Original Article

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Plant defense activator-mediated seed priming suppresses leaf rust (*Puccinia triticina*) and enhances yield in bread wheat genotypes

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Abstract

Puccinia triticina causing leaf rust is a significant pathogen of wheat because it negatively affects the canopy functioning, grain filling, and final yield. This research investigated the impact of seed priming on leaf rust severity, growth and yield of three bread wheat varieties Arooj-22, Dilkash-20 and MH-21. For this experiment, a completely randomized factorial design was used with three replications, including hormonal (jasmonic acid, salicylic acid, gibberellic acid), nutritional (KNO_3), botanical (moringa leaf extract), and biological (*Trichoderma* sp.) priming treatments. Disease severity and agronomic traits were significantly influenced by seed priming, with treatment and varietal differences in response. Arooj-22 was lowest in severity with GA 25 ppm and Dilkash-20 with *Trichoderma* 50 %, but the highest severity was in diseased control, especially in MH-21. Arooj-22 had the greatest yield of plants in *Trichoderma* 50 % and KNO3 0.5 % had the best yield response uniformly in all three varieties. Correlation analysis demonstrated that 1000 grains weight, leaf area and plant yield had a negative relationship with disease severity, but multivariate analysis differentiated between favorable lower dose treatments and weaker higher dose treatments. In general, the results indicate that seed priming has the potential to lessen the severity of leaf rust and significantly enhance the productivity of wheat, although its effectiveness requires attention to the choice of the primer, dose, and host genotype in the field pot condition.

Keywords: Bread wheat; leaf rust; seed priming

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Introduction

Wheat is one of the most important crop in the world, as it is the main source of calories and protein in human food (1). In 2024,25 season the amount of wheat produced globally was estimated to be approximately 799.9 million metric tons. Pakistan was 8th among the producers of 3.44 million metric tons and 38 % of the total land under cultivation in Pakistan was covered by wheat (2, 3). Wheat is major crop in Pakistan which is cultivated on approximately 40 % of agricultural land in Pakistan (4). It is a staple food, as it provides almost 60 % of daily food consumption, 10 % of the agricultural value and approximately 3 % of the national GDP. Therefore, an increase of wheat production is necessary to address the needs of the population and improve the economy (5).

Wheat contributes to about a fifth of the total dietary protein and calories of the world (6). It is composed of about 78 % carbohydrates, 14 % proteins, 2 % fats and 2.5 % minerals, trace levels of thiamine, vitamin B, zinc, iron, selenium and magnesium (7). These are the nutrients that render wheat as a good source of energy and amino acids important for human growth and development (8). In addition, magnesium especially helps to control blood pressure and aid in antioxidant activity by producing glutathione (9).

Among the fungal attacks in the world wheat production, rust diseases such as leaf, stripe, and stem rust caused by *Puccinia* spp. are significant, as they cause severe losses of the wheat yield and limit its production on a sustainable basis (1). Rust infections also lower the quality of grain by lowering the protein levels, gluten strength and making the grain more likely to sprout and mycotoxin contamination. The impacts of these effects are a great loss of the economy in terms of yield, cost of managing the disease, and market value (10).

Puccinia triticina, a fungus that causes leaf rust, is represented by the appearance of orange pustules on the upper surface of leaves which increase and merge to reduce photosynthetic activity and undermine the health of plants. The existence of moderate temperatures (5-30°C), and high humidity (65-90) during the growing season provide favorable conditions that promote the rapid growth and spread of leaf rust (11, 12).

Salicylic acid (SA) is an important signaling molecule involved in the plant immunity complex and mediates local defense and prompts systemic acquired resistance (SAR) against biotrophic and hemi-biotrophic pathogens (12). *Moringa oleifera* leaf extract (MLE) is a nutrient-rich seed primer with zeatin, ascorbates and minerals which are growth regulator that could enhance plant growth by 20-35 % (13).

Gibberellic acid (GA3) is employed to enhance seed germination, early growth and crop yield. GA3 priming of seeds aids in overcoming dormancy by making the endosperm soft and embryo growth active. In sunflower and wheat, this form of treatment has increased the yield of the seed by 123.5 and 7 % respectively (14). The soil fungi commonly belong to genus *Trichoderma*, are some of the most isolated pathogenic fungi that live as symbiotic organisms in root systems where they protect the plants against destructive pathogenic fungi. Due to these protective properties, they have been a major subject of study and marketed commercially as biopesticides and soil improvers (15).

Although seed priming has been widely recognized as an encouraging approach for improving plant vigor and inducing stress tolerance, its effectiveness against wheat leaf rust may vary with primer type, concentration, and host genotype. However, the differential effects of hormonal, nutritional, botanical, and biological priming treatments on disease suppression and agronomic performance in bread wheat remain inadequately explored. Therefore, this study investigated the effects of different seed-priming treatments on leaf rust severity and examined their impact on growth and yield-related traits in three bread wheat varieties under leaf rust stress.

Materials and Methods

Plant material

This study included three bread wheat (*Triticum aestivum* L.) varieties, Arooj-22, Dilkash-20, and MH-21. Seeds were obtained from the Ayub Agricultural Research Institute, Faisalabad, Pakistan. The varieties differ in agronomic performance and were selected to assess genotype-specific responses to seed priming under leaf rust stress. The highly susceptible Morocco cultivar was used as a spreader to ensure uniform disease development.

Priming treatments and preparation of solutions

Seed-priming treatments were chosen to indicate hormonal, nutritional, botanical and biological processes. Preparations of jasmonic acid (JA), salicylic acid (SA), and gibberellic acid (GA) were done at 25 ppm and 50 ppm. Potassium nitrate (KNO3) was made in 0.5 % and 1 %. Moringa leaf extract (MLE) was made at 2.5 and 5 %. *Trichoderma* spp. suspensions were made at 50 % and 100 % strength in case of biological priming. The control treatments were treated with distilled water (16).

To prepare MLE, fresh moringa leaves were picked, rinsed under distilled water and dried a bit in air to remove surface moisture. The extract was made by mixing 30 g fresh leaves in 300 mL of distilled water. A homogenate was filtered using Whatman No. 1 filter paper to get the stock extract and the necessary concentrations were diluted with distilled water on the basis of volume to volume.

Seed priming procedure

Seeds were washed twice with distilled water followed by priming to clean the seeds off dust and other dirt in the surface. Seeds were primed with a 1:5 seed-solution ratio (w/v). The respective priming solutions were used to soak the seeds at room temperature (5 h) and the hydration ensured without any radicle emergence. The seeds were soaked and then dried under controlled laboratory conditions to restore their moisture content to approximately its natural level. The re-dried seeds were then used for sowing.

