

ENHANCING PHOSPHORUS USE EFFICIENCY IN WHEAT UNDER ALKALINE SOIL USING RHIZOBACTERIA AND INTEGRATED FERTILIZATION

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Abstract

Phosphorus (P) deficiency is a major constraint in alkaline soils, where high pH promotes the formation of insoluble calcium phosphate compounds, thereby reducing P availability to plants. This limited phosphorus accessibility restricts root uptake, disrupts physiological processes, and ultimately reduces crop productivity, highlighting the need for efficient strategies to enhance P availability and use efficiency. The present study evaluated the effects of different phosphatic fertilizer and organic manure combinations, including single super phosphate (SSP) alone (100%), SSP (25%) + farmyard manure (FYM) (75%), SSP (50%) + FYM (50%), SSP (75%) + FYM (25%), and FYM alone (100%), with and without phosphate-solubilizing bacteria (PSB) inoculation on wheat growth, physiological traits, and phosphorus use efficiency. The experiment was conducted in a randomized complete block design (RCBD) with three replications. Results showed that, compared with the control (no SSP, no FYM, no PSB), the full SSP + PSB treatment significantly improved plant performance by reducing malondialdehyde (MDA) and hydrogen peroxide (H₂O₂) by 50.7% and 55.2%, respectively. It also increased net photosynthetic rate (P_n) by 81.4%, stomatal conductance (g_s) by 90%, water use efficiency (WUE) by 39.8%, and chlorophyll a and b contents by 66.7% and 61.5%, respectively. Moreover, total phosphorus uptake and phosphorus use efficiency increased by 212.5% and 325%, respectively, under full SSP + PSB application. In conclusion, treatments combining SSP with FYM and PSB are recommended to improve wheat growth when cultivated in P-deficient soil. Further field-scale studies are required to validate this combination as an effective amendment for mitigating phosphorus stress.

Key words: Wheat; Phosphorus; Phosphate-solubilizing bacteria; Farmyard manure; Antioxidants; Photosynthesis; Phosphorus use efficiency

Introduction

Phosphorus (P) is an essential macronutrient required for essential physiological and biochemical processes in plants, including energy transfer, photosynthesis, nucleic acid synthesis, and root development (Lambers, 2022, Zulfikar *et al.*, 2025). Despite its essential role, P availability can limit crop productivity because most soil P exists in forms that are not readily available for plant uptake (Hasan *et al.*, 2016, Bechtaoui *et al.*, 2021, Yahya *et al.*, 2023). Globally, more than 5.7 billion hectares of farmland are considered P-deficient, indicating that crops across a substantial proportion of cultivated soils have inadequate P availability for adequate growth (global agricultural soil estimates). Similarly, over 40% of cultivated land worldwide exhibits low soil-available P levels that constrain plant nutrition and yield potential (Balemi & Negisho, 2012). This widespread P deficiency affects global food production and the sustainable production of staple crops such as wheat (McKenzie & Williams, 2015, Kvakić *et al.*, 2018).

In Pakistan, P deficiency is a major constraint due to the prevalence of calcareous, alkaline soils with high pH and calcium carbonate content (Aslam *et al.*, 2024, Khan *et al.*, 2025). These soil conditions promote P fixation into insoluble Ca-phosphate compounds, making a large fraction of applied fertilizer unavailable to crops (Brownrigg *et al.*, 2022, Khan *et al.*, 2022). It is estimated that a majority of agricultural soils in Pakistan suffer from P deficiency and poor availability (soil test values often below critical Olsen P thresholds), with P fixation reducing fertilizer effectiveness by up to ~80% in many soil types (Mehdi, 2018). Moreover, the socio-economic limitations faced by smallholder farmers often result in under-application of recommended P fertilizer rates, further increasing P deficiency in wheat production systems (Rafiullah *et al.*, 2020, Jamal *et al.*, 2023, Kilinçoğlu *et al.*, 2025). Combined with low P use efficiency (often below 25–30% under typical management), these factors contribute to yield differences in wheat that persist despite increased fertilizer application (Jamal *et al.*, 2023, Asfaw *et al.*, 2025, Gete *et al.*, 2026).

Use of inorganic P fertilizer as the only P source is insufficient to sustainably meet crop P demand due to low use efficiency, high P fixation, and environmental risks such as eutrophication from runoff and leaching (Talboys *et al.*, 2020, Dharmateja *et al.*, 2022). Organic amendments (e.g., composts, manure) can improve soil physical properties, increase microbial activity, and release P through P mineralization (Abbas *et al.*, 2024), yet their P contents and release patterns are often variable and slower than crop demand (Zhu *et al.*, 2018, Kilinçoğlu *et al.*, 2025). Recent agronomic research suggests that integration of organic and inorganic P sources can produce synergistic effects, improving the soil P pool and promoting sustained P availability compared with sole fertilizer application (Hu *et al.*, 2022, Lucas *et al.*, 2025).

Biological approaches, particularly inoculation with P-solubilizing bacteria (PSB), are potential strategies to address P limitation in soil (Khan *et al.*, 2023, Iftikhar *et al.*, 2024, Akpö *et al.*, 2025). PSB increase P bioavailability through secretion of organic acids and phosphatases that mobilize poorly soluble P fractions, which may increase plant P uptake and use efficiency (Li *et al.*, 2023, Afkairin *et al.*, 2024). While individual studies have demonstrated benefits of PSB in increasing P uptake and crop growth, the interactive effects of combined organic-inorganic P fertilization with PSB inoculation remain insufficiently studied across different soil environments and management conditions (Khouchi *et al.*, 2023, Zhang *et al.*, 2023). Further research is needed to quantify how such integrated strategies influence P dynamics, P use efficiency, and wheat yield under conditions typical of P-deficient soils, especially in regions such as Pakistan where both soil constraints and sub-optimal P management practices are common (Jamal *et al.*, 2023, Nadia *et al.*, 2023, Ali *et al.*, 2024).

Wheat is a major staple food crop, providing a major share of daily calories and protein to billions of people not only in Pakistan but worldwide (Jamal *et al.*, 2023). Its importance for food security is evident from its extensive cultivation and wide consumption in products such as bread, chapati, and other cereal-based foods (Waqas *et al.*, 2025, Devkota *et al.*, 2026). However, sustaining wheat productivity is increasingly affected by nutrient limitations, particularly phosphorus (P) deficiency in alkaline soils (Athar *et al.*, 2025, Gete *et al.*, 2026). Under high pH conditions, phosphorus becomes fixed in insoluble calcium forms, reducing its availability for plant uptake. P deficiency reduces root development, photosynthetic efficiency, and plant growth, which can reduce grain yield and quality (Madhusudan *et al.*, 2025, Roosta *et al.*, 2025). Therefore, improving phosphorus availability and use efficiency is important for maintaining stable wheat production and global food security.

The present study addresses a research gap in phosphorus (P) management under alkaline soils by evaluating the combined effects of inorganic P fertilizer, organic manure (FYM), and phosphate-solubilizing bacteria (PSB) on soil P availability, P uptake, and wheat growth and yield. In contrast to sole chemical fertilizer application relying solely on chemical fertilizers, this study combines biological and organic amendments to improve nutrient cycling and P availability. We hypothesized that combined application of organic and inorganic phosphorus sources with phosphate-solubilizing bacteria (PSB) would synergistically increase phosphorus bioavailability and improve wheat growth.

