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SHORT COMMUNICATION

Agronomic Efficiency of Activated Rock Phosphate Granules on Maize Plants Treated with Mycorrhiza in a Calcareous Vertisol of Kenya

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Abstract
The agronomic efficiency of activated rock phosphate (RP) fertilizers to promote maize growth in the presence or absence of mycorrhizal inoculation was evaluated. Maize plants were treated with two reactive RP grades of medium (RP_M) and low (RP_L) solubility and their activated forms which were activated with a fraction of diammonium phosphate (DAP) to increase phosphorus (P) availability. We included in the trial design a control without P application and DAP (a positive control). Maize growth and N and P were measured. Activated RP_M enhanced maize N and P uptake, biomass, and relative agronomic efficiency (RAE) to levels equivalent to DAP. These properties were unaffected by mycorrhizal inoculation. The mechanism underlying the activation of RP deserves further research investigation to promote local RP deposits too small to merit industrial transformation.

Keywords Activated rock phosphate · Arbuscular mycorrhizal fungi (AMF) · Biomass P uptake · *Zea mays* L

1 Introduction

Direct application of rock phosphate (RP) offers an alternative fertilization practice to make phosphorus (P) accessible to roots, owing to a gradual release of P in the rhizosphere (Wei et al. 2014). The advantages of direct RP application are enhanced growth of microbial communities in the soil and lower cost of application, especially relevant to smallholder farmers with limited access to water-soluble P (WSP) fertilizers. Audette et al. 2020 found that 60-70% of P applied to a calcareous alkaline soil (pH > 7.5) was rapidly converted to immobile forms by Ca and Mg ions, and became less available for plant uptake. Partially activated RP may offer a promising alternative to direct RP application by improving its solubility on through slow-release processes and minimizing P fixation, but there are few studies evaluating the agronomic efficiency of activated RP in calcareous soil conditions.

Activation of RP by granulating with a WSP source has been the subject of studies in acidic soil conditions. Menon and Chien (1996) and Agyin-Birikorang et al. (2016) reported that granulating RP with modest amounts of WSP such as 20% P coming from WSP and 80% P coming from RP increases the P availability of RP, even for poorly reactive RP sources and when used on higher pH soils. The activation process was reported to render the P in the RP almost as bioavailable as the P from DAP (Agyin-Birikorang et al. 2016; Wang et al. 2020). The process could reduce large quantities of phosphogypsum waste by-product from WSP production (Agyin-Birikorang et al. 2016; Sørensen et al. 2015; Wang et al. 2020). Further, the activation process could potentially be applied to smaller regional P deposits that do not justify large investments in phosphoric acid processes (Kawatra 2014; Guimarães et al. 2005) and where the RP is not sufficiently soluble for direct application (Wei et al. 2014). Benefits of activated RP on alkaline soils have not been widely demonstrated. RP materials differ in their solubility, with many being unsuitable for direct application (Poblete-Grant et al. 2019; Prochnow et al. 2004) and some

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requiring application months before planting to be effective (Chien and Menon 1995; Zapata and Roy 2004).

Phosphorus is the second most essential macronutrient required for plant growth and development next to nitrogen (Gemenet et al. 2015; Verde and Matusso 2014; Hinsinger et al. 2011). P regulates several cellular metabolic processes including plant photosynthesis, respiration, synthesis of nucleic acids, and energy generation (Malhotra et al. 2018; Roch et al. 2019; Bhinderwala et al. 2020; Powers et al. 2020), and is essential for vigorous root system formation and development (Sun et al. 2016; Luo et al. 2019). In many soils, the total P content is often in the range of 400 − 1,200 mg kg^{-1} but less than 0.1% of the total P exists in inorganic forms for plant uptake (Bechtaoui et al. 2021). The supply of P as soluble fertilizers is the conventional method to address P deficiency in high input production systems. An estimated 47.4 million tons of WSP as P_2O_5 was produced in 2020 (FAO 2019). While WSP fertilizers are preferred due to their high solubility and high P concentration, access and affordability to many farmers limit their use in low input production systems (Hayes et al. 2018).

