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EVALUATION OF BLACKGRAM (*VIGNA MUNGO* [L.] HEPPER) GENOTYPES AGAINST YELLOW MOSAIC AND LEAF CRINKLE DISEASES

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SUMMARY

Blackgram (*Vigna mungo* [L.] Hepper) is susceptible to some of the most destructive diseases worldwide, including the yellow mosaic disease (YMD) and leaf crinkle disease (LCD). The development of resistant or tolerant varieties is an economical and long-term solution for controlling these diseases. This study visually assessed the response of 72 blackgram genotypes against the YMD and LCD under natural field conditions for two consecutive years. Seven genotypes proved resistant to the YMD, whereas 20 were resistant to the LCD. It was also evident that four genotypes, namely, 32596, 35678, Accession-4, and Accession-7, remained resistant to both diseases during both years. Therefore, the study suggests these genotypes' use could be beneficial in breeding programs to breed multiple-disease-resistant or tolerant blackgram genotypes or could be recommended varieties for general cultivation following adaptation to different agro-climatic areas. Further verification of the virus involved in the YMD complex had the infected leave samples tested with virus-specific primer pairs of the mungbean yellow mosaic virus (MYMV), the mungbean yellow mosaic India virus (MYMIV), and the horsegram yellow mosaic virus (HgYMV). An amplification of approximately ~2700 bp resulted in all samples only with a full-length component of the DNA-A of MYMIV, the first time confirming the association of the YMD complex in blackgram in Pakistan with the MYMIV.

Keywords: Blackgram (*V. mungo* L.), germplasm, evaluation, virus, diseases

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Key findings: Seven blackgram (*V. mungo* L.) genotypes emerged resistant to the YMD, and 20 were resistant to the LCD. However, among these, four genotypes (32596, 35678, Accession-4, and Accession-7) remained resistant against both diseases. The study succeeded in being the first to report the mungbean yellow mosaic India virus (MYMIV) to cause the YMD complex in blackgram in Pakistan.

INTRODUCTION

Blackgram (*Vigna mungo* [L.] Hepper), also known as mash bean, urdbean, or sabut-mash, is a warm-season, dicotyledonous, short-duration legume crop, widely grown in Afghanistan, Bangladesh, India, Myanmar, Pakistan, Sri Lanka, Thailand, and Vietnam. A report stated a small-scale cultivation of it also prevails in Kenya, Uganda, Tanzania, Argentina, and Brazil. The global leading producer of blackgram is India, which supplies 70% of the total production, accompanied by Myanmar and Pakistan (Nair e*t al*., 2024).

The blackgram is well-known for its exceptional dietary value, containing vital nutrients such as protein (20% to 24%), vitamins, and minerals, making it a source of valuable nutrition. Blackgram, as a member of the Fabaceae family, can astonishingly fix nitrogen biologically to improve soil fertility for sustainable farming practices (Verma *et al*., 2024).

Blackgram is an important part of Pakistan's agricultural landscape, even though it is the third most planted edible legume after lentil and chickpea. Although blackgram only makes up 1.5% of the land used for pulse agriculture, it produces 1.4% of all pulses generated in the country (Qayyum *et al*., 2019; Khan *et al*., 2021; Hussain *et al*., 2022). This crop's cultivation can normally proceed in several agro-ecological zones during two seasons each year—spring and summer—demonstrating its tolerance to various cropping schedules and climatic circumstances (Qayyum *et al*., 2019). The average yield of blackgram in Pakistan is far less as compared with other countries due to some biotic and abiotic factors. Among biotic factors, viral diseases, namely, the yellow mosaic disease (YMD) and leaf crinkle disease (LCD), have the capacity to seriously impair the blackgram economically (Singh *et al*., 2022).

The YMD is a significant barrier in the production of blackgram throughout the Indian subcontinent. The documentation of the YMD has existed worldwide where legume cultivation is prevalent, excluding Australia (Mishra *et al*., 2020). Globally, the YMD has reported causes of four different types of whiteflies (*Bemisia tabaci* Gennadius) transmitted as 'Legumoviruses' (MYMV, MYMIV, HgYMV, and Dolichos yellow mosaic virus [DoYMV]). These four viruses are bipartite begomoviruses, belonging to Geminiviruses, and their genome consists of two single-stranded circular DNA molecules (DNA-A and DNA-B), having a size of approximately ~2.7 kb (Mishra *et al*., 2020). In blackgram, the first time to observe the YMD in India was in 1966. With the four 'Legumoviruses,' before only the MYMV and MYMIV have achieved confirmation as the causal agent of the YMD in blackgram from various regions (Mishra *et al*., 2020; Tripathy and Das, 2021).