Materials and Methods

Experimental site description: The field experiment was conducted during the Rabi season at the University of Agriculture, Peshawar, Pakistan. The region falls under a semi-arid subtropical climate, characterized by cool winters and mild rainfall during the wheat-growing season. The experimental soil represents the calcareous alluvial soils commonly found in the wheat-growing belt of Khyber Pakhtunkhwa, which are typically alkaline and low in available P due to strong P fixation. Before experimentation, the field had been under conventional cereal-based cropping, and no P fertilizer had been applied in the preceding season, ensuring minimal residual P effects.

Soil sampling and initial soil characterization: Before sowing, we collected composite soil samples from the experimental field at a depth of 0–20 cm using a stainless-steel soil auger. Ten cores were randomly collected from the field, thoroughly mixed to form a composite sample, air-dried in the shade, gently crushed, and passed through a 2-mm sieve for physicochemical analysis. Soil samples were analyzed for texture, pH, organic matter, total nitrogen, and AB-DTPA extractable P and potassium using standard laboratory procedures.

Soil analysis: Soil texture was determined by the hydrometer method using 10 g air-dried soil dispersed in 50 mL of 5% sodium hexametaphosphate, shaken for 16 h, and readings taken at standard intervals to calculate sand, silt, and clay. Soil pH was measured in a 1:5 soil–water suspension (10 g soil + 50 mL water) after 30 min equilibration using a digital pH meter. Organic matter was determined by the Walkley–Black method using 1 g soil oxidized with $K_2Cr_2O_7$ and H_2SO_4 , followed by titration with $FeSO_4$, and converted using a factor of 1.724. Total nitrogen was analyzed by the Kjeldahl method involving H_2SO_4 digestion, NaOH distillation, and HCl titration. Available P and K were extracted using AB-DTPA (10 g soil + 20 mL extractant, 2 h shaking); P was measured colorimetrically at 420 nm and K by flame photometer. All analyses were performed in triplicate for accuracy.

Experimental design and layout: The experiment was laid out in a randomized complete block design (RCBD) with three replications to minimize field variability. Each replication contained all treatments randomly assigned to plots. Each experimental plot measured 3 m × 4 m (12 m²) and consisted of 12 rows, each 3 m long, with a uniform row-to-row spacing of 30 cm. A 0.5 m buffer zone was maintained between plots and 1 m between replications to prevent nutrient and microbial cross-contamination.

Phosphorus sources and rates: Phosphorus was applied at a uniform rate of 75 kg P₂O₅ ha⁻¹ across treatments using single superphosphate (SSP) and farmyard manure (FYM), applied either alone or in combination.

- Single superphosphate (SSP) contained 18% P₂O₅
- Farmyard manure (FYM) contained 0.37% total P, determined by laboratory analysis before application.

Based on FYM P concentration, 18.24 t ha⁻¹ FYM was required to meet the total P requirement in the 100% FYM treatment.

Treatment details: The experiment was conducted using a randomized complete block design (RCBD) with a factorial arrangement of treatments. The treatments consisted of six phosphorus (P) source combinations, including organic and inorganic fertilizer sources, along with phosphate-solubilizing bacteria (PSB) inoculation. The P source treatments were: T₁ = Control (no P and no PSB), T₂ = 100% farmyard manure (FYM), T₃ = 75% FYM + 25% single super phosphate (SSP), T₄ = 50% FYM + 50% SSP, T₅ = 25% FYM + 75% SSP, and T₆ = 100% SSP. We further evaluated each of these six P source treatments under two microbial conditions: with PSB inoculation and without PSB inoculation. This resulted in a 6 × 2 factorial arrangement, comprising 12 treatment combinations in total. Each treatment was replicated three times to ensure statistical reliability and to account for field variability.

FYM and phosphorus application method: FYM was applied two weeks before sowing and thoroughly incorporated into the soil to allow partial mineralization. SSP was applied as a basal dose at sowing and mixed into the soil during final land preparation.

Fertilizer and irrigation management: A recommended fertilizer dose of 120 kg N ha⁻¹ and 60 kg K₂O ha⁻¹ was applied to ensure adequate N and K supply using urea and potassium sulfate. Nitrogen was applied in three split applications (basal, tillering, and booting stages) to improve nitrogen use efficiency, while potassium was applied as a basal dose at sowing. Irrigation was applied as 5–6 irrigations during the entire growing season, depending on crop growth stage and soil moisture conditions. Each irrigation was applied at an approximate depth of 1.5–2.0 inches per acre, with critical irrigations scheduled at crown root initiation, tillering, booting, flowering, and grain filling stages to avoid moisture stress. Manual weeding was performed twice during early crop growth to minimize weed competition and ensure uniform crop establishment. For disease and pest management, the crop was regularly monitored, and standard integrated agronomic practices were adopted, including manual removal of infected plant debris and timely application of recommended control measures when required to maintain uniform plant health across all treatments.

Bacterial inoculation: *Bacillus amyloliquefaciens* with a viable cell density of approximately 10⁸ CFU g⁻¹ was used for seed inoculation. Wheat seeds were uniformly spread on a clean polythene sheet and lightly moistened with a 10% (w/v) sugar solution to ensure proper adhesion of the inoculum. A PSB slurry was prepared and applied at a rate of 200 g inoculum per 10 kg of seed. Seeds were thoroughly mixed to achieve uniform coating, shade-dried for 10–15 minutes, and sown immediately thereafter.

Crop establishment and management: Wheat cultivar ‘Wadan-2017’, widely adapted to local agro-climatic conditions, was used as the test crop. Seeds were sown at a rate of 120 kg ha⁻¹ using manual drilling under field capacity

soil moisture conditions. The field was prepared by deep ploughing, followed by two cultivations and planking to achieve a fine seedbed. All agronomic practices, including irrigation, pest management, and weed control, were kept uniform across treatments to isolate treatment effects. Weed control was performed using Triultra herbicide applied at the manufacturer’s recommended dose, supplemented by manual weeding when required.

Harvesting: Wheat was harvested at physiological maturity when the plants attained full grain development, and the majority of spikes turned golden yellow, indicating complete ripening.

Measurement of agronomic parameters: At physiological maturity, plant height was measured from the soil surface to the tip of the spike (excluding awns) using a meter rod. Five plants were randomly selected from each plot, and the mean value was recorded. Spike length was measured from the base of the spike to its tip (excluding awns). Five randomly selected spikes per plot were measured, and average values were calculated. Five representative spikes from each plot were harvested, manually threshed, and the number of grains per spike was counted. The average grain number was calculated. A thousand grains from each plot were counted using an automatic grain counter and weighed using a digital analytical balance. Grain moisture was assumed uniform across treatments. Four randomly selected rows from each plot were harvested at maturity. Harvested biomass was sun-dried, tied into bundles, and weighed using a digital hanging balance. Harvested biomass was threshed using a mini thresher, and grain yield was recorded using a precision balance. Grain yield was expressed on a hectare basis using the same area conversion.

Chemical analysis: Grain and straw samples were collected separately from each plot. Samples were washed with distilled water, air-dried, and oven-dried at 70 ± 2°C for 48 hours. Dried samples were ground using a stainless-steel grinder and stored in airtight containers.

