

The 2NS Chromosomal Translocation Enhances Redox Homeostasis and Mitigates Oxidative Stress during Magnaporthe oryzae Triticum Infection in Wheat

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Abstract

Wheat blast, caused by the hemibiotrophic fungus *Magnaporthe oryzae Triticum* (MoT), is a destructive disease that poses a severe threat to global wheat production. The 2NS chromosomal translocation, introgressed from *Aegilops ventricosa* into the Bangladeshi wheat variety BARI Gom 33 (BG33), confers moderate-to-high resistance to MoT under field conditions. Despite its widespread deployment, the molecular mechanisms underlying this 2NS-mediated resistance remain largely unknown. This study aimed to elucidate the physiological and biochemical bases of resistance in BG33, specifically regarding its capacity to counteract infection-induced oxidative stress. Comparative analysis between the resistant variety (BG33) and a susceptible variety (BARI Gom 26, BG26) revealed that BG33 maintained significantly lower accumulation of reactive oxygen species (ROS), including hydrogen peroxide (H₂O₂), and exhibited reduced lipid peroxidation (malondialdehyde, MDA) and lipoxygenase (LOX) activity post-inoculation. BG33 also retained higher photosynthetic pigment integrity (chlorophyll and carotenoids), indicating superior protection against oxidative cellular damage. Crucially, BG33 displayed enhanced constitutive and MoT-induced antioxidant activity; basal levels of catalase (CAT), peroxidase (POD), glutathione peroxidase (GPX), ascorbate peroxidase (APX), glutathione-S-transferase (GST), and proline were 1.3–2.5-fold higher in BG33 than in BG26. Upon MoT infection, BG33 further upregulated enzymatic antioxidants including superoxide dismutase (SOD), CAT, APX, GPX, glutathione reductase, dehydroascorbate reductase, and monodehydroascorbate reductase and proline by 1.2–2.0-fold, establishing a robust state of redox homeostasis that was absent in BG26. These findings establish, for the first time, that the 2NS translocation bolsters MoT resistance by potentiating a multi-tiered antioxidant defense system to mitigate the oxidative burst and preserve cellular function. This study provides a novel mechanistic framework for leveraging antioxidant pathways in the development of more durable and resilient blast-resistant wheat varieties.

Background

Wheat (*Triticum aestivum* L.) is essential for global food security, accounting for approximately 20% of the caloric energy and 25% of dietary proteins consumed by humans worldwide. To meet the projected 60% increase in demand by 2050, wheat yields must rise sustainably amid escalating threats from climate change and emerging pathogens (USDA, 2022). Among these threats, wheat blast caused by the hemibiotrophic fungus *Magnaporthe oryzae Triticum* (MoT) stands out as a devastating disease. First reported in Brazil in 1985, wheat blast has since spread to many South American countries and recently to South Asia (Bangladesh, 2016) and Southern Africa (Zambia, 2020), causing yield losses of up to 100% under conducive conditions (Islam et al., 2016; Tembo et al., 2020). Its potential expansion into major wheat-producing regions like India and China, driven by climate variability and global trade, underscores the urgency of developing durable resistance strategies (Kamoun et al., 2019). Using wheat crop simulation and newly developed wheat blast model, Pequeno et al. (2024) estimated that wheat blast can reduce global wheat production by 69 million tons per year (13% decrease) by mid-century. Genetic resources for wheat breeding and molecular mechanism of wheat-MoT interactions are scarcely available (Islam et al. 2020; Bhattacharjee et al. 2025).

A critical line of defense lies in genetic resistance. The 2NS translocation, a chromosomal segment introgressed from the wild grass *Aegilops ventricosa*, has been widely deployed in wheat breeding since the 1990s for its broad-spectrum resistance to pests and diseases, including nematodes, rusts, and powdery mildew (Cruz et al., 2016; Gao et al., 2021). Recent studies suggest that 2NS also confers moderate resistance to wheat blast in field conditions (Cruz and Valent, 2017; Islam et al., 2020). For instance, the Bangladeshi variety BARI Gom 33 (BG33), harbouring the 2NS segment, exhibits a considerable levels of blast resistance and terminal heat tolerance (Hossain et al., 2019; Mohi-Ud-Din et al., 2022). Stacking of a newly cloned blast-resistance gene, *Rmg8* into the 2NS background is thought to lead a durable blast-resistant wheat variety to combat fearsome wheat blast disease in Asia, Africa and South America (Asuke et al. 2024; Islam and Azad 2024). However, the molecular mechanisms underpinning 2NS-mediated blast resistance remain unresolved. Notably, while the translocation is known to carry uncharacterized resistance-associated genes (Kolmer et al., 2007), its role in modulating host-pathogen interactions at the biochemical level, particularly during oxidative stress has not been explored.

Plant defense against pathogens involves a tightly regulated oxidative burst, where reactive oxygen species (ROS) serve as dual-edged swords: they are critical for antimicrobial signaling and cell wall reinforcement but can trigger catastrophic oxidative damage if unchecked (Torres et al., 2006; Mittler, 2017). To maintain redox homeostasis, plants deploy enzymatic antioxidants (e.g., superoxide dismutase [SOD], catalase [CAT], ascorbate peroxidase [APX]) and non-enzymatic compounds (e.g., proline, glutathione) to scavenge ROS and repair cellular damage (Mittler, 2017; Hasanuzzaman et al., 2018). Proline, in particular, is a multifunctional osmolyte that stabilizes membranes, detoxifies methylglyoxal, and enhances antioxidant capacity under biotic and abiotic stress (Christgen and Becker, 2019). Recent studies suggest that ROS management and antioxidant activity are pivotal in wheat blast resistance by the genotype S615 carrying *Rmg8* gene (Islam et al. 2025), yet their role in 2NS-associated blast resistance remains unknown.

We hypothesize that the 2NS translocation enhances the antioxidant defense system in BG33, enabling it to mitigate oxidative stress and suppress MoT colonization. To test this, we (i) compare ROS accumulation and oxidative damage in resistant (BG33) and susceptible (BARI Gom 26, BG26) cultivars during MoT infection; (ii) quantify constitutive and induced activity of key enzymatic (SOD, CAT, APX, GPX, GR, DHAR, MDHAR, GST) and non-enzymatic (proline) antioxidants; and (iii) establish a mechanistic link between antioxidant efficiency and 2NS-mediated blast resistance. By elucidating how BG33's redox machinery thwarts MoT, this study addresses a critical knowledge gap in wheat blast resistance and provides actionable insights for breeding climate-resilient, disease-tolerant wheat varieties.

Results

MoT inoculation causes visible symptoms on wheat spike

To assess the resistance of wheat to the wheat blast pathogen, we artificially inoculated wheat spikes of the varieties BG33 and BG26 at the flowering stage using a conidial suspension of *Magnaporthe oryzae tritici* (MoT). Ten days post-inoculation, BG26 displayed clear symptoms of severe spike infection. In contrast, the rachis of the moderately to highly resistant variety BG33 showed only mild symptoms, characterized by partial bleaching and shrinkage of the upper section (see Fig. 1). These findings confirm that the experimental conditions and spike inoculation assay were suitable for studying reactive oxygen species (ROS) accumulation and the responses of BG33, which carries the 2NS blast resistance chromosomal segment, to MoT infection through its antioxidant defense system.

