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Full Length Article

Influence of phosphorous fertilizer on mineral nutrition and yield attributes of wheat: Acquisition with PSTOL1 gene for arid environment

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A R T I C L E I N F O

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A B S T R A C T

To boost agricultural yields, understanding mineral nutrition's reliance on fertilizers is crucial, underscoring the need to evaluate nutrient-use efficiency for optimal fertilization. In this regard, a field study was conducted to elaborate the role of phosphorus fertilizer in enhancing growth, yield and marginal rate of return in four wheat varieties; two Zn-biofortified (Zincol-2016 and Akbar-2019) and two standard types (Anaj-2017 and FSD-2008). The study revealed that application of phosphorus significantly enhanced the uptake of nitrogen (N), phosphorous (P) and potassium (K) contents in straw (13 %, 59 % and 79 %) and grains (63 %, 92 % and 28 %) respectively. Similarly, the contents of Zinc (Zn) and iron (Fe) were reduced in straw (23 % and 19 %) and grains (19 % and 18 %) respectively after application of 80 and 120 kg P_2O_5, however at 40 kg P_2O_5 the contents of Zn and Fe were slightly enhanced. The grain yield (21 %) and harvest index (11 %) was maximum at 80 kg P_2O_5 as compared to 40 and 120 kg P_2O_5. The Marginal rate of return over investment (MRR) in P_2O_5 was maximum (241 %) in Akbar-2019 when P-fertilizer was applied at the rate of 80 kg P_2O_5 per ha^{-1}. Regression analysis showed a positive correlation between grain yield and its determinants. Biofortified wheat varieties responded better to 80 kg P_2O_5, outperforming standard varieties in yield. Additionally, our findings demonstrate a significant upregulation of *PSTOL1* gene expression at 80 kg P_2O_5 level. Concludingly the application of P-fertilizer at the rate of 80 kg P_2O_5 per ha^{-1} enhanced growth, yield and rate of return of testing wheat varieties especially the biofortified ones; Zincol-2016 and Akbar-2019.

1. Introduction

Ensuring food security is a significant challenge for developing countries, where the nutrient status of arable agricultural soils is declining (Amuda & Alabdulrahman, 2024). Historically, about 50 % increase in agricultural productivity occurred during Green Revolution that began in 1960, leading to nutrient deficiency of soil especially where the use of fertilizers was not maintained according to needs. Applying a specific quantity of macro and micro nutrients in an appropriate form, amount and at the proper time is essential for optimal growth and yield (Martre et al., 2024). Over the years, imbalanced fertilizer use and intensive crop cultivation have led to the annual depletion of essential nutrients, including nitrogen, phosphorus, potassium, zinc, and boron. As the global population is expected to swell to around nine billion by 2050 or earlier, the demand for food will double, while productivity is estimated to fall by approximately 2.3 % per year (Ganapathysubramanian et al., 2024).

Following nitrogen fertilizer, phosphorus (P) is a critical nutrient that plants need in large amounts for optimal growth, as it plays a key role in forming many vital biomolecules, including nucleic acids and

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ATP (Anjum et al., 2024). P participates in a series of physiological, biochemical and metabolomic processes in plants such as photosynthesis, respiration, energy generation, nucleic acid synthesis, nitrogen fixation and redox reactions (Wang et al., 2024). Therefore, P is crucial across all plant developmental stages, from seed germination to the growth of roots, leaves, and stems, as well as flower and seed formation, yet despite its abundance, its availability to plants is severely limited as its significant portion is bounded in insoluble complexes by soil minerals like aluminum or iron, making it largely inaccessible to plants (Santoro et al., 2024).

Globally over 60 million tons of P-derived fertilizers was used in 2020, marking an increase of more than 40 % as compared to year 2000 (Clayhills, 2024). Despite widespread application of P-fertilizers, only 10–30 % is utilized by plants, with majority of P being potentially leaching from soil to water bodies (Wendimu et al., 2023). Elevated agricultural productivity can be achieved through application of optimal level of P-fertilizer that fulfils the crop's physiological requisites (Haydar et al., 2024). Grain yield has been reported to improve by 3.9–5.6 tons ha^{-1} with the application of 120 kg P_2O_5 ha^{-1}. Despite the fact that nutrient −use-efficiency (NUE) is increased with usage of fertilizers but still farmers are more dispirit to enhance crop profit (Sharma et al., 2023).

In molecular ways, *PSTOL1*, a key gene identified as the Pup1-specific protein kinase and named the Phosphorus Starvation Tolerance gene in rice (Prakasam et al., 2023). The empirical observation revealed that the overexpression of the *PSTOL1* gene in plants, cultivated within phosphorus-impoverished substrates, precipitated a notable enhancement in both the yield and biomass of plants (Balooch et al., 2024). The mechanism through which the *PSTOL1* gene exerts its influence involves the augmentation of root growth at the nascent stages, thereby capacitating the flora to assimilate an increased quantum of phosphorus from the nutrient-deficient terrain (Yang et al., 2024). In this regard a field-oriented study was designed to the understand the effect of P-fertilizer on expression of *PSTOL1* gene. Similarly, the role of P-fertilizer in enhancing yield and economic rate of return of wheat varieties was also evaluated.

2. Material and method

A field-oriented experiment was carried out at field area (71°28′31.78″ E and 71°28′31.75″ E) of Soil Science Department, Bahauddin Zakariya University Multan, Pakistan. The climatic conditions of the area are arid-to semi-arid, while maximum and minimum temperatures and rainfall were around 27.39 °C, 12.08 °C, and 580 mm, respectively during Rabi (winter) crop season. The soil of the field area was tested with results of calcareous ($CaCO_3$) in nature (6.7 %), alkaline (pH 8.1), no saline (1.4 dSm^{-1}), organic matters (0.68 %), AB-DTPA extractable P (5.6 $mgkg^{-1}$), AB-DTPA extractable K (99 mg kg^{-1}), and slit loam in textural class. The source of irrigation water supply is surface canal system as well as sub-surface resources.

The treatments were (a) four levels of phosphorus (P) fertilizers (0, 40, 80, 120 kg P_2O_5 ha^{-1}) and (b) four wheat varieties [two zinc-biofortified (Akbar-2019; Zincol-2016) and two standards (Anaj-2017 and FSD-2008)]. P-fertilizer was incorporated manually in the soil before drilling the seed. Seeds of wheat varieties were collected from National Agricultural Research Centre (NARC), Islamabad and Wheat Research Institute, Faisalabad. The planting was carried out with seed rate of 125 kg ha^{-1} drilled manually in a well-prepared seedbed sub-plots. The size of each sub-plot was 1.25 m^2 with row distance of 25 cm. The irrigation frequency and other agronomic practices were carried out according to crop and weather conditions. During vegetative and reproductive development, data related to multi-dimensional aspects were collected and analyzed.

