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Research Article

# Resistance Expression in Advanced Mungbean Generations against Mungbean Yellow Mosaic Begomovirus

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**ABSTRACT**

Yellow mosaic disease (YMD), caused by begomoviruses and transmitted primarily by *Bemisia tabaci*, is a major constraint to mungbean production in South Asia. The present study investigated YMD epidemiology, whitefly population dynamics, resistance stability of advanced PRI mungbean lines, and the role of alternate hosts under natural field conditions during two consecutive growing seasons (2021-2022). In the susceptible cultivar Mung Kabuli, YMD incidence increased from 25.67% at flowering to 61.97% at maturity in 2021 and from 40.00% to 72.42% in 2022, coinciding with higher whitefly populations during the disease initiation phase. Advanced mungbean lines exhibited considerable variation in disease response; however, disease pressure increased substantially in 2022, resulting in a decline in resistance stability. Several genotypes previously categorized as resistant or moderately resistant showed increased susceptibility, with 21PRI4 shifting from moderately resistant to moderately susceptible and 21PRI3 from moderately susceptible to susceptible. Yellow mosaic symptoms were recorded on eight alternate plant hosts, among which urdbean, cowpea, pigeon pea, soybean, and jungle mat bean showed detectable begomovirus accumulation. Serological analysis revealed increased begomovirus particle concentration in Mung Kabuli and advanced lines during 2022 compared with 2021, indicating progressive virus accumulation under continuous infection pressure. The consistent presence of begomovirus in alternate hosts highlights their potential role as inoculum reservoirs. These findings demonstrate the dynamic nature of YMD epidemics and emphasize the need for durable resistance sources, molecular characterization of circulating begomoviruses, and integrated management strategies for sustainable mungbean production.

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## Introduction

Plant pathogens are major biotic constraints to agricultural productivity, adversely affecting crop growth, development, and yield through complex host-pathogen interactions. In compatible interactions, successful pathogen colonization results in visible disease symptoms, whereas incompatible interactions activate host defense mechanisms, including the hypersensitive

response, leading to localized cell death and restriction of pathogen spread (Agrios, 2005). Regardless of the interaction type, plant diseases impose substantial yield and quality losses in economically important crops.

Mungbean (*Vigna radiata* L.) is frequently challenged by a wide range of biotic stresses under field conditions, among which viral diseases constitute the most serious limitation to sustainable production. The major viral diseases affecting mungbean include yellow mosaic disease (YMD) (Akram and Singh, 2016; Akbar et al., 2019; Mishra et al., 2020), leaf crinkle disease (Sastry, 2013; Biswas et al., 2015), groundnut bud necrosis disease (Sreekanth et al., 2004), and infections caused by *Bean common mosaic virus* and *Bean common mosaic necrosis virus* (Cui et al., 2014; Worrall et al., 2015; Lee et al., 2017). In addition, insect pests, particularly whiteflies (*Bemisia tabaci* Gennadius) and aphids, exacerbate crop losses by directly damaging plants and serving as efficient vectors for several economically important viruses, thereby significantly reducing crop productivity (Sreekanth et al., 2004; Karthikeyan et al., 2014; Khaliq et al., 2017; Akbar et al., 2019).

Among these diseases, yellow mosaic disease is recognized as the most destructive viral disease of mungbean throughout South and Southeast Asia (Bashir et al., 2006; Karthikeyan et al., 2014; Binyamin et al., 2022). Besides mungbean, the disease affects several cultivated and wild leguminous species, which serve as alternative hosts and reservoirs for the virus, facilitating its persistence and spread across cropping seasons (Qazi et al., 2007; Ramesh et al., 2017; Agnihotri et al., 2019; Dikshit et al., 2020; Ansar et al., 2021). Yellow mosaic disease was first described by Nariani (1960), who identified the causal agent as *Mungbean yellow mosaic virus* (MYMV). Subsequently, Ahmad and Harwood (1973) confirmed the occurrence of the disease in Pakistan, establishing its significance as a major production constraint in the country.

Yellow mosaic disease is caused by a complex of bipartite begomoviruses, predominantly *Mungbean yellow mosaic virus* (MYMV) and *Mungbean yellow mosaic India virus* (MYMIV) (Mishra et al., 2020). These viruses are persistently transmitted by the whitefly *Bemisia tabaci*, which acquires the virus while feeding on infected plants and inoculates healthy plants during subsequent feeding on phloem tissues (Nair et al., 2017; Akbar et al., 2019). Viral inclusions become detectable in the nuclei of infected cells approximately two days before the appearance of visible symptoms (Thongmeearkom et al., 1981). Disease symptoms initially appear as small, bright-yellow specks on young leaves that gradually coalesce into a characteristic yellow mosaic pattern accompanied by chlorosis, mottling, leaf distortion, premature senescence, and drying. Severe infections markedly reduce photosynthetic efficiency, resulting in fewer flowers, reduced pod set, malformed pods, and small, shriveled, discolored seeds, ultimately causing substantial yield losses (Qazi et al., 2007; Khattak et al., 2008; Akhtar et al., 2009; Malathi and John, 2009; Ummad-ud-Din et al., 2019).

The persistence and severity of YMD in Pakistan are influenced by multiple epidemiological factors, including the abundance of alternative host plants, continuous availability of viruliferous whiteflies, favorable environmental conditions for vector multiplication, and the widespread cultivation of susceptible mungbean cultivars. Although considerable progress has been made in breeding YMD-resistant varieties, recent reports have documented resistance breakdown in advanced breeding lines, likely resulting from evolving viral populations and intense vector pressure during critical stages of crop development (Akbar et al., 2019; Mishra et al., 2020). Consequently, YMD remains one of the principal constraints to mungbean production, with reported yield losses ranging from 10% to complete crop failure depending on disease severity, host resistance, crop growth stage, and prevailing environmental conditions (Marimuthu et al., 1981; Bashir et al., 2006; Mishra et al., 2020).

These challenges highlight the necessity for continuous disease surveillance, identification of alternative host plants that contribute to virus survival and dissemination, and rigorous evaluation of resistance in advanced mungbean breeding materials. Such efforts are essential for developing durable resistance, strengthening integrated disease management strategies, and ensuring sustainable mungbean production under diverse agroecological conditions.

Accordingly, the present study was undertaken to (i) investigate the temporal progression of yellow mosaic disease across different cropping seasons and crop growth stages, (ii) identify alternative host plants contributing to the epidemiology and spread of the disease, and (iii) evaluate the resistance responses of locally developed advanced mungbean breeding lines against yellow mosaic begomoviruses under natural

field conditions. These findings are expected to provide valuable information for resistance breeding and the development of effective disease management strategies for sustainable mungbean production.

