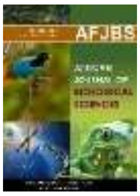
https://doi.org/10.48047/AFJBS.6.13.2024. 3587-3601

African Journal of Biological Sciences



EFFECTS OF PHOSPHORUS AND LIQUID BIOFERTILIZER ON GROWTH AND YIELD OF BLACK WHEAT (*TRITICUM AESTIVUM* L.)

Rishabh Singh, Sushila Arya[*], Tapor Pakpu, Satendra Kumar
School of Agriculture, Dev Bhoomi Uttarakhand University, Dehradun-248007, Uttarakhand, India
Corresponding address:
***Address for correspondence**
Dr. Sushila Arya
Assistant Professor, School of Agriculture, Dev Bhoomi Uttarakhand University, Dehradun-248007, Uttarakhand, India
E mail: sushilaarya.sa2626@gmail.com
Contact no.: 76177 95313, 9528660766

Article History
Volume 6, Issue 13, 2024
Received: 18June 2024
Accepted: 02July 2024
doi:*10.48047/AFJBS.6.13.2024.* 3587-3601

Abstract

Objective: This study investigates the effects of phosphorus levels (55-70 $kgha^{-1}$) and liquid biofertilizers (PSB and VAM) on the growth, yield, and economics of black wheat (*Triticum aestivum* L.).

Methods: The field experiments employed a randomized block design with 12 treatments, each replicated three times. Growth parameters and yield attributes were evaluated, and statistical analysis was conducted using ANOVA. Additionally, a comparative study of pre- and post-harvest data was performed using heat map clustering.

Results: Phosphorus at 65 $kgha^{-1}$ (P3) significantly improved plant height, dry weight, tiller number, and yield attributes. The combination of PSB + VAM (B3) resulted in the highest growth and yield, with treatment T9 (PSB + VAM + 65 $kgha^{-1}$ phosphorus) showing superior performance in tiller number, grain yield, test weight, and economic returns. **Conclusions:** Optimizing phosphorus levels and integrating PSB + VAM biofertilizers enhance black wheat productivity and economic profitability. Treatment T9 emerged as the most effective strategy, emphasizing the importance of nutrient management for sustainable crop cultivation.

Keywords: Phosphorous, Biofertilizer, Yield, growth, *Triticum aestivum* L.

1. Introduction

Black wheat (*Triticum aestivum* L.), known for its dark-colored grains, has garnered significant attention due to its high anthocyanin content and potential health benefits, including anti-inflammatory and anti-cancer properties[1]. This nutritionally enriched wheat variety offers an attractive alternative to conventional wheat, yet optimizing its cultivation requires a comprehensive understanding of its nutrient needs and the integration of sustainable practices[2].

Phosphorus (P), an essential macronutrient, plays a critical role in plant growth and development. It is a key component of ATP (adenosine triphosphate), which is vital for energy transfer during photosynthesis and respiration[3]. Adequate phosphorus levels promote extensive root growth and enhance the plant's capacity to absorb water and nutrients, thus influencing flowering, seed production, and overall crop yield and quality. However, phosphorus in the soil often becomes fixed in forms that plants cannot readily absorb, necessitating the use of fertilizers to meet crop demands[4]. While synthetic phosphorus fertilizers can boost productivity, their excessive use can cause environmental issues such as soil degradation, water pollution, and reduced biodiversity[5].

Liquid biofertilizers, containing beneficial microorganisms like nitrogen-fixing bacteria, phosphorus-solubilizing bacteria, and plant growth-promoting rhizobacteria, offer an eco-friendly alternative[6]. Phosphorus-solubilizing bacteria convert insoluble phosphorus compounds into forms that plants can readily absorb, while nitrogen-fixing bacteria transform atmospheric nitrogen into a usable form, decreasing the need for synthetic nitrogen fertilizers[7]. Plant growth-promoting rhizobacteria produce hormones and enzymes that stimulate growth and enhance stress resistance. Integrating liquid biofertilizers with conventional fertilization practices can reduce dependence on synthetic fertilizers, lower production costs, and minimize environmental impact[8]. This approach is particularly crucial for black wheat cultivation, where effective nutrient management is key to improving yield and quality.

This study explores the combined effects of varying phosphorus levels and liquid biofertilizer applications on the growth and productivity of black wheat. The study seeks to provide valuable insights into effective nutrient management strategies for black wheat, promoting sustainable agricultural practices and contributing to enhanced food security.

2. Materials and Methods

2.1. Experimental Site

The trial took place at the Crop Research Farm of Dev Bhoomi Uttarakhand University in Dehradun, Uttarakhand, in the Rabi Season of 2023–2024. Temperatures range from 35 to 39 ^{0}C in summer and drop to 0.5^{0}C in winter season. On average, the majority of the annual 1040.4 mm of rain falls between November and the end of April.

