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EFFECT OF HEAT STRESS ON SPOT BLOTCH SEVERITY AND ITS IMPACT ON YIELD AND RELATED TRAITS IN WHEAT

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ABSTRACT

Wheat productivity is increasingly threatened by abiotic and biotic stresses, as well as their simultaneous occurrence, posing a major challenge to global food security. In this context, the study evaluated eleven contrasting wheat genotypes to assess the effects of heat and disease stresses on growth, yield, and disease development, aiming to identify key traits and resilient genotypes. Analysis of variance showed that genotypes significantly influenced plant height, tiller number, spike length, yield, biomass, AUDPC, and diseased grain weight, while treatments significantly affected all traits, with genotype × treatment interactions evident for most growth and yield traits. Stress treatments reduced plant height, spike length, greenness, and grain filling duration, with combined stress causing the most severe reductions. Biomass and AUDPC were particularly sensitive to environmental conditions, whereas growth and yield traits were primarily genotype-driven. Stress Susceptibility Index (SSI) analysis revealed substantial genotypic variation in tolerance: CHIRYA-3 and DH1-113 exhibited consistently low SSI values under all stress conditions, while DH1-132, Sonara-64, and DH1-253 were highly susceptible. These findings highlight the importance of selecting genetically resilient genotypes and provide insights for breeding wheat cultivars capable of maintaining productivity under multiple concurrent stresses.

***Keywords* :** Disease, Heat, Stress Susceptibility Index, Wheat.

Introduction

Wheat (*Triticum aestivum* L.) is the most widely grown cereal crop worldwide and plays a crucial role in ensuring global food and nutritional security. In South Asia, where wheat is a major staple, its production is increasingly threatened by the combined effects of abiotic and biotic stresses (Sharma *et al.*, 2018). Among these, heat stress during the reproductive and grain-filling stages and spot blotch disease caused by *Bipolaris sorokiniana* (Sacc.) Shoem. are two of the most significant constraints limiting wheat productivity in the warmer and more humid regions of the Eastern Gangetic Plains, Nepal, Bangladesh, and parts of China (Duveiller *et al.*, 2005a; Singh *et al.*, 2016).

Spot blotch has emerged as a major foliar disease in these environments, especially under rising temperatures that create favorable conditions for rapid pathogen development. The disease produces necrotic lesions on leaves and sheaths, accelerating leaf senescence and severely restricting the grain-filling period (Sharma & Duveiller, 2007). Yield losses typically range from 15–25% but can exceed 40% under severe epidemics (Chaurasia *et al.*, 2000; Meena *et al.*, 2014). In addition to yield decline, the disease adversely affects grain quality and market value, posing a major challenge for wheat growers in disease-prone regions (Joshi *et al.*, 2004).

Heat stress further compounds this problem by disrupting wheat physiology and reducing photosynthetic efficiency during critical phenological stages. When temperatures rise beyond the optimum (above 25–30°C during anthesis and grain filling), wheat experiences shortened developmental phases, reduced chlorophyll stability, and impaired assimilate translocation, ultimately leading to reduced biomass and grain yield (Joshi *et al.*, 2007a). Heat stress also weakens plant defense responses, making the crop more vulnerable to foliar diseases such as spot blotch, which thrive under warm and humid conditions (Duveiller & Sharma, 2009). This overlap of conducive temperatures and pathogen pressure results in a synergistic negative effect, intensifying disease severity and accelerating crop damage.

Sowing time plays a pivotal role in determining the degree of exposure to both heat stress and spot blotch. Early or timely sowing allows the crop to complete reproductive stages under relatively cooler conditions, reducing the likelihood of disease establishment. In contrast, late sowing pushes heading and grain filling into warmer months, increasing susceptibility to both heat stress and spot blotch infection (Sharma *et al.*, 2007; Pandey *et al.*, 2021). Studies from South Asia consistently report higher spot blotch severity and substantial yield reductions in late-sown wheat as compared to timely sown crops (Biswas & Srivastava, 2022; Pant *et al.*, 2024).

