RESEARCH

Comparative Study on the Seed Health of Five Commonly Cultivated Wheat Varieties (*Triticum aestivum* L.) in Nepal

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Abstract

Seed-borne pathogens can negatively affect wheat crop germination, plant health, and yield, making it essential to routinely test and treat seeds. Therefore, identifying seed-borne pathogens in commonly cultivated wheat varieties is vital to ensuring sustainable food production. The study sought at the Central Agriculture Laboratory in Lalitpur, Nepal, aimed to identify seed-borne pathogens in five commonly cultivated wheat varieties and evaluate their seed health. The study utilized the Standard Blotter Method to assess various parameters, including germination percentage, pathogen incidence percentage, shoot length, and seedling vigor index, in a controlled environment. The experiment used a complete randomized design with four replications and five treatments. Five wheat varieties (Gautam, Aaditya, Bijaya, Dhaulagiri, and NL971) were sown in Petri dishes containing blotting paper wetted with sterilized distilled water to assess the incidence and severity of *Bipolaris sorokiniana*. The data obtained were tabulated in Microsoft Excel and analyzed using Gen Stat. The study found that Gautam had the highest *Bipolaris* infection (18.25%), while NL971 had the lowest (11.25%), followed by Bijaya, Dhaulagiri, and Aaditya. Dhaulagiri had the highest germination percentage (99.50%), followed by Aaditya, Bijaya, and NL971, while Gautam had the lowest (79%). Aaditya demonstrated the highest shoot and root weight, shoot length, and seedling vigor index, while Gautam had the lowest. The study concluded that Gautam was vulnerable to low seed health, while Aaditya and other varieties demonstrated stronger seed health and resistance to the pathogen. These findings are crucial for improving seed health and ensuring sustainable food production in Nepal.

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Statement of Sustainability: This comparative analysis of the seed health of five widely grown wheat cultivars in Nepal contributes to the Sustainable Development Goals (SDGs). Firstly, by evaluating the health of wheat seeds, we support SDG 2 (Zero Hunger) by enhancing agricultural yield and ensuring food security. Secondly, the research contributes to SDG 3 (Good Health and Well-being) by revealing important information about diseases that can affect human health and are transmitted through seeds. Additionally, the research examines the sustainability of wheat farming and helps to preserve biodiversity, it is consistent with SDG 15 (Life on Land).

1. Introduction

Wheat (*Triticum aestivum* L.) is a grass that is frequently grown for its seed, known as a caryopsis and belongs to the family Poaceae and tribe Hordeae (Ashraf et al., 2022; Liu et al., 2022). Wheat has 11.50% protein, 59.40% carbohydrate, 9.10% fat, 10.60% crude fiber, and 1.80% ash per 100 g of edible wheat, providing the human diet with the necessary amino acids, minerals, vitamins, advantageous phytochemicals, and dietary fiber (Jama et al., 2018; Shewry et al., 2020; Xu et al., 2021). Mid-November is the ideal time to sow wheat, as the weather conditions are highly conducive to the growth of this crop (Hasanah et al., 2019; Thapa et al., 2020; Dubey et al., 2020; Daloz et al., 2021). The fundamental cause of the demand-supply imbalance is population expansion. The population is projected to double by 2050, necessitating an increase in food production to feed the expanding population.

Pathogens that survive and act as principal sources of infection in following generations may be carried by seeds as passive carriers. These contaminated seeds have the potential to induce seed abortion, rot, necrosis, poor germination, and seedling damage, which may lead to disease development later in plant growth through systemic or local infections (Adhikari et al., 2016). Seed health is critical for ecological agricultural production, ensuring normal plant stand in the field, and preventing plant pests and pathogens from affecting seed quality, germination, and seedling vigor (Amza, 2018; Saeed et al., 2020). Numerous plant pathogenic fungi, viruses, microbes, nematodes, and additional angiosperms pests are transported in seeds. The majority of the time, seeds with an infection display a variety of symptoms. These signs include seed spoilage, kernel shriveling, tissue necrosis, and seed pigmentation (Momtaz et al., 2022).

