

To assess the efficacy of different chemical fungicides for the management of Wheat Rust

Submitted by: Thinley Wangmo and Ugyen Dema

Plant Protection Unit

TSS, ARDC Bajo

1. Introduction

Wheat (*Triticum aestivum* L.) is the world's most extensively grown cereal crop and ranks third after rice and maize in total global production. It serves as a staple food in both developed and developing countries, contributing significantly to global food security (Getie, 2015). Wheat provides nourishment for a substantial proportion of the global population and is estimated to support more than one-third of humanity (Ahanger et al., 2014; Dreisigacker, 2004). Due to its high gluten content, wheat is widely used in the production of flour for bread, pasta, and various baked and processed food products (Doty, 2012). Given the rising global population, sustainable wheat production is essential to meet increasing food demand.

In Bhutan, wheat, together with barley, constitutes the third most important cereal in terms of consumption and production. Despite its importance, wheat productivity in Bhutan remains relatively low. Earlier reports indicated an average productivity of 1.76 Mg ha⁻¹ (DoA, 2014), which is significantly lower than the global average yield of approximately 3 Mg ha⁻¹ (Tshewang et al., 2017). Wheat production has shown modest fluctuations over the years. For instance, in 2013, total production was estimated at 4,286 metric tons from 5,441 acres (MoAF, 2015).

More recent estimates suggest that wheat continues to be cultivated on approximately 4,000–6,000 hectares annually, with national production ranging between 3,500 and 5,000 metric tons, depending on seasonal and climatic conditions (MoAF, 2023; NSB, 2023). However, domestic production remains insufficient to meet national demand, leading to substantial imports. For example, Bhutan imported over 2,326 metric tons of wheat worth Nu. 416 million in 2014 alone (NSB, 2015), and import dependency persists due to limited productivity and production constraints.

The relatively low productivity of wheat in Bhutan is attributed to a combination of abiotic and biotic stresses, including poor soil fertility, suboptimal agronomic practices, and increasing disease pressure. Among the biotic constraints, wheat rust diseases are considered the most significant threats to wheat production worldwide (Aquino et al., 2002; Singh et al., 2004). Wheat rusts are caused by fungal pathogens belonging to the class Basidiomycetes and order Pucciniales (McIntosh et al., 1995; Agrios, 2005). The three major types of rust diseases affecting wheat are: stem rust (*Puccinia graminis* f. sp. *tritici*), stripe rust (*Puccinia striiformis* f. sp. *tritici*), and leaf rust (*Puccinia triticina*).

These rust pathogens differ in their morphology, life cycles, and environmental requirements. Stem rust thrives under warm and humid conditions, stripe rust prefers cool and moist climates, and leaf rust is adapted to a broader range of environmental conditions, making it the most widespread of the three (Bariana et al., 2007; Getie, 2015).

In Bhutan, stripe rust and leaf rust are the most prevalent and recurring diseases affecting wheat production, whereas stem rust has not been reported in recent decades. Historical records indicate that severe stripe rust epidemics in 1985 and 1986 caused yield losses of more than 50% in the Punakha–Wangdue valley (Mann & Hobbs, 1988; Tshewang, 2014). Subsequent surveillance studies have consistently reported the presence of stripe and leaf rust across major wheat-growing regions.

Efforts to manage wheat rust in Bhutan have included the evaluation and deployment of resistant varieties adapted to different agro-ecological zones (Chophel et al., 2019). However, the durability of host resistance is often compromised by the emergence of new pathogen races and changing climatic conditions. As a result, chemical control through fungicide application remains an important component of integrated disease management, particularly during epidemic years.

Despite the availability of fungicides, there is limited field-based evidence in Bhutan regarding their efficacy, optimal application timing, and economic feasibility under local conditions. Therefore, considering the importance of wheat as a food crop and the significant yield losses caused by rust diseases, the present study is designed to assess the efficacy of selected chemical fungicides for the management of wheat rust under Bhutanese conditions.

1.1 Problem Statement

Wheat production in Bhutan is constrained by recurring rust epidemics, leading to significant yield losses and reduced farmer adoption. Despite the availability of fungicides, there is insufficient localized evidence on their effectiveness, appropriate application timing, and economic feasibility. This limits the development of integrated disease management strategies.

1.2 Objective

- To assess the effectiveness of different fungicides in reducing wheat rust severity.
- To determine the impact of fungicide application on wheat yield and yield components.