2.2. Soil

Pre-sowing soil samples were collected from 0 to 15 cm in depth using an auger. The samples were then completely mixed together to form a composite sample. The composite samples were used for the chemical analysis. The soil had a sandy loam texture with low organic carbon and moderate levels of easily accessible phosphorus, nitrogen, and potassium. The soil was moist, well drained with uniform plane topography. The soil of the experimental field was alluvial in origin, sandy loam in texture and slightly alkaline in reaction having pH 7.6 (1:2.5 soil: water suspension method given by Jackson in 1973[9], electrical conductivity 0.45 and 0.44 dSm^{-1} (1:2.5 soil: water suspension method given by Jackson in 1973[9] , Organic carbon percentage in soil is 0.24% (Walkley & Black's rapid titration method given by Walkley & Black (1934)[10] with available nitrogen 237 kgha^{-1} (Alkaline permanganate method given by Subbiah & Asija [11], available phosphorus as sodium bicarbonate-extractable P was 19.60 kgha^{-1} (Olsen's calorimetrically method, Olsen in 1954[12].

2.3. Experimental Design

The research used a random block format with 12 treatment combinations repeated three times. Treatments were randomly assigned to 36 plots in every replication. The treatments specify a combination of layout specifications and more.

2.4. Details of Treatments:

Phosphorus levels	Biofertilizers levels
P1- 55 kg Pha^{-1}	**B1**- PSB
P2- 60 kg Pha^{-1}	**B2**- VAM
P3- 65 kg Pha^{-1}	**B3**- PSB + VAM

P4- 70 kg Pha^{-1}

2.5. Harvesting and Threshing:

The crop was harvested once it reached the appropriate level of maturity as determined through visual evaluations on (20th April, 2024). In order to avoid mistakes, two border rows were eliminated on both sides of the field, along with reducing half a meter from the length of each plot. The harvest from the enclosed area was gathered for the purpose of determining yield data. Produce was gathered and measured to determine biomass yield.

2.6. Data Collection

2.6.1. Growth Attributes

2.6.1.1. Plant height (cm)

For each plot, five plants were selected at random and marked for measuring their height at different time intervals. Height was measured at 30, 60, and 90 DAS and also at harvest by using a meter scale from the ground to the top leaf pre-heading, and from the ear head base post-heading.

2.6.1.2. Leaf area index (LAI)

The leaf area index was determined by measuring the leaf area 30, 60, and 90 days post-sowing. Plants were chosen with a row length of 0.25m, and their green leaves were separated to measure surface area with an automatic leaf area meter.

$$\text{Leaf area index} = \frac{\text{Leaf area}}{\text{Ground area}}$$

2.6.1.3. Crop growth rate (g m^{-2} day^{-1})

It refers to the quantity of plant matter obtained by a designated space of a crop during a set time frame, recorded in grams per square meter per day. The crop growth rate was determined by analyzing the dry matter production data gathered for each treatment at 30, 60, and 90 DAS. The formula provided was used for the calculation.

Rishabh Singh, Sushila Arya /Afr.J.Bio.Sc. 6(13)(2024).3587-3601

$$CGR = \frac{W_2 - W_1}{t_2 - t_1}$$

2.6.1.4. Relative growth rate (g g^{-1} day^{-1})

RGE defined as the growth rate of dry weight per unit dry matter during a specific time period and it can be calculated using the equation below:

$$\text{Relative growth rate (RGR)} = \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1}$$

2.6.2. Yield attributes

2.6.2.1. Harvest index (%)

The recovery of grains in total dry matter was considered as harvest index, expressed in percentage. It has been calculated by following formula:

Harvest Index (%) = [Seed Yield (qha^{-1}) / Biological Yield (qha^{-1})] x 100

2.6.3. Economics

2.6.3.1. Cost of cultivation (□ ha^{-1})

The cost of cultivation was worked out on the basis of input rates at the farm. Treatments cost was calculated separately. The common cost of cultivation (□ ha-1) was worked out by considering all the expenses incurred in the cultivation and added variable cost due to treatments (including interest of working capital) in order to get total cost of cultivation.

2.6.3.2. Gross return (□ ha^{-1})

The overall income was determined by multiplying the crop and straw production with the prevailing market rate in various conditions. The total income (□ /ha) was calculated by adding up the earnings from both the grain and straw harvest.

Gross return (□ ha^{-1}) = Total income from the grain and straw harvest

2.6.3.3. Net return (□ ha^{-1})

Net profit is the outcome received by subtracting the cost of cultivation from gross income (□ ha^{-1}). The net return was worked out by using following formula:

Net return (□ ha^{-1}) = Gross return (□ ha^{-1}) - Cost of cultivation (□ ha^{-1})

2.6.3.4. B: C ratio (□ ha^{-1}):

$$B{:}C = \frac{\text{Net return (□ } ha^{-1})}{\text{Cost of cultivation (□ } ha^{-1})}$$

2.7. Statistical analysis

The data collected for various characteristics underwent statistical analysis using Fisher's method of analysis of variance (ANOVA). Critical difference (CD) values were calculated when the 'F' test was found significant at the 5% level. Heatmap clustering using SRPLOT (http://www.bioinformatics.com.cn/en?keywords=heatmap) was employed to visually summarize the data, highlighting key features and their interrelationships.