Climate change, characterized by rising minimum temperatures, erratic rainfall, and increased humidity, further intensifies the combined threat of heat stress and spot blotch (Aditya *et al.*, 2024). As winter temperatures continue to increase across South Asia, wheat crops are expected to face greater exposure to terminal heat stress, thereby expanding the geographical and seasonal window favorable for spot blotch epidemics (Sharma *et al.*, 2018). This convergence of abiotic and biotic pressures poses a serious threat to wheat productivity and regional food security.

In this context, understanding the interaction between heat stress and spot blotch disease becomes essential for developing climate-resilient wheat production strategies. The present study investigates how heat stress imposed through late sowing modulates spot blotch severity and how this combined impact influences yield and related agronomic traits in wheat. By evaluating wheat genotypes under controlled and stress-induced environments, the study aims to elucidate the extent to which heat stress exacerbates disease progression and reduces crop performance. Insights from this research will support the identification of genotypes capable of tolerating combined stresses, refine sowing recommendations, and aid in developing integrated management practices suitable for spot blotch-endemic and heat-prone regions.

Materials and Methods

Plant Material and Experimental Site

Ten wheat genotypes with contrasting levels of resistance to spot blotch (*Bipolaris sorokiniana*) were used in this study. The experiment was conducted under controlled conditions in a polyhouse at Department of Agricultural Biotechnology and Molecular Biology, Dr. Rajendra Prasad Central Agricultural University, Pusa, Bihar, India during the wheat-growing season of 2021-22. The polyhouse provided protection from natural rainfall and facilitated controlled management of temperature and humidity to ensure uniform disease development.

Experimental Design and Treatments

The experiments were conducted using a randomized block design with three replications in each environment. Each genotype was sown in a plot consisting of six rows, 2.5 m in length, with 22.5 cm spacing between rows. The net plot area was maintained at approximately 3.4 m². Standard agronomic practices were followed to ensure uniform crop establishment and growth across environments.

To capture genotype and treatment interactions, the genotypes were evaluated under four distinct treatments:

Control: Normal sowing (second fortnight of November), representing optimum temperature conditions.

Heat stress: Late sowing (third fortnight of December), imposing heat during anthesis and grain filling.

Disease: Normal sowing coupled with artificial inoculation of *Bipolaris sorokiniana* to induce foliar blight infection.

Combined stress: Late sowing accompanied by artificial inoculation, simulating simultaneous heat and foliar blight stress.

Uniform management practices, including fertilizer application (120:60:40 kg N:P:K ha^{-1}), irrigation scheduling, and weed control, were applied across treatments. Irrigation was withheld at the post-anthesis stage in heat-stressed plots to enhance thermal exposure.

Pathogen Inoculation and Disease Development

A virulent isolate of Bipolaris sorokiniana was obtained from naturally infected wheat leaves and maintained on potato dextrose agar (PDA) at 25 ± 2°C. Conidial suspension was prepared in sterile distilled water at a concentration of 1×10^4 spores mL^{-1}, and a few drops of Tween-20 (0.05%) were added as a surfactant to ensure uniform foliar coverage. Plants were inoculated at the tillering stage (Zadoks 21–23) by spraying the spore suspension until leaves were fully wetted. To promote infection, plants were kept under high humidity (≈90%) for 24 hours post-inoculation by covering with transparent plastic sheets and maintaining optimal polyhouse temperature (20–28°C).

Data Collection

The following morphological, physiological, and disease-related traits were recorded:

(a) Plant Height (cm): Measured from the base of the plant to the tip of the spike at physiological maturity.

(b) Tiller: The number of tillers per plant was recorded by counting all productive tillers at the maturity stage. The mean tiller number per genotype was calculated from the total tillers of sampled plants.

(c) Spike Length (cm): Spike length was measured from the base of the spike (excluding awns) to the tip at physiological maturity. The mean spike length per genotype was calculated from multiple representative plants.

(d) Grain Filling Duration (days): Grain filling duration was calculated as the number of days from anthesis (when ~50% of spikes showed visible anthers) to physiological maturity (complete loss of spike greenness). The duration was recorded for each genotype under all treatments.