Crop establishment and experimental design

The field experiment was carried out in pots in the Plant Pathology Research Area, University of Agriculture, Faisalabad, Pakistan. The research was organized under factorial completely randomized design (CRD) with three replications. The experiment contained three wheat varieties and fourteen treatment levels, twelve seed priming treatments and two controls. On 19 November 2022, three treated seeds were sown in each pot. The pots were maintained under uniform agronomic and environmental conditions throughout the experiment to minimize external variation.

Development of leaf rust and disease evaluation

Naturally disease was developed in experimental area by sowing Morocco as spreader, exempting healthy control. Disease symptoms were observed in plants, and pathogen was identified using microscope. The final disease severity was determined by modified Cobb scale and represented as percent severity.

Growth and yield characteristics measured

Plant height and flag leaf area were measured to evaluate vegetative growth since the two measures indicate plant health and canopy activity. The reproductive performance was assessed using spike length and spike weight. Yield related traits included spikelets per spike, 1000 grains weight and plant-1 yield. The flag leaves area was measured in cm^2 and the grain yield was measured in g $plant^{-1}$ The standard agronomic procedures were used to make all the observations at the relevant stages of crop development.

Statistical analysis

All statistical analyses were carried out in RStudio. Data were grouped according to wheat varieties, treatments and replications. In the key response variables, the variety × treatment means was computed and presented to graphically depict interaction effects. Under the factorial CRD, analysis of variance (ANOVA) was performed to determine the significance of varieties, seed-priming treatment and their interaction. The least significant difference (LSD) test at P=0.05 was used to determine the mean separation among variety × treatment combinations. The interaction pattern of disease severity was presented in the same way of mean-grouping. Pearson correlation coefficients determined the relationships between measured traits. After standardizing the traits values, a heatmap was created based on the treatment-wise means to compare the performance patterns across measured traits on different scales. The standardized trait matrix was subjected to principal component analysis (PCA) to summarize the multivariate data structure. Moreover, the correlation of leaf area with the yield of the plant was analyzed by scatter-plotting with a linear regression line fitted in each variety. The within-variety treatment distributions in detail and full interaction means groupings are given in the supplementary file with the main text presenting the major interaction, correlation, clustering, and multivariate analyses.

Results

Influence of Seed Priming Treatments on Leaf Rust Severity Across Varieties

The leaf rust severity varied significantly among seed-priming levels, with the extent of this reaction being highly dependent on varietal background (Figure 1). The diseased control always occupied the most vulnerable end of the response range with severity of 70 % in MH-21, 60 % in Arooj-22 and 55 % in Dilkash-20 which confirms the fact that the disease pressure was adequate to classify between favorable and unfavorable responses to treatments.

On the other hand, disease levels were confined to a significantly smaller set of treatments, meaning that protection was selective, but not general. Arooj-22 was found to be most suppressed under GA 25 ppm and Dilkash-20 under *Trichoderma* 50 % where disease severity was reduced to 5 %. Other lower intensity responses were recorded at JA 25 ppm and Moringa 2.5 % in Arooj-22, KNO3 0.5 % in Dilkash-20 and GA 25 ppm, GA 50 ppm and *Trichoderma* 50 % in MH-21.

An activity that was consistent throughout the dataset, the lower concentrations often performed better than the higher concentrations of the same priming agent, in particular *Trichoderma* and KNO3. Therefore, there was no straightforward dose-response pattern of the disease response. Somewhat, the data suggests that the combination of the type of primer, concentration, and host genotype was necessary to successful rust suppression. Response and full treatment mean and groupings variety-wise are given in Supplementary Figure S1.

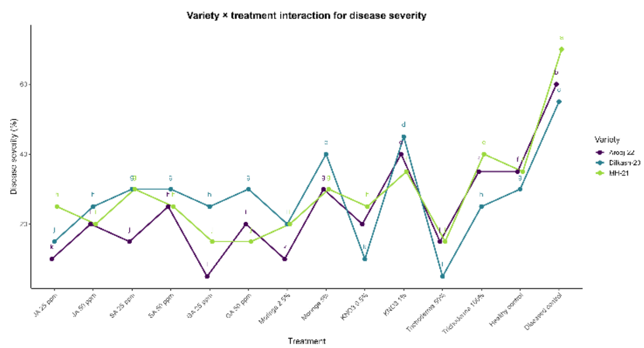


Figure 1. Interaction of variety x treatment on the level of the severity of leaf rust in three bread wheat varieties receiving the seed-priming treatments. Points indicate the mean disease severity (%) of Arooj-22, Dilkash-20 and MH-21 in the priming treatments. The LSD test suggests that there are noteworthy differences among the combinations of variety and treatment at P = 0.05 as indicated by the different lowercase letters.

Plant yield was also treatment and variety-specific in response to priming

Plant yield reflected the pattern of treatment observed regarding the severity of the disease, but agronomic response was selective compared to the pathological response (Figure 2). Arooj-22 with *Trichoderma* 50 % (29.37 g plant^{-1}) showed the highest yield in the experiment, which put this combination first in the interaction matrix. A second, more consistent high-yield response was observed with 0.5 % KNO_3 treatment, which produced the same average yield (26.17 g plant^{-1}) in Arooj-22, Dilkash-20, and MH-21, indicating a remarkably uniform response across these genotypes.

Yield response, however, was still obviously genotype dependent. The range of responses was also greatest in Arooj-22 with a range of 29.37 g plant^{-1} under *Trichoderma* 50 % as the lowest to 17.41 g plant-1 under *Trichoderma* 100 % as the highest. The highest response was recorded with KNO3 0.5% (26.17 g plant^{-1}) and Moringa 2.5 % (25.07 g plant^{-1}), and the lowest yield with KNO3 1.0 % (16.69 g plant^{-1}). In MH-21, the upper part of the response range was occupied by KNO3 0.5 % (26.17 g plant^{-1}), healthy control (25.77 g plant^{-1}), Moringa 2.5 % (25.67 g plant^{-1}), and SA 25 ppm (25.17 g plant^{-1}), while the diseased control gave the lowest yield (17.31 g plant^{-1}). Like the disease data, lower concentrations tended to work better than higher concentrations especially with *Trichoderma*, KNO3 and Moringa. Combined, the yield interaction reveals that the agronomic value of seed priming was defined by the interaction of treatment identity, concentration and the varietal background. The within-variety response pattern is provided in Supplementary Figure S2, and more general treatment distributions are provided in Supplementary Figure S3.

