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Exploring the physiological, biochemical, and enzymatic responses of *Vigna mungo* varieties to Mungbean Yellow Mosaic India Virus (MYMIV) infection

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This study aims to enhance sustainable disease management in black gram by identifying varieties resistant to Mungbean Yellow Mosaic India Virus (MYMIV). We screened sixteen black gram genotypes, assessing physiological, biochemical and enzymatic basis. Results revealed a range of resistance levels, with PANT URD-19 showing the highest resistance (PDI 0.47%) and Pejua the lowest (PDI 37.24%). Seven genotypes demonstrated strong resistance, highlighting the necessity for targeted breeding. Variations in leaf thickness, trichome density, stomatal frequency, and epicuticular wax content were significant, with resistant varieties like LBG-888 and PANT URD-19 exhibiting thicker leaves, higher trichome density, and more wax, correlating with reduced susceptibility. Chlorophyll content was higher in resistant varieties, while susceptible ones had reduced levels and increased sugar content, which may exacerbate MYMIV impact by attracting more whiteflies. Enzymatic analysis showed that resistant genotypes had elevated POD, SOD, and PAL activities, supporting their enhanced defense mechanisms. Multivariate analysis confirmed these findings, with leaf thickness, trichome density, and wax content negatively correlating with disease severity, while stomatal frequency and total sugar content were positively correlated. These results emphasize the potential of these traits in developing MYMIV-resistant black gram varieties and support the advancement of eco-friendly agricultural practices.

Vigna mungo (L.) Hepper var. mungo commonly known as blackgram is a vital leguminous crop, primarily grown for its protein-rich seeds. Originating in South Asia, it is an excellent source of digestible protein (25–26%), carbohydrates (60%), fats (1.5%), minerals, amino acids, and vitamins, making it a highly valued pulse crop[1]. Blackgram thrives in climates with temperatures between 27–30 °C, moderate rainfall, and loamy soils with high water-holding capacity. India is the largest producer and consumer, contributing around 54% of global production and accounting for 17% of the total area under pulse cultivation[2,3]. The crop is also widely grown in Bangladesh, Pakistan, Myanmar, Sri Lanka, Thailand, Nepal and Philippines[4]. Despite its nutritional significance, black gram production in India remains unstable, with annual grain yields ranging from 1.7 to 3.49 million tons over the last decade, while average productivity has stagnated at approximately 0.5 tons per hectare[5,6].

The crop is highly vulnerable to several viral diseases, with yellow mosaic disease (YMD) being the most severe[7]. YMD, caused by begomoviruses of the family Geminiviridae, poses a major threat to blackgram production. These viruses, collectively known as yellow mosaic viruses (YMVs), are transmitted by the whitefly (*Bemisia tabaci*) and are characterized by para-icosahedral geminate particles encapsulating a circular single-stranded DNA genome[8]. YMVs are classified into four types based on nucleotide sequence identity: Mungbean yellow mosaic virus (MYMV), Mungbean yellow mosaic India virus (MYMIV), Dolichos yellow mosaic virus

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(DoYMV), and Horsegram yellow mosaic virus (HgYMV)[9,10]. Of these, MYMIV is particularly problematic in South and Southeast Asia, causing significant yield losses that can range from 5% to complete crop failure, depending on infection severity[11]. MYMIV is notably prevalent in northern, central, and eastern India, posing a critical challenge for blackgram cultivation in these regions[12].

In this study, evaluating black gram varieties through physiological, biochemical, and enzymatic parameters is essential for understanding their resistance to MYMIV. As, physiological traits, such as plant height, leaf thickness, and trichome density, indicate overall plant health and structural defenses[13]. The biochemical parameters like, chlorophyll content, total sugar content, and phenolic compounds play crucial roles in plant health and disease resistance[14]. Functionally, chlorophyll content indicates photosynthetic efficiency and plant health, while elevated total sugar levels, often a stress response, can worsen disease by attracting vectors. Phenolic compounds are key in plant defense, enhancing resistance through antimicrobial activity and structural reinforcement. As, chlorophyll degradation, a hallmark of MYMIV infection, impairs photosynthesis and activates stress signaling pathways that trigger defense mechanisms[15]. Accumulation of phenolic compounds during infection acts as signaling molecules, reinforcing structural barriers and enhancing plant resistance[16]. Phenylalanine ammonia lyase (PAL) is vital in the phenylpropanoid pathway, facilitating the synthesis of phenolic compounds and other defense-related metabolites essential for the immune response[17]. Pathogens modify plant defense mechanisms by manipulating key signaling pathways, including salicylic acid, jasmonic acid, ethylene, MAPK, NPR1, and abscisic acid, to suppress immunity and disrupt reactive oxygen species (ROS) and calcium signaling[18]. ROS are crucial for plant defense, triggering localized cell death and immune responses; however, pathogens can manipulate or detoxify ROS to evade these defenses[19]. In this study the chlorophyll, sugar content, phenolic compounds, PAL, peroxidase (POD), and superoxide dismutase (SOD) involvement in plant defense signaling pathways is utilized for screening blackgram varieties resistant to MYMIV. Elevated POD activity helps manage oxidative stress and fortify cell walls through lignification, contributing to the plant's defense network[20]. SOD mitigates oxidative damage by converting superoxide radicals into less harmful substances, thus supporting overall defense signaling[21]. Collectively, these components interact within signaling pathways to orchestrate a robust defense response, mitigating the impact of MYMIV and enhancing plant resilience.

Materials and methods

Experimental site

This study comprised both field and laboratory experiments conducted at Bidhan Chandra Krishi Viswavidyalaya (BCKV), West Bengal, India. Field trials were carried out during the kharif seasons of 2022 and 2023 at the research fields of the College of Agriculture, Burdwan extended campus, BCKV, located at 23°14′ N latitude, 87°51′ E longitude, and 30 m above mean sea level. Laboratory analyses were subsequently performed in the Molecular Virology and Plant Genomics Laboratory at BCKV, Nadia, West Bengal, supported by funding from ICAR and DBT, Government of India.

Black gram genotypes and field trial setup

Sixteen genotypes, including LBG-888, TAU-1, TU-67, VBN-8, IPU-13-03, ADT-5, T-9, RU-02-13, VAMBAN-5, VBG-12-062, INDIRA URD-1, Pant Urd-19, AZAD URD-2, Pant Urd-22, and UH-1, along with the susceptible check variety PEJUA, were tested for resistance to Mungbean Yellow Mosaic India Virus (MYMIV) under field conditions. Seeds of these genotypes were sown in the second week of July during two consecutive years (2022 and 2023). The experiment was conducted in well-pulverized sandy loam soil using a Randomized Block Design (RBD) with three replications. The recommended plant spacing of 10 cm (plant-to-plant) and 30 cm (row-to-row) was maintained. To ensure uniform disease pressure, a row of the susceptible check was planted after every two rows of the test entries, with two additional rows of the susceptible check variety sown around the perimeter of the experimental plot. Good agricultural practices were followed to achieve a healthy crop stand; however, no plant protection measures were taken to ensure that the plants were exposed to natural infection.

Evaluation of MYMIV severity and associated physiological characteristics

Observation on sixteen black gram varieties for MYMIV infection at different stages (30 days after sowing and 60 days after sowing) of the crop were done at weekly interval throughout the growing season in both the years. MYMIV disease severity was scored by counting the total number of plants infected in each row. Percent Disease Index (PDI) was recorded from three randomly selected plants of each variety tagged in the plot. The cultivars were scored for MYMIV disease severity and grouped into five different categories from resistant to highly susceptible on a scale of 0 to 9 disease ratings at different days after planting.

Yellow mosaic disease severity was scored by counting the total number of plants infected in each row and per cent disease severity was calculated by using the following formula:

$$\text{Percent disease severity (PDI)} = (\text{Sum of all the numerical ratings}) / (\text{Number of observations} \times \text{Maximum disease rating}) \times 100$$

The severity of MYMIV on black gram will be assessed on a plot basis using a 0–9 disease scoring scale, as outlined by Gantait and Kantidas[22], based on the PDI.

Scale	PDI	Category	Reaction group
1	0.1–5	Resistant	R
3	5.1–15	Moderately resistant	MR
5	15.1–30	Moderately susceptible	MS
7	30.1–75	Susceptible	S
9	75.1–100	Highly susceptible	HS

Morphological parameters such as leaf thickness, trichome density, epicuticular wax content, stomatal frequency, and plant height were measured during peak activity periods, specifically at 30 and 60 days after sowing (DAS) in the second year of the study. Leaf thickness was measured using a digital micrometer. Trichome density was assessed by cutting thin leaf sections (approximately 1 cm^2) and examining them under a Zeiss Stereo Discovery V8 microscope to count the number of trichomes, with the average trichome density calculated per cm^2 of leaf area[23]. Stomatal frequency was determined following the method of Varadarajan and Wilson[24], and leaf epicuticular wax content was quantified using the procedure of Fernandes et al.[25]. Plant height was measured by calculating the average height from five randomly selected plants per cultivar using a simple arithmetic mean.

Quantifying the biochemical parameters like chlorophyll content, total sugar and phenol in MYMIV response by black gram

Chlorophyll content was determined using a modified assay protocol of Arnon[26]. The leaves were washed, dried, and chopped into small pieces. A 250 mg sample was ground in 80% acetone and centrifuged at 5000 rpm for 10 min. The supernatant was diluted to a final volume of 25 ml, and the absorbance was measured at 663 nm and 645 nm using a double-beam UV spectrophotometer.

$$\text{Total Chlorophyll } \left(\text{mg g}^{-1} \text{ tissue}\right) = [20.2\,(\text{D645}) + 8.02\,(\text{D663})] \times \text{V}/1000 \times \text{W}$$

where D = Optical density at respective wave length (nm); V = Final volume of chlorophyll extract in 80% acetone; W = Fresh weight of the tissue extracted.

