Research Square

Preprints are preliminary reports that have not undergone peer review. They should not be considered conclusive, used to inform clinical practice, or referenced by the media as validated information.

Discriminating wheat genotypes for barley yellow dwarf virus tolerance through multivariate integration of chlorophyll fluorescence and agronomic traits

Samara Campos do Nascimento

samara.camposnascimento@hotmail.com

Universidade do Estado de Santa Catarina Centro de Ciencias Agroveterinarias
https://orcid.org/0000-0001-7329-2540

Luan Tiago dos Santos Carbonari
Vanucci Marcos Santi
Douglas Lau
Daniel Schurt
Ricardo Trezzi Casa
Giselle Camargo Mendes
Fabio Nascimento da Silva

Research Article

Keywords: Triticum aestivum L., BYDV, genotype × infection interaction, chlorophyll fluorescence, multivariate analysis, resistance vs tolerance

Posted Date: October 15th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10578132/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Abstract

Barley yellow dwarf virus (BYDV; *Luteovirus pavhordei*) is one of the most economically important viral pathogens of wheat, causing substantial losses in plant growth, physiology, and grain yield. This study evaluated the combined effects of wheat genotype, BYDV infection, and their interaction on agronomic performance and photosynthetic physiology using an integrative multivariate approach. The experiment employed a completely randomized design with four genotypes and two conditions (with and without virus), using controlled inoculation via aphids and infection confirmation by RT-PCR. Growth and yield components, as well as chlorophyll fluorescence variables, were measured, enabling co-analysis using multivariate analysis of variance, canonical discriminant analysis, and principal component analysis to explore patterns of association. The results revealed marked effects of genotype and viral infection on the set of variables, as well as differential responses among genotypes. BYDV infection altered the photosynthetic apparatus, with enhanced energy dissipation mechanisms and reduced photosynthetic efficiency, lowering agronomic performance. Multivariate analyses enabled the identification of association patterns among variables and the discrimination of genotypes based on their tolerance levels to the BYDV. Tolerant genotypes maintained photosynthetic efficiency, accompanied by vegetative growth and yield stability. In contrast, intolerant genotypes exhibited stronger photoprotective responses, greater impairment of PSII photochemistry, and more pronounced reductions in agronomic performance. Overall, this study demonstrates that viral infection reorganizes the relationships among physiological and productive processes, highlighting the importance of integrative approaches for better understanding plant–virus interactions and further supporting breeding programs.

Introduction

Plant viruses, as obligate intracellular parasites, depend entirely on the host cell machinery for replication, movement, and systemic colonization. Following infection, viruses may remain localized in resistant species or spread systemically through tissues in susceptible hosts, ultimately causing to extensive physiological disruption and interfering with processes essential for growth and development (Roger Hull 2004). However, resistance is not necessarily associated with the complete prevention of systemic infection. In cereal–virus interactions, resistant genotypes may restrict viral replication and movement despite systemic infection, whereas tolerant genotypes may sustain relatively high virus accumulation while maintaining growth and productivity, highlighting that resistance and tolerance represent distinct host responses (Comeau and Haber 2002).

Among the viruses affecting cereals, Barley yellow dwarf virus (BYDV) stands out; barley yellow dwarf disease, caused by a complex of aphid-transmitted BYDV viruses, is one of the most economically destructive diseases of wheat (*Triticum aestivum* L.) worldwide (Choudhury et al. 2019).Among these viruses, BYDV-PAV (*Luteovirus pavhordei*, *Tombusviridae*) is the predominant species reported from the wheat-growing regions of southern Brazil (Parizoto et al. 2013; Lau et al. 2021, 2022b). BYDVs are phloem-limited and persistently transmitted by aphids, particularly *Rhopalosiphum padi* L.; they are widely distributed across major wheat-producing regions (Parizoto et al. 2013; Kaddachi et al. 2014;

Rebonatto et al. 2015). In Brazil, the disease has been reported for decades and remains a recurrent constraint to enhancing yield, especially under conditions favorable to vector proliferation (Comeau et al. 2001; Lau et al. 2021, 2022a).

Typical symptoms of BYDV infection include leaf chlorosis or reddening, plant stunting, reduced tillering, impaired spike development, and, in some wheat genotypes, leaf margin serration or deformation, frequently resulting in significant yield declines, particularly when infection occurs at early developmental stages (Lau et al. 2021). At the physiological level, plant virus infections generally induce profound alterations in host metabolism, including reductions in photosynthetic capacity, chlorophyll content, stomatal conductance, and overall carbon assimilation, ultimately impairing plant growth and development (Choudhury et al. 2019; Liu et al. 2020). In the case of BYDV, these physiological alterations are likely associated with virus-induced degeneration of phloem tissues, which may impair the translocation of photoassimilates and disrupt source–sink relationships, potentially leading to carbohydrate accumulation in source tissues and reduced assimilate availability for developing sink organs. However, the magnitude of these effects may vary considerably among wheat genotypes, reflecting differences in resistance, tolerance, and susceptibility to infection.

Despite this knowledge and the importance of the pathosystem, the impacts of BYDV infection remain poorly characterized in an integrative context, particularly regarding the relationships between physiological performance and agronomic outcomes. Most studies have evaluated physiological, agronomic, or biochemical traits in isolation, thereby limiting the understanding of how these processes interact to determine performance under infection. Given that phytoresponses to viral infection are inherently complex and involve coordinated changes across multiple biological levels, integrative approaches are required to capture the systemic nature of plant–virus interactions. This perspective is particularly relevant for BYDV, in which host responses such as resistance and tolerance are considered the result of multiple interacting physiological processes rather than individual traits alone (Comeau and Haber 2002). In this context, multivariate statistical analyses provide a robust framework for integrating physiological and agronomic variables, enabling the identification of response patterns, the exploration of relationships among traits, and the discrimination of genotypes based on overall performance.

Therefore, this study evaluated the combined effects of wheat genotype, BYDV infection, and their interaction on agronomic performance and photosynthetic physiology using an integrative multivariate approach.

Materials and methods

Virus inoculation and experimental design

The experimental design employed a completely randomized factorial arrangement with two factors: genotype (four levels) and infection condition (BYDV-PAV-inoculated and non-inoculated plants), with four biological replicates for each treatment combination. For agronomic and physiological variables,

measurements obtained from the five plants within each experimental unit were averaged prior to statistical analyses. The resulting mean value was considered a single biological replicate for all subsequent analyses.

The aphid *R. padi* served as the virus vector. The BYDV-PAV isolate, designated 40Rp (GenBank accession MT345895), employed here was sourced from black oat (*Avena strigosa* Schreb.) collected from Passo Fundo, Rio Grande do Sul, Brazil, in 2007 (Lau et al. 2021, 2022a). Viral inoculum was maintained and amplified in the BYDV-PAV-infected black oat plants, which were then used to produce viruliferous *R. padi* colonies.

Virus was inoculated at the early vegetative stage (Z12–Z13) of seedlings, corresponding to the two- to three-leaf stages. Pots assigned to the infected group were transferred to an adjacent aphid-proof screenhouse compartment with similar environmental conditions and management practices. Each plant was inoculated by placing a leaf fragment containing approximately ten viruliferous *R. padi* at the junction of the two expanded leaves. Plants containing fewer than ten aphids were reinfested two days after inoculation, to ensure uniform viral pressure among experimental units. The virus transmission period lasted 7 days, after which aphid populations were eliminated by applying the insecticide dichlorvos at 1 kg L^{-1}, preventing secondary spread and ensuring that infection was the exclusive result of controlled inoculation.

Plant material and growth conditions

The experiments were conducted using four wheat genotypes: BR 35, BR 14, Embrapa 16, and BRS Timbaúva. These exhibit contrasting responses to BYDV-PAV infection under Brazilian environmental conditions, with screening assays classifying BR 35 and BRS Timbaúva as tolerant and BR 14 and Embrapa 16 as intolerant controls, respectively (Lau et al. 2022a).