Grain filling, canopy maintenance and final yield were shown to have a negative relationship with disease severity

The correlation structure revealed that the burden of disease was associated with the widespread reduction of crop performance as opposed to the single traits (Figure 3). Throughout the dataset, all the measured agronomic variables were negatively correlated with disease severity. This negative relationship was strongest for 1000-grain weight (r = -0.54), followed by plant yield (r = -0.47), leaf area (r = -0.46), and spike weight (r = -0.45). The correlation with length of spikes was relatively weak (r = -0.18), which shows that the increase of disease pressure was more related to grain filling and the loss of functional yield rather than all structural traits to an equivalent extent.

Meanwhile, the agronomic characteristics were highly and positively correlated with each other. Plant yield was most significantly associated with 1000-grain weight (r = 0.80) then plant height (r = 0.72), spike weight (r = 0.69), spikelets per spike (r = 0.64) and spike length (r = 0.62). Similarly, the spike length and spike weight were highly correlated (r = 0.81), as well as the spike weight and the 1000-grain weight (r = 0.78). These associations suggest that positive treatment responses were manifested in the form of integrated enhancement in various elements of crop performance. In general, the correlation matrix outlines the definite performance gradient where the less severe a disease was, the more consistent was the canopy maintenance, the stronger was the reproductive development, the higher was the weight of grains, and the better was the final yield.

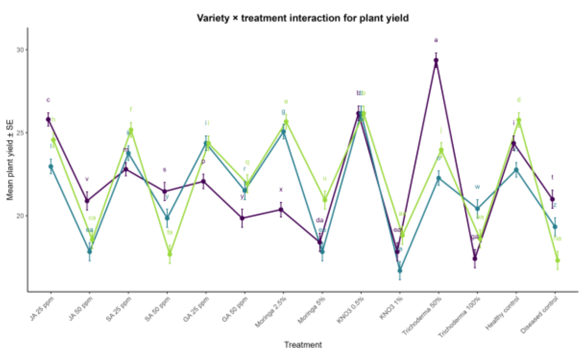


Figure 2. Interaction between variety and treatment in the yield of plant in three varieties of bread wheat receiving seed-priming treatments. The points indicate mean plant yield (g plant^{-1}), and the error bars indicate standard error over three replicas. The variety × treatment combinations differ significantly when different lowercase letters are used and the LSD test is used at P 0.05 or less.

Favorable and unfavorable priming profiles were segregated into treatment level integration

When the means of the treatments were compared in the complete set of traits, the answers had been sorted into two large performance categories instead of a random gradient (Figure 4).

The group of favorable performance was dominated by GA 25 ppm, JA 25 ppm, Trichoderma 50 %, Moringa 2.5, KNO3 0.5 %, and SA 25 ppm. These treatments were found to be always in line with positive standardized values of yield-related traits and have retained the association with lower level of disease severity. In comparison, the less-performance group consisted of disease control and some higher dose treatments, especially Moringa 5 %, KNO3 1%, GA 50 ppm, JA 50 ppm, SA 50 ppm and Trichoderma 100 %.

This cluster effect thus supports a key finding already apparent in the interaction figures: The lower-dose treatments were more frequently linked to the favorable response space, but higher-dose treatments moved more often to the weaker response space.

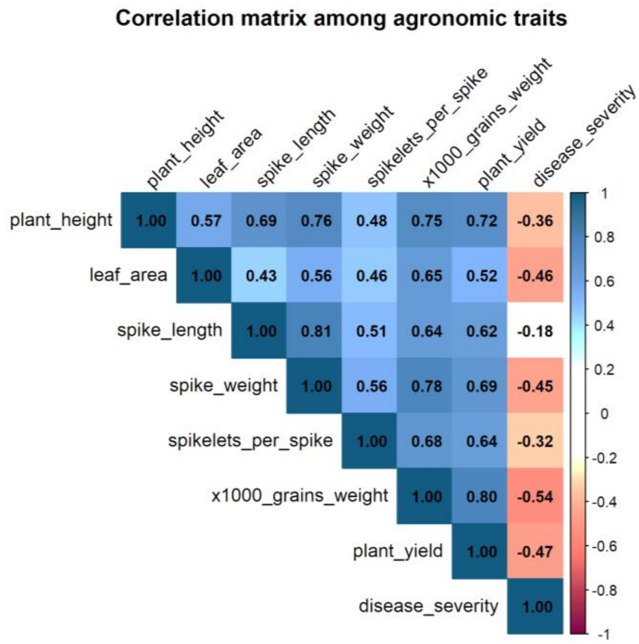


Figure 3. Correlation coefficients between severity of the disease, growth, and yield characteristics of seed-primed wheat under stress of leaf rust. Cell values are Pearson correlation coefficients. The blue color represents positive correlations whereas the red color represents negative correlations.

Multivariate variation was predominated by resistance between disease severity and positive agronomic performance

Principal Component Analysis (PCA) revealed that the overall structure of the dataset was largely governed by a few major biological gradients associated with disease severity and crop performance (Figure 5). The first two principal components accounted for 75.4 % of the total variation, with PC1 and PC2 explaining 63.9 % and 11.5 % of the variation, respectively. This indicates that most of the treatment-induced variability was effectively captured within a two-dimensional space, providing a reliable representation of the relationships among the measured traits.

Severity of the disease was loaded out of the plant yield, 1000-grain weight, leaf area, spike weight, plant height and spike length, but the other agronomic properties were generally oriented in the same direction. There was varietal dispersion in the PCA space, albeit without being absolute. Though the three varieties had slightly different localizations in the ordination, the ellipses were largely overlapping, meaning that they had a common multivariate response framework. Therefore, the differences between the varietal manifestations were more of magnitude of the response and ranking of treatment rather than an absolute division into separate trait syndromes.

