



## Vigor and Viability of Harvested Barley Seeds as Affected by Phosphorus Levels and Nitrogen Fixing Bacteria

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### ABSTRACT

Out of three macro nutrients, phosphorus (P) is one of the main nutrients and necessary for plant growth. N<sub>2</sub> fixing bacteria are microorganisms capable of transforming atmospheric nitrogen into fixed nitrogen. The presented research was conducted to estimate the effect of phosphorus levels and nitrogen fixing bacteria on the vigor and viability of harvested barley seeds at Agronomy Research Laboratory, The University of Agriculture Peshawar during Rabi season 2021-2022. The experiment was laid out in randomized complete block design having four replications. The experiment is consisted of two-factors in which one factor was nitrogen fixing bacteria (with and without) and the other was phosphorus levels (40, 60 and 80 kg ha<sup>-1</sup>) with control. Analysis of variance revealed significant differences among all parameters and interaction. Based on experimental results phosphorus applied at the rate of 80 kg ha<sup>-1</sup> significantly increased germination (86.25 %), germination rate (6.16), seedling length (21.82 cm) and root fresh weight (0.04 g). The application of nitrogen fixing bacteria shows positive response in all the parameters. N<sub>2</sub> fixing bacteria significantly increased germination (86.50 %), germination rate (6.17), seedling length (20.60 cm) and root fresh weight (0.04 g). Interaction between phosphorus level and nitrogen fixing bacteria was found significant for seedling length and shoot fresh weight. It was concluded that phosphorus applied at the rate of 80 kg ha<sup>-1</sup> with nitrogen fixing bacteria is recommended for increasing vigor and viability of harvested barley seeds.

## INTRODUCTION

Barley (*Hordeum vulgare* L.) is a member of the grass family. It is a self-pollinating, diploid species (2n) with 14 chromosomes. It is an important cereal crop in the world ranking next to maize, wheat and rice. It is one of the earliest domesticated food crops since the start of civilization. Barley is an important cereal in winter after wheat in both area and production. Due to its very hard nature, barley is successfully cultivated in adverse environments like drought, salinity, alkalinity etc. in varied topographical conditions like plains and hilly areas under rainfed and irrigated conditions. Barley is generally considered as a poor man's crop because of its input requirement and better adaptability to harsh environments. (Kumar et. al., 2012). It is originated in Ethiopia and South Asia while it is domesticated in Nile River Valley of Egypt about 17,000 years ago. Barley is being used as model crop for many disciplines like virology, pathology and genetics (Reddy et. al., 2006). In connection with uses and nutritional value, barley grain is used primarily as a malt source for alcoholic beverages, especially in the beer industry. Also, barley is used in bread, soups, stews, and health products since the barley grain is rich in several health-boosting components. As such, barley is high in protein, fiber, vitamins and natural bioactive antioxidants such as phenolic and lipids. (Badea and Wijekoon, 2021) Barley grain contains 12.1% albumins, 8.4% globulins, 25% prolamins and 54.5% glutelins. The content of each fraction has a significant influence on the technological, nutritional and, indirectly, the hygienic quality of grain (Kuktaite, 2004). Barley is also used as feed in ruminant, poultry, and aquaculture production. Compared to feed grain corn, besides offering greater protein, barley grain is also richer in methionine, lysine, cysteine, and tryptophan. (Nikkhah, A.,2012). Barley production traditionally has been important in the world. The annual world harvest of barley in the late century was approximately 140 million tons from about 55 million hectares. It is very versatile in every way and has been well adapted through its evolution. In fact, it is the most adaptable of the cereals. Much of the world's barley is produced outside of the regions where cereals such as maize and rice can grow well. The total area harvested each year is around 50 - 80 million ha and ranked 4th important cereal crop after wheat (200 million ha), rice (120 -150 million ha) and corn (100 -150 million ha). (Zhou, 2009) In Pakistan barley crop occupied about 86 thousand hectares area, the production of which was 81.5 thousand tones and the average yield was 948 kg ha<sup>-1</sup>. While in Khyber Pakhtunkhwa, it occupied 30.5 thousand hectares area, the production was 27.5 tones and the average yield was 902 kg ha<sup>-1</sup> (MNFS & R, 2012).