In June 2025, seeds were sown in 7-L plastic pots at 10 seeds per pot. After seedling emergence, seedlings were thinned by uniform distribution to five per pot, ensuring a homogeneous density. Each pot was considered one experimental unit throughout the study. They were maintained under regular screenhouse conditions and standard management practices standard crop management practices (Cunha and Caierão 2023).

Molecular detection of viral infection

The infection status of each experimental unit was confirmed by reverse transcription polymerase reaction (RT-PCR) using total RNA extracted from pooled leaf samples. Leaf tissues from the five plants within each experimental unit (one pot) were combined to form a single bulk sample for RNA extraction using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. Leaves were sampled ~ 90 days post- virus inoculation, flash-frozen in liquid nitrogen, and stored at − 80°C. For total RNA was extraction, 100 mg of leaf tissue was ground in liquid nitrogen and homogenized in TRIzol. This was

followed by phase separation with chloroform, RNA precipitation with isopropanol, and ethanol washing. The RNA pellet was resuspended in RNase-free water. cDNA was synthesized using M-MLV Reverse Transcriptase (Promega, Madison, WI, USA) and the reverse primer Yan-R (5'-TGTTGAGGAGTCTACCTATTTG-3'), following the protocol described by Malmstrom and Shu (2004) for luteovirus detection.

PCR was performed using GoTaq® DNA Polymerase (Promega, Madison, WI, USA) and the primer pair Shu-F (5'-TACGGTAAGTGCCCAACTCC-3')/Yan-R (5'-TGTTGAGGAGTCTACCTATTTG-3') (Malmstrom and Shu 2004), which amplifies an 830-bp fragment of the polymerase gene (ORF2), the intergenic region, and part of the coat protein gene (ORF3), enabling BYDV isolates detection. PCR was performed at an annealing temperature of 55°C. The amplicons were separated by electrophoresis on a 1% agarose gel using SYBR Safe DNA Gel Stain (Invitrogen) and visualized under UV light. Samples producing the expected amplicon (~ 830 bp) were considered positive for BYDV infection. In addition, non-inoculated plants (control treatment) were analyzed using the same RT-PCR protocol to confirm the absence of viral infection, ensuring the reliability of the experimentally derived contrasts between infected and non-infected treatments.

Crop management practices

To minimize interference from other biotic and abiotic stresses, standard crop management practices were applied uniformly across treatments. Nitrogen fertilizer was added at the beginning of tillering using urea (45-00-00) at a rate of 2 g per pot. Preventive applications of fungicides and insecticides during crop development covered tillering (Z25–Z29), stem elongation (Z30–Z31), flag leaf emergence (Z37–Z39), and heading stages (Z49–Z55), ensuring that plant performance was not affected by other pests or diseases.

Chlorophyll fluorescence measurements

Chlorophyll fluorescence was quantified at the late vegetative to early reproductive stages (Zadoks stages Z49–Z55), corresponding to the booting and heading phase, 90 days after virus inoculation. All five plants within each experimental unit were evaluated. For each plant, a fully expanded leaf at a comparable canopy position was selected to minimize variation associated with leaf age and canopy position. After physiological evaluations, leaf tissues from the five plants within each experimental unit were pooled and subsequently used for total RNA extraction and molecular confirmation of viral infection.

Physiological analyses were conducted using a portable pulse-amplitude modulated fluorometer (MINIPAM, Walz, Germany), following the principles described by Maxwell1 and Johnson (2000) and Baker (2008). Measurements were performed on previously marked symptomatic leaves under natural

light and temperature, enabling evaluation of photochemical performance under screenhouse conditions.

The following fluorescence variables were determined: effective quantum yield of PSII [Y(II)], non-photochemical quenching (NPQ), minimum fluorescence in the light-adapted state (Fo'), quantum yield of regulated energy dissipation [Y(NPQ)], electron transport rate (ETR), photochemical quenching coefficient (qP), non-photochemical quantum yield [Y(NO)_lake and Y(NO)_alt], fraction of open PSII reaction centers (qL), total non-photochemical quenching coefficient (qN), and maximum quantum efficiency of PSII (Fv/Fm). All variables were calculated using the saturation pulse method, as described by Klughammer (2008).

Harvest and agronomic evaluation

Fully mature plants were harvested manually at Zadoks stage Z92, when grains were completely dry and plants had reached physiological maturity, allowing the cumulative influence of viral infection on growth and yield to be fully expressed. Agronomic traits were evaluated individually in all five plants within each experimental unit to characterize plant development, biomass accumulation, and yield components.

The following agronomic variables were determined: plant height (PH), root dry weight (RDW), shoot dry weight (SDW), number of tillers (NT), number of spikes (NS), total grain number (TGN), and total grain weight (TGW). Plant height was measured from the base to the apex of the main stem. Root and shoot dry weights were determined after separating below- and aboveground tissues, followed by oven-drying at 65°C for 72 h until constant weight. The number of tillers and spikes per plant was recorded, and yield components were assessed by determining the total number of grains per plant and total grain weight.

Statistical analyses

The multivariate analysis was performed using the GLM procedure of SAS OnDemand for Academics (SAS Institute Inc., Cary, NC, USA) through multivariate analysis of variance (MANOVA) to simultaneously evaluate the agronomic and physiological responses of wheat genotypes under virus presence or absence. Graphical representations were generated in R software using the ggplot2 package.

The multivariate general linear model adopted can be expressed as: $\mathrm{Y} = \mathrm{X}\beta + \epsilon, \epsilon \sim N_{n\times v}(0, \mathrm{I}_n \otimes \Sigma)$, where Y is the $(n \times v)$ matrix of observations for the v response variables, X is the design matrix associated with the effects of genotype (G), infection (I) (plants with or without viral infection), and the genotype × infection (G × I) interaction, and β and ε are the matrices of unknown parameters and random errors, respectively. Errors were assumed to be independent across observations but correlated across variables, with variance–covariance matrix Σ. The multivariate hypotheses were formulated in the general form: $H_0 : \mathrm{L}\beta\,\mathrm{M} = 0$, where L and M are contrast matrices associated with model effects and linear combinations of response variables, respectively, allowing simultaneous testing of genotype, condition, and interaction. The significance of

multivariate effects was assessed using Wilks' lambda, Pillai's trace, Hotelling–Lawley trace, and Roy's greatest root, based on the hypothesis (H) and error (E) matrices. The significance level adopted was 5%.

The dependent variables jointly analyzed were plant height (PH), root dry weight (RDW), shoot dry weight (SDW), number of tillers (NT), number of spikes (NS), total grain number (TGN), total grain weight (TGW), and chlorophyll fluorescence variables: effective quantum yield of PSII (Y(II)), non-photochemical quenching (NPQ), minimum fluorescence (Fo), quantum yield of regulated energy dissipation (Y(NPQ)), electron transport rate (ETR), quantum yield of non-regulated energy dissipation (Y(NO)), and maximum quantum efficiency of PSII (Fv/Fm).

To support interpretation, canonical discriminant analysis was employed to reduce data dimensionality and obtain linear combinations of variables that maximize treatment separation. Multivariate patterns were visualized with a canonical biplot based on the first two canonical variables. In this plot, variable and genotype scores under virus-infected and non-infected conditions were co-represented, with interpretation conditioned on the significance of the G × I interaction. Additionally, principal component analysis (PCA) was performed to explore the structure of correlations among variables and identify the association between agronomic and physiological traits.

Results

Infection confirmation

BYDV-PAV infection was confirmed by RT-PCR using the primer pair Shu-F/Yan-R. Amplification of a fragment of expected size of ~ 830 bp was consistently detected in inoculated plants, whereas none was observed in non-inoculated controls (Fig. 1). These results confirmed the successful transmission of BYDV-PAV by viruliferous *R. padi* and the effectiveness of the inoculation procedure. Furthermore, the absence of viral amplification in control plants indicated the non-occurrence of unintended virus transmission during the experimental period, ensuring a clear distinction between infected and non-infected treatments for subsequent physiological and agronomic analyses.