- To identify the most cost-effective fungicide treatment.
- To develop recommendations for integrated wheat rust management in Bhutan.

2. MATERIALS AND METHODS

2.1 Experimental Site/Location

The field experiment was conducted on-station at the Agricultural Research and Development Centre (ARDC), Bajo, located at an altitude of approximately 1200 masl.

2.2 Study Design and Layout

The experiment was laid out in a randomized complete block design (RCBD). The study consisted of five treatments: Propiconazole, Hexaconazole, Triazole, Carbendazim, and an untreated control. Each treatment was replicated three times, resulting in a total of 15 experimental plots.

Each plot measured 4 m × 2 m (6 m²). A buffer zone of 50 cm was maintained between plots and blocks to minimize spray drift and inter-plot interference. Wheat was sown in rows at a spacing of 10 cm × 10 cm (row-to-row and plant-to-plant), and seeds were line-sown to ensure uniform plant population.

2.3 Treatments

The treatments evaluated were as follows:

- T1 = Propiconazole (1 ml per litre of water)
- T2 = Hexaconazole (1 ml per litre of water)
- T3 = Triazole (2 g per litre of water)
- T4 = Carbendazim (2 g per litre of water)
- T5 = Control (no fungicide application)

2.4 Fungicide Application

Fungicides were applied as foliar sprays using a knapsack sprayer at recommended doses. The first application was made at the early appearance of disease symptoms during the stem elongation to flag leaf emergence stage (GS32–39). A second application was carried out 14 days after the first spray, depending on disease development and prevailing weather conditions.

2.5 Disease Assessment

Disease severity of wheat rust was assessed following CIMMYT rust scoring guidelines using the Modified Cobb scale, which estimates the percentage of leaf area affected. Observations were recorded from 18 randomly selected hills per plot, assessing the top three leaves (flag leaf, flag-1, and flag-2).

Disease assessments were conducted at 14-day intervals, starting from the first appearance of rust symptoms until the milk-dough stage. For each plot and assessment date, mean disease severity values were calculated and used for subsequent statistical analysis.

2.6 Disease Progress and Statistical Analysis

Disease progress was quantified using the area under the disease progress curve (AUDPC), calculated from sequential severity observations for each plot.

To account for the experimental design and repeated measurements over time, data were analysed using a linear mixed-effects model (LMM). The model incorporated:

- **Fixed effects:** Treatment, Date (time), and Treatment $\times$ Date interaction
- **Random effects:** Block (replication) and Plot (to account for repeated measurements over time)

This model allowed assessment of both overall treatment effects and temporal disease dynamics while accounting for correlation among repeated observations within plots.

Additionally, the experiment followed a randomized complete block design (RCBD), which was incorporated into the mixed model framework through block as a random effect.

Treatment effects on AUDPC and final disease severity were assessed using a linear mixed-effects model (LMM), incorporating treatment as a fixed effect and block (replication) as a random effect, with repeated measurements accounted for within plots. Where significant effects were observed, pairwise comparisons of estimated marginal means were conducted, and differences among treatments were declared significant at $p < 0.05$.

3. Results and discussion

3.1 Effect of different fungicides on disease progress

A linear mixed-effects model revealed that wheat rust severity was significantly influenced by treatment, assessment date, and their interaction. Treatment had a highly significant effect on disease severity ($F_{4,263} = 128.85$, $P < 0.001$), indicating differences among fungicides in suppressing rust development. Disease severity also varied significantly across assessment dates ($F_{1,263} = 331.73$, $P < 0.001$), reflecting temporal disease progression. Importantly, a significant Treatment $\times$ Date interaction ($F_{4,263} = 129.03$, $P < 0.001$) indicated that the effectiveness of fungicides changed over time, demonstrating differential disease suppression dynamics among treatments.

Pairwise comparisons showed that fungicides significantly reduced disease severity compared to the untreated control (T5), particularly at later assessment dates. Treatments T1 and T2 consistently maintained lower severity across all time points, while T3 and T4 showed weaker suppression, approaching control levels in later assessments.