## Materials and Methods

### Yellow mosaic disease monitoring

Field experiments were conducted during the summer cropping seasons of 2021 and 2022 at the experimental farm of the Agricultural Biotechnology Research Institute (ABRI), Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan, following recommended agronomic practices for mungbean cultivation. To facilitate natural development of yellow mosaic disease (YMD), no insecticides were applied throughout the growing season, thereby maintaining natural populations of the whitefly vector (*Bemisia tabaci* Genn.). In addition, no weeding or hoeing was performed around the experimental plots to minimize disturbance of the vector population and promote natural virus transmission.

The highly YMD-susceptible mungbean genotype Mung Kabuli was used as an indicator host to monitor disease progression from flowering until crop maturity (Ummad-ud-Din et al., 2019; Binyamin et al., 2022). The genotype was planted under open-field conditions in a randomized complete block design (RCBD) using 3-m-long rows with 30 cm inter-row and 15 cm intra-row spacing. The experimental field was established adjacent to urd bean (*Vigna mungo* L. Hepper) plots to facilitate natural whitefly movement and virus dissemination between host species.

Yellow mosaic disease incidence was assessed based on characteristic field symptoms (Figure 1), including chlorotic yellow specks, yellow mosaic, and necrotic mottling, following the diagnostic criteria described by Bashir (2005), Qazi et al. (2007), Akhtar et al. (2009), Malathi and John (2009), Kaur (2018), Ummad-ud-Din et al. (2019), Mishra et al. (2020), and Binyamin et al. (2022). Disease observations were recorded from three randomly tagged plants at the flowering and physiological maturity stages. Disease incidence (%) was calculated using the following equation:

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

Whitefly populations were recorded simultaneously at the flowering and maturity stages by visually counting adult insects on five randomly selected plants during the early morning hours, when insect activity was relatively low.

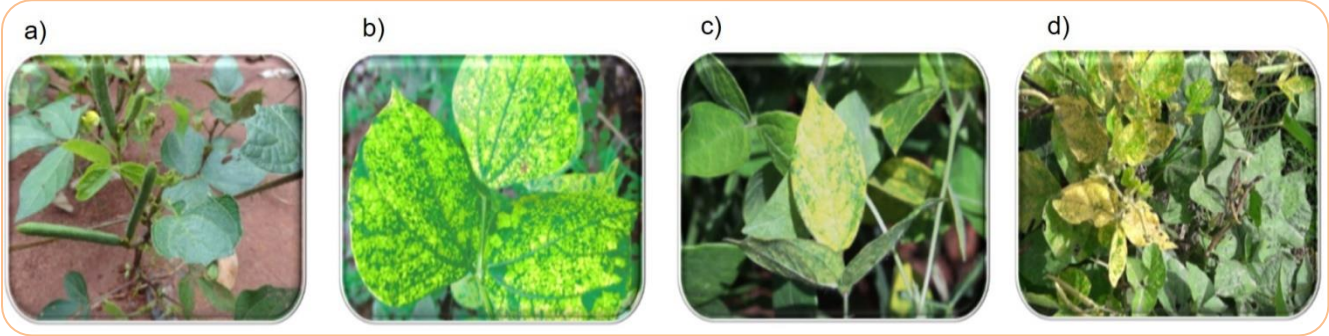


Figure 1. YMD symptoms expression in mungbean: a) Healthy plant, b) Yellow specks on infected leaves, c) Yellow mosaic leaves and d) Necrotic mottled leaves.

### Evaluation of resistance in advanced mungbean lines

Twenty advanced mungbean breeding lines (Table 1) were obtained from the Pulses Research Institute, Ayub Agricultural Research Institute, Faisalabad, Pakistan, to evaluate their resistance against YMD. The experiment was conducted under natural epiphytotic conditions during the summer seasons of 2021 and 2022. The entries were planted in an augmented experimental design, with each genotype sown in two 3-m-long rows, maintaining 30 cm between rows and 15 cm between plants.

The highly susceptible cultivar Mung Kabuli was planted after every two test entries as a spreader row and susceptible disease check to ensure uniform disease pressure. Standard agronomic practices were followed throughout the experiment, except that no insecticides were applied to preserve natural whitefly populations and virus transmission.

Disease incidence and resistance evaluation were performed using the same methodology described for the susceptible check (Habib, 2012; Ummad-ud-Din et al., 2019). Disease reactions were classified according to the 0–9 disease rating scale proposed by Bashir (2005) (Table 2). Whitefly

populations on each genotype were assessed following the same procedure adopted for the susceptible check.

Table 1. Mungbean genotypes used in the study.

| Sr. # | Genotype | Sr. # | Genotype |
|---|---|---|---|
| 1 | 21PRI1 | 11 | 21PRI11 |
| 2 | 21PRI2 | 12 | 21PRI12 |
| 3 | 21PRI3 | 13 | 21PRI13 |
| 4 | 21PRI4 | 14 | 21PRI14 |
| 5 | 21PRI5 | 15 | 21PRI15 |
| 6 | 21PRI6 | 16 | 21PRI16 |
| 7 | 21PRI7 | 17 | 21PRI17 |
| 8 | 21PRI8 | 18 | 21PRI18 |
| 9 | 21PRI9 | 19 | 21PRI19 |
| 10 | 21PRI10 | 20 | 21PRI20 |

**Collection of YMD-infected plant samples**

Young leaves exhibiting typical YMD symptoms, including bright yellow specks and mosaic patterns, were collected from the susceptible cultivar Mung Kabuli, advanced mungbean breeding lines, and cultivated leguminous hosts, including urd bean (*Vigna mungo*), pigeon pea (*Cajanus cajan*), soybean (*Glycine max*), and cowpea (*Vigna unguiculata*). Symptomatic samples were also collected from neighboring weed species, including brown top millet, itsit, jungle mat bean, and tandala, growing adjacent to mungbean fields to investigate their potential role as alternate virus reservoirs. All samples were properly labeled and stored at 4°C until serological analysis.

Table 2. YMD disease rating scale used to assess resistance reaction.

| Disease scale | Infection % | Reaction |
|---|---|---|
| 0 | All plants free of disease symptoms | Highly resistant (HR) |
| 1 | 1-10% infection | Resistant (R) |
| 2 | 11-20% infection | Moderately Resistant (MR) |
| 3 | 21-30% infection | Moderately Susceptible (MS) |
| 4 | 30-50% infection | Susceptible (S) |
| 5 | More than 50% infection | Highly Susceptible (HS) |

**Detection and quantification of YMD-associated begomoviruses**

The presence of YMD-associated begomoviruses was determined using Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA). A commercially available polyclonal antibody raised against Cotton Leaf Curl Virus (CLCuV) (Art. No. 151475; Bioreba AG, Reinach, Switzerland), known to cross-react with several begomoviruses, was used for virus detection.

The assay was performed according to the protocol of Clark and Adams (1977), with modifications described by Farooq et al. (2018) and Ummad-ud-Din et al. (2019). For each sample, a young symptomatic leaf displaying characteristic yellow specks was used for antigen extraction. Leaf extracts were loaded into duplicate wells together with appropriate positive and negative controls. All ELISA procedures were carried out according to the manufacturer's instructions. Absorbance values were measured at 405 nm using a BioTek uQuant Microplate Reader (Model MQX200, BioTek Instruments, Winooski, VT, USA). Samples exhibiting absorbance values exceeding twice those of the negative control were considered positive for YMD-associated begomoviruses.