Multivariate analyses of variance for agronomic and physiological traits

The multivariate analyses of variance revealed differential influence of the evaluated factors on the set of agronomic and physiological variables (Table 1). In general, the genotype plays a significant role in most multivariate tests, indicating substantial genetic variability among the evaluated materials. Wilks' Lambda ($p = 0.0089$), Pillai's Trace ($p = 0.0022$), and Roy's largest root ($p = 0.0008$) tests indicated significant overall variations among genotypes; however, the Hotelling–Lawley trace test yielded a marginal value ($p = 0.0545$), still suggesting a tendency toward differentiation. These results confirm that genotypes exhibit distinct responses when growth, yield-related traits, and photosynthetic efficiency variables are considered simultaneously.

Table 1
Results of the multivariate analysis of variance (MANOVA) for the effects of genotype, condition (with and without virus), and the genotype × infection interaction on the set of agronomic and physiological variables

Effect	Statistic	Value	F Value	Pr > F
Genotype	Wilks' Lambda	0.00013	4.41	0.0089
	Pillai's Trace	2.76338	4.17	0.0022
	Hotelling-Lawley Trace	98.5166	10.43	0.0545
	Roy's Greatest Root	79.99438	28.57	0.0008
Infection	Wilks' Lambda	0.01170	18.10	0.0178
	Pillai's Trace	0.98829	18.10	0.0178
	Hotelling-Lawley Trace	84.44866	18.10	0.0178
	Roy's Greatest Root	84.44866	18.10	0.0178
Genotype x Infection	Wilks' Lambda	0.00134	1.91	0.1411
	Pillai's Trace	2.38104	1.37	0.2570
	Hotelling-Lawley Trace	45.46903	4.81	0.1361
	Roy's Greatest Root	35.33442	12.62	0.0056

[1] / $H_0 : \mu_1 = \mu_2 = ... = \mu_k$, $H_1 : \mu_1 \neq \mu_2 \neq ... \neq \mu_k$

For infection (virus presence or absence), a significant impact was also observed across all multivariate tests (*p = 0.0178*), indicating that viral infection alters the set of variables. Convergence among the findings of the four tests reinforces the robustness of this effect, suggesting that the virus influences not only agronomic traits but also physiological variables associated with chlorophyll fluorescence, consistent with expected alterations in the photosynthetic apparatus under conditions of biotic stress typical of plant–virus interactions.

Regarding the G × I interaction, the results were less consistent across tests. While Wilks' Lambda (*p = 0.1411*), Pillai's Trace (*p = 0.2570*), and the Hotelling–Lawley trace (*p = 0.1361*) did not indicate statistical significance in variations, in contrast with Roy's test results (*p = 0.0056*). This pattern suggests that the interaction may be associated with specific differences primarily captured by the largest characteristic roots, indicating that certain genotypes respond variably to viral infection in at least one linear combination of the evaluated variables.

Considering that the null hypothesis tested (H_0: $\mu_1 = \mu_2 = \cdots = \mu$) was rejected for the main effects of genotype and infection, it can be inferred that differences were based on the multivariate structure considered for the levels of these factors. Evidence of a significant interaction highlights the need for

complementary analyses, such as canonical methods, to understand in-depth the patterns of differential genotype responses to virus-induced stress.

Discriminant analysis of genotypes under virus-infected and virus-free conditions

Canonical discriminant analysis enabled the characterization of multivariate variations among wheat genotypes under contrasting conditions of virus presence or absence (Figs. 2 and 3). The first two canonical variables accounted for 97.73% of the total variation, with the first explaining 77.71% (Can1) and the second 20.02% (Can2), indicating that a two-dimensional representation is sufficient to describe the data patterns.

The canonical biplot of variables showed that most of the differences were explained along the first axis, where agronomic traits such as TGN, TGW, RDW, and SDW exhibit the greatest contributions, positioned toward the positive direction of this axis (Fig. 2). PH also contributes, although more moderately, remaining closer to the origin and indicating a secondary role in discrimination along this dimension. In contrast, physiological variables associated with chlorophyll fluorescence, such as NPQ, Y(NPQ), and Y(NO), prominently contribute in direction negative to the first axis, underscoring their importance in defining this dimension in opposition to yield-related traits. The maximum quantum efficiency of PSII (Fv/Fm) also contributes in this direction, albeit with lower magnitude.

Along the second axis, Y(NPQ) showed the maximal positive projection, followed by Y(NO) and Y(II), indicating that these variables are key drivers of the variation explained by this component. Conversely, variables such as NPQ and number of tillers (NT) contributed in the opposite direction, supporting vertical data discrimination. The ETR also exhibited moderate negative contributions to this dimension (Can2); however, initial fluorescence (Fo) and NS presented lower contributions and were located closer to the origin. Overall, the second dimension represented a complementary axis that could capture secondary variations, predominantly associated with physiological traits.

The coefficients of variation revealed differences in the magnitude of variability among the evaluated traits, as shown in Table S1 (Supplementary file 1). Among the agronomic traits, RDW (57.98%), TGW (57.48%), SDW (35.95%), and TGN (32.68%) exhibited the highest coefficients of variation, whereas, among the physiological variables, NPQ (43.15%) and Y(NPQ) (41.07%) showed the greatest relative dispersion. Consistently, these same traits contributed most strongly to genotype discrimination along the canonical axes, indicating that the multivariate analysis captured the major sources of variability among genotypes and infection conditions. This pattern suggests that fluctuations in the mean values of these variables played a key role in distinguishing the observed agronomic and physiological responses.

The distribution of genotypes under virus-infected and virus-free conditions revealed a prominent separation between conditions along the first axis (Fig. 3). Genotypes in the absence of virus were predominantly clustered in the positive region of Can1; in contrast, infected genotypes shifted toward the

negative region, indicating that this axis captures the primary impact of viral infection on the set of evaluated variables.

The magnitude of displacement between points representing the same genotype under the two conditions reflects the intensity of virus-infection response. In this context, the genotypes BRS Timbaúva and BR 35 exhibited the least distances between conditions, indicating greater stability and, consequently, higher tolerance to infection. In contrast, the genotypes Embrapa 16 and BR 14 demonstrated greater displacements between virus-infected and non-infected conditions, characterizing enhanced sensitivity to virus-induced stress. Additionally, a part of this variation, particularly that for BR 14, was also expressed along the second axis (Can2), indicating additional physiological responses captured by this component. Overall, canonical discriminant analysis demonstrated that viral infection remarkably altered genotype positioning, allowing differentiation between tolerance and susceptibility, with BR 35 identified as more tolerant and Embrapa 16 and BR 14 as more susceptible.

Association among traits

PCA revealed distinct patterns of association between agronomic variables and chlorophyll fluorescence variables under virus-infected (With) and virus-free (Without) conditions (Fig. 4). Under both scenarios, the first two principal components explained a substantial proportion of the total variation, allowing a consistent interpretation of relationships among the evaluated traits.

Under virus-infected conditions (With), a prominent association was observed among agronomic variables, such as TGN, TGW, NS, NT, and SDW, which showed vectors oriented in similar directions, indicating positive interrelationships. Such clustering suggests that even under virus-induced stress, yield-related components maintain coordinated behavior. In addition, these variables were associated with NPQ and Y(NPQ), indicating that, under viral infection, mechanisms of non-photochemical energy dissipation are linked to agronomic performance. In contrast, variables such as initial fluorescence (Fo) and Y(NO) were positioned in the opposite direction, indicating a contrasting association relative to the main group. Furthermore, Y(II), ETR, and maximum quantum efficiency of PSII (Fv/Fm) were associated with each other, forming a group related to photosynthetic efficiency; however, their orientation differed from that of yield-related variables, suggesting adjustments in photosynthetic apparatus functioning under biotic stress conditions typical of plant–virus interactions.

In the absence of the virus (Without), the pattern of associations changed. Agronomic variables (TGN, TGW, NS, and NT) remained positively associated with each other, reinforcing their structural relationship with yield. However, unlike under stress conditions, these variables exhibited a closer relationship with Fv/Fm and ETR, indicating that, in the absence of stress, productivity was more directly related to photosynthetic efficiency. In contrast, NPQ and Y(NPQ) were positioned oppositely to agronomic variables, indicating a negative association a diminished role of energy dissipation mechanisms under favorable conditions.