Total sugar content was estimated colorimetrically using the anthrone reagent method[27]. A 100 mg sample of powdered leaf tissue was hydrolyzed in 5 ml of 25N HCl by boiling for 3 h. After cooling, the acid was neutralized with sodium carbonate. The volume was adjusted to 100 ml, and the solution was centrifuged at 10,000 rpm. A 0.1 ml aliquot was then used for the calculation of total sugar.

$$\text{Total sugars } \left(\text{mg g}^{-1}\right) = \frac{\text{Concentration from the graph (mg)}}{\text{Aliquot sample used}} \times \frac{\text{Total volume of extract}}{\text{Weight of the sample (g)}}$$

Total phenol content was estimated following the procedure of Folin and Ciocalteu[28]. One gram of plant material was ground, extracted with 80% ethanol, and centrifuged. The pooled extracts were evaporated, and the residue was reconstituted in water. Folin-Ciocalteu reagent and sodium carbonate were added, and the absorbance was measured at 660 nm. Total phenol concentration was determined using a standard curve and expressed as mg per gram of fresh tissue.

Quantifying the impact of MYMIV on oxidative stress and defense enzyme responses in black gram

Biochemical parameters were assessed using middle leaves of black gram varieties collected at 30 and 60 DAS. The leaves were washed thoroughly to remove surface contaminants, then dried in an oven and ground into a fine powder through a 300-mesh sieve. The powdered samples were analyzed for total phenols and total soluble sugars using standard methods. For enzyme activity estimation, freshly harvested leaf samples were used to measure PAL, SOD, and POD. The extraction buffer was prepared following the procedure described by Nayyar[29] with slight modifications, consisting of sodium phosphate buffer (pH 7.5), polyvinyl pyrrolidone (PVP), and Triton × detergent. Freshly washed and finely chopped leaf tissues were macerated with this buffer using a mortar and pestle. The mixture was centrifuged at 10,000 rpm for 30 min at 4 °C, and the supernatant was used as the enzyme source. The study included the evaluation of six biochemical parameters: chlorophyll content, total sugars, total phenols, PAL, SOD, and POD spectrophotometrically by using UV vis-spectrophotometer (UV-1900i), Shimadzu, Japan. These analyses were performed during the second year of the study.

Peroxidase activity was determined following the modified assay protocol of Lin and Kao[30]. Enzyme activity was measured spectrophotometrically by monitoring the formation of tetraguaiacol. The reaction mixture included the enzyme extract, 0.1 M sodium phosphate buffer (pH 7.5), 4% (v/v) guaiacol, and 1% (v/v) hydrogen peroxide. Guaiacol acted as a substrate, which, in the presence of hydrogen peroxide and peroxidase was oxidized to tetraguaiacol, resulting in a brown coloration. The reaction was initiated by the addition of hydrogen peroxide, and the increase in absorbance was recorded at 470 nm. Peroxidase activity was calculated based on the extinction coefficient of tetraguaiacol and expressed as micromoles (µmoles) of tetraguaiacol formed per second per gram of fresh leaf tissue.

SOD activity was determined by assessing its ability to inhibit the photoreduction of nitro blue tetrazolium (NBT) to formazan, following Beauchamp and Fridovich protocol with slight modification[31]. The reduction of NBT to formazan, which produces a blue color, was measured spectrophotometrically at 560 nm. The reaction

mixture was composed of 0.05 M phosphate buffer (pH 7.8), 20 mM methionine, 1.5 mM NBT, 1.5 mM EDTA, the enzyme extract, and 75 mM riboflavin. The mixture was then exposed to light from two 15 W fluorescent lamps to initiate the reaction. The SOD enzyme inhibits the formation of formazan by reducing the available superoxide radicals. The percent inhibition of NBT reduction was calculated using the formula:

$$\% \text{ inhibition} = [(A_0 - A_e)/A_0] \times 100,$$

where A_e represents the absorbance of the sample and A_0 represents the absorbance of the control.

Enzyme activity was expressed as units per mg of fresh leaf weight, with one unit of SOD defined as the amount of enzyme required to achieve 50% inhibition of NBT photoreduction.

The activity of PAL was determined by following Brueske's protocol with slight modification[32]. In this reaction, PAL catalyzes the conversion of L-phenylalanine into trans-cinnamic acid. To measure PAL activity, a reaction mixture was prepared containing 0.1 M Tris–HCl buffer at a pH of 8.8, 10 mM L-phenylalanine, and the enzyme extract obtained from fresh plant tissues. The reaction was initiated by adding the enzyme extract to the mixture. The reaction progress was monitored spectrophotometrically by measuring the absorbance at 270 nm, which corresponds to the production of trans-cinnamic acid. Absorbance readings were recorded over a period of 3 min, at intervals of 30 s. The enzymatic activity of PAL calculated and expressed as nanomoles of trans-cinnamate formed per hour per gram of fresh plant tissue.

Statistical analysis

Biochemical and morphological parameters, except plant height which was analyzed using randomized block design (RBD), were analyzed using a completely randomized design (CRD) with replication. Analysis of variance (ANOVA) was applied to identify significant variations across treatments, followed by Duncan's Multiple Range Test (DMRT) at a 5% probability level for mean comparisons. To explore the relationships between the morphological and biochemical characteristics of black gram and the severity of MYMIV infection, correlation analysis was carried out. In addition, principal component analysis (PCA) was performed on two sets of variables based on their correlation direction with MYMIV disease severity. This allowed for a clearer understanding of how these variables interact with one another and contribute to disease resistance. Visualization of the correlogram and PCA biplot matrices was done using the R statistical software (Version 3.6.1) and GRAPES version 1.0.0. All graphical representations, including trend analysis and comparisons, were generated using OriginPro 2024b version 9.0 for enhanced clarity and precision.

Result

Assessing physiological responses of black gram to MYMIV infection

Sixteen black gram genotypes were assessed for their MYMIV under field conditions, and the varieties were categorized into five reaction groups based on the PDI using a 0–9 disease scale. In 2022, the PDI values at 30 DAS showed considerable variation, ranging from 0.47% in Pant Urd-19 to 37.24% in Pejua (Table 1). By 60 DAS, seven genotypes-Azad Urd-2, Indira Urd-1, IPU-13-03, LBG-888, Pant Urd-19, Pant Urd-22, and RU-02-13-exhibited low disease severity, with PDI values between 2.11% and 4.68%, and were classified as resistant. Moderate resistance was observed in three genotypes, VBG-12-062, UH-1, and VBN-5-with PDI values ranging from 8.69–13.16%. Conversely, genotypes TAU-1, ADT-5, and T-9, with PDI values between 54.27% and 65.27%, were categorized as susceptible, while VBN-8 and TU-67 were moderately susceptible. Pejua consistently showed the highest PDI, recording 85.41% in 2022 and 87.85% in 2023 (Table 1). The trend in disease severity was similar in 2023, although some genotypes exhibited slightly higher PDI values compared to 2022 (Fig. 1 and Table 1S).

Leaf thickness varied significantly at both 30 and 60 DAS, as shown in Tables 2 and 3. The highest leaf thickness was observed in the least affected genotypes, including LBG-888, Indira Urd-1, Pant Urd-19, Azad Urd-2, and IPU-13-03. Moderately resistant genotypes, such as UH-1, VBG-12-062, and VBG-5, along with resistant varieties Pant Urd-22 and RU-02-13, and moderately susceptible VBN-8, exhibited relatively lower leaf thickness compared to the highly resistant varieties. Among the susceptible genotypes, leaf thickness ranged from 105.81 µm in T-9 to 137.13 µm in TAU-1 at 60 DAS. Pejua, the most severely infected variety, recorded the smallest leaf thickness at 117.25 µm (Fig. 2).

Trichome density showed significant variation across the black gram genotypes (Tables 2 and 3). The resistant genotypes, including LBG-888, Indira Urd-1, Pant Urd-19, Azad Urd-2, IPU-13-03, Pant Urd-22, and RU-02-13, exhibited high trichome densities, with counts exceeding 14 trichomes per 5 mm^2 at 60 DAS. In comparison, moderate trichome densities were found in the moderately resistant genotypes UH-1, VBG-12-062, and VBG-5, as well as in the susceptible genotype T-9. In contrast, the highly infected genotypes, such as TAU-1, ADT-5, and TU-67, showed significantly lower trichome densities. The lowest density, recorded at 5.21 trichomes per 5 mm^2, was observed in the highly susceptible variety Pejua (Fig. 2).

At 30 DAS, significant variations in stomatal frequency were observed among the black gram genotypes. The highest stomatal frequency was found in the susceptible genotype T-9, with 142 stomata per mm^2, followed closely by Pejua, a highly susceptible variety, with 136.23 stomata/mm^2. Moderately susceptible TU-67 exhibited a stomatal frequency of 132.41 stomata/mm^2, while moderately resistant VBG-12-062 recorded 127.67 stomata/mm^2. This pattern persisted at 60 DAS (Fig. 2). On the other hand, the lowest stomatal frequencies were noted in the least infected genotypes, with RU-02-13 registering 88.09 stomata/mm^2, Pant Urd-22 at 88.84 stomata/mm^2, and Pant Urd-19 at 93.51 stomata/mm^2.