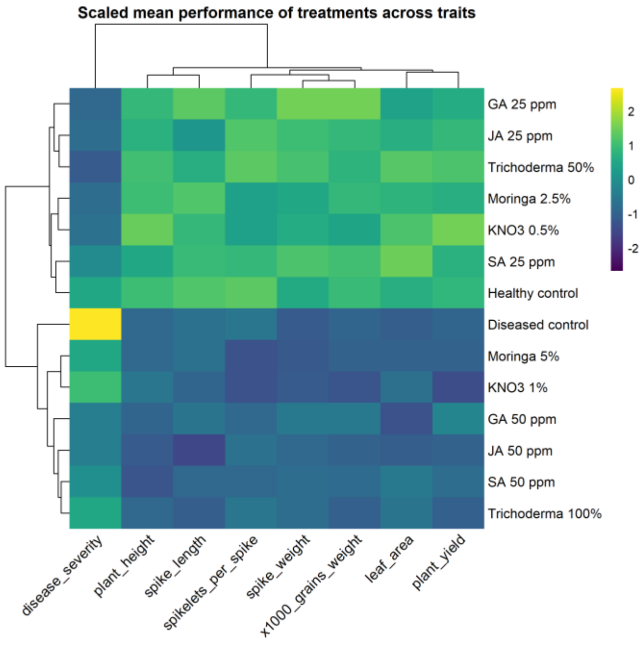


Figure 4. Heat map of scaled mean treatment performance of wheat seed primed with disease, growth, and yield traits. Standardization and clustering of trait values were implemented to compare responses to treatment across variables that had different measurement scales. Colors that are warmer depict a relatively higher standardized value, and the cooler colors depict a relatively lower standardized value.

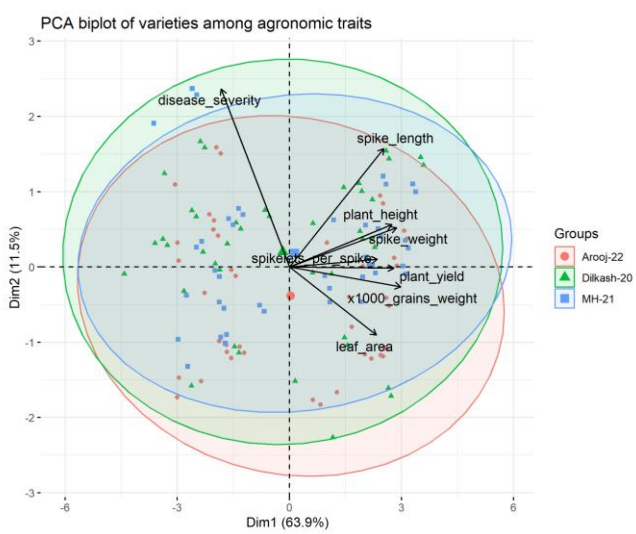


Figure 5. Principal component analysis (PCA) biplot that depicts the multivariate relations between wheat varieties and the measured traits in seed-priming treatments. Points denote single observations, ellipses denote varietal dispersion and arrows denote trait loadings. PC1 and PC2 explained 63.9 % and 11.5 % of the total variation, respectively.

Canopy maintenance was also found to aid in preserving yields, although the strength of this relationship varied among varieties

There was a positive correlation between leaf area and plant yield in the experimental population, which implied that the treatments that maintained a greater photosynthetic surface tended to contribute to high productivity in the presence of leaf rust stress (Figure 6). This relationship offers a physiological correlation of the disease and yield responses, since the treatments which had worked agronomically also worked more well to retain canopy functioning.

The association, however, was not so strong in the three varieties. In Dilkash-20 and MH-21 the positive trend was more pronounced, as the increase in the leaf area was observed with a more evident increase in plant yield. In Arooj-22, the association was positive but not as strong, implying that yield performance in this variety depended on other factors other than maintenance of the leaf-area.

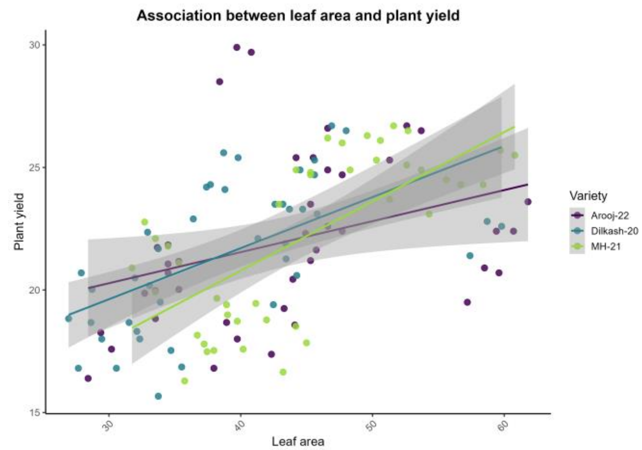


Figure 6. Correlation between plant yield and leaf area in three seed-primed wheat varieties at the conditions of leaf rust. The points denote single observations, fitted lines depict variety-wise linear trends and shaded areas denote 95 % confidence intervals.

Discussion

The current research demonstrates that seed priming did not have a homogenous effect on the leaf rust in experimental system. Relatively the reduction of the disease, yield retention was focused on the selected combination of treatment, and GA 25 ppm and *Trichoderma* 50 % have shown the best overall response and have lower concentrations outperforming high concentrations. This trend is correlated with recent direct evidence that biological and signal-based priming can suppress wheat leaf rust as well as enhance host physiology and productivity. However, the extent of such benefit is highly genotype dependent (17, 18, 19). It is also biologically consistent with available wheat leaf-rust genetics that have demonstrated that resistance is constructed on a variety of host architecture, such as Lr9, Yr87/Lr21-associated recognition and Lr30/Lr.ace-4A which implies that priming treatments are predicted to interact in different ways with different varietal defense backgrounds (20, 21, 22, 23).

One of the most notable findings in the current study was the consistent higher efficacy of lower dose treatment compared to higher dose treatment. This indicates that priming efficacy was dependent upon an optimal response window instead of a linear dose effect. Lack of Zn in wheat may support a hormetic response, but an excess of Zn will be harmful at the ultrastructural and physiological level (24). Similarly, the concentration of the methyl jasmonate responses in wheat is sensitive, and only in a narrow range, there are positive effects (25). In a more general sense, more recent studies of wheat susceptibility regulators indicate that salicylic-acid-linked defense systems can be repressed to enhance compatibility with biotrophic fungi, which supports the notion that defense signaling should be tuned, but not maximized in a blind manner (26, 27, 28). The advantage of lower dose in that case is probably the result of a condition where priming increased defensive preparedness without causing an unduly growth or signal cost.