2.1. Growth and development characteristics

The different growth and development parameters including plant height, leaf area index, heat unit efficiency and SPAD were recorded at vegetative stage.

1. Plant height was measured as a distance in centimeter from the base to the tip of spike excluding the awns at the physiological maturity stage.
2. Leaf area index was measured from randomly selected five flag leaves according to techniques of (Ahmad et al., 2015)

$$Leafareaindex(LAI) = L \times W \times K$$

where L = Length of the leaf; W = width of the leaf; K = constant factor ~ 0.75

3. Heat-use efficiency (HUE) [kg ha^{-1} °C/day] was recorded according (Nandini & Sridhara, 2019) technique

$$HUE\left[kgha^{-1}{}^{\circ}Cday^{-1}\right] = \left[Grainyield\left(kgha^{-1}\right)/accumulatedheatunits\left({}^{\circ}Cday^{-1}\right)\right]$$

4. SPAD value/ total chlorophyll contents were measured using portable chlorophyll meter "SPAD-502 DL Meter" (Minolta Camera co. Ltd. Osaka, Japan)

2.2. Yield

The wheat crop was harvested at its physiological maturity stage (when the green colour from the glumes and kernels had disappeared completely) during 2nd week of April. The grain yield and its determinants; number of productive tillers; spike length; number of spikelets $spike^{-1}$; 1000 grain weight; number of grains $spike^{-1}$; straw yield, grain yield and harvest index were quantified by employing standard protocols.

2.3. Protein, fat and ash content

The protein, fat and ash contents in wheat grains were calculated by (Rehman et al., 2020) methodology using following equations.

$$Protein(\%) = N_2(\%) \times 6.25$$

$$Fatcontents(\%) = \frac{\text{weight of beaker with fatty material (g)} - \text{weight of empty beaker (g)}}{\text{weight of original sample (g)}} \times 100$$

$$Ashcontents(\%) = \frac{\text{weight of sample after ashing (g)}}{\text{weight of original sample (g)}} \times 100$$

2.4. Ionic Constituents

The N, P, K, Zn and Fe contents in wheat grains and straw was analyzed after digestion of said plant material in con. H_2SO_4. The N concentration was determined by Kjeldahl method (Method 7.1.3) and P by colorimetry (ammonium vanadate-ammonium molybdate [Method 7.2]. The contents of K, Zn, and Fe were analyzed by wet digestion method (Ryan et al., 2001; Ryan et al., 2009).

2.5. Nutrients-use-efficiency

The different components of nutrient-use-efficiency; partial factor productivity, internal use efficiency, partial nutrient budget and agronomic efficiency (Sarkar et al., 2021; Rawal et al., 2022) for applied P-fertilizers in wheat crop was calculated at after harvest.

2.6. Economic analysis

The partial budget analysis was carried out, according to open marketing of fertilizers and grain produces at the time of planting and harvesting. The marginal rate of return (MRR) was measured according to protocol proposed by (Byerlee & Siddiq, 1994).

$$Ashcontents(\%) = \frac{\text{Change in NB (NBb} - \text{NBa)}}{\text{Change in TVC (TVCb} - \text{TVCa)}} \times 100$$

Where; NB = net profit (ha^{-1}) for each treatment [difference between the gross field benefit (GFB) and total variable cost (TVC); TVC = total variable cost is the sum of expenses incurred on costs and application of fertilizer in the field.

2.7. RNA synthesis, DNA extraction and qPCR analysis

To investigate the expression of *PSTOL1* at 80 Kg P_2O_5 ha^{-1}P-fertilizer level in wheat varieties, the mature leaves were harvested and RNA extracted for advanced analysis. *PSTOL1* sequences, originally isolated from *Oryza sativa*, were acquired from the NCBI database for BLAST analysis, excluding duplicate entries and retaining sequences that exhibited over 50 % identity. Complementary DNA was synthesized employing a Thermos Scientific Revert Aid Reverse Transcriptase kit, and primers were crafted using Beacon designer software. The expression levels after P-fertilization was quantified *via* real-time quantitative PCR (Applied Biosystems® 7900 HT Fast RT-PCR), with Actin serving as the reference gene. Expression quantification was conducted employing the $2^{-\Delta\Delta CT}$ method (Ma et al., 2006).

2.8. Statistical analysis

The data was analyzed statically using computer-based software COSTAT (6.311, USA). To evaluate the relationships among the studied traits, we employed multivariate analysis (PCA via biplot), correlation matrix (with ggplot2), and constructed a heatmap using customized code (heatmap) within the R statistical software.

3. Results

3.1. Physiological and morphological parameters

An increase in plant height of wheat varieties was observed after application of different doses of Phosphorous (P) fertilizer. The increase in plant height at 40 Kg P_2O_5 ha^{-1} was 5 % in Anaj-2017; at 80 Kg P_2O_5 ha^{-1} this enhancement was maximum of all doses; 11 % in Zincol-2016, Akbar-2019 and FSD-2008, while at 120 Kg P_2O_5 ha^{-1} highest increase was 9 % in Anaj-2017 (Fig. 1).

Total leaf chlorophyll contents (SPAD) were remarkedly enhanced in all wheat varieties after application of different doses of P-fertilizer. The increase in SPAD at 40,80, and 120 Kg P_2O_5 ha^{-1} was observed to 8 %, 21 % and 15 % respectively in wheat variety Akbar-2019 (Fig. 1).