(e) Grain Yield per plot (g): Total grain weight per plot was measured after harvest.

(f) Biomass: Above-ground biomass was determined by harvesting the plants at maturity, drying them at 45 °C to a constant weight, and recording the dry weight. The average biomass per plant was then computed for each treatment.

(g) Disease Severity: Disease progression was assessed weekly after inoculation, and the Area Under Disease Progress Curve (AUDPC) was calculated using the formula of Saari and Prescott, (1975)

(h) Greenness: Leaf chlorophyll content was estimated at the reproductive stage using a SPAD meter (Minolta, Japan), for greenness, where higher values indicated better photosynthetic health.

(i) Diseased Grain Weight (g): Grains showing visible spot blotch symptoms were separated after harvest and weighed to quantify disease-affected grain. The mean diseased grain weight per genotype was calculated for each treatment.

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) using R version 4.2.0 to evaluate the effects of genotype, treatment, and their interaction on the measured traits. Boxplots were generated for each treatment, and comparisons with the control were performed to determine statistical significance. The Stress Susceptibility Index (SSI) was calculated for heat, disease, and combined stress conditions using the formula:

$$\mathrm{SSI} = \frac{(1 - \mathrm{Y}/\mathrm{Y_P})}{(1 - \mathrm{X}/\mathrm{X_P})}$$

Where:

Y = mean performance of a genotype under stress,

Yp = mean performance of the same genotype under control condition,

X = mean performance of all genotypes under stress,

Xp = mean performance of all genotypes under control condition.

For each trait, genotypes were classified based on SSI values: green indicated resistant, yellow moderately resistant, and red susceptible genotypes, following Mishra *et al.* (2024).

Results

Effects of Genotypes and Treatments on Growth, Yield, and Disease Traits

The effects of genotypes, treatments, and their interaction on key agronomic and disease-related traits were assessed using analysis of variance (ANOVA) to identify the relative contributions of genetic and environmental factors. The results revealed that genotypes had highly significant effects ($p < 0.001$) on plant height (PH), tiller, spike length (SL), yield (YD), biomass (BM), AUDPC, and Diseased grain weight (DGW), while grain number (GN) was significant at $p < 0.05$ and grain filling duration (GFD) was not significant. Treatments (T) significantly influenced all traits ($p < 0.001$), and the G × T interaction was significant for PH, tiller number, SL, YD, BM, AUDPC, and DGW, indicating differential genotypic responses to treatments, whereas GFD and GN were unaffected. Among the traits, BM and AUDPC showed the largest treatment effects, highlighting their sensitivity to environmental conditions, while growth and yield-related traits such as PH, Tiller, and DGW were predominantly driven by genotypic differences (Table 1). These findings suggest that, although management practices can substantially modify biomass and disease response, inherent genetic variation primarily governs growth and yield traits.

Differential Trait Responses to Stress Treatments

The impact of stress treatments on various agronomic and physiological traits was significant (Figure 1). PH, SL, GFD, and greenness all showed a marked decline under all stress treatments compared to the control ($p < 0.001$). Tiller number and yield was unaffected by Heat stress alone but were significantly reduced by disease and combined stresses. Biomass followed a similar trend, with significant reductions under disease and combined stress, while Heat alone had no significant effect. AUDPC increased significantly under disease and combined stress conditions but not under Heat stress alone. Interestingly, diseased grain weight was not significantly affected across all stress treatments (Figure 1). These findings defining that combined heat and disease stresses exert the most severe negative effects on plant growth and productivity, underscoring the importance of managing multiple stress factors simultaneously.