We also find that the decrease in disease was only converted to agronomic value when reproductive performance was maintained. The severity of the diseases in this experiment had the strongest and most negative relationship with 1000-grain weight and then with the plant yield and leaf area which showed that the main agronomic cost of rust was through grain filling and source functioning rather than a consistent reduction of all structural characteristics. This parallels a current literature indicating that leaf rust may have a potent influence on such traits as grain, in particular, grain weight and yield formation (29). It is also consistent with larger-scale wheat physiology studies that stable yield is maintained through the coordination of source-sink interactions in the process of grain filling and that growth-defense trade-offs are maintained by common pathways, but not independent systems (30, 31). Therefore, the high performance of *Trichoderma* 50 % and the stable nature of KNO3 0.5 % in our experiment is perhaps indicative of the fact that the two treatments were not only suppressive to the pathogen but also spared the crop the ability to continue with it assimilate supply and grain deposition during the stress.

PCA and the heatmap show that the effects of priming were manifested as a unity of biological response and not as the changes in particular traits. The more favorable treatments shared an ordinary performance arena with less seriousness of the disease coupled with improved growth, grain weight, and yield, and the weaker treatments concentrated to the opposite side of the response profile. This type of system-level organization coincides with more recent evidence that useful microbial interventions and other priming strategies can affect several levels of plant functionality simultaneously, such as phytohormone regulation, rhizosphere architecture, stress signaling and productivity (32, 33, 34). It also supports more recent wheat research which has indicated that, in stress conditions, moringa-based or biofortification-based priming can enhance antioxidant status, grain quality, and productivity; and that better treatments are better operating on a performance network, not on a single endpoint (35, 36). The multivariate form in our data thus supports the conclusion that the most effective treatments moved the crop towards a more expanded condition of functional durability other than just reducing noticeable disease severity.

The fact that there is a positive correlation between leaf area and plant yield provides this interpretation with an important physiological aspect. It suggests that priming safeguarded productivity amid leaf rust stress by one way, canopy maintenance. This opinion is highly supported by recent studies on wheat. The characteristics of flag-leaf are highly associated with the potential of yield, the form of the canopy, and the grain structure, and the decrease in photosynthesis, transpiration, stomatal conductance, and the leaf-area index due to rust have been found to be translated into worse crop performance at the canopy scale (37, 38). Our data build upon that framework by revealing that the relationship between canopy and yield was genotype specific: the relationship was steeper in Dilkash-20 and MH-21 than in Arooj-22 suggesting that the equivalent increase in the leaf area did not yield an equivalent agronomic payoff in all three varieties. This is consistent with recent wheat work which demonstrates that source sink relations in grain filling are context sensitive in themselves and that the role of upper-canopy photosynthesis in ultimate grain development is genotype dependent (30, 39).

When combined, the discussion points towards a single consistent result: the stress of priming of the seed under the influence of the stress of leaf rust was viable only in case of the physiological balance and the similarity of the genotype. The most effective treatments in this experiment were not the most robust-input treatments; rather, they were those that enhanced resistance without causing the crop to change to nonproductive growth status. This explains why the chosen lower-dose treatments dominated the desirable response space across the severity of the disease, grain weight, canopy maintenance and final yield. To apply, this implies that the priming strategies are not to be evaluated based on visual disease suppression alone and should not be optimized based on the concentration escalation alone. The most promising therapies are those that match the primer class, the dose and the host genotype in such a way that rust restraint, the canopy functioning, the grain filling and the yield are in a state of coordination (40, 41, 42, 43).

Conclusion

This research shows that seed priming could minimize the severity of leaf rust and safeguard wheat production, yet the impact of the method is highly contingent on the combination of the priming method, concentration, and variety. Improved responses were always linked to the chosen lower dose treatments, especially GA 25 ppm, *Trichoderma* 50 % and KNO3 0.5 %, with other higher-dose treatments remaining relatively ineffective. The findings also revealed that successful priming was correlated not only to reduced disease severity but also maintained leaf area, grain weight and final yield, which means that the merit of priming is in the maintenance of a desirable ratio between resistance and crop performance. Collectively, these results indicate that seed priming ought to be established as a dose-optimal and genotype-specific disease management approach in wheat leaf rust, with a focus being placed on those treatments capable of reducing disease and maintaining yield formation against stress.

Supplementary Data:
Supplementary Figure S1
Supplementary Figure S2
Supplementary Figure S3

Disclaimer: No
Conflicts of Interest: The authors declare no conflict of interest.
Data availability: The data generated and materials used in this study are available from the corresponding author on reasonable request.
Source of Funding:
Ethics approval statement: The authors declare that this research did not involve human participants or animals requiring ethical approval. All experiments were conducted in accordance with institutional guidelines for good laboratory and greenhouse practices. No genetically modified organisms were released into the environment during the course of this study.
Authors' Contribution Statements: Shafique, I.: Writing, original draft preparation, review and editing, Formal analysis. Zulfiqar, M. W.: Writing, original draft preparation, review and editing, Formal analysis. Rehman, M. U.: Writing, original draft preparation, review and editing, Formal analysis. Zulfiqar, L.: Data analysis, review and editing. Abbas, A.: Review and editing. Ali, M. A.: Conceptualization, Validation, Supervision, Resources. All authors have read and approved the final manuscript.