3. Results and Discussion

3.1. Growth attributes

It was observed that treatment B3 consistently outperformed treatments B1 and B2 across multiple growth parameters. Notably, B3, incorporating phosphorus-solubilizing bacteria (PSB) and vesicular-arbuscular mycorrhiza (VAM), demonstrated approximately 10% increases in plant height, leaf area index, number of tillers, crop growth rate (CGR), and relative growth rate (RGR) at various stages post-sowing compared to B1 and B2. Among the phosphorus treatments, T9 (65kg Pha^{-1}) exhibited superior performance, showcasing the highest plant height, leaf area index, number of tillers, and CGR and RGR at harvest. T6 (60kg Pha^{-1}) and T8 (65kg Pha^{-1}) also yielded commendable results comparable to T9. Conversely, T10 (70 kg Pha^{-1}) generally resulted in lower growth parameters relative to T9, T6, and T8 **(Table 1 and Fig. 1).** The application of phosphorus up to 65 $kgha^{-1}$ significantly enhanced vegetative growth, attributable to its role in stimulating cell division, assimilation, and photosynthesis. This nutritional enhancement, along with the benefits conferred by PSB and VAM biofertilizers, contributed synergistically to increased plant height, leaf area, tiller count, and overall growth rates. These findings underscored the beneficial impact of phosphorus management on wheat growth and development, aligning with previous research [1,4,6].

3.2. Yield Attributes

The study unveiled significant variations in yield parameters across diverse growing conditions. T9 (PSB + VAM) emerged as the top performer in several key metrics: it boasted the highest number of ears per plant (4.65), the significant seed yield (54.72 seeds per plant), the highest grain yield (39.87 qha^{-1}), and the greatest stover yield (3.25 qha^{-1}). Moreover, T9 exhibited the highest test weight (42.55 g) and achieved the highest harvest index (44.90%). In contrast, treatments T1 and T10 (PSB, VAM) consistently yielded lower across these metrics **(Table 2 and Fig. 2)**. These findings underscored the critical role of nutrient management, particularly phosphorus and biofertilizers, in augmenting plant growth, yield, and overall productivity in wheat cultivation. They corroborated earlier research findings, emphasizing the potential for optimizing agricultural practices to enhance crop yield and efficiency, thereby contributing to sustainable agriculture and food security initiatives[1,3,4,6].

3.3. Economics

Treatment T9 (65 kg ha^{-1} P + PSB+VAM) showed the highest gross return (56085 □ha^{-1}), net return (36275 □ha^{-1}) and B:C ratio (2.92), whereas treatment T1 had the lowest gross return (49690 □ha^{-1}) and net return (31774 □ha^{-1}) **(Table 3 and Fig. 3).** None the less, T6 had similar statistical results as treatment T8. The higher economic benefits observed in treatment T9 (with 60 $kgha^{-1}$ phosphorus+ PSB+VAM) could be attributed to the combination of biofertilizers, as well as half doses of organic and chemical fertilizers, leading to the highest gross and net returns.

Table 1. Effect of different treatment combination on growth parameters of wheat

Treatments	Treatment combination	Plant height (cm)	Plant dry weight(g)	No. of tiller $plant^{-1}$	Leaf area index (%)	CGR ($g\ m^{-2}\ day^{-1}$)	RGR ($g\ g^{-1}\ day^{-1}$)
T1	PSB + Phosphorus 55 kg ha^{-1}	67.56	16.76	4.42	1.22	16.36	16.36
T2	VAM + Phosphorus 55 kg ha^{-1}	70.53	17.87	4.77	1.29	17.87	17.87

T3	PSB +VAM + Phosphorus 55 kg ha^{-1}	74.89	18.98	5.06	1.38	18.98	18.98
T4	PSB + Phosphorus 60 kg ha^{-1}	69.56	17.63	4.70	1.28	17.63	17.63
T5	VAM + Phosphorus 60 kg ha^{-1}	74.63	18.91	5.04	1.37	18.91	18.91
T6	PSB +VAM + Phosphorus 60 kg ha^{-1}	78.62	19.92	5.31	1.44	19.92	19.92
T7	PSB + Phosphorus 65 kg ha^{-1}	72.70	18.42	4.91	1.33	18.42	18.42
T8	VAM + Phosphorus 65 kg ha^{-1}	77.20	19.56	5.22	1.42	19.56	19.56
T9	**PSB + VAM + Phosphorus 65 kg ha^{-1}**	**79.21**	**20.07**	**5.35**	**1.45**	**20.07**	**20.07**
T10	PSB + Phosphorus 70 kg ha^{-1}	65.28	16.54	4.41	1.20	16.54	16.54
T11	VAM +Phosphorus 70 kg ha^{-1}	72.18	18.29	4.88	1.33	18.29	18.29
T12	PSB + VAM + Phosphorus 70 kg ha^{-1}	73.33	18.58	4.95	1.35	18.58	18.58
F test		NS	NS	NS	NS	NS	NS
SEd (±)		2.95	0.75	0.20	0.05	3.73	3.73
CD (P= 0.05)		6.11	1.56	0.42	0.11	7.73	7.73