The application of different doses of P-fertilizer affected LAI in all

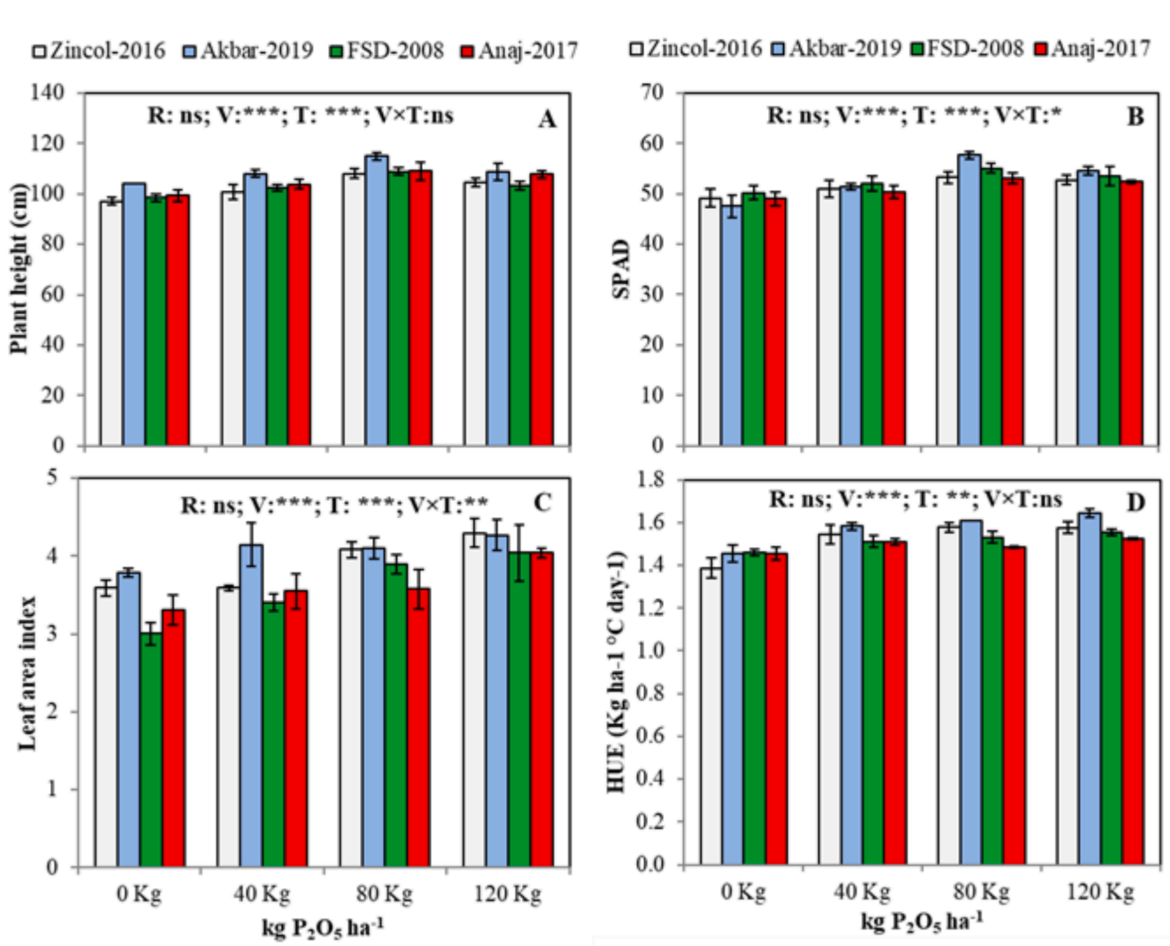

Fig. 1. The Impact of Different Phosphorus Fertilizer Doses (40, 80, and 120 kg P2O5 per hectare) on Four Wheat Varieties (Zincol-2016, Akbar-2019, FSD-2008, and Anaj-2017) in Terms of (A) Plant Height (cm), (B) SPAD (Soil Plant Analysis Development) Values, (C) Leaf Area Index, and (D) Heat Unit Efficiency (HUE) (kg per hectare per °C Day). R = Replication; V = Varieties; T = Phosphorus Fertilizer Doses; *** = Highly Significant at P ≤ 0.001; ** = Significant at P ≤ 0.01; ns = Not Significant.

wheat varieties. The maximum increase in LAI over control at 40, 80, and 120 Kg P_2O_5 ha^{-1} was observed to 13 %, 30 % and 34 % respectively in wheat variety FSD-2008.

Application of Phosphorous fertilizer affected heat unit efficiency (HUE) in almost all wheat varieties. As compared to control maximum HUE at 40,80, and120 Kg P_2O_5 ha^{-1} was recorded to 8 %, 21 % and 15 % respectively in wheat variety Akbar-2019 (Fig. 1).

3.2. Yield

The number of productive tillers of wheat varieties were remarked enhanced after application of P_2O_5 fertilizer. As compared to control plants maximum increase (11 %) in productive tillers was observed in Zincol-2016 at 80 Kg P_2O_5 ha^{-1} fertilizer application level. However, at the same fertilizer level Anaj-2017 showed 10 % increase in productive tillers as compared to control (Table 1). As compared to control plants maximum increase (12 %) in spike length was observed in Zincol-2016 at 80 Kg P_2O_5 ha^{-1} fertilizer level. However, at the same fertilizer level FSD-2008 showed 10 % increase in spike length as compared to control (Table 1).

A maximum enhancement in the number of spikelet's $spike^{-1}$ was observed at 80 Kg P_2O_5 ha^{-1} application. As compared to un-fertilized wheat plants, an increase of 9 %, 13 %, 10 % and 8 % was recorded in Zincol-2016, Akbar-2019, FSD-2008 and Anaj-2017 respectively. However, at 120 Kg P_2O_5 ha^{-1} fertilizer level, Akbar-2019 showed 9 % increase in number of spikelet's $spike^{-1}$ (Table 1). An increase in 1000 grain weight was observed after application of P-fertilizer in all wheat varieties. As compared to control maximum increase of 16 % and 12 % was recorded in Akbar-2019 and Zincol-2016 respectively at 80 Kg P_2O_5 ha^{-1} (Table 1).

Higher enhancement in number of grains $spike^{-1}$ was observed after application of P-fertilizer. At 80 Kg P_2O_5 ha^{-1}, maximum enhancement in number of grains $spike^{-1}$ was observed in Akbar-2019 (14 %) and Zincol-2016 (11 %) (Table 1). A slight change in straw yield was observed in all wheat varieties after application of P_2O_5 fertilizer. As compared to control 4 % increase in straw yield was observed in Zincol-2016, Akbar-2019 and FSD-2008 at 80 Kg P_2O_5 ha^{-1} (Table 1). The application of P-fertilizer enhanced grain yield of wheat varieties. As compared to control plants highest grain yield (21 %) was observed in Akbar-2019 at 80 Kg P_2O_5 ha^{-1}. However, at the same fertilizer level Zincol-2016 showed 13 % increase in grain yield as compared to control. The data showed that as compared to unfertilized wheat plants, highest harvest index (HI) at 40 Kg P_2O_5 ha^{-1} was observed in Zincol-2016 (5 %), at 80 Kg P_2O_5 ha^{-1} Akbar-2019 (11 %) and at 120 Kg P_2O_5 ha^{-1} Zincol-2016 (3 %) (Table 1).