MoT inoculation induces differential oxidative stress and lipid peroxidation in wheat rachis

To evaluate the induction of reactive oxygen species (ROS) and subsequent cellular damage during infection, we quantified hydrogen peroxide (H_2O_2), malondialdehyde (MDA) content, and lipoxygenase (LOX) activity in the rachis tissues of BG33 (resistant) and BG26 (susceptible) following inoculation with *MoT* conidia. While H_2O_2 acts as a vital signaling molecule under normal physiological conditions, its excessive accumulation is a hallmark of oxidative stress. Following *MoT* inoculation, a progressive increase in H_2O_2 levels was observed in both varieties (Fig. 2A). However, the resistant variety, BG33, maintained significantly tighter control over ROS accumulation. At 24 hours after inoculation (hai), H_2O_2 content increased by only 6% in BG33 compared to 12% in BG26 relative to their respective untreated controls. This trend intensified by 48 hai, with H_2O_2 levels rising to 30% in BG26, while BG33 limited the increase to 19% (Fig. 2D). Untreated rachis tissues in both varieties maintained low baseline H_2O_2 concentrations, confirming that the observed oxidative surge was a direct response to fungal infection.

The extent of oxidative damage was further assessed through lipid peroxidation markers. *MoT* infection significantly enhanced the accumulation of MDA, a byproduct of polyunsaturated fatty acid oxidation. Throughout the infection period, BG33 consistently exhibited lower MDA levels than BG26 (Fig. 2B). Specifically, at 24 hai, MDA levels rose by 45% in BG26 but only by 27% in BG33. By 48 hai, this disparity widened, with MDA levels escalating by 79% in the susceptible BG26 compared to 54% in the resistant BG33 (Fig. 2D). Parallel to the MDA trends, *MoT* inoculation triggered a sharp rise in LOX activity, which facilitates the oxidation of lipids. At 24 hai, LOX activity increased by 116% in both genotypes relative to controls. However, by 48 hai, LOX activity escalated further to 158% in BG26, whereas BG33 showed a more tempered increase of 143% (Fig. 2C, 2D). Collectively, these results demonstrate that the resistant BG33 variety effectively mitigates *MoT*-induced oxidative stress and preserves membrane integrity more efficiently than the susceptible BG26.

MoT inoculation impacts chlorophyll and carotenoid content in wheat rachis

Chlorophyll is the primary pigment driving photosynthesis and is essential for maintaining plant vigor and systemic resistance to pathogens. To evaluate the physiological impact of *MoT* infection, we quantified the levels of chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids in the rachis of the resistant variety BG33 and the susceptible variety BG26 following inoculation. Analysis of variance (ANOVA) revealed that while variety (G) and hours after inoculation (hai) had significant effects on pigment levels, the most pronounced declines were observed at 48 hai across both varieties (Fig. 3). However, the degradation of photosynthetic pigments was notably more severe in the susceptible variety BG26 compared to the resistant variety BG33. At 24 hai, chlorophyll *a* levels decreased by 22% in BG26, whereas BG33 restricted this loss to 14%. By 48 hai, this disparity widened significantly; chlorophyll *a* levels plummeted by 55% in BG26 but only by 36% in BG33.

A similar trend was observed for chlorophyll *b* and total chlorophyll content. Chlorophyll *b* levels were reduced by 58% in BG26 at 48 hai, while BG33 maintained higher integrity with a 40% reduction. Total chlorophyll followed this pattern, showing a 56% decrease in BG26 compared to a more tempered 37% decline in BG33 (Fig. 3C, E). Furthermore, *MoT* inoculation significantly depleted carotenoid levels—pigments critical for mitigating photo-oxidative damage. [cite_start]At 24 hai, carotenoid content dropped by 20% in BG26 but only by 8% in BG33. This trend continued through 48 hai, with a 48% reduction in BG26 compared to a 26% reduction in BG33. Collectively, these findings demonstrate that the resistant variety BG33, which carries the 2NS chromosomal segment, exhibits superior preservation of photosynthetic pigments following *MoT* challenge. This ability to maintain pigment integrity likely supports the robust antioxidant defense system observed in BG33, contrasting with the rapid pigment degradation and subsequent oxidative vulnerability seen in the susceptible BG26.

MoT inoculation differentially enhances proline accumulation in wheat rachis

Proline is a multifunctional amino acid that plays a pivotal role in osmotic adjustment and ROS scavenging, particularly during the early stages of pathogen infection. To determine whether proline contributes to the 2NS-mediated resistance in BG33, we quantified its accumulation in the rachis of both BG33 and BG26 following *MoT* inoculation.

Proline levels increased progressively in both varieties at 24 and 48 hours after inoculation (hai) (Fig. 4A). Notably, the resistant variety BG33 maintained significantly higher proline concentrations than the susceptible BG26 across all time points, including a higher constitutive (basal) level prior to inoculation. At 24 hai, proline content increased by 36% in BG33 compared to a 26% increase in BG26 relative to their respective untreated controls. This trend became more pronounced by 48 hai, with proline levels surging by 63% in BG33 and 56% in BG26 (Fig. 4E). These results indicate that BG33 not only possesses a higher baseline of this protective osmolyte but also triggers a more robust proline-mediated response upon *MoT* challenge, likely contributing to its superior redox stability.

Enhanced antioxidant enzyme activities in the blast-resistant wheat variety carrying the 2NS chromosomal segment

Antioxidant enzymes are fundamental to plant defense, providing a critical buffer against pathogen-induced oxidative bursts. To determine if the 2NS-mediated resistance in BG33 is linked to a more efficient enzymatic response, we assayed the activities of major antioxidant enzymes in the rachis tissues of both resistant (BG33) and susceptible (BG26) varieties following *MoT* inoculation. Our results indicate that BG33 maintains a significantly more robust and sustained antioxidant enzymatic profile compared to BG26 (Figs. 4 and 5).

Upon *MoT* challenge, both varieties exhibited an upward trend in primary antioxidant defense enzyme activities. However, the induction was consistently more pronounced in the resistant BG33. Superoxide dismutase (SOD) activity, which catalyzes the first step of ROS detoxification, rose by 23% in BG33 at 48 hours after inoculation (hai), compared to a more modest 15% increase in BG26 (Fig. 4B, E). Even more striking differences were observed in hydrogen peroxide-scavenging enzymes. Catalase (CAT) activity in BG33 surged by 46% at 48 hai, more than double the 17% increase observed in BG26 (Fig. 4C, E). Similarly, peroxidase (POD) activity showed a sharp differential response; while BG26 reached a 36% increase at 48 hai, BG33 exhibited a substantial 68% rise, nearly doubling the activity of the susceptible variety (Fig. 4D, E).

The AsA–GSH cycle is vital for maintaining cellular redox homeostasis. We observed significant upregulation across all enzymes in this pathway including ascorbate peroxidase (APX), glutathione peroxidase (GPX), glutathione reductase (GR), dehydroascorbate reductase (DHAR), and monodehydroascorbate reductase (MDHAR) with BG33 consistently outperforming BG26.

At 48 hai, APX and GPX activities in BG33 rose by 66% and 41%, respectively, significantly higher than the 55% and 23% increases seen in BG26 (Fig. 5A, B, G). The recycling enzymes of the cycle also showed superior efficiency in the resistant variety; GR and MDHAR activities in BG33 reached increases of 58% and 31%, respectively, compared to 44% and 12% in BG26 (Fig. 5C, E, G). Notably, DHAR activity in BG26 showed a sharp decline in induction between 24 and 48 hai (dropping from 13% to 5%), whereas BG33 maintained a high induction of 31%, suggesting a sustained capacity for ascorbate regeneration in the resistant variety. Finally, glutathione S-transferase (GST) activity, which facilitates the detoxification of lipid hydroperoxides, was enhanced by 31% in BG33 at 48 hai, nearly twice the induction level of 16% observed in BG26 (Fig. 5F, G). Collectively, these data demonstrate that the 2NS translocation in BG33 confers resistance by priming a broad-spectrum and highly vigorous antioxidant enzymatic network.