Various seed-borne fungi, either alone or in combination, cause poor germination and sick seedlings in wheat (Deb and Khair, 2018)**.** Among the 120 diseases that affect wheat, 42 are seed-borne and 35 are fungal (Jama et al., 2018). *Bipolaris sorokiniana* is the most common seed-borne pathogen causing black point discoloration, which results in germination loss, root decay, and spot blotch in various cereal crops, including wheat (Sharma et al., 2021; Al-Sadi, 2021). The rice-wheat cultivation system in Nepal creates a favorable environment for the survival and multiplication of *B. sorokiniana* (Singh et al., 2021), which causes 100% yield loss during the late post-anthesis phase (Kumar et al., 2020; Chowdhury, 2021). *B. sorokiniana* has a high prevalence of 65% in shriveled seeds and causes an 87% germination failure rate in vitro conditions. In the eastern plains of Nepal, a 100% foliar blight incidence with yield losses of up to 52% has been reported (Nepal et al., 2020). The pathogen also affects the quality of the harvested wheat grains and reduces farmers' income (Gupta et al., 2018; Simón et al., 2020). Thus, even a marginal reduction in disease severity would have a significant impact on farmers' income. It causes root infection, coleoptile sickness, and failure to germinate in wheat seeds and spreads from kernel to crop (Gaur et al., 2020; Nallathambi et al., 2020). Farmers must understand the prevalence of *Bipolaris* and its impact on the germination failure of various wheat varieties due to spot blotch. Farmers are deprived of information about the seed health of commonly cultivated wheat varieties, and there is an absence of a comparative study on their seed health. The novelty of this study lies in the comparison of the seed health of different wheat varieties, which has not been explored previously in Nepal. Early detection of seed-borne infections is critical for disease control and the prevention of outbreaks. The study's significance is to aid in developing seed certification criteria and identifying better wheat varieties to improve crop yield and farmers' income.

This study aims to provide useful information to farmers, seed producers, and policymakers so they can make informed decisions about seed selection and management practices. It is also critical to conduct seed health testing to prevent the spread of numerous seed-borne illnesses to new locations (Adhikari et al., 2016). Furthermore, the study aims to identify the most susceptible wheat variety to seed-borne diseases caused by *B. sorokiniana*, and this information can help farmers and seed producers in Nepal to choose the appropriate wheat variety that is less susceptible to seed-borne diseases and can improve the seed quality and yield of the crops. A marginal reduction in disease severity would have a significant impact on farmers' income, making this study significant.

2. Materials and Methods

2.1. Experimental Site

The experiment was performed at the Central Agricultural Laboratory, Hariharbhawan, Lalitpur, Nepal, under homogenous environmental conditions from April 8 to June 19, 2022. The experimental site's coordinates are 27°40′43′′ N latitude and 85°18′58′′ E longitude, with an elevation of 1,420 meters above sea level. The site experiences a mild, generally warm-temperate climate, with an average annual temperature of 18.1 °C and total annual precipitation of 1,505 mm.

2.2. Experimental Details

The experiment was sought using a single factorial Completely Randomized Design (CRD) with 5 treatments (T1-Gautam, T2-Aaditya, T3-Bijaya, T4-Dhaulagiri, and T5-NL971) and 4 replications, using seed specimens acquired from the seed lab of Central Agriculture Laboratory, Hariharbhawan, Lalitpur. The research used 40 Petri dishes in total, with each replication including 10 Petri dishes and each treatment within each replication containing 8 Petri dishes (Figure 1).

