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Research Article
(Araştırma Makalesi)

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Luteovirus pavhordei (BYDV-P0) in durum wheat: molecular diagnosis, disease intensity and impact on yield components

Makarnalık buğdayda *Luteovirus pavhordei* (*BYDV-P0*): moleküler tanısı, hastalık şiddeti ve verim bileşenleri üzerindeki etkisi

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ABSTRACT

Objective: *Luteovirus pavhordei is increasingly caus*ing significant crop losses worldwide, affecting several crops, including durum wheat (*Triticum turgidum* L. var. *durum*), which is grown extensively. Plants possess innate defense mechanisms such as resistance genes against viral diseases. Among these, the *Bdv2* gene, which occurs naturally in wild wheat varieties, provides resistance specifically to *BYDV-P0*. In this study, it was observed infected durum wheat varieties exhibited lower chlorophyll (SPAD), number of grains per ear (grains/ear) and ear yield (g/ear).

Material and Methods: The visual assessment revealed a high symptomatic infection among *Luteovirus pavhordei* infected durum wheat, underscoring the severity of the disease. Using *Bdv2*-specific primers (*BYAgi*), it was screened for the presence of the *Bdv2* gene in five different durum wheat varieties; however, none harbored this resistance gene.

Results: The findings indicate that the absence of the *Bdv2* gene in the studied durum wheat varieties renders them susceptible to *BYDV-P0*. The virus-induced reductions in chlorophyll and ear yield formation further highlight the detrimental impact of *BYDV-P0* on durum wheat.

Conclusion: These results underscore the urgency of developing strategies to mitigate the effects of this viral disease in wheat cultivation

ÖZ

Amaç: *Luteovirus pavhordei* (*BYDV-P0*), yaygın olarak yetiştirilen makarnalık buğday (*Triticum turgidum* L. var. *durum*) dahil olmak üzere çeşitli ürünleri etkileyerek dünya çapında giderek daha fazla ürün kaybına neden olmaktadır. Bitkiler, viral hastalıklara karşı dayanıklılık genleri gibi doğuştan gelen savunma mekanizmalarına sahiptir. Bunlar arasında, yabani buğday çeşitlerinde doğal olarak bulunan *Bdv2* geni, özellikle *BYDV-P0*'a karşı dayanıklılık sağlamaktadır. Bu çalışmada, enfekteli makarnalık buğday çeşitlerinin daha düşük klorofil (SPAD), başak başına tane sayısı (tane/başak) ve başak verimi (g/başak) sergilediği gözlemlenmiştir.

Materyal ve Yöntem: Görsel değerlendirme, *BYDV-P0* ile enfekte makarnalık buğday çeşitleri arasında yüksek simptomatik enfeksiyon olduğunu ortaya koyarak hastalığın ciddiyetini vurgulamaktadır. *Bdv2*'ye özgü primerler *(BYAgi)* kullanarak, beş farklı makarnalık buğday çeşidinde *Bdv2* geninin varlığını taranmıştır; ancak çeşitlerde dayanıklılık geni saptanmamıştır.

Araştırma Bulguları: Bulgular, *Bdv2* geninin makarnalık buğday çeşitlerinde bulunmamasının çeşitleri *BYDV-P0*'a karşı hassas hale getirdiğini göstermektedir. Virüsün klorofil oluşumu ve başak veriminde neden olduğu azalmalar, *BYDV-P0*'ın makarnalık buğday çeşitleri üzerindeki olumsuz etkisini daha da ön plana çıkarmaktadır.

Sonuç: Bu sonuçlar, buğday yetiştiriciliğinde bu viral hastalığın etkilerini kontrolü ve neden olduğu ürün kayıplarını azaltmak için yeni stratejiler geliştirmenin aciliyetini vurgulamaktadır.