Phosphorus is key element for increasing yield of field crops. The main problem associated with the use of phosphorus fertilizers is their low availability primarily due to immobilization and fixation in soil and therefore, the crops utilize only 10-25 per cent of applied phosphorus. The availability of phosphorus in soil is very critical as a portion of soil phosphorus is found in inorganic form such as phytin, phospholipids and nucleoproteins, which can be available to plant only after its mineralization by soil microorganisms (Singh et al., 2020). Low availability of phosphorus (P) limits crop production worldwide. Increasing seed P content can improve plant establishment and increase yields.

This is thought to be a consequence of faster initial root growth, which gives seedlings earlier access to growth-limiting resources, such as water and mineral elements. It can be calculated that seed P reserves can sustain maximal growth of cereal seedlings for several weeks after germination, until the plant has three or more leaves and an extensive root system (White and Veneklaas, 2012).

The PGPR may promote the plant growth either by using their own metabolism (solubilizing phosphates, producing hormones or fixing nitrogen) or directly affecting the plant metabolism (increasing the uptake of water and minerals), enhancing root development, increasing the enzymatic activity of the plant or “helping” other beneficial microorganisms to enhance their action on the plants or may promote the plant growth by suppressing plant pathogens (Montaño, F. P, 2014). *Azotobacter* sp, *Azospirillum* sp, blue green algae, *Azolla*, *Rhizobium* sp are few of the commonly used as nutrient solubilizing microorganisms which are also used as bio fertilizers (Selvakumar et al, 2009). The genus *Azotobacter*, belonging to family *Azotobacteriaceae*, represents the main group of heterotrophic non-symbiotic nitrogen- fixing bacteria principally inhabiting neutral or alkaline soils (Sartaj et al., 2013). The non-symbiotic free living *azotobacter* is largely associated with nitrogen fixation in plant rhizosphere (Lakshminarayana et al, 2000). Vessey (2003) reported *Azotobacter* sp. And *Azospirillum* sp. are the most commonly used bio fertilizers in different agricultural crops either as combined or as single inoculation. The estimated contribution of these free-living nitrogen fixing bacteria to the nitrogen input of soil ranges from 0–60kg/ha per year. Studies have shown that inoculation of maize crop with *Azotobacter* significantly increased its plant height, grain weight and grain yield. Inoculation of *Azotobacter* increased grain yield in maize up to 35% over the non- inoculated treatment (Bandhu and Parbati, 2013). Nitrogen fixation process is highly sensitive to O<sub>2</sub>, but *azotobacter* sp. have special mechanism of oxidases and catalases to reduce the concentration of O<sub>2</sub> in the cells (Shank et al., 2005). *Azotobacter* species have two types of nitrogenases viz., molybdenum–iron nitrogenase, vanadium–iron nitrogenase (Neeru et al.,2000).

*Azospirillum* spp. is one of the most important genera of plant growth-promoting rhizobacteria which forms the associative symbiosis with the plant roots. *Acetobacter diazotrophicus*, *Herbaspirillum seropedicae*, *Azoarcus* spp. and *Azotobacter* spp. are other free-living diazotrophs which can be found in association with plant roots. *Azospirillum* spp. also helps in the biosynthesis of plant growth hormones. *Azospirillum* was isolated from the rhizosphere of many grasses and cereals all over the world, in tropical as well as in temperate climates (Dobereiner and Day, 1976); Patriquin et al.,1983). *Azospirillum* has very positive results both under the field conditions and greenhouse conditions. *Azospirillum* shows the very stimulatory effect on plant growth through biological nitrogen fixation and auxin production. There is an alteration in the root morphology observed under the inoculation of *Azospirillum*. The number of lateral roots has been increased and enlargement of the root hairs takes place. These changes in the root morphology help in the absorption of nutrients and water from the lower layers of the soil which results in improved growth of the

plants (Yemen and Mehta, 2019). Keeping the above points in view, an investigation was conducted to check the effect of different Phosphorus levels and nitrogen fixing bacteria on the vigor and viability of harvested barley seeds.