The leaf crinkle disease (LCD) also appears as the second most destructive viral disease of blackgram in Pakistan, India, and across the world. In blackgram, the first documentation of LCD came from India during 1966, while in Pakistan, it was during 1985. LCD-infected plants can show various symptoms like leaf crinkling, twisting, rugosity, deformation, reduced plant growth, decreased pollen grain fertility, and greening/thickening of flower sepals (Iftikhar *et al*., 2020). Although confirmation through different attributes of the malady has the urdbean leaf crinkle virus (ULCV) as the causal agent of the LCD, exact identification of the virus/es involved in the LCD following molecular analysis of infected plants still needs establishing (Kamaal *et al*., 2023). However, only one study conducted by Baranwal *et al*. (2015), following next-generation sequencing, showed the presence of whiteflies transmitting the cowpea mild mottle virus (Carlavirus) in mung bean and blackgram plants showing LCD symptoms under natural field conditions. On the other hand, a closer survey of literature showed transmission of the ULCV virus results from beetles (*Henosepilachna dodecastigma*) and various aphid species (*Aphis craccivora* Koch, *A. gossypii* Glov, *Acyrthosiphon pisum*, and *Myzus persicae*) (Kamaal *et al*., 2023).

Both the YMD and LCD are tough to manage using chemicals or cultural practices alone. Therefore, the cultivation of resistant varieties is the only viable, safe, and economical control measure for these diseases (Pandey *et al*., 2023). Although blackgram has prevailed in cultivation in Pakistan for a long time, no systematic research on characterizing its germplasm for resistance to YMD and LCD materialized, nor on identifying the viruses responsible for the YMD. Yet, while global studies on these diseases exist, research in the Pakistani context remains limited. This study aimed to fill this gap by identifying the virus or viruses causing YMD in the blackgram in Pakistan and by evaluating local germplasms for resistance to both the YMD and LCD under natural field conditions. This work represents one of the first comprehensive efforts in Pakistan to address these critical issues.

MATERIALS AND METHODS

Germplasm screening

Seeds of 72 blackgram genotypes, including both commercial varieties and diverse accessions, came from the Plant Genetic Resources Institute (PGRI), National Agricultural Research Centre (NARC), Islamabad. These genotypes' selection represents a wide range of genetic diversity within the blackgram germplasm maintained at PGRI. The selection aimed to include genotypes with varying origins, agro-climatic adaptations, and morphological traits, ensuring a comprehensive evaluation of resistance to the YMD and LCD. All genotypes succeeded in their sowing in the field at the Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan (a hot spot for YMD and LCD), during the first week of July, 2022 and 2023. Two rows sown of each genotype had 20 plants per row, following a complete randomized block design in triplicate. The row-to-row and plant-to-plant distances were kept at 30.48 and 15.24 cm, respectively, for each genotype. No application of plant protection measures occurred against insect vectors to ensure high inoculum pressure during the experiment. However, all other normal agronomic practices (irrigation, fertilizers, etc.) took place to keep the crop in good condition and uniform. Blackgram field visitation continued daily for observing the development of YMD and LCD. Data recording for both diseases used the rating system described in Tables 1 and 2 to determine the infection type range (ITR), percent disease index (%DI), and the response of the genotypes. The %DI calculation utilized the formula (the sum of all disease ratings/the total number of plants observed) × (100/the maximum disease severity rating) (Akhtar *et al*., 2009, 2025).

Molecular Identification of viruses in YMD complex

The collected blackgram leaf samples from naturally infected YMD symptomatic plants served to confirm the viruses associated following molecular characterization. Total DNA extraction from the collected leaf samples used the cetyl trimethyl ammonium bromide (CTAB) method (Doyle, 1990). For this purpose, nearly 100 to 200 mg of leaf samples underwent grinding into fine powder with liquid nitrogen. The resulting crushed sample proceeded into a centrifuge tube containing 700 µl CTAB buffer before incubating in a water bath at 65 °C. After every 10 min, mixing the samples well continued, and after 30 min, cooling the samples at room temperature ensued before adding chloroform-isoamyl-alcohol in the ratio of 24:1 (700 ul) to each tube. Again, the samples received thorough mixing before their centrifugation for 15 min at 13000 rpm. After centrifugation, the upper layer, as transferred into a new sterile centrifuge tube containing 500 µl of isopropanol, remained at -20 °C for half an hour. After 30–45 min, the tubes

Table 1. Disease rating scale for the screening of blackgram germplasm against the yellow mosaic disease (YMD).