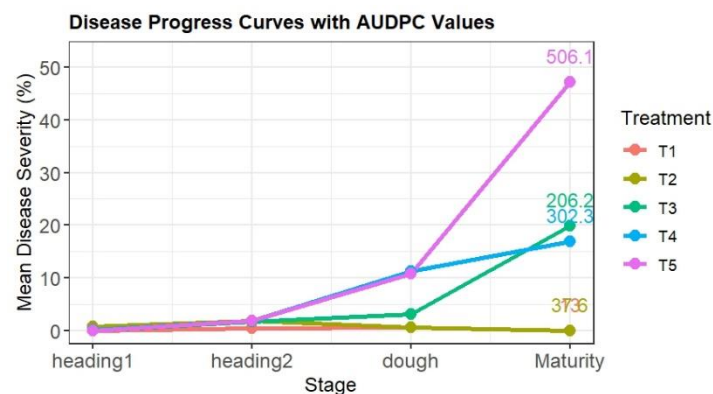


Figure 1. Disease progress curves of wheat rust severity under different fungicide treatments during the assessment period.

3.2 Effect of different fungicides on AUDPC analysis (season-long disease development)

The area under the disease progress curve (AUDPC) differed significantly among treatments ($F_{4,263} = 26.76$, $P < 0.001$), indicating strong treatment effects on overall disease development throughout the season. Mean AUDPC values showed a clear gradient of disease suppression among treatments.

Based on estimated marginal means from the mixed model, T1 recorded the lowest AUDPC, followed by T2, while T3 and T4 exhibited intermediate values. The untreated control (T5) recorded the highest AUDPC, indicating the greatest cumulative disease pressure.

Tukey-adjusted pairwise comparisons confirmed that T1 and T2 significantly reduced AUDPC compared to all other treatments ($P < 0.001$). T3 and T4 did not differ significantly from the control, indicating limited efficacy under the conditions of this study.

Log-transformed AUDPC analysis [$\log(\text{AUDPC} + 1)$] confirmed these patterns and improved model assumptions, with treatment effects remaining highly significant ($F_{4,263} = 48.12$, $P < 0.001$). In this model, T1 and T2 showed significantly lower values than T3, T4, and T5, whereas no significant differences were observed among T3, T4, and the control.

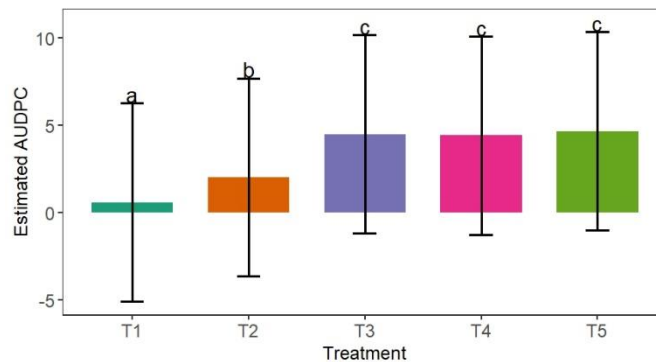


Figure 2. Area under the disease progress curve (AUDPC) of wheat rust under different fungicide treatments. Error bars represent standard errors of the mean. Different letters indicate significant differences among treatments at $p < 0.05$.

The repeated-measures linear mixed model (LMM) revealed a significant Treatment $\times$ Date interaction ($p < 0.05$), indicating that fungicide effects on disease severity were not constant over time but varied significantly across the epidemic period. This interaction suggests that treatments differed not only in overall disease reduction but also in the rate of disease progression (epidemic slope). In practical terms, this means that some fungicides were more effective at delaying epidemic development, particularly during early infection stages, which is critical in rust management (Campbell & Madden, 1990).

The significant main effect of Treatment ($p < 0.05$) further confirms that disease severity differed among fungicides when averaged across all assessment dates. Likewise, the

significant effect of Date ($p < 0.001$) indicates a strong temporal increase in disease severity across all treatments, reflecting typical exponential or logistic rust epidemic development.

Estimated marginal means from the LMM showed that T1 (propiconazole) and T2 (hexaconazole) consistently had significantly lower disease severity than T3, T4, and the control (T5) across most evaluation dates. The effect sizes (mean differences between treatments) indicate that T1 and T2 reduced disease severity by a substantial margin relative to the control, demonstrating strong biological efficacy. This strong suppression is consistent with the known activity of DMI (demethylation inhibitor) fungicides, which inhibit ergosterol biosynthesis and restrict fungal growth and sporulation (Lucas et al., 2015; FRAC, 2023).

In contrast, T3 and T4 showed non-significant or weaker reductions in disease severity ($p > 0.05$ or marginal significance depending on comparisons) and progressively converged toward the control over time. This suggests a lower effect size and reduced biological impact on epidemic suppression under field conditions, possibly due to environmental degradation, suboptimal timing, or reduced pathogen sensitivity (McGrath, 2004).