Variables such as RDW and SDW were behaviorally intermediate, with shifts in their correlations depending on infection condition, indicating that biomass allocation responded differently to virus presence. The variable Fo was also distinctly positioned between conditions, reinforcing its sensitivity to physiological changes induced by biotic stress. Overall, these associations demonstrate that viral infection significantly altered the pattern of relationships among variables, promoting a reorganization between agronomic and physiological traits. Under virus-free conditions, productivity was more closely linked to photosynthetic efficiency; however, under viral infection, yield-related traits and energy dissipation mechanisms were most robustly integrated, reflecting acclimation to stress.

Discussion

This study evaluated the impacts of BYDV-PAV infection on the physiological and agronomic traits of wheat genotypes with contrasting responses to the virus. Overall, viral infection altered plant performance, with the extent of changes varying among genotypes. Multivariate analyses revealed that BYDV-PAV infection not only affected physiological and agronomic traits but also their interrelationships. Generally, infected plants shifted from patterns associated with photosynthetic efficiency to those more closely related to photoprotection. However, tolerant genotypes maintained better physiological performance when infected. These findings suggest that BYDV-PAV exerted impacts beyond symptoms and involved a broader reorganization of plant function, affecting resource utilization and overall productivity.

The photosynthetic system is fundamental to growth and yield. One of the most noticeable impact of BYDV-PAV infection was an alteration in chlorophyll fluorescence-associated variables, indicating that the virus markedly affected the photosynthetic apparatus. Phytoviruses disrupt chloroplast structure and function, impeding photosynthetic electron transport, carbon assimilation, and energy metabolism (Zhao et al. 2016). Since BYDV primarily affects the phloem, such a decline in photosynthetic performance was not likely due to any direct effects on the photosynthetic cells themselves but rather to broader physiological disturbances, which can impact assimilate transport, source-sink relationships, hormonal homeostasis, and redox status. Such changes have been linked to declined photosynthetic efficiency and the emergence of BYDV-characteristic symptoms, including chlorosis, stunting, and reduced vigor (Choudhury et al. 2019; Lau et al. 2021, 2022a, b)

The remarkable role of NPQ and quantum yield of regulated energy dissipation (Y(NPQ) in distinguishing infected from non-infected plants indicates that BYDV-PAV activates photoprotective mechanisms that dissipate excess excitation energy (Maxwell1 and Johnson2 2000). Under stressful conditions, plants typically enhance thermal energy dissipation to prevent photosystem II (PSII) overexcitation and limit reactive oxygen species (ROS) formation; ROS can oxidatively damage cell structures (Baker 2008). In this context, the robust association of NPQ-related variables with infected plants suggests that viruses imposes a physiological burden, causing a greater proportion of absorbed light energy to be redirected toward photoprotection rather than utilization in photochemistry. Such an interpretation aligns with studies indicating that BYDV infection suppresses photosynthesis, reduces chlorophyll content, and

affects gas exchange and chlorophyll fluorescence in wheat, particularly the intolerant genotypes (Choudhury et al. 2019). Similar responses have been observed with other plant–virus interactions, where disruptions in photosynthetic metabolism result in excess energy absorption and activation of protective mechanisms that maintain photosystem integrity (Liu et al. 2020). In a recent review, Grishina et al. (2024) reported that increases in NPQ, accompanied by reductions in PSII photochemical efficiency, represent a response common across viral pathosystems, reflecting a shift from photochemical energy use to photoprotective energy dissipation.

While the activation of NPQ-related processes may protect the photosynthetic apparatus from photodamage, it also reflects a shift in energy allocation away from photochemical processes. Such a trade-off was evidenced by the contribution of Y(II), ETR, and Fv/Fm to the discrimination between infected and non-infected plants, indicating that BYDV-PAV affected light energy conversion and electron transport efficiencies within PSII. Reductions in these variables can be commonly interpreted as indicators of impaired photosynthetic performance and carbon assimilation capacity, as Y(II) and ETR are strongly associated with the efficiencies of photochemical energy conversion and electron transport operating through PSII (Baker 2008). Consequently, a smaller fraction of the absorbed light energy is available for photochemistry, thereby limiting electron transport and the synthesis of assimilates required for growth. Collectively, these findings suggest that photoprotective mechanisms are activated under viral infection at the expense of photosynthetic efficiency, ultimately constraining the physiological processes that sustain biomass accumulation and grain production.

The altered physiology of infected plants is likely to directly influence crop performance, given the primary role of photosynthesis in biomass accumulation and grain production. Carbon-based assimilates generated through photosynthesis provide the substrates and energy required for tiller development, spike growth, grain filling, and other yield-associated processes. Therefore, any reduction in photosynthetic efficiency due to viral infection may compromise the allocation of resources to reproductive structures, ultimately affecting growth and productivity. This interpretation is supported by studies demonstrating that BYDV infection can remarkably reduce biomass accumulation, grain yield, and yield components in wheat and other cereals, particularly when infection occurs during the early stages of development (Bank et al. 1995; Edwards et al. 2001; Lau et al. 2021, 2022a).In Brazilian wheat cultivars, yield losses caused by BYDV-PAV have reached up to 60.8% in susceptible genotypes, accompanied by reductions in plant height, dry matter accumulation, tillering, grain number, and thousand-kernel weight (Lanzarini et al. 2007). More recently, long-term evaluations of commercial cultivars grown in southern Brazil have shown that even the most tolerant cultivars generally exhibit average yield losses of approximately 20%, whereas susceptible cultivars frequently suffer reductions approaching 80% (Lau et al. 2022a). Such effects are consistent with the broader impact of pathogen infection on photosynthetic carbon assimilation and source–sink relationships, which ultimately limits the availability of assimilates for plant growth and grain production (Berger et al. 2007). Together, these studies reinforce the close link between virus-induced physiological impairment and losses in crop productivity.

Beyond the individual effects of BYDV-PAV on physiological and agronomic traits, the PCA revealed vital changes in the relationships among these variables. Under non-infected conditions, yield-related traits were more closely associated with variables linked to photosynthetic efficiency, indicating that crop productivity largely depended on the effective conversion of absorbed light energy into assimilates *via* photochemical processes. In contrast, under viral infection, productive traits were more robustly associated with photoprotection-linked variables, particularly those related to energy dissipation. Such a shift suggests that BYDV-PAV promoted a reorganization of plant function, in which maintaining photosystem stability was vital for sustaining growth and productivity under stress conditions.

This altered pattern among trait associations suggests that BYDV-PAV changes resource allocation priorities. Under favorable conditions, they were primarily directed toward carbon assimilation and carbon translocation, biomass accumulation, and reproductive development. However, when challenged by viral infection, plants invest a greater proportion of their metabolic resources in protective mechanisms that preserve cell integrity and photosynthetic functionality. Such reallocations are common during plant–pathogen interactions, where resources that would otherwise support growth and reproduction are redirected toward defense, maintenance, and stress adaptation processes, generating trade-offs between yield and protection (Berger et al. 2007). This shift reflects a classic adaptation common under biotic stresses, in which maintaining physiological stability becomes a prerequisite for sustaining growth. Although such adjustments may enhance survival and reduce stress-induced damage, they also impose energy costs that can ultimately restrict yield.