**Meteorological conditions**

Meteorological data for Faisalabad during the mungbean growing seasons (July–September) of 2021 and 2022 were obtained from World Weather Online (2023). During 2021, the mean maximum temperature ranged from 36 to 38°C, whereas the mean minimum temperature ranged from 26 to 28°C. Total rainfall varied between 180 and 250 mm, with relative humidity ranging from 60 to 75%.

In 2022, the mean maximum temperature ranged from 34 to 36°C, while the mean minimum temperature ranged from 26 to 27°C. Seasonal rainfall increased substantially to 400–550 mm, and relative humidity ranged from 65 to 80%. These contrasting environmental conditions were considered when interpreting annual variations in YMD incidence and whitefly population dynamics.

**Statistical analysis**

Data on YMD incidence and whitefly population density were subjected to analysis of variance (ANOVA) using Statistix version 8.1 (Analytical Software, Tallahassee, FL, USA). Treatment means were separated using Tukey's Honest Significant Difference (HSD) test at $P \leq 0.05$.

Mean ELISA absorbance values (A405), representing the relative accumulation of YMD-associated begomoviruses, were visualized using heat-map analysis generated in GraphPad Prism version 8.0.1 (GraphPad Software, San Diego, CA, USA).

## Results

### YMD incidence and whitefly population in the susceptible mung kabuli variety

In the susceptible mung kabuli variety, YMD incidence at the flowering stage was 25.67% in 2021, with a corresponding mean whitefly population of 8.47 insects per plant. In 2022, YMD incidence increased to 40.00%, accompanied by a higher mean whitefly population of 9.64 insects per plant. Disease severity increased substantially by crop maturity. In 2021, YMD incidence reached 61.97%, despite a decline in the mean whitefly population to 3.55 insects per plant. Similarly, in 2022, YMD incidence further increased to 72.42%, while the average whitefly population decreased to 4.64 insects per plant (Figure 2; Table 3). The observations revealed a progressive increase in both YMD incidence and whitefly population density, particularly during the 2022 growing season, indicating sustained susceptibility of Mung Kabuli under natural disease pressure and vector-mediated infection conditions.

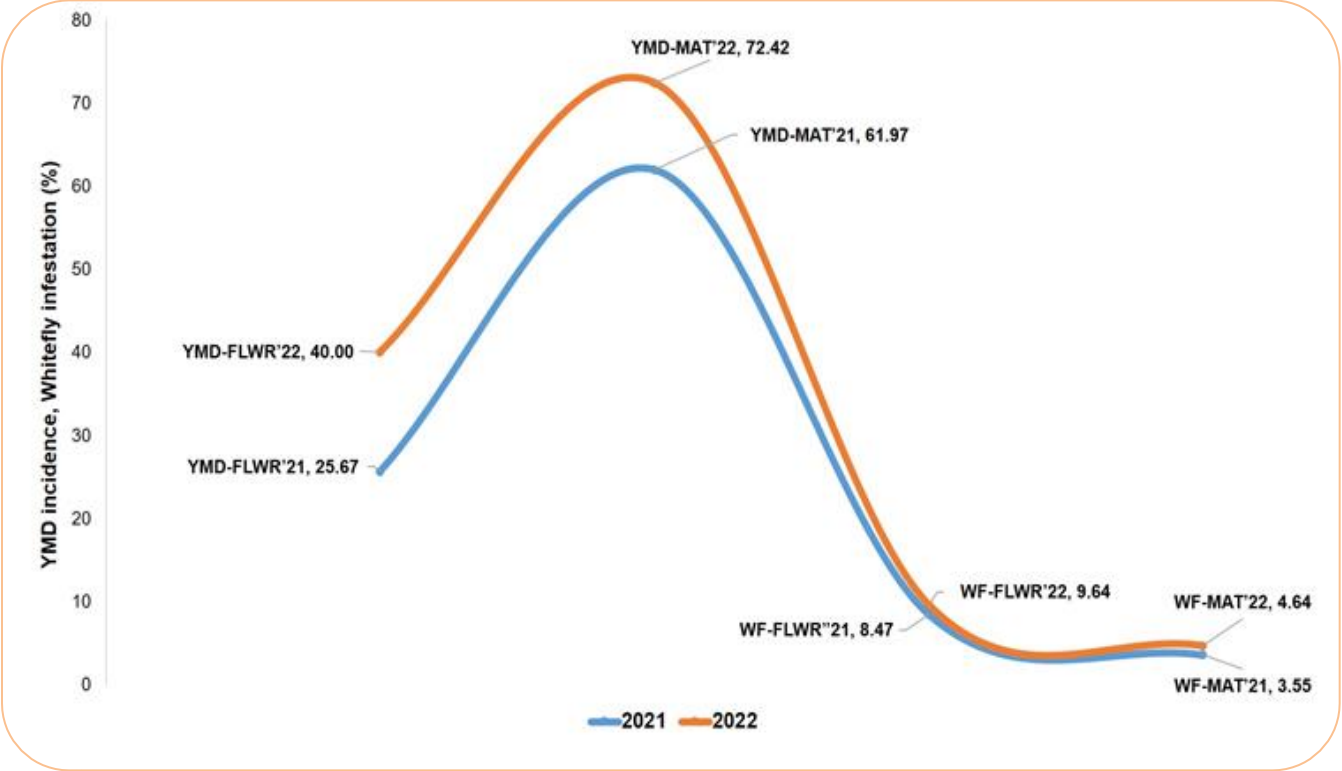


Figure 2. Temporal progression of YMD incidence and whitefly infestation in susceptible mungbean cultivar Mung Kabuli.

Table 3. Incidence of YMD and whitefly infestation levels in susceptible mungbean cultivar Mung Kabuli.

| Crop stage | Year 2021 | | Year 2022 | |
|---|---|---|---|---|
| | YMD incidence | Whitefly infestation | YMD incidence | Whitefly infestation |
| Flowering stage | 25.666[b] | 8.4727[a] | 40.002[b] | 9.6364[a] |
| Maturity stage | 61.970[a] | 3.5455[b] | 72.425[a] | 4.6364[b] |
| Statistics | F = 86.16, P = 0.0000, LSD = 8.7198 | F = 76.17, P = 0.0000, LSD = 1.2587 | F = 87.33, P = 0.0000, LSD = 7.7354 | F = 204.61, P = 0.0000, LSD = 0.7793 |

Means followed by different letters are significantly different from each other at the 5% probability level, according to Tukey's HSD all-pairwise comparison test.