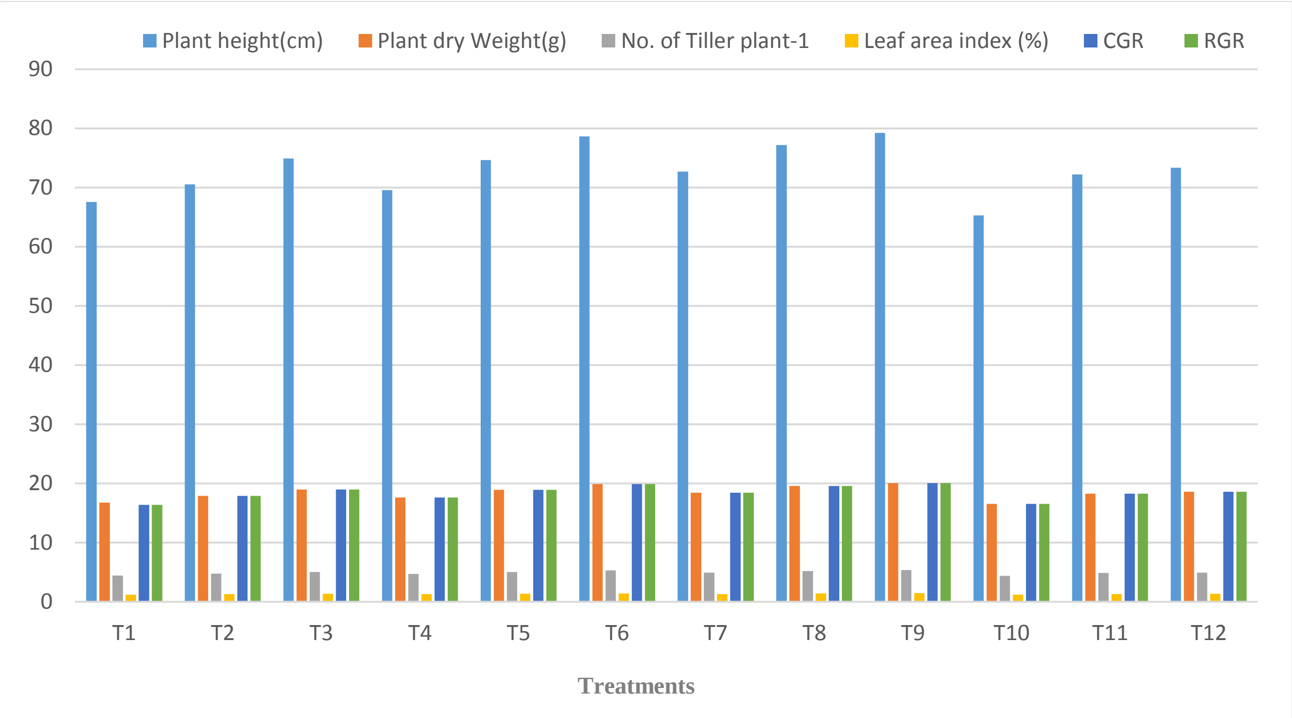


Figure 1. Effects of varied phosphorus levels and biofertilizers (PSB and VAM) on growth attributes of wheat.

Table 2. Effect of different treatment combination on yield parameters of wheat

S.No	Treatment Combination	Ear per plant	Seed per plant	Grain yield (qha 1)	Straw yield (qha^{-1})	Test weight (g)	Harvest index (%)
T1	PSB + Phosphorus 55 kg ha^{-1}	3.91	46.28	32.96	NS	37.34	44.33
T2	VAM + Phosphorus 55 kg ha^{-1}	4.14	48.73	35.50	15.2	37.88	44.88
T3	PSB +VAM + Phosphorus 55 kg ha^{-1}	4.39	51.74	37.70	31.2	40.23	44.88
T4	PSB + Phosphorus 60 kg ha^{-1}	4.08	48.06	35.02	NS	37.37	44.90
T5	VAM + Phosphorus 60 kg ha^{-1}	4.38	51.56	37.57	15.2	40.09	44.90
T6	PSB +VAM + Phosphorus 60 kg ha^{-1}	4.61	54.32	39.57	31.0	42.23	44.88
T7	PSB + Phosphorus 65 kg ha^{-1}	4.26	50.23	36.59	NS	39.05	44.87
T8	VAM + Phosphorus 65 kg ha^{-1}	4.53	53.33	38.86	15.2	41.47	44.86
T9	**PSB + VAM + Phosphorus 65 kg ha^{-1}**	**4.65**	**54.72**	**39.87**	**32.5**	**42.55**	**44.90**
T10	PSB + Phosphorus 70 kg ha^{-1}	3.83	45.10	32.86	NS	35.06	44.84
T11	VAM +Phosphorus 70 kg ha^{-1}	4.23	49.87	36.34	15.2	38.77	44.89
T12	PSB + VAM + Phosphorus 70 kg ha^{-1}	4.30	50.66	36.91	31.5	39.39	44.88
	F-test	NS	NS	NS	NS	NS	NS
	S.Ed(±)	0.17	2.03	1.52	1.82	1.66	1.75
	CD(P=0.05)	0.36	4.22	3.15	3.77	3.43	3.62