AUDPC analysis supported the LMM results by integrating disease severity over time. There were significant differences in AUDPC among treatments ($p < 0.05$), confirming that cumulative disease pressure differed strongly across fungicides.

T1 (propiconazole) and T2 (hexaconazole) recorded significantly lower AUDPC values compared to T3, T4, and T5, indicating strong season-long suppression of disease development. The magnitude of reduction in AUDPC represents a meaningful biological effect, showing that these treatments not only reduced peak severity but also limited overall epidemic duration and intensity.

In contrast, T5 (control) recorded the highest AUDPC, reflecting unrestricted disease development throughout the season. The absence of fungicide protection allowed continuous disease buildup, resulting in maximum epidemic pressure. Treatments T3 and T4 did not differ significantly from the control ($p > 0.05$), indicating negligible effect sizes and weak overall field performance under the conditions of this study.

The convergence between LMM and AUDPC results strengthens the robustness of the findings. While the LMM quantified temporal dynamics and interaction effects, AUDPC captured the integrated epidemic outcome, and both analyses consistently identified T1 (propiconazole) and T2 (hexaconazole) as the most effective treatments.

3.3 Effect of different fungicides on yield

The linear mixed-effects model indicated that fungicide treatments had no significant effect on yield ($F = 1.0952$, $p = 0.4375$). Although mean yield varied slightly among treatments, these differences were not statistically meaningful, suggesting that yield was not strongly influenced by fungicide application under the conditions of this study.

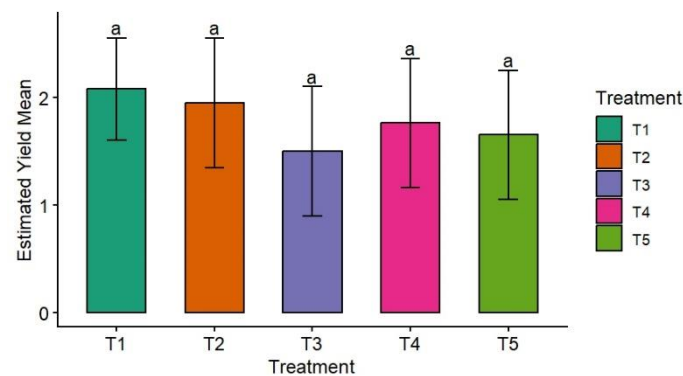


Figure 3. Effect of fungicide treatments on wheat grain yield. Error bars represent 95% confidence intervals.

3.4 Effect of different treatments on Plant height

Plant height was also not significantly affected by treatments ($F = 0.3339$, $p = 0.8461$), indicating that vegetative growth was largely independent of fungicide application. This suggests that the fungicides primarily acted on the pathogen rather than influencing plant growth processes directly.

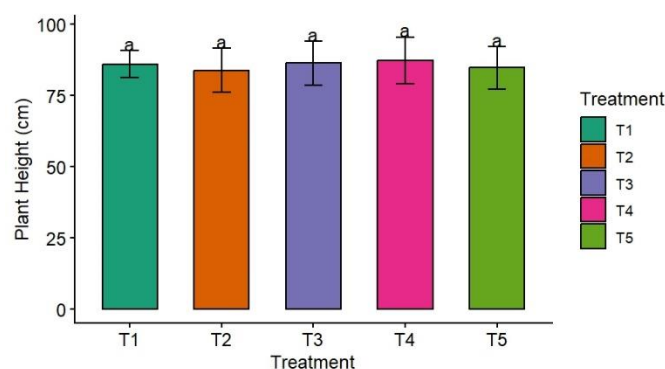


Figure 4. Effect of fungicide treatments on plant height of wheat. Error bars represent 95% confidence intervals.

3.5 Effect of different fungicide on spike length

Similarly, spike length showed no significant response to fungicide treatments ($F = 0.9028$, $p = 0.526$), indicating uniform reproductive development across treatments. This suggests that fungicide application did not influence spike formation or sink development in wheat.

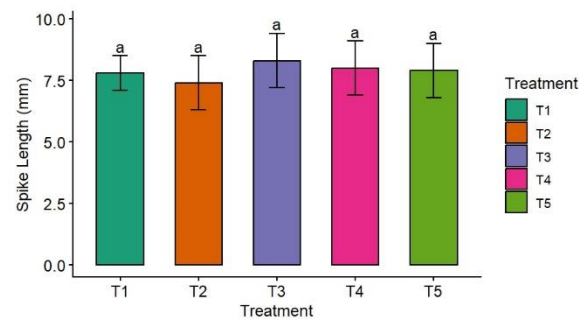


Figure 5. Effect of fungicide treatments on spike length of wheat. Error bars represent 95% confidence intervals.