Influence of variety and post-inoculation time on physiological and biochemical parameters

To validate the observed physiological changes, a two-way analysis of variance (ANOVA) was performed to evaluate the influence of variety (G), hours after inoculation (H), and their interaction (G×H) on the

measured parameters (Table 1). The analysis revealed that the variety (G) had a significant main effect ($p < 0.05$) on nearly all parameters examined, with the exception of chlorophyll *b* (Chl *b*) and carotenoids (Caro), indicating that the genetic background (2NS vs. non-2NS) is a primary determinant of the wheat's physiological state. Furthermore, the duration of infection (hai) demonstrated a highly significant effect ($p < 0.01$) on every parameter, confirming a dynamic physiological shift over the course of the *MoT* challenge. Notably, significant interaction effects between variety and time (G×H) were observed for the majority of the biochemical markers, including ROS accumulation and antioxidant enzyme activities. However, no significant G×H interaction was found for leaf pigments and proline content (Table 1), suggesting that while these specific parameters were significantly affected by both variety and time independently, their rate of change followed a similar trend in both genotypes post-inoculation.

Table 1
Variance components of analysis of variance (ANOVA) and their
significance level of studied traits using the general linear model

Trait	Variety (G)	hai (H)	G×H	Residual
DF	1	2	2	15
H ₂ O ₂	3.705 ^{**}	4.057 ^{**}	0.261 [*]	0.059
MDA	53.104 ^{**}	581.588 ^{**}	19.168 ^{**}	2.447
LOX	0.451 ^{**}	3.227 ^{**}	0.106 ^{**}	0.011
Chl <i>a</i>	0.015 ^{***}	0.016467 ^{***}	0.0002 ^{ns}	0.00043
Chl <i>b</i>	0.000417 ^{ns}	0.004517 ^{***}	0.000317 ^{ns}	0.000147
TChl	0.01000 ^{**}	0.03750 ^{***}	0.00113 ^{ns}	0.00091
Caro	0.0000667 ^{ns}	0.0026792 ^{***}	0.0003792 ^{ns}	0.0001083
Proline	27.714 ^{**}	15.366 ^{**}	0.779 ^{ns}	0.293
SOD	567.648 ^{**}	2147.051 ^{**}	332.778 ^{**}	12.641
CAT	4737.66 ^{**}	866.552 ^{**}	263.981 ^{**}	17.551
POD	0.269 ^{**}	0.093 ^{**}	0.018 ^{**}	0.002
APX	3.832 ^{**}	4.957 ^{**}	0.173 ^{**}	0.024
GPX	4243.232 ^{**}	3978.647 ^{**}	450.344 ^{**}	33.782
GR	36.605 ^{**}	773.906 ^{**}	13.305 [*]	2.299
DHAR	267.534 ^{**}	2668.343 ^{**}	663.226 ^{**}	29.4
MDHAR	301.467 [*]	1639.949 ^{**}	328.472 ^{**}	42.499
GST	14818.055 ^{**}	15375.623 ^{**}	1641.025 ^{**}	64.282

hai- hours after inoculation, DF- degrees of freedom, Chl *a*- chlorophyll *a*, Chl *b*- chlorophyll *b*, TChl- total chlorophyll, Caro- carotenoids, H₂O₂- hydrogen peroxide, MDA- malondialdehyde, LOX- lipoxxygenase, SOD- superoxide dismutase, CAT- catalase, POD- peroxidase, GPX- glutathione peroxidase, GST- glutathione-S-transferase, APX- ascorbate peroxidase, GR- glutathione reductase, DHAR- dehydroascorbate reductase, MDHAR- monodehydroascorbate reductase. ^{ns}, ^{*}, and ^{**} indicate statistically non-significant, significant at $p < 0.05$, and 0.01, respectively.

MoT resistance in wheat rachis: A multifaceted defense

To integrate the physiological and biochemical responses observed, a comparative heatmap analysis was performed. The analysis categorized the evaluated traits into three distinct functional clusters: prooxidants and markers of oxidative damage (Cluster I), photosynthetic pigments (Cluster II), and antioxidant enzymes alongside the osmoprotectant proline (Cluster III) (Fig. 6). Under control conditions, both BG26 and BG33 exhibited similar pigment profiles and biochemical baselines, clustering closely together. However, following *MoT* inoculation, the two varieties diverged significantly, reflecting their contrasting strategies for managing infection-induced oxidative stress.

The superior resistance to *MoT* in BG33 appears to be derived from a coordinated, multifaceted defense mechanism. Unlike the susceptible BG26, the resistant variety BG33 effectively restricted the accumulation of reactive oxygen species (ROS), resulting in significantly lower levels of hydrogen peroxide (H₂O₂), malondialdehyde (MDA), and lipoxygenase (LOX) activity post-inoculation. This mitigation of oxidative damage was strongly associated with the robust upregulation of Cluster III components. BG33 maintained substantially higher activities of key antioxidant enzymes and accumulated greater levels of proline, which likely served as both a ROS scavenger and a vital osmoprotectant. Collectively, these findings suggest that the 2NS chromosomal segment in BG33 confers resistance by "priming" a more resilient antioxidant network, which preserves cellular integrity and limits the physiological impact of wheat blast infection.

Discussion

Plant resistance to pathogens involves complex metabolic recalibrations centered largely on the activation of robust antioxidant systems to maintain cellular homeostasis. This study reveals that the wheat blast-resistant variety BARI Gom 33 (BG33), which carries the 2NS chromosomal translocation, exhibits a significantly more vigorous antioxidant defense network against *M. oryzae* *Triticum* (*MoT*) than the susceptible variety BARI Gom 26 (BG26). Our findings establish a clear mechanistic link between 2NS-mediated resistance and superior redox management. By effectively neutralizing the oxidative burst during infection, BG33 preserves cellular integrity and restricts fungal colonization, providing a physiological explanation for the lower blast severity previously documented in the rachis of this variety (Hossain et al., 2019). While antioxidant activation was recently linked to the *Rmg8* gene, our work extends this paradigm to the widely utilized 2NS segment, demonstrating its fundamental role in biochemical defense (Islam et al., 2025).

A critical observation in this study is that BG33 accumulated 40–60% less reactive oxygen species (ROS), specifically hydrogen peroxide (H₂O₂) and malondialdehyde (MDA), following *MoT* challenge compared to BG26. This reduction indicates minimal lipid peroxidation and superior membrane stability. Notably, the 2NS translocation appears to "prime" BG33 for a rapid ROS detoxification response, a capacity that is strikingly weak in BG26 suggesting that the 2NS segment confers a form of preemptive stress adaptation. Furthermore, BG33 maintained 30–50% higher levels of chlorophyll and carotenoids

post-infection, mitigating the photo-oxidative damage that typically accompanies the hemibiotrophic invasion of *MoT*. The

decline in chlorophyll content observed during *MoT* infection in this study is consistent with earlier reports from various plant-pathogen interactions. Similar reductions have been documented in Brassica species infected with *Alternaria* spp. (Munir et al., 2020) and *Alternaria brassicicola* (Macioszek et al., 2020), in eggplant affected by leaf spot-associated pathogenic fungi (Kaniyassery et al., 2024), and in wheat challenged by *Pyricularia oryzae* (Debona et al., 2016) particularly during the early stages of infection, such as 48 hours post-inoculation. The preservation of carotenoids, which are vital for quenching singlet oxygen, points to a synergistic relationship between enzymatic antioxidants and pigment-based chemical defenses. In contrast, the rapid pigment degradation in BG26 likely exacerbated ROS accumulation, mirroring the physiological collapse seen in other susceptible cereal-pathogen interactions (Chrpová et al., 2021).