Disease Rating	Symptoms	Percent Disease Index (PDI)	Disease response
0	Complete absence of symptoms	0	Highly resistant
1	Few small yellow specks or spots on few leaves seen after careful observations.	0.01– 10	Resistant
2	Bright yellow specks or spots common on leaves, easily observed and some coalesced.	10.01–25	Moderately resistant
3	Mostly coalesced bright yellow specks or spots common on leaves, but no or minor reduction in yield.	25.01–40	Moderately susceptible
4	Plants showing coalesced bright yellow specks or spots on all leaves, with no or minor stunting and set fewer normal pods.	40.01–60	Susceptible
5	Yellowing or chlorosis of all leaves on whole plant followed by necrosis, shortening of internode, severe stunting of plants, with no yield or few flowers and deformed pods produced with small, immature, and shriveled seeds.	$>$ 60.01	Highly susceptible

PDI = (sum of all disease ratings/the total number of plants observed) × 20.

Table 2. Disease rating scale for the screening of blackgram germplasm against the urdbean leaf crinkle disease (ULCD).

Disease Rating	Symptoms	Percent Disease Index (PDI)	Disease response
0	No visible symptoms	0	Highly resistant
1	Mild symptoms	0.01–10	Resistant
2	Moderate symptoms and set moderate number of pods	10.01–25	Tolerant
3	Severe disease, no pod formation	$>$25.01	Susceptible

PDI = (sum of all disease ratings/the total number of plants observed) × 33.33.

underwent spinning for 15 min at 13,000 rpm to precipitate DNA. DNA pellets acquired a washing of 200 µl of 70% ethanol for 2 min at 13000 rpm before drying them at 37 °C for 15 min. Then, their re-dissolving in 70 µl of sterile ddH_2O followed. The confirmation of the presence of viruses entailed the PCR using virus-specific primer pairs targeting MYMV, MYMIV, and HgYMV, as recommended by Singh *et al*. (2022). These primers were options due to their established specificity and reliability in identifying different strains of begomoviruses commonly associated with the YMD in legumes. Previous studies have validated these primers for accurately distinguishing between closely related viral species, making them suitable tools for molecular identification in blackgram. After PCR completion, loading 2 µl of the product ensued on 1% agarose gel stained with ethidium bromide before visualization using the UV gel documentation system (Bio-Rad, USA).

RESULTS

Genotype evaluation against the YMD

The response of 72 blackgram genotypes to YMD, as assessed under natural field conditions, determined their resistance or tolerance levels. All tested genotypes displayed varying degrees of disease response, ranging from resistant to highly susceptible, depending upon their genetic makeup (Table 3).

Whiteflies (the insect vector of the virus complex causing YMD) were first noticeable 10 days after germination and continued their upsurge throughout the crop development period. The first YMD symptom

Table 3. Screening of blackgram genotypes against the YMD under natural field conditions during 2022 and 2023.