Straw and grain phosphorus: Phosphorus concentration was determined after wet acid digestion using the vanadomolybdate (yellow color) method. Briefly, 0.5 g of finely ground, oven-dried straw sample was weighed into a digestion flask and pre-digested overnight with 10 mL concentrated nitric acid (HNO₃, 65–68%). The samples were then heated on a hot plate at 120–140 °C until brown fumes ceased; then 4 mL perchloric acid (HClO₄, 70%) was added, and digestion continued until the solution became clear and colorless with white fumes, indicating complete oxidation. After cooling, the digest was quantitatively transferred, diluted to 100 mL with distilled water, and filtered through Whatman No. 42 filter paper. For P determination, 5 mL of the clear filtrate was pipetted into a 50 mL volumetric flask, and 10 mL vanadomolybdate reagent (prepared by dissolving 25 g ammonium molybdate in distilled water and mixing with 1.25 g ammonium metavanadate dissolved in hot distilled water containing 330 mL concentrated HNO₃, and diluting to 1 L) was added. The volume was made up to the mark with distilled water, mixed thoroughly, and allowed to stand for 10–15 minutes for development of a stable yellow color.

Absorbance was measured using a UV–visible spectrophotometer at 420 nm, with distilled water as the blank. Phosphorus concentration in straw was calculated from a standard curve prepared using KH_2PO_4 solutions and expressed as a percentage on a dry weight basis.

$$\text{Phosphorus (\%)} = \frac{\text{Instrumental reading} \times \text{Volume made}}{\text{Weight of sample} \times \text{Volume taken} \times 10000} \times 100$$

$$\text{PUE (\%)} = \frac{\text{Total P uptake in fertilized plot (kg ha}^{-1}\text{)} - \text{Total P uptake in control plot (kg ha}^{-1}\text{)}}{\text{Applied dose of P (kg ha}^{-1}\text{)}} \times 100$$

Statistical analysis

All experimental data were analyzed using analysis of variance (ANOVA) appropriate for a factorial randomized complete block design (RCBD) in Origin software. We evaluated the effects of phosphorus (P) sources, phosphate-solubilizing bacteria (PSB) inoculation, and their interactions. Before analysis, we checked data for normality and homogeneity of variance to ensure the validity of ANOVA assumptions. Treatment means were compared using the least significant difference (LSD) test at a 5% probability level ($p \leq 0.05$).

Results

The analysis of variance (Table 1) showed that P-solubilizing bacteria (PSB), SSP + FYM treatments, and their interaction significantly influenced most of the growth, yield, and P-related parameters of wheat. PSB application significantly affected ($p < 0.001$) plant height ($F = 23.85$), spike length ($F = 22.09$), biological yield ($F = 26.26$), grain yield ($F = 26.26$), straw P ($F = 30.04$), grain P ($F = 23.29$), and P use efficiency ($F = 14.19$, $p < 0.01$). A significant effect was also observed for 100-grain weight ($F = 4.52$, $p < 0.05$) and total P uptake ($F = 4.68$, $p < 0.05$). Similarly, SSP + FYM treatments significantly ($p < 0.001$) affected plant height ($F = 14.02$), spike length ($F = 21.34$), biological yield ($F = 8.52$), grain yield ($F = 8.52$), straw P ($F = 35.09$), grain P ($F = 13.94$), total P uptake ($F = 12.34$), and P use efficiency ($F = 23.23$). For 100-grain weight, the effect was significant at $p < 0.01$ ($F = 4.57$). The interaction between PSB and SSP + FYM ($T \times P$) was significant for

Total phosphorus uptake: Total P uptake was calculated as the sum of grain and straw P uptake.

Total phosphorus uptake = Grain P uptake + Straw P uptake

Phosphorus use efficiency: Phosphorus use efficiency was calculated as:

most traits, including plant height ($F = 6.08$, $p < 0.01$), spike length ($F = 13.29$, $p < 0.001$), 100-grain weight ($F = 4.91$, $p < 0.01$), biological yield ($F = 4.16$, $p < 0.01$), grain yield ($F = 4.16$, $p < 0.01$), grain P ($F = 8.06$, $p < 0.001$), total P uptake ($F = 5.10$, $p < 0.01$), and P use efficiency ($F = 3.32$, $p < 0.05$). The interaction had no significant effect on straw P. The coefficient of variation (CV%) ranged from 3.18% (spike length) to 15.6% (P use efficiency), indicating acceptable experimental variability.

PSB significantly ($p < 0.001$) affected soil pH ($F = 96.59$), soil EC ($F = 14.44$), chlorophyll a ($F = 55.1$), and chlorophyll b ($F = 48.7$). Significant effects were also observed for soil organic matter ($F = 12.25$, $p < 0.01$), AB-DTPA extractable P ($F = 10.86$, $p < 0.01$), and AB-DTPA extractable K ($F = 7.13$, $p < 0.05$). Mineral N was significantly affected at $p < 0.05$ ($F = 5.06$). SSP + FYM treatments significantly ($p < 0.001$) influenced soil pH ($F = 64.01$), soil EC ($F = 7.01$), soil organic matter ($F = 28.61$), AB-DTPA P ($F = 7.34$), chlorophyll a ($F = 45.6$), and chlorophyll b ($F = 39.8$). The interaction between PSB and SSP + FYM ($T \times P$) was significant ($p < 0.001$) for soil pH ($F = 6.90$), soil organic matter ($F = 16.87$), and AB-DTPA P ($F = 6.86$). A significant interaction occurred for soil EC ($F = 4.70$, $p < 0.01$), chlorophyll a ($F = 15.8$, $p < 0.01$), and chlorophyll b ($F = 12.4$, $p < 0.01$). In contrast, the interaction effect was non-significant for mineral N ($F = 0.06$) and AB-DTPA K ($F = 1.06$). The coefficient of variation (CV%) ranged from 0.3% for soil pH to 13.1% for AB-DTPA P, indicating acceptable experimental variation. The relatively low CV values for chlorophyll a (4.5%) and chlorophyll b (4%) indicate consistent physiological measurements (Table 2).

Table 1. Analysis of variance (ANOVA) for the effects of P-solubilizing bacteria (PSBs), SSP + FYM treatments, and their interaction on growth, yield, and physiology of wheat. F-values, p-values, and significance levels are presented for each attribute.

Source of variation	Plant height	Spike length	100 Grain weight	Biological yield	Grain yield	Straw P	Grain P	Total P uptake	P use efficiency
PSB	23.85 ***	22.09 ***	4.52 *	26.26 ***	26.26 ***	30.04 ***	23.29 ***	4.68 *	14.19 **
Treatment	14.02 ***	21.34 ***	4.57 **	8.52 ***	8.52 ***	35.09 ***	13.94 ***	12.34 ***	23.23 ***
T × P	6.08 **	13.29 ***	4.91 **	4.16 **	4.16 **	2.36 ns	8.06 ***	5.10 **	3.32 *
Error	12.36	0.13	4.94	300179	300179	0	0	2.73	3.99
CV (%)	3.8	3.18	5.5	5.6	5.6	3.8	5.4	11.8	15.6
Source of variation	POD	SOD	CAT	APx	MDA	H ₂ O ₂	Proline	Free amino acids	Total soluble protein
PSB	45.21 ***	52.34 ***	41.12 ***	48.56 ***	30.45 ***	22.33 ***	39.21 ***	34.18 ***	28.56 ***
Treatment	34.12 ***	41.25 ***	32.18 ***	36.44 ***	22.34 ***	18.56 ***	27.45 ***	25.12 ***	20.18 ***
T × P	8.45 **	10.21 **	7.33 **	9.12 **	5.44 *	4.33 *	6.78 **	5.12 *	4.78 *
Error	1.23	1.45	1.12	1.34	0.89	0.78	1.06	1.12	0.98
CV (%)	3.8	3.6	3.9	3.7	4	4.2	3.5	3.8	3.9

Significance codes: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns = Not significant

Table 2. Analysis of variance (ANOVA) for the effects of P-solubilizing bacteria (PSBs), SSP + FYM treatments, and their interaction on soil and ion attributes. F-values, p-values, and significance levels are presented for each attribute.

