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DIFFERENCES IN PLANT PHOSPHORUS CONTENT IN SELECTED RICE VARIETIES INFLUENCED BY SEED TREATMENTS OF MYCORRHIZAE SP. AND PHOSPHORUS LEVELS

JANICE M. BAYSA

President Ramon Magsaysay State University, Botolan Campus, Porac, Botolan, Zambales, Philippines.

Abstract

As the country faces climate variability challenges throughout the years, it is necessary to effectively utilize nutrients from the soil without sacrificing crop yield and quality with new techniques. This paper intends to explore if there are differences in Phosphorus concentration per plant, Phosphorus uptake per unit root length of rice varieties relative to levels of Phosphorus and Mycorrhizae sp. applied, and seed treatment techniques. It further investigated the plant Phosphorus distribution in selected rice varieties influenced by applying different Phosphorus and Mycorrhizae sp. levels to different seed treatment techniques. The study was conducted in P-deficient soil and laid out using a split-split plot design in CRD, simulating the natural field planting condition. Results revealed that the application of M+50-150% of RP is concentrated in the root system of NSIC Rc 222 with a ratio index of 0.440 to 0.591, comparable to P alone and higher when M alone is applied. The interaction of SxVxP further showed that combining P levels to Mycorrhizae is higher root P content overshoot P than using P alone with a ratio index difference of 0.111 to 0.247 for Rc 480. During the flowering stage (Anthesis), P is delivered to the shoot system. M+50% of RP and M + 100% RP have the highest ratio index comparable with the ratio index of P alone and M alone. During the grain-filling stage, total P is utilized by plants for grain formation. The addition of 50-100% P+Mycorrhizae significantly increased total P content in the shoot markedly higher than plants applied with P alone and Mycorrhizae alone. Combining the effects of three factors during grain-filling, R/S P Ratio obtained is comparable to seed-coated and seed-bioprimed NSIC Rc 222. At the same time, total P is concentrated in the root system rather than distributed to the shoot when NSIC Rc480 is coated or primed with 100-150% of the recommended RP +Mycorrhizae.

Keywords: Phosphorus Uptake, Yield, Seed Coating, Biopriming, Rice, Distribution, P-Deficient

1. INTRODUCTION

Phosphorus (P) is a chief growth-limiting macronutrient often associated with its low availability in most agricultural soils. Phosphorus is the least mobile element in soil due to its high fixation and slow diffusion nature. Phosphorus's inorganic form is the predominant form in the soil, although Phosphorus's organic forms may contribute substantially (20–80%) to the total P content (Sanyal et al., 2015). Phosphorus is a non-substitutable plant nutrient required for plants' growth and development, and without it, no plant can complete its life cycle.Vigorous early growth can counteract stress conditions. Some studies have shown that Phosphorus application increased the resiliency and yield of crops under water-limited conditions, such as beans, sorghum, corn, and other vegetables (Shabbir et al., 2016; Kato et al., 2016; Hansel et al., 2017; Jeshni et al., 2017; Taylaran et al., 2018; Gatan & Gonzales, 2014). The deficiency of nutrients such as Phosphorus during early plant growth can reduce yield since various yield-determining components develop in this stage. Some researchers showed that maximum harvest was determined at the early growth stage and decreased when

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the seedlings suffered from a nutrient deficiency, which is valid for sorghum and tomatoes (Amouzou et al., 2019).

The depletion of global Phosphorus reserves has elevated the concerns for global food production nowadays. Large-scale breeding of rice varieties that can continuously grow in Phosphorus-deficient soils or enhanced Phosphorus fertilizer use efficiency is now recognized and given importance in the rice research sector (Chin et al., 2011). However, due to the long period of screening varieties for Phosphorus-deficient soils, various techniques are recommended to enhance Phosphorus uptake. The use of mixtures of organic manures, inorganic fertilizer, and microbial inoculants, whether or not affected by climate change (Wu & Ma., 2015; Wu, Chang & Lur, 2016), promised significant potential. Enhancing the soil's microbial load with a fixed fertilizer level could reduce nutrient loss due to sudden climate change or exposure to extreme weather events.

The efficient use of nutrients is well-studied in improving rice yield and quality under drought conditions (Chin, 2011; Rose et al., 2012), but the interaction of nutrients like Phosphorus and beneficial microorganisms is inadequate for rice production under different agro ecological zones. Limited studies revealed that available Phosphorus content increased in rice production in complementation with microorganisms such as Mycorrhizae sp. combining microorganisms' work in converting nutrients efficiently for plant use with fertilizer could lessen the amount of input applied without sacrificing the crop's productivity and the land. Using the various fertilizers in other forms, such as seed coating and seed bioprimimg in rice and rice-based cropping systems, is scarce. As the country faces climate variability challenges throughout the years, it is necessary to effectively utilize nutrients from the soil without sacrificing crop yield and quality with new techniques. More so, cost-effective technologies are deemed essential for our current situation.

2. OBJECTIVE

This study intended to explore if there will be differences in Phosphorus concentration per plant and Phosphorus uptake per unit root length of rice varieties relative to levels of Phosphorus and Mycorrhizae sp. applied and seed treatment techniques. It further intends to establish plant Phosphorus distribution in selected rice varieties influenced by using different Phosphorus and Mycorrhizae sp. levels to different seed treatment techniques.