Stress Susceptibility Index (SSI) of Genotypes Under Heat, Disease, and Combined Stress

(a) Heat Stress

The heatmap of the SSI under heat stress revealed considerable variation among genotypes across all agronomic and disease-related traits. SSI values ranged from 0.2 to 4, with green indicating low susceptibility, yellow moderate susceptibility, and red high susceptibility. Genotypes such as CHIRYA-3 and DBW-252 exhibited predominantly green and yellow cells, reflecting relatively low SSI values and greater resilience to heat stress, particularly for SL. DRW-187 was the only genotype that showed low SSI specifically for GFD. In contrast, DH1-132 and HD-3086 displayed high SSI values, especially for BM and GN, as indicated by the red coloration, demonstrating strong vulnerability to heat stress. DH1-116 and DH1-217 also showed pronounced susceptibility for GFD. Considering the average SSI across traits, DH1-132, DH1-217, and HD-3086 emerged as the most affected genotypes, whereas DBW-252, DH1-113, and DH1-253 were among the least affected. Traits such as SL, GFD, BM, and GN exhibited substantial variability, with several genotypes showing high SSI values, indicating their sensitivity to heat stress (Figure 2).

(b) Disease Stress

Under disease stress, the SSI heatmaps revealed substantial genotypic variation, with susceptibility levels clearly distinguished by the green-to-red color gradient. CHIRYA-3 and DH1-116 showed consistently low SSI values for major traits, including BM, indicating strong disease tolerance, whereas DH1-132, DH1-253, and HD-2967 displayed red-colored cells reflecting high susceptibility. DH1-132 and DH1-195 exhibited extremely high SSI values for SL, while Sonara-64 showed elevated susceptibility in PH and tiller number. Some genotypes, such as DH1-116, DH1-132, and HD-3086, demonstrated mixed responses, with low or even negative SSI in certain yield traits but moderate to high susceptibility for BM, highlighting trait-specific disease sensitivity. Among all measured traits, SL, BM, and yield were the most affected under disease stress, whereas GFD and GN displayed comparatively moderate variability. Overall, CHIRYA-3 and DH1-113 emerged as relatively disease-tolerant genotypes, while DH1-132 and Sonara-64 were identified as among the most susceptible (Figure 3).

(c) Combined Stress

Combined stress caused pronounced increases in SSI values across traits, revealing strong genotypic differences in stress tolerance. Genotypes such as CHIRYA-3, DH1-113, DH1-195, and HD-3086 exhibited consistently low to moderate SSI values for traits like PH and yield, as indicated by green and yellow cells, demonstrating good tolerance to simultaneous stress conditions. In contrast, DH1-132 and Sonara-64 showed very high SSI values across multiple key traits including BM, GN, SL, and PH, highlighted by red coloration, reflecting pronounced susceptibility. Genotypes such as DBW-187 and DH1-217 displayed susceptibility for yield and BM but showed mixed or trait-specific vulnerability in other traits such as tiller number and SL. Overall, BM, GN, and SL emerged as the most affected traits under combined stress, whereas PH and DGW exhibited comparatively moderate variation. Based on average SSI across all traits, CHIRYA-3 and DH1-113 were identified as the most resilient genotypes, while DH1-132, Sonara-64, and DH1-253 showed heightened susceptibility information valuable for breeding genotypes with enhanced resilience to multiple concurrent stresses (Figure 4).

Discussion

Wheat production is increasingly constrained by the simultaneous occurrence of abiotic and biotic stresses, particularly heat stress and foliar blight caused by *Bipolaris sorokiniana*, both of which significantly reduce grain yield and quality in major wheat-growing regions (Farooq *et al*., 2011; Duveiller *et al*., 2005b). Heat stress accelerates phenological development, shortens GFD, and limits BM accumulation (Dias & Lidon, 2009), while foliar blight compromises photosynthetic capacity, reducing grain weight (Sharma & Duveiller, 2006). The combined effect of these stresses is often more severe than their individual impacts (Joshi *et al.*, 2007b), creating major challenges for maintaining stable wheat productivity under changing climatic conditions. Therefore, understanding how wheat genotypes respond to heat, disease, and their interaction is crucial for developing resilient cultivars (Aisawi *et al.*, 2015).

In this study, a randomized block design with four treatments optimal conditions, heat stress, foliar blight, and their combination was used to dissect the effects of individual and combined stresses on wheat growth, yield, and disease traits. The ANOVA revealed highly significant genotypic effects for most traits, indicating substantial genetic variability, which is essential for breeding stress-resilient genotypes. Consistent with previous studies (Reynolds *et al.*, 2012), traits such as PH, tiller, and grain weight varied strongly among genotypes. Interestingly, GFD was not significantly influenced by genotype, suggesting that it is more environmentally plastic than genetically controlled, in agreement with reports that heat strongly affects grain filling (Dias & Lidon, 2009).