Source of variation	Soil pH	Soil EC	Soil organic matter	Mineral N
PSB	96.59 ***	14.44 ***	12.25 **	5.06 *
Treatment	64.01 ***	7.01 ***	28.61 ***	1.39 ns
T × P	6.90 ***	4.70 **	16.87 ***	0.06 ns
Error	0	0	0	0
CV (%)	0.3	6	7	10.3

Source of variation	AB-DTPA P	AB-DTPA K	Chlorophyll a	Chlorophyll b
PSB	10.86 **	7.13 *	55.1 ***	48.7 ***
Treatment	7.34 ***	0.76 ns	45.6 ***	39.8 ***
T × P	6.86 ***	1.06 ns	15.8 **	12.4 **
Error	0.5	48.56	2.48	2.16
CV (%)	13.1	6.6	4.5	4

Significance codes: *** p<0.001, ** p<0.01, * p<0.05, ns = Not significant

Table 3. Effect of different phosphatic fertilizer levels and FYM with and without phosphate-solubilizing bacteria (PSB) inoculation on antioxidant enzyme activities in wheat.

SSP	PSB	POD (U/mg)	SOD (U/mg)	CAT (U/mg)	APx (U/mg)	MDA (nmol/g FW)	H ₂ O ₂ (μmol/g FW)
Control (No SSP)	No PSB	24.50 ± 0.40 a	55.20 ± 1.10 a	28.40 ± 0.70 a	19.90 ± 0.50 a	6.90 ± 0.15 a	2.90 ± 0.10 a
Control (No SSP)	PSB	22.10 ± 0.35 b	50.80 ± 1.00 b	25.70 ± 0.60 b	17.90 ± 0.40 b	5.80 ± 0.12 b	2.40 ± 0.08 b
Full SSP	No PSB	18.00 ± 0.22 de	41.50 ± 0.68 de	20.20 ± 0.30 de	12.80 ± 0.22 de	4.00 ± 0.06 de	1.60 ± 0.04 de
Full SSP	PSB	15.50 ± 0.18 f	38.00 ± 0.60 f	18.00 ± 0.25 f	11.20 ± 0.18 f	3.40 ± 0.04 f	1.30 ± 0.03 f
SSP25:FYM75	No PSB	20.50 ± 0.28 c	47.50 ± 0.85 c	23.80 ± 0.45 c	15.80 ± 0.30 c	5.20 ± 0.09 c	2.10 ± 0.06 c
SSP25:FYM75	PSB	18.90 ± 0.25 d	44.50 ± 0.75 d	21.90 ± 0.35 d	14.20 ± 0.25 d	4.60 ± 0.07 d	1.90 ± 0.05 d
SSP50:FYM50	No PSB	19.60 ± 0.27 cd	45.60 ± 0.78 cd	22.50 ± 0.38 cd	14.80 ± 0.28 cd	4.90 ± 0.08 cd	2.00 ± 0.05 cd
SSP50:FYM50	PSB	17.80 ± 0.23 de	42.00 ± 0.70 de	20.50 ± 0.32 de	13.20 ± 0.22 de	4.20 ± 0.06 de	1.70 ± 0.04 de
Full FYM	No PSB	21.30 ± 0.30 bc	49.10 ± 0.90 bc	24.90 ± 0.50 bc	16.80 ± 0.35 bc	5.60 ± 0.10 bc	2.30 ± 0.08 bc
Full FYM	PSB	19.80 ± 0.25 cd	46.50 ± 0.80 cd	23.20 ± 0.40 cd	15.30 ± 0.30 cd	5.00 ± 0.08 cd	2.00 ± 0.06 cd
SSP75:FYM25	No PSB	18.90 ± 0.25 d	43.50 ± 0.72 d	21.20 ± 0.34 d	13.80 ± 0.25 d	4.50 ± 0.07 d	1.80 ± 0.04 d
SSP75:FYM25	PSB	16.80 ± 0.20 e	40.50 ± 0.65 e	19.80 ± 0.28 e	12.50 ± 0.20 e	3.90 ± 0.05 e	1.50 ± 0.03 e

Means are the average of n= 3 ±SD. Different letters showed significant changes at p≤0.05

The effect of different P and organic amendments on plant height was assessed with and without phosphate-solubilizing bacteria (PSB), with Control No PSB (83.33 cm) as the reference treatment. Inoculation with PSB increased plant height across all treatments. Among the inorganic and organic combinations, Full SSP with PSB produced the highest increase in plant height, showing a 30.0% increase compared to Control No PSB, whereas Full SSP without PSB showed a 10.0% increase. The integrated application of SSP50:FYM50 and SSP75:FYM25 with PSB increased plant height by 20.4% and 24.8%, respectively, while their corresponding treatments without PSB resulted in lower increases of 5.2% and 7.6%. SSP25:FYM75 with PSB increased plant height by 17.2%, compared to 4.0% without PSB. Full FYM with PSB showed a 14.0% increase, whereas Full FYM without PSB increased height by only 2.4%. Even the Control with PSB exhibited a 12.8% enhancement in plant height relative to Control No PSB, highlighting the effect of PSB inoculation in increasing plant growth across varying nutrient management regimes (Fig. 1A).

Inoculation with PSB consistently increased spike length across all treatments. Full SSP with PSB produced the highest value, reaching 12.9 cm, which corresponds to a 28.6% increase relative to Control No PSB, while Full SSP without PSB showed a lower increase of 7.0% (10.73 cm). Integrated nutrient applications, SSP50:FYM50 and SSP75:FYM25 with PSB, increased spike length by 20.6%

(12.1 cm) and 26.2% (12.67 cm), respectively, whereas the corresponding treatments without PSB resulted in smaller increases of 5.3% and 5.7%. SSP25:FYM75 with PSB enhanced spike length by 16.3% (11.67 cm) compared to 4.6% (10.5 cm) without PSB. Full FYM with PSB increased spike length by 14.3% (11.47 cm), while Full FYM without PSB achieved only a 2.7% increase (10.3 cm). Even the Control treatment with PSB showed an 11.6% increase in spike length (11.2 cm) relative to Control No PSB, indicating the effective role of PSB inoculation in promoting reproductive growth across all nutrient management strategies (Fig. 1B).

Among the treatments, Full SSP combined with PSB showed the highest value, reaching 48.13 g, which is 31.9% higher than Control No PSB, while Full SSP without PSB showed a lower increase of 9.6% (39.97 g). The integrated treatments SSP50:FYM50 and SSP75:FYM25 with PSB raised 100-grain weight by 17.2% (42.77 g) and 20.2% (43.83 g), respectively, whereas their corresponding treatments without PSB had comparatively lower gains of 6.2% and 8.1%. SSP25:FYM75 with PSB increased grain weight by 15.9% (42.27 g) in comparison to 4.9% (38.23 g) without PSB. Full FYM with PSB increased by 13.2% (41.27 g), while Full FYM without PSB showed only a 3.2% rise (37.63 g). Even the Control treatment inoculated with PSB showed an 11.2% increase (40.57 g), indicating PSB effect on improving grain filling and productivity (Fig. 1C).