Plant roots form beneficial interactions with soil microbes such as growth-promoting bacteria or arbuscular mycorrhizal fungi (AMF) to further increase P uptake. AMF aid in increasing P availability to plants, especially in low P soils, by extending their hyphae out of the root zone and transferring P to roots (Jemo et al. 2014). Symbiotic AMF colonizes plant roots, and their hyphae exploit soil P several centimeters (cm) from the roots, thus effectively increasing the volume of soil exploited for P uptake (Jemo et al. 2014; Cobb et al. 2016; Sawers et al. 2017; Xie et al. 2019). In our study, we hypothesize that RP and activated RP may have better AMF colonization compared to WSP sources. The objectives of this research were 1) to assess if RP activated by the addition of a modest amount of 20% of P from DAP increases P availability and uptake by maize, and 2) to determine the relative efficacy of AMF in increasing P uptake when applied with RP, activated RP, or DAP.

2 Materials and Methods

2.1 Seeds of Maize and Mycorrhizal Inoculum

Short cycle seeds of maize, variety Amnika WH 505 produced by Western Seed Company, Kenya were used to establish the greenhouse experiment. The mycorrhizal inoculum was obtained from Dudutech Limited in Kenya. The inoculum contained spores and mycelial fragments of AMF isolates from the species *Funneliformis mosseae, Claroideoglomus etunicatus, Rhizophagus intraradices* and *R. aggregatus* (50 propagules cm^{-3}).

2.2 Soil Sampling and Analysis

A composite core topsoil layer (0−20 cm depth) was collected from a calcareous site in the Kitengela area, Kenya. The soil, classified as Vertisol, was air-dried, sieved through a 4 mm screen, thoroughly mixed for uniformity, and used to establish the greenhouse experiment. The soil had the following characteristics: pH (soil/water 1:2.5 v/v) 8.1, organic matter 2.6%, $CaCO_3$ (Zhu et al. 2021) 0.8%, Olsen P (Olsen et al. 1954) 1.98 mg kg^{-1}, total N concentration (Novozamsky et al. 1983) 0.089%, exchangeable potassium and calcium, 578 mg kg^{-1} and 9730 mg kg^{-1}, respectivly, boron (B) 1.32 mg kg^{-1}, and cation exchange capacity (CEC) 60.1 meq/100g.

2.3 Activation of Rock Phosphate

In the case of RP_L (the low reactivity RP), activation with DAP was achieved with a lab-scale compaction granulator. For RP_M (the medium reactivity RP), activation was achieved using a lab-scale pan granulator by spraying a fixed quantity of a concentrated solution of DAP onto the correct proportion of finely ground RP powders to form granules of between 1−4 mm in diameter. In the activated treatments, 80% of P in the final product came from the specific RP source and 20% from DAP.

2.4 Experimental Design

The experiment design was a factorial with three main factors and four replicates. Factor 1 was two natural RP sources of low and medium citrate solubility (RP_L and RP_M, respectively). Factor 2 was the activation process using 20% P from DAP. Factor 3 was mycorrhizal inoculation designated as AMF inoculated and uninoculated. Inoculation was achieved by adding 50 g of inoculum to the maize seed bed at planting to each pot. Negative (No-P) and positive (DAP) controls were included, with and without AMF inoculation.

Air-dried soil of 6 kg was filled into 21 cm × 22 cm diameter × 26 cm height plastic pots. All P-containing treatments added with 1.25 g P_2O_5 pot^{-1}, which required (per pot) 2.72 g DAP, 3.83 g RP_L, 4.58 g RP_M, and 3.30 g and 4.21 g for the respective activated forms of RP_L and RP_M. Along with P forms, urea was applied at planting to No-P control and all RP treatments such that all pots received the same amount of N as the DAP treatment (1.06 g N pot^{-1}). Four maize seeds per pot were thinned to two plants after emergence. Two urea topdresses at 2.19 g pot^{-1} were applied to all treatments at 4 and 8 weeks after planting. Plants were watered regularly

with deionized water to maintain 60% water holding capacity in the soil until harvest.