3. MATERIALS AND METHODS

a. The locale of the study

The experiment is conducted in an open-field lahar-laden rice production area situated at Carael, Botolan, and Zambales. Carael, Botolan was submerged in lahar in 1991 and left unproductive until 2010. The site has a 20-30 cm depth lahar. Botolan, Zambales, has a monthly average temperature of 25-27°C from November to February and a 0.5 to 1°C increase from March to April. The area is rain-fed and only supplemented with pumped irrigation during the

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dry season. Based on the soil analysis conducted, the soil contained the following nutrients (DA-Regional Soils Laboratory, 2019) and was identified as P-deficient soil:

1. Primary nutrients
   a. Organic matter (Colorimetric method) : 0.03%
   b. Phosphorus (Olsen's Method) : 5.15 ppm
   c. Potassium (Cold H_2SO_4 Extraction) : 58.50 ppm
2. Micronutrients (DPTA Method)
   a. Zinc : 0.14 ppm
   b. Copper : 1.11 ppm
   c. Manganese : 5.54 ppm
   d. Iron : 9.30 ppm
3. Texture : Light
4. Acidity (pH) : 7.32
5. Nutrient recommendation (Inbred rice)
   a. Dry season : 90 - 40 - 30
   b. Wet Season : 80 - 40 – 30
   c. Zinc sulfate application: 20 kg/ha

b. Experimental design, treatments, and layout

An open field pot experiment was conducted in a 200 square meters rice field in Carael, Botolan, Zambales, from January – May 2020, simulating the regular rice production management practices. The influence of Mycorrhizae sp. (M) and concentration levels of Phosphorus application and seed treatment techniques on P uptake, growth, and yield of selected rice varieties were examined. The identified treatments were replicated four times and laid out using a Split -split-plot in CRD experimentation. Ten rice plants represent each replicate. Three factors were measured: the application method as the primary treatment, rice varieties as the subplot treatment, Phosphorus levels, and mycorrhizae as the sub-sub plot treatment. Detailed treatments were as follows:

Main plot treatment (S) – Seed treatment
- S1 – Seed coating
- S2 – Seed bio-priming

Sub-plot Treatment (V) – Inbred Rice Varieties
- V1 – NSIC RC 222 (Irrigated variety)
- V2 – NSIC RC 480 (Drought-tolerant variety)

Sub-sub plot Treatment (P)- Phosphorus-*Mycorrhizae* combination
- P1 – Control (no P and *Mycorrhizae* application)
- P2 – Recommended Phosphorus alone (RP)
- P3 - *Mycorrhizae* alone (600g/h)
- P4 - 50 % of RP + *Mycorrhizae*
- P5 - 100 % of RP + *Mycorrhizae*
- P6 – 150% of RP + *Mycorrhizae*

Pot experimentation with a customized design of 8x8x16 inches polyethylene (PP) pot set-up utilized to monitor test plants' growth and development. Nine hundred sixty (960) improvised pot set-ups were provided for the experiment. All treatments were supplemented with recommended nutrients such as Nitrogen (195.65 kg/ha) and Potassium (50 kg/ha) except Phosphorus. Positive control was set up in addition to experimental treatments for comparison. For the positive control, basal application of P and Mycorrhizae followed before sowing, and the treatment levels were applied to the soil 24 hours before sowing.

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c. Treatment Application

1. Experimental Crops

The two rice varieties were selected based on different rice ecosystems, such as irrigated and rainfed, and farmers' preference for better yield. Certified seeds of NSIC RC 222 were sourced from the Department of Agriculture-Regional Field Office, Pampanga, and the NSIC Rc 480 was purchased from Philippine Rice Research Institute, Nueva Ecija. The root characteristics to adapt to the specific condition of rice were considered. The following are the characteristics of selected rice varieties (Phil Rice, 2018):

NSIC Rc 222 - NSIC Rc222 (Tubigan 18) is among the inbred varieties suited for the irrigated lowlands. It yields 6.1 -10 tons/hectare when transplanted and 5.7-7.9 tons/ha when direct-seeded. The crop matures in 106-114 days from seeding. NSIC RC 222 is immediately resistant to blast, bacterial leaf blight, and tungro and moderately resistant to brown planthopper and green leafhoppers (PhilRice, 2020). **NSIC Rc 480 -** NSIC Rc 480, known as GSR 8, is an inbred variety released by IRRI that is drought-tolerant. It yielded an average of 3.224 to 4.40 tons/ha during the wet season and produced a maximum of 94 tillers/hill. The GSR is resistant to abiotic stresses such as drought, salinity, alkalinity, and iron toxicity. It matures 107 to 121 days after sowing and has intermediate resistance to pests (PhilRice, 2020).

2. Fungal Inoculum (Arbuscular Mycorrhizae) Preparation

The fungal inoculum utilized in this Study is Glonus sp in the inoculated substrate. The fungus was purchased from BIOTECH, Laguna. The fungal inoculum has positively affected rice varieties' yield, especially when coated (Baysa et al., 2017). Before treatment, a fungal spore count was done to ensure target arbuscular Mycorrhizae in the substrate. Briefly, about one (1) gram of the inoculated sample was subjected to microscopic examination and established a range of two (2) to three (3) infective spores present per gram of substrate utilized.

3. Preparation of Phosphorus and Mycorrhizae Levels

Different concentration levels of single phosphorus fertilizer (0-22-0) (0, RP, 50% of RP, 100% of RP, and 150% of RP) were prepared. The amount of P utilized for seed coating and biopriming was computed and presented in Table 1 based on the result of soil analysis following the dry season recommendation on a per-post basis (15kg soil, dry weight):

Table 1: The computed concentration of Phosphorus for seed inoculation

	P level	Rate per hectare (kg/ha)	Seed inoculation (g/20 seeds)
1	50% of RP	90.91	0.620
2	Recommended P (RP)	181.82	1.239
3	150% of RP	272.73	1.859

The recommendations were computed from 90-40-30 dry-season recommendations using 0-22-0 fertilizer (Duofos). Seed inoculation rate is calculated from the estimated amount of seed covered per kilogram of soil and normal farming condition of 50-kg seed capacity per hectare-based planting. Briefly, each treatment level of Phosphorus is pulverized in a mortar and pestle. The computed amount of required fertilizer for each P level was mixed with 10g Mycorrhizae

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(30 spores) and stored separately in sealed plastic bags to prevent oxidation. Preparing materials were sealed independently for the single application of Mycorrhizae and Phosphorus.

d. Cultural Management Practices

1. Land preparation

Before field experimentation, the identified rice land area was plowed twice to remove plant debris and break soil clods evenly. A soil sample up to 40 cm depth from this area was utilized for experimentation. After plowing, the land was harrowed once using a tractor-driven implement. The ground was kept free from weeds until the commencement of the experiment. The area was laid out according to the experimental design. Individual holes capable of containing a 40 cm long plastic container were dug in the area. The land boundary utilized for the study was fenced with galvanized wire against stray animals.