INTRODUCTION

Wheat plays a crucial role in global and national nutrition due to its rich content of essential nutrients such as vitamins, minerals, carbohydrates and fiber. As a fundamental cereal crop, it significantly contributes to addressing food security challenges and alleviating hunger worldwide (Shiferaw et al., 2013; Tonk et al., 2017). *Luteovirus pavhordei* is a very destructive viral disease that has significant economic impact for cereal crops worldwide. This problem significantly decreases the yield of important cereal crops such as wheat, barley, rice, maize and oats (Perry et al., 2000). The occurrence of *Luteovirus pavhordei* in barley plants was initially documented in 1951 in California, USA (Oswald & Houston, 1953). The disease observed in barley and other crops in the northern states of the USA in 1961 has been identified as *Luteovirus pavhordei* disease (Rochow, 1961). *BYDV-P0*, a member of the *Luteovirus* genus, is the most common and harmful virus in the group of *Luteovirus pavhordei*. It can be transmitted by at least 25 different species of aphid vectors. Almost every plant species in the *Poaceae* family can be infected, with over 150 possible sources of pathogens (Hewings, 1995; Power & Gray, 1995). Many different strategies, such as the use of insecticides to decrease aphid populations and the cultivation of resistant plant varieties, have been suggested to reduce the severe impact of *BYDV-P0* on major cereal crops worldwide (Jarošová et al., 2013). The resistance to BYDV is determined and associated to a complex ability that is inherited by multiple genes. Within these genes, four major resistance genes (*Bdv1*, *Bdv2, Bdv3* and *Bdv4*) have been identified. The most effective gene that has been successfully introduced in wheat varieties is *Bdv2*, which has demonstrated high levels of resistance to *BYDV-P0* (Jarošová et al., 2016). Among all wild relatives of wheat, *Thinopyrum intermedium* was found to have BYDV resistance genes (*Bdv2, Bdv3*, and *Bdv4*) and was the most successful wild wheat relative in resistance. It has been reported that BYDV resistance obtained from the *Thinopyrum intermedium* plant is imparted to cultivated wheat in the form of chromosome addition, substitution line, and translocation line,' which can be detected with different molecular markers (Zhang et al., 2009). The *Bdv2* gene was detected in *Thinopyrum intermedium* plant *7Ai1*(7X) in the chromosome (Brettell et al., 1988; Xin et al., 1988). To identify the *Bdv2* gene conferring effective resistance to *BYDV-P0*, the SCAR marker *BYAgi* was designed to amplify its recombinant translocation at the disomic *L1* arm and *7Ai1 L* position in the wheat genome (Stoutjesdijk et al., 2001).

Limited research exists regarding the influence of specific plant viruses on chlorophyll levels in different plant species and the corresponding symptomatology (Lehto et al., 2003). A notable example is the impact of rice stripe virus (RSV) on rice plants, where the down-regulation of certain genes involved in chlorophyll biosynthesis, such as magnesium chelatase subunit I (CHLI) and subunit D (CHLD), can lead to a decrease in chlorophyll content and the occurrence of leaf chlorosis in rice (Wang et al., 2015). Similarly, in the case of African cassava mosaic virus (ACMV) infection in cassava plants, the manifestation of leaf yellowing symptoms can be attributed to both chlorophyll degradation and reduced expression levels of genes responsible for encoding the major apoproteins in the light-harvesting complex II (Liu et al., 2014). Currently, there is a paucity of research examining the detection of chlorophyll contents in wheat plants consequent to the infection of *Luteovirus pavhordei*.

In this study, the effects on yield and quality of some durum wheat (*Triticum turgidum* L. var. *durum*) varieties infected with *BYDV-P0* were investigated for the first time according to our knowledge. Furthermore, the *Bdv2* gene, which confers resistance to *BYDV-P0*, was assessed.

MATERIALS and METHODS

Plant materials

In 2023, this experiment was conducted in greenhouse conditions where the temperature was maintained between 24-28°C during the day and 18-22°C at night, with 60-70% relative humidity and a 16/8-hour light/dark photoperiod at the Faculty of Agriculture of Aydın Adnan Menderes University. The

key aspect of this study was the selection of durum-wheat varieties "Tüten, Alatay, Ç1252, Poyraz, Şölen" as plant material. Furthermore, *Thinopyrum intermedium* was employed as a positive control for the detection of the *Bdv2* gene. For each variety, four plants were considered, and the experiment was conducted with three repetitions. Sterilized plastic containers (200 x 180 mm) were used to place wheat seedlings into a 1:1 mixture of perlite and soil. The wheat varieties were cultivated in the greenhouse, and the plants were irrigated with tap water every three days (Yıldırım et al., 2020).

Aphid colony

A culture of Bird-cherry aphid*, Rhopalosiphum padi,* was collected from a wheat and barley field in Aydın. *R. padi* were transferred to healthy wheat plants. The populations were then reared on wheat in a cage in a controlled environment in the autumn of 2023. *R. padi* were kept on *BYDV-P0*-infected wheat plants for 48 hours (Acquisition Access Period) to acquire the *BYDV-P0* virus. Twenty-one-day-old plants were then inoculated with *BYDV-P0* using the viruliferous individuals of *R. padi* for 72 hours (Infection Access Period). The Aphox insecticide (Adama, UK) (0.88 mg/per plastic containers) was applied to the plants to control the aphid vector (Parizoto et al., 2013). Plants were incubated for symptom expression in separate insect-proof net cages at a greenhouse.