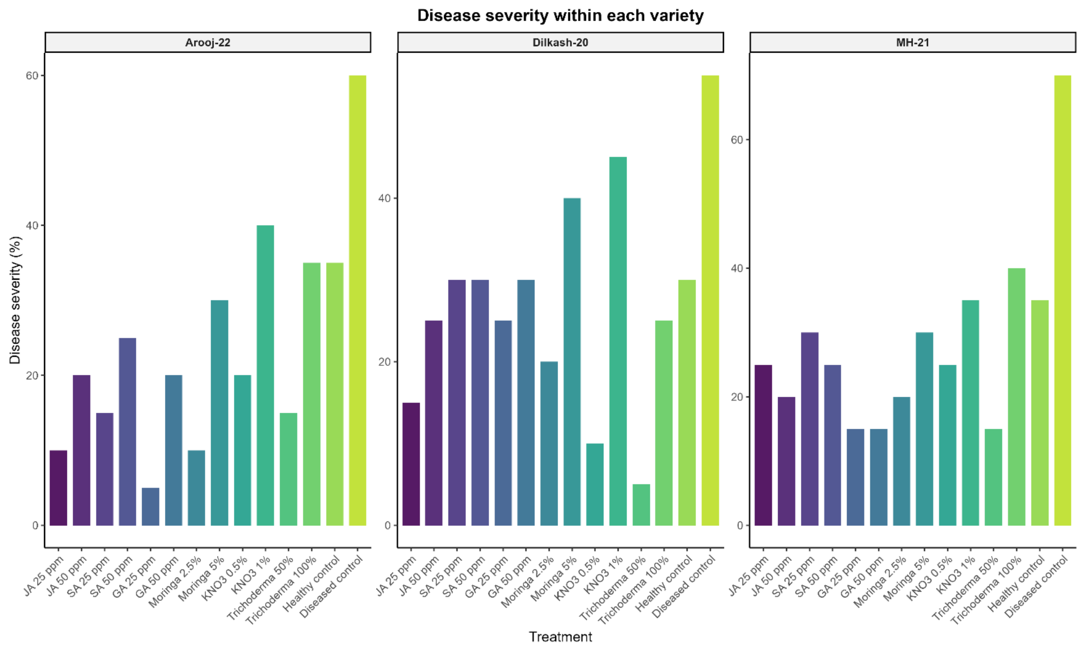
References

1. Figueroa, M., Hammond-Kosack, K. E., & Solomon, P. S. (2018). A review of wheat diseases a field perspective. Molecular plant pathology, 19(6), 1523-1536.
2. Food and Agriculture Organization of the United Nations – FAOSTAT. 2024-25.
3. USDA. Department of Agriculture, Foreign Agricultural Service. (2024-25). Production Commodity 0410000 accessed on 26 September, 2025. https://www.fas.usda.gov/data/production/commodity/0410000.
4. Prikhodko, D., & Zrilyi, O. (2013). Pakistan: Review of the wheat sector and grain storage issues country highlights. Rome: Food and Agriculture Organisation, 1-81.
5. Shafi, U., Mumtaz, R., Shafaq, Z., Zaidi, S. M. H., Kaifi, M. O., Mahmood, Z., & Zaidi, S. A. R. (2022). Wheat rust disease detection techniques: a technical perspective. Journal of Plant Diseases and Protection, 129(3), 489-504.
6. Yousafzai, S. N., Nasir, I. M., Tehsin, S., Malik, D. S., Keshta, I., Fitriyani, N. L., ... & Syafrudin, M. (2025). Multi-stage neural network-based ensemble learning approach for wheat leaf disease classification. IEEE Access.Yousafzai, S. N., Nasir, I. M., Tehsin, S., Malik, D. S., Keshta, I., Fitriyani, N. L., & Syafrudin, M. (2025). Multi-stage neural network-based ensemble learning approach for wheat leaf disease classification. IEEE Access.
7. Kumamaru, T., Ogawa, M., Satoh, H., & Okita, T. W. (2007). Protein body biogenesis in cereal endosperms. In Endosperm: developmental and molecular biology (pp. 141-158). Berlin, Heidelberg: Springer Berlin Heidelberg.
8. Moore, J., & Fung, J. (2016). The complete guide to fasting: Heal your body through intermittent, alternate-day, and extended fasting. Simon and Schuster.
9. Soetan, K. O., Olaiya, C. O., & Oyewole, O. E. (2010). The importance of mineral elements for humans, domestic animals and plants: A review. African journal of food science, 4(5), 200-222.
10. Soko, T., Bender, C. M., Prins, R., & Pretorius, Z. A. (2018). Yield loss associated with different levels of stem rust resistance in bread wheat. Plant Disease, 102(12), 2531-2538.
11. Fahim, M. A., Khadegah, M. A., Thabet, M., Negm, S. S., & El-Deeb, S. H. (2021). Early warning of wheat leaf rust disease and prediction disease status based on the modeling weather. Journal of Applied Plant Protection, 10(1), 87-95.
12. Khalil, H. B., Lutfi, A. M., Sayed, A. R., Mahmoud, M. T., Mostafa, S. A., Ibrahim, Z. A., ... & Thabet, M. (2024). Gamma-Aminobutyric Acid (GABA) as a Defense Booster for Wheat against Leaf Rust Pathogen (Puccinia triticina). Plants, 13(19), 2792.
13. Nihayati, E., & Najah, M. W. (2021). Comparative assessment of the effect of Moringa oleifera leaf extract (MLE) and zeatin on in vitro regeneration response of Pogostemon cablin bud explants. AIMS Agriculture and Food, 6(1), 308–320.
14. Khan, M. N., Z. Khan, T. Luo, J. Liu, M. Rizwan, J. Zhang, and Hu. L 2020. Seed priming with gibberellic acid and melatonin in rapeseed: consequences for improving yield and seed quality under drought and non-stress conditions. Ind. Crops Prod. 156: 112850.
15. Vinale, F., Sivasithamparam, K., Ghisalberti, E. L., Marra, R., Woo, S. L., & Lorito, M. (2008). Trichoderma–plant–pathogen interactions. Soil biology and Biochemistry, 40(1), 1-10.
16. Irshad, R., Aslam, A. H., Tariq, M., Shafique, I., Usman, M., Naveed, M., & Abbas, A. (2026). Synergetic effect of seed-primed biocontrol agents and biochar soil amendment on wheat productivity and leaf rust suppression. Integrative Plant Biotechnology, 4, 73-83.
17. Razzaq, M. U., Shafique, I., Naz, E., Jamil, S., Anas, M., Javed, A., Murtaza, U., & Abbas, A. (2024). Seed priming with bacterial and fungal biocontrol agents alter physio-chemical parameters to suppress leaf rust disease in bread wheat. Integrative Plant Biotechnology, 02, 51–62.
18. Soleimani, B.; Lehnert, H.; Schikora, A.; Stahl, A.; Matros, A.; Wehner, G. Bacterial N-Acyl Homoserine Lactone Priming Enhances Leaf-Rust Resistance in Winter Wheat and Some Genomic Regions Are