Although fungicide treatments, particularly propiconazole and hexaconazole, effectively reduced disease severity in earlier analyses, this reduction did not translate into significant differences in yield, plant height, or spike length. This disconnect between disease suppression and yield response is commonly reported in cereal pathology and suggests that reductions in disease intensity do not always correspond to measurable agronomic benefits (Madden et al., 2007).

One possible explanation is that disease pressure, although reduced by fungicide application, may not have exceeded the economic threshold required to cause significant yield loss. In such cases, wheat plants are often able to compensate for moderate foliar damage through enhanced photosynthetic activity in remaining healthy leaf tissue, thereby maintaining yield stability across treatments (Madden et al., 2007). Additionally, environmental factors such as soil fertility, rainfall distribution, and temperature variability may have further masked potential treatment effects on yield-related traits.

The timing of disease onset relative to critical growth stages is also a key determinant of yield response. If fungicide applications successfully delayed disease development until after sensitive stages such as grain filling, then yield differences among treatments would be expected to remain minimal despite clear differences in disease severity (Chen, 2005). Similar patterns have been documented in wheat rust studies where fungicide application reduced

disease levels but did not consistently improve yield under moderate disease pressure (Chen, 2005).

Furthermore, plant height was not significantly affected by fungicide treatments, which is consistent with the mode of action of DMI fungicides such as propiconazole and hexaconazole. These fungicides primarily inhibit fungal sterol biosynthesis and are not known to stimulate plant growth at recommended field doses (Lucas et al., 2015). The absence of height differences therefore confirms that the observed fungicides acted specifically on the pathogen rather than influencing vegetative growth processes.

Similarly, spike length showed no significant response to treatments, indicating uniform reproductive development across all plots. Spike formation is largely governed by genetic and environmental factors, and moderate levels of disease control may not be sufficient to influence spike morphology unless severe infection significantly reduces assimilate availability during reproductive stages (Reynolds et al., 2012). Overall, these results suggest that although fungicides effectively suppressed disease development, the level of disease pressure under the experimental conditions was insufficient to induce measurable changes in yield and yield components.

4. Conclusion

The present study demonstrated that fungicide treatments had a clear effect on wheat rust disease development, with propiconazole and hexaconazole consistently showing superior performance in reducing disease severity over time. Both the repeated-measures linear mixed model and AUDPC analysis confirmed that these two treatments effectively suppressed disease progression and maintained lower overall epidemic pressure compared to triazole, carbendazim, and the untreated control.

However, this strong disease suppression did not translate into significant improvements in agronomic traits, including yield, plant height, and spike length. The absence of a yield response suggests that the level of disease observed under the experimental conditions may not have exceeded the threshold required to cause measurable yield loss, or that wheat plants were able to compensate for moderate foliar damage through physiological adaptation and environmental buffering. Additionally, the timing of disease development relative to critical growth stages likely reduced the potential impact of fungicide treatments on final productivity.

Overall, the findings indicate that while propiconazole and hexaconazole are effective for managing wheat rust under field conditions, disease reduction alone does not necessarily guarantee yield gains. This highlights the importance of integrating disease management decisions with economic threshold levels, crop growth stage, and environmental conditions when recommending fungicide applications. The study emphasizes that effective disease control should be evaluated not only in terms of pathogen suppression but also in relation to its actual impact on agronomic performance.