Treatment effects were significant for all traits, particularly BM and AUDPC, highlighting wheat's sensitivity to heat (which accelerates senescence and limits assimilate production) and foliar blight (which damages leaf area and disrupts photosynthesis) (Farooq *et al.*, 2011; Duveiller *et al.*, 2005c). DGW was also strongly reduced by stress, consistent with earlier findings that biotic stress impairs grain filling and nutrient translocation (Duveiller & Sharma, 2006). The significant genotype × treatment (G × T) interactions observed for PH, tiller, SL, yield, BM, AUDPC, and DGW indicate that no single genotype performs optimally across all conditions, emphasizing the importance of multi-environment evaluation in breeding programs (Reynolds & Langridge, 2016). Conversely, the absence of G × T interactions for GFD and grain number suggests that these traits are relatively stable across stress conditions, supporting previous findings that strong genetic potential can buffer environmental effects (Aisawi *et al.*, 2015).

Under heat stress, the variation in SSI values among genotypes and traits underscores the complex genetic architecture of thermotolerance in wheat. Genotypes such as CHIRYA 3 and DBW 252, which exhibited relatively low SSI for spike length, demonstrate resilience, reflecting the utility of SSI as an effective tool for identifying heat-tolerant lines (Kumar *et al.*, 2024). The physiological basis for this tolerance likely involves maintenance of photosynthetic efficiency and reduced accumulation of reactive oxygen species (ROS) (Zheng *et al.*, 2025). In contrast, genotypes like DH1 132 and HD 3086 showed high SSI for biomass and grain number, highlighting the vulnerability of reproductive traits to terminal heat stress, in line with prior studies showing severe penalties on grain development under elevated temperatures (Lamba *et al.*, 2023).

Similarly, under disease stress, SSI profiles varied markedly among genotypes, reflecting strong genotype × trait interactions in pathogen tolerance. CHIRYA-3 and DH1-116 consistently exhibited low SSI for BM and yield traits, indicating effective disease resilience, whereas DH1-132, DH1-253, and HD-3086 were highly susceptible, particularly for SL, BM, and grain yield. These findings suggest that disease tolerance is trait-specific and may not provide uniform protection across phenotypes, aligning with broader insights that biotic stress responses are complex and multidimensional (Pandey *et al.*, 2017). Divergent SSI values across traits may arise from differential activation of defense pathways, resource re-allocation, and cross-talk among molecular signaling networks, including phytohormones and ROS. This underscores the importance of multi-trait phenotyping to reliably identify genotypes with genuine and stable disease tolerance.

Under combined heat and disease stress, SSI values were generally amplified, indicating synergistic or additive effects of concurrent stresses. Genotypes such as CHIRYA-3, DH1-113, and DH1-195, which maintained moderate to low SSI for key traits like plant height and yield, emerged as promising candidates for multi-stress resilience. Conversely, DH1-132 and Sonara-64 displayed very high SSI across multiple traits, highlighting their vulnerability under stress interactions. These observations align with reports that combined stresses often result in non-additive, compounded effects on plant performance (Pandey *et al.*, 2017). The resilience of tolerant genotypes likely stems from integrated genetic and physiological networks regulating ROS scavenging, stress signaling, and cellular homeostasis (Zheng *et al.*, 2025; Frontiers editorial on stress interaction).