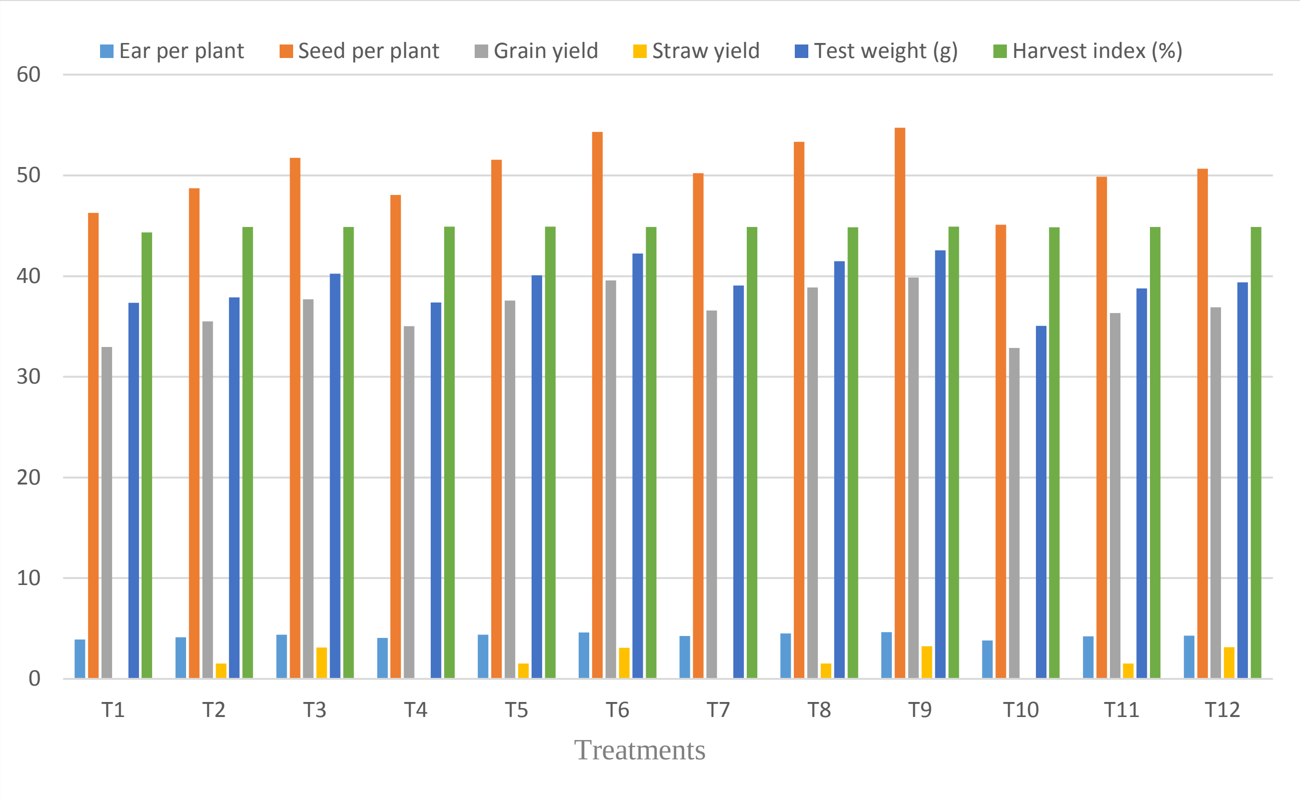


Figure 2: Effects of Varied Phosphorus Levels and Biofertilizers (VAM and PSB) on yield attributes of wheat

Table 3. Effect of different treatment combination on economics parameters of wheat