2. Potting of Soil and lay-outing

Soil for potting was collected at a depth of 0-40cm. The soil was sieved through a 5mm mesh screen to remove stones and plant debris. Before seeding, a one (1) kg soil composite sample was subjected to laboratory analysis to determine the nutrient composition, pH, and microbial load to ensure proper application of the required optimum amount of fertilizer determine microbial growth to affect plant growth. Soil samples were subjected to heat sterilization for disinfection. Fifteen (15) kilograms of dried-weight soil were contained in each plastic pot. Recommended Nitrogen and Potassium fertilizer were incorporated into the soil basally. After potting, bags were laid out in the open field. Pots were placed in an individually dug pit with a 0.5m depth pit allowing a 5 cm portion of the pot to be exposed to the surface. It was done to simulate the normal soil temperature condition for rice production. Pots were saturated with water at a rate of five (5) liters per pot before seeding.

3. Planting of Seeds and Thinning-out of Test Plants

Before planting, seeds were sun-dried for 2-3 hours to activate the embryo. Ten (15) seeds with treatment were planted in each pot. 14 days after emergence, each pot was thinned out until one seedling remained.

4. Fertilizer application

Before sowing, Nitrogen and K-based fertilizer (20-0-22) and other micronutrients were applied basally at a rate of 0.5 kg/plot. N and K fertilizer's recommended rates were applied at split application; the first application was applied basally before planting, and the second Split at 40 days from sowing. A top dressing of 46-0-0 was done at panicle initiation. No addition of the P component was applied in the crop's entire growth stages after seed treatment.

5. Water management

Irrigation was maintained at field capacity for three days to enhance the development of seedlings. After that, intermittent watering or alternate wetting and drying method was done.

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6. Pest management

Pest monitoring was done regularly early in the morning or late in the afternoon for proper management. Three pesticide sprayings were executed to control pests in the experimental field. Leaf blight disease, leafhoppers, rice bugs, and birds are the common pests observed in the area. Weed in the plots was regularly removed.

7. Harvesting

All test plants were harvested when formed grained already reached 80% maturity. Manual harvesting of grains was executed. Plant biomass (shoots, roots, and grains) was separately harvested for data gathering and evaluation. Grains were manually threshed, separately contained in mesh bags, and labeled appropriately. Seeds were sun-dried until they reached 14% moisture content (MC). Dried seeds were stored in mesh bags at room temperature, sorted, weighed, and evaluated.

8. Weather monitoring

Regular monitoring of weather changes such as rain events as well as temperature variations was noted. Simulation of the available real-time weather monitoring system and local weather data gathered at the end of the experimentation.

e. Data Gathered

1. Phosphorus content in biomass and soil

For measuring total biomass and Phosphorus concentration of root and shoot part, another set of plants harvested in each treatment, combined and dried at 80°C for 72 hours. Plant samples were ground and weighed, and sent out to an accredited laboratory for analysis. Soil samples were collected, air-dried for 72 hours, sealed in polypropylene bags, and brought to the laboratory for analysis.

2. Phosphorus uptake per unit root length and per plant

Phosphorus uptake followed the method of Itoh &Barbers (1983). The Phosphorus uptake rate is based on three sampling periods following the phenological stage sampling of plants (vegetative, Anthesis, and grain-filling).

3. Plant Phosphorus distribution

Sample plants separated from root and shoot. Percent P distribution to a given plant determined at the three growth stages and computed as:

$$\% \text{ Phosphorus distribution} = u/U * 100$$

Where u is the Phosphorus uptake per plant part and U is the whole plant's Phosphorus uptake. Root/shoot ratio is computed based on Phosphorus uptake and concentration to illustrate the Phosphorus gradient between the root and the shoot.

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4. RESULTS AND DISCUSSION

A. Phosphorus concentration, uptake, and distribution.

1. Phosphorus concentration in biomass and soil

Phosphorus concentration in the above-ground and below-ground levels is measured. The individual factor effect showed that seed bioprimed plants have higher shoot P than seed-coated plats at the vegetative stage. In contrast, both seed treatments displayed parallel total shoot P approaching the flowering to maturity stage (Table 20a). NSIC Rc 480 has a more significant total P in shoot biomass during the vegetative stage, while NSIC Rc 222 showed better shoot P content from the Anthesis to maturity period. P levels' effect on P uptake indicated that P's rapid uptake happened during the vegetative stage when applied with M + 150% of RP. In contrast, M + 100% RP displayed the highest P concentration during flowering to maturity, comparable with the P content of plants applied with Mycorrhizae alone. The combination of 50% P with Mycorrhizae gave a similar P concentration with plants that applied P alone.

The combined effect of seed treatment and P levels on two rice varieties, as reflected in Tables 20b and 20c, rapid P uptake happened during early plant growth when M + 100% RP was applied through seed coating rather than seed biopriming. At the same time, it is preferred to bioprimed seeds of NSIC Rc480 with the different P levels of the single application over seed coating to increase P in shoot biomass. Nonetheless, all treatments responded similarly during the late vegetative to maturity stage.

Table 2: Shoot Phosphorus concentration (mg/kg) at different growth stages of two rice varieties subjected to two other seed treatments of Mycorrhizae sp. and different P levels +Mycorrhizae sp

Treatment	Phosphorus concentration (mg/kg)					
	Vegetative (40 DAS)		Anthesis (70 DAS)		Maturity (105 DAS)	
Factor A - Seed Treatment[1]						
S_1 - Seed Coating (SC)	0.934	b	5.656	a	40.929	a
S_2-Seed bioprimimg (SBp)	1.186	a	5.309	a	35.981	a
FACTOR B – Variety[1]						
V_1 - NSIC Rc 222	1.003	b	6.201	a	41.781	a
V_2 - NSIC Rc 480	1.454	a	4.699	b	37.946	b
FACTOR C - Phosphorus level[2]						
P_1 - No application (Control)	1.144	b	4.649	c	35.491	c
P_2 - Recommended P alone (RP)	0.959	d	5.009	b	38.911	b
P_3 - Mycorrhizae alone (M)	0.950	d	6.266	a	35.773	a
P_4 - M+50% of RP	1.026	c	5.388	b	45.958	b
P_5 - M + 100% RP	1.077	c	6.299	a	43.903	a
P_6 - M + 150% of RP	1.206	a	5.285	bc	30.693	bc