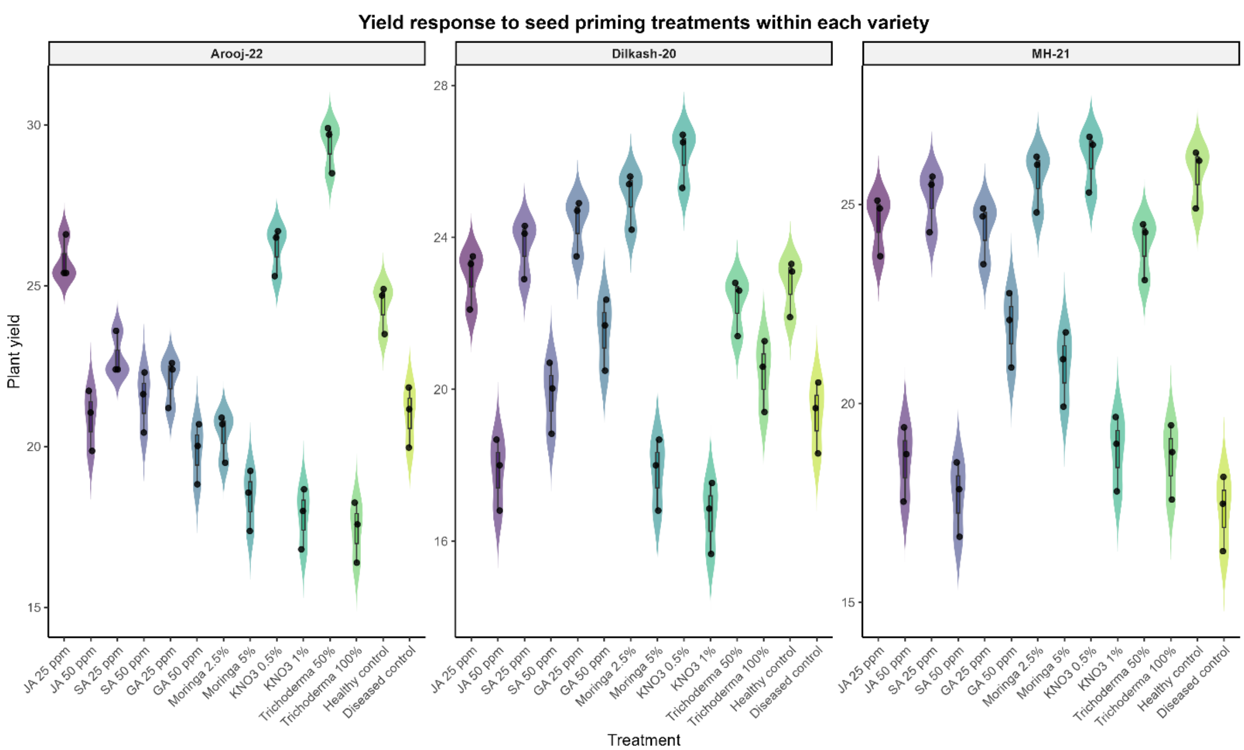
Associated with Priming Efficiency. Microorganisms 2024, 12, 1936.
19. Almanzalawi, E. A. (2026). Seed and foliar application of Trichoderma harzianum HE22 and Bacillus spp. prolong leaf rust latency, induce host defense responses, and improve wheat productivity. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 54(1), 14893-14893.
20. Wang, Y., Abrouk, M., Gourdoupis, S. et al. An unusual tandem kinase fusion protein confers leaf rust resistance in wheat. Nat Genet 55, 914–920 (2023).
21. Sharma, D., Avni, R., Gutierrez-Gonzalez, J. et al. A single NLR gene confers resistance to leaf and stripe rust in wheat. Nat Commun 15, 9925 (2024).
22. Shen, S., Wang, F., Cui, Z., Yuan, S., Meng, L., Liu, D., ... & Wang, H. (2024). Puccinia triticina avirulence protein AvrLr21 directly interacts with wheat resistance protein Lr21 to activate wheat immune response. Communications Biology, 7(1), 1170.
23. Yang, J., Li, H., Li, M., Song, R., Shen, T., Wang, G., ... & Chen, S. (2025). Genome-assisted identification of wheat leaf rust resistance gene Lr. ace-4A/Lr30. Nature Communications, 16(1), 9339.
24. Jiao, Q., Fan, L., Zhang, H., Zhang, J., Jiang, Y., Yang, J., ... & Liu, H. (2025). Transcriptomic and ultrastructural insights into zinc-induced hormesis in wheat seedlings: Glutathione-mediated antioxidant defense in zinc toxicity regulation. Plant Stress, 16, 100820.
25. Zhang, D., Wang, H., Zhang, Y., Su, Z., Hu, T., Liu, J., ... & Ma, L. (2024). Methyl jasmonate enhances the safe production ability of Cd-stressed wheat by regulating the antioxidant capacity, Cd absorption, and distribution in wheat. Plant Physiology and Biochemistry, 212, 108788.
26. Fu, Y., Chen, W., Zhang, M., Wang, X., & Chang, C. (2025). Chromatin remodeler TaSWI3D controls wheat susceptibility to pathogenic fungus Blumeria graminis forma specialis tritici. Microorganisms, 13(12), 2779.
27. Ge, P., Chen, W., Liu, J., Wang, X., & Chang, C. (2025). Wheat DNA methyltransferase TaMET1 negatively regulates salicylic acid biosynthesis to facilitate powdery mildew susceptibility. Journal of Fungi, 11(12), 876.
28. Chen, W., Fu, Y., Zhang, M., Zhao, W., Zhi, P., & Chang, C. (2026). Wheat SWI3B Subunit of SWI/SNF Chromatin Remodeling Complex Governs Powdery Mildew Susceptibility by Suppressing Salicylic Acid Biosynthesis. Journal of Fungi, 12(1), 68.
29. Bakhshi, T., Mehrabi, R., Sarbarzeh, M. A., Türkoğlu, A., Demirel, F., Haliloğlu, K., ... & Bocianowski, J. (2025). Monitoring wheat leaf rust severity using machine learning techniques. Scientific Reports.
30. Fang, L., Struik, P. C., Girousse, C., Yin, X., & Martre, P. (2024). Source–sink relationships during grain filling in wheat in response to various temperature, water deficit, and nitrogen deficit regimes. Journal of Experimental Botany, 75(20), 6563-6578.
31. Liu, S., Liu, H., Guo, M., Pan, Y., Hao, C., Hou, J., ... & Li, T. (2024). Knockout of GRAIN WIDTH2 has a dual effect on enhancing leaf rust resistance and increasing grain weight in wheat. Plant biotechnology journal, 22(7), 2007.
32. Sui, L., Li, J., Philp, J., Yang, K., Wei, Y., Li, H., ... & Wang, Y. (2022). Trichoderma atroviride seed dressing influenced the fungal community and pathogenic fungi in the wheat rhizosphere. Scientific reports, 12(1), 9677.
33. Boamah, S., Zhang, S., Xu, B., Zhu, N., & Li, E. (2025). Trichoderma longibrachiatum TG1 colonization and signal pathway in alleviating salinity and Fusarium pseudograminearum stress in wheat. International Journal of Molecular Sciences, 26(9), 4018.
34. Breedt, G., Korsten, L., & Gokul, J. K. (2025). Enhancing multi-season wheat yield through plant growth-promoting rhizobacteria using consortium and individual isolate applications. Folia Microbiologica, 1-10.
35. Nawaz, H., Rehman, H. U., Ihsan, M. Z., Rizwan, M. S., Hussain, N., Ali, B., ... & Arslan, M. (2024a). Organic seed priming with curtailed seed rate compensated wheat grains productivity by upgrading anti-oxidant status against terminal drought at flowering and milking. Scientific Reports, 14(1), 4941.
36. Nawaz, H., Rehman, H. U., Yousef, M. M., Ali, L., Hussain, N., Abdullah, M., ... & Ayaz, M. (2024b). Zinc-priming via moringa leaf extract (MLE30) achieved Zn biofortified wheat grains and drought tolerance by efficient anti-oxidant status. Plant Stress, 12, 100501.
37. Singh, R. N., Krishnan, P., Singh, V. K., Sah, S., & Das, B. (2023). Combining biophysical parameters with thermal and RGB indices using machine learning models for predicting yield in yellow rust affected wheat crop. Scientific Reports, 13(1), 18814.
38. Kumar, P., Gill, H. S., Singh, M., Kaur, K., Koupal, D., Talukder, S., ... & Sehgal, S. K. (2024). Characterization of flag leaf morphology identifies a major genomic region controlling flag leaf angle in the US winter wheat (Triticum aestivum L.). Theoretical and Applied Genetics, 137(9), 205.
39. Song, K., Hong, S., & Shim, S. (2025). Responses of Photosynthetic Activity in Flag Leaves and Spikes as well as Seed Development of Wheat (Triticum aestivum L.) to Artificial Shading. Agronomy, 15(11), 2577.
40. Porras, R., Miguel-Rojas, C., Lorite, I. J., Pérez-de-Luque, A., & Sillero, J. C. (2023). Characterization of durum wheat resistance against leaf rust under climate change conditions of increasing temperature and [CO2]. Scientific Reports, 13(1), 22001.
41. Atwa, A. A., Ahmed, S. S., El-Aziz, G. H. A., Abou-Zeid, M. A., Omara, R. I., Atwa, N. A., & Fahmy, A. H. (2025). Leaf rust resistance in wheat and interpretation of the antifungal activity of silver and copper nanoparticles. Scientific Reports, 15(1), 9429.
42. Bagwell, J. W., Mergoum, M., Subedi, M., Sapkota, S., Ghimire, B., Lopez, B., ... & Bahri, B. A. (2025). Discovering leaf and stripe rust resistance in soft red winter wheat through genome-wide association studies. The Plant Genome, 18(2), e70055.