Detection of *BYDV-P0* in durum wheat

A diseased plant of wheat, showing yellowing, stunting, and dwarfing symptoms, was collected from the field in xxx. Total nucleic acid was extracted from diseased leaf (Foissac et al., 2001) and one-step RT-PCR analysis was performed using Hotstart RT master mix and RTase (Ampliqon, Odense, Denmark). The following *BYDV-P0* specific primer pairs, F-5'ATGAATTCAGTAGGTCGTAG'3 and R-5'GAGGAGTCTCTATTTGGC'3 (Usta, 2013) was used. The PCR conditions for the reaction were as follows: 95ºC for 5 min, 94ºC for 1 min, 42ºC for 1 min, and 72ºC for 1 min for 35 cycles; 5 min for 72ºC and for each 25 µl sample mixture. The data was analyzed using the electrophoresis with 1.4% 1X TBE.

Isolation of genomic DNA and *Bdv2* gene detection

Total DNA samples were extracted from the durum wheat leaf samples to determine the presence of the *Bdv2* resistance gene against *BYDV-P0*. Total DNA was isolated from durum wheat tissue (Doyle and Doyle, 1987), and total DNA was used in PCR to detect the *Bdv2* gene. PCR analysis was performed using Taq OptiMix CLEAR 2x Master Mix® (Ampliqon, Odense, Denmark). *Thinopyrum intermedium* wild wheat containing *Bdv2* was used as a positive control. The following gene-specific SCAR marker of *BYAgi* (F: 5'-CATGGATAATTCAGGGAGCATTCTG-3' and R: 5'-CTGAACACGAATTTGCTGAGGTTG-3') was used (Stoutjesdijk et al., 2001). The amplification conditions for the PCR reaction were as follows: 95ºC for 5 min, 94ºC for 30 s, 59ºC for 30 s, and 72ºC for 30 s for 35 cycles; 5 min for 72ºC and each 25 µl sample mixture. The data was analyzed using the electrophoresis with 1.4% 1X TBE.

Detection of BVDV-PAV and visual assessment of infection

The virus status of individual plants was determined approximately three weeks after infection by isolating total RNA from plants and using a pair of *BYDV-P0*-specific primers.

Disease severity, representing the percentage of leaf tissue exhibiting symptoms, was evaluated for all plants in each pot at 2-, 3-, and 4-weeks post-inoculation. The severity of symptom manifestation was gauged by the extent of yellow discoloration, dwarfism, and stunting in the inoculated plants. A 0-5 scale was employed to score these symptoms, where: 0 indicated a symptom-free plant; 1 indicated a few leaves with discoloration, dwarfism, and stunting; 2 indicated roughly 20% of leaves affected; 3 indicated 40% of leaves affected; 4 indicated 60% of leaves affected; and 5 indicated almost the entire plant affected (Choudhury et al., 2019). The percentage of disease severity was then calculated from these scale values using the Townsend-Heuberger formula. (Choudhury et al., 2018).

SPAD chlorophyll content and ear yield formation

The SPAD chlorophyll values were measured using a SPAD chlorophyll meter (Konica Minolta 502, Japan) in the 7th, 14th, and 21st day post-inoculation (DAI) of plants (grown under the same conditions in the greenhouse). The calibrated SPAD meter was carefully clamped over a durum wheat variety leaf to obtain the chlorophyll readings. We calculated the SPAD values of three healthy and three *BYDV-P0*-infected wheat varieties of all varieties as the sum of three separate measurements taken on durum wheat leaves. Three healthy replicates (15 total leaves) and three infected replicates (15 total leaves) for each durum wheat variety were used to obtain the SPAD chlorophyll averages once a week during the measurement period (Uddling et al., 2007).

At maturity, durum wheat grains were harvested from *BYDV-P0*-infected and healthy plants while Ç1252 had no ear and yield formation (no vernalization response). The harvested grains were cleaned and air-dried to a uniform moisture content then ear yield (g/ear) and number of grains per ear (GN, grain/ear) was measured.

Statistical analysis

The experimental design was based on a randomized plot design. For each variety, four plants were considered, and the experiment was conducted with three repetitions. ANOVA was conducted to identify statistically significant infection-induced variances across the measured parameters. Statistical analysis of the experimental data was carried out according to randomized plot design and Duncan's multiple range test in SPSS Statistical Program and LSD method was performed in SAS V9.