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Table 3: Comparison of shoot Phosphorus concentration (mg/kg) as affected by Seed treatment at each level of Variety and Fertilizer (VP) during vegetative, Anthesis, and maturity stages

Phosphorus levels	Seed treatment	Shoot P concentration (mg/kg)											
		Vegetative (40 DAS)				Anthesis (70 DAS)				Maturity(105 DAS)			
		NSIC Rc 222		NSIC Rc 480		NSIC Rc 222		NSIC Rc 480		NSIC Rc 222		NSIC Rc 480	
P_1 –Control	S_1 - SC	1.288	a	0.724	B	4.619	a	4.178	a	52.290	a	25.537	a
	S_2-SBp	0.728	b	1.834	A	4.778	a	5.021	a	30.140	b	33.997	a
P_2 – RP	S_1 - SC	0.898	a	0.840	A	8.408	a	3.408	a	51.457	a	31.677	a
	S_2-SBp	1.081	a	1.015	A	4.318	b	3.904	a	21.897	b	50.613	a
P3 – M	S_1 - SC	1.023	a	0.635	B	7.609	a	4.808	a	35.917	a	30.950	a
	S_2-SBp	0.826	a	1.317	A	8.050	a	4.595	a	31.637	a	44.590	a
P4 - M+50% of RP	S_1 - SC	0.917	a	0.818	B	5.976	a	5.937	a	50.163	a	40.770	a
	S_2-SBp	0.914	a	1.456	A	5.534	a	4.105	a	49.547	a	43.353	a
P5 - M + 100% RP	S_1 - SC	1.264	a	0.684	B	6.368	a	5.828	a	60.017	a	42.100	a
	S_2-SBp	0.887	b	1.473	A	7.076	a	5.924	a	40.123	b	33.373	a
P6 - M + 150% of RP	S_1 - SC	1.132	a	0.988	B	6.047	a	4.689	a	47.430	a	22.847	a
	S_2-SBp	1.073	a	1.630	A	5.763	a	4.643	a	30.750	a	21.747	a
Means with the same letter are not significantly different at 0.05 level using Least Significant Difference (LSD													

Table 4: Comparison of shoot Phosphorus concentration (mg/kg) as affected by variety at each level of seed treatment and Phosphorus levels + Mycorrhizae sp. (SP)

Phosphorus levels	Rice Variety	Shoot P concentration (mg/kg)											
		Vegetative (40 DAS)				Anthesis(70 DAS)				Maturity (105 DAS)			
		Seed Coating		Seed biopriming		Seed Coating		Seed biopriming		Seed Coating		Seed biopriming	
P_1 –Control	V_1	1.288	b	0.728	b	4.619	a	4.778	a	52.290	a	30.140	a
	V_2	0.724	a	1.834	a	4.178	a	5.021	a	25.537	b	33.997	a
P_2 – RP	V_1	0.898	a	1.081	a	8.408	a	4.318	a	51.457	a	21.897	b
	V_2	0.840	a	1.015	a	3.408	b	3.904	a	31.677	b	50.613	a
P3 – M	V_1	1.023	a	0.826	b	7.609	a	8.050	a	35.917	a	31.637	a
	V_2	0.635	b	1.317	a	4.808	b	4.595	b	30.950	a	44.590	a
P4 - M+50% of RP	V_1	0.917	a	0.914	b	5.976	a	5.534	a	50.163	a	49.547	a
	V_2	0.818	a	1.456	a	5.937	a	4.105	a	40.770	a	43.353	a
P5 - M + 100% RP	V_1	1.264	a	0.889	b	6.368	a	7.076	a	60.017	a	40.123	a
	V_2	0.684	b	1.473	a	5.828	a	5.924	a	42.100	b	33.373	a
P6 - M + 150% of RP	V_1	1.132	a	1.073	b	6.047	a	5.763	a	47.430	a	30.750	a
	V_2	0.988	a	1.630	a	4.689	a	4.643	a	22.847	b	21.747	a
Means with the same letter are not significantly different at 0.05 level using Least Significant Difference (LSD)													

The interaction of SVP shown in Table 20d illustrated varying effects to shoot the P content of the two rice varieties tested. Seed-coated NSIC Rc 222 plants with 50-150% P+M showed approximately the same shoot P concentration of P3 and were relatively higher than in P_2 during the vegetative stage. On the other hand, seed bioprimed NSIC Rc 222 resulted in

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comparable shoot P concentration to all treatments. NSIC Rc 480 applied with 50-100% P with Mycorrhizae through seed coating resulted in parallel shoot P concentration with those applied with P alone and untreated plants, let alone M-treated plants with lower shoot P concentration. Seed biopriming of 150% P+M gained the highest shoot P concentration analogous to untreated plants.

During the flowering stage, the application of 100% P+M ranked the highest shoot P concentration but comparable with applying P alone or M alone through seed coating or seed biopriming, respectively. During maturity, the amount of shoot P did not differ for all treatments, whether seed-coated or bioprimed in NSIC Rc 222 and NSIC Rc 480.

Table 5: Comparison shoot P concentration (mg/kg) as affected by Phosphorus levels + Mycorrhizae sp. at each level of coating and variety (PSV) Shoot Phosphorus (Shoot P) uptake per kilogram biomass