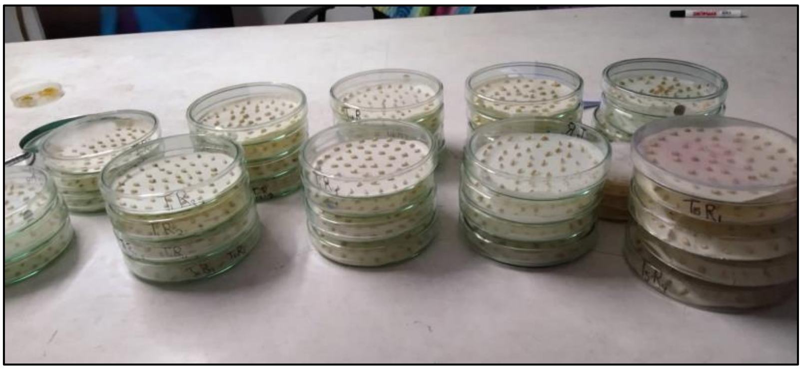

Figure 1. Petri dish configuration revealing experimental layout for seed specimens' analysis.

2.3. Preparation of Experimental Setup

A seed health test was performed using the standard blotter technique to evaluate the pathogens identified in the seed samples (Momtaz et al., 2022; Khan et al., 2023). Each sample was tested with 400 seeds. Petri dishes were collected from the pathology lab of the Central Agriculture Laboratory, washed, and sterilized with a 1% ethanol solution. To determine the incidence of seed infection, 50 seeds were plated in equidistant places on each Petri plate, which was made up of three layers of blotting paper that had been wetted with sterilized distilled water. Each replication consisted of 100 seeds on 2 Petri plates, which were incubated at 24 °C for 8 days in a seed germinator (Figure 2).

Figure 2. Incubator set up for seed health testing using blotter technique.

2.4. Observation Parameter

Two types of parameters were assessed, namely vegetative parameters (germination percentage, shoot length, seedling vigor index (SVI), and shoot-root weight) and disease parameters (disease incidence percentage). The observations were recorded on the 3rd, 5th, and 8th days after planting the seeds in the Petri dishes. The plates were observed on the third day of incubation under a binocular microscope to detect infected seeds for estimating disease incidence percentage (DIP) with *Bipolaris* infection and to monitor seed germination. DIP was calculated using the modified version of the formula provided by Kuyu and Tola (2018):

$$\text{DIP} = \frac{\text{Number of infected seeds}}{\text{Total seed sown}} \times 100$$

The germination percentage (GP) was recorded on the 3rd and 5th days after plating, using the formula mentioned by Momtaz et al. (2022) and Ullah et al. (2022). GP is the total number of seeds that germinated out of all the seeds planted on each Petri plate.

$$\text{Germination percentage (seed viability)} = \frac{\text{Final count of emerged seedlings}}{\text{Total seed sown}} \times 100$$

The shoot length of 25 randomly chosen seedlings from each replication was measured. The newly acquired weight of the shoot as well as the root was also measured using a digital balance after the root and shoot sections were divided using a sharp knife (Momtaz et al., 2022). After determining the pedicle and radicle lengths, SVI was calculated after the growth experiment. The SVI was determined using the formula proposed by Ebrahimi (2018):

$$\text{Vigor index} = \text{Mean of shoot length} \times \text{Percentage of seed germination}$$

Figure 3. Seed germination observation in Petri plate during the experiment.

2.5. Statistical Analysis

The data collected was categorized, summarized, and put into MS Excel (Microsoft Corp., USA) before being evaluated using the G-Stat (15th Edition) analysis system. For mean comparison, Duncan's Multiple Range Test (DMRT) was used for all of the evaluated data. A 5% level of significance was used for the analysis of variance (ANOVA). The variation coefficient was calculated, and the findings for the specified parameters were discussed and evaluated.