RESULTS

Bdv2 gene detection

Thinopyrum intermedium, known to carry the *Bdv2* gene and used as a positive control, showed a 566 bp long *Bdv2* gene-specific band. No durum wheat varieties with the *Bdv2* gene were found when PCR was performed using *BYAgi* markers specific to the *Bdv2* gene from genomic DNAs isolated from 5 different durum wheat varieties used in the study (Figure 1).

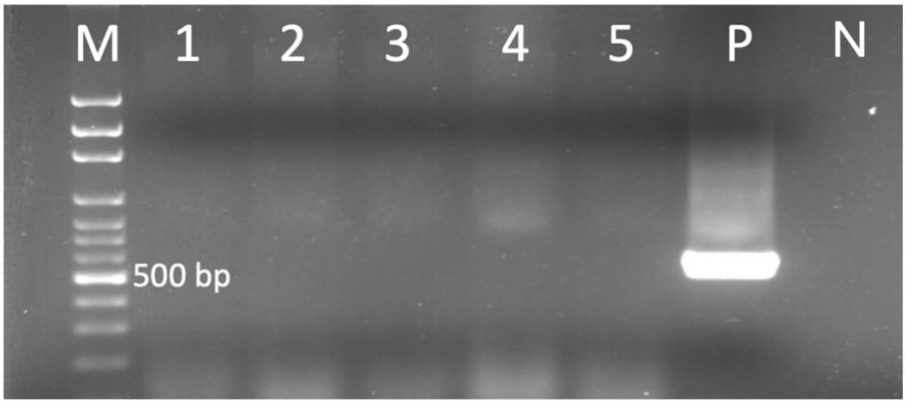

Figure 1. PCR analysis of *Bdv2* gene-specific primers. M; Marker (100 bp), 1,2,3,4,5; Tüten, Atalay, Ç1252, Poyraz, Şölen, P; positive control, N; negative control.

Şekil 1. *Bdv2* genine özgü primerler ile PCR analizi. M; Marker (100 bp), 1,2,3,4,5; Tüten, Atalay, Ç1252, Poyraz, Şölen, P; pozitif kontrol, N; negatif kontrol.

Visual assessment of viral infection

The visual assessment of the infection rate varied depending on the infection and different varieties in 30 tested samples. The rate of *BYDV-P0* infected samples was positive (Figure 2).

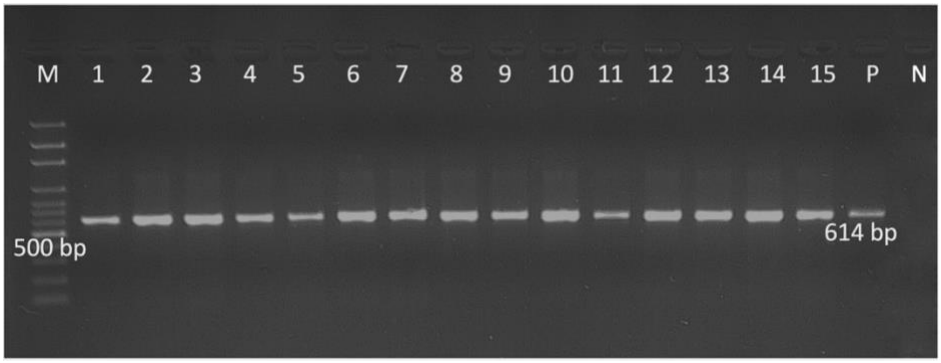

Figure 2. PCR analysis of durum wheat *BYDV-P0*-specific primers. M; Marker (100 bp), 1,2,3; Tüten, 4,5,6; Atalay, 7,8,9; Ç1252, 10,11,12; Poyraz, 13,14,15; Şölen (*BYDV-P0* positive samples), P; positive control, N; negative control.

Şekil 2. Makarnalık buğdaylarda *BYDV-P0*-spesifik primerlerinin PCR analizi. M; Marker (100 bp), 1,2,3; Tüten, 4,5,6; Atalay, 7,8,9; Ç1252, 10,11,12; Poyraz, 13,14,15; Şölen (*BYDV-P0* pozitif örnekler), P; pozitif kontrol, N; negative kontrol.

The visual assessment rate varied from 26% to 43% based on the different varieties. The visual assessment is primarily related to leaf color changes (yellowing and deformation) in the early stages and reducing straw length and tillering in the infected plants (Figure 3).