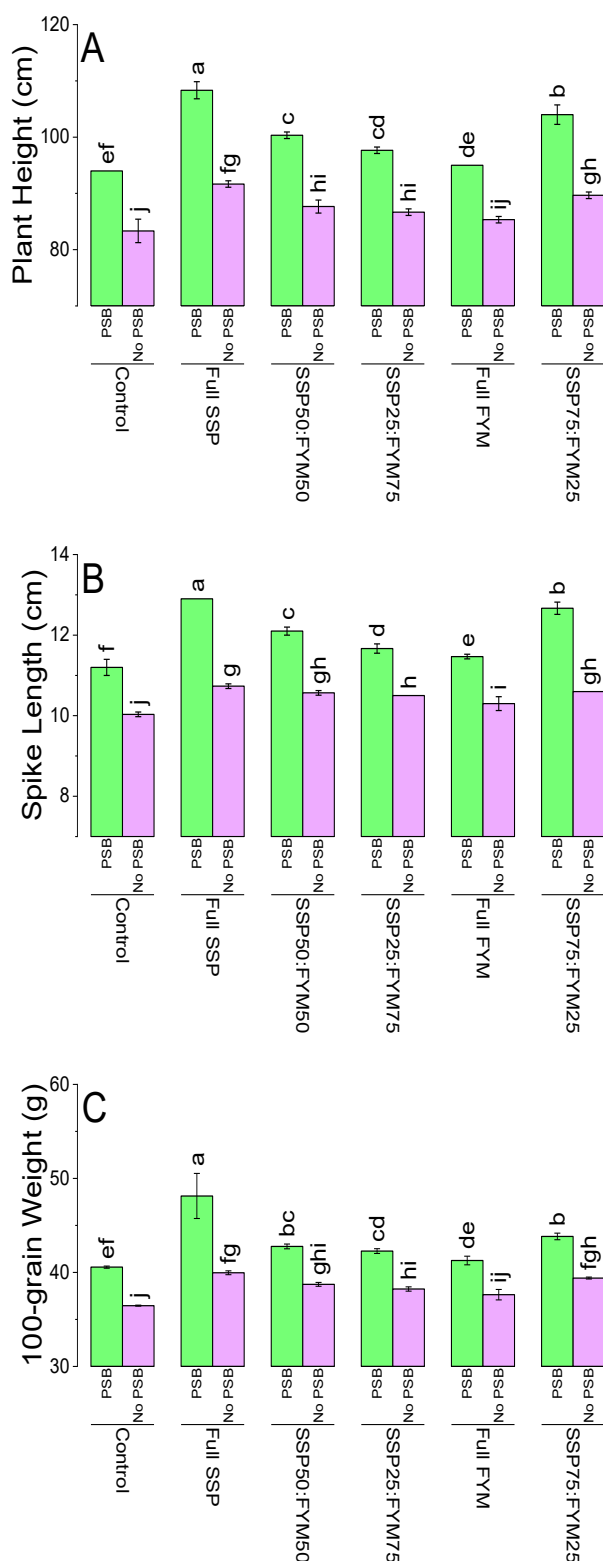


Fig. 1. Effect of different phosphatic fertilizer levels and FYM with and without phosphate-solubilizing bacteria (PSB) inoculation on plant height (A), spike length (B), and 100-grain weight (C) in wheat. Bars are means of 3 replicates $\pm$ SD, compared with the Tukey test. Different letters showed significant changes at $p \leq 0.05$.

Full SSP with PSB showed the highest increase, with 10,044 grains, which represents a 304% increase over Control No PSB (2,487.67 grains), whereas Full SSP without PSB showed a 23.5% (3,072 grains) increase.

Integrated nutrient applications SSP50:FYM50 and SSP75:FYM25 combined with PSB increased grain numbers by 241.4% (8,493 grains) and 263.5% (9,027 grains), respectively, while the corresponding treatments without PSB showed lower increases of 19.3% (2,967.7 grains) and 21.3% (3,017 grains). SSP25:FYM75 with PSB increased grain count by 196.4% (7,376 grains) compared with a 13.8% increase (2,831.3 grains) without PSB. Full FYM with PSB resulted in a 46.6% increase (3,647 grains) relative to Control No PSB, whereas Full FYM without PSB showed a 12.0% improvement (2,784 grains). The Control treatment inoculated with PSB had 3,157.7 grains, representing a 26.9% increase over Control No PSB (Fig. 2A).

PSB application increased biological yield across all nutrient treatments. The Full SSP treatment with PSB recorded the highest yield of 33,423 g, which is approximately three times higher than the Control without PSB (8,441.3 g), whereas Full SSP without PSB produced a lower value of 10,076 g. Among integrated nutrient combinations, SSP50:FYM50 and SSP75:FYM25 with PSB resulted in 27,902.7 g and 29,509 g, respectively, while the same treatments without PSB recorded 9,695.3 g and 9,758 g. SSP25:FYM75 with PSB increased yield to 26,609 g, in contrast to 9,423.7 g without PSB. Full FYM combined with PSB produced 11,707.7 g, whereas the corresponding treatment without PSB yielded 8,928.7 g. Even the control inoculated with PSB improved, reaching 11,007.7 g (Fig. 2B).

The Full SSP treatment with PSB recorded the highest P concentration at 0.383%, representing a 41.9% increase over Control No PSB (0.27%), whereas Full SSP without PSB showed a smaller increase of 11.1% (0.30%). Integrated nutrient applications SSP50:FYM50 and SSP75:FYM25 with PSB raised straw P concentration to 0.333% and 0.353%, corresponding to increases of 23.3% and 30.7%, respectively, while the same treatments without PSB resulted in lower increases of 8.6% (0.293%) and 11.1% (0.30%). SSP25:FYM75 with PSB enhanced P concentration to 0.323% compared to 0.29% without PSB. Full FYM with PSB achieved 0.3167%, whereas Full FYM without PSB had 0.2767%. Even the control inoculated with PSB showed an increase to 0.31%, indicating the effect of PSB in improving P uptake in straw (Fig. 3C).

Full SSP with PSB recorded the highest grain P concentration at 0.1867%, corresponding to a 47.3% increase over Control No PSB (0.1267%), whereas Full SSP without PSB reached 0.15%, showing an 18.4% increase. Integrated treatments SSP50:FYM50 and SSP75:FYM25 with PSB enhanced grain P content to 0.1633% and 0.1767%, representing increases of 28.9% and 39.3%, respectively, while the same treatments without PSB showed smaller gains of 10.5% (0.14%) and 10.5% (0.14%). SSP25:FYM75 with PSB increased grain P to 0.1567% compared to 0.14% without PSB. Full FYM with PSB resulted in 0.15%, whereas the same treatment without PSB had 0.1333%. Even the Control inoculated with PSB exhibited 0.15%, demonstrating the positive effect of PSB on P accumulation in grains (Fig. 2D).