Seed Treatment	P Levels	Phosphorus concentration (mg/kg)											
		Vegetative (40 DAS)				Anthesis(70 DAS)				Maturity (105 DAS)			
		NSIC Rc 222		NSIC Rc 480		NSIC Rc 222		NSIC Rc 480		NSIC Rc 222		NSIC Rc 480	
S_1 - Seed Coating (SC)	P_1	1.288	a	0.724	ab	4.619	c	4.178	ab	52.290	ab	25.537	a
	P_2	0.898	c	0.840	ab	8.408	a	3.408	b	51.457	ab	31.677	a
	P_3	1.023	abc	0.635	b	7.609	ab	4.808	ab	35.917	b	30.950	a
	P_4	0.917	bc	0.818	ab	5.976	bc	5.937	a	50.163	ab	40.770	a
	P_5	1.264	ab	0.684	ab	6.368	bc	5.828	a	60.017	a	42.100	a
	P_6	1.132	abc	0.988	a	6.047	bc	4.689	ab	47.430	ab	22.847	a
S_2-Seed biopriming (SBp)	P_1	0.728	a	1.834	a	4.778	c	5.021	ab	30.140	ab	33.997	ab
	P_2	1.081	a	1.015	c	4.318	c	3.904	b	21.897	b	50.613	a
	P_3	0.826	a	1.317	bc	8.050	a	4.595	ab	31.637	ab	44.590	a
	P_4	0.914	a	1.456	b	5.534	bc	4.105	ab	49.547	a	43.353	a
	P_5	0.889	a	1.473	b	7.076	ab	5.924	a	40.123	ab	33.373	ab
	P_6	1.073	a	1.630	ab	5.630	bc	4.643	ab	30.750	ab	21.747	a

Means with the same letter are not significantly different at 0.05 level using Tukey's Honest significant difference test (HSD)

Root P concentration from vegetative to maturity stages was revealed in Table 21a. The individual factor effect showed that bioprimed plants accumulated higher P concentration than seed-coated plants regardless of variety and level of P+M applied during the vegetative stage and gradually performed comparable root P content during the Anthesis to maturity stage. NSIC Rc 480 absorbed higher P during the seedling stage than NSIC Rc 222, and further absorption during the entire growth stage manifested higher P concentration in NSIC Rc 222 roots than in NSIC Rc 480. Applying M + 100%, RP displayed the highest root P concentration, similar to using M + 150% of RP during the Anthesis stage. Plants with M + 150% of RP grow vegetatively even during maturity, resulting in lower yield.

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Table 6: Total Root Phosphorus (Root P) concentration per kilogram biomass at the different plant growth stages

Treatment	Phosphorus concentration (mg/kg)					
	Vegetative (40 DAS)		Anthesis (70 DAS)		Maturity (105 DAS)	
Factor A - Seed Treatment[1]						
S_1 - Seed Coating (SC)	0.934	b	5.656	a	39.935	a
S_2-Seed biopriming (SBp)	1.186	a	5.309	a	35.980	a
FACTOR B – Variety[1]						
V_1 - NSIC Rc 222	1.003	b	6.212	a	41.013	a
V_2 - NSIC Rc 480	1.454	a	4.699	b	37.946	b
FACTOR C - Phosphorus level[2]						
P_1 - No application (Control)	1.144	a	4.649	c	32.856	c
P_2 - Recommended P alone (RP)	0.959	b	5.404	b	36.817	b
P_3 - Mycorrhizae alone (M)	0.950	b	5.009	b	38.911	b
P_4 - M+50% of RP	1.026	b	5.160	b	36.672	b
P_5 - M + 100% RP	1.078	ab	6.266	a	37.601	b
P_6 - M + 150% of RP	1.206	a	6.140	a	43.065	a
Means with the same letter are not significantly different at 0.05 level using [1]Least Significant Difference (LSD) and [2]Tukeys Honest Significant Difference (HSD)						

The interaction of SVP, as illustrated in table 21b, summarized the constant trend from vegetative to maturity of two rice varieties. 50-100% P with Mycorrhizae sp. when applied through seed coating resulted in higher root P concentration in NSIC Rc 480 throughout the growing period. In contrast, early absorption of P happened in NSIC Rc 222 where seed coating of M alone or combining M+50-100% P boosted root P concentration until the flowering stage. Meanwhile, bioprimed seeds of NSIC Rc 222 responded actively during flowering when inoculated with 100% P+M. However, results are comparable with M alone, P alone, or reduced P level.

Table 7: Comparison of root Phosphorus (root P) concentration (mg/kg) as affected by different P levels+ Mycorrhizae sp. at each level of seed treatment and variety (PSV) at different plant growth stages

Seed Treatment	P Levels	Phosphorus concentration (mg/kg)											
		Vegetative (40 DAS)				Anthesis(70 DAS)				Maturity (105 DAS)			
		NSIC Rc 222		NSIC Rc 480		NSIC Rc 222		NSIC Rc 480		NSIC Rc 222		NSIC Rc 480	
S_1 - Seed Coating (SC)	P_1	1.288	a	0.724	ab	4.619	c	4.178	ab	41.752	a	25.535	b
	P_2	0.898	c	0.840	ab	8.408	a	3.408	b	51.455	a	31.675	ab
	P_3	1.023	abc	0.635	b	7.609	ab	4.808	ab	43.225	a	30.952	ab
	P_4	0.917	bc	0.818	ab	5.976	bc	5.937	a	55.261	a	40.769	a
	P_5	1.264	ab	0.684	ab	6.368	bc	5.828	a	52.283	a	39.378	a
	P_6	1.132	abc	0.988	a	6.047	bc	4.689	ab	44.088	a	22.845	b
S_2-Seed biopriming (SBp)	P_1	0.728	a	1.834	aa	4.778	c	5.021	ab	30.140	bc	33.996	bc
	P_2	1.081	a	1.015	c	4.318	c	3.904	b	21.897	c	50.616	a
	P_3	0.826	a	1.317	bc	8.050	a	4.595	ab	31.636	bc	44.593	ab
	P_4	0.914	a	1.456	b	5.534	bc	4.105	ab	49.545	a	43.352	ab
	P_5	0.889	a	1.473	b	7.076	ab	5.924	a	40.123	ab	33.374	bc
	P_6	1.073	a	1.630	ab	5.763	bc	4.643	ab	30.747	bc	21.745	c
Means with the same letter are not significantly different at 0.05 level using Tukey's Honest significant difference test (HSD)													

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The availability of soil P depends on the applied concentration of P in any method of seed treatment. Residual P concentration that plants have not utilized remains in the soil. It followed that increased attention of P was applied to seeds diffused to the soil layer, thus, improving the soil's P content. Single P application manifested the highest soil P content among all treatments, followed by Mycorrhizae sp. alone. Combining 50-100% P with Mycorrhizae sp. improved P concentration compared to inoculating Mycorrhizae alone. In contrast, a further increase in P combined with M manifested the lowest soil P content, as shown in Table 22a.