**YMD incidence and whitefly population in advanced mungbean breeding lines**

In the advanced mungbean breeding lines, YMD incidence at the flowering stage during the 2021 growing season ranged from 6.67% to 30.00%, while the corresponding mean whitefly population varied from 3.40 to 11.80 insects per plant. Disease incidence increased markedly by the maturity stage, ranging from 20.00% to 76.67%, whereas mean whitefly populations declined to 1.00–4.00 insects per plant. During the 2022 growing season, disease pressure was more severe across all breeding lines. At the flowering stage, YMD incidence ranged from 16.67% to 36.67%, with mean whitefly populations of 5.40–12.00 insects per plant. By the maturity stage, disease incidence further increased to 25.00–83.33%, while mean whitefly populations ranged from 3.00 to 5.60 insects per plant (Figure 3; Table 4). YMD incidence and whitefly population density were consistently higher in 2022 than in 2021, indicating that prevailing environmental conditions during 2022 were more conducive to disease development and whitefly multiplication.

**Evaluation of resistance potential in advanced mungbean lines**

Evaluation of the resistance potential of advanced PRI mungbean lines revealed a marked decline in resistance stability across successive growing seasons. Both disease incidence and severity increased substantially in almost all genotypes during the 2022 evaluation compared with 2021. Lines 21PRI5–21PRI7, 21PRI12–21PRI15, and 21PRI19 consistently exhibited high disease incidence and severity and were classified as highly susceptible (HS) in both seasons, indicating a lack of effective resistance to YMD. In contrast, 21PRI4, which was rated as moderately resistant (MR) in 2021, deteriorated to moderately susceptible (MS) in 2022. Likewise, 21PRI3 shifted from moderately susceptible (MS) in 2021 to susceptible (S) in 2022, reflecting a further loss of resistance. Moreover, several lines that were categorized as susceptible in 2021, including 21PRI1, 21PRI2, 21PRI8–21PRI11, 21PRI16–21PRI18, and 21PRI20, progressed to the highly susceptible (HS) category during the 2022 growing season. Collectively, these findings demonstrate a progressive erosion of resistance in advanced mungbean breeding lines under natural YMD infection, suggesting that the resistance genes deployed in these genotypes may not provide durable protection under variable environmental conditions and fluctuating populations of the whitefly vector (Table 5).

**YMD occurrence on alternate plant hosts**

Yellow mosaic disease symptoms observed on mungbean leaves were also recorded on eight alternate plant hosts, including commonly occurring weed species. The detailed occurrence and symptom expression in these alternate hosts are provided in Table 6; Figure 4.

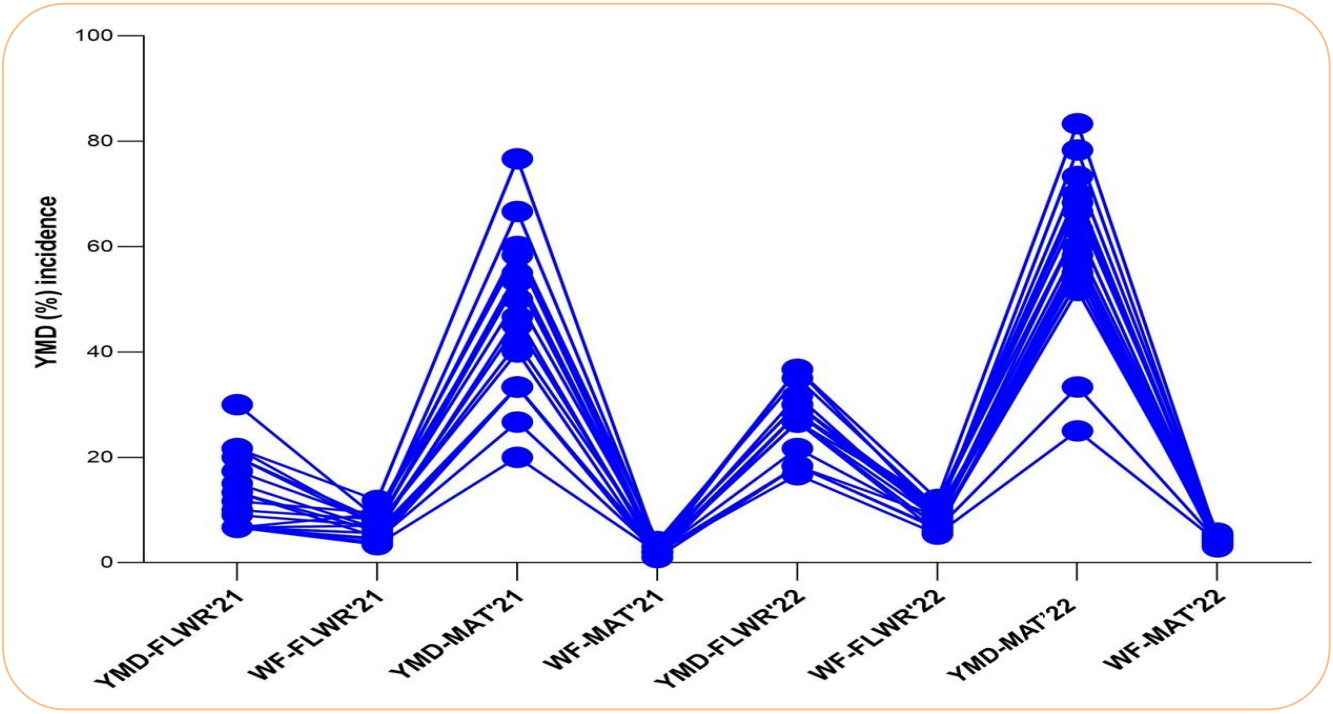


Figure 3. YMD incidence and whitefly infestation in advanced mungbean breeding lines during 2021–2022.

Table 4. YMD incidence and whitefly infestation levels in advanced mungbean breeding lines during 2021–2022.

