quantitative measurement of added nutrient which was absorbed by plant. That was also showed plants ability in adsorbing nutrients coming from the fertilizer under different environmental conditions. (Hakim 2002; Sisworo *et al.* 2006).

The kind of P source added, influenced the residual P in the soil. There was found that the amount residual P from P-sources was still high. In Andisol, the residual P from the applied sources

Table 2. The interaction of the soil types and phosphate solubilizing agents on Plant P sorption.

Soil type	Phosphate solubilizing agents		
	Control	Organic matter	Phosphate solubilizing bacteria
	……………………….…… mg kg-1 ………………………		
Oxisol	4.33 c	27.53 b	1.47 b
	(C)	(A)	(B)
Inceptisol	38.65 a	51.25 a	6.64 a
	(B)	(A)	(B)
Andisol	17.99 b	32.30 b	1.58 b
	(B)	(A)	(B)

Means value with the same letter in each column or row are not significantly different in confidential to 95% by DMRT. Small letters are read horintal and capital letter in paranthese are read verical.

Table 3. The Percentage of P and plant-P sorption (come from the added materials), total sorption and phosphate use efficiency by plants.

Treatments	P-dff (%)	Total adsorption (mg P g^{-1})	Adsorption of the material added (mg P g^{-1})	PUE (%)
Andisol				
Rock phosphate (RP)	37.46	17.88	6.71	1.50
Oganic matter (OM)	44.61	26.31	11.74	4.05
Phosphate solubilizing bacteria (PSB)	40.00	7.13	2.85	2.85
OM × RP	51.70	38.28	19.79	2.68
OM × RP	59.33	20.53	12.18	2.72
SP-36	27.19	19.93	5.42	1.25
Inceptisol				
Rock phosphate (RP)	10.89	35.12	3.83	0.85
Oganic matter (OM)	23.58	55.82	13.16	4.54
Phosphate solubilizing bacteria (PSB)	21.95	42.53	9.34	9.34
OM × RP	32.01	46.67	14.94	2.02
OM × RP	34.10	38.34	13.07	2.92
SP-36	33.66	39.36	13.25	3.07
Oxisol				
Rock phosphate (RP)	27.41	16.05	4.40	0.98
Oganic matter (OM)	12.34	20.74	2.56	0.88
Phosphate solubilizing bacteria (PSB)	34.63	5.68	1.97	1.97
OM × RP	40.26	37.23	14.99	2.03
OM × RP	45.95	22.40	10.29	2.30
SP-36	35.31	8.01	2.83	0.65

Note: dff = derived from fertilizer and PUE = phosphate use efficiency.

Table 4. The interaction between phosphate solubilizing agents and rock phosphate on the plant-P sorption.

Rate of rock phosphate	Phosphate solubilizing agents		
	Control	Oganic matter	Phosphate solubilizing bacteria
	……………………………… mg g^{-1} ……………………………….		
0 kg ha^{-1}	19.54 a (B)	34.29 a (A)	18.44 b (B)
400 kg ha^{-1}	21.11 a (B)	39.76 a (A)	27.08 a (B)

Means value with the same letter in each column or row are not significantly different in confidential to 95% by DMRT. Small letters are read horintal and capital letter in paranthese are read verical.

(OM and RP) or even from PSB activity was from 93.94 - 95.84%. While, in Inceptisol was from 91.55 - 97.17%; and in Oxisol was from 95.9 - 98.49%, (Table 5). It meant that the plants only absorbed a small portion (5 - 10%) of the added P sources. The residue was left in the soil.

The large amount of residual P left in the soil could occur as affected by several factors. Those factors were: (a) the existing of metals binding or with allophane mineral; (b) not fully decomposed added materials; and (c) immobilization of inorganic P. Recognizing that there were some residual P left in the soils, it could become a beneficial P source for the following crops.

CONCLUSIONS

The results of the study revealed that there was an interaction between soil type and phosphate solubilizing agents, phosphate solubilizing agents

Table 5. Residual P from added organic materials, rock phosphate, SP-36, and its combinations on three type of soils.

Treatments	Andisol		Inceptisol		Oxisol	
	Total P (%)	Residual P (%)	Total P (%)	Residual P (%)	Total P (%)	Residual P (%)
RP	17.26	95.69	17.53	97.17	17.52	97.15
OM	23.49	93.94	23.28	93.12	24.62	98.49
OM + RP	40.63	94.40	41.13	95.56	41.28	95.90
PSB + RP	34.58	95.84	34.56	95.80	34.69	96.16
SP-36	16.86	95.67	16.13	91.55	17.32	98.31

Note: RP= Rock Phosphate; OM= Organic Matter; PSB= Phosphate Solubilizing Bacteria.

and rock phosphate as well as soil types and rock phosphate in providing available P in the soil. The addition of organic matter increased the soil-P availability and plant-P sorption. The significant increase was occurred in Inceptisol.

Plant-P Use efficiency (PUE) at 60 days after planting was still low. Accordingly, the residual P in soils originated from the phosphate solubilizing agents was still high in all three soils: Oxisol, Inceptisol, and Andisol. The implication of the results of this research was that the application of P-natural resources had a better long term impact which could be utilized for the following crops.

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