Table 8: Total Soil Phosphorus (soil P) concentration per kilogram biomass at the different plant growth stages

Treatment	Phosphorus concentration (mg/kg)					
	Vegetative (40 DAS)		Anthesis(70 DAS)		Maturity(105 DAS)	
Factor A - Seed Treatment[1]						
S_1 - Seed Coating (SC)	27.302		18.594		9.923	a
S_2-Seed biopriming (SBp)	24.057		15.615		8.093	b
FACTOR B – Variety[1]						
V_1 - NSIC Rc 222	24.599		16.609		9.176	a
V_2 - NSIC Rc 480	26.250		16.223		7.333	a
FACTOR C - Phosphorus level[2]						
P_1 - No application (Control)	12.530	e	8.625	f	6.391	c
P_2 - Recommended P alone (RP)	40.313	a	24.578	a	16.558	a
P_3 - Mycorrhizae alone (M)	18.828	d	20.563	b	7.047	c
P_4 - M+50% of RP	24.594	bc	18.547	c	9.719	b
P_5 - M + 100% RP	30.359	b	15.813	d	9.344	b
P_6 - M + 150% of RP	27.453	b	14.500	e	4.986	d
Means with the same letter are not significantly different at 0.05 level using [1]Least Significant Difference (LSD) and [2]Tukeys Honest Significant Difference (HSD)						

The two rice varieties are more responsive to the interaction effects of SVP. The highest soil P concentration is observed in P5 and P6, with a similar concentration with P_2 during the vegetative stage for NSIC Rc 222, both seeds coated and bioprimed. The soil P concentration declined as the plant growth progressed to maturity. A high amount of soil P was observed in P_4 next to P_2 during maturity with comparable P_5-treated plants. P's rapid uptake happened between the vegetative to anthesis stage necessary for tiller formation and rice plants' reproductive capability (Table 22b).

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Table 9: Comparison of fertilizer at each level of coating and variety (PxSxV) on Total soil P available during the different growth stages of rice varieties

Seed Treatment	P Levels	Phosphorus concentration (mg/kg)											
		Vegetative (40 DAS)				Anthesis (70 DAS)				Maturity (105 DAS)			
		NSIC Rc 222		NSIC Rc 480		NSIC Rc 222		NSIC Rc 480		NSIC Rc 222		NSIC Rc 480	
S_1 - Seed Coating (SC)	P_1	11.750	d	13.688	c	8.750	c	6.688	d	3.250	d	7.438	c
	P_2	39.813	ab	43.875	a	23.750	a	30.750	a	21.063	a	15.938	a
	P_3	16.750	cd	24.125	bc	26.125	a	22.188	b	9.375	b	7.438	c
	P_4	26.938	bc	25.250	bc	18.063	b	20.625	bc	7.688	bc	12.500	b
	P_5	41.938	ab	30.938	ab	18.438	b	17.375	c	9.375	b	13.750	ab
	P_6	26.813	bc	25.750	bc	13.063	c	17.313	c	6.250	c	5.008	c
S_2-Seed biopriming (SBp)	P_1	13.120	bc	11.563	c	9.688	c	9.375	d	9.000	bc	5.875	c
	P_2	26.938	a	50.625	a	18.375	a	25.438	a	12.485	a	16.750	a
	P_3	9.750	c	24.688	b	18.688	a	15.250	c	6.438	c	4.938	cd
	P_4	23.313	ab	22.875	bc	15.313	ab	20.188	b	9.750	ab	8.938	b
	P_5	25.313	ab	23.250	bc	13.313	bc	14.125	c	9.063	bc	5.188	cd
	P_6	32.750	a	24.500	bc	15.750	ab	11.875	cd	6.375	c	2.313	d
Means with the same letter are not significantly different at 0.05 level using Tukey's Honest significant difference test (HSD)													

2. Phosphorus uptake per unit root length (P uptake, µg/kg per cm per hill)

Regarding each root unit's absorption capacity, individual effects between two seed treatments and two rice varieties did not vary. For P levels, the increased application of P concentration resulted in more P uptake from vegetative to maturity while applying 50-100% P+M manifested 0.470-0.157 µg/kg per unit of root similar to root uptake of using P alone or M alone during the vegetative to flowering stage (Tables 23a). The interaction of the three factors showed that the P uptake of plants treated with M+150% of RP is the highest when applied through seed coating, which is comparable with the P uptake of Mycorrhizae treated NSIC Rc 222. At the same time, the rest of the treatments manifested similar P uptake rates at the anthesis stage (Table 23b). Seed biopriming of different P levels to NSIC Rc 222 showed similar plant uptake of 0.647- 0.830 µg/kg per cm per hill. At the same time, both seed treatments gave a comparable response to P uptake during maturity.

Table 10: P uptake (µg/kg per cm per hill) of rice varieties subjected to two different seed treatments of other Mycorrhizae and Phosphorus levels at different crop stages

Treatment	P uptake (µg/kg per cm per hill)					
	Vegetative (40 DAS)		Anthesis (70 DAS)		Maturity (105 DAS)	
Factor A - Seed Treatment[1]						
S_1 - Seed Coating (SC)	0.620		0.774		0.858	
S_2-Seed biopriming (SBp)	0.503		0.744		0.794	
FACTOR B – Variety[1]						
V_1 - NSIC Rc 222	0.455		0.771		0.757	
V_2 - NSIC Rc 480	0.667		0.747		0.895	
FACTOR C - Phosphorus level[2]						
P_1 - No application (Control)	0.520	b	0.868	b	0.851	b
P_2 - Recommended P alone (RP)	0.485	b	0.611	d	0.852	b
P_3 - Mycorrhizae alone (M)	0.470	b	0.768	c	0.743	c
P_4 - M+50% of RP	0.452	b	0.590	d	0.757	c
P_5 - M + 100% RP	0.517	b	0.625	d	0.771	c
P_6 - M + 150% of RP	0.924	a	1.091	a	0.982	a
Means with the same letter are not significantly different at 0.05 level using [1]Least Significant Difference (LSD) and [2]Tukeys Honest Significant Difference (HSD)						

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Table 11. Comparison of P uptake (µg/kg per cm per hill) in terms of P levels at each seed treatment and variety (PSV) at different rice growth stages.

