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Humic acid and phosphate solubilizing bacteria led phosphorous bioavailability for enhancing photosynthetic efficiency and productivity of direct-seeded rice

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Abstract

Phosphorus deficiency is a major limitation in rice production ecosystems across the globe, restricting photosynthetic productivity and overall crop yields in spite of its very vital function in energy transfer and metabolic processes. Direct-seeded rice tends to experience phosphorus fixation in the soil, which lowers the bioavailability of this vital nutrient and restricts its key functions in ATP synthesis and photosynthesis. In the view of these concerns, field experiment conducted during the kharif seasons of 2022 and 2023 at Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand (India), with 12 treatment combinations tested in factorial randomized block design. The treatments consisted of 3 phosphorus levels (control, 50%, and 100%, corresponding to 0, 30, and 60 kg of P_2O_5 ha^{-1}) and 4 phosphate solubilizers (control, humic acid (HA) @ 10 kg ha^{-1}, carrier-based phosphate solubilizing bacteria (PSB) biofertilizer @ 10 g kg^{-1} seed, and HA @ 10 ha^{-1} + carrier-based PSB biofertilizer @ 10 g kg^{-1} seed). Results showed that higher phosphorus application significantly enhanced Normalized Difference Vegetation Index (NDVI) values across all growth stages, with notable increases of 9.33% at 60 days after sowing (DAS) and 26.53% by 120 DAS under 100% phosphorus (60 kg P_2O_5 ha^{-1}) compared to the control. Similarly, chlorophyll-a content improved by 19.17% and 22.81% at 60 and 90 days after sowing (DAS), respectively, while chlorophyll-b and total chlorophyll content exhibited comparable enhancements. The application of phosphate solubilizers also enhanced NDVI values, with the combination of humic acid and PSB yielding the highest NDVI of 0.82 at 60 DAS, representing an 11.11% increase over the control. Regarding chlorophyll content, humic acid + PSB application resulted in chlorophyll-a levels of 1.38 mg g^{-1} fresh weight (FW) at 60 DAS, marking a significant increase of 12.19% compared to the control. The same treatment also resulted in 13.51% higher chlorophyll-b content. These positive findings demonstrate the potential of incorporating phosphorus management with natural solubilizers to significantly improve photosynthetic efficiency and overall performance in direct-seeded rice, providing an auspicious, real-world solution for farmers confronted with nutrient constraints.

Keywords: Direct-seeded rice, phosphate solubilizers, chlorophyll content, nutrient management, crop productivity

Introduction

Rice (*Oryza sativa* L.) is a fundamental food source for over half of the world's population and is cultivated across a vast area of 168.36 million hectares (Mha) globally during the 2023-24 season (Statista, 2025). India stands out as a major rice producer, growing the crop on 47.6 Mha (Statista, 2025a), and yielding 137.82 million tonnes (Mt) during the same period (PIB, 2024). Phosphorus (P) is the 2nd most important macronutrient after nitrogen and is vital for numerous physiological and biochemical processes in rice. It plays a key role in photosynthesis, energy storage, cell division, signal transduction, and the synthesis of nucleic acids and phospholipids (Khan *et al.*, 2023). Despite the extensive use of P fertilizers in many intensive agricultural systems, only about 10-25% of the applied phosphorus is effectively utilized by crops in the year it is applied (Kaur *et al.*, 2024). The excess phosphorus either accumulates in the soil or leaches into water bodies, contributing to eutrophication, which is characterized by excessive algal blooms and subsequent oxygen depletion in aquatic ecosystems. Additionally, global phosphorus resources are becoming increasingly threatened due to the depletion of high-quality rock phosphate reserves. It is projected that these high-quality reserves could be exhausted within this century,

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compelling the agricultural sector to depend on lower-grade rock phosphate, which would incur higher processing costs (Baker *et al.*, 2024). In light of these challenges - both environmental and resource-related, it is crucial to optimize fertilizer usage and improve phosphorus use efficiency (PUE). One promising approach to enhancing PUE is the use of phosphate solubilizers, such as humic acid (HA) and phosphate solubilizing bacteria (PSB) with reduced P fertilizer without jeopardizing crop yield (Ali *et al.*, 2024). Evidence from various field studies suggested the use of HA and PSB along with P fertilizers has the potential to cut down the use of P fertilizers by up to 50% (Rawat *et al.*, 2022; Gao *et al.*, 2023). Considering the above facts, this study on humic acid and phosphate solubilizing bacteria with phosphorus levels effects on NDVI and chlorophyll content of direct-seeded rice was undertaken.