Variety	Disease reaction	Percent disease index (PDI)					
		2022		2023		Pooled	
		30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS
ADT-5	S	24.6 (29.66)	58.36 (49.82)	26.24 (30.73)	58.29 (49.79)	25.42 (30.20)^{b}	58.33 (49.77)^{d}
Azad Urd-2	R	1.06 (5.68)	3.62 (10.84)	1.68 (7.44)	3.39 (10.43)	1.37 (6.69)^{g}	3.51 (10.72)^{f}
Indira Urd-1	R	1.05 (4.72)	2.11 (8.34)	1.09 (5.98)	2.89 (9.63)	1.07 (5.77)^{g}	2.50 (9.03)^{f}
IPU-13-03	R	0.95 (4.56)	2.95 (9.81)	1.96 (7.81)	3.36 (10.42)	1.45 (6.59)^{g}	3.16 (10.17)^{f}
LBG-888	R	2.06 (8.15)	4.05 (11.44)	2.21 (8.49)	4.17 (11.54)	2.13 (8.53)^{fg}	4.11 (11.50)^{f}
Pant Urd-19	R	0.47 (3.17)	2.51 (8.98)	0.59 (4.38)	2.57 (8.51)	0.53 (4.07)^{g}	2.39 (8.77)^{f}
Pant Urd-22	R	1.41 (6.80)	3.41 (10.47)	1.31 (6.53)	4.09 (11.48)	1.36 (6.69)^{g}	3.75 (11.15)^{f}
RU 02-13	R	2.18 (8.33)	4.68 (12.43)	2.33 (8.77)	4.32 (11.90)	2.25 (8.60)^{fg}	4.50 (12.19)^{f}
T-9	S	21.62 (27.62)	65.27 (53.95)	28.02 (31.85)	68.97 (56.16)	24.82 (29.80)^{b}	67.12 (55.00)^{b}
TAU-1	S	19.97 (26.51)	54.27 (47.45)	25.02 (89.93)	61.63 (51.73)	22.50 (28.26)^{b}	57.95 (49.56)^{c}
TU 67	MS	11.42 (19.71)	22.91 (28.42)	14.05 (21.97)	27.56 (31.58)	12.74 (20.87)^{c}	25.24 (30.09)^{d}
UH-1	MR	4.68 (12.48)	10.69 (18.92)	5.33 (13.33)	11.58 (19.81)	5.01 (12.87)^{ef}	11.14 (19.37)^{e}
VBG 12-062	MR	4.33 (11.83)	8.89 (17.12)	5.74 (13.71)	10.19 (18.57)	5.04 (12.91)^{ef}	9.54 (17.95)^{e}
VBN-5	MR	5.59 (13.63)	13.16 (21.21)	6.01 (14.18)	13.29 (21.22)	5.80 (13.92)^{e}	13.23 (21.26)^{e}
VBN-8	MS	9.25 (17.69)	19.6 (26.15)	13.41 (17.85)	23.91 (29.92)	9.33 (17.74)^{d}	22.26 (28.10)^{c}
PEJUA**	HS	37.24 (37.58)	85.41 (67.63)	42.82 (40.85)	87.85 (69.81)	40.03 (39.23)^{a}	86.63 (68.69)^{a}
SEm ±		1.34	1.77	1.10	1.63	0.97	1.51
C.D. ($p < 0.05$)		3.89	5.14	3.19	4.74	2.82	3.33

Table 1. Response of different black gram, *Vigna mungo,* varieties under field conditions against MYMIV infestation. Means with different letters within a column are significantly ($p \leq 0.05$) different. *Values in parentheses are angular transformed values (mean of three replication). **Indicates highly susceptible check.

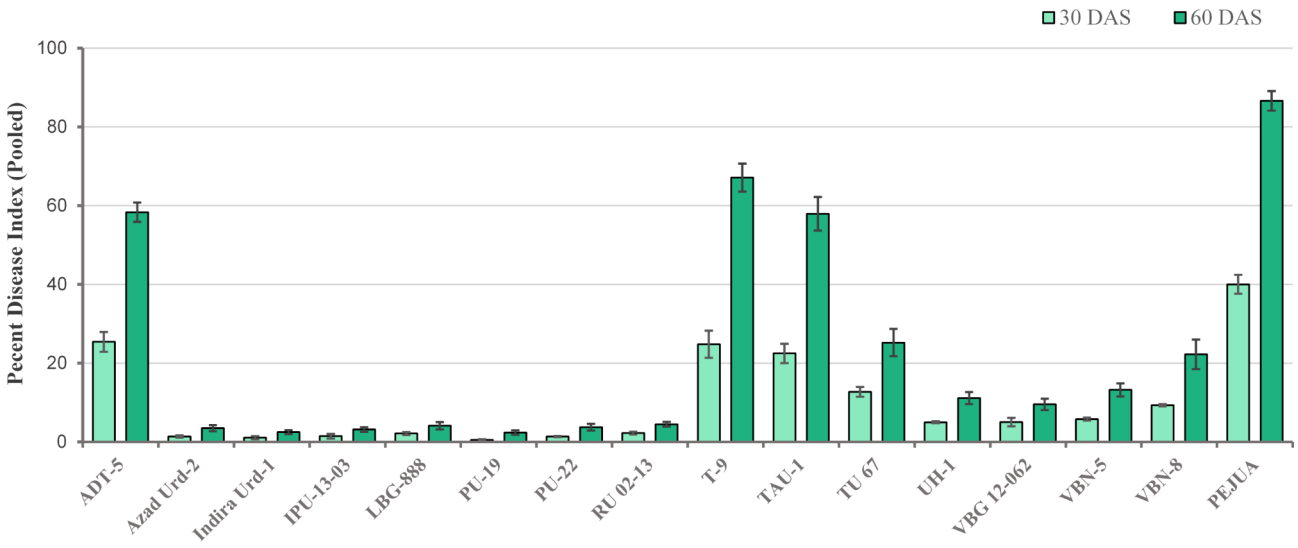

Fig. 1. MYMIV disease severity of black gram varieties (pooled over two years) at different time intervals (30 DAS and 60 DAS) in kharif -2022 and 2023. Bars represent the standard error of the mean (SEM).

Epicuticular wax content showed significant variation across the black gram varieties (Tables 2 and 3). At 30 DAS, the varieties exhibiting the lowest levels of infection, such as Pant Urd-19 (0.44 mg/dm^2), UH-1 (0.44 mg/dm^2), LBG-888 (0.42 mg/dm^2), and Azad Urd-2 (0.41 mg/dm^2), were characterized by higher epicuticular wax content. This trend persisted at 60 DAS, with the highest wax levels recorded in resistant varieties, including LBG-888 (0.87 mg/dm^2), IPU-13-03 (0.84 mg/dm^2), Azad Urd-2 (0.83 mg/dm^2), and Pant Urd-19 (0.81 mg/dm^2). Conversely, highly infected genotypes such as T-9, TU-67, ADT-5, and Pejua displayed significantly lower wax content, with values below 0.52 mg/dm^2 at 60 DAS (Fig. 2). The lower epicuticular wax content in susceptible genotypes is likely associated with increased susceptibility to MYMIV infection, potentially by facilitating insect contact with the host plant, thereby enhancing virus transmission.

Plant height was assessed at 30 and 60 DAS. At 30 DAS, most varieties exceeded 20 cm, with the exception of Pejua, RU-02-13, and VBN-8. By 60 DAS, the majority of the genotypes had surpassed 40 cm in height (Fig. 2).