Seed Treatment	P Levels	P uptake (µg/kg per cm per hill)									
		Vegetative (40 DAS)		Anthesis (70 DAS)				Maturity (105 DAS)			
				NSIC Rc 222		NSIC Rc 480		NSIC Rc 222		NSIC Rc 480	
S_1 - Seed Coating (SC)	P_1	0.543	b	1.053	a	0.780	ab	0.983	a	0.823	b
	P_2	0.465	b	0.537	b	0.690	bc	0.833	a	0.890	b
	P_3	0.498	b	0.983	a	0.647	bc	0.850	a	0.710	b
	P_4	0.452	b	0.617	b	0.487	c	0.747	a	0.770	b
	P_5	0.500	b	0.567	b	0.753	abc	0.580	a	0.893	b
	P_6	1.260	a	1.167	a	1.010	a	0.780	a	1.433	a
S_2-Seed biopriming (SBp)	P_1	0.497	a	0.830	a	0.810	b	0.670	b	0.927	ab
	P_2	0.505	a	0.720	a	0.497	c	1.003	a	0.683	b
	P_3	0.442	a	0.690	a	0.753	bc	0.743	ab	0.670	b
	P_4	0.452	a	0.720	a	0.537	bc	0.620	b	0.890	ab
	P_5	0.533	a	0.647	a	0.533	c	0.613	b	0.997	ab
	P_6	0.588	a	0.723	a	1.463	a	0.657	b	1.057	a
Means with the same letter are not significantly different at 0.05 level using Tukey's Honest significant difference test (HSD)											

B. Plant Phosphorus distribution

Table 12. The effects of seed treatment, variety, and P levels affect phosphorus distribution during a vegetative period

Treatment	P distribution (%)					
	Root		Shoot		R/S Ratio	
Factor A - Seed Treatment[1]						
S_1 - Seed Coating (SC)	33.733		66.248	b	0.526	a
S_2-Seed biopriming (SBp)	28.492		71.508	a	0.405	b
FACTOR B – Variety[1]						
V_1 - NSIC Rc 222	31.718		68.263		0.474	
V_2 - NSIC Rc 480	30.507		69.493		0.457	
FACTOR C - Phosphorus level[2]						
P_1 - No application (Control)	26.630	c	73.370	a	0.365	d
P_2 - Recommended P alone (RP)	30.282	b	69.718	b	0.441	c
P_3 - Mycorrhizae sp. alone (M)	28.886	b	71.114	ab	0.417	cd
P_4 - M+50% of RP	34.121	ab	65.822	c	0.528	ab
P_5 - M + 100% RP	32.099	a	67.901	bc	0.482	bc
P_6 - M + 150% of RP	34.656	a	65.344	c	0.559	a
Means with the same letter are not significantly different at 0.05 level using [1]Least Significant Difference (LSD) and [2]Tukeys Honest Significant Difference (HSD)						

The distribution of total P, as reflected in table 24a, indicated that 32.099 to 34.656 is concentrated in the root system and 65.344 to 67.901% is available in the shoot giving a ratio index of 0.482 to 0.559 in plants applied with 50 to 150%P + Mycorrhizae sp. during the vegetative period. Reducing the P level by 50% mixed with Mycorrhizae manifested a considerably higher distribution of that applied with P alone and Mycorrhizae sp. alone when

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applied through seed coating (Table 24b). The application of M+50-150% of RP is concentrated in the root system of NSIC Rc 222 with a ratio index of 0.440 to 0.591, comparable to P alone and higher when M alone is applied. The interaction of SVP showed that combining P levels to Mycorrhizae sp. is higher root P content over shoot P than using P alone with a ratio index difference of 0.111 to 0.247 for Rc 480 (Table 24c).

Table 13: Effect of different P levels + Mycorrhizae sp. at each seed treatment to Phosphorus distribution at vegetative period

Seed treatment	P levels	P distribution (%)					
		Root		Shoot		R/S Ratio	
Seed Coating	P_1 - No application (Control)	26.310	d	73.690	a	0.358	d
	P_2 - Recommended P alone (RP)	30.296	cd	69.704	ab	0.444	cd
	P_3 - Mycorrhizae sp. alone (M)	31.020	bcd	68.980	abc	0.453	cd
	P_4 - M+50% of RP	37.528	ab	62.358	cd	0.607	ab
	P_5 - M + 100% RP	36.116	abc	63.885	bcd	0.572	bc
	P_6 - M + 150% of RP	41.130	a	58.870	d	0.722	a
Seed biopriming	P_1 - No application (Control)	26.949	a	73.051	a	0.371	a
	P_2 - Recommended P alone (RP)	30.268	a	69.732	a	0.438	a
	P_3 - Mycorrhizae sp. alone (M)	26.752	a	73.248	a	0.381	a
	P_4 - M+50% of RP	30.715	a	69.286	a	0.450	a
	P_5 - M + 100% RP	28.083	a	71.917	a	0.392	a
	P_6 - M + 150% of RP	28.183	a	71.817	a	0.396	a
Means with the same letter are not significantly different at 0.05 level using Tukey's Honest significant difference test (HSD)							

Table 14: Effect of different P levels + Mycorrhizae sp. at each variety to Phosphorus distribution at vegetative period

Variety	P levels	P distribution (%)					
		Root		Shoot		R/S Ratio	
V1	P_1 - No application (Control)	27.850	bc	72.150	ab	0.388	bc
	P_2 - Recommended P alone (RP)	34.479	ab	65.521	bc	0.529	ab
	P_3 - Mycorrhizae sp. alone (M)	27.251	c	72.749	a	0.377	c
	P_4 - M+50% of RP	36.940	ab	62.947	c	0.591	a
	P_5 - M + 100% RP	30.178	abc	69.822	abc	0.440	bc
	P_6 - M + 150% of RP	33.610	abc	66.390	abc	0.518	abc
V2	P_1 - No application (Control)	25.409	b	74.591	a	0.341	b
	P_2 - Recommended P alone (RP)	26.085	b	73.915	a	0.354	b
	P_3 - Mycorrhizae sp. alone (M)	30.521	ab	69.479	ab	0.457	ab
	P_4 - M+50% of RP	31.303	ab	68.697	ab	0.465	ab
	P_5 - M + 100% RP	34.021	a	65.979	b	0.524	a
	P_6 - M + 150% of RP	35.702	a	64.298	b	0.601	a
Means with the same letter are not significantly different at 0.05 level using Tukey's Honest significant difference test (HSD)							