3.3. Ionic constituents

The application of P-fertilizer enhanced the uptake of N-contents in straw and grains of all wheat varieties. In Zincol-2016 as compared to control plants, 8 %, 11 % and 13 % straw N-contents were observed at 40, 80 and 120 Kg P_2O_5 ha^{-1} respectively (Table 2). Similarly at 40 Kg P_2O_5 ha^{-1} maximum N-contents (22 %) in grains were observed in Anaj-2017 while at 80, and 120 kg P_2O_5 ha^{-1} this was 42 % and 63 % respectively in Zincol-2016. As compared to control the maximum increase in straw P-contents at 40, 80 and 120 Kg P_2O_5 ha^{-1} was observed

Table 1

Impact of Varying Phosphorus Fertilizer Doses (40, 80, and 120 kg P_2O_5 per hectare) on the Yield Components of Four Wheat Varieties (Zincol-2016, Akbar-2019, FSD-2008, and Anaj-2017).

Yield	P_2O_5 (kg ha^{-1})	Zincol-2016		Akbar-2019		FSD-2008		Anaj-2017	
		Mean	Difference	Mean	Difference	Mean	Difference	Mean	Difference
No. of Productive tillers	0	281		299		278		275	
	40	293	4 %	308	3 %	279	1 %	290	6 %
	80	313	11 %	320	7 %	293	6 %	301	10 %
	120	299	7 %	307	3 %	288	4 %	298	8 %
Spike length	0	10.8	%	12.2		11.2		10.8	
	40	11.7	8 %	12.5	3 %	11.5	3 %	11.3	5 %
	80	12.2	12 %	13.2	8 %	12.3	10 %	11.7	8 %
	120	11.2	3 %	12.3	1 %	11.5	3 %	11.0	2 %
No. of spikelets $spike^{-1}$	0	18.0		18.3		17.0		17.7	
	40	18.0	0 %	18.7	2 %	17.7	4 %	18.0	2 %
	80	19.7	9 %	20.7	13 %	18.7	10 %	19.0	8 %
	120	19.0	6 %	20.0	9 %	18.0	6 %	18.3	4 %
1000 grainweight	0	39.2		39.1		36.0		35.8	
	40	41.1	5 %	42.8	9 %	37.6	4 %	37.1	4 %
	80	43.8	12 %	45.2	16 %	39.0	8 %	38.2	7 %
	120	41.6	6 %	41.9	7 %	37.5	4 %	36.5	2 %
No. of grains $spike^{-1}$	0	42.7		45.7		41.7		44.0	
	40	45.7	7 %	49.0	7 %	44.3	6 %	45.0	2 %
	80	47.3	11 %	52.0	14 %	45.0	8 %	47.3	8 %
	120	46.0	8 %	49.3	8 %	43.7	5 %	45.3	3 %
straw yield	0	4.03		4.10		3.97		3.93	
	40	4.07	1 %	4.17	2 %	4.03	2 %	3.97	1 %
	80	4.20	4 %	4.27	4 %	4.13	4 %	4.07	3 %
	120	4.10	2 %	4.13	1 %	4.00	1 %	4.00	2 %
grain yield	0	3.30		3.33		3.33		3.23	
	40	3.43	4 %	3.57	7 %	3.50	5 %	3.40	5 %
	80	3.73	13 %	4.03	21 %	3.63	9 %	3.50	8 %
	120	3.57	8 %	3.73	12 %	3.50	5 %	3.43	6 %
Harvest Index	0	45.4		46.4		44.7		44.1	
	40	47.5	5 %	47.5	2 %	45.9	3 %	45.0	2 %
	80	49.5	9 %	51.3	11 %	47.7	7 %	46.8	6 %
	120	47.4	4 %	48.0	3 %	45.8	2 %	45.7	4 %

Table 2
The impact of varying doses of phosphorus fertilizer (40, 80, and 120 kg P_2O_5 per hectare) on the content of N, P, K, Zn, and Fe in four wheat varieties (Zincol-2016, Akbar-2019, FSD-2008, and Anaj-2017).

Ioniccontents		P_2O_5 (kg ha^{-1})	Zincol-2016		Akbar-2019		FSD-2008		Anaj-2017	
			Mean	Diff.	Mean	Diff.	Mean	Diff.	Mean	Diff.
N-contents	Straw	0	79.2		85.5		81.3		79.1	
		40	85.3	8 %	90.2	5 %	84.7	4 %	83.9	6 %
		80	87.9	11 %	89.3	4 %	83.4	3 %	83.5	6 %
		120	89.5	13 %	90.9	6 %	85.0	5 %	83.2	5 %
	Grain	0	1.92		2.04		1.73		1.80	
		40	2.07	8 %	2.19	7 %	1.90	10 %	2.19	22 %
		80	2.72	42 %	2.84	39 %	2.30	33 %	2.34	30 %
		120	3.13	63 %	3.12	53 %	2.68	55 %	2.79	55 %
P-contents	Straw	0	0.08		0.08		0.07		0.06	
		40	0.09	16 %	0.10	27 %	0.08	22 %	0.08	36 %
		80	0.10	25 %	0.10	29 %	0.09	32 %	0.08	35 %
		120	0.12	51 %	0.12	52 %	0.12	69 %	0.11	95 %
	Grain	0	0.15		0.19		0.16		0.17	
		40	0.20	32 %	0.21	15 %	0.21	37 %	0.19	9 %
		80	0.25	64 %	0.23	22 %	0.25	58 %	0.26	54 %
		120	0.29	92 %	0.31	66 %	0.26	70 %	0.28	65 %
K-contents	Straw	0	0.70		0.78		0.61		0.68	
		40	0.89	28 %	0.95	21 %	0.80	33 %	0.85	26 %
		80	1.02	47 %	1.04	33 %	1.02	69 %	1.01	49 %
		120	1.04	49 %	1.07	37 %	1.09	79 %	1.11	64 %
	Grain	0	1.33		1.43		1.34		1.34	
		40	1.48	11 %	1.50	5 %	1.45	8 %	1.45	8 %
		80	1.55	17 %	1.62	13 %	1.54	16 %	1.48	11 %
		120	1.70	28 %	1.76	23 %	1.60	20 %	1.57	17 %
Zn-contents	Straw	0	11.7		14.5		13.7		10.5	
		40	13.7	17 %	15.0	3 %	14.5	6 %	12.7	22 %
		80	10.2	−13 %	12.3	−15 %	11.6	−15 %	8.60	−18 %
		120	10.3	−12 %	11.2	–23 %	10.9	−20 %	9.00	−14 %
	Grain	0	24.2		24.8		22.2		22.8	
		40	29.4	22 %	29.9	21 %	23.9	8 %	24.2	6 %
		80	21.3	−12 %	22.3	−10 %	18.5	−16 %	20.1	−12 %
		120	20.7	−14 %	22.1	−11 %	17.9	−19 %	18.5	−19 %
Fe-contents	Straw	0	150.6		154.8		141.8		145.3	
		40	161.6	7 %	169.1	9 %	149.8	6 %	151.2	4 %
		80	145.3	−4%	137.4	−11 %	137.5	−3%	136.4	−6%
		120	130.5	−13 %	125.3	−19 %	120.4	−15 %	130.2	−10 %
	Grain	0	88.0		92.78		84.8		89.5	
		40	97.0	10 %	102.6	11 %	92.8	10 %	97.5	9 %
		80	85.4	−3%	90.30	−3%	78.6	−7%	84.0	−6%
		120	82.6	−6%	86.40	−7%	69.4	−18 %	78.2	−13 %