## LITERATURE REVIEW

Marinho et al. (2022) conducted an experiment to see the effect of mineral nutrients in the composition of seeds, influencing the germination rate and the production of vigorous seedlings, which is fundamental for the establishment of the plant stand and crop yield. This work aimed to evaluate the effect of phosphate fertilization on phosphorus (P) content and the physiological quality of seeds of wheat cultivars. The evaluation includes: first count, germination, seedling emergence and length, P content in seeds, and thousand-seed weight. The data of P rates and wheat genotypes were subject to analysis of variance and to the comparison of means by the F-test and Scott-Knott test, respectively. Phosphate fertilization provides the production of wheat seeds with higher P content and physiological quality, but it does not influence the thousand-seed weight of most wheat cultivars.

Divyanshu et al. (2022) conducted an investigation for the screening of potent PGPR strains for enhancing seed germination and vigor index of *Hordeum vulgare* (commonly called barley). Rhizobacterial strains were isolated and screened for various plant growth promoting traits, their effect on seed germination and vigor index of barley plant through pot trial, and resistant ability under various temperature and pH range. Phosphate solubilization, ammonia production, catalase activity, siderophore production and MR-VP test. Barley plants treated with P. showed highest seed germination respectively in comparison to the control plant. The above increase in fold in vigor index and seed germination is much higher as compared to earlier reports. Collectively, the data underpin that addition of these PGPRs to barley rhizosphere appears a promising strategy to enhance root and shoot biomass of this important agriculture crop.

Munareto et al. (2018) conducted research on seed treatment that helps the initial establishment of the crop without the effects caused by pests and diseases. The association of diazotrophic bacteria with grasses has been used in the supply of nitrogen to plants; however, these microorganisms produce growth-promoting substances, which promote benefits in the growth and development of the crops. Thus, this study was to evaluate the compatibility of *Azospirillum brasilense* associated with the fungicide difenoconazole and the insecticide thiamethoxam by observing the effects on the quality of seed emergence of three wheat cultivars (*Triticum aestivum* L.). Three wheat cultivars, were tested. Bacteria from the *Azospirillum* genus were used in the inoculation. The wheat seed retains its quality when it checks the germination, vigor and independent accelerated aging, whether or not fungicide, insecticide and *A. brasilense* were used. The insecticide thiamethoxam increased the length of shoots and roots and provided compatibility with *A. brasilense*, and fungicide inhibited the length of shoots and roots and was antagonistic to the bacterium *A. brasilense*.

Naseer and Asim. (2018) conducted an experiment at Agronomy laboratory, The University of Agriculture, Peshawar. To study the effect of different nitrogen fixing microbes with nitrogen ratio on vigor and stand establishment of harvested wheat seeds. Two wheat varieties i.e., Pirsabak-2013 and Shahrkar 2013 were sown in Agronomy Research Farm of The University of Agriculture Peshawar. Wheat seeds were pretreated with nitrogen fixing microbes (azospirillum and azotobacter) and various nitrogen ratios (25%, 50%, 75% and 100%) were applied to the seed bed. After harvesting wheat seeds in June, 2015 were subjected to different vigor and viability tests. Nitrogen fixing microbes with nitrogen ratios increased water imbibition (89.1%), growth rate (90.1%), germination (88.4%), seed vigor index (89.2%), mean germination time (88.4%), shoot dry weight (89.4%), root dry weight (90.7%), seedling dry weight (89.9%), shoot length (88.6%), seedling length (88.3%), relative water content (92.2%), root/shoot dry weight ratio (89.9%) as compared to control wheat varieties. Azotobacter with 50% nitrogen showed highest germination percentage (95.7%), growth rate (13) and mean germination time (6.6). Azospirillum with 25 % nitrogen increased seed vigor index (340.7), mean germination time (6.6), longest shoots (3.3cm), lengthy roots (3.6cm), seedling length (6.9cm), shoot dry weight (1g), root dry weight (1.7g) and seedling dry weight (3.6g). Alone nitrogen and alone nitrogen fixing microbes did not increase vigor and viability of wheat varieties. Maximum vigor and viability were observed in Pirsabak-2013 as compared to Shahrkar- 2013. It is concluded that Azospirillum with 25% nitrogen increased vigor and stand establishment in Pirsabak-2013 wheat variety.