2.5 Plant Growth and Harvest

Plant girth was determined at 3, 6, and 10 weeks. The length and width of the fully exposed and extended eight leaf was measured at 10 weeks and the leaf surface area (LSA) was calculated. Plant girth was measured at 10 weeks about 2 cm from the base using a caliper. Plants were harvested at 12 weeks and their roots separated from the shoots using a scalpel. Roots were carefully rinsed and root dry weight determined after oven drying at 70°C for 72 h.

2.6 N and P Analysis

The shoot dry matter was finely ground and passed through a 1-mm mesh sieve. A subsample of about 0.5 g was digested in concentrated H_2SO_4 at 500°C. The concentration of N was measured according to the method described by Novozamsky et al. (1983). The concentration of P was measured using the Murphy and Riley (1962) method.

2.7 AMF Root Colonisation and Quantification

At harvest, root samples were stained to observe the AMF structures inside the root. A portion of roots preserved in 70% ethanol was assessed for mycorrhizal colonization. The quantification of AMF colonisation intensity and frequency rate was done using the grid intersection method of McGonigle et al. (1990). Estimation of percentage root mycorrhizal fungi colonization frequency and intensity was done using the subjective visual technique by Kormanik and McGraw (1982). The mycorrhizal colonization frequency (MCF) and intensity (MCI) were determined as described by Toubali et al. (2020).

2.8 Calculations and Statistics

The relative agronomy efficiency (RAE) of the various treatments was calculated using the equation:

$$\mathrm{RAE} = \left[(\text{Treated} - \text{no-P})/(\text{DAP} - \text{no-P})\right] \times 100 \quad (1)$$

"Treated" is the biomass (root plus shoot) of the treatment for which the RAE is being determined, "DAP" is the biomass of the DAP treatment without inoculation, and "no-P" is the biomass of the no-P treatment without inoculation. Statistical analysis was done in R software (R version 4.0.2, 2020). Three-factor analysis of variance using the factors RP source, activation, and inoculation was performed to determine significant factors and their interactions with the measured factors. Tukey's method was used to separate means that were different at $P \leq 0.05$.

3 Results and Discussion

3.1 Effects of RP Sources, Activation Treatments and Mycorrhizal Inoculation on N and P Uptake, Biomass (Shoot and Root) Dry Weight of Maize

Table 1 shows that PR activation interacted significantly with PR source to affect shoot P and N uptake, shoot dry weight, total biomass, and RAE. The nature of this interaction is shown in Table 2. Activated RP_M resulted in significantly greater values than did non-activated RP_M, whereas the differences between RP_L and non-activated RP_L were less pronounced and generally not significant. This shows that RP_M was more responsive to activation than RP_L.

Table 1 Analysis of variance (ANOVA) probabilities for the effects of rock phosphate (RP) sources, activation treatment, mycorrhizal inoculation, and their interactions on shoot phosphorus and nitrogen uptake, shoot and root dry weight, total biomass, and relative agronomic efficiency. Bold numbers are significant at *t*-test: $P < 0.05$

	P value					
	Shoot P uptake (mg P pot^{-1})	Shoot N uptake (g N pot^{-1})	Shoot dry weight (g pot^{-1})	Root dry weight	Biomass dry weight (g pot^{-1})	Relative Agronomic Efficiency (%)
RP sources (RPS)	0.59	0.77	0.82	**0.05**	0.80	0.80
RP activation (RPA)	**0.001**	**< 0.001**	**< 0.001**	**0.001**	**< 0.001**	**< 0.001**
Mycorrhiza (Myc) inoculation	0.84	0.54	0.69	0.06	0.95	0.95
RPS × RPA	**0.04**	**0.04**	**0.02**	0.62	**0.04**	**0.04**
RPS × Myc	0.06	0.07	0.61	0.99	0.65	0.65
RPA × Myc	0.62	0.98	0.66	0.92	0.68	0.68
RPS × RPA × Myc	0.69	0.10	0.33	**0.04**	0.69	0.69