Although BYDV-PAV induced similar physiological responses across the experimental population, the magnitude of changes differed among genotypes, underscoring the importance of genetic background in determining performance under viral stress. The significant influence of genotype detected *via* multivariate analyses, together with distinct clustering patterns observed in the discriminant analysis, indicated that wheat genotypes adopted varied response strategies following infection. Genotypes exhibiting smaller shifts between infected and non-infected conditions within the multivariate space appeared to maintain physiological stability more effectively, suggesting enhanced tolerance to BYDV-PAV. Conversely, genotypes showing larger displacements were characterized by more pronounced alterations in physiological and agronomic traits, reflecting enhanced susceptibility to virus-induced stress. This pattern is consistent with the historical development of wheat breeding for BYDV in Brazil. Early studies by Caetano (1972) established a conceptual distinction between resistance, defined by the proportion of infected plants, and tolerance, evaluated according to the ability of infected plants to maintain vegetative growth despite infection. These studies demonstrated that most Brazilian wheat genotypes were susceptible and intolerant to BYDV, while only a small proportion exhibited moderate to high levels of tolerance. Consequently, Brazilian breeding programs have predominantly selected for tolerance rather than resistance, using tolerant sources such as IAC 5 Maringá and Pel 14410-64 Londrina, from which cultivars such as BR 35 were subsequently developed (Lía Barbieri et al. 2001). More recent studies have reinforced this concept by showing that cultivars such as BR 35 and BRS Timbaúva remain susceptible to viral infection but consistently exhibit lower reductions in growth and grain yield than more intolerant genotypes, indicating that tolerance has been the predominant adaptive

strategy selected for the wheat–BYDV pathosystem in Brazil (Lau et al. 2021, 2022a). This interpretation is further supported by the observations of Comeau and Haber (2002), who demonstrated that virus accumulation alone is not necessarily associated with symptom severity or yield loss, emphasizing that the ability of infected plants to maintain physiological performance is a more appropriate indicator of tolerance than viral titer per se. Similar genotype-dependent responses have been reported in other plant–pathogen interactions, in which the influence of infection on photosynthesis, growth, and productivity varied with each host's ability to cope with stress-induced physiological disturbances (Berger et al. 2007).

Such apparent maintenance of physiological performance observed in certain genotypes is particularly relevant because tolerance and resistance do not necessarily share mechanisms. Classical resistance is generally associated with restricting virus infection, replication, or systemic movement, lowering pathogen accumulation within host tissues; however tolerant plants sustain infection while exhibiting limited reductions in growth and productivity (Cooper 1983). Studies on BYDV infection in wheat have demonstrated that virus accumulation, symptom expression, and yield losses often poorly correlate, indicating that tolerance is largely determined by the plant's ability to mitigate the physiological consequences of infection rather than by its capacity to restrict viral multiplication (Comeau and Haber 2002). From this perspective, the reduced multivariate displacement observed in genotypes such as BR 35 suggests mechanisms that help preserve physiological functionality under stress, allowing plants to maintain a more balanced functional state despite viral infection.

The differential responses among genotypes also highlight the complexity of wheat–BYDV-PAV interactions. Although infection induced common physiological adjustments across genotypes, multivariate analyses indicated that the magnitude and combination of these responses differed based on host's genetic background. Such an interpretation is supported by the G × I interaction detected by Roy's Greatest Root, suggesting that infection effects were non-uniformly expressed across evaluated traits. Rather than affecting individual variables in isolation, BYDV-PAV appeared to influence interconnected physiological and agronomic processes with varied relative relevance among genotypes. An explanation for this pattern may lie in the integration of multiple processes, including photosynthesis, photoprotection, assimilate partitioning, growth, and resource allocation, operating simultaneously during viral infection. Consequently, the interaction between genotype and infection cannot be fully explained by a single trait; rather, it reflects the combined behavior of numerous physiological and agronomic components.

These findings also emphasize the value of multivariate approaches for investigating plant–virus interactions. While univariate analyses are useful for identifying changes in individual traits, they may overlook broader patterns that emerge from relationships among variables. In this study, integration of physiological and agronomic data helped identify genotype-specific response patterns and detect shifts in trait associations that would be difficult to achieve through isolated analyses. Consequently, the multivariate framework provided a more comprehensive view of how BYDV-PAV affects plant functioning and how different genotypes cope with viral stress.

From an applied perspective, these findings have crucial implications for wheat breeding and BYDV management. The identification of genotypes capable of maintaining physiological processes under infection reinforces the importance of tolerance as a complementary target to classical resistance-based breeding. In pathosystems such as BYDV, where complete resistance is limited and often controlled by quantitative inheritance, breeding strategies have increasingly focused on identifying genotypes that sustain growth and yield despite infection rather than preventing virus establishment. Such a concept of tolerance has long been recognized as vital breeding objective because it contributes directly to yield stability under disease pressure and reduces the risk of rapid silencing of major resistance-conferring genes (Allard 1960; Parlevliet 1979). In this context, the smaller multivariate displacement observed in BR 35 and BRS Timbaúva indicated that these genotypes preserved physiological and productive performance following infection, reinforcing their potential as wheat genetic sources for breeding BYDV tolerance. Indeed, as previously discussed, tolerance appears to have been the primary trait selected for coping with barley yellow dwarf in Brazil (Lía Barbieri et al. 2001; Lau et al. 2022a).

One of the main findings of this study is that genotype discrimination can be improved substantially by integrating agronomic and physiological variables. Traditional breeding programs have relied primarily on grain yield or symptom severity as selection criteria. However, as tolerance results from the coordinated regulation of multiple physiological processes, integrative approaches such as the one adopted here may more realistically represent performance under viral stress. The evaluation of chlorophyll fluorescence-associated variables and productivity-related traits enabled the identification of genotypes that maintained photosynthetic function while minimizing yield losses, thereby providing complementary selection criteria helpful in segregating populations. Chlorophyll fluorescence-associated variables indicating photochemical efficiency and energy dissipation represent informative secondary traits for the indirect selection of BYDV tolerance. As these assessments are rapid, non-destructive, and highly reproducible, they are promising phenotyping tools, especially when combined with multivariate statistical approaches that integrate complex physiological responses. Rather than replacing conventional agronomic evaluations, they complement these examinations by revealing the functional mechanisms underlying tolerance, thereby facilitating the efficient identification of wheat germplasm valuable for breeding programs. Similar concepts have been advocated for physiological breeding, in which secondary traits associated with stress adaptation are incorporated to improve crop efficiency (Araus et al. 2008; Reynolds and Langridge 2016)

Collectively, these findings demonstrate that the effects of BYDV-PAV infection extend beyond isolated physiological or agronomic alterations and involve a coordinated reorganization of plant functioning. Rather than simply reducing performance, viral infection reshaped the relationships among physiological and productive traits, reflecting adaptive adjustments to biotic stress. The multivariate approach adopted in this study was particularly effective in capturing this complexity, revealing response patterns that would likely have remained undetected by analyzing individual variables alone. Furthermore, the contrasting responses among wheat genotypes signify the importance of genetic variability in determining plant resilience to viral infection and reinforce the concept that tolerance must be

interpreted as an integrated physiological phenomenon rather than just an expression of isolated traits. This interpretation is consistent with the hypothesis proposed by Comeau and Haber (2002), who suggested that tolerance to BYDV may represent the expression of broader mechanisms of physiological efficiency and stress adaptation rather than responses restricted to viral infection itself. According to this concept, genotypes capable of maintaining physiological function under BYDV infection are also more likely to preserve yield stability under other biotic and abiotic stresses because they share common pathways involved in carbon allocation, energy metabolism, signaling, and resource-use efficiency. The greater physiological stability observed in the tolerant genotypes evaluated here provides physiological evidence consistent with this hypothesis, suggesting that maintenance of photosynthetic performance may represent one of the mechanisms underlying this broader adaptive response. Accordingly, BYDV has been proposed not only as a disease of economic importance but also as a valuable biological tool for identifying germplasm with superior yield stability and adaptation to complex stress environments (Comeau and Haber, 2002). By integrating multivariate statistics with physiological and agronomic evaluations, the present study advances our understanding of wheat–BYDV interactions and provides a robust framework for identifying physiological mechanisms associated with tolerance and for developing cultivars with greater yield stability in environments where BYDV is endemic.

Although the present study evaluated plants at advanced developmental stages, where the cumulative effects of infection were fully expressed, future investigations should determine how early these physiological differences become detectable following BYDV infection. Identifying the onset of genotype-specific physiological responses may provide valuable opportunities for earlier phenotyping in breeding programs. Likewise, integrating chlorophyll fluorescence with histological analyses of phloem degeneration and carbohydrate partitioning would help clarify whether the superior physiological performance of tolerant genotypes primarily results from reduced disruption of phloem function or from enhanced capacity to maintain photosynthetic activity despite comparable levels of vascular damage.