Mašauskas et al. (2008) conducted two experiments with malting barley (*Hordeum vulgare* L.) cultivars in 2003 and 2004 in central, western and southern Lithuania to test phosphorus (P) availability at early seedling growth stage with P seed coating. Phosphorus seed coating resulted in alteration to plant stand structure traits. Despite the fact that seedling emergence sometimes decreased, the number of total and productive stems, number of grains per ear and 1000-grain weight increased. The positive effect of P seed coating on grain yield was revealed when the growth conditions during post-anthesis were favorable for exploiting the potential that was obtained during the pre-anthesis phase. In experiment favorable conditions were considered those that generated a grain yield over 6 t ha<sup>-1</sup>. According to analysis the significant positive effect of P seed coating on malting barley yield increase was related to seed weight increase.

## METHODOLOGY

The experiment was carried out in Agronomy Research Laboratory, The University of Agriculture Peshawar during Rabi season 2021-2022. The experiment was designed in a randomized complete block design having four replications. Materials that used in this research were four replications of barley seeds grown under two-factors in which one factor was beneficial microbes (with and without) and the other was phosphorus levels (40, 60 and 80 kg ha<sup>-1</sup>) with control. The harvested barley was subjected to test for the vigor and viability test. The following factors were studied in the experiments:

**Factor A: Phosphorus level (kg ha<sup>-1</sup>)**

P0	=	0
P1	=	40
P2	=	60
P3	=	80

**Factor B: Beneficial Microbes (BM)**

BM	=	With
BM	=	Without

Data was recorded based on the following parameters:

1. Germination percentage (%)
2. Germination rate
3. Seedling length (cm)
4. Root fresh weight (g)

**Standard Germination Test**

Standard germination test was conducted for seeds as described in (ISTA, 1999), Four replicates × 50 seed of each line were tested. Wet filter paper was used as germination medium for barley seeds. Number of normal seedlings was estimated after a 7-day incubation period at 25°C.

**Germination Percentage (%)**

To calculate germination percentage the formula is, divide the number of healthy seedlings by the total number of seeds in the test and multiply by 100.

$$\text{Germination \%} = \frac{\text{Total sprouted seeds}}{\text{Total seeds}} \times 100$$

**Germination Rate**

The total number of seeds germinated as per total number of days for germination.

$$\text{Germination Rate} = \frac{\text{Total number of seeds sprouted}}{\text{Total days for germination}}$$

**Seedling Length (Cm)**

Five seedlings were selected from each treatment to measure the seedling length with measuring tape and their averages were recorded for statistical analysis.

**Root Fresh Weight (G)**

After incubated for 7 days, 5 seedlings from each treatment were selected to measure the root fresh weight for this purpose at first seedling fresh weight is measured and then the selected seedlings are separated into root then fresh weight of root is taken.

**Statistical Analysis**

Data were analyzed statistically according to the procedure relevant to randomized complete block design design in MS Excel. Least significance difference test was used (Jan et al., 2009).