Material and Methods

Experimental site, soil and treatment details

The study was conducted during the K*harif* 2022 and 2023 at the Norman E. Borlaug Crop Research Centre, located at Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand (India). The soil at the research site was classified as sandy loam and had the following nutrient content: 0.77% organic carbon, 141.3 kg ha^{-1} of available N, 18.1 kg ha^{-1} of available P, and 174.6 kg ha^{-1} of available K. Additionally, the soil had a neutral pH of 7.1. The experiment comprised of 12 treatment combinations, including 3 phosphorus levels (control, 50%, and 100%, corresponding to 0, 30, and 60 kg of P_2O_5 ha^{-1}) and 4 phosphate solubilizers (control, humic acid (HA) @ 10 kg ha^{-1}, carrier-based phosphate solubilizing bacteria (PSB) biofertilizer @ 10 g ha^{-1} seed, and HA @ 10 kg ha^{-1} + carrier-based PSB biofertilizer @ 10 g ha^{-1} seed). This was laid in a factorial randomized block design (FRBD) with three replications. The recommended fertilizer application rate was 150 kg of N, 60 kg of P_2O_5, and 40 kg of K_2O ha^{-1}. One-third of the nitrogen, along with phosphorus according to the treatments, and the full amount of potassium was applied as a basal dose before sowing followed by soil application of humic acid. The remaining nitrogen was applied in two equal splits, with the first application at 30 days after sowing (DAS) and the second at 60 DAS as top dressing. Seeds of the rice variety NDR-359, treated with carrier-based PSB biofertilizer (NE10 strain) and untreated seeds, were manually sown in furrows spaced 20 cm apart. A seed rate of 35 kg ha^{-1} was used, and the seeds were immediately covered with soil to enhance seed-soil contact. All recommended agricultural practices, except for the treatments, were adopted throughout the experiment for raising a successful direct-seeded rice (DSR).

Normalized Difference Vegetation Index (NDVI)

The NDVI values were recorded at 60, 90, and 120 days after sowing (DAS) using a Trimble Green Seeker. The sensor emits brief bursts of red and near-infrared (NIR) light and then measures the amount of each type of light that is reflected back from the plant. The sensor continues to sample the scanned area as long as the trigger remains engaged. The sensor displays the measured value in terms of an NDVI reading (ranging from 0.00 to 0.99) on its LCD display screen.

$$NDVI = \frac{NIR - Red}{NIR + Red}$$

Chlorophyll content

Chlorophyll was extracted from leaves at 60 and 90 DAS using dimethyl sulfoxide (DMSO), following the method described by Hiscox and Israelstam in 1979. About 25 mg (W) of finely chopped fresh leaf sample was put to each test tube containing 10 mL DMSO (V) for this study. The tubes were then placed in a dark oven for 5 hours at 65 °C. After complete extraction of photosynthetic pigments from leaf tissue, test tubes were removed from the oven, refrigerated at room temperature, and absorbance at 645 and 663 nm were measured using DMSO as a blank.

$$Chl - a = [(12.7 \times OD\ at\ 663\ nm) - (2.69 \times OD\ at\ 645\ nm)] \times \frac{V}{1000 \times W}$$

$$Chl - b = [(22.9 \times OD\ at\ 645\ nm) - (4.68 \times OD\ at\ 663\ nm)] \times \frac{V}{1000 \times W}$$

$$Total\ chlorophyll = [(8.02 \times OD\ at\ 663\ nm) - (20.2 \times OD\ at\ 645\ nm)] \times \frac{V}{1000 \times W}$$

[where, OD = optical density, V = volume of supernatant (mL), W = weight of the leaf sample (g)]