The role of proline as a multifaceted metabolic sentinel is also highlighted by our data, as BG33 exhibited constitutive proline levels 2.1-fold higher than BG26, which surged further upon infection. Beyond its role as an osmoprotectant, proline may participate in reactive nitrogen species (RNS) signaling to enhance pathogen defense (Christgen & Becker, 2021), positioning it as a key metabolite in the 2NS-mediated resistance framework. Our analysis of the enzymatic landscape further revealed that primary scavengers, superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) exhibited 1.5- to 2.5-fold higher activity in BG33. The rapid conversion of superoxide anions to H₂O₂ by SOD, followed by efficient neutralization by CAT and APX, suggests a highly synchronized ROS-scavenging cascade. Interestingly, GST activity in BG33 exceeded that of BG26 by 80%, a trend reminiscent of *Fhb7*-mediated resistance to Fusarium head blight (Wang et al., 2020). These enzymes may also play a specialized role in detoxifying *MoT*-derived phytotoxins, a hypothesis that warrants further investigation through toxin-profiling experiments.

The ascorbate-glutathione (AsA-GSH) cycle emerged as a "hyperactive" engine of resistance in BG33. While BG33 increased the activities of APX, GR, MDHAR, and DHAR by up to 2.2-fold post-infection, BG26 showed a suppression of these enzymes, likely leading to the depletion of the protective AsA/GSH pool. This sustained recycling capacity is a hallmark of durable resistance, ensuring that the plant can withstand prolonged oxidative pressure. While the 2NS segment is a known driver of heat tolerance (Mohi-Ud-Din et al., 2022), this study is the first to explicitly link it to antioxidant-mediated resistance against wheat blast. This functional overlap between abiotic and biotic stress pathways underscores the evolutionary versatility of the 2NS translocation, suggesting it harbors regulatory elements that broaden the expression of antioxidant gene families.

Our findings establish the 2NS translocation as a master regulator of oxidative stress management, providing a "dual-defense" strategy against both fungal pathogens and environmental stressors. To build upon this mechanistic framework, future research should prioritize the genetic dissection of the 2NS segment using CRISPR-based approaches to clone specific genes that modulate these antioxidant

pathways (Islam 2019; Khayer et al. 2026). Additionally, assessing the stability of these traits across diverse agroclimatic zones and investigating the crosstalk between physiological antioxidant pathways and classical *R-gene* signaling will be essential for developing "stacked" resistance models. By shifting the breeding focus toward enhancing antioxidant capacity alongside vertical resistance, this study contributes a sustainable and robust strategy for safeguarding global wheat production against the escalating threat of wheat blast.

Conclusion

This study provides comprehensive evidence that the 2NS chromosomal translocation in the wheat variety BARI Gom 33 (BG33) confers resistance to *M. oryzae Triticum* (MoT) by activating a sophisticated and multi-tiered antioxidant defense system. Unlike the susceptible variety BARI Gom 26 (BG26), BG33 effectively mitigates infection-induced oxidative stress by maintaining significantly lower levels of reactive oxygen species (ROS) and lipid peroxidation markers. This physiological resilience is driven by the robust upregulation of key enzymatic antioxidants including SOD, CAT, and POD alongside a highly efficient ascorbate-glutathione (AsA-GSH) cycle. Furthermore, the elevated accumulation of proline and the superior preservation of photosynthetic pigments in BG33 suggest a coordinated mechanism that protects cellular integrity and maintains metabolic vigor under pathogen pressure. These findings establish the 2NS segment as a critical genetic resource not only for blast resistance but also for enhancing redox homeostasis in wheat. Future research should prioritize the identification and cloning of the specific regulatory genes within the 2NS segment that modulate these antioxidant pathways. Elucidating these molecular foundations will be instrumental in developing more resilient, high-yielding wheat varieties capable of withstanding the increasing global threat of wheat blast.

Materials and methods

Plant Materials and MoT Isolate

Wheat seeds of cultivars BARI Gom 26 (BG26) and BARI Gom 33 (BG33) were procured from the Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh. A randomized complete block design with four replications was employed for field sowing in late November at the research field of Gazipur Agricultural University (GAU), Gazipur, Bangladesh.

The wheat blast isolate BTJP 4–5, obtained from the Institute of Biotechnology and Genetic Engineering (IBGE) at GAU, was reactivated from – 80°C storage on potato dextrose agar (PDA) medium for subsequent experimentation.

Preparation of MoT Inoculum and Inoculation Assay

MoT conidia were obtained as described by Gupta et al. (2020). Briefly, mycelium blocks were cultured on PDA amended with 0.05 g/L phenoxymethylpenicillin at 25°C for 8 days in the dark. Subsequent incubation under blue light for 48 hours induced sporulation. Conidia were harvested by gently scraping

the plates with a paintbrush while flooded with 0.01% Tween 20 in sterile water. The suspension was filtered through cheesecloth and adjusted to a concentration of 1×10^5 conidia/mL.

Wheat plants were thoroughly sprayed with the conidial suspension, while controls received a sterile water-Tween 20 solution. High humidity (80–100%) was maintained for 48 hours by covering the plants with sterilized polyethylene. Plants were incubated at 25–30°C to facilitate blast symptom development. Infected spikes were collected at 0-, 24-, and 48-hours post-inoculation. Spikelets were removed, and the rachis was sectioned, flash-frozen in liquid nitrogen, and stored at -80°C for subsequent analyses.

Determination of photosynthetic pigments and osmolyte proline

Fresh plant samples (approximately 100 mg) were extracted with 5 mL of 80% acetone in glass vials. The vials were stored at 4°C in the dark for 24 hours. Subsequently, absorbance was measured at 663 nm, 646 nm, and 470 nm. Chlorophyll *a* (Chl *a*), Chlorophyll *b* (Chl *b*), total chlorophyll (TChl), and carotenoid content were quantified using the formulas of Arnon (1949) and expressed as mg g⁻¹ fresh weight (FW). Proline content in the rachis was determined according to the method of Bates et al. (1973) and expressed as µmol g⁻¹ FW.

Determination of H₂O₂ and MDA content

Hydrogen peroxide (H₂O₂) levels were quantified using the method described by Yu et al. (2003). The absorbance of the supernatant was measured at 410 nm using a spectrophotometer. H₂O₂ content was calculated using an extinction coefficient of 0.28 µM⁻¹ cm⁻¹ and expressed as µmol g⁻¹ fresh weight (FW).

Membrane lipid peroxidation, assessed by malondialdehyde (MDA) levels, was determined using the thiobarbituric acid (TBA) method of Heath and Packer (1968). MDA concentration was calculated using an extinction coefficient of 155 mM⁻¹ cm⁻¹ and expressed as nmol g⁻¹ FW.