## **RESULT AND DISCUSSION**

### **Germination Percentage (%)**

Data regarding germination percentage of barley are given in Table 1. Statistical analysis of the data revealed that phosphorus level (P level) and beneficial microbes (BM) significantly affected germination percentage of barley. The interaction was non-significant for germination percentage of barley. Phosphorus when applied at the rate of 80 kg ha<sup>-1</sup> and 60 kg ha<sup>-1</sup> produced higher germination percentage (86.25 and 84.87 %, respectively). Phosphorus at the rate of 0 kg ha<sup>-1</sup> and 40 kg ha<sup>-1</sup> produced comparatively lower germination percentage (81.37 and 84.37 %) respectively. The germination percentage (86.50 %) was higher under with beneficial microbes conditions as compared to without beneficial microbes conditions (81.93%). The results are supported by Lott et al. (2000) who reported that the sufficient store of phosphorus in seeds is vital for seed germination and successful seedling growth. The results are supported by Chandra et al. (2015) who reported that the application of nitrogen fixing bacteria helps to increase the synthesis of hormones like gibberellins, which would have triggered the activity of specific enzymes that promoted early germination.

### **Germination Rate**

Data regarding germination rate of barley are given in Table 2. Statistical analysis of the data revealed that phosphorus level (P level) and beneficial microbes (BM) significantly affected germination rate of barley. The interaction was non-significant for germination rate of barley. Phosphorus when applied at the rate of 80 kg ha<sup>-1</sup> and 60 kg ha<sup>-1</sup> produced higher germination rate (6.16 and 6.06, respectively). However, Phosphorus at the rate of 0 kg ha<sup>-1</sup> and 40 kg ha<sup>-1</sup> produced comparatively lower germination rate (5.81 and 6.02, respectively). The germination rate (6.17) was higher under beneficial microbes' conditions as compared to without beneficial microbes' conditions (5.85). The results are supported by Scott et al. (1985) who reported that the early vigor of barley seedlings was ensured by promotion of the formation of photosynthetic pigments and accordingly, higher rates of photosynthesis. The results are supported by Bákonyi et al. (2013) The possible mechanism of PGPB on the germination process is that these useful bacteria can excrete phytohormones such as auxins and gibberellins, etc., thereby improving seed germination and early development.

Table 1. Effect of Phosphorous Levels and Nitrogen Fixing Bacteria on Germination Percentage (%) of Barley

P (kg ha <sup>-1</sup> )	Beneficial microbes		Mean
	With	Without	
0	85.250	77.500	81.375 b
40	85.500	83.250	84.375 a
60	86.000	83.250	84.875 a
80	89.250	83.750	86.250 a
Mean	86.500a	81.938b	

LSD value ( $P \leq 0.05$ ) for Phosphorus level (P) = 2.71LSD value ( $P \leq 0.05$ ) for Beneficial Microbes (Bm) = 1.92

Interaction of P x Bm = NS

Means of the same category followed by different lettering are significantly different.

Table 2. Effect of Phosphorous Levels and Nitrogen Fixing Bacteria on Germination Rate of Barley.

P (kg ha <sup>-1</sup> )	Beneficial microbes		Mean
	With	Without	
0	6.089	5.536	5.813 b
40	6.107	5.942	6.027 a
60	6.143	5.946	6.063 a
80	6.375	5.986	6.161 a
Mean	6.179 a	5.853 b	

LSD value ( $P \leq 0.05$ ) for Phosphorus level (P) = 0.19LSD value ( $P \leq 0.05$ ) for Beneficial Microbes (Bm) = 0.13

Interaction of P x Bm = NS

Means of the same category followed by different lettering are significantly different.

### Seedling Length (Cm)

Data regarding seedling length of barley are given in Table 3. Statistical analysis of the data revealed that phosphorus level (P level) and beneficial microbes (BM) non-significantly affected seedling length of barley. The interaction was significant for seedling length of barley. Phosphorus when applied at the rate of 80 kg ha<sup>-1</sup> and 60 kg ha<sup>-1</sup> produced higher seedling length (21.82 and 20.88 cm, respectively). However, Phosphorus applied at the rate of 0 kg ha<sup>-1</sup> and 40 kg ha<sup>-1</sup> produced comparatively lower seedling length (19.36

and 19.80 cm, respectively). The seedling length (20.60 cm) was higher under with beneficial microbes conditions as compared to without beneficial microbes conditions (20.33 cm). The results are supported by White and Veneklaas. (2012). Furthermore, our results are supported by Beck and Ziegler. (1989) who reported that increases of seed germination percentage and seedling shoot length were considered typical gibberellins-like responses. They mimic the effect of exogenous GA3 application. Initially, inoculated plants showed higher emergence which might be due to the production of phytohormone as phytohormone influences seed germination.