Conclusion

BYDV-PAV infection induced significant changes in the physiological and agronomic performance of wheat genotypes, affecting photosynthetic efficiency and yield-related traits. Viral infection reorganized functional integration between physiological and productive processes, and multivariate analyses enabled discrimination of wheat genotypes based on their responses to infection. The smaller multivariate displacement observed for BR 35 and BRS Timbaúva indicated the maintenance of physiological responses under virus pressure, reinforcing their potential as genetic sources of tolerance in wheat breeding programs. Furthermore, integrating chlorophyll fluorescence measurements with multivariate statistical approaches proved to be effective for characterizing genotype-specific responses and identifying physiological mechanisms associated with tolerance to BYDV-PAV. These findings contribute to a broader understanding of wheat–virus interactions and provide valuable support for breeding programs aimed at developing cultivars with greater resilience to viral infection stress.

Declarations

Competing interests:

The authors declare no competing interests.

Ethical Approval:

Not applicable.

Consent to participate:

Not applicable.

Consent to publish:

Not applicable.

Funding

This work was supported by the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) (Project “Melhoramento genético de trigo para o Brasil – 2023 a 2026”, grant 20.22.01.001.00.00), the Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina (FAPESC) (Grant 2024TR002600 and 2023TR332), and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (Grant 304883/2025-5). FNS is a research productivity fellow of the CNPq. SCN, LTC, and VMS received doctoral scholarships from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Finance Code 001.

Author contributions

SCN, LTC, and VMS coordinated sample collection and laboratory activities, analyzed the data, and wrote the manuscript. DL, DS, GCM, RTC, and FNS designed the study, supervised the research activities, contributed to data interpretation, and critically revised the manuscript. All authors read and approved the final manuscript.

Acknowledgements

The authors thank the technical staff of Embrapa Trigo for their assistance during the greenhouse and laboratory experiments.

Data Availability

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

References

1. Allard RW (1960) Principles of Plant Breeding. Wiley, New York
2. Araus JL, Slafer GA, Royo C, Serret MD (2008) Breeding for Yield Potential and Stress Adaptation in Cereals. CRC Crit Rev Plant Sci 27:377–412. https://doi.org/10.1080/07352680802467736
3. Baker NR (2008) Chlorophyll fluorescence: A probe of photosynthesis in vivo. Annu Rev Plant Biol 59:89–113
4. Bank PM, Barianaa ~ H, Larkina PJ (1995) Effects of Barley Yellow. Dwarf Virus on the Yield of Winter Wheat
5. Berger S, Sinha AK, Roitsch T (2007) Plant physiology meets phytopathology: Plant primary metabolism and plant-pathogen interactions. J Exp Bot 58:4019–4026
6. Choudhury S, Larkin P, Meinke H et al (2019) Barley yellow dwarf virus infection affects physiology, morphology, grain yield and flour pasting properties of wheat. Crop Pasture Sci 70:16–25. https://doi.org/10.1071/CP18364
7. Comeau A, Caetano VR, St-Pierre CA, Haber S (2001) Accelerated Breeding: Dream or Reality? Developments in Plant Breeding 671–679. https://doi.org/10.1007/978-94-017-3674-9_90
8. Comeau A, Haber S (2002) Breeding for BYDV Tolerance in Wheat as a Basis for a Multiple Stress Tolerance Strategy. In: Lapierre H, Signoret P (eds) Barley Yellow Dwarf Disease. Recent Advances and Future Strategies, Éditions INRA
9. Cooper JI (1983) Responses of Plants to Viruses: Proposals for the Use of Terms. Phytopathology 73:127. https://doi.org/10.1094/Phyto-73-127
10. Cunha GR da, Caierão E (2023) Informações Técnicas para 15ª Reunião da Comissão Brasileira de Pesquisa de Trigo e Triticale
11. Edwards MC, Fetch TG, Schwarz PB, Steffenson BJ (2001) Effect of *Barley yellow dwarf virus* Infection on Yield and Malting Quality of Barley. Plant Dis 85:202–207. https://doi.org/10.1094/PDIS.2001.85.2.202
12. Kaddachi I, Souiden Y, Achouri D, Chéour F (2014) BARLEY YELLOW DWARF VIRUS (BYDV): CHARACTERISTICS, HOSTS, VECTORS, DISEASE SYMPTOMS AND DIAGNOSIS. Int J Phytopathol 155–160
13. Klughammer CSU (2008) Complementary PS II quantum yields calculated from simple fluorescence parameters measured by. PAM fluorometry and the Saturation Pulse method
14. Lanzarini AC, Schons J, Salvadori JR et al (2007) Avaliação de Danos Causados pelo Barley yellow dwarf virus-PAV em Cultivares de Trigo no Brasil. Fitopatol Bras 32:6
15. Lau D, Bernardon Mar T, Ribeiro dos Santos CD et al (2021) Advances in understanding the biology and epidemiology of barley yellow dwarf virus (BYDV). pp 709–746

16. Lau D, Mar TB, De Lima R (2022a) Reação ao BYDV-PAV de cultivares de trigo do ensaio estadual do Rio Grande do Sul, em 2021
17. Lau D, Mar TB, Silva FN et al (2022b) Barley yellow dwarf virus em trigo no Brasil. Revisão Anual de Patologia de Plantas 216–239. https://doi.org/10.31976/0104-038321v280010
18. Lía Barbieri R, Irajá Félix de Carvalho F, Fernandes Barbosa Neto J et al (2001) Análise dialélica para tolerância ao vírus-do-nanismo-amarelo-da-cevada em cultivares brasileiras de trigo (1)
19. Liu Y, Liu Y, Spetz C et al (2020) Comparative transcriptome analysis in Triticum aestivum infecting wheat dwarf virus reveals the effects of viral infection on phytohormone and photosynthesis metabolism pathways. https://doi.org/10.1186/s42483-019-0042-6. Phytopathology Research 2:
20. Malmstrom CM, Shu R (2004) Multiplexed RT-PCR for streamlined detection and separation of barley and cereal yellow dwarf viruses. J Virol Methods 120:69–78. https://doi.org/10.1016/j.jviromet.2004.04.005
21. Maxwell K 1, Johnson2 GN (2000) Chlorophyll fluorescence-a practical guide
22. Parizoto G, Rebonatto A, Schons J, Lau D (2013) Barley yellow dwarf virus-PAV in Brazil. Seasonal fluctuation and biological characteristics
23. Parlevliet JE (1979) Components of Resistance that Reduce the Rate of Epidemic Development. Annu Rev Phytopathol 17:203–222. https://doi.org/10.1146/annurev.py.17.090179.001223
24. Rebonatto A, Salvadori JR, Lau D (2015) Temporal Changes in Cereal Aphids (Hemiptera: Aphididae) Populations in Northern Rio Grande do Sul, Brazil. J Agric Sci 7. https://doi.org/10.5539/jas.v7n10p71
25. Reynolds M, Langridge P (2016) Physiological breeding. Curr Opin Plant Biol 31:162–171. https://doi.org/10.1016/j.pbi.2016.04.005
26. Roger H (2004) Matthews' Plant Virology, Fourth
27. Zhao J, Zhang X, Hong Y, Liu Y (2016) Chloroplast in plant-virus interaction. Front Microbiol 7