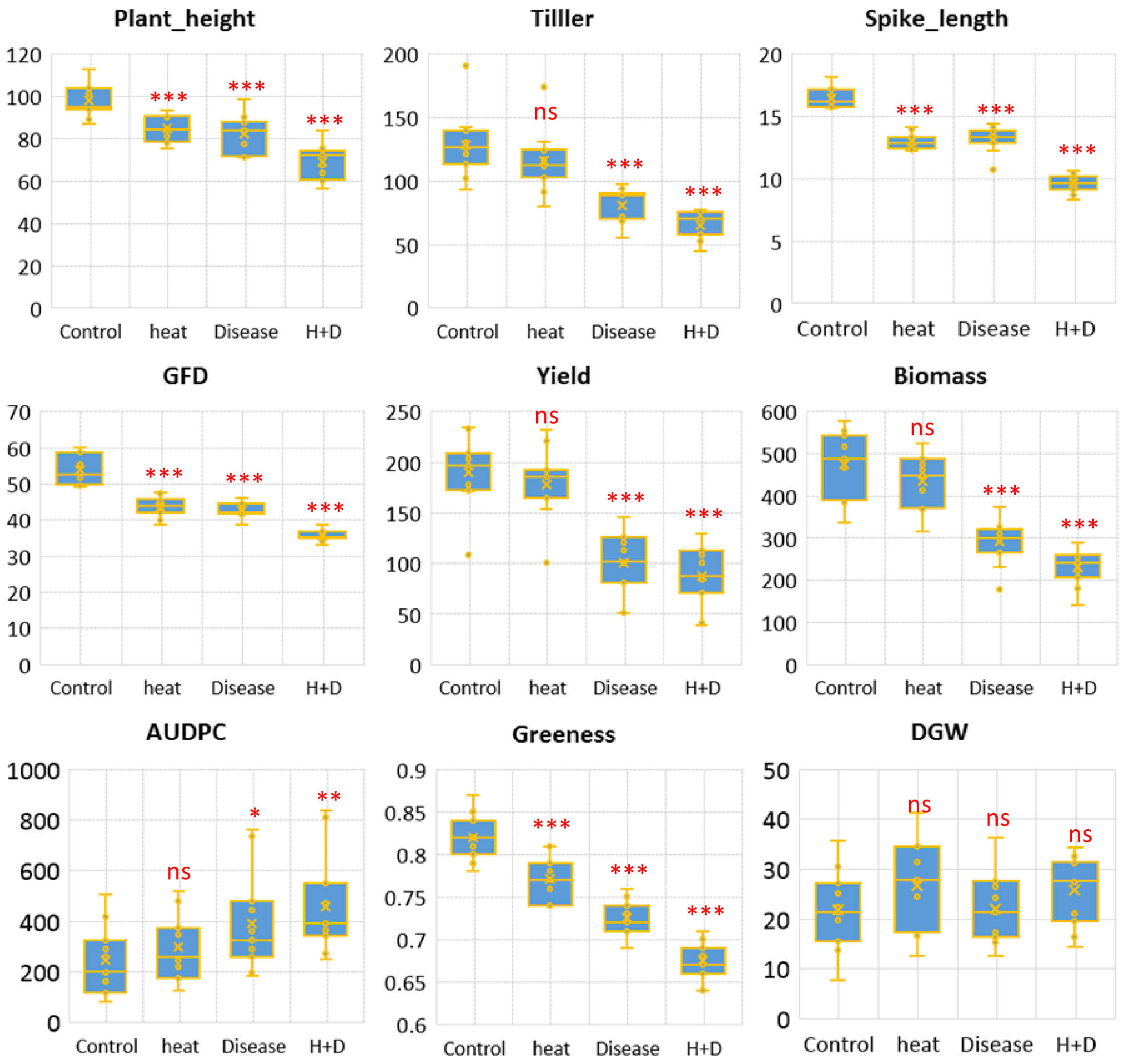
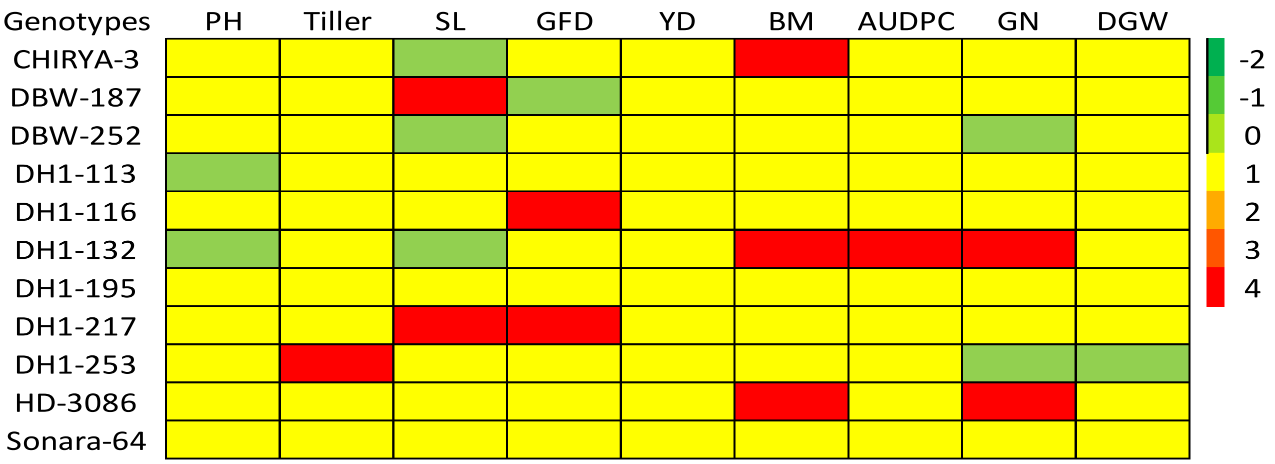