Results

Effect on NDVI values

The results of this study highlight the effect of varying phosphorus levels and phosphate solubilizers on NDVI recorded at 60, 90 and 120 DAS (Table 1). The NDVI values revealed a

Table 1: Effect of phosphorus levels and phosphate solubilizers on NDVI values (Mean of 2-years)

Treatments	NDVI values		
	60 DAS	90 DAS	120 DAS
Phosphorus levels			
Control	0.75	0.71	0.49
50% phosphorus	0.80	0.76	0.55
100% phosphorus	0.82	0.80	0.62
SEm±	0.01	0.01	0.01
CD (p=0.05)	0.04	0.03	0.03
Phosphate solubilizers			
Control	0.74	0.72	0.50
HA	0.80	0.76	0.57
PSB	0.80	0.75	0.55
HA + PSB	0.82	0.80	0.59
SEm±	0.02	0.01	0.01
CD (p=0.05)	0.04	0.04	0.04

Table 2: Effect of phosphorus levels and phosphate solubilizers on chlorophyll content (Mean of 2-years)

Treatments	Chlorophyll content (mg g^{-1} FW)					
	chl-a		*chl-b*		Total chlorophyll	
	60 DAS	90 DAS	60 DAS	90 DAS	60 DAS	90 DAS
Phosphorus levels						
Control	1.2	1.14	0.35	0.34	1.55	1.48
50% phosphorus	1.32	1.28	0.36	0.35	1.68	1.64
100% phosphorus	1.43	1.40	0.48	0.47	1.91	1.87
SEm±	0.008	0.021	0.005	0.06	0.009	0.022
CD (p=0.05)	0.024	0.060	0.016	0.019	0.026	0.063
Phosphate solubilizers						
Control	1.23	1.20	0.37	0.36	1.60	1.56
HA	1.33	1.30	0.42	0.40	1.75	1.70
PSB	1.31	1.27	0.38	0.36	1.70	1.63
HA + PSB	1.38	1.34	0.42	0.42	1.79	1.76
SEm±	0.009	0.024	0.006	0.007	0.01	0.025
CD (p=0.05)	0.028	0.070	0.018	0.022	0.030	0.073

consistent increase with increased phosphorus application at all growth stages. At 60 DAS, the lowest NDVI of 0.75 was recorded in control treatment. The application of 50% phosphorus increased the NDVI to 0.80, marking a 6.67% increase over the control. The 100% phosphorus treatment resulted in an NDVI of 0.82, reflecting an additional 2.50% increase compared to the 50% phosphorus treatment. This trend continued at 90 DAS, where the NDVI values for the control, 50%, and 100% phosphorus treatments were 0.71, 0.76, and 0.80, respectively. The 50% phosphorus application showed a 7.04% increase over the control, and the 100% phosphorus treatment resulted in a 5.26% increase compared to the 50% phosphorus treatment. At 120 DAS, the NDVI values for each phosphorus treatment demonstrated further significant differences. The control sustained a lower NDVI of 0.49, while the 50% phosphorus treatment increased the NDVI to 0.55 over the control, reflecting a 12.24% increase. The 100% phosphorus application yielded the highest NDVI value of 0.62, representing a 12.73% improvement compared to the 50% phosphorus treatment.

Similarly, the use of phosphate solubilizers also enhanced NDVI values across all growth stages. For the control, NDVI of 0.74 was recorded at 60 DAS. The application of sole HA and sole PSB raised this value to 0.80, resulting in a percentage increase of 8.11%. Moreover, the combination of HA and PSB yielded the highest NDVI value of 0.82, demonstrating an additional increase of 2.50% compared to humic acid alone or PSB alone. At 90 DAS, the NDVI values for the control, HA, and PSB were 0.72, 0.76, and 0.75, respectively. The HA treatment showed a 5.56% increase over the control, whereas the PSB exhibited a 4.17% increase. The combined application of HA and PSB again yielded the highest NDVI of 0.80, indicating a 11.1% increase over the control. At 120 DAS, the NDVI values were 0.50 for the control, 0.57 for humic acid (a 14.00% increase), 0.55 for phosphate solubilizing bacteria (a 10.00% increase), and 0.59 for the combined treatment, showing an 18.00% increase over the control.