No.	Genotype	ITR	PDI	DR	No.	Genotype	ITR	PDI	DR
1	32587	1-2	11.1	MR	37	38283	2-3	30.6	MS
2	32589	1-2	20.0	MR	38	38284	1-3	41.8	S
3	32590	1-2	12.1	MR	39	38285	2-3	34.3	MS
4	32591	1-2	13.3	MR	40	38286	2-3	39.4	MS
5	32592	1-2	4.7	R	41	38287	1-4	37.8	MS
6	32593	1-2	14.3	MR	42	38288	2-3	20.0	MR
7	32594	2	2.5	R	43	38289	2-3	41.6	S
8	32595	1-2	13.6	MR	44	38290	2-3	34.1	MS
9	32596	1-2	4.1	R	45	38291	2-3	44.8	S
10	32769	3	73.3	HS	46	38292	1-3	34.3	MS
11	32770	4	80	HS	47	38293	2-3	27.9	MS
12	35677	2-4	32.1	MS	48	38294	2-3	35.6	MS
13	35678	1-2	6.7	R	49	38297	4	55.0	S
14	36842	1-3	15.6	MR	50	38298	2-4	38.6	MS
15	38222	3-5	81.7	HS	51	38299	2-3	40.5	S
16	38256	1-3	35.0	S	52	39124	2-4	38.8	MS
17	38257	2-3	25.9	MS	53	39126	4-5	69.0	HS
18	38258	2-3	37.4	MS	54	39132	3-5	84.1	HS
19	38261	1-3	26.7	MS	55	39133	4-5	65.3	HS
20	38262	2-3	40.6	S	56	40810	1-3	27.4	MS
21	38263	2-3	41.5	S	57	40827	1-2	15.3	MR
22	38267	1-3	33.3	MS	58	40828	1-2	16.4	MR
23	38268	2-3	47.7	S	59	41296	1-3	40.0	MS
24	38269	1-3	43.3	S	60	Accession-1	1-2	7.6	R
25	38270	1-3	34.5	MS	61	Accession-4	1-2	6.5	R
26	38272	2-3	25.2	MS	62	Accession-5	1-3	29.4	MS
27	38273	2-3	54.3	S	63	Accession-6	1-3	34.9	MS
28	38274	2-3	31.1	MS	64	Accession-7	1-2	6.5	R
29	38275	2-3	34.1	MS	65	Accession-9	1-2	19.4	MR
30	38276	1-3	27.6	MS	66	Local Mash	1-2	26.7	MS
31	38277	2-3	35.2	MS	67	Arooj	1-2	17.1	MR
32	38278	1-3	29.3	MS	68	Mash-2	1-2	14.7	MR
33	38279	2-3	44.2	S	69	Mash-3	1-2	20.0	MR
34	38280	2-3	39.2	MS	70	Mash-97	1-3	15.3	MR
35	38281	1-3	38.3	MS	71	Barani Mash	1-2	16.1	MR
36	38282	2-3	31.1	MS	72	Chakwal Mash	1-2	20.0	MR

ITR=Infection type range; PDI=Percent disease index; DR=Disease response; HR=Highly resistant; R=Resistant; MR=Moderately resistant; MR=Moderately susceptible; S=Susceptible; HS=Highly susceptible.

was visible as a few dispersed tiny yellow specks on some fresh leaves of susceptible genotypes 18 days post germination. The number of diseased plants and YMD severity rose through time, depending on the genetic structure of the tested genotypes. None of the tested genotypes appeared highly resistant (completely free from symptoms). However, seven genotypes (32592, 32594, 32596, 35678, Accession-1, Accession-4, and Accession-7) were resistant with percent disease index (PDI) values ranging from 0.1% to 10% and infection type range (ITR) values of 1–2. Meanwhile, 17 genotypes (32587, 32589, 32590, 32591, 32593, 32595, 36842, 38288, 40827, 40828, Accession-9, Arooj, Mash-2, Mash-3, Mash-97, Barani Mash, and Chakwal Mash) were moderately resistant, with PDI values ranging between 10.01% and 25% and ITR values of 2–3. Moreover, 30 genotypes (35677, 38257, 38258, 38261, 38267, 38270, 38272, 38274, 38275, 38276, 38277, 38278, 38280, 38281, 38282, 38283, 38285, 38286, 38287, 38290, 38292, 38293, 38294, 38298, 39124, 40810, 41296, Accession-5, Accession-6, and Local Mash) were moderately susceptible. Their PDI values ranged from 25.01% to 40%, with ITR values of 2–4. Twelve genotypes (38256, 38262, 38263, 38268, 38269, 38273, 38279, 38284, 38289, 38291, 38297, and 38299) were susceptible, with PDI values ranging between 40.01% and 60% and ITR values of 2–4. Only six genotypes (32769, 32770, 38222, 39126, 39132, and 39133) were highly susceptible with PDI values of more than 60% and ITR values of 3–5 (Table 3).

In resistant genotypes, mild YMD symptoms manifested as a few tiny dispersed chlorotic specks on about 5–10 leaves per plant (Figure 1a). Interestingly, many plants of these resistant genotypes exhibited full recovery from the disease at advanced stages. In genotypes with moderate resistance, these symptoms appeared as more pronounced, easily observable bright yellow specks or spots common on leaves (Figure 1b). Mostly coalesced bright yellow specks or spots common on leaves, but no or minor reduction in yield resulted in the case of moderately susceptible genotypes (Figure 1c). Similarly, in susceptible genotypes, most plants surfaced with coalesced bright yellow specks or spots on all leaves, with no or minor stunting and a fewer set of normal pods (Figure 1d). However, highly susceptible genotypes had YMD-infected plants with severe symptoms. These include chlorosis of all leaves on the entire plant, followed by necrosis, shortening of internodes, acute stunting of plants with no yield or few flowers, and deformed pods with small, immature, and shriveled seeds (Figure 1e-f).