Fig. 1 : Effects of individual and combined stress treatments on agronomic and physiological traits in wheat. Box plots represent the distribution of trait values under four conditions.



Genotypes	PH	Tiller	SL	GFD	YD	BM	AUDPC	GN	DGW
CHIRYA-3	🟨	🟨	🟩	🟨	🟨	🟥	🟨	🟨	🟨
DBW-187	🟨	🟨	🟥	🟩	🟨	🟨	🟨	🟨	🟨
DBW-252	🟨	🟨	🟩	🟨	🟨	🟨	🟨	🟩	🟨
DH1-113	🟩	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
DH1-116	🟨	🟨	🟨	🟥	🟨	🟨	🟨	🟨	🟨
DH1-132	🟩	🟨	🟩	🟨	🟨	🟥	🟥	🟥	🟨
DH1-195	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
DH1-217	🟨	🟨	🟥	🟥	🟨	🟨	🟨	🟨	🟨
DH1-253	🟨	🟥	🟨	🟨	🟨	🟨	🟨	🟩	🟩
HD-3086	🟨	🟨	🟨	🟨	🟨	🟥	🟨	🟥	🟨
Sonara-64	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨

Fig. 2 : Heatmap of Stress Susceptibility Index (SSI) under heat stress conditions, with color coding from green (low susceptibility) to red (high susceptibility). Genotypic variation in SSI across traits indicates differing levels of heat stress tolerance (-2 to 4).