| Mung bean advanced lines | Year 2021 | | | | Year 2022 | | | |
|---|---|---|---|---|---|---|---|---|
| | Flowering stage | | Maturity stage | | Flowering stage | | Maturity stage | |
| | YMD incidence | WF infestation | YMD incidence | WF infestation | YMD incidence | WF infestation | YMD incidence | WF infestation |
| 21PRI1 | 6.67$^{b}$ | 4.60$^{efgh}$ | 33.33$^{ghi}$ | 1.00$^{a}$ | 18.33$^{cd}$ | 6.60$^{defg}$ | 51.67$^{g}$ | 4.00$^{a}$ |
| 21PRI2 | 6.67$^{b}$ | 7.00$^{bcdefg}$ | 40.00$^{fgh}$ | 1.00$^{a}$ | 28.33$^{abcd}$ | 9.00$^{abcdefg}$ | 53.33$^{g}$ | 3.00$^{a}$ |
| 21PRI3 | 6.67$^{b}$ | 4.40$^{fgh}$ | 26.67$^{hi}$ | 2.00$^{a}$ | 18.33$^{cd}$ | 6.40$^{efg}$ | 33.33$^{h}$ | 4.00$^{a}$ |
| 21PRI4 | 6.67$^{b}$ | 3.40$^{h}$ | 20.00$^{i}$ | 2.00$^{a}$ | 16.67$^{d}$ | 5.40$^{g}$ | 25.00$^{h}$ | 4.00$^{a}$ |
| 21PRI5 | 30.00$^{a}$ | 8.40$^{abc}$ | 60.00$^{bc}$ | 4.00$^{a}$ | 35.00$^{ab}$ | 10.40$^{abc}$ | 66.67$^{bcdef}$ | 5.60$^{a}$ |
| 21PRI6 | 10.00$^{b}$ | 8.20$^{bcd}$ | 60.00$^{bc}$ | 2.00$^{a}$ | 28.33$^{abcd}$ | 10.20$^{abcd}$ | 70.00$^{bcd}$ | 4.00$^{a}$ |
| 21PRI7 | 20.00$^{ab}$ | 8.00$^{bcde}$ | 66.67$^{ab}$ | 3.00$^{a}$ | 30.00$^{abcd}$ | 10.00$^{abcde}$ | 73.33$^{abc}$ | 5.00$^{a}$ |
| 21PRI8 | 6.67$^{b}$ | 5.60$^{cdefg}$ | 33.33$^{ghi}$ | 2.00$^{a}$ | 21.67$^{bcd}$ | 7.60$^{bcdefg}$ | 58.33$^{defg}$ | 4.00$^{a}$ |
| 21PRI9 | 13.33$^{ab}$ | 4.80$^{defgh}$ | 33.33$^{ghi}$ | 2.00$^{a}$ | 26.67$^{abcd}$ | 6.80$^{cdefg}$ | 53.33$^{g}$ | 4.00$^{a}$ |
| 21PRI10 | 6.67$^{b}$ | 3.80$^{gh}$ | 33.33$^{ghi}$ | 2.00$^{a}$ | 26.67$^{abcd}$ | 5.80$^{fg}$ | 56.67$^{efg}$ | 4.00$^{a}$ |
| 21PRI11 | 17.33$^{ab}$ | 7.80$^{bcdef}$ | 50.00$^{cdef}$ | 3.00$^{a}$ | 30.00$^{abcd}$ | 9.80$^{abcde}$ | 55.00$^{fg}$ | 5.00$^{a}$ |
| 21PRI12 | 6.67$^{b}$ | 9.20$^{ab}$ | 53.33$^{bcdef}$ | 2.00$^{a}$ | 28.33$^{abcd}$ | 11.20$^{ab}$ | 68.33$^{bcde}$ | 4.00$^{a}$ |
| 21PRI13 | 11.67$^{b}$ | 9.40$^{ab}$ | 58.33$^{bcd}$ | 2.00$^{a}$ | 26.67$^{abcd}$ | 11.00$^{ab}$ | 63.33$^{cdefg}$ | 4.00$^{a}$ |
| 21PRI14 | 21.67$^{ab}$ | 11.80$^{a}$ | 76.67$^{a}$ | 3.00$^{a}$ | 36.67$^{a}$ | 12.00$^{a}$ | 83.33$^{a}$ | 4.60$^{a}$ |
| 21PRI15 | 20.00$^{ab}$ | 7.00$^{bcdefg}$ | 53.33$^{bcdef}$ | 3.00$^{a}$ | 30.00$^{abcd}$ | 9.40$^{abcdef}$ | 56.67$^{efg}$ | 5.00$^{a}$ |
| 21PRI16 | 6.67$^{b}$ | 5.60$^{cdefg}$ | 46.67$^{cdefg}$ | 1.00$^{a}$ | 18.33$^{cd}$ | 8.80$^{abcdefg}$ | 55.00$^{fg}$ | 3.40$^{a}$ |
| 21PRI17 | 21.67$^{ab}$ | 7.00$^{bcdefg}$ | 41.67$^{efg}$ | 3.00$^{a}$ | 30.00$^{abcd}$ | 8.60$^{abcdefg}$ | 60.00$^{defg}$ | 5.00$^{a}$ |
| 21PRI18 | 13.33$^{ab}$ | 6.00$^{bcdefgh}$ | 33.33$^{ghi}$ | 2.00$^{a}$ | 31.67$^{abc}$ | 7.80$^{bcdefg}$ | 63.33$^{cdefg}$ | 4.00$^{a}$ |
| 21PRI19 | 9.00$^{b}$ | 7.00$^{bcdefg}$ | 55.00$^{bcde}$ | 2.00$^{a}$ | 36.67$^{a}$ | 9.00$^{abcdefg}$ | 78.33$^{ab}$ | 3.80$^{a}$ |
| 21PRI20 | 15.00$^{ab}$ | 6.40$^{bcdefgh}$ | 45.00$^{defg}$ | 2.00$^{a}$ | 26.67$^{abcd}$ | 8.40$^{abcdefg}$ | 55.00$^{fg}$ | 4.00$^{a}$ |
| Statistics | F: 4.93, p:0.0000, LSD: 16.714 | F: 9.23, p:0.0000, LSD: 3.5774 | F: 27.94, p:0.0000, LSD: 14.789 | F: 1.38, p:0.3536, LSD: 3.3860 | F: 5.17, p:0.0000, LSD: 14.073 | F: 6.77, p:0.0000, LSD: 3.7107 | F: 38.48, p:0.0000, LSD: 11.700 | F: 0.95, p:0.5250, LSD: 3.3071 |

Means followed by different letters are significantly different from each other, except for whitefly infestation at the maturity stage of the crop, at the 5% probability level according to Tukey's honestly significant difference (HSD) all-pairwise comparisons test.

Table 5. Resistance potential of advanced mungbean lines against yellow mosaic begomovirus.

| Sr. # | Entry name | Year 2021 | | | Year 2022 | | |
|---|---|---|---|---|---|---|---|
| | | Mean incidence | Rating scale | Reaction | Mean incidence | Rating scale | Reaction |
| 1 | 21PRI1 | 33.33 | 4 | S | 51.67 | 5 | HS |
| 2 | 21PRI2 | 40.00 | 4 | S | 53.33 | 5 | HS |
| 3 | 21PRI3 | 26.67 | 3 | MS | 33.33 | 4 | S |
| 4 | 21PRI4 | 20.00 | 2 | MR | 25.00 | 3 | MS |
| 5 | 21PRI5 | 60.00 | 5 | HS | 66.67 | 5 | HS |
| 6 | 21PRI6 | 60.00 | 5 | HS | 70.00 | 5 | HS |
| 7 | 21PRI7 | 66.67 | 5 | HS | 73.33 | 5 | HS |
| 8 | 21PRI8 | 33.33 | 4 | S | 58.33 | 5 | HS |
| 9 | 21PRI9 | 33.33 | 4 | S | 53.33 | 5 | HS |
| 10 | 21PRI10 | 33.33 | 4 | S | 56.67 | 5 | HS |
| 11 | 21PRI11 | 50.00 | 4 | S | 55.00 | 5 | HS |
| 12 | 21PRI12 | 53.33 | 5 | HS | 68.33 | 5 | HS |
| 13 | 21PRI13 | 58.33 | 5 | HS | 63.33 | 5 | HS |
| 14 | 21PRI14 | 76.67 | 5 | HS | 83.33 | 5 | HS |
| 15 | 21PRI15 | 53.33 | 5 | HS | 56.67 | 5 | HS |
| 16 | 21PRI16 | 46.67 | 4 | S | 55.00 | 5 | HS |
| 17 | 21PRI17 | 41.67 | 4 | S | 60.00 | 5 | HS |
| 18 | 21PRI18 | 33.33 | 4 | S | 63.33 | 5 | HS |
| 19 | 21PRI19 | 55.00 | 5 | HS | 78.33 | 5 | HS |
| 20 | 21PRI20 | 45.00 | 4 | S | 55.00 | 5 | HS |