Table 2 Phosphorus, nitrogen, shoot, root, and total biomass dry weights of maize under rock phosphate sources (RP_M and RP_L), their activated and non-activated forms, the control (No-P), and the diammonium phosphate (DAP) treatment with and without AMF inoculation. Means ± s.e followed by different lowercase letter are significantly different at $P < 0.05$

RP sources	RP activation treatment	Mycorrhizal Inoculation	Shoot P uptake	Shoot N uptake	Shoot dry weight	Root dry weight	Biomass dry weight
			(mg P pot $^{-1}$)	(g N pot $^{-1}$)	(g pot^{-1})		
NoP		Uninoculated	69.2 ±13.5 c	1.22 ±0.18 b	46.1 ±6.2 c	11.1 ±1.9 b	57.3 ±6.3 c
		Inoculated				11.3 ±1.1 b	
RP_M	Activated	Uninoculated	134.9 ±14.5 a	2.15 ±0.16 a	87.2 ±2.5 a	16.4 ±1.2 ab	105.8 ±2.6 a
		Inoculated				20.8 ±0.72 a	
	Non-activated	Uninoculated	74.8 ±6.2 bc	1.22 ±0.08 b	50.0 ±3.5 c	15.3 ±1.2 ab	65.1 ±3.7 c
		Inoculated				15.0 ±0.6ab	
RP_L	Activated	Uninoculated	118.5 ±9.1 ab	1.92 ±0.12 a	77.0 ±5.2 ab	17.0 ±1.9 ab	93.9 ±6.1 ab
		Inoculated				16.8 ±2.1 ab	
	Non-activated	Uninoculated	102.1 ±8.7 abc	1.53 ±0.15 ab	62.2 ±4.9 bc	10.3 ±1.8 b	74.5 ±5.2 bc
		Inoculated				14.5 ±1.8 ab	
DAP		Uninoculated	109.5 ±14.8 abc	1.99 ±0.12 ab	80.4 ±5.5 ab	16.9 ±1.2 ab	98.9 ±5.8a
		Inoculated				20.1 ±1.3 a	

Activated RP_M resulted in increases in all these parameters on par with DAP.

Inoculation had no significant effect on P and N uptake, shoot dry weight or biomass dry weight, but did interact with RP source and activation to influence root dry weight. No-P treatments with and without inoculation and non-activated, non-inoculated RP_L had significantly lower root dry weight than did treatments with activated RP_M or inoculation and DAP. Results are in accordance with those obtained by previous authors in acidic conditions on sorghum (He et al. 2005; Mao et al. 2017; Wang et al. 2020). Using activated RP from central Florida, Mao et al. (2017) reported that available P release for maize and P uptake by millet increased by 3 to 7 times compared to the original RP. N and P uptake and biomass responses from activated RP_M were greater compared to RP_L sources used, which we attribute to the greater solubility of RP_M applied, with RP_M being more soluble than the RP_L (Binh and Zapata 2002).

3.2 Maize Height, Mycorrhizal Colonisation Intensity and Frequency, Leaf Surface Area, and Girth

RP source had no significant effect on mycorrhizal colonization intensity or frequency, plant height or girth, or leaf surface area (Table 3). However, RP activation significantly increased plant height, girth, and LSA. Mycorrhizal inoculation significantly increased the colonisation intensity and leaf surface area, with the inoculated plants showing greater colonization intensity than the uninoculated plants, except for the No-P treatment (Table 4). Among the tested

Table 3 Analysis of variance (ANOVA) probabilities for the effects of rock phosphate (RP) sources, activation treatment, mycorrhizal inoculation, and their interactions on mycorrhizal colonization intensity and frequency, plant height and girth, and leaf surface area. Bold numbers are significant at *t*-test: $P < 0.05$