3. Results and Discussion

3.1. Disease Incidence Percentage (DIP)

The *Bipolaris* incidence percentage was found to be significantly different in different wheat varieties. The highest Bipolaris incidence (18.25%) was recorded in the Gautam variety. The lowest disease incidence was observed in NL971 (11.25%), followed by Bijaya and Aaditya. Table 1 showed that the Gautam variety was susceptible to *Bipolaris*, while NL971, Bijaya, Aaditya, and Dhaulagiri were moderately resistant to *Bipolaris*. The findings align with the findings of Pandey et al. (2018), where the Gautam variety showed the maximum disease severity among various varieties due to *Bipolaris*. It might imply that the genotypes employed were more resilient and tolerable than Gautam.

Table 1. Disease Incidence Percentage of several wheat species

Wheat Varieties	Number of Seeds Infected
Gautam	18.25a
Aaditya	12.50b
Bijaya	12.25b
Dhaulagiri	13.00b
NL971	11.25b
Grand Mean	13.45
SEm(±)	0.994
LSD	2.995
CV%	14.80%
F test	***

Means in the column with the same letter(s) indicate no significant difference between treatments at 0.05 level of significance; SEm(±) = Standard Error of Mean; CV = Coefficient of Variation; LSD = Least Significant Difference.; '***' Significant at 0.001 level of Significance.

3.2. Germination Percentage

All of the wheat varieties used in this study showed a significant variation in GP (Table 2). The highest germination percentage 3 days after plating (DAP) was shown in Dhaulagiri (98.5%), which is practically equivalent to Bijaya, Aaditya, and NL971, whereas the minimum GP was shown in Gautam (72%) variety. The wheat seed germination 5 days after plating (DAP) was different from the 3 DAP; however, the trend was similar. The highest germination percentage after 5 DAP (99.50%) was recorded in the variety of Dhaulagiri, whereas the lowest germination percentage after 5 days (79.00%) was recorded in the Gautam variety. Variations in genetic makeup or the existence of diseases caused by seeds may have contributed to variations in the germination percentage. But it was discovered that a lower germination rate was associated with a more serious black point infection. Numerous studies have also revealed a connection between the severity of black spot infection and the reduced sprouting of grain seeds (Minaeva et al., 2018; Al-Sadi, 2021; Rysbekova and Sultanova, 2022; Sharma et al., 2021). When infections from seed-borne sources are present in the seed, they may harm the embryo and ultimately destroy the seedlings. Crop plants' growth and productivity have been proven to be impacted by seed-borne diseases (Momtaz et al., 2022).

Table 2. Germination percentage of several wheat species on different days after plating

Wheat Varieties	3 DAP	5 DAP
Gautam	72.00b	79.00b
Aaditya	93.75a	97.50a
Bijaya	97.75a	98.25a
Dhaulagiri	98.50a	99.50a
NL971	93.00a	95.50a
Grand mean	91.1	93.95
SEm(±)	2.064	1.611
LSD	6.22	4.857
CV%	4.50%	3.40%
F test	***	***

Means in the column with the same letter(s) indicate no significant difference between treatments at 0.05 level of significance; SEm(±) = Standard Error of Mean; CV = Coefficient of Variation; LSD = Least Significant Difference.; '***' Significant at 0.001 level of Significance.

3.3. Shoot Length (cm)

All of the wheat cultivars tested in this study had highly significant differences in shoot length (Table 3). The highest shoot length (3 DAP) (2.395 cm) was recorded in the Aaditya variety, which was statistically akin to NL971 and Dhaulagiri. In contrast, the variety with the lowest shoot length (1.035 cm) was Gautam. In eight DAP, the highest shoot length (6.176 cm) was again observed in the Aaditya variety, following a similar trend, whereas the lowest shoot length (5.245 cm) was observed in the Gautam variety. The healthy cultivars showed the maximum shoot length. The result is in line with the findings of Islam et al. (2019); Momtaz et al. (2022) who reported that *Bipolaris* susceptible cultivars had low shoot length as the disease causes cellular breakdown and restricts the growth of the seedlings.