Resistance potential was assessed based on the disease rating scale described by Bashir et al. (2005).

Table 6. YMD symptoms observed on leaves of alternate host plants identified in the vicinity of mungbean fields.

| Alternate plant | Indication | Prevalence |
|---|---|---|
| Urdbean (*Vigna mungo*) | Yellow specks, yellowing, mottling, necrosis | Crop sown adjacent to mung bean advanced lines. |
| Cowpea (*Vigna unguiculata*) | Yellow specks, yellowing, mottling, necrosis | Plants noted as weeds within vicinity of the mung bean. |
| Pigeon peas (*Cajanus cajan*) | Yellow specks, yellowing, mottling, necrosis | Plants recorded as weeds within vicinity of the mung bean. |
| Soybeans (*Glycine max*) | Yellow specks, yellowing, mottling, necrosis | Plants present as weeds within vicinity of the mung bean. |
| Jungle mat bean (*Vigna trilobata*) | Yellow specks | Weed plants observed within vicinity of the mung bean. |
| Brown top millet (*Bracaria ramosa*) | Yellow specks | Weed plants observed within the vicinity of the mung bean. |
| Itsit (*Trianthema portulacastrum*) | Yellowing | Weed plants noticed within the vicinity of the mung bean. |
| Tandla (*Digera arvensis*) | Yellowing | Weed plants noticed within the vicinity of the mung bean. |

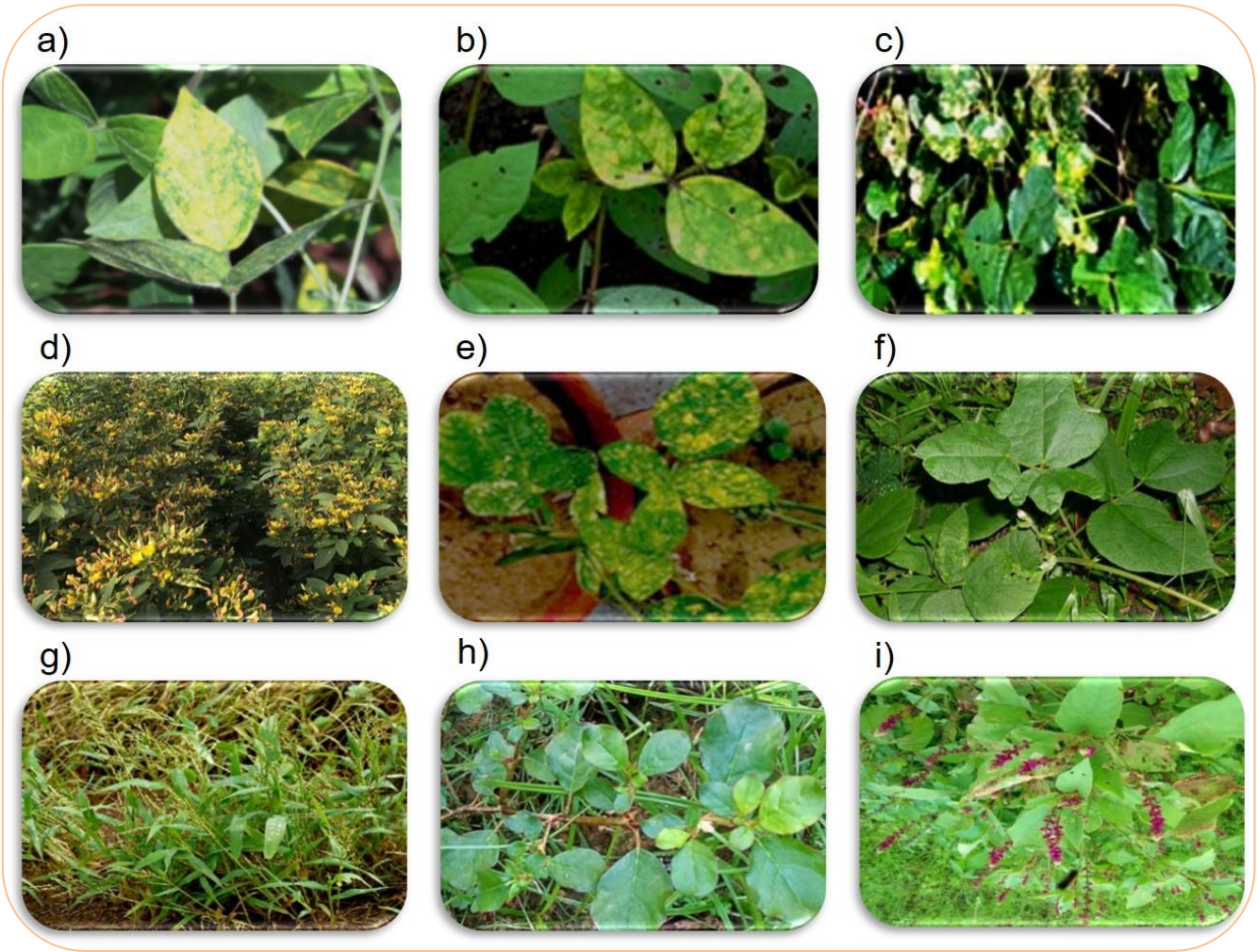


Figure 4. YMD symptoms on leaves of alternate host plants identified in the vicinity of mungbean fields: (a) Mungbean, (b) Urdbean, (c) Cowpea, (d) Pigeon pea, (e) Soybean, (f) Jungle mat bean, (g) Brown top millet, (h) Itsit, and (i) Tandla.

**Temporal dynamics of YMD begomovirus infection in mungbean and alternate hosts**

In the susceptible mungbean variety Mung Kabuli, samples collected and assayed in 2021 for the presence of YMD-associated begomovirus exhibited a mean virion concentration of 0.348. In 2022, samples from the same variety showed a slightly increased mean virion concentration of 0.360 (Figure 5; Table 7). These findings suggest that repeated cultivation of a susceptible mungbean genotype across consecutive seasons may contribute to the progressive enhancement of YMD incidence and begomovirus accumulation. The higher virion concentration and disease incidence recorded in Mung Kabuli during 2022 compared with 2021 indicate a gradual intensification of YMD infection over time.