	Mycorrhizal colonization intensity (%)	Mycorrhizal colonization frequency	Plant height (cm)	Plant girth (mm)	Leaf surface area (cm^2)
RP sources (RPS)	0.81	0.06	0.24	0.47	0.15
RP activation (RPA)	0.68	0.40	**0.01**	**< 0.001**	**< 0.001**
Mycorrhiza (Myc) inoculation	**0.001**	0.06	0.49	0.25	**< 0.001**
RPS × RPA	0.90	0.69	0.44	0.36	0.40
RPS × Myc	0.66	0.40	0.34	**0.02**	0.59
RPA × Myc	0.07	0.31	0.51	0.25	**0.001**
RPS × RPA × Myc	0.98	0.96	0.14	**0.02**	0.82

Table 4 Plant height, mycorrhizal colonisation intensity and frequency, leaf surface area and plant girth under rock phosphate sources (RP_M and RP_L), their activated and non-activated forms, the control (No-P), and the diammonium phosphate (DAP) treatment with and without AMF inoculation. Means ± s.e followed by different lowercase letter are significantly different at $P < 0.05$

RP sources	RP activation treatment	Mycorrhizal Inoculation	Mycorrhizal colonization intensity	Mycorrhizal colonization frequency	Plant height	Plant girth	Leaf surface area
			(%)		(cm)	(mm)	(cm^2)
NoP		Inoculated	17.4 ±1.0 a	83.6 ±2.1*	104 ±3 b	9.3 ±0.2 cd	205 ±4 ef
RP_M	Activated				121 ±2 abc	12.2 ±0.4 a	358 ±11 a
RP_L						10.7 ±.01 abcd	
RP_M	Non-activated				110 ±4 bc	10.2 ±0.5 abcd	245 ±5 de
RP_L						9.6 ±1.0 bcd	
DAP					119 ±3 abc	11.9 ±0.2 ab	329 ±27 ab
NoP		Uninoculated	13.4 ±1.1 b		111 ±5 abc	8.1 ±0.2 d	175 ±5 f
RP_M	Activated				117 ±1abc	9.9 ±0.9 abcd	279 ±3 cd
RP_L						11.6 ±0.3 abc	
RP_M	Non-activated				110 ±4 c	10.2 ±0.1 abcd	232 ±4 ef
RP_L						9.6 ±0.3 bcd	
DAP					128 ±4 a	11.5 ±0.1 abc	297 ±19 bc

*No significant treatment effects

interactions, the RPA × Myc interaction was significant ($P < 0.001$) on leaf surface area while the RPS × Myc, and the triple interaction RPS × RPA × Myc were significant on the girth of maize.

Activation positively interacted with mycorrhizal inoculation to increase LSA (Table 3), equivalent to the DAP treatment with inoculation (Table 4) and exceeding all other treatments. Increased LSA is correlated with plant photosynthesis, and P deficiency can limit photosynthesis (Mora et al. 2016). Previous research supports the benefits of AMF inoculation on LSA and increased plant photosynthesis (Lin et al. 2017; Mathur et al. 2019). AMF can induce protection of photosynthetic apparatus via and increase in photosynthetic pigments and chlorophyll content (Mathur et al. 2019).

3.3 Relative Agronomic Efficiency

Figure 1 shows the effects of P source, activation and inoculation on RAE (%) based on biomass production. By definition, RAE of the DAP without inoculation is 100% and RAE of the no-P treatment without inoculation is 0%. The effect of inoculation was not significant on RAE (Table 1). RAE was similar for activated RP_M, with or without inoculation, to DAP with inoculation, and was significantly more than DAP without inoculation. While activated RP_L had a higher average RAE than non-activated RP_L, the difference was not significant at $P < 0.05$ due to high variability.