Extraction of Enzymes and Soluble Proteins, and Measurement of Enzymatic Activity

Plant tissue samples (1 g each) were meticulously homogenized in an ice-cold extraction buffer. This buffer, a carefully formulated solution of 50 mM potassium phosphate (pH 7.0), incorporated 100 mM KCl to maintain ionic balance, 1 mM ascorbate to protect against oxidation, 5 mM β-mercaptoethanol to preserve thiol groups, and 10% (w/v) glycerol to prevent enzyme denaturation. The resulting homogenates were then subjected to centrifugation at 11,500×g for 12 minutes, effectively separating the cellular debris from the soluble protein fraction. The supernatant, enriched with the enzymes of interest, was carefully collected for subsequent enzymatic activity assays.

To quantify the total protein content in the enzyme extracts, the Bradford method was employed, a well-established colorimetric technique (Bradford, 1976). This method relies on the binding of Coomassie

Brilliant Blue dye to proteins, resulting in a shift in the dye's absorption spectrum, allowing for a sensitive and accurate determination of protein concentration.

The activity of key antioxidant enzymes was then meticulously measured. Superoxide dismutase (SOD) activity, crucial for neutralizing superoxide radicals, was determined using the xanthine-xanthine oxidase system, following the established protocol of Spitz and Oberley (1989). Lipoygenase (LOX) activity, involved in fatty acid oxidation, was measured according to the method of Doderer et al. (1992), utilizing linoleic acid as the substrate. Catalase (CAT) activity, responsible for decomposing hydrogen peroxide, was assessed as described by Noctor et al. (2016), with the reaction initiated by the addition of the enzyme extract and activity calculated based on the characteristic absorption of the reaction product.

Peroxidase (POD) activity, another key enzyme in the antioxidant defense system, was determined using the established method of Castillo et al. (1984). The activity of glutathione peroxidase (GPX), an essential enzyme for reducing hydrogen peroxide, was measured using H_2O_2 as the substrate, following the protocol of Elia et al. (2003).

Furthermore, the activities of several other crucial antioxidant enzymes were spectrophotometrically determined according to the established protocol of Hasanuzzaman et al. (2014). These enzymes included glutathione S-transferase (GST), involved in detoxification processes, glutathione reductase (GR) essential for maintaining the reduced form of glutathione, ascorbate peroxidase (APX) crucial for scavenging hydrogen peroxide, monodehydroascorbate reductase (MDHAR) involved in recycling ascorbate, and dehydroascorbate reductase (DHAR) also essential for ascorbate regeneration.

Statistical Analysis

Data analysis was conducted using a linear model within an R-4.1.0 for Windows environment (R Core Team, 2013) framework for a factorial randomized complete block design. Statistical analyses, including two-way analysis of variance (ANOVA) and subsequent mean comparisons using Tukey's Honestly Significant Difference (HSD) test, were performed with the aid of R packages: ggplot2, ggthemes, multcompView, dplyr, stats, and patchwork (Wickham, 2016). Boxplots were generated using these packages to visually represent the data distribution. Mean differences were considered statistically significant at a significance level of $p < 0.05$. Furthermore, a heatmap was created using the R package pheatmap (Kolde, 2019) to visualize the relationships between variables.

Abbreviations

APX

Ascorbate peroxidase

BARI

Bangladesh Agricultural Research Institute

CAT

Catalase

GPX
Glutathione peroxidase
GST
Glutathione-S-transferase
LOX
Lipoxygenase
MDA
Malondialdehyde
MoT
Magnaporthe oryzae Triticum
POD
Peroxidase
ROS
Reactive Oxygen Species

Declarations

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Author contributions statement

M.S.I., M.M. and D.R.G. conducted the experiments, curated the data, performed data analysis, and wrote the first draft. T.I., D.R.G and M.M. contributed to experimental design and editing; T.I., and D.R.G. supervised the research and edited the manuscript. D.R.G., M.S.I., M.M.R. and M.M. contributed to biochemical analysis. T.I. conceptualized the idea, supervised experiments, and reviewed and edited the manuscript. M.R. reviewed and edited the manuscript

Declaration of competing interest

The authors declare no conflict of interests.

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Figures

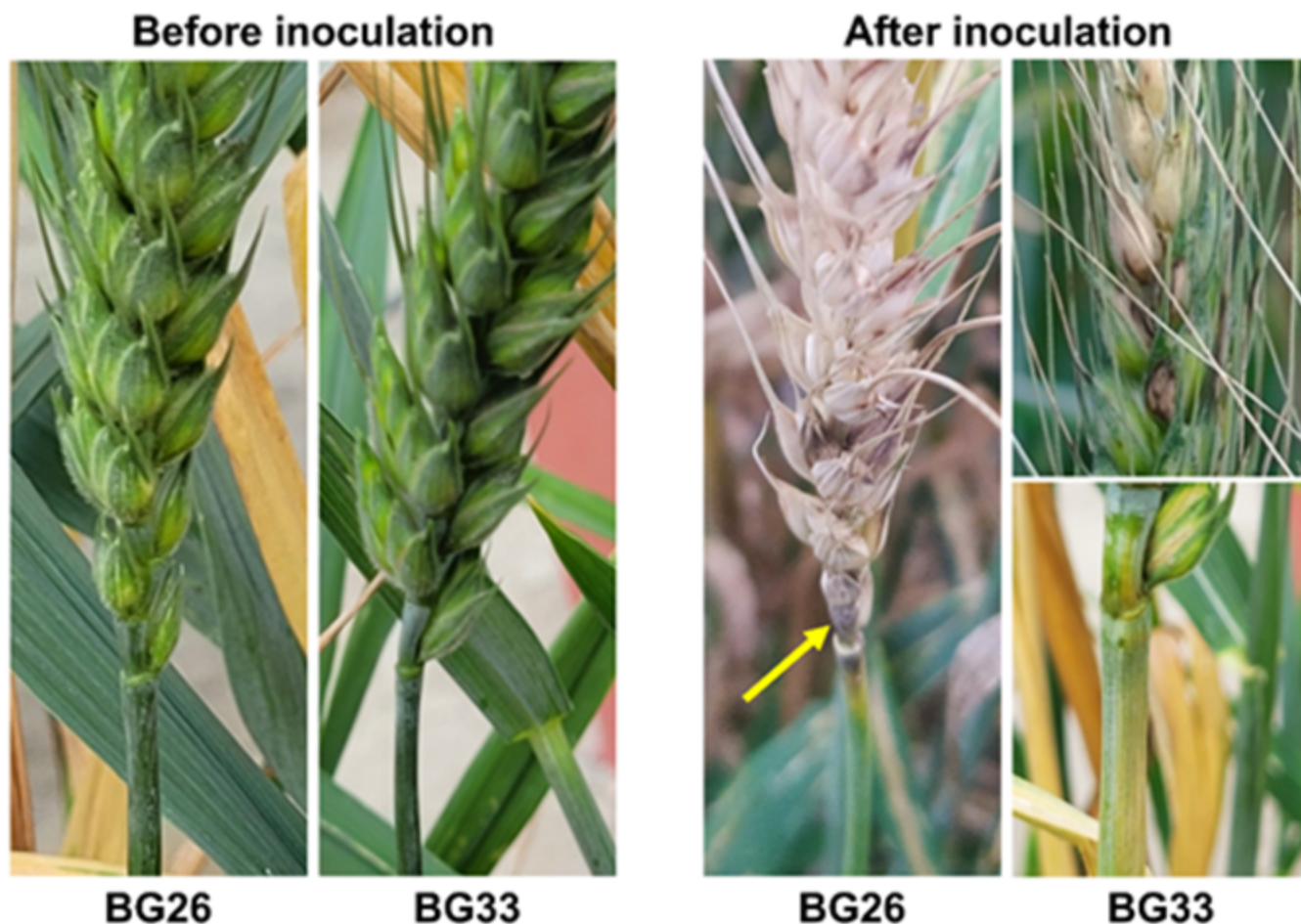


Figure 1

Development of blast disease symptoms on wheat rachis. Wheat rachis were inoculated with a conidial suspension of MoT (*Magnaporthe oryzae triticum*) at a concentration of 4×10^5 conidia/mL. The inoculated rachis were then covered with a polyethylene bag for 48 hours to maintain high humidity. Disease symptom development was monitored, and photographs were captured 10 days post-inoculation. The arrow in the image indicates the area of infection caused by MoT.