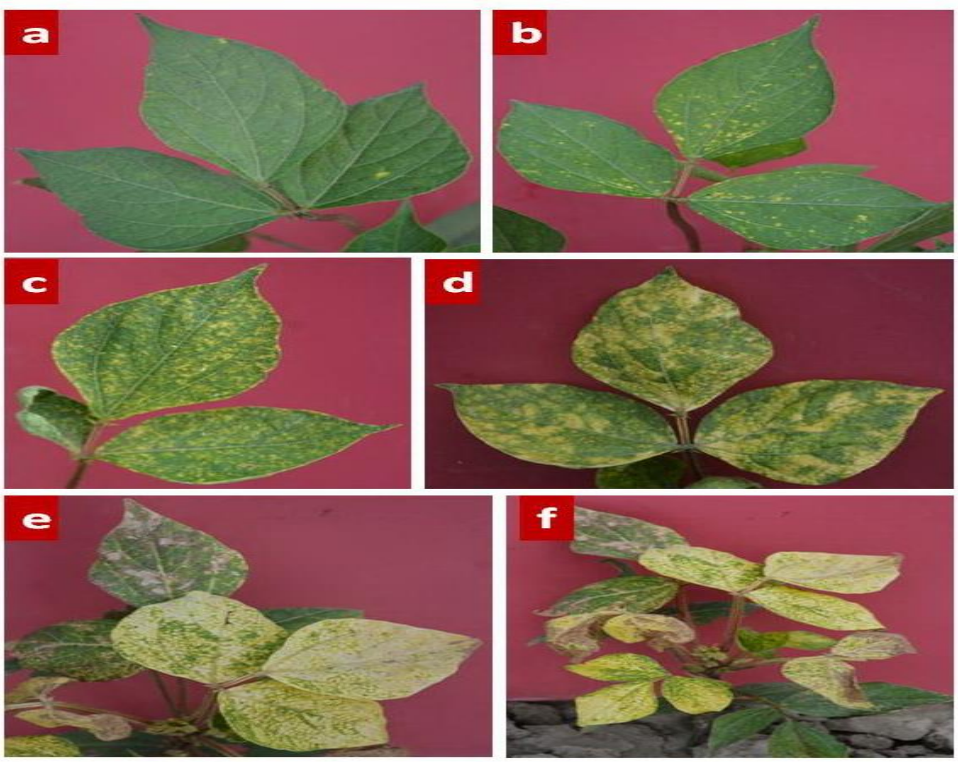

Figure 1. Photographic representation of disease scale for the rating of YMD. a) Highly resistant with no symptoms; b) Resistant with small yellow spots on few leaves; c) Moderately resistant with easily observable bright yellow spots; d) Moderately susceptible with mostly coalesced yellow spots; e) Susceptible with coalesced yellow spots on the entire plant, and f) Highly susceptible with severe chlorosis, necrosis, and plant stunting.

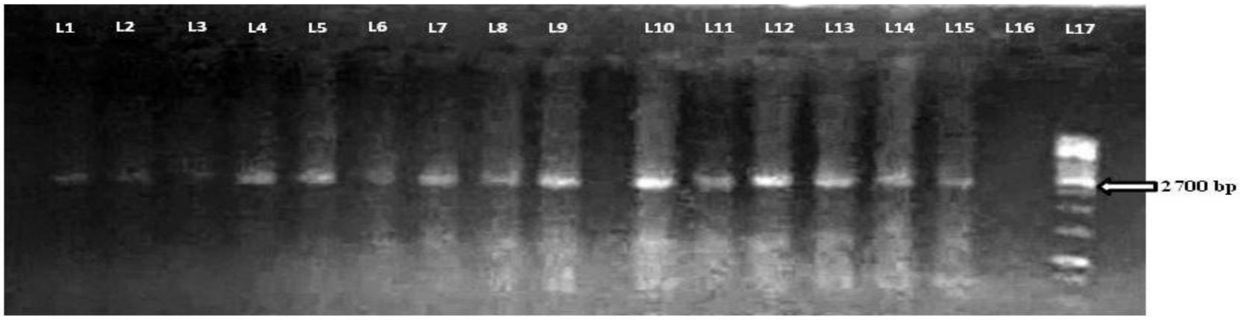

Figure 2. Confirmation of the MYMIV in YMD-infected leaf samples of blackgram using species specific primers. L1 to L15 infected samples showing PCR amplification of 2700 bp, L16 –control, and L17 showing the DNA ladder.