Studies on rice, wheat and on soybean evaluated on three different soils, including an alkaline soil revealed comparable results where significant enhancement of RAE was observed using activated RP compared to non-activated RP (Agyin-Birikorang et al. 2016). Mao et al. (2017) observed that the RAE of activated RP was equal to that of MAP and SSP applied to maize. Plant height with RP activation was greater than that from non-activated RP sources (110 cm) and the no-P control (108 cm). This is consistent with the results of Fang et al. (2022) who reported an increase in maize straw in plants fertilized with activated RP which was indistinguishable from those fertilized with WSP.

4 Conclusion

Rock phosphate activation increased growth, N and P uptake and relative agronomic efficiency of maize, irrespective of mycorrhizal inoculation. Activation of the medium solubility phosphate rock significantly improved its performance compared to its non-activated for. This effect was less pronounced with the low solubility RP source. The results show that activation RP sources with 20% DAP can be effective in increasing P availability even on high pH soils. The mechanisms underlying the increased availability of P from RP sources activated with DAP merit further investigation in order to further the exploitation of small local RP deposits for commercial use without necessitating large industrial investments.

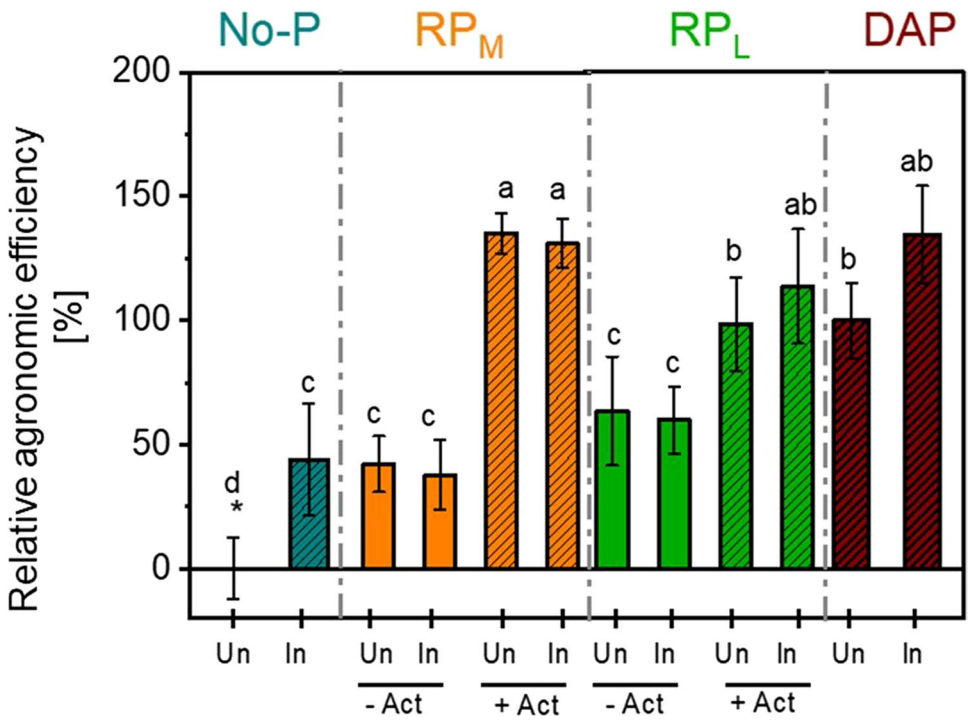

Fig. 1 Relative agronomic efficiency (RAE, %) of maize biomass production of the six P sources: two rock phosphate sources of P (RP_M and RP_L) activated (+Act) and non-activated (-Act) dammonium phopshate (DAP) and without P addition, inoculated (In) or uninoculated (Un). Bars with different lowercase letters are significantly different at $P < 0.05$

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Code availability Not applicable

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial competing interests or personal relationships that could be construed as a potential conflict of interest to influence the work reported in this paper. All authors validated the content and approved the manuscript quality before submission and declare that they have no conflict of interest.

Ethical approval All authors agreed to contribute to the development of the present manuscript.

Consent to participate All authors validated the contents and approved the manuscript contents before submission.

Consent for publication All authors validated and consent to submit this manuscript for publication.

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