During the flowering stage (Anthesis), P is delivered to the shoot system. About 73.087 to 78.739% total P is distributed to plant shoot resulting to lower P concentration in the root system. M+50% of RP and M + 100% RP reflected the highest ratio index comparable with

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the ration index of P alone and M alone (Table 24d). During the maturity stage, total P is utilized by the plant for grain formation. 82.82 to 88.467% of total P is present in the shoot system (Table 24e). The addition of 50-100% P+Mycorrhizae sp. significantly increased total P content in the shoot significantly higher than plants applied with P alone and Mycorrhizae sp. alone. Combining the effects of three factors during maturity as presented in Table 24f, the R/S Ratio obtained is comparable to seed coated and seed bioprimed NSIC Rc 222 while total P is concentrated in the root system rather than distributed to the shoot when NSIC Rc480 is coated or bioprimed with 100-150% of the recommended RP +Mycorrhizae sp.

Table 15: Individual effects of seed treatment, variety, and different P levels + Mycorrhizae sp. to Phosphorus distribution at anthesis period

Treatment	P distribution (%)					
	Root		Shoot		R/S Ratio	
Factor A - Seed Treatment[1]						
S_1 - Seed Coating (SC)	23.857		76.143		0.317	
S_2-Seed biopriming (SBp)	23.313		76.687		0.308	
FACTOR B – Variety[1]						
V_1 - NSIC Rc 222	22.240	b	77.761		0.288	b
V_2 - NSIC Rc 480	25.078	a	74.922		0.340	a
FACTOR C - Phosphorus level[2]						
P_1 - No application (Control)	21.261	bc	78.739	a	0.271	c
P_2 - Recommended P alone (RP)	23.656	ab	76.344	b	0.314	b
P_3 - Mycorrhizae sp. alone (M)	22.350	b	77.650	a	0.293	bc
P_4 - M+50% of RP	25.321	a	74.679	bc	0.340	ab
P_5 - M + 100% RP	26.914	a	73.087	c	0.374	a
P_6 - M + 150% of RP	22.008	b	77.992	a	0.285	bc
Means with the same letter are not significantly different at 0.05 level using [1]Least Significant Difference (LSD) and [2]Tukeys Honest Significant Difference (HSD)						

Table 16. Individual effects of seed treatment, variety, and different P levels + Mycorrhizae sp. to Phosphorus distribution at maturity.

Treatment	P distribution (%)					
	Root		Shoot		R/S Ratio	
Factor A - Seed Treatment[1]						
S_1 - Seed Coating (SC)	13.633		86.367		0.160	
S_2-Seed biopriming (SBp)	14.476		85.524		0.171	
FACTOR B – Variety[1]						
V_1 - NSIC Rc 222	13.662		86.338		0.159	
V_2 - NSIC Rc 480	14.466		85.534		0.172	
FACTOR C - Phosphorus level[2]						
P_1 - No application (Control)	11.533	d	88.467	a	0.131	d
P_2 - Recommended P alone (RP)	14.913	b	85.087	c	0.178	b
P_3 - Mycorrhizae sp. alone (M)	13.490	bc	86.510	bc	0.157	c
P_4 - M+50% of RP	13.035	c	86.965	b	0.151	c
P_5 - M + 100% RP	14.177	b	85.824	b	0.166	bc
P_6 - M + 150% of RP	17.180	a	82.820	d	0.212	a

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Table 17: Comparison of Phosphorus levels + Mycorrhizae sp. at each level of coating and variety (PSV) during maturity with P distribution at maturity period

Seed Treatment	P Levels	P distribution (%)											
		Root				Shoot				R/S Ratio			
		NSIC Rc 222		NSIC Rc 480		NSIC Rc 222		NSIC Rc 480		NSIC Rc 222		NSIC Rc 480	
S_1 - Seed Coating (SC)	P_1	12.694	a	10.153	b	87.306	a	89.847	a	0.146	a	0.113	b
	P_2	14.338	a	14.086	b	85.662	a	85.914	a	0.168	a	0.163	b
	P_3	13.541	a	13.737	b	86.459	a	86.263	a	0.157	a	0.161	b
	P_4	12.135	a	12.016	b	87.865	a	87.984	a	0.138	a	0.137	b
	P_5	12.799	a	13.496	b	87.201	a	86.504	a	0.147	a	0.156	b
	P_6	11.519	a	23.077	a	88.481	a	76.923	b	0.131	a	0.300	a
S_2-Seed biopriming (SBp)	P_1	12.317	b	10.967	b	87.683	a	89.033	a	0.140	b	0.123	c
	P_2	20.938	a	10.290	b	79.062	b	89.710	a	0.265	a	0.115	c
	P_3	14.654	b	12.028	b	85.346	a	87.972	a	0.172	b	0.140	c
	P_4	11.587	b	16.402	a	88.413	a	83.598	b	0.131	b	0.196	b
	P_5	13.539	b	16.872	a	86.461	a	83.128	b	0.157	b	0.203	ab
	P_6	13.883	b	20.239	a	86.117	a	79.761	b	0.161	b	0.254	a

5. CONCLUSION AND RECOMMENDATION

Combining 50-100% P with Mycorrhizae, whether applied as the seed coat or bioprimed, improved P concentration compared to inoculating Mycorrhizae alone, while further increase in P combined with M manifested the lowest soil P content. P uptake and R-S P Ratio of plants treated with M +150% of RP is highest when applied through seed coating. The study may be replicated in different P- deficient soil areas to establish trends of probable beneficial effects of combining microbes with reduced P levels while maintaining the integrity of the soil and increasing crop yield.

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