Sl. no	Variety	Leaf thickness (µm) (30 DAS)	Stomatal frequency (mm^2) (30 DAS)	Trichomes density (per 5 mm^2) (30 DAS)	Epicuticular wax content (mg/dm2) (30 DAS)	Plant height (cm) (30 DAS)
1	ADT-5	$116.25^{efg} \pm 7.17$	$119.88^{cd} \pm 5.06$	$8.87^{de} \pm 0.48$	$0.37^{abc} \pm 0.02$	$19.58^{cd} \pm 0.56$
2	Azad Urd-2	$141.24^{abcd} \pm 4.71$	$98.54^{efg} \pm 2.70$	$13.54^{ab} \pm 0.63$	$0.41^{ab} \pm 0.01$	$23.18^{abc} \pm 0.97$
3	Indira Urd-1	$151.87^{ab} \pm 4.35$	$95.36^{efgh} \pm 2.54$	$12.87^{abc} \pm 0.46$	$0.31^{cdef} \pm 0.05$	$22.68^{abc} \pm 1.25$
4	IPU-13-03	$143.22^{abc} \pm 5.03$	$101.49^{ef} \pm 7.22$	$13.71^{ab} \pm 0.53$	$0.39^{abc} \pm 0.01$	$20.57^{bcd} \pm 1.29$
5	LBG-888	$155.39^{a} \pm 4.53$	$102.21^{e} \pm 4.25$	13.21 ± 0.80	$0.42^{ab} \pm 0.01$	$20.11^{cd} \pm 1.26$
6	Pant Urd-19	$139.27^{abcd} \pm 4.89$	$84.25^{gh} \pm 3.79$	$14.47^{a} \pm 0.30$	$0.44^{a} \pm 0.03$	$25.01^{a} \pm 0.64$
7	Pant Urd-22	$128.25^{cdef} \pm 4.24$	$80.04^{h} \pm 3.44$	$11.28^{bcd} \pm 1.26$	$0.34^{bcde} \pm 0.03$	$24.53^{ab} \pm 0.84$
8	RU 02-13	$134.34^{bcde} \pm 5.47$	$84.71^{fgh} \pm 2.79$	$12.17^{abc} \pm 0.38$	$0.43^{ab} \pm 0.01$	$19.64^{cd} \pm 1.08$
9	T-9	$93.64^{h} \pm 5.43$	$142.21^{a} \pm 4.32$	$10.67^{cd} \pm 0.98$	$0.27^{def} \pm 0.02$	$20.69^{bcd} \pm 1.36$
10	TAU-1	$121.36^{defg} \pm 5.66$	$106.25^{de} \pm 3.57$	$7.64^{e} \pm 0.70$	$0.42^{ab} \pm 0.03$	$24.37^{ab} \pm 0.51$
11	TU 67	$108.47^{fgh} \pm 5.87$	$132.41^{abc} \pm 3.30$	$7.58^{e} \pm 0.71$	$0.25^{ef} \pm 0.02$	$23.38^{abc} \pm 1.32$
12	UH-1	$138.28^{abcd} \pm 3.71$	$95.28^{efgh} \pm 5.23$	$8.75^{de} \pm 0.51$	$0.44^{a} \pm 0.04$	$24.69^{ab} \pm 1.36$
13	VBG 12-062	$132.54^{bcde} \pm 3.80$	$127.67^{abc} \pm 5.72$	$9.28^{de} \pm 0.56$	$0.37^{abc} \pm 0.01$	$23.48^{abc} \pm 0.44$
14	VBN-5	$127.64^{cdef} \pm 5.39$	$121.74^{bcd} \pm 3.46$	$11.32^{bcd} \pm 0.97$	$0.35^{abcd} \pm 0.01$	$23.75^{abc} \pm 0.49$
15	VBN-8	$121.51^{defg} \pm 2.52$	$102.69^{e} \pm 3.55$	$12.87^{abc} \pm 0.57$	$0.38^{abc} \pm 0.03$	$20.61^{bcd} \pm 1.18$
16	PEJUA*	$101.69^{gh} \pm 5.24$	$136.23^{ab} \pm 4.50$	$6.94^{e} \pm 0.20$	$0.24^{f} \pm 0.02$	$18.09^{d} \pm 1.06$
SEm ±	6.13	5.30	0.83	0.03	1.23	
C.D	17.67	14.95	2.43	0.08	3.58	
C.V %	8.21	8.32	13.21	13.74	9.87	

Table 2. Morphological characteristics of black gram varieties during 30 DAS. Means with different letters within a column are significantly ($p \leq 0.05$) different. Table representing mean ± standard error. *Indicates highly susceptible check. DAS- Days after sowing.

Sl. no	Variety	Leaf thickness (µm) (60 DAS)	Stomatal frequency (mm^2) (60 DAS)	Trichomes density (per 5 mm^2) (60 DAS)	Epicuticular wax content (mg/dm^2) (60 DAS)	Plant height (cm) (60 DAS)
1	ADT-5	$130.2^{def} \pm 5.81$	$124.67^{bc} \pm 3.75$	$8.66^{e} \pm 0.55$	$0.49^{d} \pm 0.04$	$37.94^{cd} \pm 1.67$
2	Azad Urd-2	$156.77^{abc} \pm 5.94$	$111.35^{cd} \pm 3.84$	$17.51^{ab} \pm 1.09$	$0.83^{a} \pm 0.06$	$44.63^{abc} \pm 1.26$
3	Indira Urd-1	$168.57^{ab} \pm 5.80$	$105.84^{cde} \pm 3.63$	$16.70^{abc} \pm 0.96$	$0.81^{ab} \pm 0.02$	$45.81^{abc} \pm 0.96$
4	IPU-13-03	$158.97^{abc} \pm 5.05$	$112.65^{cd} \pm 2.68$	$17.01^{abc} \pm 1.24$	$0.84^{a} \pm 0.06$	$39.41^{bcd} \pm 1.36$
5	LBG-888	$172.48^{a} \pm 7.36$	$115.49^{c} \pm 2.68$	$16.89^{abc} \pm 0.78$	$0.87^{a} \pm 0.06$	$40.4^{abcd} \pm 2.74$
6	Pant Urd-19	$154.58^{abcd} \pm 7.65$	$93.51^{de} \pm 4.32$	$18.65^{a} \pm 0.97$	$0.81^{ab} \pm 0.04$	$47.54^{a} \pm 1.06$
7	Pant Urd-22	$138.92^{cdef} \pm 3.52$	$88.84^{e} \pm 6.72$	$14.56^{bcd} \pm 1.18$	$0.69^{abc} \pm 0.05$	$45.45^{abc} \pm 2.24$
8	RU 02-13	$141.80^{cde} \pm 5.17$	$88.09^{e} \pm 1.77$	$15.34^{abc} \pm 0.98$	$0.58^{cd} \pm 0.04$	$38.44^{cd} \pm 1.66$
9	T-9	$105.81^{g} \pm 6.86$	$148.04^{a} \pm 3.88$	$10.01^{e} \pm 1.24$	$0.51^{d} \pm 0.08$	$41.19^{abcd} \pm 2.58$
10	TAU-1	$137.13^{cdef} \pm 5.21$	$111.56^{cd} \pm 8.98$	$9.21^{e} \pm 0.63$	$0.75^{abc} \pm 0.03$	$45.83^{abc} \pm 2.17$
11	TU-67	$121.48^{efg} \pm 4.77$	$141.67^{ab} \pm 4.72$	$9.24^{e} \pm 0.31$	$0.47^{de} \pm 0.02$	$40.54^{abcd} \pm 0.47$
12	UH-1	$154.87^{abc} \pm 6.92$	$105.76^{cde} \pm 8.84$	$8.89^{e} \pm 0.48$	$0.63^{bcd} \pm 0.02$	$46.61^{ab} \pm 1.92$
13	VBG 12-062	$153.44^{abcd} \pm 4.76$	$141.71^{ab} \pm 6.08$	$11.69^{de} \pm 0.75$	$0.81^{ab} \pm 0.03$	$44.69^{abc} \pm 2.94$
14	VBN-5	$142.95^{cde} \pm 7.97$	$135.13^{ab} \pm 3.50$	$13.53^{cd} \pm 1.29$	$0.74^{abc} \pm 0.06$	$45.61^{abc} \pm 1.98$
15	VBN-8	$144.87^{bcde} \pm 4.64$	$110.90^{cd} \pm 2.89$	$11.70^{de} \pm 0.73$	$0.75^{abc} \pm 0.04$	$40.16^{abcd} \pm 1.76$
16	PEJUA*	$117.25^{fg} \pm 6.95$	$142.72^{ab} \pm 4.83$	$5.21^{f} \pm 0.70$	$0.31^{e} \pm 0.03$	$35.69^{d} \pm 1.85$
SEm ±	7.38	6.04	0.122	0.05	2.27	
C.D. ($p < 0.05$)	20.91	17.37	0.354	0.16	6.54	

Table 3. Morphological characteristics of black gram varieties during 60 DAS. Means with different letters within a column are significantly ($p \leq 0.05$) different. Table representing mean ± standard error. *Indicates highly susceptible check. DAS- Days after sowing.

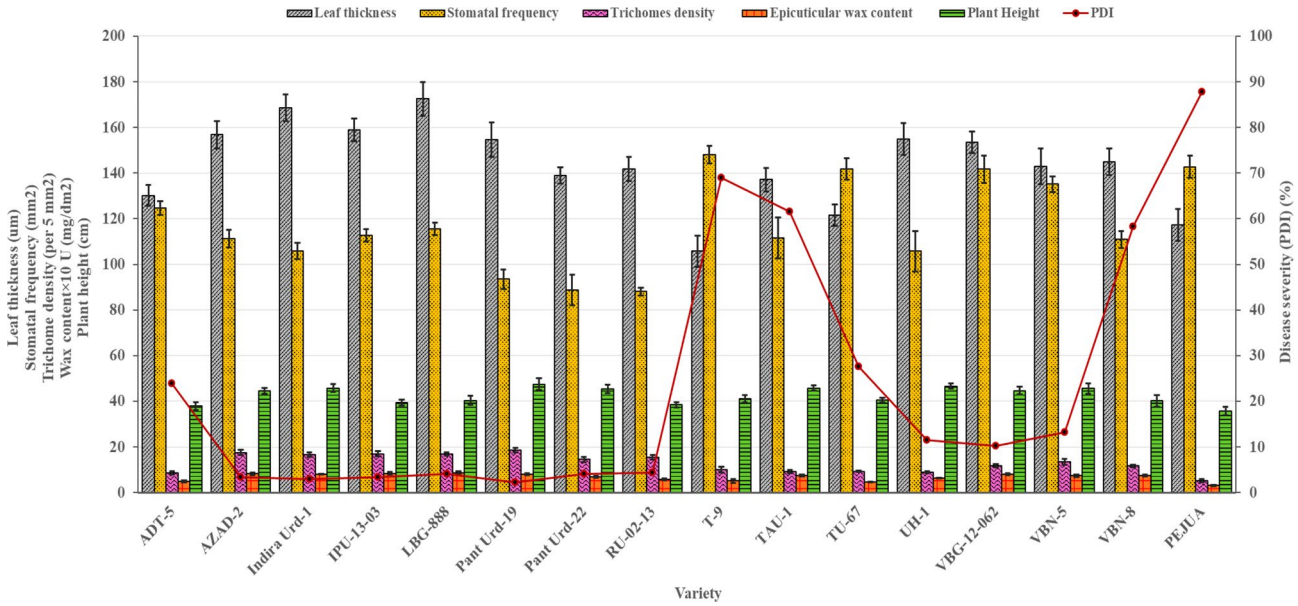

Fig. 2. Morphological characteristics of black gram varieties during peak activity of MYMIV severity (60 DAS). Bars represent the standard error of the mean (SEM).