Figures

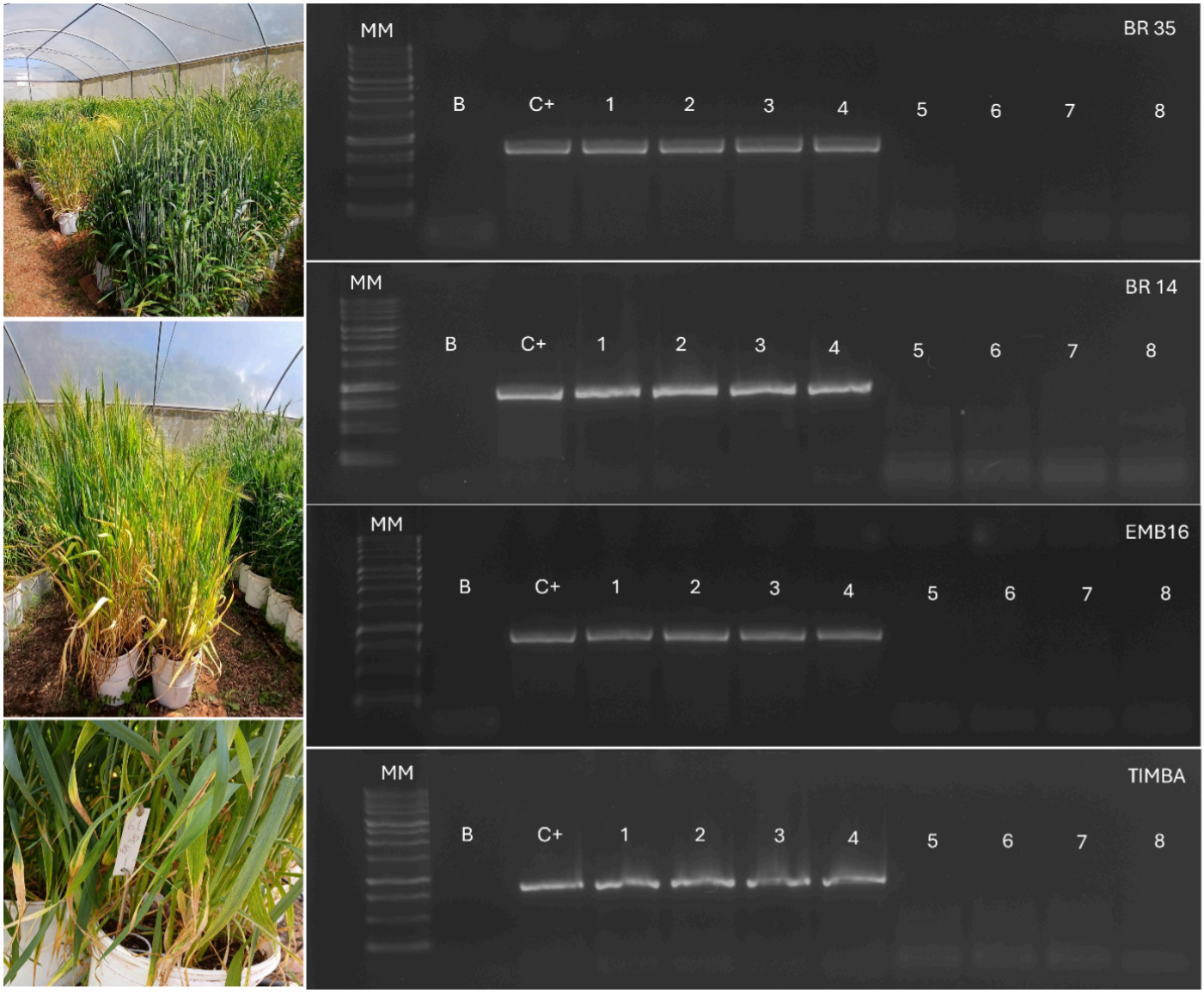

Figure 1

Symptom expression and molecular detection of barley yellow dwarf virus-PAV (BYDV-PAV) in wheat genotypes with contrasting responses to infection. Representative photographs (left) showing symptom development following aphid-mediated inoculation of BYDV-PAV under greenhouse conditions and RT-PCR detection of the virus (right). Four wheat genotypes were evaluated: BR 35, BR 14, Embrapa 16 (EMB16), and BRS Timbaúva (TIMBA). Lanes 1–4 indicate inoculated plants, whereas lanes 5–8 represent non-inoculated controls. MM, molecular size marker; B, negative control; C+, positive control

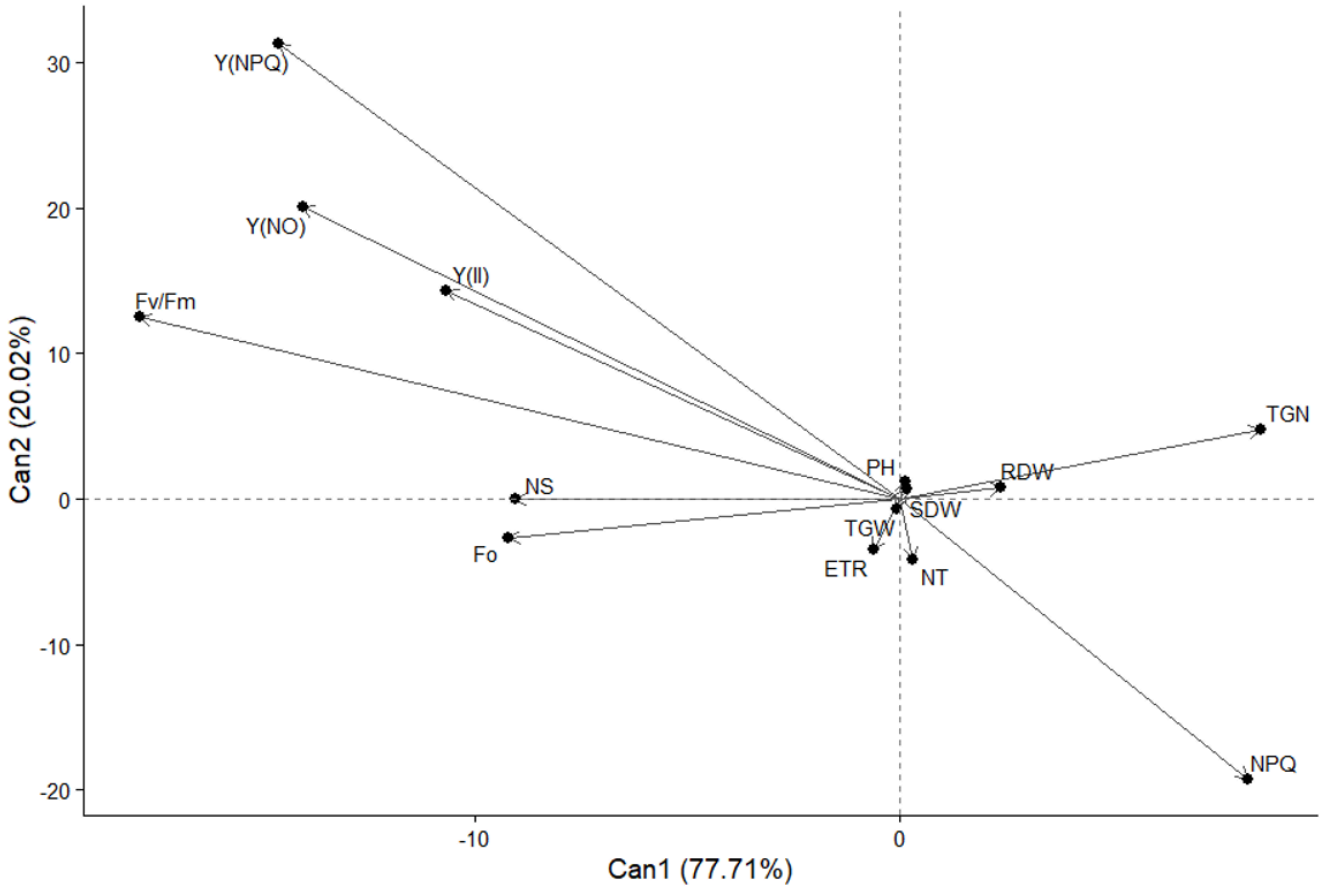

Figure 2

Canonical biplot of agronomic and chlorophyll fluorescence variables, including PH (plant height), RDW (root dry weight), SDW (shoot dry weight), NT (number of tillers), NS (number of spikes), TGN (total grain number), TGW (total grain weight), Y(II) (effective quantum yield of PSII), NPQ (non-photochemical quenching), Fo (minimum fluorescence), Y(NPQ) (quantum yield of regulated energy dissipation), ETR (electron transport rate), Y(NO) (quantum yield of non-regulated energy dissipation), and Fv/Fm (maximum quantum efficiency of PSII), based on the first two canonical variables (Can1 = 77.71% and Can2 = 20.02%)