The confirmation of the virus involved in the YMD complex in Pakistan, the research tested leaves from 15 blackgram plants with severe YMD symptoms using virus-specific primer pairs of the MYMIV, MYMV, and HgYMV. Only the MYMIV primers yielded amplification of approximately ~2700 bp, indicating that the MYMIV is the predominant virus associated with YMD in the tested blackgram genotypes in Pakistan (Figure 2). No amplification was evident with the MYMV and HgYMV primer pairs.

Genotypes evaluation against LCD

The responses of the same 72 blackgram genotypes also involved detection to see their resistance or tolerance level against the LCD under natural field conditions during two consecutive years (2022 and 2023). The combined data from both years showed all tested genotypes displayed varied degrees of disease response, ranging from resistant to susceptible, depending upon their genetic makeup (Table 4).

The first indication of LCD appeared at 22 days post germination as curly manifestations on the third trifoliate leaves of susceptible genotypes. The number of diseased plants and LCD severity increased through time, depending on the genetic structure of the tested genotypes. None of the tested genotypes emerged highly resistant against the LCD. However, 20 genotypes (32590, 32593, 32596, 35678, 38258, 38261, 38270, 38272, 38281, 38285, 38288, 38292, 38293, 40810, 40827, Accession-4, Accession-7, Accession-9, Mash-2, and Mash-3) proved resistant, with PDI values ranging from 0.1% to 10% and ITR values between 1 and 2. The 44 genotypes (38282, 38280, 38279, 38278, 38277, 38276, 38275, 38274, 38273, 38269, 38268, 38267, 38263, 38262, 38257, 38256, 38222, 36842, 35677, 32595, 32594, 32592, 38283, 38284, 38286, 38287, 38289, 38290, 38291, 38294, 38298, 38299, 39124, 39126, 39133, 41296, Accession-1, Accession-5, Accession-6, Local Mash, Arooj, Mash-97, Barani Mash, and Chakwal Mash) occurred as tolerant. Their PDI values ranged between 10.01% and 25%, with ITR values between 1 and 3. Furthermore, five genotypes (32587, 32589, 32591, 39132, and 40828) were evidently susceptible, with PDI values more than 25.01% and ITR values from 2 to 3 (Table 4). Notably, LCD symptoms were not visible in three genotypes (32769, 32770, and 38297), probably due to early and severe infections of the YMD, which may have masked the onset or progression of LCD symptoms. LCD-infected plants of susceptible genotypes showed severe symptoms, including leaf crinkling, twisting, rugosity, deformation, reduced petioles, lamina crinkling, leaf crowding, greening/thickening of flower sepals more than normal, and reduced plant growth. Early infection of LCD caused total sterility, inflorescence deformity, and flower malformation (Figure 3a-e).

Table 4. Screening of blackgram genotypes against the leaf crinkle disease (LCD) under natural field conditions during 2022 and 2023.

No.	Genotype	ITR	PDI	DR	No.	Genotype	ITR	PDI	DR
1	32587	1-3	27.1	S	37	38283	2-3	15.7	T
2	32589	1-3	31.9	S	38	38284	1-2	13.7	T
3	32590	1-2	9.1	R	39	38285	1	7.9	R
4	32591	1-3	25.9	S	40	38286	1-2	10.8	T
5	32592	1-3	15.5	T	41	38287	1-3	25.0	T
6	32593	1-2	4.8	R	42	38288	1-2	8.3	R
7	32594	1-2	10.4	T	43	38289	2	14.4	T
8	32595	1-3	17.3	T	44	38290	1-2	10.8	T
9	32596	1-2	9.4	R	45	38291	1-2	11.5	T
10	32769	No LCD due to sever YMD			46	38292	1-2	7.9	R
11	32770	No LCD due to sever YMD			47	38293	1-2	8.1	R
12	35677	1-3	14.3	T	48	38294	1-2	10.2	T
13	35678	1-2	9.9	R	49	38297	No LCD due to severe YMD		
14	36842	2-3	18.7	T	50	38298	1-2	11.9	T
15	38222	2-3	20.0	T	51	38299	2	11.4	T
16	38256	2-3	12.0	T	52	39124	2	19.6	T
17	38257	2	11.8	T	53	39126	2-3	19.5	T
18	38258	1	4.3	R	54	39132	2-3	25.5	S
19	38261	1-2	8.9	R	55	39133	2-3	17.6	T
20	38262	1-2	11.8	T	56	40810	1-2	7.9	R
21	38263	1-2	10.2	T	57	40827	1-2	6.9	R
22	38267	2	15.5	T	58	40828	2-3	29.9	S
23	38268	2	23.1	T	59	41296	1-3	13.7	T
24	38269	1-2	11.1	T	60	Accession-1	2-3	18.2	T
25	38270	1-2	8.0	R	61	Accession-4	1	2.3	R
26	38272	1	4.3	R	62	Accession-5	1-3	13.5	T
27	38273	2-3	22.6	T	63	Accession-6	1-3	15.2	T
28	38274	2-3	16.7	T	64	Accession-7	1-2	8.1	R
29	38275	1-3	12.3	T	65	Accession-9	1-2	5.7	R
30	38276	1-3	10.3	T	66	Local Mash	2	25.0	T
31	38277	1-2	19.5	T	67	Arooj	1-3	11.1	T
32	38278	1-2	12.2	T	68	Mash-2	1-2	5.6	R
33	38279	1-3	15.8	T	69	Mash-3	1-2	9.1	R
34	38280	2	13.3	T	70	Mash-97	2-3	14.7	T
35	38281	1-2	9.7	R	71	Barani Mash	2	14.8	T
36	38282	1-2	13.6	T	72	Chakwal Mash	2-3	20.5	T