The highest plant height was recorded in the MYMIV-resistant genotype PANT URD-19, reaching 47.54 cm, while the lowest height was observed in the susceptible variety Pejua, measuring 35.69 cm (Tables 2 and 3). However, no significant differences in plant height were detected across the genotypes.

Changes in key biochemical parameters under MYMIV infection

Leaf chlorophyll content is a key indicator of stress physiology, impacting growth and yield. Infection with MYMIV led to significant changes in leaf pigmentation, evident in the drastic reduction of chlorophyll observed in infected black gram cultivars. The total chlorophyll content varied significantly among genotypes at both 30 DAS and 60 DAS (Tables 2S and 3). The cultivars Indira Urd-1, Azad Urd-2, Pant Urd-19, Pant Urd-22, RU 02–13, IPU 13-03, and LBG-888 exhibited the lowest levels of MYMIV infection and maintained the highest total chlorophyll content on both observation days. Moderately infected varieties, such as UH-1, VBG 12–062, VBN-5, TU-67, and VBN-8, showed intermediate levels of chlorophyll. In contrast, susceptible varieties like TAU-1, ADT-5, T-9, and PEJUA experienced a marked decline in total chlorophyll content, with PEJUA recording the lowest value of 0.45 mg/g at 60 DAS. Total chlorophyll content ranged from 0.74 mg/g to 1.74 mg/g at 30 DAS and from 0.45 mg/g to 1.76 mg/g at 60 DAS among the different cultivars (Fig. 3A). These results underscore the correlation between MYMIV infection severity and chlorophyll levels, with resistant cultivars exhibiting higher chlorophyll retention compared to susceptible ones.

The impact of plant pathogenic viruses on the sugar metabolism of infected hosts is crucial for understanding the economic damage to the host. MYMIV infection in black gram varieties led to increased total sugar content, which, in turn, intensified the whitefly populations and exacerbated disease severity in susceptible varieties. Significant variations in sugar content were observed across different genotypes (Tables 2S and 3S). In MYMIV-resistant genotypes, total sugar content ranged from 28.39 mg/g (LBG-888) to 34.31 mg/g (Pant Urd-22) on a fresh weight basis. Conversely, in susceptible genotypes, sugar content ranged from 41.22 mg/g (T-9) to 41.41 mg/g (ADT-5), with the highest sugar content recorded in the highly susceptible check, PEJUA, at 41.79 mg/g FW. At 60 DAS, total sugar content decreased in most cultivars, except for some susceptible ones. The trend at 60 DAS showed that total sugar content remained highest in susceptible genotypes, ranging from 37.04 mg/g FW (ADT-5) to 46.31 mg/g FW (T-9), (Fig. 3B). The most heavily infected variety, PEJUA, recorded the highest sugar content at 46.86 mg/g FW.

Following pathogen infection, host phenolic compounds may increase, contributing to enhanced mechanical strength of host cell walls through the production of lignin and suberin, which form physical barriers that impede pathogen spread. In this study, significant variation in leaf total phenolic content was observed among the black gram varieties at 30 days after sowing (DAS) and 60 DAS during the kharif season of 2023 (Tables 2S and 3S). At 30 DAS, the lowest phenolic content was found in susceptible genotypes, ranging from 1.87 mg GAE/g (TAU-1) to 2.09 mg GAE/g (VBN-8), with the susceptible check PEJUA recording the lowest value of 1.56 mg GAE/g. By 60 DAS, total phenolic content increased significantly across all varieties (Fig. 3C). The highest phenolic content was observed in the MYMIV-resistant varieties Azad Urd-2, Indira Urd-1, IPU-13-03, LBG-888, Pant Urd-19, Pant Urd-22, and RU-02-13, which exhibited the least infection. Conversely, moderately resistant to moderately susceptible varieties displayed moderate levels of phenolic content, ranging from 3.58 mg GAE/g to 4.71 mg GAE/g at 60 DAS. The lowest phenolic content at this stage was observed in TAU-1, with 2.36 mg GAE/g.

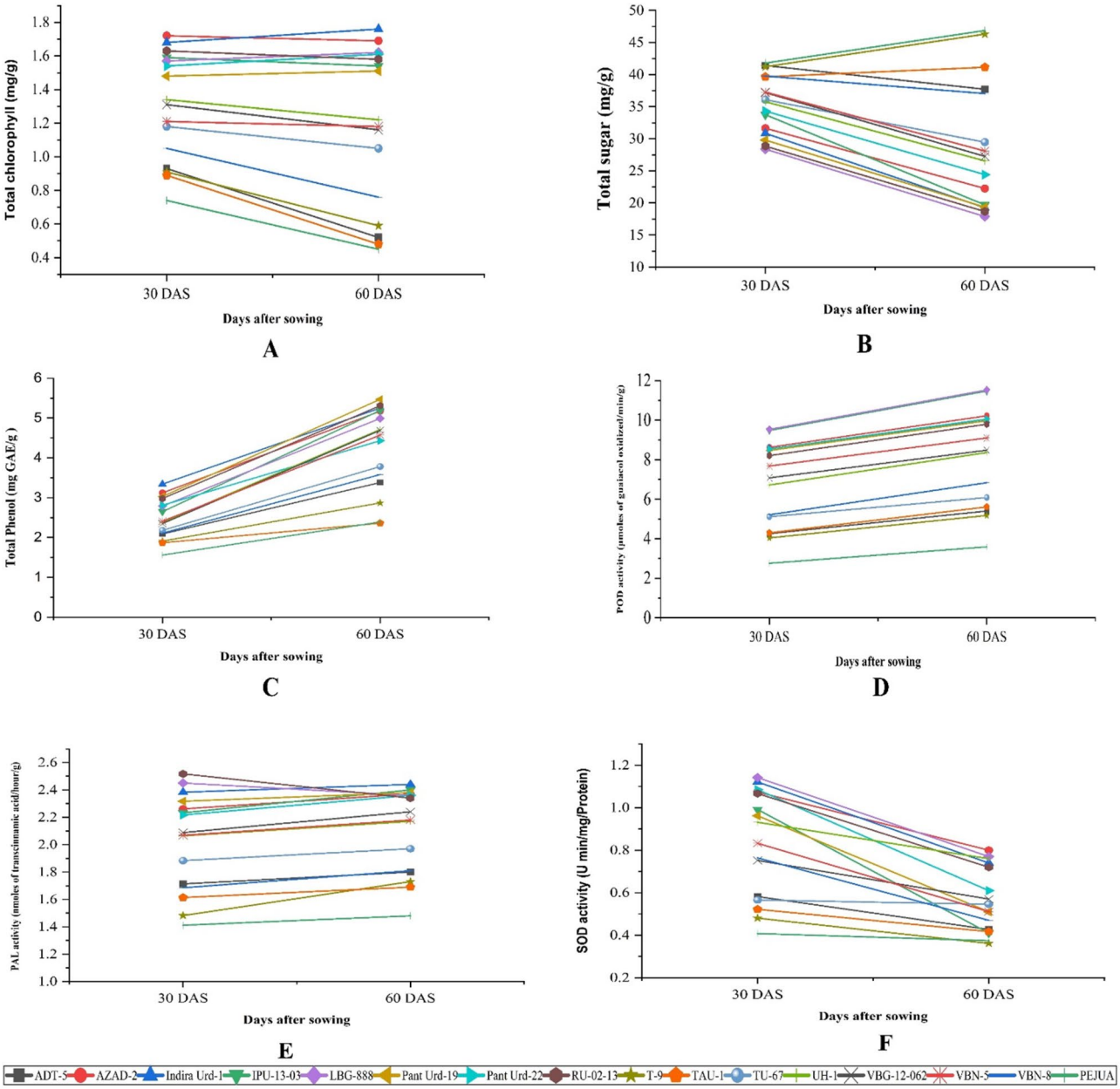

Fig. 3. Total chlorophyll (**A**), total sugar (**B**), total phenol (TSS) (**C**), peroxidase (POD) (**D**), PAL (**E**), and superoxide dismutase (SOD) (**F**) activity in the leaves of different black gram varieties at 30 DAS and 60 DAS.

Oxidative stress and defense enzyme responses in black gram under MYMIV infection

The peroxidase (POD) activity in different black gram varieties showed a notable increase in response to MYMIV infection. Varieties that were more resistant to MYMIV exhibited higher enzyme activity across all growth stages. Significant differences in peroxidase activity were observed among the varieties, as detailed in Tables 2S and 3S. At 30 DAS, the highest POD activities were recorded in LBG-888 and IPU-13-03, with values of 9.52 and 9.47 units, respectively, followed by other resistant varieties with activity ranging from 8.20 to 8.54 units. By 60 DAS, the highest POD activity was again observed in LBG-888 (11.52 units), closely followed by IPU-13-03 (11.47 units). In contrast, the lowest POD activities were found in the susceptible variety PEJUA, with values of 2.75 and 3.84 units at the respective growth stages (Fig. 3D). High levels of superoxide dismutase (SOD) activity were consistently detected in Indira Urd-1, LBG-888, PANT URD-22, RU 02–13, PANT URD-19 and Azad-2 at all stages of insect infestation (30 and 60 DAS) (Fig. 3F). Meanwhile a decreasing trend of SOD activity were noticed at 60 DAS (Fig. 3F). Indira Urd-1 had SOD activity of 0.88 and 0.85 units, while LBG-888 had 0.79 and 0.78 units at 40, 60 DAS, respectively. Varieties having heavily infected with the virus, indicative of their ability to withstand oxidative stress generated by MYMIV infection. ADT-5, T-9, TAU-1 and PEJUA have significantly decreased SOD concentrations at various growth stages. (Tables 2S and 3S). Least activity of SOD was recorded in T-9 (0.36 unit) at 60 DAS.