Table 3. Shoot length of several wheat species on different days after plating

Wheat Varieties	5 DAP	8 DAP
Gautam	1.035c	5.245b
Aaditya	2.395a	6.176a
Bijaya	1.201bc	5.521ab
Dhaulagiri	1.699abc	5.696ab
NL971	1.988ab	5.898ab
Grand mean	1.66	5.71
SEm(±)	0.36	0.254
LSD	0.767	0.766
CV%	30.60%	8.90%
F test	**	*

Means in the column with the same letter(s) indicate no significant difference between treatments at 0.05 level of significance; SEm(±) = Standard Error of Mean; CV = Coefficient of Variation; LSD = Least Significant Difference.; '**' Significant at 0.01 level of Significance; '*' Significant at 0.05 level of Significance.

3.4. Shoot and Root Weight (g)

Table 4 revealed that the highest roots, as well as shoot mass (4.692 and 2.500 g), were recorded in the Aaditya variety, which was statistically akin to the varieties NL971 (3.917 g and 2.223 g), Dhaulagiri (3.490 g and 2.380 g) and

Bijaya (3.020 g and 2.147 g) and the lowest (2.942 g and 1.778 g) was observed in Gautam variety. Black point infection has a significant impact on seedling root and shoots growth (Guo et al., 2019; Sharma et al., 2021; Al-Sadi, 2021), due to which the weight ultimately decreases.

Table 4. Root and shoot weight at 8 days after plating.

Wheat Varieties	Shoot Weight	Root Weight
Gautam	2.942c	1.778a
Aaditya	4.692a	2.500a
Bijaya	3.020bc	2.147a
Dhaulagiri	3.490bc	2.380a
NL971	3.917ab	2.223a
Grand mean	3.61	2.21
SEm(±)	0.285	0.274
LSD	0.859	0.825
CV%	15.80%	24.80%
F test	**	NS

Means in the column with the same letter(s) indicate no significant difference between treatments at 0.05 level of significance; SEm(±) = Standard Error of Mean; CV = Coefficient of Variation; LSD = Least Significant Difference.; '**' Significant at 0.01 level of Significance; NS = Non-significant.

3.5. Seedling Vigor Index

The potential for a speedy and uniform emergence of plants is determined by seed vigor, a crucial indicator of seed quality (Wen et al., 2018). Low-vigor seed planting resulted in shorter plants, later panicle exertion and anthesis, lower tillering ability, and lower yield. The most favorable seedling vigor rating was obtained in Aaditya (602.20), which was at par with Dhaulagiri (566.70), followed by NL971 (563.90) and Bijaya (542.30). The variety Gautam displayed the highest percentage of overall fungal association (18.25%) and the lowest SVI (412.70) (Table 5). The result is in close conformity with the findings of Momtaz et al. (2022), where *Bipolaris*- infested cultivars with high disease susceptibility had low SVI. *B. sorokiniana* infection increment led the seedling vigor index to decline significantly.

This leads to the conclusion that the lowest vigor index was also caused by the highest level of fungal connection. In this instance, there was probably a considerably higher load of harmful fungal inoculum in the seeds. The SVI was affected by the infection percentage of seed, as with the increase in seed infection percentage came a decrease in seedling vigor.

Table 5. Seedling vigor index of several wheat species.

Wheat Varieties	Seedling Vigor Index
Gautam	412.70b
Aaditya	602.20a
Bijaya	542.30a
Dhaulagiri	566.70a
NL971	563.90a
Grand mean	538
SEm(±)	24.5
LSD	73.9
CV%	9.10%
F test	***

Means in the column with the same letter(s) indicate no significant difference between treatments at 0.05 level of significance; SEm(±) = Standard Error of Mean; CV = Coefficient of Variation; LSD = Least Significant Difference.; '***' Significant at 0.001 level of Significance.