ITR=Infection type range; PDI=Percent disease index; DR=Disease response; R=Resistant; T=Tolerant; S=Susceptible.

DISCUSSION

Blackgram, a nutritionally rich pulse crop, incurs adverse effects from the YMD and LCD at all growth stages, drastically limiting its production. The successful management of these menaces requires breeding for high-yielding, multiple-disease-resistant genotypes as the only most sustainable and promising way (Tripathi *et al*., 2023). Although reports of extensive work have come out from countries like India and Myanmar, where blackgram is also a widely grown plant, in Pakistan, few studies have proliferated on the screening of blackgram germplasm against both diseases.

In the presented study, 72 indigenous blackgram genotypes sustained visual assessments under natural field conditions to see their resistance or tolerance level against both viral diseases. Observations showed seven genotypes were resistant, 17 were moderately resistant, 30 were moderately

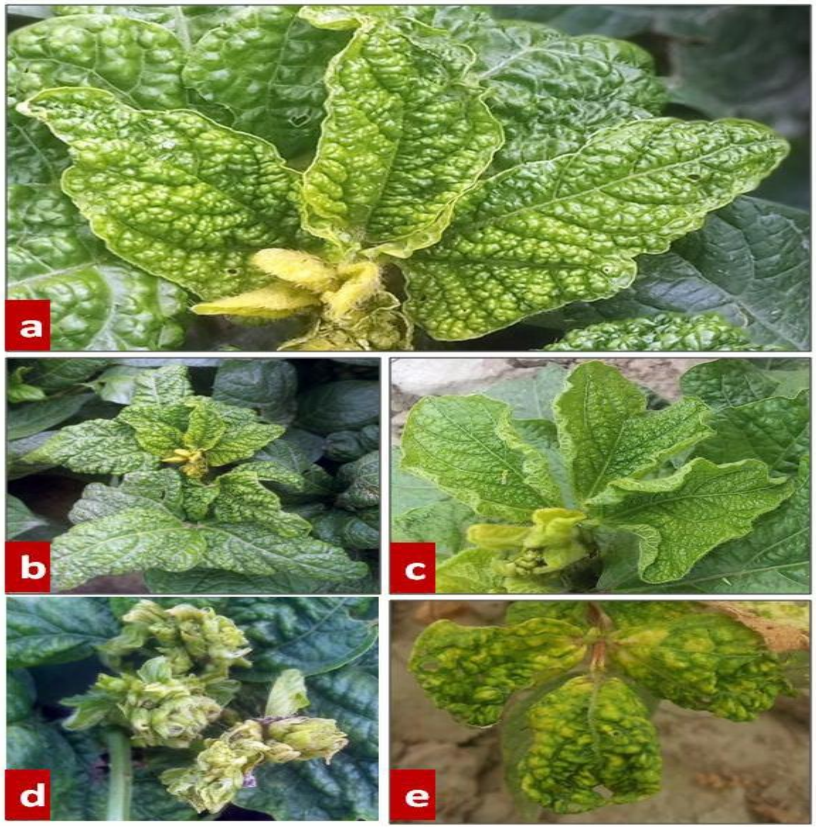

Figure 3. a-e) Severe leaf crinkling, twisting, rugosity, deformation, greening/thickening of sepals and malformation of flowers due to LCD on the blackgram under natural field conditions.