### Root Fresh Weight (G)

Data regarding root fresh weight of barley are given in Table 4. Statistical analysis of the data revealed that phosphorus level (P level) was non-significant while beneficial microbes (BM) significantly affected root fresh weight of barley. The interaction was found non-significant for root fresh weight of barley. Phosphorus at the rate of 80 kg ha<sup>-1</sup> and 60 kg ha<sup>-1</sup> produced higher seedling fresh weight (0.04 and 0.04 g, respectively). However, Phosphorus at the rate of 0 kg ha<sup>-1</sup> and 40 kg ha<sup>-1</sup> produced comparatively lower root fresh weight (0.03 and 0.03 g, respectively). The root fresh weight (0.04 g) was higher under beneficial microbes' conditions as compared to with without beneficial microbes' conditions (0.03 g). Phosphorous application and nitrogen fixing bacteria had significant effect the root fresh weight of harvested barley seeds. The results are supported by Zhu and Smith (2012), when studying the influence of seed P content on wheat plant growth and development, found that increasing the P reserve in the seed improved plant growth. In The results are supported by Turan et al. (2012) Such an improvement might be attributed to N<sub>2</sub>-fixing and phosphate solubilizing capacity of bacteria as well as the ability of these microorganisms to produce growth promoting substances such as IAA.

Table 3. Effect of Phosphorous Levels and Nitrogen Fixing Bacteria on Seedling Length (Cm) of Barley.

P (kg ha <sup>-1</sup> )	Beneficial microbes		Mean
	With	Without	
0	23.425	15.300	19.363
40	18.075	21.525	19.800
60	20.300	21.475	20.888
80	20.625	23.025	21.825
Mean	20.606	20.331	

LSD value ( $P \leq 0.05$ ) for Phosphorus level (P) = NS

LSD value ( $P \leq 0.05$ ) for Beneficial Microbes (Bm) = NS

Interaction of P x Bm = 4.41

Means of the same category followed by same lettering are non-significantly different.

Table 4. Effect of Phosphorous Levels and Nitrogen Fixing Bacteria on Root Fresh Weight (G) of Barley.

P (kg ha <sup>-1</sup> )	Beneficial microbes		Mean
	With	Without	
0	0.047	0.029	0.035
40	0.048	0.042	0.038
60	0.048	0.040	0.044
80	0.045	0.032	0.045
Mean	0.047a	0.036b	

LSD value ( $P \leq 0.05$ ) for Phosphorus level (P) = NS

LSD value ( $P \leq 0.05$ ) for Beneficial Microbes (Bm) = 7.96

Interaction of P x Bm = NS

Means of the same category followed by different lettering are significantly different.

## CONCLUSIONS AND RECOMMENDATIONS

It is concluded from the results phosphorus application of 80 kg ha<sup>-1</sup> increased vigour and viability of harvested barley seeds as compared to control. In case of nitrogen fixing bacteria application, the nitrogen fixing bacteria condition enhanced overall vigor and viability of harvested barley seeds. It is recommended that phosphorus application at the rate of 80 kg ha<sup>-1</sup> with nitrogen fixing bacteria application is recommended for increasing vigor and viability of harvested barley seeds.

## FURTHER RESEARCH

To bolster the robustness of the research findings on “Vigor and Viability of Harvested Barley Seeds as Affected by Phosphorus Levels and Nitrogen-Fixing Bacteria” it is imperative to address the current study's limitations. Additional research endeavors will not only enhance the reliability of this investigation but also offer readers greater insights into the subject matter.

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