in Anaj-2017 as 36 %, 35 % and 95 % respectively. However, grain P-contents at 40 Kg P_2O_5 ha^{-1} were higher in FSD-2008, while at 80 and 120 kg P_2O_5 ha^{-1} this increase was highest 64 % and 92 % respectively in Zincol-2016 compared to control (Table 2). The uptake of straw and grains of K-contents was remarkably increased after application of P-fertilizer in all wheat varieties. As compared to control plants maximum straw K-contents were recorded in FSD-2008, 33 %, 69 % and 79 % at 40, 80 and 120 Kg P_2O_5 ha^{-1} respectively. On the other hand, as compared to non-fertilized wheat plants, maximum increase in grain K-contents at 40, 80 and 120 Kg P_2O_5 ha^{-1} was recorded in Zincol-2016, 11 %, 17 % and 28 % respectively (Table 2).

Zn-contents in straw and grains of all wheat varieties were remarked reduced after application of P-fertilizer (80 and 120 Kg P_2O_5 ha^{-1}). At 40 Kg P_2O_5 ha^{-1} the Zn straw and grain contents were enhanced to 22 % in Anaj-2017 and Zincol-2016 respectively. While as compared to control plants maximum decrease (23 %) in Zn straw contents was observed in Akbar-2019 at 120 P_2O_5 ha^{-1}. Similarly, Zn-grain contents were reduced to 19 % at 120 Kg P_2O_5 ha^{-1} in FSD-2008 and Anaj-2017 (Table 2). Data showed that Fe-contents in straw and grains were also reduced after application of P-fertilizer (80 and 120 kg P_2O_5 ha^{-1}), while enhanced at 40 kg P_2O_5 ha^{-1}. As compared to control the maximum increase in P-contents was observed in Akbar-2019 (9 % and 11 %) in straw and grain respectively at 40 kg P_2O_5 ha^{-1}. However, at 80 and 120 kg P_2O_5 ha^{-1}, Fe-contents in straw and grain were reduced to 11 % and 19 % respectively in Akbar-2019. Similarly, grain Fe-contents were lowered to 9 % and 18 % in FSD-2008 at 80 and 120 Kg P_2O_5 ha^{-1} respectively (Table 2).

3.4. Protein, fat and ash content

Data for wheat grain protein, fat and ash contents differed statistically in response to application of P.fertilizer. As compared to non-fertilized plants an increase in grain protein contents to 16 % and 13 % at 40 and 120 Kg P_2O_5 ha^{-1} respectively was recorded in Akbar-2019, however at 80 Kg P_2O_5 ha^{-1} maximum increase (11 %) was observed in FSD-2008 (Table 3). At 40 Kg P_2O_5 ha^{-1} maximum increase (10 %) in fat contents was observed in Zincol-2016, however at 80 and 120 Kg P_2O_5 ha^{-1}, FSD-2008 achieved maximum 12 % and 23 % grain fat contents respectively as compared to un-treated plants. It was observed that as compared to other wheat varieties, the Anaj-2017 produced higher (5.9 %) grain ash contents at 40, 80, 120 Kg P_2O_5 ha^{-1} respectively (Table 3).

3.5. Phosphorus nutrient use efficiency

The application of P-fertilizer significantly enhanced nutrient use efficiency of wheat varieties. As compared to control, Anaj-2017 exhibited maximum 301 % followed by FSD-2008 (280 %), Zincol-2016 (264 %) and Akbar-2019 (258 %) partial factor productivity (PFP) at 80 Kg P_2O_5 ha^{-1} (Fig. 2). The maximum internal use efficiency

Table 3
The influence of varying phosphorus fertilizer doses (40, 80 and 120 kg P_2O_5 ha^{-1}) on the percentage of protein, fat, and ash content in the grains of four wheat varieties (Zincol-2016, Akbar-2019, FSD-2008, and Anaj-2017) at maturity.

P_2O_5 kg ha^{-1}	Zincol-2016		Akbar-2019		FSD-2008		Anaj-2017	
	Mean	Diff.	Mean	Diff.	Mean	Diff.	Mean	Diff.
Protein contents								
0	12.3		12.0		11.1		11.2	
40	12.9	5 %	13.9	16 %	11.9	7 %	11.7	4 %
80	13.1	7 %	13.1	9 %	12.3	11 %	12.3	10 %
120	13.4	9 %	13.6	13 %	12.4	12 %	12.5	12 %
Fat contents								
0	1.9		2.0		1.7		1.8	
40	2.1	10 %	2.1	5 %	1.8	6 %	1.9	6 %
80	2.1	10 %	2.2	10 %	1.9	12 %	1.9	6 %
120	2.2	16 %	2.3	15 %	2.1	23 %	2.0	11 %
Ash contents								
0	1.8		1.8		1.7		1.7	
40	1.9	5.6 %	1.9	5.6 %	1.7	0.0 %	1.8	5.9 %
80	1.9	5.6 %	1.9	5.6 %	1.8	5.9 %	1.8	5.9 %
120	1.9	5.6 %	1.9	5.6 %	1.8	5.9 %	1.8	5.9 %