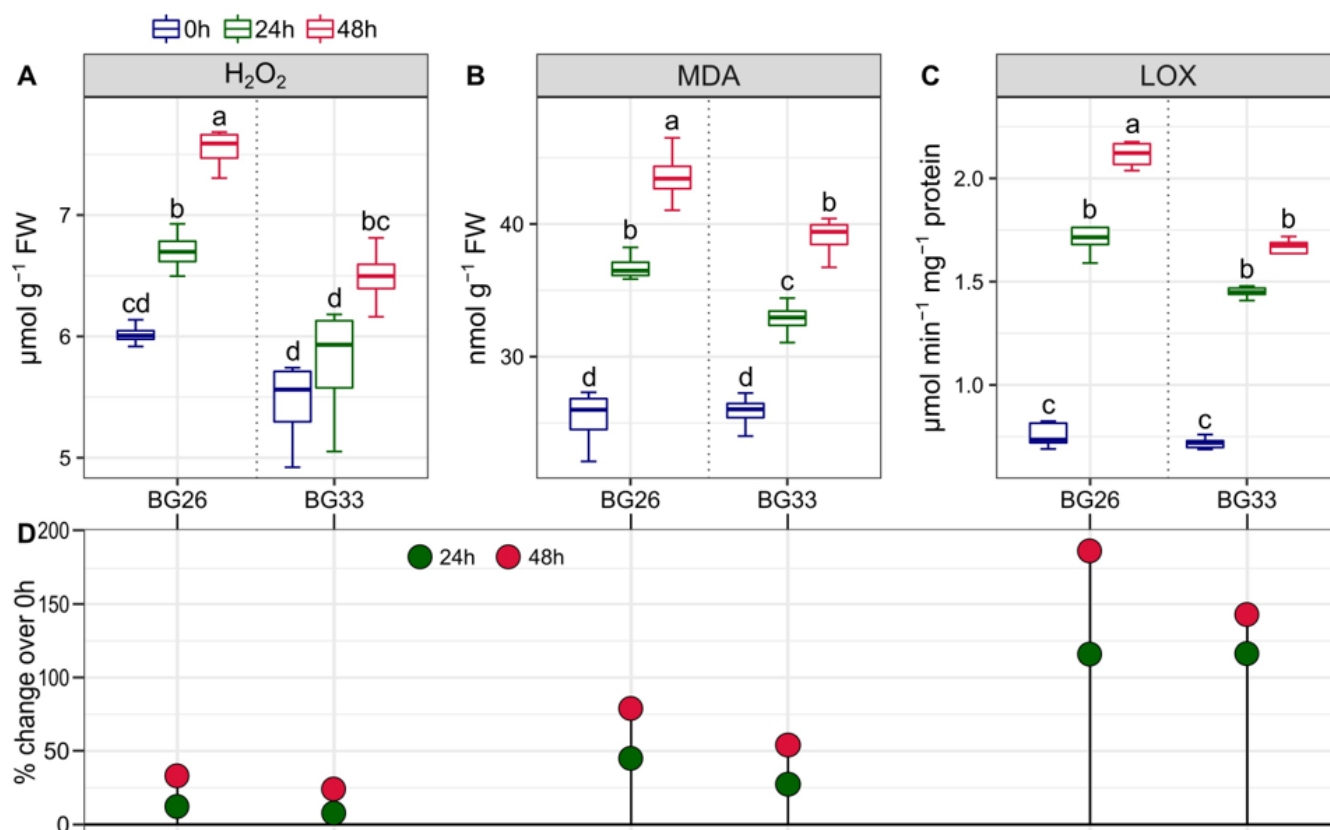


Figure 2

(A) hydrogen peroxide (H₂O₂) content, (B) malondialdehyde (MDA) content, (C) lipoxygenase (LOX) activity of the rachis at different hours after inoculation and (D) the relative change of the prooxidants at 24 and 48 hai over control (0 h) in both wheat varieties. Box and whisker represent descriptive summary of the data. Thickened horizontal line within the box indicate median of data. Different letter(s) denote a significant difference at $p < 0.05$. FW– Fresh weight. Additional details are shown in Table 1.