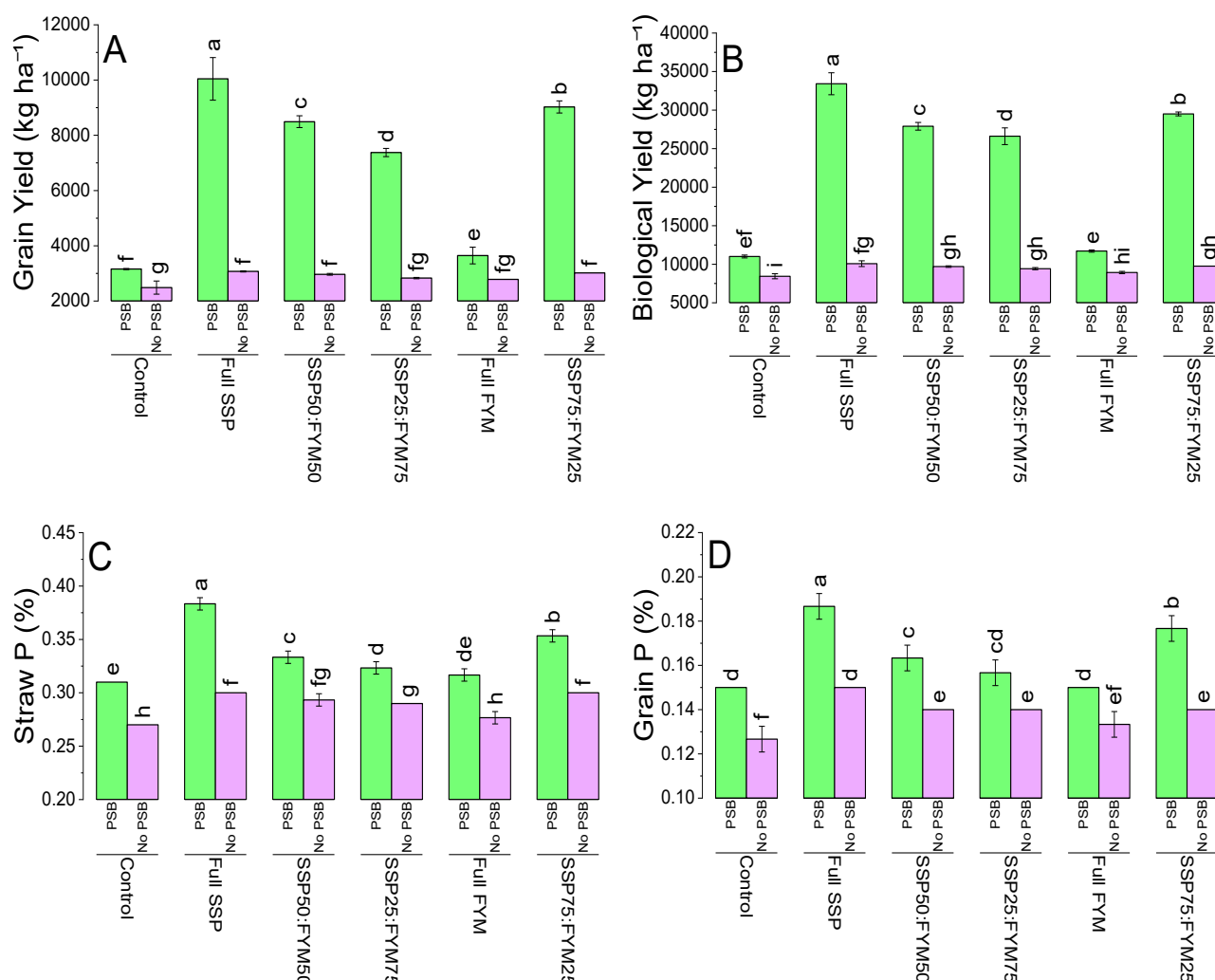


Fig. 2. Effect of different phosphatic fertilizer levels and FYM with and without phosphate-solubilizing bacteria (PSB) inoculation on grain yield (A), biological yield (B), straw P (C) and grain P (D) concentration in wheat. Bars are means of 3 replicates $\pm$ SD compared with the Tukey test. Different letters showed significant changes at $p \leq 0.05$.

Compared to the control (No SSP, No PSB), PSB inoculation alone slightly reduced enzyme activities, with POD, SOD, CAT, and APx decreasing by 9.8%, 7.97%, 9.5%, and 10.1%, respectively, while MDA and H₂O₂ decreased by 15.9% and 17.2%. Full SSP treatment caused the most pronounced reductions, with POD, SOD, CAT, and APx decreasing by 26.5%, 24.8%, 28.9%, and 35.7%, respectively, and MDA and H₂O₂ declining by 42.0% and 44.8%. The combined use of SSP with FYM mitigated these reductions; for example, SSP25:FYM75 without PSB decreased POD, SOD, CAT, and APx by 16.3%, 13.9%, 16.2%, and 20.1%, while MDA and H₂O₂ declined by 24.6% and 27.6%. PSB inoculation further reduced enzyme activities in all treatments, with SSP75:FYM25 + PSB causing POD, SOD, CAT, and APx to decrease by 31.4%, 26.6%, 30.3%, and 37.2%, respectively, and MDA and H₂O₂ by 43.5% and 48.3%. Overall, oxidative stress markers (MDA and H₂O₂) exhibited the highest percentage decreases, indicating that the treatments, particularly those combining FYM with SSP, effectively alleviated oxidative stress in wheat (Table 3).

Compared to the control, PSB inoculation alone increased net photosynthetic rate (Pn) by 20.6%,

transpiration rate (E) by 6.9%, stomatal conductance (gs) by 25%, and WUE by 12.5%, while chlorophyll a and b increased by 16.7% and 15.4%, respectively. Full SSP treatment resulted in the greatest improvements, with Pn, E, gs, and WUE increasing by 52.0%, 20.7%, 60.0%, and 25.6%, respectively, and chlorophyll a and b rising by 44.4% and 38.5%. Combined SSP and FYM treatments also enhanced these parameters, with SSP25:FYM75 + PSB increasing Pn by 58.8%, E by 22.4%, gs by 65%, WUE by 29.5%, and chlorophyll a and b by 47.2% and 43.1%, respectively. Similar trends were observed for SSP50:FYM50 and SSP75:FYM25 treatments, where PSB inoculation consistently amplified the positive effects of nutrient amendments (Table 4).

In comparison to the control (No SSP, No PSB), inoculation with PSB alone led to an increase in net photosynthetic rate (Pn) by 20.6%, transpiration rate (E) by 6.9%, stomatal conductance (gs) by 25%, and water use efficiency (WUE) by 12.5%, while chlorophyll a and b contents rose by 16.7% and 15.4%, respectively. The greatest improvements were observed under full SSP treatment, where Pn, E, gs, and WUE increased by 52.0%, 20.7%, 60.0%, and 25.6%, respectively, and chlorophyll a and b increased by 44.4% and 38.5%. Treatments

combining SSP with FYM further enhanced these physiological parameters; for example, the SSP25:FYM75 + PSB treatment boosted Pn by 58.8%, E by 22.4%, gs by 65%, WUE by 29.5%, and chlorophyll a and b by 47.2% and 43.1%, respectively (Table 4).

Compared to the control (No SSP, No PSB), PSB inoculation alone increased soil AB-DTPA P from 5.36 to 5.72 mg kg⁻¹, total P uptake from 16.80 to 18.00 kg ha⁻¹, and P use efficiency from 15.43% to 17.92%. Full SSP treatment resulted in the highest increases, with soil P

reaching 7.03 mg kg⁻¹, P uptake 47.89 kg ha⁻¹, and P use efficiency 41.52%, which were further enhanced by PSB inoculation to 7.87 mg kg⁻¹, 52.49 kg ha⁻¹, and 65.73%, respectively. Treatments combining SSP with FYM also improved P nutrition; for example, SSP25:FYM75 + PSB increased soil P to 6.45 mg kg⁻¹, total P uptake to 39.66 kg ha⁻¹, and P use efficiency to 34.13%. Similarly, SSP75:FYM25 + PSB showed substantial enhancements in soil P (7.15 mg kg⁻¹), P uptake (48.47 kg ha⁻¹), and P use efficiency (48.47%) (Table 5).