Figure 3. Symptoms of *BYDV-P0* infection in five durum-wheat varieties and negative control plant.

Şekil 3. Beş makarnalık buğday çeşidinde *BYDV-P0* enfeksiyonunun simptomları ve negatif kontrol bitkisi.

The disease scale calculated from the data obtained with the symptom scale varied between 25% and 45% in the five varieties. Higher susceptibility was recorded on average for the Tüten variety (Table 1).

Table 1. *BYDV-P0* disease scale in five different durum-wheat genotypes

Çizelge 1. *Beş farklı makarnalık buğday genotipinde BYDV-P0 hastalık skalası*

Durum-wheat genotypes	Total plant	Disease Scale	Duncan[a]
Tüten	3	43%	1,430
Alatay	3	31%	1,102
Ç1252	3	28%	0,982
Poyraz	3	36%	1,205
Şölen	3	26%	0,879
Negative control	3	0%	0,000

SPAD analysis and grain weight

SPAD chlorophyll readings in the leaves of Tüten, Alatay 2000, Poyraz and Şölen were examined with *BYDV-P0* at 7, 14, and 21 DAI and the same in non-inoculated plants. The chlorophyll contents in the *BYDV-P0*-inoculated leaves were significantly lower than those in non-inoculated varieties. The significance level for the differences observed in chlorophyll content per ear between control and infected plants was $p < 0.01$. The research findings have revealed that inoculated plants significantly had lower SPAD values than non-inoculated plants in post-inoculation days with a decreasing trend (**-Δ%** from 16% to 42%). At the DAI-3 measurement, the highest SPAD chlorophyll loss was observed in infected plants. The number of grains per ear (GN) was affected adversely by *BYDV-P0* inoculation. With the emergence of the effect of the inoculation, GN values decreased by 63.1%. This situation also observed and reflected to ear yield (g/ear) values. The durum wheat leaves with inoculation of *BYDV-P0* had the highest chlorophyll loss and this situation caused faster senescence of plants and were not able to stay green (Table 2).

Table 2. Grain weight and chlorophyll content in *BYDV-P0*-infected durum wheat plants

Çizelge 2. *BYDV-P0* ile enfekte olmuş makarnalık buğday bitkilerinde dane ağırlığı ve klorofil içeriği

	Spad DAI-1			Spad DAI-2			Spad DAI-3		
Varieties	Control	Infected	-Δ%	Control	Infected	-Δ%	Control	Infected	-Δ%
Tüten	41.3±1.5	34.9±4.1	15	44.5±2.2	32.6±0.3	27	41.4±3.0	25.1±2.3	39
Alatay	36.6±2.8	28.1±5.8	23	43.0±3.8	32.2±1.4	25	44.3±4.1	24.7±0.6	44
Poyraz	40.9±2.5	37.0±3.3	10	43.2±4.6	33.0±4.8	24	44.5±1.7	27.6±6.7	38
Şölen	38.7±1.7	32.3±3.4	17	45.2±3.6	31.0±2.1	31	46.9±2.7	25.6±1.2	45
Mean App.	39,4 a	33,1 b	16	43,9 a	32,2 b	27	44,2	25,7	42
Lsd App.		3,02**			2,67**			3,09**	

-Δ%: reduction values in chlorophyll content of infected plants.

This situation also observed in ear yield formation values. The *BYDV-P0* inoculated plants had lower ear yield (0.265 g/ear) and decreased by 58.2% (Table 3). The significance level for the differences observed in ear yield and grain number (GN) per ear between control and infected plants was $p < 0.01$.

Table 3. Ear yield (g/ear) and number of grains per ear values (GN) and reduction in values (-Δ%) for *BYDV-P0*-infected vs control in durum wheat varieties

Çizelge 3. *BYDV-P0* ile enfekteli ve kontrol makarnalık buğday çeşitlerinde başak verimi (g/başak) ve başak başına dane sayısı (GN) değerleri ve değerlerdeki azalma (-Δ%)

	Ear Yield (g/ear)			GN (grains/ear)		
Varieties	Control	Infected	-Δ%	Control	Infected	-Δ%
Tüten	0.560	0.296	47	20	16	21
Alatay	0.453	0.301	34	24	12	49
Poyraz	0.406	0.193	52	19	8	57
Şölen	0.403	0.273	32	14	13	7
Mean App.	0.455 a	0.265 b	42	19 a	12 b	36
Lsd App.		0.075**			3.45**	

-Δ%: reduction values in chlorophyll content of infected plants.