S.No	Treatment Combination	Gross return (□ ha^{-1})	Net return (□ ha^{-1})	B:C ratio
T1	PSB + Phosphorus 55 kg ha^{-1}	49690	31774	2.57
T2	VAM + Phosphorus 55 kg ha^{-1}	51940	33623	2.84
T3	PSB +VAM + Phosphorus 55 kg ha^{-1}	54230	35480	2.89
T4	PSB + Phosphorus 60 kg ha^{-1}	51530	33154	2.80
T5	VAM + Phosphorus 60 kg ha^{-1}	53780	35003	2.86
T6	PSB +VAM + Phosphorus 60 kg ha^{-1}	56070	36067	2.90
T7	PSB + Phosphorus 65 kg ha^{-1}	51955	33019	2.74
T8	VAM + Phosphorus 65 kg ha^{-1}	54205	34008	2.80

T9	**PSB + VAM + Phosphorus 65 kg ha^{-1}**	**56085**	**36275**	**2.92**
T10	PSB + Phosphorus 70 kg ha^{-1}	52430	32834	2.68
T11	VAM +Phosphorus 70 kg ha^{-1}	54608	34683	2.73
T12	PSB + VAM + Phosphorus 70 kg ha^{-1}	56020	36090	2.77

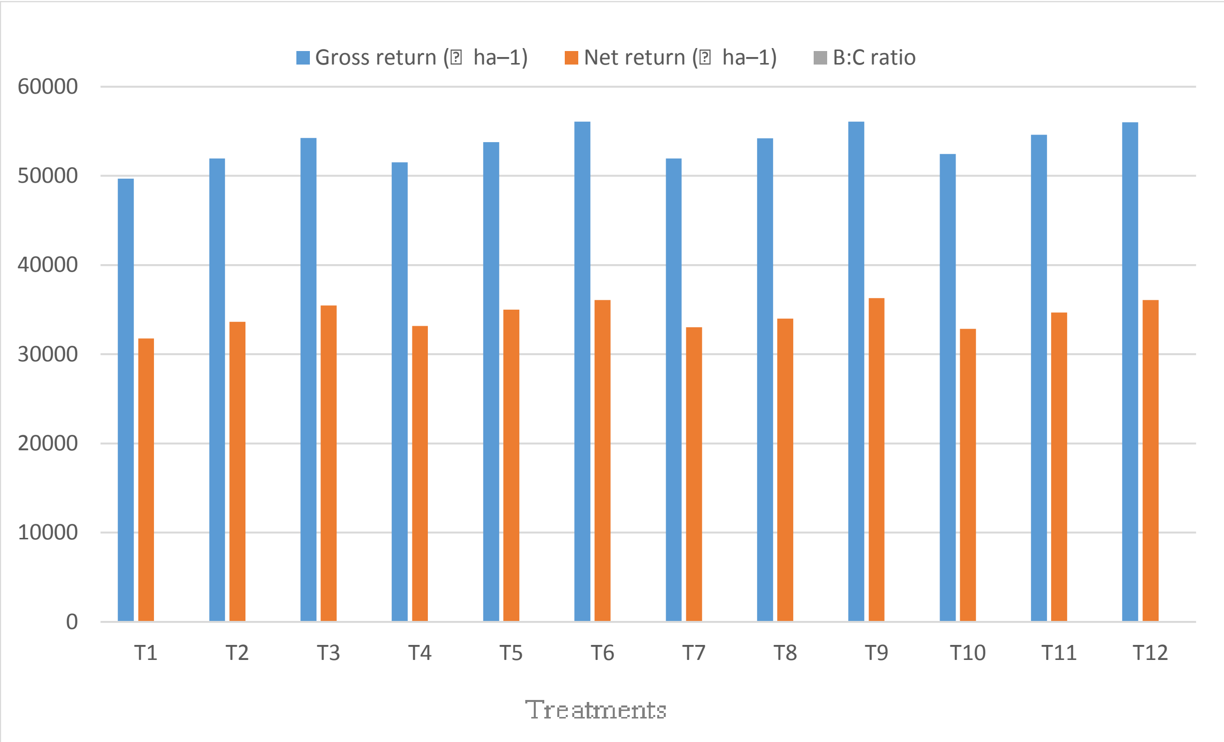


Figure 3. Effect of Different Levels of Phosphorus and Biofertilizers (PSB and VAM) on Gross return, Net return and B: C ratio