Effect on chlorophyll content

The study demonstrated a significant enhancement in chlorophyll content with the application of phosphorus (Table 2). At 60 DAS, chlorophyll-a (*chl-a*) content increased from 1.20 mg g^{-1} FW in the control to 1.43 mg g^{-1} FW under 100% phosphorus application, representing a percentage increase of 19.17%. At 90 DAS, *chl-a* exhibited a reduction across phosphorus levels but still showed considerable variation, with the 100% phosphorus application recording 1.40 mg g^{-1} FW compared to 1.14 mg g^{-1} FW in the control, reflecting a percentage increase of 22.81%. A similar trend was also observed for chlorophyll-b (*chl-b*) and total chlorophyll content. At 60 DAS, plants receiving 100% phosphorus exhibited an increase in *chl-b* content of 37.14% and total chlorophyll content of 23.23% compared to the control. Similarly, at 90 DAS, *chl-b* content increased by 38.24%, while total chlorophyll content showed an increase of 26.35% compared to the control.

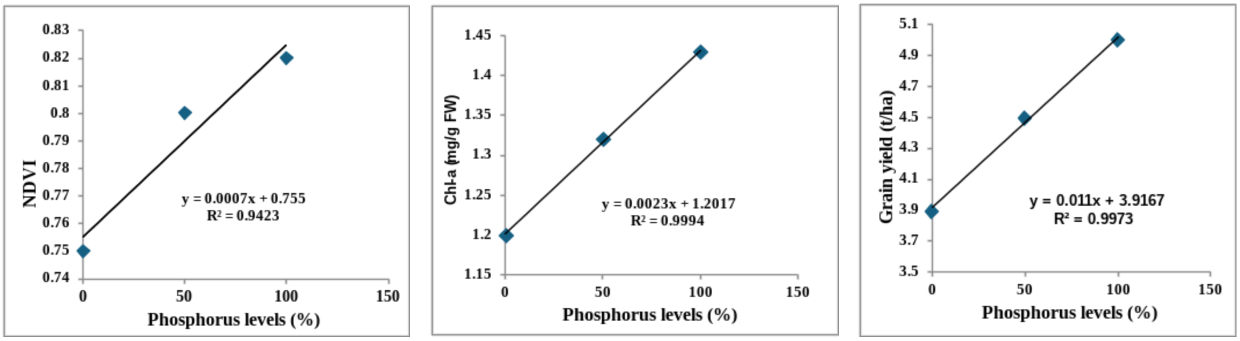

Figure 1: Pearson correlation matrix of phosphorus levels, NDVI, chlorophyll content and productivity DSR *(p <0.01)*

The application of phosphate solubilizers positively influenced chlorophyll content; for instance, at 60 DAS, the combination of HA and PSB, sole application of HA, and sole application of PSB resulted in *chl-a* content of 1.38, 1.33, and 1.31 mg g^{-1} FW, respectively, representing an increase of 12.20%, 8.13%, and 6.50% compared to the control. At 90 DAS, chlorophyll-a content decreased across phosphate solubilizers but continued to display significant variation. Among the treatments, the combination of HA and PSB recorded the highest *chl-a* content, followed by sole HA and sole PSB application, all of which were significantly higher than the control. Interestingly, the sole application of HA exhibited results similar to those of sole PSB in terms of *chl-a* content. *chl-b* and total chlorophyll content also exhibited a similar trend. At 60 DAS, HA + PSB, sole HA, and sole PSB treatments resulted in increases in *chl-b* content of 13.51%, 13.51%, and 2.70%, respectively, while total chlorophyll content increased by 11.80%, 9.30%, and 6.25%, respectively, compared to the control. At 90 DAS, HA + PSB, sole HA, and sole PSB exhibited increases in *chl-b* content of 16.60%, 11.10%, and 0%, respectively, while total chlorophyll content increased by 12.80%, 8.97%, and 4.48%, respectively, compared to the control.