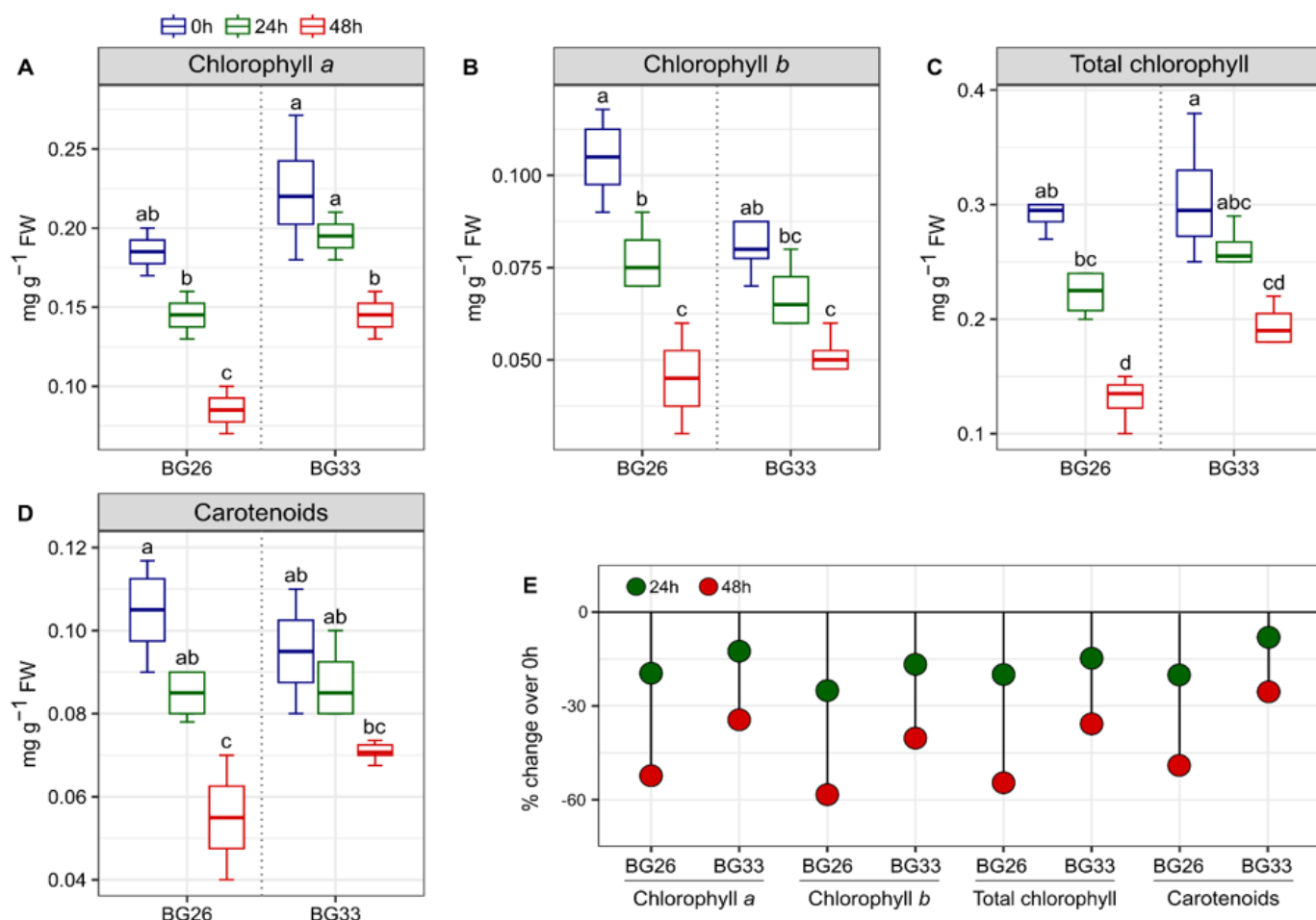


Figure 3

(A) chlorophyll a, (B) chlorophyll b, (C) total chlorophyll, (D) carotenoid contents of the rachis at different hours after inoculation and (E) the relative change of the pigments at 24 and 48 hours after inoculation over control (0 h) in both wheat varieties. Box and whisker represent descriptive summary of the data. Thickened horizontal line within the box indicate median of data. Different letter(s) denote a significant difference at $p < 0.05$. FW- Fresh weight. Additional details are shown in Table 1.

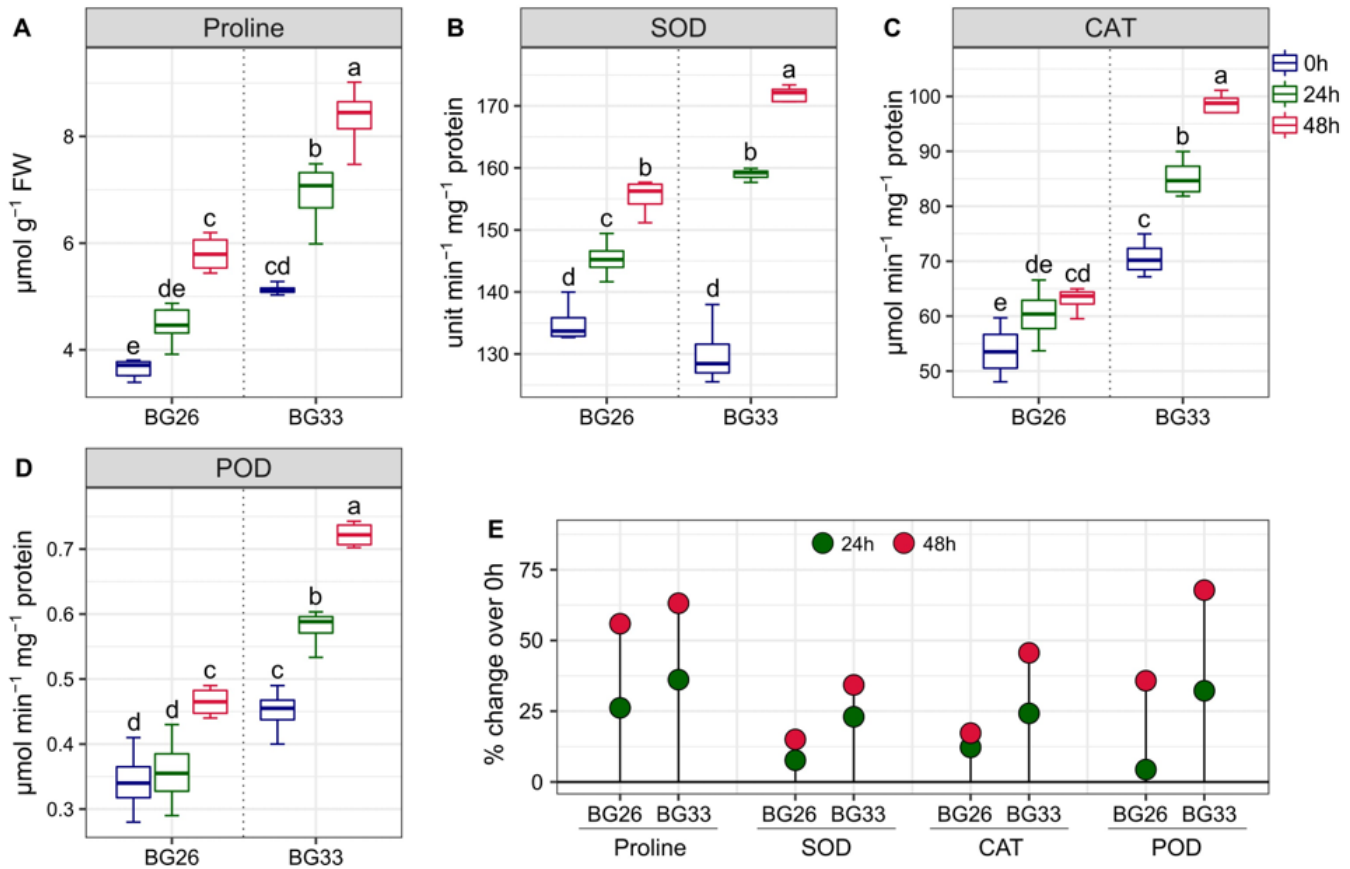


Figure 4

(A) proline content, and specific activities of (B) SOD, (C) CAT, (D) POD of the rachis at different hours after inoculation and (E) the relative change of the antioxidants at 24 and 48 hours after inoculation over control (0 h) in both wheat varieties. Box and whisker represent descriptive summary of the data. Thickened horizontal line within the box indicate median of data. Different letter(s) denote a significant difference at $p < 0.05$. FW– Fresh weight. Additional details are shown in Table 1.