3.4. Heat map clustering

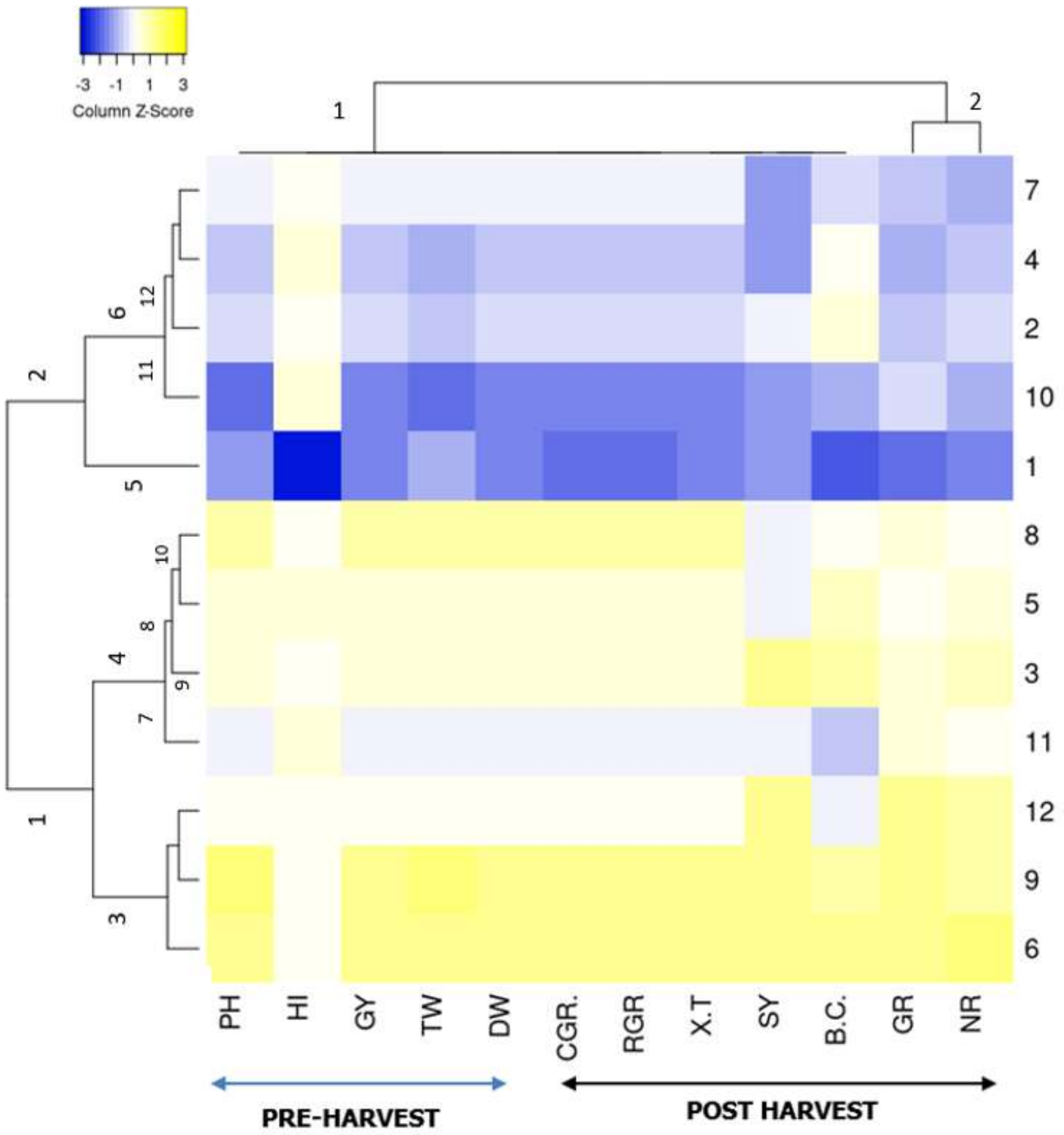


Figure 4. Clustered heat map of the effect of different treatment combination on growth, yield and economic parameters of wheat; PH= Plant height; HI= Harvest Index; GY= Grain yield; TW= Test weight; DW= Dry weight; CGR= Crop growth rate; RGR= Relative growth rate; XT= No. of tillers; SY= Straw yield; B.C= Benefit cost ratio; GR= Gross return rate; NR= Net return rate

To observe the variation in plant characteristics and treatment effects, heat map clustering was performed **(Fig. 4)**[13]. The results illustrate two distinct groups of data: pre-harvest and post-harvest. This classification is represented by two types of dendrograms: a horizontally oriented plant character dendrogram and a vertically oriented treatment dendrogram (Figure 4). In the heat map, traits are visually represented by colors, with blue indicating the maximum value and yellow the minimum. The plant character dendrogram shows two primary clusters: one containing only the traits GR and NR, and the other encompassing all remaining plant characteristics.

The treatment dendrogram, positioned vertically, distinctly separates all samples into two main clusters based on their treatments. The first main cluster further divides into two sub-clusters (Cluster 3 and Cluster 4). Similarly, the second main cluster splits into two sub-clusters (Cluster 5 and Cluster 6). Within Cluster 3, there are two sub-groups: one with a single treatment (Treatment 6) and another with two treatments (Treatments 9 and 12). Cluster 4 also divides into two sub-clusters (Cluster 7 and Cluster 8). Cluster 7 includes only Treatment 11. Cluster 8 splits again into two sub-clusters (Cluster 9 and Cluster 10), where Cluster 9 contains only Treatment 3, while Cluster 10 includes Treatments 5 and 8. In Cluster 5, there is only one treatment (Treatment 1). Cluster 6 divides into two sub-clusters (Cluster 11 and Cluster 12): Cluster 11 contains only Treatment 10, whereas Cluster 12 further subdivides into two groups, with one sub-cluster containing only Treatment 2, and the other including Treatments 4 and 7.