43. Mohan, R., Singh, V. K., Chetan, K. K., Rani, L. U., Sameriya, K. K., Kumar, S., ... & Saharan, M. S. (2025). Multiple patho-phenotyping and molecular analysis to characterize wide-spectrum durable leaf rust resistance in wheat collections from India. Frontiers in Microbiology, 16, 1596282.

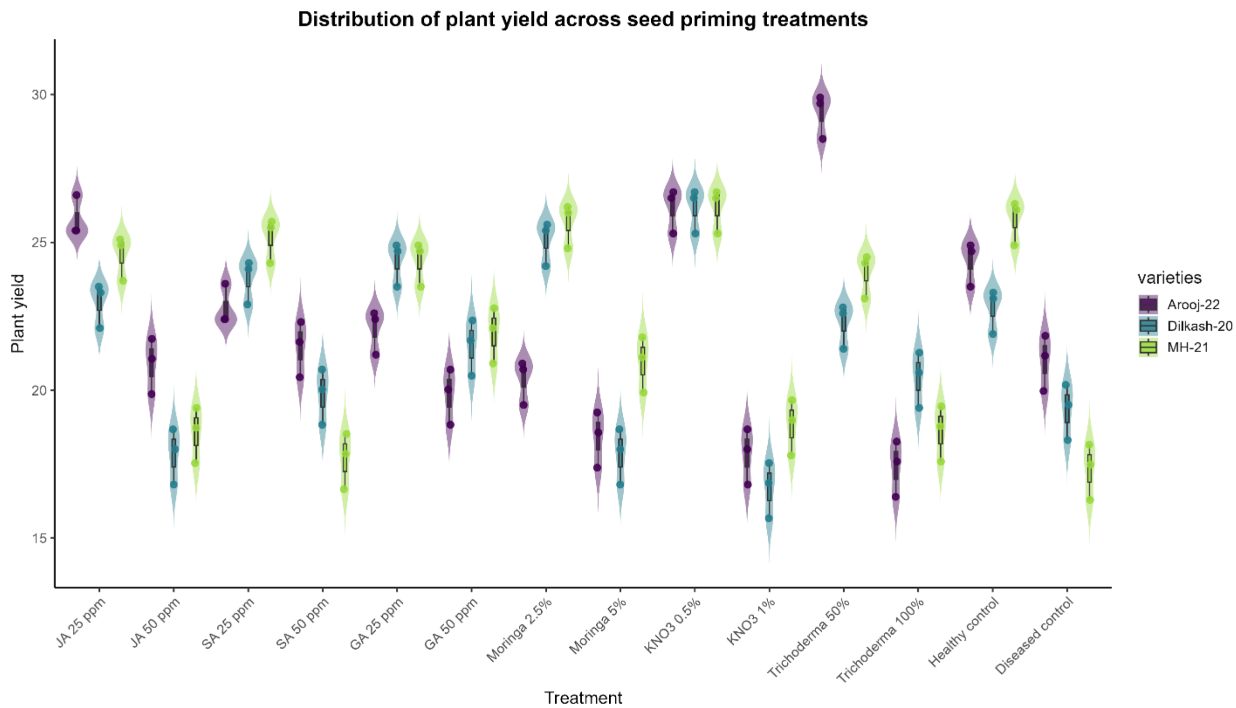
Supplementary Data



Supplementary Figure S1. Variety-wise distribution of leaf rust severity across seed-priming treatments in Arooj-22, Dilkash-20, and MH-21. Bars represent mean disease severity (%) for each treatment within each variety



Supplementary Figure S2. Variety-wise distribution of plant yield across seed-priming treatments in Arooj-22, Dilkash-20, and MH-21. Violin plots summarize the distribution of plant yield within each treatment, with overlaid boxplots and points showing variation among replications.



Supplementary Figure S3. Distribution of plant yield across seed-priming treatments in the three wheat varieties. Violin plots summarize treatment-level dispersion, while overlaid points represent replicate observations.