Table 4. Effect of different phosphatic fertilizer levels and FYM with and without phosphate-solubilizing bacteria (PSB) inoculation on gas exchange attributes, water use efficiency and chlorophyll contents in wheat.

SSP	PSB	Pn ($\mu\text{mol CO}_2/\text{m}^2/\text{s}$)	E ($\text{mmol H}_2\text{O}/\text{m}^2/\text{s}$)	gs ($\text{mol H}_2\text{O}/\text{m}^2/\text{s}$)	WUE (Pn/E)	Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)
Control (No SSP)	No PSB	10.20 $\pm$ 0.40 e	5.80 $\pm$ 0.25 e	0.20 $\pm$ 0.01 e	1.76 $\pm$ 0.06 e	1.80 $\pm$ 0.08 e	0.65 $\pm$ 0.04 e
Control (No SSP)	PSB	12.30 $\pm$ 0.45 d	6.20 $\pm$ 0.25 d	0.25 $\pm$ 0.01 d	1.98 $\pm$ 0.06 d	2.10 $\pm$ 0.09 d	0.75 $\pm$ 0.04 d
Full SSP	No PSB	15.50 $\pm$ 0.50 b	7.00 $\pm$ 0.28 b	0.32 $\pm$ 0.01 b	2.21 $\pm$ 0.06 b	2.60 $\pm$ 0.10 b	0.90 $\pm$ 0.04 b
Full SSP	PSB	18.50 $\pm$ 0.60 a	7.50 $\pm$ 0.30 a	0.38 $\pm$ 0.01 a	2.46 $\pm$ 0.06 a	3.00 $\pm$ 0.12 a	1.05 $\pm$ 0.05 a
SSP25:FYM75	No PSB	14.20 $\pm$ 0.48 c	6.70 $\pm$ 0.28 c	0.28 $\pm$ 0.01 c	2.12 $\pm$ 0.06 c	2.35 $\pm$ 0.09 c	0.83 $\pm$ 0.04 c
SSP25:FYM75	PSB	16.20 $\pm$ 0.50 b	7.10 $\pm$ 0.30 b	0.33 $\pm$ 0.01 b	2.28 $\pm$ 0.06 b	2.65 $\pm$ 0.10 b	0.93 $\pm$ 0.05 b
SSP50:FYM50	No PSB	14.80 $\pm$ 0.50 c	6.80 $\pm$ 0.28 c	0.30 $\pm$ 0.01 c	2.18 $\pm$ 0.06 c	2.45 $\pm$ 0.10 c	0.87 $\pm$ 0.04 c
SSP50:FYM50	PSB	16.80 $\pm$ 0.55 b	7.20 $\pm$ 0.30 b	0.35 $\pm$ 0.01 b	2.33 $\pm$ 0.06 b	2.75 $\pm$ 0.11 b	0.97 $\pm$ 0.05 b
Full FYM	No PSB	15.50 $\pm$ 0.50 b	7.00 $\pm$ 0.28 b	0.32 $\pm$ 0.01 b	2.21 $\pm$ 0.06 b	2.40 $\pm$ 0.10 c	0.85 $\pm$ 0.04 c
Full FYM	PSB	18.50 $\pm$ 0.60 a	7.50 $\pm$ 0.30 a	0.38 $\pm$ 0.01 a	2.46 $\pm$ 0.06 a	2.70 $\pm$ 0.11 b	0.95 $\pm$ 0.05 b
SSP75:FYM25	No PSB	15.00 $\pm$ 0.50 c	6.90 $\pm$ 0.28 c	0.31 $\pm$ 0.01 c	2.17 $\pm$ 0.06 c	2.50 $\pm$ 0.10 c	0.88 $\pm$ 0.04 c
SSP75:FYM25	PSB	17.00 $\pm$ 0.55 b	7.30 $\pm$ 0.30 b	0.36 $\pm$ 0.01 b	2.33 $\pm$ 0.06 b	2.80 $\pm$ 0.11 b	0.98 $\pm$ 0.05 b

Means are the average of n = 3 $\pm$ SD. Different letters showed significant changes at p $\leq$ 0.05

Table 5. Effect of different phosphatic fertilizer levels and FYM with and without phosphate-solubilizing bacteria (PSB) inoculation on soil P, plant P uptake and P use efficiency in wheat.

SSP	PSB	Soil AB-DTPA P (mg kg ⁻¹)	Total P uptake (kg ha ⁻¹)	P use efficiency (%)
Control (No SSP)	No PSB	5.36 $\pm$ 0.10 f	16.80 $\pm$ 0.50 f	15.43 $\pm$ 0.40 f
Control (No SSP)	PSB	5.72 $\pm$ 0.12 ef	18.00 $\pm$ 0.55 ef	17.92 $\pm$ 0.45 ef
Full SSP	No PSB	7.03 $\pm$ 0.17 b	47.89 $\pm$ 1.30 b	41.52 $\pm$ 1.10 b
Full SSP	PSB	7.87 $\pm$ 0.18 a	52.49 $\pm$ 1.40 a	65.73 $\pm$ 1.20 a
SSP25:FYM75	No PSB	5.88 $\pm$ 0.12 e	23.36 $\pm$ 0.70 e	21.78 $\pm$ 0.50 e
SSP25:FYM75	PSB	6.45 $\pm$ 0.14 cd	39.66 $\pm$ 1.15 cd	34.13 $\pm$ 1.00 cd
SSP50:FYM50	No PSB	5.85 $\pm$ 0.12 e	19.10 $\pm$ 0.60 e	21.54 $\pm$ 0.50 e
SSP50:FYM50	PSB	6.94 $\pm$ 0.16 bc	42.40 $\pm$ 1.25 bc	35.25 $\pm$ 1.05 bc
Full FYM	No PSB	6.12 $\pm$ 0.15 d	37.77 $\pm$ 1.10 d	33.09 $\pm$ 1.00 d
Full FYM	PSB	6.47 $\pm$ 0.16 cd	40.79 $\pm$ 1.20 cd	34.65 $\pm$ 1.05 cd
SSP75:FYM25	No PSB	5.88 $\pm$ 0.12 e	32.06 $\pm$ 1.00 d	23.65 $\pm$ 0.55 de
SSP75:FYM25	PSB	7.15 $\pm$ 0.17 b	48.47 $\pm$ 1.35 b	48.47 $\pm$ 1.15 b

Means are the average of n = 3 $\pm$ SD. Different letters showed significant changes at p $\leq$ 0.05