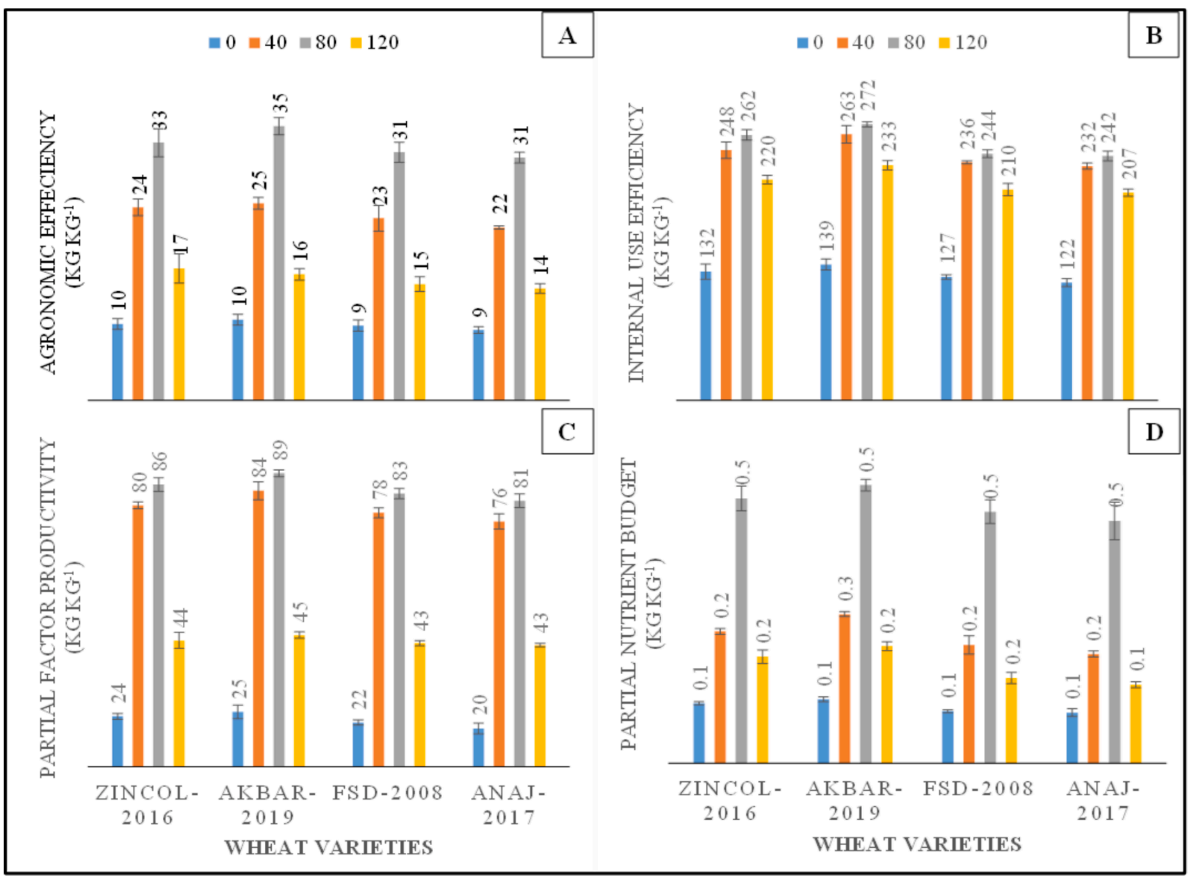

Fig. 2. Influence of Various Phosphorus Fertilizer Doses (40, 80, and 120 kg P2O5 per hectare) on Four Wheat Varieties (Zincol-2016, Akbar-2019, FSD-2008, and Anaj-2017) Regarding (A) Agronomic Efficiency, (B) Internal Use Efficiency, (C) Partial Factor Productivity, and (D) Partial Nutrient Budget (kg per kg).

(IUE) was recorded as 91 %, 99 % and 70 % at 40, 80 and 120 Kg P_2O_5 ha^{-1} respectively in Anaj-2017 (Fig. 2). A remarked increase in agronomic efficiency (AE) was observed in in Anaj-2017 (245 %) at 80 Kg P_2O_5 ha^{-1} fertilizer level as compared to control. Similarly, at the same fertilizer level Akbar-2019 (240 %), Zincol-2016 (237 %) and FSD-2008 (232 %) also showed better AE as compared to their respective controls and other two P-fertilizer levels (40 and 120 Kg P_2O_5 ha^{-1}) (Fig. 2). At 80 Kg P_2O_5 ha^{-1} a maximum value for partial nutrient budget (PNB) was recorded in Anaj-2017 (379 %) followed by FSD-2008 (385 %), Zincol-2016 (342 %) and Akbar-2019 (335 %) (Table 4).

3.6. Economic analysis

For economic analysis all the input costs excluding P-fertilizer were bought from market. Grain yield was settled to 12.50 % downward to reduce farm management losses during experimental yield and farmer's yield. The results uncovered that marginal rate of return (MRR) by P-fertilizer application was minimum in FSD-2008 (125.3 %) at 120 kg

Table 4
Partial budget analysis regarding marginal rate of return (MRR) of phosphorus fertilizer applied to wheat crop.

Treatments		Total input cost that varies (PKR ha^{-1})	Wheat grain yield (t/ha)	Grain yield increase over control (t/ha)	Gross value of total grain yield (PKR ha^{-1})	Marginal benefit over cost and control (PKR ha^{-1})	Marginal rate of return over investment (MRR) in P_2O_5 (%)
(P_2O_5 kg ha^{-1})	Variety						
0	Zincol-2016	—	2.6	—	150,800	—	—
	Akbar-2019	—	2.7	—	156,600	—	—
	FSD-2008	—	2.5	—	145,000	—	—
	Anaj-2017	—	2.4	—	139,200	—	—
40	Zincol-2016	10,800	2.9	0.3	168,200	17,400	161.1
	Akbar-2019	10,800	3.1	0.4	179,800	23,200	214.8
	FSD-2008	10,800	2.7	0.2	156,600	11,600	107.4
	Anaj-2017	10,800	2.6	0.2	150,800	11,600	107.4
80	Zincol-2016	21,600	3.4	0.8	197,200	46,400	214.8
	Akbar-2019	21,600	3.6	0.9	208,800	52,200	241.7
	FSD-2008	21,600	3.0	0.5	174,000	29,000	134.3
	Anaj-2017	21,600	3.0	0.6	174,000	34,800	161.1
120	Zincol-2016	32,400	3.7	1.1	214,600	63,800	196.9
	Akbar-2019	32,400	4.0	1.3	232,000	75,400	232.7
	FSD-2008	32,400	3.2	0.7	185,600	40,600	125.3
	Anaj-2017	32,400	3.2	0.8	185,600	46,400	143.2