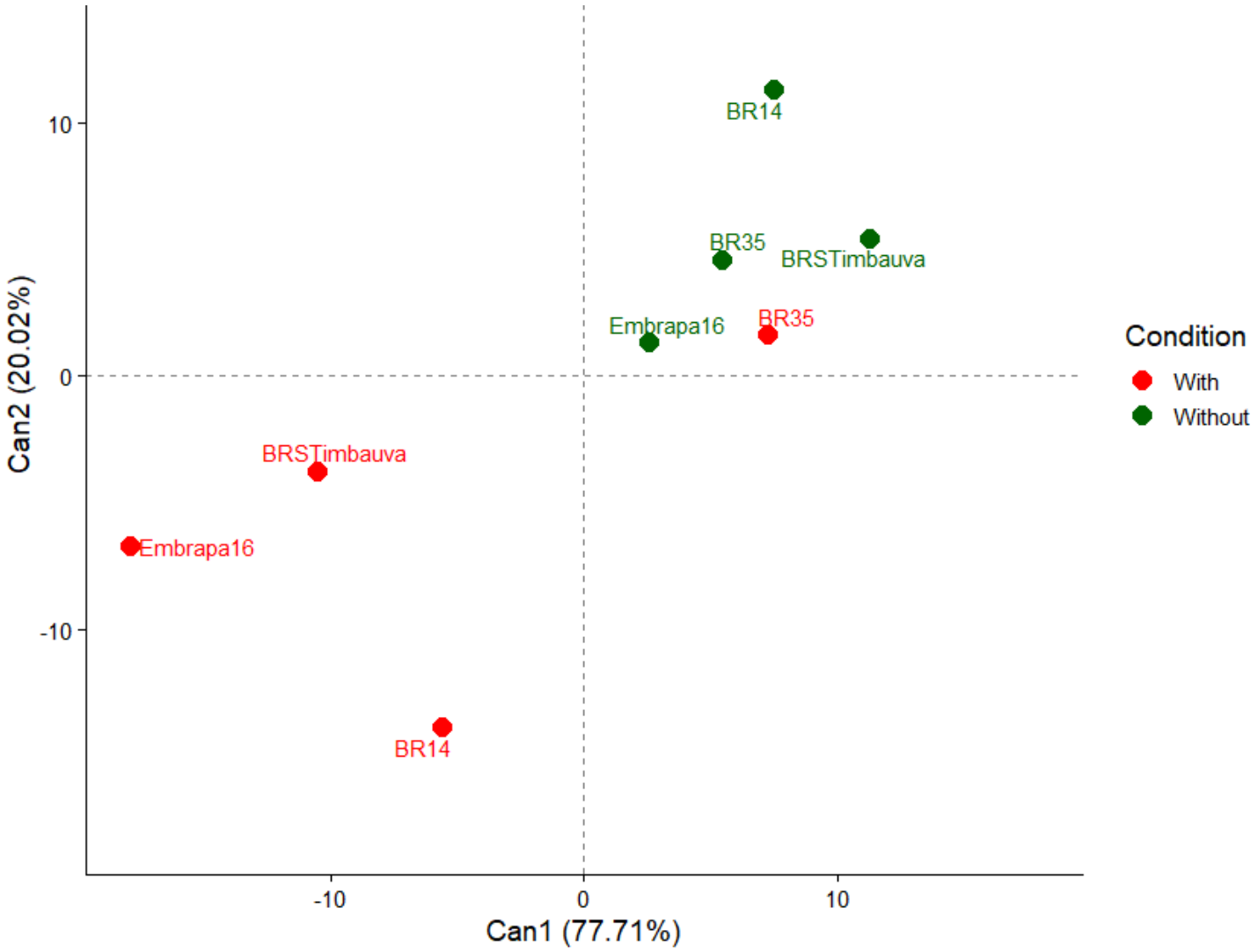

Figure 3

Canonical biplot based on the first two canonical variables (Can1 = 77.71% and Can2 = 20.02%) showing the multivariate response of wheat genotypes under contrasting conditions of virus presence. Points represent the mean values of each genotype under With (red, virus-infected) or Without (green, non-infected) conditions. The spatial separation between points indicates differences in overall agronomic and physiological performance, whereas the distance between paired points with the same genotype reflects the magnitude of response to virus infection. Dashed lines indicate the origin of the canonical axes

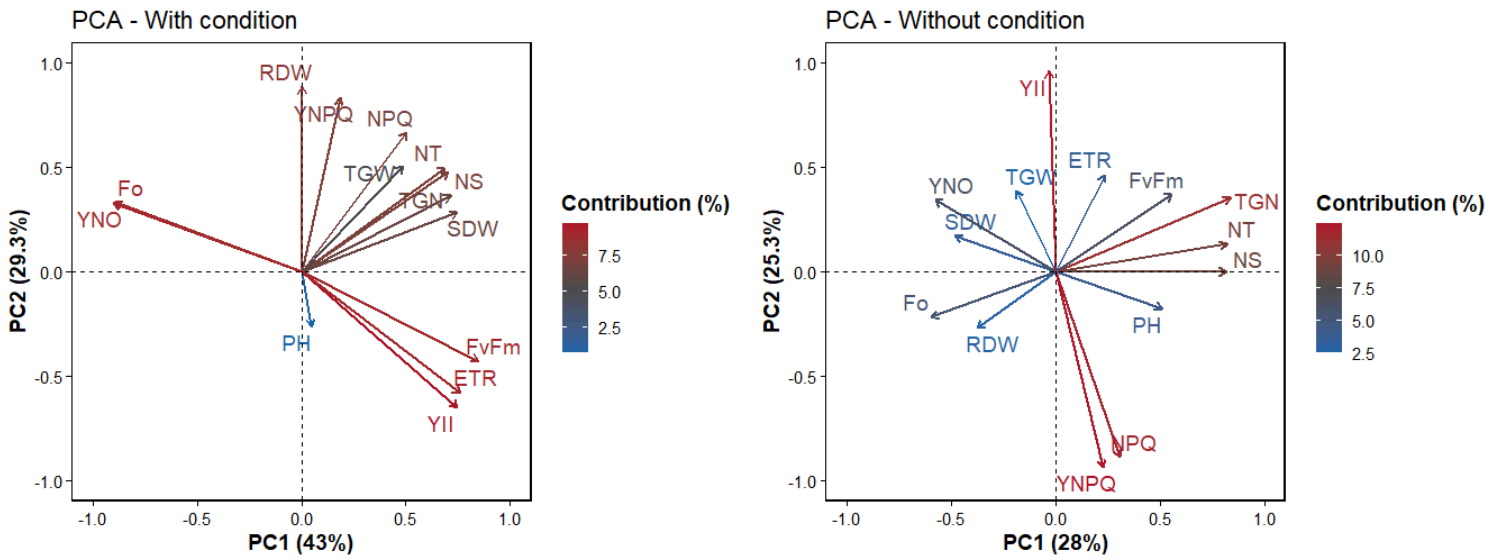

Figure 4

Principal component analysis (PCA) of agronomic and chlorophyll fluorescence traits, including PH (plant height), RDW (root dry weight), SDW (shoot dry weight), NT (number of tillers), NS (number of spikes), TGN (total grain number), TGW (total grain weight), Y(II) (effective quantum yield of PSII), NPQ (non-photochemical quenching), Fo (minimum fluorescence), Y(NPQ) (quantum yield of regulated energy dissipation), ETR (electron transport rate), Y(NO) (quantum yield of non-regulated energy dissipation), and Fv/Fm (maximum quantum efficiency of PSII), in wheat genotypes under virus-infected (With) and non-infected (Without) conditions, based on the first two principal components (PC1 and PC2)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Suplementaryfile.docx