Morphological characters	r	P	R^2	r	P	R^2
	30 DAS			60 DAS		
Relationship with MYMIV disease severity						
Leaf thickness	-0.82	0.000	0.67	-0.80	0.000	0.63
Trichome density	-0.71	0.000	0.50	-0.82	0.000	0.67
Stomatal frequency	0.71	0.002	0.50	0.59	0.017	0.34
Epicuticular wax content	-0.56	0.024	0.31	-0.74	0.001	0.54
Plant height	-0.48	NS	0.24	-0.47	NS	0.23

Table 4. Relation of MYMIV disease severity, with the morphological parameters of different black gram varieties at different time (30DAS and 60DAS) interval. *DAS- Days after sowing.

Biochemical properties	r	P	R^2	r	P	R^2
	30 DAS			60 DAS		
Relationship with MYMIV disease severity						
POD	-0.94	0.000	0.88	-0.92	0.000	0.85
Phenol	-0.88	0.000	0.77	-0.94	0.000	0.88
PAL	-0.92	0.000	0.85	-0.95	0.000	0.90
Sugar	0.83	0.000	0.68	0.94	0.000	0.88
SOD	-0.91	0.000	0.82	-0.68	0.004	0.47
Chlorophyll	-0.92	0.000	0.84	-0.92	0.000	0.84

Table 5. Relation of MYMIV disease severity, with the biochemical parameters of different black gram varieties at different time (30DAS and 60DAS) interval. *DAS- Days after sowing.

PAL activity was highest in Indira Urd-1, with a measurement of 2.32 units at 30 DAS, and in IPU-13-03, with 2.40 units at 60 DAS (Tables 2S and 3S). Conversely, the lowest PAL activities were observed in the susceptible variety PEJUA, followed by TAU-1, T-9, and ADT-5, across the different growth stages (Fig. 3E). Thus, PAL activity has been shown to increase in resistant cultivars upon virus infection.

Integrative multivariate analysis of physiological, biochemical, and enzymatic trait metrics

The correlations between MYMIV disease severity, morphological attributes, and various parameters were analyzed (Tables 4 and 5). The data revealed significant negative correlations between leaf thickness, trichome density, and wax content with disease severity at different time intervals (Fig. 4A and B). Conversely, a significant positive correlation was found between stomatal frequency and disease severity. No significant correlation was observed between plant height and disease severity at both 30 DAS and 60 DAS. This indicates that increased leaf thickness, higher trichome density, and greater wax content are associated with enhanced resistance to MYMIV. Additionally, biochemical parameters such as total phenols, total chlorophyll content, PAL, POD, and SOD showed negative correlations with disease severity, suggesting these factors contribute to resistance against the virus. In contrast, total sugar content exhibited a significant positive correlation with MYMIV severity at both 30 DAS and 60 DAS (Fig. 4C and D), indicating that higher sugar levels may act as feeding stimulants for whiteflies, making the varieties more susceptible to MYMIV.

A regression model was developed with MYMIV disease severity as the dependent variable and various morphological and biochemical parameters as independent variables for different time intervals (30 DAS & 60 DAS). As shown in Tables 6 and 7, the coefficient of determination (R^2) for morphological parameters was comparatively higher at 30 DAS, with a value of 0.84, whereas the R^2 for biochemical parameters was lower at 0.91 for MYMIV severity. This trend reversed at 60 DAS, indicating that the explanatory power of the parameters differed between the two time points.

Principal Component Analysis (PCA) of twenty-five variables negatively correlated with disease severity, with eigenvalues greater than 1, accounted for 82.14% of the significant variation. The first three principal components (PCs) explained 95.58% of the cumulative variation, with the first two components alone contributing 90.31%. The biplot of the first two components (Fig. 5A) revealed that PEJUA, followed by ADT-5 and T-9, exhibited the highest variation, while VBG-12-062 and UH-1 showed the least variation. For the remaining two variables positively correlated with disease severity (Fig. 5B), PCA generated a single PC with an eigenvalue greater than 1, explaining 81.10% of the significant variation. The biplot of the first two PCs indicated that PEJUA, followed by T-9, TAU-1, Indira Urd-1, LBG-888, PANT URD-19, UH-1, VBN-5, VBG-12-062, TU-67, Azad Urd-2, and RU-02-13, experienced the highest variation, while ADT-5 exhibited the least variation.

Discussion

In pursuit of sustainable disease management in black gram, identifying resistant varieties is essential. This study systematically screens sixteen black gram varieties, assessing key physiological, biochemical and enzymatic

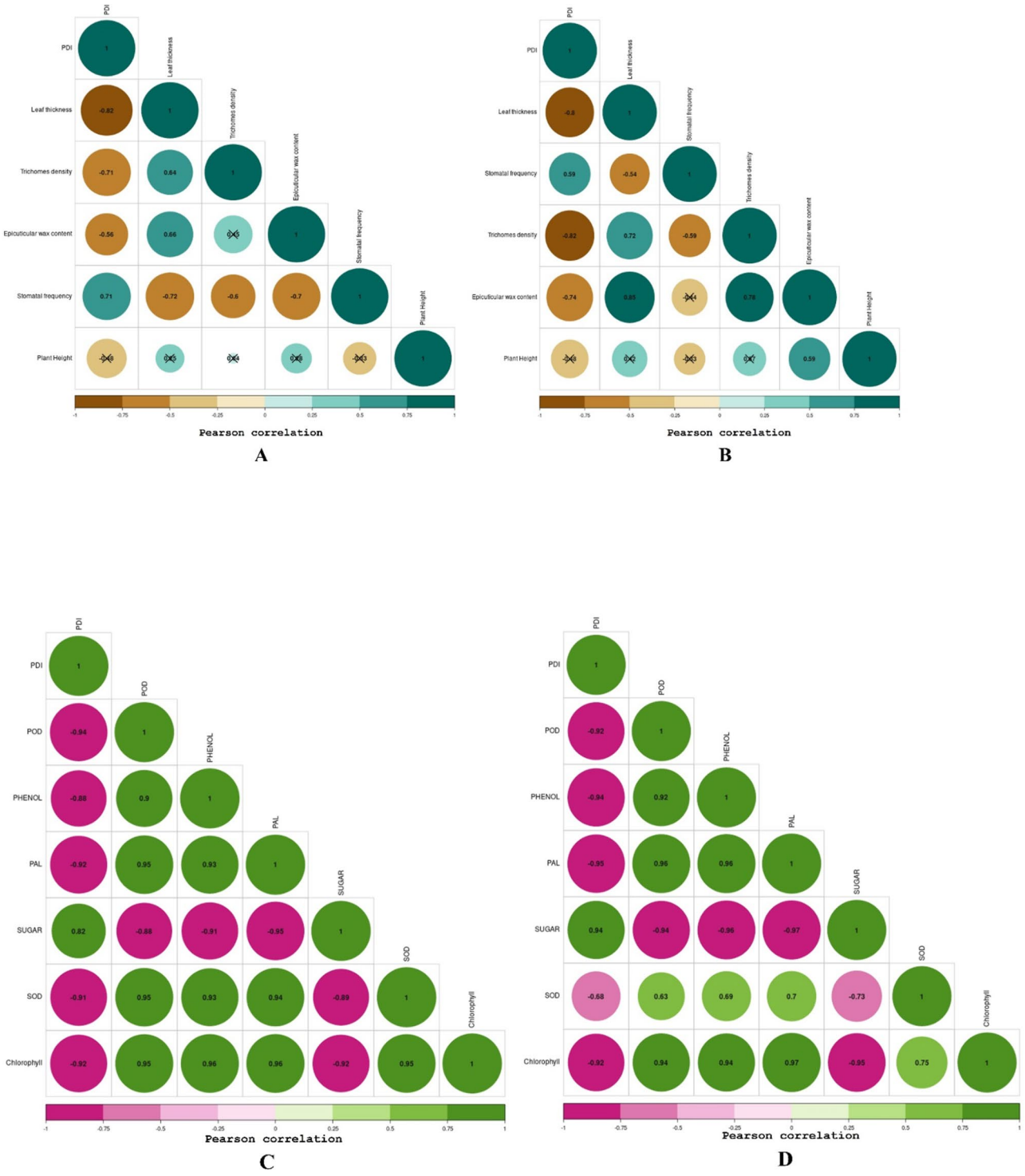

Fig. 4. Correlation of disease severity with morphological (A and B) and biochemical parameters (C and D) of black gram at 30 DAS and 60 DAS (days after sowing). (**A**) Correlation with morphological parameters at 30 DAS, (**B**) Correlation with morphological parameters at 60 DAS, (**C**) Correlation with biochemical parameters at 30 DAS and (**D**) Correlation with morphological parameters at 60 DAS ('X' represents non-significant).

parameters such as disease severity, chlorophyll degradation, phenolic compound levels, and the activities of enzymes like PAL, POD, and SOD. By elucidating these mechanisms, we aim to identify varieties with strong resistance to MYMIV, thereby supporting the advancement of eco-friendly agricultural practices. In the current assessment, sixteen black gram genotypes reveals a broad spectrum of resistance to MYMIV. In 2022, genotypes ranged from highly resistant, like PANT URD-19 with a PDI of 0.47%, to highly susceptible, such as Pejua with a PDI of 37.24%. Seven genotypes demonstrated strong resistance, underscoring the critical need to select and

Regression equation	R^2	Activity periods
$Y = 114.475 - 0.372\ a1 + 0.044\ a2 - 1.938\ a3 + 15.451\ a4 - 2.018\ a5$	0.84	(30 DAS)
$Y = 224.328 - 0.821\ b1 + 0.026\ b2 - 4.123\ b3 + 50.680\ b4 - 1.599\ b5$	0.79	(60 DAS)

Table 6. Regression models and coefficient of determination between MYMIV disease severity and morphological parameters at different growth stage of black gram (30 DAS and 60 DAS). *DAS-Days after sowing.a1: Leaf thickness at 30 DAS b1: Leaf thickness at 60 DAS a2: Stomatal frequency at 30 DAS b2: Stomatal frequency at 60 a3: Trichome density at 30 DAS b3: Trichome density at 60 DAS a4: Epicuticular wax at 30 DAS b4: Epicuticular wax at 60 DAS a5: Plant height at 30 DAS b5: Plant height at 60 DAS.