5. References

- Agrios, G. N. (2005). Disease caused by Basidiomycetes: Cereal rusts. In *Plant Pathology* (5th ed., pp. 565-571). Florida: Elsevier Academic Press Publication.
- Aquino, P., Carrion, F., & Calvo, R. (2002). Selected Wheat Statistics. In J. Ekboir (Ed.), *World Wheat overview and outlook 2001-2002: Developing No-Tillage packages for small scale farmers* (pp. 52-62). Mexico, D.F.: CIMMYT.
- Bariana, H. S., Brown, G. N., Bansal, U. K., Miah, H., Standen, G. E., & Lu, M. (2007). Breeding triple rust resistance wheat cultivars for Australia using conventional and marker-assisted selection technologies. *Australian Journal of Agricultural research*, 58, 576-587.
- Bolton, M. D., Kolmer, J. A., & Garvin, D. F. (2008). Wheat leaf rust caused by *Puccinia triticina*. *Molecular Plant Pathology*, 9(5), 563–575.
- Brar, G. S. (2015). Population structure of *Puccinia striiformis* f. sp. tritici, the cause of wheat stripe rust, in western Canada. (M.Sc. in Plant Science Master's thesis), University of Saskatchewan, Saskatoon, Canada.
- Campbell, C. L., & Madden, L. V. (1990). *Introduction to plant disease epidemiology*. Wiley.
- Campbell, C. L., & Madden, L. V. (1990). *Introduction to plant disease epidemiology*. Wiley.
- Chen, X. M. (2005). Epidemiology and control of stripe rust of wheat. *Canadian Journal of Plant Pathology*, 27(3), 314–337.
- Chen, X. M. (2005). Epidemiology and control of stripe rust of wheat. *Canadian Journal of Plant Pathology*, 27(3), 314–337.
- FRAC (Fungicide Resistance Action Committee). (2023). *FRAC code list 2023: Fungicides sorted by mode of action*. <https://www.frac.info>

John Innes Centre. (2025). MARPLE diagnostics hub launched in Bhutan.

Lucas, J. A., Hawkins, N. J., & Fraaije, B. A. (2015). The evolution of fungicide resistance. *Advances in Applied Microbiology*, 90, 29–92.
<https://doi.org/10.1016/bs.aambs.2014.09.001>

Lucas, J. A., Hawkins, N. J., & Fraaije, B. A. (2015). The evolution of fungicide resistance. *Advances in Applied Microbiology*, 90, 29–92.
<https://doi.org/10.1016/bs.aambs.2014.09.001>

Madden, L. V., Hughes, G., & van den Bosch, F. (2007). *The study of plant disease epidemics*. APS Press.

Madden, L. V., Hughes, G., & van den Bosch, F. (2007). *The study of plant disease epidemics*. APS Press.

Mann, C. E., & Hobbs, P. R. (1988). Wheat and wheat development in Bhutan. Mexico: CIMMYT.

McGrath, M. T. (2004). What are fungicides and how do they work? *The Plant Health Instructor*. <https://doi.org/10.1094/PHI-I-2004-0825-01>

McIntosh, R. A., Wellings, C. R., & Park, R. F. (1995). Wheat Rusts: An atlas of resistance genes. Dordrecht, Australia: Commonwealth Scientific and Industrial Research Organisation (CSIRO), Kluwer Academic Publishers.

Ministry of Agriculture and Forests (MoAF). (2023). *Agricultural statistics of Bhutan*. Thimphu, Bhutan.

National Plant Protection Centre (NPPC). (2026). *Wheat rust advisory and risk assessment in Bhutan*.

National Statistics Bureau (NSB). (2023). *Statistical yearbook of Bhutan*. Thimphu, Bhutan.

- Om, et al. (2012). Wheat rust survey in Bhutan. National Plant Protection Centre.
- Om, N., Thinlay., Tshewang, S., Dorji, S., Dorji, G., Tashi, K. (2012). Bhutan Wheat Rust Surveys - April 2012 - Summary Report. Thimphu. Thimphu, Bhutan: National Plant Protection Centre (NPPC), Ministry of Agriculture & Forests.
- Reynolds, M. P., Foulkes, M. J., Furbank, R. T., et al. (2012). Achieving yield gains in wheat. *Functional Plant Biology*, 39(3), 1–16. <https://doi.org/10.1071/FP11139>
- Sathguru Management Consultants. (2018). *Wheat in India, Bangladesh, Nepal and Bhutan*.
- Shaner, G., & Finney, R. E. (1977). The effect of nitrogen fertilization on slow-mildewing resistance in wheat. *Phytopathology*, 67, 1051–1056.
- Singh, S., Sethi, G. S., & Chaudhary, H. K. (2004). Differential responsiveness of winter and spring wheat genotypes to maize-mediated production of haploids. *Cereal Research Communications*, 32(2), 201-207.
- Tshewang, S. (2014). Why farmers should not grow a wheat variety, Sonalika. Retrieved from <http://www.moaf.gov.bt/why-farmers-should-not-grow-a-wheat-variety-sonalika/> 96
- Tshewang, S., & Ghimirey, M. (2016). Assessment of wheat rust research at RDC Bajo, Wangdue. Wangdue: ARDC-Bajo.