3.6. Relationship between Infection and Germination Percentage

The germination percentage of seed shows a strong negative correlation with the *Bipolaris* infection percentage, i.e., it is highly significant (r = -0.69). As the percentage of infected seeds increases, the germination percentage decreases, as shown in Figure 4.

The coefficient of determination value, i.e., R^2 = 0.4709, indicates that *Bipolaris* infection contributes 47.09% of the variation in germination, while the remaining variation may be attributed to other factors that were not accounted for in the study. Seed-borne fungi have a considerable effect on lowering germination.

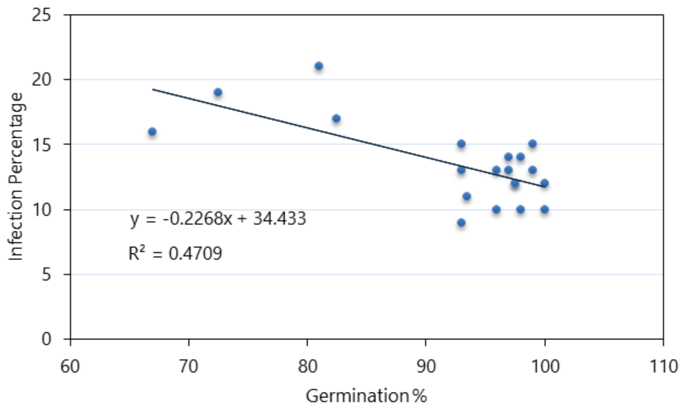

Figure 4. Correlation between germination percentage and infected seed percentage.

4. Conclusion

The study investigated the seed health and susceptibility of various wheat varieties to *B. sorokiniana*, a seed-borne pathogen. The results revealed significant variations among the tested varieties. Gautam exhibited the highest infection rate of *Bipolaris* (18.25%), while NL971 had the lowest (11.25%), followed by Bijaya, Dhaulagiri, and Aaditya. Dhaulagiri displayed the highest germination percentage (99.50%), followed by Aaditya, Bijaya, and NL971, whereas Gautam had the lowest (79%). In terms of growth parameters, Aaditya demonstrated superior performance with the highest shoot and root weights (4.692 g and 2.500 g), shoot length (6.176 cm), and seedling vigor index (602.20). Alternatively, Gautam exhibited the lowest values across these metrics. The study highlighted that *Bipolaris* infection led to poor germination, reduced shoot length, lower shoot root weight, and decreased seedling vigor. Susceptible varieties were more prone to high infections, resulting in compromised germination and seedling vigor due to cellular desiccation and metabolic activity limitations. Notably, the Gautam variety displayed high susceptibility to *B. sorokiniana*, resulting in reduced germination and seedling vigor. In contrast, the Aaditya variety performed well in all measured parameters, comparable to the NL971, Dhaulagiri, and Bijaya varieties. These findings underscore the importance of selecting resistant wheat varieties to enhance productivity and mitigate losses caused by seed-borne fungal diseases. Based on the study's results, practical recommendations for managing seed-borne pathogens in wheat cultivation include implementing seed treatments with appropriate fungicides targeting *B. sorokiniana* to significantly reduce its incidence and safeguard seed health. Furthermore, adopting cultural practices such as crop rotation, residue management, and maintaining field hygiene can minimize the risk of seed-borne pathogen contamination. Regular monitoring and testing of seeds, along with the use of disease-free planting materials, are crucial for preventing pathogen spread. Future research endeavors can explore the efficacy of various seed treatments in controlling seed-borne pathogens and enhancing seed health. These findings serve as a foundation for further investigations and the development of strategies to effectively manage seed-borne fungal diseases in wheat. The outcomes of this study hold significant implications for farmers, seed producers, and policymakers, providing them with valuable insights to make informed decisions regarding seed selection and treatment. Implementing the recommended strategies will contribute to the sustainability of wheat production in Nepal.

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Data/Supplementary Information Availability: Not applicable.

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