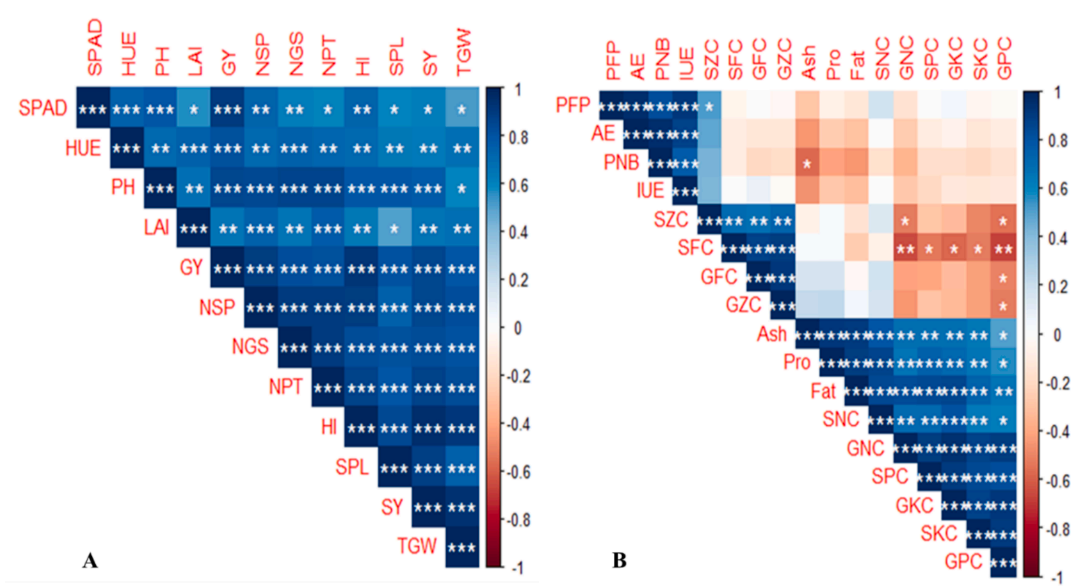

Fig. 3. Correlation among (A) growth and yield parameters, (B) ionic contents, nutritional components and phosphorus nutrient use efficiency of four wheat varieties (Zincol-2016, Akbar-2019, FSD-2008 and Anaj-2017) grown under influence of different doses of P-fertilizer (40, 80 and 120 Kg P_2O_5 ha^{-1}). Ash-ash, Pro-protein, Fat-fat, GNC-grain nitrogen content, SNC- straw nitrogen content, SKC-straw potassium content, GKC- grain potassium content, GPC-grain phosphorus content, SPC- straw phosphorus content, GFC-grain iron content, SFC-straw iron content, GZC-grain zinc content, SZC-straw zinc content, AE-agronomic efficiency, IUE- internal use efficiency, PFP- partial factor productivity, PNB- partial nutrient budget, GY-grain yield, SY-straw yield, PH-plant height, SPAD-soil plant analysis development, LAI-leaf area index, HUE-heat unit efficiency, NPT-number of productive tillers, SPL-spike length, NSP-number of spikelet's per spike, TGW-1000 grain weight, NGS-number of grains per spike, HI-harvest index.

P_2O_5 ha^{-1} while maximum in Akbar-2019 (241.7 %) at 80 kg P_2O_5 ha^{-1}. Among all wheat varieties Akbar-2019 was more efficient in utilizing phosphorus nutrient and had better marginal benefit over cost (PKR 52,200 ha^{-1}) at 80 kg P_2O_5 ha^{-1} (Table 4).

3.7. Multivariate analysis

The traits studied under P-fertilizer showed a significant correlation among different studied parameters. The pH was strongly correlated with GY, NPT, NGSA, HI, SPL, and SY, while the SPAD was correlated with HUE and PH. The HI was remarked and positively correlated with SPL, SY, and TGW. Ash, Pro, and Fat were positively correlated with SNC, GNC, SPC, GPC, SKC, and GKC, whereas PFP, AE, and PNB were correlated with IUE. A significant negative correlation was observed among GNC, SKC, GKC, SPC, SNC, PFP, PNB, and AE (Fig. 3).

3.8. Gene expression analysis

The study examined the expression profile of *PSTOL1* of two wheat varieties at 80 Kg P_2O_5 ha^{-1}. The *PSTOL1* exhibited a significant increase in expression compared to the control after application of P-fertilizer. As compared to control, 217 %, 129 %, 100 % and 93 % increase was observed in Zincol-2016, Akbar-2019, FSD-2008 and Anaj-2017 respectively (Fig. 4).

4. Discussion

Phosphorus is crucial for plant growth, playing a key role in photosynthesis, disease resistance and yield enhancement (Thiruvengadam et al., 2024). This study aimed to explore the effects of varying phosphorus (P_2O_5) fertilizer doses on wheat growth, nutritional balance and yield. Results showed that phosphorus application significantly boosted wheat height, root health and nutrient uptake leading to stronger plant growth (Karnwal et al., 2023). Moreover, increased leaf area index (LAI) and chlorophyll content (SPAD) were observed, indicating improved photosynthetic efficiency and potentially higher grain yields mirroring findings in buckwheat and maize studies (Nie et al., 2023). Heat unit efficiency (HUE) measures the conversion of light energy to dry matter is affected by environmental and soil conditions, planting patterns and sowing timing (Pieper et al., 2024). Specifically, HUE saw notable improvement in wheat varieties with 80 kg P_2O_5 ha^{-1} of phosphorus (P) fertilizer, enhancing nutrient absorption, LAI and SPAD values (George et al., 2024). Previous studies confirmed that 60 and 120 kg P_2O_5 ha^{-1} applications increased HUE in wheat and pearl millet.

The various grain yield components were positively correlated with *i. e.* seed index, number of grains spike^{-1}, number of tillers per unit land area, length of spike, grain and straw yield were positively correlated with grain yield (Liang et al., 2024). The cumulative effects of increased

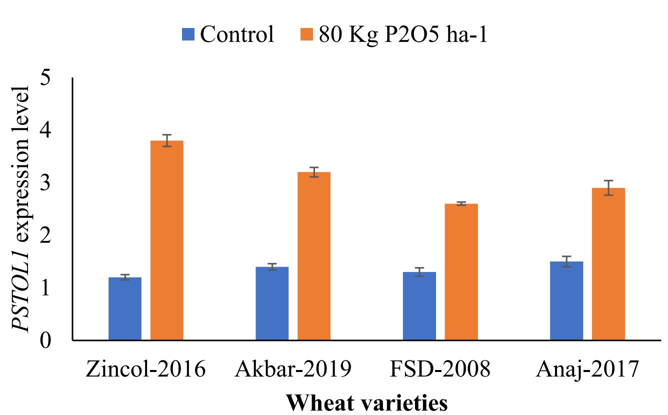

Fig. 4. Relative expression levels of *PSTOL1* in in leaves of wheat varieties after application of 80 Kg P_2O_5 ha^{-1}.

components of grain yield resulted in enhancement of yield due to P-fertilization. The results of this study also manifested a positive correlation of number of tillers m^{-2}, 1000-grain weight, number of grains spike^{-1} with grain yield (Alam *et al.,* 2000). Amongst determinants, the number of productive tillers m^{-2} are the primary ones that contributes towards grain yield of wheat and a significant correlation with grain yield (Chen et al., 2019).