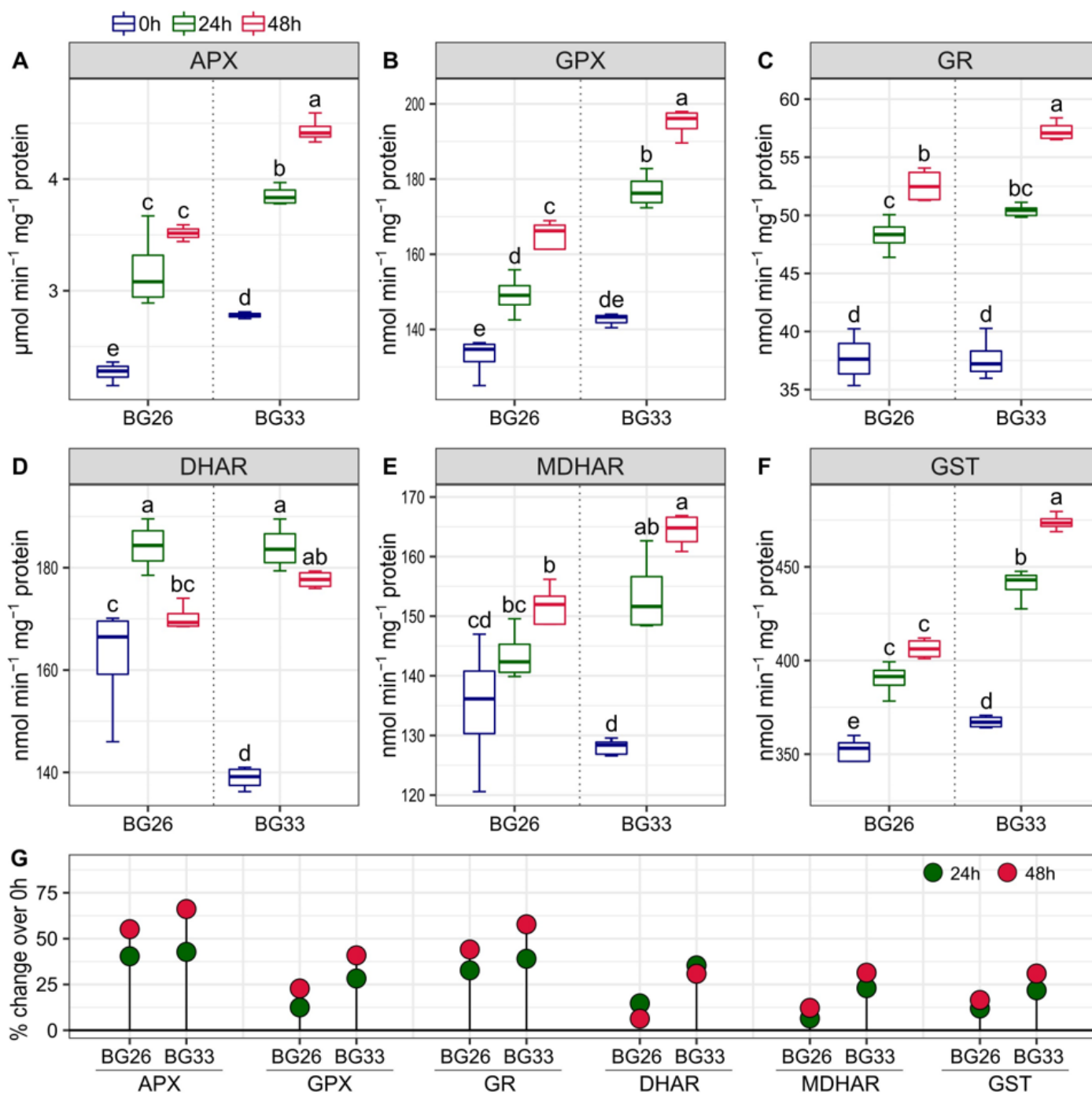


Figure 5

Specific activities of (A) APX, (B) GPX, (C) GR, (D) DHAR, (E) MDHAR, and (F) GST of the rachis at different hours after inoculation and (G) the relative change of the antioxidants at 24 and 48 hours after inoculation over control (0 h) in both wheat varieties. Box and whisker represent descriptive summary of the data. Thickened horizontal line within the box indicate median of data. Different letter(s) denote a significant difference at $p < 0.05$. Additional details are shown in Table 1.

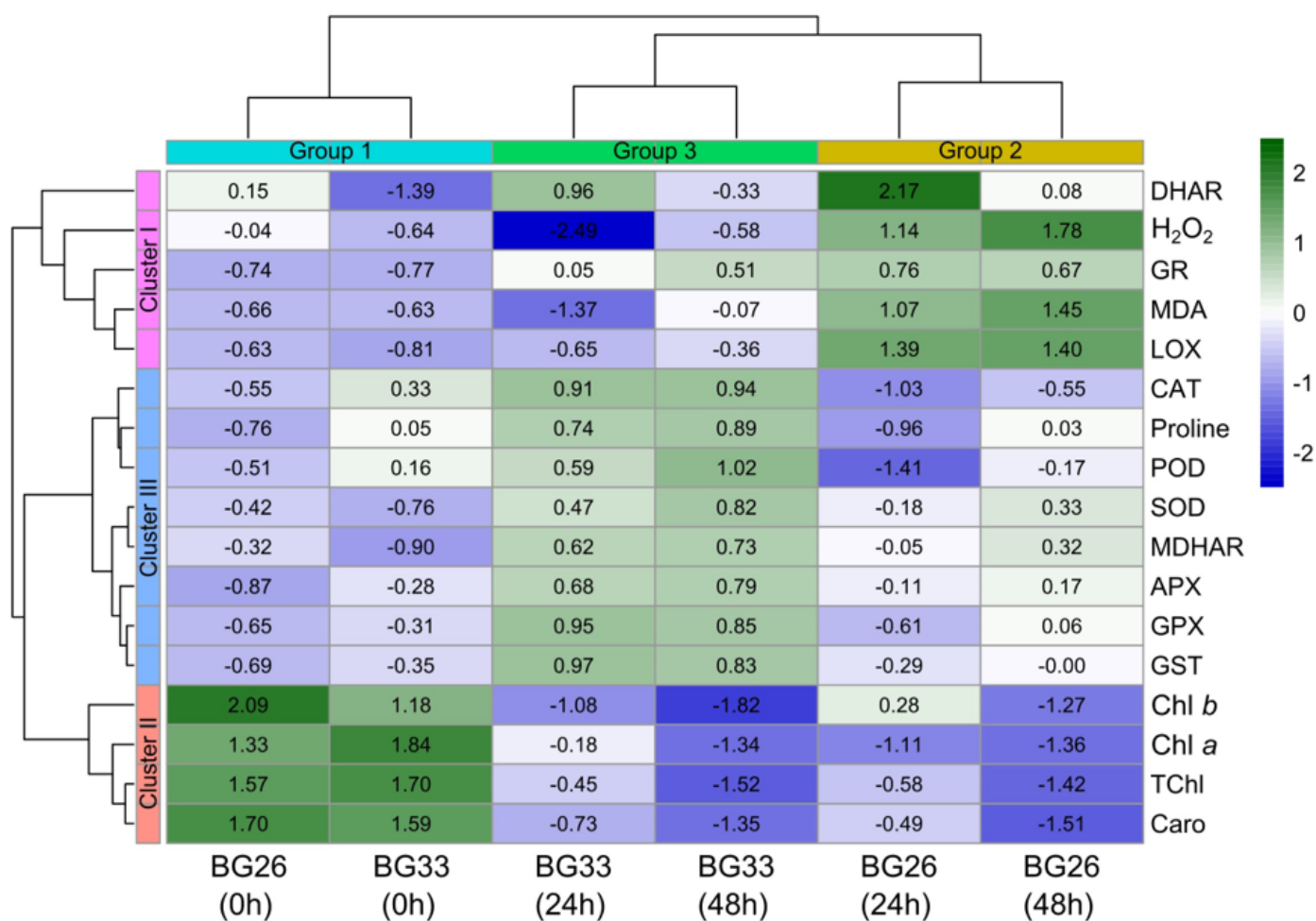


Figure 6

Heatmap-cluster analysis (**A**) and trait-by-variety PCA-biplot (**B**) showed the genotypic performance at different hours after inoculation of MoT, classified treatment combinations and traits according to the relative contribution, and depicted correlation among the traits and variety-treatment combinations.