Regression equation	R^2	Activity periods
$Y = 141.447 - 2.421\ c1 - 2.022\ c2 - 25.651\ c3 - 1.303\ c4 + 1.113\ c5 - 9.004\ c6$	0.91	(30 DAS)
$Y = 133.282 - 2.967\ d1 - 9.080\ d2 - 30.001\ d3 + 0.439\ d4 - 10.291\ d5 + 8.848\ d6$	0.92	(60 DAS)

Table 7. Regression models and coefficient of determination between MYMIV disease severity and biochemical parameters at different growth stage of black gram (30 DAS and 60 DAS). *DAS-Days after sowing. c1: POD at 30 DAS d1: POD at 60 DAS. c2: Phenol at 30 DAS d2: Phenol at 60 DAS. c3: PAL at 30 DAS d3: PAL at 60 DAS. c4: Sugar at 30 DAS d4: Sugar at 60 DAS. c5: SOD at 30 DAS d5: SOD at 60 DAS. c6: Chlorophyll at30DAS d6:Chlorophyll at 60DAS.

utilize resistant varieties for effective disease management. This variation highlights the importance of continued breeding efforts to enhance resistance traits in black gram, crucial for mitigating the impact of MYMIV and improving overall crop resilience[33]. Further Leaf thickness varied significantly among black gram genotypes, with thicker leaves observed in less infected varieties such as LBG-888 and PANT URD-19. Susceptible genotypes, including T-9 and TAU-1, had thinner leaves, indicating a possible link between increased leaf thickness and enhanced resistance to MYMIV. As, thicker leaves may provide better physical barriers against viral entry and damage, enhance overall defense mechanisms, and support higher levels of defensive compounds. The reduction in leaf thickness observed in infected genotypes may result from the secretion of hydrolytic enzymes by MYMIV, which can lead to cell wall degradation and the formation of large intercellular spaces. This enzymatic activity undermines leaf structure, making it more susceptible to viral damage. Our findings are consistent with Pilic et al.[11] and Tamilzharasi et al.[34] study, where similar changes in leaf thickness due to Cucumber Mosaic Virus (CMV) and Chilli Leaf Curl Virus infections, respectively. These studies reinforce the importance of leaf thickness as a key factor in plant resistance to viral infections.

Trichome density varied significantly, with high density in resistant varieties such as LBG-888 and PANT URD-19, and low density in susceptible varieties like Pejua. Leaf trichome density plays a protective role by hindering whitefly establishment, feeding, and oviposition[35]. This result is analogous to Arora et al.[36], who observed higher trichome density in tomato genotypes resistant to Tomato leaf curl virus. Similarly, Khan et al.[37] found that ash gourd varieties with lower trichome density were more severely affected by aphids and viruses compared to those with higher trichome density. Stomatal frequency is crucial as it influences gas exchange and pathogen entry. Higher stomatal density can facilitate increased virus infection and entry, while lower stomatal frequency in resistant genotypes may limit pathogen access, thereby enhancing plant defense and reducing susceptibility to diseases like MYMIV. In this study higher stomatal frequency was observed in susceptible genotypes like T-9 and PEJUA, indicating a potential link between higher stomatal numbers and increased susceptibility to MYMIV. Conversely, lower stomatal frequency in resistant genotypes suggests enhanced resistance. As, stomatal frequency is crucial as it influences pathogen entry. Higher stomatal density can facilitate increased virus infection and entry, while lower stomatal frequency in resistant genotypes may limit pathogen access, thereby enhancing plant defense and reducing susceptibility to diseases like MYMIV. The results were similar with the findings of Gagandeep et al.[38], where significantly less number of stomata present in CMV resistant watermelon genotypes and vice versa. Then Devi et al.[39] also observed the similar type of trend due to MYMV infection in black gram plant. Epicuticular wax content varied significantly among black gram varieties, with higher wax levels in resistant genotypes like LBG-888 and Azad Urd-2 at 60 DAS. In contrast, highly infected varieties such as T-9 and Pejua exhibited lower wax content, indicating a potential link between wax levels and disease resistance. The susceptibility of certain black gram genotypes to MYMIV infection may be attributed to insufficient epicuticular wax, which facilitates insect contact and subsequent virus transmission. The cuticular wax layer serves as a physical barrier, restricting the entry of plant pathogenic viruses and potentially acting as a reservoir for signals that trigger plant defense responses[24]. These findings align with research by Zafar et al.[40] and Majid et al.[9], who reported that a thicker cuticle can effectively act as a barrier against cotton leaf curl virus (CLCuV) infection. The evaluation showed that most black gram varieties exceeded 20 cm in height by 30 DAS and 40 cm by 60 DAS. PANT URD-19, a MYMIV-resistant variety, had the greatest height, while Pejua had the least, with no significant differences among varieties. Thus, physiological parameters are critical for evaluating plant health and resistance. Disease severity measures the extent of infection, while plant height and leaf thickness provide insights into overall growth and structural defenses. Trichome density and stomatal frequency help assess physical barriers and gas exchange capabilities, respectively. Epicuticular wax

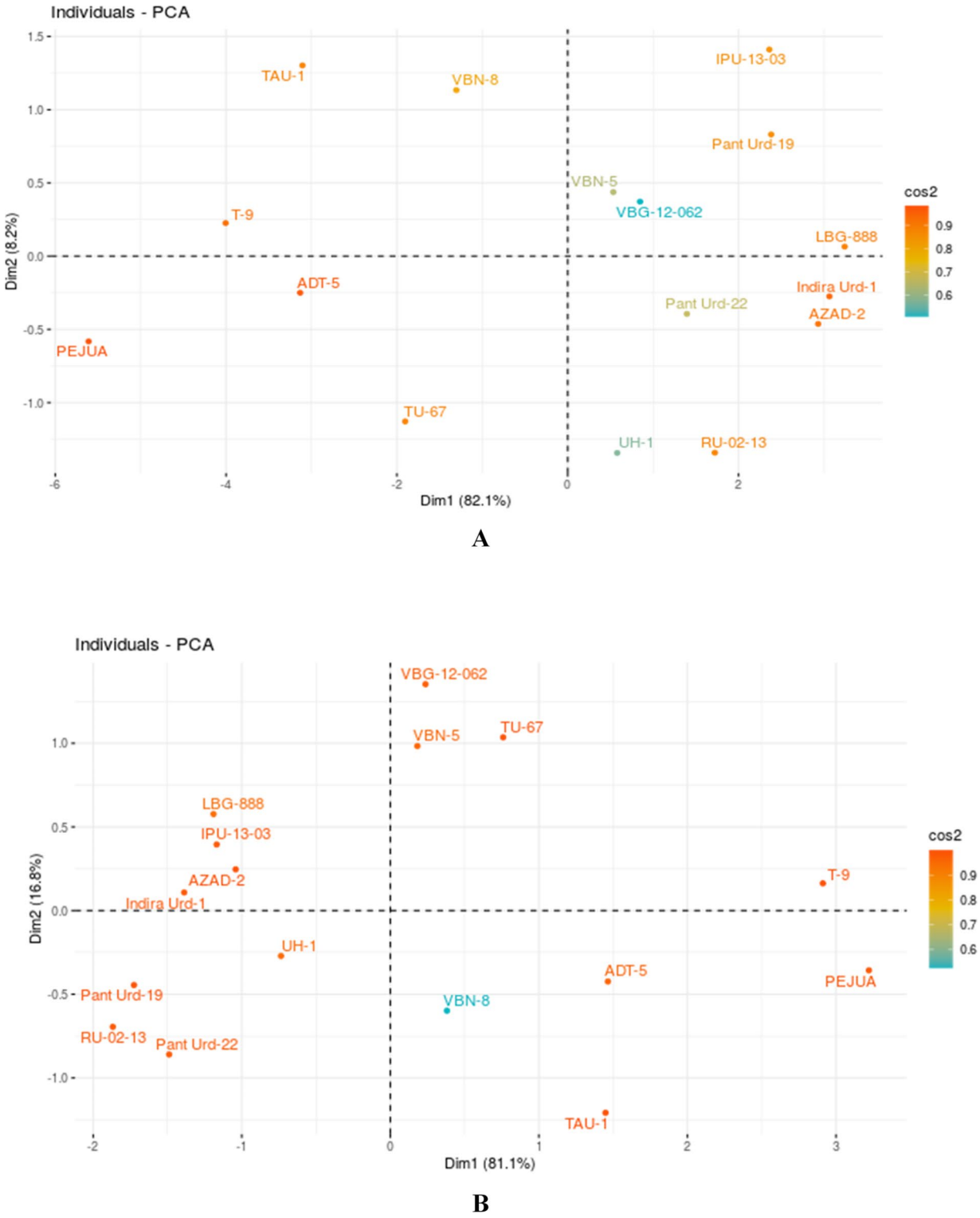

Fig. 5. PCA bioplots of the varieties based on the variation of negatively (**A**) and positively. (**B**) correlated variables with MYMIV disease severity.

levels are indicative of the plant's ability to repel pathogens and reduce water loss. Together, these parameters offer a comprehensive view of a plant's physiological response to stress and its potential resistance to diseases, guiding effective management strategies and breeding programs for improved crop resilience.