susceptible, 12 were susceptible, and six genotypes were highly susceptible to the YMD. These results are in line with the findings of previous studies from India and Myanmar, where researchers have also documented a scarcity of strong resistance in blackgram against the YMD (Bag *et al*., 2014; Naik *et al*., 2017; Tripathi *et al*., 2023). In this study, it was also evident that many plants of resistant genotypes having mild symptoms exhibited full recovery from the disease at advanced growth stages. This suggested the existence of recovery mechanisms from viral infections, probably governed by specific resistance (R) genes or defense pathways being activated post-infection. This phenomenon of recovery may involve virus multiplication suppression or enhanced systemic acquired resistance, which warrants additional molecular study to understand its genetic basis.

The leaf crinkle disease is the second economically important and destructive problem in blackgram after the YMD, also posing a severe hazard to crop production worldwide (Gautam *et al*., 2016). In this study, none of the 72 tested blackgram genotypes proved completely free from LCD symptoms. However, 20 genotypes appeared to be resistant, 44 were tolerant, and five were susceptible to the LCD. Our findings correspond with previous results of many other researchers who found blackgram resistance against LCD is few (Gautam *et al*., 2016; Sravika *et al*., 2018). Some reports from India indicated the presence of moderately resistant germplasm under specific regional conditions, while our results reflect the ongoing challenge of identifying strong resistance sources under diverse agro-climatic environments such as those in Pakistan.

In Pakistan, most commercially grown varieties of blackgram are susceptible to both viral diseases (YMD and LCD). Although few previous studies found resistant varieties to a particular disease, a dire need to find blackgram genotypes with multiple resistance against these diseases is crucial. This study also identified four genotypes (32596, 35678, Accession-4, and Accession-7) with resistance against both diseases. The detection of such multiple-disease-resistant blackgram genotypes is particularly helpful for breeding programs intended at developing superior cultivars. Given the polygenic nature of resistance to both diseases, these genotypes could serve as promising donor parents for the introgression of resistance traits into elite breeding lines. The integration of these resistant traits into elite breeding lines can provide significant speed through the application of marker-assisted selection (MAS). Future research is essential to identify molecular markers linked with disease resistance in blackgram and exploit them for MAS and genomics-assisted breeding. This approach will ultimately facilitate the researchers' ability to reduce crop losses by breeding high-yielding, multiple disease-resistant varieties with desirable characteristics.

Furthermore, accurate information on virus species causing the YMD in blackgram is necessary to devise appropriate disease management approaches. Internationally, the YMD in blackgram has reports as caused by two closely related whitefly-transmitted begomoviruses, namely, the mungbean yellow mosaic virus (MYMV) and the mungbean yellow mosaic India virus (MYMIV). Both these virus species can cause phenotypically the same disease symptoms in infected plants (Bag *et al*., 2014). In this study, PCR amplification with a virus-specific pair of primers identified only MYMIV as present in the blackgram plants showing severe YMD symptoms. This is the first molecular confirmation that MYMIV is the principal virus involved in the YMD complex in blackgram in Pakistan. Our results align with many other studies, where researchers have confirmed the association of the MYMV and MYMIV as causal agents of the YMD in blackgram from various regions (Tripathy and Das, 2021; Akram *et al*., 2024). The identification of the specific virus involved helps in resistance breeding as well as informs targeted management strategies. Therefore, a combined approach involving host plant resistance, efficient vector control, and advanced molecular techniques is essential for sustainable management of the YMD in blackgram cultivation.

Moreover, the recognition of LCD has long existed in blackgram in the Indian subcontinent, including Pakistan; however, its etiology remains unclear. Although there are confirmations through different attributes of the malady that ULCV is the causal agent of the LCD, the exact identification of the virus/es involved in LCD following molecular analysis of infected plants still requires its establishment.

CONCLUSIONS

This study concludes seven genotypes are resistant to the YMD, 20 genotypes to the LCD, and four genotypes (32596, 35678, Accession-4, and Accession-7) to both diseases. These findings emphasize the genetic variation in YMD resistance among blackgram genotypes. Likewise, they suggest a strong potential for using resistant genotypes in breeding programs to develop varieties with resistance to multiple diseases or for direct recommendation of their general cultivation after testing in different agro-climatic regions.

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