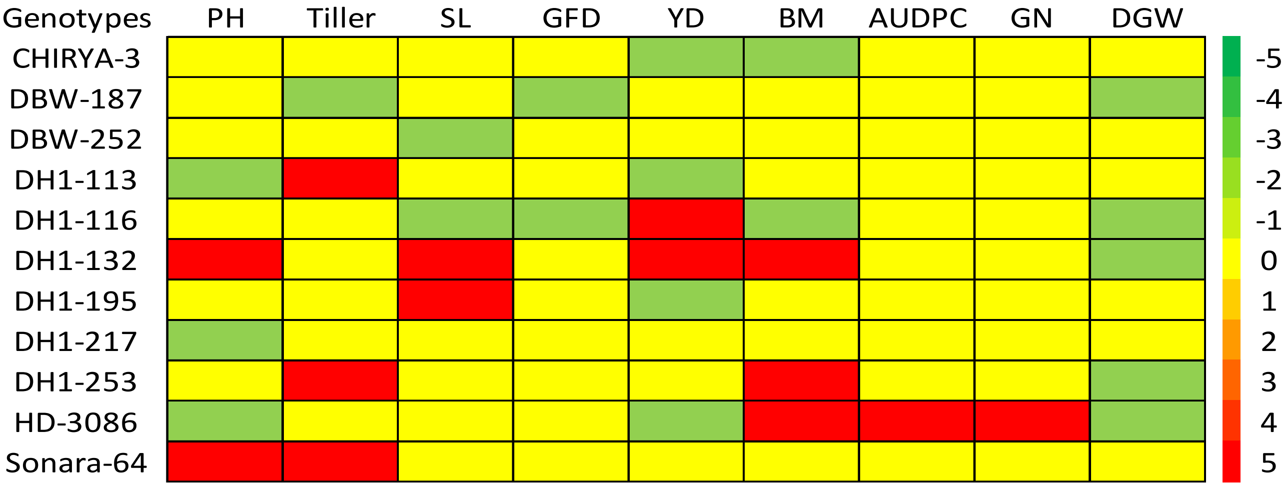


Fig. 3 : Heatmap of Stress Susceptibility Index (SSI) under disease conditions, with color coding from green (low susceptibility) to red (high susceptibility). Genotypic variation across traits

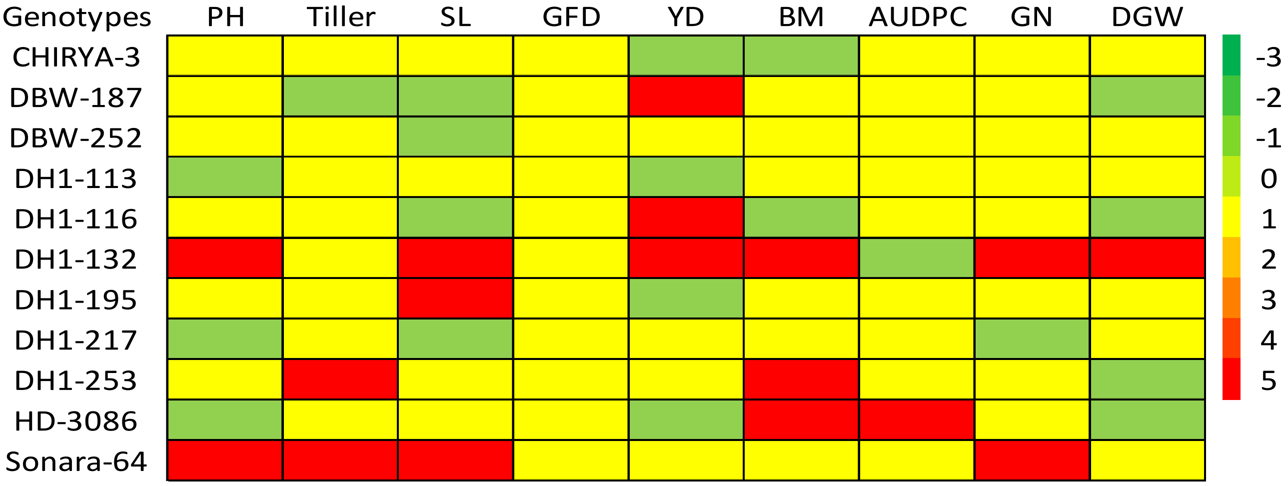


Fig. 4 : Heatmap of Stress Susceptibility Index (SSI) under combined stress, with color coding from green (low susceptibility) to red (high susceptibility). Genotypic variation across traits

Table 1: Analysis of variance for agronomic and disease related traits

Source of variation	df	PH	Tiller	SL	GFD	YD	BM	AUDPC	GN	DGW
Genotypes (G)	10	5670.89***	35028.1***	47.02***	395.18	95891.89***	297202.90***	3023767***	0.034364*	4410.54***
Treatments (T)	3	14028.12***	85047.48***	772.17***	5661.95***	272839.23***	1313677***	497918***	0.386614***	629.65***
G + T	30	1883.83*	12037.64***	30.41*	543.51	33253.78***	132166***	790743.2***	0.035661	2709.99***
Residual	88	3218.04	5080.14	53.65	2262.27	5650.28	56476.62	44478.28	0.130333	145.7

Note: ***significant P<0.001; **significant P<0.01; *significant at P<0.05

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