P-fertilizer not only boosts nutrient (N, P, K, Fe and Zn) in wheat straw and grains, particularly at 80 kg P_2O_5 ha^{-1}, but also supports root growth for better nutrient uptake due to higher soil phosphorus availability (Abou Seeda et al., 2024). Improved root systems and P-fertilizer application (20 Kg P_2O_5 ha^{-1}) in wheat plants led to a 6 % increase in K-contents in both straw and grain by enhancing mineral nutrient absorption (Rawal et al., 2023). However, Zn and Fe levels increased only at a lower P-fertilizer rate (40 Kg P_2O_5 ha^{-1}), while higher rates (80 and 120 Kg P_2O_5 ha^{-1}) decreased these minerals. Fe uptake reduction is linked to ferrous phosphate formation in the rhizosphere and adequate soil phosphorous lowered Fe content in grains and straw by 8.70 % and 14.91 % respectively (Rawal et al., 2023). P-fertilizer application significantly boosts wheat yield, especially in P-deficient soils, by improving key factors like reproductive tillers, grains per spike, and harvest index, with increases of up to 21 % (Zhang et al., 2024). This enhancement is linked to better vegetative growth, extensive root development, and improved nutrient uptake, leading to more efficient biochemical processes in leaves and higher overall yield (Ciriello et al., 2024). Phosphorous efficiency, measured by partial factor productivity, internal use efficiency, and partial nutrient budget, peaks at moderate fertilizer levels (40 and 80 Kg P_2O_5 ha^{-1}) but declines at higher doses (120 Kg P_2O_5 ha^{-1}) (Mustaffa et al., 2024).

The partial factor productivity (PFP), which evaluates the economic return on fertilizer during a crop's growing season, can rise significantly with optimal phosphorous use (Kebede et al., 2024). The internal use efficiency (IUE) assesses a crop's ability to utilize soil resources for maximum yield, while the partial nutrient budget (PNB) considers the balance between nutrient inputs and crop outputs. Proper phosphorous management significantly contributes to economic benefits (Kaushik & Grichar, 2024). Economic analysis highlights the profitability of different strategies to boost grain yield, guiding decisions for improved outcomes (Colaço et al., 2024). The minimum rate of return measures returns per investment, showing a higher minimum run rate of 185 % with 80 kg P_2O_5 ha^{-1} fertilizer use compared to 152 % at 120 kg P_2O_5 ha^{-1}, indicating that optimal fertilizer application can significantly increase economic returns by up to 20 %. Thus, achieving a higher MRR supports adopting site-specific fertilizer recommendations for better profitability (Munnaf et al., 2024).

The *PSTOL1* gene, belonging to the receptor-like kinases (RLK) family and identified through sequencing in rice varieties, crucial for stimulating early root growth in phosphorus-deficient soils and significantly boosts grain yield (Tanaka et al., 2024). In contrast to the limited number of *PSTOL1* orthologs identified in *Sorghum bicolor* and *Zea mays* and *Triticum aestivum* exhibits a significantly greater diversity, suggesting that whole-genome duplication and natural selection have enriched the functional complexity of PSTOL1 genes in wheat compared to other species (Tanaka et al., 2024). Our study revealed that the expression levels of *PSTOL1* enhanced after application of P-fertilizer in all wheat varieties, especially in biofortified varieties. Our research indicates that the wall-associated kinases (WAKs) domain not only likely enhances root surface area and phosphorus uptake under scarcity but also underscores the pivotal role of *PSTOL1* genes in boosting phosphorus efficiency and modifying root architecture (Ma et al., 2024) thus enhancing wheat growth and yield.

5. Conclusion

The balanced and optimum application of mineral fertilizers is not only enhances growth but also crucial for yield improvement. Achieving the food nutrient security is the challenging task to addresses in meeting the food production of ~10 billion people by 2050 or even earlier to this period. The findings of this study have evidence that wheat crop maintains higher nitrogen, phosphorus, potassium, zinc and iron contents and also their total uptake by fertilizing wheat crop at 80 kg P_2O_5 ha^{-1}. The maximum yield production of wheat crop was achieved by application of 80 kg P_2O_5 ha^{-1}. The zinc biofortified wheat varieties (Zincol-2016 and Akbar-2019) provided significant in producing higher grain yield compared to non-biofortified (FSD-2008 and Anaj-2017) ones. Maximum phosphorus use efficiency indices were also achieved by fertilizing with 80 kg P_2O_5 ha^{-1} compared to higher doses to this amount. The application of 80 kg P_2O_5 ha^{-1} resulted in increased economic return rate *i.e.* marginal rate of return (MRR) as compared to unfertilized wheat crop. It is recommended that biofortified wheat variety *viz.* Zincol-2016 and Akbar-2019 may be cultivated to achieve higher grain yield, nutritional values and having better rate of economic returns.

6. Consent to participate

All authors consent to participate in the manuscript publication

7. Consent for publication

All authors approved the manuscript to be published.

Ethics approval

Not applicable.

CRediT authorship contribution statement

Muhammad Iqbal Makhdum: Writing – original draft, Methodology, Formal analysis, Conceptualization. **Muhammad Abid:** Writing – review & editing, Supervision, Conceptualization. **Rashida Hameed:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Sidra Balooch:** Software, Conceptualization. **Sibgha Noreen:** Data curation, Conceptualization. **Muhammad Salim Akhter:** Writing – review & editing, Supervision, Conceptualization. **Ummar Iqbal:** Investigation, Conceptualization. **Adeel Abbas:** Validation, Software, Conceptualization. **Mohammad Abul Farah:** Writing – review & editing, Resources, Conceptualization. **Rattandeep Singh:** Writing – review & editing, Validation, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jksus.2024.103485.

Data availability

Data will be made available on request.

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