The analysis of heat maps and dendrograms offers valuable insights into the relationships between plant characteristics and treatment effects. The plant character dendrogram groups traits like Growth Rate (GR) and Nutrient Retention (NR), highlighting their similarities and importance for plant health and growth. The treatment dendrogram clusters treatments based on their effects, identifying the most effective and redundant options, which aids in optimizing agricultural practices and reducing costs. Temporal data comparison, distinguishing pre-harvest from post-harvest, provides a deeper understanding of how traits evolve over time, guiding the timing of interventions for maximum benefit. By revealing which traits are influenced similarly by various treatments, we can pinpoint key genetic or physiological pathways, aiding in breeding for desirable traits and stress resistance. This precise visualization simplifies complex data, enabling farmers, agronomists, and researchers to make informed decisions that promote sustainable and effective agricultural practices.

4. Conclusion

This study concluded that applying PSB + VAM + 65 $kgha^{-1}$ of phosphorus along with liquid biofertilizers PSB + VAM + phosphorus significantly improved all growth and yield attributes of black wheat. Treatment T9 (PSB + VAM + 65 $kgha^{-1}$ phosphorus) consistently showed the highest number of tillers per plant, grain and test weight, seed yield, and straw yield. It also achieved the maximum gross return. These results suggest that cultivating black wheat with PSB + VAM + phosphorus biofertilizers is optimal under Western Uttar Pradesh and Dehradun, Uttarakhandagro-ecological conditions. The study highlights the pivotal role of phosphorus levels and biofertilizer integration in enhancing black wheat productivity and quality. Treatment T9 stood out across various parameters such as plant height, dry weight, and harvest index, indicating its potential to boost agronomic efficiency and profitability. Future research could delve deeper into nutrient uptake mechanisms in black wheat, further refining nutrient management strategies for better crop production outcomes.

5. Acknowledgement

The authors are thankful to Department of Agronomy, Dev Bhoomi Campus, Chakrata Road, Manduwala, Naugaon, Uttarakhand 248007**.** India for providing us necessary facilities to undertake the studies.

6. References

1. Nguyen DH, El-Ramady H, Llanaj X, Törős G, Hajdú P, Prokisch J. Chemical composition and health attributes of agri-foods: A scientific overview on black foods. Sustainability. 2023; 15(4): 3852.
2. Rebouh NY, Khugaev CV, Utkina AO, Isaev KV, Mohamed ES, Kucher DE. Contribution of eco-friendly agricultural practices in improving and stabilizing wheat crop yield. A review. Agronomy. 2023; 13(9), 2400.
3. Khan F. Siddique AB, Shabala S, Zhou M, Zhao C. Phosphorus plays key roles in regulating plants physiological responses to abiotic stresses. Plants. 2023; 12(15): 2861.
4. Weeks J, Hettiarachchi GM. A review of the latest in phosphorus fertilizer technology: possibilities and pragmatism. J. Environ. Quality. 2019; 48(5):1300-1313.

5. Tripathi S, Srivastava P, Devi RS, Bhadouria R. Influence of synthetic fertilizers and pesticides on soil health and soil microbiology. In Agrochemicals detection, treatment and remediation. 2020; 25-54.
6. Kumar S, Sindhu SS, Kumar R. Biofertilizers: An ecofriendly technology for nutrient recycling and environmental sustainability. Curr. Res. Microb. Sci. 2022; 3: 100094.
7. Sendhil R, Kiran Kumar TM, Singh GP. Wheat Production in India: Trends and Prospects. Recent Advances in Grain Crop. 2019.
8. Styles D, Adams P, Thelin G, Vaneeckhaute C. Chadwick D. Withers PJ. Life cycle assessment of biofertilizer production and use compared with conventional liquid digestate management. Environ. Sci. Tech. 2018; *52*(13): 7468-7476.
9. Jackson ML. Soil chemical analysis. New Delhi: Prentice Hall of India Pvt. Ltd, 1973.
10. Walkley A, Black IA. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil science. 1934; 37(1): 29-38.
11. Subbiah BV, Asija CL. A rapid procedure for the estimation of available N in Soil. Curr Sci. 1956; 25:259-60.
12. Olsen SR, Cole CV, Watanable FS, Dean LA. Estimation of available phosphorous in soil by extraction with sodium bicarbonate. USDA Cric. (1954); 930:19-23.
13. Arya S, Kumar R. Prakash O, Kumar S, Mahawer SK, Chamoli S, de Oliveira MS. Chemical composition and biological activities of *Hedychium coccineum* Buch.- Ham. ex Sm. essential oils from Kumaun hills of Uttarakhand. Molecules, 2022; 27(15): 4833.