DISCUSSION

This study has determined that *BYDV-P0* infection caused significant ear yield losses together with SPAD chlorophyll content and GN (grains/ear) values in durum-wheat varieties, and intriguingly, the *Bdv2* resistance gene was not detected in these varieties. This is a unique contribution to the field, as few studies have delved into the effects of BYDV on durum wheat and tested the resistance/tolerance status of BYDV in different durum wheat varieties (Gill, 1967; Cheour et al., 1993).

The visual impact of *BYDV-P0* infection on durum wheat is not just noticeable, it's crucial. Yellowing, redness, and growth deformation were starkly observed in five durum-wheat varieties infected with *BYDV-P0*. This aligns with a similar study that reported the most visible symptoms of BYDV in plants, including the loss of green color in the leaves and the yellowing observed in wheat fields (D'arcy, 1995). Another study conducted in wheat production areas observed chlorotic lines starting from the tip of wheat leaves, chlorotic spots, line mosaics, and hard-structured upright leaves (Çapkan, 2016).

Symptomological observations, while informative, are often a preliminary step for molecular diagnostic methods. In this study, we used virus-specific primers to definitively diagnose the presence of *BYDV-P0* in infected durum wheat. Total nucleic acid (TNA) extraction from *BYDV-P0*-infected durum wheat leaves was obtained (Foissac et al., 2000) and virus detection was performed using specific primers. The specific primer sequence was used to diagnose *BYDV-P0*, and similar studies have shown that bands with a length of 614 bp specific to *BYDV-P0* have obtained (Usta, 2013). The successful amplification of the primers used for *BYDV-P0* (PAV) and BYDV-MAV in wheat samples indicates the presence of the agent in the samples.

It is known that BYDV resistance in wheat is a complex multi-gene trait (Ayala et al., 2002) and is controlled by four significant genes (*Bdv1, 2, 3, 4*) (Jarošová et al., 2013). The Mackellar (TC14) and Glover (TC6) wheat genotypes, both found to carry the *Bdv2* resistance gene in Australia, have proven invaluable in wheat breeding studies. Their resistance to both *BYDV-P0* (PAV) and BYDV-MAV species, as documented by (Ayala et al., 2007) highlights their potential for enhancing resistance in future wheat genotypes (Choudhury et al., 2019) investigated only *Bdv2* and *Bdv3* resistance genes associated with BYDV-PAV resistance in 335 different Chinese and Australian wheat genotypes. Also, they examined the presence of new resistance regions in wheat genotypes.

In this study, the absence of the BYDV-PAV resistance gene *Bdv2* in five durum-wheat genotypes with SCAR marker is a significant finding. This absence suggests a potential vulnerability to BYDV-PAV infection in these genotypes, which could impact future breeding efforts.

This study, conducted on specific durum wheat genotypes has revealed a significant decrease in ear yield, GN and SPAD chlorophyll content in infected durum wheat samples, underscoring the profound impact of *BYDV-P0* infection on these crucial parameters. Similar studies determined that BYDV-infected plants significantly decreased plant biomass, leaf chlorophyll content, and grain yield (Jensen & D'Arcy, 1995; McKirdy et al., 2002). Ayala et al., (2002) revealed that plant height, biomass, yield, and TKW (kernel weight) values of BYDV-infected genotypes differed from healthy control plants.

The experiments conducted in this study quantified some ear yield losses associated with BYDV-PAV infection in the evaluated durum wheat varieties. The infection of BYDV-PAV virus to durum wheat plants was found to be the main limiting factor affecting first leaf chlorophyll content in leaves then ear yield formation. The results of these experiments vividly illustrate the potential threat to durum-wheat that can result from infection with BYDV, emphasizing the gravity of the situation.

The data obtained from our study provide a better understanding of BYDV, which resulting in negative effects on plant growth and causes significant economic damage by inducing yield losses in durum wheat. Also, our study highlights the pressing need for immediate and effective disease management strategies in the future.

Data Availability

Data will be made available upon reasonable request.

Author Contributions

Conception and design of the study: YEU, AY; sample collection: YEU, AY, NY, NY; analysis and interpretation of data: YEU, AY, NY, NY; statistical analysis: AY, YEU; visualization: YEU, AY, SY; writing manuscript: YEU, AY, SY.

Conflict of Interest

There is no conflict of interest between the authors in this study.

Ethical Statement

We declare that there is no need for an ethics committee for this research.

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