A comparable trend in begomovirus accumulation was observed among the advanced mungbean lines. During 2021, the concentration of YMD-associated begomovirus particles ranged from 0.303 to 0.395, whereas in 2022, it varied between 0.311 and 0.397 (Figure 5; Table 7). The increased virus accumulation and corresponding rise in disease incidence in 2022 suggest that the evaluated advanced mungbean lines are exhibiting a gradual shift towards susceptibility under continuous disease pressure. These results highlight the importance of long-term evaluation of breeding materials to ensure durable resistance against YMD-associated begomoviruses.

Among the surveyed weed and alternate host plants, leaf samples of urdbean, cowpea, pigeon pea, soybean, and jungle mat bean showed detectable begomovirus accumulation comparable to that observed in susceptible Mung Kabuli and advanced mungbean lines. In contrast, brown top millet, itsit, and tandala showed no detectable virion particles, indicating their possible non-host status under the prevailing conditions. For the positive alternate hosts, mean absorbance values recorded in 2021 ranged from 0.336 to 0.372, while values in 2022 increased slightly and ranged from 0.363 to 0.380 (Figure 5; Table 7). The consistent detection of begomovirus particles in these alternate hosts across seasons suggests their potential role as reservoirs contributing to the persistence and seasonal spread of YMD in mungbean-growing regions.

**Discussion**

The present study was undertaken to elucidate the epidemiological dynamics of YMD in mungbean, identify prevalent alternate host plants, and evaluate the resistance potential of advanced PRI mungbean lines under natural field conditions. The integrated assessment of disease incidence, begomovirus particle concentration, and whitefly population dynamics from flowering to maturity provides valuable insights into the multifaceted factors contributing to YMD development and its persistent threat to mungbean production in Pakistan.

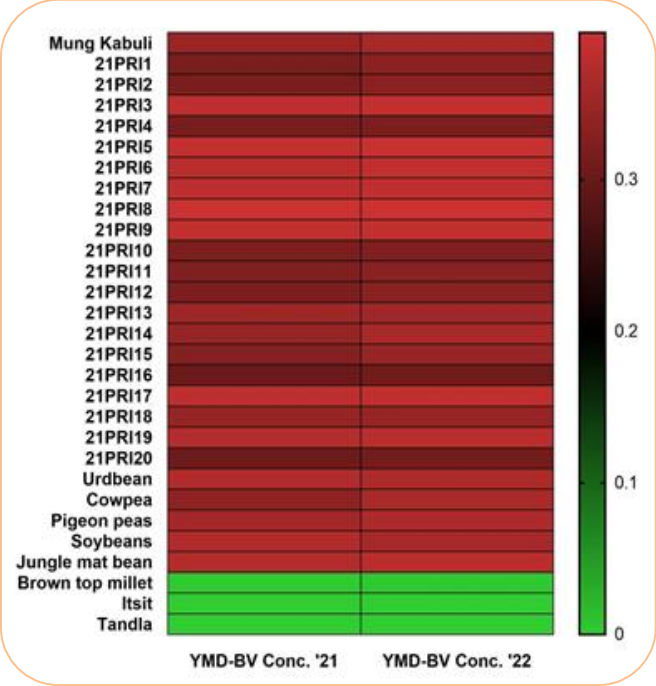


Figure 5. Heat map analysis illustrating begomovirus virion particle concentrations in young leaves of YMD-infected plants.

Throughout the study period, YMD incidence exhibited a progressive increase during crop development. Under natural field conditions, the susceptible cultivar Mung Kabuli showed continuous symptom development from flowering until harvest, confirming its high vulnerability to begomovirus infection. The consistent susceptibility of Mung Kabuli makes it a useful reference genotype for evaluating disease pressure, monitoring virus epidemiology, and validating the resistance performance of breeding materials under natural inoculum conditions (Abbas et al., 2018; Bakhtawar et al., 2018). However, the available resistance resources appear to be gradually diminishing, as previously resistant or moderately resistant genotypes have shown variable responses over time. For instance, Abbas Mung, NM-2011, and Ramzan Mung exhibited moderate resistance with 6%, 9%, and 7% disease incidence, respectively, whereas AZRI 2006 was categorized as moderately susceptible (Naseer et al.,

2025). The higher YMD incidence recorded at crop maturity compared with flowering is consistent with previous reports indicating that disease severity generally intensifies during pod development and maturity due to enhanced virus multiplication, prolonged vector feeding activity, and cumulative infection pressure (Akbar et al., 2019; Mishra et al., 2020; Nawaz et al., 2023). Similarly, recent studies from Uttar Pradesh, India, reported maximum MYMV incidence in Moth tehsil (41.85%), followed by Rajapur (36.88%), highlighting the increasing threat of yellow mosaic begomoviruses across the Indo-Pak region (Singh et al., 2025).

In the present investigation, YMD incidence was substantially higher during 2022 compared with 2021, which corresponds with earlier observations from major mungbean-producing regions of Pakistan, including Mianwali, Bhakkar, and Layyah, where disease severity increased considerably during 2022 (Nawaz et al., 2023). This seasonal escalation may be attributed to prolonged monsoon rainfall, increased relative humidity, and delayed crop establishment, which collectively create favorable ecological conditions for whitefly multiplication and efficient virus transmission. These findings emphasize the strong influence of climatic variability on YMD epidemiology and highlight the necessity of incorporating weather-based disease forecasting models into future management programs.

The observed dynamics of whitefly infestation further support previous epidemiological studies. In the current study, whitefly populations reached their maximum density during the flowering stage and subsequently declined at maturity, following patterns reported by Abdullah-Al-Rahad et al. (2018) and Alam et al. (2021). The 2022 cropping season experienced relatively cooler and more humid conditions due to extended rainfall events compared with 2021, thereby facilitating vector population build-up and enhancing begomovirus dissemination. The synchronization between peak whitefly abundance and increased disease development suggests that vector management during early and mid-crop stages is critical for reducing YMD epidemics. Therefore, effective management strategies should prioritize early intervention and continuous monitoring of vector populations, particularly in regions experiencing prolonged humid conditions and elevated whitefly pressure.