Discussion

Phosphorus plays a crucial role in various physiological processes, including photosynthesis, energy transfer, and root development. As phosphorus application increases, these processes are enhanced, resulting in improved growth parameters and better nutrient uptake. This is reflected in the increased NDVI values, which serve as an indicator of plant health and photosynthetic activity. Healthier plants with higher chlorophyll content absorb more near-infrared light and reflect less red light, leading to higher NDVI readings (Li *et al.*, 2022). Phosphate solubilizers not only improved the NDVI values but also enhanced the efficacy of phosphorus uptake by the plants, which is critical for their growth and productivity. Humic acid stimulates root growth and soil microbial activity, which, when coupled with PSB, increases phosphorus, allowing plants to absorb nutrients more effectively. This synergy promotes better chlorophyll production, essential for NDVI measurement, leading to healthier, greener crops (da Silva *et al.*, 2021).

Phosphorus is crucial for chlorophyll synthesis, which is critical for optimizing the light-harvesting capacity of plants. The observed increases in chlorophyll content imply enhanced photosynthetic activity and improved overall plant vigor under higher phosphorus levels. This effect is likely correlated with better leaf development and nutrient uptake, as phosphorus is essential for energy transfer and root development (Li *et al.*, 2020). The combined application of HA and PSB significantly improves chlorophyll content by enhancing cell membrane permeability, maintaining PS II integrity, and increasing ATP formation (Kaya *et al.*, 2020). Additionally, humic acid increases $NH4^+$ availability, which directly enhances nitrogen uptake and chlorophyll synthesis (Mindari *et al.*, 2018). Phosphate solubilizing bacteria, on the other hand, promotes the solubilization of soil organic phosphorus, which contributes to improved root development, enhanced nutrient uptake, and increased chlorophyll content (Marathe *et al.*, 2017).

The correlation coefficients reveal strong relationships between physiological plant characteristics and grain yield, which indicates phosphorus management is critical in enhancing rice productivity as depicted in Figure 1. Moreover, NDVI being a very strong positive correlator ($r = 0.942$) of phosphorous levels, as well as other indicators of crop health, photosynthetic activity chlorophyll ($r = 0.999$) and grain yield ($r = 0.997$), suggests that the availability of phosphorous is highly related to the functioning of crops. The associations imply that higher pigment concentration, being descriptive of an active photosynthetic apparatus, substantially enhances grain filling and productivity overall (Mindari *et al.*, 2018). Study of da Silva *et al.* (2021), reported positive correlation with greater pigment deposition suggests that there is a more active photosynthetic machinery which aids greatly towards productivity and grain filling. The yield of grain increases with the amount 0.9973 R^2 value indicates yield variation is more likely to be the result of applied phosphorus. Without phosphorus, the minimum yield resting at 3916.7 kg ha^{-1} is estimated to increase by 11 kg ha^{-1} for every 1% increase in phosphorus. Study by Li *et al* (2022) raised the possibility of NDVI serving as a non-invasive metric for physical crop condition under nutrient oversight, which is additionally evidenced by the strong correlation with chlorophyll indices.

Conclusion

The study highlights the significant impact of phosphorus levels and phosphate-solubilizing bacteria (PSB) on NDVI and chlorophyll content in direct-seeded rice. The combined application of humic acid (HA) and PSB proved most effective, enhancing phosphorus uptake, photosynthetic activity, and overall plant vigor. Notably, HA and PSB with 50% phosphorus (30 kg P_2O_5/ha) yielded results comparable to full phosphorus application, demonstrating their potential to cut synthetic fertilizer use by half. These findings underscore the value of bio-based solutions in improving nutrient efficiency, sustaining yields, and promoting environmentally sustainable agriculture. The enhanced correlation underscores the value of a holistic approach that incorporates both remote sensing and direct biochemical assessments. It also reinforces that precise phosphorus management, by boosting both canopy development and chlorophyll synthesis, is crucial in optimizing yield in direct-seeded rice systems. These findings provide a strong quantitative basis for recommending integrated nutrient and crop monitoring strategies.

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