The chlorophyll content study revealed that MYMIV-resistant black gram genotypes, such as Indira Urd-1 and Azad Urd-2, maintained higher chlorophyll levels. In contrast, susceptible varieties, particularly PEJUA, experienced a significant reduction in chlorophyll, indicating severe stress and infection impact. The results

indicate that increased disease severity leads to a significant reduction in chlorophyll content in infected black gram leaves. Related findings were noted by Singh and Mall[41] in *Phaseolus mungo* and by Rajitha et al.[42] in black gram. Mantesh et al.[43] suggested that this reduction in chlorophyll content might be due to MYMV infection, which inhibits plastid formation in young leaves. This inhibition impairs the plant's photosynthetic ability, ultimately reducing chlorophyll levels in infected leaves. The decreased chlorophyll content is a clear indicator of the impact of viral infection on the plant's overall health and productivity. The study revealed that MYMIV infection significantly increased total sugar content in black gram, with susceptible genotypes like PEJUA showing the highest levels. This sugar accumulation may attract more whiteflies, exacerbating disease severity and impacting crop health. Thus, varieties with higher sugar content exhibited greater MYMIV severity and higher whitefly populations. Tamilzharasi et al.[34] found similar trends, and Ramarao et al.[44] noted a positive correlation between sugar content and whitefly populations. Gurumurthy et al.[45] and Barman et al.[35] reported that increased sugar content supports higher whitefly populations, leading to greater disease severity. This underscores the role of sugar content in exacerbating MYMIV impact through enhanced vector populations. Leaves of resistant black gram genotypes exhibited higher phenolic content compared to susceptible ones, highlighting the role of phenolics in MYMIV resistance. Elevated phenolic levels at 30 and 60 DAS suggest an adaptive response to increased defense demands, enhancing cell wall integrity through lignin and suberin production, which creates physical barriers against virus transmission[46]. Additionally, phenolic compounds may stimulate antioxidant enzyme activities like phenylalanine lyase, catalase, and peroxidase, aiding in further defense[47]. The increase in total phenol content from 30 to 60 DAS this outcome corresponds to Mahjabeen et al.[48], who observed rising phenol levels in tomato post-CMV infection, and similar trends were reported in black gram by Kumar et al.[18], and in mungbean by Patel et al.[49] and Nagaraj et al.[50].

Enzymatic activity parameters are essential for understanding plant responses to oxidative stress and pathogen attack, particularly in the context of MYMIV infection. POD activity plays a vital role in the oxidative stress response by breaking down hydrogen peroxide, ROS that accumulates during pathogen infection. While, SOD activity is the first line of defense against oxidative stress, converting superoxide radicals into hydrogen peroxide[51]. PAL activity is crucial in the phenylpropanoid pathway, leading to the production of phenolic compounds that reinforce plant defenses[52]. Elevated levels of these enzymes can indicate enhanced plant defense mechanisms, mitigating the impact of MYMIV and improving resistance. POD is a key enzyme in plant defense, providing rapid responses to infections by catalyzing the formation of reactive oxygen species and contributing to lignification, suberification, and the polymerization of cell wall components. It also regulates cell wall elongation, aids in wound healing, and enhances disease resistance[53,54]. In the current study MYMIV-resistant black gram varieties exhibited higher POD activity, with significant increases observed at 30 and 60 DAS, particularly in LBG-888 and IPU-13-03. Conversely, susceptible variety PEJUA showed markedly lower POD activity throughout. Higher POD activity in resistant black gram varieties indicates a robust defense mechanism against MYMIV infection. POD is crucial for defense as it participates in cell wall modifications such as lignification and suberization, enhancing structural barriers[54,55]. It also catalyzes the oxidation of phenolic compounds, increasing antimicrobial activity, and induces programmed cell death near infection sites to halt pathogen spread[56]. This response is evident as resistant varieties like LBG-888 and IPU-13-03 showed significantly higher POD activity compared to susceptible varieties like PEJUA[57]. This is consistent with findings by Sahhafi et al.[58] in wheat and Dieng et al.[59] in brinjal, where higher peroxidase activity was observed in tolerant plants. The increased POD activity aligns with the role of peroxidases in enhancing plant defense and resistance to viral infections. While, SOD is a crucial scavenging enzyme that converts superoxide radicals into hydrogen peroxide, which is then further detoxified by other enzymes[60]. This process helps mitigate oxidative stress and protect cells from damage caused by reactive oxygen species, playing a vital role in maintaining cellular health and defense against various stressors. High SOD activity was observed in resistant varieties like Indira Urd-1 and LBG-888, indicating effective oxidative stress management. Conversely, susceptible varieties such as T-9 exhibited significantly lower SOD activity, reflecting reduced stress tolerance. As MYMIV infection progressed to 60 DAS, oxidative stress increased, and resistant varieties showed better management of superoxide radicals through high SOD activity, which converts superoxide ions to hydrogen peroxide. This reduction in superoxide radicals helps mitigate oxidative stress. In contrast, susceptible cultivars exhibited a significant drop in SOD activity, leading to higher oxidative stress and virus damage. The observed lower SOD activity in susceptible varieties reflects their reduced ability to handle oxidative stress and the resulting severe disease symptoms. SOD plays a crucial role in defending against oxidative stress by scavenging ROS generated during biotic stress[61]. Higher SOD activity in resistant varieties like Indira Urd-1 and LBG-888 indicates their strategy to limit virus colonization by efficiently managing ROS[62]. This is consistent with Ashfaq et al.[63] and Singh et al.[64], who reported decreased SOD in susceptible genotypes, and Yergaliyev et al.[65] in tomato. Plants have evolved multiple defence signalling pathways to cope with adverse environmental conditions and pathogen attack[66].

Varieties with high PAL activity, such as Indira Urd-1 and IPU 13-03, exhibited lower virus infection at various growth stages. In contrast, varieties like PEJUA, TAU-1, T-9, and ADT-5 showed significantly lower PAL activities and higher infection levels. In resistant cultivars, higher PAL activity indicates a robust defense against virus infection. PAL catalyzes the conversion of phenylalanine to t-cinnamic acid, a precursor for salicylic acid, a key signaling molecule in systemic acquired resistance[67,68]. This mechanism enhances antiviral defenses by accumulating lignin and salicylic acid, thereby preventing infection[10,69]. Our findings align with Devi et al.[70–72], who reported increased PAL activity in mungbean under MYMV infection.

Further, multivariate analyses, including Pearson correlation, regression models, and PCA, validated the study findings. Pearson correlation and regression models assessed relationships between variables and MYMIV severity, while PCA identified significant variation among varieties. Leaf thickness, trichome density, and wax content negatively correlated with MYMIV severity, indicating resistance, while stomatal frequency and total sugar content positively correlated, suggesting susceptibility. Biochemical parameters like total phenols,

chlorophyll, PAL, POD, and SOD also showed negative associations with disease severity. Regression analysis revealed that morphological parameters had a higher R^2 value (0.84) at 30 DAS, while biochemical parameters had a higher R^2 (0.91) at 60 DAS, indicating their varying influence over time. PCA showed that variables negatively correlated with disease severity explained 95.58% of variation, with Pejua, ADT-5, and T-9 showing the most variation, while positively correlated variables explained 81.10%, with Pejua, T-9, and TAU-1 showing the most variation. These findings underscore the importance of these traits in developing MYMIV-resistant varieties for effective disease management.

Conclusion

This study identified black gram varieties with strong resistance to MYMIV. Varieties such as PANT URD-19 and LBG-888 demonstrated significant resistance, marked by lower disease severity and higher levels of chlorophyll, phenolic compounds, and key defense enzymes like POD, SOD, and PAL. Resistant varieties also showed thicker leaves, higher trichome density, and greater epicuticular wax content, which may contribute to their enhanced defense mechanisms. Conversely, susceptible varieties like Pejua exhibited higher disease severity, reduced chlorophyll, and increased sugar content. These findings highlight the importance of selecting and breeding for these traits to develop MYMIV-resistant varieties, advancing sustainable disease management in black gram cultivation.

Data availability

All the related data is provided in the manuscript.

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Author contributions

Conceptualization: S.D. and J.T.; Methodology: S.D., M.B., B.A. and M.G.D.; Data curation: S.D.; Formal analysis: S.D., and B.A; Investigation: S.D.; Software: S.D. and B.A.; Writing – original draft: S.D., and M.G.D.; supervision: P.S., and J.T.; review-writing & editing: P.S., M.B., P.R., M.P., T.A., T.W.D., M.G.D. and J.T.; funding acquisition: J.T. All authors contributed to the final paper

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Declarations

Competing interests

The authors declare no competing interests.

Ethical approval

Not applicable, as no approval of research ethics committees was required to accomplish the goals of this study because experimental work was conducted with an unregulated plant varieties.

Additional information

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