Discussion

Antioxidant activity and oxidative stress alleviation: The data show that antioxidant enzymes (POD, SOD, CAT, APx) and oxidative stress markers (MDA and H₂O₂) were significantly influenced by phosphatic fertilizer levels, FYM, and PSB application. Control plants exhibited higher ROS accumulation, leading to increased antioxidant enzyme activity as a defense response. In contrast, full SSP combined with FYM and PSB markedly reduced MDA and H₂O₂ levels along with lower antioxidant enzyme activities, indicating effective mitigation of oxidative stress. These findings are consistent with previous studies reporting that improved phosphorus nutrition reduces ROS accumulation and stabilizes cellular redox balance in crops grown under nutrient stress conditions (Yahya *et al.*, 2021, Alvi *et al.*, 2025). Mechanistically, P plays a pivotal role in energy metabolism

through ATP synthesis, which is required for ROS detoxification and maintenance of redox homeostasis (Bechtaoui *et al.*, 2021, Lacerda-Abreu & Meyer-Fernandes, 2021). As a key component of ATP, phosphorus provides the energy required for the synthesis and activation of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidases (POD and APx), thereby strengthening the plant's defense system against reactive oxygen species (ROS). Adequate P supply also ensures efficient functioning of the photosynthetic electron transport chain, facilitating balanced production of ATP and NADPH and preventing the leakage of excess electrons that can generate ROS such as hydrogen peroxide (H₂O₂) and superoxide radicals (Waraich *et al.*, 2011). Adequate P supply ensures proper functioning of the photosynthetic electron transport chain, preventing overproduction of ROS. Similar reductions in oxidative stress markers under integrated

nutrient management have been reported in wheat and other cereals, where combined organic and inorganic fertilization improved antioxidant balance (Zhang *et al.*, 2025). FYM contributes organic molecules, such as humic acids and phenolics, which have inherent antioxidant properties and enhance microbial activity in the rhizosphere, further mitigating oxidative stress (Akhtar *et al.*, 2023, Naseem *et al.*, 2023). Previous studies have also demonstrated that organic amendments enhance soil microbial activity and antioxidant defense systems, thereby reducing lipid peroxidation (Shah *et al.*, 2020, Shreevastav *et al.*, 2022). PSB inoculation increases P solubilization, improving P availability and thereby strengthening the plant's metabolic capacity to cope with oxidative challenges (Wang *et al.*, 2025). This agrees with earlier reports highlighting the role of PSB in alleviating oxidative stress through enhanced nutrient availability and metabolic regulation (Danish *et al.*, 2024, Onte *et al.*, 2025). The synergistic effect of single super phosphate, farmyard, and PSB highlights that optimized nutrient availability (Boutheina *et al.*, 2019, Adnan *et al.*, 2022) reduces oxidative damage, lessening the need for high antioxidant enzyme activity (Hasanuzzaman *et al.*, 2021, Sani & Yong, 2021, Yahya *et al.*, 2021, 2023). Such synergistic interactions between fertilizers and beneficial microbes have been widely documented to improve plant resilience under stress conditions (Nadia *et al.*, 2023, Ali *et al.*, 2025). This mechanism supports plant energy conservation, as the resources can be allocated to growth and photosynthesis rather than continuous defense responses (de Queiroz *et al.*, 2023).

Photosynthetic performance and chlorophyll enhancement: The treatments significantly enhanced net photosynthetic rate (P_n), transpiration rate (E), stomatal conductance (g_s), water use efficiency (WUE), and chlorophyll content, with the combined application of full SSP, FYM, and PSB showing the greatest improvement. These findings are consistent with previous studies reporting that integrated nutrient management improves photosynthetic efficiency and gas exchange characteristics in wheat (Ranjan *et al.*, 2023, Darjee *et al.*, 2024). The increased P_n can be explained by enhanced P-mediated ATP and NADPH production, which fuels the Calvin cycle and carbon assimilation (Harish *et al.*, 2022, Khourchi *et al.*, 2023). Improved chlorophyll content reflects better chloroplast development and increased light-harvesting capacity, contributing to higher photosynthetic efficiency (Meneses-Lazo *et al.*, 2020, Bechtaoui *et al.*, 2021). Earlier findings also indicate that improved phosphorus availability promotes chlorophyll synthesis and maintains chloroplast integrity under stress conditions (Danish *et al.*, 2015, Zafar-ul-Hye *et al.*, 2021). The enhancement in g_s and E indicates improved stomatal function (Morales *et al.*, 2020), which facilitates CO_2 uptake and optimizes transpiration (Morales *et al.*, 2020). Improved WUE under these treatments suggests that plants are using water more efficiently for carbon fixation, likely due to better osmotic regulation and nutrient balance provided by FYM and PSB (Boutasknit *et al.*, 2021). Consistent with these findings, previous research has shown that organic amendments improve soil moisture retention, while PSB enhances nutrient uptake, collectively improving water relations (Duarah *et al.*, 2011, Yahya *et al.*, 2023). FYM contributes to soil structure and moisture retention (Onte *et al.*, 2025), whereas PSB ensures P is readily available to support metabolic activities (Dev *et al.*,

2020). Together, these factors enhance photosynthetic efficiency and improve plant water relations. The positive relationship between chlorophyll content and phosphorus availability further indicates its key role in chlorophyll synthesis and chloroplast structural stability (Eisvand *et al.*, 2018, Khourchi *et al.*, 2023, Lu *et al.*, 2025).

Phosphorus uptake and use efficiency: Phosphorus uptake, soil available P, and P use efficiency were significantly enhanced by the treatments, with the highest values recorded in the full SSP + PSB treatment. The application of FYM alongside SSP improved P solubilization and uptake, demonstrating that organic amendments and microbial inoculation synergistically enhance P availability. PSB produces organic acids and phosphatases that mobilize otherwise unavailable P, while FYM contributes humic substances that chelate cations and prevent P fixation in the soil (Dev *et al.*, 2020, Naemullah *et al.*, 2024). Enhanced P availability supports multiple physiological and biochemical processes, including energy metabolism, nucleic acid synthesis, and membrane phospholipid formation (Faucon *et al.*, 2015, Dev *et al.*, 2020, Jindo *et al.*, 2023). This improved nutrient status explains the higher photosynthetic rates, reduced oxidative stress, and better morphological growth observed in treated plants. The increase in P use efficiency indicates that plants are effectively converting absorbed P into biomass and yield, which is crucial for sustainable crop production and minimizing fertilizer losses (Priyam *et al.*, 2022, Wang *et al.*, 2025, Zulfiqar *et al.*, 2025).

Morphological growth improvement: Morphological traits, including plant height, leaf number, leaf area, and biomass, were significantly enhanced by phosphatic fertilizer, FYM, and PSB application, with the highest improvement observed under SSP + FYM + PSB. This response is attributed to improved phosphorus availability, which stimulates cell division and elongation in meristematic tissues, resulting in enhanced vegetative growth and leaf development (Potdar *et al.*, 2021, Nadia *et al.*, 2023, Tariq & Ahmed, 2024). Enhanced photosynthesis provides the necessary energy and assimilates for biomass accumulation, while reduced oxidative stress ensures better tissue development and reduced cellular damage (Kalve *et al.*, 2014). FYM contributes to soil aeration, water retention, and micronutrient availability (Cevheri *et al.*, 2025), which support root growth and nutrient absorption (Meena *et al.*, 2018, Akhtar *et al.*, 2023). PSB inoculation improves root architecture by enhancing lateral root formation, thereby increasing nutrient and water uptake. When combined with phosphatic fertilizer and FYM, this results in stronger shoot and root growth, reflecting improved plant vigor and yield potential (Khourchi *et al.*, 2023, Jie *et al.*, 2025).

Conclusion

In conclusion, 100% SSP combined with PSB inoculation consistently produced the best results, with reduced oxidative stress, enhanced photosynthesis and water use efficiency, higher chlorophyll content, and improved growth traits. It also increased soil P availability, plant P uptake, and P use efficiency, indicating efficient nutrient utilization. These findings highlight the synergistic

effect of chemical and microbial inputs in improving wheat productivity. Future studies should evaluate long-term impacts, underlying molecular mechanisms, and field-scale economic and environmental sustainability of this integrated nutrient management approach.

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