Table 7. Quantification of YMD-associated begomovirus virion concentrations in mungbean, kabuli mungbean, advanced mungbean lines, and alternative host plants.

| Test samples Name | Mean concentration value of YMD begumovirus virion particles | | Test samples Name | Mean concentration value of YMD begumovirus virion particles | |
|---|---|---|---|---|---|
| | Year 2021 | Year 2022 | | Year 2021 | Year 2022 |
| Mung kabuli | 0.348 | 0.360 | 21PRI15 | 0.327 | 0.346 |
| 21PRI1 | 0.317 | 0.333 | 21PRI16 | 0.303 | 0.309 |
| 21PRI2 | 0.316 | 0.332 | 21PRI17 | 0.384 | 0.385 |
| 21PRI3 | 0.383 | 0.387 | 21PRI18 | 0.344 | 0.344 |
| 21PRI4 | 0.314 | 0.319 | 21PRI19 | 0.374 | 0.380 |
| 21PRI5 | 0.388 | 0.391 | 21PRI20 | 0.305 | 0.311 |
| 21PRI6 | 0.379 | 0.385 | Urd bean | 0.370 | 0.366 |
| 21PRI7 | 0.384 | 0.385 | Cowpea | 0.336 | 0.363 |
| 21PRI8 | 0.395 | 0.397 | Pigeon peas | 0.357 | 0.367 |
| 21PRI9 | 0.386 | 0.388 | Soybeans | 0.371 | 0.360 |
| 21PRI10 | 0.319 | 0.323 | Jungle mat bean | 0.372 | 0.380 |
| 21PRI11 | 0.322 | 0.333 | Brown top millet | 0.003 | 0.002 |
| 21PRI12 | 0.320 | 0.331 | Itsit | 0.000 | 0.000 |
| 21PRI13 | 0.353 | 0.354 | Tandla | 0.000 | 0.000 |
| 21PRI14 | 0.345 | 0.363 | | | |

Virion particle concentration is at $A^{405}$ value.

Evaluation of advanced PRI mungbean lines revealed seasonal variation in resistance expression, with several genotypes showing reduced resistance stability under increased disease pressure. Some lines previously categorized as resistant or moderately resistant exhibited higher susceptibility during the 2022 season. Specifically, 21PRI4 shifted from moderately resistant to moderately susceptible, whereas 21PRI3 progressed to the susceptible category. Such fluctuations may result from genotype × environment interactions, changes in vector pressure, and environmental effects on host defense mechanisms. Similar seasonal variability in YMD resistance expression among mungbean genotypes has been documented by Rohilla et al. (2022), who reported that resistance levels can vary depending on growing season and environmental conditions. Malik et al. (2022) further demonstrated that YMD resistance is influenced by crop growth stage and may deteriorate under high whitefly populations or prolonged rainfall conditions. Moreover, genome-wide association studies have indicated that biochemical and physiological traits associated with YMD resistance are dynamic across environments, resulting in inconsistent disease responses among previously resistant genotypes (Kohli et al., 2024). These findings highlight the importance of evaluating breeding materials across multiple seasons and diverse environments to identify durable resistance sources.

Typical YMD symptoms, including bright yellow mottling and mosaic patterns, were observed not only in mungbean genotypes (Mung Kabuli and advanced PRI lines) but also in several alternate hosts, including urdbean, pigeon pea, soybean, cowpea, and jungle mat bean. The occurrence of these hosts in and around mungbean-growing areas may provide continuous reservoirs for begomovirus survival and facilitate pathogen carryover between cropping seasons (Suruthi et al., 2018; Singh et al., 2019; Mishra et al., 2020). Detection of begomovirus particles using polyclonal antibodies against Cotton Leaf Curl Virus (CLCuV) through DAS-ELISA confirmed infection in these alternate hosts, demonstrating their potential epidemiological significance. Similar serological approaches have been successfully employed for begomovirus detection using antibodies against Tomato Yellow Leaf Curl Begomovirus (Ummad-ud-Din et al., 2019) and African Cassava Mosaic Virus (Kothandaraman et al., 2016). Likewise, Swamy et al. (2023) used DAC-ELISA to detect begomovirus infection in resistant and susceptible mungbean cultivars.

The present study demonstrated consistent detection of begomovirus particles in Mung Kabuli, advanced mungbean lines, and alternate host species during both cropping seasons, with only minor annual variations. The relatively low virus titre detected across tested hosts suggests limited viral accumulation at the sampling stages or differences in host–virus interactions (Swamy et al., 2023). However, further molecular characterization is required to determine the diversity and prevalence of begomovirus species involved in YMD development. In particular, the occurrence of other important begomoviruses, such as Mungbean Yellow Mosaic India Virus (MYMIV), requires investigation because the durability and effectiveness of existing resistance sources remain uncertain against diverse viral populations (Rana et al., 2026).

The simultaneous occurrence of efficient whitefly vectors, unstable host resistance, and persistent alternate host reservoirs highlights the complex epidemiological nature of YMD. The findings demonstrate that reliance solely on host resistance may not provide sustainable disease suppression. Therefore, future management strategies should adopt an integrated approach involving deployment of durable resistance genes, suppression of vector populations, removal or management of alternate hosts, and consideration of environmental factors influencing disease development.

**Conclusion**

(YMD, caused by begomoviruses and primarily transmitted by the whitefly *B. tabaci*, remains a significant limitation to mungbean productivity in Pakistan. The progressive increase in disease incidence, seasonal variability in resistance expression, and persistence of infection sources demonstrate the complexity and evolving nature of YMD epidemiology under changing climatic and vector conditions. The breakdown of resistance in advanced mungbean lines indicates the need for continuous evaluation and identification of more durable resistance sources. Furthermore, the widespread occurrence of alternate host plants around mungbean fields may enhance virus survival and contribute to recurrent disease outbreaks.

Future research should focus on molecular characterization of prevailing begomovirus species, identification and pyramiding of durable resistance genes through molecular breeding approaches, and genome-wide association studies for resistance improvement. In addition, integrated disease

management programs combining resistant cultivars, timely whitefly suppression, elimination of alternate hosts, crop diversification, and ecological surveillance are essential for sustainable management of YMD and safeguarding mungbean production systems in Pakistan.

**Author Contributions**

MN, AM, and AB conceptualized and designed the study. SN, MS, and MZN conducted field investigations and performed the experimental work. MN and MUS provided overall supervision and ensured methodological rigor, data quality, and analytical consistency throughout the study. SN, IH, and MAH contributed as co-supervisors, providing critical guidance on experimental design, data interpretation, and scientific refinement at various stages of the research. MN, MUS, AB, MAH, and MS contributed to the preparation of the initial manuscript draft by organizing the structure, compiling the results, and integrating preliminary interpretations. Subsequently, all authors critically reviewed and revised the manuscript, contributed to its intellectual improvement, verified the data and analyses, and approved the final version for submission.

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**Conflict of Interest**

The authors declare no conflict of interest.

**Sustainable Development Goals Targeted**

SDG 2: Zero Hunger
SDG 12: Responsible Consumption and Production
SDG 13: Climate Action

**Policy Addressed**

The study highlights the urgent requirement for a coordinated national strategy integrating durable resistance breeding, molecular diagnostics, virus surveillance, vector management, and sustainable seed dissemination systems to mitigate the increasing threat of Mungbean Yellow Mosaic Begomovirus and ensure long-term mungbean productivity.

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